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Editorial Support
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Email: CIAT-TGFT-Journal@cgiar.org

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Research Paper

Evaluation of herbaceous legumes for crop-livestock systems in eastern Indonesia

Evaluación de leguminosas herbáceas para sistemas cultivos-ganadería en el este de Indonesia

KENDRICK COX[1], JACOB NULIK[2], NEAL DALGLIESH[3], ESNAWAN BUDISANTOSO[4], PASKALIS FERNANDES[5], JEFRIANUS PRAING[6], PHILIP DIDA[7], JAK URAN[5], THE LATE MARCEL MEOMUKU[7], DEBORA KANA HAU[8], TONY BASUKI[8] AND LINDSAY BELL[9]

[1]*Department Primary Industries, Queensland, Australia.* daf.qld.gov.au
[2]*National Research and Innovation Agency, Kupang, Indonesia.* brin.go.id
[3]*Formerly Commonwealth Scientific and Industrial Research Organisation, Toowoomba, Qld, Australia.* csiro.au
[4]*Private consultant for Livestock and Cattle Breeding Investment (Agroforestry), Jakarta, Indonesia.*
[5]*Department of Agriculture Agricultural Technology and Assessment, East Nusa Tenggara, Kupang, Indonesia.*
[6]*Department of Agriculture and Food, East Sumba Regency, Waingapu, Indonesia.*
[7]*Department for the Application of Agricultural Intrument Standards, Kupang, Indonesia.* ntt.bsip.pertanian.go.id
[8]*Research Organization for Agriculture and Food, National Research and Innovation Agency, Bogor, Indonesia.* brin.go.id
[9]*Commonwealth Scientific and Industrial Research Organisation, Toowoomba, Qld, Australia.* csiro.au

Abstract

The sale of cattle presents a significant opportunity to improve livelihoods for smallholder farmers in eastern Indonesia. An opportunity was identified to grow herbaceous forage legumes either in rotation with, or sown within, staple grain crops (maize, rice) to use surplus soil moisture to produce a feed bank for (mostly penned) cattle and potentially increase nitrogen supply to the grain crops. A series of experiments was conducted on Vertisol, Alfisol and Inceptisol soils in lowland and upland districts of eastern Indonesia to identify legumes from 18 taxa suitable for integrated crop-forage systems. *Clitoria ternatea* and *Centrosema pascuorum* were found to have best potential for these systems as they established reliably using local methods, consistently ranked highly for herbage yields 2–4 months after sowing and were relatively easy to harvest. Yields were highest on the Vertisol (greatest number of sites) and Inceptisol soils. *Lablab purpureus*, *Macroptilium bracteatum* and *Mucuna pruriens* also ranked high for yield on Vertisol soils. *Clitoria ternatea* regrew consistently after cutting and forage removal providing potential for extended forage production as growing conditions allow.

Keywords: Cattle, centro, clitoria, forage.

Resumen

La venta de ganado representa una oportunidad importante para mejorar los medios de vida de los pequeños agricultores en el este de Indonesia. Se identificó una oportunidad para cultivar leguminosas forrajeras herbáceas en rotación con, o sembradas en asocio, con cereales (maíz, arroz), para utilizar la humedad excedente del suelo para producir un banco de alimento para el ganado (en su mayoría estabulados) y potencialmente aumentar el suministro de nitrógeno a los cultivos de cereales. Se realizó una serie de experimentos en suelos Vertisol, Alfisol e Inceptisol en distritos de tierras bajas y altas del este de Indonesia para identificar leguminosas de 18 taxones adecuadas para sistemas integrados de cultivos y forrajes. Se encontró que *Clitoria ternatea* y *Centrosema pascuorum* tenían el mejor potencial para estos sistemas, ya que

Correspondence: Kendrick Cox, DPI, 28 Peters Street, Mareeba,
Queensland 4880, Australia. Email: Kendrick.cox@daf.qld.gov.au

se establecieron de manera confiable utilizando métodos locales, ocuparon un lugar destacado en cuanto a rendimiento de forraje de 2 a 4 meses después de la siembra y fueron relativamente fáciles de cosechar. Los rendimientos fueron más altos en los Vertisoles (mayor número de sitios) e Inceptisoles. *Lablab purpureus*, *Macroptilium bracteatum* y *Mucuna pruriens* también obtuvieron una alta calificación en cuanto a rendimiento en Vertisoles. *Clitoria ternatea* fue consistente en rebrotar después del corte y la extracción del forraje, lo que brinda potencial para una producción prolongada de forraje cuando se presentan condiciones adecuadas para su crecimiento.

Palabras clave: Centrosema, clitoria, forraje, ganado.

Introduction

In many tropical countries, including Indonesia, the low yield and poor nutritive value of forages limits the productivity of cattle and other ruminants. Legumes have a demonstrated capacity to improve animal productivity in the tropics through the provision of high-quality feed (particularly digestible protein) while increasing the level of plant available nitrogen in soil for companion forages through symbiotic nitrogen fixation (Mannetje 1997). Herbaceous forage legumes present an opportunity to produce high quality forage for livestock through integration within cropping systems (multi-purpose system).

Cattle are a key live export for farmers in the East Nusa Tenggara [(Nusa Tengarra Timur (NTT)] province of eastern Indonesia. Cattle production was historically low and cattle mostly grazed on native grasslands (*Bothriochloa*, *Heteropogon*, *Themeda*) with only short-term nutritive value and productivity limited by extended dry periods (Ayre-Smith 1991; Piggin et al. 1991).

The integration of herbaceous forages directly into cropping systems could significantly increase cattle production within NTT and benefit resource-poor farmers in eastern Indonesia through the provision of affordable high quality fresh or conserved feed for ruminants. For success, herbaceous forage legumes ideally need to integrate simply and easily into mixed crop and livestock systems.

The testing of herbaceous and shrub forage legumes in NTT commenced on the island of Sumba (Nulik 1987) and on West Timor during the early 1980s (Piggin et al. 1987; Piggin et al. 1991). Both evaluations targeted legumes considered to have potential for introduction into native grass pastures, but demonstrated the potential for *Aeschynomene*, *Chamaecrista rotundifolia* (formerly *Cassia*), *Centrosema*, *Clitoria*, *Lablab*, *Macroptilium*, *Macrotyloma*, *Neonotonia*, *Stylosanthes* and *Vigna*.

In collaborative Indonesian-Australian research (2006–2017), plant evaluation was undertaken to identify herbaceous legumes likely to fit into crop-livestock farming systems in NTT (Bell et al. 2020; Dalgliesh et al. 2014). Legumes were sought for growth principally within maize (the principal dry-land crop) systems, either in rotation (sown on the same area but in sequence with the maize crop) or as a relay (sown into the maize crop with forage harvested after the maize harvest). This followed the measurement of surplus soil moisture after traditional maize crops had been harvested, presenting a potential resource for forage production (Budisantoso et al. 2008; Dalgliesh et al. 2010). The aim was to produce fresh forage for direct use in the early dry season or stored hay to supplement other low quality forage resources at other times of the year. As legumes in these systems would need to be resown regularly, ease of seed production and harvest was also sought to better enable uptake by farmers. The research was completed on 3 islands in NTT (West Timor, Flores and Sumba) to ensure wide adaptability of the target species.

This aim of this paper is to report consolidated results of field trials conducted in NTT over 10 years of collaborative research to identify legumes suitable for maize/rice-livestock systems in a range of environments in eastern Indonesia.

Materials and Methods

Experimental design, forages and locations

The study was a multi-site assessment of tropical forage legumes using plant biomass as the key indicator of plant performance under a range of growing environments in eastern Indonesia. Sixteen experiments lasting 1- or 2-years were used to evaluate legume productivity at 14 locations in 3 islands within the NTT province (Figure 1, Table 1). Experimental sites were either on-farm (12), at a government research station (Naibonat) or at an agricultural college (Soe). Soils at the sites comprised Alfisol, Inceptosol and Vertisol (Soil Survey Staff 1999) with elevations from near sea level to 900 masl, within a narrow latitude range from 8.4-10.1 °S (Table 1). The soils are of marine origin on West Timor and Sumba and volcanic origin on Flores. They represent a range in production capacity: Alfisols - erodible alkaline and typically low in organic carbon and key nutrients including nitrogen, phosphorous and potassium and low water holding capacity; Inceptisols - heavy alkaline clays of moderate fertility and water holding capacity; Vertisols - very heavy, cracking alkaline clay soils of moderate fertility and high water holding capacity (Dalgliesh et al. 2014). All of the soils selected are used for cropping. All the on-farm sites were located on mixed enterprise farms including crop (maize or rice) and cattle production. Each site and sowing year combination was treated as a separate experiment. Legume accessions were grown individually in small plots using a randomised complete block design with 2, 3 or 4 replicates per site (Table 1).

A total of 34 legume lines were tested with up to 17 assessed at an individual site. The legumes were mostly herbaceous, with either twining/trailing (*Centrosema*, *Clitoria*, *Lablab*, *Macroptilium*, *Mucuna*, *Vigna*), non-twining (*Alysicarpus*, *Arachis*) or sub-shrub (*Desmanthus*, *Stylosanthes*) growth habits. They were mostly Australian cultivars used for long and short-term pastures or conserved fodder production with similar soils and climate to the study area.

The experiments were completed over 10 years beginning in 2006. The assessments were initially completed on West Timor, where a broad range of taxa were tested at 6 sites during the 2006/07 season. More detailed appraisal on West Timor during 2010 and 2011 focussed on promising species from the previous studies, including additional cultivars within these species as well as some more recently commercialised cultivars representing new taxa to Indonesia. Plant evaluation was extended to the islands of Flores and Sumba in 2011 and 2016, respectively, focussing on lines which performed well on West Timor under similar growing environments. The exception was an upland site with a shallow Alfisol soil on Sumba (Milpinga), where a range of *Desmanthus* and *Stylosanthes seabrana* were tested in addition to the higher-performing lines tested from West Timor.

Figure 1. Locations of field sites used to test the growth of selected tropical forage legumes in eastern Indonesia, 2006–2017.

Table 1. Experimental site characteristics, management and biomass sampling.

Location	Site characteristics			Timetable of key experimental events			Experimental design and sowing rate		
	Coordinates °S, °E	Soil type	Elevation (m)	Sowing date	Cut-back date[1]	Biomass sampling dates	Plot size (m × m)	Replicates	Plant spacing (cm × cm)
West Timor									
Naibonat	10.08, 123.86	Vertisol	50	23 Mar. 2006	22 May	23 Mar., 20 Apr., 5, 18 May, 15 Jun., 12 Jul., 28 Aug.	6 × 10	3	Broadcast[3]
Biloto	9.87, 124.22	Inceptisol	560	18 Feb. 2007	NA	3, 16, 29 Mar., 13, 27 Apr., 23 May, 18 Jul., 3 Sep.	1 × 1	3	40 × 20
Kletek	9.58, 124.93	Inceptisol	70	28 Mar. 2007	NA	11, 26 Apr., 11, 25 May, 7, 20 Jun., 30 Aug., 19 Oct.	1 × 1	3	40 × 20
Kakanuik	9.58, 124.84	Inceptisol	48	12 Apr. 2007	NA	26 Apr., 11, 25 May, 7, 21 Jun., 10 Jul., 30 Aug., 7 Nov.	1 × 1	3	40 × 20
Sillu	10.05, 123.96	Alfisol	440	16 Feb. 2007	NA	2, 16, 29 Mar., 13, 28 Apr., 3 May, 18 Jul., 4 Sep.	1 × 1	3	40 × 20
Usapininot	9.45, 124.54	Inceptisol	360	14 Feb. 2007	NA	28 Feb., 14, 28 Mar., 12, 27 Apr., 9 May, 17 Jul., 30 Aug., 7 Nov.	1 × 1	3	40 × 20
Naibonat	10.08, 123.86	Vertisol	50	12 Mar. 2010	11 Jan.	12 May, 14 Jul., 15 Oct. 4 May, 3 Jul.	8 × 3	3	50 × 10
Naibonat	10.08, 123.86	Vertisol	50	22 Feb. 2011	NA	4 May, 4 Jul.	8 × 4	3	20 × 20
Soe	9.86, 124.26	Alfisol	900	27 Mar. 2010	NA	29 May, 29 Jul.	5 × 2	2	50 × 10
Flores									
Marapokot	8.53, 121.31	Vertisol	15	22 Feb. 2011	19 Apr., 14 Jun.	19 Apr., 14 Jun., 9 Aug.[2]			20 × 20
Nakuramba	8.75, 121.58	Inceptisol	380	24 Feb. 2011	21 Apr., 14 Jun.	21 Apr., 14 Jun., 11 Aug.			20 × 20
Ulupulu	8.72, 121.32	Inceptisol	530	22 Feb. 2011	19 Apr., 14 Jun.	19 Apr., 14 Jun., 9 Aug.	5 × 10[3]	3	20 × 20
Wolomasi	8.77, 121.73	Vertisol	715	25 Feb. 2011	22 Apr., 14 Jun.	22 Apr., 14 Jun., 12 Aug.			20 × 20
Ranokolo	8.46, 121.70	Vertisol	300	21 Feb. 2017	16 May	16 May, 8 Aug.	4 × 2	4	20 × 20
Sumba									
Milpinga	9.79, 120.34	Alfisol	240	23 Jan. 2016	28 Apr.	28 Apr.[2]	4 × 2	4	Rows 30 cm[4]
Wangga	9.68, 120.26	Vertisol	85	22 Jan. 2016	27 Apr.	27 Apr, 3 Aug.			Rows 30 cm[4]

[1]Whole plots were cut back to 5–10 cm after biomass sampling.
[2]Plots failed to regrow after cutting due to inadequate soil moisture so not sampled after cutting.
[3]Sowing rates: 5–7 kg scarified uncoated seed/ha or 6–12 kg coated seed/ha. This site was fertilised with 15.7% P, 5% Sulphur broadcast at 50 kg/ha on 12 April.
[4]Sowing rates: *Desmanthus* and *Stylosanthes* 10 kg/ha, *Centrosema* 12 kg/ha, *Clitoria* and *Vigna* 15 kg/ha and *Lablab* 25 kg/ha.

Climate

The climate of the study area can be considered semi-arid tropical with a dominant wet season and an extended dry season of 5–7 months. Rainfall patterns for most of the study areas were poorly described prior to the study, but broadly represent annual rainfall between 800 and 1,200 mm with lower values on Sumba and higher values on Flores. Highest rainfall occurs from November–March, but is potentially bi-modal on the southern coast of West Timor (Dalgliesh et al. 2014). Rainfall data were collected using automated tipping bucket and logging equipment, along with continuous temperature measurements (Table 2). A limited number of weather stations meant rainfall was occasionally not measured at a particular site, but nearby within the region. The equipment was mostly reliable, although equipment failure occasionally resulted in incomplete rainfall records. Rainfall was summer dominant at all sites with significant rainfall prior to sowing in January to March and more than 200 mm in the months after sowing at most sites. Exceptions were Naibonat (lowland) during 2010 and Usapininot (midland) during 2007 (both on West Timor), where sowing was followed by little rainfall between April and October. Rainfall was low overall at the sites on Sumba, with an extended dry season. Relatively high dry-season rainfall totals were recorded at Kakanuik (lowland, West Timor) and Nakuramba (middle elevation, Flores), with moderate (40–70 mm per month) rainfall recorded at Soe (upland, West Timor). The dry season rainfall at Soe coincided with extended periods of low radiation (data not presented) believed to have contributed to poor plant growth. Temperature records collected at sites on West Timor showed mean minima ranging from 16–24 °C depending on elevation and time of year and maxima ranging from 25–36 °C (Dalgliesh et al. 2014). These temperatures tend to be moderated during winter months and with elevation (Central Bureau of Statistics 2019).

Table 2. Rainfall (mm/month) at the legume assessment sites. Month of sowing in bold. Less reliable data in italics.

Island, District	Site	Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
West Timor, Kupang	Naibonat	2006	533	185	**284**	33	2	3	0	0	6	51	89	276
	Naibonat	2010	261	191	**146**	81	15	34	1	1	0	0	63	302
	Naibonat	2011	500	453	256	455	66	0	3	0	0	66	228	257
	Sillu	2007	279	**192**	120	91	17	40	9	3	0	28	42	77
West Timor, TTS	Biloto	2007	204	**313**	191	0	3	0	0	0	0	0	19	353
	Soe	2010		170	**128**	236	337	40	48	48	73	206	299	131
West Timor, Belu	Kakaniuk	2007	81	129	376	**71**	45	455	49	122	1	4	34	159
	Kletek[1]	2007	*81*	*129*	***376***	*71*	*45*	*455*	*49*	*122*	*1*	*4*	*34*	*159*
West Timor, TTU	Usapinonot	2007	57	**148**	91	15	2	57	0	5	0	2	8	34
Flores, Ende	Wolomasi[3]	2011	284	**247**	334	289	98	22	F[4]					
	Nakuramba	2011	-	**213**	174	122	194	45	76	269	235	180	217	256
	Ranokolo	2017	*107*	***38***	*36*F[4]									
Sumba, Waingapu	Wangga[2]	2016	***123***	*149*	*150*	*28*	*31*	*40*	*9*	*18*	*4*	*76*	*5*	*123*
	Milipinga	2016	**123**	149	150	28	31	40	9	18	4	76	5	123

[1]Measured at Kakaniuk, ~10 km away

[2]Measured at Milipinga, ~13 km away

[3]Measured at Wolomasi, ~35 km away

[4]F=failure of rainfall logger

Experimental procedures

Each site was fenced to exclude grazing animals and cultivated manually or by using powered machinery (tractor and discs) to kill plants from previous cropping and prepare a relatively level site with a weed-free and friable seed bed. Any remaining weeds were controlled by hand-weeding or herbicide application (knapsack, glyphosate at label rates). The chosen sowing times and plant populations were those considered suitable for growing herbaceous legumes in eastern Indonesia based on researcher and farmer experience at the time.

Seeds for planting were mostly sourced from Australia and tested (water, 20/35 °C for up to 10 days) for germination to adjust sowing rates. Seeds of some lines were sourced within Indonesia in the latter stages of the study. Seed lots with high levels of hard seed dormancy were manually scarified using sand paper prior to sowing. Seed lots were inoculated in the first round of experiments (2006–07) with commercial *Bradyrhizobium* bacterial inoculum sourced from Australia, but not thereafter because farmers in this region are unlikely to have access to inoculum.

Seeds were sown by either 'dibbling' (directly into small holes made with a pole (dibble stick) and covering with soil) (most sites), sowing into shallow furrows or broadcasting by hand onto the soil surface and covering seed with soil using a rake (Table 3). Two to 5 seeds (fewer for large seeded taxa) were sown per dibble hole to ensure good establishment, and a range of spacing configurations used (20 or 25 holes/m^2). Seedling plants were not thinned. No fertiliser was applied and irrigation used only once at Soe to support establishment under unusually dry conditions at that time. Weeds were removed by hand. Establishment was successful at all sites except for Ulupulu and Sillu, which were excluded from subsequent analyses.

Plots were either grown continuously over a full growing season (December/January to July/August) at Naibonat and Soe (2010/11) or in a series of (typically 2 or 3) growth cycles ('re-growth' plots) initiated by sowing and thereafter cutting to 5–10 cm height and removing the cut material (all other sites) (Table 1). There was no attempt to control damage by diseases or insects which may have otherwise affected herbage production.

Plant performance measurements

Plant populations were measured by counting individual plants in central rows of plots 2 to 4 weeks after sowing to confirm satisfactory establishment. There were no further assessments of plant populations, but percentage cover was estimated at some sites immediately prior to biomass sampling. Above-ground biomass was measured approximately 8, 16 and 24 weeks after sowing in the continuously grown plots and immediately before cutting for the other experiments (specific timings for each site provided in Table 1). One or 2 randomly placed quadrats (either 0.5 or 1.0 m^2) were used to define sampling areas in each plot. Permanently marked areas were used for 're-growth' areas, whereas different locations in the plots were sampled for the continuously grown experiments, avoiding previously sampled areas. Plants were cut at 5–10 cm using a sharp bush knife and the fresh material weighed. Sub-samples were collected where biomass samples were large (>400 g/quadrat) and also weighed fresh (immediately after cutting). The whole or sub-sample fresh material was transported to a weather-proof central location and either air- or oven-dried (70 °C) until constant weight to determine dry matter content and calculation of herbage yield dry matter (DM) per unit area.

The incidence of flowering was monitored at selected sites in the 2006–07 series of assessments and at all sites after 2010 through regular visits. The date of the first fully expanded inflorescence in each plot was recorded and numbers of fully expanded inflorescences counted within marked areas (0.5 or 1.0 m^2 quadrat) areas every 1–2 weeks (detailed results not presented in this paper). Observations relating to the incidence of pests and diseases and other factors which could affect plant growth (e.g. soil moisture deficit) were recorded to aid the interpretation of results.

Statistical analysis

The field data were converted to standardised units to check for inconsistencies attributable to errors of sampling measurements. All statistical analyses were conducted using Genstat™ software (VSN International 2014). Each site was analysed as a separate experiment. Simple one-way analysis of variance was used to compare means. Those with a significant F value were compared with Fischer's least significant difference (P=0.05) procedure.

Table 3. Mean seedling density (plants/m^2 2–4 weeks after sowing) of herbaceous legumes sown using broad-cast (B), 'dibble' (spaced) and row planting in small-plots in eastern Indonesia.

Species	Identifier[1]	West Timor									Flores				Sumba		
		Naibonat	Biloto	Kletek	Kakaniuk	Sillu	Usapinonot	Soe	Naibonat	Naibonat	Marapokot	Nakuramba	Ulupulu	Wolomasi	Ranokolo	Milpinga	Wangga
		2006	2007	2007	2007	2007	2007	2010	2010	2011	2011	2011	2011	2011	2017	2016	2016
		B	'Dibble' pole													Row	
Aeschynomene americana	Glenn	S[2]															
Alysicarpus vaginalis	NS	P															
Arachis pintoi	Amarillo					2											
Centrosema molle	Cardillo							20	24	40	27	57	57	76	8	42	38
Centrosema pascuorum	Cavalcade	S	15	19	14	10	19	6	24	52				52	21		
C. pascuorum	Bundey							18	20	51							
Clitoria ternatea	APG52830							7	9						21	25	19
C. ternatea	Milgarra	S	12	19	12	11	7	14	26	73		55		32		7	5
Desmanthus leptophyllus	APG84431																
D. pernambucanus	NS	S	10	51	21	12	68										
D. virgatus	NS															12	11
D. virgatus	Marc		13	77	24	21	21									1	
D. virgatus	APG84369														6.5	57	
D. virgatus	APG48643															39	
D. virgatus + bicornutus + leptophyllus	Progardes															9	6
Lablab purpureus	Highworth		5	7		5	5	14	10								
L. purpureus	APG13483								5	11							
L. purpureus	Endurance							13	9								
L. purpureus	Rongai							20	12								
Macroptilium bracteatum	Cadarga	S	16	72	20	11	8	20	47								
M. bracteatum	Juanita							23	44	13	91	35	61				25
Macroptilium gracile	Maldonado					8			24								

Continue

Species	Identifier[1]	West Timor									Flores				Sumba		
		Naibonat	Biloto	Kletek	Kakaniuk	Sillu	Usapinonot	Soe	Naibonat	Naibonat	Marapokot	Nakuramba	Ulupulu	Wolomasi	Ranokolo	Milpinga	Wangga
		2006	2007	2007	2007	2007	2007	2010	2010	2011	2011	2011	2011	2011	2017	2016	2016
		B	'Dibble' pole													Row	
Mucuna pruriens	APG51786									2							
Stylosanthes hamata	Verano		P			39	53										
Stylosanthes guianensis	NS*			32	102	17	26	25									
S. guianensis	ATF3308									38							
S. guianensis	ATF3309									41							
Stylosanthes seabrana	NS		15	40	7	16	12										
S. seabrana	Primar														6	54	
S. seabrana	Unica															54	24
Vigna luteola	Dalrymple							13	18.0	71					21	25	32
Vigna unguiculata	Arafura							13	12.5								
P-value								0.013	<0.001	0.002	0.017	0.451	0.205	0.024	<0.001	<0.001	<0.001
LSD (P=0.05)								7	10	21	36	49	148	26	4	19	7

[1]Taxon=a cultivar unless prefixed where APG refers to current Australia Pasture Genebank records. NS=taxon not specified (not specifically known). NS*=Brazilian type.
[2]Plant populations were not measured at Naibonat in 2006, but success of establishment recorded by visual assessment: successful (S) or poor (P)

Results

Plant establishment

Plant establishment was highly successful at most sites with either 'dibble' or row (furrow) sowing techniques with mean values of between 20 and 90 plants/m^2 for most legumes and few with less than 5 (Table 3). Of the 'dibble' planted sites, plant populations were highest overall at Naibonat (lowland, West Timor), despite low levels of rainfall recorded after sowing, and the small sites on Flores where rainfall was higher after sowing (Table 3). Row planting resulted in suitable populations for forage production (15–50 plants/m^2) at the 2 Sumba sites with no obvious differences in establishment performance between legumes originating from the same seed lot and sown at both sites. Broadcast planting at Naibonat (2006) resulted in poor establishment of some legumes (*A. vaginalis* and *S. hamata*) but those with larger seeds established well (*C. pascuorum* and *C. ternatea*).

There were differences in plant population density between legume species. Legumes with seeds of moderate size and moderate sowing rates (*Centrosema*, *Clitoria* and *Macroptilium*) mostly had higher plant populations when both sowing methods were used. However, the largest seeded legumes (*Lablab*, *Mucuna*), which were sown at lower sowing numbers, tended towards low populations. Plant populations of the small, typically hardseeded legumes (*Desmanthus* and *Stylosanthes*) were highly variable, and performance was generally better using row planting, although comparisons were over differing years between the 'dibble' (2017), row-planted (2016) and broadcast-sown (2006) sites so the results are not definitive.

Biomass production without cutting (cumulative yields)

In the first experiment sown in late March 2006, 7 legume genera (*Aeschynomene*, *Alysicarpus*, *Centrosema*, *Clitoria*, *Desmanthus*, *Macroptilium* and *Stylosanthes*) were assessed on a Vertisol in a lowland environment on West Timor (Naibonat) (Table 4). *A. vaginalis* established poorly and seedlings grew slowly (yellowed plants). All legumes established and grew vigorously, except *S. hamata* 'Verano' (seedlings remained yellow and stunted). Early to mid-season biomass was greatest in *C. pascuorum* and *C. ternatea* with yields greater than 3 t DM/ha by 111 days after sowing. Subsequent yields declined slightly under drying soil conditions as growth slowed and leaves were shed, whereas *D. pernambucanus* continued to grow to yield 5.7 t DM/ha by the end of the experiment: much of this late season growth was stem. Verano stylo recovered to yield nearly 3 t DM/ha by 111 days with little measurable growth thereafter, whereas *A. americana* and *M. bracteatum* produced low yields.

The cumulative biomass yields of a similar suite of legumes were also measured on West Timor on Inceptisol soils in 2007. The 4 sites represent upland (Biloto and Usapininot) and lowland (Kletek and Kakaniuk) environments, the latter having a high winter rainfall component (Table 2). All were sown in February or March and assessed over ~200 days. All established well using the 'dibble pole' method, but plant populations 28 days after sowing varied considerably between lines. Small-seeded legumes (*Desmanthus*, *Macroptilium* and *Stylosanthes*) were particularly variable, ranging from ~10 to ~70 plants/m^2 (Table 3).

High cumulative biomass yields were measured at all sites (Table 4). The shrub legume *S. seabrana* accumulated very high biomass yields (5.9–9.8 t DM/ha) over the entire growing season. The *Desmanthus* spp. exhibited a similar growth pattern, with *D. pernambucanus* tending to out yield *D. virgatus*. The herbaceous legumes had lower yields. Cavalcade (*C. pascuorum*) and Milgarra (*C. ternatea*) performed similarly to results in the Vertisol site assessed the previous year, producing maximum herbage yields of 2–4 t DM/ha of leaf and (non-woody) stem. Highworth (*L. purpureus*), an annual or short-lived herbaceous legume, produced moderate levels of early-season biomass at 140–150 days after sowing but growth slowed towards the end of the season and herbage yield declined in one instance as leaves were shed. Cadarga (*M. bracteatum*), a short-lived perennial legume, grew well on most sites and accumulated biomass over the entire season to produce 2.2–4.9 t DM/ha.

Seventeen legumes were grown during 2010 without cutting at Naibonat (similar Vertisol soil as for the 2006/07 study) and 13 at an upland Alfisol site (Soe) (Table 5). Two *S. guianensis* lines established successfully at Naibonat but failed to grow beyond seedlings and were omitted from the experiment.

Table 4. Mean above-ground biomass (oven-dried material t/ha) of herbaceous legumes grown continuously (no cutting) at the first round of plant evaluation on West Timor[1].

Species	Identifier	Naibonat				Biloto				Kletek				Usapinonot			
		Vertisol / 50[2]				Inceptisol / 560				Inceptisol / 70				Inceptisol / 360			
		2006 (sown 23 Mar.)[3]				2007 (18 Feb.)				2007 (28 Mar.)				2007 (14 Feb.)			
		28[4]	56	111	159	94	150	197	90	155	205	90	140	209	84	153	197
A. americana	Glenn	0.10	0.40	1.15	0.95												
C. pascuorum	Cavalcade	0.20	2.50	3.60	3.20	3.51	2.88		0.86	1.62		2.44	3.27		2.70	2.93	4.40
C. ternatea	Milgarra	0.05	1.45	3.25		3.25	3.05	3.80	0.81	1.93	3.48	2.38	2.86	4.17	1.31	2.06	2.31
D. pernambucanus	NS	0.10	0.52	4.45	5.75	0.00	0.47		0.43	1.09	2.41	0.83	1.79	7.10	0.89	2.61	4.16
D. virgatus	Marc					0.88	0.08		0.28	0.05	1.98	1.50	1.17	2.17	0.51	0.40	1.60
L. purpureus	Highworth					4.25	1.76		0.98	1.42		1.86	2.95		1.12	1.40	1.19
M. bracteatum	Cadarga	0.02	0.95	1.00		1.50	1.76	4.95	1.81	2.23	3.05	0.65	0.96	4.85	0.26	1.36	2.22
S. guianensis	NS*					1.36	2.32	6.48	0.05	1.28	3.09	0.05	0.39	3.92	0.75	2.14	4.61
S. hamata	Verano				3.05												
S. seabrana	NS					1.93	4.02	9.80	0.26	4.08	5.05	0.54	2.65	7.87	0.23	3.50	5.90

[1]Herbage yields not presented for Sillu due to poor growth after establishment.
[2]Soil type / elevation (masl)
[3]Year of biomass assessment
[4]Weeks after sowing or cutting
Source: Dalgliesh et al. 2014 (project final report) and Budisantoso et al. 2006 (Indonesian national seminar series).

Table 5. Mean above-ground biomass (air-dried material t/ha) of herbaceous legumes grown continuously (no cutting) at lowland Vertisol (Naibonat) and upland Alfisol (Soe) sites on West Timor.

Species	Identifier	Naibonat (1)						Naibonat (2)		Soe	
		2010 (sown 11 Mar.)[1]			2011 (cut back 11 Jan.)			2011 (sown 22 Feb.)		2010 (sown 27 Mar.)	
		61[2]	124	217	52	113	173	71	132	63	124
C. molle	Cardillo	0.74	2.07	5.83	0.91	1.13	0.83	0.09	0.45	0.02	0.34
C. pascuorum	Bundey	2.29	7.24	6.61	0.75	2.77	1.50	2.25	2.20	0.03	0.23
C. pascuorum	Cavalcade	3.04	7.95	4.32	1.62	4.30	1.60	3.30	1.85	0.02	0.28
C. ternatea	APG52830	2.10	3.23	5.48	1.91	2.07	1.27	-	-	0.05	0.20
C. ternatea	Milgarra	2.41	3.85	5.20	1.98	2.27	1.17	1.85	1.40	0.02	0.17
L. purpureus	APG13483	4.46	4.62	6.75	-	-	-	1.60	0.30	-	-
L. purpureus	Endurance	2.62	2.91	3.92	-	-	-	-	-	0.07	0.16
L. purpureus	Highworth	3.24	4.90	3.84	-	-	-	-	-	0.64	0.39
L. purpureus	Rongai	3.58	3.86	5.90	-	-	-	-	-	0.45	0.37
M. bracteatum	Cadarga	4.00	5.34	4.91	-	-	-	-	-	0.04	0.12
M. bracteatum	Juanita	3.28	5.45	5.89	-	-	-	0.14	0.60	0.03	0.21
M. gracile	Maldonado	0.99	1.96	2.32	-	-	-	-	-	-	-
M. pruriens	APG51786	3.54	7.52	3.31	-	-	-	-	-	0.02	0.17
V. luteola	Dalrymple	1.83	3.37	4.97	-	-	-	-	-	0.21	0.11
V. unguiculata	Arafura	2.09	2.98	2.30	-	-	-	1.70	0.55	-	-
P-value		<0.001	<0.001	0.007	<0.001	0.094	0.632	0.005	0.033	0.001	0.045
LSD (P=0.05)		0.94	1.56	2.37	0.48	2.23	1.20	1.20	1.15	0.24	0.17

[1]Year of biomass assessment
[2]Days after sowing or cutting

High yields of air-dried biomass were measured 16 and 24 weeks after sowing at the lowland site where mean monthly daily temperatures ranged from 25.8 to 27.7 °C. The highest yielding taxa were *Centrosema*, *Clitoria*, *Lablab*, *Mucuna*, *Macroptilium* and *Vigna* which all produced 5–8 t DM/ha over the growing season. Some produced maximum biomass values 16 weeks after sowing (*C. pascuorum*, some *L. purpureus*, *M. bracteatum* and *Mucuna pruriens*) with little subsequent increase in herbage yield (or decline in yield as they dry). Others continued to accumulate biomass until the final measurement 24 weeks after sowing (*C. molle*, *C. tenatea* and *Vigna luteola*). *C. molle*, *C. pascuorum* and *C. clitoria* survived the dry season and were cut to ~10 cm during January 2011. Second year herbage yields were variable, potentially due to extremely wet conditions during February, March and April (Table 3). Sixteen-week herbage yields ranged from 1 t/ha (*C. molle*) to 4 t/ha (*C. pascuorum* Cavalcade). The same species, plus the better performing *L. purpureus* (APG13483), were re-sown at Naibonat in late February 2011 and growth monitored for 24 weeks. Cavalcade (*C. pascuorum*) again produced the highest biomass yields, with 3.3 t DM/ha produced 10 weeks after sowing, with the others having low (*C. molle* Cardillo) to moderate yields.

Herbage yields on the Alfisol upland site (Soe) after sowing in late March were poor. Highworth (0.64 t DM/ha) and Rongai (0.45 t DM/ha) (*L. purpureus*) had the highest yields 9 weeks after sowing. Cool (mean daily temperatures of ~20 °C) and wet conditions between May and August were associated with poor plant growth in all legumes, with most dying out over this period. The exception was Cardillo (*C. molle*) which persisted until November, although herbage yields were low when measured in July.

Biomass production with cutting

Herbage production of a range of legumes was measured after sowing and plant regrowth after a series of cutting cycles on Vertisol and Alfisol soils in Sumba and Flores (Table 6). Milgarra (*C. ternatea*) had the highest overall herbage yields (4.7 and 7.1 t air-dried material/ha) on the 2 Vertisol sites, due to a combination of rapid growth after establishment and regrowth after cutting.

Table 6. Mean[1] above-ground biomass (air-dried material t/ha) of herbaceous legumes grown in cutting cycles at lowland (Wangga) and upland (Milipinga) sites on Sumba and Flores.

Species	Identifier	Sumba						Flores		
		Milipinga (sown 23 Jan.) Alfisol, 240 masl			Wangga (sown 22 Jan.) Vertisol, 85 masl			Ranokolo (sown 21 Feb.) Vertisol, 300 masl		
		96 DAS	98 DAC	Flower	96 DAS	98 DAC	Flower	84 DAS	84 DAS	Flower
C. pascuorum	Bundey							2.18	0.32	Weak
C. pascuorum	Cavalcade	0.30^{de}		Strong	2.66	0.59	Strong	1.04	0	Strong
C. ternatea	Milgarra	0.19^{cd}		Strong	3.31	3.79	Strong	2.31	2.35	Strong
D. leptophyllus	APG84431	0.09^{bc}		Weak	1.28	1.19	Strong			
D. virgatus	Marc	0.04^{b}		Weak	1.30	0.71	Strong			
D. virgatus	APG84369	0.01^{a}		Weak						
D. virgatus	APG48643	0.41^{de}		Strong				0.21	1.73	Strong
D. virgatus	Progardes	0.39^{de}		Weak						
L. purpureus	Highworth	0.09^{bc}		Weak	1.48	0	None			
M. bracteatum	Juanita				2.86	0.71	Strong			
S. seabrana	Primar	0.69^{e}		Strong				0.14	0.49	Strong
S. seabrana	Unica	0.75^{e}		Strong	2.31	2.25	Strong			
V. luteola	Dalrymple	0.22^{cd}		Weak	1.92	0.63	Weak	3.24	1.17	None
P-value		<0.001			0.050	<0.001		<0.001	<0.001	
LSD (P=0.05)					1.43	568		0.41	0.64	

[1]Back transformed means (log_{10} transformation) are presented for Milipinga. Backtransformed means with the same letter are not considered to be different at the 95% confidence level. DAS=Days after sowing; DAC=Days after cutting Strong=flowers in all plots before biomass sampling; Weak=flowers in few plots before biomass sampling; None=flowers not detected

The sub-shrub legumes *S. seabrana* and *D. leptophyllus* also regrew well after cutting, but overall biomass yields were lower than for Milgarra. Bundey and Cavalcade (*C. pascuorum*), Juanita (*M. bracteatum*) and Dalrymple (*V. luteola*) initially yielded well, but regrew poorly after cutting, while Highworth (*L. purpureus*) did not regrow at all (one site).

Herbage yields were low 14 weeks after sowing at the upland Alfisol site on Sumba despite receiving over 300 mm rainfall. Regrowth after cutting was also poor with insufficient biomass to complete meaningful assessments. This regrowth period coincided with low monthly rainfall and notably dry soil conditions. The best performing legumes under this growing environment were the sub-shrub legumes (*Stylosanthes* and *Desmanthus*), which were the only legumes to persist through the dry season (observation only).

Peak season biomass in different environments

Total seasonal herbage production after a January-March sowing, either grown continuously or with 8- to 14-week cycles of defoliation, are presented for all sites in Table 7. The length of the measured growing season for continuously grown plants varied from 63 days at Soe, where poor growth curtailed biomass measurements, to 217 days at Naibonat.

The highest (7–8 t DM/ha) herbage yields were achieved by Bundey and Cavalcade (*C. pascuorum*) assessed at many sites and *M. pruriens* at Naibonat and Milgarra (*C. ternatea*) at Wangga. These were both lowland Vertisol sites where biomass was accumulated over an extended period. Legumes which achieved 4–6 t DM/ha included *C. molle*, *C. ternatea*, *L. purpureus*, *M. bracteatum* and *V. luteola*, also on Vertisol soils either on West Timor (Naibonat) or Flores (Ranokolo). Biomass yields tended to be lower at the sites subjected to cutting cycles, although not in all instances. For example, the highest *C. ternatea* yield was achieved under cutting. Unica (*S. seabrana*) also yielded well, producing a total of 4.5 t air-dried biomass /ha on a Vertisol under cutting (one site only).

The legumes which ranked highly across a range of sites were *C. pascuorum* and *C. ternatea*. Cavalcade (*C. pascuorum*) ranked highest when grown continuously on Vertisols, while *C. ternatea* tended to rank best under cutting. Other legumes ranked highly across a limited number of sites or cutting regimes. *C. ternatea* consistently ranked highly when grown on Vertisols on Sumba and Flores, while *C. pascuorum* grew well on both Vertisols and Alfisols. The cyclical harvesting and removal of biomass appeared to also influence total biomass of some other legumes which ranked highly for total seasonal yield. *L. purpureus* and *M. pruriens*, both short-lived (annual or biennial) twining legumes, produced high amounts of biomass when grown without cutting on West Timor but had low overall biomass when grown in cutting cycles on Sumba. *C. molle*, another twining legume but with a longer life cycle, ranked highly for total seasonal biomass under both continuous growth and cutting regimes.

The site at Milipinga on Sumba represented a quite different growing environment (upland Alfisol and low rainfall) to the other sites. The highest ranked legumes were the 2 *S. seabrana* cultivars and 2 of the 5 *Desmanthus* sp. cultivars or lines (these had the highest plant populations). Cavalcade (*C. pascuorum*) also grew well initially.

Insects and disease

C. ternatea was occasionally damaged by the leaf-chewing larvae of a butterfly (*Catopsilia* sp.) commonly observed during the wet and early-dry seasons. Damage (60–80% of leaves) was first recorded at Naibonat in 2006 and endosulfan was applied to control the caterpillar larvae. Endosulfan was not used in any of the other experiments and it was not considered a useful option for smallholder farmers. Moderate damage was recorded at Naibonat during 2010 to both lines assessed, and this may have reduced biomass over the season. Lesser damage was noted on leaves of *L. purpureus* and *V. luteola* at the same site, but this was caused by other insects. Leaf damage was also noted on *C. pascuorum* grown at Nakuramba but the cause was unknown. Pod borers (suspected to be *Helicoverpa* sp.) were also observed on *L. purpureus* but are not expected to have affected biomass yields. There was no obvious disease damage at any of the sites although extremely poor growth of *S. guianensis* at Naibonat in 2010 could possibly be attributed to soil-borne pathogens following wet conditions.

Table 7. Peak above-ground biomass (oven or air dried material t/ha) of herbaceous legumes grown in eastern Indonesia either continuously or in cycles of cutting and regrowth.

Species	Identifier	Continuously grown[1]									Cutting and regrowth cycles[2]						
		West Timor									Flores					Sumba	
		Naibonat Vertisol, 50 masl	Biloto Inceptisol, 560 masl	Kletek Inceptisol, 70 masl	Kakaniuk Inceptisol, 50 masl	Usapinonot Inceptisol, 360 masl	Soe Alfisol, 900 masl	Naibonat Vertisol, 50 masl	Naibonat Vertisol, 50 masl	Naibonat Vertisol, 50 masl	Marapokot Vertisol, 15 masl	Nakuramba Vertisol, 50 masl	Ulupulu Insceptisol 530 masl	Wolomasi Vertisol, 715 masl	Ranokolo Vertisol, 300 masl	Milipinga Alfisol, 240 masl	Wangga Vertisol, 85 masl
		2006	2007	2007	2007	2007	2010	2010 (year 1)	2010 (year 2)	2011	2011	2011	2011	2011	2017	2016	2016
		111 159[3]	*150* 197	205	*140* 209	*153* 197	*63* 124	*124* 217	113	*71* 132	112	168	112	168	168	96	194
		1[4](o)	1(o)	1(o)	1(o)	1(o)	1(o)	1(a)	1(a)	1(a)	2(a)	3(a)	2(a)	3(a)	2(a)	1(a)	2(a)
A. americana	Glenn	1.15															
C. molle	Cardillo						0.34	5.83	1.13	0.45	0.91*	1.72	0.68*	1.39			
C. pascuorum	Bundey						0.23	7.24	2.77	2.25					2.50		
C. pascuorum	Cavalcade	3.60	2.88	1.62	3.27	4.40	0.28	7.95*	4.30	3.30*				2.07	1.04	0.36	3.25
C. ternatea	APG52830						0.20	5.48	2.07								
C. ternatea	Milgarra	3.25	3.80	3.48	4.17	2.31	0.17	5.20	2.27	1.85		2.84*		2.45*	4.67*	0.19	7.11*
D. leptophyllus	APG84431															0.09	2.47
D. pernambucanus	NS	5.75*	0.47	2.41	7.10	4.16											
D. virgatus	Marc		0.08	1.98	2.17	1.60										0.04	2.02
D. virgatus	APG84369															0.01	
D. virgatus	APG48643														1.94	0.41	
D. virgatus	Progardes															0.40	
L. purpureus	APG13483							6.75		1.60							
L. purpureus	Endurance						0.16	3.92									
L. purpureus	Highworth		1.76	1.42	2.95	1.40	0.64*	4.90								0.10	1.48

Continue

Species	Identifier	Continuously grown[1]									Cutting and regrowth cycles[2]						
		West Timor									Flores					Sumba	
		Naibonat Vertisol, 50 masl	Biloto Inceptisol, 560 masl	Kletek Inceptisol, 70 masl	Kakaniuk Inceptisol, 50 masl	Usapinonot Inceptisol, 360 masl	Soe Alfisol, 900 masl	Naibonat Vertisol, 50 masl	Naibonat Vertisol, 50 masl	Naibonat Vertisol, 50 masl	Marapokot Vertisol, 15 masl	Nakuramba Vertisol, 50 masl	Ulupulu Insceptisol 530 masl	Wolomasi Vertisol, 715 masl	Ranokolo Vertisol, 300 masl	Milipinga Alfisol, 240 masl	Wangga Vertisol, 85 masl
		2006	2007	2007	2007	2007	2010	2010 (year 1)	2010 (year 2)	2011	2011	2011	2011	2011	2017	2016	2016
		111 159[3]	*150* 197	205	*140* 209	*153* 197	*63* 124	*124* 217	113	*71* 132	112	168	112	168	168	96	194
		1[4](o)	1(o)	1(o)	1(o)	1(o)	1(o)	1(a)	1(a)	1(a)	2(a)	3(a)	2(a)	3(a)	2(a)	1(a)	2(a)
L. purpureus	Rongai						0.45	5.90									
M. bracteatum	Cadarga		1.76	3.05	4.85	2.22	0.12	5.34									
M. bracteatum	Juanita						0.30	5.89			0.32	2.00	0.35				3.58
M. gracile	Maldonado							2.32									
M. pruriens	APG51786							7.52									
S. guianensis	NS		6.48	3.09	3.92	4.61											
S. hamata	Verano	3.05															
S. seabrana	NS		9.80*	5.05*	7.87*	5.90*											
S. seabrana	Primar														0.63	0.69	
S. seabrana	Unica															0.76*	4.56
V. luteola	Dalrymple						0.17	4.97		1.70					4.40	0.22	2.56
V. unguiculata	Arafura						0.21	2.98									

Shaded panels represent legumes with mean total biomass >20% of the site mean; Panels with an * represent the highest value for that site; Drying method (o=oven; a=air)
[1]Where continuously grown, the maximum value of sampled biomass is presented (normal font=end of season; italics=mid-season)
[2]Where grown in cycles of cutting and regrowth, total values of biomass measured at the end of each growth cycle are presented
[3]Growth period (days)
[4]Number of growth cycles

Table 8. Summary of better-performing legumes under a range of growing environments and management in eastern Indonesia.

Legume species	Australian cultivars (lines) tested	Growing environment(s)[1]	Successful establishment method(s)	Regrowth after cutting	Typical herbage yields under cutting[2]	Typical herbage yields without cutting[2]	Recommended system	Other considerations
Herbaceous legumes								
C. pascuorum	Cavalcade Bundey	Vertisols, Inceptisols; upland and lowland	Dibble pole, row, broadcast	Inconsistent, poor when mature or under dry conditions	1–3	2–8	Single crop; fresh cut or hay	Easy to harvest fresh or for hay
C. molle	Cardillo	Vertisols, Inceptisols; best on an Alfisol (low yields overall); upland and lowland	Dibble pole	Moderate	0.5–1.5	0.5–5.5	Regrowth fodder crop (fresh or hay) or graze directly	Low growing and difficult to cut
C. ternatea	Milgarra APG52830	Vertisols, Inceptisols; upland and lowland	Dibble pole, row, broadcast	Excellent under a range of conditions	2–7	2–5	Cutting cycles or as a single crop; fresh cut or hay	Easy to harvest. Defoliation by Catopsilia butterfly larvae.
L. purpureus	Highworth Rongai	Vertisols, Inceptisols; high rank on Alfisol (low yields overall); upland and lowland	Dibble pole	Poor	0–1.5	2–6	Grow as a single crop forage crop; fresh cut	Leaves shatter when dry
M. bracteatum	Cadarga Juanita	Vertisols, Inceptisols; lowland	Dibble pole, row, broadcast	Inconsistent, regrew well on a Vertisol	0.5–3.5	2–5	Single crop, regrowth on Vertisols; fresh cut	Leaves shatter when dry
M. pruriens	(APG51786)	Vertisol; lowland	Dibble pole	Not tested	–	7.5	Single crop forage crop; fresh cut or hay	Leaves fell when mature
V. luteola	Dalrymple	Vertisol; lowland	Dibble pole, row	Moderate under good growing conditions	2.5–4.5	2–5	Single crop forage crop; fresh cut or hay	Susceptible to insects
Shrub legumes								
D. leptophyllus	(APG84431)	Vertisol; lowland	Row	Slow regrowth	0–2.5	2.5	Semi-permanent fodder banks/pasture	Leaf drop when dry, stemmy when mature
D. pernambucanus	'Common'	Vertisol, Inceptisol; lowland and upland	Broadcast	Not tested	–	2–7	Semi-permanent fodder banks/pasture	Leaf drop when dry, stemmy when mature
D. virgatus	Marc Progardes (APG84369) (APG48643)	Vertisol; lowland	Row, dibble pole	Slow regrowth	0–2	0–2	Semi-permanent fodder banks/pasture	Leaf drop when dry
S. guianensis	(Brazilian)	Inceptisol; lowland and upland	Dibble pole	Not tested	–	3–6.5	Single crop; fresh cut or hay	Stemmy when mature
S. hamata	Verano	Vertisol (limited evaluation)	Dibble pole	Not tested	–	3	Semi-permanent fodder banks/pasture	Low growing
S. seabrana	Primar Unica (common)	Inceptisol, Vertisol, best on Alfisols overall; lowland and upland	Row	Moderate regrowth	4.5–6.5	5–9	Semi-permanent fodder banks/pasture	Stemmy when mature

[1]Herbage yields were low, overall, on Alfisols
[2](t DM/ha)

Discussion

Application and scope of the research program

The overarching aim of the plant evaluation was to identify legumes which could be grown for animal (principally cattle, but also for goats) production within mixed cropping systems. A suite of mostly herbaceous legumes were assessed (as opposed to tree) legumes because these could conceivably be grown in rotation with other food crops or as an inter-crop, for example as a 'relay' where the legumes are sown into the developing food crop (maize) and the fodder grown and utilised after the food crop is harvested. Such systems were not obvious at the onset of the study, but were identified over the course of experimentation and farmer extension resulting in productive grain (maize, rice) crop-fodder systems in which the use of forages did not reduce or even increased grain crop yields (through increased nitrogen supply) (Bell et al. 2022).

Legume selection was biased to those previously developed and adopted in northern Australia. The recently updated Tropical Forages selection tool (Cook et al. 2020) also provided a useful reference for identifying suitable species and cultivars/lines for assessment. The legumes were assessed mostly over one growing season with establishment and assessment in the same year. Establishment was during the higher rainfall summer months when conditions were conducive to establishment and growth under rainfall, similar to other annual cash or staple food crops (mung beans, maize). There is an opportunity, however, to also grow crops during the dry season after rice production (paddy systems) (Bell et al. 2022). These systems seem particularly well-suited to herbaceous legumes with larger seeds such as *C. pascuorum* and *C. ternatea*.

Relevance and interpretation of data for identifying suitable legumes

Dried herbage biomass over 1 or (in one case) 2 growing seasons (as opposed to persistence and herbage yield over time), was used as the principal measure to rank legumes across environments. Biomass production under continuous growth was initially used to rate the adaptation of legumes to various soils and climates. Subsequent experiments using the more promising legumes measured regrowth following cutting and removal of the herbage as would conceivably be used for feeding livestock, either fresh or conserved as hay. Oven dried biomass was determined for the initial experiments when there was good access to ovens and air-dried biomass (i.e. hay) thereafter at more remote sites. In a similar growing environment (Northern Territory of northern Australia) Cavalcade (*C. pascuorum*) hay (March harvest) was found to have a moisture content of 13% (i.e. 87% oven DM) after sun drying, declining to 8% in dry-season storage (Regan 1997). This is safely below the 18% recommended for safe storage in eastern Indonesia (Nulik et al. 2013). It is reasonable to expect, therefore that the legumes grown and air-dried in NTT had dry matter contents in the order of 10–15% lower than oven results. These were not considered to have influenced rankings of the legumes.

Secondary criteria for selection included ease of cutting and baling and seed collection. Larger seeds and lower levels of pod shattering are considered advantageous in smallholder systems where self-harvest of seeds can improve the sustainability of forage systems requiring regular sowing. Plant nutrient content was not assessed in these experiments. However, as most of the taxa tested have previously been well-researched, values of digestible energy and protein at different growth stages can readily be sourced in most instances (Cook et al. 2020). All of the legumes assessed are considered palatable to livestock, but vary in feed value based on plant structure and growth stage.

Legumes as short-term fodder crops in eastern Indonesia

The best overall legumes for growing between annual grain crops were *C. pascuorum* and *C. ternatea*. Both are twining herbaceous legumes with high feed value. Whereas *C. ternatea* has an erect, climbing habit, *C. pascuorum* has decumbent, sprawling growth (Cook et al. 2020). Other legumes which ranked highly in some environments included *C. molle*, *L. purpureus*, *M. bracteatum*, *M. pruriens* and *V. luteloa*.

C. pascuorum cultivars (Cavalcade and Bundey) produced high annual yields (up to 8 t air dried herbage/ha). Herbage yields compared favourably with a range of *C. pascuorum* accessions grown in a similar climatic zone in northern Australia (Katherine, Northern Territory) where fertiliser phosphorous and sulphur were applied prior to sowing by broadcasting (Clements et al. 1984). Both varieties are grown commercially for hay production in the Northern Territory where they are mostly incorporated into pellets for feeding cattle during live export (Cameron 2005), potentially providing an option for NTT to support live export between islands. In the

NTT experiments Cavalcade tended to out yield Bundey when compared at the same site, indicating Cavalcade may be best suited to eastern Indonesia; however, there may a role for Bundey in wetter environments. Although *C. pascuorum* grew vigorously at most sites, regrowth after cutting and removing herbage was inconsistent and relatively poor after cutting under dry conditions. These results indicate *C. pascuorum* is best grown as a single forage crop and regrowing after cutting should only be attempted under favourable growing conditions (high soil moisture or access to irrigation).

C. ternatea produced up to 5 t air dried material/ha over the growing season when grown continuously on Vertisols and up to 7 t air dried material/ha under cutting cycles. Production also ranked highly compared to other legumes on Inceptisol and Alfisol soils, the broad adaptation being consistent with previous adaptation studies in northern Australia (Hall 1985). The yields achieved in NTT were higher than recorded in replicated grazing-scale experiments on cropping soils in southern Queensland including Milgarra, where it took 3 years to achieve comparable yields on fertilised (P and S) Vertisols (Whitbread et al. 2005). The demonstrated capacity to regrow vigorously after cutting enables a series of forage crops to be harvested over a season as required and either fed fresh or conserved as hay. A perennial growth habit also means it can perform a useful role in longer-term grazing leys in cropping systems to provide high-quality feed and rejuvenate soils (Pengelly and Conway 2000). One potential management limitation, however, is occasional severe defoliation by larvae of butterflies (*Catopsilia* sp.) encountered over short periods during the key growing season. Given the capacity for *C. ternatea* to regrow after cutting, a strategy to avoid damage by larvae is to harvest fodder when large numbers of larvae are detected and allow the crop to regrow.

Other high-yielding legumes for short-term fodder production included *L. purpureus*, *M. bracteatum*, *M. pruriens* and *V. luteloa*, which all showed some potential as legumes which can easily be established and grow rapidly to provide useful amounts of biomass in eastern Indonesia. These legumes produced in the order of 5–7 t air dried material/ha under optimal conditions and were generally most productive on Vertisols. Herbage yields were comparable with those achieved under good growing conditions in Queensland (Pengelly and Conway 2000; Whitbread et al. 2005), but higher yields have been reported under optimum growing conditions (Cook et al. 2020). These legumes provide a useful forage option where high amounts of biomass are sought over short periods. All are recognised as annual or short-duration legumes of high forage value which can perennate under favourable conditions (Cook et al. 2020). Although yields were relatively low, Cardillo (*C. molle*) showed potential where other legumes had failed. Cardillo appears broadly adapted and robust, but the dense, low growth habit could interfere with hand harvesting.

A range of *Desmanthus* (3 species) and *Stylosanthes* (3 lines) were tested on Vertisol and Inceptisol soils on West Timor and later on an Alfisol and Vertisol soils on Sumba to identify robust, well adapted sub-shrub legumes which might have a role in permanent grazing (common grazing land). These perform well as permanent pastures in northern Australia (Clem and Hall 1994; Jones and Brandon 1998).

Desmanthus spp. proved particularly well-adapted to NTT. When initially tested on West Timor, *D. pernambucanus* grew slowly after establishment but continued to grow through the dry season to produce over 5 t DM/ha by the end of the season. There was a high proportion of stem, however, so feed value would have been relatively low. Establishment of *D. virgatus* was variable when later tested on Sumba and this appeared to influence biomass production. However, *Desmanthus* persisted on the Alfisol site whereas most other legumes died.

The performance of the *Stylosanthes* lines was more variable. *S. seabrana* was best adapted overall, similar to previous experience in northern Australia (Edye et al. 1998). A 'common' line produced exceptionally high yields (5.9 to 9.8 t DM/ha) over ~200 days on an Inceptisol on West Timor and later, Primar and Unica produced high-ranked yields on an Alfisol and Vertisol on Flores. These results indicate *S. seabrana* is broadly adapted to key soils in Eastern Indonesia. The other stylos grew poorly overall, except *S. guianensis* which yielded well (6.5 t DM/ha) on an Inceptisol.

Forage value

Forage value was not assessed during the studies, but nutrient values for the tested legumes are well documented in literature. The better performing legumes (*C. pascuorum* and *C. ternatea*) identified for short-term systems are known to have excellent feed value. Under small plot assessment conducted in north Queensland a range of *C. pascuorum* leaves had a mean nitrogen content of 2.4% (15% crude protein) and phosphorous and sulphur contents ranging from 0.15–0.18% and 0.19–0.23%, respectively (Clements et al. 1984; Clem and Hall 1994). Nitrogen contents of 2.8% and dry matter digestibilities of 66–74% were reported for irrigated *C. ternatea*, *C. pascuorum*, *L. purpureus* and *M. bracteatum* 90 days after planting on a Vertisol soil lowland site in West Timor (Hartutik et al. 2012). The other herbaceous legumes listed above also produce high quality fodder and none have any nutritional limitations, although low palatability has been reported for *M. pruriens* (Cook et al. 2020). Comparative studies found the feed value of the sub-shrub legumes *Desmanthus* spp. and *S. seabrana* tend to be lower than for *C. ternatea* (Jones et al. 2000) and the shrub legumes are considered of moderate feed value, however, and may have a useful role as fodder banks once established and managed appropriately.

Cattle production studies were completed in NTT within the broader research program using locally grown *C. ternatea* fed fresh or conserved as hay (Mayberry et al. 2021). It was found relatively small amounts of legume added to a typical local diet (native grasses and supplementation types) overcame weight loss in cows and heifers, decreased mortality in unweaned bulls and increased liveweight gain in bulls.

Forage systems and considerations for adoption in eastern Indonesia

Legumes with a range of growth habits and capacity to regrow after cutting were identified for Vertisols in eastern Indonesia, with fewer options for Alfisols and Inceptisols. The legumes which produced high amounts of biomass shortly after establishment but exhibited poorer regrowth after cutting (principally *C. pascuorum* but also *L. purpureus* and, potentially *M. pruriens*) have best potential for short-period intercrop systems, such as maize production with shorter growing seasons or 'Rice-Rice-Legume' or 'Rice-Legume-Rice' sequences using irrigation to supplement rainfall during the dry season. In complementary research conducted within the research program it was demonstrated *C. ternatea* could also be used in these systems because of its capacity to rapidly produce biomass after sowing (Bell et al. 2022). *V. luteola* and *M. bracteatum* could be used as an alternative to these species if longer growth periods are warranted.

C. ternatea provides the greatest flexibility for successive crop-forage systems because of rapid growth after establishment and the best capacity to regrow after cutting. Potential roles include forage crop production in rotation with rice in the lowland areas and with maize in the upland or drier areas with no additional irrigation. Multiple forage crops can be harvested and either fed fresh or conserved as hay and fed during times of fodder scarcity during the dry season in upland cropping systems when soil moisture limits fodder production or during the wet season in rice production areas where most available land is being used for grain production.

Another potential option to integrate legumes into crop production systems is to grow a 'living mulch' by sowing a vigorous grain such as maize into a live legume sward. Such methods can be used to suppress weeds and modify the soil environment to benefit crop production. In Nigeria, Akobundu and Okigbo (1984) demonstrated *C. pascuorum* effectively smothered weeds and maize could be successfully grown in the legume crop if the legume was suppressed before planting by cutting or defoliating with herbicide, although legume regrowth after cutting caused some reduction of yields. Such systems may benefit crop and cattle components in eastern Indonesia where they meet agronomic and cultural needs, but this requires further testing.

Conservation of fodder as legume hay to target feed gaps or for sale was demonstrated by the research team (Dalgliesh et al. 2014). *C. pascuorum* hay is routinely mechanically produced on a commercial scale in the Northern Territory of Australia (Cameron 2005) and *C. ternatea* is known to produce excellent hay (Cook et al. 2020). Both have been shown to produce excellent hay in eastern Indonesia with little loss of leaf when incorporated into small bales (Nulik et al. 2013). Hay can also be produced by most of the other legumes tested, but often with difficulty due to thick stems or the shedding of leaves when dry (Cook et al. 2020).

Two legume taxa (*Desmanthus* spp. and *S. seabrana*) were identified as having potential for longer term fodder banks or permanent pastures. They were well-adapted to the 3 soil types tested and *D. virgatus* and *S. seabrana* stood out as options on the Alfisol on Sumba, potentially providing options for extensive grass pastures used for common grazing systems. Higher yields of *D. pernambucanus*, an erect legume suited to fodder banks, than *D. virgatus*, a smaller type more commonly used in grass-pastures (Cook et al. 2020), in earlier studies indicates there might be merit in considering this species for grazing. Adoption would require further research into establishment systems under eastern Indonesian conditions due to the difficulty establishing these small-seeded legumes with high levels of hard seed dormancy (Burrows and Porter 1993; Peck et al. 2011).

Forage crop establishment and growth

The adoption by smallholder farmers of legumes within crop-forage systems (rotation, relay) is contingent on the ready supply of seeds for sowing and comparative ease of establishment. Legumes with larger seed and low levels of hard seed dormancy are generally easier to establish using smallholder methods (e.g. 'dibble' pole into rough tilth) as they will emerge from a range of sowing depths and do not require processing prior to sowing to overcome hard seed dormancy. *C. ternatea*, *C. pascuorum*, *L. purpueus* and *M. pruriens* all have relatively large seeds and low to moderate levels of hard seed dormancy compared to other legumes (*Desmanthus*, *Stylosanthes*) (Cook et al. 2020). In a series of small-lot experiments conducted within the research program, the 'dibble' pole and furrow sowing methods were found to provide higher plant populations and legume biomass of *C. ternatea* than broadcasting seeds (Nulik et al. 2017) supporting the results from the current study.

A component of the program investigated flowering for seed production of the more promising legumes identified in the program in a series of replicated experiments in northern Australia and 4 sites in eastern Indonesia (Nulik et al. 2016). Milgarra (*C. ternatea*) was found to be the most flexible seed crop due to indeterminate flowering, low levels of pod shattering (dehiscing) and the capacity to increase hand harvested yields by using trellises. Pods also present well for hand picking. Cavalcade and Bundey (*C. pascuorum*) had strong photoperiodic control over flowering which restricted the opportunity to harvest seeds during the year and pods were located within the crop canopy making harvest by hand-picking more difficult. These pods can also dehisce when dry, requiring more complex management to harvest seeds. Flowering of Highworth (*L. purpureus*) was under weaker photoperiodic control in eastern Indonesia than in northern Australia resulting in weak flowering, although good presentation and low levels of pod shattering mean hand harvest is relatively easy. Overall, seed production of *C. ternatea* is considered best suited for opportunistic harvesting by small holders as seeds can be readily hand-picked from forage crops over most of the year. The other species require special management for seed production to encourage vigorous flowering and recovery of seeds.

The legumes were grown without inoculating seeds with nitrogen fixing bacteria in most of the experiments. There were no obvious deficiencies for nitrogen (yellowing and poor growth) except possibly for *S. guianensis* grown at Naibonat in 2010. Specific bacterial strains are recommended in Australia for *M. bracteatum*, *Desmanthus* spp. and *S. seabrana*, but effective nodulation can often be achieved with native rhizobia for *C. ternatea*, *C. pascuorum* and *L. purpureus,* although application on a precautionary basis is often recommended when these, or closely related, species have not been grown previously (Cook et al. 2020). It is possible that inoculation with specific rhizobia could have increased yields, but the high forage yields often achieved indicate there was sufficient nitrogen in the soil or effective associations were formed with local bacterial strains. The use of rhizobia strains for use of the higher-performing legumes in Indonesia warrants further investigation, but is not considered a research priority.

Conclusions

A broad range of legumes have now been tested in eastern Indonesia for use in crop-livestock systems and a range of suitable legumes identified. They all establish readily from seed using local sowing practices and produced high levels of biomass without rhizobium seed inoculum.

C. pascuorum, *L. purpueus* and *M. pruriens* produced high amounts of biomass shortly after establishment during summer (January–March) but regrew poorly after cutting in most instances. These legumes are considered suitable for integrating into crop systems characterised by short intercrop periods, such as 'Rice-Rice-Legume' or 'Rice-Legume-Rice' sequences using irrigation to supplement rainfall during the dry season. *V. luteola* and *M. bracteatum* could be used as an alternative to these if longer growth periods are possible.

C. ternatea produced high biomass yields after summer sowing on Vertisol soils. Regrowth after cutting was vigorous across a range of environments and high fodder yields were accumulated over the growing season. These features present farmers with a range of options including forage production in rotation with rice in lowland areas or with maize in the upland or drier areas with no additional irrigation. Multiple forage crops can be harvested and either fed fresh or conserved as hay and fed during times of fodder scarcity.

Desmanthus species and *S. seabrana* show potential for use for longer-term fodder banks (*D. pernambucanus*) or pastures (*D. virgatus*, *S. seabrana*) on Alfisol, Inceptisol and Vertisol soils where forage production is produced outside of cropping areas.

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Research Paper

Thermal limits to stoloniferous leaves and root growth in *Paspalum notatum*, a south American native grass

Límites térmicos para el crecimiento de hojas estoloníferas y raíces en Paspalum notatum*, una gramínea nativa de América del Sur*

ÁUREA RODRIGUES CORDEIRO, ALEXANDRE APARECIDO DUARTE, ANA PAULA DE FARIA, JOSÉ PIRES DE LEMOS FILHO AND MARCEL GIOVANNI COSTA FRANÇA

Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil. icb.ufmg.br

Abstract

In tropical regions the cultivation of African grasses for animal forage is extensive and an ecophysiological alternative is to stimulate the use of native species especially in a scenario of global temperature change. The thermal limits to leaf and root growth in *Paspalum notatum* a South American native grass were evaluated. Stolon fragments with roots and dry parts removed and the same number of nodes were placed in transparent plastic boxes on moistened filter papers and transferred to chambers at constant temperatures of 15, 20, 25, 30, 35 °C and alternating temperatures of 25/15 °C and 30/20 °C, all in a 12 h photoperiod. Leaf production was evaluated daily for 30 days. Stolon fragments showed leaf growth in all temperatures, except at 15 °C. The thermal range limits were 14.3 °C as base temperature and 39.2°C as ceiling temperature. Results showed that 50 degree days were necessary for 50% of leaf growth by the stolons. The largest leaf area occurred at 25 to 30 °C and the largest specific leaf area was at 25 °C. The optimal temperature for growth was 30 °C with higher root growth at 20 °C and in alternating temperatures. Results indicate that *P. notatum* has potential to grow in a wide range of temperatures and that the increase of global average temperature should not affect its distribution in its current habitat, presenting promising traits as an option for pastures in all tropical regions.

Keywords: Climate change, grass propagation, leaf growth, native grass, thermal time model.

Resumen

En regiones tropicales el cultivo de gramíneas africanas para forraje animal es amplio y una alternativa ecofisiológica es estimular el uso de especies nativas especialmente en un escenario de cambio de la temperatura a nivel global. Se evaluaron los límites térmicos para el crecimiento de hojas y raíces en *Paspalum notatum*, una gramínea nativa de América del Sur. En cajas plásticas transparentes, sobre papeles de filtro humedecido, se colocaron fragmentos de estolones sin raíces ni partes secas, y con igual número de nudos y se transfirieron a cámaras a temperaturas constantes de 15, 20, 25, 30, 35 °C y temperaturas alternas de 25/15 °C y 30/20 °C, todo en un fotoperíodo de 12 h. La producción de hojas se evaluó diariamente durante 30 días. Los fragmentos de estolones mostraron crecimiento de hojas en todas las temperaturas, excepto a 15 °C. Los límites del rango térmico estuvieron entre 14.3 y 39.2 ºC. Los resultados mostraron que 50 grados-día fueron necesarios para el 50% del crecimiento de las hojas de los estolones. La mayor área foliar se presentó entre 25 y 30 °C y la mayor área foliar específica se presentó a 25 °C. La temperatura óptima para el crecimiento fue 30 ºC, con un mayor crecimiento de las raíces a 20 °C y en temperaturas alternadas. Los resultados indican que

Correspondence: Marcel Giovanni Costa França, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627, CEP 31270-901, Belo Horizonte, Minas Gerais, Brasil. Email: marcel@icb.ufmg.br

P. notatum tiene potencial para crecer en un amplio rango de temperaturas y que el aumento de la temperatura media a nivel global no debería afectar su distribución en su hábitat actual, presentando atributos prometedores como una opción para pasturas en todas las regiones tropicales.

Palabras clave: Cambio climático, crecimiento de hojas, modelo de tiempo térmico, pasto nativo, propagación de pastos.

Introduction

Environmental temperature is an important driver of plant growth and development (Hodges and Evans 1992; Bykova et al. 2012; Yamori et al. 2014). An extrapolation of the thermal range tolerated by a species can induce thermal stress in many plants (Porter 2005; Kotak et al. 2007). Therefore, it is one of the environmental conditions which determines vegetation distribution in an area (Scherrer and Korner 2011).

Grasses are an important plant group widely distributed in different biomes (Kellogg 2001). These plants are the largest group with C4 photosynthetic metabolism, which give them an advantage in water use efficiency and high photosynthetic rates at high temperatures over C3 plants (Sage 2004). C4 grasses present higher growth in warm and wet environments, some survive with water shortage (Yamori et al. 2014), some tolerate shading (Martuscello et al. 2009) and low nutrient availability while some are capable of post fire regeneration (Leite et al. 1998). These traits ensure grasses establish in new environments (Tinoco-Ojanguren et al. 2016). Despite the adaptation of most C4 grass species to environments with high average temperatures, it is necessary to understand how specific grass species will respond to the increase of average temperatures predicted by the intergovernmental panel on climate change (Edenhofer et al. 2014). It has been shown that, although some grasses operate through the C4 metabolic route, they do not present a response pattern to temperature increases (Faria et al. 2015), so these results indicate that responses are species-specific. In Brazil, there is wide use of African grasses for grazing, however, such species can escape their cultivation areas and become invasive competing with and replacing native species due to their vigorous growth and large-scale propagation (Martins et al. 2007; Martuscello et al. 2009). Faria et al. (2015) showed that the increase of average temperatures would not affect alien species development. These species already have invasive ability, occupying a niche of native species, because they are highly competitive for natural resources.

Over time, there was natural variation in global temperatures, with alternation of colder and warmer seasons (Ghil 2002). However, what has been observed since the industrial revolution is an increment of average temperatures at an unprecedented speed due to the exponential increase of greenhouse gas in the atmosphere, resulting from the use of fossil fuels (Edenhofer et al. 2014), as well as other factors associated with deforestation and misuse of land (Buizer et al. 2014). The question related to climate change being widely researched is whether plant species will resist climate change and remain in their habitats, or will suffer redistribution impact. Extreme temperatures exceeding the thermal limit inactivate enzymes such as Rubisco activase and compromise photosynthetic yield (Law and Crafts-Brandner 1999; Sharkey 2005). Changes in long-term average temperatures can exceed the ideal thermal limits for plants, therefore compromising their growth and development, directly impacting their productivity, and changing landscapes as we know them today, due to possible changes in the composition of plant communities (Klanderud and Totland 2005; Dieleman et al. 2015; Shi Zheng et al. 2015).

In recent decades, researchers have highlighted temperature influences in leaf emergence (Moles et al. 2014; Nagelmuller et al. 2016; Egan et al. 2017) and the need to understand the minimum heat requirement for each species (Xue Qingwu et al. 2004; Andrade et al. 2005). The thermal time model is an important tool for physiological studies, and in this study, it is used to predict plant growth under thermal conditions. Equally relevant is the assessment of thermal limits for plant development, using base temperature (T_b) and ceiling temperature (T_c), which are important for determining an optimum range or even an optimum growth temperature (T_o) for the establishment of plant species (Daibes and Cardoso 2018; Duarte et al. 2019). The use of this model will make it possible to estimate the chances of species remaining in their current habitat or if they can be redistributed (Walther et al. 2002; Thomas et al. 2004).

Brazil has many native grasses that present high forage potential (Nabinger et al. 2009), but are not used for pastures due to the superior growth of African grasses (D'Antonio and Vitousek 1992). The *Paspalum* genus has 350 species that consist of both annual and perennial species (Ravikesavan et al. 2023) with potential use for forage throughout the country (Ribeiro et al. 2006; Figure 1). Some species are stoloniferous and both seeds and stolons are used for propagation (Batista and Neto 2000; Aliscioni and Denham 2008; Pimenta et al. 2013). The hypothesis for the study is that since *Paspalum* is widely distributed in South America, this grass presents broad thermal adaptation. In this study the thermal time and cardinal temperatures for leaf emergence were determined and root growth in a thermal gradient was evaluated for *Paspalum notatum* Flugge.

Materials and Methods

Plant material and growth conditions

Stolons of *Paspalum notatum* Flugge were obtained from plants growing on the lawns of the Federal University of Minas Gerais. Immediately after collecting stolons, remaining roots, leaves and dried parts were removed. Stolon fragments were prepared with 4 nodes. Stolons were moistened with nystatin solution (2%) for disinfestation to prevent fungal contamination and placed in transparent plastic boxes (11 × 11 × 3.5 cm) on 3 sheets of filter paper moistened with the same solution to keep papers moist. The experimental design used 42 boxes, each one containing 10 stolons. Six boxes were placed in germination chambers with different temperatures, using 10 stolon fragments from the same plant from 6 individual plants per treatment. Germination chambers were maintained at constant temperatures of 15, 20, 25, 30 and 35 °C and alternating temperatures of 25/15 °C and 30/20 °C with a photoperiod for all treatments of 12 h. The light intensity in the germination chambers was approximately 80 µmol/m^2/s.

Leaf emergence and growth

Leaf emergence (defined as leaves >2 mm) and number of leaves were measured daily for 30 days and final percentage of leaf emergence determined. Considering the initial number of leaves at the end of the experimental period, the relative growth rate (RGR) for leaves during the experimental period was determined according to McGraw and Garbutt (1990), using the equation:

$$RGR=(\ln LN_2 - \ln LN_1)/(t_2 - t_1),$$

where:

LN is total leaf number at each time;

t_1 and t_2 measurements were taken weekly for 30 days (n=6).

To estimate the leaf area (LA), all fully expanded leaves were digitalized while still fresh (HP Scanjet G4050), and the total LA was integrated by using the ImageJ software. In order to obtain the specific leaf area (SLA) the digitalized leaves were, then, placed in paper bags and dried in an oven at 60 °C until constant weight (Fanem Model 320-SE). Dry leaves were weighed on a precision scale (Shimadzu, Model AY220). The SLA was estimated through the ratio between the LA and the leaf dry mass (cm^2/g). For both parameters, we sampled 5 individuals per treatment (n=5). To determine the biomass accumulation over 35 days, leaves and roots were placed in paper bags and dried in an oven until constant weight (Fanem Model 320-SE) at 60 °C. After this period, they were weighed (n=5) on a precision scale (Shimadzu Model AY220).

Cardinal temperatures and thermal time for leaf emergence

The cardinal temperature was established to determine the thermal time of leaf emergence (t_g) of the first leaf of each percent fraction of 10% of the stolon population. Following that, the leaf emergence rate (LER) ($1/t_g$) was determined. Temperatures below the sub-optimal range (T_o) were used to calculate base temperature (T_b). LER was plotted for each treatment linked to temperature for different emergence percentiles for each 10% emergence increase (Covell et al. 1986). The intersection point of this line with the x axis determined the base temperature (T_b) of the tested fraction. The chosen T_b value was obtained from linear regression, from the fraction with the highest R^2. The same procedure was used for the supra optimal portion to determine the ceiling temperature (T_c). The optimal temperature (T_o) was obtained through the intersection of the lines used to obtain T_b and T_c and the value which corresponds to this point in the temperature axis.

Once the linearity relation between LER and the temperature was evident, and T_b showed a general tendency to converge between percentile fractions, the probit model was used to calculate the thermal time for the emergence of the first leaves from stolons in infra-optimal temperatures (below T_o) using the equation Ellis et al. (1986):

$$\theta_{(sub)}=(T - T_b) \cdot t(_g),$$

where:

T is the growth temperature;

T_b is base temperature;

t_g is the necessary time for leaf emergence of a given fraction (%) from the stolon population.

Statistical analyses

The design was completely randomized for the 7 temperature treatments. Data were analyzed by Generalized Linear Models (GLM) and the means were compared by contrast test at 5% probability using the software R 3.3.1 (R Core Team 2015). For the parameter percentage of leaf emergence, growth temperatures were used as explanatory variables. Data of relative growth were submitted to a one-way ANOVA and the means compared by Tukey test. For growth analysis, the response variables used were leaf biomass, leaf area and specific leaf area.

Results

Leaf growth

Stolon leaf emergence was observed at all tested temperatures, except for 15 °C where leaf growth was not sufficient to be analyzed (Figure 1 and 2). Emergence of up to the 6th leaf was common in all the other 6 treatments where leaf growth was observed, and no statistical difference was found between them (Figure 2a1, 2a2 and 2a3). The final leaf emergence percentage was above 70% up to the 4th leaf and below 70% for the 5th and 6th leaves (Figure 2a1, 2a2 and 2a3). For the 1st leaf, only at 30 °C, there was a higher speed of leaf emergence, however, for the following leaves there was no difference in leaf emergence (Figure 2b1, 2b2 and 2b3).

The highest relative growth rates (RGR) were observed in plants submitted to constant temperatures of 20, 25, and 30 °C (0.1128 to 0.1219 g/g/day) and alternating temperatures of 25/15 °C and 30/20 °C (0.1219 and 0.1200 g/g/day, respectively) (Figure 3a). Plants growing at 35 °C showed the lowest relative growth rate (0.1127 g/g/day) (Figure 3a). Considering leaf, root and total biomass, there was no difference in production, however, there were differences for root production (Figure 3b). The 20 °C temperature induced higher root growth, with no differences among other temperatures (Figure 3b).

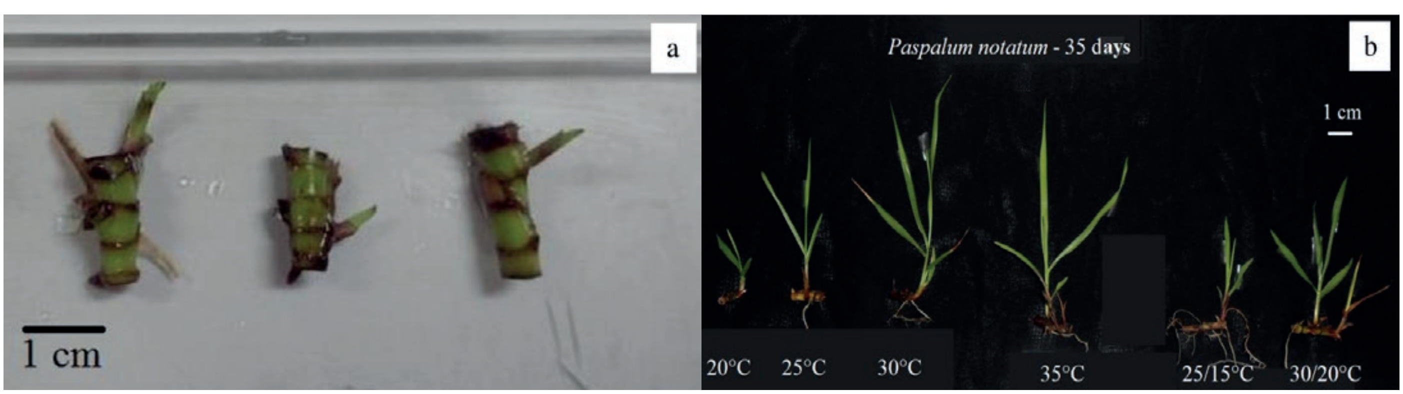

Figure 1. *Paspalum notatum* stolons (a) 4 days after moistening and (b) seedlings at 35 days of growth at different temperatures.

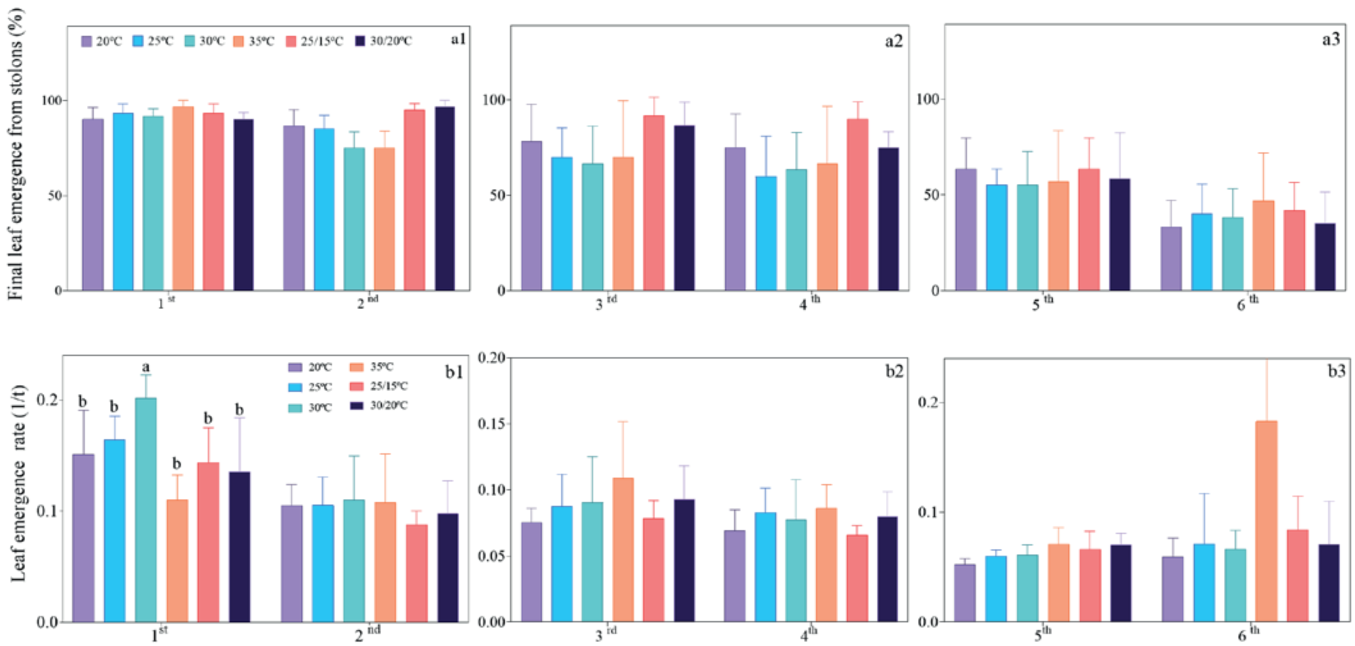

Figure 2. Final leaf emergence from *Paspalum notatum* stolons growing at different temperatures. (a1) stolons leaves 1 and 2; (a2) leaves 3 and 4; (a3) leaves 5 and 6; (b1) leaf emergence rate of leaves 1 and 2; (b2) leaves 3 and 4; (b3) leaves 5 and 6. Same letters in (b1) indicate no difference between growth and temperatures (n=10; P≤0.05). Bars show the average ± standard deviation.

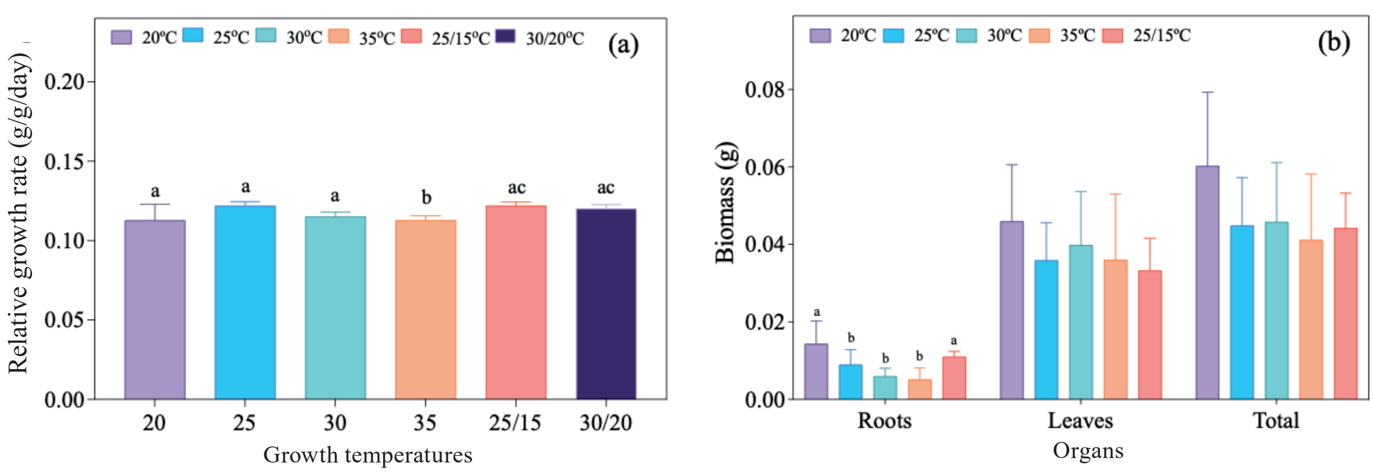

Figure 3. Leaf relative growth rate (a) and (b) root, leaf and total biomass accumulation of *Paspalum notatum* stolons after growth at different temperatures. Different letters in (a) and (b) indicate differences between growth temperatures and same letters indicate no difference between growth temperatures (n=6; P≤0.05). Bars show the average ± standard deviation.

Leaf area and specific leaf area

Leaf area and specific leaf area presented differences among thermal treatments (Figure 4). Larger leaf area was observed in plants grown in temperatures from 25, 30 and 35 °C (P≤0.05), surpassing 10 cm^2, and the most extreme temperatures tested resulted in smaller leaf area, varying from 4.6 to 10 cm^2 (Figure 4a). The specific leaf area was only larger at 25 °C (P≤0.001), with average values of 121 cm^2/g (Figure 4b). The plants from the other thermal treatments presented equivalent values, not surpassing 101 cm^2/g (Figure 4b).

Thermal time for leaf emergence

Cardinal temperatures determined for the 1st leaf emergence were 14.3 °C (T_b) 39.2 °C (T_c) and T_0 was 29.9 °C (Figure 5a). The necessary thermal time for 50% (θ_{50}) of the stolons to form the 1st leaf was determined in 50 degree days and 110 and 180 degree days to form the 2nd and 3rd leaves, respectively (Figure 5b).

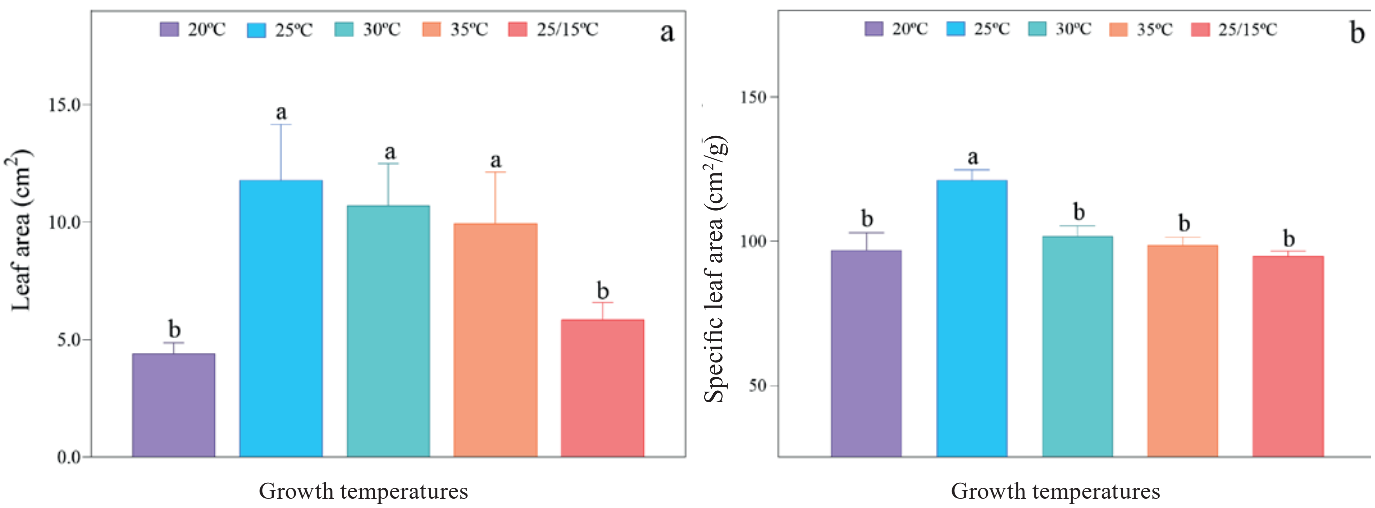

Figure 4. Leaf area (a) and specific leaf area (b) of *Paspalum notatum* stolons at 30 days of growth. Different letters in (a) and (b) indicate differences between growth temperatures and same letters indicate no difference between growth temperatures (n=5; P≤0.05). Bars show the average ± standard deviation.

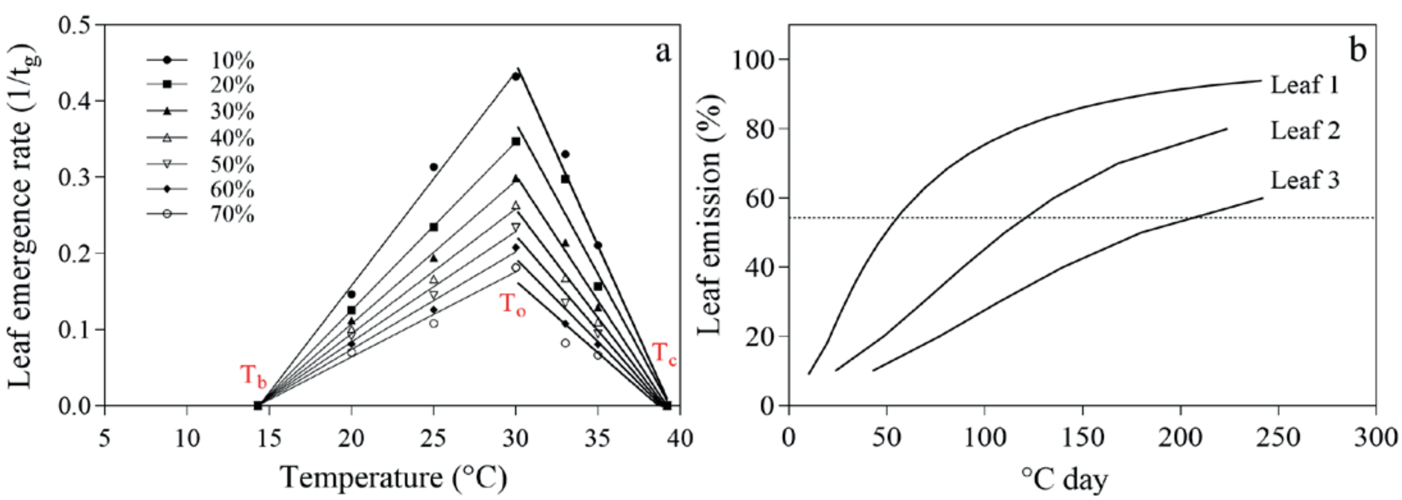

Figure 5. Necessary cardinal temperatures for leaf emergence from *Paspalum notatum* stolons. (a) Leaf emergence rate of first leaf at isothermal temperatures, were T_b=14.3 °C, T_0=29.9 °C and T_c=39.2 °C and (b) Leaf emission of the first leaves of P*aspalum notatum* whose stolons were submitted to different temperature requirements in degree days (°C). Values show the average ± standard deviation (n=6; P≤0.05).

Discussion

Leaf growth

The stolon is a reserve organ used for vegetative propagation in grasses (Donaghy and Fulkerson 1998), because it provides the ability to re-sprout through the mobilization of reserves until the sprout reaches its photosynthetic autotrophy (Fulkerson and Donaghy 2001). It was observed that the percentage of leaf emergence decreased from the 7th leaf (data not shown) as stolon reserves were depleted with possible rooting incapacity. In this study, there was neither substrate nor nutrient solution or soil to obtain nutrients, sustain newly formed roots and favor plant growth. In a natural environment, this pause would probably not have occurred due to the availability of soil for the roots to take hold and for the plant to continue its development.

Leaf emergence is associated with leaf expansion, and directly related to significant capacity for light interception, photosynthesis and, consequently, early growth (Streck et al. 2002; Streck 2002). Cardinal temperatures vary within the same species, according to the development phase of the plant (Bykova et al. 2012; Sanchez et al. 2014). When temperature reaches a value beyond this optimal range, leaf emergence and elongation become stagnated due to the discontinuance of cell division and elongation, as shown for wheat, corn and rice (Sanchez et al. 2014). Considering all stages of plant development, the extrapolation of T_0 in the field can favor other physiological phases, such as reproduction, for example. According to Sanchez et al. (2014), the T_0 for the reproductive phase is higher than the T_0 for the vegetative development phase for wheat, corn and rice, therefore, it is feasible that the T_0 for *P. notatum* grain filling to be higher than 30 °C.

Although the aim of this study was to assess the influence of temperature on the thermal time for leaf emergence, root growth is also essential for plant establishment. The study showed that the optimum temperature for root growth was different from the optimum temperature for leaf growth. The highest accumulation of root biomass at 20 °C can be related to the need for lower temperatures for its development, once the soil temperature is generally lower than air (Kaspar and Bland 1992). Different temperatures cause different responses in biomass accumulation in plants (Gunn and Farrar 1999; Moles et al. 2014), however, in *P. notatum* different responses were observed only in the roots and not for the leaves in the tested temperatures. Under low average temperatures, metabolism becomes slower and therefore it is expected that plants grown in these conditions accumulate less biomass than plants grown in higher temperatures. This was observed during the experiment through leaf area increases, however, during the period when the dry mass quantification was performed, time was only standardized in calendar days and the number of degree days accumulated were not the same for all treatments, which might have influenced the response (Gunn and Farrar 1999). Plants which grew faster may have had their growth stabilized due to the lack of resources to continue development and those which had a slower growth reached values equivalent to biomass accumulation (Gunn and Farrar 1999).

Such results reaffirm that this grass adapts to higher temperature averages. Exotic African grass species are attractive forages due to the higher germination rate of seeds, fast growth (D´Antonio and Vitousek 1992; Pivello et al. 1999) and commercial seed supply. *P. notatum* can also be attractive due to its forage potential, ability to grow in environments with high average temperatures and, as a native species, it poses no threat to other populations in natural areas.

Leaf area and specific leaf area

A larger leaf area confers a greater photosynthetic activity, production and photoassimilate accumulation in the culms. This carbon reserve is mobilized for initial leaf growth (Corre et al. 1996). SLA is an appropriate parameter to verify the effect of environmental factors such as temperature because the SLA value is proportional to plant growth (Poorter et al. 2010). Considering that stolons growing under temperatures of 25, 30 and 35 °C had larger LA, this can confer advantages in producing photoassimilate and ensure a higher carbohydrate reserve for the re-sprout. Such responses may be similar to those observed in the field where water availability is not limiting, because leaf expansion is extremely sensitive to water deficit (Liu Mengzhou et al. 2017). Kikuyu grass, a tropical species, presented shorter leaves at lower temperature than its T_b, and there was an increase in leaf thickness to compensate for the smaller size indicating that there is not an interruption in metabolism but a change in growth (Acero-Camelo et al. 2021).

Specific leaf area is a very efficient parameter to measure plant growth (Liu Mengzhou et al. 2017), but its relation to temperature has not been studied. A study to evaluate the best method of SLA analysis found that tropical species showed greater plasticity when growing in areas with temperature variation than plants growing in areas with mild weather (Poorter et al. 2010). In the present study, the SLA results might indicate plasticity. In agreement with Poorter et al. (2010) cold temperatures may have induced less cell production resulting in a lower SLA. This can explain values obtained at low temperatures but does not explain results from the treatments at high temperatures. Results show that, although this grass is able to grow in a wide temperature range, the growth in either colder or extremely hot environments will result in a reduced SLA.

Determining thermal time for leaf emergence

The thermal limit found for the leaf growth of *P. notatum* (average values between 14.5 to 39.5 °C) explains why the species grows in several vegetation types and altitudinal gradients on the American continent (Batista and Neto 2000). Based on information from SpeciesLink (2021) system it also indicates its adaptation in regions with mild temperatures. Although a low leaf emergence rate was observed in a 15 °C constant temperature, it is important to highlight temperatures vary during the day and seasons of the year (Fogliatto et al. 2020) and stolons subjected to alternating temperatures of 25/15 °C showed higher leaf emergence, similar to higher temperatures, evidencing the ability of *P. notatum* to establish in regions with this thermal range. The values determined for T_b and T_c indicated the influence of temperature on leaf emergence, as highlighted by Martins et al. (2007). Responses to the thermal gradient can be attributed to the phenotypic plasticity of the species related to the natural environment thermal variation.

High phenotypic plasticity related to temperature was observed in the C4 grass *Urochloa brizantha*, highlighting that this grass suffers more under low temperatures (Nakao and Cardoso 2016). Considering the leaf emergence percentage and leaf emergence speed did not present differences between growth temperatures, this high plasticity for leaf emergence can be considered as a positive attribute of *P. notatum* conferring advantages in environments with milder temperatures when compared to invasive species. However, the T_0 of 30 °C indicates that *P. notatum* presents faster growth in spring/summer periods, when there is more water and nutrient availability and higher average air temperature.

The thermal requirement for 50% (θ_{50}) of the population to produce first leaves was 50 degree days, indicating a low thermal requirement for half of the stolon population to emit the first leaves compared to other grasses. Higher values of θ_{50} were found for *Pennisetum purpureum* (Andrade et al. 2005) with the thermal requirement estimated as 84 degree days. In a study with *Panicum virgatum*, there was a strong relation between the necessary thermal requirement to produce leaves and the beginning of the reproductive phase (van Esbroeck et al. 1997). According to these authors, higher or lower thermal requirements matched with the late or early start of the reproductive phase, respectively. Therefore, it is possible to predict that *P. notatum* plants which present low thermal requirements, grown in average temperatures around 30 °C could be early flowering compared to plants grown in other temperature ranges. This can represent a growth strategy of the species at the beginning of the rainy season.

Determining the T_b indicated that *P. notatum* stolons have potential to sprout and grow even in colder months, which may represent a competitive advantage for the species when compared to others that have higher thermal requirement and a narrower thermal niche (Bykova et al. 2012). It suggests that this plant is able to remain green and keep an active metabolism throughout the entire year, if the only limiting resource is temperature.

In most Brazilian states, higher temperature averages occur in the summer when rainfall is more abundant and therefore it is the most favorable period for fast growth. The highest averages of air temperatures occur in the period from January to March and in November and December (INMET 2021). In 2020, the region that presented the lowest temperature average was the South, with 13 °C in July and rainfall of 262 mm and total annual rainfall of 1,425.2 mm. In this region, there are many records of *P. notatum* (Figure 1) in the SpeciesLink (2021) system, indicating that the species grows better in milder temperatures. The Midwest presented the highest averages the same year with 32.4 °C in September and only 10.2 mm rainfall with fewer records of the species compared to the South. Despite the low volume of rainfall, stolons ensure the persistence of the grass until conditions become more favorable.

Using the average monthly and daily temperatures, it is possible to estimate the periods when stoloniferous leaf emergence of *P. notatum* will take place. It is also possible to predict the time for this in a future scenario of climate change, considering the forecast of temperature averages increase, where there is a possibility of an increase up to 6 °C in the worst scenario (Edenhofer et al. 2014). Considering such increase (scenario A), it is probable there will be a reduction of approximately 35% of the time taken by *P. notatum* stolons to emit leaves. With the increase of 4 °C in temperature averages (scenario B), this time can be reduced by approximately 30% compared to present. Colder temperatures in winter may compromise growth, which becomes slower or stops, especially in low rainfall seasons in its native environment. Some authors highlighted that progressive increase in averages of air temperatures may benefit some species (Gunn and Farrar 1999; Faria et al. 2015). However, regardless of an increase of 4 °C or 6 °C, the forecast of maximum average temperatures will still remain within the thermal gradient suitable for *P. notatum*, which indicates that the species has potential to grow in environments with thermal averages around 30 °C.

Besides the capacity to grow in a wide thermal range, the stolon remaining in the soil is important to ensure *P. notatum* regrowth because their stolons are not dormant and present low thermal requirements compared to other grasses (Andrade et al. 2005). Different to stolons, seeds from this species, show some traits which make propagation difficult, such as physiological maturity, low viability and physical dormancy (Gates et al. 2004), requiring higher average temperatures (around 30 °C) to germinate, which is a disadvantage compared to the species with easily germinated seeds.

Conclusions

P. notatum demonstrated the potential to develop stolon leaves in environments with a wide thermal range from around 15 °C lower average up to around 39.5 °C higher average. *P. notatum* can grow in environments with a high thermal gradient throughout tropical regions outside of its native distribution showing potential as a forage alternative to exotic African grasses. Results show that within the current temperature averages in Brazil and the forecast of increased averages of 6 °C, establishment and development of *P. notatum* will not be affected by climate change.

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(Note of the editors: All hyperlinks were verified 22 January 2025).

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Research Paper

Effect of single and repeated waterlogging events on tropical forage grasses for cut and carry systems

Efecto de eventos únicos y repetidos de anegamiento sobre gramíneas forrajeras tropicales en sistemas de corte y acarreo

ROWAN W. SMITH[1], NGUYEN THI MUI[1,2], NGUYEN XUAN BA[2], NGUYEN HUU VAN[2], JEFF P. CORFIELD[3] AND DAVID PARSONS[4]

[1]*Tasmanian Institute of Agriculture, University of Tasmania, Tasmania, Australia.* utas.edu.au/tia
[2]*Faculty of Animal Sciences and Veterinary Medicine, University of Agriculture and Forestry, Hue City, Vietnam.* en.huaf.edu.vn
[3]*Corfield Consultants, Townsville, Australia.*
[4]*Swedish University of Agricultural Sciences, Umeå, Sweden.* slu.se/en

Abstract

The introduction of improved forage cultivars has enhanced nutrition and performance of beef cattle in stall feeding cut and carry systems in Vietnam and other Southeast Asian countries. However, the persistence of these forages can be reduced by flooding and waterlogging conditions in low-lying land during the monsoon season. This pot study was established with 5 waterlogging treatments (control, 10-day single, 10-day cycle, 20-day single, and continuous waterlogging) for an 84-day period following establishment to evaluate the waterlogging tolerance of 7 improved grass cultivars. *Urochloa humidicola* (Rendle) Morrone & Zuloaga, *Paspalum atratum* Swallen. and *Digitaria eriantha* Streud. were most tolerant of waterlogging. Tiller number and dry matter production were negatively affected by cycling and continuous waterlogging in less tolerant species *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, *Urochloa* hybrid 'Mulato II' and *Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Crins. This information will assist in providing recommendations for smallholder farmers about which species to grow under repeated or continuous waterlogging.

Keywords: Production, tiller survival.

Resumen

La introducción de cultivares de forrajes mejorados ha mejorado la nutrición y el rendimiento del ganado de carne en sistemas de alimentación estabulada en sistemas de corte y acarreo en Vietnam y otros países del sudeste asiático. Sin embargo, la persistencia de estos forrajes puede reducirse por las condiciones de inundación y anegamiento en tierras bajas durante la temporada de monzones. Este estudio en macetas se estableció con 5 tratamientos de anegamiento (control, tratamiento único de 10 días, en ciclos de 10 días, tratamiento único de 20 días y anegamiento continuo) durante un período de 84 días después del establecimiento para evaluar la tolerancia al anegamiento de 7 cultivares de pastos mejorados. *Urochloa humidicola* (Rendle) Morrone & Zuloaga, *Paspalum atratum* Swallen. y *Digitaria eriantha* Streud. fueron los más tolerantes al anegamiento. El número de macollos y la producción de materia seca se vieron afectados negativamente por el anegamiento cíclico y contínuo en las especies menos tolerantes *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, *Urochloa* híbrido 'Mulato II' y *Urochloa ruziziensis* (R. Germ. y C.M. Evrard) Crins. Esta información ayudará a proponer recomendaciones a los pequeños agricultores sobre qué especies cultivar en condiciones de anegamiento continuo o repetido.

Palabras clave: producción, sobrevivencia de macollos.

Correspondence: Rowan W. Smith, Tasmanian Institute of Agriculture, University of Tasmania, Launceston, Tasmania, Australia. Email: Rowan.Smith@utas.edu.au

Introduction

Beef production in Vietnam, like other parts of Southeast Asia, has traditionally been based on extensive grazing, utilizing native or naturally occurring grasses, legumes and herbs along roadsides or in common areas, and stubble of recently harvested crops. Diets are supplemented with crop residues fed in stalls and supplements offered by cooking 'porridge' (Ba et al. 2014). This system is characterised by high labour inputs (Khanh et al. 2015) and low feed quality (Ba et al. 2015). A lack of both quantity and quality of feed resources is one of the major constraints for beef production in smallholder beef enterprises in South Central Coastal Vietnam (Parsons et al. 2013). Competition for feed resources on common lands (Ba et al. 2013) and the reduction in the area of common lands due to intensification of agriculture and urbanisation (Khanh et al. 2014) have encouraged smallholders to investigate more intensive systems of beef production.

A significant transformation of such systems has occurred in the Ea Kar region of Dak Lak province in the central highlands (Stür et al. 2013). One approach for intensifying the production system has been to grow and harvest improved forages and stall-feed them to cattle. Reallocation of some of the cropping land to intensively grown high yielding and high quality forages has been part of a Vietnamese Government strategy to develop livestock systems. Introduction of improved tropical forage cultivars has been part of research projects for improving beef production in smallholder beef enterprises in Southeast Asia (Lisson et al. 2010; Stür et al. 2013; Ba et al. 2014). A theme of these studies is that establishing forage plantations in close proximity to cattle shelters enables an evolution in the production system from predominantly controlled grazing and feeding of crop residues to stall-feeding of improved forages.

Smallholders that do grow forages in this region of Vietnam most commonly grow local varieties of elephant grass (*Cenchrus purpureus* (Schumach.) Morrone). Farmers who have been exposed to projects focusing on improved species prefer to grow 3 main grasses: Guinea grass (*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W. L. Jacobs) 'TD58', hybrid elephant grass (*C. purpureus* × *C. americanus*) 'VA06' and the *Urochloa* hybrid (*U. ruziziensis* × *U. decumbens* × *U. brizantha*) 'Mulato II' (Ba et al. 2014). Other grass and legume species have been introduced, including *Paspalum atratum* Swallen. 'Ubon', *Stylosanthes guianensis* (Aubl.) Sw. CIAT 184 and *Leucaena leucocephala* (Lam.) De Wit ' Tarramba', but are less preferred by smallholders (Ba et al. 2013; Ba et al. 2014). These smallholder preferences are influenced by the responses of these forage species to the local growing conditions, palatability for cattle and ease of establishment (Ba et al. 2014). In the case of *S. guianensis*, the inability of farmers to extend their forage area with vegetative material and the necessity to save seed are major constraints for smallholders. For leucaena 'Tarramba', inexperience with tree legume forages, need for seedling protection, a relatively long lead time between establishment and use, and difficulties with cutting management in smallholder situations all contributed to poor adoption in some areas (Nam et al. 2015). Rapid establishment and regeneration is one of the key traits leading to the success of the grass family Poaceae (Linder et al. 2018), and is one of the key reasons why introduced grasses have been more successful than legumes.

Soils in the South Central Coastal region of Vietnam are typically deep sands (<5% clay) and deep loamy sands and sandy loams (5–18% clay) (Bell et al. 2015) with low levels of organic carbon. Sandy soils with low clay content and low organic matter tend to have low nutrient holding capacity. This means that regular fertiliser needs to be applied to sustain production. However, the risk of leaching of nutrients applied is high when coupled with heavy rainfall events. These heavy rainfall events can also lead to seasonal inundation, where plants and the soil surface are submerged or flooded for short periods; or waterlogging, where the soil profile remains saturated for long periods. This study focuses on the effects of waterlogging with waterlogging tolerance defined as the plant's ability to survive (maintenance of live tillers) and grow (maintain reasonable growth rates) under waterlogged conditions. Recent studies by Tan and Thanh (2013) have suggested that total rainfall in Vietnam and the incidence of heavy rainfall events is increasing, and that flooding (and hence waterlogging) is occurring more frequently, especially in the South Central Coastal region. Furthermore, flooding is expected to increase in the Southeast Asia region more generally (Hirabayashi et al. 2013).

Under waterlogged conditions oxygen is displaced from the soil pores, and the remainder is rapidly used by plants and other organisms (Setter and Belford 1990; Blom and Voesenek 1996). The reduction in the availability of oxygen in the soil profile is a major constraint to plant growth under waterlogging conditions. Respiration is the most efficient form of energy production for root growth and maintenance and this is reliant on oxygen (Wegner 2010). Setter and Waters (2003) categorised the mechanisms for waterlogging tolerance by trait as; 1. Phenology, 2. Morphology and anatomy, 3. Nutrition,

4. Metabolism including anaerobic catabolism and anoxia tolerance, and 5. Post anoxic damage and recovery. An important anatomic mechanism by which some plants are able to adapt to waterlogging conditions is the formation of aerenchyma cells, which allow greater oxygen flow in the plant (Colmer 2003; Ayi et al. 2019).

Waterlogging can have a number of impacts on tropical forages including reducing dry matter (DM) production and tiller and plant death (Hare et al. 2003). Following the evaluation of several grasses and legume species, Hare et al. (1999) recommended *P. atratum* 'Ubon' for dairy systems in the seasonally waterlogged and seasonally dry regions of northeast Thailand, being more productive than *U. ruziziensis* (R. Germ. and C.M. Evrard) Crins, whose productivity declined over time. Later, Hare et al. (2004) showed that *P. atratum* 'Ubon' had good tolerance to waterlogging, *M. maximus* 'Purple panic' moderate tolerance and *U. ruziziensis* had poor tolerance. *U. humidicola* (Rendle) Morrone & Zuloaga. also exhibits tolerance to waterlogged soils (Dias-Filho and Carvalho 2000; Cardoso et al. 2013).

Forages growing in this region must be tolerant of high temperatures, and the 'toolbox' of forages should include species with tolerance to extremes of both drought and waterlogging, depending on the season. Although it may be possible to have these characteristics in a single species, diversifying the forage species grown could help to reduce risk for smallholders. Each of the above-mentioned forages vary in their adaptation to soil and moisture constraints. Most studies of waterlogging in tropical forages have focussed on a single waterlogging event but there is a paucity of information regarding the effects of repeated events, which may be more reflective of some climatic locations and position within the landscape. The study objectives were to evaluate the effects of both single and repeated waterlogging events on 7 tropical forage grass cultivars and provide an assessment of their suitability for use in Central Coastal Vietnam. The study outputs support the development of guidelines for use of these species and cultivars. The hypotheses were:

- *B. humidicola*, *P. atratum* and *Digitaria eriantha* Streud. are the most tolerant based on previous studies and expert opinion and will be least affected by waterlogging.
- For plants that are sensitive to waterlogging, a repeated cycle of waterlogging is more damaging than the length of time of the waterlogging.

Materials and Methods

Study location

The study was conducted at the Hue University of Agriculture and Forestry (HUAF) campus (16°28' N, 107°34' E), Hue province, in the Central Coastal region of Vietnam. The experiment was conducted in a greenhouse covered in transparent plastic between September and December 2015.

Establishing forages

The 7 grasses used in the experiment were *C. purpureus* × *C. americanus* 'VA06', *Urochloa* hybrid 'Mulato II', *M. maximus* 'TD58', *P. atratum* 'Ubon', *D. eriantha*, *U. ruziziensis* (each collected from a farmer field in Binh Dinh province) and *U. humidicola* (collected from a farmer field in Phu Yen province. Grasses were first established in soil at the HUAF campus and stabilised for a period of 2–3 months, depending on when the tillers were obtained. Healthy tillers were then transferred to 300 mm tall and 270 mm diameter pots. The number of live tillers for each species was recorded at day 0 prior to treatments being applied and is presented in the results section. There were no significant differences in the number of tillers in each pot/treatment of the same species. Pots were filled with a sandy loam textured soil sourced from a local nursery supplier in Hue. Chemical analysis of the soil was undertaken at the Hue University of Agriculture and Forestry soil science department following AOAC International standard methods (AOAC 2012). The results were: pH (KCl) 7.16; OM 2.74%; CEC 12.3 cmol/kg; Total N 0.02 g/kg; Extractable P 0.007 g/kg; Extractable K 0.074 g/kg, indicating low levels of organic matter and major nutrients.

Experimental design

The experiment was arranged in a split-plot design with waterlogging as the main factor (5 levels), grass species as sub-plots (7 species) and 4 replications (blocks). The 5 waterlogging treatments were: 1. Control - water level maintained at 30 mm from the bottom of the pot; 2. 10-day single saturation (10-day single) - water level raised to the soil surface for 10 days, then returned to 30 mm from the bottom of the pot for the remainder of the experiment; 3. 10-day repeated saturation (10-day cycle) - water level raised to the soil surface for 10 days, then returned to 30 mm from the bottom of the pot for 10 days, this cycle was then repeated; 4. 20-day single saturation (20-day single) - water level raised to the soil surface for 20 days, then returned to 30 mm from the bottom of the pot for the remainder of the experiment; 5. Continuous saturation (Continuous) - water level raised to soil surface for the entirety of the experiment (Figure 1). Each of the 4 blocks included 5 randomly placed waterproof polystyrene boxes (with the dimensions; length 1,100 mm, width 700 mm, and depth 500 mm) each with a different waterlogging treatment. Each box (main plot) contained 1 pot of each of the 7 grass species treatments (sub-plots), arranged randomly. The experiment was conducted over 4 × 21 day cycles.

Forage measurement

At the completion of each cycle, the following plant measurements were recorded: biomass production, the total number of live tillers (both primary and secondary), and length of longest vegetative tiller (from crown to tip of the longest expanded leaf).

Grasses were cut to a height of 150 mm above the soil surface on day 21 of each cycle. Tillers were gathered up and extended into a vertical position for measurement of the 150 mm cutting height. After cutting, stem and leaf components of the harvested forage were separated and dried at 105 °C for 48 hours to determine the dry matter yield. At the completion of each cycle, granulated fertiliser (N-P_2O_5-K_2O: 16-16-8) was applied at 0.7 g/pot.

Data analysis

Each output variable was analysed using mixed model procedures in PROC MIXED (SAS 2003) using the REPEATED statement. Waterlogging and species were treated as fixed effects. Non-normal distributions were identified by visual assessment of quantile-quantile plots and distribution of model residuals. Log transformation was applied for DM yields and tiller counts, while a cubed power transformation was used for quantum yield. Significant 3-way interactions were evaluated using the slice option in the lsmeans statement of PROC MIXED, which uses an F test to assess interaction slices, but does not control for family-wise error. For 2-way interactions, the SIMULATE option was used to separate significantly different means. Geometric means used were calculated for transformed data, in lieu of true means.

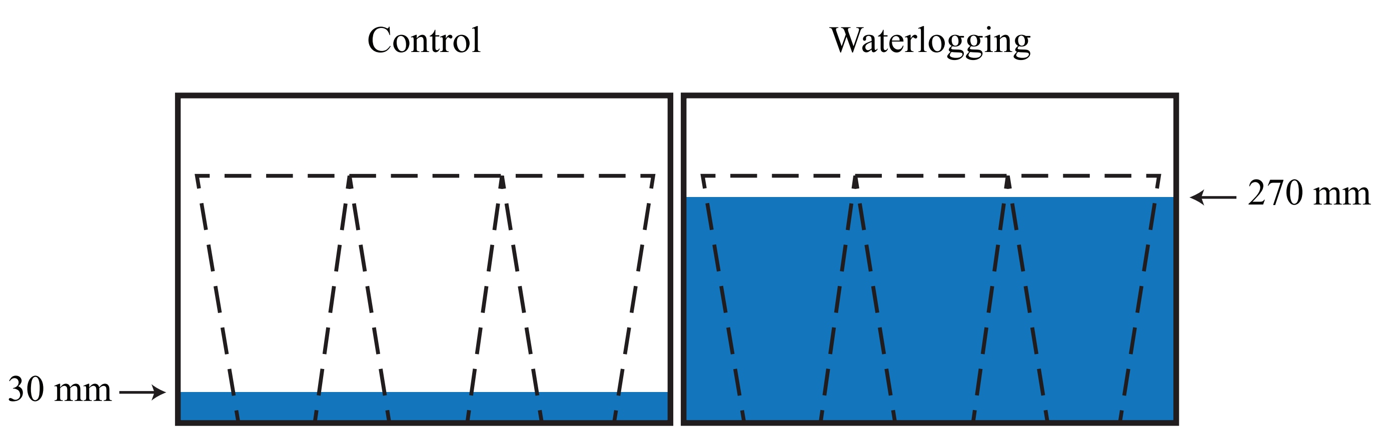

Figure 1. Representation of the water levels under control and waterlogging conditions.

Results

Overview of outputs

There was a significant (P<0.001) species*waterlogging treatment*time interaction on the above ground DM yield, leaf length, leaf DM yield, and the number of live tillers. In addition, all main effects and 2-way interactions were significant at P<0.001. Because 3-way interactions are complex to depict, we present outputs for these variables individually for each species (Figures 2–7). These figures include significances from the SLICE outputs. Significances on the side of the figures show the probability that the output variable for each waterlogging treatment changes over time. Significances on the top of the figures show the probability that the output variable from waterlogging treatments are different at a specific point in time.

Megathyrsus maximus ‘TD58’

The above ground DM yield of *M. maximus* ‘TD58’ in the control treatment appeared to decrease by half between day 21 and day 84; however, this was not significant (P=0.074) (Figure 2a). In contrast, there were significant (P<0.001) decreases in DM yield in both the 10-day cycle and continuous waterlogging treatments throughout the study to <2 g DM/pot by day 84. There were similar patterns for 10-day and 20-day single waterlogging treatments (P=0.053 and P=0.046 respectively), showing a drop in above ground DM yield at the day 63 harvest. The leaf DM results (Figure 2b) reflect those for total above ground DM yield.

The DM yield of the 10-day cycle and continuous waterlogging treatments was reflective of a significant (P<0.001) reduction in live tillers, falling to 2 in the 10-day cycle and 1 in the continuous treatments by day 84 (Figure 2c). Significant (P<0.01) reductions in tiller number were also observed in the control and 20-day single waterlogging treatments but not to the same degree as the 10-day cycle and continuous waterlogging treatments (Figure 2c).

Leaf length was also significantly (P<0.001) negatively affected in both the 10-day cycle and continuous waterlogging treatments (Figure 2d). In contrast, the leaf length significantly (P<0.001) increased in both of the single waterlogging treatments, while there was no significant (P>0.05) change in the control.

Cenchrus purpureus × C. americanus ‘VA06’

There was no significant (P>0.05) change in above ground DM yield observed in the control, 10-day single and continuous waterlogging treatments (Figure 3a). However, both the 10-day cycle (P<0.05) and the 20-day single (P<0.001) waterlogging treatments had a positive effect on DM yield over time. The DM yield of the 20-day single treatment increased from 2.4 g DM/pot at day 21 to 10.7 g DM/pot at day 84. The leaf DM results (Figure 3b) broadly reflect those for total above ground DM yield.

In contrast, there was a significant (P<0.01) reduction in live tillers between day 0 and day 84 in all waterlogging and control treatments (Figure 3c), but no significant (P>0.05) difference between treatments at any of the harvest dates, suggesting that there was no effect of waterlogging treatment on live tiller number. The reduction of live tillers occurred mainly between day zero and the first harvest at day 21.

There were significant (P<0.05) effects of all treatments on leaf length over time, although with no clear trend, and no significant (P>0.05) difference between waterlogging treatments post day 21 (Figure 3d).

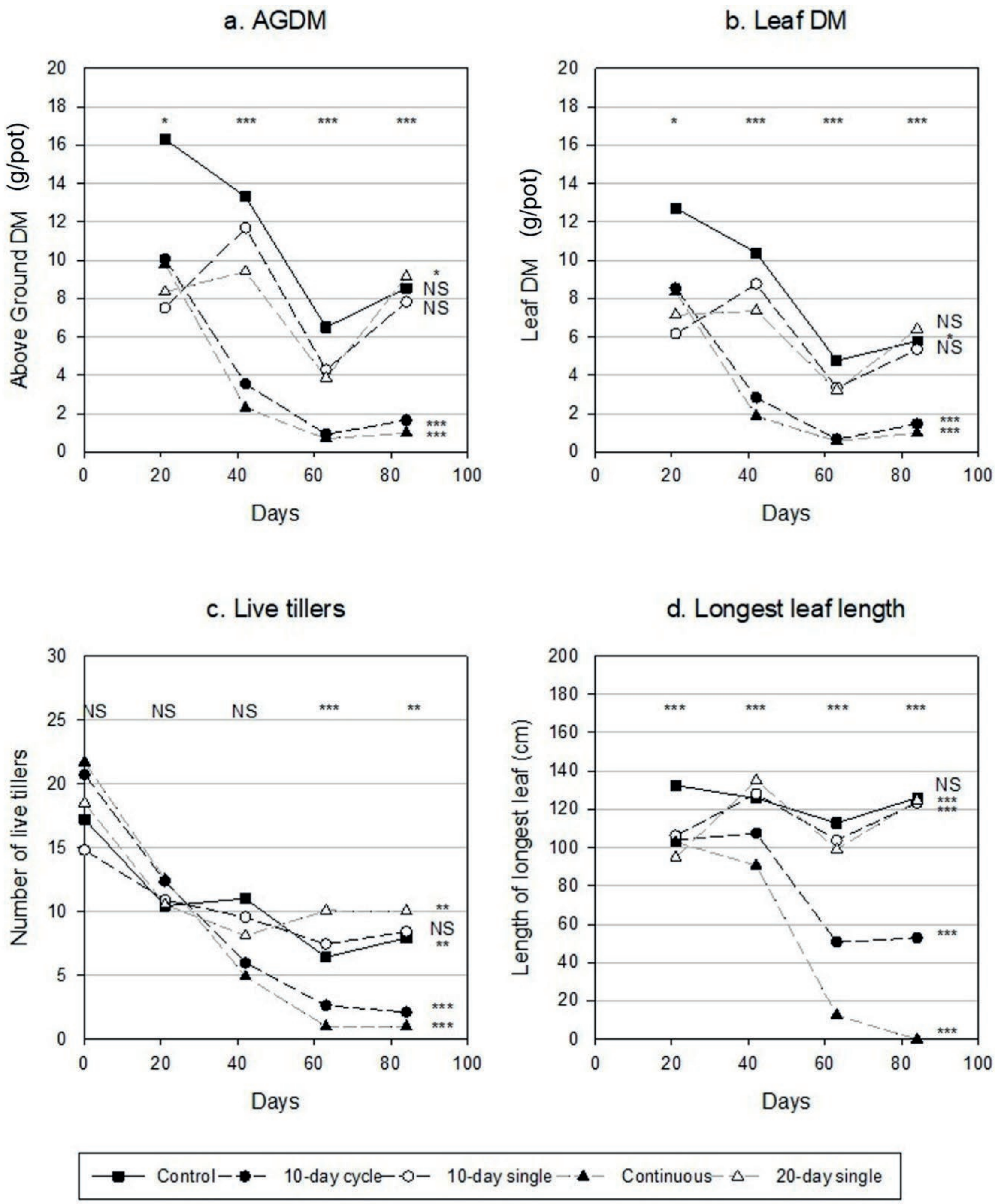

Figure 2. Effects of waterlogging treatments on *Megathyrsus maximus* 'TD58' (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

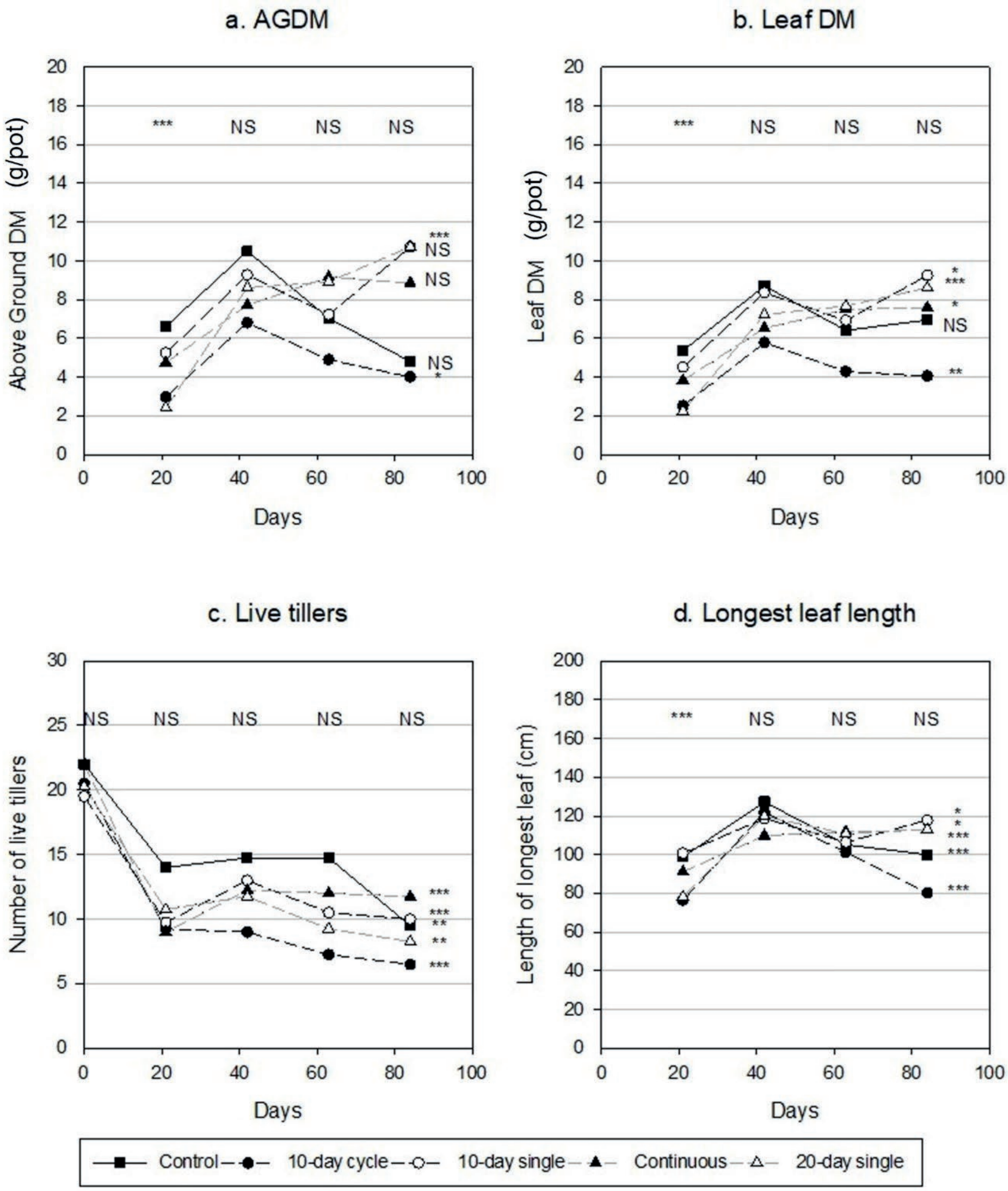

Figure 3. Effects of waterlogging treatments on *Cenchrus purpureus* × *C. americanus* 'VA06' (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20- single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

Paspalum atratum 'Ubon'

There was a significant reduction in above ground DM yield in control ($P<0.05$), 10-day single ($P<0.001$) and 20-day single ($P<0.01$) waterlogging treatments (Figure 4a). There also appeared to be a reduction in 10-day cycle and continuous waterlogging treatments, although this was not significant. The DM yield in the control dropped from 12.4 to 5.6 g DM/pot between day 21 and day 84. However, importantly, there was no significant ($P>0.05$) difference between waterlogging treatments at any of the individual harvest dates. The leaf DM results (Figure 4b) reflect those for total above ground DM yield.

Although there was an apparent reduction in mean total live tillers over time, statistically there was only a significant ($P<0.05$) reduction of tillers in the 10-day single waterlogging treatment (Figure 4c). In this treatment, the number of live tillers fell from 11.7 at day 0 to 2.9 at day 84. There was no significant ($P>0.05$) difference between waterlogging treatments at any of the individual harvest dates.

There were significant ($P<0.01$) differences in leaf length over time in all treatments (Figure 4d). In general, there was a 5–15% reduction in leaf length between day 21 and day 84, with the exception of the 10-day single waterlogging treatment which decreased by approximately 40%. There was no significant ($P>0.05$) difference between treatments at any individual harvest date.

Urochloa hybrid 'Mulato II'

Above ground DM yield significantly ($P<0.05$) decreased over time in all treatments except the 20-day single treatment (Figure 5a). Significant ($P<0.05$) differences were detected between waterlogging treatments at each of the harvest times. The 10-day cycle and continuous waterlogging treatments caused the greatest effect, with above ground DM yield falling to less than 2 g DM/pot by day 84. The leaf DM results (Figure 5b) reflect those for total above ground DM yield.

The reductions in above ground DM yield were reflective of reductions in live tiller number, with significant ($P<0.001$) reductions observed in the 10-day cycle and continuous waterlogging treatments but not in the single waterlogging treatments (Figure 5c). Differences between waterlogging treatments were only significant ($P<0.001$) from harvest day 42 onwards. The total number of live tillers at day 84 were 6.3 and 1.0 for the 10-day cycle and continuous waterlogging treatments respectively.

The effect of the continuous waterlogging treatment is evident, with leaf length falling from 57.1 at day 21 to 12.1 cm at day 84 (Figure 5d). Significant ($P<0.01$) differences between the continuous and other treatments were noticeable from harvest day 42 onwards.

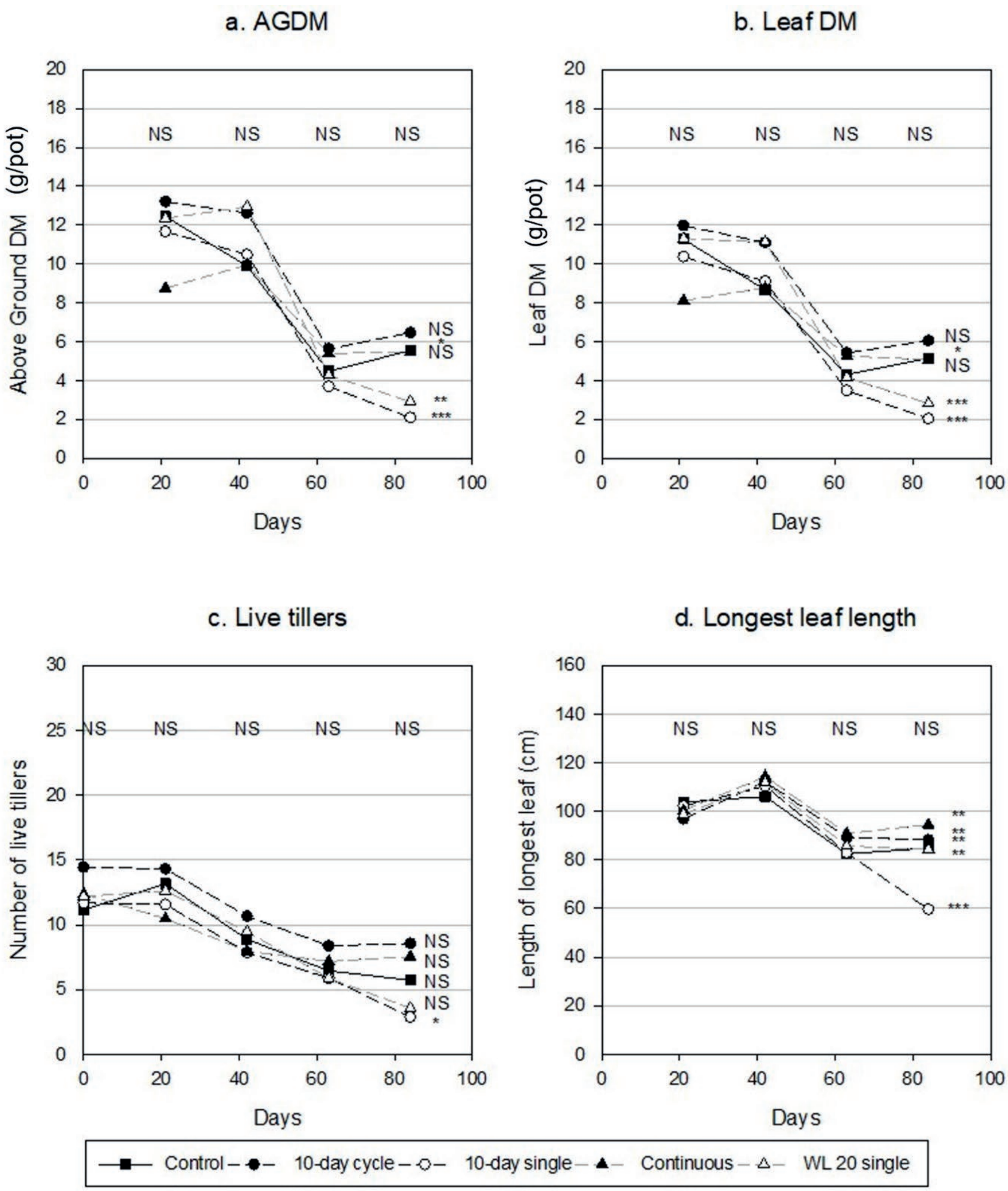

Figure 4. Effects of waterlogging treatments on *Paspalum atratum* (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

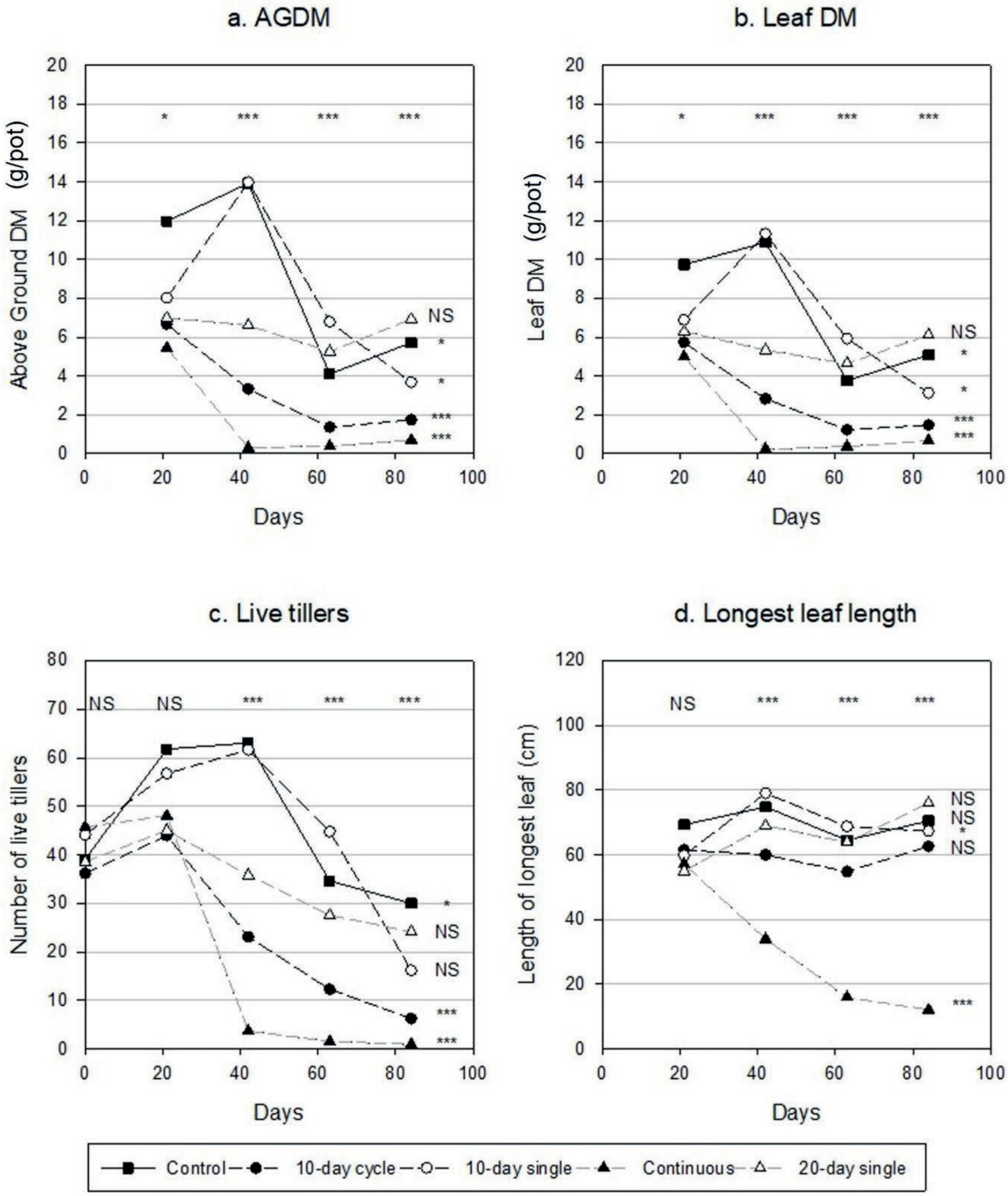

Figure 5. Effects of waterlogging treatments on *Urochloa* Hybrid 'Mulato II' (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

Urochloa humidicola

The above ground DM yield of *U. humidicola* decreased significantly (P<0.001) over time in all waterlogging treatments (Figure 6a). The DM yield of all waterlogging treatments decreased from >15 g DM/pot at day 21 to <3 g DM/pot by day 84. No significant differences were found between waterlogging treatments from day 42 onwards. The lower above ground dry matter for the 20-day single treatment appears to be due to initial differences between plants that were randomly assigned to the same treatment. The leaf DM results (Figure 6b) reflect those for total above ground DM yield.

Above ground DM yields were reflective of significant (P<0.001) reductions in the number of live tillers (Figure 6c). Total live tillers decreased from >90 at day 0 to <18 by day 84 in all waterlogging treatments. No significant differences were found between waterlogging treatments at any of the measurement days.

Longest leaf length followed the same decreasing trend (Figure 6d), declining significantly (P<0.01) between day 0 and 84 from >80 to <50cm. There was no significant (P>0.05) difference in leaf length between waterlogging treatments at any of the harvest dates.

Urochloa ruziziensis

The amount of above ground DM changed significantly (P<0.001) between harvests for both the 10-day cycle and continuous waterlogging treatments (Figure 7a). The above ground DM yield of the control and single waterlogging treatments did not significantly change over time but were higher than the 10-day cycle and continuous waterlogging treatments at the 42-, 63-, and 84-day harvest times. The leaf DM results (Figure 7b) reflect those for total above ground DM yield.

There was a general decreasing trend in the number of live tillers over the experimental period (Figure 7c), with the exception of the 20-day single waterlogging treatment (P>0.05). Significant (P<0.001) differences between waterlogging treatments were detected at days 42 and 63 only, with the 10-day cycle and continuous waterlogging treatments resulting in less live tillers.

There was no significant (P>0.05) change in the length of the longest leaf throughout the experiment in any of the waterlogging treatments (Figure 7d). There were significant differences in longest leaf length between species at days 42 and 63, with the 10-day cycle and continuous waterlogging treatments tending to result in shorter leaves.

Digitaria eriantha

The amount of above ground dry matter decreased significantly (P<0.05) over time in the control and 10-day single waterlogging treatments, i.e. the treatments representing the least amount of waterlogging (Figure 8a). There was only a significant (P<0.05) difference between waterlogging treatments at the 63-day harvest point. The leaf DM results (Figure 8b) reflect those for total above ground DM yield. Due to the growth characteristics of *D. eriantha*, the number of live tillers and the longest leaf length were not recorded because this was difficult to determine.

Total harvested dry matter

Figure 9 shows the total dry matter harvest from the 4 cuts, not including the first cut before treatments were applied. Under control conditions the only significant difference was between *M. maximus* and *D. eriantha*. Under the 10-day single waterlogging event, all species were higher yielding than *D. eriantha*. Under the 20-day single waterlogging event there was no difference between treatments. Under the 10-day cycle, *P. atratum* and *U. humidicola* were higher yielding than *M. maximus* 'TD58', *U. ruziziensis* and *Urochloa* hybrid 'Mulato II', with the other species not significantly different. Under continuous waterlogging there was a clear difference between the 4 waterlogging tolerant species, and the 3 less-tolerant species, with 'Mulato II' the lowest yielding.

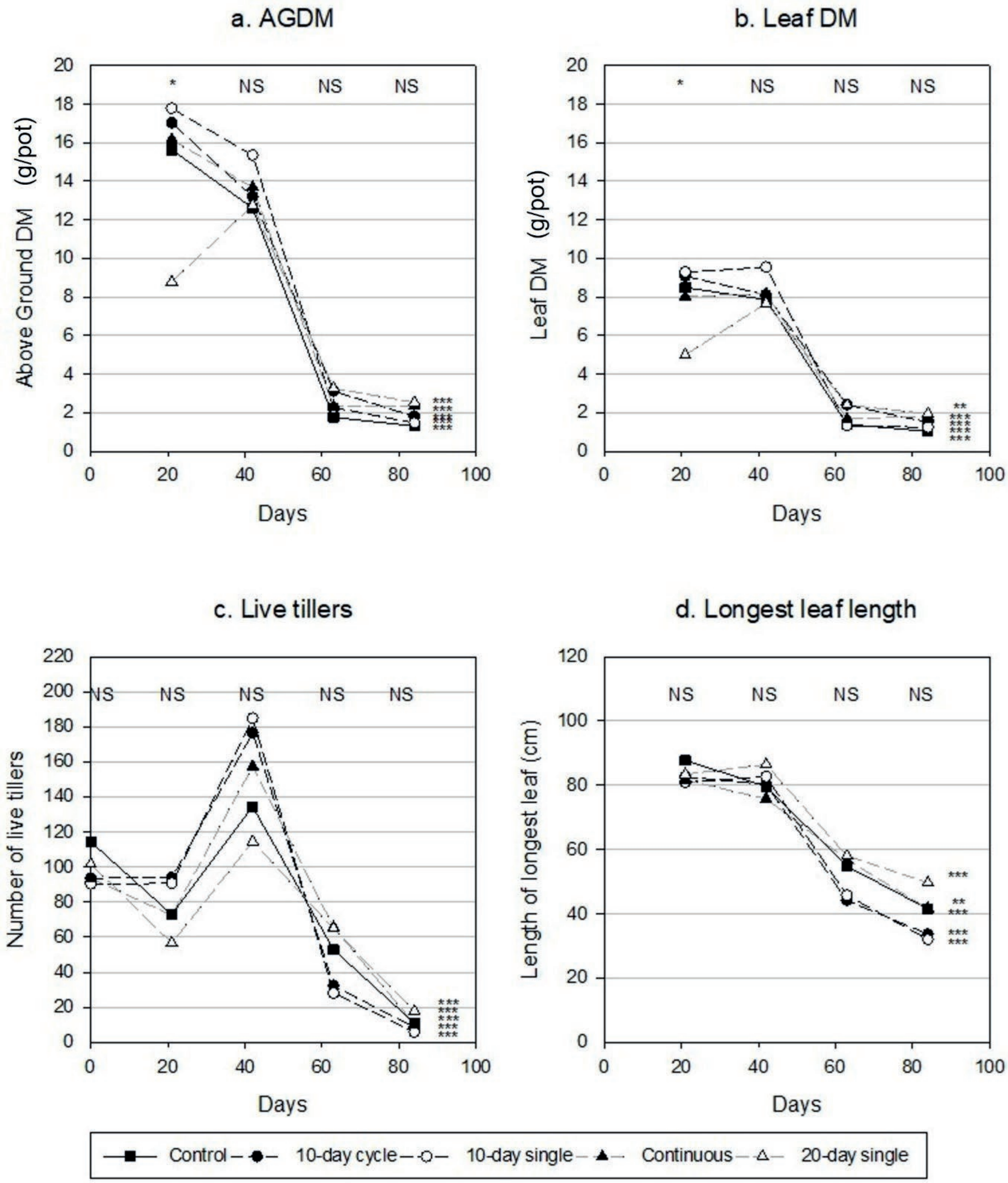

Figure 6. Effects of waterlogging treatments on *Urochloa humidicola* (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

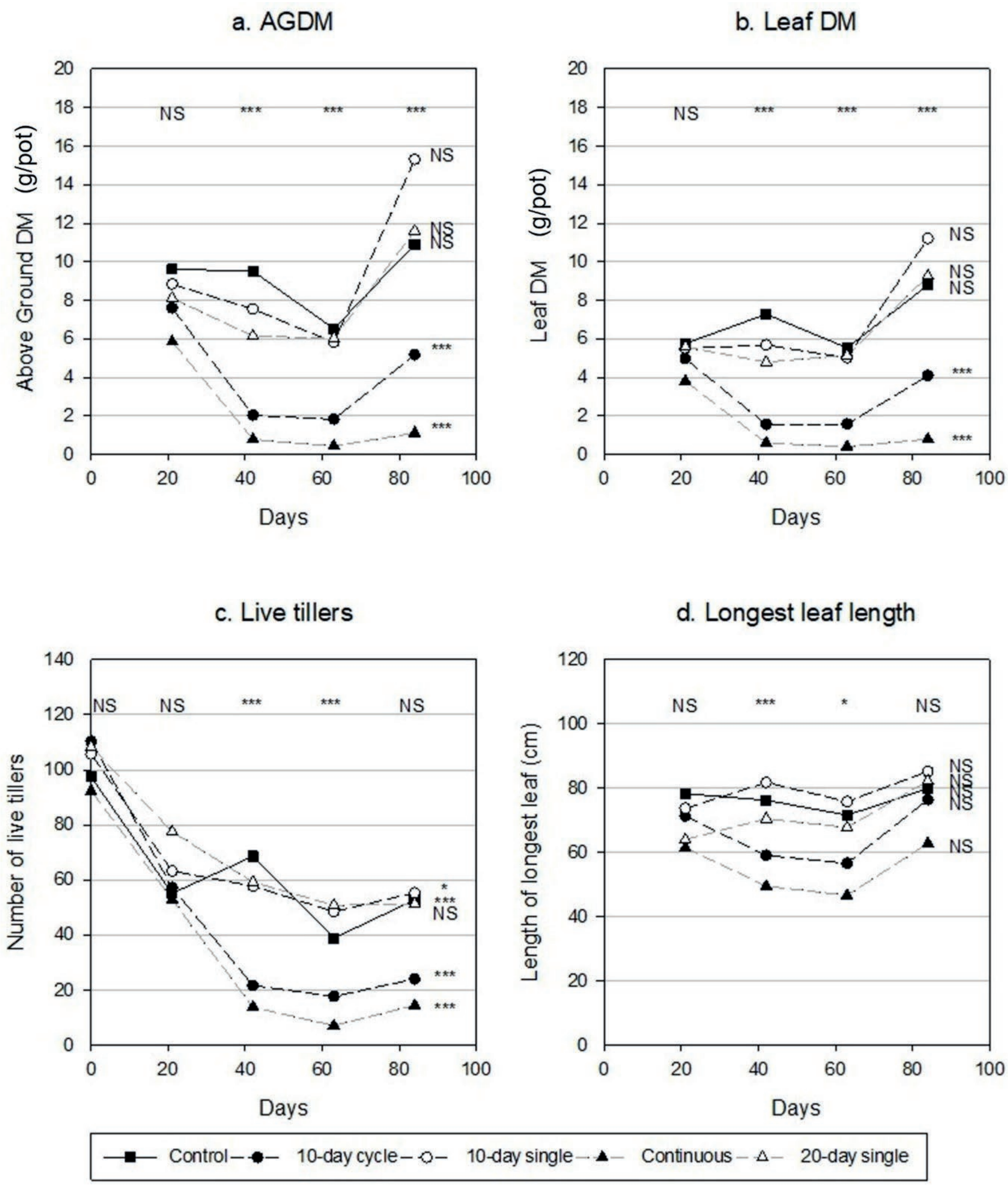

Figure 7. Effects of waterlogging treatments on *Urochloa ruziziensis* (a) above ground DM yield, (b) leaf DM yield, (c) number of live tillers, and (d) length of longest leaf. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

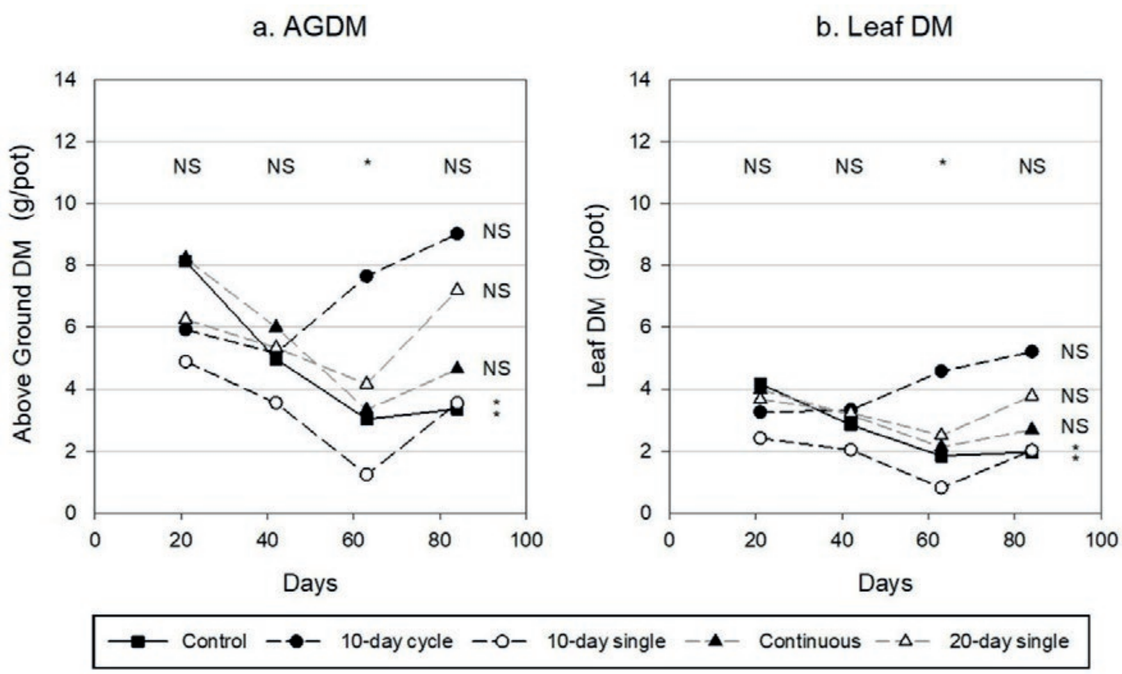

Figure 8. Effects of waterlogging treatments on *Digitaria eriantha* (a) above ground DM yield, (b) leaf DM yield. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20- single=20 days of waterlogging then control conditions throughout. Significant differences between waterlogging treatments at each harvest date (x axis) and change within each waterlogging treatment over time (y axis) are represented by NS=not significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

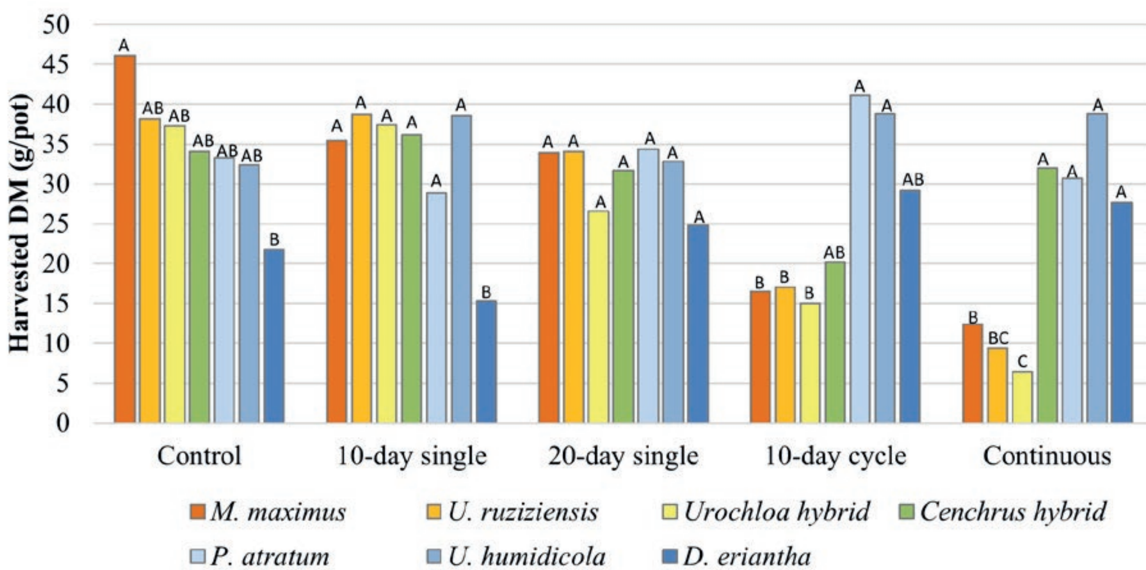

Figure 9. Effect of waterlogging treatments on total above ground DM harvest (4 harvest dates) of *Megathyrsus maximus* 'TD58', *Urochloa ruziziensis*, *Urochloa* hybrid 'Mulato II', *Cenchrus purpureus* × *C. americanus* 'VA06', *Paspalum atratum* 'Ubon', *Urochloa humidicola* and *Digitaria eriantha*. Waterlogging treatments were Control=nil waterlogging; 10-day cycle=repeated 10 days waterlogging then 10 days control conditions; 10-day single=10 days of waterlogging then control conditions throughout; continuous waterlogging conditions; 20-single=20 days of waterlogging then control conditions throughout. Within each waterlogging treatment, species means with the same letter are not significantly (P>0.05) different to each other. Colours for different species reflect typical experience of waterlogging tolerance, with blue colours reflecting waterlogging tolerance, yellow colours reflecting waterlogging susceptibility, and green in-between.

Discussion

The results for the *Urochloa* species support our first hypothesis of which would be the most waterlogging tolerant species. The International Centre for Tropical Agriculture (CIAT) commercialised the *Urochloa* hybrids 'Mulato' and 'Mulato II' but noted that these cultivars are not tolerant of waterlogging (Cardoso et al. 2013). This was confirmed by the results in the current study where repeated waterlogging events reduced the number of live tillers and the above ground dry matter of 'Mulato II' over time. Shoot dry matter (g DM/pot) of 'Mulato II' was also reduced after the third regrowth cycle (80 days) of waterlogging in a study reported by Junior et al. (2016). The number of live tillers was not affected by the single waterlogging event. Thus, 'Mulato II' may persist in areas where short seasonal waterlogging occurs but is not recommended for areas that are waterlogging prone.

Similarly, the number of live tillers, leaf DM and above ground dry matter of *U. ruziziensis* was significantly affected by repeated waterlogging events. This is in agreement with studies by Hare et al. (2004) showing low tolerance of *U. ruziziensis* to waterlogging, with high plant mortality (>50%) and lower plant DM compared with control plants after 20 days. Jiménez et al. (2015a) also noted a reduction in green leaf area and green leaf DM in waterlogged *U. ruziziensis*. In addition, Hare et al. (1999) found that productivity declined on waterlogged soils after the first year of production. The results for 'Mulato II' and *U. ruziziensis* support our second hypothesis that the repeated cycling or continuous of waterlogging is more damaging than the length of time of the waterlogging.

In contrast, there was no difference between the control and waterlogging treatments in *U. humidicola*. This compares with other studies that have also reported that *U. humidicola* has known waterlogging tolerance (Dias-Filho and Carvalho 2000; Cardoso et al. 2013) while Jiménez et al. (2015a) showed *U. humidicola* had greater tolerance than *U. ruziziensis*. The mechanisms for the greater waterlogging tolerance of *B. humidicola* over *U. ruziziensis* were elucidated by Jiménez et al. (2015b) and include greater aerenchyma formation, smaller stele proportions, and increased suberin deposition. These responses under waterlogged conditions increase the flow of oxygen to the roots and reduce the effect of reactive oxygen species in the leaves, which affects the efficiency of photosynthesis (Jiménez et al. 2015b). In addition, under waterlogged conditions *U. humidicola* increases the proportion of lateral roots close to the surface (Cardoso et al. 2014), where oxygen may be more plentiful. We also observed rooted stolons, a high proportion of which were above the waterlogged soil surface, which warrants further study. The reason for the decreasing growth of *U. humidicola* in our experiment is not clear, although the cutting interval may have been too short for plants to recover sufficiently after each cut. The reduced light in the greenhouse may have compounded this effect. As such, further experimentation under field conditions is warranted.

In addition to the existing cultivars with known tolerance for use in waterlogging prone areas, breeding for waterlogging adapted traits is a future research area for *Urochloa*. The *Urochloa* genus is one of the most widely cultivated grasses in tropical systems. CIAT has undertaken screening of *Urochloa* hybrids for waterlogging tolerance with success (Cardoso et al. 2013), which should result in new cultivar releases in the future.

Megathyrsus maximus 'TD58' was able to recover from single waterlogging events as observed with the above ground dry matter, live tillers, leaf length and leaf DM matching the control after day 21. In contrast, production following 10-day cycling and continuous waterlogging treatments was severely affected by a decline in live tillers. This supports our second hypothesis that repeated or continuous waterlogging is more damaging than the length of time of the waterlogging. A similar moderate level of tolerance was also observed by Hare et al. (2004) in *M. maximus* ('Purple panic'), where 20 days of waterlogging stunted plants and reduced plant dry weights. For this reason, *M. maximus* 'TD58' is not suitable for planting in areas where multiple or long-term waterlogging events over a season are expected.

The waterlogging tolerance of *C. purpureus* × *C. americanus* 'VA06' was one of the surprising results of the experiment. However, there was a lot of variability within treatments and the results are inconclusive. The Ba et al. (2014) study did not rank 'VA06' highly for waterlogging tolerance, however the study was only based on a visual rating. In contrast, the authors have observed this cultivar appearing to be healthily growing in inundated fields, when left in an un-cut condition. The mechanisms of waterlogging tolerance of 'VA06' and implications for its management are future areas of research that could have practical implications for such a popular cultivar. Similar to *U. humidicola*, further evaluation under field conditions is required to understand the response to waterlogging or undertaking the experiment again with more replicates.

P. atratum 'Ubon' was one of the most waterlogging tolerant species in studies by Hare et al. (2004), but their studies also showed a decline in dry weight yields the longer the waterlogging period lasted. 'Ubon' was released in Thailand following positive DM evaluation against other species in seasonally waterlogged and seasonally dry soils in northeast Thailand (Hare et al. 1999). In the current study, above ground dry matter yields declined in some treatments over time, but there was no significant difference between waterlogging treatments and control for any of the harvest dates. In addition, the number of live tillers did not change significantly over time and there was also no difference between waterlogging treatments and the control, suggesting that *P. atratum* was also one of the most waterlogging tolerant species in the current study. Beloni et al. (2017) reported variation in responses to waterlogging amongst *Paspalum* genotypes and suggested this could be exploited in further plant breeding.

Numerous factsheets report waterlogging tolerance in *D. eriantha* including Cook et al. (2020), however there are few published studies. This species performed well in experiments by Boschma et al. (2008), although the length of waterlogging was only short. Our studies concur with that general assessment of the species

Conclusions

Our results concur with our original hypothesis that *U. humidicola*, *P. atratum* and *D. eriantha* would be tolerant of waterlogging and that the production of other species would be more affected by waterlogging. Repeated waterlogging events exacerbated the effects on DM production and tiller survival in the less tolerant species *M. maximus*, *Urochloa* hybrid 'Mulato II' and *U. ruziziensis*. These results reinforce current field-based knowledge, though further field-based evaluation may strengthen recommendations.

Understanding of the response of different species and cultivars to waterlogging can help farmers to select appropriate species and cultivars for different locations in the landscape. This knowledge needs to be paired with other information about forages such as suitable soil conditions, production potential and nutritive value. Further studies are required to elucidate best management practices for forages under waterlogging conditions. For example, increasing the cutting height and cutting interval are likely methods for ameliorating some of the effects, to better manage less tolerant forages for persistence through periods of waterlogging. Farmers in the study region have shown a willingness to adopt new forage species, and clear recommendations will help them to manage their forage resources to maintain production and reduce risk.

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(Note of the editors: All hyperlinks were verified 25 January 2025).

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Nota Técnica

Parámetros fermentativos del ensilaje del pasto guinea (*Megathyrsus maximus*) cv. BRS Zuri cosechado en diferentes horarios

*Fermentation parameters of guinea grass silage (*Megathyrsus maximus*) cv. BRS Zuri harvested at different times during the day*

RAFAEL MARZALL DO AMARAL, ELIZABETH YACSIRY VEGA-CABEZAS, DORGELI SELENA MOLINA-SANTANA Y CRISTIANO EDUARDO RODRIGUES-REIS

Universidad EARTH, San José, Costa Rica. earth.ac.cr

Resumen

Este estudio investiga la influencia del horario de cosecha (06:00, 09:00, 12:00, 15:00 y 18:00 horas) sobre la calidad del ensilaje del pasto guinea (*Megathyrsus maximus*) cv. BRS Zuri. El pasto fue cosechado a los 45 días de edad de rebrote, se picó y almacenó en microsilos de PVC por 45 días. Se evaluaron parámetros de ensilabilidad en el forraje fresco, así como parámetros fermentativos y nutricionales del ensilaje. La concentración de carbohidratos solubles al momento de la cosecha se incrementó de forma lineal en función de la hora y consecuente exposición a la radiación solar, pasando de 1.07 a 2.07% a las 06:00 h y 18:00 h, respectivamente (P=0.001). Los valores más bajos para la pérdida de efluentes, pH y N-NH_3 ocurrieron a las 15:00 h, con efectos lineales y cuadráticos significativos (P≤0.05) para las tres variables. El horario de cosecha influenció en forma cuadrática el contenido de MS del ensilaje, obteniendo 17.14% a las 06:00 h y 22.64% de MS a las 15:00 h (P≤0.001). La concentración de proteína bruta fue mayor (13.78%) a las 15:00 h y el menor valor se obtuvo a las 06:00 h (10.37%) (P≤0.05). En las condiciones del estudio, la cosecha del pasto guinea cv. BRS Zuri en horas de la tarde resultó en un ensilaje con mejor calidad en términos sensoriales, fermentativos y nutricionales.

Palabras clave: Calidad de ensilaje; carbohidratos solubles; conservación de forrajes; horario de cosecha; parámetros sensoriales.

Abstract

This study investigates the influence of harvest time during the day (06:00, 09:00, 12:00, 15:00 y 18:00 hours) on the quality of guinea grass (*Megathyrsus maximus*) cv. BRS Zuri silage. The pasture was harvested 45 days after the last cut, chopped and stored in PVC microsilos for 45 days. Fresh forage ensilability parameters, as well as fermentative and nutritional parameters of silage were evaluated. The concentration of soluble carbohydrates increased linearly depending on the the harvest time and consequent exposure to solar radiation, with values ranging from 1.07 to 2.07% between 06:00 and 18:00 h, respectively (P=0.001). The lowest values for effluent losses, pH, and N-NH_3 were observed at 15:00 h, with significant linear and quadratic effects (P≤0.05) for the three variables. The harvest time influenced quadratically (P≤0.001) the DM content of the silage, with 17.14 and 22.64% DM at 06:00 h and 15:00 h, respectively. Crude protein content was also influenced by harvest time, with the highest value (13.78%) at 15:00 h and the lowest at 06:00 h (10.37%) (P≤0.05). Under the conditions of the study, harvesting guinea grass cv. BRS Zuri grass in the afternoon resulted in a better-quality silage in terms of its sensorial, fermentative, and nutritional characteristics.

Keywords: Forage conservation; harvest schedule; sensorial parameters; silage quality, soluble carbohydrates.

Correspondencia: Rafael Marzall do Amaral, Universidad EARTH,
San José 4442-1000, Costa Rica.
Correo eléctronico: rmarzall@earth.ac.cr

Introducción

Los ensilajes de pastos tropicales como *Megathyrsus maximus* y *Cenchrus purpureus* ofrecen ventajas clave con relación a los cultivos ensilados tradicionalmente, como el maíz o sorgo, tales como el alto rendimiento, carácter perene y resistencia a plagas (Jank et al. 2017; Pereira et al. 2017); sin embargo, este potencial es limitado por retos tales como las bajas concentraciones de materia seca (MS) y carbohidratos solubles, que dificultan la fermentación, resultando en una menor calidad del ensilaje.

Algunas estrategias utilizadas tradicionalmente, para mejorar estos parámetros son el pre secado y el uso de aditivos con capacidad de absorción de la humedad, que en algunos casos también aportan carbohidratos al proceso de fermentación. Estos métodos, sin embargo, pueden ser laboriosos en plantas de porte erecto y tallos gruesos, y además traen costos adicionales por la adquisición de los aditivos (Bernardes et al. 2018; Avila et al. 2022).

Algunos investigadores (Guo Gang et al. 2015; Dong Zhihao et al. 2022) han identificado fluctuaciones en la composición de los forrajes a lo largo del día, obteniendo mayores concentraciones de materia seca y de carbohidratos solubles en las plantas cosechadas en horas de la tarde. En ese contexto, la deshidratación parcial de las plantas y el acúmulo de los productos fotosintéticos potencialmente fermentables que ocurren a lo largo del día podrían ser suficientes para mejorar el proceso fermentativo y obtener un ensilaje de mejor calidad a partir de pastos tropicales tales como la guinea cv. BRS Zuri; sin que esto implique un aumento de los costos de producción, como ocurre con el pre secado o el uso de aditivos secantes. Por ello, el objetivo de este estudio fue evaluar el efecto del horario de cosecha sobre parámetros de ensilabilidad, fermentativos y nutricionales cuando se preparan ensilajes de pasto guinea (*M. maximus*) cv. BRS Zuri.

Materiales y Métodos

Ubicación del estudio y condiciones de crecimiento del forraje

El pasto guinea (*M. maximus*) cv. BRS Zuri fue cultivado en la Unidad Académica de Producción Animal de la Universidad EARTH (10° 12' 45" N y 83° 35' 39" O; con una elevación de 40 m.s.n.m.), ubicada en la provincia de Limón, Costa Rica. Durante el periodo de crecimiento vegetativo (20 de abril a 4 de junio de 2022) la temperatura promedio fue de 25.17 °C, la precipitación acumulada fue de 99.84 mm y la humedad relativa de 80.3%, según datos de la estación meteorológica de la Universidad EARTH. Estas condiciones no difirieron de los valores observados a lo largo de los últimos 3 años. En la Figura 1 se presenta la temperatura y la radiación solar por hora en el día en que se cosechó el pasto y se prepararon los silos.

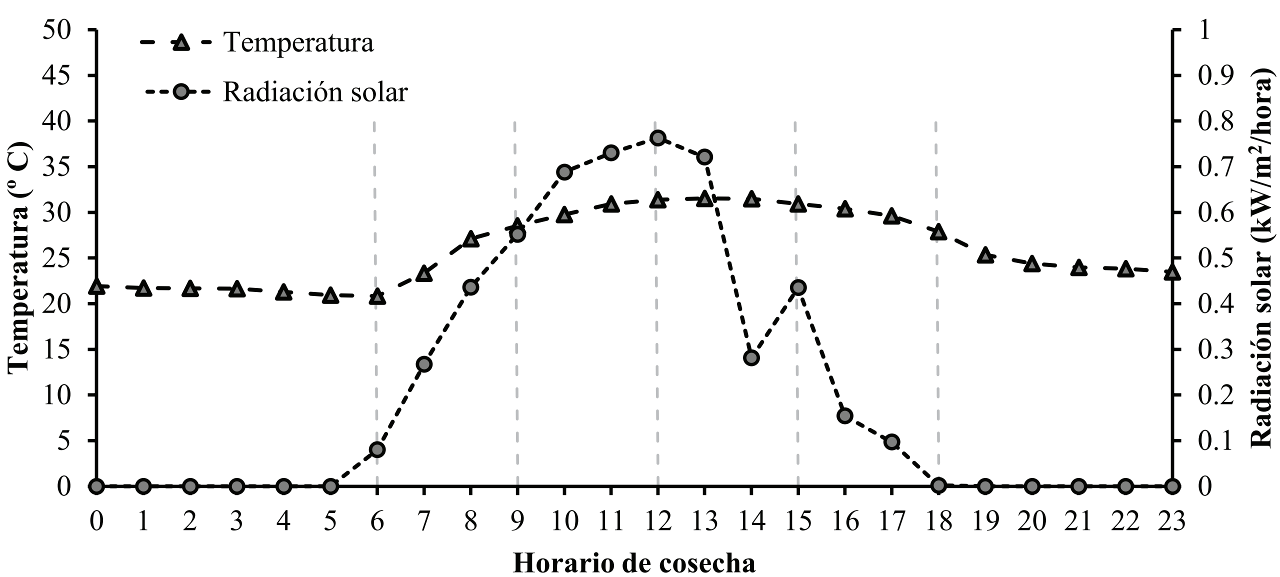

Figura 1. Temperatura (ºC) y radiación solar por m^2 (kW/m^2) por hora el día 4 de junio de 2022, fecha de la cosecha del pasto y preparación de los silos. Líneas grises verticales indican los horarios de cosecha.

Cosecha y composición nutricional inicial

En el Cuadro 1 se presenta la composición nutricional inicial del pasto en los distintos momentos de cosecha. La cosecha se realizó a los 45 días del último corte y fertilización nitrogenada (50 kg N/ha), en este momento las plantas presentaban una altura promedio de 1.72±0.22 m. El corte se realizó dejando un residuo de aproximadamente 0.30 m, asegurando elevada proporción de hojas. Las plantas de los bordes del área de cultivo se desecharon y los muestreos avanzaron sobre dos hileras paralelas dentro de una misma parcela.

Procesamiento del pasto

El pasto cosechado en los diferentes horarios se picó usando una picadora eléctrica estacionaria. El tamaño de las partículas resultantes se determinó con un Separador de Partículas de Forraje de Penn State (Heinrichs y Jones 2013), obteniendo 47.77% de partículas mayores a 19 mm, 35.26% entre 8 y 19 mm y 16.97% menores a 8 mm. En seguida se tomaron muestras del material fresco, las que se condujeron al laboratorio para la caracterización inicial y determinación de los parámetros de ensilabilidad. Posteriormente el forraje fue introducido y compactado manualmente en microsilos de PVC con capacidad para almacenar aproximadamente 2,150 kg. Los microsilos contaban con tapa dotada de una válvula para liberación de gases. Después del cierre, los microsilos se ubicaron verticalmente y se mantuvieron sin abrir por un período de 45 días para garantizar que culminara el proceso de fermentación.

Variables estudiadas

Parámetros de ensilabilidad. En las muestras de pasto recién cosechado, se determinó el contenido de materia seca luego de secado a 60 °C por 72 h, en horno de ventilación forzada, hasta alcanzar peso constante. Luego, en las muestras secas, se determinó la concentración de carbohidratos solubles (CS) expresada con base en el porcentaje de la materia seca determinada por el método fenol sulfúrico y colorimetría (DuBois et al. 1956); la capacidad de amortiguamiento (CA), presentada como miliequivalentes de NaOH por 100 g de MS, se determinó usando la metodología propuesta por Playne y McDonald (1966). Esta a su vez fue transformada a capacidad de amortiguamiento, expresada en gramos de ácido láctico por kg de materia seca, por medio de la Ecuación 1 propuesta por Clavin et al. (2017). A partir de esos datos se determinó el coeficiente de fermentación (CF) propuesto por Weissbach y Honig (1996) (Ecuación 2).

Ecuación 1:

$$\text{CA (g de ácido láctico/100 g de MS)}=0.0154\times\text{CA (mEq/100 g de MS)}-0.2115$$

Ecuación 2:

$$\text{CF}=\text{Materia seca (\%)}+8\ \text{x}\ \frac{\text{Carbohidratos solubes (\%)}}{\text{CA (g de ácido láctico/ 100 g de MS)}}$$

Cuadro 1. Composición nutricional del pasto *Megathyrsus maximus* cv. Zuri cosechado en cinco horarios distintos, previo al proceso de ensilado.

Variables	Horario de cosecha				
	06:00	09:00	12:00	15:00	18:00
Materia seca (%)	18.7	19.6	21.1	21.9	21.0
Proteína bruta (% de la MS)	16.8	18.2	17.5	14.5	16.7
Fibra detergente neutro (% de la MS)	69.4	70.2	68.6	70.4	66.9
Fibra detergente ácido (% de la MS)	38.8	37.9	36.9	40.0	35.2
Extracto etéreo (% de la MS)	3.0	3.7	3.4	3.3	3.4

Parámetros fermentativos. Al cabo de los 45 días se extrajo el ensilaje contenido en los microsilos y se pesó, para determinar el por ciento de pérdidas por lixiviación (Jobim et al. 2007). El contenido de cada microsilo fue homogeneizado y se procedió a separar en cuatro submuestras para análisis sensoriales, microbiológicos, químicos y bromatológicos.

El análisis sensorial se realizó de acuerdo con la escala descrita por Chaverra y Bernal (2000) (Cuadro 2).

La estabilidad aeróbica del ensilaje, definida como el tiempo trascurrido desde la apertura del silo hasta que la temperatura del silo sobrepase en 2 °C la temperatura ambiente, se determinó utilizando submuestras de 500 g, monitoreadas por un periodo de 5 días con toma de datos cada 30 minutos, usando registradores automáticos de temperatura (Temperature Data Logger RC-5, Elitech, San Jose, CA).

El recuento del total de mohos y levaduras se realizó en placas de Petri comerciales (3M™ Petrifilm™), para lo cual se utilizaron disoluciones de cada una de las muestras al 10^{-3}, 10^{-4} y 10^{-5} en solución salina (cloruro de sodio al 0.85%), seguida de la inoculación y posterior incubación de las placas Petri por 5 días a 25 ºC (Bird et al. 2015). El recuento de las poblaciones microbianas se realizó como unidades formadoras de colonias por gramo de forraje, y se usó la la transformación logarítmica previo al análisis estadístico.

El pH se determinó a partir de una extracción de 20 g de ensilaje fresco, la cual se diluyó en 80 ml de agua destilada y la mezcla se agitó por 20 minutos a una frecuencia de 160 rpm, y luego se dejó en reposo por una hora, para luego filtrar a través de una gasa, y del filtrado se tomó 100 ml en el cual se determinó el pH (Bernardes et al. 2019). El nitrógeno amoniacal (NH_3-N) se determinó por el método de Neesler (Kozłowska et al. 2021), y la lectura por espectrofotometría (HANNA HI801-01; Hanna Instruments Italia Srl).

Parámetros nutricionales

El contenido de materia seca en muestras de forraje fresco y ensilado se determinó secando muestras de aproximadamente 250 g en horno de aire forzado a 60 ºC por 72 horas, hasta alcanzar peso constante. La concentración de nitrógeno se determinó con el método de Dumas (Wendt Thiex 2023), y la proteína bruta (PB) se calculó multiplicando la concentración de N por la constante 6.25. La fibra detergente neutro (FDN) y la detergente ácido (ADF) se determinaron por el método de Van Soest et al. (1991), a través de la técnica de bolsas filtrantes, utilizando sulfito de sodio, sin la adición de alfa amilasa. El contenido de cenizas se determinó por calcinación en mufla a 550 °C por 3 h y la materia orgánica fue obtenida por diferencia de la MS menos la ceniza. El extracto etéreo se cuantificó según la metodología descrita por Barbosa et al. (2017).

Diseño experimental y Análisis estadísticos

Los tratamientos consistieron en la cosecha del pasto a las 06:00, 09:00, 12:00, 15:00 y 18:00 horas de un mismo día. Se utilizó un diseño irrestricto al azar considerando cinco tiempos de cosecha, con tres repeticiones para los parámetros de ensilabilidad y cuatro repeticiones (microsilos) para los ensilajes.

La respuesta a los horarios de cosecha en los parámetros de ensilabilidad, fermentativos y nutricionales se ajustaron por medio de análisis de regresión.

Cuadro 2. Parámetros considerados en el análisis sensorial (Chaverra y Bernal 2000).

Indicadores	Calidad			
	Mala (1)	Regular (2)	Buena (3)	Excelente (4)
Color	Marrón oscuro casi negro o negro.	Verde Oscuro	Verde amarillento, tallos con tonalidad más pálida que las hojas.	Verde aceituno o amarillo oscuro
Olor	Desagradable, a mantequilla rancia.	Fuerte, acido, semejante al vinagre	Agradable, con ligero olor a vinagre	A miel o azucarado de frutas maduras
Textura	No se puede diferenciar entre hojas y tallos, masa amorfa, jabonosa al tacto, húmeda y brillante.	Las hojas se separan fácilmente de los tallos. Las hojas tienden hacer transparentes y los vasos venosos muy amarillos.	Las hojas se separan fácilmente de los tallos. Las hojas tienden hacer transparentes y los vasos venosos muy amarillos.	El forraje conserva sus contornos continuos, bien definidos. Las hojas permanecen unidas al tallo.

Resultados

Parámetros de ensilabilidad

Los datos relativos a la ensilabilidad son presentados en la Figura 2. La concentración de materia seca presentó una tendencia lineal creciente (P=0.014), con su menor valor (18.74%) a las 06:00 horas. La concentración de carbohidratos solubles también tendió a incrementar linealmente en función de las horas de cosecha (P<0.001); donde los pastos cosechados a las 15:00 y 18:00 horas presentaron una concentración superior en un 66.35 y 93.45%, respecto a la obtenida en el primer muestreo (06:00 horas). La capacidad de amortiguamiento presentó un efecto cúbico (P=0.006), con el valor más bajo a las 15:00 horas y el más alto a las 18:00 horas (37.33 y 85.66 mEq/100g de MS, respectivamente). El coeficiente de fermentación (CF) varió en función de la hora de cosecha presentando un efecto cuadrático (P=0.004), siendo el valor más bajo (19.61) a las 6:00 horas y el más alto (24.52) a las 15:00 horas, estando ambos por debajo del rango considerado como crítico que es de 35.0, mientras que aquellos con valores superiores a 45 son considerados de fácil ensilabilidad (Wang Musen et al. 2020).

Parámetros fermentativos

Los parámetros sensoriales de color y olor de los ensilajes estuvieron influenciados de forma cuadrática (P≤0.05) por los horarios de cosecha, siendo los mejores puntajes obtenidos para los ensilajes de los pastos cosechados a las 12:00 h y 15:00 h (Cuadro 3). El ensilaje del pasto cosechado a las 15:00 h obtuvo la mejor calificación para el olor, considerado agradable y con ligero olor a vinagre. Independientemente del horario de cosecha, el proceso fermentativo fue suficientemente eficiente para preservar la estructura del material, los contornos se mantuvieron contínuos y bien definidos, recibiendo todas las muestras la calificación 4.

Los horarios de cosecha no afectaron los recuentos de mohos y levaduras (Cuadro 3), presentando valores similares para todos los horarios (P>0.05), lo que sugiere que no hubo diferencias en la estabilidad aeróbica de los ensilajes. Los bajos valores observados para estas variables permitieron que la temperatura de los ensilajes, después de la apertura del silo y consecuente exposición al aire, no sobrepasase en 2 °C la temperatura ambiental al cabo de 120 h después de abierto el silo, lo que demuestra que estos tuvieron una estabilidad elevada.

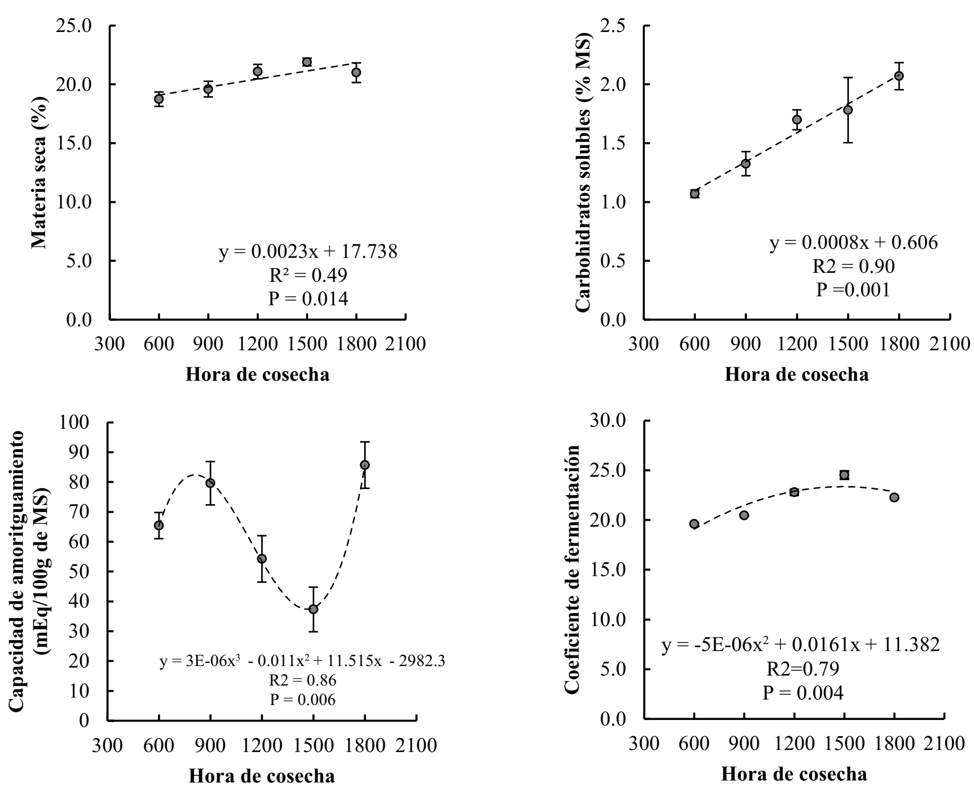

Figura 2. Contenidos de materia seca, carbohidratos solubles, capacidad de amortiguamiento y coeficiente de fermentación del pasto *Megathyrsus maximus* cv. Zuri cosechado a diferentes horas del día. Las barras verticales indican el error estándar de la media.

Cuadro 3. Parámetros sensoriales y recuento microbiano del ensilaje del pasto *Megathyrsus maximus* cv. Zuri cosechado a diferentes horas del día.

Variables	Horario de cosecha					Valor P		
	06:00	09:00	12:00	15:00	18:00	Lineal	Cuadrática	E.E.
Parámetros sensoriales								
Color	3.0	3.0	3.9	3.6	3.1	0.214	0.007	0.10
Olor	2.5	2.8	2.9	3.1	2.8	0.052	0.009	0.06
Textura	4.0	4.0	4.0	4.0	4.0	-	-	0.00
Recuento microbiano								
Mohos (log_{10} UFC/g de MF[1])	0.0	2.3	1.2	0.0	0.0	0.405	0.235	0.19
Levaduras (log_{10} UFC/g de MF)	3.1	3.0	4.5	0.0	2.9	0.431	0.743	0.26

[1]MF=Materia fresca

La Figura 3 resume los datos obtenidos para pérdida de efluentes, pH final y nitrógeno amoniacal. El ensilaje de los pastos cosechados a las 12:00 y 15:00 h presentaron menores pérdidas de efluentes, con pérdidas inferiores a 1.6%, valores sustancialmente inferiores a los 9.9% de pérdidas del pasto cosechado a las 06:00 h. El pH del ensilaje de los pastos cosechados después de las 12:00 h fue menor al obtenido en forrajes cosechados en horas de la mañana ($P \leq 0.05$), resultando en valores de 4.52 y 4.67, para los pastos cosechados a las 15:00 h y 18:00 h, respectivamente. Así mismo, los pastos cosechados antes del mediodía presentaron mayor degradación de la proteína, con valores de $N\text{-}NH_3$ que sobrepasaron el 14%, que se considera como crítico (Kung et al. 2018).

Parámetros nutricionales

Se detectó un efecto cuadrático ($P<0.01$) para los cambios en la concentración de materia seca de los ensilajes en función de la hora en que se cosechó el forraje para ser ensilado. Los pastos cosechados en las horas de la mañana resultaron en ensilajes con inferior concentración de MS (Cuadro 4); ésta tendió a incrementar hasta las 15:00 h (22.6%), para luego declinar. La concentración de proteína en función de la hora de cosecha también presentó una tendencia cuadrática, alcanzando el valor más alto (13.8%) a las 15:00 h. Las concentraciones de fibra detergente neutro, fibra detergente ácido, materia orgánica y cenizas no fueron influenciadas por los horarios de cosecha ($P>0.05$); en cambio, la concentración del extracto etéreo alcanzó su valor más alto a las 9:00 h (6.7%) para luego tender a declinar ($P<0.01$).

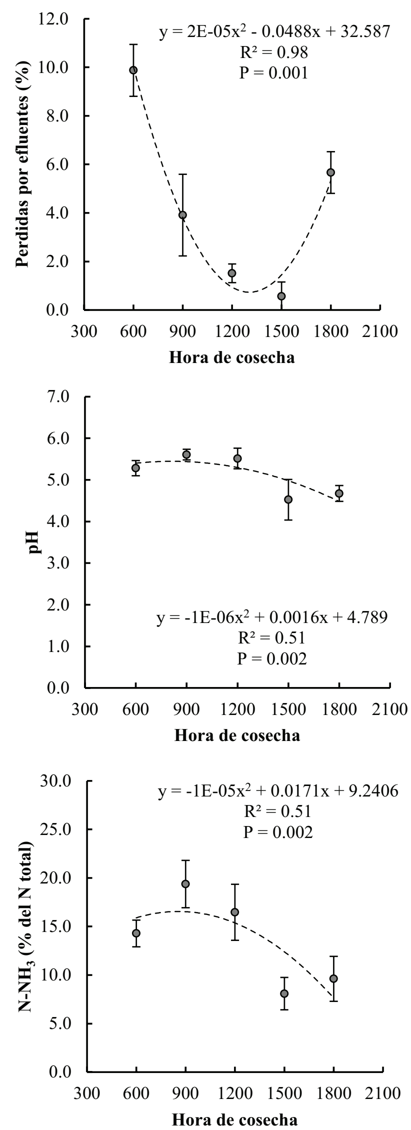

Figura 3. Pérdidas por efluentes, pH y contenido de nitrógeno amoniacal ($N\text{-}NH_3$) del ensilaje del pasto *Megathyrsus maximus* cv. Zuri cosechado a diferentes horas del día. Las barras verticales indican el error estándar de la media.

Cuadro 4. Parámetros nutricionales del ensilaje *Megathyrsus maximus* cv. Zuri cosechado a diferentes horas del día.

Variables	Horario de cosecha					Valor P		
	06:00	09:00	12:00	15:00	18:00	Lineal	Cuadrática	E.E.
Materia seca (% de la MF[1])	17.1	19.8	20.2	22.6	20.3	0.001	0.000	0.43
Proteína bruta (% de la MS)	10.4	10.8	11.1	13.8	12.3	0.002	0.005	0.34
FDN (% de la MS)	69.1	72.0	70.6	66.3	69.1	0.345	0.600	0.83
FDA (% de la MS)	41.8	46.1	41.4	37.3	39.9	0.111	0.296	2.89
Materia orgánica (% de la MS)	87.8	83.5	85.7	87.8	84.1	0.491	0.782	0.62
Extracto etéreo (% de la MS)	6.3	6.7	6.3	5.7	4.6	0.001	0.003	0.21

[1]MF=Materia fresca

Discusión

Parámetros de ensilabilidad

La concentración más baja de materia seca en las primeras horas del día puede atribuirse a la presencia del rocío nocturno que queda adherido a la parte externa de la planta, pero debe ser más importante aún el efecto de secado a medida progresan las horas diurnas, como consecuencia de la transpiración de las plantas, producto del incremento en temperatura. Un efecto similar de la hora del día sobre el contenido de materia seca fue reportado por Dong Zhihao et al. (2022) en el hibrido de *Sorghum bicolor* × *Sorghum sudanense*, quienes encontraron 2.2 unidades en porciento más cuando el forraje se cosechó a las 15:00 vs. 07:00 h. Las bajas concentraciones de materia seca de los pastos, en el momento de la preparación del ensilaje, tienen implicaciones negativas en el proceso, pues fermentaciones indeseables conducen a pérdidas de materia seca, destrucción de hemicelulosa, celulosa, carbohidratos solubles, proteínas y resultan en un mayor pH final, con una concentración de ácido láctico reducida (Yahaya et al. 2020; Chen Rong et al. 2022; Dong Zhihao et al. 2022).

El incremento en la concentración de carbohidratos solubles en el ensilaje como producto de demoras en la hora de cosecha (Figura 2) confirma lo reportado por Dong Zhihao et al. (2022). trabajando con el hibrido de *S. bicolor* × *S. sudanense* cosechado a las 07:00 h, 12:00 h y 17:00 h, con concentraciones crecientes de 7.8, 10.6 y 13.9% de carbohidratos solubles, respectivamente. También Guo Gang et al. (2015) obtuvieron un incremento de 24.7% en la concentración de carbohidratos solubles en el pasto Napier (*C. purpureus*) cosechado a las 18:00 h respecto al cosechado a las 08:00 h. Este efecto es el resultado del proceso fotosintético, que en gramíneas tropicales corresponden principalmente a la síntesis de glucosa y acumulación de sacarosa (Volenec y Nelson 2020). La variación observada en este estudio en la concentración de carbohidratos solubles en función de la hora de cosecha debe corresponder al almacenamiento de corto plazo de estos compuestos durante el día y a la utilización de estos en las diferentes rutas metabólicas que aportan glucosa para la respiración celular (Hopkins y Hüner 2008).

La capacidad de las plantas para resistir la reducción del pH está influenciada por diversos factores, incluyendo la presencia de ácidos orgánicos, sulfato, nitratos y la concentración de proteínas (Buxton y O'Kiely 2003). Además, se ha observado una variación diurna en esta capacidad de amortiguamiento, como lo demostraron Guo Gang et al. (2015), quienes encontraron resultados más altos durante las horas de la tarde. Los resultados obtenidos por esos autores fueron más consistentes en comparación con los del presente estudio, quizás por el período corto de sombra que ocurrió entre las 13:00 y las 15:00 h, perceptible por la brusca reducción en la radiación solar (Figura 1), lo cual puede haber influido en este parámetro, ya que durante estas horas los procesos fotosintéticos tienden a disminuir alterando la composición de las plantas.

Investigando opciones para mejorar el proceso fermentativo del ensilaje en el pasto *M. maximus* cv. Mombaça, Tomaz et al. (2018) encontraron que la altura de la cosecha no solo influyó en el valor nutricional, sino también en la concentración de carbohidratos solubles y materia seca, lo que les llevó a concluir que la cosecha del pasto Mombaça a una altura de 130 cm resultó en un coeficiente de fermentación de 31.0, mejor al encontrado en este estudio, pero todavía por debajo del mínimo recomendado que es de 35.

Parámetros fermentativos

El incremento observado en la concentración de materia seca y de carbohidratos solubles como resultado de la demora en el horario de cosecha (Figura 2) parece fue suficiente para mejorar los parámetros fermentativos, favoreciendo la multiplicación de los microrganismos deseables y la colonización del substrato (Guo Gang et al. 2015), resultando en ensilajes de mejor calidad sensorial y química.

De acuerdo con Bernardes et al. (2018) los ensilajes de pastos tropicales tienden a ser de menor calidad que los preparados con forrajes de zona templada, esto por las bajas concentraciones de ácido láctico y mayores valores finales de pH. Esto se reflejó en el olor final de los ensilajes, que presentaron olores ligeros a vinagre debido a la presencia del ácido acético, el cual es volátil y está presente en mayor proporción en ensilajes de pastos tropicales.

Las horas de exposición al sol permitieron incrementos significativos en la concentración de materia seca y de los carbohidratos solubles. Este punto está ampliamente discutido en la literatura, especialmente en investigaciones que evalúan la operación de secado parcial o marchitamiento y sus consecuencias en el proceso fermentativo (Wan Jiang Chun et al. 2021; Chen Rong et al. 2022). El aumento en la concentración de materia seca, en función de los horarios de cosecha, fue suficiente para reducir las pérdidas por efluentes llegando a un valor mínimo de 0.57% a las 15:00 h.

Solamente el pH de los ensilajes con los pastos cosechados a las 15:00 h (4.52) y 18:00 h (4.67) (Figura 3) estuvo dentro de los rangos establecidos por Kung et al. (2018) (pH 4.30–4.70) para ensilajes de pastos. Los pH obtenidos fueron suficientes para minimizar la degradación del forraje y la multiplicación de microrganismos no deseables. Como consecuencia de la reducción en el pH, los únicos ensilajes que tuvieron concentraciones de N-NH_3 dentro de los rangos establecidos como aceptables por Kung et al. (2018) (8 a 12 % de N-NH_3) fueron los ensilajes de los pastos cosechados a las 15:00 y 18:00 h (8.08 y 9.61%, respectivamente), todos los demás superaron los 14.2% N-NH_3 del N total (Figura 3). Franco y Rinne (2023) trabajando con las gramíneas de zona templada, pastos Timothy (*Phleum pratense*) y Festuca (*Festuca pratensis*), observaron mayores concentraciones de amoníaco (NH_3) en los pastos con menor concentración de materia seca, lo cual se ha atribuido a que una reducción lenta del pH permite la actividad de los clostridios, los cuales realizan la degradación de las proteínas de las plantas a NH_3 (McDonald et al. 1991; Franco y Rinne 2023).

Parámetros nutricionales

Las variaciones en la composición nutricional de los ensilajes como resultado de los horarios de cosecha se explican por cambios en la composición de las plantas al momento de cosecha y su efecto respectivo sobre el proceso fermentativo. Las variables nutricionales MS, PB y EE en los ensilajes fueron las únicas influenciadas por los horarios de cosecha de los forrajes. Los contenidos de materia seca inicial y final estuvieron directamente relacionados e influenciaron las pérdidas por lixiviación. Las mayores concentraciones de PB encontradas en los ensilajes de pastos cosechados a las 15:00 y 18:00 h (13.8 y 12.3%, respectivamenente) están relacionadas con los procesos metabólicos diurnos y nocturnos de las plantas, los cuales redundan en el proceso fermentativo cuando son ensilados. Buxton y O'Kiely (2003) sugieren que las proteínas de las plantas tienden a degradarse durante el periodo nocturno y a sintetizarse nuevamente a lo largo del día, lo que genera su acumulación. Además, las condiciones fermentativas observadas en los horarios de la tarde (Figura 3) resultaron en una menor degradación de las proteínas, permitiendo así su preservación.

Posibilidad de aplicación de los resultados

Los hallazgos de esta investigación evidencian los beneficios de cosechar en horas de la tarde los pastos que se van a ensilar. Sin embargo, deben considerarse otros factores que inciden en la realización de la cosecha y la elaboración del silo. Por ejemplo, es importante tener en cuenta elementos climáticos, como los horarios habituales de las lluvias y las previsiones meteorológicas. Además, los horarios de la mano de obra también son importantes, ya que mover la cosecha a horas de la tarde podría requerir ajustes en los horarios laborales, la contratación de horas extras o de trabajadores externos, dependiendo del manejo individual de cada unidad de producción.

Conclusiones

Cosechar el pasto BRS Zuri (*Megathyrsus maximus*) en horas de la tarde resulta en ensilajes de mejor calidad en aspectos fermentativos y nutricionales. El incremento en el contenido de materia seca y la acumulación de carbohidratos solubles en el pasto a ser ensilado resultaron en menores perdidas por efluentes, menor pH, menor degradación de la proteína y mayor concentración final de proteína bruta.

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TGFT Editorial Team
A.A. 6713, Km 17 Recta Cali-Palmira.
Cali, Valle del Cauca, Colombia.

Phone: +57 2 4450100 Ext. 3084
Email: CIAT-TGFT-Journal@cgiar.org