Biennial Report 1994-1995

Tropical Forages

Working Document No. 152, 1995

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Index

Introduction

This is the second Biennial Report of the Tropical Forages Program, CIAT. It has been prepared as a report to the Program Committee of the Board of Trustees of CIAT and will serve as a basis for discussion at the Internal Program Review.

It is also being sent to colleagues in the RIEPT, SEAFRAD and ILRI supported AFRNET networks and to others with whom we collaborate. We invite you to provide feedback on the relevance of our current activities and how we can achieve closer coordination and collaboration with you. Feedback is important at this time as CIAT will be preparing a new medium term plan for the period 1998-2000 in 1996.

1.1 An evolving strategy

Tropical forage research at CIAT began with selection of grasses and legumes for the humid tropical lowland savannas, firstly in the Llanos of Colombia in collaboration with ICA. The acid infertile soils and high incidence of disease and pests make this is a harsh environment for plants; pasture plants selected for other tropical environments failed to survive when introduced there.

This was followed by the evaluation of forages for the Cerrados, an environment with similar soils but a longer and more harsh dry season, in collaboration with EMBRAPA.

This was a period of active collection of new forage species from South America, Africa and Asia which was led by CIAT but with active collaboration of NARS and support of IPGRI. The emphasis on selection was on improvement of forage yield and quality, in addition to climatic and edaphic adaptation, as the native grasses of the savannas have very low feed value.

Evaluation was then extended to the forest margins through research sites at Guápiles, Costa Rica, and Pucallpa, Peru, and in close liaison with Brazilian researchers in the Amazon. In the forest margins, the emphasis was on identifying stable grass-legume associations as an alternative component for stabilizing land use in deforested areas.

Evaluation of germplasm identified by CIAT that had proved successful in these ecoregions was then extended to smallholder mixed-farming systems in the hillsides of Cauca, Colombia, and humid and subhumid areas of South East Asia and West Africa.

In addition to direct evaluation by CIAT personnel, hundreds of NARS researchers in tropical America were collaborating in the evaluation of promising forage accessions through the RIEPT network.

Thus by the time of the formalization of natural resource management research in 1992, CIAT was engaged in research on forage evaluation and production on a global scale throughout the subhumid and humid tropics and with specialist staff in a wide range of disciplines - plant geography, genetics, pathology, entomology, ecology, plant nutrition, animal nutrition, agronomy and socio-economics.

1.2 A new strategy

A new CIAT. In July 1992, CIAT introduced natural resource management research to complement germplasm development in the mandate crops --Beans, Cassava and Tropical Forages -- and Rice.

Natural resource management research (NRM) in CIAT is focused on three ecoregions in tropical America - the savannas, forest margins and mid-altitude hillsides. Process research is carried out at specific sites: savannas -Llanos, Colombia, and Cerrados, Brazil (with associated activities beginning in Santa Cruz, Bolivia); forest margins - Acre-Rondonia, Brazil and Pucallpa, Peru; Mid-altitude hillsides - Cauca, Colombia and a site in Honduras-Nicaragua, Central America.

Research at these sites is carried out in collaboration with other international and national organizations through research consortia. The Tropical Forage Program (TFP) interacts with the NRM Programs in provision of forage components for production system research. In fact, virtually all the advanced germplasm lines and forage components that have been developed in the TFP can be used in one or other of the niches in the ecoregions in which the NRM Programs operate.

However, the TFP also has a mandate to collaborate with NARS throughout tropical America, and in the subhumid and humid regions of South East Asia and Africa in identifying forage components for a much broader range of farming systems than occur within the CIAT NRM mandate ecoregions. At the same time, we are rationalizing our activities with other IARC's of the CGIAR system that have global responsibilities e.g. IPGRI in genetic resources and ILRI in livestock research.

An on-going forage program. The goal of the Tropical Forages Program is to acquire, identify and improve tropical forage germplasm that has a role in increasing the efficiency of livestock production and sustainable land use in production systems of the subhumid and humid tropics.

Research has been organized into 3 research areas and 8 projects:

i) Forage Genetic Resources
   Project: Genetic Resources & Evaluation
ii) Forage Improvement
   Projects: *Brachiaria*
   Arachis
   Stylosanthes
iii) Forage Adaptation and Utilization
   Projects: Forage quality
   Forage adaptation
   Forage component development
   Institutional linkages

with 9 senior staff located at Palmira (6), Brazil (1), Costa Rica (1) and Philippines (1).

Particular attention is focused on the above three genera in the expectation of overcoming major limitations, namely, *Brachiaria* - susceptibility to spittlebug, *Arachis* - limited availability of germplasm and *Stylosanthes* - susceptibility to anthracnose. However, there is a considerable amount of adapted germplasm available for other genera and on-going research in identifying promising new germplasm.

The Program would like to see more research undertaken in *Centrosema* and *Desmodium*, to remove limitations of seed production in *C. acutifolium*, *C. macrocarpum* and *C. pubescens* and disease susceptibility in *C. brasilianum* and *C. pubescens*, and to reduce tannins in *D. ovalifolium* to take advantage of its excellent agronomic characteristics.

A revised strategic focus. Firstly, the advent of NRM research in CIAT has meant that we now look on forages as having a much wider application than feed for livestock. Forages can contribute to soil improvement and thus to more sustainable land use through their use in agroforestry systems, as covers under tree crops, soil erosion barriers and, green manures. The reality is that there is often more widespread adoption of legumes for these other purposes than for feed.

Secondly, our focus is more demand driven, paying attention to identification of forage germplasm for particular production niches where there is real need, interest and ability of producers to adopt new technology. This may be for fruit producers, intensive mixed crop-livestock systems (see 8.12), or smallholder dual-purpose cattle farmers.

Thirdly, this means that we are involving producers at an earlier stage in selection of germplasm (see 2.16) and in the development of germplasm into a forage component technology (see 8.15) appropriate to particular production systems.

Fourthly, because of limited resources, a greater proportion of research is being carried out in collaboration with the NRM programs at their research sites and with government and private organizations at other sites (see 8.10).

New alliances. Much of the active research and development activity in many countries has now moved to universities, cooperatives and NGO's from the official government sector. We are actively developing linkages with these new groups through collaborative research and by provision of training.

Active RIEPT activities are still maintained in Mexico, Central America and the Caribbean through an out-posted scientist, who provides germplasm for regional evaluation, produces a regional newsletter and conducts regional workshops with the collaboration of Palmira-based staff. In South America contact is maintained through a newsletter and occasional visits.

Countries are encouraged to form national forage networks. This is taking place in Cuba, Colombia, Mexico and Panama. Brazil has an established network for forage evaluation.

However, effective collaboration with NARS is best achieved through jointly prepared bi-lateral and regional projects. One example is the project 'Preservation of wild Arachis species' between CENARGEN, ICRISAT and CIAT with CENARGEN as the lead organization. Others are the project with CORPOICA, to develop new forage components for smallholder dual-purpose cattle production in the forest margin area of Caquetá, Colombia, and the 'Forage for Smallholders Project' in South East Asia. Our own Project coordinators are also developing strong linkages with Advanced Research Organizations in developed countries. This collaboration is acknowledged in the summaries of research activities in the main body of the report

Again donors are increasingly funding research in CIAT through restricted core funding as with the case of the Government of Colombia whose grant is linked to forage, rice and natural resource management research.

1.3 Program activities 1994-1995 - highlights

The main activities and outcomes have been summarized at the beginning of each Project report with anticipated activities for the coming year listed at the end of the report. Here we highlight only a few of the major research outcomes and activities in which the TFP has been involved in the last two years.

Research developments

Genetic Resources

- Collection activities have greatly increased the number of available accessions of *Arachis pintoi* to (113) and the promising shrub legume *Cratylia argentea* to 57 accessions.
- There is now an agreed plan for upgrading the GRU.
- A working group initiated the establishment of a tropical forage genetic resources network
- Germplasm identified through multi-locational evaluation has resulted in widespread adaptation and multi-purpose end uses, e.g. the suitability of *Stylosanthes guianensis* CIAT 184 and *Centrosema macrocarpum* CIAT 5713 for cover crop and fallow improvement in the humid forest margins of Peru, protein banks in the subhumid savannas of West Africa and feed meal production in China.

Forage Improvement

- Natural accessions and advanced lines of *Brachiaria* resistant to spittlebug are available for regional evaluation.
- AFLP, as well as RAPD markers, have been linked to the apomixis loci in *Brachiaria*.
- Evaluation activities have identified at least 3 new *A. pintoi* accessions that have an advantage over the present commercial cultivar for particular production niches.
- Advanced lines of *Stylosanthes guianensis* resistant to anthracnose are available for regional evaluation.
- *S. guianensis* genotypes appropriate for typing the virulence of *Colletotrichum gloeosporioides*, the causal fungus of anthracnose, have been developed, and 52 pathogen types identified.

Forage adaptation and utilization

- Considerable advances have been made of the knowledge of the nature of condensed tannins in legumes as a step in development of appropriate screening methodology.
- Large responses in milk yield to pastures containing new hybrid *Stylosanthes* have been demonstrated.
- Leaf area and P uptake efficiency have been identified as possible selection indices to evaluate low fertility adaptation in grasses.
- Legume-based pastures have been shown to stimulate cycling of nutrients other than nitrogen.
- The potential of *Arachis pintoi* both as a forage and cover has become much more widely accepted. Evidence of this is that 26 papers on *Arachis* were presented at a RIEPT-MCAC workshop on subjects from research evaluation to on-farm adoption by smallholder dairy farmers and use as covers in banana plantations to minimize herbicide use; and increased commercial production of seed, 3 tons in Colombia and 15 tons in Bolivia.
- In general, the utility of forage legumes as pasture, as fodder, for fallow improvement and soil erosion control has now been well demonstrated through on -farm research in the development of components for different production systems.

Collaborative activities
Examples of collaborative activities are:

- Collective responsibilities for acquisition and conservation of wild *Arachis* species have been agreed on with CENARGEN and ICRISAT.

- A workshop was held in collaboration with CENARGEN to review research activities with

Cratylia argentea.

- Collaborative research and development on *Arachis* in Mexico, Central America and the Caribbean was reviewed at a regional workshop of the RIEPT-MCAC.
- R & D linkages with China, Lao and Vietnam were commenced in addition to those with Indonesia, Malaysia, Philippines and Thailand through the AusAID funded project 'Forages for Smallholders' Project and SEAFRAD network.
- The results of evaluation of forage grasses and legumes in 11 West African countries through the RABAOC-AFRNET network was compiled and distributed.
- Collaborative research was initiated with CORPOICA to evaluate legume-based forage options for use in the forest margin area of Caquetá with support from Nestlé.

CGIAR Systemwide Programs and Initiatives

A project 'Improved legume-based feeding systems for smallholder dual-purpose dairy cattle in tropical Latin America' was approved for funding under the Systemwide Livestock initiative.

1.4 Future developments

Effective research in an institution such as CIAT depends on having adequate support for first-rate scientists who themselves are able to interact effectively with support staff and NARS collaborators. They also need to be able to respond to the mission of CIAT to reduce poverty and improve equity. Unfortunately core funding has declined in the last three years and we anticipate a further decline in the coming year.

The continued high output of the last two years under such uncertain conditions attests to the dedication of all staff, both internationally and locally recruited. While the strategy for future changes will be determined outside the Program, there is scope for input on how changes might best be made to ensure that we maintain our global mandate of conservation and distribution of germplasm of mandate crops and continued development of natural resource management research.

The advent of the project system has helped us to prioritize our activities and demonstrate accountability. Multi-disciplinary input into projects has had a synergistic effect on output. However, the present projects still are rather disciplinary oriented and not focusing sufficient expertise on a particular problem. Coordinators have also found projects difficult to manage because of lack of transparency and credibility in many administrative procedures.

Nevertheless, recent experiences in project development have provided some useful examples of how we can effectively operate with reduced resources through increased collaboration. When core funding became unavailable for a position in South East Asia, we combined resources with CSIRO, an advanced research organization in the region, and obtained Special Project funding for a 5-year project built around collaborative research between CIAT and CSIRO and national organizations from 7 countries.

To ensure participation in the Systemwide Livestock Program, we developed a collaborative project, Tropileche, with other IARC's and NARS. It focuses on 'Utilization of legume-based forages for dual-purpose cattle production' in two countries but gives the opportunity for liaison with research groups from other countries. It includes many of our core activities in the area of adaptation and utilization of forages and involves collaboration between the TFP and the NRM programs. Indeed it could be a forerunner of how we might operate more effectively in larger projects in the future. Such projects also offer an opportunity to attract Special Project funding, something the CGIAR would prefer not to have but in a pragmatic sense that we need.

One can envisage three projects:

- Forage genetic diversity and improvement

  With specialists in plant geography, genetics, pathology, entomology and plant nutrition.

- Forage utilization in tropical America

  With specialists in plant ecology, forage agronomy, animal nutrition, livestock production and socio-economics.

- Forage utilization in Asia and Africa

  With specialists in forage agronomy, animal nutrition, soil fertility and socio-economics.

Expertise may reside in different Programs and Institutions and include senior and support staff recruited internationally. Project leaders would be coordinators with financial responsibility for management of core funds and given responsibility to generate additional Special Project funding. Such a structure would allow flexibility but also give stability to long-term research. Our role in communication and training would need to be maintained with responsibility for different aspects vested in the projects.

Project: Forage Genetic Resources

Project Coordinator: Brigitte L. Maass

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 a comprehensive germplasm collection of wild legume and grass species with forage potential will remain important activities in increasing the availability of tropical forage species for use in livestock feeding and natural resource management.

The mandate of the Tropical Forages Program (TFP) is to identify useful legumes and grasses for the humid (>1500 mm) and subhumid tropics (750-1500 mm). Historically, the emphasis in the Program was on the selection of grasses and legumes as improved pastures for very acid, infertile soils. There is now a strong demand for the use of legumes and grasses for soil improvement and erosion control in addition to forage and as components in many different production systems. Thus new areas of focus include identification of germplasm for use in crop-pasture rotations for the savannas; for multiple purpose use in the mid-altitude hillsides; as multipurpose shrubs (MPTS); and for intensive smallholder systems in Southeast Asia and West and Central Africa. As most of the germplasm that is well adapted to acid, infertile soils also performs well on more fertile soils, initial evaluation of most of the new germplasm is still evaluated on acid, infertile soils. Large rhizobia and mycorrhizae working collections are maintained for use in research.

Objective

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

Main Activities

Main output-related activities are focused in two broad areas:

1. Forage genetic resources, which activities are largely carried out by the Genetic Resources Unit (GRU) in collaboration with the TFP, and
2. Forage evaluation for environmental adaptation.

Outputs relating to the key genera *Brachiaria, Arachis* and *Stylosanthes* are reported under specific projects pertaining to these species.

A. Forage Genetic Resources

Main management and research activities

1. Acquisition
2. Conservation
3. Characterization and identification
4. Data management and documentation
5. Rhizobia and mycorrhizae
6. Forage genetic resources network

Highlights (1994-1995)

- Acquisition of new *Arachis* and *Cratylia argentea* germplasm widened the available genetic base of these species.
- Publication and distribution of catalog of germplasm that originated from Colombia.
- International working group meeting held to establish a forage genetic resources network.
- Needs for up-grading the CIAT GRU were documented during internal and external reviews of genebank operations. The main needs were determined in the area of conservation: processing of backlog accessions, multiplication, safety duplication, seed viability and seed health testing, long-term storage, and research into seed physiology.

Events and new Initiatives

Reviews. A large part of the tropical forage genetic resources kept in the CIAT germplasm bank (15,448 accessions from 631 species) and assembled prior to the enactment of the Convention of Biological Diversity, was put under the trusteeship of the United Nation's Food and Agriculture Organization (FAO), when an agreement was signed between FAO and the CGIAR in

October 1994. This agreement provides free access to this germplasm, and commits CIAT to conserve the designated germplasm according to internationally agreed standards.

At CIAT, genetic resources activities were reviewed twice during 1994-1995, (i) internally by the Genetic Diversity-Scientific Resource Group, that conducted a diagnostic study on the status of genetic resources at CIAT, and (ii) externally within the CGIAR System-wide Genebank Review, which was commissioned by the Inter Center Working Group on Genetic Resources and coordinated by the International Plant Genetic Resources Institute (IPGRI). The internal review resulted in a document that specifies the needs for upgrading the CIAT genebank operations and facilities to meet international standards agreed on by IPGRI/FAO. The external review suggested that CIAT "review carefully the large number of grass and legume species at present in the tropical forage collection with a view to concentrating on these species most relevant to its research needs or that are in danger of genetic erosion". The two reviews should lead to considerable discussion both at CIAT and among tropical forages resources centers, worldwide.

SINGER. The CGIAR's System-wide Information Network for Genetic Resources (SINGER) is being developed to provide a mechanism for the management and use of genetic resources data across the System. CIAT participated in a SINGER planning meeting held in October 1995 in Mexico. Agreement was achieved among participants about the objectives for SINGER, who the users will be, and on which data shall be fed into the system. CIAT has been developing a mechanism for revising germplasm passport and distribution data that will be delivered to SINGER.

Germplasm management and research

1. Acquisition

The research strategy of the TFP is to exploit the natural genetic variability of undomesticated species, especially legumes. In recent years, acquisition of new germplasm was focused on filling in geographic and genetic gaps and responding to requests for germplasm for specific needs. In the last years, acquisition has concentrated on collection of *Arachis* species with forage potential (reported under the Arachis project) and *Cratylia argentea* and introduction from a previous collection in Vietnam.

1.1 Collection

Genetic resources of *Cratylia*. During 1993-1994, 11 new *Cratylia argentea* accessions were collected in collaboration with the Empresa Brasileira de Pesquisa Agropecuária/Centro Nacional de Recursos Genéticos e Biotecnologia (EMBRAPA/CENARGEN) in Brazil and initial seed increased in the Centro de Pesquisa dos Cerrados (CPAC) of EMBRAPA at Planaltina, DF.

During 1995, two trips were carried out: in May-June in order to locate *in situ* the size, vigor and some agronomic characteristics of populations in the Brazilian states of Goiás, Mato Grosso, Minas Gerais and Tocantins. During this trip, soil samples and vegetative material were collected and the populations located (latitude, longitude, altitude) with a GPS receiver. Sixty two new populations were identified, three with white flowers (one in Brazil and two in Yapacani-Bolivia).

In September, a joint trip with R. Schultze-Kraft (University of Hohenheim, Germany) was carried out under the leadership of L. Coradin (EMBRAPA/CENARGEN). Thirty four new populations of *C. argentea* were collected.

At present, a total number of 57 accessions are available in Brazil, the new material still not being introduced to CIAT. The main characteristics of the populations are described in Table 1. **[E. A. Pizarro and L. Coradin]**

Table 1. Important characteristics of new population of *Cratylia argentea* in Brazil.

Characteristic	Range
Geographical	
Latitude, longitude	12° to 16° S 46° to 58° W
Altitude	180 to 810 masl.
Soils	Sandy and clay soils
Biological	
Population size	Highly variable: from isolated plants to populations with more than 500 plants
Flowering	Variable in flowering cycle and flower color
Pest and disease susceptibility	Variable

1.2 Post-entry phytosanitary follow-up

Traditionally, the tropical forage germplasm received by CIAT undergoes a post-entry phytosanitary follow-up conducted by the Instituto Colombiano Agropecuario (ICA), in collaboration with the TFP's Phytopathology Section, before initial seed multiplication can be undertaken. Plants are grown in a special glasshouse until flowering and are visually checked at different growth stages. If any peculiarity appears, additional laboratory tests are carried out with the assistance of CIAT's Virology Research Unit (VRU). In 1994 only 89 and in 1995 only 33 grass and legume accessions entered the phytosanitary follow-up; 131 accessions were released and a CIAT accession number was assigned to them.

The VRU has taken on the responsibility to index all germplasm accessions of tropical forage species being introduced into Colombia by CIAT. In 1995, the VRU indexed 157 individual plants by serology, electron microscopy and gel electrophoresis of viral nucleic acids. The different viruses encountered in *Arachis, Calopogonium, Stylosanthes, Brachiaria,* and *Paspalum*, are being characterized to develop reliable diagnostic techniques to facilitate the international exchange of tropical forages germplasm.

The post-entry phytosanitary follow-up has become a severe bottle-neck for the introduction of new germplasm and for clearing a backlog of accessions which have not yet been increased. Phytosanitary and seed health testing procedures had been compiled in 1993 (Kelemu, 1993). In 1995 a working group was formed to assist ICA in establishing proper procedures for the introduction of tropical forage germplasm to Colombia. This may include making use of the ICA quarantine facility at Mosquera, Bogota, if it can be demonstrated that tropical materials will grow in this temperate environment. [**A. Ortiz, S. Kelemu, F. J. Morales, and B. L. Maass**]

1.3 Introductions to the CIAT germplasm bank

During 1994-1995, the collection was increased by 131 accessions, much less than the 717 accessions introduced in 1992-1993 (Table 2). The main introductions were *Arachis pintoi* and other *Arachis* species received from EMBRAPA/CENARGEN and germplasm collected in Vietnam. [**A. Ortiz and B. L. Maass**]

1.4 Revision of species with forage potential

Colombian grass species at the Royal Botanic Gardens (R.B.G.), Kew, UK. To enhance the knowledge of native grass genera with forage potential, a list was made of Colombian species of the subfamilies Panicoideae and Chloridoideae held by the Herbarium of the Royal Botanic Gardens, Kew, England. These subfamilies include the perennial grasses of tropical origin that have evolved in grassland-herbivore ecosystems, especially in Africa. Almost all useful tropical forage species belong to these two subfamilies. The information on each sample consists of locality, altitude, number and collector, date of collection, and field notes. The R.B.G. database includes 329 species originating from Colombia, with more than 1,400 herbarium specimens, of which genera and species with forage value are listed in Table 3.

Eighty percent of the species belong to the subfamily Panicoideae, reflecting its broad degree of speciation in Colombia. Within these two subfamilies at Kew, there are type specimens of 12 species, i.e., as the first botanical record of the species, including species from genera: *Ichnanthus*, *Paspalum*, *Axonopus*, *Digitaria*, *Arundinella*, *Andropogon*, *Schizachyrium*, and *Hyparrhenia* which all belong to the subfamily Panicoideae.

The database contains records of samples collected since the late 1700's. The geographical areas with the highest number of species are the Atlantic coastal region, inter-Andean valleys, and the regions of Orinoquia and Amazonia. Forty-six percent of the species were collected from altitudes between 1,000 and 2,000 masl. [**A. M. Torres**]

2. Conservation

2.1 Status of the collection

At present, a total of 20,634 accessions are conserved (Table 2); these accessions are maintained either by original and/or multiplied seed, or. where seed could not be stored in field collections. The total number of accessions conserved is lower than listed in 1992-1993 because some of the accessions planted out for initial seed increase did not germinate. Not all of the conserved accessions are immediately available for distribution because the original seed has not yet been increased.

Table 2. Acquisition, inventory, and distribution of tropical forage germplasm by CIAT's Genetic Resources Unit in 1994 and 1995 (no. of accessions as of 31.10.1995).

Genus	Acquisition		Short-term storage	Long-term storage	Distribution in 1994-1995 (no. samples)
	1994	1995	Inventory 1995	Inventory 1995	
<u>Legumes</u>					
Aeschynomene	-	-	998	293	91
Arachis	48	8	75	30	280
Cajanus	25	3	131	61	128
Calopogonium	-	-	536	122	45
Centrosema	-	-	2,451	1,051	266
Chamaecrista	-	1	300	236	117
Codariocalyx	-	-	37	1	39
Cratylia	-	-	13	10	58
Desmodium	-	14	2,904	746	156
Flemingia	-	-	146	57	74
Galactia	-	2	571	378	70
Leucaena	-	-	199	151	177
Macroptilium	1	-	615	466	43
Pueraria	-	5	258	116	46
Rhynchosia	-	-	444	33	8
Stylosanthes	1	2	3,609	1,109	1,642
Teramnus	-	-	382	94	38
Vigna	-	-	741	338	114
Zornia	-	-	1,027	78	7
Other	-	20	3,201	1,008	485
Total legumes	75	55	18,638	6,378	3,884
<u>Grasses</u>					
Andropogon	-	-	91	0	40
Brachiaria	-	-	654	135	273
Hyparrhenia	-	-	53	4	5
Panicum	-	-	598	35	89
Paspalum	-	-	105	24	64
Pennisetum	-	-	54	0	9
Other	1	-	440	1	5
Total grasses	1	0	1,995	199	485
Other families	0	0	1	0	0
Grand total	76	55	20,634[a]	6,577	4,369

a. The total figure of conserved germplasm is lower than in 1993 because several backlog accessions planted for initial increase did not germinate, and are thus not anymore held at CIAT.

Table 3. Grass genera with forage value from Colombia held at the R.B.G. Kew Herbarium.

Subfamily, genus	Species (no.)
Chloridoideae	
Bouteloua	3
Eragrostis	19
Panicoideae	
Andropogon	9
Axonopus	17
Bothriochloa	4
Digitaria	13
Ichnanthus	9
Melinis	2
Panicum	39
Paspalum	54
Pennisetum	5
Tripsacum	1

2.2 Initial seed increase

After acquisition and release from post-entry phytosanitary follow-up, germplasm has to be multiplied. Initial increase is carried out in CIAT's green- and screenhouses and fields at Palmira, Quilichao and Popayán.

So far, 77% (15,927 accessions) of the tropical forage germplasm collection maintained at CIAT have been increased, 50% with more than 5,000 seeds, 31% between 1,000 and 5,000, and 18% less than 1,000 seeds. At least 30% of these were increased more than 10 years ago. Only 49% of the accessions meet the FAO/IPGRI standards with more than 2,000 seeds stored in either medium-term (8-10 °C, 35% R.H.) or long-term (-20 °C, no humidity control) storage conditions.

More than 1,000 accessions were put out for initial seed increase in 1994 and 1995. Original accessions which have not been multiplied are classified as backlog samples. Even though the backlog was greatly reduced during 1994-1995, there are still 4,935 accessions to undergo initial multiplication, being mainly from the key genera *Desmodium* and *Stylosanthes*. About 41% of the backlog originated from Brazil and Colombia. In addition, seed stored in cold rooms has to be regenerated periodically because germinability decreases with time.

Because most tropical species are not annual, and as wild species they do not have a synchronized flowering time, seed needs to be hand-harvested over time in order to obtain sufficient for conservation and evaluation trials. Priority is given to the key legume genera *Arachis, Centrosema, Desmodium* and *Stylosanthes*, and the grass, *Brachiaria*, seed of which is now produced at Popayán.

Because there has been difficulty in producing seed of high quality, entire collections of the genera *Brachiaria, Hyparrhenia*, and *Panicum maximum* are maintained as field collections at Quilichao, and *Andropogon* at Palmira. These field collections are used for seed increase, obtaining vegetative material for germplasm distribution, and characterization. However, because it is expensive, difficult and risky to keep the filed collections, the possibility of conserving them as *in vitro* material needs to be explored.

2.3 Long-term storage

The key species need to be conserved as a base collection stored under long-term storage conditions and duplicated in at least one other institution as a safeguard against loss. During 1994-1995, a total of 1,612 samples were put under long-term storage, which increased the total to 6,577 samples (Table 2). More than one-third of the legume germplasm is now conserved in the base collection under long-term storage conditions. Since 1994, we have commenced placing grass accessions in to long-term storage as seed of high quality (germination - 70% to 80%) was obtained by moving seed production from Quilichao (1000 masl.) to the cooler area of Popayán (1800 masl.). Ten percent of the grasses are now stored in the base collection.

2.4 Reproductive biology

Research was continued in the area of reproductive biology in order to develop seed multiplication protocols for proper genetic resources management. The study of outcrossing rate in *Centrosema brasilianum* by using a white-flowered accession was completed. The previous data obtained at Palmira were confirmed in other environments (Quilichao and Palmira), that *C. brasilianum* has high outcrossing rates between 31% and 57%. In *C. virginianum*, outcrossing was 18% at Palmira (plants with small flowers being cleistogamous). In *Desmodium heterocarpon*, only 4% outcrossing occurred at Quilichao, based on a couple of white-flowered accessions. Seed coat color is being used to study outcrossing in *Centrosema plumieri*, *C. acutifolium*, and *C. macrocarpum*. **[A. Ortiz, A. M. Torres, B. L. Maass]**

3. Characterization and Identification

3.1 Morphologicaland biochemicalcharacterization

Both morphological and biochemical characterizations were carried out on key species from the genera *Arachis* and *Stylosanthes*, as reported under those projects.

Validation of *Centrosema macrocarpum* var. *andinum*. Two botanical varieties of *Centrosema macrocarpum* were recognized in previous studies. These varieties were morphologically described (Schultze-Kraft and Belalcázar, n.d.). Biochemical characterization was carried out to facilitate taxonomic classification of var. *andinum*, the accession CIAT 25008 being used as the type specimen. Twenty-four accessions of *C. macrocarpum*, being 8 of var. *andinum*, were characterized by a PAGE (polyacrylamide gel electrophoresis) of the isozymes EST (α-esterase), DIA (diaphorase) and GOT (glutamate oxaloacetate). The band 4 of DIA served as an indicator to differentiate the var. *andinum* accessions.

Strong polymorphism existed among the accessions tested: 18 different banding patterns were detected across the three isozymes; discrimination among the 24 accessions being 75%. The 8 var. *andinum* accessions were included in 4 of these patterns (50% discrimination) and the other 16 accessions of *C. macrocarpum* in the remaining 14 patterns (88% discrimination). The polymorphism displayed by these three markers is therefore a valuable tool for characterizing the intra-specific variation within *C. macrocarpum*. **[A. Ortiz, C. H. Ocampo, and J. Belalcázar]**

Identification of accessions of *Panicum maximum*. *Panicum maximum* accessions evaluated under the numbers, CIAT 6799 and 6944 were outstanding in agronomic and grazing trials carried out at Carimagua in the Llanos Orientales of Colombia. However, it was observed that the morphological characteristics of vegetative material of these accessions brought from Carimagua differed from that of the original material maintained at Quilichao. Two hypotheses were raised: these materials, until then considered as apomictic, could have some percentage of sexuality or a mix-up could have occurred.

Biochemical (isozymes), cytological (embryo-sac), and morphological analyses are being conducted to establish the identity of these two accessions and of other similar accessions (CIAT 6177, 6144, and 6977) using vegetative material from all planting sites at Quilichao and Carimagua.

A series of 40 morphological descriptors has been established for *P. maximum*. Three tissues (root, leaf lamina and sheath) and 6 enzymes, α-EST, ß-EST, ACP (αß-acid phosphatase), GOT, DIA, and PRX (peroxidase), were tested in order to standardize the extraction procedure of native proteins (isozymes) for this species. The best band quality was obtained with leaf lamina and α- and ß-EST isozymes. There is large intra-accession morphological variation for several of the so called 'provenances'. It appears that a 'mix-up' ocurred and the so called accessions CIAT 6799 and 6944 evaluated at Carimagua are probably only one accession which will be given a new accession number. **[A. Ortiz, Claudia Flórez, and C. H. Ocampo]**

3.2. Taxonomic identification

Many, important tropical genera lack a modern taxonomical treatment (e.g., the monograph of *Arachis* by Krapovickas and Gregory was only published in 1994). Proper identification of material is often difficult, and would be impossible without the collaboration of a large number of specialists (Annex). Close collaboration with taxonomists has a steady decrease in ill-identified accessions in the collection. Nevertheless, 2,624 accessions (12.7%) are still not identified at the species level, in particular in the genera *Crotalaria*, *Desmodium*, *Indigofera*, *Phyllodium*, *Tephrosia*, and *Zornia* (229, 115, 139, 112, 118, and 854 unidentified accessions, respectively). Collaboration has been sought for development of expertise in the genus *Zornia* for which, worldwide, no taxonomic competence is available.

Identification and species confirmation were made for 586 accession of *Desmodium* by B. Schubert of the Arnold Arboretum, USA. In 1994, 87 specimens of different species were sent to 12 other taxonomists. In 1995, identification of 60 of the accessions was received, including 51 grass specimens identified by S.

A. Renvoize, R.B.G., Kew, England. [**A. Ortiz and A. M. Torres**]

4. Data management and documentation

4.1 Data management

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, in 1992, and assists in maintenance and development. The integrated data management system covers all aspects of germplasm management. [**A. Ortiz, M. A. Franco, and A. Ciprián**]

4.2 Passport data

The revision of passport data continued, and was completed for those accessions which originated from Colombia and Brazil. A catalog of the 4,361 accessions collected from Colombia was published and distributed in 1994 (Belalcázar and Schultze-Kraft, 1994). Data of germplasm which originated from Africa is currently being revised. Catalogues have now been published for germplasm from Southeast Asia, Venezuela, and Mexico, Central America and the Caribbean (Schultze-Kraft, 1990; 1991a; 1991b). The remaining catalogues to be published are for germplasm acquired from Brazil, Africa and Other Countries. [**A. Ortiz, B. L. Maass, B. Hincapié, and A. Ciprián**]

4.3 Herbarium

640 herbarium specimens were added during 1994-95. The reference herbarium now has a total of 16,313 specimens with 12,112 or 50% covering the registered accessions. 120 of the 168 genera and 551 of the 831 species registered in the collection are represented in the herbarium. Computerized labels were developed for the herbarium specimens.

The herbarium received visitors from several countries who carried out taxonomic and other botanical studies.

Training. A CIAT staff member was trained in herbarium techniques at the Herbarium of the R.B.B. Kew. The course covered issues related to herbarium management and techniques, and provided a botanical background in taxonomy, nomenclature, morphology, plant identification, collection, photography, illustrations, computer data management, germplasm banks, botanical gardens, and economic botany. Practical work was also conducted with the major families, Leguminosae, Gramineae, Compositae and Rubiaceae. [**A. M. Torres and A. Ortiz**]

5. Rhizobia and mycorrhizae

5.1 Rhizobium

The collection of over 4,000 strains is maintained and added to when a specific need has been identified.

Arachis. Fifteen samples of nodules obtained from *Arachis pintoi* in Brazil (collected by E. A. Pizarro and collaborators) were examined for *Bradyrhizobium* strains. A total of 10 strains were isolated (coded 5080-5089) and compared with the recommended strain 3101 in an undisturbed soil core experiment in the glasshouse. Six strains had growth characteristics similar to strain 3101 on agar media. However in the glasshouse experiment none of the strains out-performed 3101 in total plant dry weight or plant N.

Shrub legumes. Six promising species of shrub legumes were analyzed for the need to inoculate with rhizobium strains in an acid soil (pH 4.8, 76% Al saturation, 8.2% OM, 2.1 ppm P-Bray II) from the CVC San Emigdio Station, Palmira. The "need-to-inoculate test" method was used (i.e., one uninoculated treatment, one inoculated, and one receiving N fertilizer). The species and in parentheses, the Bradyrhizobium strain used were: *Calliandra* sp. CIAT 20400 (strains 4099+4910), *Cratylia argentea* CIAT 18516 (strain 3561), *Desmodium velutinum* CIAT 23984 (strain 4099), *Erythrina fusca* CVC (strain 035), *Flemingia macrophylla* CIAT 17412 (strains 4099+4203), and *Gliricidia sepium* CIAT 21290 (strain 3920).

Table 4 shows the dry weight, nitrogen and nodule numbers after 145 days. *G. sepium* failed to grow in this acid soil while all other species grew relatively well except for *D. velutinum*, which grew extremely slowly. *Calliandra* sp. CIAT 20400 (probably a new species according to H. M. Hernández) grew best and accumulated most total plant nitrogen. This genotype also showed the greatest response to inoculation compared to the other materials (Table 4). Plant dry weight and total N was increased 6-fold by inoculation and leaf area 5-fold (results not shown) compared to the uninoculated control. Biomass production surpassed the N fertilized treatment. The only other plant which showed a significant effect of inoculation on plant N was *C. argentea*. With *D. velutinum*, *E. fusca*, and *F. macrophylla* there was no response to inoculation but plants responded greatly to N fertilizer indicating that neither the indigenous strains nor the inoculated strains were effective in N_2-fixation. This was in contrast to *Calliandra* sp. CIAT 20400 and *C. argentea* which showed equal or greater N contents of inoculated plants compared to N fertilized plants, i.e., the *Bradyrhizobium* strains used were effective. N fertilizer did not appear to inhibit nodulation by native strains in any of the species tested.

The results suggest that *Calliandra* sp. CIAT 20400 and *C. argentea* are promising shrub legumes which require inoculation with rhizobia in acid hillside soils. *E. fusca* and *F. macrophylla* also appear to be adapted to the soils but may require inoculation and further strain selection studies. **[R. J. Thomas]**

Inoculant production. A total of 20.6 kg of *Bradyrhizobium* inoculants were produced during the period November 1994-September 1995 for research and commercial purposes. This included species of *Centrosema, Arachis, Pueraria, Stylosanthes, Mucuna* and *Chamaecrista*. **[R. J. Thomas]**.

Antibiotic-producing strains of *Bradyrhizobium*. Fifteen strains of *Bradyrhizobium* from the CIAT collection were screened for their antifungal activities on nutrient agar (Difco) plates. The following *in vitro* effects of strains of *Bradyrhizobium* or their antibiotic products on *Rhizoctonia solani* inocula were observed: (i) inhibition of mycelial growth of the pathogen; (ii) reduction or prevention of sclerotia formation; and (iii) inhibition of sclerotia germination. In addition, cell-free culture filtrates of three selected strains of *Bradyrhizobium* had inhibitory effects on the growth of the bacteria *Escherichia coli* DH5α and *Xanthomonas campestris* pv. *phaseoli* CIAT No. 555. This is the first time that fungal/bacterial inhibitory activities of strains of *Bradyrhizobium* isolated from tropical forage legumes has been demonstrated. **[S. Kelemu, R. J. Thomas, Claudia X. Moreno and Gloria I. Ocampo]**

5.2 Mycorrhizae

The CIAT collection of vesicular-arbuscular mycorrhizae (VAM) consists of 432 cultures of which 68 cultures were improved (considered as pure), during the last two years. The improved cultures (soil mixed with spores and hyphae of VAM) include 28 species in 5 genera (*Acaulospora, Entrophosphora, Gigaspora,*

Table 4. Effect of inoculation on shrub legumes after 145 days growth.

Parameter	Treatment[a]	Calliandra sp. CIAT 20400	*Cratylia argentea*	*Desmodium velutinum*	*Erythrina fusca*	*Flemingia macrophylla*
Dry weight (g)	I	13.8 a	9.1 a	1.5 a	9.2 a	8.1 a
	N	8.3 b	10.2 a	3.4 b	14.4 b	14.5 b
	UI	2.1 c	7.0 a	1.1 a	10.6 a	8.3 a
Nitrogen (mg)	I	236.5 a	182.0 a	25.8 a	157.4 a	142.5 a
	N	101.7 b	218.7 a	54.8 b	260.8 b	212.2 b
	UI	36.3 c	125.1 b	20.9 a	165.4 a	142.2 a
Number of nodules	I	250 a	24 a	20.a	47 a	106 a
	N	11b	33 a	31 a	33 a	200 b
	UI	16 b	28 a	32 a	50 a	97 a

a. I = inoculated; N = 150 kg N/ha fertilizer; UI = uninoculated control. For each column, numbers followed by the same letter are not significantly different ($P < 0.05$).

Glomus and *Scutullospora*). Colonization of roots by VAM is important for P supply to forage legumes and grasses in tropical Oxisols. Previous research has demonstrated that VAM species differ considerably in their ability to improve plant growth and P absorption of a number of tropical forage legumes and grasses.

A glasshouse study was conducted to evaluate the response of *Arachis pintoi* CIAT 17434 to inoculation of 4 different species of VAM (*G. clarum, E. colombiana, A. laevis* and *S. pellucida*). Response to VAM inoculation was tested at two levels of P (20 and 50 kg/ha) using two contrasting oxisols (sandy loam and clay loam) from Carimagua. Plants were grown in pots (4 kg of soil/pot). A basal nutrient mixture was supplied before planting (kg/ha: 20 N, 100 K, 66 Ca, 28.4 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). At the time of harvest (63 days of growth), shoot biomass, shoot P uptake and root colonization of VAM were measured.

Results summarized in Table 5 show that there was significant plant growth response to VAM inoculation in clay loam but not in sandy loam soil at 20 kg/ha of P supply. There were also significant differences among 4 VAM species tested: *S. pellucida* was not effective in clay loam soil. There was no response to VAM inoculation in sandy loam soil because the indigenous VAM species were effective in colonization of roots. Infected root length of *A. pintoi* by VAM species in sandy loam soil was 65-85% compared to that of 11-34% in clay loam soil. There was a significant increase in shoot P uptake by inoculation with *G. clarum* species in clay loam soil. These results indicate that root colonization by indigenous VAM species is a limitation to *A. pintoi* establishment in clay loam soil. There is a need to further determine the most effective VAM species for use in clay loam soils and determine if these will give more rapid establishment of *A. pintoi* in clay loam soils in a field situation. **[I. M. Rao, C. Cano and A. M. Jiménez]**

6. Forage genetic resources network

In 1994, a meeting was organized by CIAT to discuss the formation of a Tropical Forage Genetic Resources Network. It was attended by representatives from CSIRO, CENARGEN, ILCA and IPGRI, and a document was produced outlining the strategy for forming the network (Maass et al., 1995). The Systemwide Genetic Resources Initiative, led by IPGRI, has made resources available to initiate the network but suggested that it should include all forages. There is some debate as to whether a single network is desirable when separate networks have already been established for temperate and Mediterranean forage germplasm. **[B. L. Maass and P. C. Kerridge]**

Table 5. Effect of VAM inoculation on plant growth and shoot P uptake of *A. pintoi* grown in pots using oxisols from Carimagua (Phosphorus rate: 20 kg P/ha).

VAM species	Shoot biomass (g/pot)		Root biomass (g/pot)		Shoot P uptake (mg/pot)	
	Sandy loam	Clay loam	Sandy loam	Clay loam	Sandy loam	Clay loam
Indigenous VAM	6.6	4.9	5.5	3.5	9.6	11.8
G. clarum	7.0	7.5	5.8	5.0	10.6	15.2
E. colombiana	6.5	6.9	5.4	4.7	10.3	13.4
A. laevis	7.1	7.8	5.9	5.9	10.4	11.5
S. pellucida	6.2	3.6	4.4	3.9	9.8	8.8
$LSD_{0.05}$	NS	1.5	NS	0.7	NS	2.8

NS = not significant

Annex. Specialists consulted for identification of some genera.

Specialist	Institution	Genera identified
Arroyo, J. E.	Universidad del Valle, Cali, Colombia	*Galactia*
Barneby, R. C.	The New York Botanical Garden, Bronx, NY, U.S.A.	*Albizia, Ateleia, Calopogonium, Cassia, Chamaecrista, Dalea, Mimosa, Senna*
Baudoin, J. P.	Université de Gembloux, Belgium	*Dolichopsis, Macroptilium, Pachyrhizus, Vigna*
Clayton, W. D.	Royal Botanic Gardens, Kew, England	*Brachiaria, Cynodon*
Clements, R. J.	CSIRO, Brisbane, Australia	*Centrosema*
Costa, N. M. S.	EPAMIG, Sete Lagoas, MG, Brazil	*Stylosanthes*
Debouck, D. G.	CIAT/IBPGR, Cali, Colombia	*Lablab, Macroptilium, Macrotyloma, Phaseolus, Vigna*
Edye, L. A.	CSIRO, Townsville, Australia	*Stylosanthes*
Fantz, P. R.	North Carolina State University, Raleigh, U.S.A.	*Clitoria*
Fortunato, R. H.	INTA, Castelar, Buenos Aires, Argentina	*Galactia, Eriosema*
Geesink, R.	Leiden, The Netherlands	*Derris, Millettia*
Hernández, H. M.	Universidad Nacional Autónoma de México, México	*Calliandra, Zapoteca*
Krapovickas, A.	Universidad Nacional del Nordeste, Corrientes, Argentina	*Arachis*
Lavin, M.	Montana State University, Bozeman, U.S.A.	*Coursetia, Cracca*
Lewis, G. P.	Royal Botanic Gardens, Kew, England	*Camptosema, Clitoria, Periandra, Senna*, Leguminosae
Luckow, M.	Bailey Hortorium, Cornell University, Ithaca NY, U.S.A.	*Desmanthus*
Maesen, L. J. G. van der	Wageningen Agricultural University, The Netherlands	*Cajanus, Cratylia, Dicerma, Dolichos, Dunbaria, Dysolobium, Flemingia, Pueraria, Shuteria, Sinodolichos, Stylosanthes, Teyleria, Uraria*
Marechal, R. J. M.	Université de Gembloux, Belgium	*Dysolobium, Glycine, Lablab, Macroptilium, Macrotyloma, Phaseolus, Vigna*
Maxwell, R. H.	University of Louisville, NY, U.S.A.	*Aeschynomene, Dioclea*
Monteiro, R.	Universidade Estadual Paulista (UNESP), Rio Claro SP, Brasil	*Sesbania*
Ohashi, H.	Tohoku University, Japan	*Desmodium, Phyllodium, Tadehagi*
Queiroz, L. P.	Universidade Federal de Feira de Santana, BA, Brasil	*Camptosema, Canavalia, Cratylia, Galactia*
Renvoize, S. A.	Royal Botanic Gardens, Kew, England	*Brachiaria, Chloris, Eragrostis, Hyparrhenia, Paspalum, Pennisetum, Setaria*, Gramineae
Rico-Arce, M. L.	Royal Botanic Gardens, Kew, England	*Acacia*, Mimosoideae
Rudd, V. E.	Reseda, CA, U.S.A.	*Aeschynomene, Chaetocalyx, Poiretia, Zornia*
Rugolo de A., Z. E.	Darwinión, Buenos Aires, Argentina	*Cynodon, Digitaria*
Schultze-Kraft, R.	Universität Hohenheim, Stuttgart, Germany	*Centrosema*
Sorensson, Ch.	University of Hawaii, U.S.A.	*Leucaena*
Valls, J. F. M.	CENARGEN/EMBRAPA, Brasilia, Brazil	*Arachis*
Verdcourt, B.	Royal Botanic Gardens, Kew, England	*Abrus, Alysicarpus, Dioclea, Mucuna, Smithia, Teramnus*
Vijai Kumar, B. K.	Hyderabad, India	*Indigofera*
Williams, R. J.	CSIRO, Brisbane, Australia	*Centrosema*

Project: Forage Genetic Resources

Proposed activities for 1996

1. Acquisition

- Establish proper procedures for post-entry phytosanitary follow-up in collaboration with ICA, including provision of additional glasshouse space.

- Continue to index tropical forage germplasm for diseases (VRU).

- Participate in collection of *Cratylia* germplasm in the Brazilian Cerrados and *Arachis* spp. in Paraguay.

- Introduce new germplasm donated by ILRI (for mid-altitude highlands), OFI (*Calliandra*), and EMBRAPA/CENARGEN (*Cratylia* and *Arachis*) to Colombia.

- Prepare a list of grasses from the tribes Chloridoideae and Panicoideae with information available from Colombian herbaria.

2. Conservation

To make any progress in the up-grading of the CIAT genetic resources activities, conservation needs to be emphasized, especially processing of backlog accessions, multiplication, safety duplication, seed viability and seed health testing, long-term storage, and research into seed physiology. However, the up-grading can only be achieved if resources are available.

- Participate in the "Field and *in vitro* genebanks" Consultation Workshop to take place at CIAT in January 1996.

- Continue determination of mode of reproduction in *Brachiaria* germplasm accessions and start a study of outcrossing of sexual accessions of *Brachiaria*.

3. Characterization

- Continue characterization of key species with emphasis on new germplasm of *Arachis* and *Cratylia*.

- Participate in the biochemical characterization of *Desmodium heterocarpon* subsp. *ovalifolium* germplasm accessions with the GTZ-collaborative project.

4. Data management and documentation

- Participate actively in the data revision and standardization in the SINGER project.

- Complete passport data revision for Africa and remainder of the germplasm from Other Countries.

- Edit and publish germplasm catalog of Brazil, Africa and Other Countries.

5. Rhizobium and Mycorrhizae

- Continue the maintenance of the collections.

- Increase the number of pure strains.

- Provide sufficient inoculum for CIAT researchers.

- Offer a training course on VAM to technicians and scientists from national programs.

- Conduct a glasshouse trial to identify effective VAM species for establishment of *A. pintoi* accessions (CIAT 17434 and 22160) in clay loam soils.

6. Forage genetic resources network

- Arrive at a decision about potential participants in a new forage genetic resources network.

- Arrive at a decision how best to use the funds made available by the Systemwide Genetic Resources Initiative as 'seed' funds to attract more long-term support.

B. Evaluation for environmental adaptation

Main research activities

1. Forages for savannas and seasonally dry environments (Carimagua, Colombia and Planaltina, Brazil)
2. Forages for mid-altitude hillsides (Cauca, Colombia and Costa Rica)
3. Forages for lowland humid tropics (San Isidro and Atenas, Costa Rica and Caquetá, Colombia)
4. Forages for crop-pasture systems (Uberlândia, Brazil and Carimagua, Colombia)
5. Forages for Southeast Asia
6. Forages for West Africa
7. Forages with high feed value
8. Information systems on adaptation

Highlights (1994-1995)

- A workshop on *Cratylia* was held to set priorities for future research into this genus.

- Accessions of *Arachis pintoi, Centrosema* species, *Desmodium ovalifolium*, *Canavalia* species, *Mucuna pruriens*, *Cajanus cajan*, and *Brachiaria* species with cool temperature tolerance were identified for the mid-altitude hillsides in the Cauca Department of Colombia.

- For the humid tropical lowlands, promising accessions of *Panicum maximum* were identified in Costa Rica and Colombia; and of *Hyparrhenia* species in Colombia.

- Suitable MPTS have not yet been identified for the mid-altitude hillsides.

- Accessions of different legume species have been identified for crop-pasture production systems both for the Brazilian Cerrados and the Colombian Llanos.

- Six species were found to have wide environmental adaptation in Southeast Asia.

- New forage species evaluation sites were established in Lao PDR and Vietnam as part of the "Forages for Smallholders Project (FSP)" in January 1995.

- New forage species identified for West Africa.

- Accessions recommended for regional evaluation are listed in Tables 10 and 11.

1. Forages for savannas and seasonally dry environments

1.1 Llanos, Colombia

In 1993, 380 accessions of *Galactia striata* and about 80 accessions of *Chamaecrista rotundifolia* (syn. *Cassia rotundifolia*) were established at Carimagua for evaluating environmental adaptation. Both collections showed a wide range of variation with regard to morphological characteristics and general adaptation. Under low soil fertility conditions, neither species, *Galactia striata* (foliar blight) and *Chamaecrista rotundifolia* (anthracnose) are suitable for the Llanos because of disease susceptibility.

Galactia striata. Accessions were mainly of postrate growth habit, different from the traditionally used accession CIAT 964, that is erect. There was a lot intra-accession variation which, together with insect visitation observed, suggest cross-pollination. A few white-flowered accessions may serve for establishing the outcrossing rate of this species. Almost all germplasm suffered heavily from *Rhizoctonia* blight. The surviving and most vigorous accessions (Table 1) originated mostly from Casanare, Colombia. The best performing accessions were selected for inclusion in trials at Carimagua and in the Colombian hillsides (Cauca), where a new small agronomic trial was established late in 1995. This trial also includes a local ecotype from "San Vicente" in the mid-altitude hillsides of the Cauca department, which grows there abundantly in a *Brachiaria humidicola* pasture.

***Chamaecrista rotundifolia* (syn. *Cassia rotundifolia*).** *Chamaecrista rotundifolia* includes two botanical varieties, var. *rotundifolia* and var. *grandiflora*, which differ in plant growth habit (postrate vs. erect) and size of plant, leaflets, and flowers (small vs. big) (Irwin and Barneby, 1982). The germplasm maintained at CIAT and evaluated at Carimagua contains fewer accessions

Table 1. Evaluation of herbaceous legumes for savannas at Carimagua, Llanos of Colombia, 1993-1994.

Germplasm evaluated		Most outstanding germplasm (CIAT accession no.)
Species	Accessions (no.)	
Galactia striata	380	CIAT 8151, 20786, 20787, 7236, 8139, 8143, 8148, 8749, 17971, 20758
Chamaecrista rotundifolia	80	
var. *rotundifolia*		CIAT 8156, 8158, 8391;
var. *grandiflora* (erect, big leaves)		CIAT 8992, 17000, 17001

of the latter variety. Most materials of the collection of *C. rotundifolia* suffered heavily from anthracnose, before a regular cutting regime could be started. Nevertheless, the most remarkable feature of this species is its seed production potential, and easy and rapid establishment of new seedlings. Two and a half years after establishment, the erect type of var. *grandiflora* accessions has survived in the experiment area, which was abandoned in late 1994. The best performing accessions (Table 1) have been recommended for seed production and regional testing at other sites than the Llanos Orientales. In the mid-altitude hillsides in the Colombian Cauca department, *C. rotundifolia* accessions were shown to be a promising soil cover under cassava (K. Müller-Sämann, personal communication). **[B. L. Maass and Edgar A. Cárdenas]**

1.2 Atenas, Costa Rica

Chamaecrista rotundifolia. Seventeen accessions of *Ch. rotundifolia* var. *rotundifolia* (prostrate type) and var. *grandiflora* (erect, big leaves) selected on the basis of geographic origin and preliminary adaptation data at Carimagua, Colombia, were planted late in 1994 to study adaptation to the subhumid tropics of Atenas in Costa Rica (Inceptisol soils, 1860 mm mean annual rainfall with a 5-6 month dry season).

Significant differences between accessions have been observed with respect to drought tolerance and leaf retention during the dry period. Accessions of the erect type (var. *grandiflora*) CIAT 8992, 18252, 17000, 7792 and 8201 showed outstanding tolerance to dry conditions and adequate leaf retention during this period, particularly CIAT 8992 and 17000. Similar behavior was observed for the semi-erect types CIAT 8202 and 17002; meanwhile, the only accessions of the prostrate type (var. *rotundifolia*), that showed both acceptable drought tolerance and leaf retention, were CIAT 17007 and 8368.

Incidence of pests and diseases has been negligible. There was little disease except for presence of the foliar fungus, *Phyllosticta* sp.

Mid-season cumulative DMY was higher for CIAT 18252 (7670 kg DM/ha) followed by CIAT 8992 and 17000 (respectively 5950 and 4820 kg DM/ha). All these accessions are late flowering types and CIAT 18252 not only retains leaves during the dry period but also regrows from the base of the stem. At the beginning of the wet season, 70 seedlings/m^2 were observed in this accession.

The prostrate types CIAT 8398, 17517, 17518, 9735 and 7091 showed very poor adaptation to these subhumid conditions. They all defoliate heavily during the dry period, and recovery with rains is slow. DMY of these accessions ranged from nil to 3200 kg DM/ha. However, most of them are early flowering and good regeneration from seed was observed for CIAT 8389 and 9735.

Similar to Carimagua, Colombia (see Table 1), the best adapted accessions were from the erect type, var.

grandiflora, particularly, CIAT 8992 and 17000. **[P. J. Argel, A. Valerio, and G. Pérez]**

Shrubs. Reported in Section 3, Forages for the Lowland Humid Tropics.

1.3 Cerrados, Brazil[1]

Shrubs. During 1991-1995, 73 accessions from ten genera of shrub legumes were evaluated in the Cerrado ecosystem at Planaltina, DF (EMBRAPA/CPAC), a site with 5 to 6 months dry season. The accumulated DMY in one year varied significantly (P <0.05) among materials, and ranged from 0.5 t/ha for *Cratylia argentea* CIAT 18516 to 2.5 t/ha for the spineless *Mimosa* sp. CNPAB 0040. DMY among *C. argentea* accessions varied from 0.5 t/ha for CIAT 18516, to 2 t/ha for CIAT 18675. Leaf:stem ratio varied significantly (P <0.05) from 1:1 (*C. argentea* CIAT 18666 and 18668) up to 3:1 (*C. argentea* CIAT 18667, 18673, 18674, and 18675).

The depth of the root system between *C. argentea* and *Leucaena leucephala* is highly different. Rooting depth in *C. argentea* was 1.80 m in CIAT 18516 and 1.30 m in CIAT 18675, whereas in *L. leucocephala* it was only 0.65 m for the hybrid BRA-001911 and 0.40 m for cv. Texas. Root biomass and its distribution will be estimated.

Cratylia argentea has a number of positive agronomic attributes: it grows well on acid, low-fertility soils. To date, accessions evaluated are pest and disease free; they show high leaf retention until the end of the dry season; they are deep-rooted, and have confirmed fire tolerance. Regional evaluation of accessions CIAT 18667, 18673, 18674, and 18675 is suggested. **[E. A. Pizarro, A. K. B. Ramos, and Margarida A. Carvalho]**

***Cartylia* workshop.** In July 1995, a workshop was held in Brasília, DF, Brazil, to set priorities for future research in *Cratylia*. Five major areas of research were identified:

(i) botanical information;
(ii) location and collection of new germplasm;
(iii) characterizationand agronomic evaluation;
(iv) nutritional value; and
(v) utilization and management.

In each of these areas, specific research issues were determined. The proceedings of this workshop are being edited and will be published in Spanish and Portuguese by CIAT and EMBRAPA. **[E. A. Pizarro and L. Coradin]**

Paspalum. Tropical and subtropical America are a center of diversity of the grass genus *Paspalum*. At EMBRAPA/CENARGEN a considerable *Paspalum* germplasm collection was assembled. A small plot-clipping trial with the available germplasm from CENARGEN (93 accessions) was established at Planaltina, DF, in 1992. Controls were selected accessions of *Paspalum*, *Brachiaria brizantha* CIAT 16315 and 16488, in addition to commercial grasses used in the area. Accessions were planted in single row plots (5.5 m) with two levels of fertilizer and two replications. Low (L) and high (H) fertilizer levels were in kg/ha: P (L = 34, H = 86), K (L = 50, H = 83), FTE (L = 30, H = 60), and lime (L = 1700, H = 3000). During establishment and the first year of production, significant differences in DMY (P <0.001) were found among accessions at the first regrowth (45 days with 270 mm) and in accumulated annual DMY. There was also a significant (P <0.001) interaction between fertilizer level and genotype. According to fertilizer level, DMY at 45 days regrowth the *Paspalum* collection were 1 t/ha (L) and 2.3 t/ha (H) and of the control species 2 t/ha (L) and 6 t/ha (H), respectively.

Composite samples of all plant parts were analysed for crude protein (CP) and in vitro dry matter digestibility (IVDMD) at 45 days of regrowth. Mean CP was 9.5% and 11%, IVDMD was 46% and 57%, for the *Paspalum* accessions and the controls, respectively. The lowest IVDMD was 30% for *Paspalum* sp. BRA-012602, the highest 63% for *P. guenoarum* BRA-003824.

Mean DMY during the two rainy seasons was 23 t/ha for the controls (ranging from 14 t/ha for *B. brizantha* CIAT 16488 to 30 t/ha for *B. brizantha* CIAT 16315, and an average of 8 t/ha for the *Paspalum* accessions, ranging from 1.5 t/ha for *P. compressifolium* BRA-005088 to 17 t/ha for *Paspalum* sp. BRA-012793. The most productive *Paspalum* accessions were: BRA-012793 and Pantaneiro with 17 t/ha; BRA-012602 with 16 t/ha; *P. atratum* BRA-009610 with 16 t/ha, and BRA-009652 with 15 t/ha. Mean DMY during the minimum precipitation period (May-October, 260 mm) was 2 t/ha for the controls with a range from 1.5 for *Andropogon gayanus* cv. Planaltina (= CIAT 621) to 2.7 t/ha for *Panicum maximum* cv. Vencedor (= CIAT

[1] Respective BRA- and CIAT numbers are given in an annex.

26290) and 1.2 t/ha for the *Paspalum* accessions with a range from 0.01 t/ha for *P. plicatulum* BRA-014729 to 3 t/ha for BRA-012602, BRA-012793 and V11884 and V11802/1, respectively.

Morphological characterization and seed yield potential will be estimated in the next rainy season. The experiment will be completed during 1996. **[E. A. Pizarro and Allan K. B. Ramos]**

2. Forages for mid-altitude hillsides

2.1 Cauca, Colombia

Investigation into new forage options for the mid-altitude hillsides was commenced in May 1993 in northern Cauca department, Colombia, in order to provide forage components for the research site of the Tropical Hillsides Program. Germplasm evaluation trials were carried out at farm sites at two altitudes, 1,200 m ("San Vicente") and 1,600 m ("El Melcho"). Both sites are characterized by acid soils with low cation contents, intermediate Al saturation, and high P fixing capacity. Annual rainfall (1800 mm) distribution is bimodal, and because "El Melcho", has a lower temperature due to its higher altitude, precipitation may fall as hail.

A comprehensive germplasm set was screened, comprising 43 species and 101 accessions of herbaceous legumes, 18 species and 30 accessions of shrub legumes and 29 species and 41 accessions of grasses. The materials were chosen on the basis of their passport data and advanced accessions selected in other ecosystems. In April 1994, in collaboration with the Hillsides Program, farmers were exposed to contrasting legume plants, with the objective to (1) identify farmers' selection criteria for green manure and forage legumes, (2) identify management systems envisioned by farmers for different legumes, (3) qualify the potential of legumes for certain production systems, and (4) to test best bets in a farming situation. This exercise will be repeated in December 1995 to assist in guiding the selection of the most appropriate species.

The most severe overall pest and disease problem seems to be leaf-cutter ants which may impede the establishment of plants. There also have been attacks from "chiza" (coleoptera larvae of Melolonthidae species), which is a major pest in several crops in the region (A. Gaigl, 1995, personal communication). Little is known of the resistance or tolerance of different legumes and grasses to these pests.

Herbaceous legumes. Three trials were established in 1993, one each with *Centrosema* and *Desmodium* species and another with other species.

Centrosema. Among 10 *Centrosema* species (45 accessions) evaluated, *C. grazielae, C. acutifolium* and *C. brasilianum* established fastest at the higher site and initially produced the highest biomass; *C. acutifolium* was the best performer at the lower site. During the second and third cut, *C. macrocarpum, C. schiedeanum* and *C. acutifolium* were the best performers at the higher site. The annual, *C. pascuorum*, did not re-establish from seed set in the first season.

In general, the most vigorous species was *C. macrocarpum* with several outstanding accessions: CIAT 5713, 15047, 5911, 5744, and 15014. *Centrosema acutifolium* CIAT 15249 was very dense and provided good cover; CIAT 15160 also performed well. *Centrosema schiedeanum*, in particular CIAT 15727, was vigorous and provided very dense cover. *Centrosema grazielae* CIAT 5402 and 25398 were less vigorous than *C. schiedeanum*, but produced dense cover. Other species, such as *C. pascuorum, C. virginianum* or *C. brasilianum* were initially very vigorous and provided high DM production; however, they did not persist. There was significant interaction between species and sites. The overall production level was much lower at the higher site. **[B. L. Maass, Fernando Díaz Bolívar, and C. G. Meléndez]**

Desmodium. Most species of the genus were very slow to establish, the fastest being *D. intortum, D. distortum*, and *D. cajanifolium*. However, most outstanding in performance were *D. ovalifolium* CIAT 13115, 13307, 13089, in particular, on steeper slopes. The only other species that established well was *D. barbatum* which is native and abundant in the region; it is not very productive, but formed dense rows which may be useful in erosion control. **[B. L. Maass, E. A. Cárdenas, and Carlos G. Meléndez]**

Other species. Within the other herbaceous legumes, the best performance at the higher site, in terms of rapidity of soil cover, persistence, and DMY, was achieved by *Arachis pintoi* (CIAT 18748, 18744, and 22160), *Canavalia ensiformis* (CIAT 9108 and 715), *Canavalia brasiliensis* (CIAT 17009), and *Mucuna pruriens* (CIAT 9349).

Some accessions had relatively slower establishment but improved remarkably during the dry season: *Pueraria phaseoloides* (CIAT 9900, 17307, and 18382), *Macrotyloma axillare* (CIAT 823), *Chamaecrista rotundifolia* (CIAT 8990), *Galactia striata* (CIAT 964 and 8151), *Stylosanthes guianensis* (CIAT 11844), and *Vigna adenantha* (CIAT 4222). Accessions of *Calopogonium mucunoides*, *Macroptilium atropurpureum*, and most species of *Vigna*, *Dolichos*, and *Lablab* either did not establish well or did not persist for more than one year.

Seed of selected materials is being increased for further regional testing.

Vigna,1995. A set of new accessions of cowpea, *Vigna unguiculata*, and *V. adenantha* was established at "El Melcho" early in 1995. It included both short-lived erect grain types and perennial prostrate forage types (P). Grain types had rapid initial growth, however, they succumbed to disease, in particular, the fungal leaf spot disease caused by *Ascochyta*. The forage types of *V. unguiculata*, and also *V. adenantha* were slow to establish and do not appear to be a serious alternative to vigorous species, such as *Centrosema macrocarpum* or *Canavalia* species, unless more favorable agronomic practices of higher levels of fertilizer and disease control are used.

Shrub legumes. Among the initial evaluation of a large collection of legume shrubs and trees, only *Cajanus cajan* (pigeon pea) established and yielded well. There was little difference in performance between the two sites. All three accessions of *Cajanus cajan* evaluated, CIAT 17522, 913, and 9739, performed well. However, there was severe attack by ants during establishment. The only other species that established from seed at the higher site was *Clitoria fairchildiana* which grows very slowly.

Cratylia argentea is probably limited by relatively low temperatures, as growth was severely restricted at the higher site. Plant mortality was very high at both sites (75% at "El Melcho" and 80% at "San Vicente"). No significant difference between sites was detected because of high variation in growth 14 months after establishment (Table 2). Other observations in the Cauca region confirm that *C. argentea* is a species better adapted to sites with higher temperatures. It would be useful to establish whether it is the mean daily or low night temperature which most limits growth. It does perform well at Quilichao at 1000 masl.

A comprehensive collection of new *Cajanus cajan* germplasm was established in May 1995, containing forage types donated by ICRISAT. The materials are quite variable in terms of growth habit, flowering time, and flower and seed colour. Observations to date suggest the traditional accession used by CIAT, CIAT 913, is the most vigorous and leafiest type. **[B. L. Maass and E. A. Cárdenas]**

Grasses. Among the grasses, *Brachiaria decumbens*, *B. brizantha* (CIAT 6780) and *B. humidicola* (CIAT 16886, 6369, and cv. Llanero) showed rapid soil cover and were very vigorous. However, *B. humidicola* did not reach the levels of DMY of *B. brizantha* or *B. decumbens*. *Brachiaria brizantha* CIAT 16774

Table 2. Plant height (cm) of *Cratylia argentea*, 14 months after transplanting to mid-altitude hillsides in north Cauca, Colombia.

Accession	Site and altitude (m.a.s.l.)									
	San Vicente (1200)			El Pital (1350)			El Melcho (1600)			
	Mean	Range	CV	Mean	Range	CV	Mean	Range	CV	
CIAT 18673[b]	n.d.[c]	n.d.	-	-	-	-	72.3	20-140	0.52	-
CIAT 18516[b]	41.8	9-110	1.06	-	-	-	46.8	10-123	0.85	ns[a]
CIAT 18516[d]	-	-	-	114.9	22-210	0.48	63.7	10-160	0.67	**[a]

a. According to t-test: ns = there are no differences between sites, ** = sites significantly different.
b. Mean only of surviving plants (5 and 6 of the 24 plants established per site, respectively).
c. No plant survived.
d. Mean of 20 plants randomly chosen.

(decumbent) and CIAT 16549 (erect) showed green leaf still at the end of the dry season. Guatemala grass (*Tripsacum andersonii*) CIAT 6051 was particularly vigorous and is now being widely adopted in the region as an erosion barrier. None of the other erect grasses tested had comparable DMY. *Panicum maximum* CIAT 16081 and 6172 were of intermediate productivity, much higher than cv. Tobiatá (CIAT 6299) and CIAT 6799. None of these fodder grasses showed particular drought tolerance. **[B. L. Maass and E. A. Cárdenas]**

3. Forages for the lowland humid tropics

3.1 Central America

Panicum maximum. Evaluation was carried out at San Isidro, where the soil is an acid clay loam (pH 4.6) with high aluminum saturation (78%), low exchangeable basic cations (me/100g): Ca -0.3, Mg -0.2 and K -0.06, low extractable P (2.1 ppm Bray), Mn (2.7ppm) and Zn (0.9 ppm) but with a relatively high organic matter content of 6.7%.

Seventeen selected accessions of *P. maximum*, including the commercial cultivars Vencedor, Tanzania, Centenario, Tobiatá and the common commercial material (CIAT 604), were evaluated under cutting at San Isidro. Plants were established by transplanting 1-month-old seedlings, that were fertilized once with N-P-K (10-30-10) fertilizer at 50 kg/ha. At the beginning of the wet season in the second year the plots were given an additional 50 kg/ha of N.

A complete randomized block design was used. The plots consisted of 15 plants established in three rows at a distance of 0.5 m and 0.5 m between plants, with 3 replicates. For evaluation, only the 5 central plants were taken into account, cutting at a height of 0.25 m above the soil level and at a frequency of 6 and 8 weeks of regrowth for the wet and dry periods, respectively.

After 8 evaluation cuts (5 during the wet season and 3 during the dry period), significant differences in dry matter yield (DMY) existed (Table 3). CIAT 16061, 16051, cv. Tobiatá (= CIAT 6299), CIAT 6969 and 6798 are among the best adapted accessions of *P. maximum* to this acid soil site. The accessions CIAT 6799 and 6944 obtained directly from the GRU-CIAT, did not perform as well as accessions of these numbers from Carimagua (which we now believe are from one different accession, see 2-6) (see Genetic Resources 3.1). The commercial cultivar Centenario had intermediate DMY, while cvs. Tanzania and Vencedor were not well adapted at this site. The proportion of DMY produced during the 3 to 4 months of the dry period averaged 60% of that produced during the wet season. **[P. J. Argel, Alfredo Valerio, and Ronald Quiroz]**

Table 3. Dry matter yields per plant of *Panicum maximum* accessions established in the acid soils of San Isidro, Costa Rica (mean of 8 cuts, 5 during wet period and 3 during dry season).

CIAT accession number (cultivar)	Dry matter yield (g/plant) Wet season	Dry Season	Mean*
16061	108	50	86 a
16051	101	57	84 ab
6299 (cv. Tobiatá)	91	50	76 abc
6969	84	49	71 abc
6798	83	50	70 abc
16028	71	52	64 bcd
6177	71	45	61 bd
6871	70	33	56 bcde
(Centenario)	62	36	52 cde
6968	61	36	51 cde
6799	62	34	51 cde
16031 (Tanzania)	53	29	44 def
6215	40	36	39 ef
604 (Common)	48	25	39 ef
6944	44	25	37 ef
622	33	22	29 f
26900 (Vencedor)	27	19	24 f

a. Means followed by the same letter are not significantly different ($P<0.05$)

Shrubs. Twenty one accessions of both *Desmodium velutinum* and *Uraria* species, 11 accessions of *Cratylia argentea* and 3 accessions of *Calliandra* species are being evaluated under cutting at Atenas (dry season tropics) and San Isidro (seasonal humid tropics).

Cuts are carried out every 6 to 8 weeks depending on the season of the year. There are significant differences in adaptation and DMY between and within species. Neither *D. velutinum* nor the subshrub *Uraria* are adapted at San Isidro. Transplanted plants failed to grow well and DMY was negligible. High plant mortality occurred during the first 6 months after planting and the evaluation was suspended at this site. Meanwhile, at Atenas, there was a wide variation in DMY within the two species, but this was only produced during the 6-month wet season. During the dry season, plants defoliated completely and some died.

Calliandra species are well adapted at both sites; however, DMY of *Calliandra* sp. (CIAT 20400; probably a new species according to H. M. Hernández) was higher than that of *Calliandra calothyrsus*. *Cratylia argentea* showed good adaptation at both sites, although there has been interaction between site and accessions (Table 4). DMY was more variable at San Isidro than at Atenas. However, it is interesting to note that accessions CIAT 18516, 18666, 18667, 18668, and 18676 performed similar or better on the acid soil of San Isidro than the less acid soil of Atenas.

Table 4. Dry matter yields[1] per plant on *Cratylia argentea* established at Atenas and San Isidro, Costa Rica.

CIAT accession (no.)	Dry Matter Yield (g/plant) Atenas	San Isidro	Difference between sites[2]
18667	193 a	133 ab	ns
18676	184 ab	200 a	ns
18673	184 ab	28 c	**
18674	183 ab	73 bc	**
18668	172 abc	206 a	ns
18672	174 bcd	48 bc	**
18666	163 bcd	191 a	ns
18957	154 cd	40 c	**
18516	145 de	117 abc	ns
18675	126 e	35 c	**
18671	121 e	30 c	**
Mean	163 a	100 b	**

1. Mean of 7 cuts during the wet period and 4 during the dry season in Atenas, and mean of 3 and 2 cuts during the wet and dry period in San Isidro.
2. Means followed by the same letter are not significantly different (P<0.05).

An outstanding characteristic of *Cratylia argentea* is the high leaf retention during dry season, particularly of young shoots. Nearly 90% of the leaves were retained at Atenas that has 6 months of severe dry season, and this accounts for about 30% of DMY during that period. These results indicate the potential of this shrub for farming systems in the dry season ecosystem that is predominant along the Pacific Coast of Central America. **[P. J. Argel, A. Valerio and Guillermo Pérez]**

3.2 Caquetá, Colombia

From early 1992 to mid 1995, germplasm screening activities were carried out at the "La Rueda" ranch near Florencia, 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 and shrub legumes, and grasses were evaluated in a 9-week cutting regime, with DMY measured in the periods of minimum and maximum rainfall (Table 5). Results of the legumes *Arachis* and *Stylosanthes*, and the grass *Brachiaria* are reported under the respective projects.

A field day was held in December 1994 in collaboration with CORPOICA and the "Fondo Ganadero del Valle" to make the results known to a wider public. A total of more than 120 participants demonstrated the interest in new forage options for this region.

Herbaceous legumes. The best adapted herbaceous legumes are, in descending order, *Desmodium heterocarpon* subsp. *ovalifolium*, *Arachis pintoi* (3 accessions), *Centrosema acutifolium* and *C. tetragonolobum*, and *Pueraria phaseoloides*. Specific accessions were selected (Table 5) and seed multiplication has started.

Shrub legumes. The perforance of *Codariocalyx gyroides* and *Flemingia macrophylla* were outstanding among the shrubs. Cutting height of 100 cm and cutting frequency of two months seem to be appropriate to achieve persistence and optimum performance of *C. gyroides*. Large variation among accessions was observed for flowering time, growth habit, diameter and height, leafiness, and DMY. Plant morphology did not differ to the same degree.

The most outstanding accessions of *C. gyroides*, in both periods of low and high rainfall, originated from northern Thailand (CIAT 33130 and 33131) and Hainan, China (CIAT 13547 and 13548), all of them from environments with pronounced dry season; CIAT 3001 also performed well. From Indonesia, only CIAT

23746 and 23737 performed well, while almost all materials that originated from Papua New Guinea were unproductive, CIAT 13395 and 13979 being the best performing accessions.

All materials with a relatively high proportion of DMY produced during the minimum rainfall period had overall low production, except for CIAT 3001 in the first and CIAT 33130 in the second year. Generally, proportion of leaf DM was high, 53% and 56% in the two maximum rainfall periods and 73% and 75% in the two minimum rainfall periods.

Three years after establishment, there was considerable variation among accessions in survival. There was 100% survival of the promising accessions, CIAT 3001, 13547, and 33131, while CIAT 33129, 23745, and 23746 had 80-95% survival.

There was high acceptability of *C. gyroides* by lactating cows, with little difference among accessions, while grass was available. However, cattle rejected the legume when all other vegetation was removed.

Cratylia argentea was moderately well adapted, but produced much less biomass than *C. gyroides* or *Flemingia macrophylla*. Leaf DM yield of *C. argentea* averaged 33 g/plant in the minimum and 86 g/plant in maximum rainfall periods, over two years. Leaf proportion ranged from 64% to 90%, without major differences between rainfall periods. All accessions had high vigor three years after establishment.

Although of much higher forage value, only two accessions of *Desmodium velutinum* showed moderate adaptation (Table 5).

Grasses. Twenty nine pre-selected accessions of *Panicum maximum* and 21 of *Hyparrhenia* species were established for evaluation under cutting early in 1993. Within *Panicum maximum* large morphological variation was observed. In general, the accessions were not very productive and suffered from several diseases. Only cv. Tobiatá (CIAT 6299) had an acceptable performance, in particular, in the period of minimum rainfall (Table 6). Other accessions, such as CIAT 16024, 6629, and 6799 yielded considerably less, in particular, in the low

Table 5. Evaluation of herbaceous and shrub legumes in the lowland humid tropics, Caquetá, Colombian during 1992-1994 and promising material selected.

Germplasm evaluated		Most outstanding germplasm	
Species	Accessions (no.)	Mean DM yield[1]	CIAT accession (no.) in descending order
Shrub legumes		- g/plant -	
Codariocalyx gyroides	27	246-267	CIAT 33131, 13547, 3001, 33130
Cratylia argentea	11	81-80	CIAT 18668, 18674, 18672, 18676
Desmodium velutinum	83	57-59	CIAT 33249, 33138, 33242, 23996
Flemingia macrophylla	55	266-320	CIAT 17400, 17405, 17409, 17407, 17412
Herbaceous legumes		- g/m^2 -	
Arachis pintoi	8	49-64	CIAT 18747, 18748, 18751
Centrosema acutifolium	11	83-87	CIAT 15814, 15446, 5278
Centrosema capitatum	3	20-29	CIAT 5114, 15680
Desmodium ovalifolium	11	113-124	CIAT 13125, 350, 13400
Pueraria phaseoloides	8	42-45	CIAT 17765, 17292, 7978
Other species of *Arachis, Cajanus, Centrosema, Desmodium, Stylosanthes, Zornia*	134	not presented	not selected
Total	351		

1. Mean of four cuts of 9-week-old regrowth, two during minimum and two during maximum rainfall season; for shrub legumes only leaf DM yield is presented.

Table 6. Performance of *Panicum maximum* accessions in the Colombian humid tropics, at "La Rueda" farm, Caquetá, 1993-1994.

Characteristic	Maximum rainfall period[a]		Minimum rainfall period[a]		Relation minimum : maximum[a]	
	Mean	Range	Mean	Range	Mean	Range
Total DM yield (g/pl)	48.5***	9.4-113.2	30.3***	5.75-93.75	0.66	0.27-1.35
Leaf proportion	0.67	0.27-1.00	0.75	0.27-1.00	-	-
Leaf DM yield (g/pl)	33.8***	2.5-113.2	22.8***	1.57-93.75	0.70	0.27-1.27
Most vigorous accessions						
CIAT 6299 (cv. Tobiatá)[b]	113.2	-	93.8	-	0.83	-
CIAT 6799	62.7	-	57.9	-	0.92	-
CIAT 16024	72.6	-	34.9	-	0.48	-
CIAT 6629	45.6	-	52.4	-	1.15	-
CIAT 6798[b]	39.3	-	25.6	-	0.65	-
$LSD_{0.05}$	21.1	-	19.3	-	-	-

*** = Means between accessions are significantly different ($P < 0.001$) by F-test.

a. Only data of the first year are presented here because afterwards cattle entered the trial by accident.

b. Among best five accessions in Costa Rica.

rainfall period. Accessions, CIAT 6799 and 6944 performed poorly (accessions, evaluated under these numbers at Carimagua and that performed well there, are now regarded as being only one accession which will be given a new accession number (see 2-6). Other outstanding accessions at San Isidro, such as CIAT 16061 and 16051 were not included in the germplasm evaluated in Caquetá. There is a need for multilocational evaluation of a common set of accessions of *P. maximum*.

Several *Hyparrhenia* accessions showed excellent vigor, health, and both total and seasonal DM production (Table 7) with a high proportion of leaf. As *H. dregeana*, *H. filipendula*, *H. diplandra* and several unidentified species were evaluated in addition to the traditional *H. rufa*, considerable morphological variation was observed among accessions. Among the highest yielding accessions, CIAT 26234, 26231, 26230, and 16401, there appears to be scope for selecting those with a relative higher DMY in the minimum rainfall period. In comparison to these leafy, high-yielding accessions, the common check *H. rufa*, CIAT 601, performed poorly. It would be worthwile to analyze the forage quality of the vigorous materials, and objectively observe their acceptability. Nevertheless, when cattle entered the plot accidentally on two occasions, they grazed all accessions to the ground, except two, *H. diplandra* CIAT 26231 and *Hyparrhenia* sp. 26234. The same rejection of these two vigorous accessions was observed later, when the trial was routinely grazed by cattle. **[B. L. Maass, E. A. Cárdenas, and A. Betancourth]**

4. Forages for crop-pasture systems

4.1 Cerrados, Brazil

The more intensive use of the Cerrado ecosystem makes possible the introduction of forage legumes into crop-pasture systems with high inputs. For that reason the TFP initiated the introduction and preliminary evaluation of two genera *Neonotonia* and *Macroptilium*, that require higher soil fertility than that offered by the natural Cerrado soils.

Table 7. Performance of *Hyparrhenia* accessions during the first year of production, in the humid tropics, at "La Rueda" farm, Caquetá, Colombia.

Characteristic	Maximum rainfall period[a]		Minimum rainfall period[a]		Relation minimum : maximum[a]	
	Mean	Range	Mean	Range	Mean	Range
Total DM yield (g/pl)	89.3***	35.9-238.3	70.6***	25.8-156.7	1.12	0.26-4.31
Leaf proportion	0.63	0.35-0.92	0.49	0.11-0.93	-	-
Leaf DM yield (g/pl)	58.3***	12.2-166.1	34.9***	5.0-76.4	0.73	0.17-1.97
Most vigorous accessions						
Hyparrhenia sp. CIAT 16401	94.3	-	76.4	-	0.83	-
H. diplandra CIAT 26230	166.1	-	74.3	-	0.92	-
H. diplandra CIAT 26231	161.8	-	74.0	-	0.48	-
Hyparrhenia sp. CIAT 26234	144.6	-	69.9	-	1.15	-
H. hirta CIAT 26727	56.9	-	65.7	-	1.16	-
H. rufa CIAT 601 (common)	34.3	-	16.4	-	0.98	-
$LSD_{0.05}$	28.9	-	22.1	-	-	-

*** = Means between accessions are significantly different (P <0.001) by F-test.
a. Only data of the first year are presented here because afterwards cattle entered the trial by accident.

Neonotonia. In January 1994, thirty six accessions of *Neonotonia* species, being mainly *N. wightii*, were planted for preliminary evaluation and seed multiplication in 5 m single row plots. One year after establishment, only 3% of the accessions had died. At the end of the present dry period (May to October 1995: rainfall 3.4 mm), three accessions are outstanding in vigor and leaf retention: *N. wightii* CIAT 204, 235, and 19105.

In 1994, mean seed production was 60 g/plot (equivalent to 40 kg/ha), and 328 g/plot (equivalent to 219 kg/ha) in 1995. Pure seed production in 1995 varied from 0 to 850 kg/ha. Accessions with the highest seed yield were: *N. wightii* CIAT 18912, 206, and 233, in descending order.

Macroptilium. A collection of 75 accessions of *Macroptilium* species (mainly *M. atropurpureum*), representative of the geographic range of origin of germplasm maintained at CIAT, was planted in January 1994, in 5 m single row plots for preliminary evaluation and seed multiplication. One year after establishment, 15% of the accessions had died. At the end of the second dry period (May to October 1995: rainfall 3.4 mm), four accessions of *M. atropurpureum* were outstanding in vigor and leaf retention: CIAT 4004, 4048, 4083, and 4091. Pure seed yield in 1994 ranged from 0 to 480 kg/ha. Accessions with the highest seed yield were: *M. atropurpureum* CIAT 4615, 24118, and 561, in descending order. Seed harvesting for 1995 is still in progress. **[E. A. Pizarro and A. K. B. Ramos]**

Regional Evaluation. A modified regional trial B was established in December 1993 at the Universidad Federal de Uberlândia (UFU), Minas Gerais. Twenty five preselected legumes, comprising three species (*Arachis pintoi*, 4 accessions; *Centrosema brasilianum*, 16; and *Calopogonium mucunoides*, 5) were planted in a sandy soil with a common grass, *B. brizantha* CIAT 16488.

Simultaneously, nineteen preselected accessions from the species *B. brizantha* (11), *Paspalum* spp. (6), and *Panicum maximum* (2), were planted with a common legume, *S. guianensis* cv. Mineirão (= CIAT 2950).

Ground cover was estimated during establishment, and DMY was estimated over two growing seasons. At the end of the second growing season grazing with steers was initiated.

Legumes. Ground cover, estimated at 77, 98, and 144 days after establishment (Table 8), was significantly different (P <0.01) between accessions. At 144 days, two groups were distinguished: one with *Centrosema brasilianum* and *Calopogonium mucunoides* accessions with 100% ground cover, and another with *A. pintoi* accessions, attaining only 58% ground cover.

However, only *A. pintoi* and *Centrosema brasilianum* accessions persisted over two years. The botanical composition of the four *A. pintoi* accessions: CIAT 22160, 18750, 17434, and 18747 was 30, 15, 13, and 10%, respectively.

Grasses. Ground cover, estimated at 77, 98, and 131 days after establishment (Table 9), was different (P <0.0001) between accessions. At 131 days, two groups were distinguished: one, with *B. brizantha* and *P. maximum* accessions showing a mean ground cover ranging from 83 to 98%, and another, less agressive group represented by *Paspalum* accessions showing a mean ground cover of 26%.

For *B. brizantha* and *P. maximum* accessions, mean DMY ranged from 4-5 t/ha, with a legume proportion of 17 to 37%. DMY and botanical composition were similar for *B. brizantha* and *P. maximum* accessions since establishment. In the less agressive group, *Paspalum* accessions the mean DMY was less than 1 t/ha and *S. guianensis* cv. Mineirão dominated the sward (4 t/ha and 82% in botanical composition).

Mean DMY during the dry season was very small, in the order of 1 t/ha for the *B. brizantha* and *P. maximum* accessions and 0.5 t/ha for *Paspalum* accessions. Oustanding grasses were *B. brizantha* CIAT 16488, 26110, 16306, 16467, 16473 and *P. maximum* BRA-008761. **[E. A. Pizarro, M. A. Ayarza and A. K. B. Ramos]**

4.2 Llanos, Colombia

A trial containing 17 legume species and a total of 45 accessions was established under rice at Carimagua on a clay loam soil that contained considerable residual fertility from a previous maize crop. The legumes were associated with *B. decumbens*, and six months after establishment, persistence was evaluated under grazing. Unfortunately, the rice was grazed twice to the ground by Chigüiros, and thus, there was no chance to evaluate

Table 8. Mean ground cover during establishment and DMY during rainy season in preselected legumes associated with *Brachiaria brizantha* CIAT 16488 in Uberlândia, MG, Brazil.

Species (number of accessions)	Ground cover (%) during establishment (days)			DMY during rainy season (t/ha)	
	77	98	144	Legume (%)	Grass
Arachis pintoi (4)	20	41	58	2.0 (26)	6.0
Centrosema brasilianum (16)	33	64	100	3.0 (33)	5.0
Calopogonium mucunoides (5)	45	76	100	0 (0)	7.0

Table 9. Mean ground cover after establishment, DMY of first regrowth at 70 days, and DMY during rainy season in preselected grasses associated with *Stylosanthes guianensis* cv. Mineirão in Uberlândia, MG, Brazil.

Species (number of accessions)	Ground cover (%) during establishment (days)			DMY first regrowth (t/ha)		DMY during rainy season (t/ha)	
	77	98	131	Grass	Legume (%)	Grass	Legume (%)
B. brizantha (11)	55	65	86	5.0	1.9 (29)	3.9	1.9 (30)
P. maximum (2)	42	58	83	4.0	2.0 (37)	4.0	2.0 (32)
Paspalum spp. (6)	16	33	26	0.8	4.0 (82)	0.5	6.0 (91)

the compatibility of legumes with the crop. Nevertheless, there were several materials that initially responded well to the higher residual fertility, in particular, (in descending order for each species), *Pueraria phaseoloides* accessions CIAT 9900, 7182, 17296 and 20024; *Stylosanthes guianensis* Line 3, CIAT 2950 (cv. Mineirão), CIAT 11844 and 11833; *Arachis pintoi* CIAT 22160; *Galactia striata* CIAT 18018 and 8143; *Desmodium ovalifolium* CIAT 13089, *Calopogonium mucunoides* CIAT 9454, 822, 20676, 20709 and 709.

In comparison, other species performed rather poorly, which means that they were poorly adapted or could not take advantage of the increased soil fertility. This included *Centrosema* species, which suffered heavily from leaf-eating insects, *Macroptilium atropurpureum*, and *Neonotonia wightii*. After two years, the most persistent and best performing accessions were *A. pintoi* CIAT 22160, *D. ovalifolium* CIAT 13089, *D. strigillosum* CIAT 13661, *Pueraria phaseoloides*, and *C. macrocarpum* (CIAT 5713), the latter recovering from initial poor performance. A few accessions have been selected and were established in a new trial under a maize crop which was grazed immediately after the grain was harvested. **[B. L. Maass, E. A. Cárdenas, and C. Plazas]**

5. Forages for Southeast Asia

Research in Southeast Asia commenced in 1992 under the "Southeast Asian Regional Forage Seeds Project" (1992-1994). This was a joint project with the Division of Tropical Crops and Pasture, CSIRO, funded by AIDAB (now AusAID) and worked collaboratively with forage scientists in Indonesia, Malaysia, Philippines and Thailand.

Following initial species evaluation at Los Baños and Cavinti in the Philippines during 1992-1993, seed and planting material of the most promising species was made available to collaborators for multilocational testing. A total of 22 sites was established with a varying number of accessions at each site depending on local needs. The number of accessions evaluated at one or more sites was 32 grasses and 89 legumes.

The results of these multi-locational evaluations were summarized at the Third Regional Meeting of the project in Samarinda, Kalimantan, Indonesia, in October 1994. Six new species with wide adaptation were identified, and information leaflets produced for each of them:

- *Andropogon gayanus* CIAT 621, cv. Kent (Australia).
- *Brachiaria brizantha* CIAT 6780, cv. Marandu (Brazil).
- *Brachiaria decumbens* cv. Basilisk (Australia).
- *Brachiaria humidicola* cv. Tully (Australia), CIAT 6369 and CIAT 6133 (also referred to as *B. dictyoneura*).
- *Centrosema pubescens* CIAT 15160.
- *Stylosanthes guianensis* CIAT 184, cv. Pucallpa (Peru).

Several other forage accessions have shown potential but were only included in some sites or performed well at specific sites. These will be further evaluated before recommendations are made for widespread adoption:

- *Aeschynomene histrix* CIAT 9690.
- *Arachis pintoi* CIAT 17434, 18744, 18747, 18748, 18750.
- *Desmodium heterophyllum* CIAT 349.
- *Desmodium rensonii* (ex. Davao).
- *Panicum maximum* CIAT 6299, cv. Tobiatá (Brazil).
- *Paspalum atratum* BRA-009610.
- *Pennisetum* species (locally available).
- *Stylosanthes guianensis* selected lines from breeding project at CIAT (FM series).

In January 1995, the 5-year "Forages for Smallholders Project (FSP)" commenced as a follow-up to the "Southeast Asian Regional Forage Seeds Project". The FSP is also funded by the Australian Government through AusAID, and is managed jointly with CSIRO. The project has been expanded to include Lao PDR, Vietnam and southern China, in addition to Indonesia, Malaysia, Philippines and Thailand.

Multiplication of promising forage accessions has commenced in Los Baños, Philippines and at selected project sites, and these will be made available for on-farm evaluation. Multiplication of a wider range of species was also undertaken to service new evaluation sites in Laos and Vietnam. In 1995, four new evaluation sites were established in Laos and Vietnam.

In September 1995, a collection of 126 forage tree legume accessions (mainly *Leucaena* spp.) was established for evaluation at Los Baños, Philippines. This research forms part of a joint project with the

University of Queensland entitled "New Leucaenas for Southeast Asian, Pacific and Australian Agriculture" and is sponsored by ACIAR. [**W. W. Stür**]

6. Forages for West and Central Africa

RABAOC meetings held in Guinea in May 1994 and in Togo in April 1995 were attended by TFP staff. A final analysis and report of the multi-locational evaluation of grasses and herbaceous and shrub legumes at 18 sites in 11 countries in West and Central Africa was carried out at CIAT (RABAOC-AFRNET, 1995) and distributed to all participants.

The outcome was similar to the preliminary evaluation published in the Biennial Report for 1992-1993. The outstanding features of this collaborative evaluation with CIRAD and ILRI are:

i) A completely new set of forage germplasm was identified and made available to agronomists and development agencies in the subhumid and humid areas of West and Central Africa. In many cases, the new germplasm proved more productive than the commercial grasses and forages available from Australia.

ii) Effectiveness of many accessions in providing dry season feed, e.g., the grasses (*Andropogon gayanus* CIAT 621, *Brachiaria brizantha* CIAT 6780 and 26646 and *B. decumbens* CIAT 606); herbaceous legumes (*Stylosanthes guianensis* CIAT 10136 and 184, *Centrosema macrocarpum* CIAT 5452 and 5713, and *C. pubescens* 5172), and shrub legumes (*Leucaena leucocephala* CIAT 17502 and *Flemingia macrophylla* CIAT 17403).

iii) The potential role for fallow improvement through fixation of nitrogen for a following crop.

iv) The widespread adaptation of many of the species across a wide range of soil and climatic environments.

v) Demonstrated ability to produce seed locally (in particular, in Côte d'Ivoire and Guinea) and adoption of some grass and legume species by crop-livestock farmers in Guinea.

It was agreed by participants that the next stage of investigation should be that of on-farm research in selected production systems where some interest in the new germplasm has been shown by agronomists and farmers. A project is presently being developed by CIRAD (France) and NRI (UK) for submission for EU funding. CIAT and ILRI will collaborate in this project with CIRAD and NRI. [**P. C. Kerridge and B. Peyre de Fabrègues (CIRAD)**]

7. Forages of high feed value

As a complement to the evaluation of forage germplasm for environmental adaptation, the chemical composition and IVDMD of different shrub and legume species planted in contrasting environments was examined during 1994-1995. Among the shrub legumes evaluated in savanna (Llanos of Colombia) it was found that *Cratylia argentea, Desmodium velutinum* and *Uraria* spp. had higher IVDMD than *Codariocalyx gyroides, Flemingia macrophylla* and *Tadehagi* species. These differences in IVDMD were associated with the presence of condensed tannins (CT) in the latter legumes. Subsequent laboratory analysis of mature leaves from 27 accessions of *Codariocalyx gyroides* planted in a humid forest environment (Florencia, Caquetá, Colombia) showed differences in IVDMD (range: 33 to 45%) and CT (range 8 to 14%) among accessions. Even though, IVDMD of *C. gyroides* is relatively low, there would be merit in advancing for further evaluation the accessions CIAT 23737 and 23748. These two accessions have consistently exhibited higher IVDMD (45%) and lower extractable CT (9%) as compared to other accessions, regardless of the season of the year. [**C. E. Lascano and N. Narváez**]

8. Information systems on forage adaptation

The germplasm passport database has been fully implemented in the database management system ORACLE. This allows easy access to these data for mapping and geographic analysis.

In 1994 a map was prepared of potential areas for use of *Leucaena* in relation to adaptation to soil and climate in South America and Central America, using information on adaptive characteristics of *Leucaena* and GIS information on soils and climates. This map of potential adaptation of *Leucaena* needs to be validated.

Maps of high probability for finding five *Stylosanthes* species were developed in 1995. These maps of *S. guianensis, S. capitata, S. hamata, S. scabra,* and *Stylosanthes* sp. aff. *scabra* were developed on the basis of passport data from germplasm collection sites.

To make possible future routine development of this kind of map, both passport information and data on agronomic performance need to be revised, completed where necessary, and standardized. Data standardization was carried out for for all germplasm evaluation trials conducted in Caquetá and for two large collections of *Brachiaria* species and *Brachiaria humidicola* at Carimagua and linked to the germplasm database. **[P. C. Kerridge, P. Jones, B. L. Maass, and B. Hincapié]**

Evaluation - References

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RABAOC-AFRNET (Réseau de Recherche en Alimentation du Bétail en Afrique Occidentale et Centrale). 1995. Results 1990-1994. 5th annual meeting, Lomé, Togo, April 3-9, 1995. CIAT working document no. 145. NARS-CIRAD/EMVT-CIAT-ILCA, Cali, Colombia. 258 p.

Table 10. Germplasm for evaluation of environmental adaptation in regional trials - list of accessions (CIAT number) by ecosystem. Underlined: most promising material; bold: check.

Genus and species[a]	Savannas/ Llanos	Savannas/ Cerrados	Mid-altitude hillsides	Sub-humid forest	Humid forest	SE-Asia	W-Africa (RABAOC)
Herbaceous legumes							
Arachis pintoi	18748, 18744, 18750, 22160, **17434**	18748, 18744, 18750, 22160, **17434**	18748, 18744, 18750, 22160, **17434**	18748, 18744, 18750, 22160, **17434**	18748, 18744, 18750, 18747, 18751, **17434**	18748, 18744, 18750, 22160, **17434**	22160, **17434**
Calopogonium mucunoides	-	822, 20709, 20676, 19519, 9450, **719**	-	-	-	-	-
Canavalia brasiliensis	-	-	17009	-	-	-	-
Canavalia ensiformis	-	-	9108, 715	-	-	-	-
Centrosema acutifolium	5568, **5277**	-	15249	5568, 15086, 15816, 5112, **5277**	5568, 15086, 15816, 5112, **5277**	-	5568
Centrosema brasilianum	-	**5234**	-	5657, 15387, **5234**	-	-	5234
Centrosema macrocarpum	25522	25522	**5713**, 15014, 15047, 5911, 5744	25522	25522	-	5452, **5713**
Centrosema pubescens	-	5172, 5634, 5189, 15160, 15470, **438**	-	5172, 5189, 5634	15160, 15872	15160	5172
Centrosema schiedeanum	-	-	15727	-	-	-	-
Chamaecrista rotundifolia	-	-	8992, 17000, **8990**	8992, 17000, **8990**	-	-	8992, 17000
Desmodium heterocarpon subsp. *ovalifolium*	-	-	13115, 13307, 13089, **350**	-	13125, **350**	13305	-
Desmodium heterophyllum	-	-	-	-	-	349	
Galactia striata	-	-	8151, 20786, 8143, **964**, 20787	-	-	-	-
Macrotyloma axillare	-	-	823	-	-	-	-
Pueraria phaseoloides	20024, 17296, 18381	7182, 8042, 17296, 17300, 17307, **9900**	**9900**, 17307, 18382	7182, 8042, 17296, 17300, 17307, **9900**	17765, 7182, 8042, 17296, 17300, 17307, **9900**	-	-
Stylosanthes capitata	-	2320, 2353, 2546, 2542	-	1078, 1097	-	-	-
S. guianensis var. *vulgaris*	-	2950	-	21, 136	184	184	184
Hybrids of *Stylosanthes*	11833, 11844	-	11844	-	-	FM07-3, FM05-3	-
Stylosanthes macrocephala	-	2133, 10007, 10009	-	2133, 2756	-	-	-
Teramnus uncinatus	-	-	-	-	-	7315, 9012	-
Zornia spp.	-	-	-	-	-	8088, 14049, **728**, 9925, 8886, 9179	-

Genus and species[a]	Savannas/ Llanos	Savannas/ Cerrados	Mid-altitude hillsides	Sub-humid forest	Humid forest	SE-Asia	W-Africa (RABAOC)
Shrub legumes							
Cajanus cajan	-	-	**913**, 17522, 9739	-	-	-	-
Codariocalyx cyroides	-	-	-	-	33131, 13547, **3001**, 33130, 33129, 23746	-	-
Cratylia argentea	19668, 18676	18667, 18673, 18674, 18675	-	18668, 18676, **18516**	18674, 18668, 18957, 18676	-	18516
Desmanthus virgatus	-	-	-	474	-	-	-
Desmodium velutinum	13218, 23133, 23985	-	-	13218	13220, 23134	-	-
Flemingia macrophylla	17403, 21079, 21090	**17403**	-	801, **17403**	17400, 17405, 17413, 20626	-	17403
Leucaena diversifolia	-	-	-	-	-	-	-
Leucaena leucocephala	-	-	-	-	-	K636	17502
Leucaena pallida	-	-	-	-	-	CQ3439	-
Sesbania spp.	-	7931, 17533, 18836, 18838, 18840, 18947 19163, 19167	-	-	-	-	-
Grasses							
Andropogon gayanus	-	-	-	-	-'	**621**	
Brachiaria brizantha	16337, 16827, **26646**	16121, 16150, 16294 16306, 16307, 16315 16319, 16467, 16473 16488, 26110	**6780**	664, 667, 6387, 16322, 26110, **26646**	16305, 16337, 16827	**6780**, 26110	26646
Brachiaria decumbens	**606**	-	**606**	16497	**606**	**606**	606
Brachiaria humidicola	**6133**, 6369	-	16886, 6369, **6133**	**6133**, 6369, 26149, 16886	**6133**, 16888	**6133**, 16886	6133
Panicum maximum	6799, 6944	BRA-008761, BRA-008788	16081, 6172	16061, 6969, 16028, 16051, 6299	6299	6299	673, 16031
Paspalum atratum	-	BRA-009415, BRA-009610, BRA-009687, BRA-010537, BRA-012874	-	-	-	BRA-009610	-
Paspalum guenoarum	-	BRA-003824	-	-	-	BRA-003824	-
Setaria sphacelata	-	-	-	-	-	CPI-15899	-
Tripsacum andersonii	-	-	6051	-			

a. Cultivars: Herbaceous legumes *A. pintoi* CIAT 17434 cv. Maní Forrajero Perenne; *C. acutifolium* CIAT 5277 - cv. Vichada; *C. macrocarpum* CIAT 25522 - Compuesto Ucayali; *D. heterocarpon* ssp. *ovalifolium* CIAT 350 cv. Itabela; *D. heterophyllum* CIAT 349 - cv. Johnstone; *S. capitata* CIAT 10280 - cv. Capica; *S. guianensis* CIAT 184 - cv. Pucallpa; *S. macrocephala* CIAT 1281 -cv. Pioneiro.

Shrub legumes *L. leucocephala* CIAT 17502 - cv. Cunningham, CIAT 18477 - cv. Peru.

Grasses *A. gayanus* CIAT 621 - cv. Carimagua 1; *B. brizantha* CIAT 6780 - cv. Marandu, CIAT 26646 - cv. La Libertad; *B. decumbens* CIAT 606 - cv. Basilisk; *B. humidicola* CIAT 679 - cv. Humidícola, CIAT 6133 - cv. Llanero; *P. maximum* CIAT 622 - cv. Makueni, CIAT 6299 - cv. Tobiatá.

Table 11. Germplasm for evaluation in production systems - list of accessions (CIAT number, if not other indicated) by ecosystem.

Genus and species	Savannas/ Llanos	Savannas/ Cerrados	Sub-humid forest	Humid forest
Herbaceous legumes				
Arachis pintoi	22160	22160	18744, 18748	18748, 18744
Centrosema acutifolium	5277[a] (f)			
Centrosema brasilianum	-	5234 (BRA)	15387 (MCAC)	-
Centrosema macrocarpum	25522[b] (f)	-	25522[a]	25522[a]
Centrosema pubescens	5634, 15160 (f)	5634, 15160	5634, 15160	5634, 15160
Pueraria phaseoloides	8042 (f)	-	-	-
S. guianensis var. *vulgaris*	-	2950[c] (BRA)	-	-
Hybrids of *Stylosanthes*	11833, 11844	-	-	-
Shrub legumes				
Codariocalyx cyroides	-	-	-	33131, 13547
Cratylia argentea	-	-	18668, 18676	18674
Leucaena leucocephala	-	-	17263 (MCAC)	-
Grasses				
Brachiaria brizantha	-	16488	26110	26110
Brachiaria humidicola	-	-	16888, 26149	16886, 26149
Panicum maximum	6799, 6944[d]	BRA-008761	16061	-
Paspalum atratum	-	BRA-009610	-	-

a. cv. Vichada
b. Compuesto Ucayali.
c. cv. Mineirão.
d. There is some doubt about the identity of CIAT 6944 (see under Genetic Resources 3.1).
(f) = for crop-pasture systems with higher residual soil fertility.
(BRA) = for the Brazilian Cerrados.
(MCAC) = for Mexico, Central America and the Caribbean.

Annex. CIAT and BRA- accession numbers and cultivar names.

Species	CIAT accession (no.)	BRA- accession (no.) or cultivar	Species	CIAT accession (no.)	BRA- accession (no.) or cultivar
Grasses			Legumes		
Andropogon gayanus	621	Planaltina	*Arachis pintoi*	17434	013251
Brachiaria decumbens	606	Basilisk	*A. pintoi*	18747	015121
B. brizantha	16306	003361	*A. pintoi*	18750	015598
B. brizantha	16315	003441	*A. pintoi*	22160	031143
B. brizantha	16467	003891	*Cratylia argentea*	18516	000167
B. brizantha	16473	003948	*C. argentea*	18666	000019
B. brizantha	16488	004391	*C. argentea*	18667	000027
B. brizantha	26110	004308	*C. argentea*	18668	000035
B. brizantha	6780	Marandu	*C. argentea*	18673	000094
B. humidicola	6133	Llanero	*C. argentea*	18674	000116
Panicum maximum	6299	Tobiatá	*C. argentea*	18675	000124
P. maximum	6871	Centenario	*Stylosanthes guianensis*	184	Pucallpa
P. maximum	16031	Tanzania	*S. guianensis*	2950	Mineirão
P. maximum	26900	Vencedor			

B. Evaluation for environmental adaptation - Proposed activities for 1996

1. **Savannas and other seasonally dry season environments**

- Finalize data analysis and write technical papers.

- A new shrub-legume trial comprising 89 accessions from seven genera has been established at Planaltina, and will be evaluated in the Cerrado ecosystem between 1995-1997.

- Continue field observations of *Chamaecrista rotundifolia* in Costa Rica for an additional dry season. Select the best performers for seed multiplication and evaluate in production systems.

- A new experiment has been established at Atenas with *C. argentea* CIAT 18516 to study the effect of plant density and time of the first cut on DMY.

2. **Forages for mid-altitude hillsides**

- Continue evaluations at "San Vicente/El Melcho" established in 1995 (*Cajanus cajan* and *Vigna adenantha*); analyse data and summarize results.

- Evaluate a new comprehensive set of shrub legumes established in October 1995, in relation to temperature and moisture constraints.

- Evaluate 15 agronomically unknown species for potential use together with a similar set in the savannas at Carimagua.

- Evaluate selected accessions of *Galactia striata* for adaptation to mid-altitude hillsides (Melcho and Quilichao).

- Assemble a set of promising legume accessions for multilocational evaluation in mid-altitude hillsides in collaboration with the Tropical Hillsides Program.

- Evaluate effect of different management options on productivity and persistence of *Cratylia argentea* at Quilichao.

3. **Forages for lowland humid tropics**

- Complete data analysis and write technical paper(s) of Caquetá results.

- Continue evaluation of *Panicum maximum* at San Isidro for one more dry season (1995/1996); submit a technical paper.

- Increase seed of promising accessions for multilocational testing.

- Promising materials to Acre-Rondonia, Brazil, and Pucallpa, Peru, for regional evaluation.

4. **Forages for crop-pasture systems**

- Continue and finalize germplasm trials of *Macroptilium* and *Neonotonia* species; select promising accessions, and start seed multiplication.

- Continue trials at Uberlândia, MG, Brazil, in collaboration with the CIAT Tropical Lowlands Program; select most promising accessions, and assemble set of germplasm for multilocational testing.

5. **Forages for Southeast Asia**

- A further six evaluation sites will be established in Laos and Vietnam.

- Continuation of the tree legume evaluation at Los Baños, Philippines.

- Evaluation of a larger range of *Arachis* species.

6. **Forages for West Africa**

- Collaborate in project development by CIRAD (France) and NRI (UK) for submission for EU funding, together with ILRI.

7. Forages of high feed value

- Continue forage quality measurements of grass and legume species being evaluated for adaptation in different locations.

- Evaluate the chemical composition of selected species and accessions of *Paspalum* being screened at EMBRAPA/CPAC, Brazil.

8. Information systems on forage adaptation

- Analysis of multi-locational evaluation of different species will be carried out in collaboration with Biometrics Unit. This will provide information on essential plant characteristics to record.

- The germplasm passport database will be linked to the GIS database to produce distribution maps of occurrence and thence parameters associated with natural occurence.

- Exploration of a general program for producing maps of potential adaptation of commercially important species. Review potential distribution of *Leucaena*.

- For *Desmodium ovalifolium*, passport data of the collection sites and agronomic performance data from a large variety of environments will be utilized to develop a map for areas of potential adaptation of this species.

- Produce distribution maps of *Arachis* species from passport and herbarium data.

Project: Genetic Enhancement of *Brachiaria*

Project Coordinator: John W. Miles

Rationale

The genus *Brachiaria* is the source of the most important and widely sown tropical forage plants. *Brachiaria* cultivars are sown over an area estimated to be in excess of 50 million ha in Brazil alone.

The use of *B. decumbens* pastures on a wide scale, beginning in the early 1970s, permitted a substantial increase in animal productivity, measured either on a per area or per head basis. Unfortunately, *B. decumbens* is very susceptible to the attack of spittlebug (various species and genera in the Cercopid family (Homoptera)). *B. brizantha* cv. Marandú, released in Brazil, is highly resistant to spittlebug, but does not persist under conditions of low soil fertility. A third commercial cultivar, *B. brizantha* cv. La Libertad, released by the Colombian Agricultural Research Institute (ICA), is intermediate in both insect resistance and persistence. It is not strongly stoloniferous.

A major collecting mission, funded by IBPGR (now IPGRI), was conducted during 1984/85 by CIAT in collaboration with ILCA and national programs in six East African countries. This mission acquired nearly 800 new accessions of over 20 *Brachiaria* species. These accessions are being characterized for edaphic adaptation, reaction to key pests and diseases, as well as for other attributes.

With the introduction, by C. B. do Valle (EMBRAPA/CNPGC), to tropical America in 1985, and to CIAT in 1988, of an artificially tetraploidized biotype of the naturally sexual diploid *B. ruziziensis*, it has been possible to initiate plant breeding projects in the genus *Brachiaria*. Hybridization was previously impossible owing to the natural apomictic reproduction (asexual, but by seed) in all commercial species of *Brachiaria* except the diploid *B. ruziziensis*. The CIAT *Brachiaria* breeding work, being conducted in close collaboration with EMBRAPA in Brazil, seeks to enhance the utility and productivity of *Brachiaria* forage grasses through the use of natural genetic resources complemented by plant breeding. In the plant breeding program, we seek to combine the excellent edaphic adaptation and persistence of *B. decumbens* with the antibiotic spittlebug resistance of *B. brizantha* in new apomictic *Brachiaria* cultivars of high forage quality. This project includes associated activities that complement the selection of wild accessions of *Brachiaria* and the plant breeding program.

Objective

To improve the utility and productivity of *Brachiaria* forage grasses through utilization of natural genetic resources complemented by plant breeding.

Main activities

1. Genetic resources.
2. New *Brachiaria* gene pools.
3. Genetics of apomixis.
4. Assessment of spittlebug resistance.
5. Genetic diversity in *Brachiaria*.
6. Genetic control of key attributes.
7. Edaphic adaptation.
8. Resistance to *Rhizoctonia* foliar blight.
9. Assessment of virus resistance.
10. Seed dormancy.
11. Endophytic fungi in tropical grasses.
12. Pollen storage.
13. *Brachiaria* workshop.

Highlights (1994-1995)

- Selected accessions were advanced from agronomic clipping trials to small-plot grazing trials at Carimagua and Caquetá.
- Apomictic clones selected from the breeding population were included in small-plot grazing trials at Carimagua and Caquetá.
- AFLP, as well as RAPD markers, have now been linked to apomixis in a hybrid population.
- Hybrid segregants with resistance comparable to that in the highly resistant commercial cultivar *B. brizantha* cv. Marandú are being identified.

- Genetic diversity among and within *Brachiaria* spp. is being quantified with molecular markers.

- Initiated screening of a hybrid mapping population to assess feasibility of marking QTL's for spittlebug resistance.

- Brazilian regional *Brachiaria* trials network implemented to test 19 accessions at 10 sites.

- Initiated seed multiplication of selected accessions and hybrids at CIAT's Popayán research station for regional evaluation in 1996.

- Reliable field and glasshouse methodology for assessing genetic resistance to *Rhizoctonia* foliar blight has been used to identify sources of resistance.

- Showed marked genetic variation in forage production and acquisition of P, N, and Ca from a sandy loam oxisol at Carimagua. A close relationship was found between P acquisition and forage production.

- Developed a pot culture method to simulate declining soil fertility and to evaluate genotypic differences in tolerance to low fertility. Three plant traits -- leaf area, root length, and shoot P uptake -- were identified as indices for edaphic adaptation.

- Showed that poor persistence of *B. ruziziensis* in low fertility, acid soils could be related to its inability to partition a greater proportion of biomass to root growth.

- Identified accessions of *B. decumbens* and *B. brizantha* tolerant to waterlogging.

1. Genetic resources

1.1 Small, row-plot, agronomic clipping trials

Carimagua: Between 1991 and 1994, a set of 186 accessions from 10 different species of *Brachiaria* were evaluated in small plots at Carimagua. Considerable inter- and intraspecific variation was registered for various important plant attributes such as seasonal DM production, leaf-to-stem ratio, N, P, and Ca content of leaves, IVDMD, flowering time, and seed production.

In a separate trial, 53 *B. humidicola* accessions were evaluated in small plots between 1991 and 1994 at Carimagua along with commercial checks (*B. humidicola* CIAT 679, *B. brizantha* cv. La Libertad, *B. decumbens* cv. Basilisk, and *B. dictyoneura* cv. Llanero). The objective was to search for environmentally adapted accessions with better nutritive value (N content and IVDMD) and seed production at low latitudes than the commercial line CIAT 679. The variation registered among accessions for these attributes and DM production is presented in Table 1. **[G. Keller-Grein, B. L. Maass, C. Plazas]**

Table 1. Performance of a germplasm collection of *Brachiaria humidicola* (53 accessions) at Carimagua in the Colombian Llanos.

Characteristic	Mean	Range
DM yield, rainy season (g/m^2)[a]	65	27-133
DM yield, dry season (g/m^2)[a]	28	11-47
N content (% in leaf DM)[b]	0.94	0.76-1.16
IVDMD (% in leaf DM)[c]	61.3	54.2-66.3
Flowering onset (No. of days after standardization cut)	62	45-131
Seed production (g/m^2)	1.9	0.1-7.8

a. Means of 5 or 3 harvests in rainy or dry season, respectively, with 6 weeks of regrowth each.
b. Mean of 5 analyses. c. One analysis
Source: G. Keller-Grein et al., unpublished data.

Caquetá: Based on the selections initially made in Pucallpa, Peru, 58 accessions from seven species were evaluated during 1993-1995 in the Amazonian piedmont region near Florencia, Caquetá, Colombia. Accessions of *B. brizantha, B. decumbens*, and *B. humidicola* were particularly well adapted, and grew vigorously during the bi-monthly cutting regime. In the periods of both minimum and maximum rainfall, leaf DM yield displayed wide ranges (Table 2) among 35 accessions of *B. brizantha*, confirming CIAT 16827 and 16829 to be among the most vigorous accessions, together with CIAT 26646 (cv. La Libertad). Cultivar Marandú (CIAT 6297 or 6780) showed above average leaf DM yields. There was a wide range of leaf:stem proportion in *B. brizantha* materials. Generally, *B. humidicola* had

Table 2. Performance of different *Brachiaria* species and accessions in the humid tropics of Colombia at La Rueda farm, Caquetá, 1993-1994.

Species (no. of accessions)	Total DM yield[a]		Leaf:stem proportion[a]	
	Minimum rainfall	Maximum rainfall	Minimum rainfall	Maximum rainfall
B. brizantha	- (g/pl) -			
Mean (35)	60.4+	103.3*	0.69***	0.54***
Range (35)	30.7-90.9	60.9-162.2	0.42-0.96	0.33-0.81
Ex CIAT 16139	76.1	86.5	0.63	0.45
CIAT 16441	82.8	139.0	0.53	0.38
CIAT 16455	78.1	114.4	0.55	0.33
CIAT 16488	54.3	115.2	0.76	0.54
CIAT 16472	71.2	127.5	0.51	0.35
CIAT 16779	55.3	137.3	0.93	0.59
CIAT 6297, cv. Marandú[b]	47.2	112.5	0.90	0.68
CIAT 16827[c]	69.9	162.2	0.84	0.65
CIAT 16829[c]	56.3	101.0	0.94	0.67
CIAT 26646, cv. La Libertad[b]	84.7	148.7	0.76	0.54
B. decumbens				
CIAT 606, cv. Basilisk[b]	92.5	109.0	0.69	0.42
	- (g/m^2) -			
CIAT 6058	253.7	252.7	0.61	0.39
B. humidicola				
Mean (8)	68.9ns	153.2+	0.93***	0.91**
Range (8)	42.3-101.5	98.1-188.3	0.74-1.00	0.71-1.00
CIAT 679, cv. Humidícola[b]	94.5	174.0	0.74	0.71
CIAT 6133, cv. Llanero[b]	157.6	177.6	0.90	0.73
CIAT 16180	88.8	188.3	0.97	0.84
CIAT 16888	48.6	156.6	1.00	1.00
CIAT 16886[c]	101.5	160.9	1.00	1.00

Analysis of variance was performed among *B. brizantha* and *B. humidicola* accessions; significant differences between accessions according to F-test (ns = P>0.1; + = P<0.1; * = P<0.05; ** = P<0.01).

a. Only data of the first year are presented here

b. Commercial checks.

c. Accessions not selected for the grazing trial.

higher leaf:stem proportion than *B. brizantha*; among *B. humidicola* accessions, CIAT 16886 was particularly productive.

While spittlebug incidence was relatively low in the first year of evaluation and only few accessions were severely damaged, strong incidence of foliar blight caused by *Rhizoctonia solani* was recorded for 35 accessions during the rainy season. Heavy rust incidence was observed in *B. humidicola*, in particular, CIAT 679 and 16191. From the best performing accessions, eight were selected for further evaluation in association with *Arachis pintoi* under grazing. **[B.L. Maass, C.G. Meléndez]**

1.2 Small-plot grazing trials. Observations from the small, row-plot clipping trials at Carimagua and in Caquetá allowed selection of a small number of accessions (Table 3), which showed promise in one or other of the two environments, for further evaluation. Small plot grazing trials were established during 1995 at both Carimagua and at Caquetá to assess persistence of these selected natural accessions and a few hybrids from the breeding program under heavy periodic grazing. A similar design is used at both locations. *Brachiaria* genotypes were established vegetatively into 35 m^2 plots in alternating rows with *A. pintoi*. Plots were arranged in complete blocks with three replicates. One, or two, hybrid selections as well as appropriate commercial check cultivars were included in the trials (Table 3) **[J. W. Miles, I. M. Rao, C. Plazas]**

Table 3. List of selected genotypes of *Brachiaria* for small-plot grazing trials at two locations, Carimagua (Meta and Montañita (Caquetá).

Carimagua:	*B. humidicola* CIAT 679*, 6013, 16867, 16871, 16873, 16886, 26159, 26425, 26427. *B. brizantha* CIAT 6297, 16212, 16327, 16776, 26032, 26124, 26318, 26554, 26556G, 26562 *B. decumbens* CIAT 606*, 26180 *B. dictyoneura* CIAT 6133*, 16506 Hybrids- BR93NO/3009 FM9201/1873
Montañita:	*B. humidicola* CIAT 679*, 16180, 16888 *B. brizantha* CIAT 6297, 16139A, 16441, 16455, 16472, 16488, 16779, 26646* *B. decumbens* CIAT 606*, 6058 *B. dictyoneura* CIAT 6133* Hybrid - FM 9201/1873

* = Commercial check.

1.3 Regional agronomic evaluation. At CIAT's Popayán station we are multiplying seed of *Brachiaria* accessions and several hybrids selected at Carimagua. The seed produced will serve to establish a series of regional agronomic trials in 1996 in collaboration with Corpoica and the Fondo Nacional del Ganado in different agroecosystems in Colombia and other countries. The aim is to assess adaptation to a diversity of environmental conditions and to identify broadly adapted genotypes. Seed multiplication plots (of 100 m^2 or 30 m^2) were established vegetatively in early March 1995. Seed of a number of entries has already been harvested. Assessment of seed quality is being initiated. **[J. W. Miles, C. G. Meléndez]**

2. New *Brachiaria* gene pools

2.1 Breeding populations. Two breeding populations are currently being maintained: one segregates for reproductive mode (apomixis/sexuality) the second is fully sexual and breeds true for sexuality. The first population yields apomictic segregants each generation, but it requires a major investment in phenotyping reproductive mode each year. The second population will be much simpler to handle. As the sexual population is improved by cyclic selection, the genetic gain will be realized in apomictic hybrids generated by crossing the improved sexual population to an appropriate apomict(s). Evolving concepts of breeding methods effective and efficient for apomict *Brachiaria* suggest concentrating attention on the fully sexual population. **[J. W. Miles, M. L. Escandón]**

Approx. 1500 open pollinated progenies from the fully sexual population were established at Carimagua and Caquetá. Each genotype was replicated vegetatively for the two trials with a third propagule saved at CIAT-Palmira. The population has been culled to approx. 250 individual genotypes based upon vigor and freedom from foliar diseases at both sites. **[J. W. Miles, M. L. Escandón]**

2.2 Agronomic trials. Forty-three apomictic recombinants identified as single plants during the 1994 season were established in a replicated, row-plot agronomic trial at Carimagua in 1995, along with a number of selected accessions. We are attempting to generate a large population as a source of new apomictic and sexual segregants by exposing a series of selected sexual clones, as single spaced plants, to open-pollinate in the agronomic trial. The progeny of these sexual clones will provide a diversity of both sexual and apomictic genotypes and significantly broaden the genetic base of the breeding material. **[J. W. Miles, C. Plazas]**

Results of a small-plot agronomic trial established in 1994 show that hybrid recombinants exhibit segregation of attributes well beyond the range of the parental materials going into the breeding populations (See section on Edaphic Adaptation). **[I. M. Rao, J. W. Miles, C. Plazas]**

2.3 Recurrent selection on specific combining ability. We began to investigate the feasibility of conducting "recurrent selection on specific combining ability" in the sexual population (Miles & Escandón, in preparation). The major obstacle appears to bé the logistics of forming sufficient testcross progenies. This will probably have to be done by open pollination in a large (i.e. 2 ha) field plot. A small number of experimental testcross progenies have been formed. **[J. W. Miles, M. L. Escandón]**

3. Genetics of apomixis

The objectives of our *Brachiaria* genome research are:

- to tag and fine map the apomixis gene in different *Brachiaria* backgrounds;
- to construct a *Brachiaria* map based on heterologous probes from the rice, maize, and wheat RFLP maps;
- to integrate PCR-based markers in the breeding program using sexual and apomictic populations.

A hybrid population of 115 individuals was generated by crossing a tetraploidized, sexual *B. ruziziensis* clone with a natural tetraploid, apomictic *B. brizantha* clone (CIAT 26646). Individual hybrids were phenotyped for reproductive mode by microscopic observation of embryo sac structure in a minimum of 20 ovaries with classifiable sacs. To date 600 RAPDs and 64 AFLP primers have been screened using bulk segregant analysis and linked primers were identified. Good homology was detected between *Brachiaria*, on the one hand, and rice and maize, on the other. The construction of a molecular map has been initiated using AFLP primers and a set of 75 polymorphic rice and maize clones. **[J. Tohme, N. Palacios]**

4. Assessment of spittlebug resistance

A new Program Entomologist (0.5 TFP, 0.5 Rice) joined the project in June 1995, following a period of 18 months without direct entomological support.

4.1 Confirmation of field screening. Following field evaluation and selection, seventy-five clones generated through open-pollinated field crossings of *B. ruziziensis*, *B. decumbens*, and various accessions of *B. brizantha* were further evaluated using a screening assay designed to measure spittlebug resistance in the greenhouse. One clone (BR94NO/0678) possessed a level of resistance comparable to that of *B. brizantha* cv. Marandú, which is the principal source of resistance in these populations. This clone established in the field only at Caquetá in 1994. It is available at CIAT for further testing in the greenhouse. **[G. C. Yencho, G. Sotelo]**

4.2 Feasibility study of molecular marker assisted selection. In September 1995, we commenced screening the *B. ruziziensis* x *B. brizantha* (cv. Marandú) progenies, which are currently being used to identify molecular markers associated with apomixis. The parents represent extreme expressions of antibiosis: *B. ruziziensis* is susceptible and *B. brizantha* is highly resistant to spittlebug. We are investigating the possibility of using these populations to identify molecular markers linked to genes for resistance to spittlebug. If markers linked to spittlebug resistance are found and they explain a significant proportion of the variation in resistance, they could be used to improve the efficiency of current methods of evaluating resistance in segregating populations. The success of the gene tagging project is in part dependent on three factors: 1) that the progenies described above do, in fact, segregate for resistance to spittlebug; 2) that we are able clearly to discriminate between resistant and susceptible clones within these progenies; and 3) that we can identify adequate marker polymorphisms in the population with which to construct a molecular genetic map of *Brachiaria*. We are screening ca. 80 of these hybrid clones in the greenhouse to characterize their resistance. Depending upon the outcome of these experiments, we will decide whether or not to proceed with the gene tagging experiments. **[G. C. Yencho, G. Sotelo]**

4.3 Field screening. A significant constraint to the development of spittlebug-resistant *Brachiaria* clones is the fact that field populations of spittlebug vary dramatically, both spatially and temporally, making it difficult to screen large segregating populations in the field. We are evaluating various methods of establishing spittlebug populations in the field. However, none of the methods tried so far has been entirely satisfactory.

It is becoming increasingly clear that our one season of field evaluation is inadequate in that selections are advanced with insufficient information on spittlebug resistance. It seems inevitable that we shall have to go to a two-year field evaluation cycle until a reliable field infestation methodology or a sufficiently large-scale greenhouse screening method can be developed. A two-year evaluation will double generation time (from one year to two), but ought to improve efficiency and rate of genetic improvement. **[G. C. Yencho, J. W. Miles, G. Sotelo]**

4.4 Adult feeding preference study. To date, the principal sources of resistance to spittlebug, *B. brizantha* (cv. Marandú and other accessions) and *B. jubata* (CIAT 16531 and CIAT 16203) have been evaluated in assays designed to measure their antibiotic effect on spittlebug. In order more fully to characterize the mechanisms of resistance to spittlebug we have initiated studies to determine the relative preference of adult spittlebugs for these accessions. These studies are being conducted in small (1 m^3) cages in the greenhouse where we are giving adult spittlebugs feeding choices between resistant and susceptible plants grown in small pots. To measure preference we record the number of adults present and their location on each plant. At the conclusion of each assay we determine the number of eggs laid in each pot. The studies were initiated with *B. ruziziensis*. We will compare the parents of the *B. ruziziensis* x *B. brizantha* (cv. Marandú) populations and *B. decumbens* in subsequent experiments. **[G. C. Yencho, G. Sotelo]**

5. Genetic diversity in *Brachiaria*

Use of RAPD markers to determine genetic relationships

Most of the polyploid biotypes of *Brachiaria* are apomicts. Thus genetic improvement of *Brachiaria* depended on the identification, collection, and preservation of existing natural variation until a fertile, sexual tetraploid was developed from the sexual diploid *B. ruziziensis*. This allowed a breeding program aimed at improvement of species such as *B. decumbens* and *B. brizantha* to commence.

An understanding of the genetic relationships between and within species will contribute to designing better strategies for collection and conservation and provide breeders with information on the genetic base being used and allow rational selection of parents to maximize the expression of heterosis.

In describing genetic relationships, the approaches previously available have used data of comparative morphology, physiology, and isozymes. More recently, several molecular techniques for detecting polymorphism at the DNA level have been applied; DNA markers such as RFLPs or PCR-based RAPDs are being used extensively in crop plants for determining genetic relationships.

We have initiated a study of genetic relationships in *Brachiaria* species using PCR-based RAPD markers. This technique involves screening DNA for scrabble polymorphism, using a series of 10-base-pair primers of arbitrary sequence to amplify random, anonymous DNA fragments. The method typically yields polymorphism with dominance-recessive characteristics.

Genetic relationships among *Brachiaria* species were compared, using RAPD primers (Suárez, 1994). The genotypes evaluated included accessions of *B. decumbens*, *B. brizantha*, *B. ruziziensis*, *B. jubata*, *B. humidicola*, and *B. dictyoneura*, which are of major interest to forage programs in tropical America and represent a diverse range of germplasm, both for ploidy level and for agronomic characteristics. The RAPD data were generated by amplifying total DNA, using random 10-base nucleotide sequences as primers in PCR reactions. These data were analyzed by the unweighted pair-group method arithmetic average (UPGMA) and principal component analysis (PCA). The grouping pattern obtained (Figure 1) is consistent with assigning of *B. decumbens*, *B. brizantha*, and *B. ruziziensis* to one taxonomic group, and *B. jubata*, *B. humidicola*, and *B. dictyoneura* to another. This preliminary survey points to areas for further investigation:

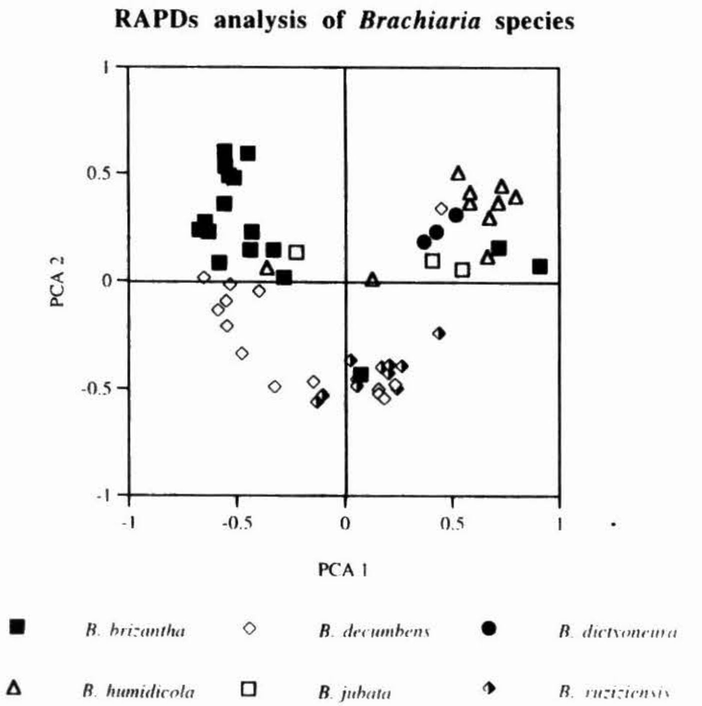


Figure 1. Genetic similarity of six *Brachiaria* species determined using principal component analysis on RAPDs data (After Suárez, 1994).

(i) The relationship between *B. decumbens* and *B. ruziziensis*: based on our RAPD data, some accessions of *B. decumbens* are closely similar to *B. ruziziensis* accessions.

(ii) The amount of diversity present in sexual versus apomictic species: the sexual *B. ruziziensis* displayed less variability than the apomictic species *B. decumbens* and *B. brizantha*.

This preliminary survey shows the usefulness of PCR-based markers, such as RAPDs, for discriminating among different species from the *Brachiaria* germplasm collection and for differentiating genotypes within species. We are pursuing this genetic characterization to understand the amount of diversity present in the collection and to obtain more information about the distribution of diversity in relation to the reproductive mode of the genotypes. **[J. Tohme, N. Palacios]**

6. Genetic control of key attributes

We are generating the genetic material needed to demonstrate genetic modification of expression of the dominant allele conferring the ability to reproduce apomictically. **[J. Miles, M. L. Escandón]**

7. Edaphic adaptation

Field observations in the Cerrados of Brazil and the Llanos of Colombia indicated that common *B. ruziziensis* and *B. brizantha* cv. Marandú are less adapted to low fertility oxisols than the widely planted *B. decumbens* cv. Basilisk, *B. ruziziensis* being the least persistent. The reasons for these differences in edaphic adaptation are not obvious.

The three cultivars are being used as parents in a breeding program to develop superior *Brachiaria* genotypes that combine the spittlebug tolerance of *B. brizantha* cv. Marandú with the high edaphic adaptation of *B. decumbens*. Previous research showed that differences in acid soil adaptation among the three parents are not due to Al toxicity, but other factors associated low soil fertility. There is a need identify plant traits of *B. decumbens* that contribute to its adaptation to low fertility acid soils so as to develop a reliable screening method. Identification of genetic variability among recombinants to acquire nutrients and produce greater amounts of forage would facilitate development of superior *Brachiaria* genotypes.

7.1 Screening under field conditions. A field trial was established on a clay loam oxisol at Carimagua in 1994 to evaluate differences in edaphic adaptation of 43 genetic recombinants of *Brachiaria* compared to that of 12 natural accessions including the 4 parents (*B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandú, *B. ruziziensis* 44-02 and *B. brizantha* cv. La Libertad). Low amounts of fertilizer was applied during establishment (kg/ha: 40 N, 20 P, 20 K, 14 Ca, 12 Mg and 12 S). After 5.5 months of plant growth, a number of plant attributes including shoot biomass (cut at 10 or 15 cm height depending on the plant type), leaf/stem ratio, leaf area index, shoot nutrient composition and shoot nutrient uptake were measured.

Results presented in Figure 2 show marked genetic variation in nutrient acquisition and forage production during pasture establishment. Linear relationships between forage yield and shoot nutrient (N, P and Ca)

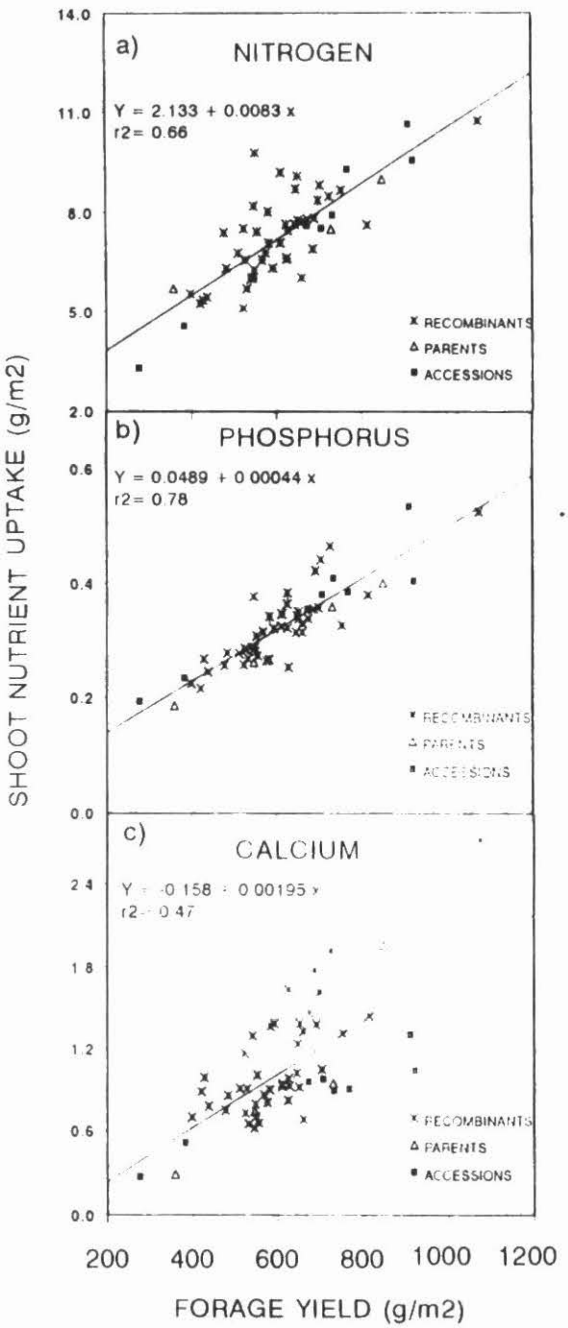


Figure 2. **Relationship between shoot nutrient uptake and forage yield of 55 genotypes of *Brachiaria* including 12 CIAT accessions (4 parents) and 43 genetic recombinants grown in a sandy loam oxisol at Carimagua.**

uptake per unit soil surface in genetic recombinants and accessions indicate the importance of the acquisition of nutrients to forage productivity. The relationship between shoot P uptake and forage yield (Figure 2b) was closer (r^2=0.78) than that of shoot N uptake (r^2=0.66) or shoot Ca uptake (r^2=0.47). This regression analysis indicates that P acquisition may be more limiting to forage production than acquisiton of N and Ca in this soil during establishment . Among the 4 parents, *B. ruziziensis* was least efficient in acquiring P and consequently least productive. Two genetic recombinants, BRN093/3009 and FM9201/1873, were outstanding in terms of both forage biomass and shoot nutrient uptake. However neither of these two recombinants was highly resistant to spittlebug infestation. It should be possible to recombine the edaphic adaptation of these two recombinants with the spittlebug tolerance of a sexual recombinant, BRN093/1371.

Significant genetic variation was also observed in several other plant attributes such as leaf area index, leaf to stem ratio and nutrient partitioning index (nutrient uptake in leaves/shoot nutrient uptake x 100). Several genetic recombinants were found to be superior to their parents in partitioning of P and N to leaves which would help to maintain higher rates of leaf expansion.

Additional genetic recombinants of *Brachiaria* together with their parents were planted in 1995 in a replicated trial on a sandy loam oxisol at Carimagua with low or high amounts of fertilizer, to continue this study of differences in edaphic adaptation and field persistence. **[I. M. Rao, J. W. Miles, J. C. Granobles]**.

7.2 Screening under controlled conditions. Field observations of *Brachiaria* pastures suggest that one of the major causes for pasture degradation in acid soils, other than spittlebug, is a decline in soil fertility over time. A glasshouse trial was conducted to evaluate differences in tolerance to diminishing nutrient supply among four parents and 53 genetic recombinants of *Brachiaria*. Plants were grown in pots (4 kg soil/pot) using a sandy loam oxisol from Carimagua. Low amounts of nutrients (kg/ha: 20 P, 20 K, 50 Ca, 14 Mg and 10 S) were supplied at planting. Nitrogen and micronutrients were not supplied. Plants were cut at different intervals (50, 96 or 192 days after standardization) and allowed to regrow after each cut without adding any more nutrients. This simulated plant growth under diminishing nutrient supply. At the time of third cut (192 days), several plant attributes (shoot biomass, leaf area, leaf/stem ratio, leaf chlorophyll, soluble leaf protein, specific leaf N, shoot nutrient uptake, N partitioning index, root biomass and root length) were measured to identify genotypic differences in tolerance to declining soil fertility.

Three plant attributes, leaf area production, root length, and shoot P uptake, were useful to identify genotypic differences in adaptation to declining soil fertility (Table 4). Among the 4 parents, *B. ruziziensis* acquired the least amount of P due to its smaller root system. The decrease in shoot P uptake had a marked effect on leaf area production compared to the other three parents. Among the 53 genetic recombinants tested, at least three showed similar or better adaptation than parents to declining soil fertility. This was evident by their ability to maintain greater root growth and leaf expansion. The three plant attributes could serve as selection indices in field trials at Carimagua in order to identify superior genotypes of *Brachiaria* for low fertility acid soils **[I. M. Rao, J. W. Miles, J. Ricaurte and R. Garcia]**.

7.3 Persistence under field conditions. A field trial was established during 1992 in a clay loam oxisol to determine edaphic adaptation differences among four cultivars of *Brachiaria* (*B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandú, *B. brizantha* cv. La Libertad and *B. ruziziensis* cv. Common) grown in monoculture or in an association with *Arachis pintoi* CIAT 17434. The experiment was designed as a split plot with soil fertility treatments as main plots and monoculture vs. association as subplots. Initial fertilizer application was at two levels: low (kg/ha: 20 P, 20 K, 50 Ca, 12 Mg and 10 S) and high (kg/ha: 50 P, 100 K, 100 Ca, 24 Mg, 20 S and micronutrients). Persistence of pastures has been evaluated under heavy periodic grazing depending on the amount of forage on offer. Maintenance fertilizer was not applied in order to evaluate persistence under declining soil fertility. The presence of a legume in the associations was expected to improve the persistence of the grass cultivars. Root and shoot attributes are being monitored in grass monocultures.

Results obtained through the 1994 rainy season on grass monocultures indicate that common *B. ruziziensis* was less persistent over time due to it's inability to partition a greater proportion of biomass toward root growth (Table 5). This allowed the native vegetation to

Table 4. Differences in low fertility tolerance among parents and genetic recombinants of *Brachiaria* grown in pots (4 kg soil) using a sandy loam oxisol from Carimagua.

Genotype	Shoot biomass (g/pot)	Leaf area (cm^2/pot)	Root length (m/pot)	Shoot P uptake (mg/pot)
Parents*:				
B. decumbens	2.0	153	177	4.6
B. brizantha - M	1.6	139	146	5.4
B. brizantha - L	2.8	109	127	4.5
B. ruziziensis	1.7	88	86	2.9
Recombinants:				
FM9302/2564	1.9	201	235	5.6
FM9302/2457	2.2	161	198	5.2
FM9302/2662	2.0	166	318	4.7
Mean**	1.9	156	183	4.6
$LSD_{0.05}$	0.4	52	77	0.9

* *B. decumbens* cv. Basilisk; *B. brizantha* cv. Marandú; *B. brizantha* cv. La Libertad; *B. ruziziensis* cv. Common.

** Mean value of 53 genetic recombinants and 4 parents dominate this grass as the soil fertility declined. There were no indication of pasture degradation in *B. brizantha* cultivars. This may be because the supply of N in this soil via mineralization is adequate for their growth. Grazing intensity was increased to induce pasture decline. The influence of the legume on pasture decline will be monitored with time. **[I. M. Rao, R. Thomas, P. Herrera, J. C. Granobles]**.

Table 5. Differences in root to shoot partitioning of biomass in four cultivars of *Brachiaria* grown in a clay loam oxisol at Carimagua with low initial fertilizer application at establishment.

Cultivar	Root/shoot ratio	
	1992	1993
B. decumbens cv. Basilisk	0.49	0.50
B. brizantha cv. Marandú	0.63	0.72
B. brizantha cv. La Libertad	0.89	0.68
B. ruziziensis cv. Common	0.32	0.36

To be productive and persistent under low nutrient supply in field conditions, an ideal genotype of *Brachiaria* would not only acquire greater amounts of limiting nutrients (e.g., P, N and Ca) by producing fine roots but also utilize nutrients to produce greater amounts of leaf area. Together with these two attributes, the ability to partition greater proportion of P and N to leaves rather than stems will contribute to the production of greater amounts of green forage. One of the strategies to develop such an ideotype of *Brachiaria* would be to screen first for tolerance to spittlebug and identify the promising genotypes and then subject those to evaluation for edaphic adaptation, forage quality, disease resistance and other desirable attributes.

7.4 Tolerance of *Brachiaria* spp. to waterlogging.

The commonly used cultivars of *B. decumbens*, cv. Basilisk, and *B. brizantha*, cv Marandú, are susceptible to water-logged conditions in the humid tropics, often succumbing to disease. However, there has been little objective comparison of inter- or intra-specific differences in tolerance to poorly drained conditions.

Nine accessions of three *Brachiaria* spp. and four commercial cultivars were evaluated along a water table gradient exhibiting from well to poorly drained conditions. At the poorly drained end of the gradient the water table level fluctuated form - 50 cm in dry periods to +10 cm during periods of heavy rainfall. Plant vigor and plant mortality were recorded at weekly intervals for one year after which the standing DMY was measured at the well drained extreme, middle and poorly drained end of the gradient.

There were significant variations between and within *Brachiaria* spp.to waterlogging tolerance (Table 6). The good adaptation of *B. humidicola* and *B. dictyoneura* to saturated soil conditions was confirmed as was the susceptibility of *B. decumbens* cv Basilisk (CIAT 606), and *B. brizantha* cv Marandú (CIAT 6780). However, there were accessions of *B. decumbens* (CIAT 16497) and *B. brizantha* (CIAT 26110) that showed good tolerance of poorly drained conditions. *B. humidicola* CIAT 16886 produced higher DMY than the commercial cultivar CIAT 679. **[P.J. Argel, A. Valerio, R. Martínez, M. Hernández (MAG)]**

8. Resistance to *Rhizoctonia* foliar blight

A field and glasshouse screening methodology, based on artificial inoculation with dried sclerotia, has been developed and a set of 42 *Brachiaria* accessions screened (Kelemu et al., in press). Disease was quantified in the glasshouse on percentage of infected leaves, percentage of leaf area infected, and number of sclerotia on infected tissue. Percentage leaf area was visually estimated on plants inoculated in the field. All disease criteria were positively correlated. Differences in resistance were not discrete, suggesting quantitative inheritance. With reliable, repeatable screening methodology such resistance can readily be managed in the breeding program. The most resistant among the sample of accessions tested were, with one exception, accessions of *B. humidicola*. An accession of *B. brizantha*, CIAT 16320, was classified as moderately resistant. Several other accessions of *B. brizantha* or *B. decumbens* were less susceptible than the commercial *B. brizantha* cv. Marandú. **[S. Kelemu, J. Badel]**

9. Assessment of virus resistance

9.1 Development of a reliable screening methodology to select *Brachiaria* genotypes resistant to potyviruses. Artificial inoculation tests conducted in 1994, yielded infection rates of up to 35 or 80% when the *Brachiaria* potyvirus was manually inoculated onto

Table 6. Plant vigor, plant mortality, and dry matter yields (DMY) of *Brachiaria* spp. established along a gradient from (1) poorly drained, (2) moderately drained to (3) well drained soil, Guápiles, Costa Rica.

Species	CIAT Accession No.	Plant vigor[1]	Plant mortality	DMY (kg/ha)		
				(1)	(2)	(3)
B. humidicola	679	4.5	0	1349a[2]	1468a	1055a
	16886	5.0	0	2840a	1987b	2768ab
	26149	4.5	0	1824a	2391a	2654a
B. dictyoneura	6133	4.5	0	2052a	3311a	2454a
B. decumbens	606	3.5	0	1425a	1531a	1841b
	16497	4.0	0	2364a	2122a	2468a
B. brizantha	6387	3.5	0	1601a	2811a	2763a
	16322	4.0	0	2100a	3129b	2458ab
	26110	3.5	0	2468a	2803a	2297a
	26646	3.0	0	1633a	2986b	2735ab
	16168	3.0	0.5	1826a	2497b	2982b
	16827	2.5	0.5	1369a	2371ab	1758b
	16835	1.5	2.0	1384a	2098b	2078b
	6780	2.0	1.5	1483a	2114a	3101b

[1] Vigor rated 0= poor vigor; 5= highly vigorous. Mortality rated 0=no mortality; 5= high mortality
[2] Within a row, means followed by the same letter are not significantly different ($P<0.1$). Duncan's.

vegetative propagules or seedlings of *Brachiaria* spp. The lower inoculation efficiency observed for vegetative propagules relative to seedlings, is not unusual for most virus-host systems, where the phenomenon known as 'adult plant resistance' is responsible for low infection rates of viruses inoculated by artificial means.

This year, a different mechanical inoculation technique (the air-brush technique) used for other potyviruses of grasses, such as sugarcane mosaic virus, was tested using *Brachiaria* seedlings. The results from this test conducted at 30 or 50 p.s.i, yielded average transmission efficiencies of 11 or 6%. Consequently, this technique was considered inadequate for screening purposes.

The mechanical inoculation technique used at CIAT has yielded infection rates of 100 % for other grasses, such as sorghum, maize, and *Paspalum* spp. While the species of *Brachiaria* tested are more difficult to inoculate with the conventional manual inoculation technique, the current infection rates of 80-90% are adequate for screening purposes, as long as test plants are inoculated as seedlings.

9.2 Characterization of a potyvirus infecting *Brachiaria* spp.: Last year, we reported on the molecular characterization on the potyvirus detected in *Brachiaria brizantha* at CIAT. This virus was shown to have a nucleotide sequence similarity of 97.3 % with the corresponding fragment analyzed of the coat protein of johnson grass mosaic virus strain JG (JGMV-JG), previously described in Australia. This year, an antiserum to JGMV-JG prepared in Australia, was tested at CIAT with the *Brachiaria* potyvirus. The antiserum reacted positively, confirming the molecular characterization of the *Brachiaria* potyvirus as a strain of johnsongrass mosaic virus. A manuscript of this publication has been submitted to the Journal of Phytopathology.

9.3 Effect of johnsongrass mosaic virus on seed production of *Brachiaria* spp. Last year, a preliminary test was conducted to determine the effect of the *Brachiaria* potyvirus on seed yield of *B. brizantha*. The average seed yield of 15 *B. brizantha* plants systemically infected by the virus was 92.4 seeds, with 4 plants producing no seed. The average yield of the virus-free plants was 213 seeds, with all plants producing seed.

The above experiment was repeated this year, increasing the number of test plants to 50 per treatment (virus-infected or virus-free). A randomized block design with five reps of 10 plants each for the two treatments, has been chosen for this experiment. The test plants were inoculated in June and we are waiting to harvest the seed.

9.4 Evaluation of the possible transmission of the *Brachiaria* strain of johnsongrass mosaic virus in *B. brizantha*. The potential seed transmissibility of the *Brachiaria* strain of johnsongrass mosaic potyvirus was investigated. A total of 1,433 seeds collected from systemically infected *B. brizantha* plants were germinated, and the resulting seedlings assayed for the presence of the virus. None of the seedlings was infected by the virus. Hence, the rate of transmission must be less than 0.07%.

9.4 Screening of *Brachiaria* germplasm for its reaction to the *Brachiaria* strain of johnsongrass mosaic virus. A total of 131 *Brachiaria* sp. selections made in Caqueta and Carimagua, have been under continuous screening by frequent rejuvenation and manual inoculation of the vegetative propagules. To date, a total of 64 hybrids have proved susceptible to the virus: The remaining 67 hybrids have escaped infection under artificial conditions.

These hybrids should be further evaluated when seed becomes available, to increase the efficiency of inoculation and distinguish between "true" virus resistance and 'adult plant resistance'. **[F. Morales]**

10. Seed dormancy

Strong seed dormancy or latency has been a recurring concern with seed of *B. dictyoneura*. An M.S. thesis project conducted by Manuel Sánchez (U. Nacional de Colombia, Sede Palmira) with support from the TFP has been completed (Sánchez Orozco, M. S. 1995). Seed from several Colombian sources was subjected to acid or mechanical scarification, and dry heat treatments whose effects on germination were monitored over a 12-month post harvest period. The effects of imbibition with KNO_3 or 'Gelvatol' (a polyvinyl alcohol) were assessed.

Two types of latency were found (as in other *Brachiaria* species): a physical restriction of germination owing to impermeable seed coat (lemma and pelea, and glumes) and a physiological dormancy of the seed embryo.

Scarification (acid or mechanical) was effective in overcoming the physical barriers to germination. While statistically significant effects were detected for several treatments, none, aside from time, had a major effect on physiological dormancy of the seed embryo. Germination of intact spikelets was low even 12 mo after harvest. Maximum germination of acid-scarified seed occurred 6 mo after harvest. Acid scarification and a 3-mo after-ripening period are recommended. **[M. Sánchez, J. W. Miles]**

11. Endophytic fungi in tropical grasses

Preliminary studies identified the presence of endophytes in native grasses from the Llanos. Activities will increase in 1996 with the funding of a new project.

12. Pollen storage

No activity

13. *Brachiaria* workshop

In October, 1994, the International Workshop on the Biology, Agronomy, and Improvement of Brachiaria was held at CIAT headquarters. Invited speakers from 13 countries of Africa, America, Asia, Australia, and Europe were present.

Editing is complete and manuscripts for the Workshop proceedings are nearly ready to go to press. We anticipate being able to begin to distribute the proceedings early in 1996. **[J. W. Miles, B. L. Maass, C. B. do Valle]**

Project: Genetic Enhancement of *Brachiaria*

Proposed activities for 1996

1. Genetic Resources

- Establish eight regional agronomic trials in collaboration with Corpoica and Fondo Nacional del Ganado in Colombia

- Establish regional trials in Bolivia, Costa Rica, and Brazil

2. New *Brachiaria* gene pools

- Make a second year's observation on field trials at Carimagua and Caquetá prior to final selections for recombination.

- Test the feasibility of recombining selected clones using potted plants at CIAT-Palmira.

- Recombination of a small number of sexual clones selected after two years of field observations.

- Continue to assess logistics of conducting "recurrent selection on specific combining ability" in sexual populations through observations of small number of testcross progenies formed in 1995 and setting up testcross crossing block (i.e. 2 ha plot of tester genotype).

3. Genetics of apomixis

- Confirmation of currently available markers in a second hybrid mapping population.

- Identification of markers more tightly linked to the apomixis locus (fine mapping).

4. Assessment of spittlebug resistance

- The gene tagging, artificial field infestation, and spittlebug feeding preference studies described above are ongoing. The preference studies should be concluded by mid-1996.

- We plan to finish the initial screening of progenies 1027 and 1028 for gene-tagging of resistance to spittlebug by March-April.

5. Genetic diversity in *Brachiaria*

- No specific activities projected for 1996.

6. Genetic control of key attributes.

- Identification of parental material for study of genetic modification of expression of major gene conferring apomictic reproduction

- Initiation of controlled crosses to produce appropriate progenies.

7. Edaphic adaptation

- Continue measurements on persistence of four parental accessions of *Brachiaria* under intensive grazing.

- Plant attributes that could be related to edaphic adaptation and persistence such as leaf area production, shoot P uptake and shoot N uptake will be monitored over time in the field trial planted in 1995. This trial will be protected with insecticide to eliminate the effect of spittlebug.

- Conduct a glasshouse trial to evaluate tolerance to low fertility in a few selected recombinants and their parents using frequent cutting regime to induce decline in soil fertility.

- Study root anatomical and morphological characteristics of accessions tolerant and susceptible to waterlogging.

8. **Resistance to *Rhizoctonia* foliar blight**

- Artificial inoculation of large field breeding nurseries at Carimagua and assessment of resistant in the breeding populations.

- Assess relationship between leaf morphology (e.g. density of leaf trichomes) and *Rhizoctonia* resistance and nutritional quality.

9. **Assessment of virus resistance.**

- Complete assessment of the effect of Johnsongrass mosaic virus on seed and forage yield.

- Continue and expand screening of breeding populations for resistance to virus diseases.

10. **Seed dormancy.**

- No specific activities projected for 1996.

11. **Endophytic fungi in tropical grasses.**

- Determine if *Brachiaria* spp. are naturally infected with endophytic fungi.

- If endophytes are detected, begin to assess effects of endophytic fungi on attributes of *Brachiaria* forage grasses, particularly with regard to spittlebug resistance and forage quality parameters.

12. **Pollen storage.**

- Undergraduate thesis finished.

- Prepare manuscript for publication.

13. ***Brachiaria* workshop.**

- Proceedings published and distributed.

Project: Enhanced genepools of forage *Arachis*

Project coordinators: P.C. Kerridge and E.A. Pizarro

Rationale

Arachis pintoi is the first herbaceous, tropical legume to give high productivity and long-term persistence with vigorous, stoloniferous grasses in the humid and subhumid tropics. It also has potential as a green cover for weed control and for soil improvement. However, the development of *A. pintoi* as a commercial forage legume is at present based essentially on a single genotype. While this line is very successful, several limitations such as slow establishment and limited drought tolerance are recognized. Additional accessions need to be acquired and evaluated so as to extend the range of adaptation of *A. pintoi* and to ensure the availability of genes for resistance to possible future disease and insect outbreaks. There is a need to assess disease and insect resistance, of all potentially promising accessions, to those disease and insects that affect the common peanut (*Arachis hypogaea*) and also assess problems that might arise as forage *Arachis* is more widely spread and larger areas are planted with *Arachis*. Other species of the genus *Arachis* which have forage potential and are likely to extend the range of *Arachis* into different environments also need to be acquired and evaluated. It is also important to increase the knowledge of the genetic variability in the potentially useful species in order to develop programs for *ex-situ* and *in-situ* conservation as many areas where this genus occurs are currently under threat from agricultural development.

Wild *Arachis* species have the potential for widespread use in grass-legume pastures or as cover crops through the humid and subhumid tropics and sub-tropics, and to play a strategic role in sustainable agricultural systems through providing soil cover and fixing nitrogen. Ex-ante studies suggest a high internal social rate of return (58%). Beneficiaries will be farmers in all major ecosystems in the subhumid and humid tropics.

A collaborative project between CENARGEN, ICRISAT and CIAT on 'Preservation of wild *Arachis* species' will commence in 1996. This should have a considerable impact on acquisition of *Arachis* and in studies on the potential for disease and insect damage in natural *Arachis* accessions. CIAT is seeking additional funding for research in the area of genetic diversity and conservation.

Objective

To extend the range of adaptation of forage *Arachis* species by broadening the available genetic base through collection and evaluation, studies of genetic diversity to assess disease and insect resistance, and to improve utility through more rapid and reliable establishment practices.

Main activities:

1. Acquisition of *Arachis* germplasm.
2. Characterization of *Arachis* germplasm.
3. Evaluation for environmental adaptation
4. Disease and insect tolerance.
5. Management for rapid establishment and high yield.
6. Investigation of optimum conditions for seed production and storage.
7. Genetic diversity and conservation of wild *Arachis* species.
8. Reproductive behavior and species compatibility.

Highlights

- *Arachis pintoi* germplasm has been collected from areas with < 1000 mm rainfall.

- There is an increase in the number of available *A. pintoi* accessions to 113 and *A. repens* to 31.

- Morphological and isozyme characterization of new *A. pintoi* germplasm showed that the range of variation has increased as accessions were added; there is good separation of groups according to geographical origin and no genotypic duplicates have been detected in the accessions held at CIAT.

- Multilocational evaluation of the newly introduced germplasm of *A. pintoi* to Colombia highlights the agronomic potential of several new accessions, CIAT 22160, 18751 and 18747.

- A new accession, BRA-031828, has shown high adaptation in the Cerrados, Brazil.

- Feed quality of new accessions appears to be as good as or higher than that of the present commercial cultivar, CIAT 17434.

- Direct planting from seed, high seed rates and fertilization increase the rate of establishment

- Seed quality is affected by where seed is grown, post-harvest handling and storage; seed deterioration can be very rapid under humid conditions but very small under low humidity.

1. Acquisition of *Arachis* germplasm

1.1 Collection in Brazil

During 1994-1995, four collection trips were made under the leadership of Dr.J.F.M.Valls (EMBRAPA/CENARGEN). Special emphasis was placed on collection of wild *Arachis* spp. with forage potential. One hundred and seventy two accessions were collected (Table 1). The available germplasm of section Caulorrhizae in Brazil is now 144 accessions (*A.pintoi*, 113; *A.repens*, 31) and in the Rhizomatosae section there are 72 *A.glabrata* accessions. All the material is being multiplied.

Seven new accessions of the 32 collected 1995 came from areas with 700-1000 mm/yr annual rainfall. All have been planted out in the nursery. **[J.F.M.Valls and E. A.Pizarro]**.

1.2 Introduction to the CIAT gene bank and post-entry phytosanitary follow-up

During 1994-1995, 56 accessions of *A. pintoi* and other *Arachis* species, largely from EMBRAPA/CENARGEN, Brazil, but also from INTA, Argentina, were added to the collection maintained at CIAT. The gene bank now holds 106 accessions of 20 wild *Arachis* species, including 61 accessions of *Arachis pintoi*. All but 14 accessions have been released from post-entry phytosanitary follow-up for evidence of pests, diseases, and virus.

Some were tested in more detail by the Virology Research Unit (VRU) which indexed 22 accessions of *A. pintoi*, 4 of *A. repens* and 7 other *Arachis* species.

Table 1. Arachis germplasm collected in Brazil during 1994 and 1995.

Section/Species	No. of accessions
ARACHIS	
A. hypogaea	11
A. cardenasii vel. aff.	2
A. decora	3
A. diogoi vel. aff.	2
A. glandulifera	1
A. helodes et aff.	3
A. kuhlmannii et aff.	3
A. magna et aff.	5
A. stenosperma	4
A. simpsonii et aff.	10
A. valida	2
A. sp. (Luis Alves)	6
A. sp. (São Félix)	1
CAULORRHIZAE	
A. pintoi	21
A. repens	10
ERECTOIDES	
A. archeri	3
A. benthamii	1
A. oteroi	1
A. paraguariensis	5
A. sp. (Pto. Murtinho)	1
EXTRANERVOSAE	
A. burchellii	6
A. lutescens	8
A. macedoi	2
A. prostrata	5
A. retusa	1
HETERANTHAE	
A. dardanii	3
A. giacomettii	1
A. pusilla	12
A. sylvestris	3
PROCUMBENTES	
A. lignosa	1
A. matiensis	3
A. vallsii	1
RHIZOMATOSAE	
A. burkartii	1
A. glabrata	24
A. pseudovillosa	3
TRIERECTOIDES	
A. guaranitica	2
TRISEMINATAE	
A. triseminata	1

EMBRAPA-CENARGEN/CPAC-CIAT/Texas A&M-USDA-ICRISAT.

The VRU is continuing an investigation of 2 accessions of *A. pintoi* from Costa Rica that were positive in ELISA tests and 1 accession of *Arachis* sp. that had a potyvirus related to Peanut Mottle Virus (PMoV), the first virus detected in *A. pintoi*. This virus is being characterized to determine its relationship to the first strain of PMoV detected. Twelve of the first 18 *A. pintoi* accessions received from Brazil have proved susceptible to PmoV using mechanical inoculation. As the remaining 6 accessions of *A. pintoi* and all of *A. repens* were not successfully infected by artificial inoculation, they are being investigated further to determine if there they may provide a source of resistance to PMoV.

Clean plants were assigned CIAT accession numbers and established in a field collection at CIAT-Palmira for initial seed increase, and morphological and biochemical characterization. **[A. Ortiz, B.L. Maass, F. Morales, S. Kelemu, M. Castaño]**

2. Characterization of *Arachis* germplasm

2.1 Brazil

The morphological variation of 51 accession of the genus *Arachis*, belonging to section Caulorrhizae were evaluated by multivariate analysis. Cluster analysis was applied to data related to 52 morphological characters of qualitative and quantitative nature. The resulting phenogram, based on the distance coefficient using 51 OTU's, correlated well with morphological characteristics. This analysis also allows the separation of the groups according to geographical regions of the San Francisco floodplains, Minas Gerais and Bahia State. **[Luci Monçato and J.F.M. Valls** (CENARGEN)**]**.

2.2 Colombia

Morphological and biochemical characterization (using isozymes and seed proteins), is being carried out with assistance of undergraduate students from Universidad del Valle, Cali, and Universidad Nacional de Colombia, Palmira.

Validation of morphological descriptors in *Arachis pintoi*. Morphological characterization of *Arachis pintoi* is based on the 32 descriptors proposed for the genus *Arachis* (IBPGR 1990), using those that proved most discriminating (Maass et al 1993; Bermúdez, 1994).

To determine the stability of these characteristics over time and in four different environments, 10 quantitative characteristics (Table 2) and 10 qualitative characteristics (Table 3) were compared for six introductions of *A. pintoi*, in four different environmental conditions. The "Gabriel" test for difference of means was used to compare the averages of each characteristic.

Some variation was observed in quantitative and qualitative characteristics between the four sites that were related to differences in vigor of growth between sites. In general, there was adequate stability of expression though there was distinct variation in stem pigmentation and petiole seta.

It is because of the interaction of morphological expression with environmental conditions that biochemical characterization is a valuable complementary tool in characterization studies.

Table 2. Quantitative characteristics of *Arachis pintoi* under four different environmental conditions.

Morphological descriptor	GH[1] Pal-90	Field[2] Pal-93	Field[3] Pal-95	Field[4] Chi-95
Petiole length (cm)	3.0B*	3.8A	2.4C	3.7A
Length apical leaflet (cm)	2.6B	3.4A	2.5B	3.4A
Diameter apical leaflet (cm)	1.8B	2.1A	1.6 C	2.1A
Hypanthium length (cm)	7.5B	9.1A	7.6B	---
Standart diameter (cm)	1.5B	1.7 A	1.5B	---
Peg length (cm)	13.4B	15.2A	11.8B	---
Length basal segment (mm)	10.9B	11.8A	10.5B	---
Isthmus length (cm)	2.4A	2.4A	1.3B	---
Seed length (mm)	9.1B	10.0A	---	---

[1]Greenhouse conditions, Palmira, 1990; Field conditions; [2]Palmira, 1993,
[3]Palmira, 1995;
[4]Chinchiná, 1995
* Characteristics followed by same letter are statistically similar.

Arachis pintoi and A. repens. 52 accessions of *A. pintoi* and 2 of *A. repens* were characterized by applying polyacrylamide gel electrophoresis (PAGE) to the isozymes α-esterase and ß-esterase (EST), diaphorase (DIA), αß-acid phosphatase (ACP), gluatamate oxaloacetate (GOT), and peroxidase (PRX).

Table 3.Qualitative characteristics of *Arachis pintoi* under four different environmental conditions.

Morphological descriptor	GH[1] Pal-90	Field[2] Pal-93	Field[3] Pal-95	Field[4] Chi-95
Stem pigmentation Stipule seta	absent	absent	present	---
Petiole seta	absent	absent	absent	absent
Leaflet color	absent	absent	few	few
	green	yellow-	green	---
Shape apical leaflet		green		
Leaflet pubescence	obovate	obovate	obovate	obovate
	glabrous	glabrous	glabrous	glabrous
Leaflet seta	/hairy*	/hairy*	/hairy*	/hairy*
Pubescence leaflet margin	absent	absent	absent	absent
Standart color	hairy	hairy	hairy	hairy
Seed color				
	yellow.	yellow	yellow	---
	grey- orange	grey- orange	---	---

[1] Greenhouse conditions, Palmira, 1990; Field conditions: [2] Palmira, 1993; [3]Palmira, 1995; [4] Chinchiná, 1995.
*Almost glabrous upper, hairy below.

The results indicated large intrasectional and intraspecific variation. No genetic duplicates were detected.

The range of morphological variation increased as new accessions were characterized, with a broad and continuous range between *A. repens* and *A. pintoi*. These two *Arachis* species of section *Caulorrhizae* could not be differentiated by isozymatic polymorphism or by morphological traits. Neither were there specific patterns that could be related to the geographical origin of the germplasm.

Arachis glabrata. The collection of 14 accessions of *A. glabrata* held at CIAT were characterized morphologically using 46 vegetative and reproductive descriptors. Fruit and seed descriptors were excluded because of the absence of fructification. Cluster and principal component analyses were used to group similar morphological plant types. By using those nine characters that contributed most to the first two principal components in the cluster analysis, seven morphological groups (similarity index 0.91) were distinguished, indicating broad genotypic variation within this germplasm. **[B.L. Maass, A. Ortiz, A.M. Torres, M.F. Bermúdez, J.M. Alarcón]**

2.3 Taxonomy of *Arachis*

A new taxonomy of *Arachis* is available:

Krapovickas, A. and W.C. Gregory. 1994. Taxonomia del genero *Arachis* (Leguminosae). Bonplandia 8:1-186.

3. Evaluation for environmental adaptation

Most of the *Arachis* germplasm has originated from Brazil where they have been assigned BRA- accession numbers. Material introduced to CIAT is assigned a CIAT accession number. An annex is attached showing the correspondence between the BRA- and CIAT numbers,

3.1 Planaltina, Brazil[1]

Section Caulorrhizae

Nursery. The recently collected 32 accessions were established for preliminary evaluation and multiplication. After eight months from establishment, there was a wide range in the agronomic adaptation index (bad to excellent), ground cover (50 - 100%) and leaf retention (low and high). The outstanding accessions are: BRA -031291; -031895; -032239; -032328; -032352; -032361; -032395; -032433 and -033430. **[E. A.Pizarro and A.K.B.Ramos]**.

[1] See annex for corresponding BRA- and CIAT accession numbers.

Small plot cutting trial. A trial of 46 accessions associated with *Paspalum atratum* BRA-009610 was established in 1992 on two soil types at CPAC, Planaltina. One site is representative of the low lying areas, and the other, the well-drained upper portions of the Cerrado landscape.

Mean dry matter yield (DMY) at 45 (118 mm received) and 180 days regrowth (1054 mm received) during the first year were similar in the well drained and in the low lying area (1.7 t/ha vs. 1.5 t/ha at 45 days regrowth and 4 t/ha vs. 3.6 t/ha at 180 days, respectively).

A large yield reduction occured in the second year in both sites. Mean DMY in the well- drained savanna at 45 days regrowth (313 mm) was 360% lower than in the lowland area (0.05 t/ha vs. 1.5 t/ha), and at 180 days regrowth (1132 mm), 54% lower (2.6 t/ha vs. 5 t/ha, respectively). The proportion of legume decreased from 47 to 15%.

The most productive and stable associations at both sites are: *A. pintoi* CIAT 17434, 18748, 22159, 22160 and BRA-031828;-031844, and -031852.

Green leaf retention was less on the upper portion of the landscape during the dry season. Green leaf retention, at the end of the dry season in September was observed in only 3 accessions in the well drained savanna (CIAT17434, 18748 and BRA-031852), while in the lower area 7 accessions were outstanding (CIAT 18750, 22160,22159 and BRA-030082;-030449;-031861;-031828).

Seed yield, at 15 months post-establishment, of the 46 accessions grown in association with *P.atratum* BRA-009610 on the seasonally flooded area ranged from 0 to 4 t/ha for the *A. pintoi* accessions and from 0 to 400 kg/ha for the *A. repens*. *A. pintoi* accessions with at least 2 t/ha seed were CIAT 22148, 22255, 22258 and 22160); with 3 t/ha - CIAT 22155 and with 4 t/ha - CIAT22150. **[M. A. Carvalho and E. A. Pizarro]**.

Promising accessions. Seven *Arachis pintoi* accessions, that had shown agronomic performance in earlier evaluation at EMBRAPA-CPAC or had been observed to be particularly vigorous when collected, were selected for evaluation with *Paspalum maritimum* BRA-028075, an accession with high green leaf retention during the dry season. The trial was planted in January 1993 on a humid gley soil, representative of low lying areas of the Cerrado, and situated at the Colegio Agricola de Brasilia.

From January 1993 to February 1995, with 1524 mm rainfall, the total annual DMY was similar with a mean of 5 t/ha, whereas there was a wide range in legume yield - from 0.2 t/ha for CIAT 22172 to 3.2 t/ha for BRA-031828. There were few weeds. From Feb-Jul 1995, (447 mm rainfall) two cuts gave DMY of 4 t/ha (CIAT 22172) to 7 t/ha (BRA-031828).

The soil seed bank reserve at 18 months post-establishment was low for all accessions (8 to 650 kg/ha) when compared with the yield of the same accessions grown in the same area but in association with *P.atratum* BRA-009610. Accession BRA-031828 appears to be a promising new accession. The area will be put under grazing from October 1995. **[E. A.Pizarro, A. K.B.Ramos and M.A.Carvalho]**.

Section Arachis.

Leafier plant types of the Section Arachis, even those with a short life cycle, may have a place in mixed agricultural systems.

Thirty five accessions of *A.hypogaea*, selected by Dr Ignacio Godoy from the Instituto Agronômico de Campinas, SaoPablo, were evaluated for their utility as a forage in a well drained area in the Cerrado ecosystem. *A. pintoi* CIAT 22160 was included for comparison.

There were significant differences ($P < 0.01$) in dry matter yield at 90 days from planting. DMY ranged from 0.75 t/ha for the *A.pintoi* to 5 t/ha for the *A. hypogaea* accession V5554. The accumulated DMY at 180 days from planting was lower ($P < 0.001$) than at 90 days, mainly due to leaf shattering.

The mean DMY of the regrowth (between 90 and 180 days, 486 mm) in the *A. hypogaea* accessions, was 50 % lower than that in *A. pintoi* CIAT 22160. Ground cover index was 3.7 for the *A.hypogaea* accessions and 1.7 for *A. pintoi*.

Pure seed yield at 180 days from planting was significantly ($P < 0.05$) different between accessions and ranged from 1.3 to 2.2 t/ha for the *A. hypogaea* accessions and 400 kg/ha for the *A. pintoi*. **[E. A. Pizarro,.M.A.Carvalho and A.K.B.Ramos]**.

3.2 Colombia

Agronomic evaluation. In 1994, the available 27 accessions of *A. pintoi* and 5 of *A. repens*, were planted at 6 sites in Colombia to evaluate genotype-environment interaction. The environments ranged from the humid tropics (Macagual and La Rueda in Caquetá), the savannas (a sandy loam (Alegría) and clay loam (Alcancía) soil sites at Carimagua, Meta) to the seasonally dry hillsides (a fertile clay loam at Chinchiná and a silty loam at Popayán). The legume was established in association with a common grass (*B. humidicola* CIAT 6133, formerly *B. dictyoneura*). After an initial harvest and standardization cut, management has been by heavy grazing at monthly intervals. At Popayán, where no cattle are available, the plots are cut every four weeks.

Because of the initial scarcity of material, rooted stolons of *Arachis* were used as planting material. They were planted 50 cm apart in two rows, between the three 1 m rows of the grass in 3m x 2 m plots (i.e. with a legume density of 40%).

Measurements. At 2-monthly intervals during establishment measurements were made of rate of stolon expansion (cm/day) (until individual plants touched at 4 months after planting); soil cover (%); rooting capacity of stolons; and observations made on the occurrence of diseases, pests and apparent nutrient deficiencies. The commencement of flowering was determined as 50% of plants at full anthesis and flowering (numbers/m^2) at two-weekly intervals was recorded. Total DM yield was determined by cutting one m^2 per plot at 10 cm stubble height.

Flowering. All accessions initiated flowering by the time of the standardization cut except *A. repens* CIAT 22165 (all sites), and CIAT 22164 at Alegría, Alcancía, and Macagual. There was large variation in intensity of flowering, *A. pintoi* CIAT 22152 being the most prolific and *A. repens* and *A. glabrata* accessions having few flowers.

Establishment. Rapidity of establishment can be assessed by soil cover and lateral expension rate of stolons. The two variables are quite different. Some stoloniferous accessions, particularly *A. repens*, expand and rapidly invade the area, but do not provide much soil cover and their stolons do not root. Both characters are related to initial plant vigour and DM yield, although the latter depends on the plant's capacity to expand vertically when grass competion is heavier.

The six sites selected provided different conditions for establishment:

At Chinchiná, soil cover was rapid, reaching an early peak around 120 days. By 160 days after planting, vigorous grass growth dominated the association. Most accessions did not cover more than 50% of the plot; however, two, CIAT 18747 and 22160, had attained more than 80% cover.

At Popayán, the reverse happened because of slow growth of the grass. *Arachis* also grew slowly, but growth was fast enough to invade the entire plot before grass competition started. Maximum soil cover was only reached after 200 days with most accessions.

In Caquetá, at La Rueda, vigor and cover development was simialr to that at Popayán, whereas at Macagual, vigor and growth was quite variable between accessions.

At Carimagua, at both sites, Alcancía and Alegría, the situation was similar to that at Chinchiná, although less pronounced. At these sites, development of *Arachis* was also heavily constrained by disease pressure, particularly anthracnose.

In summary, daily expansion, to 4 months after planing (Table 4) was most rapid at Alcancía, followed by Chinchiná, Alegría, and La Rueda, and slowest at Popayán and Macagual.

Total and legume DM production reflect the rate of expansion and soil cover due to the difference in environmental conditions at the sites (Table 5).

There is large variation among the *A. pintoi* accessions and between environments. Some accessions (CIAT 18751, 18747, 22160, 22155) have superior establishment characteristics in terms of vigor and rapid growth and other accessions (CIAT 22152, 22154, 22156, 22158), inferior characteristics. **[B.L. Maass, E.A. Cardenas]**

Table 4. Rapidity of establishment of *Arachis* species and accessions at 6 sites in Colombia as shown by lateral extension (cm/day) 4 months after planting.

Site	Alcancía	Alegría	La Rueda	Macagual	Chinchiná	Popayán
Lateral expension rate (cm/day)						
Mean	0.42**	0.29**	0.26**	0.20**	0.32**	0.17**
Range	0.10-0.61	0.04-0.49	0.06-0.44	0.04-0.36	0.10-0.53	0.05-0.31
Selected accessions						
Range	0.43-0.61	0.27-0.41	0.15-0.42	0.12-0.30	0.24-0.53	0.14-0.29
Ranking						
CIAT 17434[a]	7	3	12	9	23	15
CIAT 18744b	6	16	4	7	10	12
CIAT 18748b	11	19	6	12	7	6
CIAT 22159c	9	7	19	27	13	3
CIAT 22160c	14	4	5	4	2	7
CIAT 22154d	18	11	23	25	15	20
CIAT 18747e	2	9	3	15	1	4
CIAT 18751e	1	8	9	10	3	2

a. Control; b. Selected accessions due to good compatibility with grasses; c. Selected accessions due to dry season tolerance; d. Superior quality; e. Superior growth rate during establishment

** Differences between accessions (by site) highly significant by F-test (P >0.0001)

Table 5. Mean total and legume dry matter (DM) production during establishment for all Arachis accessions and for the 14 most productive A. pintoi accessions at 4 sites in Colombia.

Site	La Rueda	Macagual	Chinchiná	Popayán	Overall
Total grass and legume DM production (g/m2)					
Mean of 14 accessions	682.0*	776.0*	656.5ns	278.9ns	598.4**
Range of 14 accessions	540-961	499-1096	499-739	198-342	
Legume DM production (g/m2)					
Mean of total	20.9	16.7	33.3	29.5	25.1
Mean of 14 accessions	45.3**	32.7ns	69.3***	60.1*	51.8***
14 selected accessions					
CIAT 17434a	26.1 de	44.6	5.4 d	16.8 cd	23.2 e
CIAT 18744b	57.1 abcd	24.3	65.6 c	51.7 bcd	49.7 bcd
CIAT 18746	30.7 de	52.1	51.8 cd	53.9 bcd	47.1 bcde
CIAT 18747e	76.1 abc	20.7	188.4 a	75.0 abc	90.1 a
CIAT 18748b	82.8 a	33.0	54.1 cd	67.5 abcd	59.4 bc
CIAT 18751e	54.7 abcd	58.5	131.5 b	119.4 a	91.0 a
CIAT 18752	77.2 ab	38.0	7.2 d	87.8 ab	52.6 bcd
CIAT 22148	41.8 bcde	27.9	10.4 d	26.4 bcd	26.6 de
CIAT 22150	13.3 e	37.2	54.2 cd	74.1 abc	44.7 cde
CIAT 22154d	n.i.	n.i.	n.i.	n.i.	n.i.
CIAT 22155	33.1 de	13.9	159.0 ab	83.2 ab	72.3 ab
CIAT 22157	38.2 cde	42.3	31.5 cd	30.8 bcd	35.7 cde
CIAT 22159c	24.0 de	1.0	45.1 cd	84.9 ab	38.7 cde
CIAT 22160c	56.2 abcd	19.5	150.3 ab	66.3 abcd	73.1 ab
CIAT 22165f	22.7 de	45.0	15.0 cd	4.2 d	21.7 e

Differences between accessions significant by F-test (*** = P >0.0001; ** = P >0.001; * = P >0.05)

a. Control; b. Selected accessions due to good compatibility with grasses; c. Selected accessions due to dry season tolerance; d. Superior quality; not included in 14 high yielding accessions; e. Superior growth rate during establishment; f. The only *A. repens* accession.

Forage quality of new *Arachis* germplasm. Different species and accessions within species of *Arachis* planted at the humid forest site (Caquetá) were evaluated for differencies in forage quality. Leaf samples of *A. glabrata* (3 accessions), *A. repens* (6 accessions) and *A. pintoi* (21 accessions) were analyzed for N, IVDMD and tannins.

Results shown in Table 6, show only small differences among *Arachis* species in N and IVDMD. The outstanding *A. pintoi* accessions such as CIAT 18744 and 18748 (i.e., good compatibility with aggressive grasses and persistence under grazing) and CIAT 22160 (i.e., drought tolerance) have slightly higher IVDMD values than the commercial cultivar (CIAT 17434). Also worth noting is the high IVDMD of *A. pintoi* CIAT 22154, which is associated with a lower tánnin concentration (Table 6).

A. glabrata had less extractable and bound CT compared to the other species.

These results show that the ecotypes of *A. pintoi* being advanced on agronomic merit, have as high or higher feed quality than the commercial cultivar, Mani forrajero (CIAT 17434). **[C. Lascano and N. Narváez].**

4. Disease and insect tolerance

There has not been any active research on diseases or pest of wild *Arachis* species during 1994-95.

In general disease has not been considered a major limitation in the adaptation and utilization of *Arachis*. However, the common diseases of the peanut are widespread and virus has also been reported. Occasional high incidence of anthracnose has been observed, in particular, in vegetatively planted material.

Table 6. Variation in quality among accessions of Arachis species grown in a humid forest site (Macagual, Caquetá, Colombia).

Species	Number of accessions	N (%)	IVDMD (%)	Condensed tannis	
				Extractable (%)	Bound (%)
A. glabrata					
Mean	3	2.7	73.6	0.3	0.9
Range		(2.4-3.1)	(72.2-76.1)	(0.2-0.6)	(0.7-1.0)
A. pintoi					
Mean	22	2.8	74.7	2.4	2.3
Range		(2.1-3.3)	(69.6-82.4)	(0.4-3.3)	(0.8-3.0)
CIAT 17434a		2.9	71.5	2.5	2.4
CIAT 18744b		3.2	78.0	1.4	3.0
CIAT 18748b		2.9	75.8	2.5	2.1
CIAT 22159c		3.4	69.6	2.5	2.3
CIAT 22160c		3.0	74.5	1.9	1.8
CIAT 22154d		2.3	82.4	0.4	0.8
A. repens					
Mean	5	3.4	71.6	1.8	2.4
Range		(2.6-3.7)	(68.6-76.3)	(0.6-2.6)	(1.4-3.4)

a. Control; b. Selected accessions due to good compatibility with grasses; c. Selected accessions due to dry season tolerance; d. Superior quality

An insect pest, *Cyrtomenus bergi*, a polyphagous, subterannean, burrowing and sucking bug, can attack the seed through the pod and predispose plants to soil borne pathogens which in turn greatly reduce seed yield. Seed production is limited in some areas because of the presence or build up of this insect. As the insect is also a common pest on cassava, potatoes, maize, groundnut, and sugarcane, care needs to be taken in location of seed production areas for *Arachis* and using *Arachis* in association if increased damage could result to these crops.

5. Management for rapid establishment and high production

5.1 Planaltina, Brazil

Several experiments were conducted to investigate the effect of various factors on rapidity of establishment.

Seeding rate. A trial with 2, 4, 6, 8 and 10 seeds/linear meter in 0.5 width row (equivalent to 5, 10, 15, 20, and 25 kg/ha germinated pure seed (GPS)/ha) was established with *A. pintoi* CIAT 22160 in a humid gley soil at Planaltina.

The effect of increasing seed rate on yield and weed-yield was striking. During establishment (140 days, 800 mm and 70 rainy days), dry matter yield (DMY) of *A. pintoi* increased from 0.5 t/ha with 5 GPS/ha up to 1.5 t/ha with 25 GPS/ha ($P<0.001$) to give a relation of:

$$DMY(kg/ha) = 150 + 48 (GPS/ha).$$

Increasing seeding rate significantly decreased weed yield ($P<0.001$).

An amount of 10 seeds/m would be considered a minimum for the establishment of most pasture legumes but is relatively expensive for *Arachis* because of the high seed weight; for comparison, the weight of 100 seeds of *A. pintoi, C. macrocarpum, S. guianensis* is approx. 24 g, 5 g and 0.3 g, respectively. The trade-off between number of seeds and cost is thus very critical for *Arachis*. **[E. A. Pizarro, A.K.B.Ramos, M.A.Carvalho]**

Effect of P, K and lime. The importance of adequate nutrition on establishment was investigated. An experiment was sown in a well drained virgin soil at Planaltina to study the effect of lime, (at 0 and 75% base saturation), K (0 and 100 kg/ha) and P (0 and 52 kg/ha) on the establishment of *A. pintoi* CIAT 22160. P had a significant effect ($P< 0.001$) effect but lime and K no effect on ground cover and yield during establishment. Ground cover at 130 days post-establishment was 20 % with nil P and 80 % with 52 kg/ha of P and DMY 300 kg/ha and 1.200 kg/ha, respectively. **[L. Vilela and E. A. Pizarro]**.

5.2 Colombia

In a field experiment at Carimagua, it was again demonstrated that establishment is much more rapid with use of adequate phosphorus fertilizer either with small quantities placed under the seed or larger amounts broadcast.

It appears advantageous to plant *Arachis* together with other legumes that establish more rapidly, such as *Stylosanthes* or *Centrosema*.

The research on developing Arachis as a forage component (see 8.10) has clearly demonstrated the need for early heavy grazing to reduce competition from associated grasses.

5.3 Costa Rica

Establishment of *Arachis pintoi* in association with *Brachiaria dictyoneura* using different means of propagation for the legume. *A. pintoi* CIAT 17434 was planted, using either (i) 25 cm long stolons, (ii) seed or (iii) one month old seedlings, in alternative rows with *B. dictyoneura* cv. Llanero. Planting density was 0.5 m within rows and 1 m between grass and legume rows. There were 4 replications. The site was an acid soil at San Isidro.

Transplanting seedlings (locally named almácigo) is a system widely used by small farmers with *Brachiaria* spp. They argue that it is more economic (less seed is used) and a safer way to plant pastures, particularly on steep hills.

Observations made 30 days after planting indicate that there was equal establishment of legume plants for each treatment but that plants from direct seed planting were more vigorous than those from stolons or the transplanted seedlings. **[A.Valerio and R. Quiroz]**.

The effect of burning and herbicides on recovery of *A. pintoi* from stolons or soil seed

A study was undertaken to determine the effectiveness of burning and herbicides on survival of *A. pintoi* in an established stand.

The site selected was at Guápiles (humid tropics, inceptisol soils) where there were one year old stands of *A. pintoi* CIAT 17434, 18744 and 18748 with estimated soil seed of 2080, 550 and 600 kg/ha corresponding to 1977, 374 and 462 seeds/m2 respectively.

The following treatments were applied:

i) Burning
ii) Burning + Picloram + 2,4-D amine (1.5% v/v) 30 d after burning
iii) Picloram + 2,4-D amine (1.5% v/v) + Atrazine (2.5 kg i.a./ha), once
iv) Same as 3 but Picloram + 2,4-D amine repeated at monthly intervals
v) Metsulfuron metil (0.23 kg i.a./ha) + Atrazine (2.5 kg i.a./ha) once,
+ Metsulfuron- metil (0.23 kg i.a./ha) at monthly intervals

A total rainfall of 7011 mm were recorded during the 18 months of the experimental period.

All accessions had the capacity to recover from either burning or a single application of herbicides through both stolons and soil seed.

Plant recovery was closely monitored for treatments (iv) and (v) where there were repeated applications of the herbicides (Table 7). Buds from surviving stolons continued to emerge after 2 to 3 months with repeated application of Picloram + 2,4-D amine (1.5% v/v) and from 5 to 6 months with repeated application of Metsulfuron- metil (0.23 kg i.a./ha).

Seedling emergence from soil seed reserves was higher for *A. pintoi* CIAT 17434, which had the largest soil seed bank and continued for the 18 months observations were made, although with a sharp decline after month 13. CIAT 18748 had higher recovery from stolons than the other two accessions.

These observations show the high tolerance of *A. pintoi* to herbicides and the tremendous capacity of this species to recover by stolons or soil seed reserves. **[P.J. Argel, A. Valerio, R. Martinez]**

6. Investigation of optimum conditions for seed production and storage

6.1 Conditions of storage

A study was conducted to investigate the viability of four seed lots produced in different regions of Colombia under three different storage conditions-ambient (25-30^{o}C and 60-75% RH), control A (10-15^{o}C and 50-55%RH) and control B (-20^{o}Cand <10%RH).

Germination tests at 2,4,8,10 and 14 months post harvest showed there was a dormancy factor that caused abnormal seedlings and this did not disappear completely until 6 to 8 months post harvest.

Seed stored at ambient conditions at Cali began to loose viabiltiy at 10 months post harvest but there was no decline in germination under the two controlled conditions at 14 months when the study was terminated.

Natural drying reduced seed moisture content to 6.5% moisture.

There were differences in the physiological quality between seed lots produced in different production systems which were not distinguished by the tetrazolium test.

This study suggests that the dormancy factor needs to be investigated further. **[C.I. Cardozo, Univ. Nacional, Palmira].**

There are few studies on the effect of environmental conditions on maintenance of seed quality in *A. pintoi*. However, some observations are worth recording. Seed has been stored at Cochabamba in Bolivia by SEFO-SAM for five years with only a 10% loss in viability. On the other hand high quality seed stored for two months at Caquetá in the humid environment of the Amazonia lost complete viability after 2 months.

Field observations on post-handling of seed are also relevant. When seed is dried immediately there is little problem with germination, subsequently. However, temporary storage in bags prior to drying gives rise to problems with fungal attack in germination tests.

Thus there is a need to investigate the effect of fungal infestation on subsequent seed viability.

Table 7. Monthly observations of regrowth from stolons and emergemce from seed following repeated herbicides applications on *Arachis pintoi* accessions in Guápiles, Costa Rica.

CIAT Accession N°	Herbicide	Months following treatment																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	TOTAL
		Regrowth/m^2																		
17434	iv	17	13	1	0	0	0													31
	v	14	33	3	1	1	0													52
18744	1	7	11	0	0	0	0													18
	2	0	19	21	2	4	0													46
18748	1	45	21	3	0	0	0													69
	2	0	15	53	61	40	6													175
		Seedlings/m^2																		
17434	1	0	15	8	11	4	9	9	7	11	21	18	9	2	1	0	1	1	0	127
	2	0	36	6	11	2	11	9	4	5	16	11	11	24	1	0	0	0	1	148
18744	1	0	0	0	0	0	1	0	0	1	0	1	2	2	0	0	1	1	1	10
	2	0	0	0	2	1	1	0	0	1	1	1	2	4	0	0	0	0	0	13
18748	1	0	0	0	0	1	1	0	0	1	1	1	2	1	0	0	1	1	1	11
	2	0	0	0	0	1	0	2	1	1	3	2	3	6	1	0	1	0	0	21

Treatments:
1. Picloram + 2,4 D Amine + atrazine once, plus picloram + 2,4 D amine at monthly intervals
2. Metsulfuron metil + atrazine once, plus metsulfuron metil at monthly intervals.

6.2 Defoliation

The effect on seed yield of A. pintoi CIAT 22160 of frequency of defoliation (3, 6, 9 months after planting) vs no defoliation for 12 months was studied. Defoliation at 3 and 6 months after planting reduced pure seed yield to 130 kg/ha from 860 kg/ha with nil defoliation. **[E A. Pizarro and M.A. Carvalho]**

6.3 Effect of storage conditions and seed origin on seed quality of two ecotypes of *Arachis pintoi.*

Two seed lots of *A. pintoi* CIAT 18744 and 17434 harvested in January 1995 either in Guápiles (humid tropics) or Atenas (subhumid tropics) are held at two storage conditions at the latter site. Germination tests are carried out every two months of seed with or without pods and stored either in controlled (18 °C, 55 % RH) or uncontrolled room conditions (mean of 27 °C, 80 to 95% RH). Normal and abnormal seedlings are counted at 7, 14 and 21 days. With the final count rotten and not-rotten ungerminated seeds are also tabulated.

Preliminary results on germination percentages indicate a strong interaction between harvest site and the variables of seed class, storage conditions, seed age and accessions (Table 8).

Table 8. Germination percentages of two *A. pintoi* ecotypes harvested in Guápiles and Atenas and stored under controlled and uncontrolled conditions.

Variables		Atenas	Guápiles	Difference between sites
			%	
Type of seed	With pod	47 a[1]	23 b	*[2]
	Without pod	45 a	24 b	*
Storage conditions	Uncontrolled	56 c	36 e	*
	Controlled	35 d	11 f	*
Seed age	2 months	46 g	20 h	*
	4 months	45 g	27 h	*
CIAT accession	17434	51 i	28 k	*
	18744	41 j	19 l	*
Mean germination		46	24	**

[1] For each site, variable means followed by the same letter are not sig.different ($P<0.001$)
[2] $P<0.05$

Seed harvested at the humid site of Guápiles has given consistently lower germination percentages up to date, an indication of a stronger seed dormancy.

Within each site, there has been higher germination of seeds stored under uncontrolled conditions than those kept in the cool room, and of CIAT 17434 than CIAT 18744. There has not been any difference in germination of seed germinated with or without the pod, or of seed stored for 2 or 4 months. **[P.J. Argel, A. Valerio and G. Perez]**

7. Genetic diversity and conservation of wild *Arachis* species

There was no activity in 1994-1995. It is proposed to undertake a study of genetic diversity, geographical distribution and implications for conservation, once additional funding is obtained for the project 'Preservation of wild *Arachis* species'.

8. Reproductive behavior and species compatibility

There was no activity in 1994-1995.

ANNEX. List of *Arachis* accessions originated from Brazil, held at CIAT, organized by BRA-accession number.

A. pintoi	BRA-012122	= CIAT 18744
A. pintoi	BRA-013251	= CIAT 17434
A. pintoi	BRA-014931	= CIAT 18745
A. pintoi	BRA-014940	= CIAT 18746
A. pintoi	BRA-015083	= CIAT 18747
A. pintoi	BRA-015121	= CIAT 18748
A. pintoi	BRA-015253	= CIAT 18749
A. pintoi	BRA-015598	= CIAT 18750
A. pintoi	BRA-016357	= CIAT 18751
A. pintoi	BRA-016683	= CIAT 18752
A. pintoi	BRA-020401	= CIAT 20826
A. pintoi	BRA-030252	= CIAT 22148
A. pintoi	BRA-030261	= CIAT 22149
A. pintoi	BRA-030325	= CIAT 22150
A. pintoi	BRA-030333	= CIAT 22292
A. pintoi	BRA-030368	= CIAT 22172
A. pintoi	BRA-030384	= CIAT 22151
A. pintoi	BRA-030392	= CIAT 22173
A. pintoi	BRA-030465	= CIAT 22174
A. pintoi	BRA-030481	= CIAT 22152
A. pintoi	BRA-030490	= CIAT 22175
A. pintoi	BRA-030511	= CIAT 22153
A. pintoi	BRA-030520	= CIAT 22154
A. pintoi	BRA-030546	= CIAT 22256
A. pintoi	BRA-030601	= CIAT 22155
A. pintoi	BRA-030619	= CIAT 22156
A. pintoi	BRA-030872	= CIAT 22257
A. pintoi	BRA-030899	= CIAT 22231
A. pintoi	BRA-030929	= CIAT 22258
A. pintoi	BRA-030945	= CIAT 22176
A. pintoi	BRA-030988	= CIAT 22157
A. pintoi	BRA-031003	= CIAT 22158
A. pintoi	BRA-031135	= CIAT 22159
A. pintoi	BRA-031143	= CIAT 22160
A. pintoi	BRA-031194	= CIAT 22259
A. pintoi	BRA-031208	= CIAT 22232
A. pintoi	BRA-031216	= CIAT 22260
A. pintoi	BRA-031224	= CIAT 22233
A. pintoi	BRA-031267	= CIAT 22261
A. pintoi	BRA-031275	= CIAT 22239
A. pintoi	BRA-031283	= CIAT 22262
A. pintoi	BRA-031291	= CIAT 22294
A. pintoi	BRA-031305	= CIAT 22235
A. pintoi	BRA-031313	= CIAT 22263
A. pintoi	BRA-031321	= CIAT 22264
A. pintoi	BRA-031330	= CIAT 22265
A. pintoi	BRA-031348	= CIAT 22295
A. pintoi	BRA-031356	= CIAT 22289
A. pintoi	BRA-031364	= CIAT 22237
A. pintoi	BRA-031381	= CIAT 22240
A. pintoi	BRA-031399	= CIAT 22234
A. pintoi	BRA-031445	= CIAT 22266
A. pintoi	BRA-031453	= CIAT 22267
A. pintoi	BRA-031461	= CIAT 22268
A. pintoi	BRA-031496	= CIAT 22236
A. pintoi	BRA-031500	= CIAT 22241
A. pintoi	BRA-031526	= CIAT 22290
A. pintoi	BRA-031534	= CIAT 22238
A. pintoi	BRA-031542	= CIAT 22269
A. pintoi	BRA-031551	= CIAT 22270
A. pintoi	BRA-031798	= CIAT 22271
A. repens	BRA-029190	= CIAT 22161
A. repens	BRA-029220	= CIAT 22162
A. repens	BRA-029211	= CIAT 22293
A. repens	BRA-030082	= CIAT 22163
A. repens	BRA-030449	= CIAT 22164

Project: Enhanced genepools of forage *Arachis*

Proposed activities for 1996

1. Acquisition of Arachis germplasm

Collect *Arachis* in Paraguay.

Continue the introduction and phytosanitary supervision of new germplasm to CIAT.

The VRU will index all germplasm accessions by serology, electron microscopy and gel electrophoresis of viral nucleic acids.

Different viruses encountered in *Arachis* are being characterized to develop reliable diagnostic techniques to facilitate the international exchange of germplasm.

2. Characterization of Arachis germplasm

Continue morphological and biochemical characterization.

Exchange data on characterization with CENARGEN and ICRISAT

3. Evaluation for environmental adaptation

Continue assessment of genotype-environment interaction of *A. pintoi*, including the new germplasm accessions obtained by collection

Evaluate 5 new accessions of *A. pintoi* in Atenas in association with a grass under grazing management.

4. Disease and insect tolerance

A program to evaluate disease resistance among all Arachis accessions will be commenced after suitable inoculation protocols have been determined. Focus will be on the two diseases, anthracnose and Cercospora leaf spot.

Contacts will be developed with ICRISAT pathologists.

5. Management conditions for rapid establishment and high yield

Conduct establishment experiments in association with on-farm evaluation of *Arachis*.

6. Investigation of optimum conditions for seed production and storage

Continue with seed quality monitoring experiment.

Conduct experiment to monitor the effect of pathogens on seed quality over time.

Establish an experiment to explain difference in seed production between plantings made with vegetative material or seed

7. Genetic diversity and conservation of wild *Arachis* species

Commence the accurate location of accessions and herbarium specimens for entry to a GIS data base.

8. Reproductive behavior and species compatibility

Design experiments that can be carried out with the assistance of pre-graduate students from the local universities.

Project: Development of Persistent *Stylosanthes* Cultivars

Project Coordinator: Segenet Kelemu

Rationale

Species of *Stylosanthes* are diverse tropical and subtropical forage legumes naturally distributed in Central and South America. These species are among the most important forage legumes in Australia, South and Central America, Africa, Asia, and parts of the southern United States. *Stylosanthes* species are being used for pasture legumes, green manure, feed meal production and fallow improvement.

The main limitations to the extensive use of *Stylosanthes* as a tropical forage legume are the disease anthracnose and poor persistence under grazing. Traits such as high seed yield and seedling vigor are important components of a cultivar which could contribute to persistence in *Stylosanthes* pasture systems.

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., is the most important and widespread disease of *Stylosanthes*. Complete loss of plants due to anthracnose has been reported in Colombia. The pathogen is a heterogeneous species consisting of various host specific populations. It exhibits considerable range of variation in both morphology and pathogenicity.

The center of origin of the genus *Stylosanthes*, and thus the presumed center of genetic diversity of its pathogen, is in South America. However, very little is known about the physiologic race composition of the South American pathogen population, primarily because appropriate differential cultivars and/or accessions were lacking. In *S. guianensis/C. gloeosporioides* interactions, pathogen variability studies are complicated by the hosts's heterogeneity and its relatively recent domestication. Knowledge of the race composition of *C. gloeosporioides* and the geographic distribution of the various races will help in the development of effective breeding programs for anthracnose resistance and of gene deployment strategies for managing this resistance.

Objectives

- To increase and characterize the genetic diversity in species of *Stylosanthes*;
- To select and develop natural and improved hybrid *Stylosanthes* germplasm with high seed yield, seedling vigor and anthracnose resistance;
- To determine the variability in virulence pattern of South American isolates of *C. gloeosporioides* and thereby designate a set of *S. guianensis* genotypes that differentiate the pathogen's physiologic races; (iv) to determine the genetic diversity of *C. gloeosporioides* infecting *Stylosanthes* spp.

Main Activities

1. Characterization of *Stylosanthes* species.
2. Evaluation of natural *Stylosanthes* genotypes.
3. New *Stylosanthes* gene pools
4. Studies on seed yield and plant persistence.
5. Characterization of *C. gloeosporioides*
   - 5.1. Identification of *Stylosanthes* genotypes as differentials.
   - 5.2. Physiologic race identification of isolates of *C. gloeosporioides*.
   - 5.3. Characterization of *C. gloeosporioides* isolates based on molecular markers.
   - 5.4. Biochemical analysis of *C. gloeosporioides* isolates.
6. Epidemiological studies.
7. Plant transformation systems and resistance.
8. Identification of new diseases.

Highlights (1994-95)

- Detected wide diversity with distinct geographic groups in *Stylosanthes* germplasm.
- Identified high seed yielding genotypes of *Stylosanthes*.
- Identified promising vigorous and anthracnose-resistant *Stylosanthes* genotypes.
- Identified 17 differential *S. guianensis* genotypes based on virulence patterns of 45 *C. gloeosporioides* isolates.
- Determined 52 pathotypes in South American isolates of *C. gloeosporioides*.

- Found that isolates of *C. gloeosporioides* from South America are more complex and diverse than those from Australia based on pathogenicity tests and RAPD analysis.

- Showed that the Australian differentials composed of 4 *S. guianensis* cultivars are not sufficient to differentiate South American isolates of the pathogen.

- Found "unique" pathotypes among South American isolates which can cross-infect *Stylosanthes* spp.

- Showed that isolates from *Arachis pintoi* could cause anthracnose symptoms in *Stylosanthes* spp.

- Showed that isolates which cause anthracnose symptoms in *Stylosanthes* spp. produce cell-wall degrading enzymes which seem to be associated with virulence.

1. Biochemical and morphological characterization of *Stylosanthes* species

Morphological and biochemical characterizations are being conducted for the large germplasm collections of *S. capitata* and *S. guianensis* (approx. 300 and 1400 accessions, respectively) in collaboration with the CIAT Genetic Resources Unit and undergraduate students from Universidad del Valle, Cali, and Universidad Nacional de Colombia, Palmira.

Stylosanthes capitata. CIAT assembled a large *ex situ* germplasm collection of *S. capitata*, part of which was agronomically evaluated in the past. Based on electrophoretic data of four isozymes (in descending order of polymorphism, α-EST, ß-EST, ACP, DIA) and native seed proteins, a high degree of intraspecific polymorphism was revealed. The most polymorphic enzymes were α- and ß-esterases (EST) and αß-acid phosphatase (ACP), however, seed proteins were even more polymorphic. Banding patterns of ACP and seed proteins showed some geographic relationship.

The marker systems investigated were useful for fingerprinting the *S. capitata* accessions in the germplasm collection maintained at CIAT. The diversity in this collection is remarkable, and only two accessions, that originated from adjacent collection sites in the Brazilian state of Minas Gerais, were suspected to be duplicates. Concerning the number of unique patterns as well as the total number of patterns resolved, regions of highest variation were found in northeastern and northern Brazil, while southeastern Brazil seems to be the least diverse region for *S. capitata* germplasm, independent of the number of accessions analized.

As a relatively large diversity was detected in the small group of germplasm from Venezuela and no evidence of any duplicate was found, future collection may especially be emphasized in this country, bearing in mind the recent observations by Fernandes *et al.* (1993) and Grof *et al.* (1993) that *S. capitata* germplasm originated from Venezuela was very little affected by anthracnose disease in the evaluations carried out at Campo Grande in the Brazilian state of Mato Grosso do Sul.

Morphological characterization of *S. capitata* show large variability among accessions, with some relationship to the origin of germplasm. For example, accessions that originated from Brazil or Venezuela were morphologically different. The data generated still need to be analyzed **[B. L. Maass, S. I. Marulanda, A. Ortiz, I. C. Vásquez]**.

Stylosanthes guianensis. To date 561 accessions of *Stylosanthes guianensis* L. collection, held at CIAT, have been characterized using biochemical genetic markers. These markers involved native seed proteins and the isozymes α-EST, ß-EST, αß-ACP, and DIA. All tests were conducted in polyacrylamide gel electrophoresis (PAGE) and isozyme markers were selected based on their polymorphism and quality of banding patterns.

The 561 accessions cover the geographical distribution range of *S. guianensis*, and represent more than one-third of the 1400 accessions held at CIAT's gene bank.

The polymorphism shown by these biochemical markers is greater for the isozyme α-EST, followed by ß-EST, and, to a lesser extent, by ACP and DIA. Seed proteins presented intermediate polymorphism, not as high as the esterases but not as low as that of ACP and DIA. Of these five markers, only the polymorphism in the isozyme ACP and that in native seed proteins were associated with geographical distribution.

Two distinct bands of native seed proteins (designated bands 13 and 15) were present in the germplasm that originated in Brazil, Argentina, and Peru, but absent in the germplasm that originated in Mexico, Central America, Colombia, Venezuela, and Bolivia, and in some accessions of Guyana. On the other hand, band 14 was absent in the germplasm from Brazil, Argentina, and Peru, and present in the germplasm from Mexico, Central America, Colombia, Venezuela, Bolivia, and in some accessions from Guyana. These are two distinct groupings.

The data definitely show an association of banding patterns with geographical origin of the germplasm. But further analysis is required for interpretation of significance in relation to the nature of the diversity and if there is significance for evaluation of the main disease, antrhacnose **[C.H. Ocampo, A. Ortiz, B.L. Maass]**.

2. Evaluation of natural *Stylosanthes* accessions.

Continuous identification of new sources of resistance in accessions of *Stylosanthes* is an integral part of an improvement program. Routine screening for general adaptation with emphasis on anthracnose resistance was carried out for those accessions of the germplasm collection of *Stylosanthes guianensis*, which were not evaluated at Carimagua in the past.

Two sets were planted in 1993 (100 acc.) and 1994 (135 acc.) at the sandy loam site Yopare, Carimagua, Colombia. The accessions were evaluated for anthracnose resistance under natural infections. Initially, anthracnose incidence was light in Yopare. In addition, the 1994-set suffered heavy infestation of a stemborer (probably a species of *Caloptilia*). After 18 months of evaluation, a large number of *S. guianensis* var. *pauciflora* accessions had high survival rates (Figure 1).

Nine accessions, CIAT 11415, 11645, 11671, 10929, 11413, 11646, 11682, 2996 and 10287, have been selected that were at least as vigorous as the improved lines CIAT 11844 and CIAT 11833. All selected accessions belong to var. *pauciflora*. These accessions along with improved lines will be screened in large plots under high anthracnose pressure in Carimagua **[B.L. Maass, E.A. Cárdenas, C. Plazas]**.

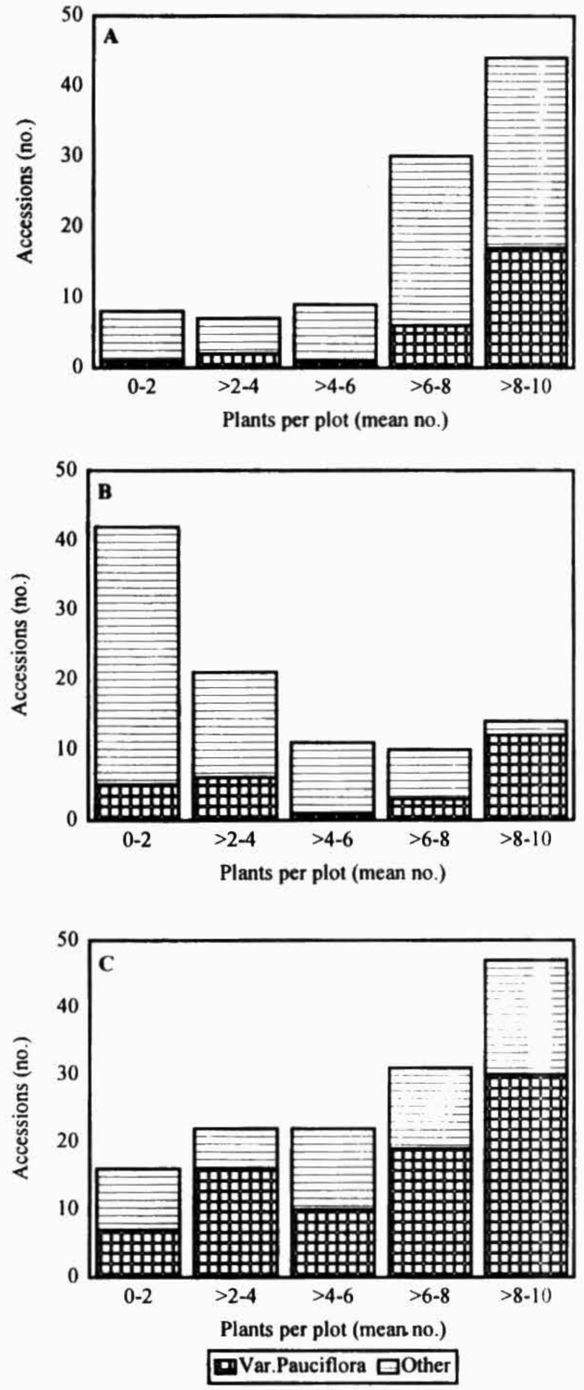


Figure 1. Mortality caused mainly by anthracnose of *Stylosanthes guianensis* (number of accessions) at Carimagua; (A) and (C) 15 months, (B) 19 after establishment of sets established (A) in 1994, and (B) and (C) in 1993.

3. New *Stylosanthes* Gene Pools

3.1 Selection

Bulk advance populations were advanced an additional generation during 1995.

3.2 Evaluation of breeding lines

A small-plot experiment was established during 1995 at Carimagua to assess the results of breeding work with *Stylosanthes*. The trial includes the 10 original parental accessions going into the hybridization program which began in 1981, as well as the products of this activity in the form of two pedigree-derived lines (now denoted CIAT 11833 and CIAT 11844), and six bulk advance populations. We expect to assess forage and seed production attributes on these materials, as well as disease reactions. Evidence of the genetic changes owing to 12 generations of bulk mass selection may be assessed using appropriate biochemical and/or molecular markers **[J. W. Miles and S. Kelemu]**.

3.3 On farm evaluation of breeding lines

Materials from the *Stylosanthes* breeding program are now receiving wider testing and are showing good performance, for example, in cropping systems work being conducted on farm in the Puerto López (Colombian Llanos) region (J. I. Sanz, personal communication; also this report (Project: Forage Components for Production Systems).

3.4 Advanced populations of *S. guianensis*

Early flowering and anthracnose resistant *S. guianensis* lines were derived from CIAT bulk advance populations in the Philippines and Brazil. In order to create heterogeneous populations, seeds of selections of similar phenology and morphological characteristics were physically mixed. Six of these populations designated GC 1575, GC 1576, GC 1577, GC 1578, GC 1579 and GC 1580 are currently being assembled together with 10 other highly productive anthracnose-resistant hybrids (CIAT 11833, CIAT 11844, bulk advance populations # 1 - 6) and two standard checks (CIAT 184, CIAT 2312) for extensive regional trials **[B. Grof, J. W. Miles and C. Fernandes]**.

3.5 Advanced population of *S. capitata* and *S. macrocephala*

A population (designated GC 1580 multiline 5) was developed from a mixture of anthracnose-resistant accessions of *S. capitata* and *S. macrocephala*. Natural selection and out-crossing among these genotypes played a major role in the development of this multiline. Both species are adapted to low fertility soils. This population will be included in the regional trials **[B. Grof and C. Fernandes, EMBRAPA/CNPGC]**.

4. Studies on seed yield and plant persistence

In the absence of disease and insect attack and with adequate nutrition, *Stylosanthes* plants still have a finite life. The half-life is likely to vary from 1 to 3 years depending on species and management. Hence, in addition to disease and insect resistance, long-term persistence of stylos in grass-legume associations will depend on the ability of the legume to set sufficient seed under grazing followed by successful seedling establishment from this seed in competition with the associated grass.

4.1 Seed yield of advanced lines

Increased seed yield has been a major aim in improvement of *S. guianensis* in addition to anthracnose resistance. This is more important in the late flowering *S. guianensis* var pauciflora than the earlier flowering *S. guianensis* var vulgaris type because the former produces less seed than the latter. However, the pauciflora types generally have higher anthracnose resistance than the vulgaris types. Selection pressure for increased seed yields has resulted in relatively high seed yields under zero defoliation (Table 1).

It still remains to be seen if seed yield under grazing is high enough to produce sufficient soil seed reserves for replenishment of the original plant stand. **[J.W. Miles]**.

An experiment was established on a dark red Latosol at Campo Grande, Brazil to determine the seed production potential of selected *S. guianensis* hybrids. Potted seedlings of 20 hybrids selected for early flowering and

Table 1. Seed yield of *Stylosanthes guianensis* genotypes in row-plot trials.

Entry	Type	Yield (s.e.)
		(g/3 m)
FM-103 (CIAT 136)	*vulgaris*	0.2 (0.1)
FM-104 (CIAT 184)	*vulgaris*	0.3 (0.1)
FM0186/06 (cv. Minerao?)	?	1.0 (0.2)
FM9205/04	*pauciflora*	9.4 (0.8)
MF-23 (CIAT 10136)	*pauciflora*	9.5
FM9205/05	*pauciflora*	13.6 (1.4)
FM9205/06	*pauciflora*	18.6 (2.3)
Line 41 (CIAT 11833)	*pauciflora*	19.4 (2.1)
FM-20 (CIAT 2031)	*pauciflora*	22.9 (3.2)
Line 44 (CIAT 11844)	*pauciflora*	23.6 (3.2)
FM9205/03	*vulgaris*	35.0 (6.0)
FM9205/02	*vulgaris*	38.0 (4.2)

two control genotypes, cv. Mineirao (released in 1993), GC 348 (a locally collected accession), were transplanted in the field. The range of clean seed yield was from 9.95 kg/ha to 199.69 kg/ha. *S. guianensis* GC 1518, GC 1517 and GC 1561 were the top seed yielders. Cultivar Mineirao and Gc 348 yielded 10.42 kg/ha and 19.00 kg/ha seed-in-pod **[B. Grof and C. Fernandes (EMBRAPA/CNPGC)]**.

4.2 Survival of seedlings in grass associations.

Seedling survival in grass-legume associations will be influenced by the competition of the associated grass for nutrients and moisture and to a more limited extent for light. The stoloniferous grasses, *Brachiaria dictyonuera* and *B. decumbens*, are generally regarded as more competitive than tussock grasses, such as *Andropogon gayanus* and *P. maximum*.

Survival was determined by oversowing legume seed into established grass pastures and observing survival of established seedlings with time (in the absence of having established stands of the grass-stylo pastures and hence soil seed reserves of the legumes). To evaluate the effectiveness of the procedure, two of the advanced *S. guianensis* var pauciflora lines, CIAT 11833 and CIAT 11844, were compared with *S. capitata* cv Capica in three different grass swards which were grazed continuously. The grasses were at different sites, both *Brachiaria* species being on a sandy loam soil and the *Andropogon* on a clay loam soil. Treatments included the addition or absence of P and K fertilizer.

Germination was quite variable between sites though the proportion of plants surviving was not effected to the same degree as was germination. Survival of CIAT 11844 was greater than that of CIAT 11833 suggesting that there may be a difference between lines in competitive ability (Table 2). There was not a consistent difference between grasses in their effect on legume survival.

This investigation will be continued over three years with a wider range of lines of *S. guianensis*. There is now good reason to evaluate seed production under grazing to determine whether availability of seed rather than seedling survival has the greater effect on long-term persistence **[P. C. Kerridge and J. C. Granobles]**.

Table 2. Seedling survival[1] of *Stylosanthes* lines associated with different grasses averaged over fertilizer

Species	Aug	Dec	Mar	Jun	Aug
			(plants/m^2)		
B. dictyoneura+					
CIAT 11833	143	45	19	13	6
CIAT 11844	148	93	105	97	68
cv. Capica	74	24	18	14	16
B. decumbens+					
CIAT 11833	7	3	1.3	0.7	0.9
CIAT 11844	19	9	6	6	5
cv. Capica	4	3	3	3	3
A. gayanus+					
CIAT 11833	65	27	6	6	5
CIAT 11844	67	25	24	20	17
cv. Capica	46	31	24	32	31

1. 200 seeds/m^2 were oversown in July 1994.

5. Characterization of *Colletotrichum gloeosporioides*

5.1 Identification of *Stylosanthes* genotypes as differentials

Very little information is available on the race composition and pathogenic diversity of South American *C. gloeosporioides* population. This is primarily because of lack of appropriate differential *Stylosanthes* cultivars and/or accessions. The reactions of 24 accessions and inbred lines of diverse morphology to isolates of the pathogen collected from various regions were used to further select host genotypes which showed differential capacity. Virulence patterns were used to select 17 *S. guianensis* genotypes as differentials. The identity (number and uniqueness) of anthracnose resistance-conferring gene(s) in each host genotype, however, is still unknown.

5.1.1 Development of *S. guianensis* inbred lines. The *S. guianensis* inbred lines were developed by single-seed descent over several generations, the number of which differed according to the genotype. That is, early flowering genotypes had more generations than late flowering ones, but none was less than five generations inbred, i.e., all were essentially 100% homozygous. Each single-seed descent line originated from a single, arbitrarily selected plant grown from seed of accessions held at CIAT. While the heritage of each plant can be traced, the genetic identity for each inbred line is not necessarily representative of the original germplasm accession **[J. W. Miles]**.

5.1.2 Isolates of *C. gloeosporioides*. A total of 45 monoconidial isolates of *C. gloeosporioides* from natural infections on various accessions and advanced lines of *S. guianensis* were selected. Thirty isolates were from Caquetá (the Colombian Amazon); 11 from Carimagua (Colombian savannas); 1 from Quilichao (Colombia); 1 from Paragominas (Brazil); and 2 from Pucallpa (Peruvian Amazon). These 45 isolates were grouped into 32 pathotypes using the 17 host genotypes as differentials. Some older isolates from the culture collection, notably CIAT 13393, 11372, 14101, 10643, 12622, and 13366, were less virulent and may have lost virulence during culturing and storage. The lower virulence, however, may also represent a shift toward increased virulence in the 10-year span.

Isolates CIAT 16093, 16094, 16135, 16162, 16176, 16133, 16140, 16134, and 13373 infected the supposedly resistant and non-host species *S. scabra* cv. Fitzroy. These isolates may represent a unique biotype. Except for isolate 13373, this group was collected from Caquetá, in the Colombian Amazon. The significance of a potentially new biotype remains to be investigated **[S. Kelemu, J. L. Badel, X. P. Bonilla]**.

In a separate experiment, fungal cultures isolated from *Arachis pintoi* plants with anthracnose symptoms were highly virulent on *Stylosanthes* in glasshouse tests **[S. Kelemu and M. S. Sanchez]**.

5.2 Physiologic race identification of isolates of C. gloeosporioides

Two sets of differential hosts were used to characterize pathogenic variability in 106 isolates from various regions of South America and Australia. Using the 17 *S. guianensis* genotypes including four Australian differentials, 72 of the Type B-like isolates (isolates specific to *S. guianensis*) tested fall into 52 pathotypes. However, using only the four Australian differentials, these isolates were grouped into 10 pathotypes.

Similarity dendrogram of these isolates based on virulence and avirulence on the 17 host genotypes revealed six virulence groups, using 80% similarity as the cut-off point (Figure 2). The isolates generally fall into two major groups by geographic origin: the savanna of Colombia and the Amazon basin of Colombia, with isolates from other origins dispersed within the two groups.

Eight of the 34 type A-like isolates (non-*S. guianensis*-specific isolates), CIAT #'s 12048, 11203, 10484, 10657, 14285, 13782, 16082 and 10738 were pathogenic to *S. guianensis* cv. Endeavour. These South American isolates may yet represent a fourth group of pathotypes which differ from type A-like and type B-like isolates. Cultivar Fitzroy, the supposedly "universal suscept" to Australian type A isolates, expressed resistance to isolates CIAT #'s 10471, 10484, 11203, 14285, 16084 and 16085. None of the differentials was resistant to all of the isolates tested. On the other hand, seven of the isolates, CIAT #'s 10351, 10480, 12048, 12414, 16086, 16088, and 16089, were pathogenic on all the differentials. It is important to note that all of these isolates, except isolate 12048, originated from Brazil, one of the centers of genetic diversity for species of *Stylosanthes*. Nineteen virulence patterns were identified among the 34 type A-like isolates on seven differentials **[S. Kelemu, J. W. Miles, J. L. Badel, C. X. Moreno and M. X. Rodriguez]**.

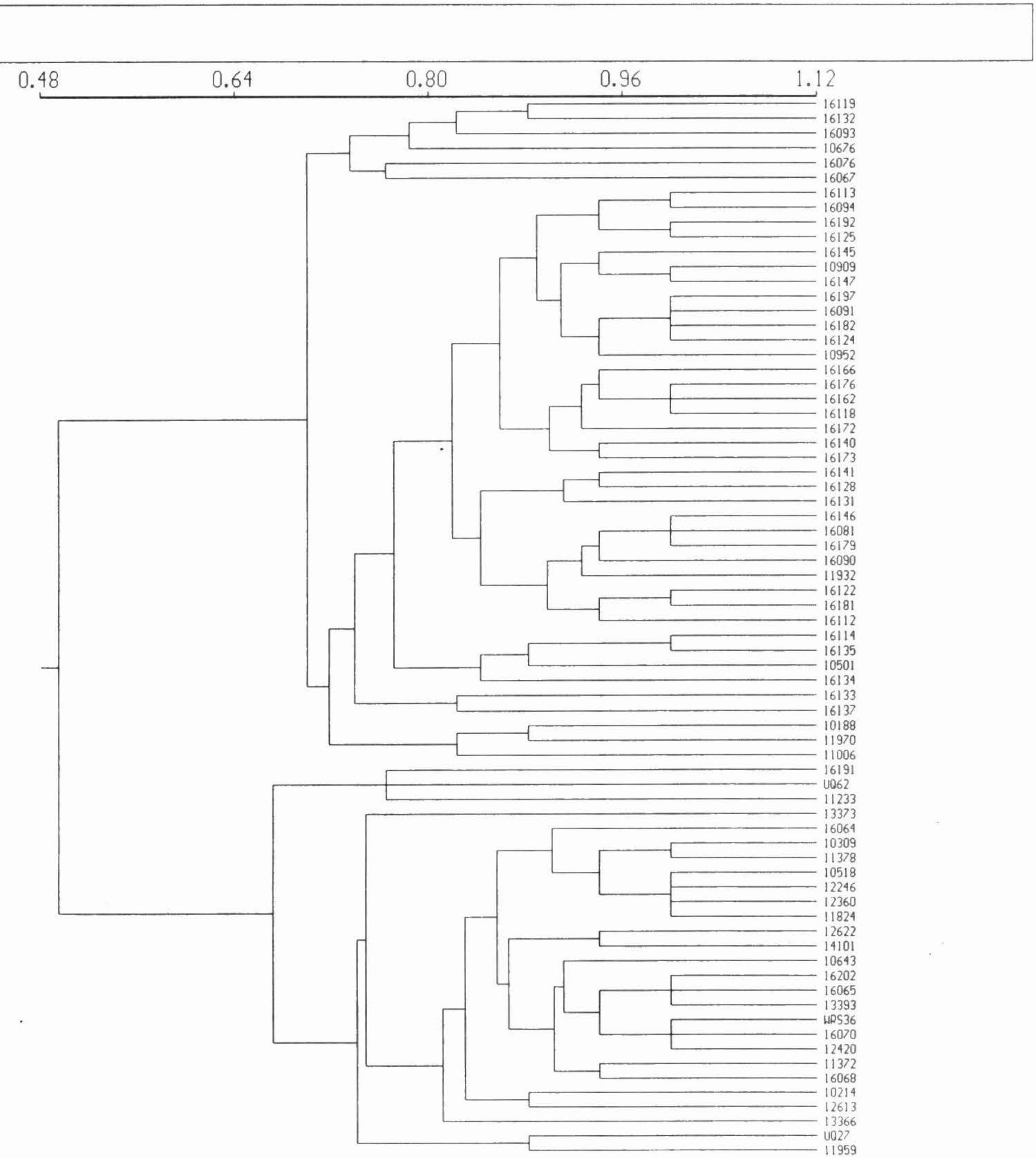


Figure 2. Similarity dendrogram of 77 monoconidial isolates of *Colletotrichum gloeosporioides* specific to *Stylosanthes guianensis* (Type B-like isolates) based on virulence and avirulence on 17 host genotypes using the unweighted pair group arithmetic mean (UPGMA) program of NTSYS-pc.

5.3 Characterization of *C. gloeosporioides* isolates based on molecular markers

The amount of genetic diversity of 138 South American and Australian isolates in two populations of *C. gloeosporioides* was measured at molecular level by polymerase chain reaction (PCR) amplification of DNA using 11 arbitrary primers of 10 bases (Kelemu *et al.* 1995). The amplifications revealed scorable polymorphism among the isolates, and a total of 125 band positions was scored (eg. Figure 3). No distinct correlations existed between genetic diversity as measured by random amplified polymorphic DNA (RAPD) and pathogen race as defined by pathogenicity pattern on the differentials. However, non-pathogenic or weakly pathogenic isolates isolated from *S. guianensis* were more genetically diverse than virulent isolates from the same host, and distinctly grouped together and separated from the more virulent isolates by RAPD analysis. Generally, isolates were clustered together by their geographic origin. *S. guianensis*-specific isolates collected from Carimagua, a long time *Stylosanthes* breeding and selection site, exhibited a wider range of genetic diversity than those from a newly opened trial site in the Amazon basin of Colombia (Florencia, Caquetá). In a separate experiment involving South American and Australian isolates, RAPD data analysis suggested that the genetic background of South American isolates of the pathogen are very divergent. However, some isolates from *S. guianensis* from Caquetá were similar to Australian biotype B strains [**S. Kelemu, C. X. Moreno, M. X. Rodriguez, J. L. Badel and Chaozu He (CSIRO)**].

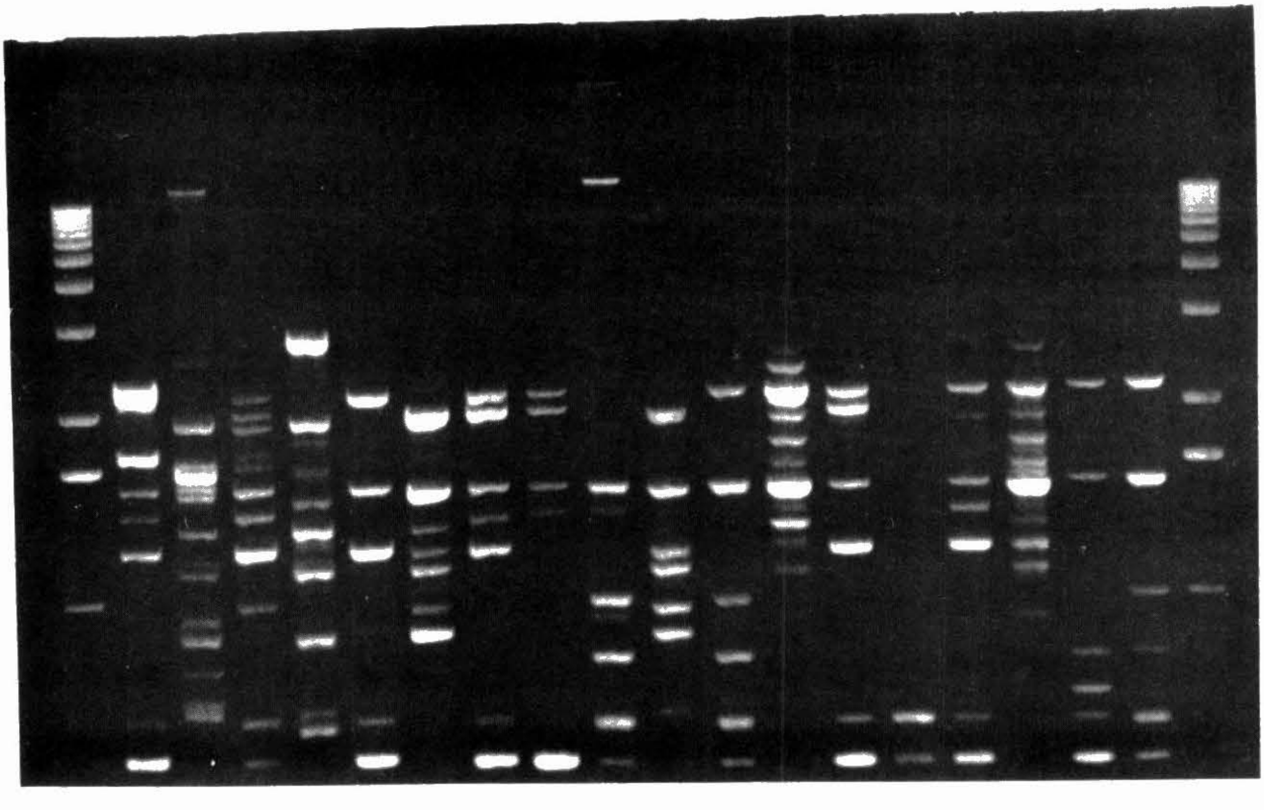


Figure 3. RAPD analysis of representative isolates of *Colletotrichum gloeosporioides* infecting species of *Stylosanthes*.

5.4 Biochemical analysis of *C. gloeosporioides* isolates

Plant pathogens produce many types of cell wall degrading enzymes, and in several plant diseases these enzymes play a major role in the infection process and in the development of symptoms. Various species of *Colletotrichum* have been shown to produce cell wall degrading enzymes in culture and/or during infection of plant tissues. However, there have been no reports of cell-wall degrading enzyme production by isolates of *C. gloeosporioides* infecting species of *Stylosanthes*. We report here the detection of pectic lyase activity in *C. gloeosporioides*-infected *Stylosanthes* tissues (Table 3). Activity-stained, ultra-thin-layer isoelectric focusing gels of these preparations revealed the presence of isozymes with pIs ranging from acidic to alkaline (Figure 4). The role of each of these isozymes in pathogensis remains to be investigated **[S. Kelemu and M. S. Sanchez]**.

Table 3. Pectic lyase activity in samples extracted from *Stylosanthes* tissues infected with isolates of *Colletotrichum gloeosporioides*[1].

Isolate	Original host	Pectate lyase activity
16331	S. capitata	0.71
16086	S. capitata	4.54
10035	S. capitata	2.85
10351	S. capitata	2.19
16088	S. capitata	1.68
16266	S. guianensis Mineirao	2.13
11378	S. guianensis	4.35
11233	S. guianensis	0.17
11372	S. guianensis	0.27
SR24	Stylosanthes spp.	2.02
16338	S. capitata	3.91
16273	S. capitata	2.53
UQ27	Stylosanthes spp.	0.58

[1] Pectate lyase activity is expressed in μmol product liberated per minute per mg totalprotein in the sample at 25 C.

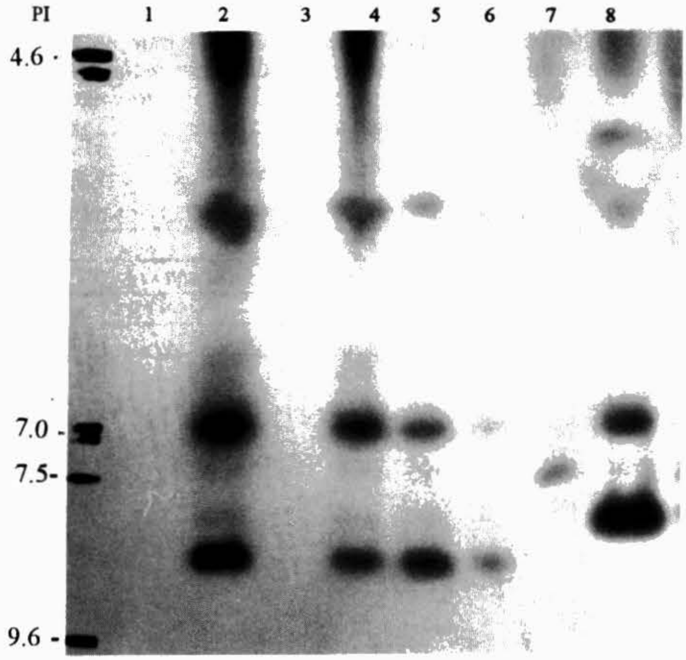


Figure 4. Activity-stained isoelectric focusing gel analysis of pectic lyase isozymes produced in plant by isolates of *Colletotrichum gloeosporioides*. Following resolution by ultra-thin-layer isoelectric focusing, the isozymes were visualized by overlaying a pectate-agarose gel which was then stained with cetyltrimethylammoniumbromide. Lanes: 1-8, negative control plant extract, isolate CIAT #'s 14412, 16088, CS89, negative control plant extract, isolate UQ397, WRS36, 16363, respectively.

6. Epidemiological studies

Thirty two selected genotypes of *S. guianensis* and *S. scabra* were planted in Carimagua and Caquetá, Colombia next to monitor sensor weather stations which measure various weather parameters. Monthly weather data (canopy temperature, leaf wetness, rainfall, air temperature) and anthracnose severity are recorded in order to understand disease progress relative to weather conditions. Table 4 shows mean anthracnose ratings of host genotypes at the two locations in Colombia for part of 1995. Similar studies are being conducted in Planaltina and Campo Grande, Brazil. *C. gloeosporioides* isolates collected from the host genotypes are characterized using differentials and at molecular level. These studies will be conducted for a total of 2-3 years **[S. Kelemu, J. Badel, C. Fernandes (EMBRAPA/CNPGC), M. Charchar (EMBRAPA/CPAC) and S. Chakraborty (CSIRO)]**.

Table 4. Mean anthracnose ratings of 32 stylosanthes genotypes planted in carimagua and caqueta, 1995.

HOST GENOTYPE	LOCATION													
	CARIMAGUA							CAQUETA						
	MONTH							MONTH						
	APR	MAY	JUN	JUL	AUG	SEP	OCT	MAR	APR	MAY	JUN	JUL	AUG	SEP
1. *S. guianensis*														
FM 2E	3.0	0.0		0.0	0.4	0.0	5.4	0.1	0.0	0.0	0.6	0.5	0.4	0.0
FM 4E	3.0			0.0	0.1	3.5	2.8	0.1	0.0	0.0	0.3	0.5	1.2	1.1
FM 42G	1.4	8.5		0.0	1.5	2.5	0.5	0.9	1.1	1.5	2.5	2.5	2.5	3.1
FM 6E	0.0			0.0	0.1	0.1	0.0	0.1	0.1	0.0	1.6	0.4	0.3	0.4
FM 7D	0.0	0.0	0.0	0.0	0.2	0.4	0.6	0.4	0.1	0.0	1.5	1.1	1.1	1.4
FM 8E	7.5	4.1	0.0	0.0	0.4	0.7	8.0	0.0	0.0	0.0	0.5	0.1	0.1	0.1
FM 9D	0.0			0.1	1.1		8.0	0.0	0.0	0.0	1.5	0.1	0.1	0.0
FM 10E	0.0	0.3	0.0	0.0	0.1	0.0	0.5	0.0	0.0	0.0	1.3	0.0	0.0	0.0
FM 12E	0.0	0.0	0.0	0.0	0.3	1.3	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.3
FM 13D	0.0	0.5	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1
FM 9205 /01	5.7	4.8	7.7	1.2	4.3	5.0	6.1	2.9	3.2	3.3	4.5	3.7	4.1	4.1
FM 9205 /02	4.3	7.4	8.5	2.0	2.0	5.1	3.3	2.4	2.9	2.8	3.9	3.1	3.2	3.7
FM 9205 /03	5.5	8.7	9.0	1.1	1.8	3.3	2.5	2.1	2.1	2.8	3.1	2.5	2.7	3.1
FM 9205 /05	0.0	0.0	0.2	0.4	0.6	1.5	0.1	0.4	0.0	0.0	0.7	0.6	0.7	1.0
FM 9205 /06	0.0	0.0	0.0	0.3	1.5	1.5	0.1	0.1	0.0	0.0	0.5	0.9	0.9	1.1
2. *S. scabra*														
45-I-1	0.4	0.2	0.9	5.8	6.1	4.8	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.1
11-V-1	1.0	0.7	2.8	0.3	1.1	5.9	0.1	0.9	0.3	0.0	0.1	1.0	0.9	0.8
2-I-4	0.1	0.2	2.0	3.4	4.3	3.9	0.0	0.1	0.1	0.0	0.6	0.1	0.0	0.1
80-V-10	0.8	0.7	3.4	4.3	4.2	7.0	0.1	0.2	0.2	0.0	0.1	0.0	0.1	0.0
86-II1-9	0.5			0.1	0.6	3.4	0.0	0.4	0.0	0.0	0.1	0.2	0.6	1.1
38-II-1	0.8	0.3	2.7	4.5	5.1	6.4	1.1	0.3	0.1	0.0	0.1	0.1	0.1	0.1
45-II-6	0.4	0.5	3.6	1.4	2.6	1.7	0.8	0.1	0.0	0.0	0.0	0.0	0.0	0.3
11-IV-1	0.1	0.0	1.6	4.8	7.3	9.0	0.0	0.3	0.1	0.0	0.0	0.2	0.2	0.3
86-I-8	0.3	0.0	0.9	0.8	1.2	0.5	0.0	0.1	0.0	0.0	0.0	0.1	0.3	0.3
42-III-6	2.6	2.6	4.2	3.6	3.9	4.1	0.1	0.2	0.1	0.0	0.1	0.0	0.0	0.0
110345	0.5	0.0	0.5	2.7	3.6		0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1
40205	0.0			0.0	1.1	6.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
55860	0.3	0.0	0.0	0.1	1.2	3.9	0.3	0.1	0.2	0.0	0.5	0.3	0.3	0.1
93116	0.3	0.7	2.5	6.0	7.0	9.0	0.6	0.0	0.0	0.0	0.7	0.1	0.1	0.0
36260	0.3	1.7	2.2	3.1	6.2	5.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Q10042	0.3	0.1	3.1	5.7	8.1		0.4	0.7	0.2	0.0	0.0	0.1	0.3	0.9
40292	0.3	0.1	1.3	3.7	2.9	3.8	0.8	0.1	0.1	0.0	1.2	1.2	1.3	0.9

Based on leaf rating scale 0, no disease incidence, 9 - high disease incidence.
Missing values represent completely dried plots.

7. Plant transformation systems and resistance

Protocols for agrobacterium-mediated transformation and plant regeneration have been established as reported earlier (see previous year annual report). Because of the ease of transformation and regeneration of *Stylosanthes*, this legume may be used as a model system to study basic biological processes in transgenic plants. Genetically engineered versions of most of the world's important food crops such as corn, rice, soybeans and cotton are available. Several applications for field tests of genetically engineered crops have been approved by the government of USA and governments of other countries. The number of cloned desirable genes is increasing steadily world wide. A project profile has been prepared in collaboration with BRU (CIAT) in order to attract funds for a special project of transferring some of the available cloned disease resistance genes from other plants to species of *Stylosanthes*.

8. Identification of new diseases

8.1 Die-back and wilt disease of *Stylosanthes* spp.

A disease showing die-back and wilt symptoms has been observed in *Stylosanthes* field plots in Brazil and Colombia for few years now. A severe case of the disease was evident at a seed multiplication lot of *S. guianensis* cv. Mineirao in Campo Grande, Brazil in 1995. The disease is also often observed in Carimagua, Colombia. We had reported earlier that we could reproduce the symptoms in the glasshouse by inoculating plants with a fungal pure culture. However, the correct identification of the fungus was not possible because it was not possible to obtain fungal spores in culture until recently.

A series of experiments with various culture media and growth conditions revealed that the fungus produced limited spores on V-8 juice agar, yeast extract agar, and oatmeal agar after 21 days of incubation at 28 C. Correct identification of the fungus is currently in progress. Symptoms were artificially reproducible both in adult and seedling plants (Figure 5) **[S. Kelemu, J. L. Badel and C. Fernandes (EMBRAPA/CNPGC)]**.

8.2 Botrytis head blight

Botrytis head blight caused by *Botrytis cinerea* was observed in *Stylosanthes* plots in Caquetá, Colombia. The wet conditions in Caquetá favored disease development characterized by severe blossom blighting and apical dieback. The disease has been detected on *S. guianensis* and *S. hamata* at San Jose del Nus, Colombia in 1979. Under environmental conditions favorable to the development of the disease, heavy yield losses of seed crops can occur in susceptible host genotypes **[S. Kelemu and J. L. Badel]**.

A

B

Figure 5. Die-back and wilt disease symptoms a) in adult, b) seedling, *Stylosanthes guianensis* plants in the glasshouse. Plants on the right inof each figure are healthy.

Project: Development of persistent *Stylosanthes* cultivars

Proposed activities for 1996

1 Characterization

- Finalize data analysis of *S. capitata* morphological characterization; supervise completion of undergraduate thesis.

- Combine results from morphological and biochemical characterization of *S. capitata* for overall analysis of diversity in this species; prepare technical paper.

- Complete data analysis of *S. guianensis* biochemical characterization data; prepare technical paper.

2 Evaluation

- Combine a set of most anthracnose tolerant accessions selected at Carimagua for agronomic trial.

- Assemble a set of accessions and bred lines of *S. guianensis* and *S. capitata* for multilocational evaluation.

3. New *Stylosanthes* gene pools:

- Further selection of advanced populations.

- Evaluations of advanced breeding lines.

- Further on farm evaluation of breeding lines.

- Regional testing of advanced and selected lines.

4. Studies on seed yield and plant persistence.

- Continue with the same experiment on survival.
- Investigate seed production under grazing.

5. Characterization of *C. gloeosporioides*

- Characterization of more isolates at molecular and biochemical level.

- Physiologic race identification of new isolates.

- Complete data analysis.

6. Epidemiological studies

- Continue with the studies and data collections.

- Data analysis.

7. Plant transformations and resistance

- Acquire appropriate cloned plant resistance genes.

- Transform a genotype of *S. guianensis*.

8. Identification of new diseases

- Continue with a look out for new and potentially trouble some diseases.

- Identify some sources of resistance to the die-back and wilt disease.

Project: Forages with High Nutritive Value

Project Coordinator: Carlos E. Lascano

Rationale

Forage plants have multiple uses (i.e. as a source of feed, to improve soil fertility and control erosion) in production systems in the tropics. However, the adoption of new forage species by farmers could be determined to a great extent by their beneficial effects on animal production (i.e. liveweight gain, milk production). As a consequence, new forage species need to be characterized in terms of feed quality.

Some herbaceous and woody leguminous species adapted to acid soils have high levels of condensed tannins, but their effect on animal nutrition has not been evaluated for many tropical species. To develop screening methods for the presence of tannins in tropical legumes and to design legume-based feeding systems, we need a better understanding of how tannins, and other polyphenols, affect intake, digestibility and nitrogen utilization by ruminants. There is a limited knowledge of the effect of environmental factors (i.e. soil, climate), on forage quality. New forage ecotypes should be tested in animal feeding trials or grazing experiments in order to determine their potential contribution to animal production.

Objective

To determine the feeding value of new grass and legume species selected for acid soils in the subhumid and humid tropics.

Main Activities

1. Screening procedures for the presence of tannins and other secondary compounds in legumes.

2. Influence of the environment on nutritive value of selected forage species.

3. Nutritive value and productivity of new forage ecotypes.

Highlights (1994-95)

- Found that different species of *Calliandra* (i.e. *C. calothyrsus, C. houstoniana, C. magdalenae*) have hydrolyzable tannins (i.e. gallic acid) in addition to condensed tannins.

- Found large differences among shrub legumes in distribution of condensed tannins within the leaf tissue. While in *Senna velutinum* and *Acacia boliviana* all CT present were extractable, in *Gliricidia sepium* all CT present were bound to protein and fiber.

- Demonstrated that in *D. ovalifolium* and *F. macrophylla* there are at least three types of CT: non-reactive with protein, and with low and high affinity to protein.

- Showed that in sheep fed contrasting legumes *D.ovalifolium* and *F. macrophylla*) the concentration of extractable CT had greater effect on N utilization than level of bound CT or tannin astringency.

- Showed that mixtures of shrub legumes with and without tannins fed to sheep as suppplements to low quality grasses increased flow and apparent absorption of N in the small intestine, but that N retention did not increase.

- Showed genotypic variation in digestibility among *Brachiaria* spp., and stability of this attribute across environments.

- Showed genotypic variation in leaf lignocellulose among species of *Brachiaria* grown with low supply of nutrients in a sandy loam oxisol.

- Showed that genotypic variation in leaf IVDMD among 18 genotypes of *Brachiaria* was greater than variation induced by changes in soil nitrogen supply to an individual genotype.

- Showed large increments in milk yield in pastures with *S. guianensis* (hybrid) in association with a grass.

- Showed that milk yield in nitrogen fertilized pastures was similar in *P. maximum* ecotypes selected for acid soils and *B. decumbens* cv. basilisk, and greater than in *B. dictyoneura* cv. Llanero.

1. Screening procedures for the presence of tannins and other secondary compounds

Research during the past two years has concentrated on: (1) screening shrub legumes in CIAT's collection for tannins; (2) studying distribution of condensed tannins (CT) in leaf tissue of different tropical legumes grown in Colombia and Australia; (3) defining interactions of method of legume forage preservation and level of PEG added to reduce CT in the forage; (4) studying the effect of level and type of CT in legumes on N utilization by sheep and (5) studying the effect of dilution of CT through legume mixtures on N utilization by sheep.

1.1 Screening woody legumes for tannins

Different genus and species of woody legumes planted at CIAT - Palmira and CIAT-Quilichao were screened for the presence of condensed and hydrolyzable tannins. Results (Table 1) indicate that *Senna spectabilis* (CIAT 20823) and *Cassia* sp. (CIAT 7975) had no tannins and this was associated with higher IVDMD as compared to other woody legumes. In contrast, all species of *Calliandra* analyzed had both hydrolyzable and condensed tannins. The significance of this finding in terms of ruminant nutrition is not well understood. Limited data suggest that tannins based on gallic acid (i.e. hydrolyzable) can be degraded by micro-organisms and that they interact with protein in a similar way as condensed tannins (i.e. tannins consisting of flavonols). However, end products of hydrolyzable tannin degradation by ruminal microbes could be potentially toxic to animals. [**N. Narváez, and C. Lascano**].

1.2 Distribution of condensed tannins in leaves of tropical legumes

The extractable and bound condensed tannins (CT) concentrations in leaves of tropical legumes grown in Quilichao, Colombia and Townsville, Northern Australia were measured (i.e. butanal - HCl method) at Massey University, New Zealand. With the exception of *Senna siamea* (CIAT 20698) all species contained CT. Most species contained 65-95% of total CT as extractable CT, with the exception of *Flemingia macrophylla* where 60% was extractable and 40% bound, and *Gliricidia sepium* where almost all the CT was bound to protein. In *Acacia boliviana* grown in Australia and in *Senna velutina* grown in Colombia, all CT were extractable.

The results of this study indicate that the use of analytical methods for measuring only extractable CT can be misleading when evaluating quality of tropical legumes because they overlook the effect of bound tannin [**F. Jackson (Massey Unv., N.Z.), T. Barry, C. Lascano and B. Palmer (CSIRO)**].

1.3 Effect of sample preparation method and PEG on concentration and astringency of condensed tannins

To implement an effective plant selection scheme for low CT we need to better define how different levels of CT affect ruminants. One approach is to reduce CT in test plants with polyethylene glycol (PEG), since CT bind to PEG in preference to protein. In addition, it is known that CT concentration in legumes can be affected

Table 1. Quality measurements on leaves of woody legumes from the Genetic Resources Unit of CIAT.

Legumes (CIAT No.)	N (%)	IVDMD	Hydrolyzable Tannis[1/] (%)	Condensed Tannins (%) Extractable	Bound
Calliandra calothyrsus (21252)	3.3	45.6	1.3	2.7	1.9
Calliandra houstoniana (20399)	2.5	28.1	0.4	4.0	1.0
Calliandra magdalenae (20401)	2.9	37.9	1.2	8.6	17.0
Calliandra sp. (21419)	2.6	22.2	0.4	9.4	13.1
Clitoria fairchildiana	2.5	29.5	-	3.1	4.2
Senna spectabilis (20823)	3.6	58.3	-	-	-
Cassia sp. (7975)	3.3	53.3	-	-	-
Mimosa colombiana (9287)	3.3	22.4	2.0	13.6	14.5
Mimosa sp. (21343)	2.1	20.6	0.8	18.4	7.3

[1/] Galic acid

by post-harvest treatment or sample preparation of the forage. The effect of PEG on concentration of CT in tropical legumes subject to different sample preparation methods has not been defined. Thus a study was conducted to determine the effects of PEG and sample preparation method on concentration and astringency (i.e. capacity of tannins to bind protein) of CT in two contrasting tropical legumes.

Leaves from *Desmodium ovalifolium* were subjected to 3 treatments: T1: fresh forage + PEG, samples frozen; T2: fresh forage + PEG; samples sun-dried and T3: sun-dried forage + PEG. Five concentrations (0, 19, 25, 35 and 45 g/kg of DM) of PEG (MW 8000) were included in each treatment. Sun-dried leaves of *Flemingia macrophylla* were also subjected to the same 5 concentrations of PEG used with *D. ovalifolium.*

Extractable and bound residual (fiber + PEG) CT were affected by PEG, but both sample preparation method and legume species x PEG interactions were observed. Extractable CT from sun-dried *D. ovalifolium* were more astringent than those from sun-dried *F. macrophylla*. However, astringency of CT from *D. ovalifolium* was lower in frozen samples as compared to sun-dried samples, regardless of PEG concentration. Results also showed that by increasing the concentration of PEG there was an exponential decline of extractable CT, but a point was reached (42 to 61 g/kg DM) where further increments of PEG resulted only in small changes in extractable CT (Figure 1A). This suggested the presence of extractable CT that were non-reactive with protein in the legumes tested. A quadratic relationship was observed between astringency of extractable CT and PEG concentration (Figure 1B), which suggested that extractable CT with different affinity for protein were also present in the legumes evaluated.

In general, results from this study indicate that PEG added to forage reduced extractable CT in fresh-frozen or sun-dried forage and that it made no difference whether PEG was added before or after sun-drying the forage. However, results did show that reduction of extractable CT with PEG was exponential and that there was a certain portion of extractable CT that did not react with protein. This implies that there is a need for quantifying the relationship between extractable CT and level of PEG in forage legumes prior to their use in feeding trials. Our results also suggest that legume species evaluated may have at least three types of extractable CT with different molecular weights and as

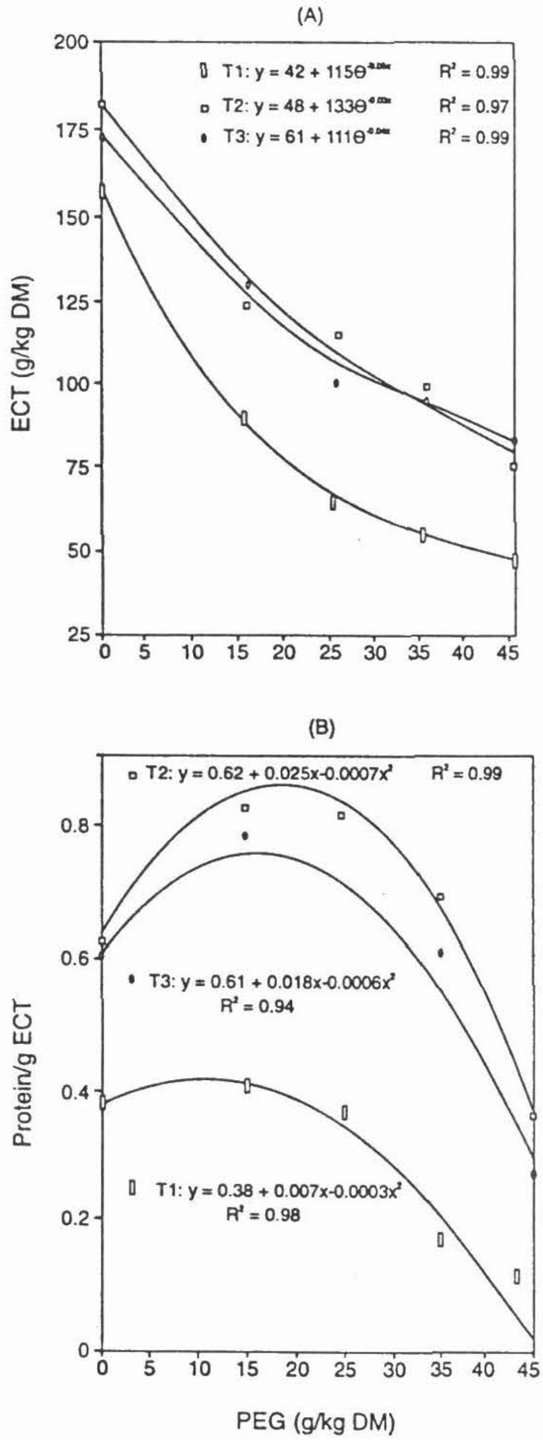


Figure 1. Effect of polythylene glycol (PEG) on level (A) and astringency (B) of extractable condensed tannins (ECT) in *D. ovalifolium* subject to different preservation treatments (T1 = Fresh + PEG, frozen; T2 = Fresh+PEG, sun-dried; T3 = sun-dried + PEG) (Barahona, R., MS Thesis).

a result different affinities for protein. It was also deduced from the results that method of forage preservation and sample processing could affect the activities of the CT present in a given legume species. This implies that in selecting leguminous species or ecotypes for low tannin, there is a need to assess not only tannin concentration in the forage, but also the structure of tannins present in the forage handled in the way it will be fed to animals. **[R. Barahona (Unv Kansas), C. Lascano, R. Cochran (Unv Kansas and J. Morrill (Univ Kansas)].**

1.4 Effect of concentration and astringency of tannins in legumes on intake and N utilization by sheep

Previous results had indicated that nutritional benefits could be realized by reducing the concentration of extractable CT in *D. ovalifolium* with PEG. Reduction of extractable CT in *D. ovalifolium* from 5 to 2% resulted in a 20% increase in fold intake and a 2.5 feed increase in N retention by lambs (Carulla, 1994). However, the extent to which these results could be generalized to other tropical legumes is unknown. Results from our laboratory had shown large differences among tropical legume species in affinity of extractable CT to protein (astringency) and in concentration and relative distribution of extractable and bound CT (Cano et al. 1994). Thus an experiment was carried out to study the influence of extractable and bound CT and tannin astringency on intake, digestion and N utilization by sheep fed two contrasting tropical legumes.

Sheep housed in metabolism crates were fed *D. ovalifolium* (D.o.) and *F. macrophylla* (F.m.) which had similar concentrations of extractable CT but different concentration of bound CT and tannin astringency (see Table 2). In addition, the legumes differed in crude protein (D.o. 17% and F.m. 23%) and indigestible cell wall content (D.o. 28% and F.m. 44%). Chopped sun-dried forage of each legume was sprayed with either water or PEG (3.5 g/kg of DM) to reduce extractable CT and fed (26 g/kg BW/day) to 8 sheep with rumen and duodenal fistulas. Animals received intraruminally a constant (4 g/kg BW/day) energy supplement (starch-extracted cassava meal).

Results summarized in Table 2, showed that intake of the two legumes was similar at similar levels of extractable CT in the forage, but was increased by 8% and 15% for *D. ovalifolium* and *F. macrophylla*, respectively when extractable CT was reduced from 9 to 5% (DM basis) in the forage by addition of PEG. However, intake of both legumes seemed to be limited not only by tannins but also by low leaf:stem ratio (1.0) in *D. ovalifolium* and high level of indigestible cell wall (44%) in *F. macrophylla*.

Digestibilities of OM and NDF were low but were higher with *D. ovalifolium* than with *F. macrophylla* However, digestibility of OM and NDF increased for both legumes due to reduction of extractable CT by addition of PEG. Greater N flow to duodenum, apparent N absorption and fecal N were observed with *F. macrophylla* than with *D. ovalifolium* and this was associated with greater N intake. Estimates of escape protein were similar for both legumes high in extractable CT, but were reduced when PEG was added to the forage. However, apparent N absorption in sheep fed the two legumes was not affected by reduction of extractable CT. (Table 2).

Table 2. Effect of level and degree of astringency of condensed tannins (CT) in tropical legumes on intake, digestibility and nitrogen (N) utilization by sheep (Barahona, R., MS Thesis).

Item	*D. ovalifolium*		*F. macrophylla*		SE
	Control	PEG	Control	PEG	
Extractable CT (%)	9.4a	5.4b	9.0a	4.7b	9.4
Bound CT (%)	10.0d	15.9c	25.9b	30.6a	0.4
Tannin astringency[1/]	0.6b	1.0a	0.3c	0.7	0.03
Intake (g DM/d)	591b	638a	555b	625a	17
OM digestibility (%)	51.4a	51.6a	45.3a	48.5b	0.9
NDF digestibility (%)	38.0b	44.6a	25.1d	34.2c	1.3
N intake (g/d)	13.9c	13.9c	21.5b	23.6a	0.5
Duodenal N flow (g/d)	14.9b	11.9c	19.9a	18.2a	0.9
N absorbed, % N duodenum	48.9	52.9	47.2	50.6	2.3
Escape N, % N intake	57.6a	39.5b	60.7a	48.2a	4.6

[1/] g protein (BSA) precipitated/g of ECT a,b,c,d means with different letters in each row are different (P<0.05)

In general, our results indicate that level of dietary protein escaping from the rumen appeared to be associated with extractable CT concentration, but was unrelated to concentration of bound CT or to tannin astringency as measured by a laboratory assay. Results from this study also showed that intake and digestibility of the two legumes tested were not only influenced by concentration of extractable CT but also by the cell wall content and composition. Thus when evaluating quality of tropical legumes with CT there is a need to examine their cell wall composition and degradability. [**R. Barahona, C. Lascano, R. Cochran and J. Morrill**].

1.5 Dilution of tannins through legume mixtures

A common feature in many woody tropical legumes is the presence of high concentrations of CT which are known to depress intake and body weight gains. However, low levels of CT are believed to be beneficial in ruminant diets since they reduce protein degradation in the rumen and increase escape protein. It follows, that mixtures of shrub legumes with and without tannin could have practical implications in smallholder feeding systems which involve supplementation of low quality grasses or agriculture by-products with cut and carry fodder from woody legume species.

To test this hypothesis we used *C. argentea* (tannin - free) and *F. macrophylla* (high tannin) alone or in mixture to supplement sheep fed a low quality grass as fed low quality *B. dictyoneura* (60% of the ration) a basal diet. In a first feeding experiment, sheep were supplemented with mixtures (80% C.a. + 20% F.m. or 60% Ca + 40% F.m.) of the two legumes (40% of the ration). Results showed that urinary N was reduced as *F. macrophylla* increased in the legume mixture, suggesting less rumen ammonia losses due to protein protection by tannins. However, this positive effect was offset by increased fecal N as the level of CT increased in the diet, and as a result N retention was not affected (Fässler and Lascano, 1995).

A second feeding experiment was carried-out to study in more detail N utilization by sheep fed a low quality grass (*B. dictyoneura*) supplemented with *C. argentea* and *F. macrophylla* alone or in mixture. Supplements of *C. argentea*, replaced with 0,25, 50 or 100% *F. macrophylla* were fed as 40% of the total ration in one meal. Measurements included intake, digestibility, rumen ammonia and N utilization.

Results showed a marked increase in rumen ammonia levels with decreasing levels of *F. macrophylla* in the legume mixture (Figure 2). In addition, duodenal N flow increased with increasing levels of *F. macrophylla* in the diet (Table 3). This was associated with a small but significant improvement in efficiency of N apparent absorption in the small intestine. However, N retained as a proportion of N intake was not affected by the different legume supplements.

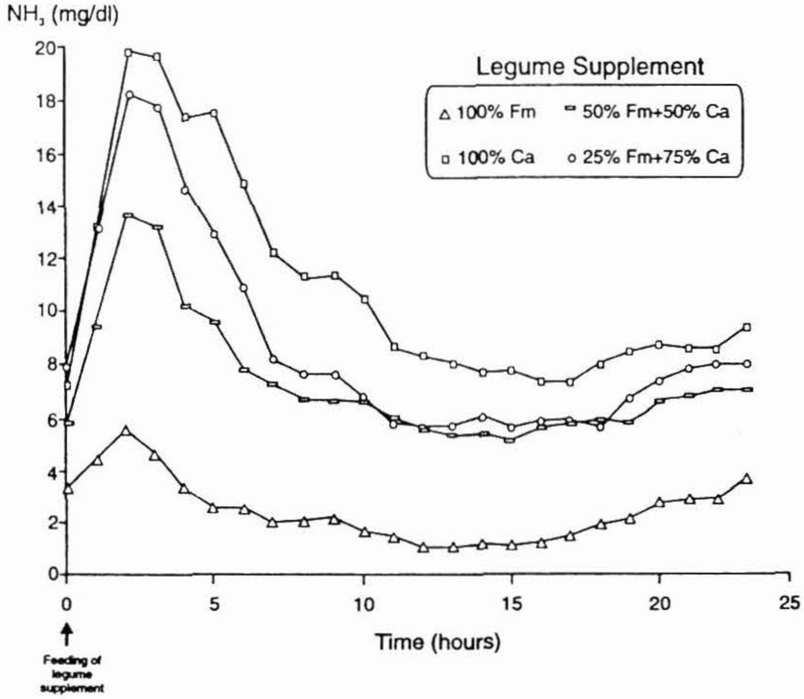


Figure 2. Changes across time in rumen ammonia concentration in sheep on a low quality grass (60% of ration) supplemented with two legumes (40% of ration) fed alone or in mixture (Powell et al., unpublished results).

Table 3. Effect of legume mixtures (*C. argentea* and *F. macrophylla*) as supplements to a low quality grass (*B. dictyoneura*) on intake, digestibility and N utilization by sheep (Powell et al., unpublished results).

Item	Legume supplements[1/]				SE
	100% Ca	75% Ca + 25% Fm	50% Ca + 50% Fm	100% Fm	
Total intake (g DM/d)	822	811	808	810	30
Dietary extractable CT (%)	-	1.2	2.4	3.9	
Digestibility					
DM (%)	55.9	55.6	55.2	57.0	1.3
NDF (%)	58.9a	56.6ab	55.0ab	54.2b	1.3
N utilization					
N intake (g/d)	15.1a	14.5a	13.8a	11.6b	0.6
Duodenal N flow (g/d)	9.0b	9.9ab	10.7a	11.3a	0.4
N absorbed, % N duodenum	42.1c	43.9bc	46.0ab	47.7a	1.1
N retained, % of N intake	31.7	31.5	29.0	30.5	3.8

[1/]Fed as 40% (DM basis) of daily ration in one morning meal.
a,b,c means with different letters in each row are different ($P < 0.05$)

Our results indicate that mixtures of legumes with and without CT fed as supplement to low quality grasses increase N flow to the small intestine of ruminants relative to a legume supplement with no tannins. However, N retention is not significantly improved either due to low supply of digestible energy in the rumen or to incomplete digestion of tannin-protein complexes in the lower GI tract. Thus in future studies formulation of legume mixtures should be based not only on tannin level, but also considering digestibility of the species involved [**C. Powell (NRI, UK) , C. Lascano, D. Romney (NRI, UK), and M. Gill (NRI, UK)**].

In summary, the foregoing results indicate that the level of extractable CT and cell wall degradability should be taken into account in screening procedures for tropical legumes based on tannins. Our results also show that legume mixtures could be a practical way of diluting CT to a level where they could be beneficial to the animal. However, it is still not clear to what extent bound CT, tannin astringency and cell wall composition affect digestion and N utilization by ruminants fed legumes high in tannins. In addition, we need to define the effect on intake, digestion and N utilization by ruminants of legumes high in digestibility in legumes mixtures formulated to dilute tannins.

2. Influence of the environment on nutritive value of selected forage species

An assessment of the interaction of genotype x environment on forage quality is important to define strategies for forage improvement and to identify "niches" for selected forage species.

2.1 G x E interaction on forage attributes of Desmodium ovalifolium

A series of agronomic and grazing trials carried-out in tropical areas showed that *D. ovalifolium* is well adapted to acid infertile soils, that it is compatible with aggressive grasses and that it tolerates heavy grazing. However, animal performance in *D. ovalifolium*-based pastures is frequently poor mainly because of low acceptability and digestibility of the legume as a result of high tannin levels. Preliminaries studies with a limited set of genotypes suggested that there was variability in tannin concentration and other agronomic attributes in the collection of *D. ovalifolium* held by CIAT. In addition, studies with temperate legumes have shown that tannin concentration can increase with environmental stresses (i.e. soil nutrient deficiencies). These environmental effects on forage quality have not been well defined with tropical legumes high in tannins.

A collaborative project funded by BMZ-Germany was initiated during 1995 to study G x E interactions in a core collection of *D. ovalifolium*. A total of 18 genotypes selected mainly on origin were planted in 4 contrasting environments in Colombia (well-drained savanna, Carimagua, Llanos; forest margins, Florencia, Caquetá; dry-hillsides, Cauca; and wet-hillsides, Chinchiná, Caldas). Two fertilizer treatments (low and high) adjusted to the specific conditions of the site were applied. Measurements to be performed at each site include: phenology, incidence of pest and disease, seasonal biomass, seasonal forage quality (CP, IVDMD, cell wall content, tannin concentration, tannin astringency), litter quantity and quality and relative acceptability to cattle of genotypes.

It is expected that the outputs of this study will help to identify superior genotypes of *D. ovalifolium* for pasture improvement and soil enhancement in selected environments. Furthermore, results obtained will broaden our knowledge on the effect of soil fertility and climate on forage/litter quality of legumes containing tannins, which is highly relevant to the work with shrub legumes for acid soils. [**A. Schmidt (Unv. Hohenheim, Germany), B. Maass, C. Lascano, P. Kerridge, R. Schultze-Kraft (Unv. Hohenheim, Germany]**.

2.2 G x E interaction on quality of *Brachiaria* spp.

In a collaborative study with the plant breeder of the Tropical Forages Program we measured the *in vitro* digestibility (IVDMD) of 20 *Brachiaria* spp. genotypes planted at three sites in Colombia with contrasting soil and climate (Palmira, Quilichao and Carimagua). Results showed that the variance in IVDMD was 4 times greater than the variance associated with G x E interaction and that there was high variability in IVDMD among accessions within species. These results suggest that there is great scope to select genotypes of *B. decumbens* and *B. brizantha* on the basis of increased forage quality [**J. Miles and C. Lascano**].

2.3 Plant nutrient supply and forage quality

A limitation of tropical grasses to animal production is their low forage quality. It is well known that grasses loose nutritional quality over time as a result of declining soil fertility. Thus an improved knowledge on genotypic variation in forage quality of grasses under low nutrient supply would allow selection for higher forage quality genotypes in the *Brachiaria* improvement program.

A glasshouse trial was conducted to test the relationship between soil nutrient supply and forage quality among 15 genotypes from 5 species of *Brachiaria* (3 ecotypes of each species: *B. decumbens, B. brizantha, B. ruziziensis, B. humidicola* and *B. dictyoneura*). The selection of genotypes was based on agronomic evaluation in the field (commonly used, very productive and less productive ecotypes). A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Nutrients were supplied before planting at two levels (low and high). Low nutrient supply (kg/ha) included 20P, 20K, 33Ca, 14Mg and 10S while the high nutrient supply included 80N, 50P, 100K, 66Ca, 28.5Mg, 20S and micronutrients at 2Zn, 2Cu, 0.1B and 0.1 Mo. At the time of harvest (49 days of growth) forage quality characteristics such as crude protein (CP), in vitro digestibility (IVDMD), neutral detergent (NDF) and acid detergent (ADF) fiber were determined in leaf and stem material. Plant samples from 3 replicates were pooled and analyzed because of small sample size for each replicate.

Results summarized in Table 4 show that nutrient supply affected forage quality of both leaf and stem fractions in genotypes within species. However, forage quality attributes of both leaf and stem were influenced to a greater extent by genotype than by level of nutrient supply.

The level of ADF (which represent lignocellulose) in leaves of *B. humidicola* and *B. dictyoneura* was greater than that of the other three species (32-39% compared to 27-30%) when grown at low nutrient supply. High ADF values were associated with low specific leaf area (leaf area per unit leaf weight) which suggest that thickness of mesophyll tissue may be greater in *B. humidicola* and *B. dictyoneura* compared to the other three species. Further work is needed to test this hypothesis.

A second glasshouse trial was conducted to test the relationship between soil N supply and forage quality among 18 genotypes of 6 species of *Brachiaria* (3 ecotypes of each species: *B. decumbens*, *B. brinzantha*, *B. ruziziensis*, *B. humidicola*, *B. dictyoneura* and *B. arrecta*). The selection of genotypes was based on agronomic evaluation in the field (commonly used, very productive and less productive ecotypes). A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). A basal nutrient mixture was supplied before planting (kg/ha: 50P, 100K, 66Ca, 28.5Mg, 20S and micronutrients at 2Zn, 2Cu, 0.1B and 0.1 Mo). N was supplied at four levels (kg/ha: 0, 40, 80 and 200). At the time of harvest (48 days of growth), forage quality measurements such as CP, IVDMD, NDF and ADF were performed in leaf and stem material.

As observed in the previous study on nutrient supply, genotypic variation in forage quality attributes was greater than the variation induced by the supply of N to soil (Figure 3). The influence of N supply on leaf quality was greater than that of stems and greatly dependent on species and genotypes within the species. Increase in N supply improved not only shoot biomass but also IVDMD and lowered cell wall (NDF) and lignocellulose (ADF) concentration in leaves. However, the effect of N was greater on IVDMD than in NDF and ADF. These results indicate that a decrease in N supply could change the composition of the cell wall to a greater extent than the content of cell wall **[I. M. Rao, C. Lascano, N. Narváez, and J. Ricaurte]**.

In summary, our results indicate that genotypic variation in forage quality characteristics is greater than variation induced by environmental factors such as soil nutrient supply. Thus there is a large scope to select for improved forage quality of *Brachiaria* ecotypes and genetic recombinants particularly under low fertility acid soils. However, to develop screening methodology to evaluate the feed quality of forages produced on low fertility soils future work should focus on defining the effect of nutrient supply and physiological age of plant tissue on cell wall composition and digestibility.

Table 4. Influence of soil nutrient supply on the range of genotypic variation in forage quality characteristics of 15 genotypes from 5 species of *Brachiaria* grown in pots using a sandy loam oxisol from Carimagua.

Plant material	Nutrient supply	Forage quality (%)			
		CP	IVDMD	NDF	ADF
Leaf	Low[1]	3.3-6.7	62-78	63-77	27-39
	High[2]	4.3-7.6	66-80	64-74	26-35
Stem	Low	1.8-3.8	46-60	77-84	39-52
	High	2.5-4.8	48-60	76-87	39-48

[1]20P, 20K, 33Ca, 14Mg and 10S (kg/ha)

[2]80N, 50P, 100K, 66Ca, 28.5Mg, 20S, 2Zn, 2Cu, 0.1B and 0.1M0 (kg/ha)

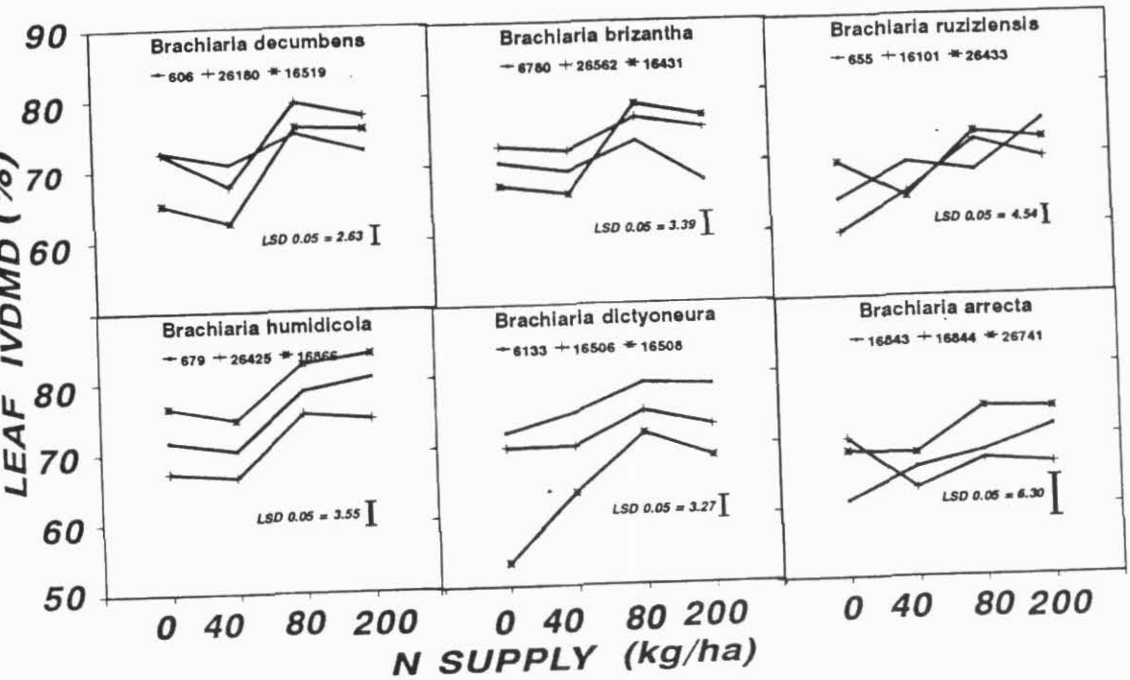


Figure 3. Relationship between soil nitrogen supply and in vitro dry matter digestibility (IVDMD) of leaves of 18 genotypes of 6 *Brachiaria* species.

3. Nutritive value and productivity of new forage ecotypes

Grazing experiments to measure milk production potential of selected genotypes of grass and legume species being developed by the TFP were carried-out in the Quilichao research station.

3.1 Milk production with *S. guianensis* hybrids

One result of the breeding efforts of the TFP has been the development of *Stylosanthes guianensis* hybrids with tolerance to anthracnose. Two lines (CIAT 11833 and 11844) of *S. guianensis* were sown in mixture with *B. dictyoneura* to determine milk production. Results on short-term milk yield of cows grazing grass alone and grass/legume pastures are summarized in Table 5. Cows grazing the grass/legume pasture produced 17 to 65% more milk than those grazing the grass alone pasture. As expected, the greatest effect of the legume on milk production was in periods with limited rainfall.

The results of 5 evaluations phases with *S. guianensis* hybrids reported in this section were recorded over a 9 months period (May 1994 to February 1995). During this period legume content in the pasture ranged from 40 to 54%. With this high legume proportion in the pasture there was a significant impact of *S. guianensis* on milk yield. It should be noted however, that since February 1995 legume content in the pasture has decreased significantly for reasons not well understood **[C. Lascano, J. Miles and P. Avila]**.

3.2 Milk production with *P. maximum*

From the ORSTOM *Panicum maximum* collection evaluated by CIAT in the acid-low fertility soils of Carimagua, two ecotypes (CIAT 6799 and 6944) were selected for further testing. Mixed seed of the two ecotypes was sown in 1994 at Quilichao either alone or in association with *C. macrocarpum* or *S. guianensis* in order to measure milk production.

Up to now, two short-term grazing experiments with milking cows have been completed. In the first experiment cows grazed *P. maximum* alone and in

Table 5. Short-term milk yield (fat-corrected) of cows grazing *B. dictyoneura* (*B. dic.*) alone and in association with *S. guianensis* (S.g.) hybrids (CIAT 11833 and 11844) (C. Lascano, J. Miles and P. Avila, unpublished results).

Measurement Phase	Hydric Balance[1] (mm)	Milk yield *B.dic.* (kg/cow/d)	*B.dic.* + *S.g.*	Difference (%)
1	4	4.6	7.6	65.2**
2	-109	6.1	8.3	36.0*
3	70	4.6	6.7	45.6**
4	398	11.5	13.5	17.4**
5	-157	8.6	10.6	23.2**

[1] Precipitation - evapotranspiration
* Significance level ($P < 0.10$)
** Significance level ($P < 0.05$)

association with legumes. A second experiment included the comparison of *P. maximum* with two *Brachiaria* species fertilized with nitrogen (100 kg/ha). Results summarized in Table 6, show that milk production with both crossbred and Holstein cows was similar in pastures of *P. maximum* alone or with legume. Levels of urea in milk were high (22 mg%) and similar in the grass alone as compared to the grass/legume pastures. This suggest that protein was not limiting in *P. maximum* and that cows selected little legume. On the other hand, milk yield of cows with different genetic potential grazing *P. maximum* fertilized with N was greater than in *B. dictyoneura* cv. Llanero + N, but similar to that was recorded in *B. decumbens* cv. Basilisk + N **[P. Avila and C. Lascano]**.

Initial results confirm that *P. maximum* is an excellent option for milk production in the tropics. There are several cultivars of *P. maximum* (i.e. Vencedor, Tanzania, Centenario) released in Brazil, which are marginally adapted to acid soils of low fertility mainly as a result of high P and N requirements. It remains to be seen if the *P. maximum* ecotypes selected by the TFP for acid soils have lower soil nutrient requirements as compared to commercial cultivars.

Table 6. Short-term milk yield (fat - corrected) of cows grazing legume based and nitrogen fertilizerd *Panicum maximum* ecotypes (CIAT 6799 + 6944) selected for acid soils (C. Lascano and P. Avila, unpublished results).

Pasture Type	Cow type Crossbred (kg/cow/d)	Holstein
Grass/legume		
P. maximum alone[1]	5.3	11.5
+ *C. macrocarpum*	5.6	11.4
+ *S. guianensis*	5.4	10.4
Nitrogen fertilized[1]		
P. maximum	5.6a	10.5a
B. decumbens (+control)	5.6a	10.9a
B. dictyoneura (-control)	5.1b	8.6b
Increment[2] (%)	10	24

[1] Fertilized with 100 kg N/ha
[2] Relative to the negative control
a, b, means with different letters in each column are different ($P< 0.05$)

Project: Forages with High Nutritive Value

Proposed activities for 1996

1. Screening procedures for the presence of tannins and other secondary compounds in legumes.

Activities planned for the next year to address some of the constraints identified in the evaluation and utilization of legumes high in tannins are:

- Studies to establish relationships between short-term intake by sheep and extractable and bound CT concentration in *F. macrophylla* as affected forage maturity and post-harvest treatment (pre-graduate thesis).

- Studies on N utilization by sheep fed legume mixtures differing in tannin concentration (*C. argentea* and *F. macrophylla*) and digestibility (*C. argentea* and *D. velutinum*) (pre-graduate thesis).

- Studies to define possible structural changes in tannins recovered from the small intestine of sheep fed contrasting legumes (Collaborative work with IGER, UK).

- Initiation of collaborative work with the Institute of Grassland and Environmental Research (IGER) in the United Kingdom through an ODA financed project. The aim is to define the influence of CT from a range of legume species on: (1) fermentation kinetics of cell wall and (2) activity of cellulolytic and proteolytic enzymes. In addition, studies will be carried-out to determine the effect of environmental factors on tannin and cell wall chemistry of *D. ovalifolium* and to assess tanninase activity of rumen microorganisms from animals on high tannin diets. A range of samples of legume species with variable tannin levels will be used to evaluate the feasibility of using NIRS to measure tannin concentration in tropical legumes (PhD thesis - U of Wales).

2. Influence of the environment on nutritive value of selected forage species.

Field and glasshouse experiments will be initiated to quantify the effect of contrasting climatic and edaphic factors on grass and legume quality at different stages of development. Specific activities planned for next year are:

- Initiation of measurements in *D. ovalifolium* planted in 4 locations. At the beginning of the production phase, measurements will be made on forage yield, leaf:stem ratio, flowering date, and forage quality. In addition, tannin concentration will be measured in 3 or 4 genotypes of *D. ovalifolium* grown in the greenhouse under different stress conditions (i.e. soil nutrients, drought) (pre-graduate thesis).

- Initiation of measurements in a field trial at Carimagua (sandy soil) to measure genotypic differences in forage quality and plant adaptive attributes of *Brachiaria* genotypes and genetic recombinants grown with two levels of fertility.

- Glasshouse study to test the influence of decreasing nutrient supply and increasing plant age on leaf anatomy and cell wall composition using contrasting genotypes from *B. brizantha* and *B. dictyoneura*.

- Follow-up a joint OFI-CIAT-ICRAF proposal entitled "Investigation of factors affecting the nutritive value of *Calliandra calothyrsus* leaf as a browse plant" which was submitted to ODA for funding. The project includes an assessment of forage quality of two genotypes of *C. calothyrsus* with low and high levels of tannins grown in two contrasting soils (Palmira and Quilichao).

3. Nutritive value and productivity of new forage ecotypes.

Activities on evaluation of animal production potential of selected forage ecotypes developed by the TFP will continue at Quilichao. However these activities are being expanded to other sites through collaboration with NARS. Specific activities proposed for 1996 include:

- Reformulation and establishment at Carimagua of a grazing experiment including ecotypes of *P. maximum* selected by the TFP for acid soils and commercial cultivars developed by EMBRAPA in Brazil with contrasting levels of fertilizer.

- Follow-up grazing experiments with *A. pintoi*-based pastures for milk production established by IVITA (Perú), the U. Federal of Uberlandia (Brazil) and CORPOICA (Macagual, Florencia).

- Collaborate in the design of a grazing experiment with contrasting legumes to measure milk production in CIAT-Santa Cruz, Bolivia.

Project: Adaptive attributes of forages to infertile soils

Project Coordinator: I. M. Rao

Rationale

The use of forages adapted to low fertility soils of the tropics is one of the most effective means of managing these soils. Considerable achievements have been made in identifying tropical forage legumes and grasses well adapted to these soils but little is known about their plant attributes for adaptation. Continued progress in the selection and genetic improvement of forages will depend upon the development of rapid and reliable techniques which facilitate screening of large numbers of genotypes for tolerance to infertile soils.

Low nutrient supply is a major limitation of forage adaptation and production in low fertility, acid soils of the tropics. Widespread adoption of forage cultivars depends on efficient acquisition of nutrients from the soil and in utilization for growth. Plant growth in these soils is not often limited by hydrogen ion (H^+) activity, but rather by Al toxicity and deficiencies of nutrients such as P, N and Ca. Adapted plants have attributes that are linked to strategies to acquire these nutrients in a low pH and high Al environment. Understanding these strategies is fundamental to developing more efficient screening procedures.

The genetic potentials of tropical forages and the environments in which they are grown influence growth and productivity. Genetic variability and plant ability to acquire, translocate, distribute, accumulate, and use mineral nutrients are important in adapting forages to infertile soils. The extent of inter- and intraspecific variation in the ability to acquire and use nutrients need to be conscientiously considered to adapt and fit forages to infertile soil conditions or to improve efficiency of nutrient gain and use. Improving adaptation of forages to infertile soils without loss of forage yield or quality will contribute to lower input requirements, lower animal production costs, and fewer environmental problems from soil degradation.

Root growth and turnover are two key components in the study of nutrient cycling in pastures and carbon sequestration in soils. Some plants make efficient use of nutrients by extracting nutrients from depth and also recycling them within the whole plant. In addition to nutrient acquisition and nutrient cycling, deep root systems can also contribute to carbon sequestration in soils. Are these the principal roles of large and extensive root systems as exhibited by many introduced tropical grasses (*Brachiaria* spp.) and legumes (*Arachis* spp.) ? Thus the role of roots in nutrient cycling and carbon sequestration processes in pastures under different management regimes need to be further evaluated.

Considerable importance is attributed to the nitrogen contribution of legumes to sustainable animal production from stable grass/legume associations. While the nitrogen fixed can largely be related to the amount of legume growth, fixation is also directly influenced by some macro- (e.g. Ca) and micronutrients (e.g. Mo) and environmental conditions. Many legumes that are well adapted to edaphic, climatic and biotic stress factors fail to persist in association with vigorous grasses. The reasons for this are obvious in some cases but not in others. There is a need to better define attributes for persistence, in particular, to be better able to predict success or otherwise during primary evaluation of forage accessions.

This project will provide a strategic research base through identification of root, shoot and reproductive attributes of forage genotypes leading to (i) improved efficiency in selection and breeding; (ii) identifying critical plant-soil, plant-plant and plant-soil-animal nutrient interactions in forage-based production systems; and (iii) assisting in identifying ecological niches for forage germplasm.

Objective

To identify plant attributes that confer tolerance to low fertility soils and contribute to efficient acquisition and utilization of nutrients in order to develop reliable screening procedures and effectively deploy forages in different ecosystems.

Main Activities

1. Plant attributes that confer tolerance to low fertility soils
2. Role of roots in nutrient cycling and carbon sequestration
3. Development of stable grass-legume associations

Highlights (1994-95)

- Showed that genotypic variation in leaf area production and leaf N partitioning of *Brachiaria* species is greater than variation in acquisition of N from soil.

- Identified three plant attributes, leaf area production, root length and P uptake efficiency per unit root length, as possible selection indices to evaluate low fertility tolerance of *Brachiaria* genotypes.

- Showed that genotypic variation in some key plant attributes was greater than variation due to change in nutrient supply.

- Showed that *B. ruziziensis* is less adapted to low fertility Oxisols due to its inability to alter partitioning of dry matter between root and shoot as the supply of nutrients decrease in soil.

- Showed that root attributes such as root length and root branching are major factors influencing P and Ca acquisition from low fertility soils.

- Demonstrated that plant growth of *A. pintoi* in clay loam soils can be improved by inoculation with vesicular-arbuscular mycorrhizae under glasshouse conditions.

- Found that P deficiency in *A. pintoi* roots can induce exudation of fumaric acid in the rhizosphere.

- Showed that root biomass production of improved pastures was greater than that of native savanna.

- Found that improved legume-based pastures can sequester significant amounts of carbon in soil.

- Showed that legume-based pastures can not only improve N cycling but also stimulate cycling of other nutrients such as Ca.

1. Plant attributes that confer tolerance to low fertility soils

It is highly desirable that the adapted forages make efficient use of naturally occurring nutrients and those added as fertilizer for growth, and in the case of legumes, for N_2 fixation. The use of adapted forage germplasm reduces the amount of fertilizer needed, but does not eliminate the need to fertilize. Previous research indicated that adapted forage legumes acquire more P and Ca from infertile soils than grasses per unit length of root. Grasses have greater capacity to utilize the acquired N, P and Ca in terms of dry matter produced per unit of nutrient absorbed. The extent of variation between and within species needs to be explored to develop nutrient efficient genotypes which could meet the mineral nutrient requirements of ruminants.

Research during the past two years has focused on: (1) identifying genotypic variation in tolerance to low supply of nutrients in soil; (2) identifying genotypic variation in acquisition and utilization of nutrients; (3) determining phosphorus dynamics in the rhizosphere; and (4) identifying the mechanisms of acid soil tolerance in *Brachiaria*.

1.1 Genotypic variation in tolerance to low nutrient supply

Intergeneric and interspecific variation in tolerance to low nutrient supply has been identified among a number of grasses and legumes when grown in soils of contrasting texture. But intraspecific variation in tolerance to low nutrient supply was not determined.

A glasshouse trial was conducted to determine genotypic differences in *Brachiaria* species for tolerance to low nutrient supply in soil. Shoot and root attributes of 15 genotypes from 5 species of *Brachiaria* (3 genotypes of each species: *B. decumbens, B. brinzantha, B. ruziziensis, B. humidicola* and *B. dictyoneura*) were measured in order to evaluate their low fertility tolerance. The selection of genotypes was based on agronomic evaluation in the field (commonly used, very productive and less productive genotypes for each species). A sandy loam Oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Nutrients were supplied before planting at three levels (nil, low and high). Low nutrient supply (kg/ha) included 20 P, 20 K, 33 Ca, 14 Mg and 10 S while the high nutrient supply included 80 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. After 49 days of growth, plants were harvested.

Measurements were made of a number of plant attributes such as forage yield, root biomass, root to shoot ratio, leaf to stem ratio, leaf area, photochemical efficiency of photosystem II, root length, specific root length, root length to leaf area, specific leaf area, specific leaf N (leaf N/shoot N x 100), soluble leaf protein, leaf N partitioning index, shoot nutrient uptake and nutrient use efficiency. A great deal of genotypic variation was found for majority of plant attributes at each level of nutrient supply (Table 1). Genotypic variation in several plant attributes was greater than the variation induced by level of nutrient supply. The extent of genotypic variation in leaf and root attributes was greater than that of forage yield.

Results summarized in Table 2 show significant genotypic differences in tolerance to low nutrient supply as revealed by forage yield, leaf area, root length and P uptake efficiency. The extent of ecotypic variation in leaf area production, root length and P uptake efficiency per unit root length was greater than that of forage yield. Among the 15 genotypes tested, *B. decumbens* CIAT 606, showed better tolerance to low nutrient supply by producing good amounts of leaf area and forage yield due to its greater ability to produce fine roots and to acquire P per unit root length. Some genotypes which showed greater root length were less efficient in acquiring P per unit root length. There is a need to verify the usefulness of these three plant attributes under field conditions in order to develop a screening strategy to identify superior genotypes of *Brachiaria* **[I. M. Rao, G. Keller-Grein, J. Ricaurte and R. Garcia]**.

1.2 Genotypic variation in nutrient acquisition and utilization

Intergeneric and interspecific differences among grasses and legumes were observed in acquisition and utilization of N, P, and Ca when grown in two Oxisols of contrasting texture with low and high amounts of fertilizer application, but genotypic variation within species has not been determined.

A glasshouse experiment examined genotypic differences in acquisition and utilization of N among 18 genotypes of 6 species of *Brachiaria* (3 genotypes of each species: *B. decumbens, B. brizantha, B. ruziziensis, B. humidicola, B. dictyoneura* and *B. arrecta*). The selection of genotypes was based on prior agronomic evaluation in the field (commonly used, very productive and less productive genotypes). A sandy loam Oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). A basal nutrient mixture was applied to soil before planting (kg/ha: 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). N was supplied at four levels (kg/ha: 0, 40, 80 and 200). At the time of harvest (48 days of growth), several shoot attributes such as forage yield, leaf/stem ratio, leaf area, leaf chlorophyll, soluble leaf protein, photochemical efficiency of photosystem II, leaf and stem nutrient composition and leaf N partitioning index were determined.

Table 1. Influence of nutrient supply on the range of genotypic variation in plant attributes of five *Brachiaria* species (15 genotypes) when grown in pots (4 kg soil) in a sandy loam Oxisol from Carimagua.

Plant attributes	Nutrient supply		
	Nil	Low[1]	High[2]
Forage yield (g/pot)	1.5 - 4.8	4.9 - 10.6	14.1 - 29.2
Root biomass (g/pot)	0.3 - 2.0	0.9 - 4.1	2.0 - 9.6
Leaf area (cm^2/pot)	32 - 202	104 - 456	295 - 1259
Leaf chlorophyll (mg/m^2)	115 - 187	63 - 171	69 - 173
Soluble leaf protein (g/m^2)	1.7 - 3.9	0.7 - 2.4	0.4 - 2.4
Leaf N partitioning index (%)	20 - 58	36 - 55	32 - 57
Root length (m/pot)	25 - 105	53 - 202	122 - 422
Root length/Leaf area (km/m^2)	3.2 - 28.9	2.3 - 12.1	1.6 - 14.7
P uptake efficiency (µg/m)	9.0 - 27.2	18.1 - 96.5	24.3 - 140.6

[1] 20 P, 20 K, 33 Ca, 14 Mg, and 10 S (kg/ha)

[2] 80 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B, and 0.1 Mo (kg/ha)

Table 2. Genotypic differences in plant attributes for tolerance to low nutrient supply in five species of *Brachiaria* grown in pots (4 kg soil) in a sandy loam Oxisol from Carimagua.

Species	CIAT accession number	Forage yield (g/pot)	Leaf area (cm^2/pot)	Root length (m/pot)	P uptake efficiency (μg/m)
B. decumbens	606	8.7	368	87	79
	26180	7.7	319	85	41
	16519	4.9	192	102	41
B. brizantha	6780	10.6	456	110	53
	26562	8.0	213	55	62
	16431	8.2	192	64	53
B. ruziziensis	655	7.1	255	90	52
	16101	8.5	339	102	48
	26433	6.3	308	94	44
B. humidicola	679	7.4	140	160	28
	26425	7.5	176	202	18
	16866	10.5	225	143	38
B.dictyoneura	6133	8.4	183	114	35
	16506	8.0	239	60	64
	16508	7.2	104	53	96
Mean		7.9	247	101	50
LSD (P = 0.05)		1.9	76	33	26

Results summarized in Table 3 show that plant attributes were influenced by level of N supply. However, plant attributes were influenced to a greater extent by genotype than by level of N supply. Some plant attributes (leaf area production, specific leaf N and soluble leaf protein) exhibited greater genotypic variation than the others (forage yield, leaf N partitioning index and shoot N uptake). As expected, increase in N supply improved forage yield as a result of stimulation of leaf area production.

Genotypic variation in forage yield in relation to N supply was greater in *B. dictyoneura* and *B. humidicola* than that of the other four species. Genotypes of *B. decumbens* and *B. arrecta* responded almost linearly to the increase in N supply. The cultivar *B. dictyoneura* cv Llanero (CIAT 6133) was outstanding in forage yield compared to the other two genotypes of this species at each level of N supply. Significant genotypic variation in leaf area production was observed in *B. brizantha*, *B. decumbens* and *B. dictyoneura*. Genotypic variation in leaf area production in relation to N supply (Figure 1) was greater than that of forage yield (Table 3). Increase in N supply improved leaf area production of genotypes from *B. decumbens*, *B. ruziziensis* and *B. arrecta* species. These results indicate that genotypic variation in leaf area production and leaf N partitioning of *Brachiaria* species was greater than variation in acquisition of N from soil **[I. M. Rao, G. Keller-Grein, J. Ricaurte, and R. Garcia]**.

Table 3. Influence of N supply on the range of genotypic variation in plant attributes of six *Brachiaria* species (18 genotypes) when grown in pots (4 kg soil) in a sandy loam Oxisol from Carimagua. Measurements on plant attributes were made after 48 days of growth.

Plant attributes	Nitrogen supply (kg/ha)			
	0	40	80	200
Forage yield (g/pot)	7.2 - 13.4	8.9 - 23.0	10.5 - 27.2	14.1 - 35.2
Leaf area (cm^2/pot)	186 - 828	236 - 1230	238 - 1520	365 - 1922
Specific leaf N (mg/m^2)	402 - 1857	525 - 2219	577 - 2641	936 - 2620
Soluble leaf protein (g/m^2)	0.54 - 2.15	0.72 - 2.80	0.83 - 2.58	1.29 - 3.19
Leaf N partitioning index (%)	44 - 86	35 - 80	41 - 74	38 - 75
Shoot N uptake (mg/pot)	36 - 75	77 - 130	115 - 200	179 - 322

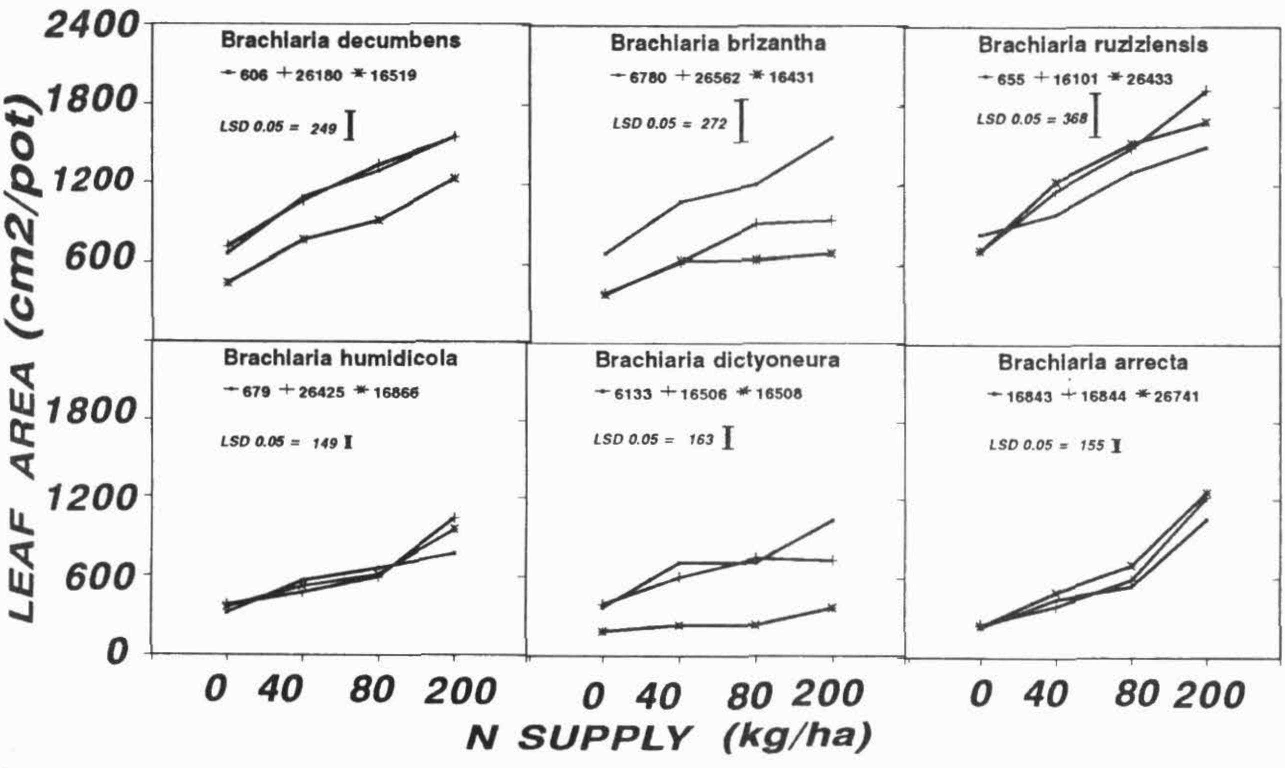


Figure 1. Genotypic differences in leaf area production of six *Brachiaria* species as influenced by nitrogen supply to a sandy loam Oxisol from Carimagua.

1.3 Phosphorus dynamics in the rhizosphere

The BMZ/GTZ-funded project undertaken in conjunction with German scientists was completed in 1995. The purpose of this project was to determine P dynamics in the rhizosphere of tropical forage grasses and legumes grown in infertile acid soils. The progress made in this project will be reported in the form of a Ph. D. thesis to be submitted to the University of Hohenheim, Germany. Research in this project was aimed to determine: (1) differences in acid soil adaptation between *B. dictyoneura* and *B. ruziziensis*; (2) the influence of P supply on root morphology and nutrient acquisition; (3) the role of vesicular-arbuscular mycorrhizae (VAM) in P acquisition; and (4) the influence of P deficiency on exudation of organic acids from roots. The main observations are summarized below.

1.3.1 Adaptation to acid soils: A field experiment was conducted at Carimagua on two contrasting soil types (clay loam and sandy loam) to compare the differences in acid soil adaptation between *B. dictyoneura* (well adapted) and *B. ruziziensis* (less adapted). The study was designed to identify causal factors of P deficiency by using two soil types, two levels of nutrient supply, and application of lime (to decrease Al toxicity) and gypsum (to improve subsoil Ca supply). These treatments allowed to evaluate whether P limitation to root and shoot growth was directly due to low supply of P in soil or indirectly due to restriction of root growth by Al toxicity or Ca deficiency. Low nutrient supply was (kg/ha): 20 P, 20 K, 47 Ca, 14 Mg, 10 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. High nutrient supply was (kg/ha): 50 P, 40 N, 100 K, 100 Ca, 28 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. Lime was applied to reduce Al saturation to 50% (t/ha: 0.5 to sandy loam and 2.0 to clay loam). Gypsum was applied to provide same amounts of Ca as in lime treatment (t/ha: 1.2 to sandy loam and 4.8 to clay loam).

Results presented in Figure 2 show that root and shoot growth of both species was not limited by Al toxicity. Root length density of *B. ruziziensis* was markedly lower than that of *B. dictyoneura* irrespective of treatments. Thus, the demand for high supply of nutrients was greater with *B. ruziziensis*. In soils of very low nutrient supply (sandy loam with no additional N supply), *B. dictyoneura* was more productive than *B. ruziziensis* with increasing age of the pasture (up to 28 months). This was mainly due to the ability of *B. dictyoneura* to alter its root to shoot partitioning of dry matter to acquire greater amounts of P together with its low requirement of N for growth [**K. Häußler, F. Tabares, R. Garcia, I. M. Rao, and H. Marschner**].

1.3.2 Root morphology and nutrient acquisition: Three glasshouse experiments were conducted to determine the influence of nutrient supply on root branching of *B. dictyoneura* and *B. ruziziensis*. Plants were grown in pots (4 kg soil) or in minirhizotrons (2 kg soil) using two soil types (sandy loam and clay loam Oxisols) from Carimagua. In the first experiment, both species were grown in pots with a basal fertilizer mixture (kg/ha: 40 N, 100 K, 28 Mg, 20 S and micronutrients). The influence of low and high levels of P and Ca supply (20 P and 50 Ca; 50 P and 100 Ca) was tested on root morphology and nutrient acquisition from two soil types. Shoot biomass and root length of both species were greater with high P and Ca supply. Acquisition of Ca per unit root length was several fold greater by *B. ruziziensis* than that of *B. dictyoneura*.

Using minirhizotrons (plexiglass root boxes), another experiment was conducted to determine the differences in root branching of both species in a clay loam soil at different levels of N supply (kg/ha: 7, 22 and 66). Although total root length was lower in *B. ruziziensis*, root branching and thus the number of root tips were greater in *B. ruziziensis* than that of *B. dictyoneura* (Plate 1). As root tips are the main uptake sites for Ca, a third experiment was conducted to determine the influence of P supply (kg/ha: 5, 10, 20 and 50) on Ca acquisition of *B. dictyoneura* from a sandy loam Oxisol. Root branching and number of root tips increased three fold with increasing P supply. Root branching was closely related to P supply (r^2= 0.89) and Ca concentration in the shoot was significantly improved by the increase in P supply (r^2= 0.72). These results indicate that improved P supply in soil increases root branching and contributes to greater acquisition of Ca from soil [**K. Häußler, F. Tabares, R. Garcia, I. M. Rao and H. Marschner**].

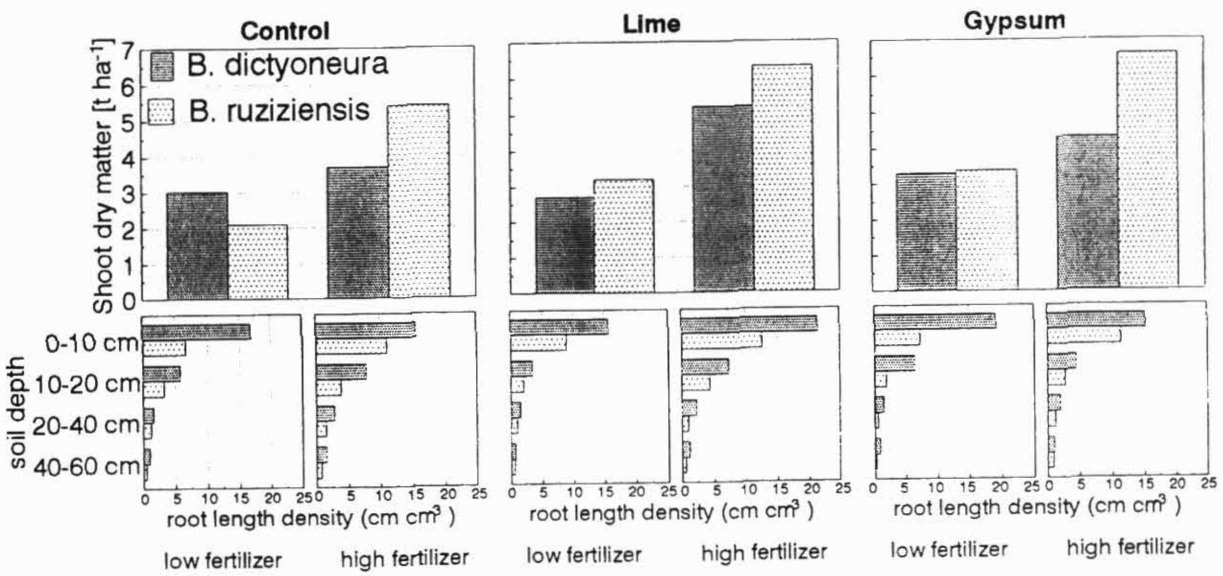


Figure 2. Differences in shoot biomass production and root length density of *B. dictyoneura* and *B. ruziziensis* as influenced by lime or gypsum application with low or high amounts of fertilizer application. (see text for details on rates of fertilizer applications).

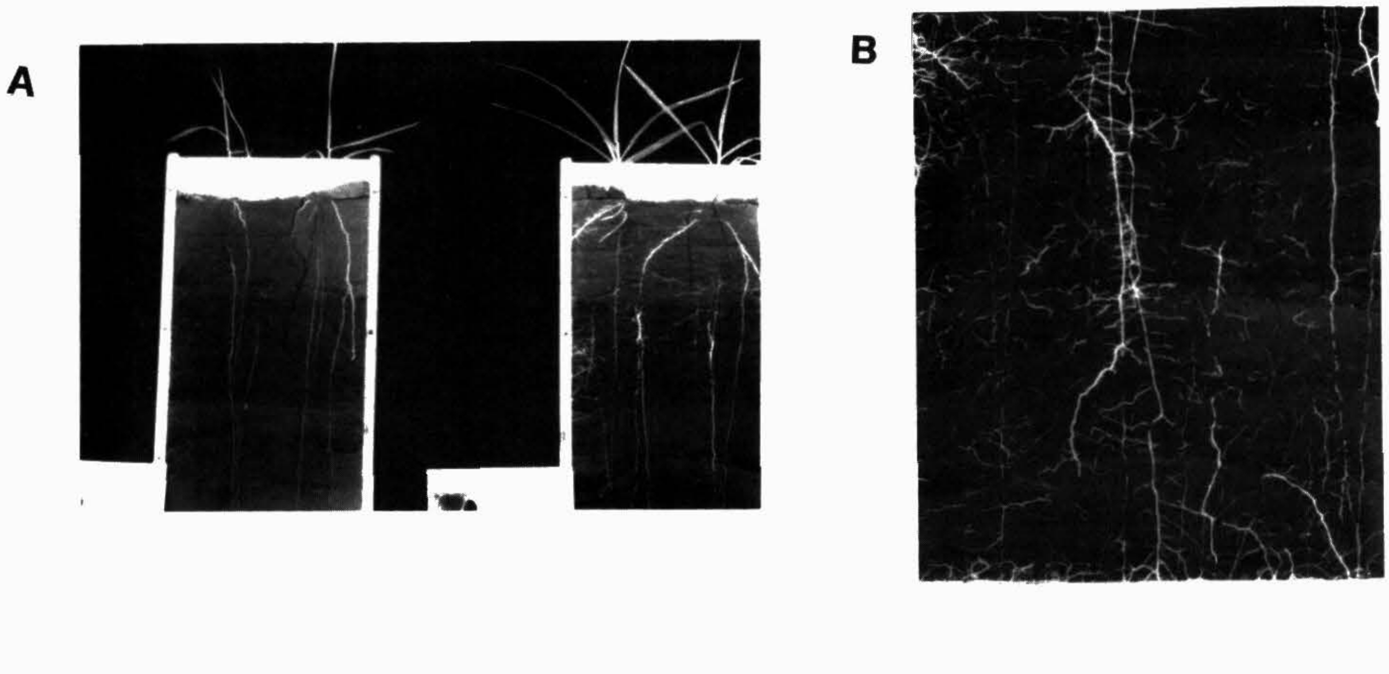


Plate 1. (A) Root growth and development of two *Brachiaria* species (*B. ruziziensis* (right) and *B. dictyoneura* (left)) grown in an Oxisol from Carimagua; and (B) Root system of *B. ruziziensis* showing extensive branching and greater number of root tips.

1.3.3 Role of VA mycorrhizae: Three glasshouse experiments were conducted to evaluate the importance of VAM association on plant growth and P acquisition of three forage legumes (*Stylosanthes capitata*, *S. sympodialis* and *Arachis pintoi*) grown at low nutrient supply. It is known that *S. sympodialis* is less adapted to acid soils. Experiment 1 examined VAM dependence of *S. capitata* and *S. sympodialis* in a sterilized sandy loam soil inoculated with *Glomus manihotis*. At low and moderate levels of P supply (kg/ha: 10, 20 and 50), both species were highly dependent on VAM colonization for plant growth and P acquisition. Experiment 2 tested the response of *A. pintoi* to VAM inoculation in two soil types (unsterilized) at different levels of P supply (0-200 kg P/ha). Although initial spore population in both soils was similar (400 spores per 100 g of soil), VAM colonization and response to P application in *A. pintoi* was greater in sandy loam than that of clay loam soil.

To verify whether soil characteristics suppress VAM colonization or indigenous VAM in clay loam soil lack the potential to colonize *A. pintoi* roots, a third experiment was conducted. *A. pintoi* plants were grown in an unsterilized clay loam soil inoculated with spores derived from sandy loam soil. Plant growth, root colonization of VAM and P acquisition were significantly improved by inoculation of spores from sandy loam soil. These results indicate the importance of VAM colonization to forage legume establishment, particularly in clay loam soils where indigenous VAM population may not be able to sustain adequate plant growth. Therefore, selection of superior *A. pintoi* accessions and/or inoculation with effective VAM species may be an important strategy for rapid establishment of *A. pintoi* in clay loam Oxisols [**K. Häußler, A. Milz, I. M. Rao, B. Dinkelaker and H. Marschner**].

1.3.4 Production and exudation of organic acids from roots: It is known that exudation of organic acids in the rhizosphere will contribute to acquisition of P from less available sources and tolerance to P deficiency. At the University of Hohenheim, using plant growth chambers and nutrient solution culture techniques, the influence of P deficiency on exudation of organic acids from roots of *A. pintoi* was investigated. P deficiency was induced by transferring plants grown in complete nutrient solution to P deficient solution. P deficiency decreased the concentrations of malic, citric and fumaric acids in roots. But exudation of fumaric acid from fresh roots was at 0.8 nmol/g/h for P deficient plants compared to very negligible amounts from P sufficient plants (< 0.07 nmol/g/h).

P deficiency is known to induce exudation of fumaric acid from roots of cultivated peanut but not from beans and soybeans. The total amount of organic acids exuded by roots of P deficient *A. pintoi* plants was not sufficient to explain the drop in pH of the nutrient solution compared to P sufficient plants, suggesting that the shift in cation/anion-balance was responsible for it. Further work is needed to determine the significance of root exudation of fumaric acid from P deficient *A. pintoi* plants on rhizosphere P dynamics [**K. Häußler, I. M. Rao and H. Marschner**].

1.4 Acid soil tolerance mechanisms in *Brachiaria*

A graduate student thesis (Ph.D.) project funded by the Austrian Academy of Sciences was initiated in collaboration with the University of Vienna, Austria; the Biotechnology Research Unit at CIAT; and the National Accelerator Center in Faure, South Africa. The aim of the project is to investigate acid soil tolerance mechanisms in *Brachiaria*.

Three species, *B. decumbens* cv Basilisk (well adapted), *B. brizantha* cv Marandú (less persistent) and *B. ruziziensis* cv Common (poorly persistent) were chosen to identify the physiological, biochemical and molecular basis of differences in tolerance to a simulated acid soil stress (Al toxicity and low supply of nutrients). Low ionic strength nutrient solutions designed with the help of GEOCHEM 2.0 model were used to simulate stress treatment. Four treatments were compared: control solution (co-Al) which contained no Al but sufficient amounts of all nutrients; control with toxic concentration of Al (Al^{3+}=44 μM) solution (co+Al); nutrient stress solution with no Al (st-Al); and nutrient stress solution with Al (st+Al) which contained low levels of N, P, K, Ca, Mg in combination with a toxic concentration of Al.

The effectiveness of these solutions was tested on three rice varieties differing in acid soil tolerance. Root and shoot dry matter and particularly root elongation were affected severely by the st+Al treatment, even in the most acid soil tolerant rice variety. In contrast to rice varieties, *Brachiaria* species, especially *B. decumbens*, were less sensitive to st+Al treatment. Results obtained so far indicate that: (i) the three *Brachiaria* species are tolerant to Al; and (ii) *B. ruziziensis* is less adapted than the other two species when grown in the st+Al treatment as judged by relative growth parameters (Table 4). Root length of *B. ruziziensis* was significantly decreased by st+Al treatment while it had no effect on *B. decumbens*.

The protocol developed to simulate acid soil stress (st+Al) is being used to investigate mechanisms of acid soil adaptation in *Brachiaria* species. Research is focused on three aspects: (1) nutrient acquisition; (2) production and exudation of toxic phenolic compounds; and (3) induction of genes.

1.4.1 Nutrient acquisition: The H^+-translocating ATPase of the plasma membrane plays a key role in plant nutrition because it creates a proton gradient that is used as driving force for nutrient uptake. Experiments were initiated to evaluate the effect of st+Al treatment on plasma membrane ATPase activity in roots. Plasma membranes were purified by means of a two-phase partition method. Studies with specific inhibitors of ATPases of different cellular membrane types revealed a high degree of purity. We are currently evaluating the extent and the mode of inhibition of H^+-ATPase activity by Al^{3+} and the influence of cations like K^+, Ca^{2+}, and Mg^{2+} on the activity of the enzyme.

Table 4. Relative differences in shoot and root attributes of three *Brachiaria* species grown in low ionic strength nutrient solutions. Values are percentages of a st+Al nutrient solution[1] compared to those of a co-Al solution[2]. Letters a,b,c indicate significant differences between species at the 0.05 level[3].

Parameter	Species		
	B. ruziziensis	***B. decumbens***	***B. brizantha***
Shoot biomass	45[a]	71[b]	73[b]
Leaf area	33[a]	61[b]	63[b]
Root biomass	81[a]	139[b]	118[b]
Root length	35[a]	100[c]	65[a]
Number of root tips	24[a]	58[b]	61[b]

[1] Composition in μM: 100 NO_3^-, 10 NH_4^+, 60 Ca^{2+}, 60 K^+, 30 Mg^{2+}, 1 $H_2PO_4^-$, 6 H_3BO_3, 1 Mn^{2+}, 1 Zn^{2+}, 0.2 Cu^{2+}, 0.001 MoO_4^{2-}, 5 Fe^{3+}, 5 $EDTA^{2-}$, 5 SiO_3^{2-}, 80 Na^+, 100 SO_4^{2-}, 268.398 Cl^-, 80 Al^{3+}, 54.68 HCl (pH=4.2)

[2] Composition in μM: 500 NO_3^-, 50 NH_4^+, 300 Ca^{2+}, 300 K^+, 150 Mg^{2+}, 5 $H_2PO_4^-$, 6 H_3BO_3, 1 Mn^{2+}, 1 Zn^{2+}, 0.2 Cu^{2+}, 0.001 MoO_4^{2-}, 5 Fe^{3+}, 5 $EDTA^{2-}$, 5 SiO_3^{2-}, 80 Na^+, 286 SO_4^{2-}, 252.398 Cl^-, 67.75 HCl (pH=4.2)

[3] The mean percentages and their corresponding variances were estimated by means of jackknifing and subsequently compared with the Tukey-Kramer method.

Nutrient uptake is generally considered to be specially active in root tips. Thus, differences in uptake mechanisms or preferences towards certain elements, e.g. in the context of the anion-cation balance, could result in differences in the levels and/or distribution of nutrients in root tip tissue. To address this issue, transverse sections of root tips were prepared with a cryotome, lyophilized, and scanned by means of "proton induced x-ray emission" (PIXE). This technique allows to visualize spatial distribution of Al, P, Ca, K, Si, S, Cl, Fe, Mn, Cu, and Zn in root tips. Preliminary results on the distribution of S, P, and K indicated good spatial resolution and sample to background ratio.

A well-known strategy for acquisition of P and tolerance of Al in acid soils involves production and exudation of organic acids. To evaluate the role of organic acid production and exudation in acid soil adaptation of *Brachiaria* species, protocols for organic acid purification and HPLC analysis are currently being developed.

1.4.2 Production and exudation of toxic phenolic compounds: Preliminary studies carried out at EMBRAPA-CPAC, Planaltina, Brazil, suggested that persistence of certain legumes when associated with *Brachiaria* species (e.g., *B. brizantha* cv. Marandú) could be affected by the accumulation of toxic (allelopathic) compounds in the soil. Production and exudation of phenolic compounds can also cause autotoxicity to *Brachiaria* species leading to poor persistence under field conditions. Medicarpin, a water soluble phenolic compound produced by alfalfa was implicated as the allelochemicalthat causes autotoxicity. Thus, phenolic compounds were extracted from roots of plants grown under control and stress conditions and analyzed by reverse-phased HPLC. In roots of *B. ruziziensis,* two major compounds were detected that accumulate at least tenfold under stress conditions. It remains to be shown to what extent these compounds are responsible for diminished root growth of *B. ruziziensis* under st+Al treatment. There is a need to develop seedling bioassays for the evaluation of the toxic potential of root and shoot extracts on seedlings of a number of grasses and legumes.

1.4.3 Induction of genes: Isolation of stress-induced genes may provide an alternative approach to gain insights on differences in acid soil adaptation among *Brachiaria* species. Comparison of the DNA-sequences of isolated genes with known genes in databases, visualization of their expression pattern in tissues by *in-situ* hybridization, and evaluation of their inducibility by different stress treatments could yield valuable information with respect to their role in acid soil adaptation. Experiments were initiated by isolation of mRNA from root tips of plants grown under st+Al treatment and used for cDNA synthesis. The cDNA was then PCR amplified and is currently being cloned into plasmid vectors for cDNA library construction **[P. Wenzl, L. Mancilla, C. Pineda, A.L. Chaves, J. Mayer, I. Rao, R. Albert, and E. Heberle-Bors]**

Results obtained through 1995 indicate that differences in acid soil adaptation and persistence in the field among *Brachiaria* species could be attributed to differences in tolerance to low availability of nutrients in Al toxic soil environment. Root attributes such as root length and root branching are major factors influencing P and Ca acquisition from low fertility soils. Improved acquisition of P from low fertility soils contributes to greater expansion of leaves and production of forage. Genotypic variation in leaf and root attributes of *Brachiaria* species was greater than variation due to change in nutrient supply. Three plant attributes, leaf area production, root length and P uptake efficiency per unit root length, were identified as possible selection indices to evaluate low fertility tolerançe of *Brachiaria* genotypes. Further research work is needed on *A. pintoi* accessions to assess the role of VA mycorrhizae and root exudation of organic acids in order to elucidate the mechanisms of P acquisition and to improve their performance during pasture establishment phase, particularly in clay loam Oxisols.

2. Role of roots in nutrient cycling and carbon sequestration

In grazed pastures, nutrients cycle from the soil to pasture plants and then back to the soil, either through the death of plant tissue or via the excreta of grazing animals. The assessment of root biomass and root length dynamics in legume-based pastures compared to grass alone or native pastures is a key component in understanding the role of nutrient cycling in pastures and carbon sequestration in soils. The differences in root biomass through time are considered to reflect the net result of new root growth and loss of roots in death and decay. Thus the turnover of roots through time contributes not only to nutrient cycling but also to soil improvement via carbon sequestration in soil.

2.1 Changes in root biomass and specific root length in pastures

In the Llanos of Colombia (at Carimagua Research Station), root biomass and root length were estimated during the growing season for four years in improved grass alone (*Brachiaria dictyoneura*) and grass + legume (*Brachiaria dictyoneura* + *Centrosema acutifolium*) pastures under grazing compared with native savanna in a clay loam Oxisol site. Details on pasture management and fertilizer applications were described before (Tropical Forages Program Biennial Report 1992-93). Low fertilizer application was (kg/ha): 20 P, 20 K, 50 Ca, 20 Mg, 12 S, 2 Zn, 2 Cu, 0.5 Bo, 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 Bo and 0.1 Mo.

Changes in root biomass and specific root length (root length per unit root weight) in relation to pasture age are shown in Figure 3. The average root biomass production of grass alone pastures was about 6.0 t/ha compared to 3.9 t/ha of the grass + legume pasture with low initial fertilizer application. In contrast to the improved pastures, the average root biomass production of the native savanna was only 1.5 tons per hectare

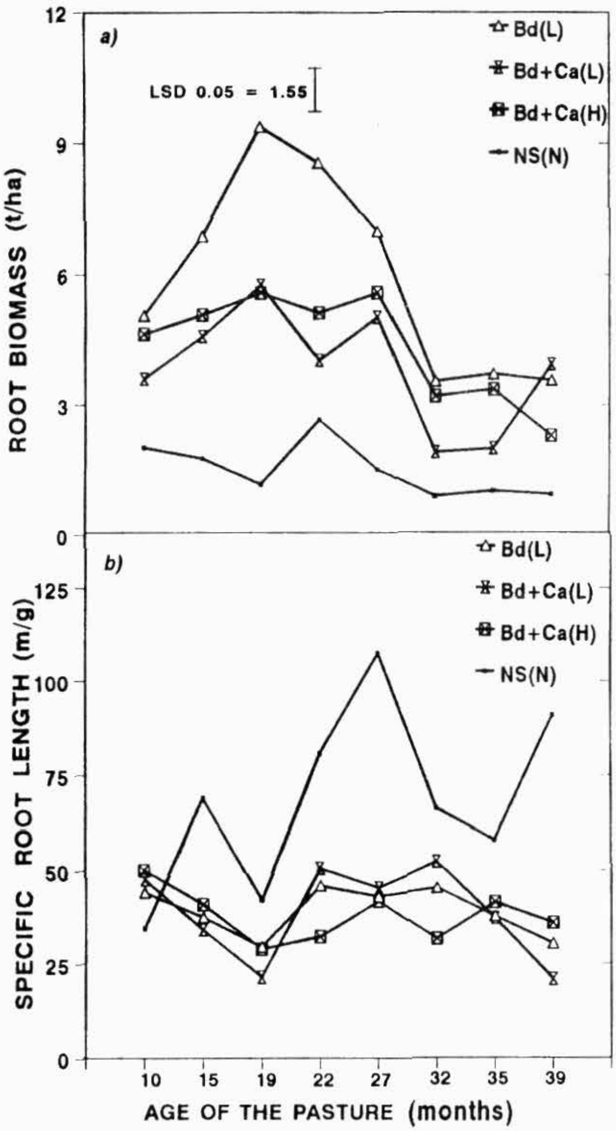


Figure 3. Changes in (a) total root biomass and (b) specific root length in several pastures established in a clay loam Oxisol site at Carimagua. Pastures were established in August 1990 and grazing began at 10 months after establishment (June 1991). Improved pastures were grazed at medium stocking rate. Bd = *B. dictyoneura*; Ca = *C. acutifolium*; NS = native savanna. L = low initial fertilizer; H = high initial fertilizer; N = no fertilizer application (see text for rates).

(Figure 3a). Estimations of root turnover (k, per year) based on the changes in root biomass over time (k = Wy/Wmax where Wy = annual increment, Wmax = maximum biomass) indicated that root turnover was 2 to 3 times greater in 3 -year-old improved pastures (0.97 to 1.65) than that of native savanna (0.48). Specific root length, which is a measure of the fineness of the root system, was greater in the native savanna pastures than that of improved pastures (Figure 3b).Further research is needed to assess root turnover and root residence time of long-term pastures (> 5 years of pasture age).

Results summarized in Table 5 show changes through time in pool size of nutrients in roots of several pastures. The changes in N and P pools reflect in part the changes in root biomass of these pastures. The amount of N present in roots of improved pastures was up to 18 kg/ha while in native savanna it was little over 6 kg/ha. The extent of P pool size in roots of improved pastures was also up to five times greater than that of native savanna. Similar trends were observed with K and Ca pool sizes in roots. These results indicate the extent of nutrient cycling via root turnover in pastures under grazing.

Determination of root biomass and root length in long-term grass alone or legume-based pastures (> 5 years-old) indicated that the total root biomass in these pastures (*B. decumbens/P. phaseoloides; B. humidicola/A. pintoi*) was between 0.8 and 1.4 t/ha. This observation indicates that well-managed pastures (with maintenance fertilizer applications for every two years) may not require extensive root system if cycling of nutrients and soil biological activity is greater in the top 20 cm soil profile. However, their small root system may exhibit a greater turnover than that of short-term pastures which are yet to reach an equilibrium status in terms of nutrient cycling. Further research work is needed to verify this hypothesis. Estimation of specific root length in these long-term pastures indicated that it was greater in *B. decumbens* than that of *B. humidicola* **[I. M. Rao, P. Herrera and J. C. Granobles]**.

2.2 Contribution of pastures to carbon sequestration in soil

In conjunction with Tropical Lowlands Program, sequestration of C in soil at depth (0-80 cm) by improved pastures compared to native savanna was estimated at Carimagua (Figure 4). Well managed

Table 5. Changes through time in pool size of nutrients in roots (kg/ha) of several pastures established in a clay loam Oxisol site at Carimagua (see Figure 3 for treatment details).

Nutrients	Pastures	Age of the pasture (months)					
		10	15	19	32	35	Mean
Nitrogen	NS (N)	5.71	6.23	4.60	3.81	2.70	4.61
	Bd (L)	9.40	16.59	15.42	13.17	7.51	12.42
	Bd+Ca (L)	10.82	18.04	12.46	8.08	6.27	11.13
	Bd+Ca (H)	15.26	17.35	16.43	14.64	7.77	14.29
Phosphorus	NS (N)	0.41	0.41	0.15	0.13	0.59	0.34
	Bd (L)	1.17	1.96	3.63	0.99	0.14	1.58
	Bd+Ca (L)	1.07	1.35	2.04	0.34	0.07	0.97
	Bd+Ca (H)	1.47	1.63	2.00	1.41	0.15	1.33

NS = native savanna; Bd = *B. dictyoneura*; Ca = *C. acutifolium*

N = no fertilizer; L = low initial fertilizer; H = high initial fertilizer application long-term (9 year-old) *Brachiaria humidicola* alone pastures increased soil C concentration up to 80 cm depth which resulted in sequestration of 25 tons of C per hectare more than that of the nearby native savanna pastures. Inclusion of a legume component (*Arachis pintoi*) for 5 years significantly increased soil C concentration and markedly improved the amount of C sequestered in soil, almost trebling the amount (75 tons per hectare more than the native savanna).

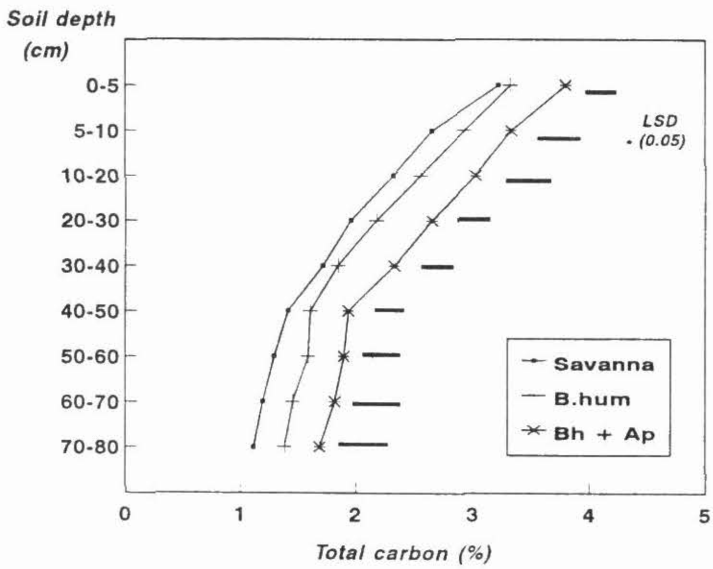


Figure 4. Soil organic carbon distribution by depth in improved grass alone (*Brachiaria humidicola* (Bh) and Bh/*Arachis pintoi* pastures compared to native savanna of a clay loam Oxisol at Carimagua.

We do not yet know the dynamics of C in the soil in these pastures. But results on C:N ratios of the soil from these pastures indicate that not only the C:N ratio of soil from native savanna unusually high at 21.5 but also it shifted to 33.2 in improved legume-based pasture. For this to occur, the C:N ratio of the newly accumulated soil organic matter must be very high. Thus there is a need to understand the dynamics of soil-plant processes associated with C sequestration in improved pastures. These results indicate that legume-based pastures not only contribute to greater animal production but also moderate the rise of atmospheric carbon dioxide, thereby reducing global warming **[I. M. Rao, M. Fisher, P. Herrera and R. Garcia]**

2.3 Root production in grass-legume associations sown with crops

Root production and turnover by legume-based pastures can contribute to soil improvement and sustainable crop production from crop-pasture rotations. Root biomass and root length distribution (up to 80 cm soil depth) have been monitored in a grass/legumes association (*B. humidicola* CIAT 679 + *A. pintoi* cv Mani forrajero + *S. capitata* cv Capica + *C. acutifolium* cv Vichada) compared to that of native savanna treatment. The grass/legumes association was sown with upland rice in May 1993. At 1.5 years after establishment (November 1994), total root biomass (t/ha) of the improved pasture was 1.96 compared to 0.93 in native savanna. Estimation of specific root length (m/g) indicated that native savanna had greater fine root production; the values were 90 for savanna, and 59 for improved pasture. **[I. M. Rao, D. K. Friesen and J. C. Granobles]**.

Results obtained through 1995 indicate that root production and turnover in legume-based pastures not only improve cycling of nutrients but also contribute to substantial sequestration of C in soil. However, the extent of root turnover in long-term pastures is yet to be determined. In addition, we need to determine the dynamics of C in soil under improved pastures.

3. Development of stable grass-legume associations

Tropical forage legumes play a key role by fixing N, by stimulating cycling of other nutrients, and by enhancing both quantity and quality of forage on offer to animals. However, legume-based pastures are often unstable. There is a need to define and model plant attributes that contribute to stable-grass legume associations.

3.1 N fixation and N transfer by legumes

Rates of N fixation by forage legumes are normally measured during the first or second year of pasture establishment. There have been only few reports of N fixation rates over longer time periods as it is generally assumed that the legume continues to fix N at similar rates to those measured during initial years of pasture establishment. However, this assumption needs to be verified because of two reasons: (1) soil fertility may be declining after the initial fertilizer application given at establishment; and (2) decreased soil fertility is known to reduce the percent of the legume's N derived from fixation.

Using ^{15}N isotope dilution technique, N fixation was measured for three successive years under field conditions in three forage legumes *Arachis pintoi, Centrosema acutifolium* and *Stylosanthes capitata*. The legumes were grown in association with *Brachiaria dictyoneura* with two levels of initial fertilizer application (low and high) on two contrasting soil types (clay loam and sandy loam) at Carimagua. Results showed that the main effect of fertility was on legume population and not on N fixation per unit biomass (Tropical Lowlands Program annual report, 1994). Thus the amounts of N fixed by tropical forage legumes can be estimated from a measurement of legume biomass in the pasture. Thus such estimates can be done very simply in farmer's fields **[R. Thomas, M. Rondon, H. F. Alarcón and N. M. Asakawa]**.

3.2 Role of legumes in nutrient cycling

Determination of shoot nutrient uptake (N, P, K and Ca) in stable grass/legume associations of *Brachiaria decumbens/Pueraria phaseoloides* and *Brachiaria humidicola/Arachis pintoi* indicated that the introduction of legumes not only improve the supply of N through biological N fixation but also contribute to greater acquisition and cycling of Ca from low fertility acid soils (Table 6) **[I. M. Rao and P. Herrera]**

Table 6. Differences in shoot nutrient uptake of long-term grass/legume pastures from a clay loam Oxisol at Carimagua.

Pasture	Age (yrs)	Nutrients (kg/ha)			
		N	P	K	Ca
B. decumbens (Bd)	13	23	2.2	27	6
Bd/P. phaseoloides	13	40	2.5	18	16
B. humidicola (Bh)	9	33	6.2	58	6
Bh/A. pintoi	9*	76	7.0	69	14

* *5 years with the legume.*

3.3 Legume persistence

Studies of the dynamics of plant survival and vigor of introduced legumes in association with vigorous grasses will provide a better understanding of the attributes needed for legume persistence.

3.3.1 Persistence of legumes on sandy infertile soils: The legumes, *Stylosanthes capitata* cv Capica and *Centrosema acutifolium* cv Vichada, sown in association with *Brachiaria dictyoneura* cv Llanero, did not persist for longer than two years when sown on a sandy loam soil at Carimagua whereas they persisted longer on a clay loam soil. This poor persistence may have been due to an inability to compete with the grass for nutrients, in particular K, and during the dry season for moisture. On the other hand, *S. capitata* cv Capica had persisted for 8 years in association with *B. decumbens* on a similar soil in the same locality, under conditions where the aggressive nature of the grass was reduced by infestation with spittlebug.

An experiment was established in June 1994 to investigate the cause of the poor persistence. Four legumes, *A. pintoi* cv Mani forrajero, *S. capitata* cv Capica, *C. acutifolium* cv Vichada and *D. ovalifolium* CIAT 13089, were introduced into established grass pastures of *B. dictyoneura* and *B. decumbens* by planting in cultivated strips and fertilizing with 4 combinations of P and K fertilizer.

There was satisfactory establishment of legumes, except for Vichada centro in *B. decumbens* due to seedlings being eaten by grasshoppers. There was a strong response to P and K at establishment. The experimental areas are situated within larger grazed paddocks and have been opened to grazing every 3- 4 weeks.

In the second season, 1995, all legumes are still persisting but with variable vigor. There is still a strong response to P. The response to K is now variable across species and within treatments of species apparently more susceptible to K deficiency. There is no response to P or K by *D. ovalifolium*. The variable response within plots is probably due to small spatial differences in soil K. The legume Capica appears to be more susceptible to K deficiency than Maní forrajero and Vichada.

The overall vigour of the plants is highest for *D. intortum* (which is grazed very little) followed by Capica stylo (which is severely grazed) and with similar vigor of Maní forrajero and Vichada centro. The performance of the *A. pintoi* cv mani forrajero (CIAT 17434) has been disappointing in 1995. It has less vigor, is more yellow and appears more susceptible to diseases such as anthracnose and leaf spot than in 1994. Newly established *A. pintoi* CIAT 22160 in the same area appears much more vigorous.

The dynamics of plant survival of the original plants and succeeding generations, plant cover and ingress of the grass into the legume rows is also being monitored. **[P. Kerridge and J. C. Granobles]**.

3.3.2 Legume establishment and persistence with different grasses:

Some new accessions of legumes and grasses are now available for regional evaluation. It was considered useful to evaluate the legumes in terms of their compatibility and persistence when grown in association with different grasses.

A field experiment was established at Carimagua in June 1995 on a sandy loam Oxisol to study associations of the legume accessions: *A. pintoi* CIAT 22160, *S. guianensis* CIAT 11833, and *S. capitata* cv Capica with the stoloniferous grass, *B. dictyoneura* cv Llanero, and tussock grass, *P. maximum* CIAT 6944. Pure grass treatments were also established to evaluate the effect of the legume on grass growth. Two fertilizer treatment levels have been included to allow a study of the interaction of nutrition on compatibility. **[P. Kerridge, Y. Saito, I. M. Rao and J. Granobles]**

3.4 Development of simulation models

Computer simulation models of stable grass-legume associations can make a valuable contribution to both furthering our understanding of the processes determining pasture productivity and predicting pasture performance in different areas. User-oriented simulation models can also greatly facilitate the task of pasture management to sustain animal production.The Decision Support System for Agrotechnology Transfer (DSSAT) Version 3.0 has 10 crop models including peanut. These models share a common input-output format, and they are similar in level of detail. They operate on a daily time step and are based on an understanding of biophysical processes. The future DSSAT 3.1 version may cover a pasture grass (Bahia grass). We have made some initial attempts to modify the peanut model to forage *Arachis* model.

CROPGRO-Peanut model simulates growth of peanut

(*Arachis hypogea*) using a FORTRAN coded program. The model has inputs from files to define species and cultivar attributes. It is a mechanistic model which includes leaf-level photosynthesis, hedge-row canopy light interception, soil N balance, N uptake, N fixation, additional energy balance options, improved crop development routines, and pest-coupling approaches.

The objective is to modify "species" and "cultivar" files of CROPGRO-Peanut to simulate growth and productivity of forage *Arachis* under grazing. Using a generic grain legume model from DSSAT v.3.0, we modified a number of species parameters and genetic coefficients of peanut. After modifying crop specific parameters or the "species" file, we modified a number of peanut genotype coefficients. Use of pest coupling subroutine allowed us to simulate rotational and continuous grazing effects on forage *Arachis* growth and productivity at Carimagua. Further modifications of the parameters and coefficients together with some FORTRAN coded program changes will be needed to complete the development of a practical forage *Arachis* model. This work is being carried out in collaboration with scientists from the University of Florida, University of Georgia and IFDC **[I. M. Rao, K. J. Boote, J. W. Jones, G. Hoogenboom and W. Bowen]**.

Results from the above studies indicate that N contribution by forage legumes is dependent on the legume biomass proportion in the pasture. Introduction of legumes can not only improve N cycling in pastures but also stimulate cycling of other nutrients such as Ca. Thus while the benefits of legumes to animal production and soil improvement are clearly demonstrated with persistent species such as *A. pintoi* and *P. phaseoloides*, it remains a challenge to identify plant attributes that will improve the persistence of *Stylosanthes* species when associated with vigorous grasses.

Project: Adaptive attributes of forages to infertile soils

Proposed activities for 1996

1. **Plant attributes that confer tolerance to low fertility soils**

- Establish a field experiment to determine genotypic differences in N and P acquisition and utilization in *Panicum maximum*.

- Determine genotypic differences in N, P, and Ca acquisition and utilization in *Arachis pintoi* taking advantage of a field experiment established in Carimagua.

- Conduct a glasshouse trial to determine genotypic differences in root and shoot attributes of 24 genotypes of *Arachis, Stylosanthes*, and *Centrosema* species when grown in a clay loam Oxisol with different amounts of fertilizer application.

- Continue studies on biochemical and molecular mechanisms of tolerance to acid soil stress in *Brachiaria* species (graduate thesis).

2. **Role of roots in nutrient cycling and carbon sequestration**

- Continue measurements on root turnover and C sequestration in soil from improved pastures compared to that of native savanna at Carimagua.

- Continue measurements on changes in root biomass and root length of grass-legume pastures sown with crops.

3. **Development of stable grass-legume associations**

- Continue field experiments to determine factors that effect persistence of legumes in grass-legume associations.

- Continue investigation of shoot and root attributes that improve nutrient acquisition and legume persistence in grass-legume associations.

- Make further modifications to the forage *Arachis* model.

Project: Forage Components of Known Performance in Production Systems

Project Coordinators: Carlos E. Lascano (Tropical America) and
Werner Stür (Southeast Asia)

Forage Components in Tropical America

Rationale

Forages are utilized as components in a diverse range of agricultural production systems. To ensure that new forage components will be acceptable to farmers, there is a need to assess their performance in targeted production systems in addition to knowing that they are adapted to a particular environment. This includes knowing whether farmers consider these new forage components as a useful addition to those they already have, are feasible to implement and give an economic return. This can be achieved using participatory research methodology.

To increase adoption of new forage cultivars, seed multiplication and delivery systems must be developed. Because some new forage technology, in particular, legume-based forage systems, may not be readily adopted, it is also important to have the collaboration of sociologists and economists in the development and on-farm evaluation of new forages.

The activities in this project in tropical America are mainly carried-out in collaboration with others, both with the Natural Resource Management Programs in CIAT, other IARC's and with NARDS. This project is viewed as an interface between germplasm development and adoption of new forage component technology by farmers.

Objective

To develop and evaluate the productivity, environmental and socioeconomic impact of forage components for different production systems.

Main Activities in Tropical America

1. Shrub legumes for acid infertile soils.
2. Legumes for fallow improvement.
3. Grass-legume associations for lowlands.
4. Grass-legume associations for crop-livestock systems.
5. Forages for soil cover, erosion and weed control.
6. Seed multiplication.
7. Socio-economic studies on adoption of new forages by farmers.

Highlights (1994-95)

- Found that *Cratylia argentea* when fed to confined sheep on low quality grass and to cows grazing pure grass pastures resulted in increased intake and milk yield, respectively.

- Demonstrated that *Centrosema macrocarpum* (CIAT 5713) was a useful legume for improvement of fallow land for cropping in hillsides.

- Found that *Arachis pintoi* (CIAT 22160) is more competitive than *A. pintoi* cv. Maní forrajero (CIAT 17434) in crop-pasture systems in savannas.

- Identified dwarf elephant grass cv. Mott (*Pennisetum purpureum*) as a promising new barrier grass option for mid altitude hillsides.

- Found that *Stylosanthes guianensis* (CIAT 184) was successful in suppressing *Imperata cylindrica* and in control of soil erosion in young forestry plantations in Southeast Asia.

- Found excellent erosion control and high yields of cassava when grown after a two year rotation with *Brachiaria decumbens*/*Centrosema macrocarpum* in an Ultisol (Quilichao).

- Showed that competition imposed to cassava by intercropped forage legumes differed among species with similar above ground biomass mainly due to below ground root density.

- Showed that grasses and legumes for fallow improvement in hillsides can be introduced directly or during the final phase of cropping with minimal soil disturbance or herbicides and small application of P.

- Initiated an on-farm Special Funded Project in forest margins of Caqueta, Colombia to test new grass-legume pastures and shrub legume options in dual purpose cattle systems.

- Found through a survey that *Arachis pintoi* is being disseminated by commercial seed companies in many regions of Colombia and that it is in an early phase of adoption by farmers.

- Found by ex-ante analysis that the marginal profitability for milk production in *Arachis pintoi* based-pastures was higher than in N fertilized pastures, even with periodic renovation of the legume-based pasture.

- Found through review of literature from Latin America that adoption of tropical forages is a highly complex long-term process involving technical, sociological and economic issues.

- Trained key personnel from Indonesia, Laos, Malaysia, Philippines, southern China, Thailand and Vietnam in participatory research methodology.

1. Shrub legumes for acid soils

The TFP undertook the task of selecting shrub legumes for acid soils given their role as a source of supplementary fodder for livestock and on soil improvement. Species that have been selected for on-station and on-farm feeding studies are *Cratylia argentea, Codariocalyx gyroides, Flemingia macrophylla* and *Desmodium velutinum*.

1.1 Utilization of *Cratylia argentea* as a supplement to low quality grasses

The shrub legume *Cratylia argentea* was nominated by the TFP as a priority species due to its high potential value as fodder in tropical livestock production systems in subhumid areas with acid soils. Initial results from feeding trials showed that intake by sheep of immature fresh leaves of *C. argentea* was low, but increased when the forage was wilted or sun-dried or when it was mature (Raaflaub and Lascano, 1995).

Subsequent feeding trials were carried-out to determine the feeding value of *C. argentea* as a supplement to low quality grasses. Initial results indicated that when *C. argentea* (50% of the ration) was fed in one meal to sheep on a low quality grass, there was no effect on intake of the total ration (Fassler and Lascano, 1995). Thus a second experiment was designed to determine the effect of continuous supplementation to sheep of different levels of *C. argentea* in combination with a low quality grass.

Sheep housed in metabolism crates were either fed grass alone (7% CP) or grass in combination with fresh wilted leaves of *C. argentea* (19% CP) at three levels (10, 20 and 40% on a dry matter basis). Results summarized in Table 1, showed that total dry matter intake increased linearly with increasing levels of *C. argentea* in the ration. However, as *C. argentea* increased in the supplement there was a reduction (8 to 34%) on grass intake (i.e. substitution effect). Similarly, total tract digestibility (DM and NDF) slightly decreased with increasing levels of *C. argentea* in the forage consumed. As a consequence, intake of digestible dry matter did not increase with supplementation of *C. argentea* fodder. In contrast, protein intake was higher in sheep supplemented with legume fodder and as a result more nitrogen was apparently absorbed in the small intestine.

Our results indicate that supplementing *C. argentea* to ruminants fed a low quality grass did not improve energy intake, mainly because of the relatively high level of indigestible cell wall in leaves of this legume. On the contrary, supplementation with *C. argentea* greatly improved the protein status of the animals. [**W. Quiñónez, P. Avila, and C. Lascano**].

1.2 Milk yield responses with shrub legumes

In our Quilichao research facility we have evaluated the use of fodder from shrub legumes as a supplement to milking cows grazing pastures of *Brachiaria* spp. Results summarized in Table 2, show that dry season supplementation with wilted leaves of *C. argentea* resulted in 13% average increase in milk yield in cows grazing *B. dictyoneura* cv. Llanero. In the same experiment, supplementation with lower quality fodder of *F. macrophylla* did not increase milk yield relative to the control. Differences in quality between the two legumes were reflected in dry matter intake (3.0 vs 6.3 g/kg BW/d) and in milk urea (9 vs 15 mg%) which is indicative of protein degradation in the rumen.

Table 1. Voluntary intake, digestibility and N utilization by sheep fed a low quality grass (G) supplemented with *C. argentea* (L) (W. Quiñonez, pre-graduate thesis).

Item	Treatments				SE
	G	G + 10% L	G + 20% L	G + 40% L	
Intake					
Total (DM/kg BW/d)	21.6b	23.5ab	24.7a	25.5a	0.9
Grass (DM/kg BW/d)	21.6a	19.9ab	18.2b	14.3c	1.0
Digestibility					
DM (%)	57.5a	55.6ab	55.1ab	52.1b	0.9
NDF (%)	63.7a	60.1b	58.6b	54.3c	0.9
N utilization					
Intake (g/d)	8.6d	11.9a	14.2b	17.6a	0.4
Duodenal N (g/d)	8.4d	10.8cd	12.5b	14.2a	0.4
Fecal N (g/d)	3.6a	4.7b	5.2b	6.0a	0.2
Absorbed N (g/d)	4.8c	6.1b	7.3a	8.2a	0.3

a,b,c,d means with different letters in the same row are different (P<0.05).

Supplementation of *C. argentea* during the wet season to cows grazing *B. dictyoneura* cv. Llanero resulted in little or no milk yield increment (Table 2). However, it should be noted that milk yield did not increase with supplementation of a high quality legume such *Centrosema macrocarpum*.

The increment observed during the dry season in milk yield with supplementation of *C. argentea* is similar to what we found when cows grazed pastures of *B. decumbens* complemented with protein banks of *Centrosema* spp. However, in the same location milk yield during the dry season increased by 30% in cows

Table 2. Short-term milk yield (fat corrected) of cows grazing *Brachiaria dictyoneura* pastures with and without supplementation of legumes (Lascano, C. and Avila, P., unpublished results).

Season	Treatments	Legume		Milk yield
		Offered	Consumed	
		(gDM/kgBW/d)		(kg/cow/d)
Dry	Control	--	--	6.2b
	F. macrophylla	8.4	3.0	6.4b
	C. argentea	8.1	6.3	7.0a
Wet	Control	--	--	6.8
	C. macrocarpum	13.5	10.5	7.0
	C. argentea	11.3	7.8	6.9

a,b means for each season are different (p<0.10).

grazing pastures of *Brachiaria* spp. in association with *Centrosema* spp. This greater milk yield response in grass/legume pastures could be related to increased intake as suggested by our results with sheep and to better energy:protein balance in the diet. Supplementation of *C. argentea* with high levels of degradable protein, results in excess rumen ammonia due to lack of an energy source. Thus future studies on supplementation of milking cows will consider the combination of legume fodder with cut and carry grasses as energy sources **[P. Avila and C. Lascano]**.

1.3 On-farm testing of shrub legumes

During 1994 we established *C. argentea* at two sites (1200 and 1400 m.a.s.l.) in the hillsides of Cauca, Colombia with the idea of supplementing milking cows during the dry season. However, it soon became clear that *C. argentea* was not adapted to the higher site and that it had slow establishment in the lower site. Thus there is a clear need to better define climatic (i.e. temperature) and edaphic (i.e. fertilization) factors related to the establishment and productivity of *C. argentea*.

Results from agronomic evaluation of shrub legumes in Caqueta, Colombia showed that *C. gyroides* was well adapted to this humid environment. As a result, we introduced this legume in two farms in Caqueta with the idea of providing protein-rich fodder to milking cows being supplemented with elephant grass or sugar cane planted near the milking corrals. Establishment of *C. gyroides* with seed was not successful because seed was unburied and consumed by chicken raised by most farmers of the region near the house and corral facilities of the farm. As a consequence, the areas were fenced-out and replanted with seedlings of *C. gyroides* grown in a nursery. These activities represent an extra cost to the farmer and need to be considered in the economic analysis of supplementation practices involving fodder from shrub legumes.

On-station and on-farm testing of shrub legumes is also being carried-out in a subhumid environment in Atenas, Costa Rica. Results showed that *C. argentea* is an excellent option for dry season supplementation of milking cows in dual-purpose systems. Consequently, plots of *C. argentea* are being established in 10 dual purpose cattle farms, which participate in a joint (ECAG-MAG-ODA) project that is looking for improved feeding alternatives for the dry season in the Central Pacific region of Costa Rica. In addition, *C. argentea* (CIAT 8516) was established as contour barriers together with improved grass-legume pastures in a water-shed reclamation project (MAG-MIRENEM-U. of Costa Rica) in the puriscal region of Costa Rica.

We have identified *C. argentea* as a promising source of protein-rich fodder for dual purpose cattle in locations with long dry seasons. However, we still have to demonstrate the potential value of *C. argentea* under farm conditions. Thus priority should be given to on-farm evaluation of *C. argentea* in terms of establishment, cutting management and supplementation to milking cows in the dry season. In addition, it is important that we identify the antinutritional factor present in young leaves of *C. argentea* in order to screen new germplasm that will soon become available to the TFP **[P. Avila, G. Ruiz, C. Lascano, P. Argel and S. McLean, ODA, UK]**.

2. Legumes for Fallow Improvement

Many smallholder farmers in upland farming systems follow a rotational system in which a period of cropping is followed by a period where the land is left idle or in fallow. The natural vegetation is generally unproductive even for grazing though the fallow does serve to reduce disease incidence and increase soil organic matter. Introduction of legume mulches into the fallow could have a large impact on overall productivity through soil improvement and provision of quality feed to livestock.

2.1 Effect of managed legume fallow on subsequent crop yield

Site: North Cauca, Colombia; $2^0 47'$ N, $76^0 32'$W, 1500 masl. The terrain is highly dissected with steep slopes with the land used for cropping cassava, beans and maize, vegetables and grazing by dual purpose cattle. Rainfall is bi-modal and occurs during April-July and October-December with an average total of 1800 mm per annum. The soil was a sandy clay loam of pH 5.4, organic matter (%) 11.5, extractable P (Bray 1 ppm) 1.3, exchangeable bases (me/100g) Ca 1.09 , Mg 0.25 and K 0.23. The on-farm site was under fallow and had not received any fertilizer for five years.

Experiment: An experiment was conducted to observe if a legume cover would indeed influence subsequent crop yields and if length of fallow was important. Different P fertilizer levels were included as little legume growth was expected without P addition and

poultry manure included as it is the most commonly used fertilizer used for cash crops in the area.
Treatments:
Covers-
(i) *Centrosema macrocarpum* (CIAT 5713),
(ii) Cowpea followed by pigeon pea after 6 months
(iii) Natural cover

Fertilizer (kg/ha) applied to legume covers only -
(i) 20 P
(ii) 100 P
(iii) 100 P + 1000 lime,50 K, 20 S, 20 Mg + CuBZn
(iv) Poultry manure 8 t/ha, equiv. to 240 N, 110 P, 150 Ca, 30 Mg, 150 K

Additional Ca was added to the 20 P treatment equivalent to that added in the 100 P treatment. The existing natural fallow system was left untouched as a control for comparison with the two legume fallow systems.

The covers were planted in rows in October 1993 followed by maize crops in October 1994 and April 1995. The covers were incorporated into the soil 2-3 weeks before planting the maize which was fertilized with a basal application of (kg/ha):50 P, 50 K, 20 Mg, 30 ZnBCu mixture and either 0, 25, 50 or 100 N.

Results:

Cover: While there was rapid establishment of cowpea, the other legumes took about 6 months to form a good cover after planting. The cowpea, which was a grain type, matured and died within 4 months regardless of fertilizer treatment. The other legumes formed dense foliage and dominated the natural vegetation. There was a large effect of fertilizer on rapidity of establishment and yield of the legumes (Table 4). The amount of herbage at the time of incorporation does not fully reflect the legume yields as there was considerable leaf fall each dry season.

Soil nutrients: Fertilized legume covers increased the amount of available soil nitrogen for the following crop (Table 3). The amount of labile N (NO_3+NH_4) was more than double that in the natural fallow. Samples were taken prior to N fertilization of the maize. There was no movement of N below 20 cm at 30 days after planting as shown by data from samples taken to 40 cm depth. Surface soil samples (0-10 cm) taken at the time of harvest of maize showed that fertilizer treatments had no effect on pH whereas soil P had increased from 1.03 ppm to 2.6 ppm in the 100 kg/ha P treatment, to 3.3 ppm with the manure treatment and to 1.8 ppm where only 50 kg/ha P had been applied to the maize crop.

Maize yield: Fertilized legume covers increased the yield of the following maize crops over that of the natural fallow (Table 4). The effect was greater where the legume was fertilized with 100 cf. 20 kg/ha P. Maize yields were even higher where additional nutrients had been added by fertilizer or manure.

Table 3. Total labile N (NO_3+NH_4) at the time of planting and at 30 days after planting the maize crops for the legume treatments receiving 100 kg/ha P and for the unfertilized natural fallow.

Cover	Depth	Crop 1[1]		Crop 2[2]	
		Planting	30 days	Planting	30 days
	(cm)		(ppm)		
Natural	0-10	7.4	11.2	7.9	8.5
	10-20	3.8	6.2	3.8	4.2
Centro	0-10	16.8	14.1	25.1	17.0
	10-20	5.2	16.9	6.6	46.8
Pigeon	0-10	13.4	18.0	19.6	9.3
	10-20	6.5	19.4	10.7	13.1

[1] After 12 months of legume cover
[2] After 18 months of legume cover

Table 4. Amount of above ground cover at time of incorporation, total dry matter yields and grain yield of maize (averaged over N rates).

Cover	Fert. Applic.	Cover 12mth	Cover 18mth	Crop 1 (12 mth) Grain	Crop 1 (12 mth) Total	Crop 2 (18 month) Grain	Crop 2 (18 month) Total
				(kg/ha			
Natural		6000	9950	350	1500	350	1500
C.m.	P20	2750	4300	750	2300	700	2850
	P100	5100	8200	1550	4250	1850	5700
	P100+	4300	5950	2550	6800	1600	5100
	Manure	4000	6600	3100	7450	2900	7850
Pigeon[2]	P20	400	9750	450	1750	350	1700
pea	P100	550	13400	450	2150	1100	3950
	P100+	600	8000	1550	4200	1450	4500
	Manure	1950	18200	1700	4550	2900	9250

[1]*Centrosema macrocarpum* (CIAT 5713)
[2]*Cajanus cajan* (CIAT 18701)

The maximum effect of *C. macrocarpum* was evident in the first maize due to high yield and coverage of the legume within 12 months. The later development of pigeon pea, which was planted at 6 months after the cowpea matured and died, meant that the maximum effect of the legume was not seen until the second planting of maize at 18 months. There was apparently little residual effect of the cowpea.

Interpretation: There are three reasons for the increase in maize yields in the legume treatments over that in the natural fallow treatments. The first is that incorporation of the natural fallow vegetation tied up some available soil N. In the second maize crop it was shown that the mean grain yields (kg/ha) of maize with vegetation incorporated or burnt were 280 and 420 kg/ha, respectively. The second is an effect of N contributed by the legume which would be expected to be proportional to the legume growth. The third is due to the additional nutrients in the '100 P + additional nutrients' and 'manure' treatments. The response to N was higher in these treatments (Figures 1). However, it is not possible to separate the latter two effects with the design that was used.

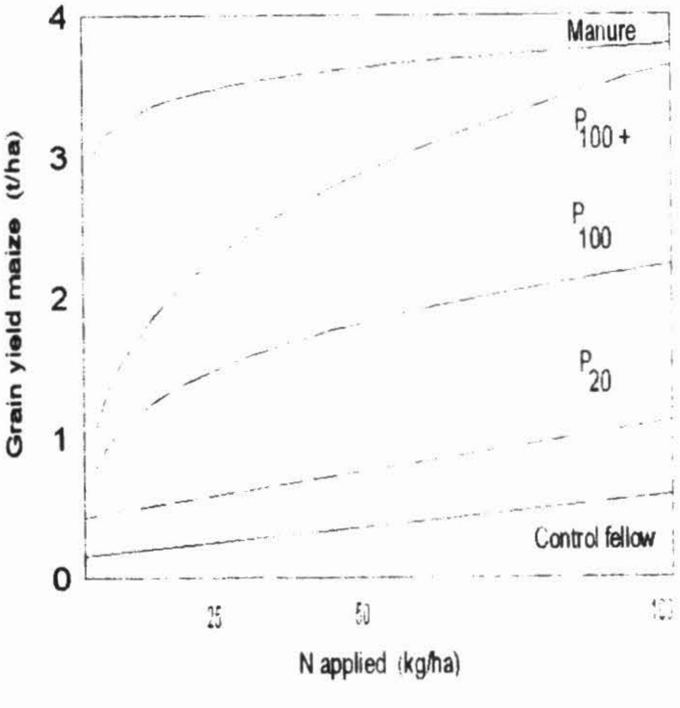


Figure 1. Response of maize to nitrogen for the different fallow treatments.

There was an interaction between fertilizer N applied to the corn and the legume covers which might be explained by the temporal difference in development of two cover crops. There was also an interaction between rates of fertilizer N applied to the corn and the fertilizer treatments applied at planting which can be assumed to be due to some deficiency in the amount or type of basal nutrients applied to the corn.

The results do demonstrate that a system where the fallow is oversown with legume has the potential to increase overall productivity of these hillside soils. The improvement occurs with six months of vigorous legume growth. Further investigation is needed to determine the appropriate management practices for legume fallows.

Incorporation of legumes is a viable practice in the hillsides where land is prepared by cultivation with oxen. A limitation to the introduction of the practice is to devise a feasible method of establishment. We believe that this might be done at the time of the second weeding or at harvest of the last crop prior to spelling of the land under fallow.

This research has also demonstrated the need for rapid establishment of legumes either through a combination of choice of species or species mixture and appropriate management practices. Further legumes are being evaluated in the Forage Genetic Resources Project for potential use as legumes for fallow improvement. [**L. H. Franco and P.C. Kerridge**].

2.2 Phosphorus fertilizer management -residual effect of applied phosphorus

Phosphorus is the most limiting nutrient for legume growth on the soils of north Cauca. A better knowledge of the residual value of applied P in these soils would assist in fertilizer management.

Two P rate trials, planted with a *Brachiaria decumbens-Centrosema macrocarpum* association, were established at the same sites used for evaluation of forages for environmental adaptation (see 2.16 for site details) in October 1993. They will be maintained under defoliation by grazing for 4-5 years to obtain a measure of the residual value of initially applied P. A harvest for dry matter yield and sampling for soil P analysis will be made every 12 months. The response surface in the first year is shown for *C. macrocarpum*

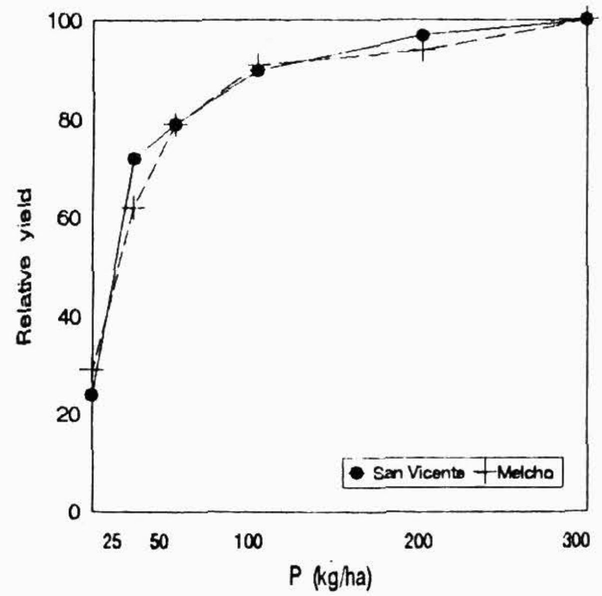


Figure 2. Response of *Centrosema macrocarpum* to phosphorus at two hillsides sites.

2.3 Establishment of improved fallow, Cauca, Colombia

Time of establishment. Introduction of legumes and grasses into improved fallow will depend on a method that can be readily used by farmers and involve minimal expenditure of time and money. It seemed appropriate to establish an improved fallow following cassava as it is often the last crop in a cropping cycle.

Site: El Pital, Cauca, Colombia; 1300 masl; 1700 mm annual rainfall. The soil was a clay loam of pH 4.0, extractable P (Bray 1 ppm) 24.1, exchangeable bases (me/100g) Ca 1.33 , Mg 0.5 and K 0.22. Farm site with cassava crop that had been fertilized with poultry manure.

Experiment: An establishment experiment was planted at the time of harvesting a cassava crop during the October-December rainy season to determine if it was feasible to establish a pasture without any soil preparation. Planting was carried out immediately the cassava crop was harvested either on 4 Nov 1994 or one month later with a mixture of legumes and the grass *Panicum maximum*. Four levels of fertilizer were applied (kg/ha) Nil, 25 P, 100 P and 4000 poultry manure. The experiment was not weeded or defoliate until 6 June when it was cut back to obtain an estimate of regrowth on 30 Aug 1995. A control area was left unplanted.

Results: There was better establishment of legumes at the first sowing date but no effect on the grass. There was also heavy establishment and growth of broad-leaf weeds, such that at the time of defoliation in June, the area appeared weed dominant. Subsequent regrowth was grass dominant (Table 5). The low legume yield was due to heavy shading by weeds during the establishment period and grass during the regrowth period while the relatively high grass yield was no doubt due to the residual effect of poultry manure applied to the cassava crop. There was little effect of an additional application of manure over that obtained with 25 kg/ha P. Weed yields in areas outside the planted area were estimated at 8-10,000 kg/ha DM.

Interpretation: It is preferable to plant the improved fallow mixture at harvest rather than to delay planting. Establishment of the legumes during the last weeding of the cassava and of the grass at harvest would probably result in a higher proportion of legume. Legume survival would also be improved by early defoliation by grazing **[L.H. Franco and P.C. Kerridge]**.

Method of Establishment. In some instances it may be desirable to establish improved legume-based fallow directly into an existing fallow that is severely degraded. An experiment was conducted investigate different methods of land preparation, sowing and fertilizer application in severely degraded fallow land that had been abandoned for cropping.

Treatments: These are shown in Table 6. The 'disturbance' treatment was a slight soil disturbance with a hoe to simulate a light discing, whereas the 'cultivation' treatment was carried out with a small rotovator. The herbicide was an application of round-up. For the 'band' treatment, seed was sown in a row. A mixture of the grass, *Brachiaria dictyoneura* (CIAT 6133), and legumes, *Centrosema macrocarpum* (CIAT 5713), *C. acutifolium* (CIAT 15249), *Vigna unguiculata* (CIAT 4537) and *Stylosanthes guianensis* (CIAT 11844) were sown on 25 Nov 1994. Early observations were made of plant numbers and cover of the sown species and a harvest was made on 4 Oct 1995. There was occasional defoliation by cattle during the year but otherwise no attempt was made to manage the pasture.

Results: There was good establishment and persistence of the *B. dictyoneura* and legumes, *V. unguiculata* and *S. guianensis*, in particular, in the herbicide and cultivated treatments that had received at least 25 kg/ha P (Table 6). The centrosemas established but did not persist. The reason is not clear. There was some insect attack but it is possible that disappearance was due to a trace element deficiency. Mo response has been obtained in a pot experiment with a similar soil. There was no grass establishment without a herbicide treatment or some disturbance. This suggests that the grass cannot germinate and survive in an existing grass sward due to competition from established plants.

Table 5. Regrowth of grass, legume and weeds following their establishment at the time of harvest of a cassava crop.

Planting date	Fertilizer	Dry Matter Yield (kg/ha)		
		Grass	Legume	Weed
4 Nov 94	P_0	3800	160	1500
	P_{25}	7000	200	600
	P_{100}	9200	200	100
	4 ton Manure	5900	220	600
4 Dec 94	P_0	4800	200	1800
	P_{25}	8500	0	400
	P_{100}	9900	0	0
	4 ton Manure	10300	170	1200

Table 6. Dry matter yields of sown grass and legume and other (native grass and broad-leaf) species as influenced by various establishment treatments.

Treatments			Yield (kg/ha DM)			
Land preparation	Sowing	Fertilizer	Sown Grass	Sown Legume	Other	Total
Nil	Band	P_0	40	150	1700	1890
		P_{25}	0	250	2200	2450
		P_{100}	0	320	3730	4050
	Broadcast	P_0	0	0	1190	1190
		P_{25}	0	180	2230	2410
		P_{100}	0	250	4170	4420
Herbicide	Band	P_0	63	140	1850	2050
		P_{25}	250	440	3580	4270
		P_{100}	150	620	3530	4300
	Broadcast	P_0	200	230	2310	2730
		P_{25}	210	700	3590	4500
		P_{100}	170	760	3630	4560
Disturbance	Band	P_0	210	150	1060	1420
		P_{25}	580	610	1940	3140
		P_{100}	400	980	2340	3730
	Broadcast	P_0	60	80	1050	1190
		P_{25}	170	810	2590	3570
		P_{100}	990	1400	2400	4790
Cultivation	Band	P_0	300	290	280	870
		P_{25}	970	650	570	2190
		P_{100}	790	850	1250	2880
	Broadcast	P_0	220	120	620	950
		P_{25}	390	380	1410	2180
		P_{100}	770	780	1880	3430

There was stimulation of growth of native legumes and grasses by fertilizer application. However, the amount of native legume was always much less than sown legume. Nevertheless, this observation suggests that it would be worth while to investigate the effect of fertilizer application on native pastures, in particular, those used for milk production, to determine if the there is an increase in abundance of native legumes with time.

The conclusion from this study is that a low application of P fertilizer and set back of native pasture with herbicide or some slight disturbance will enhance establishment on degraded fallow land. **[L. H. Franco and P.C. Kerridge].**

3. Grass-Legume Associations for Tropical Lowlands

Grass-legume associations selected in small plot grazing experiments and with known animal production potential are tested in medium to large scale pastures with participatory methods in order to enhance adoption by farmers and provide feed-back to the germplasm development group in the TFP.

On-farm pasture evaluation activities by the TFP are currently under-way in dual-purpose cattle farms in the forest margins of Florencia, Caqueta and in Costa Rica. In addition, the Tropical Lowlands Program is evaluating grass-legume pastures in crop-pasture rotation systems in the Llanos of Colombia and Cerrados of Brazil.

Legume-based pastures in forest margins of Caqueta, Colombia

The Andean piedmont of the Amazon basin in Caqueta, Colombia with acid soils (Ultisol) and high rainfall (3600 mm/year) has been subject to an intensive process of colonization. As a consequence, there are an estimated 1 million hectares totally deforested with much of it dedicated to dual-cattle production. Beef and milk are produced exclusively on grazed pastures mostly covered by native grasses (i.e. *Homolepsis aturensis*) of low carrying capacity and by *Brachiaria decumbens* in different stages of degradation as a result of spittlebug and soil nutrient deficiencies. Milk production is in the order of 3-4 liters/cow/day and 600-700 liters/hectare/ year.

Over the period 1987-1990, CIAT collaborated with several institutions (ICA, U. de la Amazonia, Fondo Ganadero del Valle and NESTLE) present in the region in the on-station and on-farm evaluation of forage germplasm with potential for reclaiming degraded pastures. The most successful pasture legume was *Arachis pintoi* in association with different *Brachiaria* species.

As a follow-up to this initial effort, a collaborative project on technology transfer to farmers of legume-based pastures is currently underway with financial support of Nestle de Colombia. The aim of the project is to demonstrate the feasibility of intensifying dual cattle production in the region, through reclamation of degraded pastures in already cleared forest land. The major outputs of the 4-year project will be:

1. New forage options to farmers.
2. Alternative methods for the establishment of grass-legume pastures.
3. Established grass-legume pastures in 10 farms with direct assistance of the project and in other 20-30 farms by the initiative of farmers.
4. Quantified effect of grass-legume pastures in animal production and soil enhancement.
5. Trained professionals in forage agronomy and in participatory methods of on-farm pasture evaluation.

The project was initiated in February 1995 with the appointment of a coordinator (G. A. Ruiz) to supervise day to day activities and with the conformation of an inter-institutional committee (TFP-CIAT, CORPOICA-Macagual, U. de la Amazonia and Nestle) to plan and review activities. What follows is a summary of major activities carried-out so far by the project.

3.1 Establishment of grass-legume pasture

The goal for 1995 was to introduce *A. pintoi* (CIAT 17434) in 5 farms that hand land prepared to plant *Brachiaria* spp. A total of 40 ha of *A. pintoi* were planted with sexual seed (12 kg/ha-40% germination) in 4 farms (farm 1: 6 ha; farm 2: 6 ha; farm 3: 15 ha and farm 4: 13 ha). A pure grass pasture was left as control in each farm. A major cost in the establishment of *A. pintoi* has been labour (range 3 to 5 man-days/ha), since planting has been done manually due to lack of mechanical planters in the region. Initial establishment of *A. pintoi* was excellent in all farms. However, in one of the farms the legume was lost soon after establishment due to heavy competition from *B. decumbens* and *B. brizantha*, both of which establish very fast. It was not possible to graze the newly established pasture to eliminate competition, since the farmer felt that this would be detrimental to the grass, a belief shared by many farmers. In other farms, early grazing of pastures with *A. pintoi* in association with *B. decumbens* resulted in excellent legume development. Another problem encountered, was poor seed germination of *B. humidicola*, which resulted in heavy weed infestation. The pasture has been grazed to trample the weeds and allow re-planting of the grass with vegetative material.

Given that in the region there is lack of machinery, we examined the use of herbicides to establish *A. pintoi* with sexual seed in native pastures. Results showed that seedlings emerged but lacked vigour, probably associated with soil compaction and soil nutrient deficiencies. In soils with 4.1 to 4.8% OM and 6 to 11 PPM of P, both *B. decumbens* and *A. pintoi* responded to fertilization (60 P, 40 K, 20 S and 20 Mg, kg/ha).

3.2 Technical short-courses and field days

An important objective of the project is to exchange technical information on factors related to pasture establishment and productivity. Three short-courses were held in the CORPOICA-Macagual research station. The course were: (1) Soil physical properties and methodology to measure compaction (Dr. E. Amezquita, TLP-CIAT), (2) Role of roots in nutrient cycling and methods to sample roots (Dr. I. M. Rao, TFP-CIAT) and (3) Biological activity in soils and methods to measure macro-fauna (Dr. Juan Jose Jimenez, Universidad Complutense de Madrid-CIAT). All three courses were well attended by professionals of the region working in research and transfer of technology.

A field day attended by 80 farmers was held in one of the farms participating in the project. The program included information on establishment and on benefits of *Arachis* in animal production and a visit to a recently established grass-legume pasture. As a result of this field day a number of farmers expressed interest in planting *A. pintoi* in the next rainy season. This information will be passed-on to commercial suppliers of *Arachis* seed and technical assistance will be provided when requested.

3.3 Base line information

Detailed information on infrastructure, cattle inventory, cattle and pasture management and level of production is being recorded for each farm participating in the project. Information is also being gathered on number of farms that have sown *A. pintoi* in association with grasses and that have seed plots of *A. pintoi* for vegetative propagation. This information will be useful to quantify adoption of *A. pintoi* as a pasture legume **[G. A. Ruiz, C. Lascano, J. Velásquez, N. Gacharná and V. Carrillo]**.

3.4 Monitoring *A. pintoi* in a dairy farm of Canan, Pérez Zeledón, Costa Rica

This farm is located on steep hills at 1,300 masl on soils of volcanic origin with a mean temperature of 23 °C and 2,500 mm annual rainfall. The farmer planted the forage legume *A. pintoi* CIAT 17434 vegetatively in association with Gamalote grass (*Axonopus micay*) in 4 paddocks of approximately 2,500 m^2 each.

Eight months after establishment, estimations of forage availability gave the following results: 10,120 kg/ha DM (54% grass, 46% legume) at ground level, and 2,810 kg/ha DM (62% grass,38% legume) above 10 cm height. This is evidence of the dense mat of stolons that *A. pintoi* produces close to the soil surface at this site, characterized by high rainfall and mild temperatures.

Dairy cows graze the pasture in a rotation system of one day grazing and 30 days rest. 15 to 18 animals cows are monitored in the morning for milk yield production the day they enter the grass/legume association and again on the fourth day. Four cycles of measurements have been taken to date. The results show that the cows consistently increased milk yield by 0.5 kg/cow/day in a single milking when they graze the legume grass/association, although the daily increase would be greater as cows are milked twice per day (Table 7). **[Horacio Chi Chan (MAG), A. Valerio, R. Quiroz (CIAT)]**

Table 7. Daily milk production (one single milking) of dairy cows grazing the grass Gamalote alone and in association with *Arachis pintoi*.

Pasture	Gamalote	Gamalote + Arachis	Difference
Period		(kg milk/cow)	
1	7.1	7.5	+ 0.4
2	7.2	7.7	+ 0.5
3	7.0	7.6	+ 0.6
4	6.5	7.0	+0.5
Mean	6.9 a*	7.5 b	+ 0.6

* $P < 0.05$

3.5 Reclamation of degraded areas in the high part of the Rio Picagres watershed, Puriscal, Costa Rica

This is a joint project between with Programa de Investigación en Sinecología y Restauración de Ecosistemas Terrestres of the University of Costa Rica, the Ministry of Agriculture (MAG), the Ministry of Natural Resources (MIRENEM) and the Programa de Desarrollo Agrícola y Forestal MAG/MIRENEM/GTZ. Input by CIAT is in supply and assessment of the value of improved forages.

The following treatments were established in 2 farms in the Rio Picagres' watershed in areas of degraded native pasture:

i) Native pasture (dominated by *Hyparrhenia rufa* and *Paspalum* spp.).
ii) Native pasture + introduction of 153 trees/ha of *Schizolobium parahibum*.
iii) Improved pasture.
iv) Improved pasture + trees as above.

The improved pasture consists of *Brachiaria dictyoneura* cv. Brunca (CIAT 6133) and a cocktail of legumes -*Centrosema macrocarpum* CIAT 5713, *Desmodium ovalifolium* CIAT 350, *Stylosanthes guianensis* CIAT 184 and *Arachis pintoi* cv. Maní Forrajero (CIAT 17434). It was successful introduced using minimum tillage. Trees have established slowly because of damage by the animals.

Pasture, liveweight and biological changes in the soil, soil compaction and soil erosion are being made.

Significant changes in pasture availability and botanical composition have been observed. Forage yields in native pastures range from 800 to 1100 kg DM/ ha, while in the improved pastures the yields range from 3800 to 4100 kg DM/ ha. Legumes contribute 12 % of total DM and are ranked as follows: *S. guianensis*> *D. ovalifolium* > *A. pintoi* > *C. macrocarpum*.

The results show that planting improved grazed on native, degraded pastures is feasible with minimum tillage systems and appropriate germplasm. **[P.J. Argel]**

4. Grass-Legume Associations for Crop-Livestock Systems

Cerrados

The Brazilian Cerrado have contributed significantly to the country's crop and livestock economy during the last three decades. However, the intensive use of some areas in the cerrado particularly for monocropping and pasture development, has given rise to forms of land use that are neither environmentally nor economically sustainable. Thus alternative land use systems are needed to halt and revert declining productivity and losses of soil and water. Among technologies with potential to do this are the combination of crops and pastures in space and time.

4.1 Tradeoffs between crop and pasture components

The introduction of forage legumes in crop-pasture systems with high inputs has been difficult so far. *S. guianensis* cv. Mineirao, which can be established in rice-pasture systems with low inputs, suffers from severe competition in crop-pasture systems with high inputs. Therefore, aggressive and productive legume species are needed for high input agropastoril systems.

A small plot experiment was carried out in a sandy soil of Uberlandia, MG, Brazil to test the establishment ability of 3 preselected accessions of *C. mucunoides*, *P. phaseoloides* and *A. pintoi*. Reference species were *S. guianensis* cv. Mineirao, commercial *C. mucunoides* and *Neonotonia wightii*. All accessions were planted alone and simultaneously with corn and *P. maximum* cv.Tanzania in 2 m rows with four replicates. Grain production and yields of pasture components were estimated at corn harvesting (120 days from planting). Measurements of legume plant population were done 50 days after planting and at harvesting time.

Results showed a decreased in plant population and yield of selected legume accessions and control legume cultivars planted either with the crop or with the crop and the grass. However, reduction of legume population in the crop-legume-grass treatment was lower in the case of *A. pintoi* accessions BRA/CIAT-031143/ 22160 and higher for *S. guianensis* cv. Mineirao (Table 8). Reduction of the new *C. mucunoides* and *P. phaseoloides* accessions varied between 30 to 60%.

Average grain yield in the corn-legume system was 9 t/ha and 8.5 t/ha in the corn-legume-grass system. DMY of the *P. maximum* cv. Tanzania at harvesting time was 3 t/ha.

From the results of this experiment we concluded that competition is a major factor interfering with forage legume establishment in crop-pasture systems with high inputs. Competition results in population loss and yield reduction. However, it was evident that *A. pintoi* (BRA/CIAT-031143/22160) was the only legume capable of maintaining its original population through the crop cycle. Subsequent observations indicated that some legumes came back after harvesting the crop and initial grazing of the grass. These observations will be quantified next year **[M. Ayarza, L. Vilela and E. A. Pizarro]**.

Table 8. Relative decrease of plant populations of 10 forage legumes when planted alone and in association with corn and *P. maximum* cv. Tanzania in a sandy soil of Uberlandia.

Species	Monoculture[1]	Corn+Leg[1] (%)	Corn+Leg+Grass[1] (%)
A. pintoi (BRA31143)	0	0	24
C. mucunoides (BRA3147)	19	19	50
C. mucunoides (BRA3174)	16	13	35
C. mucunoides (BRA0477)	11	17	45
Commercial *P. phaseoloides*	15	16	56
P. phaseoloides (BRA582)	7	17	53
P. phaseoloides (BRA 761)	52	25	29
P. phaseoloides (BRA817)	11	26	32
Stylosanthes Mineirao	25	42	66
Commercial soybeans	31	9	24

[1]Numbers correspond to the percentage decrease between two dates (53 and 141 days after planting).

4.2 On-farm testing of improved agropastoral systems

Large satellite plots of crop-pasture systems were established in two soil types in several farms of Uberlandia in 1992. The objective was, to complement the results of the long term crop-pasture integration experiment on progress at CPAC-Planaltina and to measure the potential impact of crop-pasture integration on agricultural output and soil fertility under farm conditions. The work consisted on the reclamation of degraded *Brachiaria* pastures in livestock production systems with rice and a cocktail of forage legumes including *S. guyanensis* cv. Mineirao and the commercial cultivars of *C. mucunoides* and perennial soybeans. The same cocktail of legumes was planted with *P. maximum* cv. Vencedor and corn in a rotation systems with continuous cropping.

Establishment of the legumes in the livestock production system with low inputs was excellent while it was poor in the crop-pasture rotation system with high inputs. After 3 years of grazing, *S. guianensis* cv. Mineirao was still contributing to the total biomass of the reclaimed rice-grass-legume pasture in both the sandy and clay sites. Legume proportion has been around 50-60% and it has remained green and available for grazing during the dry season. Overall animal performance data showed that there was a 50% increase in liveweight gains in this system compared to the rice-grass pasture (Table 9).

In spite of the lack of legumes, the performance of animals on the *P. maximum* pasture in the clay site has been outstanding (507 kg/ha/year). This contrasts with the lower animal gain in the sandy site where *P. maximum* is losing vigor rapidly. This could be related to the higher content of soil OM and greater N supply over time in the clay site. Such finding indicate the need to identify forage legumes for crop-pasture rotation systems in clay soils with high inputs. Factors such as soil type, OM content and length of the pasture cycle must be considered in the selection process **[M. Ayarza and L. Vilela]**.

Carimagua, Llanos

An intensive crop-livestock system, in which crops are well fertilized, provides a more fertile soil for grass and legume establishment and production. It is likely that species that do not persist well on infertile soils with low fertilizer (the improved grass and grass-legume systems for the Llanos) or even on infertile soils more heavily fertilized for growing rice (rice-pasture system) may persist and be productive on soils heavily fertilized for maize and soybean production.

Table 9. Animal liveweight gains (LWG) in several agropastoral systems established in Uberlandia in 1992.

Management system	Treatments	Clay soil		Sandy soil	
		LWG[1] g/day	LWG[1] kg/ha	LWG[1] g/day	LWG[1] kg/ha
Livestock	Unreclaimed pasture	384±184	n.d.	399±97	180
Livestock	Rice + grass pasture	354±145	226	437±97	180
Livestock	Rice + grass + legume	456±180	364	567±80	272
Continuous cropping	Corn + grass pasture	411±98	517	282±136	253
Continuous cropping	Corn + grass + legume	n.d.	n.d.	404±149	308

[1]Individual gains (g/day) corresponds to the mean value of eight evaluations during the whole evaluation period (220-300 days).

Recognized species that have been selected for poorer soils of the Llanos have been shown to establish and are productive on more heavily fertilized soils, viz. the grasses, *B. dictyoneura* and *B. humidicola,* and the legumes, *Stylosanthes capitata* cv Capica, *Centrosema acutifolium, Desmodium ovalifolium* and *Arachis pintoi* (Tropical Lowlands Program Annual Report 1994). However, there has been a request to provide additional alternatives. There is also an additional requirement that species should be able to establish and persist under a crop.

4.3 Establishment under maize (1994 trial)

A common grass, a new accession of *Panicum maximum*, was established with 5 legume species and a standard 'cocktail' of legumes under a maize crop which received the normal fertilization of (kg/ha) 60 P, 100 K, 120 N, 20 S, 10 Zn, 2 B. The land had been limed and heavily fertilized the previous year for a maize crop. The legumes included in the trial were: *A. pintoi* cv. Maní forrajero, *S. captitata* cv. Capica, *C. acutifolium* cv Vichada, *C. pubescens* (CIAT 438), *N. wightii* cv. Cooper, *A. pintoi* (CIAT 22160), *S. guianensis* hybrid lines, *P. phaseoloides* (CIAT 8042).

There was good establishment of the grass and legumes but the latter were severely shaded at the time of harvest of the maize (1.5 ton/ha); the grass being the same height as the maize. After harvest, the maize stubble and pasture were heavily grazed during the remainder of the wet season. The pasture was not grazed over the dry season. Legume recovery was very good in all species except *N. wightii* which had established poorly.

The area has been grazed moderately every 21-28 days during 1995. The relative persistence and productivity of the legumes is, from good to poor, *A. pintoi* CIAT 22160 = *P. phaseoloides* CIAT 8042 > *C. pubscens* CIAT 438 = the 'cocktail' > *S. guianensis* hybrid lines > *N. wightii* cv. Cooper.

The performance of *A. pintoi* (CIAT 22160) is particularly promising in that, though planted vegetatively with < 4 cuttings/m^2, it has spread more rapidly and is more vigorous than *A. pintoi* cv. Maní forrajero (CIAT 17343) included in the 'cocktail'. It covers most of the space between the *P. maximum* tussocks. The performance of *P. phaseoloides* (CIAT 8042) is also promising but then this species has persisted well for 18 years with *B.decumbens* at

Carimagua. *C. pubscens* (CIAT 438) would also be considered a promising accession except for the large amount of leaf damage by insects and disease. The *P. maximum* appears to be well adapted but is in general quite yellow signifying a limitation of N.

This trial will be maintained for another two years and then cropped again cropped with maize to establish if there are any differences between legume treatments.

4.4 Establishment under maize (1995 trial)

A similar trial was established using a revised suite of legumes and two grasses, *P. maximum* and *B. brizantha* cv. Marandu. It was considered that *B. brizantha* would be less competitive against the legumes than the taller and more vigorous *P. maximum*.
The legumes included in the trial were: *S. guianènsis* (CIAT 11844), *P. phaseoloides* (CIAT 8042), *A. pintoi* (CIAT 22160), *C. pubescens* (CIAT 5634), *G. striata* (CIAT 8143), *S. guianensis* (CIAT 11844), *A. pintoi* (CIAT 22160), *C. pubescens* (CIAT 5634), *A. pintoi* cv. mani forrajero, *S. captitata* cv Capica, *C. acutifolium* cv Vichada.

G. striata (CIAT 8143) was selected from an evaluation trial in 1994 (see Forage Genetic Resources Project). Initial establishment of grasses and legumes was satisfactory. The corn was harvested in September and the pasture was first grazed at the beginning of October 1995 **[P. Kerridge, J. C. Granobles and C. Plazas]**.

5. Forages for soil cover, erosion- and weed control

Properly managed grassland or dense populations of forage plants figure amongst the forms of land use with highest levels stability as regards to soil erosion on sloping lands.

Grass and legume species can be used not only as a source of feed for animals but also as components in cropping systems with annual and perennial crops. When used as soil covers, legumes help to protect soils against erosion and to maintain soil fertility. Cut and carry grasses offer the opportunity to make use of sloping lands or can be planted as barriers to improve sustainability of arable land in hillsides.

5.1. Forage legumes as cover crops

Cassava is a crop frequently found on low fertility hillsides, due to high levels of tolerance against environmental stress. Under these conditions it can cause high levels of soil erosion when planted as a sole crop. This is mainly because of slow formation of a soil cover to protect the soil against raindrop impact.

On slopes with gradients of 10-15% intercropped forage legumes, once they are established, have shown to reduce soil annual losses from levels of 10-20 t/ha to values as low as 1-3 t/ha. However, due to slow initial growth of most of the legumes tested, efficient erosion control could only be obtained in the second growth cycle of cassava when legumes were already well established from the very beginning of the growing season. In the year of legume establishment, soil losses were equal or even higher than those of only cassava.

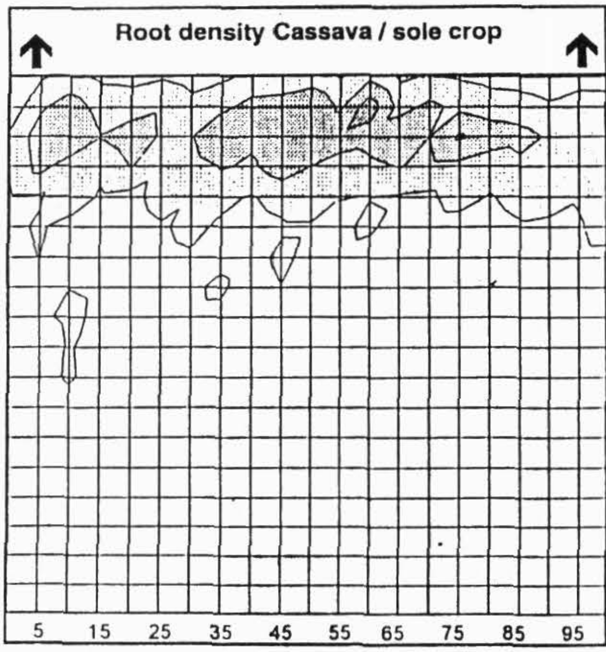
Vigorous legumes like *Centrosema macrocarpum*, *Pueraria phaseoloides*, *Galactia striata*, *Zornia glabra*, *Centrosema acutifolium* and *Galactia striata*, well adapted to acid low fertility conditions, exercised relatively high levels of competition resulting in 15-30% yield reductions in cassava in the first year and between 30-50% in the second year (data not shown).

Yield reductions were compensated to some degree by forage yields between 2-4 t/ha of dry matter. Nevertheless, considering the levels of competition, forage intercropping in this form is probably only attractive to farmers which in addition to the need for forages intend to use a second cassava/forage crop as a means to establish legumes for later use in improved pastures. For all other situations efforts had to be made to select legumes less competitive with crops.

Observation made in large field experiments indicated that *G. striata* (CIAT 964), a forage legume with a very extensive grass like root system, was very competitive with cassava. Thus we hypothesized that below ground competition of cover legumes had a major influence in cassava/legume systems.

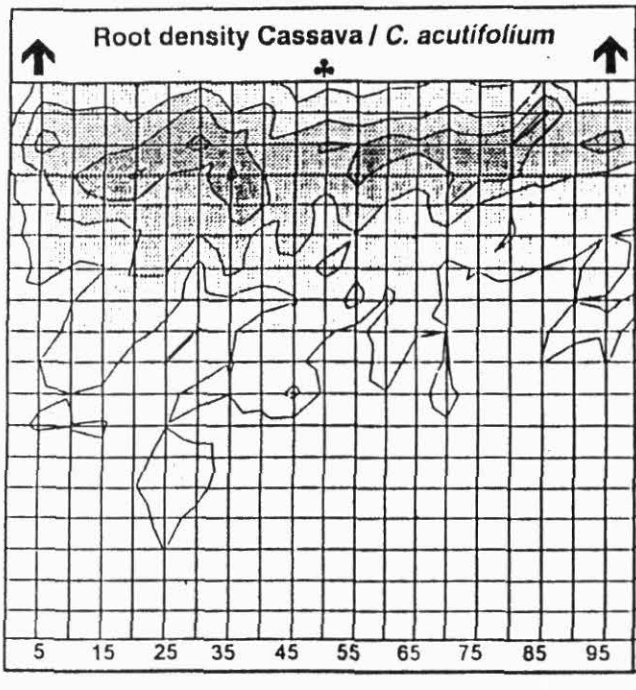
Two out of seven cassava/legume systems tested in a long term agronomic intercropping trial were selected for direct root observation on soil profiles of 1 m width and 90 cm depth in the second year after establishment. Cassava was about 6 months old at the time the observations were made.

Counts of root tips per cm^2 were digitized and fed into a computer program. Compared with cassava alone (Figure 3) with highest root densities of about 0,5-0,6 roots per cm^2 (R cm^2) the introduction of *C. acutifolium* (CIAT 5277) to the system led to higher root densities and deeper root extension (Figure 4).



Soil depth (cm)	Ø RD No. / cm³	% roots / layer
5	0,26	24%
10	0,53	
15	0,68	35%
20	0,51	
25	0,34	18%
30	0,22	
35	0,22	12%
40	0,19	
45	0,15	7%
50	0,10	
55	0,07	3%
60	0,03	
65	0,02	1%
70	0,01	
75	0	0%
80	0,01	
85	0,01	0%
90	0	
cm		100%

Figure 3. Root densities (RD) in number of roots per cm³ and root distribution in different soil layers of cassava (CM 849-1) in a natural soil profile (Mean of three replications). ↑ = Cassava plant.



Soil depth (cm)	Ø RD No. / cm³	% roots / layer
5	0,31	10%
10	0,62	
15	0,89	28%
20	0,78	
25	0,59	20%
30	0,46	
35	0,39	13%
40	0,29	
45	0,29	9%
50	0,24	
55	0,23	8%
60	0,19	
65	0,17	6%
70	0,15	
75	0,14	4%
80	0,07	
85	0,06	2%
90	0,03	
cm		100%

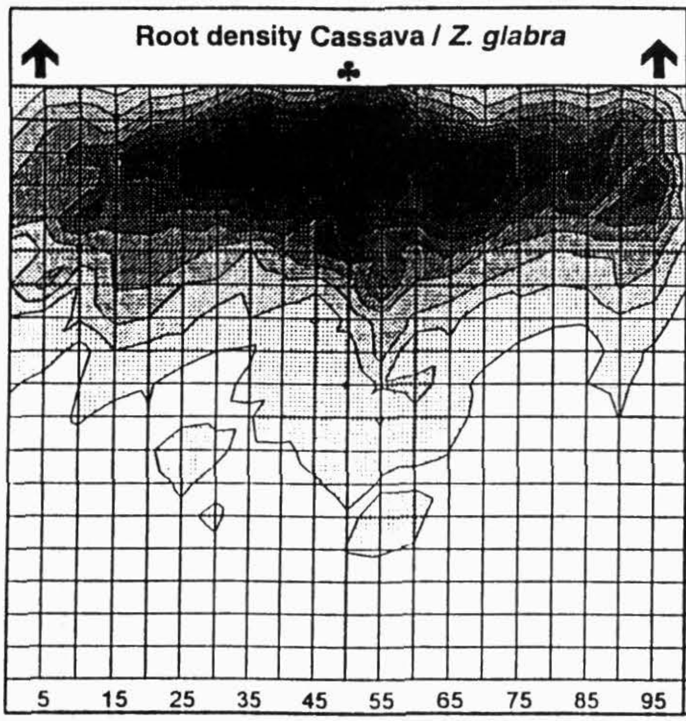
Figure 4. Root densities (RD) in number of roots per cm² and root distribution in different soil layers of a mixed cropping systems of cassava (CM 849-1) with *Centrosema acutifolium* (CIAT 5277) in a natural soil profile in Santander de Quilichao. (Mean of three replications). ↑ = Cassava plant; ♣ = *Centrosema.*

Highest root densities however, were obtained by combining cassava with *Z. glabra* (CIAT 8283) as it is shown in Figure 5.

Total number of roots in the profile (0,9 x 1 m) were 1696, 3060 and 6376 for cassava alone, Cassava/Centrosema and Cassava/Zornia respectively. Values for Zornia were significantly higher than in the other cropping systems. Mean above ground biomass production for Zornia was 4.7 $t.ha^{-1}$ DM which again was significantly higher than yield of Centrosema (3,4 t). Both legumes provided good soil cover between cassava rows, but reduced yield. Compared with tilled sole cassava yielding 32,4 t/ha of fresh roots the yield of cassava grown with minimum tillage and legumes decreased to 17,6 t with Zornia and 17,2 t with *Centrosema*.

Higher root density and biomass production with the Zornia treatment did not cause a greater loss in cassava yield when compared to *Centrosema*. Thus, it can not be concluded that legumes with a higher root density and similar or slightly higher biomass production necessarily lead to more competition. Attributes such as response to water stress, water use efficiency, levels of inter and instraspecific competition and perhaps micorrhiza infection could also be important in cassava/ legume systems.

As a result of screening activities to identify low competing and fast covering forage legumes, *Chamaecrista rotundifolia*, (CIAT 8990) was identified as a very promising species for altitudes up to 1200 m.a.s.l. Out of 22 species tested *C. rotundifolia* exhibited outstanding soil cover capacity and did not



Soil depth (cm)	Ø RD No. / cm²	% roots / layer
5	0,80	19%
10	1,61	
15	2,17	33%
20	1,90	
25	1,61	22%
30	1,11	
35	0,88	12%
40	0,55	
45	0,38	6%
50	0,30	
55	0,28	4%
60	0,21	
65	0,16	2%
70	0,12	
75	0,11	1%
80	0,07	
85	0,07	1%
90	0,06	
cm		100%

R/cm²: 3,3-3,6; 3-3,3; 2,7-3; 2,4-2,7; 2,1-2,4; 1,8-2,1; 1,5-1,8; 1,2-1,5; 0,9-1,2; 0,6-0,9; 0,3-0,6; 0-0,3

Figure 5. Root densities (RD) in number of roots per cm^2 and root distribution in different soil layers of a mixed cropping system of cassava (CM 849) with *Zornia glabra* (CIAT 8283) in a natural soil profile in Santander de Quilichao. (Mean of three replications). ↑ = Cassava; ♣ = *Zornia*.

lead to yield reductions in cassava under favorable moisture conditions or to high levels of competition under extremely unfavorable conditions. The latter case is presented in Table 10. In spite of a disease attack which led to a complete defoliation of cassava at the end of the first rainy season (3 months after planting), competition from the undersown legume only led to a yield reduction of about 20% which was much less than could have been expected from experience with other legumes.

So far little is known about the reasons for this outstanding performance of *C. rotundifolia*. A deep but fine root system, low nutrient requirements, immediate physiological response to water stress and economic use of water might be responsible for this performance and merit further attention. Other characteristics contributing to the attractiveness of *C. rotundifolia* as a cover crop at the altitudes tested, were low postrate growth habit, early vigor, early and abundant seed production, resistance to pests and diseases, tolerance to soil acidity and low fertility. Another important attribute is that it does not interfere with harvest of the root crop which is an important criteria for cassava farmers **[K. Müller-Sämann, J. Castillo, C. Gallego, L. Muhr (Univ. Hohenheim, Germany and Anna Haering (Univ. Hohenheim, Germany]**.

5.2. Use of grasses in soil conservation

Arable land is usually related with physical degradation of soil properties when compared to soil under natural vegetation. Similar effects can be observed, when productive, dense grassland is converted to crop land.

Trials conducted on erosion plots in the Quilichao research station revealed that managed grass/legume rotations have a high potential to restore soil structure and stability. Seven years after plots had been converted from grassland to arable land, major differences could be observed in response to previous cropping history.

Compared to continuous cassava, soil characteristic could be greatly improved with a 2 year grass-legume rotation. This system, which was also superior to natural fallowing, led to the highest level of erosion control, crop yield and soil structural stability (Table 11). Thus traditional weed fallow, commonly used to restore soil fertility and soil health, can be replaced by the more productive managed grass/legume cover which was eliminated with a herbicide after two years.

The grass-legume technology matched the dual objective of increasing food production at the same time conserving or even enhancing the natural resource base. In addition, this technology indicates a good potential ley farming elements to improve land management in peasant hillside farming.

Another approach to make use of grasses for improved soil management in hillsides is the planting of hedgerows or grass barriers along contour lines. If planted at regular intervals on steep lands with annual crops they reduce slope length and thus contribute to control soil and water losses resulting from runoff.

Table 10. Results from growing cassava with and without *Chamaecrista rotundifolia* (CIAT No. 8990) as an undersown legume cover crop[1] in Santander de Quilichao 1000 m.a.s.l.

	% soil cover of legume[1]			Legume yield[2]	Cassava root yield[3]	Harvest index
	1	2	3	t DM/ha	ta/ha	(fresh
	(Months after planting)					weight)
Cassava sole crop	-	-	-	-	20,54 a (100%)	0.60 a
Cassava plus *Chamaecrista*	1,63	22,87	67,5	4,265	15,92 b (77,5%)	0.59 a

[1]Sown in a single row between cassava (1,0 x 0,25 m).
[2]11 Months after planting.
[3]Because of total defoliation of cassava by CBB between July to September, undersown legume was completely exposed to sunlight and competition strongly increased compared to normal conditions were growth and transpiration is reduced due to canopy shade.
a,b means are different ($P<0.05$).

Table 11. Dry soil losses, cassava fresh root yields and mean aggregate sizes with contrasting land management practices on a 10% slope in Santander de Quilichao (Evaluation of residual effects of previous land use).

Previous land use (1992-1994)	Cropping treatment period 1994-1995	Dry soil loss (t/ha)	MWD[3] (mm)	Fresh root yield (t/ha)
1. Bare plot [1]	Bare	127,50	2,00	-
2. Cassava continuous	Cassava	5,15	2,85	16,93
3. Two year weed fallow	Cassava	0,84	3,15	20,63
4. Kudzu/Brachiaria mixture	Cassava	0,55	3,39	26,38

[1]Plots were left bare (1) or used for arable crops between 1986 and 1992.
[2]*Pueraria phaseoloides/Brachiaria decumbens*
[3]Mean Weight Diameter of soil aggregates

Forage grasses with potential to form dense and persistent barriers and that compete little with adjacent crops, are of special interest for soil conservation, because they allow farmers to derive some benefit from soil conservation.

In on-station and on farm-trials Dwarf elefant grass (*Pennisetum purpureum Schum.*) cv. Mott exhibited excellent characteristics as a grass for barriers: low growth height, high tillering, high leaf/stem ratio, good fodder quality and convenience of being cut with machete.

Based on these findings on-farm evaluation of Dwarf Elephant was initiated in 1994 and multiplication plots were installed to satisfy the increasing demand from local organizations and farmer communities. When planted as a barrier every 8-10 m on hillsides in the northern Cauca Department of Colombia (1000-1600 m.a.s.l.), the grass showed good adaptation and outyielded other grasses commonly used in the area (Table 12).

Promotion of Dwarf elephant as an alternative and complement to already existing barrier grasses in Andean mid-altitudes is therefore recommended **[K. Müller-Sämann, J. Castillo, C. Gallego, L. Muhr and Anna Hearing (Univ. Hohenheim, Germany]**.

6. Seed multiplication

Lack of seed is a major constraint in the evaluation of promising forage germplasm in regional trials and on-farm work. Thus the TFP has assigned resources to ensure adequate availability of experimental seed of promising forage species.

6.1 Seed Unit in CIAT-Palmira

A small Seed Multiplication Unit was created in 1995, within the Tropical Forages Program with the mandate to produce and distribute seed of selected forage plant accessions to meet both internal and external experimental needs. The Unit comprises two assistants, one technician, and one laborer.

Existing seed multiplication areas are located on the properties of CENICAFE (Chinchiná, Caldas), and at CIAT-managed field sites at Headquarters, CIAT-Quilichao, and CIAT-Popayán.

In early 1995, the Unit inherited 38 seed multiplication plots, with a total area of just over 2.5 ha, from the former Seed Biology Section and from the TFP Germplasm Section. The bulk of this "inheritance" was 2 ha of *Stylosanthes guianensis* planted late in 1994 at CIAT-Quilichao.

The Unit established 127 new plots in 1995, with a total area of nearly 2.4 ha. Over half the plots established in 1995 were of *Brachiaria* accessions at the CIAT-Popayán station. Significant areas of *Panicum maximum* (0.7 ha, 2 accessions) and of *Arachis pintoi* (0.5 ha, 7 accessions) were sown during 1995 at CIAT-Quilichao or at Chinchiná, respectively.

The Unit received 598 requests for seed during 1995, of which 457 have been delivered, representing a total of

Table 12. Forage production of barrier grasses, based on two cuttings of 3 month old regrowth between April and September 1995 on farms in the northern Cauca Department of Colombia.

Grass species	Mean 3 monthly yield	Annual yield equivalent
	(t DM/1000 linear meters)[1]	
Dwarf elefant (*Pennisetum purpureum* cv. Mott.)	3,37	13,46
Imperial grass (*Axonopus scoparius*; local "Telembi")	2,33	9,32
Guatemala grass (*Tripsacum andersonii*)	2,31	9,26
Vetiver grass [2] (*Vetiveria zizanioides*)	1,99	7,96
Citronella grass [2] (*Cymbopogon nardus*)	3,14	12,55

[1]1000 linear meters/ha is equivalent to a grass barrier every 10 meters recommended for slopes with a gradient of approximately 15%.
[2]Not a forage

1.35 T. The policy of the Unit has been to attempt to cover a major portion of the cost of larger quantities of seed dispatched, although donations have also been made under special conditions. **[C. G. Meléndez, R. Mosquera, J W.Miles, B.L. Maass]**.

6.2 Seed multiplication in the cerrados

The TLP and TFP are working together to develop mechanisms of forage seed multiplication in Uberlandia with the participation of the UFU and farmers. Two legumes are currently under multiplication: *S. guianensis* cv. Mineirao and *A. pintoi* (BRA/CIAT-031143/22160).

One hectare of *S. guianensis* cv. Mineirao was established in a farm during 1993. Seed produced was used to establish multiplication plots, one with irrigation facilities at the UFU farm and two other seed-plots in private farms. A similar strategy was followed for *A. pintoi*. At this moment we have 25 kg of *S. guianensis* cv. Mineirao and 250 kg of *A. pintoi* (BRA/CIAT-031143/22160) for on-farm research activities. Additionally, one of the collaborating farmers produced 200 kg of *Paspalum atratum* (BRA-009610) **[M. Ayarza, E.A. Pizarro, and Seed Production Units of the UFU and EMBRAPA-CPAC]**.

7. Socioeconomic Studies of Adoption of Forage Species

During this period, activities of the Impact Studies Unit in the area of Tropical Forages focused on: (1) Initiation of a study on early adoption or acceptability in Colombia of the perennial forage peanut, *Arachis pintoi* (CIAT 17434), released by ICA in 1992; (2)*ex ante* economic assessment, at the micro level, of new pasture technology; (3) review and documentation of the dissemination and impact of pastures technology in Latin America and elsewhere; and (4) continued monitoring of economic trends in the cattle industry of Latin America.

7.1 Study of early adoption-acceptability by farmers of *A. pintoi* in Colombia

Acceptability to farmers of *A. pintoi* (CIAT 1743) was studied in Colombia in order to make an assessment of factors contributing to adoption of this legume. The legume has been promoted mainly in the country's coffee-growing regions by CENICAFE, (National Coffee Research Center), CODEGAR (Cooperative of Farmers and Cattle Raisers of Risaralda), and the Coffee Growers Committee of Antioquia. Commercial seed of *A. pintoi* is produced in Colombia mainly by SERVISEMILLAS, which controls nearly 90% of the domestic market. The company sold a little over 5 MT of seed during 1993-1994, and, during 1995, sales have been close to 3 MT (at time of writing).

A sample of producers from different regions of the country were selected from the SERVISEMILLAS sales list to conduct a telephone survey. The sample included 50 producers throughout Colombia, which provided information on the following aspects: (1) source of information on the legume; (2) number of plots and area planted; (3) problems encountered during establishment; (4) alternative uses; (5) advantages and disadvantages of the legume; and (6) expectations with the legume.

Results of the survey showed that:

- *A. pintoi* is being disseminated throughout the entire country, even in areas with unsuitable growing conditions (i.e. long dry season).

- Of 95 plots established, *A. pintoi* was associated with grasses in 37 (39%); used as soil cover in crops such as coffee, citrus fruits, palm, and banana in 24 plots (26%); and planted alone, as seed beds for subsequent vegetative propagation, in the remaining 34 plots (36%).

- Adoption of *A. pintoi* by farmers is in a very early phase, since farmers are basically learning how to establish, use, and manage the legume, as indicated by the average plot size. The average size of seed plots is 1.1 ha (range 0.01 to 20 ha). When associated with grasses, the average plot size is 4.8 ha (range 0.04 to 30 ha). The size of plots in which *A. pintoi* is used as cover averages 1.8 ha (range 0.02 to 42 ha).

- Farmers obtain information on *A. pintoi*, mainly through technical bulletins and other written information from CIAT and the coffee growers associations.

- The main reason why producers tested *A. pintoi* was to increase pasture productivity (42% of the farmers interviewed). This reflects a high potential demand for high quality forage legumes. Nearly 1/4 of the producers (14) surveyed felt that *A. pintoi* was a new alternative for soil cover.

- Approximately 1/3 of the producers (17) who planted *A. pintoi* reported problems during establishment. The most frequent difficulty encountered was weed control (53%), dry season stress (29%), and slow establishment (24%).

- The experience with *A.pintoi* has been positive for 69% of those who have planted it. However, 1/4 of the farmers were not satisfied with the results obtained. Of those who planted *A. pintoi*, 69% are willing to expand the area planted to the legume.

- Twenty-seven (54%) of the producers surveyed have already grazed *A. pintoi*; of these, 23 considered it palatable for animals, while the remaining 4 said it was not.

- In 1993 and 1994 sales of *A. pintoi* seed were mostly to farmers in hillside areas of the coffee-growing region. This is in part, explained by the low coffee prices and the reduction in area planted to coffee. In 1995 the demand for seed grew in Caquetá region dedicated mostly to dual-purpose cattle.

We will continue monitoring adoption of *A. pintoi* in Colombia, since the information obtained could be useful to national programs in other countries and also for subsequent studies on socioeconomic impact of this legume **[L. Rivas]**.

7.2 *Ex-ante* analysis of new technological alternatives at the micro level

An *ex-ante* analysis was conducted at the micro level, within the context of economic assessment of new forage alternatives. Two technological alternatives that could eventually be used as substitutes were considered: (a) use of grasses alone, with N applications at establishment and maintenance, and (b) use of grasses-legumes pastures.

The productivity indicators used in the analysis were: (a) milk yield in star grass, with 100 kg/ha of N applied at establishment and as maintenance every year, and (b) milk yield in star grass in association with *A. pintoi*, without N fertilization. Result on milk yield correspond to 5 years averages, recorded in experimental pastures in Turrialba, Costa Rica.

Experimental results showed significant differences in milk yield/cow per day in the pastures evaluated. Daily milk production in dual-purpose cows increased from 9.5 to 10.8 kg/cow when changed from the N fertilized grass pasture to the associated grass-legume pasture (Argel, 1994).

The economic analysis was based on the following assumptions: (a) 10 years of continuous production; (b) the star grass pasture is fertilized each year with 100 kg of N; (c) after 3 years of continuous production, star grass + *A. pintoi* is resown, and (d) costs associated with management of the legume-based pasture assumed to be 50% higher than those of grass alone.

In terms of marginal profitability, the two alternatives are highly attractive. However, even with periodic renovation of the legume-based pasture, returns are somewhat higher with the legume alternative (73%) than with the N alternative (67%). The *A. pintoi*-based pasture can loose up to 10% productivity per year and still continue to be more profitable than the N-fertilized grass pasture.

The two pasture alternatives are highly attractive from the viewpoint of profit. However, constraints to large-scale use of these technologies could be related to economic and technical viability. Some of these constraints could be: (a) high levels of investment in pastures and cattle; (b) limited tradition of fertilizing pastures, both for establishment and maintenance; (c) a growing deficit in phosphate and nitrogen fertilizer production in Latin America (a massive use of fertilizers in pastures would increase this deficit, implying a rise in imports and/or prices); (d) low persistence of legumes in several regions; and (e) markets that are not sufficiently dynamic to absorb the additional production **[L. Rivas]**.

7.3 Documentation of experiences on impact assessment of tropical forages

To gather information and criteria for studies on adoption and impact assessment of pastures technology, empirical studies conducted in Latin America on this topic were reviewed. The main conclusions of the review were:

- Adoption and impact studies on tropical forages in Latin America are scarce and mainly focussed on the early phases of adoption of new grass and legume cultivars. In addition, researchers have used different methodologies, which limits comparison of results.

- Adoption and dissemination of new forage technology are highly complex, long-term processes, involving technical, psychological, sociological, and economic issues.

- It is difficult to extrapolate the results of adoption studies. Nevertheless many forage technologies are extensively recommended, despite large variability in soil, climates, and socioeconomic set-ups in tropical America.

- Although *ex-ante* indicators of economic returns to investment are necessary, they are insufficient to promote high levels of adoption and dissemination of new forage technology.

- Unlike the processes of adoption and dissemination of improved crops, in which a variety or cultural practice can be rapidly changed for another, adoption of new grasses and legumes is a long-term process involving testing and evaluation by progressive farmers, before definitive decisions are made on the wide adoption of any given cultivar.

- Empirical evidence suggests that there is a strong trend to overestimate the potential area in which a new forage technology could have an impact. Consequently, a greater effort should be made to identify environmental and production "niches" for different grass and legume ecotypes.

In general, it is important to recognize that there are multiple constraints affecting adoption of new forage technology, which vary in importance and intensity and depend on specific environmental and socioeconomic conditions **[L. Rivas]**.

7.4 *Ex-ante* economic evaluation of research projects in the Tropical Forages Program

This study formed part of an *ex-ante* economic evaluation and prioritization of CIAT's research projects. In Table 13, we summarize the main results obtained in terms of internal rates of return to funds invested in different projects and of expected current net value of social profits (surplus goes to producers and consumers).

Our results confirm the high potential profitability (range 22 to 48%) of improved forage-based technologies which have been observed in other areas of the world. For example, in Australia the internal rate of return of improved forage-based technologies ranged between 17 and 78% per year (Davies, 1993).

The TFP Projects with the greatest socio-economical impact are does dealing with *A. pintoi*-based pastures, improved *Brachiaria* spp. pastures and crop-pasture rotation systems for low lands. On the other hand, grass-legume pastures for hillsides appear to have low economical return.

Given the importance of meat and milk in the diet of consumers of tropical America and high elasticity of demand for these products, technological improvements aimed at improving feed resources for livestock will benefit mostly poor urban consumers. However, these potential benefits will only be reached with efficient technology transfer systems, adequate supply of critical inputs such as seed of improved forage cultivars, and a stable socioeconomical environment that stimulates adoption of new and improved technology by farmers **[L. Rivas]**.

Table 13. Indicators of social benefit (attributable to CIAT) derived from the use of new forages for meat and milk production in tropical Latin America[1] (Evaluation period: 1994 to 2020).

Type of System	Expected current net value of social benefits		Internal rate of return (%)	
	WIS[2]	WOIS[3]	WIS[2]	WOIS[3]
Brachiaria pastures	1014	766	50.4	43.4
Arachis-based pastures	1228	924	61.5	53.6
Stylosanthes-based pastures	313	235	48.6	43.1
Centrosema- and *Desmodium*-based pastures	247	185	49.0	42.7
Pasture-crop systems	1023	772	84.3	73.6
Mixed legume systems	11	8	21.9	19.3
Mixed systems including crops, grasses, and shrub and nonshrub legumes	211	164	50.2	44.4
Grass-based systems	1014	766	50.4	43.4
Legume-based systems	1795	1348	51.4	44.7
Pasture-crop systems	1234	936	71.9	62.5
Total	4046	3053	55.3	48.4
Current net value of Institutional Support	903[3]		71.8	

[1]Excluding US$90 million attributable to the Tropical Lowlands Program.
[2]With Institutional Support.
[3]Without Institutional Support.

Sub-Project: Forage Components in Tropical America

Planned activities for 1996

1. Shrub legumes for acid infertile soils

- Identify the anti-nutritional factors responsible for the low intake of immature-fresh *C. argentea* forage (CIAT-IGER Project). Priority will be given to examining non-protein amino acids, given the exceptional high levels of CP (42%) in young leaves of *C. argentea* that was reported by researchers of CNPGL-EMBRAPA, Brazil (MS Thesis of a UK student).

- Evaluate the effect of using *C. argentea* alone or in combination with cut and carry grasses (i.e. king grass, sugar cane) as supplements to cows grazing pastures with contrasting levels of forage on offer both in the dry and wet season (MS Thesis of a Colombian student).

- Measure with farmer participation the effect of supplementing legume fodder on milk yield during the dry and wet seasons (Cauca and Caqueta).

2. Legumes for fallow improvement

- Continue the maintenance of the P fertilizer residual trials.
- Determine if there is a field response by *Centrosema* species to Mo application on degraded acid hillside soils.
- Establish a grass-legume mixture on a sufficiently large area of fallow land in order to evaluate the effect of grazing vs no grazing on fallow improvement as measured by yield of a subsequent crop.
- Continue investigation of establishment of legumes into the final cassava crop of a cropping cycle.
- Establish and evaluate new *A. pintoi* accessions for spread and persistence in heavily grazed *B. dictyoneura* pastures in north Cauca.

3. Grass-legume associations for lowlands

The following activities are planned for the on-farm project in dual purpose cattle in Caqueta, Colombia:

- Monitor the botanical composition and milk production in grass alone and *A. pintoi*-based pastures.
- Establish in 5 farms *A. pintoi* in association with *Brachiaria* spp. following conventional land preparation.
- Establish in 3 farms strips of *A. pintoi* (seed and vegetative material) in *Brachiaria* spp. pastures.
- Compare different tillage systems (disk, chisel, disk + chisel), and fertilizer treatments in the establishment of *A. pintoi* in association with *Brachiaria* spp.
- Establish seed plots of new ecotypes (CIAT 18744, 18748, 22160) of *A. pintoi* for later distribution of vegetative material to farmers collaborating in the on-farm project.
- Introduce *S. guianensis* cv. Pucallpa in steep hills to improve native and degraded *Brachiaria* pastures.
- Make an inventory of farmers that have or which to have stands of *A. pintoi* for vegetative propagation and assistance in legume introduction in pastures.
- Program field days on establishment of *A. pintoi* in association with grasses, on grazing management and on pasture productivity.

4. Grass-legume associations for crop-livestock systems

Cerrados

- Test the potential of new selected forage germplasm as components of agropastoral systems to improve milk production in small farms.

- Complement the grazing experiment established by the University of Uberlandia with establishment of satellite pastures in smallholder farms in the dairy area at Prata, MG.

Carimagua-Llanos

- Maintain existing trials and plant an additional suite of legumes as they are identified and seed is available.

5. Forages for soil cover, erosion and weed control

- Widen the forage germplasm genetic base for lower altitudes, for which promising legume species have already been identified. New forage germplasm screening activities should rely on criteria already identified for legume covers in cropping systems in hillsides.

- Catalyze development of mechanized low cost sowing equipment that will make large scale use of small seeded legumes for cover a feasible option for smallholder farmers in hillside farming systems.

- Improve knowledge based on ecophysiological requirements of dwarf elephant grass to provide guidelines for cost effective management of the grass as a barrier.

- Quantify soil and water runoff from superior fields with dwarf elephant grass grown as a crop or as barriers.

6. Seed multiplication

- Continue operation of seed unit to multiply promising accessions and new breeding lines for regional evaluation

- Develop a Special Project for strengthening commercial legume seed production in Latin America.

7. Socio-economic studies on adoption of new forages by farmers

Activities planned for 1996 are those which have been prioritized by the TFP. However, the execution of these activities will depend on adequate financial and human resources and on decisions made by CIAT's Impact Assessment Unit.

- Continue study on adoption of *Arachis pintoi* in Colombia and initiate a similar study in Central America.

- Determine potential impact of *Arachis pintoi*-based technology on dual-purpose cattle systems in the Forest Margins of Caqueta, Colombia, as part of the on-going Nestle Project. Specific activities include: literature review, rapid rural appraisal and mathematical modelling.

- Support studies on socioeconomic characterization of the Central Pacific region of Costa Rica as part of the Tropileche project.

Forage Components in Southeast Asia

Rationale

In Southeast Asia, smallholder livestock production systems are generally part of intensive, mixed farming systems. There are few smallholder farmers who are specialized livestock producers and, those who are, tend to be concentrated on the few remaining extensive grazing lands. The poorer section of the farming community grows crops for subsistence purposes and crops are therefore considered of paramount importance. In these traditional systems livestock are used for draught and transport, a way of preserving money which can be liquidated easily, and to generate income. In upland farming systems, livestock may account for well over 50% of the cash income of smallholder families. In recent years, prices for meat have increased sharply, making cattle and goat production increasingly attractive.

Naturally occurring feed resources are becoming increasingly scarce and farmers, who want to breed or fatten ruminants for income generation, are looking for forages which fit into their farming system to supply the required feed. In upland areas, forages are important also for controlling soil erosion, suppressing weeds and soil amelioration during crop fallows. There is a need to develop sustainable farming systems in the uplands which are catchment areas for water used in downstream agriculture and for human consumption.

For forages to be adopted by smallholder farmers, species must not only be well adapted to the particular environment but be compatible with and complement other farm activities. The special funded (AusAID) project "Forages for Smallholders Project (FSP)" is working with farmers and scientists using participatory research methods to identify forages suitable for integration into target farming systems. The 5-year project commenced in January 1995 and collaborates with forage R&D workers in Indonesia, Laos, Malaysia, Philippines, southern China, Thailand and Vietnam.

Main activities in Southeast Asia

In July 1995, a 4-week "International training course for Trainers: Participatory Research with Farmers in Feed Resources" was held in the Philippines to introduce the concept of farmer participatory research to forage researchers in the region. Participants will conduct similar courses in their own countries. Field activities have commenced at some sites while others are still being discussed. The activities described below are at an early stage of implementation and there are few results at this stage:

1. Legumes for control of weeds and erosion in agroforestry and plantation systems.
2. Grass-legume associations for grazing in coconut plantations.
3. Legumes and grass-legume associations for fallow improvement in upland cropping systems.
4. Forages for cut and carry feeding systems grown as hedgerows or fodder banks in rainfed lowland and upland cropping systems.
5. Grass-legume associations to improve natural/induced grasslands.
6. Multipurpose tree legumes for fodder banks, fence lines, contour hedgerows in lowland, upland, grasslands, agroforestry and plantation systems .
7. Legumes for leaf meal production in upland cropping systems.
8. Seed and vegetative planting material supply systems for commercial cultivars.

1. Legumes for control of weeds and erosion in agroforestry and plantation systems

Legumes may be used to control weeds and erosion in forestry, agroforestry and plantation systems. In the Philippines, *Stylosanthes guianensis* (CIAT 184) was found to effectively control erosion during planting and suppress growth of *Imperata cylindrica* in planting strips of young forestry plantations **[Bukidnon Forest Inc., Mindanao]**.

2. Grass legume associations for grazing in coconut plantations

A grazing trial has been established comparing liveweight gain of cattle grazing *Brachiaria humidicola* cv. Tully monoculture with a *B. humidicola* plus *Arachis pintoi* (CIAT 17434 and CIAT 18750) pasture at 2 head/ha. Species were established using cuttings. Vegetative establishment of *A. pintoi* (CIAT 18750) was less successful than for the other two species. Poor establishment of this accession was reported also by other collaborators. Grazing has commenced and botanical composition and liveweight gain of cattle will be recorded **[Bureau of Animal Industry, Bicol]**.

An on-farm experiment commenced comparing different forage options. The following treatments were established in 4 x 4 m plots:

- *Brachiaria decumbens* (local) monoculture
- *B. decumbens* (local) monoculture with 200 kg/ha/year N fertilizer
- *B. decumbens* (local) + legume mixture[1]
- *B. humidicola* (local) + legume mixture[1]
- *B. humidicola* CIAT 16886 + legume mixture[1]
- *B. dictyoneura* CIAT 6133 + legume mixture[1]

[1]Legume mixture: *A. pintoi* and *C. pubescens*

Plots are sampled for yield and botanical composition every 6 weeks and then grazed by cattle and buffalo for 2-3 days to consume all edible material.

All species established well. However, 6 months after establishment the proportion of legumes was less than 20% of total yield in the grass legume associations with *A. pintoi* contributing less than *C. pubescens*. The farmer expressed a preference for *B. dictyoneura* which was preferred by cattle. This experiment will be continued for at least two years **[Bureau of Animal Industry, Bicol, and Provincial Veterinary Service, Albay]**.

3. Legumes and grass-legume associations for fallow improvement in upland cropping systems

In October 1994, a range of grasses and legumes were undersown into an upland rice crop in Matalom, Philippines, 4 weeks before the rice harvest. Dry conditions after undersowing resulted in poor establishment and low yields of the undersown forage species. The best performing species were *S. guianensis* (CIAT 184) and FM-series, *Aeschynomene histrix* (CIAT 9690), *Paspalum atratum* (BRA 9610) and *B. humidicola* cv. Tully and *B. decumbens* cv. Basilisk. Future undersowing of forages into upland rice will be done earlier to ensure better establishment **[Visayas State College of Agriculture, Leyte, Philippines]**.

In September 1995, an on-farm experiment was established in Matalom to demonstrate the positive effect of one and two-year legume fallows (*S. guianensis* CIAT 184) when compared to natural fallow. Upland rice will be used as the indicator crop **[Visayas State College of Agriculture, Leyte, Philippines]**.

4. Forages for cut and carry feeding systems grown as hedgerows or fodder banks in rainfed lowland and upland cropping systems

Nine grasses and eight legumes were established as contour hedgerows in an upland cropping area in Matalom in late 1994. These were designed to control soil erosion during the cropping period as well as providing fodder for ruminants in the dry season. Criteria for suitability include effectiveness in reducing runoff, production in and maintenance of green leaf in the dry season, and time required to control hedgerow species during the crop growing season (to minimize competition with crops) **[Visayas State College of Agriculture, Leyte, Philippines]**.

5. Grass legume associations to improve natural/induced grasslands

Multiplication of adapted forage species is in progress at a new research site situated in an *Imperata cylindrica* grassland in East Kalimantan. Grasses and legumes are required which are able to successfully compete with *I. cylindrica*. This work follows on from species evaluations in East Kalimantan which identified a range of well adapted forage species. These are now being propagated for on-farm research starting in late 1995 **[Provincial Livestock Service, Samarinda, Indonesia]**.

6. Multipurpose tree legumes for fodder banks, fence lines, contour hedgerows in lowland, upland, grasslands, agroforestry and plantation systems

Multipurpose tree and shrub legumes (MPTS) may have a role in almost all land use systems because of their versatility. So far MPTS have been included as one of the forage species in contour hedgerows at Matalom.

7. Legumes for leaf meal production in upland cropping systems

Using legumes for leaf meal production for poultry and pigs is a viable option for farmers. Several thousand hectares of *S. guianensis* (CIAT 184) and *S. scabra* cv. Seca are planted for this purpose in southern China.

8. Seed and vegetative planting material supply systems for commercial cultivars

Commercial seed production of forages is often difficult in the humid tropics. In Southeast Asia there are few examples of successful seed production schemes with almost no commercial seed produced in Indonesia, Philippines, Laos and Vietnam.

Two seed production sites were selected in Isabela and Quirino in northern Luzon, Philippines in 1995. These were chosen on the basis of a distinct dry period during short days which will promote seed production of most legumes and some of grasses. Six forage species, recommended for release in countries in Southeast Asia by the S.E. Asian Regional Forage Seeds Project (1992-94), were established in 1,000m^2 plots for realistic production conditions. The six species are *Andropogon gayanus* (CIAT 621), *Brachiaria brizantha* (CIAT 6780), *Brachiaria decumbens* cv. Basilisk, *B. humidicola* cv. Tully, *Centrosema pubescens* (CIAT 15160) and *Stylosanthes guianensis* (CIAT 184). Measurements include inflorescence density, seed set, seed yield and labour requirements **[Regional Department of Agriculture, Region 2, Philippines]**.

Forage Components in Southeast Asia

Planned activities for 1996

1. Select legumes for soil cover in young coconut plantations [Philippine Coconut Authority, Davao, Philippines] and fruit tree plantations [University of Southern Mindanao, Philippines].

2. Evaluate various forage options for coconut plantations with farmers who will also be offered some or all of these forage options for testing on their farms. New research sites are planned in collaboration with the Philippine Coconut Authority in Mindanao and the Provincial Livestock Service in northern Sulawesi, Indonesia.

3. Continue on-farm experiment on fallow improvement at Matalom and a new research site planned in Laos.

4. Select additional research sites to test forage for cut and carry feeding systems in East Kalimantan, Indonesia, at the University of Southern Mindanao, Philippines, and in Laos.

5. Select additional research sites in Indonesia, Laos and Vietnam to test grass-legume associations to improve natural grasslands.

6. Include MPTS at several research sites as one of the forage options for farmers.

7. Commence collaborative research with the Chinese Academy of Tropical Agricultural Science in Hainan to improve persistence of *S. guianensis* swards, broaden the germplasm base and improve harvesting technology.

8. Extend seed production activities to include smallholder farmers in Region 2 in the Philippines.

Project: Institutional support

Project coordinators: P. C.Kerridge and P. J. Argel

Rationale

The Tropical Forage program aims to develop technologies that have wide application. This includes identification of new forage species and accessions, production of genetically enhanced genepools of commercial grasses and legumes and developing forage components with the potential to be used in different farming systems. Some aspects of the development and certainly the transfer of this technology will be carried out in conjunction with the NARS in the different regions. This involves maintaining close contact and working with them in the development of their technical skills.

The most effective way to maintain contact is through regional R&D networks. Where possible, TFP staff will facilitate the development of national networks which can serve as channels for internal information flow to government and non-government sectors and represent countries in regional networks which in turn are used for dissemination of information, new forage germplasm and forage component technologies and for training of national staff. Regional networks not only assist with the rapid flow of germplasm, but in commercial release which is the responsibility of individual countries (Appendix).

Ideally regional networks should be constituted and managed by national representatives from the region with CIAT or other contracted staff acting as facilitator. They can also be used as platforms to attract and manage regional development projects.

The TFP has always actively supported forage networks; the two main networks being the RIEPT (Red Internacional para Evaluation de Pastos Tropicales) for tropical America and SEAFRAD (South East Asia Forage and Feed Resources Research and Development Network) for South East Asia. However, it is likely there will be an opportunity to integrate these the existing forage networks with networks that have a wider focus. One of these is the Agropastoral network in which the Tropical Lowlands Program is active. As ILRI extends its activities to Latin America there may be opportunity to develop a Livestock Network. In Southeast Asia, we have integrated network activities with those of FAO in the region and assume that this will happen with those of ILRI.

The TFP also endeavors to maintain communication channels for scientists in the networks through newsletters and publication of an international journal, Pasturas Tropicales. For scientists in many countries, this is the only avenue for publication of research findings.

Effective institutional support also works for the Program in providing feedback from the ecoregions in which we work and contacts for collaborative research activities.

Objective: To facilitate interaction with and between national organizations involved in tropical forage R&D through active regional networks that exchange information, new germplasm and forage-based technologies and to provide assistance with non-degree training.

Main Activities:

1. Red Internacional para Evaluation de Pastos Tropicales sede Mexico, Centroamerica y el Caribe (RIEPT-MCAC).
2. Red Internacional para Evaluation de Pastos Tropicales sede Sud America (RIEPT-SA).
3. South East Asia Forage and Feed Resources Research and Development Network (SEAFRAD).
4. Training.
5. Research communication.

Highlights

- Regional workshop n Costa Rica on 'Experience with *Arachis pintoi*'.
- Regular publication of three newsletters
  - RIEPT-MCAC, also distributed on Internet
  - RIEPT-SA
  - SEAFRAD
- Scientists trained at workshops and by in-country training.

- Continued publication of Pasturas Tropicales.

- Releases of *Arachis pintoi* CIAT 17434 in Brazil and Costa Rica.

1. Red Internacional para Evaluation de Pastos Tropicales-MCAC

This branch of the RIEPT has been quite active through having a core CIAT scientist outposted in Costa Rica who spends considerable time on network activities. We consider that this is justified because of the many countries in the region with limited resources for forage research and development.

Regional workshop. A workshop on 'Regional experiences with *Arachis pintoi* and . future collaboration in evaluation and promotion' was held in Costa Rica from the 9-13 October in collaboration with the Research Station 'Alfredo Volio Mata' of the University of Costa Rica. It was attended by 30 participants from Mexico, Guatemala, Cuba, El Salvador, Nicaragua, Panama, Colombia, Dominican Republic and Costa Rica and staff from CIAT, Palmira, Colombia.

The workshop was a follow up to a regional workshop held in 1993 on 'Methodology for on-farm pasture evaluation', where each country developed a project with *A. pintoi* as the main forage component.

A total of 26 technical reports were presented covering topics on utilization of A. pintoi as a forage plant and as a cover crop. The group of participants represented national institutions, private companies and international development projects.

Significant advances in the utilization of *A. pintoi* as cover crop in banana and coffee plantations were presented, from experiences in Costa Rica and Nicaragua. The accession CIAT 18744 is performing better than the cv. Amarillo (CIAT 17434) as a cover crop because it produces more stolons and covers the soil more rapidly. It also associates well with stoloniferous grasses and grows well on steep land for the control of soil erosion. This accession will be considered for release in Costa Rica in the near future. Plans for seed multiplication and regional promotion were drawn up.

Research priorities were also defined for establishment studies, characterization of new ecotypes, particularly for tolerance to shade and prolonged dry conditions, and postplanting management. Additional studies on N fixation, utilization as hay, the effect of the legume on cow reproduction and alternative uses of the legume.

The proceedings of the workshop will be published and distributed early in 1996.

Newsletter. Three issues of 250 copies of the Hoja Informativa RIEPT-MCAC/CIAT-TFP were produced and distributed during 1995. The contents of this newsletter covered advances on forage germplasm evaluations, seed availability of new promising forages and short communications on regional events of interest to the pasture network. The newsletter is well recognized in the region. It is also available via INTERNET and records held at CIAT central office in San Jose, indicate that it is accessed worldwide (for access: http://ciat-pc3.iica.ac.cr/riept.htm).

Country visits. During 1995 visits were made to national institutions in Mexico, Honduras, Nicaragua, Panama and El Salvador. The visits had the objective to follow up advances in identifying new forage germplasm and promotion of it at the country level. These visits also permit the TFP to become acquainted with capacity of technical personnel in each country to strengthen and to identify opportunities for collaboration. The opportunity is taken to present technical talks to both technicians and farmers on different aspects of pasture research and promotion.

Germplasm evaluation and exchange. The majority of seed for the research and development needs of countries that participate in the RIEPT-MCAC is produced and distributed from the regional CIAT office in Costa Rica. A small seed unit is maintained at the Escuela Centroamerica de Ganaderia (ECAG). Between August 1993 and September 1995 a total of 127 experimental and basic seed requests were received and delivered to 17 countries, including small amounts of seed forwarded to the US and the United Kingdom to be used in postgraduate studies of Latin American students in these countries. Other countries that requested seed were Barbados, Colombia, Puerto Rico, Suriname, Trinidad and Tobago and Venezuela (who are not officially members of the RIEPT).

Costa Rica was the country with the largest number of requests (78), followed by Honduras (10), Mexico (9) and Nicaragua (8). The amount of basic and experimental seed delivered was 433 kg of grasses and 456 kg of legumes. Additional to this 5 ton of vegetative material of promising new ecotypes of *Arachis pintoi* was supplied in Costa Rica. The seed was requested for use in agronomic evaluation, seed multiplication plots, for studies as a cover crop, on-farm demonstrations and for basic studies of microbiology and plant vigor.

Cultivar release. ***Arachis pintoi*** CIAT 17434 was released in Costa Rica as Mani Mejorador. **[P.J. Argel]**

2. Red Internacional para Evaluation de Pastos Tropicales sede-Sud America

Effective networking is more difficult in South America than in MCAC. Firstly, because of the area involved and the variable needs between countries, it is impractical to service all the needs by one person from one site. Secondly, we cannot justify the resources that would be required. We rely on contacts of individual CIAT scientists based in both the TFP and the Tropical Lowlands Program (TLP) and, in particular, our outposted germplasm specialist based in Brazil who maintains contacts with scientists in Brazil, Bolivia and Paraguay and produces a regional newsletter.

We do have close contact with national scientists in Colombia and have re-established direct contact with those in Pucallpa, Peru. Contact with national scientists in Venezuela and Ecuador depends on contact during country visits and at regional or specialist meetings. The Agropastoral workshops held by the TLP in different countries have proved to be a valuable point of contact as was the *Brachiaria* workshop held in 1994. Some of our staff have been able to attend national and regional agronomy or animal production meetings. Training attachments of national scientists at CIAT for periods of 2-6 months have led to valuable contacts and collaborative research activities.

Brazil-based activities

Newsletter. A 4-monthly newsletter has been distributed to 350 collaborators. A feature of these newsletters has been the focus on different themes such as the latest research findings on *Brachiaria* and *Arachis*.

Country visits. Visits were made to Bolivia, Brazil and Paraguay to discuss forage R&D programs with different national scientists and give informal seminars of CIAT activities.

Visits were also made to Acre and Rondonia to initiate collaborative evaluation activities with EMBRAPA and CIAT scientists involved in the Alternative Slash and Burn Program. The visit was also used to bring national scientists together for a general discussion on germplasm evaluation and forage seed production. Seed was supplied for evaluation in on-farm trials.

Regional germplasm evaluation and exchange. Activities have involved seed multiplication and exchange and collaboration with EMBRAPA scientists in regional evaluation trials with new accessions of *Brachiaria* and *Arachis*.

Seed of new forage accessions is multiplied and has been distributed on request (Table 1).

Table 1. Germplasm requests and seed supply to CPAC and RIEPT collaborators

	1994		1995	
Item	Request	Sent	Request	Sent
	No.	kg	No.	kg
Grasses				
CPAC	21	4	11	3
RIEPT	155	29	123	5
Legumes				
CPAC	63	270	34	11
RIEPT	259	207	79	22

Source: Ronald P. de Andrade

Between 1994-1995 a total amount of 150 kg of seed was supplied for on-farm trials; 130 kg was *Arachis pintoi* CIAT 22160 for on-farm trials.

Regional evaluation of the pre-selected *Brachiaria* accessions was initiated. The germplasm under evaluation is listed in Table 2.

Regional evaluation of the pre-selected *Arachis pintoi* accessions has also been initiated by multiplication of the 16 accessions (Table 3). This is being carried out in close collaboration with the seed unit of the Animal Production Program at CPAC **[R. P. de Andrade and E. A. Pizarro]**.

Table 2. *Brachiaria* germplasm under evaluation in the Brazilian Cerrado with alternative accession numbers.

Germplasm		BRA	CPAC	CNPGC	CIAT
B. brizantha		002801	3337	B108	16121
		002844	3341	B112	16125
		003000	3649	B187	16150
		003204	3386	B132	16288
		003247	3390	B61	16294
		003361	3401	B138	16306
		003387	3403	B70	16308
		003395	3404	B140	16309
		003441	3409	B72	16315
		003450	3410	B144	16316
		003484	3413	B146	16319
		003719	3435	B158	16441
		003824	3446	B163	16457.
		003891	3451	B166	16467
		003948	3456	B89	16473
		004308	3555	B178	26110
	C*	000591*		3099	-6294
B. decumbens		004391	3464	DI	16488
	C*	001058*		3088	- 606
B. humidicola		005118	3564	H16	26149
		005011	3545	H13	16886
	C*	Comm.	-	-	679

* Control RIEPT-BRAZIL Comm. = Commercial

Table 3. *Arachis* germplasm under multiplication for regional evaluation in Brazil.

Germplasm	BRA No.	CIAT No.
Arachis pintoi	0013251	17434 *
	0015121	18748
	0022683	-
	0030333	-
	0030368	22172
	0030546	22256
	0030872	22257
	0031135	22159
	0031143	22160
	0031496	22236
	0031534	-
	0031542	22269
	0031801	-
	0031828	-
	0031861	-
A. repens	0031801	-

* cv. Amarillo- Control RIEPT-BRAZIL

Colombia-based activities

Copies of the Biennial Report and other publications were distributed to RIEPT members.

Regional evaluation data has been entered into the RIEPT evaluation data base. The data base has been very useful in assembling additional sets of germplasm for evaluation. **[L.H. Franco]**

Visits were made to Bolivia, Ecuador, Peru and Venezuela by various TFP members to become familiar with current activities of national organizations and discuss future collaborative activities. These visits have resulted in younger national scientists coming to CIAT

for training and in development of plans for on-farm evaluation. Continuing contact with these other countries would be assisted by the presence and activities of a liaison scientist, either nominated by the country or appointed by CIAT when funding becomes available.

Cultivar release. *Arachis pintoi* CIAT 17434 has been released in the State of Sao Paulo-Brazil, as cv. Amarillo-MG-100, by a private seed company. The main target use at the moment is as a ground cover, since more than 3.5 millon ha are cultivated with citrus and coffee in Brazil.

3. South East Asia Forage and Feed Resources Research and Development Network (SEAFRAD)

The activities in the SEAFRAD network are carried out jointly with CSIRO Division of Tropical Crops and Pastures with funding provided by the Australian Government under a special project "Forages for Smallholders Project (FSP)" which commenced in January 1995. SEAFRAD collaborates with the FAO Regional Working Group on Grazing and Feed Resources.

The activities in this network are to:

- facilitate communication and networking within countries
- make new forage germplasm and forage component technology available
- develop collaborative R&D activities with national scientist in tropical areas of China, Indonesia, Lao PDR, Malaysia, Philippines, Thailand and Vietnam
- produce and distribute a regional newsletter with

- the assistance of national coordinators
- hold annual regional meetings
- conduct training in forage technology and technology transfer

Regional meeting: The 3rd Regional Meeting of the "S.E. Asian Regional Forage Seeds Project", hosted by the Indonesian Government was held in Samarinda,East Kalimantan, Indonesia, 23-28 October 1994. The proceedings were published as CIAT Working document No. 143 in 1995.

Newsletter. The first issue of the SEAFRAD Newsletter was published in October 1995 with the second issue due in December 1995.

The first issue of FSP News, an information sheet of the Forages for Smallholders Project (FSP) was distributed in September 1995. This News sheet was translated into five local languages and widely distributed in all seven countries.

Germplasm evaluation and exchange. This has been covered in the reported under the Forage Genetic Resources Project (see page 2-22).

4. Training

Training is a major feature of the CIAT activities but has been severely restrained by budget cuts. The Training Unit has continued to receive and subsidize the training of individual visitors and students to CIAT and is active in seeking additional funding for holding group courses. Training needs are being reviewed for individual countries.

Cali, Colombia. Training has been provided as short - term on-the-job training and for students undertaking B.S., M.S. and PhD degrees (Table 4).

Costa Rica. National scientists from Costa Rica and Nicaragua received in-service training. Three students from the University of Costa Rica worked on research projects for their B.S. degree.

Brazil. Five students, two for M.Sc. and three for an Agronomy degree, are currently conducting their thesis research with staff from UnB-Brasilia/EMBRAPA-CENARGEN-CPAC/CIAT on *Paspalum atratum* BRA 009610 and *Arachis pintoi* accessions.

South East Asia. As part of the AIDAB funded "S.E. Asian Regional Forage Seed Project (1992-1994)" the following training was provided for collaborators in Indonesia, Malaysia, Philippines and Thailand in practical aspects of forage evaluation, seed technology, seed production and management of forages:

- In 1994, four technicians from the Department of Livestock Development, Thailand spent one month at the Division of Tropical Crops and Pastures, CSIRO , Australia.

- In 1994, three collaborating scientists from Universities in the Philippines spent 11 weeks at the Division of Tropical Crops and Pastures, CSIRO, Australia.

- A 2-week training course was conducted for 40 staff of the Directorate General of Livestock Service and Provincial Livestock Officers of Indonesia in Baturraden in 1994 (sponsored by the Government of Indonesia).

- In the Philippines a 2-day training course was held for 15 staff of the Department of Agriculture in Northern Mindanao in 1994 (sponsored by the AIDAB funded "Pilot Provincial Agricultural Extension Project").

- In 1994, a week-long training course on seed production was conducted for 17 scientists involved in the national forage R & D network at Los Baños, Philippines (co-sponsored by the Philippines Council for Agriculture, Forestry and Natural Resources Research and Development).

- Additionally, on-the-job training was provided for collaborators by attaching them to the central forage species screening and multiplication site at Los Baños, Philippines for short period.

- In 1995, a 4-week "International Training Course for Trainers: Participatory Research with Farmers in Feed Resources" was held at the Visaya State College for Agriculture, Leyte and at the International Rice Research Institute, Los Baños, Philippines. This course was taught largely by Ms. Teresa Gracia, IPRA, CIAT

  Hillsides Program and was attended by 14 collaborators of the "Forages for Smallholders Project (FSP)" from Indonesia, Laos, Malaysia, Philippines, Southern China, Thailand and Vietnam. These participants will hold in-country courses for other collaborators involved in the FSP project.

- In October 1995, the first in-country "Training Course on Participatory Research in Forages" was held at CVARRD, Isabela State University, Philippines on 9-20 October 1995 for 18 collaborators of the FSP.

Table 4. Training provided to national scientists at CIAT, Colombia, 1994-95

Country	Area of training	Duration	No. persons
Austria	PhD-Plant nutrition	2 years	1
Bolivia	On-farm agronomy	2 months	3
	Forage agronomy	1 week	3
Brazil	Pathology	1 month	1
	Genetics	1 month	1
Colombia	M.S.-Seed biology	2 years	2
	B.S.-Plant nutrition	1 year	4
	B.S.-Genetics	1 year	1
	B.S.-Germplasm	1 year	7
	B.S.-Forage quality	1 year	4
	Forage agronomy	1 week	12
	Forage quality	2 weeks	2
	Pathology	5 months	1
China	Pathology	3 months	1
Germany	PhD-Forage agronomy	2 years	1
	PhD-Plant nutrition	2 years	
	B.S.-Plant nutrition	1 year	1
	B.S.-Germplasm	1 year	1
Honduras	M.S.-Forage quality	2 years	1
Netherlands	Forage agronomy	6 months	1
Paraguay	Germplasm	2 months	1
Switzerland	PhD-forage quality	2 years	1
United Kingdom	Forage quality	7 months	1
Venezuela	Entomology	1 month	2

4. Research Communication.

Distribution of books, bibliographies and the journal has always been a widely recognized feature of CIAT and the TFP. Distribution was restricted in the last few years due to financial cutbacks and an effort was made to achieve greater cost recovery. However, our present assessment is that this has been counter-productive in terms of both image and wide distribution of information. Hence we will attempt to resume distribution but to a more restricted and frequently updated list of active forage research and development workers and agencies involved in agricultural development.

The main activity is the editing and publication of three issues annually of Pasturas Tropicales. The journal celebrated its tenth anniversary in 1995. We gratefully acknowledge the dedication of the editor, Alberto Ramirez, for his untiring efforts in assisting authors and ensuring the high standard of the journal is maintained.

We also congratulate the CIAT Library for their efforts in bringing out an new Bibliography on Tropical Forages.

Appendix 1. Grasses and legumes released as commercial cultivars adapted to acid tropical soils (1980-1995).

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
		Maní Mejorador	1994	Costa Rica
		Amarillo-MG-100	1995	Brazil
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

Project: Institutional support

Proposed activities 1996

1. **RIEPT-MCAC**

- Continue with the publication and the distribution of the Newsletter.

- Continue seed multiplication and distribution of promising germplasm.

- Continue with in country visits to follow up forage germplasm evaluation and promotion, particularly those related to projects discussed during the regional workshop on *A. pintoi*.

- Collaborate with the implementation of two regional project proposals:
  i) Tropileche, a consortium formed by several institutions of Costa Rica and Perú to improve legume-based feeding systems for smallholder dual-purpose cattle.
  ii) a training project to strengthen sub-regional mechanisms for technology transfer through training in Mexico and Central America and the Andean zone of South America.
- Continue collaborative research with the CIAT Hillside Program in Honduras and Nicaragua.

2. **RIEPT-SA**

- Continue with publication and distribution of a newsletter
- Continue seed multiplication and distribution
- Make visits made to Bolivia, Ecuador, Paraguay, Peru and Venezuela
- Pursue development of collaborative research projects in Bolivia
- Maintain the RIEPT evaluation database
- Distribute the Biennial Report and other publications

3. **SEAFRAD**

- In January 1996, the first regional meeting of the new "Forages for Smallholders Project" will be held in Vientiane, Laos, in conjunction with the FAO "Regional Working Group on Grazing and Feed Resources" and FAO Project "Better Use of Locally Available Feed Resources".
- Continue to support the publication of the SEAFRAD Newsletter.
- Produce and distribute further FSP News sheets.
- Continue collaborative research in Indonesia, Laos, Philippines, Thailand and Vietnam and commence activities in southern China.

4. Training

Colombia

- Meet requests for training for scientists from Brazil, China and Paraguay

- Assist with in-country training courses in Bolivia

- Conduct training course in on-farm methodology for evaluation of feed resources for dual-purpose cattle production

- Continue with student training for B.S., M.S. and PhD degrees

South East Asia

- Hold in-country training courses on Participatory Research in Thailand, Indonesia Laos, and Vietnam.

5. Research communication

- Continue publication of Pasturas Tropicales

- Establish an Editorial Advisory Board for Pasturas Tropicales

- Publish and distribute proceedings of the *Brachiaria* workshop "Biology, Agronomy and Improvement of *Brachiaria*"

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Donors - Supplementary Funding[1]

Donor/Project	Project Duration	Project Total (To CIAT) USD
Complementary:		
Australia-AusAID		
Forages Seeds	1992-1994	792,600 (500,000)
Forages for Smallholders	1995-1999	3,268,000 (1,634,000)
Australia -ACIAR		
Anthracnose resistance in Stylosanthes	1993-1994	170,000 (57,000)
Austria - Academy of Sciences		
Mechanisms of acid soil tolerance	1994-1996	52,884
Colombia-Nestlé		
Pilot development program - Caquetá	1995-1999	544,960
Colombia-Fedegan		
Resistance to spittlebug in grasses	1995-1999	140,000
Germany - BMZ		
Rhizosphere P dynamics	1994-1996	131,471
Desmodium-genotype x environment	1995-1997	230,400 (103,400)
Great Britain-ODA		
Anti-quality factors in legumes (with IGER).	1991-1994	(25,000)
Japan - JIRCAS[2]		
The role of endophytes in tropical grasses	1995-1999	1,000,000
Systemwide:		
SLI - Legume-based forage systems for dual-urpose cattle[3]	1996-1998	2,555,000 (1,200,000)

[1] Funds available to the TFP are indicative as some funds are distributed to other organizations.
[2] This project sourced extra funding but is now included in the core budget from Japan.
[3] Funds will also be used in other Program areas.