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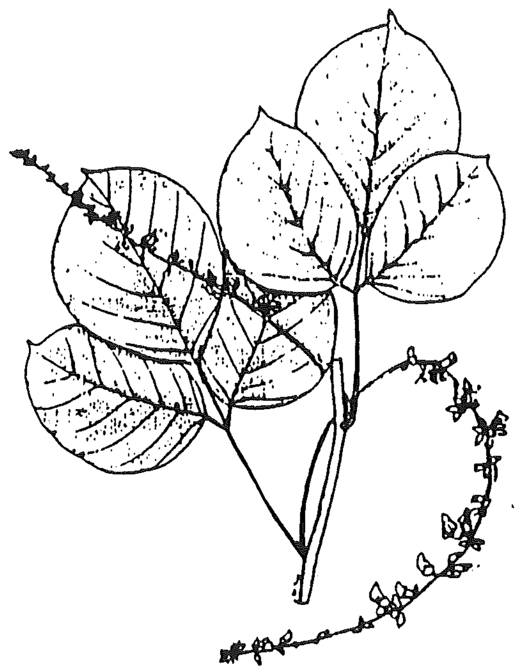
Annual Report 1996

Tropical Forages Program



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November 1996

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

ACKNOWLEDGEMENT

With the structural changes introduced in the medium term plan of CIAT (1998-2000) that eliminates the continuity of the Tropical Forages Program (TFP) as an entity within CIAT, the members of the TFP wish to acknowledge the efforts of Dr. Peter C. Kerridge in promoting and defending a continuing mandate in tropical forage research at CIAT. Peter has tirelessly led and championed the mandate of tropical forages since 1992, during difficult times of progressively eroding funding. His insistence and ability to communicate a compelling vision of the crucial role of forages in sustainable tropical agriculture has been critical in maintaining high visibility for forage research in an environment where competing activities might seem to hold greater short-term appeal. Not only the staff of the Tropical Forages Program, but all beneficiaries of CIAT's research output must be indebted to Peter's inspired leadership.

Members of the Tropical Forages Program

Tropical Forages Program

Annual Report 1996

to the Program Committee of the Board of Trustees, CIAT, November 1996

For limited circulation

Introduction

A comprehensive report of the Tropical Forages Program, in the form of a Biennial Report for 1994-95 was presented to the Program Committee of the Board of Trustees of CIAT in February 1996. At that stage we felt considerable pride in the achievements of the Program during the preceding two years and a high expectation of new achievements over the next two years. However, changes in staff numbers and time devoted to re-defining the research agenda, largely due to funding shortfalls, has severely disrupted research output during the remainder of 1996. This process of re-definition of objectives within the context of preparation for the MTP 1998-2000 is still on-going. Nonetheless both international and support staff have valiantly carried on the effort of maintaining on-going research and initiating new areas of research.

In April, three of the nine positions in the Program were cut, those of the germplasm specialist in CIAT-Palmira, and the outposted scientists in Central America and Brazil who were also conducting forage germplasm research. Research has been consolidated into two forage projects with some forage research also being conducted within cross-center projects. It is being proposed that core activities in forage germplasm research be further curtailed and that forage research activity be consolidated into a single project.

Considerable time has been devoted at the Program level to ensuring either an efficient phase-out of the research underway in the positions that were cut or attempting to put alternative structures in place that will allow prior commitments with other organizations to be met. It was proposed to carry out some of the germplasm specialist activities through a postdoctorate position in tropical forage biology. A suitable candidate has been identified, though the position is on hold pending a clarification of funding availability and perhaps a broadening of the job description to include other crop areas. In Central America, support was given at the regional level through the Council of Ministers for Agriculture (CORECA) for continuation of forage network activities (the RIEPT-MCAC) but funds still need to be identified. There is a good possibility of forage activities being incorporated into the Central America Hillsides project and for forage input into a Training Project, both of which would be funded by Special Project funds. In Brazil, CENARGEN have indicated that they might contract the germplasm specialist and CIAT would apply some funding sourced from Brazil towards operational expenditure of the activities.

At the time of the last Program Meeting and in the CIAT submission to TAC (CIAT 1997 Program Plans and Funding Requirements) three forage projects were envisaged:

Project #16: Identifying and characterizing tropical grasses and legumes for multiple uses.

Project # 4: Genetic enhancement of tropical grasses and legumes for feed and soil improvement in the subhumid and humid tropics.

Project # 9: Utilization of tropical grasses and legumes in production systems of the subhumid and humid tropics.

Following staff reductions announced in April, the forage research was consolidated into two projects and workplans re-written for an 18 month period, July 1996-December 1997:

Project # 4: Identification, characterization and enhancement of tropical grasses and legumes for multiple end uses.

Project # 9: Utilization of tropical grasses and legumes in production systems.

These two projects form the structure in which we are reporting the activities of the tropical forage research for 1996, though some activities also occur within other projects as illustrated in the work breakdown structure of the TFP. (See work breakdown figure).

Research Highlights

Research progress of the two projects is given in detail on the following pages. However, we would like to emphasize several key accomplishments that demonstrate continuing vitality of the research.

Project 4: Identification, Characterization and Enhancement of Tropical Grasses and Legumes for Multiple End Uses

- A major collaborative effort with the GRU has resulted in a full revision of data on forage germplasm held in the GRU and its incorporation into the SINGER system.

- Research commenced on the associations between geographic and biochemical characterization data of *Stylosanthes* using GIS technology.

- Several materials of *Cajanus cajan* were identified in the mid-altitude hillsides that offer high potential for forage, use as live barriers, or grain production.

- Identified new SCAR, AFLP and the RFLP markers linked to apomixis in *Brachiaria*.

- New lines of spittlebug resistant hybrid *Brachiaria* are displaying higher vigor during establishment than commercially available lines.

- Evaluation of new *Arachis* germplasm continues to show the potential of this species for wide adaptation.

- New methodology has been developed to detection of endophytes on tropical grasses

- Found differences among legume species with and without tannins in extent of dry matter degradation and end products (VFA's) of fermentation by rumen microbes

- Found differences among legume species in condensed tannin composition using HPLC, as indicated by procyanidin, delphinidin and pelargonadin ratios.

- Field evaluation of germplasm accessions and sexual recombinants of *Brachiaria* in wet and dry seasons resulted in identification of one spittlebug resistant sexual recombinant which exhibited several desirable attributes that could contribute to adaptation and persistence in low fertility acid soils.

- Showed that *Arachis pintoi* (CIAT 18748) was outstanding in it's adaptation to low P supply to soil as revealed by the extent of nodulation, leaf area production and P use efficiency.

Project 9: Utilization of tropical grasses and legumes in production systems of the subhumid and humid tropics

- Showed that the presence of *Arachis pintoi* in association with *Brachiaria dictyoneura* enhanced the ability of the grass to acquire P from rock phosphate as P source.

- Found greater compatibility and persistence of *Desmodium ovalifolium* in association with grasses in sandy loam soil than *Arachis pintoi, Centrosema acutifolium* and *Stylosanthes capitata.*

- Showed that both grazing system and stocking rate could influence total root biomass and root length in grass alone and grass + legume pastures.

- Showed a large increment in milk yield by the inclusion of *Cratylia argentea* in a forage-based supplement fed to cows grazing pastures with limited forage availability.

- Showed that milk urea nitrogen, a potential useful nutritional indicator, varied more with pasture attributes than with animal breed or climatic factors.

- Derived methodological principles for on-farm evaluation of improved feed resources with milking cows.

- Identified *Arachis pintoi* CIAT 22160 as a promising ecotype to replace the commercial ecotype (CIAT 17434) in crop-livestock systems for the Lowland Tropics.

- Demonstrated that fallow oversown with legumes has the potential to increase overall productivity of soils in hillsides of north Cauca.

- Showed through a simulation model that legume-based feed technologies could be economically attractive to smallholder dual purpose cattle farmers in subhumid areas of Costa Rica, provided labour costs were kept down or the genetic potential of cows to produce milk was increased.

- Showed through an ex-ante analysis that economical internal rates of return would increase significantly if calving rate and milk yield were improved with Arachis-based technologies in dual purpose cattle farms in forest margins of Caqueta, Colombia.

- TROPILECHE-A systemwide Livestock Project for improving feed resources in smallholders dual purpose cattle systems, is operating in two benchmark sites (Costa Rica and Peru) and has received strong support from NARS in LAC as a platform for collaborative research and information exchange.

- Forages for Smallholders Project-A Southeast Asian consortia for on-farm testing of new forage alternatives has shown that the concept of participatory research is keenly adopted by collaborators.

The Future

In coming year Project # 4 will become the single vehicle to the tropical forage mandate. Activities within Project # 9 will be incorporated in a new project entitled "Integrated Natural Resource Management for Intensifying Small Holder Farming in the Tropics" which will be directed specifically towards production systems which utilize CIAT's mandate crops of beans, cassava and forages.

We are assured that forage germplasm-based work, linked to a strong focus on natural resources management, will remain as an important component of CIAT's core research program. The emphasis of the core research activity will be in identifying and making grass and legume germplasm available as components in the development of intensive and sustainable agricultural systems. The focus on the use of forages for natural resource management will include the utilization of forages for livestock as livestock play a significant role in generating cash flow and alleviation of poverty in many smallholder production systems.

TROPICAL FORAGES PROGRAM

Program Goal

To increase the efficiency of livestock production and contribute to sustainable land use in production systems of the subhumid and humid tropics through collaborative research in the identification, improvement and deployment of muti-purpose grasses and legumes

Outputs

1. Legume and grass ecotypes identified for multi-purpose end uses and securely maintained

Activities:
- Acquire new germplasm
- Conserve germplasm securely
- Predict occurrence and potential distribution of species
- Evaluate accessions for climatic and edaphic adaptation
- Determine genotypic variation in adaptive attributes
- Evaluate potential for soil enhancement and protection
- Determine nutritive value for livestock
- Investigate the nature of anti-quality factors in legumes
- Investigate the role of endophytes in grasses
- Develop information system on adaptation and use

Through Projects: #14, #15, #16

2. Gene pools of commercial grasses and legumes with high feed value and tolerance to abiotic and biotic stresses

Activities:
- Investigate mechanisms for tolerance to main disease and pests
- Develop screening procedures for insect and disease tolerance
- Develop screening procedures for feed quality
- Identify nutrient efficient genotypes
- Develop superior gene pools of *Brachiaria*, *Panicum* and *Paspalum*
- Develop superior gene pools of *Arachis* and *Stylosanthes*
- Define genetic control of key attributes, including apomixis in *Brachiaria*
- Identify new cultivars of shrub legumes, viz. *Cratylia* and *Leucaena*
- Investigate factors affecting seed quality
- Develop efficient seed production systems

Through Projects: #4, #5

3. Forage components developed and deployed in production systems

Activities:
- Develop forage systems for dual-purpose cattle in tropical America
- Develop forage components for production systems in Asia
- Use grasses and legumes to rehabilitate degraded grazing lands
- Investigate use of legumes for fallow improvement
- Deploy grasses and legumes for soil cover and erosion control
- Investigate agro-industrial uses of grasses and legumes
- Conduct ex-ante and ex-post impact analysis of new technology
- Carry out socio-economic studies of forage adoption
- Develop indicators of sustainability for grazing lands
- Assess economic impact of forages on sustainable land use

Through Projects: #10

4. Research and training networks in Latin America and S.E. Asia

Activities:
- Coordinate a forage research network in Central America (RIEPT-MCAC)
- Facilitate a forage research network in South America (RIEPT-SA)
- Coordinate a forage and feed resources network in SE Asia (SEAFRAD)
- Assist in individual and group training with national partners
- Organize technical workshops and conferences
- Provide for exchange visits between researchers
- Publish regional newsletters
- Publish Pasturas Tropicales

Through Projects: #18

PROJECT 4

Identification, Characterization and Enhancement of Tropical Grasses and Legumes for Multiple End Uses

Project Manager: Peter C. Kerridge

The Challenge

In tropical Latin America, Asia and in Africa, smallholders with few resources are looking for diverse options to ensure greater stability of productivity and income. Options available to them include raising cattle to generate income, producing concentrate feeds for sale, and adopting low cost measures to protect and increase the value of their water and soil resources.

Grasses and legumes well adapted to climate, soil, and pests and diseases, and which require minimal inputs or management, can contribute to more efficient animal production. In addition, they can improve soil fertility, increase water retention and reduce soil loss. Wild species of grasses and legumes suitable for use in farming systems comprise a rich resource, providing that they can be characterized for their usefulness in different geographic and production system niches.

Further, where potentially useful plants lack certain advantageous characteristics, the task remains to identify those characteristics in wild relatives and transfer them to the more desirable plants.

Objective

To identify, and improve where necessary, wild grasses and legumes for the feeding of livestock and for soil improvement. These suitable and well adapted forages can then be distributed to farmers, through local and international rural organizations, to optimize the use of natural plant diversity.

Strategy

To build on the strength of previous research in forage genetic resources and the linkages with NARS, through regional networks such as RIEPT (Latin America) and SEAFRAD (S.E. Asia), and with other advanced organizations, to:

- Define adaptations required in grass and legume species (including shrubs) for those species to fill different agroecosystem niches for livestock feed and natural resource use
- Evaluate wild accessions of grasses and legumes with the potential to meet these requirements, and/or combine attributes needed to overcome existing limitations and select improved genotypes
- Develop an information system on species characteristics, and distribute new germplasm

Outputs

Project activities generate:

- New grass and legume ecotypes, characterized for their climatic and edaphic adaptation, feed quality, and role in natural resource management

- New legume cultivars of commercial grasses, herbaceous legumes and shrubs

- Identification of adaptive attributes for biotic stresses, such as spittlebug (affecting grasses *Brachiaria* and *Panicum*) and anthracnose (a threat to important herbaceous legumes *Arachis* and *Stylosanthes* and shrubs *Cratylia* and *Leucaena*), and abiotic stresses; and a greater understanding of the detrimental factors affecting forage quality

- Knowledge about the role of endophytes found in tropical grasses in reducing pest and disease incidence

- Information system on the potential suitability of grasses and legumes for different ecoregions and ecosystems

Activities

The activities of Project 4 are grouped into the following 4 subprojects:

1. Identification of grasses and legumes with known environmental adaptation and feed value for different niches
2. Development of superior grass genotypes to the level of commercial cultivars;
3. Identification of superior *Arachis* and *Stylosanthes* genotypes; and
4. Identification of new accessions of multipurpose shrub legumes.

(See work breakdown structure of Project 4).

Benefits

The project offers low income farmers access to new grass and legume resources, through government, NGO and farmer organizations. The resources are adapted for use in crop-livestock and perennial tree systems in lowland and hillside tropical America; in Asia's upland farming systems; and in intensive crop-livestock systems in Africa.

Project Team

- **International Center for Tropical Agriculture (CIAT)**
  The CIAT team consists of a germplasm specialist, geneticist, pathologist, entomologist, feed quality specialist and plant nutritionist, and collaborates with other CIAT teams in the lowland, hillside and forest margin ecosystems, to identify and evaluate species.

- **National organizations**
  Both strategic and applied aspects of the research will be undertaken in collaboration with research teams in EMBRAPA, CORPOICA and other members of the RIEPT network in LAC and the SEAFRAD network in Asia.

- **Other advanced organizations**
  Close liaison will be maintained with ILRI in Africa, CSIRO (Australia), OFI (UK), and the Universities of Hohenheim and Kassel (Germany).

Project #4: Identification, Characterization and Enhancement of Tropical Grasses and Legumes for Multiple end Uses

Project Goal

To assemble, evaluate and enhance tropical grasses and legumes with the potential for use as feed and for soil improvement in different agroecosystems of the humid and subhumid tropics

MAIN OUTPUT: Improved Grasses and Legumes with Commercial Potential

Sub-projects / *Outputs*

To identify grasses and legumes with known environmental adaptation and feed value for different niches

- New herbaceous species evaluated
- Genetic variation for adaptation to climatic, biotic and edaphic stresses quantified for promising genera (Desmodium, Arachis, Stylosanthes)
- Forage antinutritional factors of selected legumes better understood
- Information systems on forage adaptation to different agroecosystems

To develop superior grass genotypes to the level of commercial cultivars

- Spittlebug resistance in Brachiaria assessed and characterized
- Host-plant relationships, ecology and populations dynamics of spittlebug are understood
- Virus resistance in Brachiaria assessed
- Nutrient efficient grass genotypes identified
- Role of endophytes in tropical grasses assessed
- Improved sexual Brachiaria genepool with edaphic adaptation and insect resistance
- Genetic control of molecular markers for key grass attributes investigated. e.g. spittlebug resistance, edaphic adaptation expression of apomixis
- Promising accessions and lines of Brachiaria, Panicum and Paspalum for production systems evaluated

To identify superior *Arachis* and *Stylosanthes* genotypes

- Anthracnose resistance in Stylosanthes characterised
- Pathogen biodiversity in Colletotrichum gloeosporioides characterised
- Potential importance of diseases in A. pintoi assessed
- Virus resistance in Arachis investigated
- Nutrient efficiency in legumes identified
- Disease resistance in Stylosanthes enhanced
- Resistance to Wilt disease of Stylosanthes identified
- Feed value of new accessions of Arachis and lines of Stylosanthes evaluated
- Advanced accessions and lines of Stylosanthes and Arachis production systems evaluated

To identify new accessions of multipurpose shrub legumes

- Shrub legumes for different agroecosystems identified
- Promising accessions of *Cratylia*. *Leucaena* and *Calliandra* for production, disease and pest resistance evaluated
- Methodology for establishment and and growth of shrub legumes developed

Subproject 1.0

Identification of grasses and legumes with known environmental adaptation and feed value for different niches

Output 1.1

New herbaceous and shrub legume species evaluated

Main achievements

- The vigorous establishment with excellent soil cover of 10 *Galactia striata* accessions at Quilichao highlights again the advantage of selecting germplasm at Carimagua, a site with high environmental pressure.

Activity: Analysis and publication of evaluation of *Cajanus, Chamaecrista,* and *Galactia* in Colombia

Chamaecrista rotundifolia. Data of the germplasm evaluation at Carimagua have been analyzed. A technical paper on "characterization and preliminary evaluation of *Chamaecrista rotundifolia* germplasm in the Eastern Plains of Colombia" is being prepared to be submitted to "Genetic Resources Communications". In addition, contribution was made in analyzing geographic data of origin for a paper submitted to the IGC'97 in Canada on the "Origin of the world's collection of the tropical forage legume *Chamaecrista rotundifolia*" by Pengelly *et al.* **[B.L. Maass and E.A. Cárdenas]**

Galactia striata. Ten accessions of *Galactia striata*, which have previously been selected from a large germplasm collection at Carimagua, plus one native accession, that was growing vigorously in a *Brachiaria humidicola* pasture in the northern Cauca region, were sown in a small plot agronomic trial at Santander de Quilichao. The establishment of almost all materials was very vigorous and soil cover was excellent. This highlights again the advantage of selecting materials at Carimagua, which is a site with high environmental pressure. A cutting regime was started 5 months after establishment and showed large differences among accessions (Table 1). Initially, accession CIAT 17971 was outstanding but it did not maintain a high production level during the following dry season, when accessions CIAT 20786 and 8151, and the native one performed better. The experiment will continue for another year. **[B.L. Maass and E.A. Cárdenas]**

Activity: Analysis and publication of evaluation of *Calopogonium* accessions in Brazil

Two hundred fifteen accessions of the tropical forage legume *Calopogonium mucunoides* were evaluated at two fertility levels on a red-yellow latosol in the Brazilian Cerrado. A cluster analysis was performed on data for five agronomic attributes to define 12 clusters, whose dry matter yield (cluster mean) ranged from 1 to 3.6 t/ha/yr. There was a negative relation between seed yield and forage yield. Clusters with high seed yielding accessions were low in dry matter forage yield. Accessions differed also for leaf size and leaf pubescence. The presence of leaf pubescence had a negative effect on in vitro digestibility, as hairs were found

Table 1. DM yield of 11 *Galactia striata* accessions in Santander de Quilichao, Cauca.

CIAT accession		DM yield (g/m^2)	
(no.)	Origin[a]	Establishment	Regrowth
17971	Meta, COL	202 a	84 abc
20786	Cauca, COL	165 ab	135 a
native	Cauca, COL	119 bc	127 ab
8151	Casanare, COL	119 bc	127 ab
20787	Cauca, COL	109 bc	103 abc
8143	Casanare, COL	99 bc	88 abc
8148	Casanare, COL	92 c	65 bcd
7236	Monagas, VEN	88 c	14 d
20758	Valle, COL	86 c	88 abc
8749	Magdalena, COL	79 c	51 dc
8139	Casanare, COL	59 c	60 dc

Means followed by the same letter are not significantly different ($P < .05$).
a. COL = Colombia; VEN = Venezuela

to be impregnated with lignin and cutin. Of the 215 accessions, 17 were selected for more intensive evaluation. Two accessions (CIAT 822 and CIAT 20709) are outstanding for their leaf retention during the dry season. **[E.A. Pizarro and M.M. Carvalho]**

Activity: Commence evaluation of *Paspalum* species for poorly drained conditions

There is a need for additional forage species for poorly-drained situations such as vast areas of lowland savannas and lower areas in most landscapes. The genus *Paspalum* offers good possibilities for increasing the availability of improved grasses for this area.

Eight accessions of *Paspalum* spp. which showed promise in evaluation in Brazil have been passed through quarantine, multiplied in the greenhouse, and planted in a situation with poor drainage at Santander de Quilichao in October 1996, for agronomic evaluation. **[L.H. Franco, P.C. Kerridge, and E.A. Cárdenas]**

Activity: Evaluation of species for seasonally dry areas

During 1996, seeds of forage germplasm for multilocational trials were distributed to Corpoica for planting in four locations in the Caribbean Coast region of Colombia. *A. pintoi* (four accessions), *Pueraria phaseoloides* (7); *Centrosema* spp. (8), *Neonotonia wightii* (1); *B. brizantha* (6), *P. maximum* (8), and a collection of forty materials of *Leucaena* and other shrub species of interest.

Germplasm of the genera *Arachis, Aeschynomene, Stylosanthes, Pueraria, Cajanus, Cratylia, Canavalia, Centrosema, Chamaecrista, Calopogonium, Codariocalyx, Leucaena, Acacia, Brachiaria*, and *Panicum* was sent to four locations in the Orinoquia (Llanos) region. Materials will be sent to additional locations in the inter-Andean valleys and the Amazon basin for testing during 1997. **[L.H. Franco, P.C. Kerridge, and B.L. Maass]**

Output 1.2

Genetic variation for adaptation to climatic, biotic and edaphic stresses quantified for promising genera (*Desmodium*, *Arachis*, and *Stylosanthes*)

Main achievements

- New vigorous germplasm of *Arachis pintoi* with high potential for soil cover was identified in multilocational trial.
- Advanced *Arachis pintoi* accessions previously selected are among the best performing and persisting materials in multilocational trial in Colombia.
- Showed that the establishment of *A. pintoi* was more rapid when associated with a tussock forming grass, *P. maximum* than with a stoloniferous grass, *B. humidicola*.

Activity: Study G-E interactions of *D. ovalifolium* with respect to yield

From April to June 1995, field trials were established at six sites (Table 2). The core collection (18 accession, (Table 3)) was planted in 6x5 m sub-plots in a split-plot design with two fertilizer levels (main plots) and three replications at each site. Following the evaluation of seedling emergence 15 weeks after seedling, plant height and lateral extension, soil cover, plant vigor, and pest and disease incidence were recorded every six weeks for a total of 30 weeks. Flowering patterns were also observed during this period.

Table 2. Evaluation sites of *Desmodium ovalifolium.*

Site	Latt.	Long.	Elevation. (m)	Acidity	Al saturation	Fertility	Temp.	Dry season stress
El Melcho-Cauca	02°44'23"N	76°33'34"W	1555	M	L	M	Cool	H
La Romelia-Chinchiná	04°58'20''N	75°39'58''W	1360	M	L	M	Cool	L
Macagual-Caquetá	01°29'59"N	75°39'33"W	190	H	H	L	Hot	L
La Rueda-Caquetá	01°26'10"N	75°25'47"W	180	H	H	L	Hot	L
Alcancía-Carimagua	04°34'37"N	71°21'09"W	150	H	H	L	Hot	H
Maquenque-Carimagua	04°31'17"N	71°15'41"W	150	H	H	L	Hot	H

H= high M= moderate L= low

Table 3. Core collection selected for *Desmodium ovalifolium*- trials

Accession (CIAT)	Collection site	Province	Country	Latitude	Longitude	Elevation (m)	Precipitation mm/a	dry season No. months (<100mm)
350	no information available							
3788	Narathiwat	Narathiwat	Thailand	6°02' N	101°53' E	50	2770	0
3793	Thepha	Songkhla	Thailand	6°52' N	100°59' E	50	2220	1
13030	Hatyai	Songkhla	Thailand	6°56' N	100°28' E	60	1880	2
13086	Pr. Khiri Khan	Pr. Khiri Khan	Thailand	11°43' N	99°44' E	60	1160	5
13089	Chumphon	Chumphon	Thailand	10°04' N	99°04' E	40	1870	3
13105	Ipoh	Perak	Malaysia	4°45' N	101°07' E	80	2440	0
13110	Alor Gajah	Melaka	Malaysia	2°23' N	102°13' E	80	2150	0
13125	Trang	Trang	Thailand	7°30' N	99°38' E	40	2140	2
13305	Kuala Dungun	Terengganu	Malaysia	5°06' N	103°16' E	30	2900	0
13647	Rayong	Rayong	Thailand	12°45' N	101°17' E	80	1510	4
13651	Trat	Trat	Thailand	11°57' N	102°47' E	20	4540	2
13655	Lahan Sai	Buri Ram	Thailand	14°21' N	102°46' E	260	1310	5
23195	Bangkok	Jambi	Indonesia	2°43' S	102°49' E	80	3180	0
23618	Vientane	Tourakhom	Laos	18°22' N	102°38' E	200	1660	5
23665	Langgapayung	N. Sumatra	Indonesia	1°38' N	99°54' E	50	2470	0
23762	Kendari	Sulawesi	Indonesia	3°59' S	121°45' E	320		
33058	Chiang Mai	Chiang Rai	Thailand	19°04' N	99°24' E	900	1280	6

The Maquenque site at Carimagua could not be evaluated because insufficient rainfall inhibited emergence and plant growth until April 1996. Preliminary data analysis, including climate and soil data, indicates that slope and high P-fixation, together with high precipitation, were the reasons for poor establishment at the Cauca site. Plants at Chinchiná were under constant pressure of leaf-eating insects without showing subsequent limitations in growth. In Caquetá, plant performance was outstanding after relatively poor emergence caused by waterlogging at the Macagual site, and moisture deficiencies at the sandy site of La Rueda. Plants at the clay-loam soil site of Alcancía (Carimagua) which survived the dry conditions during 1995, showed high vigor ratings. After very poor emergence, plants grew slowly which resulted in a very prostrate growth habit and low plant height **[A. Schmidt, B.L. Maass, R. Schultze-Kraft and C.E. Lascano]**

Activity: Study G-E interaction of *Arachis pintoi* with respect to establishment and yield

First set of accessions, established in 1994: The evaluation of the trial with 35 accessions of *Arachis pintoi*, *A. repens*, and *A. glabrata* established at six sites in 1994 (Table 4) was continued according to the methodology described in previous reports. After a year of grazing, total forage and legume DM yield were measured in the rainy season. In Alcancía and Alegría (Llanos) and La Rueda (Humid tropics), most of the legume had disappeared, owing to heavy anthracnose stress at the beginning of the experiment, which led to poor establishment and consequently low legume percentage at these sites. Nevertheless, at

Table 4. Summary of site characteristics for study of g x e interactions of *Arachis pintoi*.

Ecosystem (Department)	Site	Year	Acidity	Al-Saturation	Soil fertility	Temperature	Dry season stress
Llanos (Meta)	Alegría/ Maquenque	1994 1995	H	H	L	H	H
	Alcancía	1994 1995	H	H	L	H	H
Humid tropics (Caquetá)	La Rueda	1994 1995	H	H	L	H	M
	Macagual	1994 1995	H	H	L	H	L
Hillsides (Cauca, Caldas)	Popayán	1994 1995	M	L	M L	L	H
	Chinchiná	1994 1995	M	L	M	L	L
Dry tropics (Valle)	Palmira	1995	—	—	H	M	H

a. H = high; M = moderate; L = low

Alcancía some accessions survived, such as CIAT 22160, 18748 and 18744. However, they do not contribute significantly to forage yield. At the sandy soil site, Alegría, only CIAT 22160 survived poor establishment and heavy drought stress with low population density. This highlights the drought resistance of accession CIAT 22160 also for the Llanos of Colombia.

For the remaining sites, DM yields are shown in Table 5. Among the 10 selected accessions, significant differences were observed among sites both in total and in legume DM yield, the level of legume biomass production being significantly higher at Popayán than at Macagual or Chinchiná. However, the overall low production level at Popayán reflects that the common grass, *Brachiaria humidicola*[1] cv. Llanero, is not very well adapted to the cooler climate of this site, and the legume could easily outcompete it. Because of high variation, no differences were detected ($P < .05$) among accessions at Popayán or Macagual, while at Chinchiná accessions with highest production were CIAT 18747 and 18751. Across the three sites, the best performing accession was CIAT 18751 followed by CIAT 22160 and 18747, which all were superior to the commercial check, CIAT 17434. Advanced selections, CIAT 18744 and 18748 performed intermediate. From all accessions across all sites, samples were taken for quality analyses to be carried out. Preliminary data on seed yield 14 months after sowing, showed high variation among accessions and sites. To conclude the evaluation of this set of germplasm, a final assessment of DM yield will be carried out at the end of 1996. **[B.L. Maass and E.A. Cárdenas]**

[1]Formerly referred to as *B. dictyoneura*

Table 5. Total and legume DM yield after one year of grazing of 10 accessions selected out of 32 *Arachis pintoi* and *A. repens* accessions associated with *Brachiaria humidicola*[2] cv. Llanero at six sites in Colombia.

	Humid tropics	Hillsides		Mean
	Macagual	Chinchiná	Popayán	
Total DM yield (g/m^2)				
Mean of 10 accessions	319.1 ns	331.7 ns	175.10 ns	275.3***
Range of 10 accessions	255-469	141-201	177-520	
Legume DM yield (g/m^2)				
Mean of 32 accessions	18.3	23.5	47.4	29.7
Mean of 10 accessions	31.0 ns	53.6***	93.5 ns	59.4***
Superior accessions (no. CIAT)				
18751	40.2	145.6 a	93.6	93.2 a
22160[b]	47.7	86.0 b	125.7	86.5 ab
18747	18.1	140.4 a	83.2	80.6 abc
18748[b]	29.3	34.6 cd	113.8	59.2 abcd
18746	17.1	54.4 c	101.7	57.7 abcd
18744[b]	21.2	30.2 cd	114.8	55.4 abcd
18752	49.0	2.1 d	88.2	46.5 bcd
22159	5.8	18.5 d	112.7	45.7 bcd
22157	63.8	11.7 d	45.4	40.3 cd
17434[a]	18.1	12.2 d	56.1	28.8 d

Differences between accessions significant according to F-test: *** = P<0.0001; ** = P<0.001; * = P<0.05; ns = not significant.
Means followed by the same letter are not significantly different (P <.05) according to Duncan test.
a. = check; b. = advanced accessions.

Second set of accessions, established in 1995: In 1995, 39 germplasm accessions belonging only to *A. pintoi* were sown at seven sites. Of this new set, 32 accessions were newly introduced from Brazil and seven were selected from the 1994 trial. In order to widen the evaluation of environmental effects on the genotypes, the CIAT experimental station at Palmira was included in addition to the six acid soil sites of the first set. The environment of CIAT-Palmira is representative of Dry Tropical Forest (according to Holdridge) with clay soils of high fertility and neutral to alkaline pH.

The legume was associated with *Brachiaria humidicola*[1] cv. Llanero at Alcancía and Maquenque (Llanos) as well as at La Rueda and Macagual (Humid tropics), and with *B. decumbens* cv. Basilisk at Palmira, Popayán and Chinchiná because of the better adaptation

[2] Formerly referred to as *B. dictyoneura*

of the grass to the respective environments. Establishment, management and evaluation of the trial were carried out using the same methodology as in the first set sown in 1994. After six months of establishment, heavy grazing of the trial was carried out every 28 days with 5 to 10 animals for 1 to 3 days depending on forage availability. At Palmira and Popayán, where no cattle are available; the plots are cut every four weeks.

Flowering. All accessions initiated flowering, except CIAT 22157, 22233, 22236 and 22238 at Alcancía and Maquenque (Llanos). A wide range in days to flower, between 29 and 156 days after establishment, was observed. Similarly, there was wide variation in flowering intensity among accessions.

Establishment. There was wide variation concerning rapidity of establishment among accessions and the different environments. Most favorable environments were La Rueda and Alcancía (Table 6).

Table 6. Rapidity of establishment of 39 accessions of *Arachis pintoi* at 7 sites in Colombia as shown by lateral extension (cm/day) four months after planting.

	Llanos		Humid tropics		Hillsides		Dry forest
	Alcancía	Maquenque	La Rueda	Macagual	Chinchiná	Popayán	Palmira
Lateral expansion rate (cm/day)							
Mean	0.37**	0.29**	0.39**	0.33**	0.32**	0.09**	0.28**
Range	0-0.62	0.04-0.74	0.16-0.75	0.05-0.71	0.10-0.51	0.03-0.20	0.11-0.44
Superior accessions							
Range	0.40-0.56	0.19-0.58	0.33-0.70	0.1-0.61	0.30-0.48	0.09-0.16	0.21-0.39
Ranking (CIAT accession no.)							
18744[b]	10	9	5	1	1	17	1
22241	5	1	18	10	3	2	2
22238	12	2	4	8	5	6	7
22233	1	4	1	17	11	4	16
22160[b]	2	8	15	16	6	5	3
22257	4	6	7	9	7	16	4
18750[b]	3	7	9	12	24	13	5
22236	17	3	3	39	2	12	9
17434[a]	8	28	6	2	16	14	34
22260	13	12	16	4	4	39	15
22240	24	10	20	5	22	12	13
18748[b]	6	33	28	23	8	10	14
22268	26	17	19	19	9	20	10
22269	14	11	23	7	25	21	31
22235	9	32	33	26	19	7	12

Differences between accessions significant according to F-test: ** = P<0.001.
a. = check; b. = advanced accessions.

Production. At the beginning of grazing, total forage and legume DM yield was highly variable among and within sites (Table 7). Highest total production was achieved at Chinchiná, while the sites with the lowest production were La Rueda, Popayán and Maquenque. The proportions of legume in the total biomass production differed among sites. The highest legume DM yields were produced in La Rueda and Palmira, while again at Popayán and Maquenque yields were lowest. Across all sites, accession CIAT 22160 is outstanding, followed by CIAT 18744, 18748, 22236 and 22241. Accessions CIAT 22241, 22238 and 22236 were all collected as cultivated material in the coastal region of Bahia, Brazil (EMBRAPA, Cruz das Almas; Jussari; Feira de Santana) in 1993, and may well have originated from the same source of germplasm. These materials were very rapid in establishment and heavily competing with the associated grass. However, they are all very late and scarcely flowering and probably do not produce much seed.

This set of *Arachis pintoi* accessions will continue to be managed under grazing. DM production and persistence of the legume will be monitored for another year. Seed production will be evaluated in late 1996. **[B.L. Maass, E.A. Cárdenas, and I.R. Moreno]**

Activity: Field studies to evaluate persistence of new legumes with different grasses

A field study is in progress at Carimagua on a sandy loam Oxisol to identify plant attributes that contribute to rapid establishment and persistence of new forage legumes in association with aggressive grasses. The trial includes three legumes (*A. pintoi* CIAT 22160, *S. guianensis* CIAT 11833, and *S. capitata* cv Capica) and two contrasting, stoloniferous (*B. humidicola*[3] cv Llanero) or tussock (*P. maximum* CIAT 36000) grasses. The trial was planted in June 1995, as a randomized complete block in a split plot arrangement (grasses as main plots and fertilizer x legumes as sub-plots). Initial application of fertilizer was at two levels (low: kg/ha, 10P, 10K, 33Ca, 14Mg, 10S and high: 40P, 40K, 66Ca, 28Mg, 20S and micronutrients). 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. A number of plant attributes including botanical composition, shoot and root production, nutrient composition, and nutrient acquisition are being monitored under periodic mob grazing. Results on pasture presentation yield and botanical composition at 13 months after establishment of grass alone and grass + legume associations indicated that the establishment of *A. pintoi* was more rapid when associated with *P. maximum* than with *B. humidicola* (Table 8). As expected, *A. pintoi* responded more than the other two legumes to high level of initial fertilizer application. Among the three legumes, *S. guianensis* was more vigorous in growth and dominated the grasses, particularly *P. maximum* during the establishment phase with low initial fertilizer application. **[Y. Saito, P.C. Kerridge, I. M. Rao, and C. Plazas]**

[3]Formerly referred to as *B. dictyoneura*

Table 7. Mean total and legume dry matter (DM) production during establishment of 39 accessions of *Arachis pintoi* and the 15 most productive accessions at seven sites in Colombia.

	Llanos		Humid forest		Hillsides		Dry forest	Mean
	Alcancía	Maquenque	La Rueda	Macagual	Chinchiná	Popayán	Palmira	
Total DM yield (g/m^2)								
Mean of 15 acc.	176.4 ns	144.0 ns	172.9 ns	311.6 ns	542.1 ns	161.0 ns	268.4 ns	253.8 ***
Range of 15 acc.	101-226	118-201	39-356	229-403	451-610	63-299	132-433	
Legume DM yield (g/m^2)								
Mean of 39 acc.	11.7	6.6	34.1	22.5	14.6	2.8	29.7	17.6
Mean of 15 acc.	21.2**	11.6**	50.0**	37.3**	29.2***	4.6 ns	55.4***	29.9***
Superior acc. (CIAT no.)								
22160[b]	66.0 a	17.7 abcd	94.5 a	17.0 d	73.1 a	9.0	194.3 a	67.4 a
18744[b]	35.4 b	10.3 bcde	76.7 ab	48.0abcd	35.5 bcd	0.0	61.7 c	38.2 b
22236	18.7 bc	24.8 ab	48.0 bcd	70.6 ab	42.3 bc	12.0	46.5 c	37.6 bc
22241	33.0 bc	26.7 a	44.8 bcd	71.9 ab	30.2 cde	0.3	55.3 c	37.5 bc
18748[b]	35.1 b	4.5 de	39.6 bcd	8.3 d	34.3 bcd	4.7	132.8 b	37.0 bc
22238	30.0 bc	21.8 abc	54.3 bcd	80.0 a	25.9 cde	1.7	25.6 c	34.2 bcd
22260	10.6 bc	1.4 e	67.8 abc	39.0 abcd	60.0 ab	0.0	50.4 c	32.8 bcd
22269	15.0 bc	12.8 abcde	59.3 abcd	36.7 abcd	19.0 cde	6.7	50.0 c	28.5 bcde
22268	4.1 bc	2.5 de	36.2 cd	24.7 cd	39.9 bc	2.3	65.7 c	25.1 bcdef
22257	11.1 bc	8.7 cde	54.0 bcd	20.3 d	42.7 bc	2.0	28.5 c	23.9 cdefg
22233	15.6 bc	9.9 bcde	46.0 bcd	67.9 abc	4.7 e	7.3	14.6 c	23.7 cdefg
22240	3.4 c	10.1 bcde	40.3 bcd	31.3 bcd	10.3 de	0.0	48.7 c	20.6 defg
22235	10.4 bc	8.4 cde	43.9 bcd	15.0 d	9.0 de	16.0	19.6 c	17.5 efg
18750[b]	8.2 bc	10.6 bcde	20.2 d	9.7 d	8.3 de	6.7	35.4 c	14.1 fg
17434[a]	21.8 bc	3.6 de	24.3 d	18.3 d	3.1 e	0.0	2.1 e	10.5 g

Differences between accessions significant according to F-test: *** = $P<0.0001$; ** = $P<0.001$; * = $P<0.05$; ns = not significant.
Means followed by the same letter are not significantly different ($P<.01$) according to Duncan test.
a. = check; b. = advanced accessions.

Table 8. The influence of initial fertilizer application on pasture presentation yield (> 10 cm height, g of dry wt/m^2) and botanical composition (%) at 13 months after establishment of grass alone and grass + legume associations in a sandy loam Oxisol at Carimagua, Colombia.

Pasture	Presentation Yield (g/m^2)		Legume content (%)	
	LIF*	HIF	LIF	HIF
Bh**	70.9	67.5	-	-
Bh + Ap	59.2	65.5	4.0	12.4
Bh + Sg	145	127	56.3	53.3
Bh + Sc	100	108	35.1	49.2
Pm	73.5	68.3	-	-
Pm + Ap	88.8	97.2	21.4	29.6
Pm + Sg	107	128	73.4	52.0
Pm + Sc	104	135	58.4	47.6

* LIF = low initial fertilizer application; HIF = high initial fertilizer application
** Bh = *Brachiaria humidicola*[4] cv. Llanero; Pm = *Panicum maximum*; Ap = *Arachis pintoi*; Sg = *Stylosanthes guianensis*; Sc = *Stylosanthes capitata.*

Output 1.3

Forage antinutritional factors of selected legumes better understood

Main achievements

- Demonstrated that extraction of condensed tannins with acetone without evaporation of the solvent resulted in overestimation of tannin content in a range of tropical legumes.
- Found differences among legume species in soluble and bound condensed tannin proportion due to maturity which could be related to changes in digestibility.
- Found differences among legume species with and without condensed tannins in extent of degradation and end products of fermentation by rumen microbes.
- Found differences among legume species in condensed tannin composition as indicated by procyanidin, delphinidin and pelargonadin proportions in leaf tissue.

A large number of tropical herbaceous and woody legume species have high levels of condensed tannins (CT), which are known to negatively affect intake, digestion and nitrogen

[4] Formerly referred to as *B. dictyoneura*

utilization by ruminants. To develop screening procedures to select tropical legumes and to better define strategies for utilization of these legumes in feeding systems, we need to understand how these and other antinutritional factors affect the nutrition of ruminants. A better understanding of the mechanisms by which CT affect ruminants and change with environmental conditions is also needed to define forage improvement strategies and agroecological niches for tropical legumes.

Studies carried out so far have shown a positive effect of reducing CT in *Desmodium ovalifolium* and *Flemingia macrophylla* on intake, digestibility and nitrogen utilization by sheep. In addition, our work has demonstrated:

- Differences among legumes species in the type of tannin present in edible forage (i.e. hydrolizale and condensed).
- Differences among legume species in the distribution of CT in leaves (i.e. soluble and bound).
- Differences among legume species in the biological activity of soluble CT (i.e. affinity to bind protein).

Based on previous results our emphasis now is to:

1. Refine laboratory assays for tannins
2. Better define the role of tannins in legumes in extent and rate of digestion and on end products of fermentation by rumen microbes.
3. Define and quantify different tannin structures (i.e. monomers, dimers) present in tropical legumes using HPLC.
4. Standardize NIRS to measure CT and other forage quality attributes in legumes and grasses.
5. Study changes in tannin levels and biological activity due to plant development.
6. Study the influence of soil and climate on tannin level and their biological activity.

A report on the above activities grouped according to the Project Work Plan follows:

<u>Activities: Studies on the influence of condensed tannins from a range of legumes on protein degradation by bacterial enzymes and on fermentation kinetics of plant cell walls</u>

These two activities form the basis of a joint IGER-CIAT Project (Amelioration of anti-nutritive factors in tropical legumes) funded by ODA and carried-out at IGER and CIAT by a PhD student from Reading University (Rolando Barahona).

To carry-out the research, a range of immature and mature leaves were harvested from seven tropical legume species with contrasting level of soluble and bound CT. The legumes used were : *Desmodium ovalifolium, Flemingia macrophylla, Leucaena leucocephala, Leucaena pallida, Leucaena macrophylla, Calliandra calothyrsus* and *Clitoria fairchildiana*. All forage samples were freeze-dried and then ground in a Willey Mill to pass a 1 mm screen. Samples were stored at room temperature and in the dark to prevent changes in tannin composition.

Study on tannin extraction with different solvents. Purified tannin extracts from the test legumes, except *L. macrophylla* which has no measurable CT, have been used for different laboratory tests. In the process of extraction and purification of CT from the test legumes, it became clear that acetone, commonly used in many laboratories as an organic solvent to extract tannin was causing interference with the Butanol-HCl tannin assay. Thus, an experiment was set-up to compare different tannin extraction procedures: (1) 70% aqueous acetone, (2) 70% aqueous methanol, (3) 70% aqueous acetone followed by evaporation and extraction with diethyl ether and dilution with methanol and (4) 70% aqueous acetone followed by evaporation of the acetone and dilution with methanol. Results shown in Table 9 indicated no differences in the determination of soluble CT when using methanol or acetone followed by evaporation. However, when acetone was not evaporated the level of soluble CT was 2 to 3 times higher than with the other extraction methods. Thus, for standard tannin determination in tropical legumes we recommend the use of methanol rather than the commonly used acetone, which is more expensive and difficult to obtain in some LAC countries **[R. Barahona, N. Narváez, and C. Lascano]**

Table 9. Effect of extraction method on the determination of soluble condensed tannins (SCT) (Barahona et al., unpublished results).

Legume species	Extraction methods			
	Acetone[1]	Methanol[2]	Acetone[3] + Ether	Acetone[4] + Evaporation
	SCT (% of DM)			
Leucaena leucocephala	18.3	8.6	8.3	8.6
Desmodium ovalifolium	24.2	8.9	9.4	9.3
Flemingia macrophylla	18.9	9.7	9.5	9.6

[1]70% aqueous acetone

[2]70% aqueous methanol

[3]70% aqueous acetone followed by evaporation and extraction with diethyl ether (dilution with methanol)

[4]70% aqueous acetone followed by evaporation (dilution with methanol)

Study on tannin distribution in leaf tissue: Results from previous studies carried-out in collaboration with Massey University, New Zealand, had shown differences among tropical legumes in the proportion of soluble and bound CT in leaf tissue. For example, while in *Senna velutinum* and *Acacia boliviana* all CT present were soluble in *Gliricidia sepium* all CT present were bound. To follow-up this work immature and mature leaves from the test legume species included in the IGER-CIAT Project were analyzed for soluble and bound tannins in the tissue. Results in Table 10 show that in all legumes species 65 to 90% of the tannins present were soluble and only 10 to 35% were bound, presumably to the cell wall. However, it was interesting to note differences in the soluble: bound tannin ratios due to maturity in some legume species but not in others. In *C. calothyrsus* and *L. leucocephala*

Table 10. Proportion of soluble and bound condensed tannins (CT) in immature and mature leaves of different legume species (Barahona et al., unpublished results).

Legume species	Maturity	% of total CT	
		Soluble	Bound
Calliandra calothyrsus	Immature	90.3	9.7
	Mature	66.3	33.7
Clitoria fairchildiana	Immature	77.4	22.6
	Mature	74.6	25.4
Desmodium ovalifolium	Immature	85.8	14.2
	Mature	85.4	14.6
Leucaena leucocephala	Immature	79.9	21.1
	Mature	71.5	28.5
Leucaena pallida	Immature	65.0	35.0
	Mature	64.9	35.1

there was an increase in bound tannins with maturity, which was not the case in the other legumes. These results could explain the large drop in in vitro DM degradability due to maturity observed in *C. calothyrsus* (44% immature to 32% mature), and in *L. leucocephala* (62% immature and 53% mature), but not in *F. macrophylla, C. fairchildiana* and *D. ovalifolium* **[R. Barahona, M. Theodorou, P. Morris, E. Owen, N. Narváez, and C. Lascano]**

Study on effect of tannin on extent of degradation and end products of fermentation of tropical legumes: A major objective of the IGER-CIAT Project is to elucidate the effect of CT in cell wall digestion of tropical legumes. To accomplish this objective a number of studies using an in vitro gas production methodology have been carried out at IGER and presently in CIAT's Forage Quality Laboratory. With the Gas Technique developed at IGER it is possible to study fermentation kinetics, extent of degradation and end products of digestion by rumen microbes in large number of legume species. Results so far have shown large differences among the 7 legumes included in the IGER-CIAT Project on in vitro fermentation parameters. For example, the extent of DM degradation of *Flemingia macrophylla* forage was extremely low (22%) when compared to other legumes (range 41 to 62%). Also the extent of degradation of *Leucaena macrophylla* (48%), with no measurable CT, was considerably lower as compared to *L. leucocephala* (62%). In fact, the DM degradability of *L. macrophylla* was similar to that of *L. pallida* (46%) which has CT. This suggest that differences in digestibility among tropical legumes is affected not only by level and distribution of CT but also by cell wall composition.

A number of in vitro gas experiments involving fermentation of test legumes using buffer solutions, a reducing agent and rumen fluid or the fermentation of glucose (energy source) and casein (protein source) with increasing levels of purified tannins have been useful to determine end products of fermentation by rumen microbes. Results summarized in Table 11, show differences among legumes in total level of volatile fatty acids (VFA) and in the proportion of straight and branched chain VFA's after 144 hours of fermentation with rumen microbes. As expected, total VFA's was positively correlated (r= 0.77) with extent of DM degradability, being lōwest in the case of *F. macrophylla* and highest in *L. leucocephala*. On the other hand, the concentration of branched-chain VFA's mostly derived from degradation of pre-formed plant protein changed among species. The highest levels of branched VFA's were recorded with legumes that have no tannins (*L. macrophylla*), or with what appear to be less reactive tannins (*L. leucocephala*). This suggest increased utilization by rumen microbes of branched VFA's for protein synthesis in the presence of tannins. Complexing of plant protein by tannins could result in less branched carbon chains which are needed for bacterial protein synthesis.

Table 11. Total straight and branched-chain volatile fatty acids (VFA's) after 144 hours incubation of different legume species with rumen microbes (Barahona et al., unpublished results).

Legumes[1]	VFA's[2]						
	Total	Straight chain[3]				Branched chain[4]	
		Ac	Pr	Bu	Va	Iso-Bu	Iso-Va
					(moles/liter)		
Flemingia macrophylla	19.5	13.8	4.9	0.9	0.1	0.01	-0.17
Desmodium ovalifolium	20.5	15.3	4.4	1.0	0.2	-0.10	-0.26
Calliandra calothyrsus	23.7	19.1	3.8	0.6	0.5	-0.07	-0.20
Leucaena pallida	28.3	20.1	6.7	1.2	0.3	0.05	-0.06
Leucaena macrophylla[5]	31.9	20.4	7.5	1.8	0.8	0.46	0.94
Clitoria fairchildiana	31.9	22.1	7.6	1.7	0.1	0:28	0.13
Leucaena leucocephala	42.6	29.2	8.7	2.5	1.1	0.36	0.76

[1]Immature leaves

[2]Values corrected for blank (fermentation media and rumen fluid)

[3]Acetic, propionic, butyric, valeric

[4]Iso-butyric, isovaleric

[5]No measurable tannins

Increasing levels of purified soluble tannins from *D. ovalifolium* in a fermentation media with glucose and casein did not affect total VFA levels after 30 hours of fermentation by rumen microbes, but did affect the molar proportion of branched-VFA's. With increasing levels of tannins, there was a linear reduction in isovalerate and isobutyrate **[R. Barahona, M. Theodorou, P. Morris, E. Owen, N. Narváez, and C. Lascano]**

Activity: Studies on the effect of plant development and environment on the production of condensed tannins

A Special Project funded by BMZ/GTZ, Germany, being executed by a PhD student (Axel Schmidt) from Hohenheim University, is currently under way to examine the effect of genotype and environmental factors on productivity and quality of different ecotypes of *Desmodium ovalifolium*. Expected outputs from this Project include: (1) an understanding of tannin production as plants develop; (2) a quantification of the effect of changes in soil fertility and climate (rainfall and temperature) on tannins and other quality parameters in different genotypes, and (3) calibration of curves for NIRS to measure tannins and other forage quality attributes.

Study on types of tannins present in tropical legumes: To get base line information on types of tannins present in *D. ovalifolium* and other tropical legumes, measurements of proanthocyanidins constituents in test legumes were carried out as part of the IGER-CIAT Project. The results from this work is providing methodology which is useful for Hohenheim University-CIAT Project on G x E interactions in quality of *D. ovalifolium*.

Previous results obtained after binding soluble CT with PEG and measuring level of extractable CT and astringency of the tannins (i.e. ability to bind protein) had suggested that in some tropical legumes there were at least three types of tannins: (1) non-reactive with protein; (2) low affinity to protein and; (3) high affinity to proteins. To verify this finding, purified tannins from 6 test legumes were analyzed at IGER using HPLC for ratios of proanthocyanidins or condensed tannins constituents. Results summarized in Table 12 show differences in ratios of tannins present in the legumes studied. In *D. ovalifolium* a high

Table 12. Proanthocyanidin ratios in purified tannins from contrasting legume species (Barahona et al., unpublished results).

Legume species	Proanthocyanidin ratios[2]				Unknown
	Cyanidin	Delphinidin	Pelargadinin	Fisetinidin	
Calliandra calothyrsus	82.0	9.1	2.6	---	6.3
Clitoria fairchildiana	39.0	24.1	27.5	---	9.4
Desmodium ovalifolium	85.2	---	1.5	2.6	10.7
Flemingia macrophylla	10.4	83.4	---	---	6.2
Leucaena leucocephala	28.2	59.2	5.5	---	6.7
Leucaena pallida	14.3	71.3	---	---	14.4

[1]Immature leaves [2]HPLC

proportion of the CT present are in the form of cyanidin and this legume had fisetinidin, which was not found in the other legumes studied. In contrast, most of the tannins in *F. macrophylla* are in the form of delphinidin. These differences could explain the higher affinity of tannins from *D. ovalifolium* to protein (BSA) as compared to the tannins from *F. macrophylla*.

In the case of *Leucaena*, there were also differences between species in cyanidin: delphinidin ratios and in presence or absence of other types of tannins. While pelargonidin was present in

L. leucocephala it was not in *L. pallida*. These differences could be related to tannin biological activity, digestibility and overall feeding value of *Leucaena* species. It is worth noting that in all test legumes analyzed for proanthocyanidin constituents, there were distinct peaks in the chromatograms for which there are no known standards. Resolution of these unknown tannin compounds present in tropical legumes will require the input from a phytochemist. A possibility is to link CIAT's work on tannins to the group at ILRI, who is planning to enhance the capacity to undertake strategic research on antinutritional factors in tropical forages **[R. Barahona, M. Theodorou, P. Morris, E. Owen, N. Narváez, C. Lascano and A. Schmidt]**

Analysis of quality and antiquality attributes in *D. ovalifolium*: During 1996 a total of 1224 samples of *D. ovalifolium* leaves were harvested from six sites in Colombia as part of the Hohenheim University-CIAT Project. Near Infrared Reflectance Spectroscopy (NIRS) was chosen as an appropriate method to perform the quality analysis given time and budgetary constraints. It is well recognized that once NIRS is calibrated it can provide accurate results on quality parameters of different forage species. Thus, the calibration of NIRS was undertaken at CIAT's Forage Quality Laboratory using a data base with different quality parameters obtained by traditional laboratory methods. Up to now calibration curves have been obtained for different quality attributes of *D. ovalifolium* such as IVDMD, CP, NDF, ADF and N-ADF. Calibration of NIRS for extractable and bound CT is presently being done in the laboratory. In addition, purified extracts of different *D. ovalifolium* ecotypes growing in two contrasting sites were used to derive tannin standard curves for the Butanol-HCL tannin colorimetric assay. Results indicated that parameters in the tannin standard curves did not change with ecotype or with location. This finding will help simplify work in the laboratory **[A. Schmidt, R. Schultze-Kraft, N. Narváez, and C. Lascano]**

Growth stage and tannin production in *D. ovalifolium*: There is limited knowledge on how plant growth affects tannin production in tropical legumes. Consequently, we lack appropriate plant sampling schemes for tannin analysis. A study was initiated, as part of the Hohenheim University-CIAT Project, to determine level of soluble and bound CT in leaves of 5 ecotypes of *D. ovalifolium* grown in the greenhouse. Sampling began 30 days after seedling emergence and from there on every 30 days. To harvest leaves for laboratory analysis, stolons from each plant are marked on the lost emerged leaf 30 days prior to sampling. In addition, to CT and other quality attributes, measurements are also being done on plant height, LAI, specific leaf weight, total biomass and leaf: stem ratio **[J. Martínez, A. Schmidt, R. Schultze-Kraft, N. Narváez, and C. Lascano]**

Activity: Effect of environment, management and provenance on feed value of *Calliandra calothyrsus*

A collaborative OFI-CIAT Project with financial support from ODA is currently underway to examine the effect of soil fertility and cutting management on fodder quality of two provenances of *C. calothyrsus*. The two provenances (Ixtapa, Mexico-low level of tannins and Union Juárez, Mexico-high level of tannins) received from OFI were planted in jiffy pots and seedlings transplanted into the field in Quilichao (low soil fertility site) and Palmira (high soil fertility site).

The OFI-CIAT Project includes two feeding trials with sheep to determine: (1) effect of soil fertility and provenance on palatability, and (2) effect of post-harvest management (i.e. fresh vs. sun-dried forage) on intake, digestibility and N utilization. In addition, we plan to examine the effect of shade and maturity on fodder quality of the two *C. calothyrsus* provenances **[J. Stewart, P. Avila, N. Narváez, and C. Lascano]**

Activity: Identification of antinutritional factors in *Cratylia argentea.*

Previous results indicated that mature leaves of *C. argentea* were well consumed by sheep regardless of post-harvest treatment (i.e. fresh, wilted or sun-dried forage) or previous adjustment of animal to the forage. In contrast, immature leaves of *C. argentea* were rejected by sheep when offered fresh, but were well consumed when wilted or sun-dried. Subsequent observations with milking cows with access to a fodder bank with mature *C. argentea* showed that cows readily consumed the legume after few days of adjustment, which was not the case when the legume was immature.

From the evidence available, we postulated that the antinutritional factor(s) present in fresh immature leaves of *C. argentea* became inactivated when the plant matured or when the forage was wilted or sun-dried.

In the IGER-CIAT Project we are attempting to identify the antinutritional factors in *C. argentea*. Thus, immature (4.4% N, 62% IVDMD, 55% NDF and 33% ADF) and mature (3.1% N, 53% IVDMD, 58% NDF and 37% ADF) freeze-dried leaves were sent to IGER. In addition, immature and mature leaves were extracted with different solvents and the extracts also sent to IGER. The results obtained so far and future direction of this research will be discussed this year at CIAT with IGER scientists **[A. Brook, M. Theodorou, P. Morris, N. Narváez, and C.E. Lascano]**

Output 1.4

Information system of forage adaptation to different agroecosystems

Main achievements

- For the first time isozyme characterization data of *S. guianensis* were integrated with geographic information systems (GIS).

Activity: GIS and *Stylosanthes*

Information systems about species adaptation: With the complete implementation of the germplasm passport database in the database management system ORACLE, easy access is given to these data for mapping and geographic analysis with the help of geographic information systems (GIS). Based on the maps of high probability of occurrence of six species of *Stylosanthes* developed in 1995, another two maps were developed for *S. humilis* and *S. viscosa*. In addition, for the first time isozyme characterization data of *S. guianensis* were integrated with GIS in a collaborative project with the University of Birmingham, UK. Relations between certain esterase bands with climate groups defined by GIS were found.

Two publications from this area of research were prepared. **[P.G. Jones, M.C. Sawkins, and B.L. Maass]**

Subproject 2.0

Development of superior grass genotypes to the level of commercial cultivars

Output 2.1

Spittlebug resistance in *Brachiaria* assessed and characterized

Main achievements

- Techniques for mass rearing of spittlebug has been streamlined and improved.

- Progress is being made in the development of an improved and faster greenhouse assay technique to screen for resistance to spittlebug.

- Progress is being made in understanding the role of host plant preference in spittlebug resistance.

Activity: Develop improved field and glasshouse screening procedures

As indicated in previous reports, assessment of resistance to spittlebug in the field is extremely difficult due to the focal, unpredictable occurrence of the insect. Attempts to develop a reliable technique have not been successful. The least we can do at present is to develop quicker, less cumbersome greenhouse tests in order to support the breeding process. Three different assay techniques were compared: 1) the standard pot assay which measures % survival of nymphs (or adults); 2) a "tubular flat" assay in which plants are sown in 40-cell tubular flats, infested with 10 eggs per cell and evaluated for plant damage due to nymphal feeding after a suitable period of time; and 3) an adult feeding assay in which plants sown in "jiffy" pots are subjected to adult feeding damage and scored on the basis of a visual 1-5 damage scale (1 being highly resistant, 5 being susceptible).

Using a set of 85 advanced clones, the assays were compared on the basis of their efficiency/precision. Preliminary results suggested that there was a significant but not strong correlation ($r = 0.513$) between the "tubular flat" assay and the standard pot assay. The correlation between the adult damage assay and the standard assay was poor ($r = 0.18$). This can be explained in terms of independence of mechanisms of resistance. In another set of experiments, again the correlation between the pot assay (nymphal survival) and the "tubular flat" technique (nymphal damage) was significant ($r = 0.561$). In spite of the fact that the "tubular flat" assay would save time and space needed to screen large numbers of materials, it was later discarded because of difficulties in the handling of vegetative material and in evaluating damage. Since then, an improved technique is being tested. This is called the "single-tube" assay. It consists of a 6 cm wide x 10 cm long PVC tube topped with a cap with a 2.5 cm hole through which the plant stem is placed (the unit can also be used to promote superficial root growth in the plants by inverting the plant for 15-25 days prior to infestation with eggs). This technique will be tested in order to compare the reliability of adult feeding damage scores as opposed to nymphal survival values in selecting for resistance to spittlebug.

Studies on the possibility of detecting resistance in single, young plants of *Brachiaria* were also initiated in July, 1996. At present, work is being conducted in two areas; 1) The effect of plant age (15 vs 30 day-old plants) and level of infestation (0, 3, 6, 9, 12 adults per plant) on resistance expression, and 2) The effect of plant age (as above) and level of nymphal infestation (0, 5, 10, 15, 20, 25 nymphs per plant) on resistance expression. Results will be presented in 1997.

At the same time, efforts are being made in order to streamline the mass breeding techniques used at present. Preliminary tests in large wooden benches that would reduce manpower and time by 50% have been successful. Alternate host plants (rice, maize, sorghum) that could be easier to handle and that would occupy substantially less space are also being tested. A full report on this subject will be presented in 1997. **[G.C. Yencho, G. Sotelo, and C. Cardona]**

Activity : Study adult feeding and oviposition behavior

In order to understand how adults select sites for oviposition and determine if adult preference is an important component of resistance (never tested before), a series of trials were conducted in order to quantify spittlebug adult host egg laying preferences. Known numbers of adults (1:1 sex ratio) were given feeding choices between plants grown in small pots. To measure preference we recorded the number of adults present on each plant and their location at predetermined intervals. The number of eggs laid per plot was also recorded. Working with *Brachiaria ruziziensis* we found that: 1) spittlebug adults prefer to feed and rest on the lower third of the plant; 2) the position of the pot in a cage may have a slight influence in the number of adults found on a plant; 3) large number of eggs were laid in the pots; 4) no major differences were observed in the number of eggs laid per pot amongst susceptible plants. This indicated that the methodology does work. At present, three more trials are being conducted using a susceptible cultivar. When the technology is fully developed, studies on adult preferences among resistant and susceptible genotypes will be conducted in order to ascertain whether host plant preferences do play a role in spittlebug resistance. **[G.C. Yencho, M. Cruz, and C. Cardona]**

Activity: Develop molecular markers for spittlebug resistance

In September 1995, the section began screening four sexual *B. ruziziensis* x *B. brizantha* (cv. Marandú) progenies [BP1016 and BP 1027 (44-2 x CIAT 6294), and BP1017 and BP1028 (44-3 x CIAT 6294)] for resistance to spittlebug which are being used by the Apomixis Project to identify molecular markers associated with apomixis.

The screening of these progenies was completed in February, 1996. A total of 78 clones were evaluated. As measured by % survival to adult and a spittlebug resistance index developed at CIAT (mean % survival on clone x - mean % survival on CIAT 6294) / (mean % survival on CIAT 606 - mean % survival on CIAT 6294), seven clones (BP1016-009, -010, -019;

BP1027-061, -110, -113; and BP1028-027) were identified to be as or more resistant than the resistant parent, CIAT 6294, while 19 clones were found to be more susceptible than the susceptible check, CIAT 606.

Survival scores over the 78 hybrid progenies showed no evidence of qualitative inheritance. The essentially continuous distribution (Figure 1) suggests that resistance is quantitatively inherited. Further use of these findings in trying to develop molecular markers for spittlebug resistance is dependent on increased funding and technical support both in the laboratory and the greenhouse. **[G.C. Yencho, G. Sotelo, and C. Cardona]**

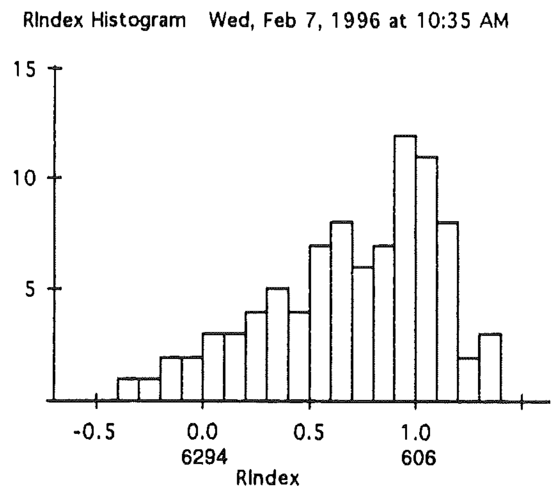


Figure 1. Distribution of indexized resistance score of 78 hybrid progeny of the cross *B. ruziziensis* x *B. brizantha* cv. Marandú. Index score is based on percent nymphal survival, where *B. decumbens* CIAT 606 = 1.0 and *B. brizantha* cv. Marandú (6294) = 0.0.

Output 2.2

Host-plant relationships, ecology and population dynamics os spittlebug are understood

On 16 September 1996, Dr. Daniel Peck joined the Program as a post-doctoral fellow in Entomology. Dr. Peck was contracted through a special project funded by the Colombian Fondo Nacional del Ganado. The project is managed by Dr. Nora Jiménez, Corpoica, Regional 2 (Montería, Córdoba). Dr. Peck's activities will be conducted at four field sites with the objective to study the bioecology and population dynamics of spittlebugs in tropical areas of Colombia.

Output 2.3

Virus resistance in *Brachiaria* assessed

Major achievements

- A reliable virus detection and screening methodology has been developed to select resistant *Brachiara* spp. germplasm.

Activity: Development of a reliable inoculation methodology

Based on artificial inoculations tests of over 100 plants per treatment, the rates of infection were as follows:

Technique	Test Plant	Infection Rate
Air Gun at 30 lbs	Seedlings	12%
Air Gun at 50 lbs	Seedlings	7%
Manual Inoculation	Vegetative P.	35%
Manual Inoculation	Seedlings	80%

It is apparent from these results that the air gun technique recommended for sugarcane mosaic virus, does not work for the *Brachiaria* strain of Johnsongrass mosaic virus (JGMV-Brac). The probable cause for this observations may be the weaker nature of *Brachiaria* seedlings as compared to the more rigid structure of sugarcane seedlings, which do not bend when shot with the air gun. Nevertheless, the mechanical inoculation technique using cheesecloth pads, can also be readily used to inoculate a large number of seedlings in a short period. An inoculation efficiency of 70-80% is considered as highly efficient for most potyviruses. Furthermore, *Brachiaria* seedlings that do not become infected following their manual inoculation, can be inoculated a second time to increase the rate of infection. **[F. Morales]**

Activity: Screening *Brachiaria* germplasm for virus resistance

To date, no immunity has been identified in *Brachiaria brizantha* (accessions 6294, 6297, 6780, 16106, 16107, 16122, 16126, 16152, 16162), *B. decumbens* (accessions 606, 26185, 26186), *B. dictyoneura* (accessions 16186, 16187, 16508), *B. humidicola* (accessions 679, 682, 6133[5], 16181, 16182), *B. jubata* (accessions 6409, 16195, 16536, 16710), *B. platynota* (accessions 26200, 26340, 26343), and *B. ruziziensis* (accessions 641, 660, 6019, 6692, and 26163). **[F. Morales]**

Activity: Selection of virus resistant breeding materials

The search for genetic resistance to JGMV-Brac in *Brachiaria*, has been a desirable but not critical objective in the improvement of this tropical grass, because the virus does not seem to significantly reduce either the biomass or quality of the forage. However, preliminary studies conducted last year, suggested that the virus reduced seed production (over 50%) in systemically infected *B. brizantha* plants. This year, a second experiment was conducted using 50 plants per treatment (virus-free and virus-infected) under screenhouse conditions to prevent secondary virus spread by aphids. The results obtained showed that the seed yield of virus-infected plants was reduced 42% as compared to the virus-free test plants. Also, the number of virus-free test plants that produced seed was 52% greater than the number of virus-infected

[5]Formerly referred to as *B. dictyoneura*

test plants that set seed. Finally, the virus-free plants produced a total of 52 panicles, while virus-infected test plants produced only 29 panicles.

So far, a total of 65 out of 131 hybrids inoculated as vegetative propagules, could not be infected by JGMV-Brac under artificial conditions. However, this result might suggest that there are other mechanisms, generically known as 'adult plant resistance', which confer resistance to mature plants. The existence of virus-free *Brachiaria* plants under field conditions, and the results presented above for the mechanical inoculation of vegetative propagules, seem to support this observation. **[F. Morales]**

Output 2.4

Nutrient efficient grass and legume genotypes identified

Main achievements

- Field evaluation of germplasm accessions and sexual recombinants of *Brachiaria* in wet and dry seasons resulted in identification of one spittlebug resistant sexual recombinant which exhibited several desirable attributes that could contribute to adaptation and persistence in low fertility acid soils.

- Showed that a number of shoot attributes of *Brachiaria* were influenced by genotype and also P supply in soil.

- Showed that root acid phosphatase activity of *B. decumbens*, when compared to that of *B. brizantha* and *B. ruziziensis*, is less sensitive to the presence of aluminum in the assay medium.

Activity: Differences in edaphic adaptation in *Brachiaria*

A field study was conducted during two seasons (wet and dry) in a medium textured Oxisol at Carimagua, Colombia. The main objective of the study was to evaluate differences in edaphic adaptation among 55 *Brachiaria* genotypes. The genotypes included 43 genetic recombinants selected from a breeding population, four parental accessions and an additional eight germplasm accessions. Low amounts of fertilizer (kg/ha: 40N, 20P, 20K, 14Ca, 12Mg and 12S) were applied during establishment. Forage yield, leaf area, shoot nutrient uptake, and other attributes were measured during both seasons. Significant genetic variation was observed in plant attributes such as forage yield, leaf area, and nutrient acquisition (Table 13). Two apomictic genetic recombinants (FM9201/1873; BRN093/3009) were productive when grown in low fertility acid soil in both wet and dry seasons. But neither of these two recombinants is highly resistant to spittlebug. One spittlebug resistant sexual recombinant (BRN093/1371) exhibited several desirable attributes that could contribute to adaptation and persistence in low fertility acid soils. This recombinant is being utilized in a breeding program to develop superior *Brachiaria* genotypes.

Table 13. Genotypic variation in plant growth and nitrogen acquisition among selected genetic recombinants, parents, and other germplasm accessions of *Brachiaria* grown during wet and dry seasons in a sandy loam Oxisol site at Carimagua, Colombia.

Genotype	Forage yield ($g\ plant^{-1}$)		Leaf area ($m^2\ plant^{-1}$)		Shoot N uptake ($mg\ plant^{-1}$)	
	Wet	Dry	Wet	Dry	Wet	Dry
Recombinants:						
BRN093/1371	175	84	0.89	0.67	3254	1001
BRN093/3009	234	159	0.60	0.71	2257	1638
FM9201/0209	140	79	0.53	0.47	1783	780
FM9201/1873	220	160	0.65	0.85	1808	1541
Parents:						
CIAT 606	246	85	0.75	0.42	2708	892
CIAT 6780	343	174	0.88	0.84	3792	1545
BRUZ/44-02	59	4.7	0.05	0.02	787	70
CIAT 26646	273	139	0.76	0.56	2884	1318
Accessions:						
CIAT 679	77	55	0.23	0.12	1227	648
CIAT 6133	108	94	0.49	0.35	1611	1038
Mean*	181	96	0.51	0.50	2202	990
LSD(p = 0.05)	80	49	0.27	0.20	981	410

* of 53 genotypes in wet season and 14 genotypes in dry season.

Another field study is in progress to evaluate differences in edaphic adaptation and persistence of *Brachiaria* genotypes and to identify key attributes of edaphic adaptation. The trial comprises 17 entries, including nine natural accessions (four parents) and eight genetic recombinants. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. The trial is submitted to periodic mob grazing according to forage on offer. Measurements of forage yield and leaf area at the end of wet season during the establishment phase indicated marked genotypic variation in forage yield and leaf area production. With low initial fertilizer application, a genetic recombinant, BRN093/3204, was outstanding in forage yield compared to other entries. **[I. M. Rao, J. W. Miles, C. Plazas, and J. Ricaurte]**

Activity: Mechanisms of acid soil tolerance in *Brachiaria*

A graduate student thesis (Ph.D.) project funded by the Austrian Academy of Sciences was continued in collaboration with the Biotechnology Research Unit at CIAT, the National Accelerator Center in Faure, South Africa, and the University of Vienna, Austria. The aim of the project is to get insights into mechanisms of acid soil tolerance of three *Brachiaria* species which are being used as parents in a breeding program at CIAT: *B. decumbens* cv. Basilisk (well adapted), *B. brizantha* cv. Marandú (less persistent), and *B. ruziziensis* cv. Common (poorly persistent).

The so-called "acid soil syndrome", a multiple edaphic stress situation consisting of Al toxicity and low availability of essential nutrients such as N, P, K, Ca, and Mg, was simulated by low ionic strength hydroponic solution cultures using GEOCHEM 2.0 model. Main treatments were: a control solution *(co)* that contained sufficient nutrients and a multiple stress solution *(st+Al)* that composed of low levels of N, P, K, Ca, and Mg in combination with a high Al concentration ($\{Al^{3+}\} = 44\ \mu M$). To separate the effects of low nutrient stress and Al-toxicity an Al-containing control solution *(co+Al)* and a nutrient stress solution without Al *(st)* were used as well. Relative growth in the *st+Al* treatment with respect to the *co* treatment was in the same order as acid soil persistence under field conditions (TFP Biennial Report, 1994-1995).

Using these four different treatments, several experimental approaches are being pursued to gain insights into physiological, biochemical, and genetic mechanisms that could play a role in interspecific difference in acid soil adaptation. These included: (i) an evaluation of plant growth responses to nutrient supply and Al toxicity with a special focus on root system architecture, (ii) measurement of activities of two key enzymes involved in nutrient uptake, (iii) mapping of nutrient distribution in root tips on a micro-scale, (iv) characterization of plant metabolites such as phenolic secondary compounds and organic acids, which could be relevant to nutrient acquisition and Al-detoxification, and (v) isolation of acid soil stress-induced genes.

(i) Plant growth responses to nutrient supply: Plants were grown in a series of solutions with decreasing levels of a single nutrient or increasing levels of Al. Several shoot and root growth characteristics including shoot biomass, leaf area, root biomass, total root length, root diameter distribution, root system topology (magnitude, total exterior path length, altitude), and root system fractal dimension were measured. Root parameters were evaluated on scanned images of root systems with the program "WinRHIZO" which helped to characterize more closely the interspecific differences in adaptive phenotypic plasticity of the root system as influenced by low levels of certain essential nutrients in culture medium.

(ii) Key enzymes involved in nutrient uptake and utilization: The H^+-translocating ATPase of the plasma membrane plays a key role in plant nutrition because it creates an electrochemical proton gradient that is used as driving force for nutrient uptake. Plants were grown under four treatment conditions described above and root plasma membranes were extracted and purified by means of a two-phase partition method. Studies with specific inhibitors of the H^+-ATPase as well as of other enzymes from other membranes revealed a high degree of purity. Measurements on enzyme kinetics yielded the following results: (i) Al stress - under high and low nutrient supply *(co+Al, st+Al)* - increased the speed of proton transport across the plasmamembrane, as monitored by the V_{max} value of ATP hydrolysis, with the notable exception of *B. ruziziensis* grown under *st+Al* conditions; (ii) the affinity of the enzyme towards ATP (described by its K_m value) decreased under Al stress for both *B. decumbens* and *B. ruziziensis* with the exception of *B. ruziziensis* grown in the *st+Al* solution where it remained high; and (iii) the H^+-ATPase of *B. brizantha* was generally less responsive.

Root acid phosphatases play an important role in the intra- and extracellular mobilization of organically bound P reserves under P-deficient conditions. Acid phosphatase activity was measured in root extracts of hydroponically grown plants. Results indicated that: (i) Al stress could induce a 5 to 10-fold increase of specific activity of the enzyme in all three species; (ii) low nutrient stress could trigger a 5-fold increase of specific activity in *B. ruziziensis* but not in the other two species; and (iii) the root acid phosphatase of *B. decumbens* plants grown under Al stress became more Al tolerant than that of those that were grown without Al, a reaction that was not observed with the other two species (Table 14).

Table 14. Characteristics of the root acid phosphatase from three *Brachiaria* species. (Mean ±S.D.).

Treatment	*B. ruziziensis* Specific activity[a]	*B. ruziziensis* Al inhibition[b]	*B. decumbens* Specific activity	*B. decumbens* Al inhibition	*B. brizantha* Specific activity	*B. brizantha* Al inhibition
co	20±1	81±3	17±1	60±0	20±3	88±4
co+Al	74±2	88±5	183±2	20±2	96±2	84±1
st	105±1	52±2	26±2	74±3	29±5	79±2
st+Al	93±1	64±2	91±0	42±1	112±2	85±2

[a] in nmole substrate•mg $protein^{-1}$•h^{-1}
[b] in %; 1mM $AlCl_3$.

(iii) Nutrient mapping in root tips: Nutrient uptake is generally considered to be specially active in root tips. Differences in uptake mechanisms or preferences towards certain elements, e.g. in the context of the anion-cation balance, could result in different levels and/or distributions of nutrients in root tip tissue. In order to map these changes, seminal root tips of plants grown under four different treatment solutions were lyophilized and scanned with "Proton Induced X-ray Emission" (PIXE) with a spatial resolution at 5 μm. The resulting 2-dimensional maps of Al, P, Ca, K, Mg, Si, S, Cl, and Fe are being analyzed.

(iv) Characterization of plant metabolites: A series of phenolic, secondary compounds have been found to act as signaling molecules for the initiation and functioning of plant-microbe symbioses (symbioses with N-fixing bacteria and arbuscular-mycorrhizae)which play a key role in nutrient acquisition from infertile soils. Thus, phenolic compounds in roots of hydroponically grown plants were analyzed by reverse-phase HPLC. A dramatic accumulation (at least 10-fold) of two major compounds was observed with *B. ruziziensis* but not with either *B. brizantha* or *B. decumbens* when grown under low nutrient stress *(st, st+Al)*. This observation was consistent with previous finding that the concentration of these compounds in soil-grown plants of *B. ruziziensis* was about 5 to 10-fold greater than that of the other two species. Further research work is in progress to determine whether lack of supply of which essential nutrient could induce this effect in *B. ruziziensis*. A purification procedure is being developed to determine the molecular structure of these two compounds.

Organic acids have been shown to be involved in Al tolerance as well as in P stress response in plants. A technique for organic acid purification and HPLC analysis was optimized to permit the quantification of 11 physiologically important acids. Application of this technique to root extracts of plants grown under four different treatments clearly demonstrated that all three species accumulated citric acid under Al stress. Accumulation and/or exudation of citric acid was implicated to confer Al tolerance in maize and beans. Interestingly, citric acid was not induced by low levels of P, which suggests that the presence of Al rather than Al-induced P deficiency triggers this response.

(v) Induction of genes under simulated acid soil stress: Isolation of stress-induced genes provides a direct approach to characterize molecular mechanisms underlying abiotic stresses. This approach is not biased towards the supposition of certain hypotheses concerning adaptation mechanisms. Comparison of the DNA-sequences of isolated genes with known genes in databases and evaluation of their inducibility by different stress factors can yield valuable information with respect to their role in acid soil adaptation. Since the offspring of an interspecific cross between the best and the less-adapted species (*B. decumbens* x *B. ruziziensis*) shows a pronounced heterosis effect, it cannot be used to isolate genes involved in acid soil adaptation by means of a segregating population-based approach. Thus, a subtractive hybridization technique is being applied to isolate genes that are specifically induced in *B. decumbens* but not in *B. ruziziensis* under simulated acid soil stress *(st+Al)* when compared to control conditions *(co)*. In order to obtain full-length clones, a number of techniques including isolation of total RNA, mRNA purification, ds-cDNA synthesis on paramagnetic beads, and PCR amplification of CDNA have been currently optimized. **[P. Wenzl, L.I. Mancilla, A.L. Chaves, C. Pineda, J. Mayer, I. M. Rao, R. Albert, and E. Heberle-Bors]**

Activity: Genotypic variation in nutrient acquisition and utilization

The extent of genotypic variation between and within species needs to be explored to develop nutrient efficient genotypes which could meet the mineral nutrient requirements of ruminants. A glasshouse experiment examined genotypic differences in acquisition and utilization of P among 15 genotypes of 5 species of *Brachiaria* (3 genotypes of each species: *B. decumbens*, *B. brizantha*, *B. ruziziensis*, *B. dictyoneura*, and *B. humidicola*). The selection of genotypes was based on prior agronomic evaluation in the field (commonly used, more productive and less productive genotypes). A clay 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: 80N, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). P was supplied at four levels (kg/ha: 0, 20, 50 and 200). All pots received inoculation with mycorrhizae (*Glomus clarum*). At the time of harvest (53 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 P partitioning index (leaf P/shoot P x 100) were determined.

Results showed that shoot attributes were influenced by genotype and also the level of P supply to soil (Table 15). As expected, increase in P supply improved forage yield as a result of stimulation of leaf area production. Data on root attributes, P acquisition and P utilization are being analyzed. Genotypic variation was greater in leaf inorganic P concentration of *B. dictyoneura* and *B. humidicola* compared to the other three species (Figure 2). **[I. M. Rao, J. Ricaurte, and R. García]**

Table 15. Influence of P supply on the range of genotypic variation in plant attributes of five species of *Brachiaria* (15 genotypes) grown in pots (4 kg soil) in a clay loam Oxisol from Carimagua. Measurements on plant attributes were made after days of growth.

Plant attributes	Phosphorus supply (kg/ha)			
	0	20	50	100
Forage yield (g/pot)	0.92 - 2.74	10.4 - 22.9	17.7 - 35.9	16.4 - 43.2
Leaf area (cm^2/pot)	87 - 265	76.7 - 1674	86.4 - 2026	776 - 2293
Specific leaf area (m^2/kg)	8.61 - 22.4	13.2 - 25.9	13.1 - 22.6	11.5 - 20.0
Inorganic P in leaves ($\mu g/cm^2$)	1.19 - 3.35	1.41 - 3.98	1.26 - 5.02	1.69 - 7.32
Total chlorophyll in leaves (mg/m^2)	260 - 513	248 - 412	201 - 467	158 - 477

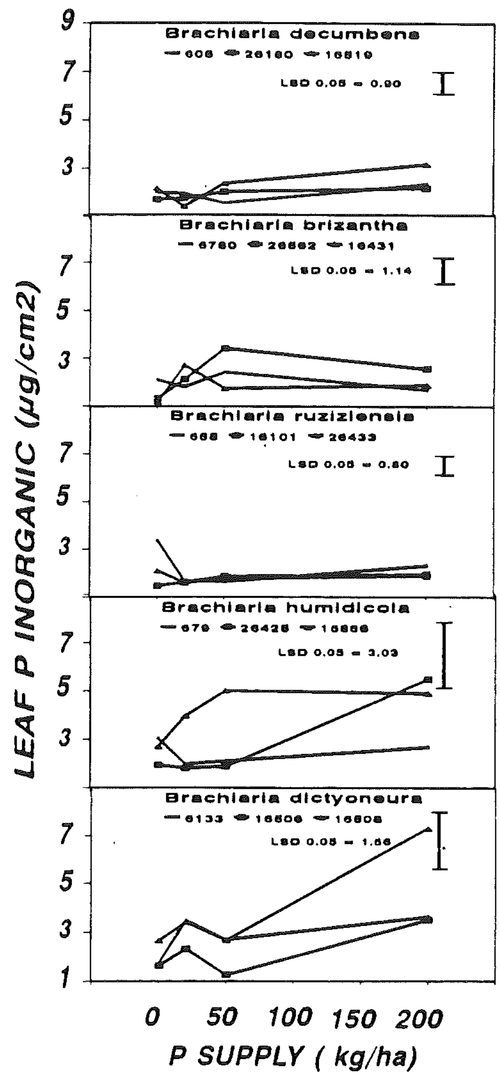


Figure 2. Genotypic differences in leaf inorganic P concentration in five *Brachiaria* species as influenced by P supply to a clay loam Oxisol from Carimagua, Colombia.

Output 2.5

Role of endophytes in tropical grasses assessed

Main achievements

- Staining protocols were developed to detect endophytic fungi in plant tissues and seeds of tropical grasses.

Endophytic fungi reside within plant tissues: These fungi are reported in grasses, shrubs, and evergreen trees, and are most likely to be found in most plants. Many grasses harbor clavicipitaceous endophytes within their leaves, stems, or seeds. Some of these endophytic fungi confer beneficial effects on infected plant hosts. In some grasses, the presence of endophytes provides increased resistance to some insects, plant pathogens, and increased tolerance to drought. In addition, endophyte-infected plants often exhibit more vigorous growth. However, they may also contain alkaloids which can be toxic to animals which graze them.

The role of endophytes in tropical grasses is largely unknown. Beneficial effects have not been established, but it is possible that some insect resistance can be attributed to endophytes. Detrimental effects have been attributed to endophytes, but there is no proof of such effects.

Tropical forage grasses are widely sown in South America, the most common being different species of *Brachiaria*, of which there are approximately 50 million hectares of sown pasture. Differences in tolerance to insects has been observed between species and accessions. Also toxicity problems have been observed in animals grazing *Brachiaria decumbens*. A role for endophytes in insect resistance and toxicity to grazing animals has been suggested, but not confirmed.

The use of naturally occurring biocontrol agents for disease and insect control will counter the adverse environmental consequences of heavy pesticide use. The use of endophytes as a natural control agent would have many advantages. One of these is that endophytes can be transmitted through ovules and seeds of their plant hosts. Results of strategic research on endophytes may have widespread application. The results are of particular significance to small farmers because of the low cost of applying the research results and savings in the reduced use of pesticides.

A staining protocol was developed to detect fungi in plant tissues (leaf blades, sheaths, stems, etc.) and seeds of various tropical grasses. An endophytic fungus was isolated and purified from *Panicum* sp., a native savanna grass collected in Carimagua. The exact identity of the fungus is currently being examined at CMI in England. A fungus which apparently resembles an endophyte has recently been consistently detected in leaf tissues of *Brachiaria arrecta* CIAT 16845 collected in Quilichao, Colombia (Figure 3). However, various tests such as fungal isolation, scanning electron microscopy and final correct identification are currently in progress. **[S. Kelemu, M.S. Sánchez, and S. França]**

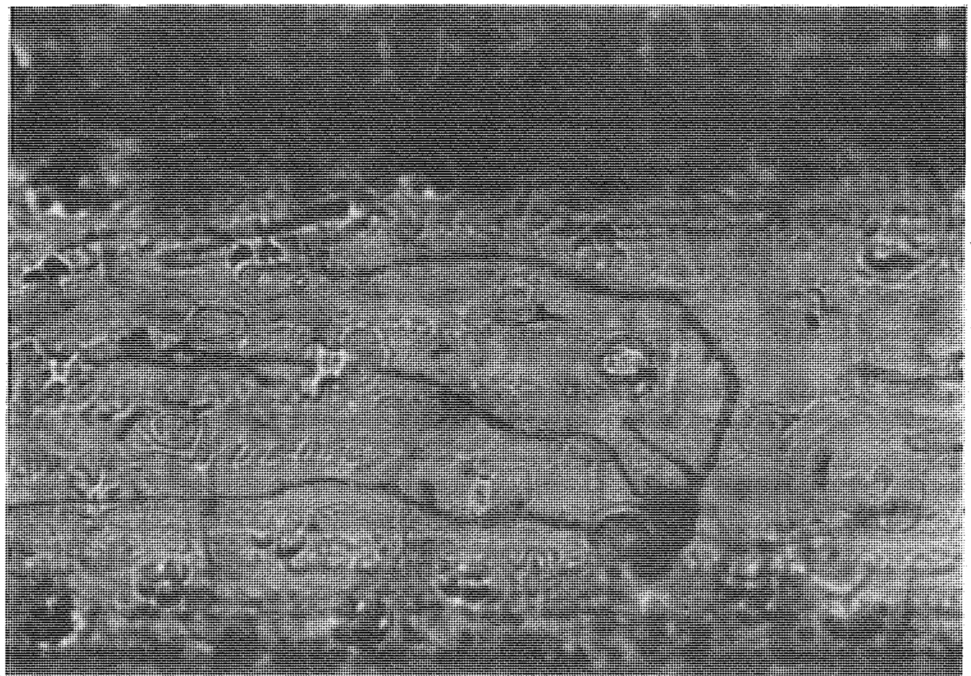


Figure 3. Fungal growth in leaf tissues of *Brachiaria arrecta* CIAT 16845.

Output 2.6:

Improved sexual *Brachiaria* gene pool with edaphic adaptation and insect resistance

Main achievements

- Sexual *Brachiaria* population (1500 individual clones) screened at two field sites and 180 individuals selected.

- Field-selected *Brachiaria* clones screened for spittlebug reaction using artificial infestation in glasshouse. Twelve clones selected.

- Crossing block established at CIAT to recombine selected *Brachiaria* clones.

<u>Activity: Implement improved field, glasshouse, and laboratory screening methodologies for spittlebug resistance</u>

Spittlebug resistance (along with edaphic adaptation) is a key attribute receiving attention in the *Brachiaria* gene pool enhancement program. We now know that assessment of resistance was deficient in early cycles of selection in which populations were advanced one cycle per year. We have adopted a two-year cycle. Recombined progenies were propagated and established in space-planted field screening trials at Carimagua and Montañita, Caquetá, in May 1995. During 1995, the initial population of approx. 1500 clones was culled to 180 on visual scoring of growth habit, leafiness, vigor, and freedom from foliar disease or deficiency symptoms. Spittlebug reaction of these 180 clones was assessed in 1996, on artificially infested plants in the glasshouse, using nymphal survival as the criterion of resistance. Most of the 180 selected clones were highly susceptible. Twelve clones were selected and propagated to a crossing block for recombination. Recombination is being achieved in isolation on pot-grown plants at CIAT. **[J.W. Miles, G. Sotelo, C. Cardona, and G.C. Yencho]**

Between 1995 and 1996, ca. 220 breeding lines were screened for resistance to spittlebug using the standard greenhouse test developed in previous years. Based on nymphal survival, only one clone (BP1016-009) was classified as highly resistant (nymphal survival significantly lower than in the standard check, CIAT 6294). The following clones were as resistant as the resistant check: BP1016-010, BP1016-019, BP1027-061, BP1027-110, BP1027-113, and BP1028-027. Two clones (BP1016-001 and BP1016-022) exhibited some degree of tolerance (low damage rating, high nymphal survival). The rest were classified as susceptible. **[C. Cardona, G.C. Yencho, and G. Sotelo]**

Output 2.7

Genetic control of key attributes investigated and QTLs identified

Main achievements

- Determined that spittlebug resistance in *Brachiaria* is not under simple genetic control.
- Identified new SCAR, AFLP, and rice RFLP markers linked to apomixis in *Brachiaria*.
- Molecular markers linked to apomixis locus in a second hybrid *Brachiaria* mapping population.

Activity: Saturate chromosome region around apomixis locus with additional markers

A modest project to tag the apomixis locus made significant progress in 1996. A SCAR (Sequence Characterized Amplified Region) was developed from a RAPD marker and it confirms linkage (approx. 13 cM) to the apomixis locus in the initial *B. ruziziensis* x *B. brizantha* mapping population. The same SCAR exhibits even closer linkage (4 cM) to the apomixis locus in a second mapping population (*B. ruziziensis* x *B. decumbens*). AFLP markers and a rice RFLP probe have also shown cosegregation with apomixis in *Brachiaria* (Figure 4). **[J. Tohmé, J.W. Miles, P. Rocha, and A. Bernal]**

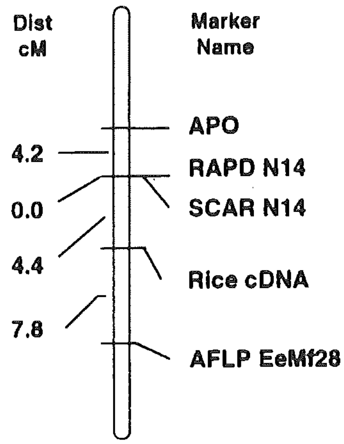


Figure 4. Preliminary genetic map in the region of the apomixis locus in *Brachiaria decumbens*. Linkage data from a population (n=87) of hybrid clones from the cross of tetraploid, sexual *B. ruziziensis* x tetraploid, apomictic *B. decumbens*.

Output 2.8

Promising accessions and lines of *Brachiaria*, *Panicum*, and *Paspalum* for production systems evaluated

Main achievements

- Seed of 20 promising *Brachiaria* accessions, two selected apomictic hybrids, and two check entries distributed to 12 collaborators for regional testing in diverse environments in Colombia.

- Two *B. humidicola* accessions selected for testing in large-plot grazing trials in Caquetá in 1997.

- Established plots to multiply seed of promising *Brachiaria* accessions for large-plot grazing trials in the Llanos Orientales in 1997.

<u>Activity: Organize and implement regional trials of promising accessions in Colombia and other countries</u>

A regional trials project aimed at testing promising accessions and hybrids of *Brachiaria* in a range of relevant environments in Colombia is being funded by the Colombian National Livestock Fund. The trials network at present comprises 12 sites, and involves collaborators from the Colombian National Agricultural Research Corporation (Corpoica), two Colombian universities, and a private producer. An organizational workshop was held in Villavicencio (Meta) and Carimagua in February. Seed of 20 selected accessions, two hybrid selections, and two checks was multiplied during 1995, and distributed to collaborators in May 1996. Following the initial establishment phase, data on dry matter production, disease and insect incidence, and phenology will be collected. From these trials we expect to identify a small number of widely adapted lines to advance to large-plot grazing trials in 1998, or 1999. This regional trials network represents an interesting model of collaborative, applied research involving a local, private NGO funding agency, and NARI, university, and private producer collaborators **[J.W. Miles, C. Plazas, and Collaborators]**

Activity: Elite accessions compared in grazing trials conducted with NARS

We expect to establish large scale grazing trials with selected *Brachiaria* accessions in the Amazon piedmont region (Caquetá), and in the Llanos Orientales during 1997.

Activity: Increase basic seed

A contract for seed multiplication of one accession of *B. brizantha* was signed and pre-basic seed delivered.

Subproject 3.0

Identification of superior *Arachis* and *Stylosanthes* genotypes

Output 3.1

Anthracnose resistance in *Stylosanthes* characterized

Main achievements

- Isolates of *C. gloeosporioides* commonly produce cell-wall degrading enzymes especially in the presence of *Stylosanthes* plant materials. Cell-free preparations of these enzymes induce anthracnose-like symptoms when leaves were infiltrated.

- Sources of anthracnose resistance were identified in *Stylosanthes guianensis*.

- Several genotypes of *S. guianensis* with high levels of resistance to a wide range of *C. gloeosporioides* pathotypes were identified.

Output 3.2

Pathogen biodiversity in *Colletotrichum gloeosporioides* characterized

Main achievements

- Determined several pathotypes of isolates of *C. gloeosporioides* in South American pathogen population.

- Isolates which can cross-infect *Stylosanthes* spp. were detected in South America.

- RAPD and RFLP analysis revealed that South America consists of a more complex and diverse population of *C. gloeosporioides* than other areas of the world.

- A statistical analysis program for molecular data was developed in collaboration with USDA/Kansas State University.

Stylosanthes guianensis (Aubl.) Sw. is a diverse forage legume species with a wide natural distribution throughout tropical and subtropical America.

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., is one of the major limitations to the extensive use of this legume as a forage. Dry matter losses of up to 100% have been reported in Colombia.

C. gloeosporioides varies in both morphology and pathogenicity. Two separate biotypes of the pathogen, designated A and B, have been described as causing distinct anthracnose symptoms on *Stylosanthes* species in Australia. Type A infects most species of the genus *Stylosanthes*, whereas Type B appears to be specific to *S. guianensis*.

The center of origin of the genus *Stylosanthes*, and thus the presumed center of genetic diversity of its pathogen, is in South America. Very little information is available on the extent of pathogenic and genetic variability among isolates of the pathogen in South America, key elements in effective breeding programs for anthracnose resistance and deployment of resistance.

The amount of genetic diversity of 127 *C. gloeosporioides* isolates originated from *S. guianensis* genotypes was measured at molecular level by polymerase chain reaction (PCR) amplifications of DNA using 11 arbitrary primers of 10 bases. The amplifications revealed scorable polymorphism among the isolates, and a total of 80 band positions was scored. A set of differentials consisting of 17 *S. guianensis* genotypes were used to characterize pathogenic variability in 104 of these isolates. Based on their virulence pattern on these differentials, the 104 isolates were grouped into various pathotypes. However, with the four *S. guianensis* genotypes used as differentials in Australia, these same isolates were grouped into very few pathotypes. No correlation existed between genetic diversity as measured by random amplified polymorphic DNA (RAPD) and pattern of pathogenicity on the differentials.

RFLP data also showed a great diversity in South American isolates (Figure 5). Generally, isolates clustered together by their geographic origin. Where isolates of various regions were grouped together, most of the isolates had identical host genotype origin. Isolates from Carimagua, Colombia, a savanna ecosystem and 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.

Several isolates of *C. gloeosporioides* produced cell-wall degrading pectolytic enzymes. The isolates generally produced higher levels of enzymes in the presence of plant materials than in artificial culture media. Stylosanthes leaves infiltrated with crude cell-free preparations of active enzymes showed anthracnose-like symptoms after few days of incubation at 28^0 C (Figure 6). **[S. Kelemu, J.L. Badel, C.X. Moreno, M.X. Rodriguez, C.D. Fernandes, M.J. Charchar, C. He, S. Chakraborty, and J.M. Manners]**

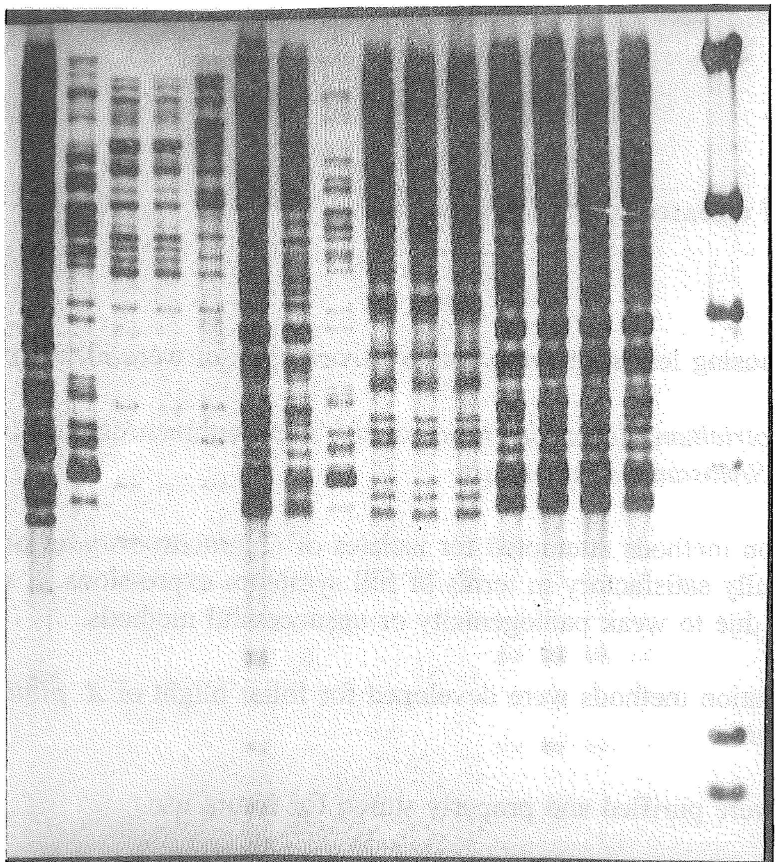


Figure 5. RFLP analysis of representative isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes guianensis* (probed with a retrotransposon DNA sequence).

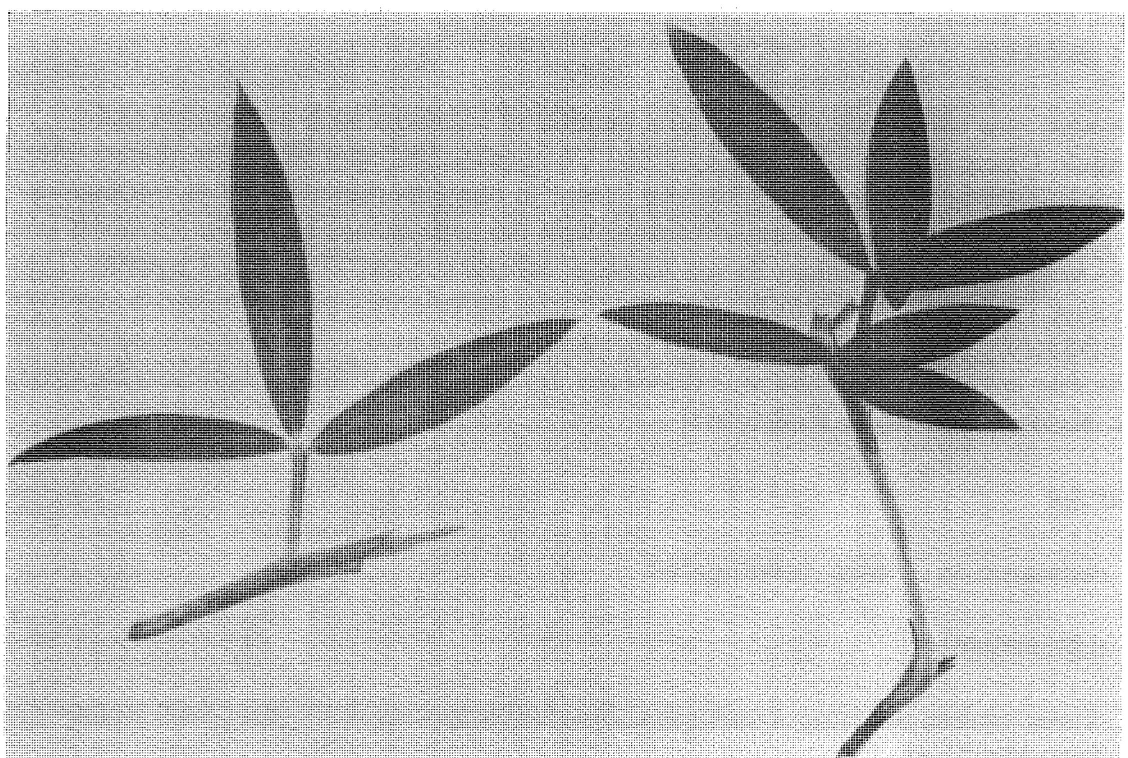


Figure 6. Leaves of *Stylosanthes guianensis* infiltrated with cell-free cell-wall degrading enzyme preparations isolated from *Colletotrichum gloeosporioides* (right) and control leaves infiltrated with liquid medium.

Output 3.3

Potential importance of diseases in *A. pintoi* assessed

Main achievements

- Few pathogens causing leaf spot symptoms of *Arachis pintoi* were identified.

- Isolates of *Colletotrichum gloeosporioides* isolated from anthracnosed tissues of *Arachis* were virulent on *Stylosanthes guianensis*

- Several inoculation methods attempted for isolates of *C. gloeosporioides* on *Arachis pintoi* were not fully satisfactory in terms of full symptom expressions. It was not clear whether this was due to weak pathogenicity or unsuccessful methods.

- Successful inoculation methods were developed for foliar blight of *A. pintoi* caused by *Rhizoctonia solani.*

- Several isolates were purified and properly stored for future use.

To date, no reports of major disease epidemics of forage *Arachis* exist. However, various diseases appear in field plots and these may eventually become limiting to production. Activities involving disease surveys, identifications, documentation, developing inoculation methods, and identification of sources of resistance will be crucial to tackling possible future disease problems.

Anthracnose was commonly observed in a number of locations such as Caquetá and Carimagua. Diseases such as pepper spot and *Cercospora* leaf spot were also documented. However, no severe damage was observed except in one experimental plot at Carimagua where some accessions were heavily infected with the anthracnose pathogen. *Arachis* was infected with *Rhizoctonia solani* in wet humid locations such as Florencia, Caquetá. Typical anthracnose symptoms were produced with artificial inoculation, but the methods were not fully satisfactory to be used to screen germplasm for resistance. However, a successful and reproducible inoculation method was developed for *Rhizoctonia* foliar blight disease (Figure 7). **[S. Kelemu and M.X. Rodríguez]**

Output 3.4

Virus resistance in *Arachis* investigated

Main achievements

- Three viruses infecting *Arachis* and *Brachiaria* species in the American tropics have been identified.

- Reliable diagnostic techniques to detect three potyviruses has been developed.

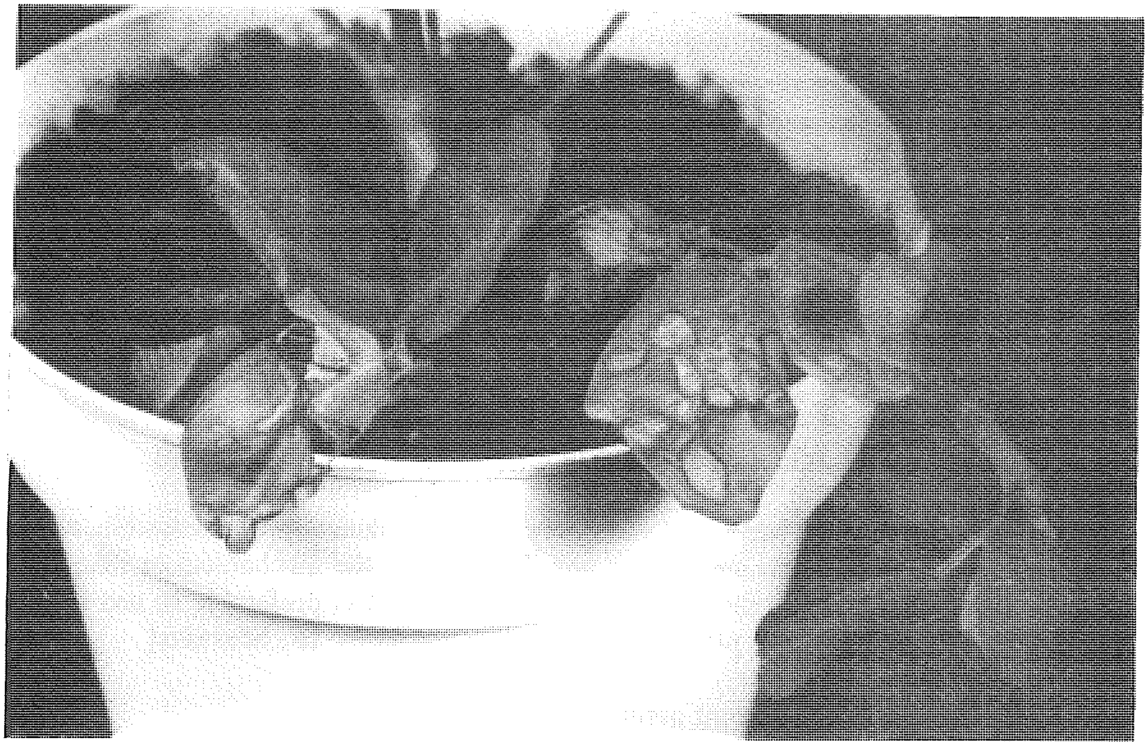


Figure 7. Symptoms of *Rhizoctonia* foliar blight in *Arachis pintoi*.

The virus that infects *Brachiaria* spp., Johnsongrass Mosaic Virus, is currently distributed in Australia, East and West Africa, and in tropical America (Brazil, Colombia, and Perú). The virus has been propagated vegetatively, mainly through infected planting material and, more recently, through tissue-cultured plants. Several aphid vectors are responsible for secondary spread of the virus.

The two potyviruses identified so far in *Arachis* species, Peanut Mottle Virus and another potyvirus related to Peanut Stripe Virus, are common pathogens of *Arachis* spp. The former virus is now distributed worldwide, wherever peanuts are cultivated. Peanut Stripe Virus probably originated in China, and was later introduced into the United States. Should the new potyvirus isolated from wild *Arachis* sp. be confirmed as Peanut Stripe Virus, it would be the first report of this virus in tropical America, namely Brazil, where the PStV-infected *Arachis major* plant [accession BRA 13196 (= CIAT 22307)] was collected. Fortunately, the new potyvirus was detected at CIAT before it had a chance to spread. Its pathogenicity in *Arachis* spp. seems limited. Studies to date indicate that potyviruses of *Arachis* and *Brachiaria* forage species can be controlled economically through the use of virus-free planting material.
[F. Morales]

<u>Activity: Indexing of available wild *Arachis* germplasm</u>

This year, a second virus was detected in an *Arachis major* accession BRA-13196 (= CIAT 22307). The pathogen has filamentous particles and cytoplasmic inclusions (pinwheels) characteristic of the potyvirus group. The biological and physico-chemical properties of the virus were studied in order to differentiate it from the Peanut Mottle Virus strain (PMoV-Arac) previously isolated from *Arachis pintoi*. Pathogenically, the new potyvirus differs from

PMoV-Arac in that it does not readily infect *A. hypogaea* or *A. pintoi*. Following the partial molecular characterization of the new potyvirus, nucleotide and amino acid sequence comparisons with the 3'untranslated region and part of the coat protein gene of other legume potyviruses, indicated that the new viral pathogen of *Arachis* sp. is closely related to Bean Common Mosaic and Peanut Stripe potyviruses, and not to Peanut Mottle Virus. However, a study of the pathogenicity range of the new *Arachis* potyvirus, does not suggest that this virus is a typical variant of either bean common mosaic or peanut stripe viruses. Fortunately, this virus was detected during the routine post-quarantine procedures established for exotic plant germplasm at CIAT and, consequently, it did not have a chance to spread in the field. **[F. Morales]**

Activity: Development of controlled inoculation methodology for selected viruses

The two viruses detected so far, PMoV and PStV, are both mechanically transmissible, and the species of *Arachis* tested to date, do not present any difficulty to the mechanical or manual inoculation process. However, as in the case of *Brachiaria* spp., it is preferable to use seedlings and not vegetative propagules as test plants for inoculation purposes. **[F. Morales]**

Activity: Identification of virus-resistant germplasm

The following wild *Arachis* germplasm accessions have been inoculated with the two potyviruses isolated so far from wild *Arachis* species:

***Arachis* sp.**	**CIAT acc. No.**	**Reaction to* PeMoV-Arac**	**New Poty**
Arachis pintoi	17434	+	-
	18744	+	-
	18745	+	-
	18746	+	-
	18747	+	-
	18748	+	-
	18751	+	-
	18752	+	-
	20826	+	-
	22148	+	-
	22149	+	-
	22150	+	
	22151	+	
	22152	+	
	22153	+	
	22154	+	
	22155	+	
	22156	+	
	22157	+	
	22158	-	
	22159	-	-
	22160	+	
	22172	+	
	22173	-	
	22174	-	
	22175	-	
	22176	-	
Arachis repens	22161	-	
	22162	-	
	22163	-	
	22164	-	
Arachis villosulicarpa	9500	+	-
Arachis kretschmeri	20693	+	-

* + = systemic infection; - = no systemic infection; blank = not tested.

The results presented here suggest that some *A. pintoi* accessions are not readily infected under artificial conditions. It is necessary to follow up their performance in the field. *A. repens*, seems to be another interesting wild forage species in relation to its resistance to PeMoV. These materials will now be evaluated under field conditions. As concluded above, the new potyvirus discovered in an unidentified *Arachis* species from Brazil, does not seem to be a major pathogen of *A. pintoi* or other species of *Arachis*. **[F. Morales]**

Output 3.5

Nutrient efficiency in legumes identified

Main achievements

- Showed that *A. pintoi*, CIAT 18748, was outstanding in it's adaptation to low P supply to soil as revealed by the extent of nodulation, leaf area production and P use efficiency.

- Demonstrated that the size of root system of *S.capitata* in a clay loam Oxisol was markedly lower than that of *S. guianensis* and *A. pintoi*.

Activity: Genotypic variation in nutrient acquisition and utilization

A glasshouse experiment examined genotypic differences in acquisition and utilization of P among 24 genotypes of 6 legume species (4 genotypes for each species: *Arachis pintoi*, *Stylosanthes guianensis*, *Stylosanthes capitata*, *Centrosema macrocarpum*, *Centrosema brasilianum* and *Centrosema pubescens*). The selection of genotypes was based on prior agronomic evaluation in the field. A clay 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: 80N, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). P was supplied at four levels (kg/ha: 0, 20, 50 and 200). All pots received inoculation with mycorrhizae (*Glomus manihotis*) and effective *Rhizobium* strains. At the time of harvest (80 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, leaf P partitioning index (leaf P/shoot P x 100), nodule number and nodule weight were determined.

Results showed that plant attributes were influenced by level of P supply and also by genotype (Table 16). The extent of nodulation exhibited greater genotypic variation than the other plant attributes. As expected, increase in P supply improved forage yield as a result of stimulation of leaf area production.

Genotypic variation in plant attributes of *A. pintoi* and *S. guianensis* when grown at 20 kg/ha of P supply is shown in Table 17. Among the four genotypes of *A. pintoi*, CIAT 18748 was outstanding in the extent of nodulation, leaf area production and P use efficiency. Among the four accessions of *S. guianensis*, CIAT 2950 (= cv. Mineirao) was outstanding in forage yield, leaf area production, extent of nodulation and P use efficiency. It is important to note that the

Table 16. Influence of P supply on the range of genotypic variation in plant attributes of six forage legumes (24 genotypes) grown in pots (4 kg soil) in a clay loam Oxisol from Carimagua. Measurements on plant attributes were made after 80 days of growth.

Plant attributes	Phosphorus supply (kg/ha)			
	0	20	50	200
Forage yield (g/pot)	1.37 - 7.07	5.43 - 16.5	7.39 - 19.7	10.0 - 25.4
Leaf area (cm^2/pot)	153 - 633	632 - 1958	850 - 2338	899 - 2992
Root length (m/pot)	44.0 - 82.8	85.3 - 321	95.4 - 227	65.1 - 304
Root length/leaf area (km/m^2)	0.98 - 4.18	0.61 - 6.42	0.54 - 2.02	0.43 - 2.28
Shoot P uptake (mg/pot)	1.56 - 6.31	6.28 - 13.4	9.92 - 24.1	16.5 - 39.6
Nodule number (no./pot)	0.0	27.3 - 396	59.0 - 330	101 - 699
P uptake efficiency (μg/m)	26.6 - 98.6	26.5 - 133	65.1 - 255	95.0 - 1100
P use efficiency (g/g)	755 - 1907	485 - 1850	383 - 1572	247 - 1506

hybrid, CIAT 11844 of *S. guianensis* had lower root length but greater P uptake efficiency per unit root length. **[I. M. Rao, J. Ricaurte, and R. García]**

Table 17. Genotypic differences in plant attributes related to P acquisition and utilization of *Arachis pintoi* and *Stylosanthes guianensis* grown with a P supply of 20 kg/ha in pots (4 kg soil) in a clay loam Oxisol from Carimagua. Measurements were made after 80 days of growth. LSD values are at the 0.05 probability level.

Species	CIAT accession number	Forage yield (g/pot)	Leaf area (cm^2/pot)	Root length (m/pot)	Nodule number (no. pot)	Shoot P uptake (mg/pot)	P uptake efficiency (μg/m)	P use eff. g/g
A. pintoi	17434	11.9	1044	102	195	12.8	126	766
	18744	16.2	1222	106	290	14.0	133	986
	18748	14.8	1432	113	396	11.2	105	1012
	22160	13.1	1258	103	301	10.8	106	946
$LSD_{0.05}$		NS	NS	NS	NS	1.4	NS	NS
S. guianensis	11844	10.7	906	162	91	9.81	65	1011
	184	10.4	874	321	229	8.18	27	1005
	21	9.81	838	201	167	8.43	45	970
	2950	12.3	1008	289	288	8.72	30	1205
$LSD_{0.05}$		2.05	NS	89	NS	1.02	19	158

NS = not significant

Using minirhizotrons (plexiglass root boxes), another experiment was conducted by an undergraduate student to compare root growth and development and to test the relationship between root growth and shoot growth during seedling establishment in two genotypes of *Arachis pintoi* (CIAT 17434 and 22160) and two species of *Stylosanthes* (*S. guianensis* CIAT 184 and *S. capitata* CIAT 1315). Plants were grown in a clay loam Oxisol with low amounts of fertilizer application (kg/ha: 20P, 20K, 33Ca, 14Mg and 10S). Among the four legume accessions, *S. capitata* exhibited markedly smaller size of the root system (Figure 8). Root development was also markedly slower with *S. capitata* than that of *S. guianensis*. The extent of root branching and total root length were also lower in *S. capitata* compared to the other three legumes. There were no significant differences in size of the root system between the two accessions of *A. pintoi* although CIAT 22160 showed greater ramification of the root system during seedling establishment. **[L.E.C. Martínez, J. Ricaurte, R. García, and I.M. Rao]**

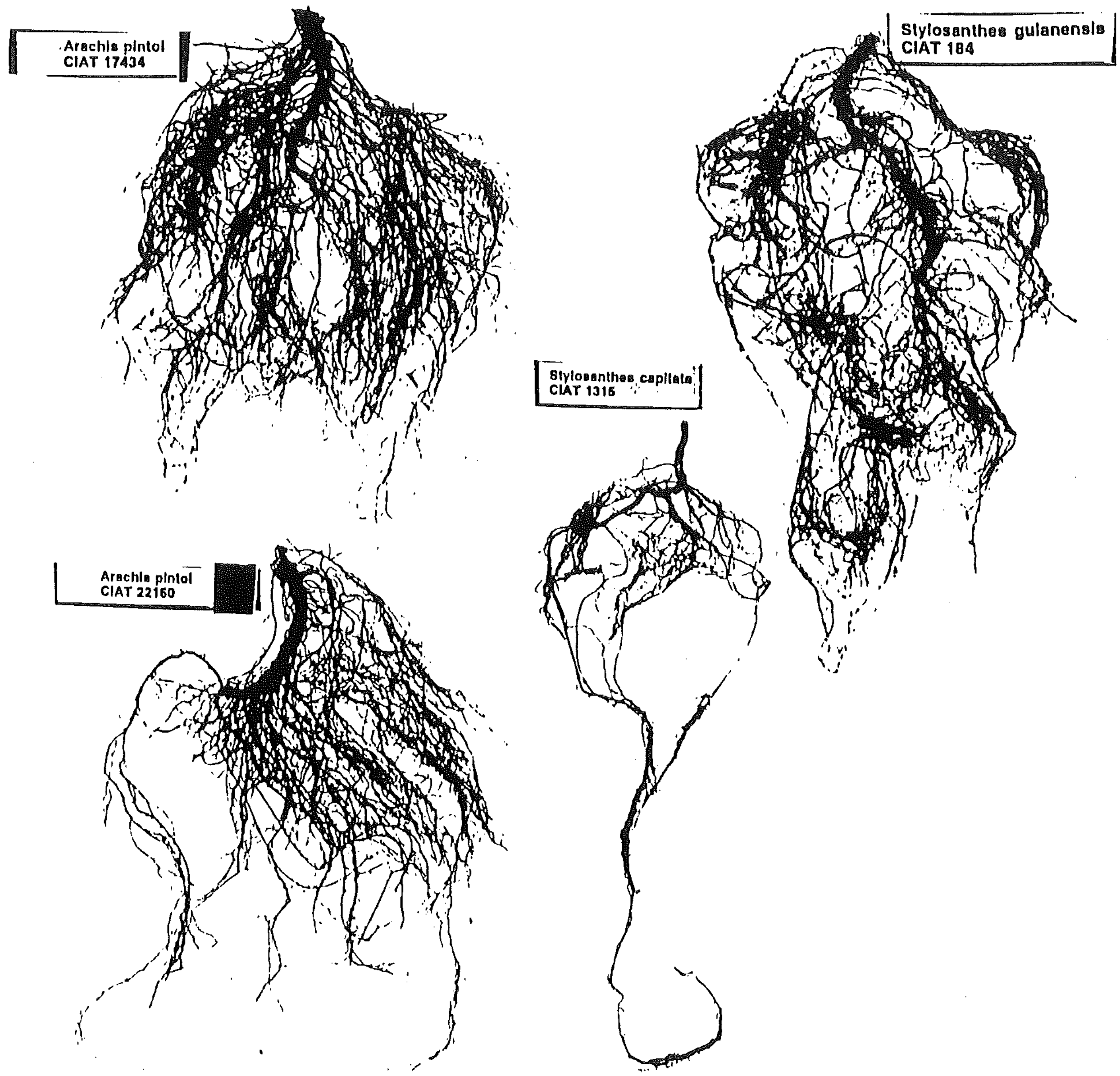


Figure 8. Differences in size of the root system among four legumes grown in minirhizotrons at low nutrient supply in a clay loam Oxisol from Carimagua, Colombia.

Output 3.6

Disease resistance in *Stylosanthes* enhanced

Activity: Recurrent population improvement

An additional cycle of bulk-mass selection is being conducted in 1996. This very low-cost project, begun 13 years ago, has generated six gene pools covering a wide range in maturities and with good anthracnose resistance and seed yield. No additional *Stylosanthes* breeding activities are in progress nor contemplated at the moment. **[J.W. Miles and C. Plazas]**

Activity: Combination of diverse resistance genes in elite lines

Progress in this area awaits outputs from the ACIAR *Stylosanthes* project in the way of cloned anthracnose-resistance genes.

Output 3.7

Die-back/wilt disease of *Stylosanthes*

Main achievements

- The causal agent of the wilt/dieback disease of *Stylosanthes* spp. was identified as *Lasiodiplodia theobromae*.
- Effective inoculation and disease evaluation methods were developed for the wilt/dieback disease.
- Enhanced sporulation of *Lasiodiplodia theobromae* was achieved in certain culture media.

Stylosanthes guianensis (Aubl.) SW., is a diverse, and economically important forage legume with a wide natural distribution throughout tropical and subtropical South America. The species is also important China.

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., has been a major constraint to the extensive use of *S. guianensis* as a tropical forage, encouraging efforts in breeding and selection among the natural populations to focus, for decades, on resistance to anthracnose. These efforts, particularly selection among natural populations, have generated a number of genotypes with high levels of anthracnose resistance. Meanwhile the sporadic occurrence of wilt/dieback disease in seed multiplication plots did not receive much attention as to the correct identification of the causal agent and subsequent control measures. As a result, the disease became prominent and widespread, especially in genotypes with anthracnose resistance.

In this study, we identify the causal agent of the disease, its culture characteristics, and host-plant reactions.

A wilt and eventual dieback disease of the forage legume *Stylosanthes guianensis* occurs widely in Colombia and Brazil (see symptoms in Figure 9). Breeding and selection efforts in *Stylosanthes* have concentrated on resistance to anthracnose, economically a more significant disease. Eventually, the wilt/dieback disease became prominent in various locations, particularly on *S. guianensis* genotypes with high levels of anthracnose resistance. The causal agent of the wilt disease has been identified as *Lasiodiplodia theobromae*, a virulent, unspecialized, facultative wound pathogen, with worldwide distribution and more than 500 different host plants. The 1-septate conidia are slow maturing, brown, thick walled, and striate (Figure 10). Genotypes of *S. guianensis* differ in their reaction to the pathogen. Of the eight important *S. guianensis* genotypes tested, the Brazilian cultivar Mineirao was the most susceptible; and CIAT 10136 and FM 9405 parcela 1 were the most resistant (Table 18). **[S. Kelemu, J. L. Badel, and C. D. Fernandes]**

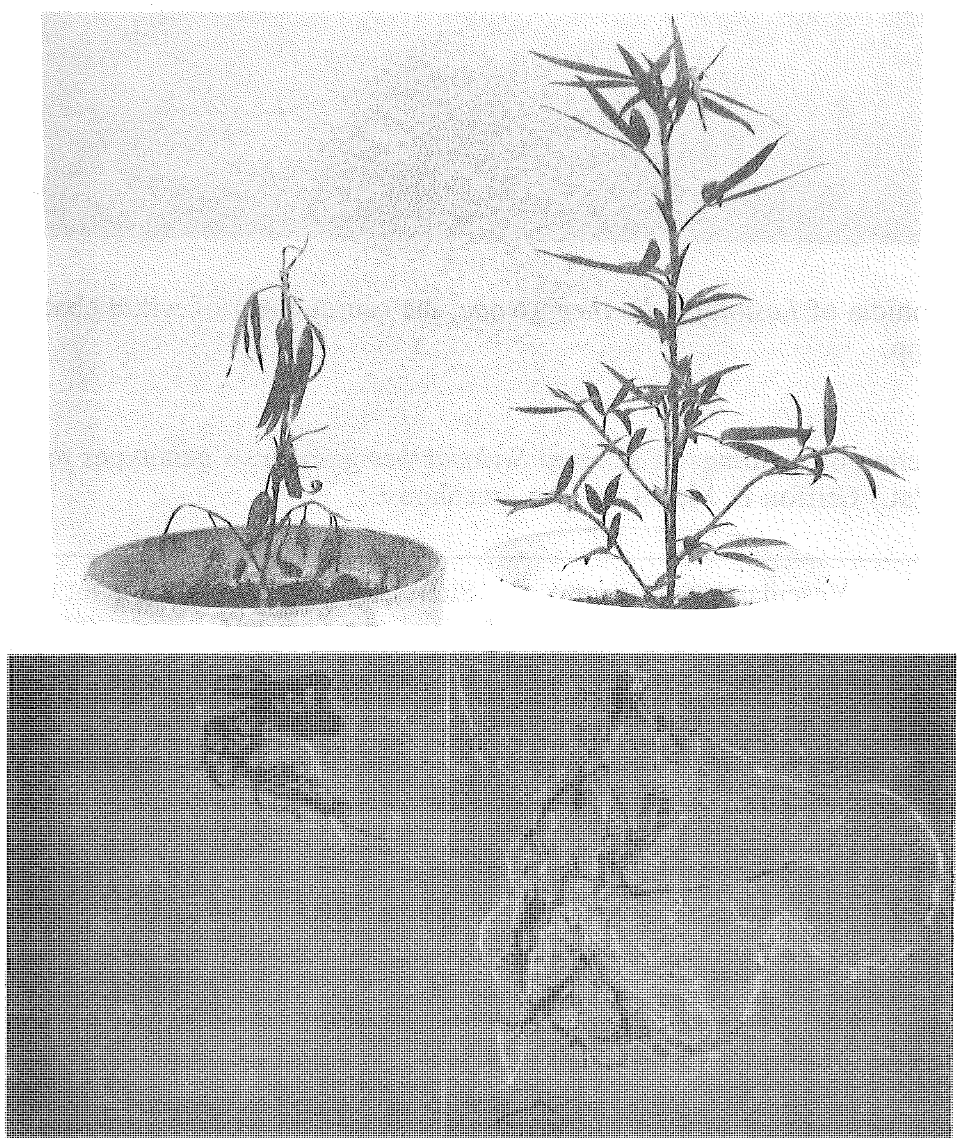


Figure 9. Symptoms of wilt/dieback disease of *Stylosanthes* spp. in both above and below ground parts of the plant (left), and healthy control parts.

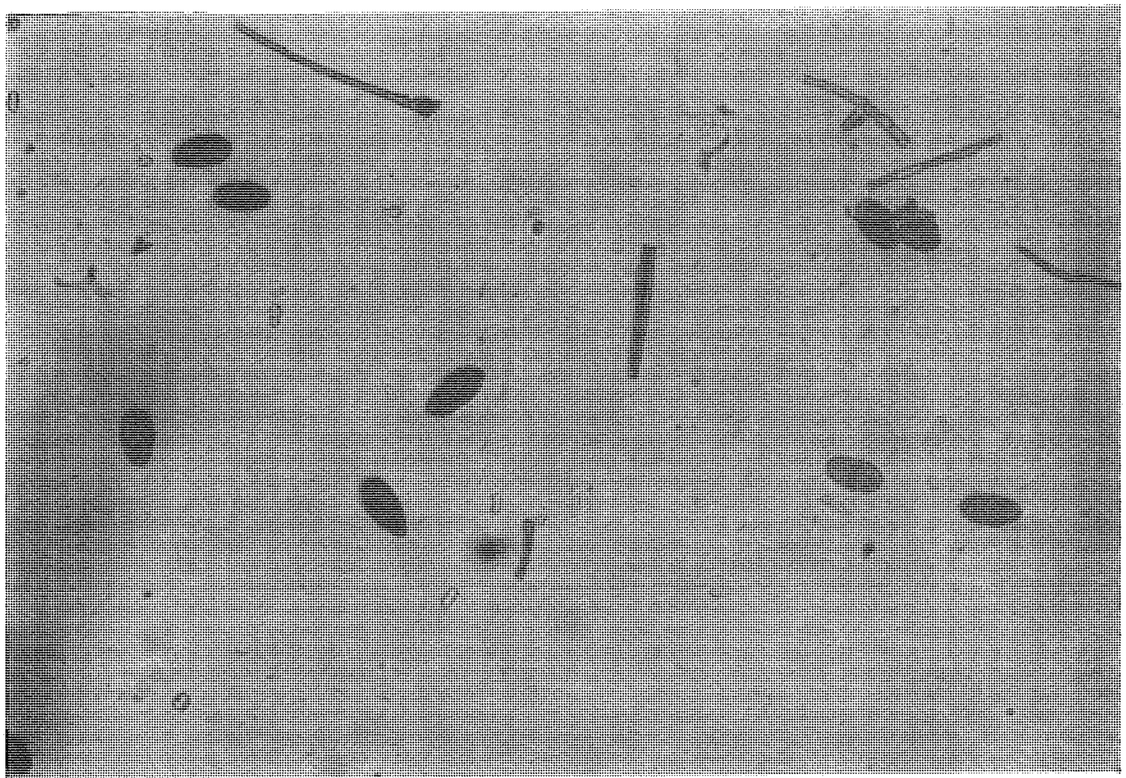


Figure 10. Conidia of *Lasiodiplodia theobromae*, the causal agent of wilt/dieback disease of *Stylosanthes* spp.

Table 18. Reaction of seedlings of selected *Stylosanthes guianensis* genotypes to *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. in the greenhouse [a]

Host	Vascular lesion length (cm)	Stem lesion size (% total plant height)	Root dry weight (% control)
Mineirão	3.7 a	38.4 a	48.8
FM9405 P6	3.1 ab	25.6 ab	96.5
CIAT 184	2.8 ab	29.6 a	100.0
CIAT 11844	1.8 abc	14.4 ab	89.4
CIAT 11833	1.4 bc	13.7 ab	90.6
CIAT 136	0.4 c	2.5 b	89.7
CIAT 10136	0.3 c	0.0 b	100.0
FM9405 P1	0.6 c	0.0 b	92.0

[a] Each value is the mean of three replicates. Data in vertical columns followed by the same letter are not significantly different (P = 0.01), according to Duncan's multiple range test.

Output 3.8

Feed value of new accessions of *Arachis* and lines of *Stylosanthes* evaluated

Main achievements

- Found differences in quality attributes among selected ecotypes of *Arachis pintoi.*
- Found differences in quality attributes of selected *Arachis pintoi* ecotypes due to site.

Forage samples from an ongoing multilocational agronomic study with ecotypes of *Arachis pintoi* were analyzed for N, IVDMD, and tannins. A summary of results on quality parameters for the commercial *A. pintoi* cultivar (CIAT 17434) and ecotypes selected on the basis of agronomic performances is presented in Table 19. In the three sites (hillsides, forest margins and savanna) there were differences in quality among ecotypes. However, it is interesting to note that in all sites *A. pintoi* (18744) had higher N, higher digestibility and less tannins than the commercial cultivar (17434). On the other hand, results suggest that quality of *A. pintoi* ecotypes was influenced by site. In the most favorable site (hillsides, Popayán) in terms of soil fertility, all ecotypes had higher N, higher digestibility and less tannins as compared to the sites in forest margins and savanna.

Small but consistent differences in quality of selected ecotypes of *A. pintoi* were also observed in the two savanna sites. In a clay loam soil (Alcancía) ecotypes of *A. pintoi* had higher digestibility and less tannins than in a sandy loam soil (Alegría) **[C.E. Lascano, B.L. Maass, E.A. Cárdenas, and N. Narváez]**

Output 3.9

Advanced accessions and lines of *Stylosanthes* and *Arachis* for production systems evaluated

Main achievements

- Seed of promising *Arachis* accessions harvested.

Small samples of seed of selected *Stylosanthes* accessions were sent to Australia for multiplication and distribution to collaborators in an ACIAR-funded project involving India, China, and Brazil, in addition to CSIRO and CIAT. Seed of *S. guianensis* selections is being multiplied for distribution and wide-scale testing in 1997.

The first large-scale seed multiplication of *Arachis* selections was accomplished in 1996, in collaboration with the Colombian Coffee Federation's Research Center (Cenicafé) in Chinchiná, Caldas. Total seed yield recorded on accession CIAT 22160 was much lower than that of two other promising accessions (CIAT 18744 and CIAT 18748) (Table 20). This is consistent with data from a small-plot agronomic trial conducted in Brazil and presented in the

Table 19. Quality attributes in selected *Arachis pintoi* ecotypes grown in contrasting sites in Colombia (Maass et al., unpublished results).

Ecotypes (CIAT No.)	Sites											
	Hillsides[1]			Forest Margins[1]			Savanna (Carimagua)[2]					
	Popayán			Macagual			Alcancía[3]			Alegría[4]		
	N	IVDMD	ECT[5]	N	IVDMD	ECT	N	IVDMD	ECT	N	IVDMD	ECT
		(%)			(%)			(%)			(%)	
17434 (control)	3.1	79.8	4.1	2.0	71.7	6.4	2.4	66.7	---	2.2	65.4	7.3
18744	3.7	81.5	3.8	2.6	73.6	5.2	2.7	70.3	5.1	2.2	67.6	6.5
18748	3.3	75.5	4.6	2.3	70.2	7.7	2.7	64.6	7.0	2.0	65.5	8.3
18751	3.6	77.7	3.5	2.1	73.6	6.5	2.3	67.3	7.7	2.4	63.1	7.8
22159	3.6	78.8	3.3	2.4	70.2	5.2	2.3	72.2	7.4	2.4	68.3	7.0
22160	3.6	78.6	3.3	2.4	72.7	6.2	2.1	66.3	6.1	2.2	63.3	7.7
Mean	3.5	78.7	3.8	2.3	72.0	6.2	2.4	67.9	6.7	2.2	65.5	7.4

[1] 4 week regrowth (leaves)

[2] 8 weeks regrowth (leaves)

[3] Heavy clay soil

[4] Sandy soil

[5] Extractable condensed tannins

1992/1993 Biennial Report. Values for 100-seed weight were similar for all three accessions (Table 20). If indeed the seed yield potential of CIAT 22160 is substantially less than other accessions, it would represent a critical defect, unless it can be rectified by agronomic practices.

These *A. pintoi* seed supplies will be used for further, basic seed production, wider agronomic testing, and on-farm demonstration/promotion such as through the Nestlé project in Caquetá, Colombia. We anticipate being able to distribute seed samples to a number of countries in 1997 for local testing. **[J.W. Miles, R. Mosquera, A. Ortega, and P.C. Kerridge]**

Table 20. Seed yields of three *Arachis pintoi* accessions, Finca La Romelia, Chinchiná, Caldas.

Accession	Area harvested m^2	Yield (kg/ha)	100-seed wt (gm)
CIAT 18744	922	2,017	17
CIAT 18748	837	1,505	18
CIAT 22160	1,600	1,025	20

Subproject 4.0

Identification of new accessions of multipurpose shrub legumes

Output 4.1

Shrub legumes for different agroecosystems identified

Main achievements

- Several materials of *Cajanus cajan* were identified in the mid-altitude hillsides that offer high potential for forage, use as live barriers, or grain production.

- The continuation of shrub legume evaluation in the mid-altitude hillsides of the Colombian Cauca department is supported by local NGO (Agroganadera del Valle)

Activity: Continue with evaluation of shrub legumes in Cauca 1995-97

To continue the search for multipurpose shrub and tree germplasm adapted to the mid-altitude hillsides in the Colombian Cauca Department, a new collection of woody legumes (Table 21) was sown at the localities of El Melcho (Piendamó, 1600 masl.) and San Vicente (Santander de Quilichao, 1200 masl.), to characterize their range of adaptation to different altitudes on hillside soils and with a minimum fertilization of 50 kg P/ha. During the establishment phase (6 months), growth was very slow at both sites. Therefore chicken manure was applied at a

Table 21. Shrub legume species sown in the mid-altitude hillsides of the Colombian Cauca Department.

Genus	No. of accessions	Species	No. of accessions
Acacia	3	*A. angustissima*	1
		A. farnesiana	1
		Acacia sp.	1
Ateleia	1	*A. ovata*	1
Calliandra	3	*C. calothyrsus*	1
		C. houstoniana	1
		Calliandra sp.	1
Cassia	1	*Cassia* sp.	1
Clitoria	3	*C. fairchildiana*	2
		C. dendrina	1
Dalea	1	*D. carthagenensis*	1
Erythrina	2	*E. burana*	1
		Erythrina sp.	1
Flemingia	6	*F. macrophylla*	6
Gliricidia	2	*G. sepium*	2
Leucaena	10	*L. diversifolia*	6
		L. leucocephala	4
Mimosa	3	*M. colombiana*	3
Pueraria	4	*P. wallichi*	4
Rynchosia	3	*R. schomburgkii*	3
Senna	3	*S. spectabilis*	1
		S. siammea	1
		S. velutina	1
Sesbania	3	*S. keniensis*	1
		S. sesban	2
Zapoteca	2	*Z. formosa*	2
*Trichantera**	1	*T. gigantea* (Nacedero)	1
Total	51		

* Belongs to family of Acantaceae

rate of 5 t/ha for 10 plants in the plot, while the remaining 10 plants were not fertilized. There was a stronger response to the fertilization at San Vicente than at El Melcho. Materials which have been performing best at both sites were: *Leucaena diversifolia* CIAT 17271, *Pueraria wallichi* CIAT 21076 y 21287, *Rhynchosia schomburgkii* CIAT 22235 y 8215 and *Sesbania sesban* CIAT 19678, and in addition, *Sesbania keniensis* CIAT 19165 at El Melcho. This trial is being conducted with the financial support of "Agroganadera del Valle" and will initiate its production phase at the end of 1996, which will last until 1998. **[E.A. Cárdenas and B.L. Maass]**

Cajanus cajan. To date, pigeon pea (*Cajanus cajan*) is the shrub which offers the highest potential for the hillside region with low-fertility soils in the Colombian Cauca department, where it also proved to be free of pest and disease problems. During 1996, the evaluation of a germplasm collection of 49 pigeon pea accessions was continued at El Melcho (1600 masl.). Both DM and seed yield were measured. To determine variation within and among accessions, additional morphological characteristics were evaluated, such as flower and seed colors, plant growth habit, and pubescence and dehiscence of the pod. Differences among accessions were obvious: some were relatively uniform, while others represent segregating populations. As this species is partially outcrossing, selection of superior individuals from the different accessions is intended. Several materials were identified that offer high potential for forage, use as live barriers, or for grain production. Data are being processed and will be published in a technical paper. It is suggested to continue the selection process for the hillside region according to the future utilization of pigeon pea. **[E.A. Cárdenas and B.L. Maass]**

<u>Activity: Publish results of evaluations of shrub legumes in Colombia, Costa Rica, Brazil.</u>

In agronomic trials, acceptability evaluation and quality analysis, *Codariocalyx gyroides* has been identified as the most promising shrub legume for the humid tropics. A leaflet on this legume was prepared within the collaborative project Nestlé de Colombia-CORPOICA-Universidad de la Amazonia-CIAT: "*Codariocalyx gyroides* (Cora-cora) - una leguminosa semi-arbustiva para la ganadería del trópico húmedo". In addition to two technical papers submitted to "Pasturas Tropicales", a poster paper on the development of this legume was presented at the Meeting of the German Society of Agronomy (Maass, Keller-Grein and Schultze-Kraft, 1996) and a paper was submitted to the IGC'97 in Canada on the identification of environmental adaptation through multilocational evaluation of *C. gyroides* by RIEPT researchers. **[B.L. Maass, E.A. Cárdenas, L.H. Franco, and G. Ramírez]**

Calliandra spp. A field experiment in Atenas (inceptisols, pH 5.6) and San Isidro (ultisols, pH 4.6) evaluated the adaptation of three accessions of *Calliandra* spp. Individual plants were cut at 70 cm height every 8 weeks and the material separated into leaves and stems.

Across sites the accessions CIAT 20400 and 20399 were better adapted and produced more leaf DM than CIAT 21252, but the proportion of DM recorded during the dry season was greater in Atenas (5-6 months dry) than in San Isidro (3-4 months dry) (Table 22). The accession CIAT 21252 had some plant mortality at the end of the evaluation period, meanwhile CIAT 20400 maintained good plant vigor and no apparent incidence of pests and diseases. It seems that this is a new species of *Calliandra* according to H. M. Hernández from Mexico. **[P.J. Argel]**

Table 22. Dry matter yields of *Calliandra* spp. under cutting in San Isidro and Atenas, Costa Rica.

CIAT No.	Yield (g DM/plant)*	
	San Isidro	**Atenas**
20400	295 (12) a**	232 (51) ab
20399	223 (12) b	337 (55) a
21252	87 (18) c	90 (84) b

* Means of 11 evaluation cuts
** P < 0.1, Duncan. In brackets % of DM yield during the dry period

Calliandra calothrysus is an alternative legume to *Leucaena* in cooler areas and where there is psyllid damage to *Leucaena*. It is performing well in cooler areas of the Cauca hillsides.

Thirteen new accessions del OFI have been cleared through quarantine and planted in the field at Santander de Quilichao for evaluation. They will be investigated for feed value as well as agronomic adaptation. **[E.A. Cárdenas, L.H. Franco, and P.C. Kerridge]**

Leucaena spp. In collaboration with the Oxford Forest Institute (OFI) of England, a trial, as part of a regional network, was established in Atenas to evaluate the adaptation of new species of the genus *Leucaena.* The species under evaluation are *L. collinsii, L. collinsii zacapana, L. diversifolia diversifolia, L. diversifolia stenocarpa, L. esculenta esculenta, L. esculenta paniculata, L. lanceolata, L. lempirana, L. leucocephala glabrata, L. macrophylla nelsoni, L. multicapitulata, L. pallida, L. pulverulenta, L. salvadorensis, L. shannoni magnifica, L. trichoides*, and one *Leucaena* hybrid. The local checks established in this trial are *L. leucocephala* CIAT 17263, *Calliandra calothyrsus* CPI 115690 and *C. argentea* CIAT 18668. Additional to plant adaptation, the experiment evaluates tolerance to Psyllid attacks and seasonal DM yields. A successful establishment was made by direct planting (10 plants per plot, replicated 4 times), and the first evaluation records have been taken. **[P.J. Argel]**

Output 4.2

Methodology for establishment and growth of shrub legumes

Main achievements

- Established differences in seedling emergence of *Cratylia argentea* due to planting depth.
- Identified an effective *Rhizobium* strain to inoculate seeds of *Cratylia argentea.*

<u>Activity: Field evaluation of shrub plant density, direct vs. transplanting, inoculated vs. non-inoculated seed and the interactin with fertilizer</u>

Evaluation of seed inoculation and planting depth of *Cratylia* seed: Seed of *C. argentea* was inoculated with two *Rhizobium* strains before planting in PVC cylinders filled with undisturbed soil from San Isidro (low fertility ultisol, 78% Al saturation and pH 4.6). The strains used were CIAT 3564 and 3566, and these were compared with an uninoculated treatment and the application of 90 kg/ha of N; plants were harvested 10 weeks after planting. The strain CIAT 3564 and the N fertilization produced similar DM yields indicating the effectiveness of this rhizobium strain, meanwhile CIAT 3566 was ineffective and yielded similar to the uninoculated treatment. However, the N treatment significantly increased ($P < 0.05$) root biomass and plant height, indicating that there is still room for selection of more effective strains to inoculate *C. argentea.* Another strain that has been tested is CIAT 3561, but it only increased the N content of the plants compared to the uninoculated treatment (see Tropical Forages Biennial Report 1994 -1995). **[A. Valerio and R. Quirós]**

Planting depth: *C. argentea* flowers and sets seed satisfactorily in Costa Rica and other parts of the tropics. However, reports exist of failure of adequate plant emergence from direct planting. A glasshouse experiment was conducted in Atenas consisting of planting seed of the legume at 0, 2, 4 and 6 cm of planting depth. Twenty pots (2 kg of soil) were used for each planting depth in a complete randomized design. Pots were watered daily to keep enough germinating moisture. Records of plant emergence were taken at 5, 10, 15, 21, 30 and 47 days after planting.

Table 23 shows that superficial planting and at 2 cm depth produced at the end of the observations, nearly twice the number of seedlings per pot compared to 4 and 6 cm planting depth. Plant emergence commenced 10 and 15 days after planting respectively in the latter treatments, meanwhile that in the superficial plantings it initiated much earlier. Total plant emergence was not statistically different ($P < 0.05$) for 0 and 2 cm planting depth, however, at 2 cm a high percentage of plant emergence was observed at 15 days of planting with small

Table 23 . Plant emergence of *C. argentea* seed planted at different depth in Atenas, Costa Rica.

Days after planting	**Planting depth (cm)**			
	0	**2**	**4**	**6**
	Cumulative % of seedlings			
5	0	0	0	0
10	33	28	0	0
15	48	80	8	0
21	55	80	48	20
30	83	83	55	38
47	93 a*	83 a	55 b	40 c

$P < 0.05$, Duncan

increases thereafter. Superficial planting had a more steady increase of seedlings along the observation period. Therefore, it is recommended to plant *C. argentea* seeds superficially - no more than 2 cm depth -. for successful direct planting. **[P.J. Argel]**

Activity: Field evaluation of cutting heights and age at first cutting

Response of *C. argentea* to P and Ca during establishment. A field experiment is being established in Atenas, Costa Rica, to study the effect of different levels of P and Ca on plant growth and DM yield during establishment of *C. argentea* CIAT 18668. The levels used of the nutrients are, P: 0, 20, 40 and 60 kg/ha, and Ca: 0, 200, 400 and 600 kg/ha. Seeds of the legume were direct planted following the application and incorporation into the soil of the nutrients. Harvesting is planned 4 months after planting. **[M. Jiménez, A. Valerio, and G. Pérez]**

According to the recommendations generated at the *Cratylia* workshop in 1995, to study the species' response in biomass production and persistence to different management forms, 3 accessions were sown at Santander de Quilichao in late 1995. The accessions CIAT 18668, 18672 and 18673 were selected because of distinct geographic origin. Different cutting regimes are being evaluated, initial cut at 5, 7, or 10 months, with two cutting frequencies at 30 or 60 days, and with three cutting heights, at 5, 40, or 80 cm. This trial is being carried out in collaboration with the Universidad Nacional de Colombia, Palmira. Preliminary results indicate that *C. argentea* may be cut at soil surface, once it is well established, without producing a reduction in leaf dry matter. **[H.A. Martínez, E.A. Cárdenas, and B.L. Maass]**

Project 9

Utilization of tropical grasses and legumes in production systems of the subhumid and humid tropics

Project Manager: Carlos E. Lascano

The Challenge

For smallholders in marginal areas of the humid and subhumid tropics, the need to increase productivity to raise living standards is accompanied by the need to address serious environmental degradation. The farmers therefore must meet these two distinct challenges. In both cases, forage grasses and legumes have the potential to assist them. Legume species provide nutritious feed and increase livestock production efficiency. They reduce the need for fertilizer inputs, cut down on concentrate supplement requirements in feeds, and thus lower production costs. They can also contribute to more sustainable land use: they help regenerate degraded soils and legumes add nitrogen to the system. The task remains to identify and document forage grass and legume combinations, for both purposes, which can be used by farmers to overcome specific, local constraints.

Objective

To stimulate the utilization of new grasses and legumes in the subhumid and humid tropics, in order to increase efficiency of animal production and produce a basis for sustainable land use. Some of the species will be selected to strengthen animal production, or in combination to reinforce soil structures or to regenerate nutrient-exhausted soils, thus increasing the standard of living of low income farmers.

Outputs

The principal output generated by project activities comprises a generic methodology for introducing improved grasses and legumes into different farming systems. Specifically:

- Stable grass-legume pastures for livestock production in the humid and subhumid tropics
- Fodder grasses and shrub legumes for dry season supplementation
- Legumes for fallow improvement
- Legumes for use as soil covers in perennial tree crops
- Forages for green manure
- Grasses and legumes for renovating degraded lands

Complementary outputs include seed for research and cultivar release by NARS; an understanding of the impact of grasses and legumes in different production systems; and an enhanced rate of adoption of new grasses and legumes.

Activities

The activities of this project are grouped into the following 5 sub-projects:

1. Forage components for livestock production systems;
2. Development of grass and legume components for multipurpose use in intensive agriculture systems;
3. Seed for research and national partners;
4. Adoption and impact analysis of legumes in dual purpose cattle systems; and
5. Adoption of new grasses and legumes

(See Workplan Breakdown structure of Project 9)

Benefits

Direct beneficiaries are those smallholders throughout the subhumid and humid tropics who depend on livestock, arable crops, arable crop-livestock combinations and perennial crops. These farmers can thus increase productivity while maintaining soil fertility, conserving water resources, and preserving the natural vegetation in critical areas of the landscape. Indirectly, positive economic returns for farmers, together with the beneficial environmental impact, ensure that the wider community also profits.

Strategy

The project features partnership with National Agricultural Research Systems (NARS) and places an emphasis on farmer participation. This approach enables the team to:

- Involve farmers in technology development
- Identify suitable species for use in stable grass-legume associations, in the context of their animal production potential
- Assess the value of selected grasses and legumes for rehabilitating degraded lands employed in intensive agriculture
- Analyze and disseminate information on the impact of introduced grasses and legumes in different production systems

Project Team

International Center for Tropical Agriculture, CIAT.

The project team at CIAT collaborates with other CIAT project teams working to develop sustainable production systems for the savannas, hillsides, and forest margins of Latin America

Two major consortia are:

TROPILECHE (LAC)

International Livestock Research Institute (ILRI)
Advanced Research Organizations
NARS in Latin America: Costa Rica, Peru, Colombia, Brazil, Bolivia.

Forages for Smallholders (SEAFRAD)

NARS in Southeast Asia: China, Indonesia (Lao PDR), Philippines, Malaysia, Thailand, and Vietnam

Project 9: Utilization of Tropical Grasses and Legumes in Production Systems of the Subhumid and Humid Tropics

Project Goal

To contribute to the development and deployment of selected grasses and legumes in production systems of the subhumid and humid tropics in collaboration with NARS

Sub-projects / Outputs

Purpose: To develop forage components for livestock production systems

- Selected grass and legume genotypes for animal production potential evaluated (CEL)
- Species and management inputs for stable grass-legume associations identified (IMR,PCK)
- Methodology developed for matching feed resources to animal requirements (CEL)
- Legume-based forage components for dual purpose cattle developed with farmer participation (CEL)
- Forage-based systems for dry season supplementation with farmer participation (CEL)
- Models on livestock systems applied to smallholder dual purpose cattle systems (CEL, FH)

Purpose: To develop grass and legume components for multipurpose use in intensive agricultural systems

- Grasses and legumes identified for rehabilitation of degraded lands (IMR, PCK, CEL)
- Grasses and legumes identified for crop-pasture rotations (PCK)
- Legumes available for fallow improvement (PCK, WSS, PJA)
- Grasses and legumes available for soil cover, erosion, weed and disease control (PCK, WSS, PJA)
- Grasses and legumes available for cut and carry feeding systems and for leaf protein meal (CEL, WSS, PJA)

Purpose: To multiply seed for research and promotion by national partners

- Promising accessions and advanced lines of grasses and legumes multiplied (JWM)
- Factors affecting seed quality investigated (PJA, JWM)
- Methodology of seed production and storage appropriate to national partners investigated (PJA, WSS)
- Seed available for use in investigation by CIAT programs and national partners (JWM, WSS)

Purpose: To determine adoption and impact of grasses and legumes in different production systems

- Ex-ante and ex-post impact analysis of legumes in dual purpose cattle farms (LR, FH)
- Socio-economic studies on legume-based feeding systems carried out in regions with dual purpose cattle systems (LR, FH, SF)
- Contribution of new legume-based systems to sustainable land use evaluated (SF, PCK)

Purpose: To enhance rate of adoption of new grasses and legumes

- Partnerships with national organizations established to identify grass and legume needs and to undertake collaborative research (PCK, CEL, PJA)
- Promising germplasm distributed through regional networks and release of new cultivars facilitated (PJA, WSS)
- Relevant methodology for legume-grass technology with farmer participation developed (JA, LAH, TG)
- Communication on grass-legume technology facilitated through newsletters, journals and workshops (PCK, CEL, PJA, AR)

Subproject 1.0

Forage components for livestock production systems

Output 1.1

Selected grass and legume genotypes for animal production potential evaluated

Activity : Evaluation with milking cows of selected grass and legume genotypes

In the last Biennial Report (1994-1995) we presented results on the evaluation of selected hybrid lines of *Stylosanthes guianensis* and *Panicum maximum* with milking cows. With hybrids of *S. guianensis* in association with *B. dictyoneura* milk yield increased by 17 to 65% over the straight grass. However, the legume had poor persistence for reasons not well understood. Milk yields with an ecotype of *P. maximum* + N selected for low fertility soils in Carimagua were greater than yields recorded in *B. dictyoneura* + N, but similar to yields recorded in *B. decumbens* + N. During 1996 we gave priority to the evaluation of the shrub legume *Cratylia argentea* as supplement for milking cows and results are presented in Output 1.5 of this report. In addition, we put a great deal effort in the analysis of results on milk yield and milk composition from a large data base from grazing trials with cows at Quilichao. The objective was to derive some methodological principles that could be applicable to the on-farm evaluation of improved feed resources. The results of the analysis are presented in Output 1.4 of this report **[C. Lascano and P. Avila]**.

Output 1.2

Species and management inputs for stable-grass legume association

Main achievements

- Found greater compatibility and persistence of *Desmodium ovalifolium* in association with grasses in sandy soil than *Arachis pintoi, Centrosema acutifolium* and *Stylosanthes capitata*.
- Showed that the presence of *Arachis pintoi* in association with *Brachiaria dictyoneura* enhanced the ability of the grass to acquire P from rock phosphate as P source.
- Application of a model on vertical root distribution to root biomass and root length data indicated that both native savanna and introduced grasses-legumes were deep rooted.
- Showed that the extent of accumulation of soil organic carbon, at 32 months after sowing, in grass alone pasture could be as high as 31.6 Mg ha^{-1} compared to native savanna.
- Showed that both grazing system and stocking rate could influence total root biomass and root length in grass alone and grass + legume associations.

Activity : Persistence of grass-legume associations

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, *Arachis pintoi* cv. mani forrajero, *Stylosanthes capitata* cv. Capica, *Centrosema acutifolium* cv. Vichada and *Desmodium 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 *C. acutifolium* in *B. decumbens* due to seedling 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, legumes were still persisting but with varying vigor. There was still a strong response to P and the response to K was variable across species and within treatments of species apparently more susceptible to K deficiency. There was no response to P or K by *D. ovalifolium*. The variable response within plots was probably due to small spatial differences in soil K. The legume Capica appeared to be more susceptible to K deficiency than *A. pintoi* and *C. acutifolium*. The overall vigor of the plants was highest for *D. ovalifolium* (which is grazed very little) followed by Capica stylo (which is severely grazed) and with similar vigor of *A. pintoi* and *C. acutifolium*. The performance of *A. pintoi* cv. Mani Forrajero (CIAT 17434) was disappointing in 1995 since it had less vigor, was yellow and appeared more susceptible to diseases such as anthracnose and leaf spot than in 1994. Newly established *A. pintoi* (CIAT 22160) in the same area appear more vigorous. In the third year (1996) the associations of *B. decumbens* and *B. dictyoneura* with *D. ovalifolium* continued with strong vigor of the legume. At the beginning of the dry season in January 1996, there was 3000 kg/ha of legume in the *B. decumbens* association and 2120 kg/ha with *B. dictyoneura*. The next most productive legume was *Stylosanthes capitata* cv. Capica with 340 kg/ha with *B. decumbens* and 50 kg/ha with *B. dictyoneura*. We did not find an effect of P or K on yield or stolon production of *D. ovalifolium*, but as noted above there was a grass species effect. Other legumes included in the experiment have drastically reduced their contribution to the total biomass. In terms of population dynamics the original population of *A. pintoi*, *C. acutifolium* and *S. capitata* has reduced greatly in both grasses. The succeeding generation of plants of *A. pintoi* and *S. capitata* have also decreased in both grasses, but more in *B. dictyoneura* than *B. decumbens*. It was interesting to note also a difference between grasses in the extent of legume invasion. In *B. decumbens*, *A. pintoi* invaded more than *D. ovalifolium* and there was a little invasion of *C. acutifolium*. In contrast, in *B. dictyoneura* there was greater invasion of *D. ovalifolium* than of the other two legumes. The advance of the grass in the rows planted with legumes has been greater with *B. dictyoneura* than *B. decumbens*. In addition, there was much greater proportion of grass into *C. acutifolium* than *A. pintoi* and little in *S. capitata* and *D. ovalifolium* **[C. Plazas, L. H. Franco and P. C. Kerridge]**.

Activity : Effects of competition of grass and legume components on nutrient acquisition and production

If plant components are grown together in an agricultural system they should possess complementary light, spatial, water and nutrient requirements, or use these resources at different times in order to maintain stable and productive associations. An appreciation of plant attributes of individual component, that are beneficial to associated components(s) in nutrient acquisition from low fertility soils, is of paramount importance in designing compatible system for enhanced productivity and resource use efficiency in an agropastoral system. In highly weathered acid soils in the tropics, low P supply is a major limitation to growth and productivity of crop and forage components. Previous research indicated that the grass (*Brachiaria dictyoneura*) and the legume (*Arachis pintoi*) vary significantly in their responsiveness to P fertilizer application in acid soils. The increased response of the grass to P application was associated with higher P use efficiency (g of forage produced per g of total P uptake). However, P uptake efficiency per unit root length was several times greater in the legume than in the grass. This greater P uptake efficiency of the legume was associated with its greater ability to mobilize less soluble forms of P and from organic P sources. The acquisition of P per plant by the grass was greater when grown in an association with the legume than when grown in monoculture.

A glasshouse study was conducted to determine to what extent the acquisition of P by grass roots is enhanced when they are allowed to intermix with the roots of the legume. A grass (*Brachiaria dictyoneura* CIAT 6133) and a legume (*Arachis pintoi* CIAT 17434) were grown in monoculture or in an association to give ten plant communities of contrasting composition, namely: (i) monoculture of grass with 20 plants per container; (ii) monoculture of grass with 10 plants per container; (iii) monoculture of grass with 5 plants per container; (iv) monoculture of legume with 20 plants per container; (v) monoculture of legume with 10 plants per container; (vi) monoculture of legume with 5 plants per container; (vii) balanced association with 10 grass + 10 legume plants per container; (viii) balanced association with 5 grass + 5 legume plants per container; (ix) grass dominant association with 15 grass plants + 5 legume plants per container; and (x) legume dominant association with 15 legume plants + 5 grass plants per container. A clay loam Oxisol from Carimagua was used to grow the plants (40 kg soil/container). A basal nutrient mixture was applied to soil before planting (kg ha^{-1}: 40N, 20K, 33Ca, 14.25Mg, 10S, 2Zn, 2Cu, 0.1Bo and 0.1Mo). P was supplied in the form of partially acidulated rock phosphate (fosfacid-S) at the rate of 50 kg ha^{-1}. The trial consisted of 3 replications of ten plant communities arranged in a randomized complete block design. All containers received mycorrhizal inoculation (*Glomus clarum*), while the legume received inoculation with an effective Rhizobium strain (CIAT 3101). At the time of harvest (84 days of growth), shoot biomass, leaf area, root biomass, root length and shoot P uptake were determined.

Differences in shoot and root growth characteristics and P acquisition between the grass and the legume when grown in monoculture or in association are shown in Table 1. Shoot biomass and leaf area index (LAI) of the grass, in both monoculture and association, increased with the increase in number of plants per container. The increase in shoot biomass and LAI was smaller in the case of the legume with the increase in number of plants. There was clear evidence for grass dominance in terms of shoot growth when grown in balanced association of

5 + 5 or 10 + 10 plants per container. Legume dominance in shoot yield and LAI was observed only with a 15 + 5 composition of legume + grass in the association. When there were only 5 plants per container as monoculture, legume produced greater amounts of leaf area than the grass. In contrast to LAI, root biomass and root growth were several times greater with the grass than those of the legume. Despite smaller root length, the legume acquired markedly greater amounts of P than the grass from rock phosphate, when grown either as monoculture or in association. The greater acquisition of P by the legume was due to higher P uptake rates per unit root length mainly through mobilization of P especially in the rhizosphere. Results from this study demonstrate the superior ability of the grass to acquire P when associated with the legume at 5 + 5 community composition (174 mg/m^2) than grown as 5 plants as monoculture (154 mg/m^2). Results from root length measurements indicate that an increased P uptake rate per unit root length could be responsible for better acquisition of P by the grass in association, rather than the increase in total root length. It appears that *A. pinto*i was able to mobilize P in the rhizosphere from rock phosphate source, in excess of its own requirements. In addition, these results demonstrate that the presence of *A. pintoi* in association with *B. dictyoneura*, can not only improve animal production via biological nitrogen fixation but also enhance the ability of the grass to acquire P from rock phosphate sources **[I. M. Rao, J. Ricaurte, R. Garcia and P. Kerridge]**.

Table 1. Differences in phosphorus acquisition between a grass (*B. dictyoneura*) and a legume (*A. pintoi*) when grown as monoculture versus association in contrasting composition of communities in a clay loam Oxisol (40 kg soil/container) from Carimagua, Colombia.

Community composition	Number of plants per container	Shoot biomass ($g\ m^{-2}$)	Leaf area index ($m^2\ m^{-2}$)	Root biomass ($g\ m^{-2}$)	Root length ($km\ m^{-2}$)	Shoot P uptake ($mg\ m^{-2}$)
Grass alone	5	388	2.18	134	11.3	154
Legume alone	5	242	3.08	52	2.0	484
Grass alone	10	578	3.74	210	15.6	243
Legume alone	10	288	3.52	65	2.4	555
Grass + Legume	5 + 5	466 (313)*	3.79 (1.92)	139	9.5	382 (174)
Grass alone	20	631	4.26	257	16.9	328
Legume alone	20	310	3.82	85	2.2	553
Grass + Legume	10 + 10	476 (299)	4.05 (1.75)	170	10.3	419 (181)
Grass + Legume	15 + 5	511 (409)	3.77 (2.46)	216	12.6	324 (208)
Grass + Legume	5 + 15	412 (136)	4.14 (0.77)	134	8.0	523 (94)
$LSD_{0.05}$		87	0.81	35	4.1	104

* Numbers in parenthesis indicate the contribution of the grass to the association

Activity : Differences in rooting pattern among native and introduced pastures

The determination 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 C sequestration in soils. The differences in root biomass and root length 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 C sequestration in soil. This study was part of a larger field experiment with a goal to model the pastures to forecast the effect of management options on long-term persistence and nutrient cycling in grazed legume-grass associations in the Latin American savannas. The specific objectives were: (i) to compare root distribution and production of introduced (grass alone and grass + legume) pastures with native savanna pastures growing on an Oxisol under grazing; and (ii) to determine the role of roots for their potential contribution to nutrient cycling in pastures and carbon sequestration in soil. A sequential root coring approach was used to assess rooting dynamics in different pasture treatments.

Description of pasture treatments: In the Llanos of Colombia, at Carimagua research station, distribution of root biomass and root length was determined during the growing season for four years (10, 15, 19, 22, 27, 32, 35,39 and 47 months after sowing), in grazed pastures compared with native savanna, in a clay loam Oxisol site. *Brachiaria dictyoneura* CIAT 6133 was sown in August 1990 alone or with *Centrosema acutifolium* CIAT 5277. Pasture treatments studied were:

B. dictyoneura alone (Bd)	-	low initial fertilizer (L)
Bd + *C. acutifolium* (Bd + Ca)	-	low initial fertilizer
Bd + *C. acutifolium* (Bd + Ca)	-	high initial fertilizer (H)
Native savanna (NS)	-	no fertilizer (N)

Low initial fertilizer application was (kg ha^{-1}): 20 P, 20 K, 50 Ca, 20 Mg, 12 S, 2 Zn, 2 Cu, 0.5 Bo and 0.1 Mo. High initial fertilizer application was (kg ha^{-1}): 60 P, 60 K, 150 Ca, 60 Mg, 24 S, 2 Zn, 2 Cu, 0.5 Bo and 0.1 Mo. The treatments form an incomplete factorial with 2 replicates. Within each replicate, treatments were divided into 4 paddocks, randomized by pairs, and each paddock is grazed from June 1991 (10 months after planting) by 3 steers in a rotation of 7 days' occupation, 21 days' rest. The stocking rates differed between treatments (0.2 head ha^{-1} for native savanna; 2.0 head ha^{-1} for introduced Bd or Bd + Ca pastures).

Dynamics of root biomass and root length distribution: Changes in standing live root biomass (kg/ha) and root length (km/m^2) with pasture age of introduced grass (Bd) alone and grass + legume (Bd + Ca) pastures with low (L) or high (H) initial fertilizer application compared to native savanna (NS) with traditional no fertilizer application (N) are quantified. Native savanna pasture had significantly lower levels of root biomass compared to introduced pastures ($P < 0.05$). Changes in total root biomass (0-80 cm depth) ranged between 781 to 2664, 2817 to 9379, 2739 to 5770, and 2000 to 5598 kg ha^{-1} for native savanna, Bd alone, Bd + Ca (L) and Bd + Ca (H) pastures, respectively. Compared with introduced pastures, native savanna pasture had also relatively lower root length. Changes in total root length (0-80 cm soil depth) ranged between 3.98 to 21.52, 7.06 to 39.42, 7.38 to 22.85, and 5.92 to 23.42 km m^{-2} for NS, Bd (L), Bd + Ca (L) and Bd + Ca (H) pastures, respectively.

Mean root biomass and root length distribution: Mean root biomass distribution (0-80 cm soil depth) between 10 and 47 months after sowing of improved pastures showed that the introduced pastures had more root biomass at depth than the native savanna pasture (Figure 1a-d). Introduction of the legume (Ca) to the improved grass markedly reduced the amount of standing live root biomass. The upper 20 cm soil profile contained 64, 73, 73 and 70 % of total root biomass of NS, Bd (L), Bd + Ca (L) and Bd + Ca (H) pastures, respectively. Root biomass decreased with increasing soil depth for all pasture treatments. Native savanna had relatively fewer roots at lower depths, particularly between 20 and 80 cm compared to introduced pastures. Mean values of total root biomass (0-80 cm soil depth) were 1.4, 5.65, 3,81 and 4.06 Mg ha^{-1} for NS, Bd (L), Bd + Ca (L) and Bd + Ca (H) pastures, respectively. Mean root length distribution by soil depth between 10 and 47 months after sowing of introduced pastures showed a similar trend to that observed for root biomass (Figure 1e-h). As much as 62, 75, 75 and 72 % of total root length was found in the upper 20 cm soil layer of NS, Bd (L), Bd + Ca (L) and Bd + Ca (H) pastures, respectively. Mean values of total root length (0-80 cm soil depth) were 9.5, 21.5, 13.8 and 15.5 km m^{-2} for NS, Bd (L), Bd + Ca (L) and Bd + Ca (H) pastures, respectively. A two-fold difference was observed between native savanna and introduced pastures in mean values of specific root length (SRL) (Figure 1i-l). This root attribute is a measure of the fineness of the root system. There was a tendency for an increase in fineness of the root system of NS pasture in subsoil layers. High SRL values of native savanna pasture maximize root surface area for nutrient uptake and ability to explore soil for available nutrients.

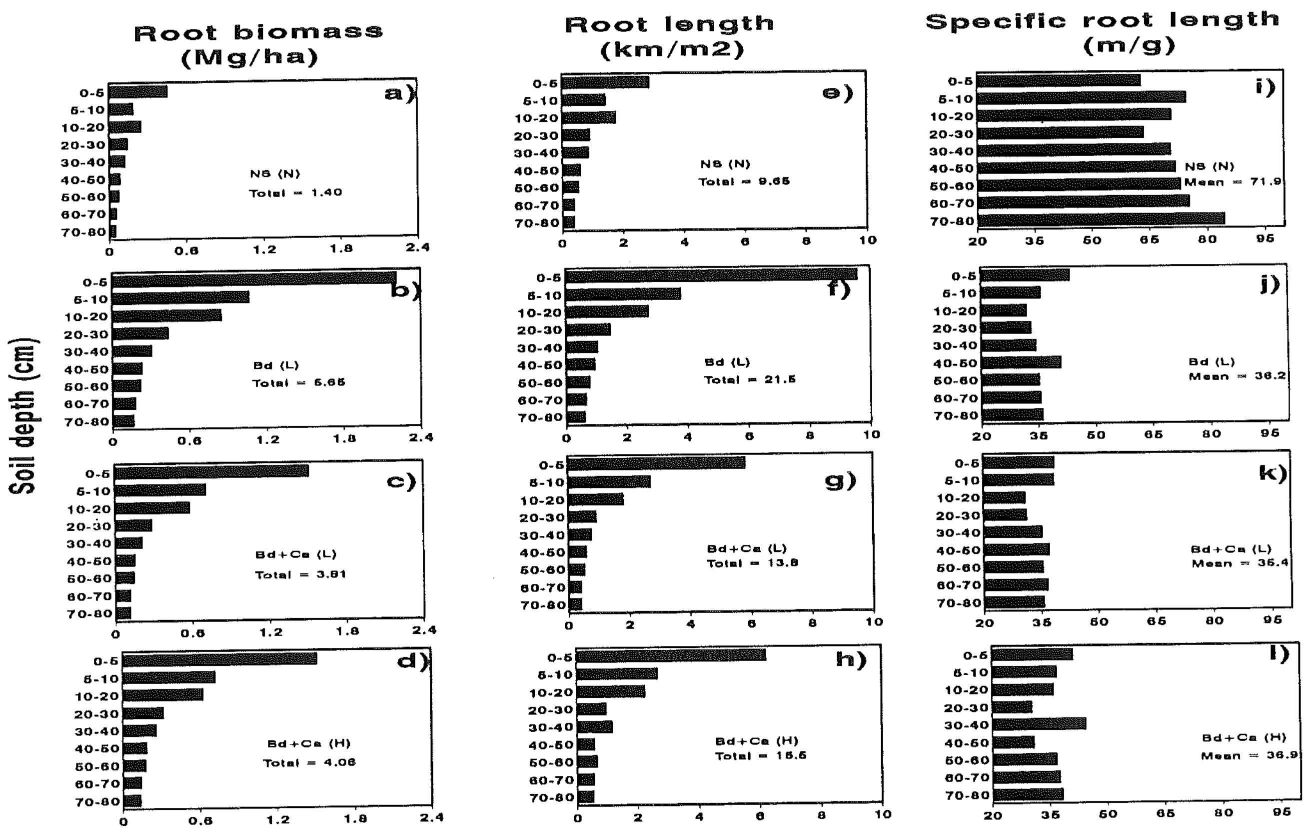


Figure 1. Differences in mean root biomass, root length and specific root length distribution by soil depth among native savanna and introduced pastures. Values are means from 10 and 47 months after sowing. Bd= *B. dictyoneura*; Ca= *C. acutifolium*; NS= native savanna; L= low initial fertilizer; H= High initial fertilizer; N= no fertilizer application.

Estimation of differences in rooting. Differences in rooting among introduced and native pastures were estimated using a model of vertical root distribution which is based on the following asymptotic equation: $Y = 1 - ß^d$, where, Y is the cumulative root biomass or root length fraction (a proportion between 0 and 1) from the soil surface to depth d (cm), and ß is the fitted "extinction coefficient". ß is the only perameter estimated in the model and provides a simple numerical index of root biomass or root length distribution, where high ß values (e.g., 0.98) correspond to a greater proportion of root biomass or root length at depth and low ß values (e.g., 0.91) imply a greater proportion of root biomass or root length near the soil surface. ß values were fitted to the data for each pasture.

To estimate differences in rooting between native savanna grasses and introduced grasses, we calculated cumulative root biomass and root length (fraction) for each sampling time for each soil layer. The estimated mean cumulative root biomass and root length fractions for introduced pastures compared to NS are shown in Figure 2. The values of ß for root biomass and root length indicate that NS and introduced pastures are both deep rooted. However, based on proportional root length distribution at depth, native savanna pastures were relatively more deep rooted. Higher values of ß, particularly for root length distribution, in NS compared to introduced pastures correspond to a greater proportion of roots with depth. Higher values of ß and greater SRL (30-80 cm soil depth) in native savanna pastures with no fertilizer application may contribute to an adaptive/survival mechanism to scavenge very low amounts of mineralized nutrients (e.g., N) leached down the soil profile in addition to tolerance to dry season. This observation indicates that understanding strategies of native vegetation may contribute to development of superior forage/crop germplasm to infertile soil conditions. There is a need to explore genotypic differences among *Brachiaria* germplasm accessions and genetic recombinants for this adaptive attribute in order to mitigate pasture degradation on infertile acid soils of the south American savannas.

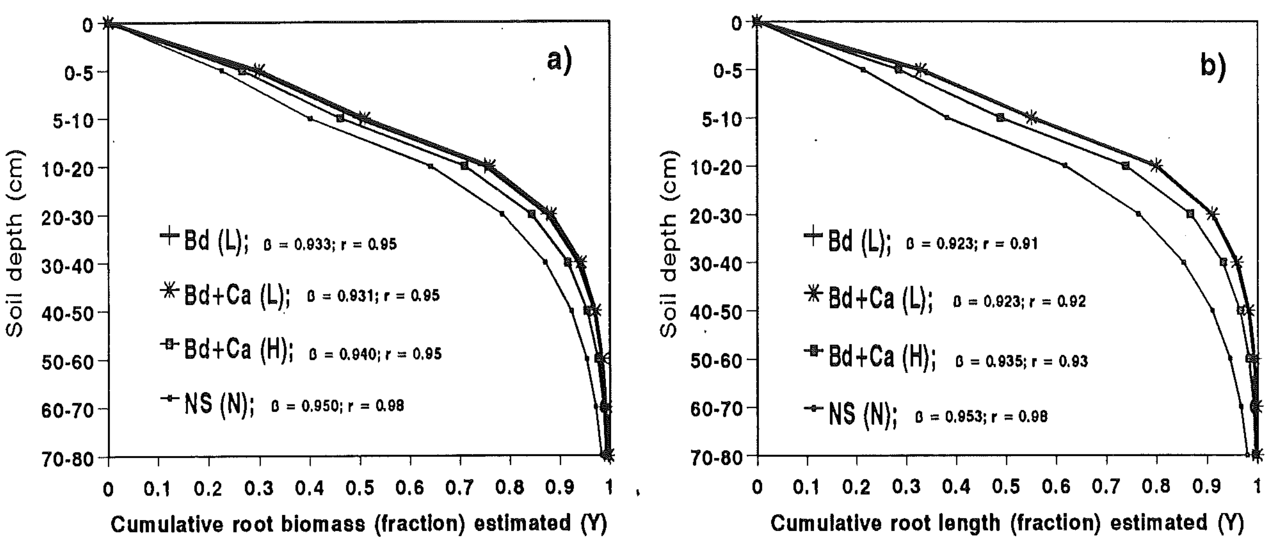


Figure 2. Vertical root biomass (a) and root length (b) distributions (cumulative proportion) for native savanna and introduced pastures . The equation is of the form $Y=1-ß^d$, where Y is the cumulative root biomass or root length fraction with depth (a proportion between 0 and 1), d is soil depth (in cm), and ß is the fitted parameter. Values are means from 10 and 47 months after sowing. Bd= *B. dictyoneura*; Ca= *C. acutifolium*; NS= native savanna; L= low initial fertilizer; H= High initial fertilizer; N= no fertilizer application.

Legume root proportion in grass-legume association: Legume root proportion in grass + legume associations was determined by using the stable carbon isotope technique. This was possible because the grass roots exhibited $\delta\ ^{13}C$ values typical of C4 species and the legume had $\delta\ ^{13}C$ values typical of C3 species. Use of this technique allowed to estimate legume root proportion by soil depth in 22 month-old Bd + Ca (L) and Bd + Ca (H) pastures. Legume root proportion was less than 10 % of the total root biomass in both legume based pastures. It was greater (11 to 21%) in deeper soil layers (60 to 80 cm) compared to 4 to 11% in top soil layers. A major consequence of the variation in size of the root system probably is the effect on competitive ability: the larger the root biomass of a plant species compared with other plant species when grown in association, the greater its competitive ability.

Differences in nutrient composition of roots: Nutrient composition of pooled root samples (0-30; 30-50; 50-80 cm) was determined. Significant differences were found in mean values of nutrient composition among native savanna grasses and introduced grasses (Table 2). Total N and C content of native savanna roots were greater than those of grass alone pasture. The ratio of C:P in native savanna roots was markedly greater than that of introduced pastures. High initial fertilizer application to introduced legume-based pasture significantly decreased total C and increased N status ($P<0.05$), thereby reducing C:N ratios of roots

Table 2. Mean nutrient composition of roots (%) from native savanna (NS), grass alone (Bd) and grass + legume (Bd + Ca) associations established with no fertilizer (N), low initial fertilizer (L) and high initial fertilizer (H) application at the time of establishment in a clay loam Oxisol at Carimagua. LSD values are at the 0.05 probability level.

Pastures	Root nutrient composition (%)							C:N	C:P
	C	N	P	K	Ca	Mg	S		
NS (N)	56.5	0.304	0.014	0.053	0.081	0.032	0.084	185	4036
Bd (L)	43.3	0.203	0.021	0.151	0.071	0.041	0.079	213	2062
Bd + Ca (L)	37.9	0.298	0.020	0.114	0.075	0.039	0.092	127	1898
Bd + Ca (H)	36.8	0.313	0.022	0.146	0.097	0.052	0.091	118	1673
$LSD_{0.05}$	3.4	0.046	0.005	0.035	0.017	0.004	NS		

Bd = *B. dictyoneura*; Ca = *C. acutifolium*

Changes in pool size of nutrients in roots: 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 introduced pastures was up to 18 kg ha^{-1} while in native savanna it was little over 6 kg ha^{-1}. The extent of P pool size in roots of introduced pastures was also up to five times greater than that of native savanna. Similar trends were observed with pool sizes of other nutrients, K, Ca, Mg and S, in roots (results not shown).

Carbon sequestration in soil: Differences in distribution of soil organic carbon (SOC) with depth (0-80 cm) at 32 months after sowing amongst introduced pastures compared to native savanna are shown in Figure 3. Greatest amounts of SOC were measured in the Bd (L) pasture and the increase in SOC levels in introduced pastures compared to native savanna pasture were to some extent reflected the differences in standing root biomass over time. The values of SOC (%) in Bd (L) pasture in subsoil layers (20-80 cm depth) ranged from 0.8 to 1.6 compared to 0.69 to 1.19 of the NS pasture. The values of SOC from Bd +Ca (L) and Bd + Ca (H) pastures were in between the Bd (L) and NS pastures. The total amount of additional SOC from 0 to 80 cm depth in Bd (L) compared with NS pasture was estimated (using bulk density values for each soil layer) to be 31.6 Mg ha^{-1}. Between 19 and 32 months of development of Bd (L) pastures, very high root turnover was observed. A greater proportion of SOC accumulation in Bd (L) pasture may be attributed to this high root turnover. These results confirm previous findings on carbon sequestration potential of introduced pastures in the Llanos of Colombia. In contrast to the Bd (L), Bd + Ca pastures explored less volume of soil and also showed less SOC accumulation in soil. This observation is in contrast to greater SOC accumulation observed with long-term, grass + legume (*Brachiaria humidicola* + *Arachis pintoi*) pastures than grass alone pastures when maintenance fertilizer was applied to both types of pastures (TFP Biennial Report, 1994-1995).

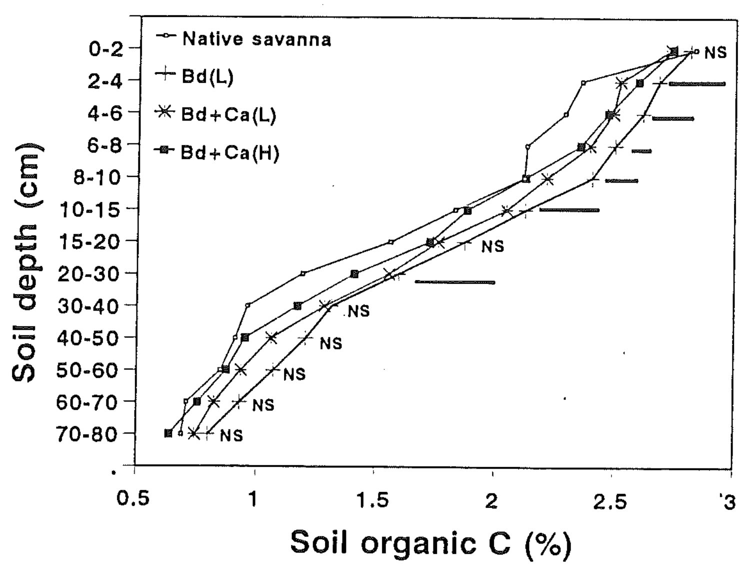


Figure 3. Differences in soil organic carbon (SOC) distribution by soil depth among native savanna and introduced pastures. Bd= *B. dictyoneura*; Ca= *C. acutifolium*; NS= native savanna; L= low initial fertilizer; H= High initial fertilizer; N= no fertilizer application (see text for rates); LSD values are at the 0.05 probability level.

Above-ground biomass production:. The values for standing above-ground biomass of NS pastures ranged from 1.71 (dry season) to 4.83 (middle of wet season) Mg ha^{-1} (G. Rippstein, unpublished data). The monthly net production of above-ground biomass of Bd (L) pastures ranged from 0.11 (dry season) to 0.80 (middle of wet season) Mg ha^{-1}. In the case of Bd + Ca (L) pasture the values for the dry season were similar to Bd (L) pasture but in the middle of wet season the values were lower (0.56 Mg ha^{-1} of which about 20% was from the legume until October 1992). With Bd + Ca (H) pasture, monthly net production of above-ground biomass of the legume improved markedly compared with Bd (L) pasture (M. Fisher, unpublished data). Mean above-ground litter production of the introduced pastures during wet season was about 40 g m^{-2} month^{-1}. The legume persisted in Bd +Ca (L) or Bd + Ca (H) pastures for about 3 years.

In summary, the results from this study indicate that introduced pastures produce greater amounts of standing live roots at depth compared to native savanna pastures. However, estimation of differences in rooting, based on proportional root length distribution at depth, indicated that native savanna pastures were more deep rooted than the introduced pastures. With their abundant root system of introduced pastures at depth, they have the potential to not only recycle nutrients from deep soil layers but also sequester greater amounts of carbon below the plough layer. **[I. M. Rao, M. A. Ayarza, P. Herrera, J. C. Granobles, C. Plazas and J. Ricaurte].**

Activity : Influence of grazing management on root production and distribution

In addition to affecting animal production and forage yield, grazing management could play a major role in cycling of nutrients in pastures via production and turnover of roots. Most studies show that any defoliation (clipping or grazing) reduces root growth. In general, plants respond to grazing by reducing root numbers, branching, root diameter and depth of soil penetration. Reduced root growth might reduce the ability of a defoliated plant to regrow since both nutrient and water uptake will be reduced. Differences between species in amount or duration of root growth inhibition might thus be an important mechanism by which grazing affects the competitive balance in grass + legume associations. Alternatively, root growth reductions might be a mechanism to reduce belowground carbon demand in defoliated plants and would thus allow greater allocation of carbon to the shoot resulting in more rapid establishment of the canopy and return to root:shoot equilibrium. In contrast to most studies, some researchers observed either no effect or stimulation of root growth in grazed pastures.

A field study was conducted to determine the influence of stocking rate on total root biomass and root length in 10 year-old *B. humidicola* alone and *B. humidicola* + *A. pintoi* (6 year-old) association in a clay loam Oxisol at Carimagua, Colombia. Both pastures were grazed at three stocking rates (2, 3 and 4 A ha^{-1}) using a flexible alternate system. The availability of *A. pintoi* on-offer in the rainy season increased over time (500 to 650 kg ha^{-1} after 4 years of grazing). Increasing in stocking rate had no effect on root biomass but markedly improved root length of *B. humidicola* alone pasture (Table 3). In contrast to grass alone pasture, increases in stocking rate markedly improved root biomass and root length of the *B. humidicola* + *A. pintoi* association. Increases in stocking rate improved fine root production, as revealed by the greater values of specific root length, of both pastures.

Table 3. Influence of stocking rate on total root biomass and root length in 10-year-old *B. humidicola* alone and *B. humidicola* + *A. pintoi* (6-year-old) association in a clay loam Oxisol at Carimagua, Colombia.

Pasture	Stocking rate (A ha^{-1})	Root biomass (Mg ha^{-1})	Root length (km m^{-2})	Specific root length (m g^{-1})
Bh	2	1.22	5.88	54.1
	3	1.65	9.73	58.5
	4	1.68	10.56	77.1
Bh + Ap	2	0.71	3.25	53.1
	3	0.91	4.19	71.7
	4	1.27	8.58	67.9

Another field study was conducted to determine the influence of grazing system and stocking rate on total root biomass and root length in 8 year-old *B. dictyoneura* alone and *B. dictyoneura* + *A. pintoi* association in a clay loam Oxisol at Carimagua, Colombia. Both pastures were managed with alternate and rotational grazing system at two stocking rates (2 and 3 A ha^{-1}). The availability of *A. pintoi* on-offer in the rainy season was markedly affected by stocking rate (730, 350 and 270 kg ha^{-1} alternate 2 A ha^{-1}, alternate 3 A ha^{-1} and rotational 3 A ha^{-1}, respectively, after 3 years of grazing). Alternate grazing system with 3 A ha^{-1} improved root biomass production over rotational grazing system with 3 A ha^{-1} (Table 4). However, the rotational grazing system markedly improved fine root production of both grass alone and grass + legume association.

Table 4. Influence of grazing system and stocking rate on total root biomass and root length in 8-year-old *B. dictyoneura* alone and *B. dictyoneura* + *A. pintoi* association in a clay loam Oxisol at Carimagua, Colombia.

Pasture	Grazing system	Stocking rate (A ha^{-1})	Root biomass (Mg ha^{-1})	Root length (km m^{-2})	Specific root length (m g^{-1})
Bd	Alternate*	3.0	5.62	10.9	18.3
		2.0	5.07	12.1	25.0
	Rotational**	3.0	3.25	11.2	33.8
Bd + Ap	Alternate	3.0	5.47	10.0	16.9
		2.0	2.67	9.8	37.7
	Rotational	3.0	1.74	7.03	43.5

*2-paddock system: 7/7 dry season; 21/21 wet season
**4-paddock system: 7/21 dry and wet seasons

Results from the above two field studies indicate that both grazing system and stocking rate can influence root biomass and root length in grass alone and grass + legume associations. It is important to note that long-term pastures of *B. dictyoneura* had markedly greater amount of root biomass than the *B. humidicola* pastures. This was mainly attributed to the coarser root system of *B. dictyoneura*. **[I. M. Rao, P. Herrera, J. C. Granobles, C. Plazas, J. Ricaurte and C.E. Lascano]**.

Output 1.3

Methodology developed for matching feed resources to animal requirements

Main achievements

- Showed that milk urea nitrogen (MUN), a potential useful nutritional indicator, varied more with pasture attributes than with animal breed or climatic factors.

<u>Activity : Matching feed resources to maxime intake and digestibility</u>

Through controlled feeding experiments with sheep we are attempting to derive basic information on matching feed resources to enhance intake and digestibility of low quality grass basal diets.

Feeding experiments with sheep on low quality grass basal diets have been either completed or are in progress at the Quilichao research station to evaluate: (a) Constant sugar cane supplementation in combination with shrub legumes alone (*Cratylia argentea* or *Flemingia macrophylla*) or in mixture and (b) Frequency and level of supplementation of sugar cane in combination with a shrub legume (*C. argentea*). Preliminary results from this work carried-out by a student (W. Quiñonez) with support from COLCIENCIAS indicate no effect of the forage-based supplement treatments on DM intake and digestibility. In fact, we have observed that sheep have substituted the low quality grass by the higher quality forage-based supplements **[W. Quiñonez, P. Avila and C. Lascano]**.

Activity : Nutritional indicators

We are looking into the use of nutritional indicators to assess energy: protein balance in the diet of grazing milking cows. Several researchers have suggested that blood urea nitrogen (BUN) or milk urea nitrogen (MUN) could be useful indicators of energy: protein status of ruminants. The utility of BUN as guide to supplement protein to grazing beef cows was demonstrated in Florida by USDA researchers. Thus, we became interested in separating sources of variation of MUN values recorded from 1992 to 1995 in controlled grazing trials at Quilichao with milking cows. The MUN values recorded varied due to N fertilization of the grass (7 to 29 mg/dL), presence or absence of legume in the pasture (11 to 20 mg/dL), breed (15 to 21 mg/dL) and dry and wet season (13 to 20 mg/dL). However, a greater proportion of the variation in MUN was associated with pasture attributes or dietary factors than with breed or climatic condition as indicated by regression coefficients in Table 5 **[C. Lascano, P. Avila, G. Ramírez and M. C. Amézquita]**.

Table 5. Significant sources of variation associated with milk urea nitrogen (MUN) values in milk from grazing cows (Lascano et al., unpublished results).

Sources of variation	Regression coefficients[1,2]	Significance level
Pasture		
Presence of *P. maximum* + N[3]	18.1	0.0001
Presence of legume	9.8	0.0001
Climate		
Presence of rains	3.6	0.05
Breed		
Presence of Holstein	-2.4	0.05

[1]Steep wise regression analysis using dummy variables (presence or

[2]R^2 of the regression model= 0.50

[3]Fertilized with 100 kg of N

Output 1.4

Legume-based forage components for dual-purpose cattle developed with farmer participation

Main achievements

- Established grass and grass-legume pastures in dual purpose farms in forest margins and subhumid hillsides as part of the NESTLE and Systemwide Livestock-TROPILECHE Projects.

Current activities to achieve the above output include on-farm establishment and monitoring of improved pastures with milking cows. On-farm pasture research is being carried-out in forest margins benchmark sites of Caqueta (NESTLE, special Project) and Pucallpa, Peru (Systemwide Livestock Program-TROPILECHE Project) and in subhumid hillsides of Costa Rica (Systemwide Livestock Program-TROPILECHE Project).

Activity : Pasture establishment and evaluation in dual purpose cattle farms in Caqueta (NESTLE, Special Project)

The NESTLE Project entered into its second year of activities in dual purpose cattle farms in forest margins of Caqueta, Colombia. During 1995 and 1996 we have established 49 ha of grass alone *Brachiaria* spp. pastures and 75 ha of grass/legume pastures (*Brachiaria* spp./*Arachis pintoi*) in 11 farms. Measurements in these pastures have included botanical composition and milk yield.

Results on botanical composition summarized in Table 6 show that in older pastures there was an increase in the proportion of *A. pintoi* in the pasture and a reduction of native grasses. However, it is interesting to note that the increase in *A. pintoi* in Farm No. 2 was associated with a large decline in *B. decumbens* due to spittlebug attack. Loss of vigor of the grass due to the insect attack favored legume stolon development in open spaces. In the recently established pastures, the contribution of *A. pintoi* has increased in a short period of time due to good legume establishment and heavy grazing imposed soon after establishment. Nevertheless, weeds have been and continue to be a problem in pasture establishment in this very humid region.

We have only been able to monitor milk yield in one of the farms participating in the Nestle Project. Frequent milk recording has been done in Farm No. 1 since October 1995 and results indicate either no increase in milk yield or a maximum increases of 0.6 liters/cow/day due to *A. pintoi*. These rather modest increasesin milk yield due to the presence of the legume in the pasture could be related to low production potential of the cows in the farm and/or to the methodology being used to measure milk production responses. In this farm cows rotate from the grass alone pasture to the grass/legume pasture and have limited adjustment periods in both pasture. Consequently we could be underestimating the effect of legume on milk yield as suggested by our analysis on effects of adjustment and sequence of rotation on pasture evaluation with milking cows as discussed under Output 1.4 of this report **[G. A. Ruiz, J. Velásquez, J. Rozo and C. Lascano]**.

Table 6. Botanical composition in grass/legume pastures sown in commercial dual purpose cattle farms in Caquetá, Colombia.

Farms	Pasture age (months)	Initial botanical composition (%)				Last botanical composition (%)			
		Brachiaria	*Arachis*	Native grass	Weeds	*Brachiaria*	*Arachis*	Native grass	Weeds
A[1]	17	42	11	43	4	40	41	15	4
B[2]	18	65	8	24	3	29	35	28	8
C[3]	10	26	10	43	21	31	15	38	16
D[4]	7	25	10	7	58	30	16	20	34
E[5]	4	29	20	41	10	35	40	17	8

[1]Pasture sown in April 1995; initial sampling-October 1995; last sampling-September 1996.

[2]Pasture sown in April 1996; initial sampling-November 1995; last sampling-october 1996.

[3]Pasture sown in October 1996; initial sampling-May 1996; last sampling-September 1996.

[4]Pasture sown in May 1996; initial sampling-April 1996; last sampling-September 1996.

[5]Pasture sown in March 1996; initial sampling-July 1996; last sampling-October 1996.

Activity : Pasture establishment in dual purpose cattle farms in Costa Rica and Peru
(Systemwide Livestock Program-TROPILECHE)

The Project entitled "Improved legume-based feeding systems for smallholders dual-purpose cattle production in tropical Latin America" (short name: TROPILECHE) which operates under de SWLP convened by ILRI began on-farm research activities in 1995 at benchmark sites in forest margins and subhumid hillsides of Peru and Costa Rica, respectively. The TFP and participating institutions in Costa Rica (MAG) and Peru (INIA) have committed resources to initiate this work in selected smallholder dual purpose farms. A major activity during 1996 was the establishment of grass and grass-legume pastures to measure milk production and calve performance. In forest margins of Pucallpa, Peru, grass (*Brachiaria* spp.) and grass in association with a legume mixture (*Arachis pintoi* and *Stylosanthes guianensis*) were sown in 4 farms (4 to 5 ha/farm) after different land uses (native grass, degraded *Brachiaria* pasture and secondary forest). Plans are to establish grass and grass/legume pastures in 3 more farms late this year. In addition, small areas of improved grass legume pastures have been sown in two farms for early weaning of calves. In general, the establishment in Pucallpa of improved pastures has been successful in spite of heavy weed infestation in most farms. Early grazing has been a key to favor *A. pintoi* development by trampling weeds and by minimizing competition from the sown grass **[K. Reátegui, J. Vela and C. Lascano]**.

Pastures were also established in smallholder dual purpose cattle farms in the central pacific region of Costa Rica . After an initial survey, 7 farms were selected by MAG in the districts of Miramar, Orolina, San Mateo, and Esparza. In three of the farms, 2 ha plots of *B. brizantha* cv. La Libertad and of *B. brizantha* in association with *A. pintoi* (18744) and *Centrosema brasilianum* (5234) were successfully established. However, in two other farms there were problems in establishing *B. dictyoneura* due to poor seed germination and ants. Thus, replanting of grass and introduction of a legume cocktail will be done next year.

Approximately 0.5 ha plots of *S. guianensis* cv. Pucallpa have been successfully established in 2 farms for early weaning of calves **[C. E. Hidalgo, M. V. Lobo, and P. Argel]**.

The TROPILECHE Project is being used by CIAT as a platform for other countries in the region interested in developing improved feed resources for smallholder dual purpose cattle systems. In Nicaragua the Proyecto de Desarrollo Lechero, financed by the World Food Program, recently joined Tropileche. The objective is to introduce and evaluate *A. pintoi* and *S. guianensis* in association with grasses (*B. dictyoneura, B. brizantha, A. gayanus*) in farms (Muy Muy and Esquipulas regions) using milking cows and calves. Planting is underway in 4 farms and establishment of *B. brizantha, A. gayanus* and *S. guianensis* has been successful. Plans are to establish *A. pintoi* and *C. argentea* at the beginning of the next rainy season **[T. Fariñas, R. Urbina, F. Holmann and P. J. Argel]**.

Output 1.5

Forage-based systems for dry season supplementation developed with farmer participation

Main achievements

- Showed a large increment in milk yield by the inclusion of *Cratylia argentea* in a forage-based supplement fed to cows grazing pastures with limited forage availability.

- Established *Cratylia argentea* fodder banks in dual purpose cattle farms in subhumid hillsides of Costa Rica collaborating with Tropileche.

<u>Activity : Supplementation of grazing cows with forage-based supplements</u>

In the seasonally dry hillsides a major constraint for increasing milk production is the lack of suitable forages from pastures during the dry season. The introduction of leguminous fodder shrubs for use as supplementation in the dry season will assist in overcoming the feed shortage and reduce grazing pressure in steep slopes.

Previous studies carried-out at Quilichao had shown that by supplementing leaves of *C. argentea* to grazing cows we could expect a 10-13% increase in milk yield in the dry season. In subsequent studies we have supplemented cows with *C. argentea* in combination with king grass and sugarcane, which are commonly used by farmers in subhumid areas as dry season supplements. The rationale behind this strategy has been that by combining king grass or sugarcane (energy source) and *C. argentea* (protein source) we could have a more balanced forage-based supplement. We have tested this hypothesis this year by supplementing (king grass) alone or in combination with *C. argentea* fodder to cows grazing *B. decumbens* pastures with contrasting forage availability. Results summarized in Table 7 show a strong interaction in milk production between forage availability in the pasture (i.e. stocking rate) and type of supplement (king grass or king grass + *Cratylia*). When forage availability was not limiting in the pasture, there was noe response in milk yield to the inclusion of *C. argentea* in the supplement. In contrast, inclusion of *C. argentea* in the supplement when forage on offer was limiting in the pasture, resulted in a 25% increase in milk yield. As expected, voluntary intake of the forage-based supplements was influenced by stocking rate and to a lesser extent by type of supplement fed **[P. Avila and C. Lascano].**

Table 7. Milk production of cows grazing *B. decumbens* pastures with contrasting stocking rates and supplemented with king grass and *C. argentea* fodder (P. Avila, unpublished results).

Stocking rate (AU/ha)	Type of supplement offered	Supplement consumed (kg DM/day)	Milk yield (l/cow/d)
2	King grass[1]	2.4 b	8.8 a
	King grass[2]+*Cratylia*	3.5 ab	7.9 ab
4	King grass[1]	4.3 ab	6.9 b
	King grass[2]+*Cratylia*	4.8 a	8.6 a

a,b Means different ($P<0.05$)

[1]Level of offer: 1.5% (DM basis) of BW

[2]Level of offer: 1.0% king grass (DM basis) + 0.5% (DM basis) of BW

Activity : Establishment of shrub legume fodder banks in dual purpose farms (NESTLE and TROPILECHE Projects)

NESTLE Project: Results from evaluation of different shrub legumes species in forest margins in Caqueta, Colombia showed that *Codariocalyx gyroides* was well adapted to poorly drained soils. As an activity in the Nestle Project we have established fodder banks of *C. gyroides* in two farms that supplement milking cows with sugarcane. To derive some cutting management practices useful to farmers, we are presently evaluating the effect of cutting frequency and height on yield. Results so far indicate that greatest yields per plant are obtained by cutting at 1 m height every 9 weeks **[G. A. Ruiz, J. Velásquez and C. Lascano]**.

TROPILECHE Project: Results from small plot evaluation of shrub legume species in ECAG-Atenas, Costa Rica (6 month dry season) had conclusively shown that *C. argentea* was an alternative for dry season supplementation. Thus, we have introduced *C. argentea* in three of the farms participating in the Tropileche Project in Costa Rica. The initial establishment of *C. argentea* fodder banks failed due to poor quality seed. However, replanting was successful and we expect to evaluate the effect of supplementing *C. argentea* in combination with sugarcane on milk yield in the next dry season **[C.E. Hidalgo, M.V. Lobo and P.J. Argel]**.

Output 1.6

Models on livestock systems applied to smallholder dual purpose cattle systems

Main achievements

- Showed through a simulation model that legume-based feed technologies could be economically attractive to smallholder dual purpose cattle farmers in subhumid areas of Costa Rica, provided labor costs were kept down or the genetic potential of cows to produce milk was improved.

Activity : Application of a simulation model to dual purpose cattle farms in Costa Rica

One of the aims of the Tropileche Project is to apply optimization models to assess the potential biological and economical impact of new forage species in smallholder dual cattle production systems.

During 1996 a simulation model developed by CATIE and RISPAL was used to estimate the marginal profitability of legume-based feeding systems as compared to traditional grass alone pastures used by dual purpose cattle farmers in the central pacific region of Costa Rica, where Tropileche operates. Results showed that marginal economical profitability of feeding systems based on legumes was dependent on labor cost, price of animal products (beef and milk) and livestock productivity. The use of *C. argentea* as a dry season cut and carry fodder improved profitability by 47% over the grass alone used by farmers when the price of milk was US$0.30/liter. However, with a lower milk price (US$0.20/liter) use of *C. argentea* was not profitable, since with this technology labor cost is increased by 233%.

In dual purpose cattle farms with cows producing 1000 liters of milk/lactation, the use of *Arachis*-based pastures in the wet season supplemented with *C. argentea* fodder banks in the dry season resulted in 45% increment in marginal returns as compared with the traditional grass pastures, when labor cost was US$10/day. Increases in labor cost above US$13/day made the traditional pasture the best economical option with the 1000 liter/lactation cows. On the other hand, the use of *Arachis*-based pastures and *C. argentea* was more profitable than the traditional pasture at all labor costs analyzed (US$10 to 18/day) with cows producing 1500 liters/lactation (Figure 4).

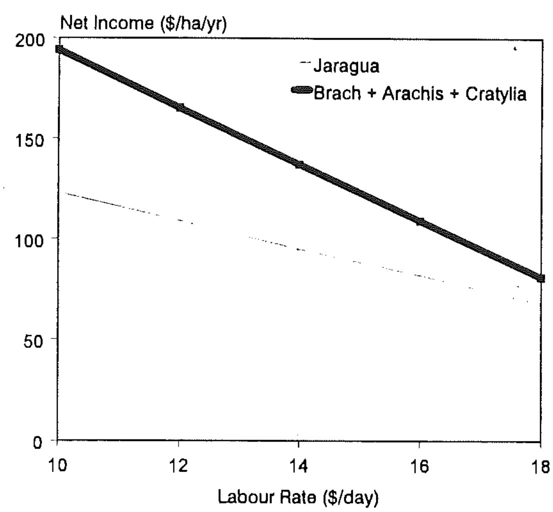


Figure 4. Net income based on different labor rates in dual purpose systems producing 1,500 kg milk/L.

This ex-ante analysis suggests that legume-based feeding systems could be economically attractive to smallholder dual purpose cattle farms in hillsides of Central America if labor costs are kept down or if cow production potential is increased **[F. Holmann and R. D. Estrada]**.

Subproject 2.0

Development of grass and legume components for multipurpose use in intensive agriculture systems

Output 2.1

Grasses and legumes identified for rehabilitation of degraded lands

Main achievements

- Initiated on-farm activities in Southeast Asia to identify suitable legumes to suppress *Imperata cylindrica.*
- Initiated on-farm activities in forest margins of Caqueta, Colombia to study root growth and distribution in degraded native and improved grass alone and legume-based pastures.

In many regions of the tropics there are large areas of degraded land in most cases associated with extensive grazing. Grasses and legumes can contribute to reclamation of degraded lands through efficient nutrient cycling, deep root systems and by displacing unproductive vegetation. We are currently evaluating the role of grasses and legumes for reclaiming degraded land in two regions: (1) Southeast Asia through the Forages for Smallholders Project financed by AUSAID and carried out by CIAT and CSIRO, and (2) Forest margins of Caqueta, Colombia through the NESTLE Project.

<u>Activity : Grasses and legumes to suppress *Imperata* in Southeast Asia</u>

In Southeast Asia there are large areas of *Imperata cylindrica* grasslands and a cultivation of larger areas under *Imperata* is beyond the means of most smallholders farmers because of the enormous effort required to control regrowth of the grass. Likewise, animal production of cattle or goats grazing *I. cylindrica* is poor. Thus rehabilitation and transformation of *Imperata* grasslands into cropping and forage areas was identified by farmers as the most important issue to be solved in a participatory research site in Sepaku, East Kalimonton. Following participatory diagnosis and planning, farmers have now established a range of forages to suppress *Imperata* using minimum cultivation. Pioneer legumes being tested by farmers include: *Stylosanthes guianensis* (184), *Centrosema acutifolium* (5277), *C. macrocarpum* (25522) snf *C. pubescens* (15160) **[Provincial Livestock Service, East Kalimanton, Indonesia, and W. Stur]**.

Activity : On-farm measurements of rooting pattern in native and introduced pastures

In the Amazonian piedmont of Caqueta, Colombia, a field study is in progress in collaboration with CORPOICA-Macagual and the University of Amazonia through pre-graduate students. The objective of this investigation is to determine root growth and distribution and to test relationships among root growth, shoot growth and nutrient acquisition in native and introduced grass only and legume-based pastures. Overgrazing, subsoil compaction and low nutrient supply are three major factors leading to degradation of native and introduced pastures in the Caqueta region. Widespread adoption and utilization of introduced legume-based pastures in different production systems in this region will depend to some extent on attributes that contribute to rapid establishment (rooting pattern) and efficient extraction and utilization of nutrients for growth. Pasture treatments selected for the study included: degraded native pasture, introduced grass pasture (*Brachiaria decumbens/B. humidicola*) and grass + legume association (*B. decumbens/B. humidicola/Arachis pintoi*). A number of plant attributes including leaf biomass, stem biomass, root biomass, root length, arbuscular-mycorrhizal colonization, and nutrient acquisition are being monitored under grazing. **[Y. Conta Diaz, H. J. Baracaldo, G. Ruiz, C. J. Escobar, I. M. Rao and C. E. Lascano].**

Output 2.2

Grasses and legumes identified for crop-pasture rotations

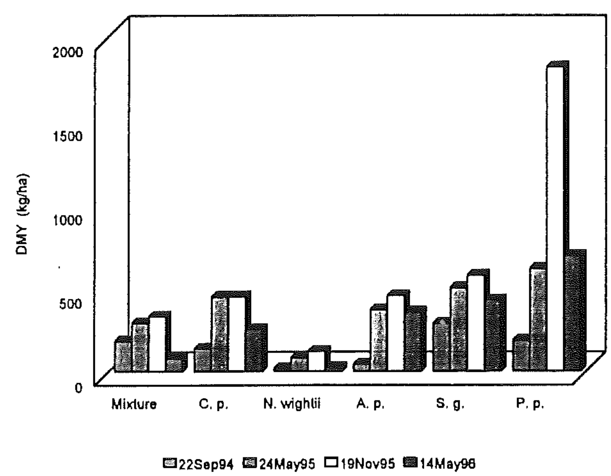
Main achievements

- Identified *Arachis pintoi* CIAT 22160 as a promising ecotype to replace the commercial ecotype (CIAT 17434) in crop-livestock systems for the Lowland Tropics.

Activity : Selection of grasses and legumes for crop-pasture systems in savannas

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 grass and legume species that do not persist well on infertile soils with low fertilizer may persist and be productive on soils heavily fertilized for maize and soybean production. Species that were selected for infertile soils of the Llanos also perform well on more heavily fertilized soils. These are the grasses, *B. dictyoneura* and *B. humidicola*, and the legumes, *Stylosanthes capitata* cv. Capica, *Centrosema acutifolium*, *Desmodium ovalifolium* and *Arachis pintoi*. An experiment was commenced in 1994 and another in 1995 to consider alternative species.

1994 trial (establishment under maize): A common grass, a new accession of *Panicum maximum* CIAT 36000 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, and 2 B. The land had been limed and heavily fertilized the previous year for a maize crop. The legumes were: (i) A mixture of *A. pintoi* cv. mani forrajero, *S. capitata* cv. Capica and *C. acutifolium* cv. Vichada; (ii) *C. pubescens* CIAT 438; (iii) *Neonotonia wightii* cv. cooper; (iv) *A. pintoi* CIAT 22160; (v) Two *Stylosanthes guianensis* hybrid lines and (vi) *Pueraria phaseoloides* CIAT 8042. At the beginning of the wet season in 1955 the legume recovery was very good in all species except *N. wightii* which had established poorly in 1994. The relative persistence and productivity of the legumes was, from good to poor, *A. pintoi* CIAT 22160 = *P. phaseoloides* CIAT 8042 > *C. pubescens* CIAT 438 = the 'cocktail' > *S. guianensis* hybrid lines > *N. wightii* cv. Cooper. The performance of *A. pintoi* CIAT 22160 was considered particularly promising in that, though planted vegetatively with < 3 plants/m^2, it spread and was more vigorous than *A. pintoi* cv. mani forrajero (CIAT 17343) included in the 'mixture'. It covered most of the space between the *P. maximum* tussocks. The performance of *P. phaseoloides* CIAT 8042 was also promising. Observations have been continued through 1996. The yield of the grass remained stable independently of the associated legume. The amount of legume was much lower in this year, associated with more constant grazing and no spelling as was practiced at the end of the first year. Figure 5 shows legume yields over time. *P. phaseoloides* CIAT 8042 continued as the best adapted and persistent legume followed by *S. guianensis* CIAT 11844 and *A. pintoi* CIAT 22160. *N. wightii* cv. Cooper has disappeared from the plots **[C. Plazas, L.H. Franco and P. C. Kerridge]**.



Mixture : *A. pintoi* cv. Maní Forrajero, cv. Capica, *C. acutifolium* cv. Vichada . *C. pubescens* CIAT 438 , *N. wightii* cv. Cooper, *A. pintoi* CIAT 22160, *S. guianensis* CIAT 11844 , *P. phaseoloides* CIAT 8042

Figure 5. Dry matter yield of legumes sown under maize at different sampling times in savannas (Carimagua, Llanos of Colombia).

The *P. maximum* appears to be well adapted but is in general quite yellow signifying a limitation of N. The grass appears greener with *P. phaseoloides* than the other legumes. This trial will be maintained for another year and then cropped again with maize to establish if there are any differences between legume treatments **[C. Plazas, L. H. Franco and P. C. Kerridge]**.

1995 trial (establishment under maize): A similar trial was established using a revised suite of legumes and two grasses, *P. maximum* CIAT 6799 and *B. brizantha* cv. Marandu. It was considered that the Brachiaria would be less competitive against the legumes than the taller and more vigorous *Panicum*. The legumes were: (i) *Stylosanthes guianensis* CIAT 11844; (ii) *Pueraria phaseoloides* CIAT 8042; (iii) *Arachis pintoi* CIAT 22160; (iv) *Centrosema pubescens* CIAT 5634; (v) *Galactia striata* CIAT 8143; and mixtures of (vi) *S. guianensis* CIAT 11844, *A. pintoi* CIAT, *C. pubescens* CIAT 5634; and (vii) *A. pintoi* cv. mani forrajero, *S. capitata* cv. Capica, *C. acutifolium* cv. Vichada.

The initial establishment of grasses and legumes in 1995 was satisfactory. The corn was harvested in September with an average production of 3 t/ha grain. There was no significant difference in grain yield between the two grasses and the pasture was first grazed at the beginning of October 1995 when it was observed that the legume population had been much reduced. Legume establishment was quite variable between species, varying from 1.5 plants/m^2 for *A. pintoi* to 45 plants/m^2 for *S. guianensis*. However, legume growth was slow and legume yields in May 1996 varied from < 10 kg/ha with *Centrosema pubescens* and *Galactia striata* to 170 kg/ha in mixture 1 **[C. Plazas, L. H. Franco and P. C. Kerridge]**.

Output 2.3

Legumes available for fallow improvement

Main achievements

- Establishment by farmers of *Stylosanthes guianensis* to improve fallows in upland rice systems in hilly areas of the Philippines.

- Demonstrated that fallow oversown with legumes has the potential to increase overall productivity of soils in hillsides of north Cauca.

- Confirmed that phosphorus is the most limiting nutrient for legume growth in fallow land in hillsides of north Cauca.

Activity : Legumes for fallow improvement in Southeast Asia

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'. In short fallows, 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 legumes into the fallow could have a large impact on overall productivity through soil improvement and provision of quality feed to livestock.

In Southeast Asia upland rice is grown by farmers in hilly and mountainous areas largely as subsistence crop. Fertilizer is seldom applied to upland rice, and the crop is usually grown for only one or two years before the field is left fallow. The reasons for this include increasing weed competition and declining soil fertility. Fields are left uncropped and revert to naturally occurring vegetation which is grazed heavily by ruminants during the fallow stage. Farmers used to leave fields for several years before coming back to the same field for cropping. Increasing population pressure forces farmers to return to the same field even more frequently and upland rice yields are decreasing.

At a farmer participatory research site in Matalom, Philippines, farmers have identified declining crop yields as a major concern and they are now testing ways to intensify production on their limited land. One of the options tested is to replace natural fallows with leguminous fallows. In previous experiments at this site, *Stylosanthes guianensis* CIAT 184 was identified as the most productive legume when undersown in upland rice. This option is now being tested by farmers in formal experiments aimed at quantifying the contribution of this legume to soil fertility and weed succession **[Visayas State College of Agriculture, Leyte, Philippines, and W. Stür]**.

Activity : Effect of managed fallow on subsequent crops in hillsides of north Cauca

The use of legumes for improving fallow land in smallholder farming systems is being studied in hillsides of Cauca, Colombia. Results from an experiment involving legume covers, fertilizer and fallow length was completed and results will be presented in the next International Grassland Congress in Canada.

In general, results showed that after 12 or 18 month of managed fallow through fertilized legume covers (*C. macrocarpum*, pigeon pea and *Cajanus cajan*), there was an increase in the amount of available soil N and in yield of following maize crops over the unfertilized natural fallow. However, the effect on maize yield was greater when the legume covers were fertilized with 100 as compared to 20 kg/ha of P. Maize yields were even higher where additional nutrients had been added by fertilizer or poultry manure.

From this preliminary study we concluded that a fallow oversown with fertilized legumes has the potential to increase overall productivity of hillsides soils of north Cauca. The improvement occurs within six months of vigorous legume growth. However, a priority for the use by farmers of legumes in the fallow is to derive a feasible method of establishment. We believe that this might be done at the time of the second weeding before the harvest of cassava, which is usually the last crop grown prior to spelling of the land under fallow. Rapid establishment of legumes is desirable to avoid undue competition from weeds **[L. H. Franco and P. C. Kerridge]**.

Activity : Residual effect of phosphorus fertilizer in hillsides of north Cauca

Phosphorus (P) is the most limiting nutrient for legume growth on hillsides soils of north Cauca. Thus, a better knowledge of the residual value of applied P in these soils would assist in fertilizer management. Two P rate trials, planted with *Brachiaria decumbens-Centrosema macrocarpum* association, were established in 1994. The association is being maintained under defoliation by grazing for 4 to 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 is carried out every 12 months and residual effect measured at the end of each year. Results so far indicate a slight loss of residual effect of P at 25 and 50 kg/ha over the results for 1994, but no reduction has been observed at 100 kg/ha **[L. H. Franco and P. C. Kerridge]**.

Activity : Methods of establishment of grass-legume pastures in hillsides of north Cauca

In an on-going experiment we are studying the effect of land preparation, sowing method and P fertilizer application on the establishment of grass-legume pastures in hillsides of north Cauca. Legume yield after 20 months of establishment is higher with mechanical cultivation than with manual cultivation, herbicide application or no tillage (Table 8). On the other hand, we have not observed differences in legume yield between the broadcast or hand sowing treatments, but there has been an effect of P fertilizer (360, 1500 and 1850 kg DM/ha of legume for P0, P25 and P100, respectively) **[L. H. Franco and P. Kerridge]**.

Table 8. Dry matter yields of sown grass and legume and other (native grass and broad-leaf) species as influenced by various establishment treatments (August 1996).

Treatments			Yield (kg/ha DM)			
Land preparation	Sowing	Fertilizer	Sown grass	Sown legume	Other	Total
Nil	Band	P0	0	100	1100	1200
		P25	0	1370	1230	2600
		P100	0	610	1200	1810
	Broadcast	P0	0	50	1330	1380
		P25	0	1090	810	1900
		P100	0	930	1280	2210
Herbicide	Band	P0	160	430	1140	1730
		P25	0	890	11250	2140
		P100	70	1780	810	2660
	Broadcast	P0	110	430	830	1370
		P25	10	850	1470	2330
		P100	20	2180	1210	3410
Disturbance	Band	P0	280	330	730	1340
		P25	220	1480	1420	3120
		P100	310	1910	1030	3250
	Broadcast	P0	170	410	730	1310
		P25	0	2480	950	3430
		P100	260	2470	920	3650
Cultivation	Band	P0	240	840	490	1570
		P25	310	1750	380	2440
		P100	290	2820	430	3540
	Broadcast	P0	730	290	190	1210
		P25	90	2120	930	3140
		P100	110	2070	990	3170

Outputs 2.4/2.5

Grasses and legumes available for soil cover erosion, weed and disease control and for intercropping in plantations

Main achievements

- Identified new ecotypes of *Arachis pintoi* with higher tolerance to drought, yield and soil cover than the commercial cultivar.

- Showed that the erect types of *Chamaecrista rotundifolia* were more drought tolerant than the prostrate types.

Activity : Grasses and legumes for soil cover, erosion and weed control and for plantations in Southeast Asia

A range of grasses, herbaceous and tree legumes have been established as contour hedgerows in an upland cropping area in Matalom, Philippines by more than 20 farmers. Selection criteria of farmers included the perceived ability of forages to control soil erosion during the cropping period as well as providing fodder for ruminants in the dry season. Farmer evaluation of these species is ongoing **[Visayas State College of Agriculture, Leyte, Philippines, and W. Stür]**.

Additional farmer research sites with particular interest in soil erosion have been established recently in southern Mindanao (University of Southern Mindanao), Cebu (Mag-uugmad Foundation-NGO) and northern Mindanao (Cagayan de Oro Veterinary Services and Department of Agriculture, Bukidnon).

Researcher-controlled on-farm experiments of performance of grass legume associations in coconut plantations were continued in Bicol, Philippines. Outputs of the research include contribution of legumes to soil and animal productivity of grazed pastures, weed invasion and soil cover **[Bureau of Animal Industries, Philippines, and W. Stür]**.

A new research site in coconut plantations has recently been established in southern Mindanao in collaboration with the Philippine Coconut Authority. Following preliminary evaluation and multiplication of forages at the Davao Research Center, a farmer research site will be established in the area to actively involve farmers in the identification and integration of forages into coconut-based farming systems **[Philippine Coconut Authority, and W. Stür]**.

Activity : Identification of legumes with dry season tolerance in Central America

Arachis pintoi: Unreplicated plots of 23 lines of *Arachis* spp. including 2 offtypes of the genus, were established in Atenas, a site with a dry spell of 5 to 6 months annually. Visual ratings of drought tolerance and leaf retention were taken at the middle and at the end of the dry season for 2 consecutive years. The more outstanding lines in the following order have been: *A. pintoi* CIAT 22157, CIAT 22159, CIAT 22155, CIAT 18744 and CIAT 22160. *A. glabrata* cv. Florigraze and *A. repens* CIAT 22161 have also performed well but produced low dry matter yields. On the other hand, *A. pintoi* CIAT 22160, 22159 and 18744 are the ecotypes with faster growth (more soil cover) and dry matter yield. The offtype lines identified in Guapiles and San Isidro have intermediate tolerance to dry conditions. Seed multiplication of the best lines is underway for further evaluation.

Chamaecrista rotundifolia: Finalized in Atenas the evaluation of adaptation of 17 accessions of *C. rotundifolia* va. *rotundifolia* (prostrate type) and var. *grandiflora* (erect type). The better adapted lines were identified within the latter group because high DM yield, high leaf retention in the dry season and high plant survival at the end of the 6 months dry period. Within this group the outstanding accessions were CIAT 18252, 8992 and 17000. None of the prostrate accessions adapted well to Atenas conditions because of heavy defoliation during the dry period and poor recovery with the initiation of rains. However, the semi-erect types CIAT 17002 and 8201 showed acceptable DM yields and acceptable drought tolerance. Seed harvesting of the better lines is underway for further evaluation **[A. Valerio, Guillermo Pérez and P. J. Argel]**.

Output 2.6

Grasses and legumes for cut and carry feeding systems and for agroindustrial use

Main achievements

- Initiation of collaborative studies in China to improve harvesting technology and identify improved *Stylosanthes guianensis* genotypes for protein leaf meal production.

- Defined plant densities to optimize fodder production in stands of *Cratylia argentea* in hillsides of Central America.

<u>Activity : Identification of grasses and legumes for cut and carry feeding systems and agroindustrial use in Southeast Asia</u>

Cut and carry feeding of forages is commonly practiced in Southeast Asia. Farmers have very limited areas for growing forages and thus, farmers are always interested in high-yielding grasses and leguminous multipurpose trees and shrubs (MPTS). A large range of potentially productive grasses and MPTS is available to farmers. Cut and carry systems involving grasses such as *Pennisetum* spp. result in high nutrient drain from the grass area. Unless these nutrients, now in the form of manure and urine, are recycled efficiently back to the grass area, soil fertility is exhausted quickly resulting in failure of such systems. Cut and carry systems involving MPTS are less draining on soil nutrient reserves because of their lower yield, deeper root system and their ability to fix nitrogen. Nevertheless, a nutrient drain occurs in legume-based cut and carry systems as well and needs to be addressed.

Previous regional evaluations have identified forages with broad adaptation and these are now being offered to farmers for testing. Ways of recycling nutrients on farms are being discussed with farmers to develop sustainable cut and carry systems. Farmer testing of cut and carry forages is on-going in farmers research sites in Matalom, Cebu, norther and southern Mindanao in the Philippines, and East Kalimantan, North Sulawesi, North Sumatra and Aceh in Indonesia **[various partners Philippines and Indonesia, and W. Stur]**.

Legume leaf meal is used in feed rations for poultry, pigs and ruminants. The most widely used legume for leaf meal has traditionally been *Leucaena leucocephala*. The devastation of Leucaena stands by psyllids in Southeast Asia in the mid 1980s resulted in a search for alternative legumes. In southern China, *Stylosanthes guianensis* CIAT 184 and *S. scabra* cv. Seca were identified as being suitable and there are now several thousand hectare of *Stylosanthes* grown for this purpose. Another species used for leaf meal in Thailand is

Desmanthus virgatus. Collaborative research commenced with the Chinese Academy of Tropical Agricultural Science in Hainan to improve persistence of *S. guianensis* swards, identify more anthracnose resistant lines by broadening the germplasm base and improve harvesting technology used by smallholder farmers **[Chinese Academy of Tropical Agricultural Science, China, and W. Stur]**.

Activity : Evaluation of shrub legumes for cut and carry feeding systems in hillsides of Central America

A field experiment is underway in Atenas, Costa Rica to study the effect on DM yield of plant density and plant age at first cut of *C. argentea* CIAT 18516. The plant densities studied are 2.0 (1 x 0.5 m), 1.0 (1 x 1 m) and 0.7 (1 x 1.5 m) plants/m^2, and the ages at first cut are 4, 6 and 8 months after transplanting. A split plot design is used in a randomized complete block. Cuts are done at 70 cm height and at 8 weeks intervals. Preliminary results after 6 cuts, shown in Table 9 indicate that single plant DM yields are affected by both plant density and by age at first cut. More spaced plants (1 x 1.5 m) and those cut at 8 months of age produce higher yields. However, estimates of DM yield/ha shows that the plant density in which greatest yields are obtained is 2 plants/m^2, which nearly doubles the DM yields observed for the other plant densities studied. This study will continue for another growing season **[A. Valerio, G. Pérez and P. J. Argel]**.

Table 9. Dry matter yields of *C. argentea* CIAT 18516 planted at three densities and cut at three different ages in Atenas, Costa Rica.

	Age at first cut (months)			Mean	Estimated
Plant density	4	6	8		
(plants/m^2)	yield (g DM/plant)				(kg/ha)
2	47	48	79	56 b*	1120
1	48	71	82	65 b	650
0.7	62	81	144	90 a	600
Mean	52 c	67 b	101 a		

*Means of 6 cut; $P<0.05$, Duncan

Subproject 3.0

Seed for research and national partners

Output 3.1

Promising accessions and advanced lines of grasses and legumes multiplied

Main achievements

- New forage seed multiplication areas established in Colombia, Central America and

Southeast Asia during 1996.

- Large quantities of new accessions of *Arachis pintoi* harvested in Colombia during 1996.

Activity : Establish, maintain and harvest field and glasshouse seed multiplication plots in Colombia

Important new seed multiplication areas were established this year at all existing production sites and at a new site near Armenia, Quindio in Colombia (Table 10). Areas of promising *Brachiaria* accessions were expanded at CIAT-Popayán, and on a Cenicafé property in Chinchiná, Caldas, to provide seed for large-scale grazing trials in 1997. Significant areas of the new *A. pintoi* accessions, which are being identified in multilocational trials, were established on a private property near Armenia, Quindío in Colombia to provide the larger volumes of seed which will be required for more extensive trials and to support eventual commercial release of some of these materials. Smaller plots of a large number of different forage legume species are being multiplied at CIAT headquarters.

Table 10. New forage seed multiplication areas established during 1996.

Site	Accessions No.	Total area (m^2)
Chinchiná	10	2,485
Popayán	11	3,670
Armenia	11	18,280
Palmira	36	1,490

Chinchiná: *Brachiaria* spp.

Popayán: *Arachis, Brachiaria, Panicum, Hyparrhenia*

Armenia: *Arachis*

Palmira: *Stylosanthes, Cratylia, Codariocalyx, Pueraria,*

A total of 107 seed lots were harvested and processed in 1996 (to mid-October), for a total of 667 kg, more than double the volume harvested the entire previous year. There was a sharp drop-off in forage seed multiplication between 1991 and 1992 (from nearly 600 kg to just over 100 kg). This is due to a sharp cutback in the resources devoted to the Seed Production Section of the Tropical Forages Program. From 1992 to 1996, there has been a steady increase in the volume of seed harvested (Figure 6). Large quantities (>100 kg) of each of three new accessions of *A. pintoi* were harvested this year at Chinchiná, and several plots remain to be harvested in November. Most other seed lots were on the order of 1 kg or less **[A. Ortega, R. Mosquera and J. W. Miles]**.

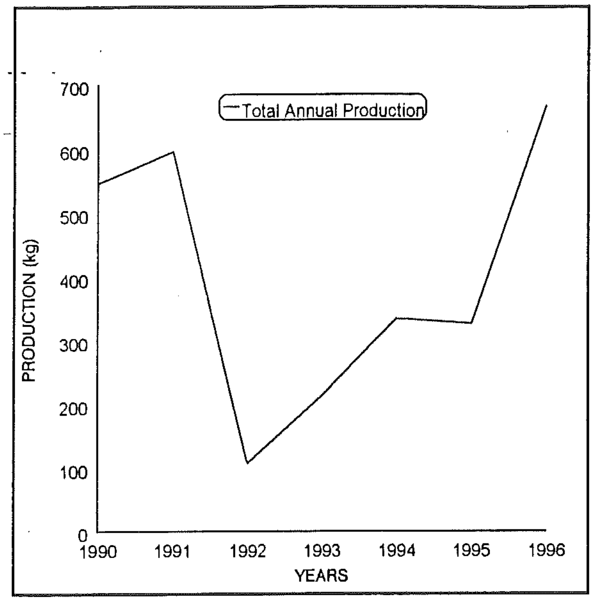


Figure 6. Forage seed multiplied by the Tropical Forages Program in the last 7 years.

Activity : Multiplication of promising accessions and advanced lines of grasses and legumes in Southeast Asia

Multiplication of approximately 45 forages species for regional evaluation and on-farm activities has been continued at Los Baños, Philippines. A new multiplication site has been established at Nam Suang in Laos. Twenty species were sown in June 1996 and seed from this site will support regional evaluations and on-farm activities in Indochina.

Activity : Contracts of large scale seed multiplication with private sector producers in Colombia

We are establishing formal agreements with private producers in Colombia for large-scale multiplication forage seeds of a very limited number of accessions to promote eventual release. These agreements serve the double purpose of exposing new materials to the private seed sector as well as to generate the large volumes of seed which will be needed for on-farm research and promotion **[A. Ortega, R. Mosquera and J. W. Miles]**.

Activity : Harvesting and processing experimental seed for national partners in Central America

Harvesting and processing seed of promising grasses and legumes is a permanent activity given the wide variability in flowering time and seed set of the pasture germplasm under multiplication. The activities are concentrated in Atenas, Costa Rica a site that has an adequate growing season period and a defined dry season that facilitates the harvesting and processing of the seed. A total of 142 kg of experimental and basic seed has been harvested during 1996, and distributed as follows: *C. argentea* (24.1 kg), *Centrosema* spp. (2.7 kg), *A.*

pintoi CIAT 18744 (27 kg), *A. pintoi* CIAT 17434 (46 kg), *C. rotundifolia* (1.6 kg), *Brachiaria* spp. (25 kg) and *P. maximum* (15 kg). The seed harvested is processed and classified before storage in a cool room at the ECAG in Atenas. This seed is later tested for quality parameters before it is distributed to regional collaborators **[P. J. Argel]** .

Outputs 3.2/3.3

Factors affecting seed quality and methodology of seed production and storage investigated

Main achievements

- Initiated on-farm studies in the Philippines on seed production of selected grasses and legumes.

The seed multiplication unit at CIAT has not had adequate resources nor the professional expertise to conduct research in the area of seed production, quality and storage. However, methodology on seed production appropriate to farmers is being investigated in Southeast Asia through the SEAFRAD consortia.

Activity : Studies on seed production methodology in Southeast Asia

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 a 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 1,000 m^2 plots for realistic production conditions by the regional Department of Agriculture. The six species are *Andropogon gayanus* (CIAT 621), *Brachiaria brizantha* CIAT (6780), *Brachiaria decumbens* cv. Basilisk, *Brachiaria humidicola* cv. Tully, *Centrosema pubescens* (CIAT 15160), and *Stylosanthes guianensis* (CIAT 184). Measurements include inflorescence density, seed set, seed yield and labor requirements. Additionally, farmer pilot seed production commenced around these two areas.

The aim is to identify constraints to smallholder seed production and work with farmers to overcome technological constraints **[Regional Department of Agriculture, Region 2, Philippines and W. Stür]**.

In November 1996, a 2-day Workshop on Forage Seed Supply Systems will be held in Thailand. This workshop will review existing seed production systems, identify factors contributing to the success or failure of these schemes, and discuss options for the improvement of existing and development of new systems for countries in Southeast Asia. Participants will come from countries in Southeast Asia, Australia, South America, India, UK and China **[all partners in Southeast Asia and W. Stür]**.

Output 3.4

Distributed seed to national research partners

Main achievements

- Continued delivery of forage seed to national research partners in Colombia, Central America and Southeast Asia.

<u>Activity: Monitoring seed quality and health in the Forage Seed Unit of CIAT</u>

A total of 280 seed germination tests were conducted during 1996, up to mid-October, to monitor the quality of seed stocks held by the TFP. Limited resources have constrained this vital activity at CIAT, although every attempt is made to avoid distributing seed of unknown quality and germinability. Thus, all seed requests for shipment outside of Colombia are routinely submitted to rigorous seed health inspection and Colombian government seed quarantine regulations **[J. W. Miles]**.

<u>Activity : Delivery of forage seed to national research partners</u>

<u>Colombia</u>: From January 1996, through 11 October, a total of 82 individual seed requests made to the Forage Seed Unit at CIAT were processed. This represents a total of 443 individual items, each of which must be weighed, packed, labeled, and delivered. For seed supplied to entities outside of Colombia, processing includes strict quarantine inspection and the issuing of a phytosanitary certificate by Colombian authorities. Seed was supplied to CIAT research programs, government research institutes, universities, NGO's (such as the Colombian Rice Grower's Federation),and several private producers.

The major institutional consumer was Colombia's national agricultural research corporation, CORPOICA. The bulk of the seed was delivered to researchers in Colombia and to other developing countries as well as to institutions in developed countries such as Japan and the USA **[J. W. Miles]**.

<u>Costa Rica</u>: Seed of new promising forage species continued to be multiplied and delivered on request from institutions members of the RIEPT-MCAC network. Between August 1995-October 1996 a total of 102 experimental and basic seed request were received and delivered to 17 countries including non-RIEPT-MCAC members such as Jamaica, Haiti, Vietnam, Venezuela, Bolivia, Puerto Rico and The Philippines. A total of 279 kg and 98 kg respectively of legume and grass seed was delivered.

This is an important figure, because during the last 2 years the requests for forage legume seed has been greater than that of grasses. The increasing request for seed of *Arachis pintoi* accounts for much of this increase. Costa Rica is the country with the largest number of requests (64), followed by Honduras (13) and Mexico (5). The seed requested had destinations for agronomic trials, seed multiplication plots and plantations cover crops studies **[P. J. Argel]**.

<u>Southeast Asia</u>: During 1996, over 1000 seed lots were distributed for evaluation at over 40 sites in Southeast Asia **[W. Stür]**.

Subproject 4.0

Adoption and impact analysis of legumes in dual purpose cattle systems

Output 4.1

Ex-ante and ex-post impact analysis at legumes in dual purpose cattle farms

Main achievements

- Showed through an ex-ante analysis that economical internal rates of return would increase significantly if calving rate and milk yield were improved with *Arachis*-based technologies in dual purpose cattle farms in forest margins of Caqueta, Colombia.

Activity : Studies on economic profitability and viability of *Arachis pintoi* in dual purpose cattle farms (NESTLE Project)

One of the objectives in the NESTLE Project that operates in forest margins of Caquetá, Colombia is to determine the economic benefits of using *Arachis*-based pastures in dual purpose cattle farms. Thus an ex-ante analysis was carried out during 1996 to determine profitability and economic viability of *Arachis*-based pastures using partial budgeting. For the analysis we assumed that economic returns associated with *Arachis*-based pastures were mostly affected by cost of establishment, milk yield/cow, stocking rate and calving rate. In addition, several technical assumptions an parameters were considered for the analysis as shown in Table 11.

Table 11. Economical and biological information used for the analysis on alternative pastures for dual purpose cattle farms in Caqueta, Colombia (1996).

Variables*	*B. decumbens* alone	*B. decumbens* +*A. pintoi*	*B. dictyoneura* + *A. pintoi*	*B. humidicola* + *A. pintoi*
Establishment cost ($/ha)	157600	281600	368000	336000
Milk yield (l/cow/day)	3.0	3.5	3.5	3.5
Price of milk on the farm ($/L)	234	234	234	234
Stocking rate (ua/ha)	1	1.5	2	2
Price of land ($/ha)	300000	300000	300000	300000
Price of cattle ($/)				
Milking cow	500000	500000	500000	500000
Culled cow	350000	350000	350000	350000
Weaned calf	180000	180000	180000	180000
Lactation (days)	240	240	240	240
Calving rate (%)	60	60	60	60
Evaluation period (years)	12	12	12	12
Labor cost ($/day)	8000	8000	8000	8000
Pasture recuperation cost ($/ha)	39400	39400	39400	39400
Frequency (years)	4	4	4	4

*All values are given in Colombian Pesos (US$1=$1.000).

Results expressed as internal rates of return (IRR) indicated that with the parameters used and assumption made *Arachis*-based pastures are economically attractive as compared to the traditional *B. decumbens* pastures used by farmers in the region (Table 12).

Table 12. Internal rates of returns (IRR) of *Arachis*-based pastures with different *Brachiaria* species in dual purpose cattle farms in Caqueta, Colombia (1996).

Pasture	Establishment cost* ($/ha)	Milk yield (L/cow/day)	Stocking rate (AU/ha)	IRR (%)
B. decumbens alone	157600	3	1	12.0
B. decumbens + *A. pintoi*	218600	3.5	1.5	19.3
B. humidicola + *A. pintoi*	336600	3.5	2.0	21.8
B. dictyoneura + *A. pintoi*	368000	3.5	2.0	21.1

*All values are given in Colombian Pesos (US$1=$1.000).

In a second phase of the study we considered the sensitivity of economical returns with *Arachis*-based pastures, due to changes in some technical parameters (calving rate, stocking rate, milk yield per cow) and cost of inputs (seed price). Results shown in Table 13, indicate that changes in calving rate had the largest effect on IRR associated with *Arachis*-based pastures. Increasing calving rate by 10% (60 to 66%) would result in 16% increase in IRR. On the other hand, it was interesting to observe that in spite the high incidence of the cost of *A. pintoi* seed on establishment (35% of the total cost), changes in seed cost had little effect on IRR. A 10% reduction in seed cost had a minor effect (1.5%) in IRR. However, it should be kept in mind that even though the cost of *A. pintoi* seed seemed to have little effect on IRR, it could have a major effect on the economic viability of the *A. pintoi* technology. At current seed prices (US$25/kg) the introduction of *A. pintoi* in association with *B. decumbens* increases the cost of establishment by 79%.

As expected changes in milk yield per cow had a large effect on the IRR associated with *Arachis*-based pastures. A 10% increment in milk yield resulted in a 9% increment in IRR, which is almost proportional. This points out once again, the importance of improving in dual purpose cattle systems not only the feed resources, but also the genetic ability of milking cows to respond to the improved legume-based feeding systems. Changes in stocking rate did not result in proportional changes in IRR. Nevertheless, our analysis showed that the economic advantage of *Arachis*-based pastures over the straight grass is obtained when the stocking rate used is above 0.9 AU/ha, which is average in the region. Finally, we analyzed the effect of the persistence of *Arachis*-based pastures on the IRR. Results indicated that if *A. pintoi* had to be sown every two years the IRR would be drastically affected and would be half of the IRR obtained with a straight grass (12%). However, our experimental results suggest that *A. pintoi* is very persistent even under poor management (i.e. overgrazing). It is acknowledged that this analysis has limitations, since it does not consider the farm as a whole (i.e. labor). Nevertheless, the results can help us identify constraints that farmers may face when adopting *Arachis*-based pastures **[L. Rivas and F. Holmann]**.

Table 13. Economic sensitivity of internal rates of return (IRR) of the *B. decumbens/A. pintoi* association due to changes of critical parameters in dual purpose cattle farms in Caqueta, Colombia (1996).

Change (%)	Stocking rate (AU/ha)	Milk yield (L/cow/day)	Cost of *A. pintoi* seed* ($/kg)*	Calving rate (%)
		IRR (%)		
+10	20.6 (1.65)	21.0 (3.86)	19.6 (22500)	22.5 (66.0)
+20	21.8 (1.80)	22.7 (4.20)	19.8 (20000)	25.8 (72.0)
+30	22.9 (1.95)	24.4 (4.55)	20.1 (17500)	29.1 (78.0)
+40	23.9 (2.10)	26.1 (4.90)	20.4 (15000)	32.6 (84.0)
+50	24.8 (2.25)	27.8 (5.25)	20.7 (12500)	36.1 (90.0)
0	19.3 (1.5)	19.3 (3.5)	19.3 (25000)	19.3 (60.0)

*The percentages in the case of *A. pintoi* correspond to seed price reduction. All values are given in Colombian Pesos (US$1=$1.000). Figures in parenthesis correspond to value of parameters.

Activity : Monitoring the adoption process of *Arachis* by farmers in dual purpose cattle systems (NESTLE Project)

A survey is being planned in dual purpose cattle farms in Caqueta, Colombia. The objective of the survey are (1) to determine the potential demand of improved grass-legume mixtures, (2) to identify limiting factors for adoption, and (3) to feedback the research and technology transfer process.

In addition, this activity will include an economic analysis utilizing farm prototypes with the objective of complementing the partial budgeting procedures performed in the previous activities by means of establishing the economic impact of new technologies in different farm sizes, geographical location, and quality of productive resources **[L. Rivas and F. Holmann]**.

Output 4.3

Contribution of new legume-based systems to sustainable land use evaluated

Main achievements

- Completed field work on land dynamic studies in benchmark sites of the Systemwide TROPILECHE Project.

Activity : Diagnostic land use of benchmark sites in hillsides of Costa Rica and forest margins of Peru (Systemwide TROPILECHE Project)

An important objective of the Systemwide Livestock-Tropileche Project is to assess the appropriateness to the smallholder sector of legume-based feeding strategies and their relevance to a wider range of farming systems. Thus, Tropileche is contributing operational expenses to complement on-going activities on land use and economic characterization of benchmark sites in forest margins of Pucallpa, Peru and subhumid areas of the Central Pacific region of Costa Rica. .. .

Analysis of land use dynamics in Pucallpa, Peru. During 1996 more than 150 interviews were conducted in the Tropileche study area in Pucallpa, Peru. At present we are analyzing the data in terms of land use dynamics in several "zones" defined by land form and resources and land use patterns. Preliminary results on some land use patterns in different "zones" follows.

1. **Small dual purpose cattle ranches along the paved Lima-Pucallpa highway and along the Campo Verde (at km 34) to Nueva Requena road**. Enterprises in these early-settled areas include annual (slash-and-burn production of rice, cassava) and perennial (e.g., citrus) crops. Most informants have an over-capacity of pasture due to herd reductions during the Sendero period. Aggregate herd size in the Department of Ucayali has decreased from some 68,000 head eight years ago to a current 21,500 head. Less cash is now available for herd rebuilding due to increased coca production control with the return of government forces in the area. Few of the Campo Verde-Nueva Requena settlers now have cattle, although many retain pastures with the hope of re-establishing herds.

2. **Slash-and-burn rice and cassava + oil palm along the seasonal road joining the highway at km 61 and Curimana on the Rio Aguaytia**. Some 100 farmers planted oil palm on up to five ha each with government loans. Slash-and-burn production follows a pattern of rice followed by two cassava plantings in (disturbed) primary forest, followed by fallow (after a total two years cropping). Farmers have high expectations regarding returns to oil palm--which have yet to come into production.

3. **Slash-and-burn rice and maize plus banana producers along the Rio Aguaytia.** These areas of lighter soils are seasonally inundated. Banana is a main cash crop. Sigatoka is a problem, and one that farmers think was exacerbated by areal spraying of coca fields. Current income also comes from harvesting the softwood *Gauzuma crinita* (locally *bolaina blanca*) which grow in fallows. Some farmers apparently increased slash-and-burn agriculture in their remaining primary forest in order to have more fallow lands on which the *bolaina* is a pioneer species.

4. **Small slash-and-burn farmers who also produce coca and charcoal in areas further from the three main roads and the river**. Reduced demand for labor for coca production because of more government control has meant an increase in charcoal production (reflected by lower prices for charcoal) and an increase in pressure on specific forest trees such as the hardwood *Dipterix oderata* (locally *chihauhauco*).

5. **Parastatal lands held by the Sociedades Agraria de Interes Social (SAIS Pachacutec and Tupac Amaru) and the government cattle ranch "San Jorge"**. Tupac Amaru has 40,000 ha in the area bounded by the roads and river defining our study area; has 450 ha of oil palm in production land plants to reach 20,000 ha of oil palm in the future. Pachacutec has 3200 ha in the study area, with 550 ha of pasture--

and 560 head of cattle (60% for meat and 40% dual purpose)--and some 2600 ha of forest. With the exception of some wage labor, the SAIS provide few benefits for local farmer-settlers (excluding "squatters" on Tupac Amaru lands). San Jorge has 5000 ha--including 1200 ha of pasture, 2400 ha of ex-pasture lands which have reverted to secondary forest, and 1400 ha of forest-and 950 head of cattle and 25 employees. The ranch is scheduled to be privatized in the near future.

Analysis of land use in Costa Rica: Farmer-ranchers of the Pacific Coast region of Costa Rica were interviewed. Holdings were small (4-20 ha), medium (21-60 ha), large (61-100 ha), and haciendas (100+ ha). About half of those interviewed had dual purpose (beef and milk) operations. Other production systems were mixed crop and livestock, cattle fattening, calf production and sale, and dairy. Many of the smallholders (50%) but few of the larger operations (0-14%) relied on non-livestock and off-farm activities such as fruit (eg., mango) and crop (eg., rice, beans, maize, coffee) production or hired labor.

The main constraint facing livestock operations was the lack of dry season feed resources. Strategies included renting pastures and providing animals with sugar cane and chicken manure supplements. A third (32%) of all respondents distributed evenly across classes rented pasture lands for part of the year, either in the rainy season to allow pastures to recover for the dry season or during the dry season when owned pasture resources were depleted. Considering all respondents, almost all provided animals with salt and minerals; 72% provided molasses (largely in the dry season); and 44% provided chicken manure. Other purchased supplements included soybean husks, concentrate feeds, and bone meal. Thirty-five percent provided sugar cane and 9% gave crop residues produced on-farm.

That nearly two-thirds of the farmers had established some improved pasture (largely Cynodon nlemfuensis or Digitaria decumbens) and a similar proportion had more recently sown Brachiaria spp. would argue for continued work on improved pasture systems. Factors identified as constraints to the adoption of improved pastures included: high price (and unavailability) of seed; low germination of the seed; difficulties in the establishment; the need to prepare land/cost of mechanization; availability of land to rent as an alternative; the slow process of seed releases by the government; and some lack of exposure to innovations.

On the other hand, other factors were identified as supporting the adoption of pasture and forage innovations: lack of feed resources in the dry season; possibly increasing prices for supplements such as chicken manure; existing substantial adoption of improved pastures; practice of establishing improved pastures following intensively managed high-valued annual crops such as watermelon; the intensive management systems of the small holders; and the assumed higher yields associated with the alternatives.

The survey team concluded that, especially with smallholders: (1) there is experience with and demand for improved pastures; (2) there would be high demand for innovations which would increase feed resources in the dry season; (3) there is negligible experience with forage legumes; (4) further adaptive research is desirable on fodder and animal yields comparing existing and improved feeding systems; (5) participatory adaptive research on management of new alternatives and technology transfer (eg., farmer-to-farmer) is needed; and finally, (6) among other constraints, MAG researchers now have overly large areas of responsibility (both in terms of geographical areas covered and in fields of expected expertise) and that local MAG technicians lack needed capacity **[Sam Fujisaka, Federico Holmann, and German Escobar for CIAT; Nils Solorzano, Leticia Badilla, Luis Umaña, Marco Lobo, and Vidal Acuña for MAG]**.

Subproject 5.0

Adoption of new grasses and legumes

Output 5.1

Partnerships with national organizations established to undertake collaborative research

Main achievements

- Obtained support from Ministers of Agriculture in Central America to continue the forage network but no funding was offered.

Activity : Support raising for continued activities of the Forage Network in Central America

During 1996 visits were made to several RIEPT-MCAC countries with two objectives: follow up advances on pasture germplasm evaluation and promotion at country level, and search for financial support for the continuation of the RIEPT-MCAC activities, since this network will not have additional financial support from CIAT beginning 1997.

A project proposal that contemplated contributions from each country member of RIEPT-MCAC was prepared and presented to different authorities in the region. The cost of maintaining the network is US$ 232,000 annually, including the cost of keeping a seed multiplication unit in Atenas. The proposal was later presented at the Reunión Extraordinaria del Consejo de Ministros de Coreca held in Mexico on the 23rd of August, 1996. Both, national institutions of each country member and Coreca showed strong support for the continuation of the RIEPT-MCAC pasture network. However, none of them pledged financial support to the network. Coreca suggested that CIAT should look for international funds for the continuation of the network.

In Nicaragua, a project proposal was submitted to Instituto Nacional de Tecnología Agropecuaria (INTA) titled "Strengthening Pasture Germplasm Evaluation and Seed Multiplication Activities in Nacaragua". The cost of the project is US$420,000 for 3 years, and includes US$25,000 per year to support the assessment of RIEPT-MCAC to assure success of the project. The money for this project will come from the World Bank, and at this moment the proposal has been revised by INTA and given tacit approval with some amendments that are being incorporated in the final document **[P. J. Argel, P. C. Kerridge, F. Holmann and R. Posada]**.

Output 5.2

Promising germplasm distributed through regional networks to undertake collaborative research

Main achievements

- Continued flow of new forage germplasm for regional testing by national researchers.

Activity : Delivery of new forage germplasm to national research partners

Agronomic evaluation of forage germplasm for adaptation to biotic and abiotic constraints has remained an important activity of the Tropical Forages Program. A list of forage germplasm for different agroecosystems in tropical America was prepared and distributed to research partners in the region. Seed packages of grasses and herbaceous legumes (16 packages) and shrub legumes (13 packages) was also distributed for regional testing on request to 7 countries of the region **[L. H. Franco, P. C. Kerridge and P. J. Argel]**.

Output 5.3

Relevant methodologies for legume-grass technology with farmer participation developed

Main achievements

- Derived methodological results that showed that legume effects on milk yield could be underestimated with a given pasture rotation sequence or if cows were not given an adjustment period.

- Carried-out a training course for researchers in forest margins of Caqueta on participatory research on dual-purpose cattle farms.

Activity : Development of methodology for on-farm pasture evaluation

On-farm evaluation of improved pastures with dual purpose cows offers some methodological challenges. One common approach is to rotate the milking herd in the farm from the grass alone pasture (farmer's or introduced) to the grass/legume pasture. However, by doing this we have to be aware of possible effects on milk yield of previous days of adjustment in the pasture being evaluated and sequence of rotation (e.g. grass $\rightarrow$ grass/legume or grass/legume $\rightarrow$ grass). To address these questions we analyzed a data set from our work with milking cows in short-term duration grazing trials at Quilichao. A total of 15 experiments were included in a mean comparison analysis using the "t" test. Results shown in Table 14 indicate that in the sequential evaluation of pastures, milk yield could be overestimated in the grass alone pasture and underestimated in the grass/legume pasture, if cows were not allowed a 7 day minimum adjustment period. For example, if in the experiments analyzed there had not been a 7 day adjustment period, the average increase in milk yield due to legume would have been only of 3%, when in fact the average effect measured was 15%.

Table 14. Comparison of milk yield means from Holstein cows during a 7 day adjustment (A) period and a 7 day measurement (M) period in grass alone and grass + legume pastures (Lascano et al., unpublished results).

Experiment	Pastures					
	Grass			Grass/Legume		
No.	A	M	Sig	A	M	Sig
	(l/cow)			(l/cow)		
1	11.5	10.9	NS	12.1	12.9	NS
2	11.8	10.9	NS	12.8	12.3	NS
3	10.4	9.6	NS	11.3	11.7	NS
4	8.8	7.8	*	8.8	9.3	*
5	5.7	4.8	*	5.0	4.9	NS
6	9.6	9.0	**	9.1	9.2	NS
7	7.1	6.2	*	6.7	7.0	NS
8	9.6	9.2	NS	9.7	9.8	NS
9	6.1	5.1	**	6.9	7.4	**
10	7.2	6.3	***	8.1	8.5	**
11	5.0	4.8	NS	5.4	6.1	***
12	13.2	12.0	***	13.0	13.9	***
13	10.3	8.9	***	11.3	11.1	NS
14	10.1	11.1	**	10.5	11.3	***
15	10.9	10.8	NS	11.1	10.8	NS
	9.2	8.5		9.5	9.8	

NS = Not significant; *P < 0.05; **P < 0.01; ***P < 0.001

The effect of sequence of rotation on milk yield is shown in Table 15. Results indicate a consistent reduction in milk yield across experiments when cows were moved from the grass-legume pastures to the grass alone pasture. However, when cows were rotated from the grass alone pasture to the grass-legume pasture milk yields increased in some experiments but not in others. On the other hand, the average changes in milk yield due to legume in the pasture were greater in the rotation from grass/legume to grass alone pasture (-2.0 L/cow/day) than in the rotation from grass alone to grass/legume pasture (+0.7 l/cow/day) **[C. Lascano, P. Avila, G. Ramírez and M. C. Amézquita]**.

Table 15. Changes in milk yield of Holstein cows in two rotation sequences when evaluating grass alone (G) and grass + legume (G+L) pastures (Lascano et al., unpublished results).

	Rotational sequence			
Experiment No.	G→G+L[1] (change, l/cow)	Sig	G+L→G [2] (Change, l/cow)	Sig
1	+3.6	***	-0.2	NS
2	-0.1	NS[3]	-3.2	**
3	+0.4	NS	-2.3	***
4	-1.2	**	-1.5	***
5	-0.4	NS	-1.1	*
6	-0.3	NS	-1.5	***
7	+0.7	*	-1.1	***
8	+1.5	**	-2.5	***
9	+1.5	***	-2.6	***
10	+1.3	***	-2.4	***
11	+0.8	*	-3.3	***
12	+0.8	*	-3.4	***
13	+1.1	***	-0.5	*
14	-0.6	NS	-0.6	*
15	+0.8	**	-3.4	***

[1]Rotation from grass to grass-legume pasture
[2]Rotation from grass-legume pasture to grass pasture
[3]NS = Not Significant; * ($P<0.05$); **($P<0.01$); ***($P<0.0001$)

Activity : Adjusting participatory research methodology to forage-based livestock systems

As part of the NESTLE Project a course on participatory research methods was offered in Florencia, Caqueta. A total of 20 professionals from NESTLE, Fondos Ganaderos, CORPOICA, UMATAS, and the local university participated in the 4 day course. Activities carried out as part of the program included: (1) presentation of the conceptual framework of participatory research, (2) interviews of dual purpose cattle owners or administrators by participants to define major constraints faced by the farmers and possible solutions, (3) farmer involvement in the selection of new forage species being evaluated at a research station and (4) video of the different activities carried out by participants. A follow-up of the course is planned for next year **[L. A. Hernández and T. Gracia]**.

Output 5.4

Facilitate communication on grass-legume technology through newsletters, journals and workshops

Major achievements

- Organized an International Workshop on methodologies for on-farm research in dual purpose cattle farms.

- Organized an international workshop on the use of BOTANAL to measure changes in vegetation in different land uses.

- Financial award from COLCIENCIAS to Pasturas Tropicales.

Activity : Workshop on Methodology for on-farm research in dual purpose cattle systems (Systemwide Livestock-TROPILECHE Project)

In order to define methodologies for biological and socio-economical research in dual purpose cattle we organized a workshop in CIAT during July of this year. The program was organized around three themes: (1) Animal measurements in dual purpose cattle farms (7 papers), (20 Application of models for optimizing use of resources in dual purpose cattle farms (5 papers) and (3) land use characterization and assessment of adoption and impact of new feed resources and management practices (7 papers).

Presentations and discussions of papers was followed by work group sessions. Invited participants (34) from 11 countries were distributed in three groups to discuss and make methodological recommendations on (1) measurements in feed resources and animals in dual purpose cattle farms, (2) measurements of indicators of sustainability in dual purpose cattle farms, and (3) measurements of acceptability and adoption by farmers of new feed resources and management practices. Some **highlights** of the workshop are summarized below:

1. Need to make a clear distinction between monitoring of performance of dual purpose cattle in farm (i.e. milk yield, calving interval, birth and weaning weights of calves, cow body condition, progesterone) with existing feed resources from the on-farm evaluation of new feed resources. For the later case, projects like Tropileche can not afford to measure but few animal responses (i.e. daily milk yield) using a methodology described by C. Lascano in the workshop as sequential (animals receive contrasting feed resources in a sequential manner over time). With this methodology it would seem possible to reconstruct lactation curves which are useful to assess the potential benefit of a given feed resource being evaluated. The use of Milk Urea Nitrogen (MUN) as a nutritional indicator of Energy/Protein status of cows as affected by diet was presented by L. Hammond (USDA). It was recommended that MUN be tested in projects like Tropileche as an on-farm guide for supplementing milking cows and to explain retrospectively responses to feeding treatments. When evaluating new feed resources it was recommended that mineral deficiencies be corrected and thus eliminate them as sources of variation.

2. Quality measurement in feed resources were grouped in two categories: (a) quality parameters (i.e. soluble protein, fast and slow degradable protein, undergradable protein, neutral detergent fiber, starch, sugars, etc.) to feed existing feed ruminant models like the Cornell Net Carbohydrate and Protein System (CNCPS) and (b) minimum measurement to describe a feed resource (i.e. CP, IVDMD), being tested in farms. It was clearly stated that Projects like Tropileche could not afford to make large number of chemicals analysis in feeds being evaluated in order to validate the CNCPS model. Detailed measurements on feed resources were recommended for on-station experiments, in order to explain animal responses and provide information to validate feed ruminant models. It was also recommended that CIAT coordinate an effort for compiling information on chemical analyses on forages and other non-traditional tropical feed resources performed by different laboratories in the region to make this information available in tabular form to researchers. In addition, it was recommended that CIAT should provide standards to calibrate NIRS in different labs of the region that have this equipment.

3. Feed ruminant models like the CNCPS were viewed as an important tool to help researchers in developing hypothesis and in selecting treatments for feeding experiments designed to study complementarily among feed resources. The results of these experiments would then be validated in on-farm trials.

4. A number of simulation models useful to provide feed-back to researchers through ex-ante analyses on alternative uses of resources in dual purpose cattle farms were identified. One of such models was developed by CATIE and RISPAL, and its use illustrated in the workshop by F. Holmann using information from the central pacific region of Costa Rica where Tropileche is currently working. Other models such as the one being developed by Cornell (O. Reynoso from Mexico) which is closely linked to the CNCPS model, still needs further refinement (i.e. environmental parameters).

5. Animal Genotype x Environment interactions were well illustrated by Lucia Vaccaro using milk yield as an indicator of environmental quality. Study herds were grouped into three classes according to milk yield/lactation (G1 761, G2 912 and G3 2563 liters/lactation) and data classified according to cow breed (Zebu type, and crossbreds with medium or high European blood). Results showed that as milk yield increased form G1 to G3 there were improvement in days in milk (17%) and days open (-9%), but there were reductions in calf weight produced/cow (-12%) and increase in losses due to stillbirths and calf mortality (279%). Significant breed x environmental group interactions were found for the majority of traits. Breed groups classified as Zebu and High European were particularly affected by the interactions. The medium European cows exceeded the other genotypes on annual production in the poor and medium environments. Thus a clear message from Vaccaro's paper was that 1/2 blood cows [Zebu x European (H)] were little affected by the genotype x environment interaction and that they are appropriate for a wide range of environments, including conditions defined as close to optimum in grazing systems in the tropics. From a methodological point of view it was pointed out that for assessing responses to environmental improvement in dual purpose cattle systems it was necessary to measure not only milk yield/lactation but also fertility and survival. These results were confirmed by M. C.

Amezquita after analyzing results from a commercial dual purpose farm in Colombia which keeps detailed records.

6. Studies involving calves calf in dual purpose cattle farms need to take into account the residual milk which has a higher energy value (fat content) than milk in the bucket. Consequently in studies designed to test different feed supplements for calves there is a need to weigh animals before and after milking to better explain responses.

7. Participatory research methods were considered as useful tools in projects like Tropileche in order to better understand farmers motivations and needs, accelerate adoption and feed-back researchers. The use of participatory methods was well illustrated by Sam Fujisaka on soil conservation practices in the Philippines. The group (Teresa Gracia and Luis Alfredo Hernández) in CIAT led by J. Ashby presented the conceptual basis of participatory research and illustrated how CIAT is trying to incorporate these methods in an on-farm project with dual purpose cattle in Caqueta, Colombia (The NESTLE Project). There is a need for short training courses in participatory methods in the Tropileche benchmark sites. It was also clear that even though participatory research methods could help in promoting early adoption of new feed technology, massive diffusion of the technology was still going to be dependent on more traditional extension methods.

8. Methodology to characterize land use systems in marginal areas was illustrated by Sam Fujisaka through a study carried out in forest margins of Brazil and Peru. Fujisaka showed how in a short period of time (2 weeks) a small team was able to gather very useful information for a research project like Tropileche. This was accomplished by asking key questions to farmers, visiting the field and by having access to secondary information. The message was that we do not need to continuously monitor farms like other projects have done in the past in order to determine the constraints faced by farmers and to identify research issues.

9. Follow-up studies on early adoption of new technologies (i.e. new forage species) developed with farmers were considered to be urgent in the region (provide feed-back to researchers and help in promoting the technology). Methodologies to carry out these studies were illustrated by S. Franzel for *Calliandra callothyrsus* in Kenya and by L. Rivas for *Arachis pintoi* in Colombia.

10. Measurement of bio-physical and socio-economic indicators in projects like Tropileche was introduced by P. Kerridge, who emphasized that sustainable land use practices in dual purpose cattle farms need to offer high profit to farmers in order to be adopted. Concepts developed by Muller (1995) on indicators of sustainability were presented to the participants. A recommendation was that in projects like Tropileche the emphasis should be in measuring indicators (bio-physical and socio-economic) at the farm level and that given the relatively short duration of such projects on should measure physical indicators (i.e. vegetation, soil) in contrasting land uses in the research benchmark sites. Methodology developed for dry areas of Australia (Tongway and Hindley, 1995) was used to illustrate how through soil monitoring it is possible to assess the relative capacity of the soil to absorb and store rainfall, store and cycle nutrients, provide good

growing conditions for plant growth and resist erosion **[C. Lascano and F. Holmann]**.

Activity: International Workshop in the use of BOTANAL

A Workshop was offered during July 1-5 at CIAT's headquarters on BOTANAL (Method and statistical package to estimate yield and botanical composition of pastures and other land uses utilizing laptop computers). The objective was to provide field technicians with theoretical and practical knowledge on quantifying attributes from pasture lands and other land uses to allow a more efficient management and utilization of forage resources. There were 29 participants from five countries (Colombia, Costa Rica, Nicaragua, Peru and Venezuela) representing research, development, and training institutions. The workshop was given by Cam McDonald, Division of Tropical Crops and Pastures of CSIRO in Australia, and organized in CIAT with the collaboration of M. A. Franco, L. H. Franco, A. Schmidt, and M. J. Fisher **[P. C. Kerridge]**.

Activity: Review of collaborative research with *Desmodium ovalifolium*

A one day review meeting took place in CIAT during 1996 with 40 participants from different institutions (CORPOICA, CENICAFE, Fondo Ganadero del Valle, University of Höhenheim and CIAT) directly involved in the U. of Höhenheim-CIAT Project on *D. ovalifolium* or with an interest in this legume. The program included short presentations by participants on research achievements with *D. ovalifolium*, followed by a comprehensive discussion on major research issues related to the G x E Project and on opportunities for advancing superior genotypes of *D. ovalifolium* in Colombia **[A. Schmidt, R. Schultze Kraft, B. Maass and C. Lascano]**.

Activity : Edit and publish newsletters and research publications on tropical forages

During 1996 the two newsletters of the TFP Forage Networks (Savannas and MCAC) were sent to collaborators in Central and South America. In addition, we have prepared the first number of the Tropileche newsletters which well be distributed to collaborators in the two benchmark sites of the Project and to researchers in the region that are actively involved in dual purpose cattle research.

The journal Pasturas Tropicales continues to be an important media to communicate research results on Tropical Forages in the region. This year Pasturas Tropicales was selected by COLCIENCIAS as one the scientific publications that they will support financially, starting in 1997. This award by COLCIENCIAS to Pasturas Tropicales is a recognition to a publication that in spite of financial limitations has had continuity in CIAT and strong support from forage researchers throughout Latin America **[P. C. Kerridge, P. J. Argel, E. A. Pizarro, A. Ramirez, F. Holmann and C. Lascano]**.

Activity : Develop new linkages with NARS and advanced institutions with interest in dual purpose cattle systems

During the TROPILECHE Workshop representatives of Brazil (EMBRAPA-CNPGL), Bolivia (CIAT-Santa Cruz), Venezuela (U. Central de Maracay), Colombia (CORPOICA), Mexico

(Yucatan-London University) and from RISPAL (IICA), CONDESAN (CIP) and CIAT met to discuss the idea of using Tropileche as a platform (i.e. information exchange, short study leaves, exchange of models, on the job training, formulation and execution of joint research projects) for institutions and group of researchers interested in dual purpose cattle systems. The idea was well accepted and should be followed-up through constant communication (i.e. Tropileche newsletter) and visits by CIAT staff to further explore how we can best collaborate to get some concrete actions in motion **[P. C. Kerridge, C. Lascano, P. J. Argel, and F. Holmann]**.

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Donors - Supplementary Funding[1]

Donor/Project	Project Duration	Project Total (To CIAT) USD
Complementary:		
Australia-AusAID		
Forages for Smallholders	1995-1999	3,268,000 (1,634,000)
Australia -ACIAR		
Anthracnose resistance in Stylosanthes	1994-1996	170,000 (57,000)
Austria - Academy of Sciences		
Mechanisms of acid soil tolerance	1994-1996	52,884
Colombia - Gobierno Colombiano	1996	685,000
Mejoramiento de Brachiaria		
Mejoramiento de Arachis		
Mejoramiento de Stylosanthes		
Ecotipos con alta calidad Forrajera		
Atributos adaptativos a suelos ácidos		
Componentes para sistemas de producción		
Ecotipos forrajeros con adaptación ambiental conocida		
Mejoramiento de la base de recursos genéticos de pastos tropicales		
Colombia - COLCIENCIAS	1996	3,500
Colombia - Agroganadera del Valle	1995-1996	8,500
Evaluación de germoplasma de leguminosas arbustivas promisorias en la zona norte del Cauca		
Colombia-Nestlé	1995-1999	544,960
Pilot development program - Caquetá		
Colombia-Fedegan	1995-1999	140,000
Resistance to spittlebug in grasses		
Colombia-CORPOICA		
Biology and comparative ecology spittlebug	1996-1998	72,250
Germany - BMZ		
Desmodium-genotype x environment	1995-1997	230,400 (103,400)
Great Britain-ODA	1991-1994	(25,000)
Anti-quality factors in legumes (with IGER).		
Great Britain - OFI		
Leucaena research (Philippines and Central America)	1996-1998	31,000
Japan - JIRCAS	1995-1999	1,000,000
The role of endophytes in tropical grasses		
Systemwide Livestock Program	1996-1998	2,555,000 (1,200,000)
Legume-based forage systems for dual-purpose cattle[2]		

[1] Funds available to the TFP are indicative as some funds are distributed to other organizations.
[2] Not funds are available.