Biennial Report 1992-1993

Tropical Forages

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CIAT Centro Internacional de Agricultura Tropical

INDEX

1. INTRODUCTION

This Biennial Report is the first report from the new Tropical Forages Program of CIAT. The last report of the old Tropical Pastures Program was published as a summation of five years research from 1986 to 1991, in a book titled 'Pastures for the Tropical Lowlands: CIAT's Contribution'. If you have not received a copy you are invited to request one.

The Biennial Report was prepared for our Internal Review and the Introduction as an Executive Summary for the Board of Trustees. They have been reproduced as a Working Document to inform colleagues in Latin America and indeed throughout the tropics of our current activities. We invite your response.

1.1 Program Strategy

The Program was formed from the forage genetic resources, plant improvement and utilization components of the previous Tropical Pastures Program and became operational in August 1992. In the re-organization of the old Tropical Pastures Program, positions in Nutrient Cycling, Ecophysiology, Farming Systems and Economics were transferred to the Savannas Program. The Tropical Forages Program was left with a mandate to develop and deliver selected forage ecotypes to the NARS and the Natural Resource Programs in CIAT. The overall strategy has not changed but the Program must now depend much more on collaborative efforts with others to evaluate new germplasm in production systems and to develop management strategies for utilization of this germplasm.

The overall goal of the Program is to identify and develop forages adapted to acid, infertile soils in the humid and subhumid tropics which will contribute to increased and more efficient meat and milk production, soil improvement and erosion control in different production systems and ecoregions.

Development of improved forage systems of high nutritive value is the key to increased and sustainable livestock productivity, as inadequate nutrition is the main constraint in ruminant productivity throughout most tropical regions. However, it is becoming clear that forages also can contribute to more sustainable agricultural systems through soil fertility improvement and control of erosion and weeds.

While the main focus of the Program is on production systems in tropical America, in CIAT's mandated agroecosystems of the Savannas, Forest Margins in the humid tropics and the mid-altitude Hillsides, the policy is to extend activities to Southeast Asia and West Africa with complementary funding.

In tropical America, the native pastures have low productivity, productivity increases but then declines with time on improved pure grass pastures and soils are degrading under cropping.

For the Savannas, it was demonstrated by the former Tropical Pastures Program that legume-grass pastures will give high and sustained animal production. Thus in the Llanos of Colombia, where native savanna only produces 20 kg/ha/year liveweight gain and improved pure grass pastures 200 kg/ha/year, improved legume-grass pastures will produce 400 kg/ha/year. Further, soil fertility is improved for subsequent crop production.

However, this success also opened up new research priorities. A different suite of forage legumes is needed for pasture-crop rotations to take advantage of the higher soil fertility due to fertilization of crops. Also as forage cultivars became more widely used, new constraints appeared, and for some it has become necessary to combine desirable attributes from different genotypes.

For the Forest Margins and Hillsides we are in the process of identifying appropriate forage germplasm to develop similar forage-based technologies as the sustainable rice-pasture system in the savannas.

In the Forest Margins, the need is for more permanent and stable legume-grass associations to prevent the run-down and weed encroachment that occurs following sowing of grasses after forest clearing, and for ground covers in horticultural and tree crops to prevent weed invasion and reduce the use of herbicides.

A suitable legume has been identified in *Arachis pintoi*. It forms stable associations with grasses for pasture situations and has proven to be an aggressive ground cover that resists weed invasion. The main limitation to increased use is that there is little tradition of the use of legumes in pastures by either technologists or farmers. Hence a major effort is required in technology transfer, i.e. training of technologists in on-farm evaluation and to seek funding for this activity.

Following the success of *Arachis pintoi* CIAT 17434, selected from a genetic base of a few accessions, a major effort is in progress with CENARGEN/ EMBRAPA to acquire additional accessions of this species to widen the genetic base with a view to extending the range of adaptation and to ensure there is sufficient genetic diversity for future recombination as constraints appear in the natural germplasm.

In the Hillsides, the main need has been defined as improving the fallow land by the use of forage legumes. In most hillside areas as much as 60% of the land is in 'crop' fallow or 'permanent' fallow. If the productivity of the fallow land can be improved this will result in increased productivity of livestock products, shorter fallow periods and higher crop yields. There are also requirements for shrub legumes for use as supplementary feed in the dry season and as a fuel source and for ground covers and live barriers to slow erosion.

In Southeast Asia, a position has now been located at IRRI. Here there is a need for forage legumes and grasses tolerant of acid soils and which can be used in association with upland rice-based farming systems. Some activities are integrated with the Upland Farming Systems Project of IRRI. The Forage germplasm selected by CIAT in tropical America is also proving to be well adapted for conditions in West Africa. However, there is a need to locate a position there in conjunction with other IARC's in the region to evaluate and move the germplasm into farming systems.

As well as providing well adapted tropical germplasm for the three ecoregions in tropical America, in Southeast Asia and in West Africa, many requests are received for forage germplasm for other regions. The Program thus has to identify a diverse portfolio of germplasm options adapted to different production niches in the acid infertile soils of the tropics, and increase, classify and distribute this germplasm.

The strategy to achieve these objectives was to organize the Program activities into four Project areas:
i) forage genetic resources;
(ii) enhancement of forage germplasm;
(iii) plant attributes for improved feed quality and soil adaptation and improvement;
(iv) utilization of forage germplasm.

In addition we have sought collaborative research arrangements with the NARS. The NARS are very much our partners in the research and development process. Also, together with the donors and farmers they are our clients.

No dramatic change is foreshadowed to the above strategy or the manner in which the Program will operate within the new Project system which will come into effect in 1994. However, the move to Project rather than Section operation and the necessity to seek more Special Project funding to maintain essential activities will require some on-going adjustment within the Program.

From a Center perspective there is a need to reduce the number of Projects from the four mentioned above to three. Essentially this will be achieved by combining activities in the present projects (iii) and (iv) and modification of projects (i) and (ii). Thus it is expected there will be three macro-projects:

Forage Biodiversity and Genetic Resources
Adaptation and Utilization of Forages, and
Enhancement of Genetic Diversity of Key Forage Species

However the structure is not as important as setting the right priorities. To some extent these are constrained by on-going activities. However, we look to more dialogue and improved coordination with NARS scientists when setting up new activities in the future. Likewise, as we strengthen collaborative research, there will be opportunity to seek joint funding for Special Projects.

1.2 Research Activities

The following summary presents some highlights and comments on research activities together with future research priorities. The presentation is based on the more detailed annual report which was prepared in the format of the four Project areas in which research is currently organized.

Project area 1. Forage Genetic Resources

The appointment of a Curator of tropical forages germplasm in the GRU together with that of the Germplasm Specialist position in the Program has been most welcome and enabled some consolidation of activities and new directions of research.

Genetic resource activities. The main advance in germplasm improvement in tropical forages will continue to be through exploiting the natural plant diversity among and within wild species. Thus acquisition by collection, conservation and exchange remain important activities of the Program.

Notable accomplishments have been:

- Acquisition by collection and exchange of a large number of accessions of *Arachis pintoi* from Brazil and *Desmodium* spp. and *Pueraria phaseoloides* from Vietnam.
- Linkage of the operations and passport data bases.
- Distribution of 4000 forage germplasm samples to 25 countries.

Future priorities:

* To complete the placement of the 21000 grass and legume accessions in long-term storage. There are now 5000 accessions in long-term storage, 1100 having been added in 1992-93.
* This means limiting immediate collection trips to that of promising species, e.g. *Arachis* spp. and *Cratylia argentea.*
* Complete the publication of the regional germplasm catalogs.

Rhizobia and mycorrhizae. The main activity has been maintenance of the 4000 rhizobia and 600 mycorrhizae strains and provision of inoculant cultures to researchers. Additional *Bradyrhizobium* strains have been acquired for *Arachis* and there is an on-going activity of evaluating native mycorrhizae in different soils.

Future priorities:

* To investigate the persistence of applied rhizobia cultures in soils under different crop-pasture systems.
* To explore the benefit of rhizobia with antifungal properties for plant protection.
* To consolidate the rhizobia collections within CIAT.

Environmental adaptation of germplasm. Data on environmental adaptation, i.e. to biotic, climatic and edaphic constraints, is necessary in addition to that of the rather limited data obtained during plant collection (the passport data) and that from the initial growing out of new accessions for morphological characterization and seed increase.

Several major evaluations have been completed:

- Savannas. Collections of the herbaceous legumes, *Arachis* spp. and *Calopogonium* spp., a collection of shrub legumes, and the grass genus, *Paspalum*, in the Cerrados of Brazil. Collections of the herbaceous legumes, *Calopogonium* and *Pueraria*, and shrub legumes, *Cratylia argentea, Desmodium velutinum, Flemingia macrophylla* and *Tadehagi* spp., in the Llanos of Colombia. The evaluation in Brazil identified an *A. pintoi* accession, BRA 31143 (CIAT 22160), that is adapted to long dry seasons and two *C. mucunoides* accessions that retain green leaf in the dry season.
- Humid tropics. Collections of the shrub legumes *Cratylia argentea, Flemingia macrophylla* and *Desmodium velutinum* were evaluated in Costa Rica and Caquetá, Colombia, and *Codariocalyx gyroides* at the latter site where it was outstanding on poorly drained soils.
- Semi-evergreen forest site, Atenas, Costa Rica. In an evaluation of 90 *Leucaena* accessions, CIAT 17263 was outstanding in dry matter production during both the wet and dry seasons.
- Multisite evaluation. 30 accessions of *Centrosema pubscens* were evaluated over 15 sites in various ecosystems.
- Southeast Asia. Accessions that have

performed outstandingly are the legumes - *Stylosanthes guianensis* CIAT 184 and some new hybrid gene pools, *Arachis pintoi* CIAT 18750 and 18744, *Centrosema pubescens* CIAT 15470 and 438, *Desmodium ovalifolium* and the grasses, *Brachiaria brizantha* CIAT 16318, Andropogon gayanus CIAT 6221 and *Panicum maximum* CIAT 6299.

- This has led to the compilation of a list of germplasm suitable for evaluation by NARS in regional trials for environmental adaptation or, after seed multiplication, direct evaluation in production systems.

Future priorities:

* Savannas. The higher soil fertility in crop-pasture rotations requires a re-evaluation of legumes for this system.
* Humid tropics. Establish a site in collaboration with EMBRAPA.
* Hillsides. Legumes suitable for improving the fallow land and shrub legumes for supplementary feed.
* Southeast Asia. Feedback is needed of the acceptability of recent selections in smallholder systems.
* Analysis of completed evaluations.
* To organize the evaluation and passport data bases for easier interrogation.

Evaluation in production systems. Once an accession has shown promise of environmental adaptation, then it is evaluated as a component of a production system where there is a perceived need for that type of forage. This activity is generally carried out with NARS or other CIAT programs.

Current evaluations are proceeding of:

- Savannas. *Stylosanthes* spp. in crop-pasture rotations and new *Panicum maximum* accessions under grazing.
- Humid tropics. *Arachis pintoi* in association with grasses under grazing and as a cover in tree crops.
- Hillsides. *Cratylia argentea* as a fodder legume.
- Southeast Asia. *S. guianensis* CIAT 184 in smallholder fallow.

Future priority:

* To move selected germplasm more rapidly to evaluation in production systems.

Project area 2. Enhancement of forage germplasm

Once forage species have found acceptance in production systems, it is desirable to overcome well-defined constraints that limit their exploitation. This may be initially by acquisition of a broader germplasm base to extend the range of adaptation and management studies to enhance their utilization as with *A. pintoi*. But where combinations of desirable attributes are not found in natural accessions, genetic recombination is necessary. This applies in the case of *Brachiaria* and *Stylosanthes*.

Improvement of Brachiaria. There have been two lines of activity (i) the acquisition and evaluation of a large collection of different *Brachiaria* species and, (ii) the development of spittlebug resistance with the retention of acid soil adaptation, high feed quality and leafcutter ant resistance by recombination.

Evaluation of the *Brachiaria* collection has taken place in the savannas in Colombia and Brazil (with EMBRAPA), and in the humid tropics in Costa Rica. Of particular interest are some lines of *B. humidicola* which appear to have higher feed value than the commercial cultivar, variations in the onset of flowering and *B. brizantha* accessions with good adaptation to acid soils.

Both the success and speed with which spittlebug resistant gene pools of *Brachiaria* will be obtained has been enhanced this year by two developments. One is improved field screening through use of a second screening site at Caquetá and new glasshouse procedures which can handle a greater quantity of material.

The second is the improved ability to manipulate apomixis through tagging the apomictic gene with PCR markers so apomixis can be detected in seedling plants. This research is being carried out with the BRU who have also been able to regenerate *Brachiaria* from callus tissue and who are working to produce dihaploid *Brachiaria* genotypes using microspore culture.

Future priorities:

* Complete evaluation of the *Brachiaria* collection, in particular for desirable attributes such as spittlebug resistance, acid soil adaptation and feed quality.
* Refine the screening procedure for spittlebug resistance.
* Complete the tagging of the apomixis gene in *Brachiaria*.
* Continue with recombination and selection for spittlebug resistance.
* Complete the anther microspore culture and use this development in conjunction with the polymorphic primers to construct a PCR - based map of *Brachiaria*.
* Host a workshop on *Brachiaria* in October 1994 to review present information on the genus and coordinate future activities.

Improvement of Arachis. *A. pintoi* is the most productive, high quality and persistent legume that has been identified for grass-legume pastures in the humid tropics and the savannas of Colombia and which has also proved useful as a green cover in tree crops. At an international workshop held by the Program in May, two main activities were suggested (i) to continue acquisition and evaluation in order to broaden the genetic base and extend the range of adaptation, and (ii) to promote utilization of the present successful accession CIAT 17343 in order to increase awareness of the value of this species. Better definition of conditions for successful establishment and lower seed costs are also prerequisites for greater utilization.

Highlights of the continued activities to achieve this are:

- The present availability of almost 80 accessions of *A.pintoi*.
- Identification of an ecotype adapted to long dry seasons.
- A regional workshop that developed plans for the on-farm evaluation of *A. pintoi* in 8 countries of Central America and the Caribbean.
- Demonstration of more rapid establishment from seeds than vegetative material.

Future priorities:

* To increase the availability of seed of the new accessions for multisite evaluation.
* To refine methods of establishment that can be used successfully by farmers.
* To obtain funding for development projects for on-farm evaluation as a means of increasing awareness of *A. pintoi*.

Improvement of *Stylosanthes*. Several cultivars of natural accessions of *Stylosanthes* have been released in tropical America but all have one or other deficiencies that detract from widespread adoption. The most important is the lack of broad based resistance to anthracnose but low seed yields and poor seedling vigor are also important. Some advanced gene pools of *S. guianensis* have been developed and show increased anthracnose resistance over earlier selected cultivars. They have yet to be evaluated widely.

However, definite advances will only be made when more is known of the distribution of races of *Colletotrichum*, and their virulence in relation to different species. These studies will be made as a prelude to a decision on further breeding.

Some highlights of the research in this activity are:

- Field screening of further *Stylosanthes* accessions.
- Ability of the new gene pools to maintain resistance to anthracnose at Carimagua.
- Development of a reliable screening procedure for anthracnose.
- Steps taken to clone a gene encoding antibiotics from a bacterium and transfer it to *Stylosanthes* as an alternative to host plant resistance.

Future priorities:

* Studies on host-pathogen relationships.
* Transfer of the bacterial gene for antibiotic production.
* Multilocational evaluation of previously selected gene pools in production systems.
* Studies on the importance of seed yield and seedling vigor on persistence.

Project area 3. Plant attributes for improved feed quality and soil adaptation and improvement

Major limitations to forage improvement in the tropics are the low digestibility of many tropical legumes, in particular shrub legumes, and low soil fertility which necessitates identifying forages with the ability to extract and utilize mineral nutrients efficiently. Improved understanding in these areas will allow development of screening procedures for use in evaluation and breeding and also suggest management practices to overcome some constraints.

Advances made in this project area were:

- Demonstration of differences between legumes in the nature of tannin complexes and identification of shrub legumes with low tannin, e.g. *Cratylia argentea* and *Desmodium velutinum*.
- Increased feed intake and nitrogen retention in sheep by decreasing tannin in *D. ovalifolium* using polyethylene glycol.
- Increased intake of *C. argentea* with mature vs.immature leaf, but with pre-wilting improving the intake of the latter.
- Demonstration that one reason for the success of *Arachis pintoi* in competing with aggressive grasses was the ability to acquire soil phosphorus more efficiently.
- Demonstration that factors other than high soil aluminum are responsible for adaptation to acid infertile soils.
- Description and photographs of foliar nutrient deficiency symptoms of major pasture species.

Future priorities:

* Improved management for the utilization of *Cratylia argentea.*
* Further elucidation of the nature of tannin complexes with the objective of an improved screening procedure for legumes containing tannin.
* Collaborative research to genetically engineer the reduced production of tannin in *Desmodium ovalifolium.*
* Identification of the causal factors for acid soil adaptation.
* Investigation of the role of organic acids on nutrient acquisition in acid soils.
* Investigation of the contribution of mineral nutrition to nitrogen fixation by legumes in acid soils.

Project area 4. Utilization of forage germplasm

The measure of success of a forage improvement program is the utilization of new forage ecotypes in production systems. The strategy of the Program is to act as a facilitator with NARDS to ensure that this happens. While improved grasses have been readily adopted by the farmers and the seed industry, legumes have not. The reasons are several (i) there is no tradition of use of legumes as sown forages (ii) the seed industry will not respond until there is a demand and (iii) different technologies are required for management and seed production of legumes than grasses. The strategy to hasten utilization involves simultaneous on-farm evaluation and development of seed systems, training, use of national and regional networks and communications and finally economic assessment.

Important activities in this area have been:

- Continued demonstration of higher liveweight gains with grass- *A. pintoi* associations than with grass alone.
- The production of forage seed as an intercrop with upland rice.
- A seed system for production of *A. pintoi* seed by small farmers.
- Development of oversowing technique.
- A RIEPT workshop on 'Expanding seed supply of tropical forages'.
- A regional workshop that developed plans for the on-farm evaluation of *A. pintoi* in 8 countries of MCAC.
- Development of a methodology for evaluating the economic and social impact of research into pasture improvement.
- Continued publication of 'Pasturas Tropicales' as a mean for NARS to publish research on tropical forages.

Future priorities:

* Study establishment and early management of *A. pintoi.*
* Develop and promote new seed systems.
* Facilitate development of national and regional networks.
* Provide training through workshops.
* Establish regional newsletters.
* Obtain Special Project funding for collaborative R & D projects.

1.3 Collaborative Research Projects

Inter-American Bank-financed project on identification of new forage species and development of improved *Brachiaria* cultivars

This project provides a major portion of the operational funds for forage evaluation, investigations into the nature of spittlebug resistance in grasses and the development of new *Brachiaria* gene pools with resistance to spittlebug, high feed quality and acid soil adaptation.

Forage seeds project - Southeast Asia

This is a joint project with the CSIRO Division of Tropical Crops and Pastures, funded by AIDAB, to introduce new forage germplasm into the region and evaluate it for use in smallholder farming systems.

Nutrient cycling and animal production

This is a joint activity, funded from core funds, with the Savannas Program in which the role of nutrients on legume and grass production and subsequent animal production is being investigated. Animal performance is closely related to the amount of legume in the pastures with the amount of labile nitrogen in the system being the main variable that drives the system.

Role of improved forages in a hillside farming system

This a joint activity being developed with the Hillsides Program. to evaluate the role that improved forages, in particular legumes, can play in increasing overall productivity. The main focus will be on the role of legumes in the fallow to increase forage quality and soil fertility. It is expected that this will be financed by Special Project Funds.

Legume establishment

TARC funded a position until October 1993 to investigate how new techniques in seed pelleting could be used to reduce the costs of legume establishment in the savannas. Successful establishment was achieved by broadcasting pasture seed, combined with fertilizer, either in the form of a pellet or in a paper bag. This technique will have application in the hillside areas. A new project area for support by TARC is under consideration.

Rhizosphere phosphorus dynamics

This is a collaborative project with the University of Hohenheim to investigate the importance of organic acid excretion and other factors in the rhizosphere on phosphorus uptake. It is funded by BMZ.

2. FORAGE GENETIC RESOURCES

Purpose

To identify, conserve, and propagate productive forage germplasm for different production systems in selected ecoregions.

Rationale

The main advances in germplasm improvement in tropical forages have been through exploiting the natural plant genetic diversity among and within species. This is still an important area for germplasm improvement. Acquisition, characterization, and conservation of wild legume and grass species with forage potential will remain important activities. The TFP will continue to screen species for the acid infertile soils of the humid and subhumid tropical lowlands for use in long-term pastures. New areas of focus are germplasm for use in crop-pasture rotations for the savannas; for multiple purposes in the mid-altitude hillsides; as multipurpose trees and shrubs (MPTS); as forages and green manures for Southeast Asia; and as forages for West and Central Africa. Rhizobia and mycorrhizae collections need to be maintained for research purposes and use with this forage germplasm.

Highlights

* Acquisition of new *Arachis* germplasm widened the genetic base of this genus considerably.

* Accessions of *Arachis pintoi* and *Calopogonium muconoides* with dry season tolerance were identified for the Cerrados.

* Outstanding performance of *Codariocalyx gyroides* in poorly drained situations in the humid tropical lowlands.

* Establishment of a wide range of germplasm for evaluation of adaptation to mid-altitude hillsides in the Cauca Department of Colombia.

* A new research focus on multipurpose tree and shrub legumes for acid soils. Morphological characterization as well as studies on acid soil adaptation in the most promising species, *Cratylia argentea*, *Leucaena leucocephala*, and *Codariocalyx gyroides*.

* The following species were identified as having potential in West Africa: Herbaceous legumes, *Stylosanthes guianensis* CIAT 184, *Centrosema brasilianum* CIAT 5234, *C. macrocarpum* CIAT 5452; shrub legumes, *Cajanus cajan* CIAT 18700, *L. leucocephala* CIAT 17502 and *Flemingia macrocephylla* CIAT 17403; grasses, accessions of *Panicum maximum* and *Brachiaria brizantha*.

* In Southeast Asia a number of forage accessions have been identified for regional evaluation and already a few for on-farm testing. Selected forages of wide-spread adaptation include: *Andropogon gayanus, B. brizantha, B. humidicola, B. dictyoneura, Paspalum atratum*, *Centrosema pubescens, Desmodium heterophyllum*, *A. pintoi* and accessions and hybrid derivatives of *S. guianensis*.

2.1 Genetic Resources Activities

Acquisition. The TFP's general research strategy is to exploit the natural genetic variability of undomesticated species, especially of legumes. In recent years, acquisition of new germplasm was strategically focused on filling in geographic and genetic gaps, and in response to international requests to specific needs. During 1992-1993, the collection increased by a total of 717 accessions (Table 2.1), which originated predominantly from the germplasm collected in 1992 in Thailand and Vietnam. Important donations of *Panicum maximum*, originating from the French ORSTOM collection, and of *Arachis pintoi* and *A. repens* were received from the Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnología (CENARGEN), Brazil. (B.L. Maass and A. Ortíz).

CIAT scientists participated in six collection trips with emphasis on wild *Arachis* species, under the leadership of Drs. J. F. M. Valls and L. Coradín from CENARGEN. Seventy-nine accessions of *Arachis* were assembled and will be duplicated at CIAT headquarters after initial increase in Brazil. (E.A. Pizarro and B.L. Maass).

New legume germplasm was acquired in two collection missions to Vietnam and Thailand under the leadership of Dr. R. Schultze-Kraft, in collaboration with different national agricultural organizations. This activity was jointly funded by CIAT and the International Board for Plant Genetic Resources (IBPGR). The collection route in Vietnam followed a north-south transect of the country from Hanoi to Ho Chi Minh City, and in Thailand, germplasm was collected from the central and northeastern regions, emphasizing species of *Desmodium* and related genera, and *Pueraria phaseoloides*. New forage type pigeon pea (*Cajanus cajan*) germplasm was donated by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and several leguminous species which may adapt well in tropical mid-altitude, including *Sesbania* species, were introduced from the International Livestock Centre for Africa (ILCA). Most of this new germplasm is presently being cleared in post-entry phytosanitary follow-up; it will then undergo initial seed increase (B.L. Maass and A. Ortíz).

Initial seed increase. After acquisition and release from phytosanitary follow-up, germplasm has to be multiplied. So far, 70% of the tropical forage germplasm collection maintained at CIAT has been increased. About one-third of the remaining backlog of almost 6300 accessions belongs to species of *Desmodium*, *Stylosanthes*, and *Aeschynomene*. *Brachiaria*, *Centrosema*, *Chamaecrista*, and *Rhynchosia* account for another 20%. In addition, seed stored in cold rooms has to be regenerated periodically because viability decreases with time. Initial increase and rejuvenation is carried out for 2150 accessions annually. Besides the key genera *Arachis*, *Centrosema*, *Desmodium*, *Pueraria*, and *Stylosanthes* collections of *Chamaecrista*, *Erythrina*, *Galactia*, *Macroptilium*, *Neonotonia*, *Phyllodium*, *Vigna*, and *Paspalum* received the most attention for seed increase.

Because there is difficulty in producing seed of reasonable quality of most grass species, entire collections of the genera *Brachiaria*, *Hyparrhenia*, and *Panicum maximum* are maintained as field collections at CIAT-Quilichao, and *Andropogon* at CIAT-Palmira. These field collections serve for seed increase, obtaining vegetative material for germplasm distribution, and characterization (A. Ortiz and B.L. Maass).

Characterization. The main objective of this work is to describe the existing intraspecific variability, which helps to classify germplasm into morphological plant types, and to develop descriptors. This is carried out with CIAT's Genetic Resources Unit (GRU). A large effort was made in characterization of the shrub legume collection. Little morphological variation was found in the small collection of *Cajanus cajan* maintained at CIAT, which originated mainly from Brazil. Among 81 accessions of *Desmodium velutinum*, however, ample variation was found, and seven morphological plant types were identified according to leaf shape, variegation, and pubescence, growth habit, and plant height. The *Flemingia* collection comprises 53 accessions of *F. macrophylla* and other species, some of which are not yet identified. The morphological variation found in *Flemingia*, led to the definition of 11 morphological plant types, which may even reflect different species. Most of the *Phyllodium* germplasm has not been identified at the species level. The five morphological types determined are probably different species.

A collection of 265 accessions in several species of *Chamaecrista* was characterized at CIAT-Quilichao. Large inter- and intra-specific variation was found in most of the 38 morphological descriptors recorded such as leaflet, stipule, and pod shape and size. Maturity type and seed production are very variable, and great differences exist in longevity: many materials died after the first year, some are perennial (J. Belalcázar and B.L. Maass).

Conservation. CIAT needs to have a base collection stored under long-term conditions and to have the collection conserved in at least one other institution as a safeguard against the risk of loss. During 1992-1993, a total of 1094 samples were put under long-term storage, which increased the total to 4965 samples (Table 2.1) (A. Ortíz, B.L. Maass, and J. Belalcázar).

Documentation. Reliable documentation and efficient data management are basic for germplasm management. CIAT's Information Management and Network Support (IMNS) implemented the new data management system, ORACLE, which now is fully operational. A system of integrated data management for all aspects of germplasm management is being completed in collaboration with the IMNS.

Table 2.1. Acquisition, inventory, and distribution of tropical forage germplasm by CIAT's Genetic Resources Unit in 1992 and 1993. (no. of accessions as of 30.09.1993)

Genus	Short-term storage		Long-term storage	Distribution in 1992-1993 (no. samples)
	New in 1992-1993	Inventory 1993	Inventory 1993	
Legumes				
Aeschynomene	20	999	252	107
Arachis	23	42	9	193
Cajanus	17	106	56	171
Calopogonium	8	535	112	232
Centrosema	32	2404	977	1128
Chamaecrista	14	305	4	288
Codariocalyx	2	37	0	116
Cratylia	0	14	0	119
Desmodium	149	2925	621	526
Flemingia	23	146	35	164
Galactia	15	565	352	446
Leucaena	4	198	85	104
Macroptilium	10	607	397	174
Pueraria	17	255	45	46
Rhynchosia	6	447	8	24
Stylosanthes	22	3586	810	808
Teramnus	10	379	77	110
Vigna	19	746	327	100
Zornia	5	1030	49	925
Other	243	3284	749	635
Total legumes	639	18610	4965	6416
Grasses				
Andropogon	0	100	0	6
Brachiaria	1	689	0	533
Hyparrhenia	0	60	0	66
Panicum	59	598	0	111
Paspalum	4	119	0	5
Pennisetum	0	53	0	62
Other	14	454	0	20
Total grasses	78	2073	0	803
Other families	0	2	0	0
Grand total	717	20685	4965	7219

In the face of the growing number of species maintained in CIAT's tropical forages germplasm bank, the need was recognized to document and standardize their scientific names. Therefore an inventory of tropical forage species maintained in the germplasm bank was prepared and published as a working tool, which may serve as a reference to avoid frequent spelling mistakes of names and authors (Torres et al., 1993)[1]. The inventory was widely distributed to collaborators.

Correct identification of germplasm was given high priority. The collaboration with leading taxonomists resulted in reducing germplasm which still lacks identification at species level from 3,700 to 2,600 accessions in the last four years. The revision of passport data continued, and was completed for those accessions which originated from Colombia and Brazil. Germplasm catalogs are presently being edited. Important institutions with which CIAT has jointly collected or exchanged large portions of germplasm, such as CENARGEN, CSIRO, ILCA, and the University of Florida, have been contacted, and data are being exchanged (B.L. Maass, A. Ortiz, A.M. Torres, and J. Belalcázar).

Herbarium. The reference herbarium grew, particularly in its representation of genera and species. At present, 111 of the 167 registered genera and 533 of the 806 species of the tropical forages germplasm collection are held in the herbarium. The represented accessions increased by more than 1700 specimens to 11,470, that is, almost 50% of the registered accessions. The herbarium laboratory received visitors from several countries who carried out taxonomic and other botanical studies (A.M. Torres and B.L. Maass).

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[1] Torres, A.M., J. Belalcázar, B.L. Maass and R. Schultze-Kraft. 1993. Inventory of tropical forage species maintained at CIAT. Working document No. 125. CIAT, Cali, Colombia, pp. 36.

[2] Franco, M.A., G.I. Ocampo, E. Melo and R. Thomas. 1993. Catalogue of Rhizobium strains for tropical forage legumes. CIAT, Cali, Colombia, pp. 123.

2.2 Rhizobia and Mycorrhizae

Bradyrhizobium **collection and activities.** The collection of over 4000 strains is maintained and occasionally added to when a specific need has been identified. Around 100 requests for forage legume inoculants and ampoules are serviced per annum. An updated fifth version of the *Bradyrhizobium* collection catalog for forage legumes was completed and published in 1993 with the further characterisation of some 2056 strains (Franco et al., 1993)[2].

This year the TFP identified a need to expand the options for forage *Arachis* and consequently strains were collected from *A. pintoi* plants grown at Manizales, Colombia and various localities in Brazil. Sixteen new strains have been added to the collection and results from experiments done at Palmira are presented in Table 2.2. No strains were superior to the recommended strain 3101 but 9 out of the 40 strains isolated were found to be as effective (R.J. Thomas and G. Ocampo).

Mycorrhizae - collection and activities. In order to evaluate the role of mycorrhizae in plant nutrition and productivity, CIAT maintains a collection of mycorrhizae from a range of tropical soils. This collection of cultures must be characterized so that their purity can be monitored. At present, there are 479 cultures from 31 described fungal species in the collection (Table 2.3), that are being propagated or are stored at 10°C in a cold room. These cultures originated from 3 Latin American and 8 countries from other continents. The collection has over 400 slide vouchers , i.e. spores are permanently mounted in polyvinyl lacto-glycerol (PVLG/PVLG + Melzeré reagent), and 120 vial vouchers. They are used for documentation, training, and by visitors for taxonomic comparisons.

As most of the cultures maintained at CIAT have not been pure cultures, they are treated as trap cultures. In this way, we can ascertain which fungi in these cultures will propagate well under our greenhouse environment, and thus be successfully subcultured. At least once during a calendar year, spores from the 600 cultures stored are extracted for monitoring. The mycorrhizal laboratory provides service to evaluate the effectiveness of native mycorrhizae in different soil types as determined by percentage of root infection and number of spores in soil (I.M. Rao and C. Cano).

Table 2.2. Effect of *Bradyrhizobium* strains isolated from nodules of *Arachis pintoi* grown at Manizales on 9-week-old plants of *A. pintoi*.

Treatment (strain no.)	mg N/plant		Nodule/plant (no.)	
	Expt. I	Expt. II	Expt. I	Expt. II
+ 90 kg/ha N[a]	22.0 a[b]	61.1 a	2.0 cd	29 e
- Inoculum	9.2 b	9.9 b	15.4 d	67 cd
3101	24.4 a	65.0 a	33.4 b	90 abcd
5036	20.8 a	-	54.4 a	-
5037	26.9 a	-	55.2 a	-
5038	-	51.0 a	-	74 bcd
5039	-	57.1 a	-	70 cd
5040	-	57.4 a	-	68 cd
5041	-	64.4 a	-	56 cd
5042	-	63.0 a	-	91 abc
5043	-	53.0 a	-	111 a
5044	-	54.3 a	-	106 ab

a. Urea-H.
b. Numbers in columns followed by the same letter are not significantly different ($P<0.001$).

Table 2.3. Present status of the mycorrhizal collection at CIAT.

Genus	Described species (no.)	Undescribed species (no. of isolates)
Acaulospora	10	41
Entrophospora	3	-
Glomus	14	94
Scutellospora	4	12

2.3 Germplasm Evaluation for Environmental Adaptation

The Program evaluated germplasm for environmenta adaptation, i.e., adaptation to abiotic (acid, infertile soils and dry season) and biotic (pests and diseases) constraints. There are 8 major screening sites Hillsides (Quilichao, Pescador in Colombia); Savannas (Carimagua in Colombia; Planaltina in Brazil); Humid tropical lowlands (San Isidro, Guápiles in Costa Rica Florencia in Colombia and Los Baños and Cavinti in Southeast Asia); Semi-evergreen forest (Atenas in Costa Rica). Large collections were evaluated at many of these sites (Table 2.4). The most important results are highlighted by ecosystem. The results for the key genera *Arachis, Brachiaria, Centrosema, Panicum, Paspalum*, and *Stylosanthes* are reported in Project 2 - Enhancement of forage germplasm.

Table 2.4. Germplasm evaluated at the major screening sites of the CIAT Tropical Forages Program during 1992-1993.

Genus, species	Hillsides	Savannas		SEG[a] forest	Humid tropical lowlands		
	Cauca, Colombia[b]	Llanos, Carimagua	Cerrados, Brazil	MCAC[c] Atenas	MCAC[c] Guápiles	Amazonia, Caquetá	SE-Asia Philippines
Herbaceous legumes							
Aeschynomene spp.	-	-	4	-	-	-	82
Arachis spp.	7	8[d]	46	-	-	8	8
Cajanus scarabaeoides	-	28	15	-	-	15	-
Calopogonium mucunoides	4	30[e]	215	-	-	34	8
Canavalia spp.	6	120	-	-	-	-	-
Centrosema rotundifolium	2	6[d]	-	-	-	-	-
other *Centrosema* spp.	43	27[d]	-	-	-	6	45
Chamaecrista rotundifolia	8	80[b]	8	-	-	49	-
D. heterocarpon ssp. *ovalifol.*	5	28[d]	-	-	-	-	5
Desmodium heterophyllum	-	-	-	-	-	11	22
other *Desmodium* spp.	24	57	-	-	-	11	39
Dioclea guianensis	-	19	-	-	-	19	-
Galactia striata	6	385[b]	-	-	-	-	21
Pseudarthria viscida	-	33	-	-	-	-	-
Pueraria phaseoloides	3	163[d]	-	-	-	8	4
other *Pueraria* spp.	2	11[d]	-	-	-	-	-
Stylosanthes spp.	5	97[b]	-	-	-	5	42
Uraria spp.	-	38	-	-	-	-	-
Zornia spp.	-	-	-	-	-	7	34
Other	39	4[e]	-	-	-	-	23
Shrub legumes							
Codariocalyx gyroides	1	27	22	-	-	27	-
Cratylia argentea	2	11[e]	11	1	1	11	2
Desmodium velutinum	3	107	-	-	1	83	14
Flemingia macrophylla	-	65	10	-	2	55	6
Leucaena spp.	5	-	23	90	-	-	-
Sesbania spp.	1	-	17	-	-	-	-
Tadehagi spp.	-	40	-	-	-	-	-
Other	15	25	6	-	-	-	3
Total legumes	181	1409	377	91	4	349	358
Grasses							
Brachiaria spp.	17	263	-	-	294	-	22
Panicum maximum	7	30[d]	-	-	-	77	13
Paspalum spp.	6	-	78	-	-	35	-
Other	17	-	-	-	-	31	-
Total grasses	47	293	78	0	294	143	35

a. SEG = Semi-evergreen forest.
b. Established in 1993.
c. MCAC = Mexico, Central America and Caribbean.
d. In both Carimagua and Villavicencio, Colombia.
e. In Villavicencio, only.

Savannas - Llanos, Colombia

Calopogonium mucunoides. Thirty accessions have been evaluated both at Carimagua and Villavicencio. Establishment was good, and most accessions produced high dry matter yields (DMY) during the first cut. At Carimagua, regrowth was poor. All accessions were completely defoliated during the dry season in the second year after establishment and did not regrow in the following rainy season. Soil seed reserves were low and no seedling regeneration occurred. At Villavicencio, performance during the dry season was also poor but there was good regrowth and seedling regeneration after the first rains. Wide variation was found with regard to DMY, stolon root development, soil cover, and seed production, which ranged between 8 and 110 g/m^2. However, the high incidence of an isometric virus in all accessions may limit the usefulness of this species in the area of Villavicencio (G. Keller-Grein, F. Díaz, and E. Cárdenas).

Pueraria phaseoloides. One hundred and sixty-three accessions of *P. phaseoloides* were evaluated at Villavicencio. Already in the early evaluation phase, a majority of the accessions showed nutritional deficiencies, and DMY declined drastically over time. After two years of evaluation under cutting, many accessions had disappeared or were of little vigor, which resulted in heavy weed invasion of the plots. A group of accessions, however, was identified for good performance, especially with regard to seasonal DMY and stolon root development (CIAT 9188, 17294, 17296, 17297, 17302, 17315, 17766, 18381, 20013, and 20224). Performance of the check CIAT 9900 was poor (G. Keller-Grein and F. Díaz).

New legume trials. In 1993, two collections of herbaceous legumes were established at Carimagua for evaluating environmental adaptation: almost 400 accessions of *Galactia striata* and about 80 accessions of *Chamaecrista rotundifolia* (syn. *Cassia rotundifolia*). Both collections show a wide range of variation regarding morphological characteristics and general adaptation (B.L. Maass and E. Cárdenas).

Savannas - Cerrados, Brazil

In addition to evaluations of *Paspalum* and *Arachis* a collection of 215 accessions of *Calopogonium mucunoides* was evaluated. The following accessions were selected for further seed increase and evaluation, CIAT 822, 884, 887, 7722, 8404, 8405, 8513, 9111, 9450, 18065, 18107, 18564, 20676, and 20709. Accessions CIAT 822 and 20709 maintained green leaf during the dry season.

Humid tropical lowlands - Caquetá, Colombia

In early 1992, new germplasm screening activities were initiated at the "La Rueda" ranch near Florençia, Caquetá, which is owned by the Fondo Ganadero del Valle. In addition to very heavy rainfall, soils at this site have poor internal drainage. Pre-selected accessions of a broad range of herbaceous legumes were evaluated in a 9-week cutting regime, with DMY measured in the period of minimum and the period of maximum rainfall.

Desmodium. Besides *Desmodium heterocarpon* subsp. *ovalifolium* no other species of *Desmodium* (*D. heterocarpon, D. heterophyllum*, and *D. strigillosum*) was adapted to the environmental conditions. Among accessions of *D. heterocarpon* subsp. *ovalifolium*, CIAT 350, 13095, 13125, 13400, and 13647 established and covered the soil rapidly, and had the best performance regarding DMY.

Pueraria phaseoloides. All 8 accessions of *P. phaseoloides* established well. The best adapted accessions were CIAT 7978 and 17765 because of rapid soil cover and highest DMY in both seasons of minimum and maximum rainfall. The check accession CIAT 9900 performed particularly poorly during the period of maximum rainfall.

Cajanus scarabaeoides* and *Zornia. A germplasm collection of 34 accessions of *Cajanus scarabaeoides*, a species native to the Asian tropics, confirmed the little promise of this species for the humid tropical lowlands. Seven selected accessions of *Zornia* almost disappeared sixteen months after establishment (B.L. Maass, C.G. Meléndez, and Gerhard Keller-Grein).

Multipurpose shrub legumes - Llanos, Colombia

Since 1989 emphasis has been given to testing shrub legume species. Evaluation of collections of *Cratylia argentea, Desmodium velutinum, Flemingia macrophylla* and species of *Tadehagi* was concluded in 1992.

C. argentea was well adapted to the environmental conditions both at Carimagua and Villavicencio. There were differences in DMY among accessions at both sites, the yields being considerably higher at Villavicencio than at Carimagua. Leaf proportion of total DMY and IVDMD were relatively high.

With regard to the evaluation of *D. velutinum*, *F. macrophylla* and *Tadehagi* spp., the intraspecific variation found for some important agronomic traits and IVDMD is summarized in Table 2.5. Leaf DMY varied considerably between seasons. All species responded to the higher soil fertility regarding leaf DMY and primary regrowth branches while there was no effect on IVDMD. Mean leaf DMY were highest for *F. macrophylla* reflecting the excellent adaptation of most of the accessions to the environment. IVDMD, however, was very low for this species and *Tadehagi*, but much higher in the accessions of *D. velutinum*.

After completing the agronomic evaluation, acceptability of the three collections to cattle was evaluated in a 12-week-old regrowth during the 1993 dry season. Each collection was fenced off separately and grazed by three steers from 0800 to 1600 hours over four days (two days per replication). The accessions eaten by the animals were recorded at ten-minute intervals. Acceptability expressed as relative palatability index (PI) varied strongly among accessions of *D. velutinum* and *Tadehagi*, the range being 0 and 4.9, and 0 and 4.5, respectively. As forage availability of preferred accessions decreased during the first day, animals were forced to graze less palatable accessions the second day, which led to significant variation of PI between the two days. Acceptability of *F. macrophylla* was so low that it was not worthwhile to calculate PI's.

Based on agronomic performance and acceptability to cattle, the most promising accessions were CIAT 23973, 23981, and 23990 for *D. velutinum*, CIAT 13269, 23227, and 33111 for *Tadehagi*, and CIAT 21095 and 21079 for F. macrophylla (G. Keller-Grein and E. Cárdenas).

Multipurpose shrub legumes - Humid tropical lowlands, Colombia

Collections of the shrub legumes *Codariocalyx gyroides*, *Cratylia argentea*, *Flemingia macrophylla*, and *Desmodium velutinum* were evaluated for environmental adaptation under a 9-week cutting regime at 100 and 50 cm cutting height, at the "La Rueda" ranch in Caquetá. The most striking was the vigor of almost the entire collection of *C. gyroides*, a species which is known to be sensitive to frequent and low cuts. Leaf DMY ranged between 51 and 221 g/plant in the season of minimum rainfall, and 73 and 466 g/plant in the season of maximum rainfall. The amount of leaf biomass left after cutting was always very high, so that the species was easily able to regrow. Accessions CIAT 3001, 13547, 13548, 23746, 33129, 33131, and 33134 performed best regarding leaf DMY in both seasons evaluated during 1992-1993. Seed production was generally also high.

F. macrophylla was similarly vigorous as *C. gyroides*. The range of leaf DMY, however, was considerably lower (10 to 379 g/plant in minimum rainfall and 20 to 509 g/plant in maximum rainfall). *Cratylia argentea* and, even more so, *D. velutinum* suffered strongly from the poorly drained soil conditions at the experimental site. Leaf DMY's of *C. argentea* ranged between 28 and 142 g/plant in the season of minimum rainfall, and 51 to 118 g/plant in the season of maximum rainfall, CIAT 18674 being the outstanding accession. *D. velutinum* accessions performed poorly (B.L. Maass, G. Keller-Grein and C.G. Meléndez).

Multipurpose shrub legumes - Costa Rica

Ninety accessions of different *Leucaena* species were evaluated under cutting every 8 weeks during the wet season and every 12 weeks during the dry period in conditions of the subhumid tropics of Atenas (Clay loam soil classified as inceptisol with pH 5.9 with low in available P content (3.6 ppm) and 1,600 mm total rainfall distributed from May to November). Other shrubs evaluated were *Desmodium velutinum* CIAT 13218 and *Flemingia macrophylla* CIAT 17403 and 17407 in the humid conditions of Guápiles, and *Cratylia argentea* CIAT 18516 in both sites of Atenas and Guápiles.

Significant differences were observed within and between species of *Leucaena*. A cluster analysis carried out on total DMY of edible forage (leaves and thin stems) of all 12 cuts, gave 7 different groups. Group 7 consisted only of *L. leucocephala* CIAT 17263, that produced higher DMY (3,6 kg/plant/cut), 20% of which was produced during the dry period; this accession also had better regrowth during the wet season (170 cm in 8 weeks). A group formed by *L. leucocephala* CIAT 18481, 18483, 17500, 7986, and 9993 and *L. diversifolia* CIAT 17503 followed in DMY (1.78 kg/plant, 18% of this during the dry period). *L. leucocephala* CIAT 17502 (cv. Cunningham) and CIAT 18477 (cv. Peru) were situated in groups of low DMY (345 and 890 g/plant respectively; 17 and 21% of which was produced during the dry season). In this experiment DMY was highly correlated with plant height (r^2 = 0.90; $P<0.001$).

C. argentea CIAT 18516 showed good adaptation to both humid and subhumid conditions, although large differences were observed in DMY for the two environments. At Guápiles, total annual DMY were 16 ton/ha, while at Atenas these were 5 ton/ha, 13% of which was produced during the dry period. Similarly, *F. macrophylla* CIAT 17403 and 17407, and *D. velutinum* CIAT 13218 were well adapted to the conditions of Guápiles; the latter produced 17 ton/ha of DMY annually. No major pests or diseases were observed, although there was mild psyllid attack on *Leucaena* in Atenas (P.J. Argel and A. Valerio).

Southeast Asia

As the population increases and general economic development proceeds, the demand for meat and dairy products will rise in Southeast Asia. If projected trends in livestock numbers are sustained to the turn of the century, the demand for forage resources in Southeast Asia will double. The provision of seed of appropriate, adapted forage species is considered as a key element in improving livestock production in Southeast Asia and improving the soil fertility of fallow land in upland systems.

The Southeast Asian Regional Forage Seed Project began in January 1992 in four participating countries, Indonesia, Malaysia, Thailand, and the Philippines. It is a joint project with the Division of Tropical Crops and Pastures of CSIRO (Commonwealth Scientific and Industrial Organisation) and is funded by the Australian International Development Bureau (AIDAB). Project headquarters and the central forage species evaluation and seed multiplication site were set up at the International Rice Research Institute (IRRI), Los Baños, Philippines. Species are evaluated for acid-soil tolerance at an acid oxisol site at Cavinti 60 km from Los Baños. The objective is to enhance animal feed supply for small holders in the four participating countries.

Introduction and evaluation of forage species. A major input was the introduction of 486 forage germplasm accessions, largely, from the collection maintained at CIAT. Twenty-three genera and 54 species of legumes and grasses are represented. At present, 67 accessions show sufficient promise for multilocational testing and seed or vegetative planting material are available of 10 grasses and 34 legumes for distribution and on-farm evaluation. In addition to the primary evaluation and multiplication sites at Los Baños and Cavinti, evaluation trials have been established at 5 other locations in the Philippines, 3 in Malaysia, 4 in Thailand, and 7 in Indonesia.

Performance of key species. Results of agronomic evaluation of the legumes *Stylosanthes*, *Arachis pintoi*, *Centrosema*, and the grasses *Brachiaria* and *Paspalum* is reported in Project 2 - Enhancement of forage germplasm. A number of late-flowering accessions of *Desmodium ovalifolium* have been selected to be planted in hedgerows, a highly suitable use for this species. *D. heterophyllum* is performing well in a coconut plantation at Bicol.

Andropogon gayanus. Accession CIAT 621 has shown widespread adaptation on low fertility soils, including a marine sand in Malaysia, a podsolic sandy soil in southern Thailand, and similar sites of low fertility in the Philippines and East Kalimantan, Indonesia (B. Grof).

West and Central Africa

Since 1991, CIAT has been cooperating with IEMVT/CIRAD and ILCA in the evaluation of forage germplasm in West and Central Africa. The germplasm was evaluated under the RABAOC network (French acronym for West and Central African Forage Network = WECAFNET). RABAOC now forms part of AFRNET (African Feed Resources Network, sponsored by IDRC and coordinated by ILCA).

All RABAOC trials are located in the humid and sub-humid zone of Western and Central Africa. Kolda in Senegal (174°55'W) and Bossembélé in the Central African Republic (17°44'E) form the longitudinal

Table 2.5. Performance of shrub legumes (*Desmodium velutinum, Flemingia macrophylla*, and *Tadehagi* spp.) at Carimagua.

Species (no. of accessions)	Characteristic[a]	Fertilization[b]	Maximum rainfall		Minimum rainfall	
			Mean	Range	Mean	Range
D. velutinum	Leaf DM yield (g/plant)	C	7.9 a[c]	3.0-18.2	1.9 a	0.5-6.1
(48)		P	6.2 b	1.3-11.2	0.9 b	0.1-2.2
	Primary regrowth (no. branches/plant)	C	22 a	7-45	13 a	4-24
		P	17 b	6-34	9 b	3-17
	Leaf IVDMD (%)[d]					
		C			51.7	45.3-59.0
		P			52.8	46.9-59.1
F. macrophylla	Leaf DM yield (g/plant)	C	23.2 a	1.1-41.7	6.2 a	0.3-13.4
(23)		P	16.3 b	2.3-33.7	3.5 b	0.2- 6.9
	Primary regrowth				9 a	
	(no. branches/plant)	C	5 a	2-9	7 b	2-23
		P	4 b	2-8		2-19
	Leaf IVDMD (%)					
		P	20.3	8.2-35.8		
Tadehagi	Leaf DM yield (g/plant)	C	12.1 a	4.7-27.9	2.1 a	0.5-4.4
(41)		P	5.4 b	1.9-10.9	1.3 b	0.4-2.9
	Primary regrowth (no. branches/plant)	C	26 a	5-51	22 a	7-36
		P	21 b	5-37	18 b	7-33
	Leaf IVDMD (%)					
		C			29.8	19.5-41.6
		P			30.7	21.9-43.2

a. Regrowth of 12 weeks.
b. Fertilization recommended for the establishment of crops (C) or pastures (P).
c. Means in each column followed by the same letter are not significantly different ($P<0.05$).
d. In vitro DM digestibility.

limits, and again Kolda (12°56'N) and N'Kolbisson in Cameroon (3°50'N) represent the most latitudinal extremes. The trials have been conducted in 9 countries, with 11 collaborators, and at 13 trial sites, including two new sites in Bénin and Guinée. The trials were established using the RIEPT protocol with 35 accessions from CIAT (6 shrub and 21 herbaceous legumes, and 8 grasses) in addition to some local germplasm.

Performance of species. There was variation in adaptation of species among sites. The performance across sites was evaluated during a workshop in April 1992, by scoring at each site the relative value from 0 to 5 of the following criteria:

- ease of establishment;
- productivity during the rainy season;
- adaptation to the dry season; and
- potential seed production.

The general performance of species is shown in Table 2.6. Performance of the different species was also reported at a workshop at Bamako in March 1993. The herbaceous legumes with the greatest potential for the subhumid areas were considered to be: *S. guianensis* CIAT 184, *C. brasilianum* CIAT 5234 and *C. macrocarpum* CIAT 5452 and 5713. At some sites *S. guianensis* CIAT 10136 was the best perfomer in the dry season and *D. ovalifolium* performed well in Ghana.

All grasses performed well at most sites but there were reported to be problems in seed production.

Shrub legumes are considered the forage component with the greatest potential for many production systems. Only a few species were evaluated in the RABAOC trials. *C. cajan* CIAT 18700 was outstanding but is only a biennial. *L. leucacephala, F. macrophylla* and *C. argentea* (for acid soils) showed potential.

Annual meetings. Annual workshops were held in April 1991, at Bethania, Togo, to initiate the investigation, and in April 1992, at Bouaké, Côte d'Ivoire and in March 1993 at Bamako, Mali for evaluation and discussion on future work. These meetings were attended by all active members of the network, the coordinator, B. Peyre de Fabrègue, and representatives from CIAT and ILCA. IEMVT provided funding for the two earlier meetings and

Table 2.6. General performance of species in RABAOC trials (20 = high; 0 = low).

Species, accession	Performance
Herbaceous legumes	
Centrosema pubescens CIAT 5172	16
Stylosanthes guianensis cv. Pucallpa	15
Cassia rotundifolia cv. Wynn	14
Centrosema macrocarpum CIAT 5452	14
Centrosema macrocarpum CIAT 5713	13
Aeschynomene histrix CIAT 9690	13
Stylosanthes guianensis CIAT 10136	12
Arachis pintoi CIAT 17434	11
Centrosema brasilianum CIAT 5234	11
Centrosema acutifolium CIAT 5568	10
Centrosema acutifolium CIAT 5277	10
Stylosanthes hamata cv. Verano	10
Centrosema pascuorum cv. Cavalcade	9
Stylosanthes capitata cv. Capica	8
Zornia glabra CIAT 8279	7
Desmodium ovalifolium CIAT 13089	6
Desmodium strigillosum CIAT 13155	5
Stylosanthes sympodiales CIAT 1044	5
Zornia latifolia CIAT 728	5
Stylosanthes macrocephala CIAT 1281	2
Shrub legumes	
Leucaena leucocephala CIAT 17502	16
Flemingia macrophylla CIAT 17403	15
Cajanus cajan CIAT 18700	12
Cratylia argentea CIAT 18516	10
Codariocalyx gyroides CIAT 3001	10
Desmodium velutinum CIAT 33138	4
Grasses	
Panicum maximum T58	16
Panicum maximum CIAT 673	16
Brachiaria brizantha CIAT 26646	14
Brachiaria decumbens CIAT 606	13
Brachiaria brizantha CIAT 6780	10
Andropogon gayanus CIAT 621	10
Brachiaria dictyoneura CIAT 6133	9
Brachiaria humidicola CIAT 6369	8

ILCA, through AFRNET, funded and organised the workshop in Bamako. The next workshop is planned for April 1994. Funding will be sought to station ar agronomist permanently in West Africa to undertakecollaborative research on evalution of forages for production systems and seed production.

The meeting in Bamako also acknowledged that while there is good knowledge on the utilization of crop residues and agro-industrial products, there is only limited knowledge on the the utilization of improved herbaceous and multipurpose trees for supplementary feeding and in pasture systems (B. Peyre de Fabrègue and C.E. Lascano).

Germplasm for regional evaluation

New germplasm to be considered for evaluation in regional trials has been compiled in Tables 2.7a-c. A final decision on sets for evaluation will be made during the annual Program Review and added as an appendum to this report (P.J. Argel, B. Grof, G. Keller-Grein, B.L. Maass, and E.A. Pizarro).

2.4 Evaluation of Selected Forage Ecotypes for Production Systems

Introduction. Evaluation for environmental adaptation will identify a set of forage ecotypes that appear to be well adapted to the soil, climate, and diseases and insects in a particular environment. But these ecotypes then need to be evaluated for productivity under a management regime in which it is anticipated they could be used. In this evaluation it is important that management of the germplasm is similar to that which would or could be applied to it by farmers in their particular farming system. It is also important that some farmer reaction is sought to the particular forage ecotypes that are being evaluated.

Grazed pastures in savannas. New species to be tested in pasture production systems at Carimagua are *Panicum maximum* (CIAT 6744 and 6977) and advanced selections of new anthracnose resistant *Stylosanthes* gene pools.

Crop-pasture rotations for the savannas. It is proposed to evaluate the dry season tolerant accession of *Arachis pintoi* CIAT 22160 and several accessions of *Calopogonium mucunoides* and *Centrosema pubescens*.

MPTS for hillsides. *Cratylia argentea* will be evaluated in Cauca, Colombia for use in supplementary feeding during the dry season.

Soil conservation in the mid-altitude hillsides. *Arachis pintoi* and *Chamaecrista rotundifolia* and *Pennisetum purpureum* (dwarf elephant grass) will be evaluated in cassava based systems.

Managed fallow. Evaluations will be made of *Codariocalyx gyroides* in the humid tropical lowlands, and *Centrosema macrocarpum, Flemingia macrophylla* and *Cratylia argentea* in the hillsides, Cauca.

Table 2.7. Germplasm for evaluation of environmental adaptation in regional trials - list of accessions (CIAT numbe no other indicated) by ecosystem. Shaded: most important species in ecosystem; underlined: new, most promising mater bold: check.

Genus and species	Savannas/ Llanos	Savannas/ Cerrados	Sub-humid forest	Humid forest	SE-Asia	W-Africa (RABAOC)
Herbaceous legumes						
Aeschynomene americana	-	-	IRFL 1725	IRFL 1725	-	-
Arachis pintoi	18748, 18744, 18745, 18750, 22160, **17434**	18748, 18744, 18745, 18750, 22160, **17434**	18748, 18744, 18745, 18750, 22160, **17434**	18748, 18744, 18745, 18750, 22160, **17434**	18748, 18744, 18745, 18750, 22160, **17434**	18748, 1874 18745, 1875 22160, **1743**
Calopogonium mucunoides	-	822, 20709, 20676, 19519, 9450, **719**	-	-	8132	-
Calopogonium coeruleum	-	-	-	-	7304	-
Centrosema acutifolium	5568, 15086, 15816, 5112, **5277**	-	5568, 15086, 15816, 5112, **5277**	5568, 15086, 15816, 5112, **5277**	-	5568
Centrosema brasilianum	-	5234	5657, 15387, **5234**	-	-	5234
Centrosema macrocarpum	25522[a]	25522	25522	25522	-	25522, **5713**
Centrosema pubescens	5172, 5634, 5189, 15160, 15470, **438**	5172, 5634, 5189, 15160, 15470, **438**	5172, 5634, 5189, 15160, 15470, **438**	5172, 5634, 5189, 15160, 15470, **438**	15160, 15470, **438**	5172
D. heterocarpon ssp. *ovalif.*	-	-	-	-	13089, 3666, 3794, **13305**	-
Galactia striata	-	-	-	-	17444, 18331, **964**	-
Pueraria phaseoloides	7182, 8042, 17296, 17300, 17307, 18381, **9900**	7182, 8042, 17296, 17300, 17307, 18381, **9900**	7182, 8042, 17296, 17300, 17307, 18381, **9900**	7182, 8042, 17296, 17300, 17307, 18381, **9900**	-	-
Stylosanthes capitata	-	2320, 2353, 2546, **10280**	-	-	-	-
Stylosanthes guianensis Hybrids	11833, 11844, FM07-3, FM05-3, **2950**	11833, 11844, FM07-3, FM05-3, **2950**	11833, 11844, FM07-3, FM05-3, **2950**	-	11833, 11844, FM07-3, FM05-3, **2950**	11833, 1184 FM07-3, FM05-3, **29**
Shrub legumes						
Calliandra calothyrsus	-	21252	21252	21252	21252	-
Codariocalyx gyroides	-	-	23748, 33129, 13979, **3001**	23748, 33129, 13979, **3001**	23748, 33129, 13979, **3001**	-
Cratylia argentea	-	18672, 18957, 18674, **18516**	18672, 18957, 18674, **18516**	18672, 18957, 18674, **18516**	18672, 18957, 18674, **18516**	18672, 1895 18674, **1851**
Desmodium velutinum	-	-	13220, 23134, **13218**	-	**13218**	-
Leucaena diversifolia	-	-	17503	-	-	-
Leucaena leucocephala	-	-	17263, 18481, 18483, 17500, 7986, **17502**	-	41/90 (CSIRO)	**17502**
Grasses						
Brachiaria brizantha	16827, **6780**	16827, **6780**	16827, **6780**	16827, **6780**	16318, **6780**	**26646**
Brachiaria humidicola	-	26149, 6707, 16886/16888, **679**	26149, 6707, 16886/16888, **679**	26149, 6707, 16886/16888, **679**	26149, 6707, 16886/16888, **679**	-
Panicum maximum	6799, 6944, 6969, 16028, 16051, **26900** BRA-008761, BRA-008788	6799, 6944, 6969, 16028, 16051, **26900**, BRA-008761, BRA-008788	6799, 6944, 6969, 16028, 16051, **26900**, BRA-008761, BRA-008788	6799, 6944, 6969, 16028, 16051, **26900**, BRA-008761, BRA-008788	6799, 6944, 6969, 16028, 16051, **26900**, BRA-008761, BRA-008788	6799, 6944, 6969, 1602 16051, **269** BRA-00876 BRA-00878

Genus and species	Savannas/ Llanos	Savannas/ Cerrados	Sub-humid forest	Humid forest	SE-Asia	W-Africa (RABAOC)
Herbaceous legumes						
Paspalum spp.	-	**BRA-009610**, BRA-009415, BRA-009687, BRA-010537, BRA-012874	-	-	**BRA-009610**	-

a. CIAT 25522 compuesto 'Ucayali' = CIAT 5432, 5447, 5452, 5674, 5713, 5735, 5740, 5887, 5959, 15014, 15047, 15097, 15098, 15102, 15115.

Table 2.8. Germplasm for evaluation in production systems - list of accessions (CIAT number) by ecosystem.

Genus and species	Savannas/ Llanos	Savannas/ Cerrados	Sub-humid forest	Humid forest
Herbaceous legumes				
Arachis pintoi	-	22160	18744, 18748	18748, 18744
Centrosema brasilianum	-	5234 (BRA)	15387 (MCAC)	-
Centrosema macrocarpum	25522[a] (f)	-	25522	25522
Centrosema pubescens	5634, 15160 (f)	5634, 15160	5634, 15160	5634, 15160
S. guianensis var. *vulgaris*	-	2950[b] (BRA)	-	-
Hybrids of *Stylosanthes*	11833, 11844	-	-	-
Shrub legumes				
Cratylia argentea	-	-	18516	-
Leucaena leucocephala	-	-	17263 (MCAC)	-
Grasses				
Brachiaria brizantha	-	16488	26110	26110, 16827
Brachiaria humidicola	-	-	16888, 26149	16886, 26149
Panicum maximum	6799, 6944	-	-	-
Paspalum spp.	-	BRA-009610	-	-

a. Compuesto 'Ucayali'.
b. cv. Mineirao.
(f) = for crop-pasture system with higher soil fertility.
(BRA) = for Brazilian Cerrados.
(MCAC) = for Mexico, Centro America and the Caribbean.

Table 2.7a-c. Germplasm under consideration for regional trials A/B - list of accessions (CIAT number, if not other indicated) by ecosystem.

Genus and species	Savannas		Semi-evergreen forest	Humid Lowland Tropics				Ecosystem?
	Llanos (Carimagua)	Cerrados (Planaltina)	CAC (Atenas)	CAC (San Isidro)	CAC (Guápiles)	Amazonia (Caquetá/ Pucallpa)	SE-Asia (Philippines)	W-Africa (RABAOC)
Herbaceous legumes								
Aeschynomene americana	-	-	-	-	1725	-	-	-
Arachis pintoi	18748	22160, 18750, 18748	18748, 18744	-	18744, 18748	18748, 18745, 18751	18744, 18750	17434
Arachis repens	-	BRA-012106	-	-	-	-	-	-
Calopogonium spp.	-	822, 20709, 20676, 19519, 9450	-	-	-	-	7304, 8132	-
Centrosema acutifolium	5568	5112, 15249, 15531	5568	5568, 5610, 15353	5564, 5568, 5610	15086, 15816, 5112	-	5568
Centrosema brasilianum	5828	5178, 5667, 15387, 15521, 15522, 15524	5178, 5657	15387	-	-	-	5234
Centrosema macrocarpum	5713, 15102	-	5452, 5674, 5713, 5740, 5887	5452, 5674, 5713, 5740, 5887	5452, 5674, 5713, 5733, 5740, 5887	'Ucayali'	-	5452, 5713
Centrosema pubescens	5172, 5634	-	5172, 5189, 5634	5172, 5189, 5634, 5878	442, 5172, 5189, 5634	15160, 15872	442, 15160, 15470	5172
Centrosema rotundifolium	5620, 25120	-	-	-	-	-	-	-
Chamaecrista rotundifolia	8389, 17518	-	8202	-	-	-	-	-
Desmodium heterocarpon	23880	-	3787	-	13178	23880	-	-
D. heterocarpon ssp. *ovalif.*	13089, 13305	-	3781	-	13129, 13400	13030, 13647, 13125, 13400	13089, 13129, 3666, 3784, 3794, 13305, 13030	-
Desmodium heterophyllum	-	-	-	-	-	349	349	-
Desmodium strigillosum	13661	-	13155	-	-	-	-	-
Galactia striata	21454, 20083	-	-	-	-	-	17444, 18331	-
Pueraria montana	-	-	-	-	17277	-	-	-
Pueraria phaseoloides	18382, 20024, 20322	7182, 8042, 17300, 17320	-	-	7182	17296, 17307, 7978	-	-
Stylosanthes capitata	10280	2320, 2353, 2546	1078, 1097	-	-	-	-	-
S. guianensis var. *pauciflora*	-	2542	-	1175	1175, 11262	-	2542	10136
S. guianensis var. *vulgaris*	-	2950	21, 136	21, 136	21, 136	184	21, SSD-12	184
Hybrids of *Stylosanthes*	11833, 11844	-	-	-	-	-	FM07-3, FM05-3	-
Stylosanthes macrocephala	-	2133, 10007, 10009	2133, 2756	-	-	-	-	-
Teramnus uncinatus	-	-	-	-	-	-	7315, 9113, 18173	-

Table 2.7a-c. Continued.

Genus and species	Savannas		Semi-evergreen forest	Humid Lowland Tropics				Ecosystem?
	Llanos (Carimagua)	Cerrados (Planaltina)	CAC (Atenas)	CAC (San Isidro)	CAC (Guápiles)	Amazonia (Caquetá/ Pucallpa)	SE-Asia (Philippines)	W-Africa (RABAOC)
Shrub legumes								
Cajanus cajan	-	-	-	-	-	18701	-	-
Codariocalyx gyroides	-	-	-	-	-	23748, 33129, 13979	-	-
Cratylia argentea	19668, 18676	18672	18516	18516	18516	18957, 18674	-	18516
Desmanthus virgatus	-	-	474	-	474	-	-	-
Desmodium velutinum	13218, 23133, 23985	-	13218	-	13218	13220, 23134	13218	-
Flemingia macrophylla	17403, 21079, 21090	17403	801, 17403	17403	801, 17403	17413, 20626	-	17403
Leucaena diversifolia	-	-	-	17503	-	-	-	-
Leucaena leucocephala	-	-	-	17263, 17502, 18477, 18481, 18483, 17500, 7986, 9993	-	-	41/90 (CSIRO)	17502
Leucaena pallida	-	-	-	-	-	-	-	-
Sesbania spp.	-	7931, 17533, 18836, 18838, 18840, 18947, 19163, 19167	-	-	-	-	-	-

Table 2.7a-c. Continued

Genus and species	Savannas		Semi-evergreen forest	Humid Lowland Tropics				Ecosystem?
	Llanos (Carimagua)	Cerrados (Planaltina)	CAC (Atenas)	CAC (San Isidro)	CAC (Guápiles)	Amazonia (Caquetá/ Pucallpa)	SE-Asia (Philippines)	W-Africa (RABAOC)
Grasses								
Andropogon gayanus	621	-	-	621	-	-	621	-
Brachiaria brizantha	16337, 16827, 26646	16121, 16150, 16294, 16306, 16307, 16315, 16319, 16467, 16473, 16488, 26110	664, 667, 6387, 16322, 26110, 26646	664, 667, 6387, 16322, 26110, 26646	664, 667, 6387, 16322, 26110, 26646, 16549	16305, 16337, 16827	16318	26646
Brachiaria decumbens	606	-	16497	16497	16491, 16497	606	-	606
Brachiaria dictyoneura	6133	-	6133	6133	6133	6133	6133	6133
Brachiaria humidicola	6369	-	6369, 26149, 16886	6707, 16886, 26149	6369, 16876, 26149, 16886	16888	16886	-
Panicum maximum	6799, 6944	BRA-008761, BRA-008788	6969, 16028, 16051	6969, 16028, 16051, 26900	6969, 16028, 16051, 16061	-	6299	673, 16031
Paspalum spp.	-	BRA-009415, 009610, 009687, 010537, 012874	-	-	-	-	BRA-009610, 'Pantaneira', BRA-003824	-
Setaria sphacelata	-	-	-	-	-	-	CPI-15899	-

Cultivars or checks are: Herbaceous legumes *Arachis pintoi* CIAT 17434 cv. Maní Forrajero Perenne; *Centrosema acutifolium* CIAT 5277 - cv. Vichada; *C. brasilianum* CIAT 5234; *C. macrocarpum* CIAT 5713; *C. pubescens* CIAT 438; *Chamaecrista rotundifolia* CIAT 7792, 8990; *Desmodium heterocarpon* ssp. *ovalifolium* CIAT 350 cv. Itabela; *D. heterophyllum* CIAT 349 - cv. Johnstone; *Galactia striata* CIAT 964; *Pueraria phaseoloides* CIAT 9900; *Stylosanthes capitata* CIAT 10280 - cv. Capica; *S. guianensis* CIAT 184 - cv. Pucallpa; *S. macrocephala* CIAT 1281 - cv. Pioneiro.

Shrub legumes *Codariocalyx gyroides* CIAT 3001; *Cratylia argentea* CIAT 18516; *Flemingia macrophylla* CIAT 801; *Leucaena leucocephala* CIAT 17502 - cv. Cunningham, CIAT 18477 - cv. Peru.

Grasses *Andropogon gayanus* CIAT 621 - cv. Carimagua 1; *Brachiaria brizantha* CIAT 6780 - cv. Marandú; *B. decumbens* CIAT 606 - Basilisk; *B. dictyoneura* CIAT 6133 - cv. Llanero; *B. humidicola* CIAT 679 - cv. Humidicola; *Panicum maximum* CIAT 622 - cv. Makueni, CIAT 6299 - cv. Tobiatá.

3. ENHANCEMENT OF FORAGE GERMPLASM

Purpose

To improve the utility of key forage species through genetic manipulation or other means.

Rationale

Evaluation of natural genetic diversity will remain important in the development of tropical forage grass and legume species. However, ideal forage cultivars are unlikely to be found in nature. Hence, once key forage species have been identified there is a need to focus attention to overcome well-defined constraints that may limit their full exploitation. This may be initially by acquisition of more diverse germplasm collections in cases, such as *A. pintoi*., where the existing germplasm base is judged to be inadequate. Directed genetic manipulation, whether by conventional (plant breeding) or more novel methods (or a combination of the two) will be needed in cases where desired attributes or character combinations are not found in large collections of natural germplasm (e.g. *Brachiaria* spp. or *Stylosanthes* spp.).

This project will generate gene pools from which superior genetic recombinants can be isolated for subsequent development to cultivar status. Specifically, combinations of critical attributes for better performance, such as edaphic adaptation and spittlebug resistance in apomictic *Brachiaria* or anthracnose resistance, seed production, and persistence under grazing in *Stylosanthes*, will be achieved by conventional population improvement methods augmented where appropriate by such biotechnologies as gene tagging (apomixis gene in *Brachiaria*) and marker assisted selection (quantitative traits in both *Brachiaria* and *Stylosanthes*).

3.1 *Brachiaria* Improvement

Introduction

Approximately 50 million hectares are planted to *Brachiaria* species in tropical America, the majority to *B. decumbens*. This species is well adapted to acid, infertile soils, but productivity is severely reduced by spittlebug (*Homoptera: Cercopidae*). On the other hand, the spittlebug resistant *B. brizantha* cv. Marandú has poor persistence on infertile soils. Also, while *B. decumbens* exhibits resistance to leaf cutter ants (*Hymenoptera: Attini*), other *Brachiaria* species do not, and hence it is important to maintain this resistance. An important germplasm collection was assembled in the mid-80's and regional evaluation of this collection is essentially complete. A breeding project will utilize new techniques in exploiting apomixis to create synthetic gene pools with resistance to spittlebug and leaf cutter ants, high feed quality and good persistence on acid, low fertility soils. An additional spin-off of this research will be an understanding of the inheritance of apomixis and tagging of the apomixis gene.

Genetic resources of *Brachiaria*

Taxonomy and genetic variability of *Brachiaria*. In a collaborative project with the Royal Botanic Gardens, Kew, U.K., taxonomy of *Brachiaria* was studied on living plant material and herbarium specimens under the leadership of Dr. S.A. Renvoize. Several plant types were found in some species, [e.g. in *B. brizantha* (7) and *B. jubata* (5)]. Types intermediate between species were identified. (B.L. Maass).

In collaboration with the University of Bristol, U.K., over 400 accessions of the five economically most important species were characterized by isozyme patterns through polyacrylamide gel electrophoresis (PAGE). Five isozyme systems revealed polymorphism. Cluster analysis was applied to every isozyme separately, and finally to all five isozymes together. Every isozyme differentiated among species. These data are being used to analyze genetic variability in the genus. (B.L. Maass and C. Meghji)

The characterization of the mode of reproduction of a number of *Brachiaria* accessions not previously studied (Valle & Glienke 1991), has been completed in a collaborative project with the Universidad del Cauca, Popayán, and the Genetics Section of the TFP. (B.L. Maass and L.A. Ortega).

Agronomic evaluation of natural Brachiaria germplasm in the Llanos, Carimagua

Brachiaria humidicola. Common *B. humidicola* (CIAT 679) which is commercially available in various Latin American countries, is well adapted to acidic, low fertility soils including poorly drained situations. It is tolerant, but not resistant, to spittlebug, and has replaced the highly susceptible *B. decumbens* in many areas. Nutritive value, however, is poor compared with *B. decumbens*.

A collection of 54 *B. humidicola* accessions was established in 1991 along with *B. humidicola* (CIAT 679), *B. brizantha* (CIAT 26646), *B. decumbens* (CIAT 606) and *B. dictyoneura* (CIAT 6133) as controls. After the establishment phase, the collection has been evaluated under a cutting regime at 12- or 6-week intervals in 1992 and 1993, respectively, to assess the agronomic performance and to study the variation with regard to nutritional quality (especially N content and IVDMD).

The collection shows considerable variation for several morphological attributes such as leaf size and form, and stolon development. Preliminary data on important agronomic attributes and nutritive value are summarized in Table 3.1. DM yields, leaf proportion and plot cover differ widely among accessions during both rainfall periods. Considerable variation also appears to exist for N concentration and IVDMD of leaves. Several new accessions show better agronomic performance than CIAT 679 and also seem to have higher nutritional quality, similar to that of *B. decumbens* CIAT 606. Two further cuts and chemical analyses of the main part of the samples harvested are still pending, before final selections are made.

***Brachiaria* spp.** As part of the characterization and evaluation of CIAT's *Brachiaria* collection which comprises about 700 accessions, a subcollection of 189 accessions of 10 *Brachiaria* species has been evaluated in small plots at Carimagua, applying the same cutting regime as in the *B. humidicola* experiment. The experiment is being conducted at two fertilization levels, one which considers the recommendation for pasture establishment in Carimagua (kg/ha: 20 P, 20 K, 12 S, 12 Mg and 40 N), and another which is recommended for upland rice (kg/ha: 50 P, 100 K, 20 S, 5 Zn, 80 N, 300 dolomitic lime) to assess germplasm response to higher soil fertility. Evaluation under cutting was interrupted in 1993 for 6 months to conduct morphological studies and record flowering onset and seed production.

Considerable intraspecific variation was observed for several morphological parameters such as growth habit; size, shape and pubescence of leaves; and various characteristics of the inflorescences. Flowering onset (50% of plants of an accession had started flowering) was extremely variable. It began for example in the case of the 95 accessions of *B.*

Table 3.1. Preliminary performance of a *Brachiaria humidicola* collection at Carimagua.

Characteristic	Regrowth period (weeks)	Maximum rainfall		Minimum rainfall	
		Mean	Range	Mean	Range
DM yield (g/m^2)	12	176.6	78.6-455.8	87.2	37.3-179.4
	6	71.6	29.8-156.9	17.9	6.0- 33.7
Leaf proportion (% DM)	12	64.7	33.7- 92.9	54.4	26.9- 80.8
	6	76.4	53.4- 95.4	85.4	57.2- 98.3
Cover (%)	12	73.8	43.8- 88.8	-	-
	6	55.2	23.3- 79.2	40.1	12.5- 66.3
IVDMD (% Leaf DM)	12	-	-	56.62	45.48-63.81
N concentration (% leaf DM)	12	0.86	0.55- 1.23	1.28	0.85- 1.80

brizantha between April and early September; almost half of the accessions, however, started flowering between late May and the end of June. Seed production was low in general; particularly accessions of *B. humidicola* produced very little seeds mainly because of a fungal infection of the spikelets.

DM yields and leaf proportion proved to be highly variable among and within species. Most of the accessions showed a response in DM production with higher soil fertility. The most promising accessions observed so far have been *B. brizantha* [CIAT 16130, 16467, 16473, 26032, 26124, 26555, 26556 and the control accessions 6297 (= cv. Marandú) and 26646 (= cv. La Libertad)) and *B. humidicola* (CIAT 16774, 16793, 16873, 16876, 26425 and 26573). However, selection of promising germplasm for further testing will only take place after analyses of the numerous data collected, including those of a final cut to be conducted at the end of the current rainy season to evaluate DM yields and nutritional quality.

Spittlebug infestation has been irregular in this trial, so that it has not been possible to record reliable field data on host resistance to date. (G. Keller-Grein, E. Cárdenas).

Agronomic evaluation in the Humid Tropical Lowlands - Guápiles, Costa Rica

Two hundred ninety-four *Brachiaria* spp. accessions were evaluated. Plots were harvested 16 times at 6-wk intervals over two years. Dry matter yield (DMY), plant height, ground cover, and incidence of pests and diseases were measured.

Cluster analysis showed differences among and within species for the parameters measured. Accessions with higher DMY than standard commercial *Brachiaria* cultivars were detected.

DMY was highly correlated with plant height and ground cover (r^2= 0.98 and 0.82, respectively; $P < 0.001$). Pests and diseases were unimportant: only mild attacks of spittle bug (*Aeneolamia* sp.) and *Rhizoctonia* foliar blight were recorded. (P.J. Argel and A. Valerio).

Agronomic evaluation at Los Baños, Philippines.

Twenty-two advanced *Brachiaria* spp. accessions were evaluated at the Central Nursery of the South East Asian Regional Forage Seeds Project. Accessions of *B. brizantha* [CIAT 16318], *B. humidicola*, and *B. dictyoneura* show good performance on low fertility soils while *B. brizantha* cv. Marandú performed well on higher fertility soils. Several recently introduced accessions are also showing promise. (B. Grof).

Spittlebug resistance

Resistance to various species of spittlebug is a major objective of the *Brachiaria* improvement project. This insect can cause severe and unpredictable losses in forage yield and quality. Genetic resistance appears the only viable control measure for economic as well as ecological reasons.

Excellent resistance exists in the genus. We have demonstrated that inheritance of resistance is probably under simple genetic control as parent-offspring correlation is high [$r=0.95$ ($P<0.0001$) or $r=0.83$ ($P<0.001$) for percentage nymphal survival or duration of nymphal stage, respectively].

The major bottleneck to the effective utilization of available resistance in the breeding project is the difficulty in reliably assessing resistance on large segregating populations. Two approaches are being taken: i) improvement of the reliability of infestation in the field and greenhouse; and ii) identification of the biochemical mechanisms of resistance so as to develop a simple laboratory screening method.

Glasshouse screening is a reliable measure of host plant antibiosis. Pot-grown plants are artificially infested and nymphal survival is assessed (Lapointe, et al. 1992). However, with available resources, the capacity of the present glasshouse screening method is limited to approx. 300 genotypes per year. As the breeding program is generating several thousand recombinants per year, the glasshouse test is clearly inadequate.

Neither natural nor artificial infestation in the field at the Carimagua research station has been reliable. The reasons for this failure are unclear, and have been variously attributed to insufficient precipitation and low relative humidity, or to predation of spittlebug eggs by ants. In any case, first year field data have not been good indicators of true resistance. Seventy-two second generation recombinants selected on the basis of first-year field nymphal counts were assessed in the glasshouse screening test. There was no relation between field nymphal counts and resistance as measured by adult emergence in the glasshouse test ($r=0.08$; $P=0.49$). Second-year ratings of spittlebug damage in the field agreed better with the glasshouse results ($r=0.30$; $P<0.01$).

However, postponing selection until the second year would halve the rate of genetic progress. Hence, a trial was planted in 1993 near Florencia, Caquetá, in the Colombian Amazon basin (annual rainfall in excess of 4,000 mm) in hopes of achieving heavy, uniform natural infestation in the first season. This planting appears to be becoming heavily infested with nymphs after only two months in the field (Sept. 1993). We expect to get reliable discrimination of host resistance by year's end.

Refinements in glasshouse screening methodologies should increase the capacity and perhaps the *precision* of this test of resistance to spittlebug nymphs. More recently investigations are underway to develop a method to assess adult damage on glasshouse-grown seedlings. (S.L. Lapointe; G. Sotelo; J.W. Miles).

Leaf cutter ants

Most commercial *Brachiaria* pastures appear to be unaffected by leaf cutting ants. Investigations have demonstrated that this resistance is owing to the grass's inhibiting the growth of the ant fungus gardens. This has been demonstrated in the laboratory where fungal growth in vitro is affected by crude aqueous extracts of different grasses (Figure 3.1) and in the field, where ant foraging was restricted to particular grass genotypes (Table 3.2). (S.L. Lapointe).

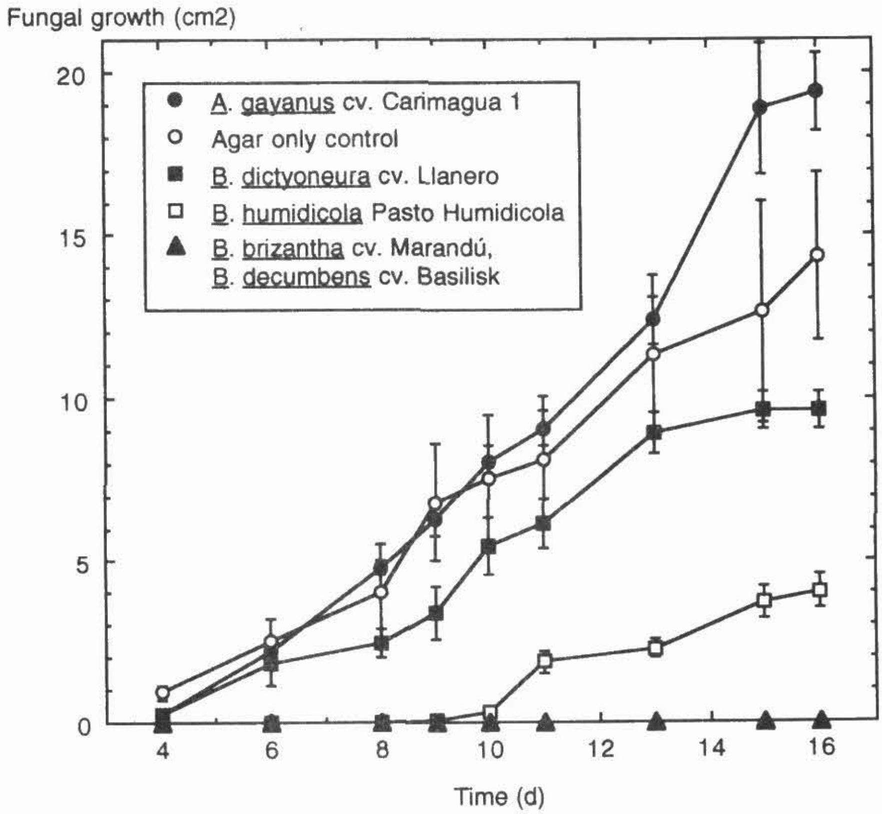

Figure 3.1 Growth of the attine symbiotic fungus on agar medium containing whole leaf homogenates of five commercial forage cultivars.

Table 3.2. Total fresh weight of fungus gardens from colonies of *Acromyrmex landolti* excavated after being restricted to cutting one of five forage cultivars during 30 days. Carimagua, 1993.

Cultivar	Fungus wt. (g)[1,2]
Marandú	5.5 a
Pasto Humidicola	7.8 a
Basilisk	10.5 a
Llanero	37.0 b
Carimagua 1	37.3 b
Control (native savanna)	50.5 b

[1] Means in columns followed by the same letter do not differ (DMRT, α = 0.05).
[2] N = 4 colonies excavated/cultivar.

Foliar blight (*Rhizoctonia solani*)

Diseases are generally of minor significance in *Brachiaria*. *Rhizoctonia* foliar blight can cause substantial foliar damage when susceptible genotypes are grown in humid areas.

Host plant resistance is the best and cheapest method of disease control. A highly reproducible and effective artificial inoculation method to identify host resistance has been developed. The method allows screening a large volume of germplasm. The key factors in the method are 100% humidity and viable fungal sclerotia produced in a peptone, sucrose, yeast extract liquid medium.

All inoculated plants were infected indicating that the method is reliable. *Brachiaria* accessions reacted differently to artificial infection by *R. solani* (Table 3.3). Forty-two accessions of *B. decumbens, B. brizantha, B. dictyoneura, B. humidicola*, B. ruziziensis, and *B. jubata* are being evaluated in the field (Caquetá, Colombia) and in the greenhouse. (S. Kelemu and X. Bonilla).

Manipulation of apomixis

Apomixis is a powerful tool for fixing even highly heterozygous, heterotic genotypes. Important commercial *Brachiaria* spp. -- *B. decumbens* and *B. brizantha* -- are tetraploid apomicts. A tetraploidized sexual *B. ruziziensis* is cross compatible with both apomictic species (Ndikumana, 1985). Analysis of embryo sacs of first and second generation hybrid populations [Valle & Glienke, 1993] suggest simple genetic control of apomixis, with a single dominant gene conditioning the potential for apomictic reproduction. (C. do Valle (EMBRAPA); J.W. Miles).

New *Brachiaria* gene pools

Two *Brachiaria* gene pools are being assembled with the objective of improving their genetic potential while exploiting apomixis in future cultivars. One population includes both sexual and apomictic genotypes in proportions managed (within limits) by choosing the parents of each cycle on the basis of reproductive mode as determined by embryo sac analysis. Selected apomictic segregants identified each cycle become candidates for future cultivar status after further testing.

A second gene pool is being formed with only sexual, first-cycle hybrids as initial parents. This population should breed true for sexual reproductive mode.

Table 3.3 Reactions of four accessions of *Brachiaria* spp. to *R. solani* 6780.

Accession	Disease incidence (%)*	Disease severity (%)**	Presence of sclerotia on plant***
606	100	10	+
6780	100	34	++
679	100	5	-
26646	100	10	+

* (Total number of plants infected/total number of plants inoculated) X 100.
** [(Total number of infected leaves) x % leaf area blighted)/(total number of leaves) X 100.
*** + = very few and small; ++ = abundant and big; - = not present.

Selection within this pool will not be complicated by the need to determine reproductive mode. Advanced cycle sexual clones will be crossed with elite apomicts to produce improved apomictic recombinants for subsequent development to cultivar status.

Field evaluation of spaced plants is being conducted near Florencia, Caquetá, where we anticipate that high rainfall will assure heavy, uniform, natural spittlebug infestation, and at Carimagua where other agronomic attributes are assessed and recombination is achieved. (J.W. Miles).

Tissue culture in *Brachiaria*

A range of media components was tested for callus induction and regeneration of five *Brachiaria* spp., including important commercial cultivars. Callus induction from immature panicles was best in the presence of 2,4-D and casein hydrolyzate. All species responded well to callus induction from immature panicles and mature seed.

After 30-40 days, embryogenic calli were transferred to a regeneration medium. Following 20-25 days in the regeneration medium, somatic embryos were clearly differentiated from the callus. Amount of callus depended on the medium and the genotype. Germinated embryos were transferred one week later to test tubes for further growth and finally into soil in the greenhouse.

Successful regeneration of *Brachiaria* opens the possibility of initiating studies on somaclonal variation or contemplating genetic transformation of *Brachiaria*. (S. Lenis, W. Roca).

Culture of isolated microspores of *Brachiaria* spp.

A project was initiated in 1992 to produce dihaploid *Brachiaria* genotypes by microspore culture of apomictic, tetraploid *Brachiaria*. These dihaploids have at least three potential uses: i) They will contribute to our understanding of the genetic control of apomixis. [If our working model of one dominant gene conditioning apomixis is true then dihaploids derived from a tetraploid apomict ought to segregate 1:1 sexual:apomictic.] ii) Sexual dihaploids will broaden the genetic base of available sexuals for the breeding work. iii) A random array of dihaploid genotypes derived from tetraploids will provide extremely useful genetic material for the development of a map of the *Brachiaria* genome.

This project has achieved several advances to date: optimum floral developmental stage for sampling microspores has been determined; duration of low temperature pretreatment of inflorescences has been evaluated; two culture media have been tested: one gave no result, while plasmolysis was observed with the other. A dilution series was tested for the second medium. Microspores have been cultured to the premitotic stage prerequisite to androgenesis. (S. Lenis; W. Roca).

Progress in mapping the apomixis gene

Apomixis in the *B. decumbens/B. brizantha/B. ruziziensis* group appears to be controlled by a single, dominant gene (Valle & Gleinke, 1993). A molecular genetic marker tightly linked to the apomixis gene, which would permit identification of reproductive mode of small seedlings, would improve the efficiency of *Brachiaria* breeding. The identification of such a marker is a necessary first step to mapping of the apomixis gene and its possible eventual transfer into other crop species. A project is underway whose objective is to identify a PCR-based marker. A DNA extraction procedure has been implemented for *Brachiaria* species based on the protocol described by McCouch, et al. (1988). DNA was bulked from three apomictic or from six sexual F_1 individuals from a cross involving a sexual tetraploid *B. ruziziensis* clone and an apomictic *B. brizantha* accession. To date 255 primers have been screened. Polymorphism was observed for 94 primers between the parental genotypes and between F_1 bulks. Of these, 20 primers produced bands which were associated only with the apomixis phenotype both in parents and in the F_1 bulks. These 20 primers are now being screened on a large F_1 population from a cross between a single sexual *B. ruziziensis* clone and an apomictic *B. brizantha* to detect PCR markers associated with the apomixis phenotype. The polymorphic primers are also being used to construct a PCR-based map of *Brachiaria*. [F. Pedraza; J. Tohme; J.W. Miles].

Virology

A *Brachiaria* potyvirus, closely resembling guinea grass mosaic virus, was isolated and identified as the casual agent of a previously unknown disease of *Brachiaria* in tropical America. It is an aphid transmitted potyvirus. (F.J. Morales).

3.2 *Arachis* Improvement

Introduction

Arachis pintoi is unique among tropical legumes in the humid tropics in its ability to form stable grass-legume associations with vigorous grasses like the brachiarias. It is persistent and even increases under heavy and continuous grazing pressure. Nutritive quality is high. Further it has proven to be a very successful cover legume in numerous horticultural and tree crops.

However, there are limitations to its use. The present widely used cultivar CIAT 17434 (cv. Amarillo in Australia) is not tolerant of dry conditions. Establishment is slow and of variable success. Underground seed production results in high seed costs, though it can also be readily established vegetatively. The other limitation is more generic. Few farmers in tropical America are accustomed to using grass-legume associations and the legume is not well known.

What is more disconcerting is that development of *A. pintoi* as a commercial forage legume resulted from only one introduction, CIAT 17434, though there had been prior selection in Brazil, the USA and Australia. Thus it is imperative to increase the genetic base of germplasm for *A. pintoi* and other *Arachis* species with potential forage value.

The Program is following a dual approach in enhancing the utility of this genus. The first is in assisting CENARGEN/EMBRAPA in increasing the genetic base through collection so as to increase the range of adaptation and ensure the availability of resistance genes in case of future disease or pests outbreaks. The second is to facilitate the utilization of the present successful cultivar of *A. pintoi* CIAT 17434 through collaboration with NARS in on-farm evaluation and training.

Arachis Workshop

There is a now a large scientific body interested in *Arachis* as a forage and cover crop legume. To tap some of this interest and to focus research on priority areas, a Workshop was held on *Arachis* in May 1993.

Scientists from Australia, Brazil, Colombia, Costa Rica, and USA participated in addition to those of CIAT.

A wide range of topics was reviewed including: distribution, taxonomy, genetic resources and diversity, reproductive biology and physiology, mineral nutrition, potential diseases and pests, nutritive value and animal production, seed biology and systems, and regional experiences in tropical America, Australia, USA, and South East Asia were summarized. Working Groups focused upon the status and needs of germplasm, evaluation and agronomic use, and adoption. The workshop proceedings will be available in late 1993.

Genetic Resources of *Arachis*

During 1992-1993, E.A. Pizarro and B.L. Maass participated in four collection trips in Brazil under the leadership of Dr. J.F.M. Valls (EMBRAPA-CENARGEN). Special emphasis was on wild *Arachis* spp. with forage potential. Seventy-nine accessions were collected and these will be duplicated at CIAT after initial propagation in Brazil. Forty accessions of *A. pintoi* and *A. repens*, partly of the new material, were received from CENARGEN and are being increased. Isozyme variation in this collection is being studied in collaboration with Universidad del Valle, Cali. (E.A. Pizarro, B.L. Maass, and A. Ortíz).

Agronomic evaluation of *Arachis* accessions

Savannas - Cerrados, Brazil. Thirty-three accessions were evaluated under conditions of high water table and seasonal flooding during the wet season. Some agronomic attributes of the best species are shown in Table 3.4. Pure seed yield of pre-selected accessions ranged from 20 to 1400 kg/ha. Pre-selected accessions differed in flower density. Flowering peaked in December and was least in April. A new trial of 46 accessions associated with *Paspalum atratum* BRA-009610 was established on two soil types representative of the low lying areas and the well

Table 3.4. Dry matter yield of shoot and roots, percentage of yield occurring in the dry season (in parentheses), root-to-shoot ratio, and seed yield of nine Arachis accessions grown on seasonally flooded land of the Cerrados, Planaltina, Brazil.[a]

Species	Accession		Shoot yield		Root yield[b]	Root to shoot ratio[c]	Seed yield months after planting	
	BRA	CIAT	Year 1	Year 2			16	28
							(kg/ha)	(kg/ha)
A. pintoi (434)	31844	-	12.8(24)[d]	9.2(38)	12.7	0.8:1	440	310
	31852	-	9.4(31)	7.6(36)	6.1	1.1:1	830	650
	13251	17434	9.4(37)	9.5(36)	10.2	0.6:1	1240	890
	15121	18748	6.9(30)	6.8(29)	9.8	0.5:1	1170	1350
	15598	-	11.1(27)	9.7(37)	12.8	0.6:1		
	31143	22160	13.0(31)	11.0(30)	13.3	0.7:1	540	580
A. repens	31861	-	5.2(53)	5.8(45)	10.6	0.2:1	5	20
	12106	-	5.8(43)	5.6(56)	14.1	0.1:1	-	-
A. glabrata	17531	-	6.2(37)	2.8(48)	5.6	0.7:1	50	30

(a). Planted January 1990; year 1, wet season November to March (1391 mm); dry season April to October (175 mm) year 2, wet season November to March (1512mm); dry season April to October (188 mm). (b). Accumulated root yield from January 1990 to May 1991. (c). Using shoot yield at time of determining root yield. (d). Percentage of yield in the dry season.

drained upper portions of the Cerrado landscape. Green leaf retention was less on this upper portion of the landscape during the dry season, though one accession, BRA-31143/CIAT 22160 was much more vigorous than others.

Humid Tropical Lowlands - Caquetá, Colombia. In 1992, eight germplasm accessions of *A. pintoi* and 15 of *A. glabrata* were established by seed or vegetatively. Spread and soil cover were greater for accessions of *A. pintoi* than for *A. glabrata*. The best accessions in terms of percent soil cover and DM production throughout the year were *A. pintoi* CIAT 18748, 18751, and 18747. (B.L. Maass and C.G. Meléndez).

Humid Tropical Lowlands - Los Baños, Philippines. Seven accessions of *A. pintoi* were evaluated by the South East Asian Regional Forage Seeds Project. CIAT 18750 is better adapted to low fertility soils than the commercial cv. Amarillo (CIAT 17434). On the Oxisol at Cavinti, Laguna, CIAT 18750 is the best of the seven accessions introduced. On a fertile soil and in the second season after establishment CIAT 17434 has recovered a normal green color and produced 1064 kg of seed in pod per ha. (B. Grof).

Establishment Agronomy

Method of propagation. Establishment by seed or vegetatively were compared at Chinchiná, Colombia. Four seedling densities (between 5-30 kg/ha) were compared with 25-cm long, basal or distal primary stolon segments sown at a single density.

Basal propagules produced twice the number of plantlets and covered the ground twice as fast as terminal propagules. Seeding density was positively associated with number of seedlings and rate of ground cover. Averaged over the four densities, seeds produced more plants and faster ground cover than stolons. Sixty days after planting, ground cover from 10 kg/ha of seeds was greater than from either basal or terminal propagules. Table (3.5). (J.E. Ferguson).

Table 3.5. The effect of establishment method on base plant population and ground cover.

Establishment Treatment	Plant Population[1]		Ground Cover	
	Density	Emergence	40 days	60 days
	no/m^2	%[2]	%	%
Seed, 5 kg/ha	3.4 e	98	2.7 d	20.9 c
Seed, 10 kg/ha	6.6 d	93	7.7 c	35.2 b
Seed, 15 kg/ha	10.3 b	97	14.2 b	48.6 a
Seed, 30 kg/ha	20.4 a	96	21.9 a	53.8 a
Basal propagules[3]	7.8 c	97	4.3 cd	24.0 c
Terminal propagules[3]	4.1 e	51	2.3 d	10.1 d

1 At 40 days post planting.
2 Based upon number of seeds or minimum number of fresh node sites.
3 Propagules were planted at a volume sufficient to provide at least the same number of fresh nodes sites as seeds in the 10 kg/ha seed treatment.
4 Means followed by the same letter do not differ.

At Guápiles, Costa Rica weed control and selectivity of several herbicides were evaluated in the establishment of *A. pintoi* CIAT 17434 using seed or vegetative material. The best weed control and selectivity were with preemergence application of Alachlor (1.4 or 2.5 kg a.i./ha) or Pendimetalin (0.8 kg a.i./ha). Preemergence application of Oxiflourfen at 1.0 kg a.i./ha or post emergence application of a mixture of Metolachlor and Gramuron (5% v/v) gave good weed control, but caused severe damage to *A. pintoi*, particularly when established by seed. (P.J. Argel).

Effect fertilizer N on establishment. The slow establishment of *A. pintoi* inhibits adoption. The reasons for slow establishment are poorly understood.

A glasshouse pot experiment was conducted to study the effect of fertilizer N, applied as KNO_3 or $(NH_4)_2SO_4$ in rates up to 150 kg N/ha, on early growth and nodulation. Seeds of *A. pintoi* were germinated in sand and transplanted to pots of a Carimagua oxisol inoculated with Bradyrhizobium strain 3101.

Eight-wk plant dry weight was approx. 30% greater with 50-100 kg/ha NO_3-N fertilization than with no applied N. Leaf number, and area also increased. Nodule number and weight at 8 weeks increased with doses of NO_3-N fertilizer up to 50 kg/ha. Higher doses inhibited nodulation. NH_4-N also improved yield but did not improve nodulation.

The results suggest that moderate doses of NO_3-N fertilizer at establishment will increase plant dry weight and nodulation, and hence should improve establishment when there are no competing plants. (R.J. Thomas and N.M. Asakawa).

Seed Quality

Germination tests were conducted on six seed lots hand harvested in Yapacini, Bolivia in 1992. Non-germinating fresh seeds were submitted to a tetrazolium test to determine their viability. Normal seedlings plus viable nongerminating seeds were taken as total viable seeds. Dormancy was then calculated as nongerminating viable seeds as a proportion of total viable seeds (Figure 3.2).

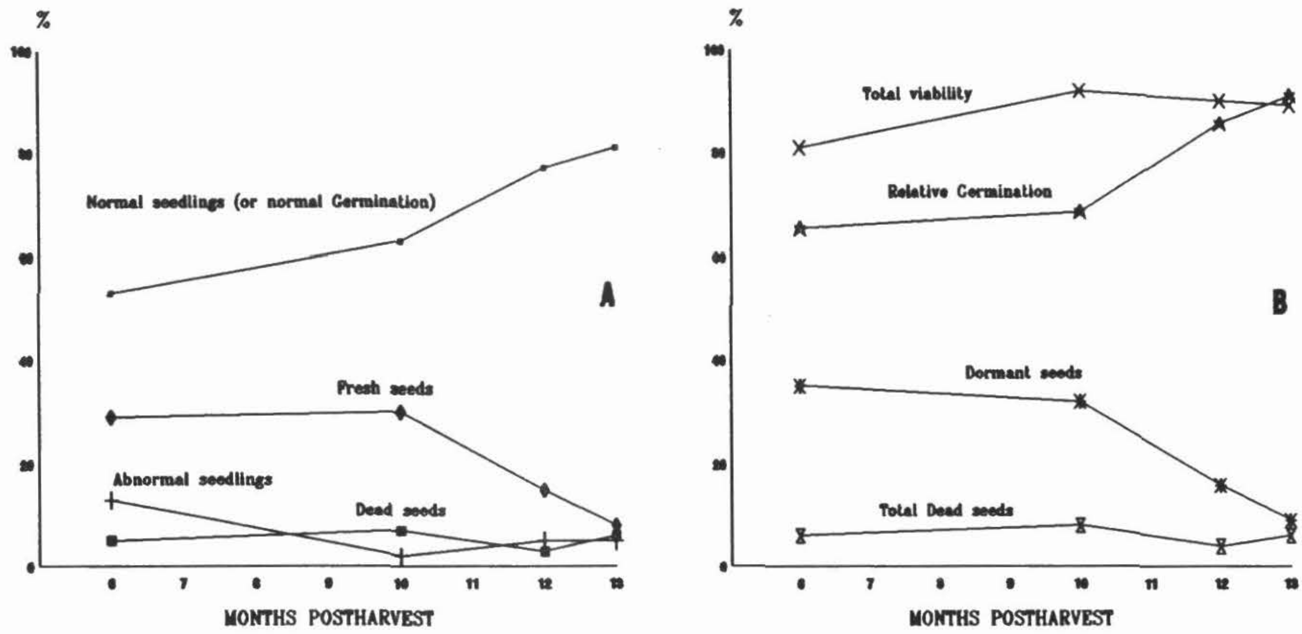

Figure 3.2 Proportions of seed quality components of *Arachis pintoi*, average values of six seeds lots from 6-13 months postharvest. A) From germination testing without pretreatment. B) Plus (i.e. after A) tetrazolium testing of non-germinating seeds.

Total viability = normal seedlings + non-germinating viables; relative germination = Normal seedlings ÷ total viability; dormant seeds = non-germinating viables ÷ total viability; total dead seeds = dead seeds + non-germinating, non-viables.

Germination was markedly improved by drying fresh seed for 14 days at 40°C (Table 3.6), indicating that this pretreatment reduces dormancy. (J.E. Ferguson).

Management and seed yields

The effect of frequency of defoliation (2, 4, or 8-mo intervals vs. uncut, over 69 weeks) on flowering, seed yield and seed quality was studied at Guápiles, Costa Rica, a site with no dry season. CIAT 17434 produced more flowers than CIAT 18744 (18 vs. 12 flowers/m^2/day). Daily flower number of CIAT 17434 was not affected by cutting frequencies. Short cutting intervals reduced daily flower production in CIAT 18744. Seed yield was higher for CIAT 17434 than for CIAT 18744 (1,240 vs. 370 kg/ha). Seed yield of CIAT 17434 was reduced only at the shortest

Table 3.6 The effect of predrying (14 days at 40°C) upon germination of seeds of *Arachis pintoi*[1] at six weeks postharvest.

Treatment	Germination[2]		
	Seed	Seed in pod	Mean
Nil (control)	25	18	22[a*]
Predried	69	71	70[b]
Mean	47[a]	45[a]	

[1] Average of one seed lot from each of three accessions. CIAT 17434, 18744, 18748.

[2] Normal seedlings at 21 days, at 25°C.

* Means followed by the same letter do not differ, P = 0.05.

cutting interval while in CIAT 18744 any defoliation reduced seed yield.

Frequency of cutting of seed multiplication plots did not affect seed quality as measured by emergence of 6-mo-old seed. Seed quality of CIAT 17434 was better than that of CIAT 18744 (53 vs. 28% emergence $P<0.05$).

Seed of *A. pintoi* CIAT 17434, CIAT 18744 and CIAT 18748 was harvested at 8, 12, 16, or 20 months after planting at Guápiles, and at 8, 16, or 20 months at San Isidro (humid seasonal tropical forest). Seed yield was not affected by date of harvest at Guápiles, but in San Isidro yield of all accessions was higher for the 16-month harvest (overall mean 1,230 kg/ha). A marked site-accession interaction was observed. At Guápiles CIAT 17434 yielded more seed (960 kg/ha, over harvests) than either CIAT 18744 or CIAT 18748 (160 or 490 kg/ha, respectively), but no difference among accessions was detected at San Isidro. Although soil and climate differ between sites, we do not understand the factor(s) affecting the difference in seed yield between the two sites. (P.J. Argel).

Release and seed production

A. pintoi has been released in additional countries. The different cultivar names assigned by different countries, all relate to a single accession, CIAT 17434 (=CPI58113).

Since the release of cv. Amarillo in Australia in 1987, the seed enterprise Sauer and Sons has expanded seed production. In 1991, seven tons were produced. Prices are on the order of US$ 13/kg.

In Bolivia SEFO-SAM first grew seed under contract to CIAT in 1990-91. After achieving high seed yields at Yapacini, SEFO involved small farmers in contract production and in 1992 manually harvested approx. three tons. Seed has been exported to Brazil and Colombia. In Colombia, 'maní forrajero perenne' was released in 1992 and four seed enterprises are expanding commercial production. Seed will enter the market in late 1993. In Honduras, cv. 'Pico Bonito' was released in August 1993, and basic seed multiplied by SRN was distributed to several farmer multipliers. (J.E. Ferguson).

Animal production studies - Brazil

Animal performance is being recorded in trials in two different regions of the Cerrado ecosystem and at one site in the humid tropics. Liveweight gains ranged from 200 to 600 g/animal/day. (E.A. Pizarro and M. Barcellos).

Animal production studies - Colombia

Effect of stocking rate on liveweight gain. An experiment is being carried out at Carimagua to compare liveweight gains (LWG) of steers grazing *B. humidicola* alone or in association with *Arachis pintoi* (CIAT 17434) at three stocking rates. In May 1987, the legume was introduced in strips into an existing stand of *B. humidicola*, and fertilized with 20 P, 100 Ca, 10 K, 11 Mg, 22 S (kg/ha). Grazing was initiated in May 1988 with the following six treatments: (1-3) grass alone with 2, 3, or 4 hd/ha and (4-6) grass + legume with 2, 3, or 4 hd/ha. After 4 years of grazing, legume content in the forage on offer has increased over time. LWG per animal has been consistently higher ($P < .05$) in the grass-legume pastures regardless of stocking rate or year of evaluation (Figure 3.3). The benefit of the legume has been observed in both the dry and wet season.

Effect of grazing system on liveweight gain. A second grazing experiment at Carimagua was designed to compare alternate vs. rotational (4-paddock) grazing of *B. dictyoneura* alone or in association with *A. pintoi* (CIAT 17434). LWG have been consistently higher in the pastures with *A. pintoi* than in pastures with grass alone, regardless of grazing system (Figure 3.4). Alternate grazing of the association at 2 hd/ha gave the highest LWG (166 kg/hd/yr) and also resulted in the highest legume content in the forage on offer. (C.E. Lascano and C. Plazas).

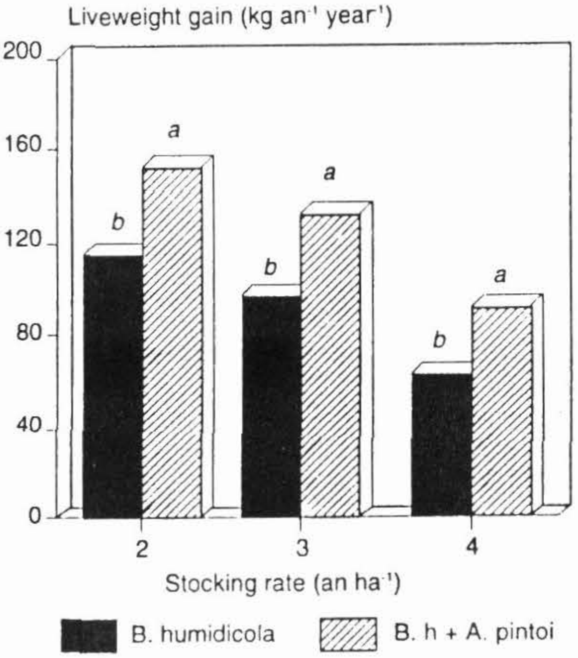

Figure 3.3 Annual liveweight gain in pastures of *B. humidicola* with on without *A. pintoi* managed with 3 stocking rates (average of 4 years of grazing).

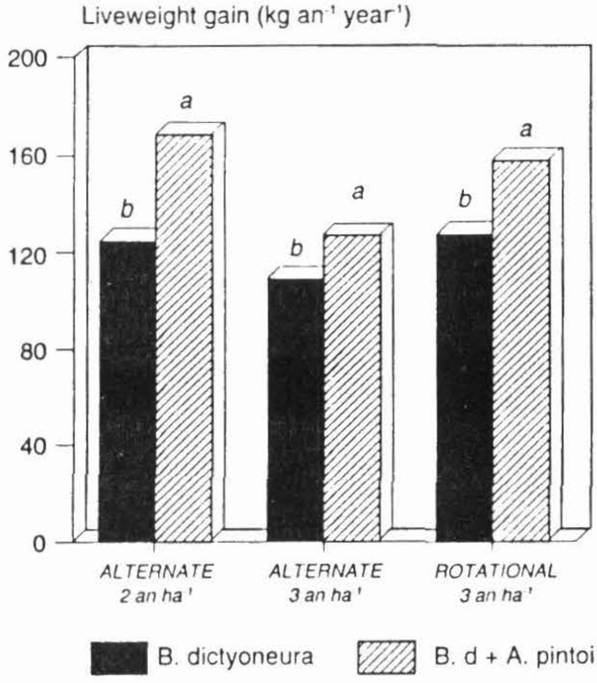

Figure 3.4 Animal liveweight gain in pastures of *B. dictyoneura* with on without *A. pintoi* under different grazing management (average of 3 years of grazing).

3.3 Stylosanthes Improvement

Introduction

Stylosanthes species have been the most successful commercial tropical legumes on a global scale. For example, 800,000 has of *S. scabra* have been sown in Australia and *S. guianensis* CIAT 184 is now grown widely in China. In tropical America the use of *Stylosanthes* species has been limited by the anthracnose disease and by poor persistence in pastures due to low seed production and poor seedling vigor.

Genetic Resources of *Stylosanthes*

Acquisition of new germplasm. During a trip in late August 1992 to Goiás and Mato Grosso, Brazil, 12 late flowering accessions of *S. capitata* were collected. This new germplasm is ready for initial seed increase. (E.A. Pizarro).

Morphological and biochemical characterization. Morphological and biochemical characterization is being conducted for the large germplasm collections of *S. capitata* and *S. guianensis* (approx. 300 or 1400 accessions, respectively) in collaboration with the CIAT GRU, Universidad del Valle, Cali, and Universidad Nacional de Colombia, Seccional Palmira. Several isozyme systems revealed strong polymorphisms which will assist in identifying the accessions, and lead to better understanding of population structure and genetic diversity of these key species. (A. Ortíz and B.L. Maass).

Agronomic evaluation of *Stylosanthes* accessions

Savannas - Llanos, Colombia. Routine screening for general adaptation, with emphasis on anthracnose resistance, was initiated for those *S. guianensis* accessions which had not previously been evaluated. One hundred accessions were established at Carimagua in 1993. (B.L. Maass and E. Cárdenas).

Savannas - Cerrados, Brazil. An agronomic trial was carried out to measure the effect of supplementary dry season irrigation on seed yield of a highly promising *S. guianensis* var. *vulgaris* line [CIAT 2950 (= BRA-017817)]. With irrigation from June until August, seed yield increased four-fold from 82 to 333 kg pure seed/ha. This accession was released by CPAC as cv. Minerao in October, 1993. (E.A. Pizarro and M. Barcellos).

Humid Tropical Lowlands - Caquetá, Colombia. In an agronomic trial in Florencia, Caquetá, all five *S. guianensis* accessions selected in the humid tropics of Pucallpa, Peru, established well and covered the soil completely. The yield data confirmed the outstanding performance of CIAT 184 (cv. Pucallpa). This line yielded particularly well during the period of minimum rainfall. The second best accession was CIAT 10136. (B.L. Maass and C.G. Meléndez).

Humid Tropical Lowlands - Los Baños, Philippines. The South East Asian Regional Forage Seeds Project at Los Baños, Philippines, introduced accessions of *S. guianensis* (12) and *S. capitata* (30). Several accessions of *S. guianensis* show good adaptation to low fertility *Imperata* grassland. Selection criteria include: resistance to anthracnose, mid-season flowering, high seed yield, high residual leaf area, and rapid recovery following close defoliation, and leaf retention after reproductive maturity. *S. guianensis* CIAT 184 is performing well at all sites in the target area. Eight ha CIAT 184 has been planted for seed production. (B. Grof).

Studies on anthracnose disease of *Stylosanthes* spp.

Anthracnose disease of *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the main factor limiting the use of this important forage legume. The pathogen exhibits considerable morphological and pathogenic variability. The distribution of races in South America is not known because of: a) a lack of reliable artificial inoculation methods; and b) a lack of differential host lines. This lack of understanding of pathogen diversity constitutes a critical bottleneck in *Stylosanthes* improvement. In order to understand pathogen diversity the following activities are undertaken:

1. Improvement of existing, or development of new inoculation methods.
2. Assembling differential inbred host lines of *S. guianensis* for testing at four field locations in Brazil and Colombia.

These studies will be conducted in conjunction with the CSIRO Division of Tropical Crops and Pastures and EMBRAPA.

***Stylosanthes* seed health**

Eight hundred seeds of each of two *S. guianensis* accessions were surface sterilized and tested for microorganisms associated with them. A number of fungi were isolated, but their incidence (% seeds infected) in no case exceeded 0.5% (S. Kelemu, C. Fernandez, J. Badel).

Growth inhibition of *Colletotrichum gloeosporioides* and other important plant pathogens by antagonistic bacteria and their extracellular products.

Although the use of host plant resistance is the best method of disease control, the use of antagonistic microorganisms and their products may be a useful alternative for highly variable pathogens. If this is to be a viable alternative, it is important to understand why, when, and how these microorganisms and their products affect the development of plant pathogens.

Twelve-day mycelial growth of *C. gloeosporioides* in the presence of cells of *Bacillus subtilis* was less than half that on a cell-free culture filtrate (Figure 3.5) (Kelemu and Badel, 1993). A number of other antagonistic bacteria, including *Rhizobium* spp. strains have been identified. (S. Kelemu, J. Badel, C. Moreno, R. Thomas).

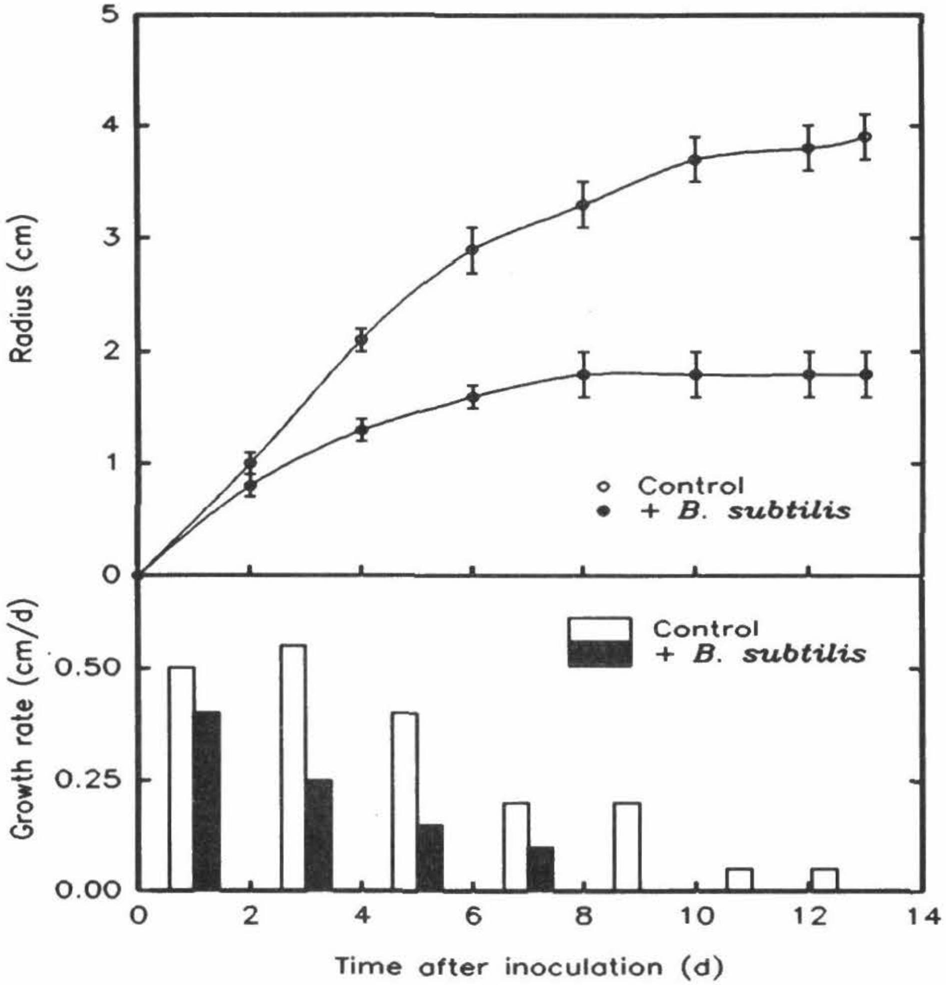

Figure 3.5 Mycelial growth inhibition of *Colletotrichum gloeosporioides* by *Bacillus subtilis* I2 on nutrient agar. Symbols represent means of five replicates and bars indicate standard deviation (Kelemu, S. and Badel, J. Australasian Plant Pathology. Accepted for publication).

Genetic manipulation of an antifungal /antibacterial, antibiotic-producing, gram- negative bacterium ('Bact. I')

Steps to molecular cloning of the gene(s) encoding antibiotics

A gram-negative bacterium tentatively designated as 'Bact.I' was isolated from seeds of *S. guianensis* harvested in 1992 at the Carimagua research station.This bacterium showed a wide range of antifungal and antibacterial properties.

Marking this bacterium for a selectable marker such as resistance to rifampicin was essential for further genetic manipulation. A rifampicin-resistant mutant with improved antibiotic production was selected on an appropriate medium. Using this selectable marker, an efficient conjugation procedure was established. Plasmid vectors carrying Tn5 were mobilized by conjugation to the recipient Rifampicin-resistant mutant Bact. I cells.

A fast bioassay for detection of antibiotic production was developed.

Since *E. coli* strains which are commonly used for cloning purposes are highly sensitive to the product of Bact.I, it was necessary either to create a mutant strain of *E. coli* which is resistant to the inhibitory product or a mutant of Bact. I which lacks the ability to produce the antifungal/ antibacterial product. A mutant strain of *E. coli* DH5 which is resistant to the inhibitory product of Bact. I was developed. However, the level of resistance is not sufficient for the mutant to be used as a cloning host.

In order to create a Tn5-mediated mutant of Bact. I, about 15,000 transconjugants carrying pSUP202::Tn5 were screened on nutrient agar with lawns of *E. coli* for loss of antibiotic production. All retained their antibiotic producing ability. Using chemical (nitrous acid) mutagenesis we recently obtained several mutants which do not produce detectable antibiotics (Figure 3.6). Secretion mutants were separated from antibiotic production mutants. We previously constructed a genomic library of Bact. I in pBluescript. A new library will be constructed in a cosmid vector in the mutant.

The gene isolated from Bact. I will be transferred to *S. guianensis* in order to create plants resistant to a wide range of fungi and bacteria.

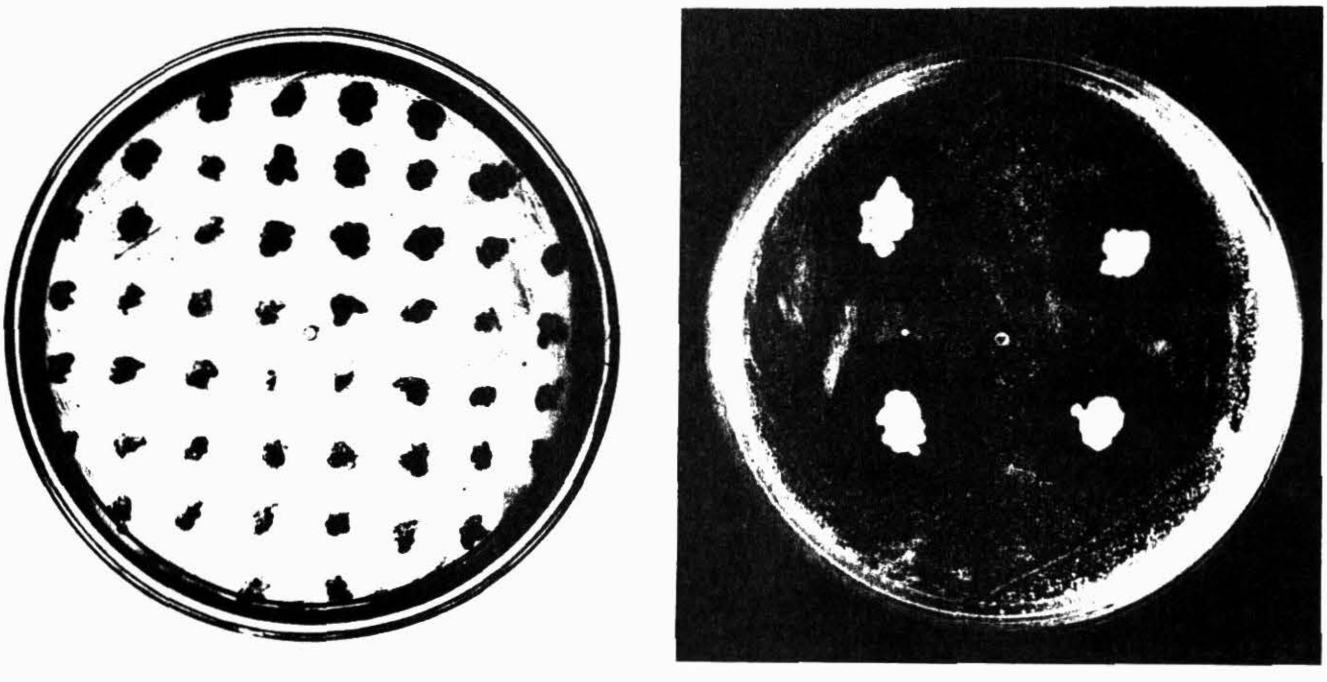

Figure 3.6 Antibiotic detection plate assay: (A) wild-type Bact. I cells and Rifampicin-resistant cells derived from Bact. I with antibiotic(s) inhibitory to *E. coli* DH5α cells; and (B) antibiotic-minus mutants and antibiotic producing cells.

Seed production

Seed production continues to be an important focus of the *S. guianensis* improvement project. Following an initial large increase in seed yield between the first and subsequent cycles of bulk generation advance, no clear trend in seed yield is apparent owing to large year-to-year variation. However, high yields (over 50 kg/ha) have been recorded for these populations under field conditions at Carimagua without insecticide protection against *Stegasta* bud worm, suggesting either that genetic yield potential has been improved over the original population or that genetic resistance to *Stegasta* bud worm has increased.

Relative high seed yields (80-100 kg/ha) were recorded with insecticide protection on larger areas (2,000 m^2) of two *S. guianensis* var. *pauciflora* pedigree-derived lines being multiplied for distribution. (J.W. Miles).

New *Stylosanthes* gene pools

Heterogeneous *S. guianensis* populations have been managed by yearly bulk generation advance since 1983, starting with an initial bulk F_2 population derived by mixing F_2 seed from the 45 biparental diallel crosses involving a selected set of 10 *S. guianensis* accessions. From the first generation advance, different harvest dates covering a range of six months were applied to subpopulations. The objective of this exercise was to assess the effect on seed yield, anthracnose resistance, and flowering maturity of a simple selection method.

The effect of the different harvest dates on maturity was striking from the first generation. While the initial set of parental accessions included five *S. guianensis* var. *vulgaris* and five *S. guianensis* var. *pauciflora* lines, the early bulk populations now appear to be essentially pure *S. guianensis* var. *vulgaris* and late populations *S. guianensis* var. *pauciflora* . Preliminary results from a small trial planted at Carimagua in 1993 show that anthracnose resistance of even the earliest bulk populations is markedly better than that of the *S. guianensis* var. *vulgaris* checks CIAT 184 and CIAT 136.

Selection of progenies from these bulk populations continued in the second season in the Philippines. The segregating populations represent a very broad genetic base and thus a good opportunity to select desirable agronomic and disease resistance traits. Two cycles of selection were carried out in each of the six hybrid bulk population for high seed production and conformity to the robust, tropical, var. vulgaris phenotype with resistance to anthracnose. Another group of transgressive segregates are early flowering, free-seeding types of short stature, and these are designed for undersowing in crops such as upland rice or cassava. Approximately, 2500 second generation progenies of these selected groups are established for seed multiplication at Los Baños and Cavinti, Laguna. [J.W. Miles, B. Grof].

3.4 *Panicum* Improvement

Genetic Resources of *Panicum*

In 1992, a donation of over 30 accessions of *Panicum maximum* germplasm, originating from the French ORSTOM collection, was received from CNPGC through CENARGEN. This new germplasm is currently in quarantine prior to initial seed increase in Colombia. (B.L. Maass).

Agronomic evaluation

The Agronomy section of the former Tropical Pastures Program selected five accessions of *P. maximum* for acid soils, based on superior forage yield under periodic clipping at two contrasting field sites (i.e. well drained savanna or piedmont) in Colombia. These accessions (CIAT 6177, 6799, 6944, 6973 and 16042) were included in a small-plot grazing experiment at Carimagua. Grass accessions, individually sown in association with *Centrosema acutifolium* (CIAT 5568) and *C. brasilianum* (CIAT 5234), have been grazed for 20 months (Oct/91 to Jun/93) with two grazing intensities (1 or 2 AU/ha).

Accessions 6799 and 6944 were more productive and persistent than the other accessions, regardless of grazing intensity or sampling period (Table 3.7). Grass on offer in pastures with accessions 6799 and

Table 3.7. Grass productivity and legume content of grazed *Panicum maximum* pastures (Carimagua).

Accession (CIAT No.)	Grass on offer* (kg DM/ha)	Legume content* (%)
6944	928	36
6799	780	37
16042	328	70
6177	158	89
6973	103	93
LSD 0.05	186	7

* Average across six evaluation periods (October 1991 to June 1993) and two grazing pressures.

6944 managed with low grazing intensity did not change over time. However, the same accessions managed at high grazing intensity suffered a 75 or 45% reduction in grass on offer, respectively, from the first to last evaluation. Legume content (% of forage on offer) in the pasture has been considerably higher with the less productive *P. maximum* accessions. In the last evaluation period (June 93) pastures sown with the accessions 6177 and 6973 were legume dominant.

The two superior accessions (6799 and 6944) were also selected in an agronomic trial at ICA-La Libertad, near Villavicencio, Colombia. Together with *P. maximum* cv. Vencedor they will be evaluated in grazing trials to measure animal performance. [C.Lascano, G. Keller-Grein, and E. Cárdenas].

3.5 *Paspalum* Improvement

Genetic resources of *Paspalum*

A major donation of 86 new *Paspalum* spp. accessions was received from CENARGEN. These are in phytosanitary quarantine prior to initial seed increase in Colombia. In addition to this new material, CIAT maintains a collection of 115 *Paspalum* spp. accessions, 79 of which were morphologically characterized by 34 descriptors during seed multiplication at CIAT-Quilichao. Most accessions were well adapted to the acid soil conditions of Quilichao. The collection is highly variable, particularly in growth habit, leaf width, and flowering maturity. (A. Ortîz, J. Belalcázar, and B.L. Maass).

Agronomic evaluation

Savannas - Cerrados, Brazil. Agronomic evaluation of 42 *Paspalum* spp. accessions on a red-yellow Latosol was completed in 1992. The average accumulated DM yield during the rainy seasons of 1991 and 1992 ranged from 0.5 to 21 t/ha. Seed yields differed greatly among accessions (from 0 to 1500 kg pure seed/ha). In 1992, a new trial was established to evaluate all *Paspalum* germplasm available at CENARGEN. The controls are selected accessions of *Paspalum* and *Brachiaria*, in addition to commercial grasses used in the area. (E.A. Pizarro and M. Barcellos).

Humid Tropical Lowlands - Los Baños, Philippines. In the evaluations conducted by the Southeast Asia Regional Forage Seeds Project two accessions of *P. atratum* and one *P. guenoarum* were productive, shade tolerant, and of high nutritive value. Other important features were tolerance to saturated soil conditions and high water table. (B. Grof).

3.6 *Centrosema* IMPROVEMENT

Agronomic evaluation of *Centrosema* accessions

Savannas - Llanos, Colombia. *C. rotundifolium* is native to environments with very sandy soils, low rainfall, and long dry season in Northeast Brazil. A stoloniferous growth habit, amphicarpic seed production, and adaptation to poor soils are valuable characteristics. Five accessions have been evaluated at Carimagua and 6 accessions at Villavicencio. Dry matter yields were relatively low and differed between rainfall periods. At Carimagua accession CIAT 25120 was the most promising with regard to DM yield and soil seed reserve. At Villavicencio, this accession as well as CIAT 5260 showed the best performance. At both sites, all accessions were susceptible to leaf-sucking insects, *Rhizoctonia* foliar blight, and Phoma leaf spot. A larger germplasm base needs to be assembled better to assess the agronomic potential of this species. Phenology and seed production of *C. rotundifolia* are being studied in collaboration with the University of Hohenheim, Germany. (G.Keller-Grein, F. Díaz, E. Cárdenas, and B.L. Maass).

Hillsides - Cauca, Colombia. Forty-five accessions of ten *Centrosema* spp. were established in 1993 in the mid-altitude hillsides in the Cauca Department of Colombia, to evaluate environmental adaptation with emphasis on adaptation to cooler temperatures. Two on-farm evaluation sites are situated at 1200 or 1600 masl. Although planting was relatively late in the rainy season, all materials established well. *C. pascuorum* and *C. plumieri* had the most rapid rate of soil cover at both sites. (B.L. Maass and F. Díaz).

Humid Tropical Lowlands - Caquetá, Colombia. Of the species of *Centrosema* tested, several accessions of *C. acutifolium* were best adapted, producing the highest DM yields both in the rainy and in the dry season. They showed rapid soil cover and thus compete with weeds. Other species, such as *C. pubescens, C. macrocarpum*, and *C. capitatum*, had one outstanding accession each. No disease problems were obvious in these species. However, all accessions were heavily attacked by leaf-eating insects. The other *Centrosema* species (*C. brasilianum, C. grazielae, C. plumieri, C. rotundifolium, C. schiedeanum*, and *C. tetragonolobum*) were not well adapted. *C. brasilianum* was severely affected by *Rhizoctonia* foliar blight. Except *C. acutifolium*, all species performed better in the period of minimum than in the period of maximum rainfall, indicating that they may be better adapted to drier environments. (B.L. Maass and C.G. Meléndez).

Humid Tropical Lowlands - Los Baños, Philippines. The Southeast Asian Regional Forage Seeds Project introduced 56 accessions of five species of *Centrosema*. Although *C. acutifolium* and *C. macrocarpum* grew well an acid soil (Matalom, Leyte and in East Kalimantan) and showed good shade tolerance under coconuts at Bicol, only CIAT 5277 (*C. acutifolium*) produced seed at Los Baños. No *C. macrocarpum* accession seeded. Four accessions of *C. pubescens* (CIAT 442, 438, 15160, and 15470) gave high DM yields and normal seed production. (B. Grof).

Rhizoctonia foliar blight

The lack of a reproducible inoculation method for this most important disease of *Centrosema* has been a bottleneck to genetic improvement of *Centrosema* (CIAT, 1984, 1987, 1988, 1989, 1990). A reliable inoculation method was developed in 1993.

Sclerotia of *Rhizoctonia solani* 5596 were produced in a liquid peptone-sucrose-yeast extract medium.

Air dried sclerotia were placed on the soil surface in contact with two-month-old, pot-grown plants (one sclerotium/plant). One hundred percent humidity was maintained during a five-day incubation period.

Disease was assessed eight days after inoculation. *C. brasilianum* (5234) and *C. tetragonolobum* (15444) reacted differently to one isolate of *R. solani* (Table 3.8).

Fourteen accessions of *C. brasilianum* are under evaluation in the greenhouse. (S. Kelemu and X. Bonilla).

Table 3.8. Reaction of two *Centrosema* species to *Rhizoctonia* solani CIAT No. 5596.

Accession	$\bar{x}$ number of blighted leaves per plant	Disease severity (%)*	Disease incidence (%)**
5234	25.7 ± 4.5	54	100
15444	9.2 ± 1.39	22	100

* (Infected leaves/total number of leaves) X 100.
** (Total number of plants infected/total number of plants inoculated) X 100.

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4. PLANT ATTRIBUTES FOR IMPROVED FEED QUALITY, PERSISTENCE, SOIL ADAPTATION AND SOIL IMPROVEMENT

Purpose

To elucidate plant attributes for feed quality, persistence, soil adaptation and soil improvement in order to develop improved evaluation procedures for forage germplasm.

Rationale

Forage quality: Some herbaceous and woody leguminous species adapted to acid soils have limited feeding value due to antiquality factors, such as tannins. An understanding of the nature of depressed intake and digestibility will assist in development of screening procedures and in design of feeding strategies.

Legume persistence: Many herbaceous legume species have good adaptation to edaphic and climatic constraints, but do not persist in association with grasses under grazing. A knowledge of plant attributes that contribute to successful survival of legumes in mixed swards would contribute to more efficient selection of legumes for use in pasture and grazing management strategies that improve persistence.

Soil adaptation: Low nutrient supply is a major limitation to forage production in acid soils of the tropics. Widespread adoption of forage species depends on efficient extraction nutrients from the soil and in utilization for growth. An understanding of mechanisms of adaptation to acid infertile soils will contribute to the development of more efficient screening procedures to develop superior forage germplasm.

Contribution to soil improvement: In the context of sustainable agriculture, forages play a role in soil improvement and erosion control in addition to their more traditional use as a feed resource. A better knowledge of factors that contribute to increased nitrogen fixation by forage legumes and soil organic matter increase will contribute to the design of improved agropastoral systems.

Highlights

Feed Quality

* Found differences in quality among and within shrub legume species. Shrub legumes such as *Cratylia argentea* and *Desmodium velutinum* have low tannin levels and higher digestibility than those of the other species evaluated.
* Verified that freeze-drying forage samples is an appropriate preservation method for tannin analysis in tropical legumes due to less complexation of these with plant protein and fiber.
* Demonstrated that decreasing tannins in *Desmodium ovalifolium* with polyethylene glycol (PEG) can result in higher intake, increased rumen ammonia and greater nitrogen retention by sheep.
* Found that short term intake and acceptability by sheep of *Cratylia argentea* is low with fresh immature forage but not with fresh mature forage. Wilting or sun-drying immature *C. argentea* partially overcomes the acceptability problem.

Soil adaptation

* *Arachis pintoi*, a tropical forage legume, was shown to be more efficient in acquiring aluminum-bound phosphorus (Al-P) and organic phosphorus from acid soil than a tropical forage grass, *Brachiaria dictyoneura*. This finding suggests that association of *A. pintoi* with *B.*
* Developed a seedling bioassay and demonstrated that the interspecific differences in acid soil adaptation among three *Brachiaria* species are not related to aluminum toxicity.
* Described and photographed the foliar symptoms of nutrient disorders in *Brachiaria decumbens*, *Arachis pintoi*, *Stylosanthes capitata* and *Centrosema acutifolium*.

Legume persistence

Studies on legume persistence were carried out by the Ecophysiologist in the Savannas Program. Those pertaining to *Arachis* have been reported in the Annual Report of that Program and the proceedings of a workshop on *Arachis* "The Biology and Agronomy of *Arachis*" published by CIAT, 1994.

Contribution to soil improvement

Studies on the contribution of legumes to soil improvement were carried out by the Nutrient Cycling Specialist in the Savannas Program and are reported in the Annual Report of that Program and the proceedings of a workshop on *Arachis* "Biology and Agronomy of *Arachis*" published by CIAT, 1994.

4.1 Studies on feed quality

Most research on leguminous shrubs and trees in tropical America has been confined to a few genera *Leucaena*, *Gliricidia*, and *Erythrina* which grow well in soils of moderate fertility and acidity. There is growing need for legume shrubs and trees with good adaptation to acid infertile soils in tropical countries. Initial work carried-out by the Tropical Forages Program has identified legume shrubs that are adapted to acid soils. However, most of the species have low feeding value due to the presence of antiquality factors, such as tannins.

Thus, research during the past two years has concentrated on: (1) screening CIAT's collection of leguminous shrubs for quality and antiquality factors, (2) comparing laboratory methods for tannin analysis, (3) evaluating the effects of method of preservation on quality of herbaceous and shrub legumes containing tannins, (4) defining the effect of tannin level on animal nutrition, and (5) evaluating the feeding value of some shrub legumes.

Screening legumes for quality and antiquality factors

A limited collection of shrub legumes planted in Carimagua and Quilichao have been screened for antiquality factors. Results showed differences in quality among and within species. Shrub legumes such as *Cratylia argentea*, *Desmodium velutinum* and *Uraria* spp. have low tannin levels and significantly higher digestibility than other species evaluated (i.e. *Flemingia macrophylla*, *Tadehagi* spp., *Calliandra* spp., *Phyllodium* spp.). In shrub legumes such as *F. macrophylla* there is variation within accessions for tannins and for in vitro digestibility. However, accessions of *F. macrophylla* with lower tannin levels (CIAT 18437, 20626, 21079 and 21090) still have very low digestibility (range 25 to 31%). This suggests that the possibility of selecting accession of *F. macrophylla* for higher quality are limited. [C. Lascano, N. Narváez, E. Cárdenas].

Laboratory methods for tannin analysis

Screening legumes for tannins require a chemical assay of high accuracy and precision and low cost. There are several laboratory methods to determine the level of condensed tannins in plant tissue. A modified Vanillin assay and the two Butanol methods were compared using a common organic solvent (70% aqueous methanol, 0.5% formic acid and 0.05% ascorbic acid) and purified tannins as standard, using samples of *Calliandra* spp. and *Tadehagi* spp. Results indicated a high positive correlation ($r = +0.9$) between Vanillin-HCl and Butanol-HCl, but negative correlations ($r = -0.7$ and -0.8) between these methods and the Butanol H_2SO_4. Variation within methods was considerably greater with Butanol-H_2SO_4, which also gave high values of extractable condensed tannins in *Calliandra* spp. This was associated with the presence of both hydrolyzable and condensed tannins in *Calliandra* spp.

Even though the Butanol-HCL method is not as fast as the modified Vanillin-HCl method, it does require less reagents and results are highly reproducible. Thus we feel that the Butanol-HCl is an acceptable method to use in screening legumes for extractable condensed tannins. Future studies will examine the effect of organic solvents on extraction of condensed tannins in some shrub legumes. [R. Cano, B. Torres, C. Lascano].

Preservation method of legumes with tannins

In the process of defining appropriate screening procedures for legumes with tannins it is important to consider sample preparation. Several reports in the literature indicate that with temperate legumes high in condensed tannins oven-drying can result in increased polymerization and binding of tannins with cell-wall proteins. In contrast, freezing forage samples in the field, followed by freeze-drying seems to reduce polymerization and complexing of tannins with plant proteins.

The effect of no drying (fresh-frozen) and of drying (oven-drying at 60°C and freeze-drying) on tannin levels and protein precipitation capacity of tannins was determined with legumes having different tannin levels. Results showed that preservation method had a significant effect on tannin extractability and protein precipitation capacity. Freeze dried forage samples had the highest extractable tannin concentrations and also the highest protein precipitation capacity, whereas fresh-frozen samples had lower tannin levels and less protein precipitation capacity.

Further we tested the effect of drying method on distribution of tannins in plant tissue and confirmed earlier findings that freeze-dried legume samples had higher extractable tannin levels than samples subject to oven-drying (Table 4.1). However, the most interesting finding was that reduction in extractable tannins in oven-dried legume samples was accompanied by an increase in tannins bound to plant protein and to a lesser extent to plant fiber. The net result being, that total tannin levels (i.e. extractable + bound) were similar in oven-dried and freeze-dried samples.

In summary, freeze-drying legume samples resulted in more extractable tannins due to less complexation of these with plant proteins. This together with ease of sample preparation and handling make freeze drying the best sample preservation method available for tannin analysis. [R. Cano, B. Torres, C. Lascano].

Effect of tannin levels on animal nutrition

To implement a genetic enhancement program for legumes with tannins or for developing feeding systems based on legumes with tannins, it is important that we understand how tannins in tropical legumes affect the nutrition of ruminant animals. With this aim we conducted a series of in vitro and in vivo experiments at CIAT.

Table 4.1. Effect of drying method on extractable and bound tannins in leaves of some tropical legumes.

Legumes (CIAT No.)	Drying method	Condensed tannins (%) Extractable	Bound to Protein	Bound to Fiber	Total
Desmodium ovalifolium (350)	Oven-dried	2.9	0.6	0.7	4.2
	Freeze-dried	4.5	0.4	0.5	5.4
Phyllodium spp. (23958)	Oven-dried	13.3	3.9	1.5	18.7
	Freeze-dried	14.5	3.6	1.7	19.8
Tadehagi spp. (13269-274)	Oven-dried	13.8	1.0	0.8	15.6
	Freeze-dried	16.0	0.3	0.2	16.4
Dioclea guianensis (19391)	Oven-dried	18.6	4.4	3.2	26.2
	Freeze-dried	20.3	3.2	1.6	25.0
Flemingia macrophylla (17403)	Oven-dried	22.5	11.5	3.2	37.3
	Freeze-dried	27.6	7.8	3.1	38.8
Mean	Oven-dried	14.2[b]	4.3[a]	1.9[a]	20.4
	Freeze-dried	16.6[a]	3.1[b]	1.4[b]	21.1
SEM		0.20	0.06	0.01	0.19

[a,b]Means in the same column for each response variable are different ($P < .05$)

Using the in vitro system we investigated how tannins in legume species differing in tannins could affectammonia production and fiber disappearance. In these studies we used polyethylene glycol (PEG) as a tannin binding agent. Ammonia levels with *A. pintoi* (CIAT 17434) with low tannin levels reached high levels after 48 hr of incubation, regardless of PEG addition (Figure 4.1). In contrast, low levels of ammonia (< 50 mg/liter) were observed with *Desmodium ovalifolium* (CIAT 350), which has high tannin levels. By binding tannins in *D. ovalifolium* with PEG, ammonia production after 48 hr of incubation was two times higher than in the untreated forage. Low ammonia levels found with *D. ovalifolium* could be a limiting factor for bacterial protein synthesis.

Results from the in vitro work with legume species of contrasting quality suggested that tannins could also depress cell-wall digestion in some legume species. Binding tannins of *D. ovalifolium* and *Tadehagi* spp. with PEG resulted in more fiber digestion after 48 hr incubation with rumen inoculum. High cell-wall residues in *D. ovalifolium* and *F. macrophylla* without PEG after 48 hr in vitro incubation, suggested that tannins formed complexes with cell-wall proteins, which were not solubilized by neutral detergent solution.

Results from the in vitro work served as a basis for designing a number of feeding trials with *D. ovalifolium* (CIAT 350) in the Quilichao research station. In these experiments we used PEG to bind tannins in the forage offered to sheep housed in metabolism crates. We found that intake of *D. ovalifolium* increased by 24% when extractable tannins were reduced with PEG from 4.6% to 1.7%. A large increase in rumen ammonia was also observed when tannins in *D. ovalifolium* were reduced with PEG. However, reducing tannins in *D. ovalifolium* with PEG did not increase digestibility of organic matter or neutral detergent fiber, as has had been predicted from the in vitro work.

We also tested the effect of tannins on nitrogen flux to the small intestine and on N excretion. Sheep fistulated in the rumen and duodenum were offered *D. ovalifolium* treated and untreated with PEG. Results showed that reducing tannins in

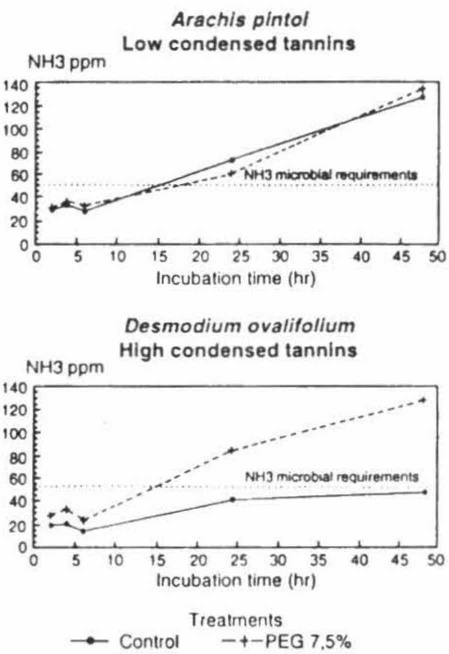


Figure 4.1. In vitro ammonia (NH3-N) levels at different incubation times of legume species varying in condensed tannin content with or without PEG.

D. ovalifolium with PEG had a large effect on nitrogen flux and excretion (Table 4.2). When *D. ovalifolium* forage with low tannin levels was fed, less nitrogen reached the small intestine, but there was less nitrogen excreted in the feces. As a result, nitrogen retained by the animal was considerably greater with *D. ovalifolium* treated with PEG.

The in vivo work showed that tannins in *D. ovalifolium* and possibly other tropical legumes have positive and negative implications in the nutrition of ruminant animals. High tannin levels in *D. ovalifolium* protected protein from being degradated by rumen microorganisms and as a result there was more nitrogen reaching the small intestine (i.e. by-pass protein). However, high tannin levels reduced intake, depressed rumen ammonia levels and incremented fecal nitrogen losses. This resulted in a significant reduction in nitrogen retention and overall low nitrogen use efficiency. It follows, that a plant improvement program aimed at lowering tannin levels in legumes such as *D. ovalifolium* could have a large impact on animal production. [J. Carulla, N. Narváez, C. Lascano].

Table 4.2. Effect of reducing tannins in *Desmodium ovalifolium* with polyethylene glycol (PEG) on nitrogen (N flux and excretion by sheep.

Measurements	Treatments			$LSD_{0.05}$	SEM
	-PEG	+PEG (3.5%)[1]	+PEG (7.5%)[1]		
Extractable tannins (%)	4.1	1.7	1.6		
N intake ($g/kg^{0.75}$/day)	0.93	0.97	1.0	0.01	0.001
N flux to duodenum (%)[2]	87	65	54	10.7	1.02
N excreted in feces (%)[2]	40	26	23	2.3	0.22
N excreted in urine (%)[2]	33	36	34		
N retained (%)[2]	27	39	43		

[1]Proportion of PEG in the forage offered. [2]As percent of N intake.

Feeding value of the shrub legume *Cratylia argentea*

Cratylia argentea is a shrub legume collected in the Brazilian Cerrados, state of Matto Grosso. This shrub legume has a number of positive agronomic attributes: it grows well on acid infertile soils, has quick establishment, tolerates drought and has vigorous regrowth after defoliation. On the other hand, *C. argentea* has good nutritional properties: high protein content, relatively high digestibility and no measurable condensed tannins.

Given the high potential forage value of *C. argentea*, there is a need to evaluate its feeding value. Preliminary observations indicated that immature *C. argentea* grown as a protein bank was rejected by grazing cows. However, cows consumed *C. argentea* when the forage was cut and wilted. Based on these preliminary observations we designed a number of experiments to determine the effect of post-harvest treatment on intake and relative acceptability of *C. argentea*. Sheep housed in metabolism crates were offered for short periods of time, fresh, wilted or sun-dried chopped leaves + fine stems of immature and mature *C. argentea*.

Results indicate that short-term intake (i.e. forage consumed in 20 minutes) was affected by post-harvest treatment when the forage offered was immature but not when it was mature (Table 4.3). Short-teri intake of immature *C. argentea* was doubled when th forage offered to sheep was wilted or sun-dried a compared to when it was offered fresh. It wa interesting to observe that intake *C. argentea* wa considerably higher with mature than with immatur forage, regardless of post-harvest treatment.

Observations made during the feedings tria suggested that age and previous experience of shee could be affecting preference for fresh or sun-dried *C argentea*. Therefore, we designed an experiment t test animal factors as well as plant factors on relativ preference of *C. argentea*. Results shown in Table 4. indicate that when sheep were given a choice of fres or dried *C. argentea* they selected more of the drie forage, regardless of forage maturity, age or previou experience of the animals. Nevertheless, it wa interesting to observe that adult sheep discriminate less between fresh and dried mature *C. argentea* tha young sheep. Also, sheep without previous experienc on *C. argentea* discriminated less between fresh an dried forage than those with previous experienc regardless of forage maturity.

In summary, our results suggest that intake an acceptability problems with *C. argentea* can b particularly serious with immature forage. Howeve wilting or sun-drying immature forage would seem t

Table 4.3. Effect of post-harvest treatment on short-term intake of *Cratylia argentea* by sheep with no previous experience.

Type of forage	Post-harvest treatment				SEM
	Fresh	Wilted 24 h	Wilted 48 h	Sun-dried	
		(g DM/20 m)			
Immature[1]	28^b	52^a	61^a	53^a	10.7
Mature[2]	97	125	--	120	15.7

[1]3-4 month regrowth

[2]3 year old stand for seed multiplication

[a,b] Means in the same row are different ($P<.05$)

Table 4.4. Effect of age and previous experience of sheep on relative preference (RP) for fresh and dried immature and mature *Cratylia argentea*.

Type of forage	Forage offered	Age[1] Young	Age[1] Adult	Sig.[2]	Previous experience[3] Without	Previous experience[3] With	Sig.
		RP (%)			RP (%)		
Immature[4]	Fresh	23^b	25^b	NS	32^a	17^d	0.05
	Dried	77^a	75^a		68^b	83^a	
Mature[5]	Fresh	23^d	32^c	0.05	37^a	17^d	0.05
	Dried	77^a	68^b		63^b	83^a	

[1]Young= 6-8 months and 17 kg average weight; Adult= 10-12 months and 26 kg average weight.

[2]Significance of the interaction type of forage with age or previous experience.

[3]Previous experience with *C. argentea*= 26 days

[4]3-4 month regrowth

[5]3 year old stand for seed multiplication

[a,b,c,d]For each type of forage, means followed by different letters are different ($P<.05$)

partially overcome the acceptability problem. As with most legumes, animal factors, such as previous experience and age, seem to have some role on acceptability of *C. argentea*. Therefore, future studies designed to measure liveweight gain and/or milk yield of animals supplemented with protein banks of *C. argentea* either in a grazing or cut and carry system need to consider plant and animal factors.

Low intake and acceptability of fresh immature and to a lesser extent fresh mature *C. argentea* suggest that some antiquality compound may be present in the edible forage. Whatever compound is present in *C. argentea* would seem to be partially lost when the forage is wilted or dried or when the plant is mature. Samples of leaves of immature and mature of *C. argentea* were analyzed for the presence of known antiquality factors in collaboration with CIAT's Biotechnology Unit. Results indicated that *C. argentea* only contained traces of condensed tannins, alkaloids, cyanogens and coumarin. However, the study revealed the presence of hydroxycoumarins other than coumarins and terpens. Whether these compounds are responsible for low intake of fresh *C. argentea* could not be determined. [M. Raaflaub, P. Avila and C. Lascano].

4.2 Studies on soil adaptation

Results obtained from glasshouse experiments conducted during the past two years indicate that in forage species adapted to nutrient-poor acid soils, fixed carbon is preferentially partitioned towards root growth at the expense of leaf expansion and shoot growth. The response to an increase in phosphorus (P) supply in acid soil was greater in a grass (*Brachiaria dictyoneura*) than in three legume species (*Arachis pintoi, Stylosanthes capitata*, and *Centrosema acutifolium*) in terms of both shoot and root growth. This increased response to P supply in the grass tested was associated with higher P use efficiency (g of forage produced for g of P in the shoots). However, P uptake efficiency (measured as mg of P uptake per unit root weight or length) was several times higher in the legumes tested than in the grass.

Phosphorus uptake by forage plants, at a given soil P supply, might be improved by: (1) a root system that provides greater contact with P; (2) a greater uptake per unit of root, due to enhanced uptake mechanisms; and/or (3) an ability to utilize insoluble organic or inorganic P forms that are relatively unavailable or poorly available to plants. Association of vesicular-arbuscular mycorrhizae can significantly affect each of these attributes. We need information on these mechanisms to explain the differences in P uptake efficiency between grass and legume species for acid soils.

Acquisition of phosphorus from different P sources

A glasshouse study was carried out to test whether the higher P uptake efficiency of acid-soil-adapted legume species was due to better ability of legumes to mobilize P from less soluble forms of phosphate (Al-P) and from organic P sources. A grass (*Brachiar dictyoneura* CIAT 6133) and a legume (*Arachis pint* CIAT 17434) were selected to grow either as monoculture or as a grass + legume association. T contrasting acid-soil types (Oxisol) from Carimag were used: sandy loam (Alegría, 65% sand) and cl loam (Pista, 18% sand). Both soils are characteriz by low pH (< 5.1) and high Al saturation (> 77% but the sandy loam had lower levels of soil orga matter and total nitrogen than the clay loam. T available P in the soil was about 2 ppm in both s types before fertilizer application. Soil was placed containers (40 kg of soil) and fertilized at the ra (kg/ha) of : 40 N, 66 Ca, 28 Mg, 100 K, 20 S, 2 Zn, Cu, 0.1 B, and 0.1 Mo. P sources used were: calcium phosphate (Ca-P), aluminum phosphate (A P), phytic acid (organic P), and cattle manure (du P). A control with no added P was included in t experiment. The rate of 20 kg/ha was chosen f each source. The dung P represents a combination inorganic and organic P forms. Twenty plants we grown in each container. Deionized water was add as needed. Containers were arranged in randomized complete block design with thr replications. All containers received mycorrhi inoculation (*Glomus occultum*), while the legur received inoculation with an effective *Rhizobium* stra (CIAT 3101). Plants were harvested after 74 days growth.

Because the differences in P acquisition were mo contrasting between the grass and the legume th between the two types of soil only the results obtain from clay loam soil are presented below. Sho biomass production per unit soil surface area of bo species was higher with a Ca-P source than with Al- organic P, and dung P (Figure 4.2). The gra produced twice as much shoot biomass as the legur with a Ca-P source. The grass plants increased sho biomass production 6.6-fold with a Ca-P sour compared with the control (no P added). T increase in shoot biomass of the legume was 2.2-fo with a Ca-P source. When no P was added, t legume and the grass + legume association produc more shoot biomass than the grass alone. T response of the grass in both root and shoot biom production with different P sources was simi (Figure 1B).

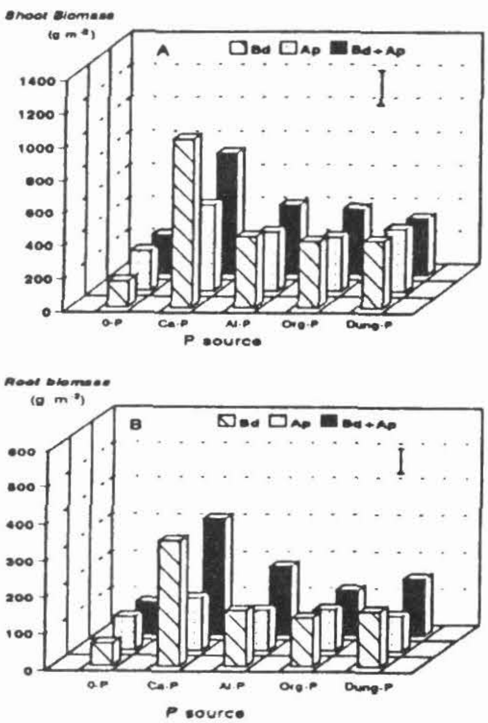


Figure 4.2 Shoot (A) and root (B) biomass production of *B. dictyoneura* (Bd), *A. pintoi* (Ap), and an association of Bd + Ap grown with different P sources in a clay loam soil.

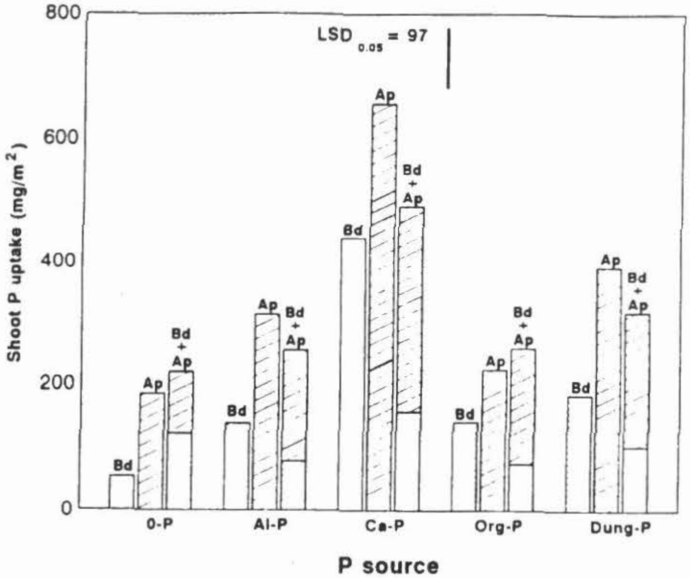


Figure 4.3. Shoot phosphorus uptake per square meter of soil surface area for *B. dictyoneura* (Bd), *A. pintoi* (Ap), and an association of Bd + Ap grown with different P sources in a clay loam soil.

However, in the case of the legume, differences in root biomass production with different P sources were not significant. The highest root biomass production was observed for both species with the Ca-P source.

In this study *B. dictyoneura* and *A. pintoi* acquired more P from the soil when it was supplied as Ca-P as compared to the other three sources (Figure 4.3).

When there was no P added to the soil, shoot P uptake per unit soil surface area by the legume or the grass + legume association was at least 3 times higher than that of the grass grown as a monoculture. Shoot P uptake of the grass in association with the legume, when no P was added to the soil, was 2.3 -fold higher than that of the grass grown as a monoculture.

The ability of *A. pintoi* to acquire more P per unit soil surface area as compared to the grass was maintained with different P sources added to the soil. It is interesting to note that *A. pintoi* plants grown either as a monoculture or in association with *B. dictyoneura* absorbed more than twice the amount of P from Al-P when compared with the grass plants. When the grass and legume were grown together, the effect was not additive, indicating that both *B. dictyoneura* and *A. pintoi* are exploiting the same source of P.

Nevertheless, this ability of *A. pintoi* to acquire P in competition with a grass could be an important attribute, that contributes to its persistence in grazed pastures.

The relationship between shoot P uptake and root length showed that the legume roots acquired more P per unit root length than the grass roots. The superior ability of the roots of *A. pintoi* to acquire P from different P sources was associated with higher levels of inorganic P in roots and to the activity of the enzyme acid phosphatase in roots (Table 4.5). The higher P use efficiency (g of forage produced per g of total P uptake) observed with the *B. dictyoneura* was associated with higher levels of the activity of the enzyme acid phosphatase in leaves.

These results indicate that the superior compatibility of *A. pintoi* with aggressive grasses such as *Brachiaria* spp. may be due to its ability to acquire phosphorus from less-available forms present in acid infertile soils. [I. M. Rao, V. Borrero and R. García].

Development of screening methods to evaluate acid soil tolerance in *Brachiaria* species

Progress in the genetic improvement of *Brachiaria* spp. (Project on forage enhancement) will depen upon the development of rapid and reliable screenin techniques which facilitate screening of large numbe of genotypes for their tolerance to acid soil condition Plant growth in acid soil is not often limited by H activity, but rather by Al toxicity and/or deficiency c essential nutrient elements such as Ca, P and N Therefore it is essential to verify whether th differences in acid soil tolerance (based on persistenc under field conditions) are related to Al toxicity or t nutrient deficiency.

Screening for tolerance to Al toxicity in soil: Thre *Brachiaria* spp. used in the breeding program (*E decumbens* cv. Basilisk, *B. brizantha* cv. Marandú an *B. ruziziensis* cv. Common) were included in a stud to test Al toxicity. In terms of persistence, *E decumbens* is much better adapted to acid so conditions than the other two species. A clay loam

Table 4.5. Leaf and root physiological characteristics of *B. dictyoneura* (Bd) and *A. pintoi* (Ap) grown with differen P sources in a clay loam soil.

Measurements	P SOURCE								
	O-P[1]		Ca-P[2]		Al-P[3]		Org-P[4]		LSD
	Bd	Ap	Bd	Ap	Bd	Ap	Bd	Ap	(P=0.05)
Leaf P (%)	0.04	0.09	0.06	0.15	0.03	0.11	0.05	0.09	0.01
Leaf acid phosphatase (μmol/m^2/s)	20.6	3.18	10.1	1.71	11.7	3.17	17.8	1.54	3.77
P use efficiency (g/g)	2370	973	2058	622	2640	863	2448	1115	349
Root P (%)	0.02	0.08	0.02	0.16	0.02	0.10	0.02	0.08	0.016
Root Pi (μmol/g fresh wt./min.)	14	46	7.5	261	15	104	15	58	22
Root acid phosphatase (μmol/g fresh wt./ min.)	2.3	2.7	0.51	2.23	1.08	1.74	1.09	1.59	0.84

[1]No P added; [2]dicalcium phosphate; [3]Aluminum phosphate; [4]Organic phosphate

Oxisol (Pista) from Carimagua was used to grow seedlings of the above three grass species in long, narrow plastic tubes (25cm long and 3cm wide) for 60 days in a glasshouse. These containers allow almost linear root systems to be produced. A removable front cover to the tube allowed repeated access to the growing root system. Soil was fertilized at the rates (kg/ha) of: 40N, 66 Ca, 28 Mg, 100 K, 50 P, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. $AlSO_4$ was added to the soil at 50, 100, 200 and 500 mg/kg soil to increase the exchangeable Al in the soil solution.

Since root elongation is very sensitive to Al in soil solution, root length density (RLD) was measured as a function of exchangeable Al in the soil. RLD of the three species decreased with the increase in exchangeable Al in soil (Figure 4.4). The extent of decrease in RLD with the increase in exchangeable Al was very similar for all the three species tested. The sensitivity of shoot growth to exchangeable Al was also very similar.

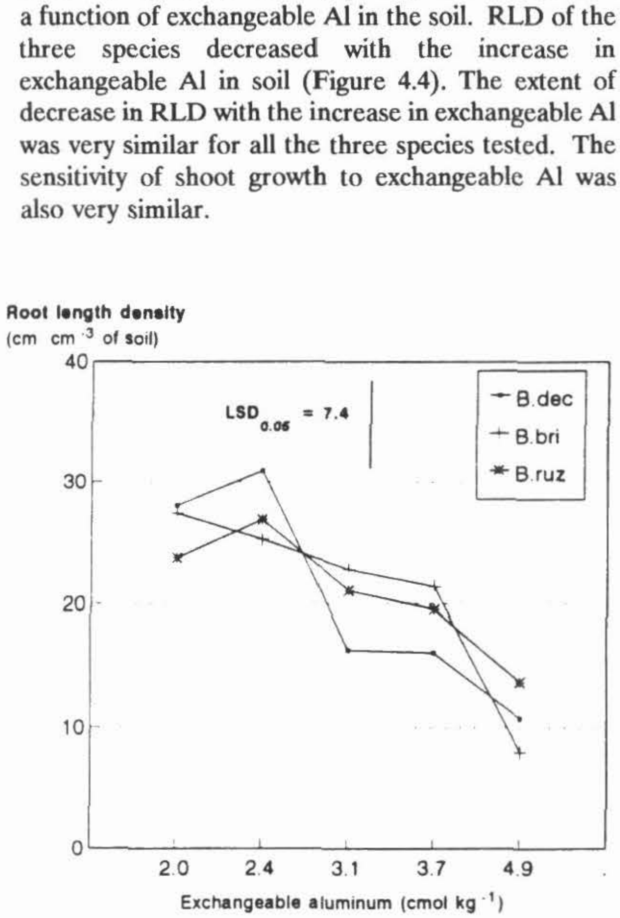


Figure 4.4 Root length density per unit soil volume of three *Brachiaria* species (*B. decumbens, B. brizantha* and *B. ruziziensis*) as affected by exchangeable aluminum in a clay loam soil.

The results of this experiment indicate that the differences in acid soil adaptation among the three *Brachiaria* species tested may not be attributed to Al toxicity, but rather to tolerance to low supply of nutrients. Preliminary results from a recent glasshouse study indicated that the differences in acid soil adaptation among three *Brachiaria* spp. may be due to changes in leaf expansion and partitioning of nitrogen in the form of soluble protein in leaves. [I. M. Rao, V. Borrero and R. García].

Foliar symptoms of nutrient disorders in some tropical grass and legume species

The symptoms of N, P, K, Mg, Ca, S, and Zn deficiencies, Mn toxicity in leaves, and Al toxicity in roots have been developed, described, and photographed for *B. decumbens* CIAT 606, *A. pintoi* CIAT 17434, *Stylosanthes capitata* CIAT 10280, and *Centrosema acutifolium* CIAT 5277.

Plant nutritional disorders are most often manifested as growth irregularities, so that distinguishing among two or more deficiencies may be difficult. Because many deficiency symptoms are similar, identifying where the deficiency occurs can be very important. We have developed a key to symptoms of nutrient deficiency in some tropical forage species to help identify a particular deficiency symptom. [I. M. Rao, V. Borrero and R. García].

5. UTILIZATION OF FORAGE GERMPLASM IN DIFFERENT ECOSYSTEMS

Purpose

To improve the utilization of forage germplasm in different production systems and ecoregions.

Rationale

As forages are utilized in such a diverse array of environments and production systems there is a need to understand germplasm x environment interactions and to define their relevant niches. With legumes, not only do they require different management to grasses, but cases of successful adoption are few and credibility is low. Thus there is a great need to define and evaluate prototype forage systems in collaboration with the agroecosystem programs and NARD's. By means of active networks in the various ecoregions, NARD's can be involved in the development and delivery of improved forage germplasm to farmers. Finally economic value of forages both to farmers and the community needs to be assessed.

Project highlights include the Workshops and the efforts therein to synchronize efforts on seed supply development and on-farm evaluation of grass-legume associations.

5.1 Forage systems and on-farm evaluation in Costa Rica

Persistence and productivity under grazing of the forage legume *Arachis pintoi* CIAT 17434 associated with stoloniferous grasses is currently under evaluation in several humid tropic sites of Costa Rica in collaboration with national and regional research institutions.

Legume persistence and compatibility is being measured at San Carlos (rainfall, 3060 mm evenly distributed throughout the year, mean temperature, 26.7 °C, and Inceptisol soils of pH 5.8, extractable P of 4.5 ppm and low Al). The experiment is under the leadership of the Instituto Tecnológico de Costa Rica (ITCR). Good compatibility of *A. pintoi* has been observed in association with the grasses *B. brizantha* CIAT 664, *B. brizantha* cv Diamantes 1 (CIAT 6780), *B. dictyoneura* CIAT 6133 and *C. nlemfuensis* (stargrass/pasto estrella). The grazing system is a rotational one of 7 days grazing and 35 days rest. After five grazing cycles legume content ranged from 10 to 21% at 3.0 AU/ha and from 5 to 9% at 1.5 AU/ha. Thus *Arachis* content is higher at the higher stocking rate. Increases in stocking rate have reduced pasture availability except for the star grass association and resulted in a higher proportion of weeds, particularly in the *B. dictyoneura* association.

Liveweight gain was measured in a *B. brizantha* (cv Diamantes 1)- *A. pintoi* pasture in a grazing trial at Guápiles under the leadership of the Ministry of Agriculture (MAG). The trial is a rotational grazing system of 7 days grazing, 21 days rest, at two stocking rates (1.5 and 3.0 AU/ha). After 16 grazing cycles, mean animal liveweight gains are above 500 g/day in the grass-legume associations; whereas with *B. brizantha* alone, liveweight gain was significant lower at the high stocking rate (345 g/an/day).

Milk production of dual purpose cows was measured by the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in an association of *A. pintoi* with star grass at Turrialba. Daily milk production increased 14% (9.5 to 10.8 kg/cow/day) in cows grazing the association compared with cows grazing the grass alone and fertilized annually with 100 kg/ha of N. The association was also effective in suppressing the invasion of weeds and native grasses.

The sylvopastoral project in Guácimo and Río Frío conducted by CATIE under the financial support of CIID, re-initiated activities in 1993. Five small dual-purpose cattle farms in the area of Río Frío and one in Guácimo continue under evaluation. These farms had been planted with either *B. brizantha* cv Diamantes 1 as monoculture or associated with *A. pintoi*. No data is yet available on milk production or on pasture components and performance. Present activities are concerned with the introduction of the tree Poró (*Erythrina berteroana*) into the existing pastures. Two farms have been planted with Poró at planting distances of 6x6 and 9x9 m in areas of 0.4 and 0.5 ha, respectively.

Another activity is the planting of 2.0 ha of Poró and Madero negro (*Gliricidia sepium*) as protein banks. It is planned to introduce *A. pintoi* as a cover legume. (P.J. Argel).

5.2 Seed systems

A seed system is regarded as that combination of components, processes and the forces at work (especially their organization, interaction, and support) involved in the production and marketing of seed of one of more species to a particular user/client group in an on-going way.

Forage seed systems are more complex than the prevalent concept of seed systems as provided by hybrid varieties of major grain crops. Forage grass seed systems of known species function well when driven by strong demand forces and when seed production is a by-product in favored geographic regions (eg. *Brachiaria* spp. in Brazil). In marked contrast, legume seed systems in South America hardly exist beyond some opportunistic harvesting of seed of *P. phaseoloides* in some years. While pioneer cultivars of a few new legume species have been released in Latin America, their seed supply at a commercial level remains incipient. Thus research is warranted to achieve successful seed systems, especially of novel species. Research on the limiting components of seed systems for both *Brachiaria* and *Arachis* has been initiated.

Intercropping for seed production

The economic feasibility of seed production can be markedly influenced by the cropping system within which seed is generated. Intercropping is often the key to viable production and lower unit costs. The agro-pastoral system of planting rice and pastures together appears highly attractive to farmers in parts of the Colombian savannas. In this system the availability and cost of forage seeds is an important consideration.

At 'Matazul' in the Colombian Llanos, a collaborative experiment with the Savanna Program, was conducted to define the opportunity to generate forage seeds within the rice-pasture system. The pasture species were *Brachiaria dictyoneura* 'Llanero' and *Stylosanthes capitata* 'Capica'. Experimental variables included a) planting system (rows vs broadcast); and planting time (simultaneous vs +15 and +30 days for the pasture species). After the rice harvest in September, the pasture was managed for seed production, first of 'Capica' in December, then of 'Llanero' in June. This also resulted in different times of commencement of grazing. Results are summarized in Table 5.1. Rice

Table 5.1. Intercropping of forage seed within a rice/pastures system, in Matazul, Colombia.

	Cropping System /Treatment	Production Yield (kg/ha) Monocrop	Intercrop[1] A	B	C	Date
1.	Rice monocrop					
	Rice grain[2]	3,200	-	-	-	Sept, 1992
2.	Rice/Pastures					
	Rice grain	-	2,800	3,000	3,000	Sept, 1992
	Pasture[3]	-		(assumed same)		Nov, 1992
3.	Rice/Pasture/Forage Seed					
	a) Rice grain	-	2,800	3,000	3,000	Sept, 1992
	Legume seed[4]	-	30	45	35	Dec, 1992
	Pasture	-		(assumed same)		Jan, 1993
	b) Rice grain	-	2,800	3,000	3,000	Sept, 1992
	Legume seed	-	30	45	35	Dec, 1992
	Grass seed[5]	-	44	26	26	July, 1993
	Pasture	-		(assumed same)		Sept, 1993

[1] Rice, *Brachiaria dictyoneura* and *Stylosanthes capitata* planted; A = simultaneously; B = pasture planted 15 days after rice; C = pasture planted 30 days after rice.; [2] "Paddy" i.e. seed with hull, 12% mc, > 90% pure seed;
[3] Established association of *B. dictyoneura/S. capitata*; [4]Pods with seed of, > 90% pure seed.
[5] Seed of , > 90% pure seed.

with pasture was lower than rice alone. The reduction was lower when pasture planting was delayed. While seedling growth of 'Capica' was improved when planted with rice, seed yields were unaffected by both planting system and time. 'Capica' seed yields were of the order of 36 kg/ha in December. 'Llanero' seed yields were favored by row planting and simultaneous planting with rice. Maximum yield was 40 kg/ha in July 1993. These results will now be submitted to an economic analysis. (J.E. Ferguson, J.I. Sanz).

5.3 Low-cost technology for establishment of legumes

Earlier studies demonstrated that a macro-pellet containing seed and fertilizer could be used to introduce legumes into improved grass pastures using minimum tillage without weed control.

More recent studies compared the use of a paper bag containing seed and fertilizer with the macro-pellet and broadcast treatments. With legumes with smaller seeds, there was higher germination with the paper bag technique than the macropellet but the reverse with legumes with large seeds e.g. *Arachis pintoi*. There was an initial higher density of plants in the 'broadcast' treatment than the other two but much poorer survival in the absence of fertilizer placement near the seed. Improved durability of the paper would have increased germination with the paper bag technique.

Another significant finding was that application of fertilizer close to the seed resulted in much more rapid establishment of *A. pintoi*. (N. Kitahara).

5.4 Institutional development and cooperation

Research training

Four PhD students are currently conducting their thesis research with Program staff while six students from European Institutions are engaged in research experience.

Nine representatives from Colombian research and development institutions received training to enable them to continue as trainers within their organizations. Seven agronomists from Latin American research institutions received specialized training within discipline areas from Program staff.

Publications

'Pasturas Tropicales'. The Communications Unit in CIAT has continued to publish this scientific journal devoted to research on tropical forages. It remains the only international publication in the field which accepts articles in Spanish or Portuguese and thus the only means for many scientists to publish their research.

However, due to budgetary cuts, editorial assistance is no longer available from core funds. The TFP is committed to finding an alternative source of funding and will support the publication in the meantime.

'Resúmenes sobre Pastos Tropicales'. The production and distribution of this regular publication of abstracts, in Spanish, of articles on tropical forage research and development has been discontinued due to budgetary cuts.

Workshops

RIEPT Savannas Network. The first meeting of the RIEPT-Savannas sub-network was held with financial and logistic support of CIID, FAO, and EMBRAPA/CPAC, from November 23-26, 1992, at Brasilia. Eighty-four members from 33 institutions of Argentina, Bolivia, Brazil, Colombia, Paraguay, and Venezuela participated in the meeting, and presented 98 papers related to evaluation of pastures in the tropical American savannas. There were 45 papers on agronomic evaluation and 23 papers on supporting topics.

On-farm research in Central America. A regional Workshop, entitled "On-farm evaluation of grass legume associations. Project design and seed procurement" was held from 7-19 June 1993 in Panamá City, Panamá. Nine countries were represented along with invited speakers and members of the Tropical Forage Program. The Workshop was coordinated by Dr. Pedro Argel.

The objective was to strengthen the Central American chapter of RIEPT by increasing the institutional capacity to conduct on farm participatory research on grass legume associations. The Workshop program included four modules, these being:

(i) Conceptual Framework for on-farm research with grass legume associations.
(ii) Case studies of regional experience.
(iii) Study tour (involving farm visits between western Costa Rica to eastern Panamá) and
(iv) Project design using the Logical Framework Matrix.

At the conclusion of the Workshop, pre-proposals for projects in 8 countries were completed. These will be developed further in the coming year. The Proceeding of the Workshop will be available in early 1994.

RIEPT Advisory Committee: focus on seeds. The eighth meeting of the Technical Advisory Committee of RIEPT was held in Villavicencio, Colombia from 18-21 of November 1992. Fifteen members, representing 14 countries, 8 invited speakers and members of the Tropical Forages Program participated actively in the event which was coordinated by Dr. John Ferguson.

The chosen theme was "Expanding seed supply of tropical forages". The program was organized into five complementary modules which dealt with:

(i) Developing seed supply and marketing systems.
(ii) Initial seed multiplication and distribution.
(iii) Production and marketing by seed enterprises.
(iv) Linkages between participants and components.
(v) Working Groups which sought to define priorities relating to (a) participant groups and their interrelations, (b) seed supply to expand on-farm research on legume grass associations, (c) a realistic seed research agenda. The proceedings of the Workshop will be available in early 1994.

Additionally, a business meeting discussed the future structure and activities of RIEPT and participants were also present at the field day organized by ICA for the release of *Arachis pintoi* CIAT 17434 with the name of 'perennial forage peanut'.

RIEPT

A meeting of the Advisory Committee and two workshops were held in 1992. There have not been any RIEPT meetings or workshops in 1993 because of the discontinuation of IDRC (CIID) funding for such activities at the end of 1992 and an inability to attract new funding.

Nevertheless, the RIEPT evaluation network is still functioning strongly as evidenced by the distribution of new germplasm for 43 regional trials in 10 countries during 1992-93. A list of new germplasm for regional evaluation is being circulated with this report.

Data continues to be sent by the RIEPT and RABAOC collaborators for entry into the germplasm evaluation data base maintained by the TFP. However, with the discontinuation of the data entry service at CIAT and the reduction in staff of the Biometrics Unit, the entry and analysis of this data is becoming an onerous task for the TFP. We are investigating the use of an electronic data entry and transfer system for NARD's cooperators.

The larger RIEPT network was decentralised into three regional networks:

(i) MCAC - Mexico, Central America and the Caribbean.
(ii) Savannas.
(iii) Humid tropics.

There has been considerable activity in the MCAC network. Some assistance is being given to the formation of National Forage networks which will nominate representatives to the MCAC Regional Committee. A MCAC newsletter is being published. A regional workshop was held on on-farm evaluation. It is planned to have the first meeting of the MCAC Regional Committee in 1994.

It is likely these new regional networks will focus on different areas of research collaboration and not only on forage evaluation.

Distribution of germplasm for ecoregional evaluation

Genetic Resources Unit (GRU). In 1992-1993, the GRU received 313 requests, and 7213 samples of 83 genera were distributed to countries (see Project 1, Table a). This distribution of germplasm included new germplasm for 43 regional trials in 10 countries. Mainly advanced materials, recommended by the Tropical Forages Program, were distributed. There was a heavy increase in requests for *Arachis* and shrub legume germplasm, such as *Cratylia argentea* and *Cajanus cajan*, and particularly for materials for soil cover and erosion control, such as *Chamaecrista rotundifolia* and the grasses *Pennisetum purpureum* cv. Mott (dwarf elefant grass) and *Vetiveria zizanoides*. (A. Ortîz and B.L. Maass)

Regional Distribution, Costa Rica. From September 1991 to August 1993, a total of 184 requests for basic and experimental seed of promising pasture germplasm were received from members of nine RIEPT-MCAC (Mexico, Central America and the Caribbean) countries. Sixty-eight percent of the requests came from Costa Rica, which is the host country of the Tropical Forages Program in the region (Table 5.2). A total of 456 kg of seed was delivered (235 kg of legume and 222 kg of grass seed), which were intended to establish regional trials type A, B, C, and D, as well as seed multiplication plots. Other purposes of requests were for cover crop and greenhouse experiments. The most requested legumes were *Arachis pintoi* CIAT 17434, 18744, and 18748, *Centrosema macrocarpum* CIAT 5713 and 5452, and *Stylosanthes guianensis* CIAT 184. The most requested grasses were *Brachiaria dictyoneura* CIAT 6133, *B. brizantha* CIAT 6780, 667, and 664, and *B. humidicola* CIAT 6369. (P.J. Argel)

Cultivar release

As a result of intensive, multilocational testing of promising germplasm, new cultivars were released by national institutions in several countries (Table 5.3). A list of released forage cultivars in South America is given in Appendix 1. (L.H. Franco and B.L. Maass).

5.5 Assessment of economic impact

As part of the prioritization process of research activities for the Operational Plan over the next decade, the programs of the center are being submitted to a socio-economic evaluation. Economic efficiency indicators such as Present Value of Expected Social Benefits and Internal Rate of Return were estimated for each project in collaboration with the Impact Evaluation Section.

Four phases were investigated. (i). Definition of economic and technical coefficients. (ii). Simulation of market evolution of the products effected for technological change, eg meat and milk. In this way

Table 5.2. Distribution of seed of promising germplasm in Central America.

Country	Requests (no.)	Legumes (kg)	Grasses (kg)
Costa Rica	125	108.3	125.0
Cuba	1	9.0	-
Dominican Republic	7	41.0	26.4
El Salvador	6	3.5	2.8
Guatemala	14	5.9	7.0
Honduras	12	11.2	30.1
México	7	20.1	0.1
Nicaragua	8	14.5	14.0
Panamá	4	21.0	16.1
Total	184	234.5	221.5

Table 5.3. New cultivars released by NARS in different countries during 1992-1993.

Species	CIAT accession (no.)	Country and year	Cultivar name
Legumes			
Arachis pintoi	CIAT 17434	Colombia (1992)	Maní Forrajero Perenne
		Honduras (1993)	Pico Bonito
Stylosanthes guianensis	CIAT 2950	Brazil (1993)	Mineirão
Grasses			
Andropogon gayanus	CIAT 621	Guatemala (1992)	ICTA-Real
Brachiaria dictyoneura	CIAT 6133	Panamá (1992)	Gualaca
Brachiaria humidicola	CIAT 679	Colombia (1992)	Pasto Humidicola

the derived social benefits for the new technology, their distribution over time and between producers and consumers according to farm size and income were estimated. (iii). Determination of the investment associated with each project. (iv). Estimation of the economic efficiency indicators of the invested funds. This type of analysis has been conducted for; the Savannas, Forest Margin and Hillsides ecosystems; for both fattening and dual purpose production systems; for pastures based on *Brachiaria spp*., for grass legume associations including *Arachis pintoi*, *Stylosanthes spp*.; *Centrosema spp*. and *Desmodium spp*. and for various crop/pastoral systems. (L. Rivas).

Appendix 1. Grasses and legumes released as commercial cultivars adapted to acid tropical soils (1980-1993).

Species	Accession CIAT No.	Name of cultivar	Year of release	Country
A. GRASSES				
Andropogon gayanus	621	Carimagua 1	1980	Colombia
		Planaltina	1980	Brasil
		Sabanero	1983	Venezuela
		Veranero	1983	Panamá
		San Martín	1984	Perú
		Llanero	1986	Mexico
		Andropogon	1988	Cuba
		Veranero	1989	Costa Rica
		Otoreño	1989	Honduras
		Gamba	1989	Nicaragua
		ICTA- Real	1992	Guatemala
Brachiaria dictyoneura	6133	Llanero	1987	Colombia
		Gualaca	1992	Panamá
		Ganadero	1993	Venezuela
Brachiaria brizantha	6780	Marandú	1984	Brasil
		Brizantha	1987	Cuba
		Gigante	1989	Venezuela
		Insurgente	1989	Mexico
		Diamantes 1	1991	Costa Rica
	26646	La Libertad	1987	Colombia
Brachiaria decumbens	606	Brachiaria	1987	Cuba
		Chontalpo	1989	Mexico
		Señal	1989	Panamá
		Pasto Peludo	1991	Costa Rica
Brachiaria humidicola	679	INIAP-Napo	1985	Ecuador
		Aguja	1989	Venezuela
		Humidicola	1989	Panamá
		Chetumal	1990	Mexico
		Humidicola	1992	Colombia
Panicum maximum	26900	Vencedor	1990	Brasil
	16031	Tanzania 1	1990	Brasil
B. LEGUMES				
Arachis pintoi	17434	Amarillo	1990	Australia
		Maní Forrajero Perenne	1992	Colombia
		Pico Bonito	1993	Honduras
Centrosema pubescens	438	El Porvenir	1990	Honduras
	-	Villanueva	1993	Cuba
Centrosema acutifolium	5277	Vichada	1987	Colombia
Clitoria ternatea	20692	Tehuana	1988	Mexico
		Clitoria	1990	Honduras
Desmodium ovalifolium	350	Itabela	1989	Brasil
Leucaena leucocephala	21888	Romelia	1991	Colombia
Pueraria phaseoloides	9900	Jarocha	1989	Mexico
Stylosanthes capitata	10280	Capica	1983	Colombia
S.guianensis var vulgaris	184	Pucallpa	1985	Perú
		Bihuadou (Zhuhuacao)	1987	China
	2950	Mineirao	1993	Brasil
S.guianensis var pauciflora	2243	Bandeirante	1983	Brasil
S.macrocephala	1281	Pioneiro	1983	Brasil

6. INTER-PROGRAM PROJECT

6.1 Nutrient cycling and animal production

Purpose

To understand the functioning of grass-legume pastures with the aim of modelling the dominant processes to enable prediction of the effect of management options on persistence of the pasture, nutrient cycling and associated animal production.

Rationale and Background

Legume-based pastures give significant increases in animal production compared with improved pure grass pastures. In these pastures, legumes play a key role by fixing nitrogen, stimulating cycling of other nutrients, and enhancing both the quantity and quality of the forage. However, legume-based pastures are often unstable, and management to maintain an adequate proportion of legume is little understood. A multidisciplinary team from the Savannas and the Tropical Forages Programs is investigating the main processes involved in grass-legume and grass pastures in a long-term experiment at the Carimagua Research Station where different grass and grass-legume pasture have been established on two soil types.

Scientists from the TFP have the rsponsibility for studies on plant-soil relations and animal intake and production and those from the the Savannas Program on pasture dynamics and nitrogen and mineral nutrient cycling. Only the detailed results of the research on plant-soil relations and animal production are presented here but a brief summary is given of the overall results. The experimental design was reported in the 1990 and 1991 reports of the old Tropical Pastures Program and details of other current research on the project are presented in the Annual Report for the Savannas Program.

Animal production on the grass-legume pastures has been closely related to the amount of legume in the pasture. Thus if there was high legume initially and then a decline, animal production closely followed this pattern and there was little residual effect of the legume. The labile nitrogen in the system appears to be the dominant driving force. Thus any factors that effect legume production have a large impact on the system. Potassium deficiency occurs on one soil type, has had a strong but diiferential effect on the tw legumes on that soil type and obviously becom another variable to be managed.

Highlights of research on soil-plant relations an animal production

* Root biomass production in improved pastur increased more in the sandy loam than in the cl loam soil.

* The proporticn of legume roots in we established legume-grass pastures was less than 20 of the total root biomass, but similar to t proportion of the legume.

* The presence of legume in an association had greater effect on animal production than the level initial fertilizer application or stocking rat particularly on the sandy loam soil.

Soil-Plant Relations

The specific research activities were: (i) quantificati of root biomass production, root distribution, and ro length; (ii) estimation of the proportion of legum root biomass in a grass-legume association; (i determination of nutrient composition of roots; (i estimation of nutrient dynamics in soil; and (determination of soil physical characteristics.

Root dynamics. A knowledge of the root dynamics a key component in the study of nutrient cyclin Sequential determination of the root biomass shou reflect the net result of new root growth and loss roots to death and decay. Heavy grazing is known cause a reduction in root growth and implications this need to be studied.

Root coring was used to determine root distributio biomass, and length in different pastures grazed medium stocking rates. Pasture treatments studi were:
sandy loam soil site -

B. dictyoneura alone (Bd)	low initial fertiliz
Bd + *C. acutifolium* (Bd + Ca)	low initial fertiliz
Bd + *S. capitata* (Bd + Sc)	low initial fertiliz
Bd + *C. acutifolium* (Bd + Ca)	high initial fertiliz
Native pasture -	no fertilizer

clay loam soil site -

B. dictyoneura alone (Bd)	low initial fertilizer
Bd + C. acutifolium (Bd+Ca)	low initial fertilizer
Bd + A. pintoi (Bd+Ap)	low initial fertilizer
Bd + A. pintoi (Bd+Ap)	high initial fertilier
Native pasture	no fertilizer

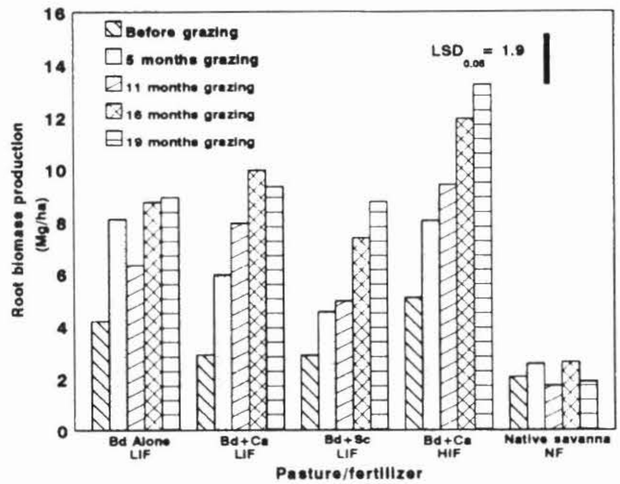
Low fertilizer application was (kg/ha): 20 P, 20 K, 50 Ca, 20 Mg, 12 S, 2 Zn, 2 Cu, 0.5 B, 0.1 Mo and high fertilizer application (kg/ha): 60 P, 60 K, 150 Ca, 60 Mg, 24 S, 2 Zn, 2 Cu, 0.5 B, 0.1 Mo.

Native savanna pastures had much lower root biomass and root length (2 Mg/ha and 8-17 km/m^2) compared to improved pastures (4 to 5 Mg/ha and 22 km/m^2). More than 60% of the total root biomass was found in the top 30 cm and more than 50% of live root length in the top 20 cm. Before grazing was initiated (early rainy season), at low initial fertilizer, total root biomass of the grass alone (Bd) treatment was higher than that of the two associations ('Bd+Ca' and 'Bd+Sc') at the sandy loam site and of one association ('Bd+Ca') at the clay loam site (Figure 6.1). Because of poor establishment of *A. pintoi* in the association at the clay loam site, total root biomass of the grass-legume pasture was very similar to that of the grass-alone pasture. The high initial fertilizer treatment increased total root biomass in both soils for the ' Bd+Ca' association.

Root biomass increased with time in all pastures but much more so on the sandy loam soil than the clay loam soil (Figure 6.1). There was a response to the higher fertilizer rate in the sandy loam but not the clay loam soil. The grass-alone pasture had more roots than the grass-legume associations in the clay loam soil but similar amounts of roots in the sandy loam soil. Root biomass remained low in the native pastures.

These changes in root biomass under grazing over time reflect the influence of several factors, including: nitrogen supply from the legume, grazing, soil type, and fertility status. The increase in the root biomass with time in sandy loam soil suggest that the grass may be exploring more soil volume for limiting nutrients such as N, K, P, and Ca and moisture. Results on root length production showed similar trends to those of root biomass production.

Sandy loam site



Clay loam site

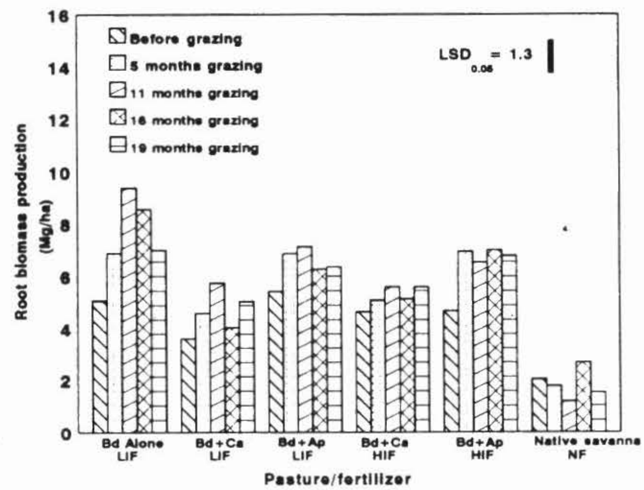
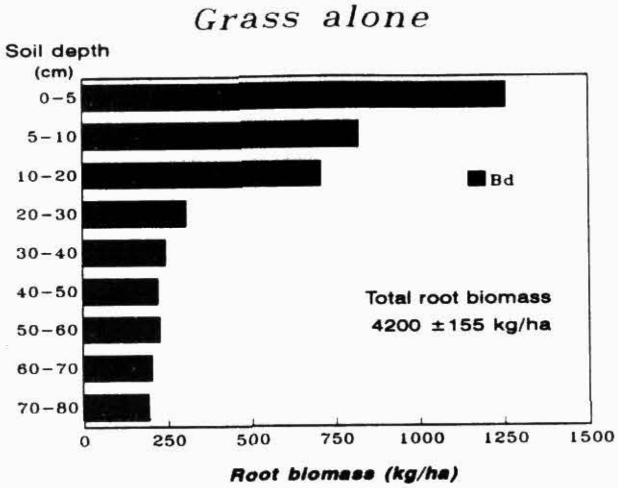


Figure 6.1. Total root biomass as influenced by soil type and fertilizer application in several pastures established at sandy loam and clay loam sites. Samples were taken before grazing began, and at 5, 11, 16, and 19 months after grazing at medium stocking rate. LIF = Low Onitial Fertilizer; HIF = High Initial Fertilizer; NF = No Fertilizer application (see text for rates).



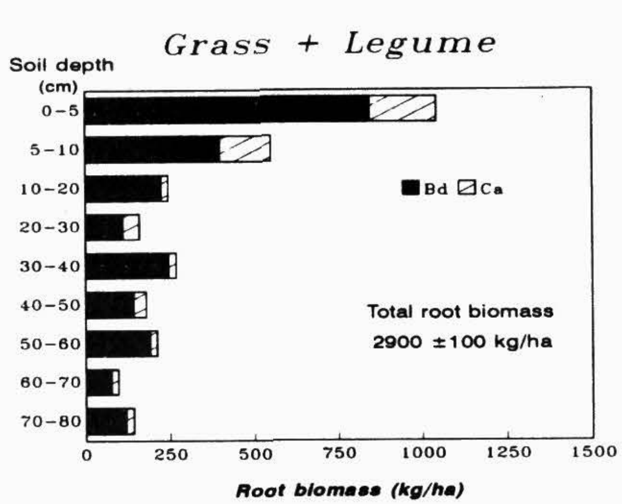


Figure 6.2. Root biomass distribution of a grass-alone (*B. dictyoneura*) and grass-legume (*B. dictyoneura* + *C. acutifolium*) pasture before the initiation of grazing in the sandy loam soil with low initial fertilizer application. The proportion of legume root in the grass-legume association was determined by stable isotope analysis.

Estimation of legume roots in an association. The proportion of legume root in the pastures of 'Bd + Ca' at low fertilizer application in the sandy loam soil was determined by stable carbon isotope analysis (Figure 6.2). The difference in the photosynthetic pathway between grass (C_4) and legume (C_3) provides a means of estimating the proportion of grass and legume. The proportion of legume root proportion was less than 20% down the soil profile. There was more root biomass in the top 20 cm of soil profile in the grass alone pasture than in that of the association. Having a greater proportion of roots in the top 20 cm of the soil may result in greater absorption of nutrients released from plant residues and thus less loss by leaching.

Nutrient composition of roots. Although the proportion of legume roots in grass-legume associations was less than 20%, there was an increase in the N concentration of the roots at low initial fertilizer application. Root Ca concentraton in the 'Bd + Ca' association in the sandy loam was increased 2-3 fold.. Roots from grass-legume associations in the clay loam soil contained higher K and lower Ca concentrations compared to roots from similar associations from sandy loam soil. Surprisingly, roots of native grasses had a higher N concentration (0.29-0.42%) than roots from the grass alone (Bd) treatment (0.2-0.29%) for both soils.

Nutrient dynamics in soil. The changes in soil extractable P and exchangeable K and Ca of a grass-alone (Bd) pasture with low initial fertilizer application and the 'Bd + Ca' grass-legume association with low and high initial fertilizer at different soil depths at both sites was monitored over time. Soil P, K, and Ca available for plant growth decreased over time in both the Bd and 'Bd + Ca' pastures at the two sites. However, the decrease was more pronounced at the clay loam site. In the 'Bd + Ca' association with high fertilizer, available P in soil, before grazing, was higher in the sandy loam than clay loam soil. But at 11 months after grazing, available P in the top 5 cm soil layer was greater than that of the value before grazing, indicating possible P cycling.

Exchangeable K was higher in the clay loam than sandy loam soil. There was also an indication of K cycling in the improved pasures in the top 5 cm soil layer in the clay loam soil. Exchangeable Ca in the 0-10 cm layer of the clay loam soil was much higher than that of the sandy loam soil for each pasture type. Monitoring the soil nutrient dynamics will allow an evaluation of the residual fertilizer in the two soils.

Soil physical characteristics. Measurements of water sorptivity, hydraulic conductivity, bulk density and structural stability were made at the beginning of the rainy season each year. A native savanna pasture was included as a reference point. Tillage effected soil physical properties on both soil types. An increase in root biomass in the sandy loam soil also appeared to decrease water sorptivity and hydraulic conductivity, e.g. in the grass-alone at low initial fertilizer and grass-legume associations at high initial fertilizer. There were small differences in bulk density among treatments. [I. M. Rao and P. Herrera].

Animal Production

Measurements of liveweight gain were recorded over 5 grazing cycles (700 grazing days) in grass and grass-legume pastures established at two sites (sandy loam and clay loam) at Carimagua (TPP Annual Report, 1990; 1991).

At the sandy loam soil site, average liveweight gains were greater ($P<.05$) in pastures with legumes (*C. acutifolium* and *S. capitata*) than in pure *B. dictyoneura* pastures (Table 6.1). However, it is interesting to observe that the liveweight gain decline over time in all pastures was associated with loss of legume from the pastures. In pastures with *C. acutifolium*, the legume practically disappeared by the end of the second grazing cycle, whereas the legume in pastures with *S. capitata* dropped significantly by the end of the third grazing cycle.

Table 6.1. Effect of legumes on average daily gain of steers grazing *Brachiaria dictyoneura* pastures at two sites with contrasting soil texture. Pastures were established with low initial fertilizer application (kg/ha: 20 P, 20 K, 50 Ca, 20 Mg, 12 S, 2 Zn, 2 Cu, 0.5 B, 0.1 Mo).

Sites	Pastures (hd/ha)	Grazing cycles* 1	2	3	4	5	Mean
		(g/hd/day)					
Sandy loam soil**	*B. dictyoneura* (1.5)	218	171	165	136	59	150
	+ *C. acutifolium* (1.5)	588	524	370	290	75	369
	+ *S. capitata* (1.5)	508	495	375	261	156	359
	SEM						39
	$LSD_{0.05}$						127
Clay loam soil***	*B. dictyoneura* (2.0)	438	179	348	173	267	281
	+ *C. acutifolium* (2.0)	519	336	486	323	459	424
	+ *A. pintoi* (2.5)	472	200	344	243	317	315
	SEM						31
	$LSD_{0.05}$						101

* Grazing cycle = 140 days

** Grazing days = 700 (30.07.91 to 25.05.93)

***Grazing days = 700 (19.09.91 to 15.07.93)

At the clay loam soil site, average liveweight gains were greater (P<.05) in pastures with *C. acutifolium* than in pastures with only grass or grass + *A. pintoi* (Table 6.1). At this site, *C. acutifolium* established well and has persisted over a range of management treatments (i.e., initial fertilizer level, stocking rate). In contrast, *A. pintoi* did not establish well and thus has made little contribution to the forage on offer. The effect of fertilizer treatment and stocking rate on animal gains was analyzed at both sites. Average liveweight gain in grass-legume pastures was not affected (P>.05) by fertilizer treatment at either site. However, at both sites animal gains were lower in *B. dictyoneura* + *C. acutifolium* pastures grazed at the highest stocking rate (2.0 hd/ha in sandy loam soil and 2.5 hd/ha in the clay loam soil).

In this experiment, legume has generally had a greater effect on liveweight gain than fertilizer treatment or stocking rate. At the site with sandy loam soil, liveweight gains were 2- to 3-fold higher in grass-legume than in the grass-alone pasture when legume content in the pasture was on the order of 13% (*C. acutifolium*) to 35% (*S. capitata*). However, as legume content declined, liveweight gains also declined, with no indication of a residual effect of the legume. At the site with clay loam soil, liveweight gains in the association with *C. acutifolium* have been 20 to 90% greater than in the grass-alone pasture, with the greatest benefit being in the dry season (grazing cycles 2 and 4). In addition, at the site with clay loam soil, animal gains decreased 40% over a 2-year period in the grass-alone pasture, but remained relatively stable in the same period in pastures with *C. acutifolium*. These results, from soils of contrasting texture and nutrient status, confirm the importance of legume persistence for sustainable animal production. [C. Lascano and C. Plazas].

7. LIST OF CIAT TROPICAL FORAGES PROGRAM STAFF PUBLICATIONS (1992-1993)

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Senior Research Fellow

GERHARD KELLER-GREIN, Dr sc. ag
Agronomist

NORIHISA KITAHARA, Ph.D., Pasture Scient
(TARC)
(Left June 1993)

Research Associates

LIBARDO RIVAS, Economist, Leader's Office

RAUL BOTERO, Veterinarian and Animal Scienti
Leader's Office

Research Assistants

LUIS HORACIO FRANCO, Agronomist, Leade
Office

JAVIER ASDRUBAL CANO, Agricultu
Economist, Leader's Office

GERARDO RAMIREZ, Statistician, Leader's Off

CARLOS IVAN CARDOZO, Agronomist, Se
Biology

MANUEL SANCHEZ, Agronomist, Seeds Biolog

Research Assistants (Cont'd.)

FERNANDO DIAZ, Agronomist, Germplasm

CARLOS GUILLERMO MELENDEZ, Agronomist, Germplasm

JORGE LUIS BADEL, Biochemist, Plant Pathology

ALEXANDRA VALDERRAMA, Genetic Biologist, Plant Pathology

MARTHA ESCANDON, Agronomist, Genetics

EDGAR CARDENAS, Zootecnist, Genetics

GUILLERMO SOTELO, Biologist, Entomology

GUILLERMO ARANGO, Biologist, Entomology (Left September 1993)

OSCAR GUZMAN, Agronomist, Entomology (Left June 1993)

BLANCA TORRES
Quality/Ruminant Nutrition
(Left October 1993)

NELMY NARVAEZ, Zootechnist, Forage Quality/Ruminant Nutrition

PATRICIA AVILA, Zootechnist, Forage Quality/Ruminant Nutrition

CAMILO PLAZAS, Zootechnist, Forage Quality/Ruminant Nutrition

AIRES C. MARCELO, Agronomist (Cerrados, Brazil)

ALFREDO VALERIO, Agronomist, Germplasm Selection (Central America, Costa Rica)

VICENTE BORRERO, Agronomist, Plant Nutrition

PEDRO HERRERA, Agronomist, Plant Nutrition

Secretaries

BLANCA NORA AGUIRRE
JULIA GOMEZ
MAGDA LAMBERT (Brasília, Brazil)
MARTHA REYES
ANA VEGA (San José, Costa Rica)
DELIA OSPINA DE FRANCO

9. Donors - Complementary Projects

Donor/Project	Duration	Total Pledge (US$)
Australia - AIDAB		
Forage network in Southeast Asia	1991-1994	773,000
Stylosanthes with stable resistance to Anthracnose	1988-1993	395,000
Canada - IDRC		
Tropical Forages Network	1988-1992	683,000
Seed Pastures Fund	1990-1992	15,000
Germany - BMZ		
Rhizosphere P dynamics	1991-1994	131,471
Great Britain - ODA		
Taxonomic revision of *Brachiaria* germplasm	1992-1993	23,158
Japan - TARC		
Improved native grassland research	1989-1994	386,000