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September 14, 1989

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Dr. Kevin A. Rushing
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Manila

Dear Dr. Rushing:

This is to submit progress report for the first year of project 936-5542.08 entitled "Improving performance of grazing dairy cattle through biological control of bovine ticks using stylosanthes spp."

Thank you.

Very truly yours,

L.T. TRUNG
Assistant Professor &
Project Leader

cc: AID/SCI, Rm. 320, SA-18
AID Reference Center
PPC/CDIE/DI, Rm. 105, SA-18

Joyce Turk, Technical Backstop Office
AID, S and T, Rm. 420, SA-18

ANNUAL REPORT

USAID PROJECT 936-5542.08 "IMPROVING THE PERFORMANCE OF GRAZING DAIRY CATTLE THROUGH BIOLOGICAL CONTROL OF BOVINE TICKS USING STYLOSANTHES SPP"

SUMMARY

During the first project year, studies 1, 2 and 4 have fully been implemented using revised methodology. Study 3 is to commence awaiting the arrival of stylo seeds ordered from Australia, hopefully by November, 1989.

Among eight species/cultivars of Stylosanthes tested, S. scabra (seca and CPI cv 55860), S. viscosa and S. guianensis cv IPB 03 were found very effective in trapping tick larvae (55 to 65% of larvae was trapped). For other legumes, Pueraria phaseoloides was found to also have the ability to trap larvae. Among 12 grass species tested, Pennisetum purpureum and Andropogon gayanus were found to have the highest larvae trapping rates. While the stylos trapped tick larvae mostly at their stems, other legumes and the grasses trapped larvae at their leaf blades. No clear-cut relationship between trapping rate and the morphological characteristics of the stylos was found, although the climbing rate of larvae was negatively correlated with bristle density.

Significant ($P/.05$) improvement in dry matter yield of grasses was observed when N-fertilizer was applied at the rate of 60 kg/ha/yr. No further increase was obtained as this rate was doubled. Andropogon gayanus and Panicum maximum had higher ($P/.05$) yield compared to Setaria splendida and Brachiaria mutica. Setaria and Andropogon were the lowest in DM and ash contents, respectively. In vitro digestibility was better in favor of para and setaria grasses.

The stylo species/cultivars under study did not respond to phosphorus fertilization in terms of dry matter yield. S. guianensis was found better than S. scabra in dry matter yield. Compared to S. guianensis, higher dry matter but lower crude protein contents were found with S. scabra.

OBJECTIVES

Only objectives in connection with this report are hereunder stated. Other objectives which have not been achieved within the first project year can be found in the proposal.

1. To identify stylo, other legume and grass species able to trap tick larvae;
2. To determine morphological traits of stylo/grass associating with trapping larvae; and
3. To determine yield and chemical composition of stylo and grass species grown singly in plots with varying fertilization rates.

MATERIALS AND METHODS

The revised methodology for the whole project has been described in the semi-annual report, hence not repeated here. Only those involved in the accomplishments during the first year will be mentioned subsequently.

Study 1. Tick larvae trapping characteristics of selected stylos and grasses.

Experiment 1a. Screen test for trapping/repelling characteristics of selected stylo and grass species

Eight stylo species/accessions, five other legume species and 12 grass species replicated 5 times were used in this experiment. The stylos were 65 days old while the other legumes and grasses, 45-day regrowth. The materials for six stylo and four grass species obtained

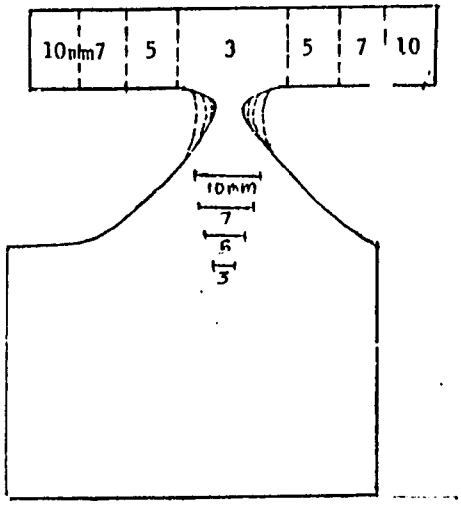
used in study 2 were from 2mx 5m experimental plots while the other legume and grass species from DTRI's 2m x 5 m demonstration plots.

Eggs of B. microplus were obtained from a culture maintained at the Dairy Training and Research Institute, UPLB. Batches of 1000 eggs (0.05 g of eggs) were held in plastic vials (3 x 1.5 cm) and incubated at 28°C, 95% RH until the larvae were 10 days old. Each vial was covered with nylon gauze to provide ventilation and to prevent larvae from escaping (Fig 1a).

The capacity of different stylo, grass spp. to trap larvae of B. microplus was tested using a standard bioassay technique (Sutherst et al, 1988). The plastic culture vials containing 1000 tick larvae were suspended from plant stems in a paper cup attached near the stem base by a bridge (Fig 1b). Larvae climbed up the bridge onto the stem. The number of larvae that were free or had been trapped on each third of the stem was recorded after 24 hr.

The bioassays were assembled on sheets of polystyrene foam (Fig. 2) surfaced with black cloth. Each board was divided into 9 tick-proof squares of 22 x 22cm with double-sided adhesive tape. A hole 2.5 cm in diameter and 3 cm deep was cut in the center of each area to hold a water-filled glass tube (7.5 cm long x 2.5 cm diameter) supporting a test stem (30-40 cm long for the stylos or the whole plant for the grasses). The stem was wedged into the tube by a cork with a vertical groove cut into its side.

After 24 hours, untrapped larvae were separated from the plant material by using separation jars (Fig 3). These were clear plastic jars (6 cm D x 5 cm H) with screw-on lids into which a brass mesh



1b. Paper cup design showing varying neck sizes depending on the stem thickness.

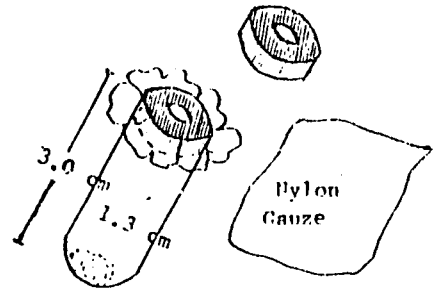


Fig. 1a. Plastic vial and cover

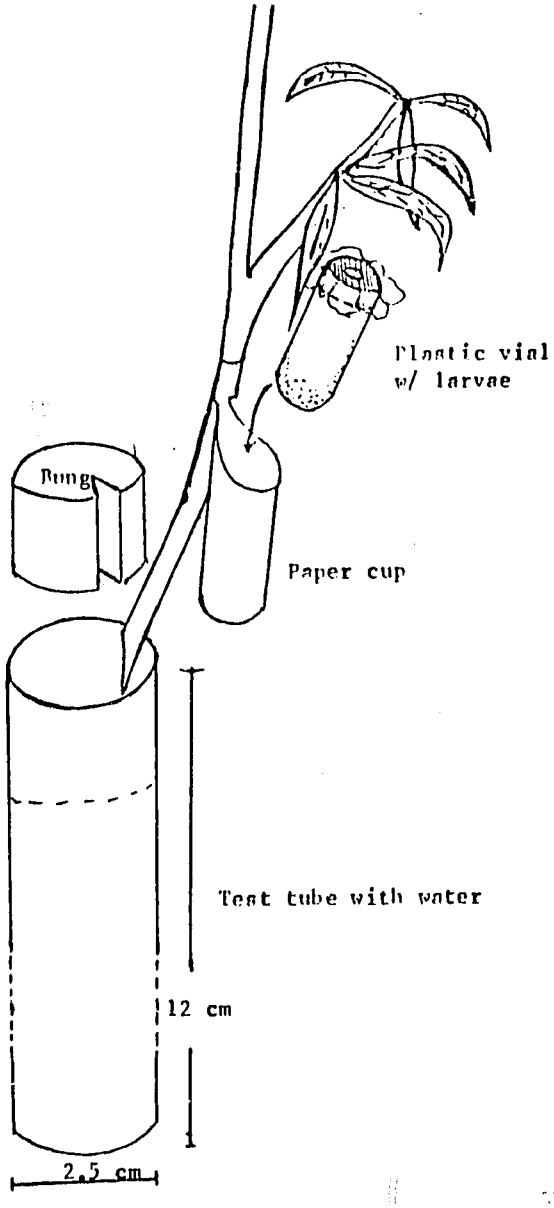


Fig. 1c. Plant testing set-up

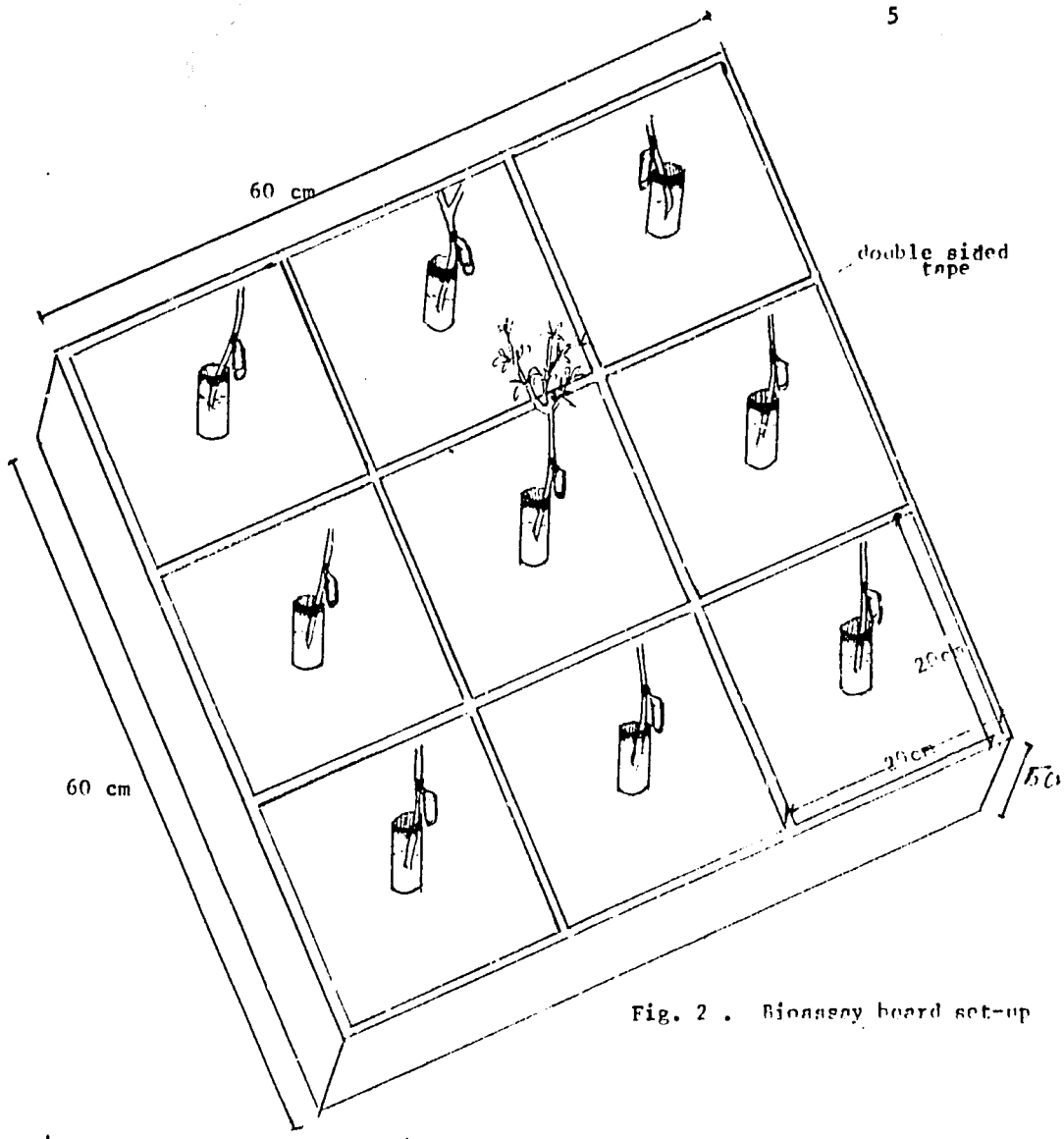


Fig. 2 . Bioassay board set-up

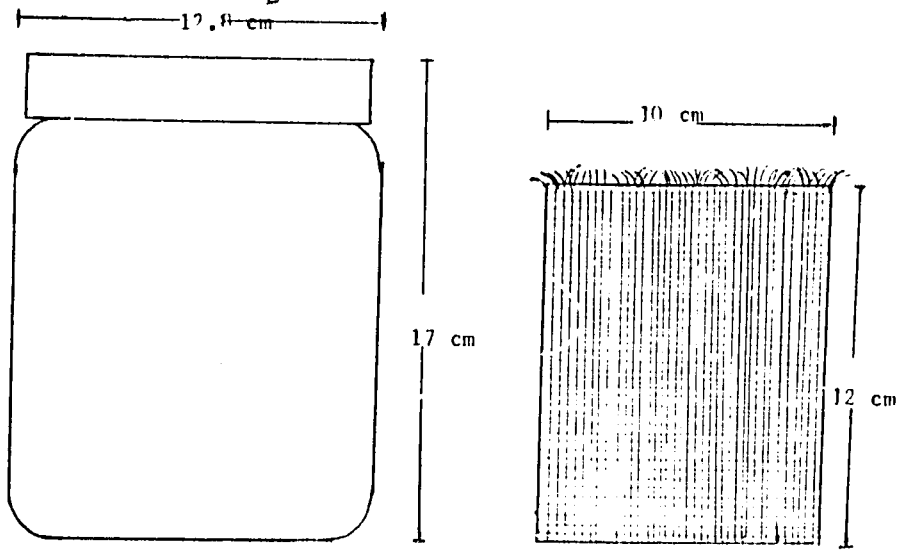


Fig. 3. Separation jar

basket (2 x 2 mm mesh, 5 cm high) was pushed a tight fit held it in place (Fig. 3). The stem was cut into 3 equal lengths and each section was placed into a separate jar. Vapona (8 drops) was put on a 1.5 cm piece of chalk which was placed in the mesh basket with the sample before the jar was capped.

The vapona took about 5 min to kill the larvae. After removal of the chalk, the jar was recapped and dropped 15 times from a height of 10 cm to dislodge all free larvae, which fell through the mesh basket and collected in the bottom of the separating jar. Trapped larvae remained on the plant stem. Free and trapped larvae were either counted immediately or stored separately in 70% ethanol until counted.

The stickiness of stem was assessed by pressing the stem between thumb and forefinger and a value of 0 was assigned to glabrous or non-sticky stems, 1 to stems with few trichomes and slightly sticky feeling, 2 to stems with a distinct tacky feeling, 3 to stems which were very tacky, and 4 to stems which were extremely tacky and almost coated with sticky secretion. Three people rated each stem independently, with good agreement. The mean rating was used in the analyses.

Morphological features were determined using a hand lens and a stereomicroscope. There were two distinct types of trichomes: stout glandular bristles, and soft thin hairs which appeared non-glandular. Both varied in density and distribution, and were described separately. The scores were:

Bristle length: the actual measurement (mm) were taken. Two bristles each from the old part and from the young part of a stem were measured against a ruler under a stereomicrospope.

Bristle location: Young and old parts of stems from representative plants were observed for the presence (+) or absence (-) of bristles. From the data collected, the bristle distribution was determined, whether they are only on the young part, only on the old part or on both young and old parts.

Bristle internode distribution: 1, all internodes; 2 upper only: 3, low mostly; 4, none.

Bristle density: The actual number of bristles on a 1 cm length of the stem, both of the young and of the old parts were counted and recorded.

Hair density: 1, none; 2, sparse; 3, medium; 4, dense

Hair distribution: 1, all over stem; 2, one side mostly; 3, scattered; 4, None.

Plant Habit: 1, erect; 2, ascending; 3, semi-erect; 4, prostate; 5, bushy.

Study 2. Productivy of tick-killing stylo spp in pure and mixed vegetations.

This study is consisted 3 experiments, viz. 2a involving pure grass stands, 2b with pure stylo stands and 2c with mixed vegetations. Only partial results of the first two experiments are available in this write-up.

In experiment 2a, four species of grass(Guinea, Para, Setaria and Andropogon) and three nitrogen fertilization levels (0, 60 and 120 kg/ha/yr) were tested in a 4 x 3 factorial experiment in a RCB design, using 2m x 5m plots as experimental units, replicated 3 times.

In experiment 2b, six species/cultivars of stylo and three phosphorus fertilization rates (0, 60 and 90 kg/ha/yr) were tested in a 6 x 3 factorial experiment in a RCB design using 2m x 5m plots replicated 3 times.

The grasses were cut at 60 days after establishment and 45 days thereafter while the stylos, 3 months after establishment and 45 days thereafter.

Sampling of each plot was done at harvest time replicated 3 times using a 1 x 1m quadrat. The samples were dried at 60°C and then ground for dry matter, crude protein (A.O.A.C., 1970), neutral detergent fiber (Goering and Van Soest, 1970) and in vitro dry matter digestibility (Minson and McLeod, 1972).

Analysis of variance was used to detect differences among the treatment means for yield while analysis of covariance for data on larvae climbed/trapped using either total larvae count or larvae climbed as covariable. Data on percentage were transformed into arcsine before analysis.

HIGHLIGHTS OF ACCOMPLISHMENTS

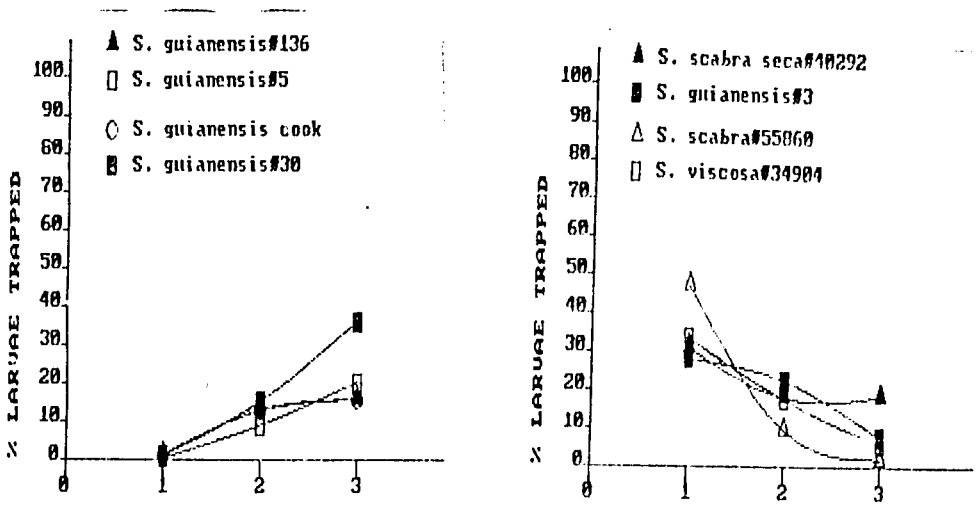
Study 1 - Experiment 1a. Tick larvae trapping/repelling characteristics of selected legume and grass species

Results of the bioassay on tick larvae trapping/repelling characteristics of the stylos are shown in Table 1a. All the cultivars/species of stylos examined were able to trap tick larvae; relatively higher trapping rates were observed among the cultivars of S. scabra and S. viscosa compared to S. guianensis (except for IPB cv #3).

A correlation analysis (Table 1b) was run to determine the relationship between trapping and those morphological characteristics that were objectively determined, i.e. the bristle length and density. While bristle density had a negative correlation ($r = -0.41$ with $P \leq .01$) with the number of larvae that climbed up the plant, the trapping ability appeared to be negatively correlated with bristle length ($r = -0.44$ with $P \leq .01$). No relationship was found between trapping rate and bristle density. The unexpected relationship between trapping rate and bristle length can be explained by the fact that the bristle length of scabra and viscosa is only 1/2 of that of guianensis, yet much higher trapping was found with the former. This relationship therefore is considered coincident and should not be generalized. There is a need to look into anatomical and chemical properties of the bristle to be able to establish more meaningful relationship with tick larvae trapping rate.

Examining the larvae distribution among the cultivars of S. scabra, and S. guianensis cv IPB #3, S. viscosa (Fig. 1), one would note that most of the trapping took place right at the basal 1/3 of the plant (the part just above the smooth old base). Trapping

TICK LARVAE TRAPPED



FREE

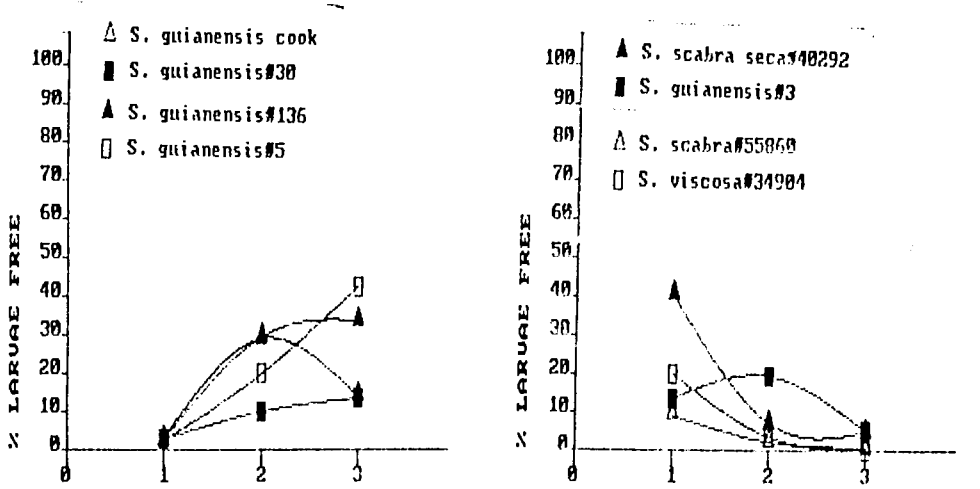


Fig. 4- Distribution of free and trapped larvae in 8 species/cultivars of stylosanthes.
(x-axis: 1- basal 1/3; 2- middle 1/3; 3- top 1/3 of each plant specie)

Table 1a. Tick larvae trapping rate and morphological characteristics of selected species/cultivars of stylosanthes

Species/ Cultivar	Larvae		Bristle Scores				Hair	Distrbn.	Stickiness	Plant Habit
	% 1/ trapped	No. on 2/ plant	Density (No/cm)		Length, mm		Density			
			Top	Base	Top	Base				
S. scabra										
seca, CP#40292	67.4	720	18	0	1.0	-	3.5	1	3	1
CPI#55860	58.3	583	56	7	1.0	1.0	3.5	1	4	1
S. viscosa #34904	53.1	649	532	41	1.0	1.0	3	1	4	5
S. guianensis										
IPB #3	58.3	791	185	51	2.0	2.0	2.5	2	2	2
#5	29.7	725	65	39	2.5	2.0	2.5	2	1	2
#30	51.3	612	118	58	2.5	2.0	2.5	2	1	2
CIAT#136	39.0	702	119	19	2.2	2.0	2.5	2	2	2
Cook	29.9	616	74	59	2.2	2.0	2.5	2	1	2

1/Adjusted to a common number of larvae climbed.

2/Adjusted to a common number of larvae released to plants

Table 1b. Correlation coefficients between number of larvae climbed/trapped and bristle density/length

	Larvae Trapped	Larvae Climbed
Bristle density	0.07^{ns}	-0.41**
Bristle length	-0.44**	0.15^{ns}

ns - non significant; ** - P/.01

continued to take place at the middle and upper portions of the plants such that at the tip 1/3, very minimal number of free larvae were counted. Since the exposure period was only 24 hr, it is postulated that had longer time been given to those larvae still at the base to climb up, much higher trapping rates would have been recorded among these cultivars.

For the guianensis cultivars, except for CV cv IPB#3, the trapping was not effective as indicated by much lower trapping rates at the basal 1/3 and much greater percentages of free tick larvae at the top 1/3 of the plants.

For other legumes and grasses, results are shown in Table 2. Among the other legume species tested, Pueraria phaseoloides (tropical Kudzu) was found with highest trapping rate (51.7% of the larvae on plant was trapped). For the grasses, Andropogon gayanus topped the list with 30% followed by Pennisetum purpureum with 27.4%. It was noted that unlike stylo wherein trapping took place at the stem, other legumes and grasses trapped larvae at the leaf blade.

It is suggested that characteristics of trapping may differ among the plant genera. It may be accomplished through simple physical contact in one genus while it may take chemical or physico-chemical reaction in another. This is a very fertile area of research to look into.

Table 2. Tick larvae trapping rate of selected legumes and grasses

Species	% Trapped	Larvae on Plant	Total Larvae
A. Other Legumes:			
1. *Centrosema pubescens*	9.0	863	1020
2. *Desmodium intortum*	25.1	1032	1085
3. *Lablab purpureus*	15.5	541	710
4. *Marcoptilium atropurpureum*	10.5	667	741
5. *Pueraria phaseoloides*	51.7	740	808
B. Grasses:			
1. *Andropogon gayanus*	30.0	852	930
2. *Brachiaria brizantha*	17.0	693	740
3. *Brachiaria decumbens*	17.3	1165	1186
4. *Brachiaria ruziziensis*	16.1	735	802
5. *Brachiaria mutica*	19.6	707	812
6. *Cenchrus ciliaris*	7.2	609	645
7. *Cynodon plectostachyus*	2.9	572	647
8. *Panicum maximum*	18.4	689	790
9. *Pennisetum purpureum*	27.4	776	942
10. *Setaria sphacelata*	1.0	555	627
11. *Setaria splendida* (Phil)	10.1	679	700
12. *Setaria splendida* (Thai)	12.1	823	853

Study 2 - Productivity of tick-killing stylo spp in pure and mixed vegetations

Experiment 2a - Pure grass stands

Dry matter yield of four grass species at three N fertilization rates is shown in Table 3.

Table 3. Dry matter yield (kg/ha) of four species of grass at three levels of N fertilization (Ave of 2 harvests)

N-fertilization (Kg/ha/yr)	0	60	120	Ave
Para grass	3381.8	3468.0	4249.2	3702.9[c]
Guinea grass	4301.3	5671.6	5748.3	5241.5[ab]
Setaria grass	4203.9	4187.2	4577.7	4322.9[c]
Andropogon	4873.0	5636.0	5895.6	5466.8[a]
Ave	4190.0[b]	4740.0[a]	5121.3[a]	

Means for the main effects within row/column without a common superscript are different ($P \leq .05$)

Significant ($P \leq .05$) improvement in dry matter yield over the control was observed when N-fertilizer was applied at the rate of 60 kg/ha/yr. Doubling this rate did not further increase yield. No significant interactions between species and fertilization level was detected. Andropogon and guinea grasses were found to be better ($P \leq .05$) in dry matter yield compared to setaria and para grasses.

Chemical composition and digestibility of the grasses are presented in Table 4. While Setaria was noted to have relatively less cell wall content and much less dry matter content compared to the other three species, Andropogon was the lowest in mineral analysis. Crude protein was slightly higher with para grass, in vitro digestibility was in favor of para and setaria grasses. Nitrogen fertilization did not appear to influence the quality traits examined in this research.

Dry matter yield of five species/cultivars of stylosanthes as affected by 3 levels of phosphorus fertilization is shown in Table 5. The trend would show negative response to P fertilization. The yield of S. guianensis cultivars was better than that of S. scabra cultivars. Because of establishment problem, yield of S. viscosa is not yet available. Data presented in table 5 have not been treated statistically and it is too early at this point of time to draw any conclusion.

Partial results on chemical analysis of the stylos are shown in Table 6. While the scabra cultivars were higher in dry matter content, the Guianensis had higher protein content.

Other experiments

Experiments 1b, 1c and 2c have all been established. No data are yet available at this time.

Study 3 is to commence as soon as pastures are established. Seeds have been ordered from Australia and pasture establishment is tentatively scheduled in November, 1989.

Table 4. Chemical composition and digestibility of the experimental grasses.

GRASS	DM	ASH	CP	NDF	IVDMD	IVOMD
	(% as is)	% Dry Matter Basis				
Para grass						
0	21.41	12.08	7.86	70.32	45.81	45.21
60	20.89	12.24	8.06	70.08	45.27	44.42
120	20.33	12.91	6.83	70.19	45.29	44.26
Guinea grass						
0	26.21	12.34	6.22	72.33	41.04	41.44
60	25.10	12.44	5.93	73.12	41.04	40.33
120	23.79	12.74	6.76	72.32	41.04	40.90
Setaria						
0	13.84	13.82	6.22	68.04	47.83	45.36
60	12.57	14.18	6.72	67.28	47.53	45.81
120	12.05	14.16	6.38	68.32	47.13	44.40
Andropogon						
0	23.60	8.70	6.20	73.32	40.80	40.76
60	22.71	9.35	7.06	69.84	41.40	41.13
120	23.24	8.92	6.86	71.66	39.88	39.04

Table 5. Dry matter yield (kg/ha/yr) of 6 stylo species/cultivars at three levels of phosphorus fertilization (first harvest only)

Phosphorus level (kg/ha/yr)		0	60	90	Ave
S. scabra	CPI #34925	2939.3	1954.7	2362.5	2418.8
Seca	CPI #40292	1471.0	1863.6	1132.4	1489.0
S. guianensis	IPB#30	3040.0	3224.4	2980.9	3081.8
"	CIAT#136	3044.9	2651.7	2843.8	2846.8
"	IPB#3	3883.2	4308.2	3374.5	3855.3
Ave		2875.7	2500.5	2538.8	

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Table 6. Chemical composition of selected species/cultivars of *stylosanthes*

Species	P. Fert'n (K_{II}/ha/yr)	DM % as is	CP % dry matter	Ash % dry matter	NDF % dry matter
S2. *S. scabra* #55860	0	20.24	13.35	8.76	49.04
	60	18.69	13.66	9.30	49.72
	90	16.76	12.52	7.48	46.95
S3. *S. scabra seca* #40292	0	19.29	14.74	8.36	50.53
	60	21.38	12.31	8.48	48.07
	90	21.96	14.68	8.50	48.94
S4- *S. guianensis* #30	0	13.89	19.14	7.46	47.90
	60	14.80	18.48	8.94	47.33
	90	14.16	18.17	8.55	46.11
S5- *S. guianensis* #126	0	14.60	18.75	9.14	47.18
	60	14.28	17.85	8.85	47.96
	90	13.30	17.16	9.56	46.79
S6- *S. guianensis* #3	0	15.54	17.98	8.78	48.50
	60	17.54	18.09	7.91	49.11
	90	15.57	18.35	8.25	49.68

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