

EXPANDING THE FARMING POTENTIAL OF NAPIER GRASS (*PENNISETUM PURPUREUM* SCHUMACH.) UNDER LOW-FERTILE CONDITIONS

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Abstract

In the face of growing water scarcity and ever-increasing energy consumption in Thailand, farmers will need to optimize farmland use instead of using it only for seasonal cropping. Climatically tolerant energy crops have the potential for year-round cropping and renewable energy sources are a growing market for the future. Napier grass (*Pennisetum purpureum* SCHUMACH.) is one such stress-tolerant energy crop, showing potential to grow best under less-than-ideal field conditions. In this study, Napier grass crops were grown under low-fertile conditions as found on unused land or during the dry season. For identification, 1 such suitable farming system with growing factors including cutting length, planting method (vertical or horizontal), plant density, and planting date, as well as management practices after planting, e.g. the intercutting regime, was investigated in terms of biomass production. Napier grass was able to thrive under non-irrigated and non-fertilized conditions. The cutting type showed a significant impact on the establishment of seedlings. With an increase in length, stem sections tended to produce more biomass than shorter setts. Matured terminal cuttings established dense canopies with high yields (16.14 Mg dry mass ha⁻¹) in contrast to setts that formed sparse populations. Frequent intercutting intervals caused almost immeasurable, low yields. Furthermore, Napier grass crops produced more biomass during the dry season than in the rainy season. These results could be a new method suitable for cropping Napier grass on unused land.

Keywords: Biomass, Napier grass, energy crop, farming system, unfertile soil

Introduction

Napier grass (*Pennisetum purpureum* SCHUMACH.) is a high-yielding grass mainly cropped in Thailand as cattle fodder. An all-season fodder supply must be guaranteed by cropping methods with quantity and quality (e.g. digestibility) being criteria which can vary with the season (Tessema *et al.*, 2010). Hence, such cropping systems have been intensely researched and optimized for management practices (e.g., intercutting intervals, optimal fertilization, and

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much more) under high-fertility conditions by supplementing naturally deficient conditions (Lounglawan *et al.*, 2014; Wijitphan *et al.*, 2009).

Thailand's increasing water scarcity makes water and its conservation the greatest challenge for agricultural management with successful yields (Sethaputra *et al.*, 2001). A deficit in water supply for field crops results in undesirable effects such as withering, growth stop, yield losses, or total failure. From establishment through to harvest, supplemental irrigation must guarantee a sufficient water supply especially in areas or seasons with limited natural precipitation (Tudsri *et al.*, 2002). Particularly, forages and food crops produce poor quality if cropped under deficit conditions. Farmers in Nakhon Ratchasima Province prefer to let their fields lie fallow due to limited supplementation. Thus, valuable acreage lies completely unused which is not effective and produces no income. Thus, drought tolerant energy crops such as Napier grass provide an interesting opportunity.

Its chemical and physical properties have proved Napier grass to be a promising energy crop. With diverse methods, however, bio-energy can be obtained from all plant parts in all developmental stages (Rengsirikul *et al.*, 2013). Its establishment in arid areas is displayed by diazotrophic and thermophilic life, drought tolerance, and a C4-photosynthetic pathway. Thus, Napier grass owns a wide range of climatic tolerances due to its favorable physiology, including a potential for biomass production under low-fertile conditions found on low-fertility soils or during the dry season (Jorgensen *et al.*, 2010).

The extraordinarily effective physiology of Napier grass is well known, mostly for the energy it produces under heavy fertilization and additional irrigation to attain maximum biomass (Wijitphan *et al.*, 2009; Rengsirikul *et al.*, 2013). However, in order to be able to establish crops on unused farmland and intensify existing acreage use by year-round cropping, Napier grass must be established under low-fertile conditions. Due to little information being available regarding the best farming methods and practices for Napier grass under low-fertile

conditions in Nakhon Ratchasima Province, it was the purpose of this study to investigate how Napier grass crops can be established successfully under low-fertile conditions, with the aim of biomass productivity, by examining the eligibility of cutting types, initiation density, frequency of intercutting intervals, and inception dates. Hence, an in-situ field experiment was carried out on the campus of Suranaree University of Technology (SUT) in Nakhon Ratchasima Province, Thailand.

Materials and Methods

Experimental Site

This study was conducted in 2012 on a site located on the campus of SUT (latitude 14.87014° N, longitude 102.03209° E, elevation 250 m). Soil on the site was classified as the Korat soil series (Oxic Paleustults) (Srisa-ard, 2007). Before the experiment started, soil samples were randomly collected and intermixed from the whole site for analysis until 5 liters were obtained (each sample weighed approximately 200 g). Samples from the first 30 cm thick horizon were classified as humic acid sand by the VDLUFA (Association of German Agricultural Analytic and Research Institutes) method (physical characteristics: 1.05% humus, 3.80% clay, 12.10% silt, and 84.10% sand with a pH-value of 5.3 (CaCl₂) and a slight deficiency of phosphorus (content 30 mg/kg, CAL (calcium-acetate-lactate method)) and potassium (content 60 mg/kg, CAL (calcium-acetate-lactate method))).

To monitor the temperature and precipitation during the study, a data logger (T-Warner, Type iMetos 2, Software Version 05.52, Pessl Instruments GmbH, Weiz, Austria), equipped with sensors for measuring the air temperature (SMT 160-30 with a convection cover) and precipitation (Joss-Tognini principle, Wilh. Lambrecht GmbH, Goettingen, Germany), was installed close to the experimental site.

Experimental Design and Set-up

The production of biomass from Napier grass commonly consists of elementary farming practices: cutting length, planting method, plant

density, planting date, and intercutting intervals after planting (Payne, 2000; Kolo *et al.*, 2005; Ferraris, 1980; Tudsri *et al.*, 2002). Each treatment has an effect or interaction on the produced yield. Hence, these treatments (T) were selected as being important for altering a farming system for Napier grass and were tested in a randomized block design. The experimental plots followed the recommended guidelines of the Federal Plant Variety Office with each plot sized 6×14 m and spaced 1 m apart (Bundessortenamt (Federal Plant Variety Office), 2000). All entries were replicated 3 times (Casler *et al.*, 2015).

Within the 4 main groups of treatments, variables were tested for specific effects under tested conditions:

- T1: intercutting intervals (120, 240, and 360 days after planting).
- T2: cutting lengths (15, 60, and 120 cm

long stem sections).

- T3: planting method (vertical or horizontal).
- T4: plant densities (6, 9, 10, and 12 setts m⁻²).
- T5: planting dates (May, September).

Produced dry mass (DM) was used as the determinant of effects from the tested variables. Taken together, 17 specific entries were organized for the Napier grass cultivar from the SUT campus and tested in the experimental set-up from 2012-2013 (Table 1). Cuttings for propagation were taken from a 2-year-old abandoned Napier grass crop from the university farm. The sections for propagation were sized with a circular saw by hand, to guarantee that the smallest unit (sett) with a size of 15 cm possessed at least 2 nodes (Table 2). For larger-sized stem sections, length-templates were used.

Table 1. Experimental set-up, treatments, and attributes of the experiment on Napier grass for testing the effects on biomass production under natural conditions

Entry	Plant density (m ²)	Treatment		
		Planting method (Cutting length)	Intercut intervals (yr ⁻¹)	Planting date
1	6	Horizontal (15 cm)	1	17/05/12
2	9	Horizontal (15 cm)	1	17/05/12
3	12	Horizontal (15 cm)	1	17/05/12
4 E	10	Horizontal (15 cm)	1	17/05/12
5 E	9	Horizontal (15 cm)	3	17/05/12
6 E	9	Horizontal (15 cm)	1	10/09/12
7 E	9	Horizontal (15 cm)	2	17/05/12
8	9	Vertical (Terminal)	1	17/05/12
9 E	9	Vertical (Terminal)	3	17/05/12
10	3	Horizontal (60 cm)	1	17/05/12
11 E	3	Horizontal (60 cm)	2	17/05/12
12	3	Horizontal (120 cm)	1	17/05/12
13 E	3	Horizontal (120 cm)	2	17/05/12
14	9	Vertical (15 cm)	1	17/05/12
15 E	9	Vertical (15 cm)	2	17/05/12
16 E	18	Vertical (15 cm)	2	17/05/12
17	3 each	Horizontal (60 cm) + Vertical (Terminal)	3	17/05/12

E = Evaluated for biometric performance

Cultivation Method

Before the study's initiation, the fields were completely cleared and ploughed and the cuttings were pruned of dry leaves. Subsequently, pursuant to entry-specific treatments, the cuttings were planted in uniform squares by hand (Table 1). Cane setts were either entry-specifically horizontally buried 5 cm deep in the ground or vertically inserted with 1 node in the ground and the other exposed. The terminal cuttings were inserted in the ground as deeply as possible but not deeper than the fresh leaf sheath. No management (additional fertilization, irrigation, pest or weed control) was practiced after planting.

Evaluation of Agronomic Important Growth Parameters

To monitor the agronomic performance of the Napier grass stands under low-fertile conditions, pre-selected entries were evaluated before harvests for seedling rate, tussock forming (=tiller per plant), tiller height, and the photosynthetic active leaf-area index (LAI) (Table 1). Agronomic and biometric data were collected from all plants in the net plot. The product of node-input (ni) (cutting-specific nodes (Table 2)) was calculated from the number of cuttings per square meter or the planting method, as shown in Table 1. Napier grass possesses 1 bud per node, thus for forming the seedling rate (Sr) (Bakker, 1999). Subsequently, the counted number of growing buds (bg) was calculated as $Sr(\%) = (bg / ni) \times 100$. Plant tillers were counted individually and their aerial heights (from ground to apical meristem) were measured with a tapeline and averaged. The attached trash (t) and leaf damage (d) (caused by *Curvularia penniseti*, *Cladosporium spec.*, and *Myloccerus*

subfasciatus Guérin-Méneville (Farrell *et al.*, 2002; Hill, 2008)) were recorded in decimal steps from 0 to 100 per plant and expressed in percent. The leaf area index (LAI) was calculated as $LAI(\%) = (100 - (\sum (t + d)/n)) \times 100$.

Plant Sampling and Data Analysis

For sampling, the aerial biomass of the full plot (6×14 m) was harvested (10 cm above ground) with the ratoon method which leaves the stubble of the tussock for re-sprouting in the ground. Above-ground harvested biomass was completely removed from plots and only the yield of the net plot (2×6 m) was analyzed. Fresh mass (FM) was weighed and 200 g of the individual samples were oven-dried at 80°C for 72 h to a constant weight and the dry mass (DM) was determined. Moisture contents (MC) were calculated as $MC = (FM - DM) / FM$ (as a percentage $\times 100$) and averaged (Av. MC) per harvest; the sample's DM was re-calculated as $DM = FM(1 - Av. MC)(5/6)$ and expressed as Mg DM ha⁻¹ in the final analysis.

Determination of Tested Propagation Material

The Napier grass hybrid was undetermined; therefore, a herbarium voucher was prepared from 1 of the clones. It was transferred to the greenhouses at the Munich Botanical Garden for long-term documentation of the material's identity and deposited at Ludwig-Maximilians-University (LMU) Munich's Herbarium of Systematic Botany (MSB). The material was classified into species levels using traditional morphology-based keys along with a DNA barcoding approach. The total DNA was extracted from approximately 0.2 mg of fresh leaf tissue using the NucleoSpin Plant II kit

Table 2. Measurements of tested cutting types before being planted in the field

	Cutting type							
	15 cm		60 cm		120 cm		Terminal	
	(n = 201)	RSD (%)	(n = 100)	RSD (%)	(n = 100)	RSD (%)	(n = 46)	RSD (%)
Size (cm)	16.73 ± 2.87	17.13	60.27 ± 2.40	3.99	121.2 ± 2.47	2.04		
FM (g)	32.17 ± 17.00	52.85	133.59 ± 43.68	32.70	249.73 ± 93.28	37.35	87.11 ± 43.23	49.62
Nodes (n)	2.42 ± 0.71	29.34	6.87 ± 3.02	43.92	13.08 ± 5.44	41.56		

(Macherey–Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's protocol. The DNA was dissolved in a 50 μ L elution buffer. After a check for quality and concentration on a 0.8% agarose gel, 1 μ L of template solution was used for the polymerase chain reaction (PCR). Two regions are frequently used for molecular authentication of plant material, ITS (Internal transcribed spacer) from the nuclear ribosomal and trnL-F from the plastid DNA, and the ITS was chosen for the purpose of this study. Amplification was performed using the primer pairs Leu1 and ITS 4 for the ITS and C and F, as described previously (White et al., 1990; Taberlet et al., 1991; Bräuchler et al., 2004, 2010). The PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey–Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. The products were sequenced bidirectionally on an automated ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Waltham, MA, USA) at the LMU sequencing service.

Statistical Analysis

The DM yields of the entries were statistically compared for significances. Data collected for various comparisons were subjected to the analysis

of variance (ANOVA) appropriate to the design as given by Munzert (1992). All statistical data analyses were performed using the IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). The least significant difference test was used to determine significant differences among treatments. The significant differences among treatments were compared with the critical difference at the 0.05 level of probability for significance.

Results and Discussion

Plant Material

Plant development and the agricultural performance of cultivars can be quite variable and make a determination of the plant material necessary (Rengsirikul et al., 2013). For the unknown Napier grass cultivar from the SUT farm, a phylogenetic determination was conducted. The resulting consensus sequences (GenBank Accession numbers: KR350689, KR350690) were checked for identity using BLAST (basic local alignment search tool) with the top scores (100% coverage, 99% sequence identity) being 2 accessions of *Pennisetum purpureum* (JX156340.1, JX156338.1) confirming its

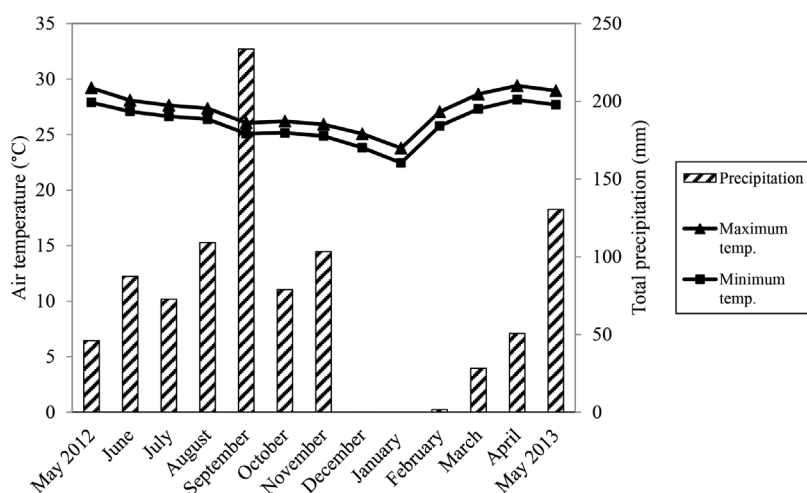


Figure 1. Monthly precipitation and temperature for the duration of the field experiment at SUT. Data were obtained from the field site. The bar chart describes the monthly total precipitation and crop water supply concurrently. The line chart shows the monthly averaged minimum and maximum temperatures

morphological determination (Zhang *et al.*, 2000).

Weather

The annual rainfall of 811 mm during the investigation period lay two thirds under the regional longtime average in this study. Nevertheless, rainfall was sufficient and all plots started to grow successfully and established stands at the specific planting time under field conditions, as the yields proved. The course of the rainy season from May until November with a precipitation peak in September is mirrored in Figure 1. The dry season was characterized by almost no rainfall from December until March and ended with a small amount of rainfall in March. The average minimum and maximum monthly temperatures ranged moderately between 25 and 30°C, very favorable for thermophilic Napier grass. The temperatures decreased continuously during the seasons, reaching the lowest values in the middle of the dry season, and increased again before the rainy season started (Figure 1).

Produced Biomass

The effect on the tested cuttings with respect to precipitation on the accumulated dry mass (DM) is shown in Figure 2; DM yields of the setts (15 cm) vary marginally and the chart

line proceeded almost parallel, whether planted vertically or horizontally. Setts showed a drastic yield increase at 800 mm of water consumption, whereas the vertically planted setts surpassed the horizontally buried ones. The longer stem sections of 60 and 120 cm produced little biomass but the latter had a higher base level. To this end, the chart lines of all various long stem sections are close together and spread moderately. The chart line of terminal cuttings is virtually straight, thus showing the closest relation between produced biomass and precipitation. In other words, terminal cuttings developed more quickly than cane setts from propagule to plantlet and, thus, used precipitation more efficiently than stem-section cuttings.

The process of bud germination (from bud to plantlet) of stem sections is described as taking up to 8 weeks and the almost parallel proceeding chart lines of the stem section cuttings are very consistent with that description (Humbert, 1963; Bakker, 1999). It was observed in the fields that terminal cuttings had already started sprouting after 5 days. Hartmann *et al.* (2013) reported on woody plants, where softwood plum cuttings from terminal shoots rooted and flushed more quickly than more lignified lateral shoots.

After completion of the study, the produced DM ranged from roughly 0.60 to 16 Mg ha⁻¹ (Figure 3). The bar chart in Figure 3 shows

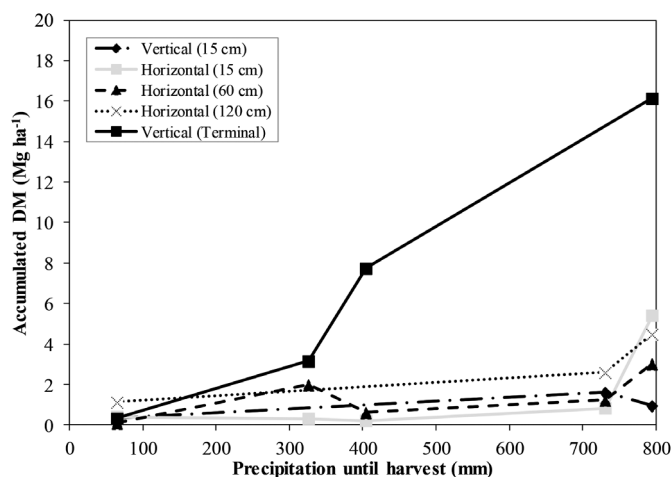


Figure 2. Produced biomass from cutting types in response to quantities of consumed rainfall. Yields and precipitation were accumulated for intercutting intervals

the produced DM with the standard deviation for individual spreads that became larger with the increasing DM yield. The outstanding DM yield of entry 8 (roughly 16 Mg DM ha⁻¹) differed highly from the others. Interestingly, most entries which were planted with setts ranged below a yield of 2 Mg DM ha⁻¹, while entries with longer stem sections or terminal cuttings produced more biomass. Basically, the yields observed in this investigation were much lower than from heavily fertilized and irrigated plots which ranged between 40 to 60 Mg DM ha⁻¹ (Wijitphan *et al.*, 2009; Rengsirikul *et al.*, 2013). In other studies, in which fertility was more limited, Napier grass, in 3 successive harvests with no application of N fertilizer, produced 26.3, 20.9, and 9.8 Mg DM ha⁻¹ biomass on average, respectively (de Moraes *et al.*, 2009). However, the yields of many entries were even less than in reports of

naturalized Napier grass (between 7 to 11 Mg DM ha⁻¹) (Ohimain *et al.*, 2014).

Intercutting Interval

In Figure 3, the DM yields in a pairwise comparison of intercut and uninterrupted grown entries (2/5, 10/11, 12/13, and 14/15) clearly indicate the negative impact of intercuts by the smaller total yields of the entries with an intercutting interval. Frequent intercutting intervals, particularly for entries 5 and 15, evinced consistently small yields. From inception to cessation (360 days after planting), small yields revealed poor development exacerbated by successive intercuts. The produced biomass from 60 cm- (entries 10 and 11) and 120 cm- (entries 12 and 13) long canes as well as the cane/terminal mixed entry 17, ranged in the middle of the experiment, albeit ratooning during a

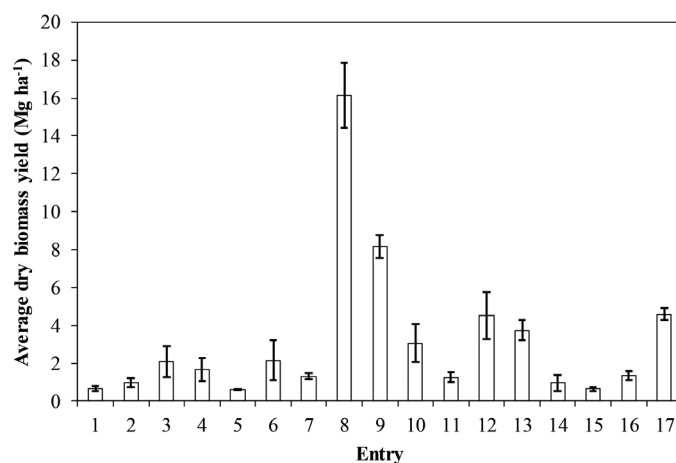


Figure 3. Distribution of dry mass yields and specific standard deviation of entries of Napier grass after completion of the study from 2012 to 2013

Table 3. Effect of cutting type on dry mass on a 4-monthly intercutting interval in the experimental period 2012 to 2013

		Mg DM ha ⁻¹		
	Cutting type Av. MC (%)	120 DAP 70.06	Intercut 240 DAP 47.84	360 DAP 59.37
Entry 5	(Horizontal 15 cm)	0.19 c, A	0.31 b, A	0.11 a, B
Entry 9	(Terminal)	5.86 a, A	1.99 a, B	0.31 a, C
Entry 17	(60 cm + terminal)	2.54 b, A	1.95 a, A	0.09 a, B

Means followed by the same lowercase letter within a column and the same uppercase letter within a row are not significantly different. DAP = Days after planting; Av. MC = Average moisture content

dry time caused a considerable yield decrease. Significantly, most biomass was produced with terminal cuttings, with entry 8 yielding the most followed by 3-times-ratooned entry 9 (with 16.14 and 8.16 Mg DM ha⁻¹, respectively).

The produced biomass amounts from entries 5, 9, and 17 with the most frequent intercutting intervals (every 120 days) are shown in Table 3. Entry 5 yielded, with 0.19 Mg DM ha⁻¹, the least 120 days after planting and significantly less than entries 9 and 17 (5.86 and 2.54 Mg DM ha⁻¹, respectively). Interestingly, the produced biomass declined from unmixed terminal cuttings (entry 9) via a mix of 60 cm long canes + terminal cuttings (entry 17) to setts (entry 5). One hundred twenty days after the first intercut, the biomass amounts of entries 9 and 17 were more or less on the same level and differed significantly from

entry 5. Entry 9 experienced a severe yield drop from 5.86 to 1.99 Mg DM ha⁻¹ after ratooning. At the final intercut, 120 days after the second intercut, biomass yields decreased further and displayed the negative impact of intercutting during the dry time. Previous intercuts did not show such a drastic biomass decrease (Figure 4). Finally, all entries yielded significantly less than in the previous intercutting interval until yields were on an almost immeasurably low level (0.09 to 0.31 Mg DM ha⁻¹), shown in Table 3.

After a cropping period of 12 months, the biomass of interrupted growing entries, shown in Table 4, ranged between 0.96 to 16.14 Mg DM ha⁻¹ with a significant yield difference from the outstanding entry 8 (16.14 Mg DM ha⁻¹) that was planted with terminal cuttings. The outstanding biomass of entry 8 results from an established

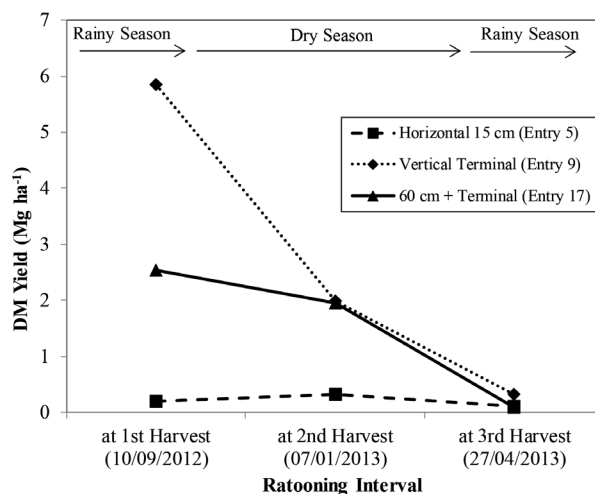


Figure 4. Biomass decrease with season and intercutting intervals

Table 4. Effect of intercuts and cutting type on dry mass in the experimental period 2012 to 2013. Yield of intercut entries was accumulated

Cutting type	Comparison	Mg DM ha ⁻¹	
		Intercut	Uninterrupted
(Horizontal 15 cm)	Entry 5 / Entry 2	0.61 b, A	0.96 b, A
(Terminal)	Entry 9 / Entry 8	8.16 a, B	16.14 a, A
(Horizontal 60 cm)	Entry 11 / Entry 10	1.26 b, A	3.05 b, A
(Horizontal 120 cm)	Entry 13 / Entry 12	3.74 b, A	4.51 b, A
(Vertical 15 cm)	Entry 15 / Entry 14	0.64 b, A	0.96 b, A

Means followed by the same lowercase letter within a column and the same uppercase letter within a row are not significantly different

dense stand with an estimated 100% seedling rate.

Yields of frequently intercut entries were accumulated and ranged between 0.61 and 8.16 Mg DM ha⁻¹, whereas setts (entries 5 and 15, 0.61 and 0.64 Mg DM ha⁻¹, respectively) yielded the least. On that occasion, the yield of entry 9 (8.16 Mg DM ha⁻¹), which was planted with terminal cuttings, differed significantly from those of the other entries.

Generally, entries which were grown uninterrupted produced more biomass than equivalents with more frequent intercuts after planting. Longer stem sections, entries 10 and 12 (60 cm and 120 cm, respectively), produced more biomass (3.05 and 4.51 Mg DM ha⁻¹, respectively) than the smaller 15 cm setts (entries 2 and 14 each produced 0.96 Mg DM ha⁻¹) and, thus, were higher-yielding as cuttings than setts. Nevertheless, the biomass amounts finally produced did not differ significantly except for entries 9 and 8 consisting of terminal cuttings. Entry 8, growing undisturbed, produced twice as much biomass (16.14 Mg DM ha⁻¹) as its ratooned equivalent entry 9 (8.16 Mg DM ha⁻¹).

Cutting Length

Table 5 shows the DM yield of various long stem-section cuttings of Napier grass. Two hundred forty days after planting, the biomass yield was in a wide range from 0.54 to 2.60 Mg DM ha⁻¹. On that occasion, the DM did not differ significantly and showed no significant effect if stem sections with increasing lengths (entries

7, 11, and 13) were used. There was an unclear pattern for increasingly produced biomass, except for entry 7, and a tendency of biomass doubling with the doubling of the stem section length from 60 to 120 cm (entries 11 and 13) was found. However, stem sections with an increasing length also produced more biomass than the shorter setts. The asexual reproduction cycle of Napier grass starts with sett roots in the ground and stimulates a bud break of the node section. Hence, double buds in the ground should bear double biomass in consequence, for instance by increasing the stem section length (from 15 to 60 and 120 cm). The biomass yield increase from more densely planted cuttings (more buds in the ground) is generally consistent with reports from the literature (Wijitphan *et al.*, 2009).

One hundred twenty days after an intercut, the biomass of all entries in Table 5 decreased drastically, even though not significantly, and ranged between 0.04 to 1.14 Mg DM ha⁻¹. Particularly, entry 11 (60 cm long stem section) produced almost immeasurably little biomass (0.04 Mg DM ha⁻¹), signaling a vital crop shrinkage close to total loss. Nevertheless, besides the drastic biomass decrease, intercutting showed no significant effect on the crops which is inconsistent with reports in which a negative impact of intercut during the dry time was found (Tudsri *et al.*, 2002).

Plant Density

More biomass should ideally be produced from increasing plant densities. This was tested

Table 5. Effect of stem section length and planting method on DM at 2 different harvesting dates in the experimental period 2012 to 2013

		Mg DM ha ⁻¹	
		Intercut	
	Cutting type	240 DAP	360 DAP
Entry 7	(Horizontal 15 cm)	1.01 a, A	0.31 a, A
Entry 11	(Horizontal 60 cm)	1.22 a, A	0.04 a, A
Entry 13	(Horizontal 120 cm)	2.60 a, A	1.14 a, A
Entry 15	(Vertical 15 cm)	0.54 a, A	0.10 a, A
Entry 16	(Vertical 15 cm)	1.09 a, A	0.26 a, A

Means followed by the same lowercase letter within a column and the same uppercase letter within a row are not significantly different. DAP = Days after planting

in the present experiment for setts and is shown in Tables 5 and 6. Previous studies of plant spacing showed significant effects of plant density per farmland unit on yields under supplemental irrigation and heavy fertilization since yields increased significantly with increasing planting densities (Miyagi, 1980; Wijitphan *et al.*, 2009). In contrast to expectations and the literature, the effects of the plant density of setts were not observed. Nevertheless, the produced biomass tended to increase when the planted sett density was increased from 6 to 12 per square meter (Table 6). Additionally, no significant effect was found for vertically planted setts (entries 15

and 16), even though entry 16 was planted with twice as many setts as entry 15, as shown in Table 5.

Planting Method

The produced biomass of vertically or horizontally planted setts is shown in Table 5. The planting method of the setts showed no significant effect on the produced biomass (entries 7, 15, and 16). Interestingly, the horizontal burying of setts (entries 7 and 16) tended to double the DM yield as this planting method placed double the number of buds in the ground compared to vertical insertion. Due to the fact

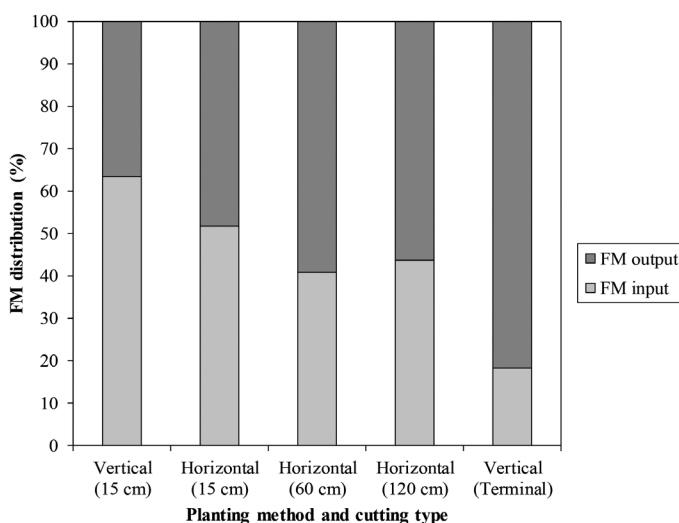


Figure 5. Relative distributions for fresh mass (FM) input and output sorted by cutting type and planting method in the experimental period 2012 to 2013

Table 6. Effect of initiation density and planting method of setts on DM yield in the experimental period 2012 to 2013

			Mg DM ha ⁻¹
	Planting density (setts m ⁻²)	Planting method	Produced biomass
Entry 1	6	(Horizontal 15 cm)	0.68 a
Entry 2	9	(Horizontal 15 cm)	0.96 a
Entry 3	12	(Horizontal 15 cm)	2.09 a
Entry 4	10	(Horizontal 15 cm)	1.66 a
Entry 14	9	(Vertical 15 cm)	0.96 a
Entry 15*	9	(Vertical 15 cm)	0.64 a
Entry 16*	18	(Vertical 15 cm)	1.35 a

Means followed by the same lowercase letter are not significantly different. * = Yield was accumulated

that Napier grass starts its vegetative reproduction from the buds in the ground, aerial buds often shrivel and are not part of the reproduction physiology and, thus, the effects of that planting method were expected. However, these results are consistent with the results of earlier studies in which no significant differences between the planting methods of lower portioned stem sections were found (Knoll and Anderson, 2012).

Productivity

The weights of the produced biomass, disregarding the weight required for planting, are imprecise data, whereas the input:output ratio is more exact. The relative distributions of used and produced biomass sorted by cuttings are shown in Figure 5. Besides the economic aspects, planting inputs of more than 50% consumed more biomass than was produced and vice versa. Figure 5 visualizes a decline of the biomass input:output ratio from setts to longer-stem sections to terminal cuttings which were the most productive cuttings. Setts, irrespective of the planting method, produced unprofitable low biomass due to higher inputs than outputs. Also longer-stem sections produced only a bit more biomass than was necessary for the initiation. Thus, stem sections with increasing lengths performed more favorably than the shorter setts, as the relative produced biomass shows. Terminal cuttings stood out for their produced biomass ratio as every cutting multiplied its biomass almost 5 times (Figure 5).

Planting Date

Locally, rain-fed cropping systems start regularly with the rainy season in May and end in September, leaving farmland unused during the ensuing dry season. Little has been reported

about an anticyclical farming strategy, starting with the end of the rainy season to produce biomass during the dry season. The produced biomass of entry 5, initiated regularly in May, and entry 6, initiated at a date late in September, differed insignificantly (Table 7). Interestingly, entry 6 produced 2.16 Mg DM ha⁻¹ 120 days after planting and, thus, much more biomass during the identical long cropping period than under the more favorable growing conditions of entry 5 (0.19 Mg DM ha⁻¹), showing that setts were able to produce biomass when planted late.

Evaluation of Parameters of Agronomic Importance Seedling rate

Previous studies under greenhouse management showed germination rates of 57.5% to 100% for horizontally buried setts, and 85% to 97.5% for vertically inserted setts (Knoll and Anderson, 2012). A quite constant seedling rate between 8.22% to 13.27% per entry was found except in entry 13 (120 cm long canes) with 2.48% (Table 8). At a second evaluation (240 days after planting) the seedling rate increased and then dropped drastically after intercutting. The best seedling rate (21.81%) was found for the late-planted entry 6. Propagation success is reported with N-supply by fertilization as well as soil moisture. Reduced germination of propagules is found under deficient conditions (Woodard and Prine, 1990; Rusland *et al.*, 1993; Veenendaal *et al.*, 1996). Hence, a reduced seedling rate was consistent with the conditions (non-fertilization and non-irrigation) in this experiment.

Plant Architecture

An effective but plant-compliant biomass-harvest method for Napier grass is ratooning which leaves tussock stubble and rootstocks

Table 7. Effect of regular and late inception date of setts on dry mass 120 days after planting in the experimental period 2012 to 2013

Planting date Yield	Mg DM ha ⁻¹	
	Entry 5 May 2012	Entry 6 September 2012
	0.19 A	2.16 A

Means followed by the same uppercase letter are not significantly different

for regeneration in the field, eliminating pests and, in consequence, competitive weeds and which supports lateral soil occupation. Large tussock formation by recruiting tillers is found under natural grazing or mechanical ratooning (Pereira *et al.*, 2015). At a first evaluation (120 days after planting), between 1.09 and 1.65 tillers per plant were counted, increasing steadily in a range from 2.32 to 6.15 tillers per plant until completion of the study. In this experiment, tiller recruitment was much less than reported from previous experiments in which, on average, between 12.3 to 23.7 tillers per plant were counted under heavy fertilization and additional irrigation (Zahid *et al.*, 2002). Drought stress after intercutting can intensify plant losses. It was found that ratooning caused plant losses particularly during the dry season after an intercut in January. Minimum rainfall in January worsened the unfavorable environmental conditions, as shown in Figure 1, and caused severe plant losses after an intercut (Table 8). Despite ratooning or not, all entries in this

experiment recruited tillers steadily and formed tussocks during maturity. In accordance with the literature, horizontally buried cane sections started lateral expansion, right after planting, by regenerating many clinched nodes below the soil surface, and intensified with maturity (Bakker, 1999). Furthermore, it was observed that terminal cuttings recruited almost no tillers and showed a significant apical dominance by forming stalks, whereas tussock forming was completely disregarded. Likewise, plant architecture was expected for vertically inserted setts, while, in contrast, tussocks were preferentially formed (Table 8). Conversely, terminal cuttings started not to form tussocks after ratooning as found with the vertically inserted setts, and the DM yield decreased through perished plants. It was observed that canes and terminal cuttings differed, specifically in regeneration found in different ratooning tolerances, whereby tussock formation of terminal cuttings could not be mechanically manipulated into forming such as in a cane-section cutting.

Table 8. Evaluation of agronomic important growth parameters of Napier grass under natural conditions in the experimental period 2012 to 2013

	Entry								
	4	5	6	7	9	11	13	15	16
1 st Evaluation (120 DAP)	✂			✂					
Seedling rate (%)	11.25	11.52		11.74	-	8.22	2.48	13.27	9.57
Tiller (plant ⁻¹)	1.09	1.12		1.15	-	1.64	1.65	1.32	1.09
Height (cm tiller ⁻¹)	29.96	20.40		24.24	-	35.43	41.44	29.60	17.00
LAI (%)	40.81	27.39		36.30	-	34.24	45.19	50.15	59.97
2 nd Evaluation (240 DAP)	✂			✂	✂	✂	✂	✂	✂
Seedling rate (%)	11.59	14.16		10.03	-	13.21	3.33	15.12	10.80
Tiller (plant ⁻¹)	1.98	1.22		1.37	-	1.84	4.14	2.33	1.93
Height (cm tiller ⁻¹)	68.63	20.43		37.80	-	38.29	58.43	33.63	22.89
LAI (%)	58.35	46.88		45.14	-	37.98	43.18	39.57	54.73
3 rd Evaluation (360 DAP)	✂	✂	✂	✂	✂	✂	✂	✂	✂
Seedling rate (%)	-	4.79	21.81	8.03	35.80	2.02	2.69	4.94	5.86
Tiller (plant ⁻¹)	-	2.59	1.24	2.32	2.55	2.53	6.15	5.89	2.32
Height (cm tiller ⁻¹)	-	6.04	47.85	12.63	11.17	10.36	18.00	11.52	7.22
LAI (%)	-	56.17	20.89	53.56	38.01	48.72	45.50	42.95	68.31

LAI = Leaf-area index; DAP = Days after planting; ✂ = Intercutting

Stem Formation

Tillers grew from planting to first evaluation between 17.00 to 41.44 cm tall, whereas entries 11 and 13 (35.43 and 41.44 cm per tiller, respectively), consisting of stem sections with increasing lengths grew taller as setts. Two hundred and forty days after planting, the height of tillers increased in a range from 22.89 to 68.63 cm per tiller; entry 4 (15 cm setts) thrived vigorously to 68.63 cm per tiller. The height of the late-planted entry 6 was measured at 21.81 cm per tiller, ranging low in the grade of entries planted in May (Table 8). The biomass yields' results from herbage production and tiller height are an important agronomic indicator to evaluate stand development. In previous studies, the shoot length of setts ranged between 40.9 to 64.8 cm after 14 days under greenhouse conditions, while conventionally managed field crops grew on average 108 to 140 cm per month (Jorgensen *et al.*, 2010; Knoll and Anderson, 2012). Thus, the evaluated tiller height in this experiment was irregularly smaller than found in other investigations where drought stress symptoms with reduced tiller height and herbage production were reported (Purbajanti *et al.*, 2012). In this experiment, crops were exposed to environmental stress factors by omitting fertilization and irrigation application, and irregular small tillers were the cause.

Herbage Health

Leaves are the organs of Napier grass containing the most chlorophyll and drive biomass production. Therefore, the leaf-area index (LAI) should be as high as possible since a low LAI is a sign of crop stunting (Kubota *et al.*, 1994; Nagasuga, 2005). The evaluated LAI (Table 8) ranged continuously between 27.39% and 59.97% during the full experiment. Interestingly, entry 6, planted in September, showed the smallest LAI (20.89%). Drought stress causes leaves to dry off and reduces the photosynthetic active leaf area, matching dry leaves as a visual sign for weak plants (Smit and Singels, 2006). Rain-fed crops are exposed to seasonal rainfall changes and thus to fluctuations of drought stress. Hence, it was expected that environmental conditions would reduce the

LAI and weaken crops during the dry season. Interestingly, the opposite effect was found and the LAI was unaffected, as shown in Table 8.

Conclusions

The cutting type from elementary farming practices showed the most significant effect under natural farm management. Terminal cuttings were able to produce high biomass yields with no application of fertilizer or irrigation, if cropped uninterrupted for a full year, in contrast to stem-section cuttings. On the other hand, late planted setts of Napier grass revealed a potential for dry-season cropping when acreage in Nakhon Ratchasima Province most often lies fallow.

Of all the investigated treatments, the least-altered system (planting setts, 3 intercutting intervals per year, inception in May), which is widely seen in Thailand, produced the lowest biomass under natural-fertile conditions. A fundamentally altered cropping system (using terminal cuttings, a full-year cropping period, single-cut instead of ratooning) resulted in a significantly higher yield. More research will be required to determine an optimized farming strategy for a wider farming region envisaging economic and distribution aspects for producers and power plant operators.

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