Morphological, phenological and reproductive trait analysis for the pasture species, siratro (*Macroptilium atropurpureum*)

J.B. MORRIS
Plant Genetic Resources Conservation Unit, USDA, ARS, University of Georgia, USA

Abstract

Variation in agronomic traits among 66 siratro (*Macroptilium atropurpureum*) accessions being regenerated in Byron and Griffin, GA, USA during May – June 2000, 2005 and 2007 – 2008 was measured. At 50% maturity, individual plants of the accessions were evaluated for branching, foliage growth, height, diameter, days to maturity (DTM) and seed numbers. Most accessions displayed prolific branching and foliage growth. Accessions exhibiting early maturity (96 DTM) and high seed production (averaging 2429 seeds per accession) included PI 451726, PI 543331 and PI 543380. The Australian accession, PI 543332, matured later (121 DTM) than the above accessions but produced slightly smaller plants and the highest number of seeds (4480). The coefficient of variation was 109% for DTM and 169% for seed number, indicating high variability for these traits. Potential exists to develop cultivars which would mature early and produce high seed numbers. These accessions require further field evaluation in the south-eastern USA for pasture value (production and persistence) under grazing conditions and variation among the remaining 82 accessions in the collection should also be examined when the opportunity arises, possibly during regeneration cycles.

Introduction

Siratro (*Macroptilium atropurpureum*) is a self-pollinated diploid ($2n$ = 22) annual legume widely grown throughout the tropics and subtropics including Australia (Cameron 1985) and is adapted to many soil types and climatic conditions (Jones and Jones 1977). It is a perennial species when grown in tropical regions, but performs as an annual when grown in subtropical climates such as north-central Georgia, USA. It is a trailing, climbing and twining species with a deep and swollen taproot. While its primary use is for grazing, it can be cut for hay.

Beef cattle liveweight gains, breeder performance and calf weaning weights have been improved when siratro has been included in native or improved pastures in Australia, Uganda and Fiji (Walker 1977). When siratro was oversown into cleared native pasture or a fully sown grass-siratro pasture was established (with addition of superphosphate), cattle liveweight gain/ha increased 2- to 3-fold (Tothill *et al.*2008a). During wet years, siratro yields were 50% of total pasture yield, but in dry years, yield of the legume decreased below 10% (Tothill *et al.* 2008b). In semi-arid areas of Kenya, siratro produced higher dry matter yields than Wynn cassia (*Chamaecrista rotundifolia* cv. Wynn), Cook stylo (*Stylosanthes guianensis* cv. Cook), Verano stylo (*S. hamata* cv. Verano) and Fitzroy stylo (*S. scabra* cv. Fitzroy) (Njarui and Wandera 2004). Furthermore, siratro was found to be the best legume supplement to complement natural pasture hay consisting of *Hyparrhenia* grasses in Zimbabwe (Matizha *et al.* 1997). Protein concentration in siratro ranged from 68 to 266 g/kg dry matter (DM) (Topps and Oliver 1993; Norton and Poppi 1995). Siratro does not contain oestrogens, toxins (Bindon and Lamond 1966) or bloat-producing compounds that can cause adverse effects in cattle (Jones and Lyttleton 1971) and does not adversely affect meat flavour in lambs (Park and Minson 1972) or milk flavour in cows (Stobbs and Frazer 1971).

It can also be used for soil conservation, cover cropping and as a fallow crop. Siratro has also shown potential as a living mulch in banana plan-

Correspondence: Dr J.B. Morris, USDA, ARS, Plant Genetic Resources Conservation Unit, University of Georgia, 1109 Experiment Street, Griffin, Georgia, USA, 30223. E-mail: Brad.Morris@ars.usda.gov

tations because it has stimulated fast vegetative banana growth as well as banana bunch weights (Espindola *et al*. 2006).

Cultivars of siratro (cv. Siratro) currently in use are often lost from pastures, especially in subtropical areas, because of late flowering, which can prevent seed set with early frosts and removal of growing points through close grazing. While variation within siratro accessions for grazing traits (McDonald and Clements 2005) has been identified, accessions which are more resistant to grazing need to be identified.

One hundred and forty-eight siratro accessions originating from Australia, Belize, Brazil, Colombia, Costa Rica, El Salvador, Guatemala, Malawi, Mexico, Panama, Taiwan and Venezuela are conserved by the Plant Genetic Resources Conservation Unit, Agricultural Research Service, United States Department of Agriculture (USDA, ARS, PGRCU) (National Plant Germplasm System 2010). Most have not been evaluated and there are limited data or research results in the USA pertaining to siratro. Periodically, accessions are regenerated because of low seed numbers or low seed viability and this provides an opportunity to record data on the regenerating plants, which could be beneficial in identifying or breeding superior accessions.

The objectives of this study were to document the morphological, phenological and seed production traits of regenerated siratro accessions from the collection at Griffin, GA, USA during the 2000, 2005 and 2007–2008 regeneration cycles and to assess variability and relatedness of accessions using principal component and cluster analysis.

Materials and methods

The 66 siratro accessions studied were conserved at the USDA, ARS, Plant Genetic Resources Conservation Unit located in Griffin, GA, USA and had been derived from Australia, Brazil, Colombia, Costa Rica, El Salvador, Malawi, Mexico, Taiwan, Venezuela and the USA (Table 1). These accessions were selected from seed storage based on origin, low seed viabilities and low seed numbers for regeneration. Seeds of all accessions were planted in 6.4 cm x 7.0 cm jiffy pots (Hummert International, Earth City, MO) containing Metro Mix 200 potting soil (Scotts Sierra Horticultural Products Company, Marysville, OH) in March to early April of 2000, 2005 and 2007–2008 in a greenhouse maintained at 21–26ºC. After 60–75 days, siratro plants were transplanted to field regeneration plots located at the USDA, ARS in Byron and Griffin, GA, USA. Twenty-five to 50 plants of each accession were transplanted in a single 6 m row with 6 m between rows. Check accessions included: PI 543330 from Australia during 2000 in Byron, GA, USA; the high seed-producing accession, PI 322580 from Brazil during 2005 in Griffin, GA, USA; the medium yielding accession, PI 322581 from Brazil during 2007 in Griffin; and PI 543338 from Australia during 2008 in Griffin. Plots were irrigated with sprinklers as necessary.

Plant and seed characteristics were recorded each year from all accessions. The characters studied were recommended by the Crop Germplasm Committee (CGC) and included: branching, foliage growth, plant height, plant diameter, days to maturity (DTM) and seed numbers. These traits were recorded for all plants in each plot at 25–50% flowering. Branching and foliage growth were based on a scale of 1–5, where: 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69% and 5 = 50–59% of each plant producing branches and/or foliage based on visual observations. Plant diameter was measured using a graduated stick at the average plant width per row. Pods were harvested from all accessions as they matured, dried at 21°C/25% RH for approximately 1 week and threshed. Threshed seeds were then counted and the number of seeds per row was recorded.

Principal component analysis and PC SAS procedure CLUSTER analysis were then used for multivariate analysis of the data. PROC PRINCOMP (SAS Institute 2003) was performed for all traits. Eigenvalues and the percentage of variance explained by each principal component were also determined. PROC CLUSTER in SAS (SAS Institute 2003) was used for cluster analysis with the unweighted paired group method using mathematical averages (UPGMA) by specifying the AVERAGE option (SAS Institute 2003). The clustering method was used to verify the true genetic relationships among accessions. The UPGMA is a clustering algorithm commonly used for germplasm analysis. Standard errors and coefficients of variation were also determined to confirm variability using principal component analysis (SAS Institute 2003).

Results

Successful plant regeneration occurred for many accessions tested. Results of observations on morphological, phenological and reproductive characteristics for the different accessions are reported in Table 1. Wide variability in branching, foliage growth, plant height, plant diameter, DTM and seed number was observed. Branching and foliage growth ranged from 1 to 5, with an average of 1.3 for all accessions, and coefficients of variation of 76 and 72%, respectively (Table 1). Plant height ranged from 1 to 87 cm with most accessions producing plants averaging 36 cm tall. Plant diameter ranged from 10 to 294 cm, with an average of 182 cm and a coefficient of variation of 47%. Even though some plants had a final diameter of 260–290 cm and were closely crowded, it did not appear to have an impact on plant development. However, 12 accessions produced small plants, averaging 27 cm in diameter.

Five Mexican accessions (PI 451726, PI 543271, PI 543313, PI 543380 and PI 543378) and the Australian accession, PI 543331, matured early at an average of 97 DTM. Twenty-four accessions displayed intermediate maturity (mean of 136 DTM), while 3 accessions (PI 415781 from Taiwan and PI 543264 and PI 543281 from Mexico) were late maturing plants, averaging 211 DTM. The coefficient of variation for DTM was 109%, indicating a high amount of variation for this trait.

Accession PI 543332 produced the most seeds (4480 seeds), while 4 other accessions (PI 322579, PI 451726, PI 543350 and PI 543351) also produced high seed numbers (mean of 3578 seeds per accession). Thirteen intermediate seed producers averaged 905 seeds per accession, 8 low seed producers averaged 57 seeds and 34 accessions failed to produce any seed. The coefficient of variation for seed number was 169%.

Principal component analysis

Principal component analysis showed that the first principal component accounted for 42% of the total variation (Table 2). When principal components 2 and 3 were added progressively, the cumulative amount of variation accounted for was 67 and 82%, respectively. The first principal component was most correlated with branching, foliage growth, plant height and plant diameter (Table 3), while the second principal component, which accounted for 25% of the variation, was mostly related to DTM and seed number. The third principal component explained 15% of the variation and was composed primarily of plant height. Branching was significantly correlated with foliage growth ($r^2 = 0.83^{***}$, n = 66), plant height ($r^2 = -0.38^{**}$, n = 66) and plant diameter ($r^2 = -0.43^{**}$, n = 66), while foliage growth was significantly correlated with plant height ($r^2 = -0.27^{*}$, n = 66) and diameter ($r^2 = -0.31^{*}$, n = 66). Plant height was significantly correlated with plant diameter ($r^2 = 0.51^{***}$, n = 66) and plant diameter was correlated with DTM ($r^2 = 0.37^{**}$, n = 66). Days to maturity was significantly correlated with seed number ($r^2 = 0.52^{***}$, n = 66).

Average linkage cluster analysis grouped the original 66 accessions into well defined phenotypes with 3 distinct groups based on number of seeds produced (Group A – 42 accessions, Group B – 13 accessions and Group C – 11 accessions; Figure 1). However, 6 sub-groups could be identified within these 3 major groups on the basis of seed production, namely: zero seeds; very low seed numbers (16–130 seeds); low seed numbers (400–830 seeds); intermediate seed numbers (980–1500 seeds); high seed numbers (1870–2800 seeds); and very high seed numbers (3390–4480 seeds). Within Group A were Sub-group 1 and Sub-group 2 (34 accessions, which failed to produce any seed), and an outlier group (8 accessions, which produced an average of 57 seeds per accession). Group B contained intermediate seed-producing accessions (Sub-group 3 of 6 accessions, producing an average of 1225 seeds; and Sub-group 4 of 7 accessions, producing an average of 631 seeds). The high to very high seed-producing Group C contained Sub-group 5 (5 accessions, producing an average of 3758 seeds per accession) and Sub-group 6 (6 accessions, producing an average of 2359 seeds). Accessions clustered in Groups B and C were more closely related genetically than those in Group A. Using the distance values indicated in Figure 1, the groupings at any similarity level can be identified. For example, PI 543342 and PI 543362, which originated from Australia and Mexico, respectively, have a phenotypic distance index of 0.0722, which indicates their close morphological similarities. (According to Cam McDonald of CSIRO, Australia, PI 543342 was

Table 1. Variability in morphological, phenological and reproductive traits in 66 siratro accessions at 2 locations during 2000, 2005 and 2007 – 2008.

Accession Number (PI)	Australian Accession Number	Origin	Branching[1]	Foliage[1]	Plant height	Plant diameter	Days to maturity	Seed no.
					(cm)	(cm)	(DTM)	
2000 – Byron, GA								
508619		Brazil	1	1	50	150	na[2]	0
543292		Mexico	1	1	2	40	na	0
543330	CPI 84997	Australia	1	1	3	30	na	0
543352		Costa Rica	1	1	2	40	na	0
543353		Costa Rica	1	1	3	50	na	0
543354		Costa Rica	1	1	1	30	na	0
543365		Mexico	5	5	1	10	na	0
543368		Mexico	5	5	1	20	na	0
543374		Mexico	5	5	1	10	na	0
543377		Mexico	1	1	1	20	na	0
543387		Mexico	1	1	2	15	na	0
543391		Mexico	5	1	2	30	na	0
543393		Mexico	1	1	10	26	na	0
2005 – Griffin, GA								
322580		Brazil	1	1	50	150	150	24
415781		Taiwan	1	1	50	150	206	16
494129		Mexico	1	1	30	130	na	0
2007 – Griffin, GA								
322578		Brazil	1	1	50	150	na	0
322581		Brazil	1	1	50	150	na	0
383436		Malawi	1	1	50	150	na	0
2008 – Griffin, GA								
322579		Brazil	1	1	45	160	119	3390
451726		Mexico	1	1	87	135	99	3390
543262		Venezuela	1	1	17	197	137	1260
543263		Mexico	1	1	28	294	na	0
543264		Mexico	3	3	21	260	200	2560
543266		Mexico	3	3	40	230	171	30
543267		Mexico	1	1	40	240	108	1190
543270		Mexico	2	3	35	240	na	0
543271		Mexico	1	3	40	231	97	525
543272		Mexico	1	1	27	270	na	0
543273		Mexico	1	1	40	236	na	0
543277		Mexico	1	1	37	210	na	0
543278		Mexico	1	1	41	214	na	0
543279		Mexico	1	1	40	210	na	0
543280		Mexico	1	1	31	282	na	0
543281		Mexico	1	1	43	216	228	130
543283		Colombia	1	1	36	261	133	830
543290		Mexico	1	1	44	213	na	0
543291		Mexico	1	1	46	216	na	0
543296		Mexico	1	1	39	236	na	0

Accession Number (PI)	Australian Accession Number	Origin	Branching[1]	Foliage[1]	Plant height	Plant diameter	Days to maturity	Seed no.
					(cm)	(cm)	(DTM)	
543297		Mexico	1	1	36	241	na	0
543298		Mexico	1	1	40	250	137	720
543301		Mexico	1	1	32	231	na	0
543302		Mexico	1	1	34	230	na	0
543304		Mexico	1	1	30	231	na	0
543309		Mexico	1	1	27	229	164	110
543313		Mexico	1	1	21	236	97	400
543324		Mexico	1	1	36	180	na	0
543326	CPI 91101	USA	1	1	30	269	na	0
543331	CPI 87532	Australia	1	1	27	241	97	1870
543332	CPI 87546	Australia	1	1	12	165	121	4480
543333	CPI 87807	Australia	1	1	20	219	126	980
543335	CPI 87849	Australia	1	1	24	291	137	2700
543338	CPI 90337	Australia	1	1	22	115	143	700
543342	CPI 90748	Australia	1	1	35	226	137	1500
543350	5816	Colombia	1	1	14	157	140	3900
543351	1981-CQ 1282	Colombia	3	2	11	106	131	3630
543362		Mexico	1	1	32	270	137	1390
543369		Mexico	1	1	25	223	108	68
543375		Mexico	1	1	21	225	160	40
543378		Mexico	1	1	24	251	97	40
543379		Mexico	1	1	32	275	137	2800
543380		Mexico	1	1	24	231	93	2026
543384		Mexico	1	1	35	280	124	2200
543394		Mexico	1	1	12	220	140	490
543395		Mexico	1	1	18	270	137	1030
553015		Australia	1	1	25	251	137	750
s.e.			0.13	0.12	2.08	10.5	8.9	142.3
CV (%)			76	72	61	47	109	169

[1] Scale of 1–5 where 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69% and 5 = 50–59% of each plant producing branches or foliage on visual observation.
[2] Plants did not flower.

Table 2. Eigenvalues and the proportion of total variability among 66 siratro accessions (2000, 2005 and 2007–2008) as explained by the principal components.

Principal component	Eigenvalue	Proportion of variance explained	Cumulative %
1	2.5315	42.2	42.2
2	1.4940	24.9	67.1
3	0.9213	15.4	82.5
4	0.5182	8.6	91.1
5	0.3794	6.3	97.4
6	0.1553	2.6	100.0

Table 3. Eigenvectors and principal components for 6 traits in 66 siratro accessions based on regeneration data in 2000, 2005 and 2007–2008.

	Principal components					
Trait	1	2	3	4	5	6
Branching[1]	-0.51	0.32	0.29	0.03	0.03	0.73
Foliage[1]	-0.47	0.33	0.46	-0.14	0.06	-0.66
Plant ht (cm)	0.41	-0.06	0.62	0.55	-0.34	0.05
Plant diameter (cm)	0.47	0.12	0.43	-0.44	0.61	0.09
DTM[2]	0.28	0.61	-0.07	-0.42	-0.59	0.007
Seed number	0.19	0.61	-0.34	0.55	0.37	-0.04

[1] Scale of 1–5 where 1 = >90%, 2 = 80–89%, 3 = 70–79%, 4 = 60–69% and 5 = 50–59% of each plant producing branches or foliage on visual observation.
[2] Days to maturity.

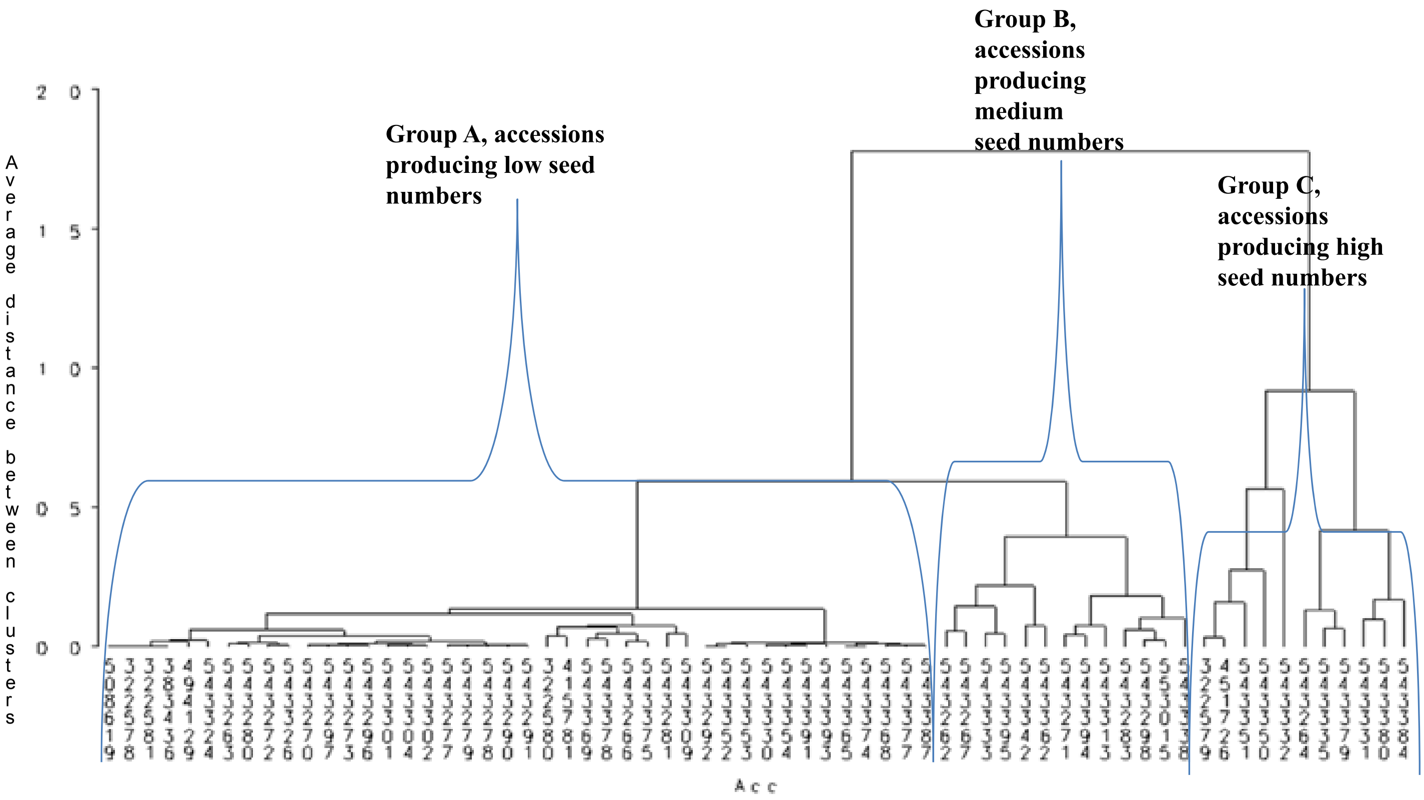
collected in Mexico, so it is not surprising they are closely related.)

Discussion

This study has highlighted the wide variability for important traits, including seed number, foliage growth and time to maturity, within accessions of siratro held by the Plant Genetic Resources Conservation Unit of the USDA. Generally, there was wide variation in all morphological, phenological and reproductive traits among the accessions which were studied, reflecting both genetic differences and the environments in which they were regenerated. With this degree of variation, there is obviously scope for selecting accessions for further testing, which are earlier maturing than cultivars currently in use (*e.g.* cv. Siratro) and which produce high seed numbers; these characteristics should enhance the chances of survival of this species under grazing. The ability to mature early would be a distinct advantage in subtropical areas, where early frosts could damage the legume before it had set seed.

The earliest maturing accessions (averaging 96 DTM), which included the Mexican accessions, PI 451726 and PI 543380, and the Australian accession, PI 543331, still produced an average of 2429 seeds per accession. However, PI 543332 from Australia matured at 121 days and produced the highest number of seeds (4480) per accession. Since seed production is an important trait to ensure the survival of the species, the 16 accessions which produced seed numbers in excess of 1000 during these evaluations are all worthy of further study. These 16 accessions outperformed the check accessions, PI 543330 and PI 543338, a normally high producing accession, PI 322580, and the medium yielding accession, PI 322581. In addition, they outperformed the Australian accession, PI 553015, in terms of seed production and DTM. Despite growing to a significant size (25 cm high and 251 cm diameter), this accession produced only 750 seeds/row. None of the 13 accessions grown in Byron in 2000 or the 3 grown at Griffin in 2007 reached maturity and set seed because they require longer

Figure 1. Dendrogram of distance between clusters based on morphological, phenological, and seed reproductive differences. Accession numbers are given (Acc). Values on the baseline indicate average phenotypic distances between accessions.



daylight hours and freedom from frost during the fall/autumn.

Clements (1989) indicated that numbers of siratro plants in grazed pastures declined greatly owing to the removal of growing points on the siratro plants, leading to reduced regrowth following grazing, lower seed production and depressed regeneration. Siratro accessions with extensive branching characteristics may help alleviate this problem. Since most of the siratro accessions studied produced high levels of branching, they could possibly be more resistant to grazing than existing cultivars in subtropical and tropical areas. Most of the accessions displayed prolific branching and only 4 Mexican accessions including PI 543365, PI 543368, PI 543374 and PI 543391 produced scores of 5, denoting poor branching. Wide ranges in branching characteristics were also observed by McDonald and Clements (2005). Although the variability for plant height was lower than the variability for branching and foliage growth, plant height still showed sufficient variability to warrant further study among these siratro accessions.

Principal component analysis findings suggest that potential exists to develop cultivars with improved architecture, early or late maturity and high seed yield. The cluster analyses conducted on the data clearly separated low seed-producing accessions from those accessions producing intermediate to high seed numbers. The analysis revealed tighter clustering among the poor seeders than among the high seeders, which indicates greater genetic variability in the intermediate and high seed-producing accessions.

Our findings support an earlier report (McDonald and Clements 2005) that several accessions with superior grazing and survival traits to cv. Siratro existed. While our study revealed sufficient variability in the 66 siratro accessions studied to justify selection for more suitable cultivars for subtropical areas, these accessions represent less than half of the genetic resources held by the Plant Genetic Resources Unit of the USDA. Examination of the remaining 82 accessions in the siratro collection, especially for early flowering time, seed yield and branching, and further examination of those accessions used in this study, including grazing studies, seem warranted to develop new cultivars for use as pasture legumes in these regions of the world.

References

Bindon, B.M. and Lamond, D.R. (1966) Examination of tropical legumes for deleterious effects on animal reproduction. *Proceedings of the Australian Society of Animal Production,* **6**, 109–116.

Cameron, D.G. (1985) Tropical and subtropical pasture legumes 5. Siratro (*Macroptilium atropurpureum*): the most widely planted subtropical legume. *Queensland Agricultural Journal*, **111**, 45–49.

Clements, R.J. (1989) Rates of destruction of growing points of pasture legumes by grazing cattle. *Proceedings of the XVI International Grassland Congress*, *Nice, France*, *1989.* **2**, 1027–1028.

Espindola, J.A.A., Marinho Guerra, J.G., Perin, A., Teixeira, M.G., De Almeida, D.L., Urquiaga, S. and Briancon Busquet, R.N. (2006) Banana plants intercropped with perennial herbaceous legumes used as living mulches. *Pesquisa Agropecuaria Brasileira*, **41**, 415–420.

Jones, R.J. and Jones, R.M. (1977) The ecology of siratro-based pastures. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* (CSIRO: Melbourne).

Jones, W.T. and Lyttleton, J.W. (1971) Bloat in cattle. XXXIV. A survey of legumes that do not produce bloat. *New Zealand Journal of Agricultural Research*, **14**, 101–107.

Matizha, W., Ngongoni, N.T. and Topps, J.H. (1997) Effect of supplementing veld hay with tropical legumes *Desmodium uncinatum*, *Stylosanthes guianensis* and *Macroptilium atropurpureum* on intake, digestibility, outflow rates, nitrogen retention and live weight gain in lambs. *Animal Feed Science Technology*, **69**, 187–193.

McDonald, C.K. and Clements, R.J. (2005) Variation within the species *Macroptilium atropurpureum* regarding adaptation to grazing. *Tropical Grasslands*, **39**, 237.

National Plant Germplasm System (2010) Germplasm Resources Information Network (GRIN). Database Management Unit (DBMU), National Plant Germplasm System, United States Department of Agriculture, Beltsville, MD.

Njarui, D.M.G. and Wandera, F.P. (2004) Effect of cutting frequency on productivity of five selected herbaceous legumes and five grasses in semi-arid tropical Kenya. *Tropical Grasslands*, **38**, 158–166.

Norton, B.W. and Poppi, D.P. (1995) Composition and nutritional attributes of pasture legumes. In: D'Mello, J.P.F. and Devendra, C. (eds) *Tropical Legumes in Animal Nutrition*. pp. 23–48. (CAB International: Wallingford, UK).

Park, R.J. and Minson, D.J. (1972) Flavour differences in meat from lambs grazed on tropical legumes. *Journal of Agricultural Science*, **79**, 473–478.

SAS Institute (2003) *SAS user's guide.* (SAS Institute: Cary, NC).

Stobbs, T.H. and Frazer, J.S. (1971) Composition and processing quality of milk produced from cows grazing some tropical pasture species. *Australian Journal of Dairy Technology*, **26**, 100–104.

Topps, J.U. and Oliver, J. (1993) Animal foods of central Asia. *Technical Handbook No. 2*. pp. 81–83.

Tothill, J.C., McDonald, C.K., McHarg, G.W. and Hargreaves, J.N.G. (2008a) Development options in *Heteropogon contortus* grasslands in south-east Queensland: Tree killing, legume oversowing and pasture replacement. 2. Animal production. *Tropical Grasslands*, **42**, 152–169.

Tothill, J.C., McDonald, C.K., McHarg, G.W. and Hargreaves, J.N.G. (2008b) Development options in *Heteropogon contortus* grasslands in south-east Queensland: Tree killing, legume oversowing and pasture replacement. 1. Pasture production and composition. *Tropical Grasslands*, **42**, 129–151.

Walker, B. (1977) Productivity of *Macroptilium atropurpureum* cv. Siratro pastures. *Tropical Grasslands*, **11**, 79–86.

(*Received for publication April 3, 2010; accepted November 14, 2010*)