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Full Length Research Paper

An alternative to complete banana mat uprooting: Assessing the effectiveness of continuous cutting at soil level of all shoots in a mat on speed for corm decay

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The complete uprooting of diseased mats/fields (CMU) is one of the recommended control options for Xanthomonas wilt of banana. CMU is labour intensive, time consuming and disturbs the soil structure, exposing fields to erosion. CMU often involves exportation of whole plant biomass, affecting soil fertility. The potential of continuous cutting at soil level of all shoots in a mat until complete corm decay *in situ* as an alternative to CMU was assessed. The first experiment was established using 224 banana mats in their third cropping cycle. All the plants were cut down at soil level, meristems were removed, and sweet potato and bush bean planted. In a repeat experiment with 180 banana mats, a wide range of treatments were applied on top by cutting and removing the apical meristems. These included the: injection of 2,4-D herbicide into the centre of each corm; removal of a cone shaped section from the center of each corm; and creation of a 20 cm deep incision in the center of each corm; in combination with the application of soil or farmyard manure substrate on cut surface. In the first experiment, re-sprouting stopped at 8 months while corms fully decayed after 25 months. Annual intercrops did not influence re-sprouting and corm decay rate. Similar re-sprouting trends occured in the repeat experiment. However, 2,4-D application significantly (P<0.05) lowered decay time, with 12-47% of corms decomposed at 8 months compared with 0-20% in other treatments without 2,4-D. In the 2,4-D treatments, 100% of corms had decomposed compared with 36-80% in other treatments by the 20th month. Deep incisions or cuts did not significantly hasten decomposition. Soil or manure substrate addition had no advantage when compared with the cut surfaces without substrates. A cost-benefit analysis showed a five times higher net income with continuous cutting of re-sprouts when compared with CMU.

Key words: Apical meristems, cost-benefit analysis, decomposition, herbicide, soil, Xanthomonas wilt.

INTRODUCTION

Banana production in the Democratic Republic of Congo (DR Congo) is severely threatened by the new and highly devastating disease, banana bacterial wilt caused by *Xanthomonas campestris* pv.*musacearum* (Xcm)

(Ndungo et al*.,* 2005). Its non-discriminate infection of all *Musa* cultivars and ability to cause up to 100% yield loss, severely compromises livelihoods and food security for banana farming households (Tushemereirwe et al., 2003; Ndungo et al*.,* 2005; Karamura et al., 2006; Blomme et al., 2014). It is a vascular disease that results in permanent wilting and eventual death of the banana plant (Yirgou and Bradbury, 1968, 1974). Transmission of this disease is through insects frequently associated with the inflorescence, infected tools, birds, bats, foraging domestic animals and movement of infected plants or plant parts (Biruma et al., 2007; Ocimati et al., 2013; Buregyeya et al., 2014).

The uprooting of diseased mats coupled with banana-free fallows (e.g. grass fallows or cultivation crops such as bush beans, sweet potatos, taro, maize and cassava) has been recommended as a control option for Xanthomonas wilt disease in well-managed banana production systems as in the highland cooking banana (AAA-EA) systems in south-western Uganda and Rwanda (Turyagyenda et al., 2008; Ssekiwoko et al., 2010; Rutikanga et al., 2013; Kubiriba et al., 2014). However, complete mat uprooting is very labour intensive, time consuming and is thus not widely practiced (Jogo et al., 2013; Blomme et al., 2014; Ocimati et al., 2015). In addition, a factor that negatively contributes to the effectiveness of complete mat uprooting is that the majority of farmers do not disinfect their garden tools and yet they often borrow/share tools. They often find disinfection of the garden tools through heating above a fire cumbersome/inconvinient, while chemical disinfection has been perceived by farmers as too expensive or hampered by lack of access (Blomme et al., 2014). Uprooting of diseased mats also results in destruction of the soil structure and exacerbates soil erosion in the affected farms. Coupled to this, the exercise often involves exporting crop debris out of fields, potentially affecting the fertility of the soils.

Herbicides have been used previously to destroy infected banana plants. In Uganda, for example, glyphosate (Roundup®) and 2,4-dichlorophenoxyacetic acid (2,4-D) pseudostem injections have been used to destroy banana plants infected by bacterial wilt in controlled experiments (Okurut et al., 2006; Blomme et al., 2008). Similarly in Martinique, glyphosate has been used to kill off Cavendish plants (*Musa* AAA group) in preparation for fallow (Quénéhervé, Pers. Comm. In Blomme et al., 2008). Glyphosate has also been used for destroying banana bunchy top infected plants (*Musa* AAA group) in Hawaii (Sommer, 2000) and Xanthomonas wilt infected plants in Kenya (Kubiriba et al., 2014). Using herbicide injections to destroy bananas has several advantages. First the systemic nature of herbicides means that the whole plant, including the corm, dies and decays in a short time. In contrast, during manual removal or when using a tractor with a set of trailing discs corm pieces can remain in the soil and this could lead to unwanted re-sprouting. The herbicide equipment needed for herbicide application is simple, affordable and requires little skill to operate by farm staff (Blomme et al., 2008). Regarding the cost of 2,4 D, evidence from published research data shows that use of herbicides can be cost-effective and less laborious as compared to physical uprooting of mats, and a small well trained team can eradicate a large acreage in a relatively short time (Blomme et al., 2008). However, some setbacks to the use of herbicides by small-scale farmers in east and central Africa includes their often limited supply, the perceived high cost of herbicides and the possible intake of treated plant material by free-ranging domestic animals (Karamura, personal comm., 2010). Since banana is mainly grown by poor small-scale farmers in east and central Africa (ECA), it is necessary to explore novel environmentally sound methods to destroy infected mats.

The objective of this study was to asses if mat removal through continuous cutting at soil level, of all shoots in a mat could hasten complete corm decay, and thus be a less labour demanding and environmentally sound alternative to manual complete mat uprooting. The effects of two common annual crops, which are often planted after complete mat uprooting to meet household food security needs, on the efficacy of continuous cutting of shoots at soil level were also evaluated.

MATERIALS AND METHODS

The first trial was carried out at the “Institut National pour l’Etude et la Recherche Agronomiques (INERA) Mulungu” research station in South Kivu, DR Congo. An existing field with highland cooking banana (AAA-EA; variety ‘Barhabesha’) mats in the 3rd cropping cycle (2nd ratoon cycle) was used. All the plants were cut down during December 2011. All pseudostems in a mat (including peepers, sword suckers and maiden suckers) were cut off with a machete at soil level. In addition, any remaining apical meristem was destroyed/removed using a machete. Two common break crops, namely, the sweet potato, ‘Mugande’ variety and the bush bean, ‘MLB49’ variety, were respectively planted in December 2011 and January 2012 to assess their potential effects on the efficacy of continuous cutting of shoots at soil level.

There were 8 replications per break crop, giving a total of 16 plots. Each plot contained 14 banana mats. Hence, an overall total

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Table 1. Average number of suckers and mature plants per mat, average diameter (cm) of the cut corm surface across suckers and mature plants, mean corm surface area of suckers and mature plants, mean mat corm surface area and percentage soil surface occupied by the corms for each of the 12 treatments. Data was collected at trial initiation.

Treatment	Plants < 2 m tall			Plants >2 m tall			Mean total corm surface area (cm^2)	Soil surface occupied by corms (%)
	Mean No. of plants/ mat	Mean corm diameter	Mean corm surface area (cm^2)	Mean No. of plants / mat	Mean corm diameter	Mean corm surface area (cm^2)		
T1*	$1.65^{cd\#}$	9.38^{c}	78.1^{c}	2.01^{a}	23.19^{b}	433.5^{bc}	1053.0^{bc}	2.63^{bc}
T2	1.78^{bcd}	10.58^{bc}	95.3^{bc}	1.93^{a}	23.35^{b}	436.4^{bc}	907.0^{c}	2.27^{c}
T3	1.89^{bcd}	10.77^{bc}	103.1^{bc}	2.07^{a}	23.41^{b}	437.9^{bc}	1011.0^{bc}	2.53^{bc}
T4	1.80^{bcd}	10.4^{bc}	89.0^{bc}	2.13^{a}	22.81^{b}	415.2^{c}	984.0^{bc}	2.46^{bc}
T5	2.78^{ab}	10.1^{bc}	85.5^{bc}	2.20^{a}	27.80^{a}	615.5^{a}	1521.0^{a}	3.80^{a}
T6	2.07^{abcd}	11.3^{a}	103.0^{bc}	2.13^{a}	25.07^{b}	498.6^{b}	1202.0^{abc}	3.01^{abc}
T7	2.69^{ab}	10.0^{bc}	80.4^{c}	2.13^{a}	23.31^{b}	437.9^{bc}	1189.0^{abc}	2.97^{abc}
T8	2.20^{abd}	10.2^{bc}	89.4^{bc}	2.37^{a}	23.30^{b}	434.1^{bc}	1001.0^{bc}	2.50^{bc}
T9	1.28^{d}	12.6^{a}	147.3^{a}	2.01^{a}	24.27^{b}	467.5^{bc}	1021.0^{bc}	2.55^{bc}
T10	2.37^{abc}	10.0^{bc}	82.4^{c}	1.79^{a}	23.39^{b}	437.8^{bc}	960.0^{bc}	2.40^{bc}
T11	2.98^{a}	10.0^{bc}	84.0^{bc}	2.39^{a}	23.98^{b}	465.6^{bc}	1358.0^{ab}	3.40^{ab}
T12	1.54^{cd}	11.6^{ab}	115.7^{ab}	1.93^{a}	24.53^{b}	479.5^{bc}	992.0^{bc}	2.48^{bc}
LSD	1.03	1.85	32.3	0.74	2.43	83.26	404.4	1.01
Fpr	0.025	0.057	0.003	0.925	0.008	0.001	0.104	0.104

[#]Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test ($P<0.05$). [*]: T1: all the apical meristems were removed and 2.4-D was injected into the centre of each large corm. no cover; T2: all the apical meristems were removed and 2.4- D was injected into the centre of each large corm. cover cut surfaces of all stems with manure; T3: all the apical meristems were removed and 2.4-D injected into the centre of each large corm and cover cut surfaces of all stems with soil; T4: all the apical meristems were removed. no cover; T5: all the apical meristems were removed and cut surfaces of all stems were covered with manure; T6: all the apical meristems were removed and cut surfaces of all stems were covered with soil; T7: all the apical meristems were removed and a cone shaped section was cut out from the center of each corm (machete was used to remove the "cone") no cover; T8: all the apical meristems were removed and a cone shaped section was cut out from the center of each corm (use machete to remove the "cone") and cover cut surfaces of all stems with manure; T9: all the apical meristems were removed and a cone shaped section was cut out from the center of each corm (machete was used to remove the "cone") and cover cut surfaces of all stems with soil; T10 all the apical meristems were removed and make a deep incision into the center of all the corms (use spear pointed tool) no cover; T11: all the apical meristems were removed and a deep incision was made in the center of all the corms (spear pointed tool was used) and cover cut surfaces of all stems with manure and T12: all the apical meristems were removed and a deep incision was made in the center of all the corms (spear pointed tool was used) and cut surfaces of all stems was covered with soil.

of 224 mats were assessed. Any re-growth (sprouting suckers) was systematically cut off at soil level, and apical meristems destroyed, at weekly intervals. Corm decay and number of emerging sprouts was assessed at monthly intervals.

In a second trial, initiated in April 2013, the dessert banana cultivar 'Giant Cavendish' was used. At the initiation of the trial, all pseudostems were cut off at soil level and any remaining apical meristem was removed using a machete. The number of suckers/plants (< 2 meter tall shoots and > 2 m tall shoots) in each mat were counted, cut off at soil level and the diameter (cm) of each plant was measured at the cut-off section (Table 1). These measurements provided information on the approximate mat and corm size and percentage soil surface area occupied by the corms and which was hence not available for annual crop production. In addition, it was hypothesized that the initial variations in total size of corms in a mat could influence its rate of decay. The treatments during this second trial consisted of i) the removal of the apical meristem of all stems ii) the removal of the apical meristem of all stems and the injection of 2,4-D into the centre of each corm, iii) the removal of a cone shaped section in the center of all corms using a machete, and iv) the insertion of a spear like pointed soil auger to at least 20 cm depth into the center of each corm. To ease/facilitate the injection of 1.6 ml of 2,4-D (using a syringe), a 10 mm wide metal rod was inserted up to 10 cm depth, into the corm tissue. In addition to the above treatments, either de-composted manure or top soil was used to cover the cut corm surfaces. Cut surfaces without manure or top soil acted as controls. Treatments iii and iv were anticipated to enhance corm decay as water would stagnate in the cut out corm sections, while micro-organisms from top soil and manure were expected to enhance corm decay. There were 5 mats for each of the 12 treatment combinations and 3 replications, giving a total of 180 mats. Re-sprouts in all the treatments were counted and removed at weekly intervals, while the level of corm and cord root decay was also assessed at monthly intervals.

Cord root decay was assessed following a procedure described by Speijer and De Waele (1997) for nematode necrosis assessment. However, instead of digging a 20 x 20 x 20 cm (8,000 cm^3) hole, we opted for a smaller 10 x 10 x 10 cm (1,000 cm^3) hole in order to minimise possible effects on corm decay. The holes were dug, using a machete, at 20 cm from the mat and all banana cord roots in the hole were collected. All functional/alive roots were counted. Cord root assessment across all treatments began five months after the initiation of the trials (when re-sprouts had stopped emerging) and was carried out monthly from September, 2013 till December,

2013. In addition, further monthly cord root assessments were carried out from May till August 2014. During the initial cord root assessment phase (first four months), all the 15 mats (total of 180 mats) were assessed per treatment while in the subsequent phase, only 6 mats were assessed per treatment (totaling 72 mats). The reduction in mats sampled for cord roots was carried out in order to minimise its potential effect on the rate of corm decay within the mats. Labour costs were computed for the different corm treatments, while 10 mats were completely uprooted in order to calculate the labour costs for manual complete mat uprooting. Corm decay was assessed monthly by slightly cutting the upper (visible) corm tissue using a machete. Only the bush bean 'CODMLB-001' variety was planted as a break crop during the second trial as no significant effects of break crop type were observed in the first trial. A cost-benefit analysis was conducted to compare these different treatments and the complete mat removal approach over four seasons of bean cropping.

All data were collated using Excel (Microsoft) and analysis of variance (ANOVA), multivariate analysis of variance (MANOVA) for repeated measurements and means separation with least significant difference at 5% were obtained using the GenStat V. 12 statistical software (VSN International Ltd, 2009). The effect of mat size on the rate of corm decay and total number of resprouts was determined through a simple linear regression of mat size to the percentage of decayed corms at 20 months and the total number of resprouts using the Excel (Microsoft) data anlysis package. Simple linear regression of mat size to corm decay was assessed at 20 months because all corms in the herbicide treatments had already decayed by the 20th month.

RESULTS AND DISCUSSION

Banana corm lateral shoot production and decay under bean and sweet potato break crops

In the first experiment, it was observed that corms continued to produce plantlets until the 8th month (Table 2) from the time the experiments were established. Most of the re-sprouts (varying between 15 and 30% per month) emerged between the first and the fourth months across the break crop treatments. Type of break crop did not significantly (P>0.05) affect re-sprouting (Table 2).

No corm decayed under both bean and sweet potato crop during the first 12 months of the experiment (Table 3). The first decayed mats were noted in the 13th month of the experiment, with most of the corms fully decaying between the 20th and 23rd months of the experiment (Table 3). It took more than 2 years (25 months) for the corms to completely decay. Mat decay was, independent of the presence of the break crops planted in the fields. No significant differences (P >0.05) were observed in corm decay between corms in bean plots and sweet potato plots (Table 3).

In corms used for macropagation, and at the site of this study, shoot emergence was reported to stop between the 7th and 9th months after corm planting in the susbtrate (Ntamwira et al. un-published data), which is in line with the observations made in this study. This could be attributed to nutrient exhaustion in the corms as a result of the continuous removal/cutting of shoots/leaves that are responsible for generating photosynthates under macro-propagation or cutting as in this study. The rate of corm decay has also been reported to be highly correlated with the number of plantlets harvested in macro-propagation experiments (Ntamwira et al., unpublished data). This can be attributed to the fact that the new shoots rely on the stored food reserves in the corm tissues, that over time get exhausted by the continuously removed plantlets. In the absence of fresh assimilates, a rapid degradation of tissues in the corm is expected.

The fact that shoots were produced till the 8th month means that by only repeatedly cutting banana pseudostems at soil level, a common practice observed within communities affected by Xanthomonas wilt, the bacteria Xcm can potentially survive within the corms/farms for at least up to 8 months. However, after 8 month with no additional shoot development and further accumulation of assimilates within the corm tissues to support the survival of the corm tissues, a gradual corm tissue decomposition process is expected, a process unsuitable for the bacteria. Xcm has been reported not to compete effectively with other organisms or survive saprophytically in decomposing plant parts. For example, in studies conducted by Mwebaze et al. (2006), Xcm did not survive in banana plant debris left on the ground surface or buried under the ground beyond 35 days.

Description of mat and corm size in the repeat experiment

The mean number of plants per mat, corm diameter and mat corm surface area of small (< 2 m tall) and big plants (> 2 m tall); mean total corm surface area and percentage surface of soil covered/occupied by corms was only determined for the treatments in the second/repeat experiment (Table 1). Significant differences (P <0.05) were observed in the distribution of small (< 2 m tall) and big (> 2 m tall) plants in terms of the mean surface areas between the different treatments (Table 1). For example, treatment T5 had a larger proportion of its surface area contributed by plants > 2 m tall, while T9 had a higher proportion of small plants (mean surface area of corms) when compared with the other treatments. However, no significant differences (P>0.05) were observed between the treatments for the total mean corm surface areas (sum of mean corm surface area of plants < 2 m tall and plants > 2 m tall) (Table 1). A simple linear regression of the mat sizes to the percentage of decayed corms at 20 months and the total numer of resprouts returned an adjusted R^2 value of -0.03 and -0.06, respectively. The F-probability for the regression and P-value for the parameter estimates were also not significant at P<0.05.

Table 2. The total, percentage and average (per corm) number of re-sprouts according to month after trial initiation and break crop. Data was collected after continuous cutting of shoots and apical meristem removal on a total of 112 banana mats per break crop type.

Variables	Break crops	Months after trial initiation								
		1	2	3	4	5	6	7	8	Total
Total number of resprouts	Beans	41	80	70	57	12	3	2	2	267
	Sweet potato	41	75	49	44	21	7	6	5	248
Resprouts (%)	Beans	15.4	30.0	26.2	21.4	4.5	1.1	0.8	0.8	100
	Sweet potato	16.5	30.2	19.8	17.7	8.5	2.8	2.4	2.0	100
Mean number of resprouts/ corm	Beans	0.47[a]*	0.71[a]	0.63[a]	0.51[a]	0.11b	0.03[a]	0.02[a]	0.02[a]	2.38[a]
	Sweet potato	0.37[a]	0.67[a]	0.44[a]	0.39[a]	0.19[a]	0.06[a]	0.05[a]	0.04[a]	2.21[a]
	LSD	0.22	0.34	0.30	0.18	0.06	0.05	0.04	0.06	0.59
	Fpr	1.000	0.780	0.192	0.173	0.010	0.125	0.060	0.357	0.537

*Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test (P<0.05).

Table 3. Monthly decay (%) of banana corms within mats after continuous cutting of the shoots and apical meristem removal in fields planted with beans and sweet potato (as break crops). No mat was completely decayed during the first 12 months of assessment. The total number of mats under each break crop type was 112.

Break crops	Number of months after trial initiation												
	13	14	15	16	17	18	19	20	21	22	23	24	25
Bean	0.9[a]*	2.7[a]	0.9[a]	0.0	0.0[a]	6.3[a]	3.6[a]	16.1[a]	16.1[b]	36.6[a]	11.6b	4.5[a]	0.9[a]
Sweet potato	0.0[a]	0.9[a]	0.0[a]	0.0	1.8[a]	2.7[a]	0.9[a]	8.0[a]	26.8[a]	31.3[a]	22.3[a]	4.5[a]	0.9[a]
LSD	1.97	3.14	1.97	-	2.65	8.2	4.23	13.02	10.19	24.08	9.02	6.27	2.90
Fpr	0.339	0.236	0.339		0.166	0.359	0.191	0.202	0.041	0.634	0.024	1.000	1.000

*Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test (P<0.05).

These findings suggest that the sizes of the mats did not influence the time to corm decay and the total number of resprouts produced per mat across the treatments. The total percentage soil surface area per hectare occupied by mat corms was limited (less than 3.8%, Table 1) and hence did not affect the available space for annual crop cultivation and the overall profits realized from the cultivation of annual crops.

Effect of additional pseudostem cutting/corm treatments in the destruction of complete banana mats

Re-sprouting in the repeat experiment stopped at 8 months after trial initiation (Table 4). A lower number of lateral shoots (130 to 191) were produced by the corms that had been injected with 2,4-D (T1, T2 and T3) while the highest (314) were produded in treatment T7 (all the apical meristems were removed and a cone shaped section was cut out from the center of each corm (machete was used to remove the "cone") and no cover) (Figure 1 and Table 4). Significant (P< 0.05) effects of 2,4-D on resprouts were observed from the second to the fourth month of experimentation when compared with the treatments without herbicide application (Figure 1A). The observed effect of 2,4-D treatments T1, T2 and T3 can be attributed to the enhanced destruction of corm tissues by the herbicide 2,4-D. For example, 2,4-D had been previously recommended for the destruction of banana mats (Blomme et al., 2008). Addition of manure or soil on the cut corm surface had no significant (P> 0.05) advantage as compared to the control without substrate (Figure 1B). Similarly, additional mechanical damaging of the corm through a cone-shaped cut on the surface or a deep incision in the corm as compared to only cutting of resprouts did not significantly affect the number of resprouts (Table 5).

Table 4. Total number of re-sprouts for all assessed mats, percentage of re-sprouts per treatment and the mean number of re-sprouts per mat over time (months) for different treatments. Fifteen mats were assessed per treatment.

Treatment		Months								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
	Resprouts	71	51	42	9	8	6	3	1	191
T1*	%	37.17	26.70	21.99	4.71	4.19	3.14	1.57	0.52	100
	Mean/mat	4.73$^{a\#}$	3.40^{bcd}	2.80^{abc}	0.60^{c}	0.53^{a}	0.40^{ab}	0.20^{bc}	0.07^{a}	12.7
	Resprouts	61	44	13	10	16	3	4	2	153
T2	%	39.87	28.76	8.50	6.54	10.46	1.96	2.61	1.31	100
	Mean/mat	4.07^{a}	2.93^{cd}	0.87^{c}	0.67^{bc}	1.07^{a}	0.20^{ab}	0.27^{bc}	0.13^{a}	10.2
	Resprouts	48	28	21	11	14	3	4	1	130
T3	%	36.92	21.54	16.15	8.46	10.77	2.31	3.08	0.77	100
	Mean/mat	3.20^{a}	1.87^{c}	1.40^{c}	0.73^{bc}	0.93^{a}	0.20^{ab}	0.27^{bc}	0.07^{a}	8.7
	Resprouts	69	79	64	29	18	13	15	3	290
T4	%	23.79	27.24	22.07	10.00	6.21	4.48	5.17	1.03	100
	Mean/mat	4.60^{a}	5.27^{abc}	4.27^{ab}	1.93^{ab}	1.20^{a}	0.87^{a}	1.00^{a}	0.20^{a}	19.3
	Resprouts	55	88	40	8	5	3	2	1	202
T5	%	27.23	43.56	19.80	3.96	2.48	1.49	0.99	0.50	100
	Mean/mat	3.67^{a}	5.87^{ab}	2.67^{abc}	0.53^{c}	0.33^{a}	0.20^{ab}	0.13^{c}	0.07^{a}	13.5
	Resprouts	57	86	63	19	7	2	7	0	241
T6	%	23.65	35.68	26.14	7.88	2.90	0.83	2.90	0.00	100
	Mean/mat	3.80^{a}	5.73^{ab}	4.20^{ab}	1.27^{bc}	0.47^{a}	0.13^{b}	0.47^{abc}	0.00^{a}	16.1
	Resprouts	70	100	77	39	12	8	6	2	314
T7	%	22.29	31.85	24.52	12.42	3.82	2.55	1.91	0.64	100
	Mean/mat	4.67^{a}	6.67^{a}	5.13^{a}	2.60^{a}	0.80^{a}	0.53^{ab}	0.4^{abc}	0.13^{a}	20.9
	Resprouts	71	83	40	16	10	8	5	0	233
T8	%	30.47	35.62	17.17	6.87	4.29	3.43	2.15	0.00	100
	Mean/mat	4.73^{a}	5.53^{abc}	2.67^{abc}	1.07^{bc}	0.67^{a}	0.53^{ab}	0.33^{bc}	0.00^{a}	15.5
	Resprouts	45	72	35	10	4	8	12	2	188
T9	%	23.94	38.30	18.62	5.32	2.13	4.26	6.38	1.06	100
	Mean/mat	3.00^{a}	4.80^{abc}	2.33^{bc}	0.67^{bc}	0.27^{a}	0.53^{ab}	0.80^{ab}	0.13^{a}	12.5
	Resprouts	70	82	49	13	13	4	3	0	234
T10	%	29.91	35.04	20.94	5.56	5.56	1.71	1.28	0.00	100
	Mean/mat	4.67^{a}	5.47^{abc}	3.27^{abc}	0.87^{bc}	0.87^{a}	0.27^{ab}	0.20^{bc}	0.00^{a}	15.6
	Resprouts	70	95	47	18	9	3	3	1	246
T11	%	28.46	38.62	19.11	7.32	3.66	1.22	1.22	0.41	100
	Mean/mat	4.67^{a}	6.33^{a}	3.13^{abc}	1.20^{bc}	0.60^{a}	0.20^{ab}	0.20^{bc}	0.07^{a}	16.4
	Resprouts	58	82	48	29	14	13	9	3	256
T12	%	22.66	32.03	18.75	11.33	5.47	5.08	3.52	1.17	100
	Mean/mat	3.87^{a}	5.47^{abc}	3.20^{abc}	1.93^{ab}	0.93^{a}	0.87^{a}	0.60^{abc}	0.20^{a}	17.1
	LSD	2.00	2.68	2.49	1.30	0.94	0.70	0.66	0.27	
	Fpr	0.666	0.012	0.062	0.032	0.690	0.400	0.222	0.878	

#Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test (P<0.05). *Table 1.

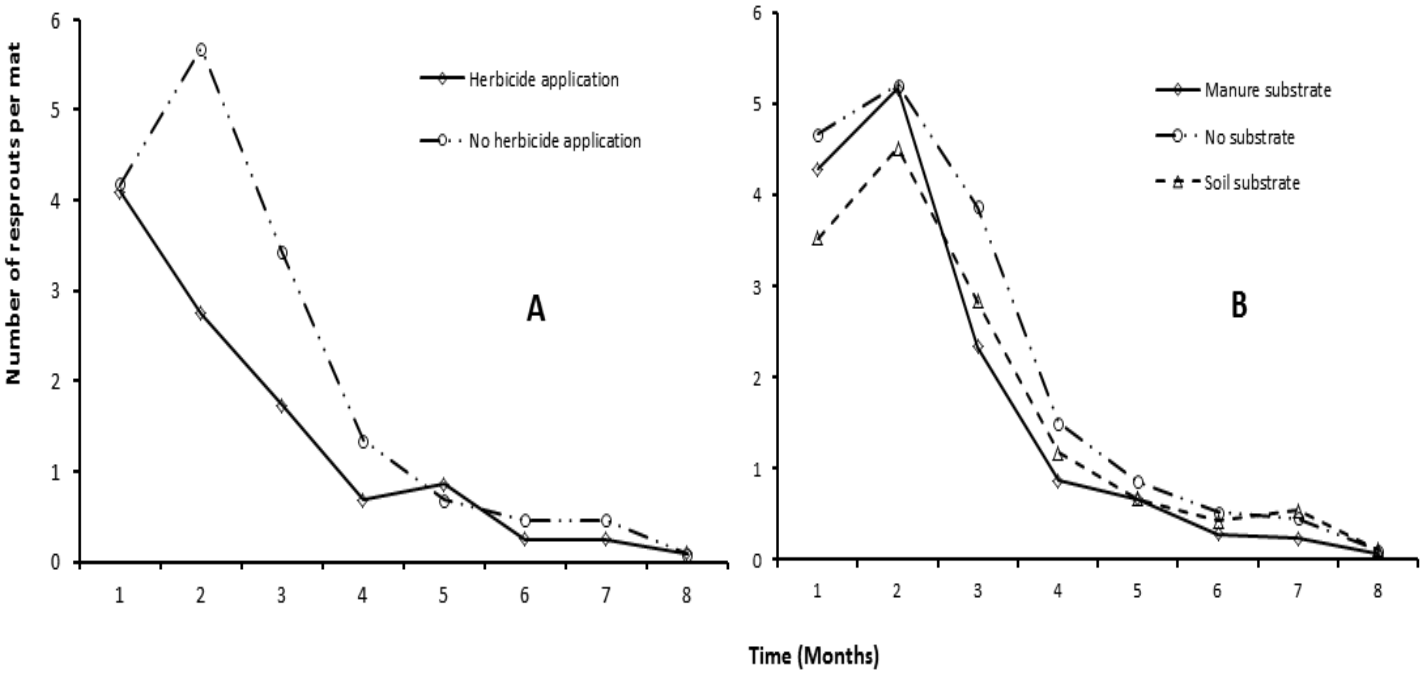

Figure 1. Plots of number of resprouts produced in mats againt time (months): A)- either injected with 2,4-D herbicide and without herbicide injection; and B)- with manure or soil substrate and without substrate on cut surface. No resprouts were observed in all treatments from the 8th month of experimentation.

The results of the effectiveness of the different methods used to destroy banana mats after cutting down the pseudostem at soil level on corm decay are presented in Table 5 and Figure 2. Across all treatments, no single corm completely decayed during the first 7 months after trial initiation. At 8 months, some corms had however completely decayed in five of the 12 treatments, with a significantly higher (47%, $P< 0.001$) decay in the treatment in which 2,4-D was injected into the center of each corm and the cut stem surfaces covered with manure (T2) (Table 5). Significantly higher ($P< 0.001$) mat decay was noted in T2 from the 8th to the 14th week, with 93% cumulative mat decay recorded. Herbicide treatment (T2) was followed in effectiveness by treatments T3 (inject cut stems with 2,4-D and cover with top soil) and T1 (inject cut stems with 2,4-D and no cover), respectively. Generally, the three herbicide treatments significantly ($P< 0.05$) outperformed the other treatments (Figure 2A). For example, at 15 months, about 73 to 93% of the corms under the herbicide treatments had decayed as compared to only 0-27% in the other treatments. Similarly, the herbicide treatments reached 100% corm decay at 20 months, 3 or 4 months earlier than the other treatments (Figure 2A and Table 5).

The deep incision and cone-shaped cut treatments did not significantly vary ($P> 0.05$) from the control in which re-sprouts were only cut (Table 5). Therefore, an additional effort of making an incision or cut on the corm is not worthwhile.

Mats with corm surfaces covered with manure generally decomposed faster than those without any substrate. However, corm decay rates under the treatments covered with manure or soil did not significantly differ ($P> 0.05$) from controls that lacked a substrate cover (Figure 2B). Thus, no added advantage with respect to corm decomposition rate is obtained from applying these substrates on cut corm surfaces. However, the 2,4-D treatments, treatment T2 that had decomposed manure applied to the cut surface, recorded significantly higher ($P< 0.05$) corm decay values relative to the other two treatments T1 (no covering) and T3 (cover with top soil).

All cord roots had already decayed at 17 months after trial initiation (Table 6). Similar to the number of re-sprouts, a lower number and faster cord root decay was observed for the treatments with 2,4-D injection. The highest number of cord roots (156 roots) were observed for the T7 treatment, while the lowest number of cord roots (51) was observed for the T2 treatment.

These results indicated that the injection of the herbicide 2,4-D is effective for destroying banana mats. The use of 2,4-D to destroy banana plants/mats has been reported by Sommer (2000), Okurut et al. (2006) and

Table 5. Percentage mean cumulative values of decayed mats accoding to different treatments (T1-T12) following continuous cutting of shoots and apical meristem removal. No mat was completely decayed during the first 7 months of assessment.

Treatment	Number of months																
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
T1*	$20^{b\#}$	33^{b}	47^{ab}	47^{ab}	60^{a}	60^{b}	67^{ab}	73^{a}	73^{a}	73^{ab}	80^{ab}	80^{ab}	100^{a}				
T2	47^{a}	67^{a}	73^{a}	73^{a}	73^{a}	93^{a}	93^{a}	93^{a}	93^{a}	93^{a}	93^{a}	93^{a}	100^{a}				
T3	13^{bc}	27^{bc}	40^{b}	47^{ab}	53^{a}	53^{b}	60^{b}	80^{a}	87^{a}	87^{a}	93^{a}	93^{a}	100^{a}				
T4	0^{c}	0^{c}	0^{d}	0^{c}	7^{b}	13^{cd}	13^{cd}	13^{b}	13^{b}	20^{c}	20^{d}	40^{bcd}	53^{bc}	60^{a}	80^{a}	93^{a}	100
T5	0^{c}	0^{c}	0^{d}	0^{c}	0^{b}	0^{d}	0^{d}	0^{c}	0^{c}	7^{c}	7^{d}	20^{d}	47^{bc}	60^{a}	80^{a}	87^{a}	100
T6	0^{c}	0^{c}	0^{d}	0^{c}	0^{b}	0^{d}	0^{d}	0^{c}	0^{c}	7^{c}	20^{d}	40^{b}	53^{bc}	67^{a}	87^{a}	100^{a}	
T7	0^{c}	0^{c}	0^{d}	7^{c}	13^{b}	13^{cd}	13^{cd}	13^{bc}	20^{bc}	27^{c}	33^{cd}	33^{cd}	47^{bc}	53^{a}	73^{a}	87^{a}	100
T8	0^{c}	0^{c}	0^{d}	0^{c}	0^{b}	0^{d}	7^{cd}	13^{bc}	13^{bc}	20^{c}	20^{d}	20^{d}	53^{bc}	67^{a}	80^{a}	100^{a}	
T9	0^{c}	0^{c}	0^{d}	0^{c}	7^{b}	7^{cd}	7^{cd}	7^{bc}	7^{bc}	13^{c}	20^{d}	40^{bcd}	60^{abc}	67^{a}	80^{a}	93^{a}	100
T10	0^{c}	0^{c}	0^{d}	13^{c}	13^{b}	13^{cd}	20^{cd}	27^{b}	33^{b}	33^{c}	47^{bcd}	47^{abcd}	67^{ab}	73^{a}	87^{a}	93^{a}	100
T11	20^{bc}	20^{bc}	20^{bcd}	20^{bc}	20^{b}	27^{c}	27^{c}	27^{b}	27^{bc}	40^{bc}	67^{abc}	73^{abc}	80^{ab}	87^{a}	94^{a}	100^{a}	
T12	7^{bc}	7^{bc}	7^{c}	7^{c}	7^{b}	7	7^{cd}	7^{bc}	7^{bc}	7^{c}	13	20^{d}	33^{c}	67^{a}	80^{a}	93^{a}	100
LSD	17	27	29	29	22	24	26	26	30	36	43	46	41	36	28	19	
CV	114	126	111	96	61	58	51	51	59	55	60	55	36	29	19.4	11	
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.012	0.018	0.098	0.283	0.725	

[#]Means in a column followed by the same letter are not significantly different from each other according to Tukey's HSD test (P<0.05). *see Table 1.

Blomme et al. (2008). Blomme et al. (2008) observed that plants injected with 2,4-D started rotting after three weeks (resulting in snapping of treated plants) and by the 10th week, a complete decay of the treated mats/corms was observed. The same authors also reported that none of the treatments with glyphosate (trade name: Roundup) killed off all the emerging daughter suckers. Although, the sucker leaf lamina edges started showing signs of drying during the first 4 weeks after application (WAA), at 6 WAA, new healthy leaves were formed. In contrast, a 2 ml 2,4-D application killed the emerging daughter suckers. However, at 10 WAA, some re-sprouting from the remaining live parts of daughter sucker corms was still observed. These re-sprouting suckers could however easily be removed from the soil with a knife/machete or even by hand, as they were not firmly attached to the decaying corms of the daughter suckers. Apart from 2,4-D, the application of manure, soil substrate and additional damaging of corms offered no added advantage with respect to the rate of resprouting and corm decay, and is thus not recommended.

Correlation analysis

A significant positive correlation was observed between the time to mat decay on one hand and the number of re-sprouts per mat (R^2= 0.718) and the number of functional cord roots per mat (R^2= 0.801) on the other hand. A larger number of resprouts would drain the corms' reserves at a faster rate and lead to earlier corm decay as reported by Ntamwira et al. (unpublished data). A higher number of functional cord roots per mat enhances water and nutrient uptake and could hence lead to more vigorous resprouting. This is in agreement with observations made by Sebuwufu et al. (2004) who reported significant positive correlations between shoot and root traits of mats during both the vegetative and reproductive phase for East African highland banana cultivars (*Musa* AAA-EA genome group).

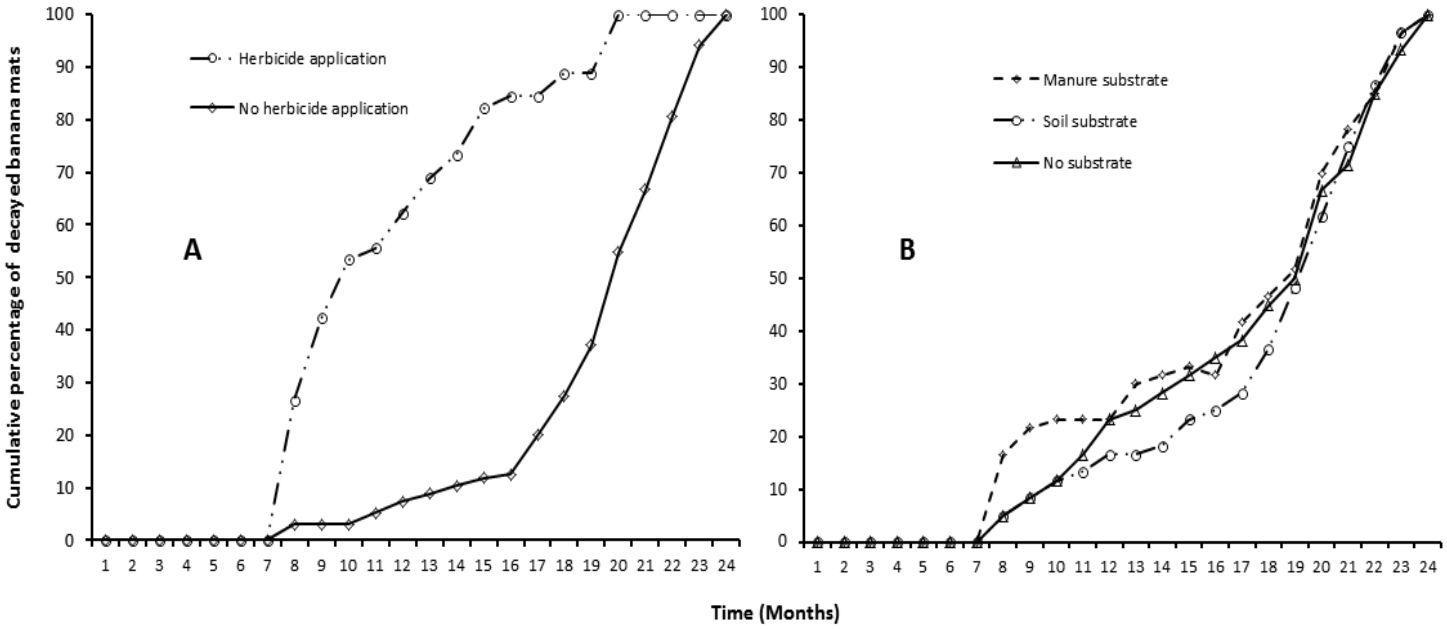

Figure 2. Plots of the cumulative percentage of decayed mats againt time (months): A)- either injected with 2,4-D herbicide and without herbicide injection; and B)- with manure or soil substrate and without substrate on cut surface.

Mean corm diameter was not correlated with other paramaters, while a significant positive relation (R^2= 0.670) was observed between the number of functional cord roots and the number of re-sprouts (Table 7).

Cost analysis (labour) comparing complete mat uprooting with continuous stem cutting

The method chosen for removing diseased mats will depend on the resources available to the farmer and the associated net benefits. Uprooting 10 mats (each mat containing an average of 4 plants) takes 4 h for one person at the rate of 10 US$ in the study region. In contrast, the cutting down at soil level of 60 mats (240 plants) takes about 3 h, at a cost of 3.3 US$. The labour cost analysis of the different treatments/methods used in this study showed that the use of these methods can be cost-effective and far less laborious as compared to the physical uprooting of complete mats, and a small well trained team can eradicate a large area in a relatively short time (Tables 8 and 9). The potential net benefit over 4 seasons of bean cultivation (US$/1 ha) that could be obtained after complete corm removal/uprooting was very small (only 226 US$) (Table 9). In constrast, the net benefit for 4 seasons (US$/1 ha) for the T1 to T12 treatments ranged from US$2164 to 2308. This however does not take into perspective the advantages offered by cutting plants to the maintenance or even improvement of the soil structure/fertility conditions, though this may hardly be recognized or considered by farmers.

Conclusion

The results of this study showed that complete corm rotting took more than 2 years, irrespective of break crop type, following the cutting of plants. However, shoot production stopped at 8 months after trial initiation across the different treatments. The cost benefit analysis of this method in comparison with complete mat removal showed that it has 5 times higher net profits. The approach is also relatively easy to apply and corms were also relatively easy to uproot as of 7-9 months after trial initiation due to the absence of new re-sprouts and a limited number of remaining functional cord roots with corresponding poor anchoring of the corms in the soil. This approach is also environmentally sound in the sense that it prevents the drastic destruction of the soil surface associated with digging of corms and the *in situ* decomposition of roots and corms maintains/enhances soil fertility. Trade-offs between immediate clearing through complete mat rouging for immediate establishment of other crops and the soil fertility and labour benefits associated with continuous cutting, however, will need intensive communication to farmers for a wider

Table 6. Total number of cord roots for all assessed mats and the mean number of functional cord roots per mat, per month and per treatment. Fifteen mats were assessed per treatment for month 6 to 9 and 6 mats for months 14 to 16.

Treatment		Number of months							
		6	7	8	9	14	15	16	Total
T1*	TN#	27.00	21.00	28.00	10.00	6.00	2.00	1.00	95.00
	MN	1.80^{cd}	1.40^{ab}	1.87^{a}	0.67^{bc}	0.40^{b}	0.13^{ab}	0.07^{a}	
T2	TN	20.00	7.00	19.00	4.00	0.00	1.00	0.00	51.00
	MN	1.33^{d}	0.47^{b}	1.27^{a}	0.27^{c}	0.00^{b}	0.07^{ab}	0.00^{a}	
T3	TN	25.00	19.00	18.00	11.00	0.00	0.00	0.00	73.00
	MN	1.67^{cd}	1.27^{ab}	1.20^{a}	0.73^{bc}	0.00^{b}	0.00^{b}	0.00^{a}	
T4	TN	30.00	28.00	29.00	21.00	2.00	2.00	0.00	112.00
	MN	2.00^{bcd}	1.87^{a}	1.93^{a}	1.40^{ab}	0.13^{b}	0.13^{ab}	0.00^{a}	
T5	TN	57.00	33.00	33.00	21.00	6.00	2.00	2.00	154.00
	MN	3.80^{a}	2.20^{a}	2.2^{a}	1.40^{ab}	0.40^{b}	0.13^{ab}	0.13^{a}	
T6	TN	43.00	25.00	22.00	16.00	1.00	1.00	0.00	108.00
	MN	2.87^{abc}	1.67^{a}	1.47^{a}	1.07^{abc}	0.07^{b}	0.07^{ab}	0.00^{a}	
T7	TN	40.00	30.00	38.00	28.00	14.00	4.00	2.00	156.00
	MN	2.67^{abcd}	2.00^{a}	2.53^{a}	1.87^{a}	0.93^{a}	0.27^{a}	0.13^{a}	
T8	TN	51.00	21.00	22.00	11.00	3.00	1.00	1.00	110.00
	MN	3.40^{ab}	1.40^{ab}	1.47^{a}	0.73^{bc}	0.20^{b}	0.07^{ab}	0.07^{a}	
T9	TN	38.00	27.00	28.00	19.00	1.00	2.00	1.00	116.00
	MN	2.53^{abcd}	1.80^{a}	1.87^{a}	1.27^{ab}	0.07^{b}	0.13^{ab}	0.07^{a}	
T10	TN	47.00	25.00	25.00	19.00	3.00	0.00	1.00	120.00
	MN	3.13^{abc}	1.67^{a}	1.67^{a}	1.27^{ab}	0.20^{b}	0.00^{b}	0.07^{a}	
T11	TN	44.00	21.00	25.00	13.00	1.00	1.00	0.00	105.00
	MN	2.93^{abc}	1.40^{ab}	1.67^{a}	0.87^{bc}	0.07^{b}	0.07^{ab}	0.00^{a}	
T12	TN	45.00	17.00	32.00	15.00	3.00	3.00	2.00	117.00
	MN	3.00^{abc}	1.13^{ab}	2.13^{a}	1.00^{abc}	0.20^{b}	0.02^{b}	0.13^{a}	
	LSD	1.49	1.00	1.29	0.94	0.44	0.24	0.17	
	Fpr	0.034	0.090	0.697	0.101	0.002	0.597	0.567	

*See Table 1. #: TN and MN respectively, denote total number of cord roots for all mats and the mean number of functional cord roots per mat.

Table 7. Correlations between the various assessed traits.

	Time to mat decay	N^{o} of mature plants/mat	Mean corm diameter	N^{o} of suckers/ mat	Total N^{o} of re-sprouts/ mat	N^{o} of functional cord roots/ mat
Time to mat decay	1					
N^{o} of mature plants/mat	0.318	1				
Mean corm diameter	0.528	-0.109	1			
N^{o} of suckers/ mat	0.127	0.471	-0.474	1		
Total N^{o} of re-sprouts/ mat	0.718**	0.220	0.087	0.304	1	
N^{o} of functional cord roots/ mat	0.801**	0.188	0.195	0.377	0.670*	1

Table 8. The cost of various inputs used in the different corm treatments (T1-T12) and for completely uprooting approximately 2500 banana mats on 1 ha of land.

Treatment	Input requirements	Quantity	Unit cost (US $)	Cost (US $)
T1-12*	Machete	1.00	3.00	3.00
T1-T3	10 mm wide metal rod with sharp tip	1.00	15.00	15.00
Complete mat uprooting	Hoe	1.00	3.00	3.00
T10-T12	Auger with spear-like pointed tip	1.00	20.00	20.00
T1-12	Labour for cutting banana plants	2500 mats	0.06	137.50
T1-T5	Labour for removing meristems	2500 mats	0.02	50.00
T1-T3	Herbicide	4 L	15.00	60.00
T1-T3	Labour for herbicide injection	2500 mats	0.01	20.00
T2,T5,T8, T11	Manure	200 kg	0.25	50.00
T2,T5,T8, T11	Labour (covering cut surface of the corm with manure)	2500 mats	0.01	25.00
T3,T6,T9, T12	Labour (covering cut surface of the corm with top soil)	2500 mats	0.01	20.00
T7-T9	Labour (cutting out a cone-shaped section in the middle of the corm)	2500 mats	0.03	80.00
T10-T12	Labour for making a deep incision in the middle of the corm	2500 mats	0.04	100.00
Complete mat uprooting	Labour for uprooting mats	2500 mats	1.00	2500.00

*See Table 1.

Table 9. Input cost for applying a treatment to 2,500 banana mats (on 1 ha), labour costs related to bean cultivation (on 1 ha), income obtained from bush bean grain yield and net benefit for each of the 12 corm treatments and when uprooting complete mats. Data are presented for four bean planting seasons. The cost of legume seed is not included as farmers set aside the required amount of seed from the previous harvest for use during the subsequent planting season.

Treatment	1st season						2nd season	3rd season	4th season	Total cost for 4 seasons (US$/ ha)	Total income for 4 seasons (US$/ ha)	Net benefit for 4 seasons (US$/ ha)
	Input costs (US$) for banana treatment application	Cost for cutting banana re-sprouts during 4 months (US$/ ha)	Labour costs for bean cultivation (land preparation, weeding, planting and harvesting) (US$/ ha)	Total labour cost for banana cutting and bean cultivation (US$/ ha)	Bean grain yield income (US$/ ha)	Net benefit (US$/ ha)	Bean grain yield income (US$/ ha)	Bean grain yield income (US$/ ha)	Bean grain yield income (US$/ ha)			
T1*	286	24	483	793	901#	108	1158	1194	1321&	2266	4573	2308
T2	361	24	483	892	904	13	1162	1198	1321	2365	4585	2221
T3	306	24	483	837	902	65	1159	1195	1321	2310	4576	2267
T4	265	24	483	796	902	106	1160	1196	1289	2269	4546	2277
T5	266	24	483	797	890	93	1144	1179	1271	2270	4484	2214
T6	294	24	483	825	897	73	1153	1189	1281	2298	4521	2223
T7	221	24	483	752	898	146	1154	1190	1282	2225	4523	2298
T8	296	24	483	827	902	75	1159	1195	1288	2300	4544	2245
T9	241	24	483	772	901	130	1159	1195	1287	2245	4542	2298
T10	260	24	483	791	903	112	1160	1197	1289	2264	4549	2285

Table 9. Contd.

T11	335	24	483	866	894	28	1149	1184	1276	2339	4503	2164
T12	281	24	483	812	902	91	1160	1196	1288	2285	4545	2261
Complete mat uprooting	2503	0	483	2986	925	-2061	1189	1226	1321	4435	4661	226

*See Table 1. #The slight reduction in bean grain yield income during all four seasons (for T1 to T12) was due to a reduced soil surface area linked to the presence of decaying corms. However, during season 4 (&) there were no remaining corms in plots under treatments T1, T2 and T3 (these treatments included herbicide injection). Input costs (US$) for banana treatment application were not included in seasons 2, 3 and 4. Cost for cutting banana re-sprouts was not included in seasons 3 and 4 because no re-sprouts were observed at 8 months after trial initiation.

adoption. It is however not clear if the long survival period of banana corms can support the survival of pathogens (e.g., Xcm or the banana bunchy top virus [BBTV]) previously present in these corms. Disease free fields were used in this study and this was as such not investigated. However, studies have shown that Xcm does not favourably compete under conditions of decomposition as is the case with these corms especially after 8 months when the corm tissues/plant no longer receive assimilates. It is however, recommended to determine the ability of Xcm to survive in such decomposing corm tissues.

Conflict of Interests

The authors have not declared any conflict of interests.

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The effects of crop rotation systems on maize agronomic traits under no-tillage in optimal and dry cropping seasons

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The purpose of this research was to evaluate the crop rotations effects on agronomic traits of maize cultivated in spring-summer in optimal (OCS) and dry cropping seasons (DCS). The experimental design was set up in a randomized complete block and the treatments were arranged in a factorial (8 x 2), consisting of 8 crop rotation systems and two cropping seasons (OCS and DCS), with four replications. The following variables were determined; plant height, height of the first ear insertion, number of kernels per ear, ear diameter and length, number of kernel per ear, 1000-kernel weight and grain yield. In OCS, 1000-kernel weight and grain yield were 20.30 and 60.80% respectively, which means higher than DCS. The crop rotation systems affected the agronomic traits of maize only in OCS. The crop rotation with soybean/niger/maize, soybean/crambe/maize, soybean/rapeseed/maize and soybean/sunflower/maize resulted in higher grain yield. The effects of drought on agronomic traits of maize resulted in higher impact than the crop rotations systems assessed in this research. The amount of 752 mm of rainfall in maize cropping season was not enough for maize development and yield. This study guided alternatives of new cover crops to insert in crop rotation system.

Key words: *Glycine max* L., maize kernel yield, cover crops, soil conservation.

INTRODUCTION

The maize crop shows great economic and social importance, due to its multiple utilization as, animal feeding like grain or silage, until its use as income in high technology industry. In Brazil, the area cultivated with

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Abbreviations: NTS, No-tillage system; **OCS,** optimal cropping season; **DCS,** dry cropping season.

Table 1. Soil chemical properties from samples collected in 0-20 and 20-40 cm depth in the experimental site.

Chemical properties	Depth (cm)	
	0-20	20-40
pH ($CaCl_2$)	5.2	4.9
CEC	11.4	5.8
P (mg dm^{-3})	12.4	1.9
Al^{3+} ($mmol_c$ dm^{-3})	0.9	4.1
K^+ ($mmol_c$ dm^{-3})	2.5	0.7
Ca^{2+} ($mmol_c$ dm^{-3})	5.5	2.4
Mg^{2+} ($mmol_c$ dm^{-3})	1.6	0.9
H+Al ($mmol_c$ dm_{-3})	1.7	1.9
BS (%)	9.6	3.9

CEC: Cation exchange capacity; total acidity pH 7.0 (H^+ +Al^{3+}); exchangeable (KCl 1 mol L^{-1}) Ca^{2+}, Mg^{2+} and Al^{3+}; BS: Base Saturation = (∑cations/CEC)x100.

maize is 15,596.6 millions hectares, and the grain yield average is 5,200 kg ha^{-1} (Conab, 2015). Nevertheless, the potential maize grain yield in Brazil may achieve values above 10,000 kg ha^{-1} (Sanghoi et al., 2001; Souza et al., 2003). However, the average grain yield in Brazil is under the maize potential that may be associated with low technology applied by farmers, which in many cases do not use conservation cropping systems. The use of cover crops to increase aboveground dry matter is an important step to implement a conservation system as no-tillage associate with crop rotation (Congreves et al., 2015). Nevertheless, in the Cerrado region, there are little options of species of cover crops (Freitas et al., 2016), which results in the intensive plantation of soybean in spring-summer and maize in fall-winter season (Rosa et al., 2015; Ensinas et al., 2015). This cultivation is limited in the Cerrado region where the weather conditions are adequate for good plant development, especially in terms of enough rainfall in fall-winter season. When the rainfall is not enough in some Cerrado regions, the area remains the fall-winter season without any crops; in this situation, eventually the sorghum or millet is used as cover crops in preceding months before sowing the spring-summer crop. In the period with absence of cover crops, the soil remains exposed to raindrop impact, which results to damage in physical (Liu et al., 2011), chemical (Ensinas et al., 2016) and biologic soil properties (Balota et al., 2014; Lourente et al., 2016). The implementation of cropping rotation system with maize in spring-summer season is an opportunity to use the oleaginous species in fall-winter season that may result in economic benefits, besides the possibility in improving the yield of succession crop (Freitas et al., 2016). Researches with oleaginous in crop rotation systems in fall-winter season in Brazilian Cerrado are scarce, which is necessary to find more cover crops species to insert in crop rotation with maize. The challenge is to find the adequate proportion and frequency of each species in rotation to maximize the biomass production, carbon sequestration and nutrients recycled in soil. Nevertheless, the climate changes specially the rainfall in some cropping seasons have worried the farmers. With decrease in rainfall amount, the consequence is the seasonality of crop yield because of the drought stress faced in Brazilian Cerrado in some cropping seasons. The deforestation in Cerrado biome occupy more than 65% in Mato Grosso do Sul State (Casella and Filho, 2013), probably, this alteration in forest can modify the rainfall in this region. Spracklen et al. (2012) reported the negative effects of forests replacement to crop or pasture, which can cause decrease in rainfall, due to reduction of evapotranspiration of moisture from soil and vegetation in comparison with forest. The purpose of this research was to measure the cropping systems effects on maize agronomic traits cultivated in spring-summer in optimal (OCS) and dry cropping seasons (DCS).

MATERIALS AND METHODS

Site and soil description

This research was carried out in 2010/2011 and 2011/2012 cropping seasons in a Rhodic Hapludox, clayey texture, clay mineralogy constituted mainly by Al/Fe oxy-hydroxides classified according to Santos et al. (2013). Located in the municipality of Dourados, State of Mato Grosso do Sul, Brazil (approximately 22°13'16" S latitude, 54°48'2" W longitude, average altitude 430 m above sea level). The soil chemical properties analyzed before the establishment of the experiment in October 2009 are in Table 1. The textural analysis showed the following results: 531, 249 and 220 g kg^{-1} of clay, silt and sand respectively, according to Claessen (1997).

Weather condition in the experimental site in optimal and dry cropping seasons

The data of rainfall and temperature in the experimental site are

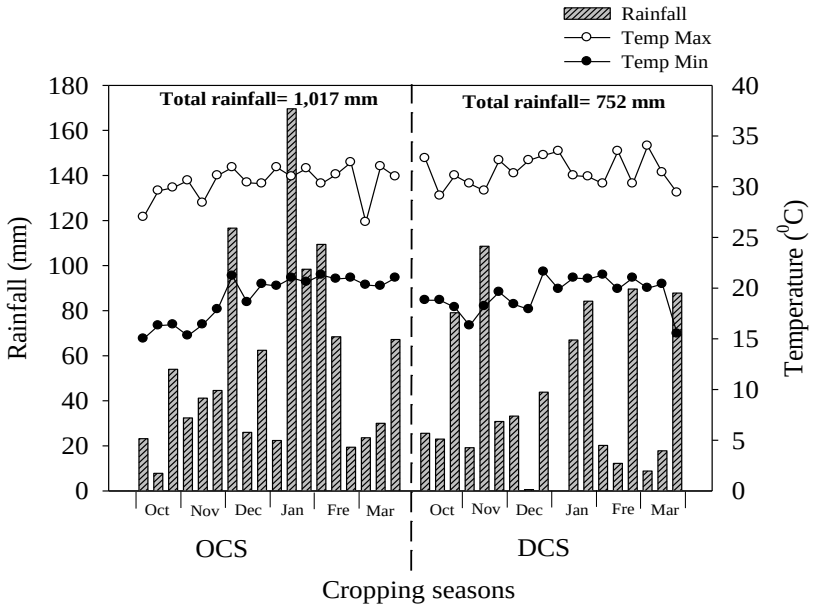

Figure 1. Rainfall, maximum and minimum temperature by each 10 days, in the period of October to March in optimal cropping season (OCS) and dry cropping season (DCS). Data from meteorological station of Universidade Federal da Grande Dourados (UFGD), Dourados city, in Brazil.

shown in Figure 1. The period of these data collection initiated in October, 2010 and ended in March, 2011 for the optimal cropping season (OCS), and initiated in October, 2011 and ended in March, 2012 for the dry cropping season (DCS). The definition of optimal and dry cropping season was based on the results of historic rainfall and the drought occurrence in the region of the study published by Arai et al. (2010). According to Köppen (1948), the region is classified as tropical climate of type Cwa, with rainy summer and dry winter.

Historic of the experimental area

Before the implementation of the experiment, the site was cultivated with soybean in spring-summer and maize crop in fall-winter seasons under no-till. Before the implementation of the experiment, the correction of soil acidity was performed in September, 2009. The recommendation of liming dose was based on the results of soil chemical analysis (Table 1), which was necessary to apply 4,000 kg ha^{-1} of liming. The dolomitic lime showed calcium carbonate equivalent (CCE) of 80% (33% calcium oxide and 15% magnesium oxide). The incorporation of the liming occurred with disk harrow of 32 inches. Right after the lime incorporation, 2,000 kg ha^{-1} gypsum was applied and incorporated with leveling disk harrow of 22 inches.

Experimental design and treatments

The experimental design was set up in a randomized complete block design and the treatments were arranged in a factorial arrangement (8 x 2), consisting of 8 crop rotation systems (Table 2) and two cropping seasons (OCS and DCS), with four replications. The experimental units had dimensions of 15 m length by 35 m width (525 m^2). All operations were executed with a tractor wheel of 112 HP (Horsepower). For the seeding procedure, the grain drill was used with the rows spaced 90 cm apart for planting maize. The cover crops are fall-winter crops species, sowed in the fall-winter season, right after the maize harvest. Despite the treatment with maize/fallow/maize, the other treatments of crop rotation systems were maize in spring-summer and fall-winter crop rotations were compiled by gramineaes and oilseed, S-S-M, S-R-M, S-W-M, S-T-M, S-C-M, S-N-M, S-M-M, M-S-S-W, M-R-S-S, M-W-S-R, M-C-S-T, M-N-S-C, M-N-S-C, and M-W-S-N (Table 2).

Plant material and measurement

The maize (*Zea mays* Hybrid DKB 390 YG) was established in crop rotation to be feasible the implementation of sustainable no-till system. The maize was sowed on October, 20th 2010 and October, 10th 2011 right after the fall-winter cover crops desiccation. The maize sowing was performed 20 days after the cover crops desiccation with glyphosate herbicide (1,296 kg a.i. ha^{-1}). The seed density of maize was 6 seeds per meter, resulting in 66,667 plants per hectare, and the dose of fertilizer was N=16, P_2O_5=60, K_2O=60, Zn=0.9 and B=0.9 kg ha^{-1}. The fertilizer application was in seeding row, with 8 cm depth that was allocated under and beside the seed to avoid contact with the seed. The topdressing nitrogen (60 kg N ha^{-1}) was applied in the development stage of V5 (maize plant with five leaves). In order to increase the N use efficiency, the fertilizer was incorporated deeper in soil with appropriate device. To control the weeds, herbicide nicosulfuron (0.7 L ha^{-1}) and atrazine (3.0 L ha^{-1}) were sprayed in the vegetative growth of maize plant, and the weed stage was in the beginning of vegetative stage. The maize was harvested and sampled on March, 5th 2011 and March, 1st 2012, cropping seasons 2010/2011 and 2011/2012, respectively. The following variables were determined: plant height (PH), the height of the first ear insertion (HFEI), number of kernels per ear (NKE), ear diameter (ED) and length (EL), 1000-kernel weight (1000 KW) and maize grain yield (MGY). The measurement of PH consisted of the distance in centimeters from the soil surface to the

Table 2. The treatments[1] of crop rotation systems evaluated in two cropping seasons.

Crop rotation systems (Abbreviation)	Cropping seasons				
	2009/2010	2010	2010/2011	2011	2011/2012
	Spring-Summer	Fall-winter	Spring-summer	Fall-winter	Spring-summer
M-F-M	**Maize**	**Fallow**	**Maize**	**Fallow**	**Maize**
S-R-M	**Soybean**	**Rapeseed**	**Maize**	Sunflower	Soybean
S-W-M	**Soybean**	**Wheat**	**Maize**	Rapeseed	Soybean
S-T-M	**Soybean**	**Forage turnip**	**Maize**	Wheat	Soybean
S-C-M	**Soybean**	**Crambe**	**Maize**	Wheat	Soybean
S-N-M	**Soybean**	**Níger**	**Maize**	Crambe	Soybean
S-M-M	**Soybean**	**Maize**	**Maize**	Níger	Soybean
S-W-M	Maize	Sunflower	**Soybean**	**Wheat**	**Maize**
S-S-M	Maize	Rapeseed	**Soybean**	**Sunflower**	**Maize**
S-R-M	Maize	Wheat	**Soybean**	**Rapeseed**	**Maize**
S-T-M	Maize	*Carthamus tinctorius* L.	**Soybean**	**Forage turnip**	**Maize**
S-M-M	Maize	Crambe	**Soybean**	**Maize**	**Maize**
S-C-M	Maize	Níger	**Soybean**	**Crambe**	**Maize**
S-N-M	Maize	Wheat	**Soybean**	**Níger**	**Maize**

[1]In bold font indicates the crop rotation systems studied in this research. Maize (*Zea mays*); soybean (*Glycine max*); Rapeseed (*Brassica napus* L.); Sunflower (*Heliathus annuus*); níger (*Guizotia abyssinica*); wheat (*Triticum durum*); crambe (*Crambe abyssinica);* (*Carthamus tinctorius* L.); forage turnip (*Brassica rapa*).

basis of flag leaf at reproductive stage. Similarly, the HFEI scored as the distance from the soil surface to the primary ear node at the same development stage. The measurement of maize grain yield was comprised by the manual harvest in the center of experimental unit in a dimension of 5 by 0.9 m. The grains were weighed and the yield was determined in kg ha^{-1}.

Statistical analysis

The variables evaluated in the experiment were submitted to the analysis of variance (ANOVA) by the *F*-test. The joint analysis was accomplished by OCS and DCS, and in the case of significant effects of the treatments in ANOVA, the mean were compared by the Scott-Knott test of mean at 0.05 levels. The correlation matrix of dependent variable was performed to obtain the degree of relationship between them. To define the strength of correlation, the following criteria were adopted: weak (r^2=0.10 to 0.30), moderate (r^2=0.31 to 0.70) and strong (r^2=0.71 to 100), and positive or negative correlation. These statistical analyses were carried out with the assistance of ASSISTAT software.

RESULTS AND DISCUSSION

Statistical analysis of all variables assessed

The variables were measured in optimal cropping season (OCS) in 2010/2011 and dry cropping season (DCS) in 2011/2012. In order to assess and compare the results in two cropping seasons, the same maize agronomic traits in both cropping seasons (OCS and DCS) was measured, then the crop rotation systems and cropping seasons were studied in a joint analysis. Based on the results in ANOVA, the crop rotation systems did not affect ($p>0.05$) the plant height, height of the first ear insertion, number of kernels per ear and stem diameter. However, the ear diameter, ear length, 1000-kernel weight and grain yield showed significant difference ($p\leq0.01$) among the crop rotation systems evaluated (Table 3). With the exceptions of stem diameter, the other maize agronomic traits showed significant difference ($p\leq0.01$) between the OCS and DCS. The crop rotation systems and cropping seasons showed interactive effects for the most variables evaluated (Table 3).

Crop rotation and cropping seasons changed the agronomic performance of maize

The cultivation of maize after sunflower, rapeseed, crambe and niger, respectively, S-S-M, S-P-M, S-C-M, and S-N-M crop rotation systems showed higher plant height of maize in OCS, on the other hand, the crop rotations evaluated did not show any resulting in plant height in dry cropping season (DCS) (Figure 2A). Despite the cropping rotation systems effects, the OCS showed the highest plant height on average (2.313 m) in all treatments in comparison with the DCS (1.592 m). In both cropping seasons, the plant height showed positive and moderate Person's correlation with stem diameter, 1000-kernel weight and grain yield (Table 4). Nevertheless, in DCS, the plant height showed positive and strong correlation with first ear insertion and moderate with ear diameter and number of kernel per ear. Even with significant correlation between these variables, most correlations showed moderate and positive, which is in

Table 3. Summary of analysis of variance (ANOVA).

Source of variation	*df*	PH	HFPI	NKE	ED	SD	EL	1000KW	MGY
		F-value							
Block	3	2.37	1.28	0.03	0.86	0.72	0.93	3.98	4.52
CR†	7	1.43^{ns}	1.06^{ns}	0.72^{ns}	3.27**	1.29^{ns}	2.46**	2.91**	6.73**
CS††	1	527.08**	13.81**	8.47**	152.36**	1.32^{ns}	2126.64**	302.16**	482.79**
CRxGS	7	3.25**	1.02^{ns}	1.22^{ns}	3.82**	1.25^{ns}	2.54**	3.78**	6.81**

Df: Degree of freedom. ^{ns}non-significant; *significant at 0.05 probability level; **significant at 0.01 probability level by *F*-value; ^{NS}no significant at 0.05 probability level by *F*-value; †CR=crop rotation; ††CS=cropping seasons (OCS and DCS). PH: plant height; HFEI: height of the first ear insertion; NKE: number of kernels per ear; ED: ear diameter; SD: stem diameter; EL: ear length; 1000KW: 1000-kernel weight; MGY: maize grain yield.

accordance with the field conditions, because other variables influence the maize agronomic traits. In order to obtain great development for this hybrid (DKB-390 YG), in a regular cropping season, the plant height of 2.2-2.4 m for DKB is expected, which is indicated by hybrid producing company. However, Mendonça et al. (2014) obtained values higher than 2.4 m for DKB-390 YG hybrid, which observed on average 2.65 m. The height of the first ear insertion (HFEI) did not change with the crop rotations systems (Figure 2B), otherwise the OCS and DCS showed significant difference in this agronomic trait, in the DCS the HFEI decreased 39.10% in relation to OCS. In DCS, the HFEI was positive and strongly correlated (r=0.901) with plant height (Table 4).

For the number of kernel per ear (NKE), no effects were observed in relation to the crop rotation systems, however in relation to the cropping seasons, the OCS was 10.74% higher than DCS for NKE (Figure 2C). In crop rotations with S-N-M, S-C-M, S-S-M and S-P-M observed higher ear length, the crop rotations remaining was equal to the M-F-M. The ear length in OCS and DCS did not differ in the crop rotations M-F-M and S-M-M, these two cropping systems placed negative effects on grain yield due to the positive correlation of ear length with grain yield. The ear diameter showed higher values in S-N-M, S-C-M, S-S-M and S-P-M crop rotations in OCS, but no effect of crop rotation system was observed in DCS (Figure 2D). The ear diameter was sensitive to the drought stress, which showed 17.78% reduction in DCS.

Optimal and dry cropping seasons and crop rotation systems affected 1000-kernel weight and grain yield

In OCS, the crop rotation system S-N-M showed the highest value of 1000-kernel yield, followed by S-C-M, S-S-M and S-P-M, on the other hand, the crop rotations did not affect the 1000-kernel weight in DCS (Figure 3A). On average, in OCS the 1000-kernel weight was 20.30% higher than DCS. The crop rotations in OCS affected the maize grain yield, which showed higher grain yield in S-S-M, S-C-M and S-N-M crop rotations (Figure 3B). In DCS, the crop rotation systems did not influence the grain yield, but the drought stress decreased on average (60.80%), the maize grain yield. As reported by Sabiel et al. (2014), the drought stress in vegetative and reproductive stages of maize may affect drastically the maize grain yield, because of alterations in the period of pre-flowering and post-flowering that imply negatively in the agronomic traits of maize.

The Person's correlation between 1000-kernel weight and grain yield was positive in both cropping seasons (Table 4), which infers that 1000-kernel weight was the most effective variable that influenced maize grain yield. Furthermore, 1000-kernel weight was moderate and positively correlated with maize stem diameter, ear length and ear diameter in OCS (Table 4). This way, the effects of drought and crop rotations on these variables mentioned above may influence indirectly on maize grain yield. In DCS, the occurrence of unfavorable weather conditions, mainly in grain filling stage contributes to decrease the biomass accumulation in grain and consequently reduction on grain yield.

The production potential of maize in DCS was decreased, and no response of the crop rotation systems was observed. Despite the cover crops evaluated, the cropping seasons on average showed 9,486 and 3,718 kg ha^{-1}, OCS and DCS, respectively. As reported by Conab (2011), the average of the maize grain yield in Mato Grosso do Sul State was 6,700 kg ha^{-1} in 2010/2011 cropping season and 6,850 kg ha^{-1} in 2011/2012 cropping season. The most crop rotation systems in OCS was average above the region, with exception of M-F-M (Figure 3B). On the other hand, in DCS, all crop rotation systems showed average yield below the region of the experiment. The drought and high air temperature influenced hardly maize yield in the local of the experiment, with the absence of cover crops effecting maize grain yield. As reported by Bassu et al. (2014), the air temperature may be one of the most limited factors in maize yield; their results showed that increasing 1°C might reduce 500 kg ha^{-1} of maize grain yield. This can imply that decreasing liquid photosynthesis

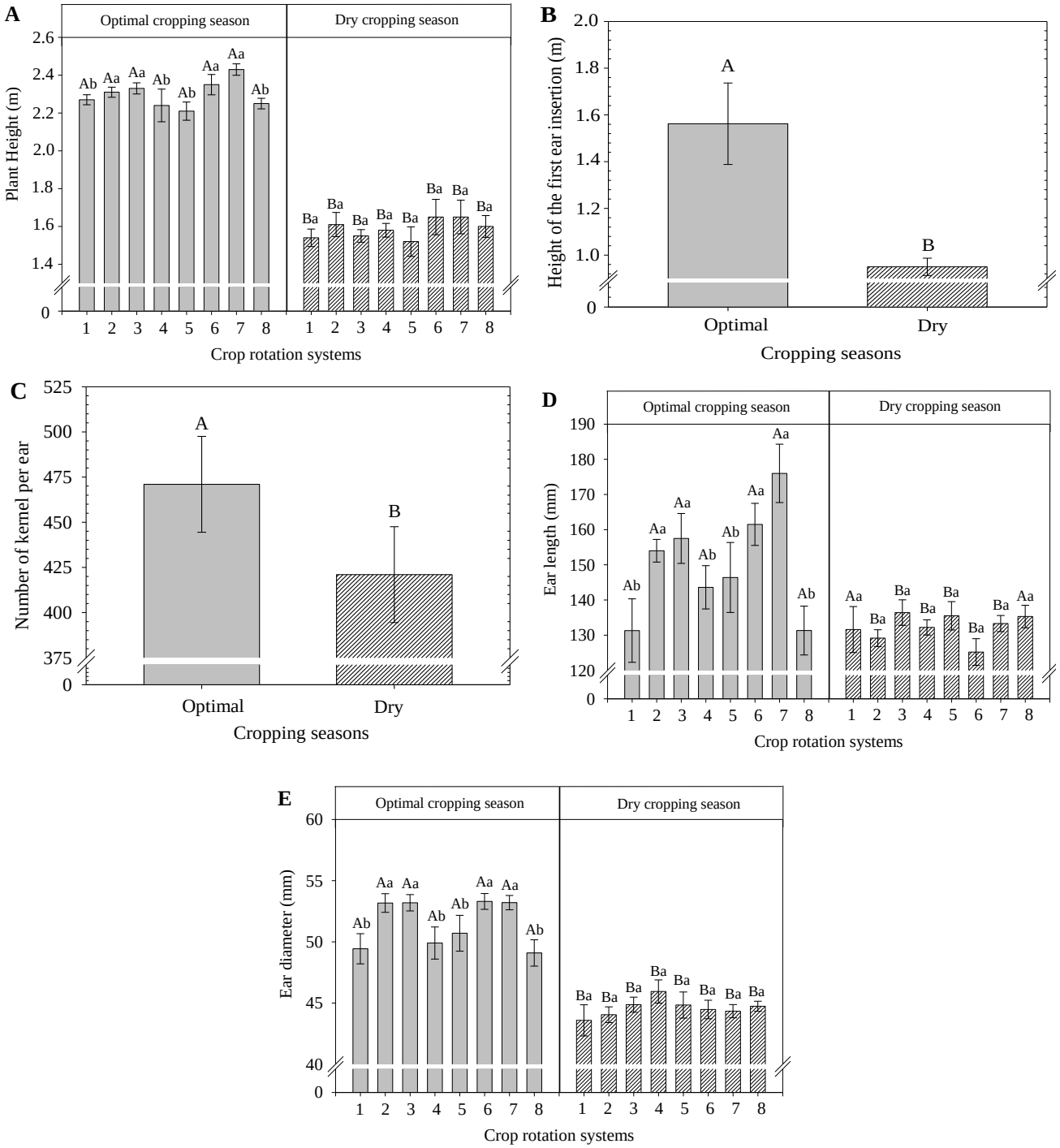

Figure 2. Agronomic traits of maize under optimal and dry cropping seasons and crop rotation systems. (A) Plant height; (B) Height of the first ear insertion; (C) Number of kernel per ear; (D) Ear length; (E) Ear diameter. 1: maize/Fallow/maize; 2_soybean/sunflower/maize; 3: soybean/rapeseed/maize; 4: Soybean/wheat/maize; 5: soybean/forage turnip/maize; 6: soybean/crambe/maize; 7: soybean/niger/maize; 8: soybean/maize/maize. Mean in each bar followed by the same capital letter are not significantly different between the cropping seasons by Scott-Knott test of means ($p<0.05$). Mean in each bar followed by the same lower case are not significantly different among the crop rotation systems by Scott-Knott test of means ($p<0.05$).

in the function of increasing breath rate, affect the biomass accumulation, and the number of eggs per ear. These negative effects of high air temperature ought to decrease 1000-kernel weight and consequently the grain yield, due to the positive and moderate correlation among these variables observed in this research (Table 4). The

Table 4. Correlation matrix of dependent variable.

	MGY	PH	SD	HFEI	EL	ED	NKE	1000-KW
Optimal cropping season								
MGY	1.000	0.405**	0.512**	-0.132^{ns}	0.583**	0.643**	0.486**	0.716**
PH		1.000	0.560**	-0.115^{ns}	0.277*	0.282*	0.284*	0.348*
SD			1.000	-0.074^{ns}	0.333*	0.417*	0.239*	0.586**
HFEI				1.000	-0.344*	-0.295*	-0.352**	-0.062^{ns}
EL					1.000	0.782**	0.848**	0.580**
ED						1.000	0.804**	0.595**
NKE							1.000	0.424*
1000-KW								1.000
Dry cropping season								
	MGY	**PH**	**SD**	**HFEI**	**EL**	**ED**	**NKE**	**1000-KW**
MGY	1.000	0.595**	0.090^{ns}	0.441**	0.448**	0.519**	0.294*	0.630**
PH		1.000	0.310**	0.901**	0.258*	0.346*	0.343*	0.508**
SD			1.000	0.277*	0.492**	0.091^{ns}	0.412**	0.295*
HFEI				1.000	0.195^{ns}	0.252*	0.262*	0.406**
EL					1.000	0.445*	0.570**	0.226*
ED						1.000	0.648**	0.275**
NKE							1.000	0.312*
1000-KW								1.000

PH: plant height; HFEI: height of the first ear insertion; NKE: number of kernels per ear; ED: ear diameter; SD: stem diameter; EL: ear length; 1000KW: 1000-kernel weight; MGY: maize grain yield. Significance effects are at P<0.05 (*), and <0.01 (**).

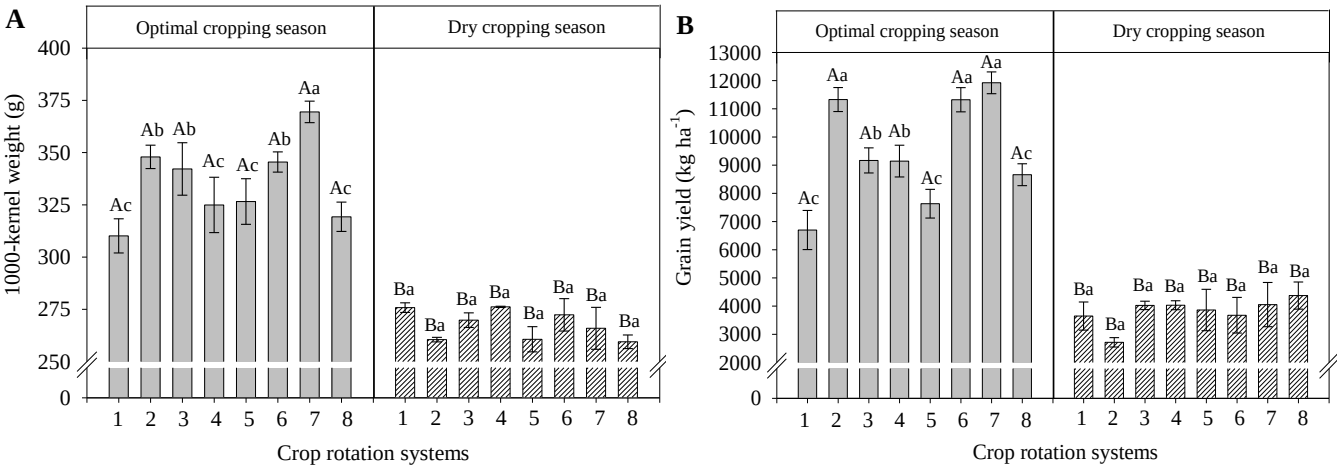

Figure 3. 1000-Kernel weight and kernel yield under optimal and dry cropping seasons and crop rotation system. (A) 1000-kernel weight; (B) maize grain yield. 1: maize/Fallow/maize (M-F-M); 2: soybean/sunflower/maize (S-S-M); 3: soybean/rapeseed/maize; 4: Soybean/wheat/maize; 5: soybean/forage turnip/maize; 6: soybean/crambe/maize; 7: soybean/niger/maize; 8: soybean/maize/maize. Mean in each bar followed by the same capital letter are not significantly different between the cropping seasons by Scott-Knott test of means (p<0.05). Mean in each bar followed by the same lower case are not significantly different among the crop rotation systems by Scott-Knott test of means (p<0.05).

adequate rain distribution on maize cycle is quite important, because reproductive stage is the most required stage for maize. Bergamaschi et al. (2006) observed that maize needs 7 mm day^{-1} in this stage. In OCS, the well-distributed rainfall, contributes to obtain higher grain yield and response of crop rotation systems on the variables evaluated. It is possible to infer that the crops in rotation contribute to increase chemical and

physical soil properties, as the case of the sunflower, crambe and niger as preceding crop in rotation. As reported by Lima et al. (2007), the root development of sunflower may break the layer compacted in deeper soil that increases the root volume and higher water uptake. With time, the root decomposition increases the bio-pores and may contribute to aggregate stability (Vezzani and Mielniczuk, 2011). In preview research developed by Bergamin et al. (2015) in the same experimental area of this research, resulting in decreasing soil bulk density in 0-10 cm in the plots cultivated with niger as preceding crop of soybean or maize. This author observed the benefits of this cover crop in soil physical properties. In crop rotation with niger and maize, Zerihun et al. (2013) observed higher grain yield (8,500 kg ha^{-1}). The effects of niger on maize grain yield may be attributed to better chemical and physical soil properties, as the case of increasing residual phosphorus content and nitrogen (Tolera et al., 2005; Lourente et al., 2007). It is possible to infer that the greater benefits from monoculture to crop rotation with these species evaluated were the improvement of maize grain yield and decreasing risk that monoculture offer.

Conclusions

In optimal cropping season (OCS), the crop rotation systems affected the agronomic traits of maize. This indicate that the crop rotation with soybean/niger/maize (S-N-M), soybean/crambe/maize (S-C-M), soybean/ rapeseed/maize (S-R-M) and soybean/sunflower/maize (S-S-M) resulted in higher kernel yield in comparison with fallow and crop rotations with preceding crops as wheat, maize and forage turnip. The effects of drought in dry cropping season (DCS) on agronomic traits of maize were highly pronounced than the crop rotations systems evaluated. In DCS, the drought reduced the plant height, height of the first ear insertion, ear diameter, 1000-kernel weight and kernel yield. The drought in reproductive stage of maize is a constraint that decreased the kernel yield in 60.80% from optimal cropping season. The amount of 752 mm of rainfall in maize cropping season is not enough for well growth and economic maize grain yield, majorly when the drought occurs in reproductive stage of maize. This research shows options for cover crops system to be viable under no-till system with maize in spring-summer season.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Chemical attributes of the soil in agroforestry systems subjected to organic fertilizations

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The reuse of organic byproducts in agroforestry systems is a sustainable proposal, since, in addition to preserving the natural resources, it has allowed the fertilization of the soils, obtaining a reduction of costs with mineral fertilizers. Therefore, the objective of this research was to evaluate the chemical attributes of the soil after two years of successive fertilizations using cattle manure and sewage sludge in agroforestry systems. The study was conducted in Goiânia, state of Goiás, Brazil. The experimental design used randomized blocks on a 2 x 4 factorial (cultivation systems and fertilizations), with four repetitions. The cultivation systems were: agroforestry and monoculture systems. The fertilizations used were: cattle manure, sewage sludge, mineral fertilizer and control (no fertilization). Regardless of the cultivation system, the fertilizations with sewage sludge increases the calcium, phosphor and zinc contents of the soil, as well as the pH values, sum of bases and cation exchange capacity, at 0-10cm depth. However, the potassium contents are lower in relation to the use of mineral fertilizers, both at 0 to 10 cm depth and at 0 to 20 cm. Teak plants in agroforestry systems presents similar heights to the monoculture plants, and they are higher on fertilizations with sewage sludge. The soybean grain productivity in the agroforestry system presents similar outputs in relation to the use of sewage sludge and mineral fertilizers. Therefore, it is recommended for farmers to adopt agroforestry systems and the organic fertilization practice with sewage sludge, associating the quality of the chemical attributes of the soil, the growth of forest species and soybean grain yields.

Key words: *Tectona grandis*, sewage sludge, cattle manure, intercropping systems, soybeans.

INTRODUCTION

The increase in the productivity of forest cultures by area unit is the current objective in order to meet the worldwide growing demand for wood (Smitha et al., 2016). However, the conventional agricultural systems

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with monocultures must undergo transformations into systems that consider associations among agroecosystems (agroforestry systems), since these are sustainable methods for the production of food and forest products (Bonaudo et al., 2014), intending to soften the emission of greenhouse gases and offer improvements to the chemical attributes of the soil (Buller et al., 2015). However, most forest species cultivated have a high capacity to extract nutrients, causing significant impacts to the chemical attributes of the soils (Pelissari et al., 2012), and this high nutrient consumption may be intensified in agroforestry systems due to the introduction of new production components, such as annual grain production cultures.

In order to reduce the high subjection to mineral fertilizers, the use of organic byproducts may be a sustainable alternative (Smitha et al., 2016). One highlight is cattle manure, since it offers benefits to the chemical attributes of the soil, helping to increase mineralization and the availability of nutrients to the plants (Tejada et al., 2008), mainly due to its capacity to interact with metals and metal oxides and hydroxides, creating organometallic compounds, in addition to potentiate the nitrogen and phosphor stocks (Muraishi et al., 2011)

Another byproduct, from an urban origin, sewage sludge has been deeply investigated regarding the potential improvements of the chemical attributes of the soil (Ricci et al., 2010; Bittencourt et al., 2012; Costa et al., 2014; Cavalcanti et al., 2015). However, concerns have emerged since it leads to a significant concentration of heavy metals on the soil (Nascimento et al., 2014; Albuquerque et al., 2015). Considering these questions, the search for solutions for an adequate destination for this byproduct has been considered vital, and agricultural recycling seems like the most promising alternative from the economic and environmental perspective (Barbosa et al., 2007).

The accelerated growth of the global population and the increase of the livestock farming production in intensive systems are responsible for the generation of large amounts of sewage sludge and cattle manure, respectively in Brazil. Managing these solid residues, specially sewage sludge, is one of the greatest challenges of the Brazilian municipalities, which face problems related to the environmental issue, combined to the financial difficulties of the country (Ricci et al., 2010). However, reusing these residues for agricultural purposes is a sustainable proposal, since it preserves human health and the environment considering that it would otherwise be likely to inadequate disposal (Bonini et al., 2015), while, at the same time, it allows the fertilization of the soils, offering cost reductions on mineral fertilizers.

The application of organic fertilizers in a successive manner during the first years of implementation of agroforestry systems may promote the growth of forest species and increase the grain yield in annual cultures. Therefore, the objective of this research was to evaluate the chemical attributes of the soil after two years of fertilizations with cattle manure and sewage sludge on agroforestry systems.

MATERIALS AND METHODS

Characterization of the experimental area

The study was conducted in Goiânia, state of Goiás, Brazil (16°36' 09.57'' S and 49°16' 52.55'' W). The region has an Aw climate (Megathermal) or tropical savanna climate, with dry winters and rainy summers, according to the Köppen classification. The studied area has an altitude of 730 m, average annual rainfall of 1600 mm, with annual minimal and maximal temperatures of 15.2 and 30.4°C, respectively.

The soil was classified as typical distroferric Red Latosol (Embrapa, 2013). The chemical analysis of the soil before implementing the experiment showed, at a 0 to 20cm depth, the following contents: Ca^{2+}: 1.0 $cmol_c\ dm^{-3}$, Mg^{2+}: 0.3 $cmol_c\ dm^{-3}$, K^{+}: 33$mgdm^{-3}$, P (Mehlich I): 2.1 mg dm^{-3}, Organic Matter: 11 g dm^{-3}, Al^{3+}: 0.1 $cmol_c\ dm^{-3}$, $H+Al^{3+}$: 2.4 $cmol_c\ dm^{-3}$ and pH ($CaCl_2$): 4.9. The textural analysis of the soil showed 430, 110 and 460 g kg^{-1} of clay, silt and sand, respectively (Embrapa, 2009).

The research area showed signs of degradation due to the reduced cultivation of the fodder species (*Urochhoa Decumbens*). In the crop season 2013 to 2014, agroforestry system was adopted and cultivation of teak (*Tectona grandis* Linn. F.) intercropped with millet (*Pennisetum glaucum*) was introduced. While in the forthcoming season (2014 to 2015), teak was intercropped with soybean (*Glycine Max*).

Experimental design

The transplanting of teak seeding was conducted with the help of a furrower to open holes (average depth of 35 to 40 cm), and seeds were manually placed. The teak forest was grown with plant-to-plant space of 2 m and row-to-row distance 6m, containing four rows with sixteen plants each, at a total population of sixty-four plants, occupying a total area of 768 m^2. The experimental design used was the randomized block design (RBD) on a 2 x 4 factorial (cultivation systems and fertilizations), with four repetitions. The cultivation systems followed were either agroforestry or monoculture. The fertilizations composed of cattle manure, sewage sludge, mineral fertilizer and control (no fertilization).

The experimental units were constituted as 6 m wide and 4 m long, at a total area of 24 m^2, and experimental units were constituted by two plants of the forest species on the central position, in addition to the spaces where the sowing of the annual cultures was conducted. For the useful area, 0.5 m on the edges of the borders was disregarded, totaling 15 m^2.

Experimentation in 2013 to 2014

The liming was conducted at the time of teat transplanting using dolomitic limestone (Total Relative Neutralizing Power of 92%) at 20 g per hole while 0.476 Mg ha^{-1} was used between rows to increase the base saturation to 50% (Souza and Lobato, 2004).

The soil fertilization for the holes where teak seedlings were planted for the treatments corresponding to mineral fertilizers was conducted using monoammonium phosphate, offering 15 g of P_2O_5 $plant^{-1}$ and, 30 days after transplanting, the fertilization was

Table 1. Chemical analysis of cattle manure and sewage sludge used for the experiment during the first (2013) and second year (2014), Goiânia, Goiás, Brazil.

Year	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	OM	H_2O
	---------- g kg^{-1}----------						---------- mg kg^{-1}----------					----- % -----	
Cattle manure													
1°	0.64	0.14	3.12	0.34	0.08	0.09	0.26	32	170	4	5	0.8	97
2°	0.54	0.08	2.16	0.42	0.05	0.08	0.25	25	145	2	3	0.6	98
Year	**Sewage sludge**												
1°	30.3	12.4	1.2	121.0	20.1	8.00	0.45	122.0	1850	112	1430	22	64
2°	21.6	7.3	1.6	89.0	19.0	10.0	0.80	151.0	1500	148	3500	19	67

OM: Organic matter; H_20: Umidade.

conducted with 15 g of N (urea) and 10 g of K_2O $plant^{-1}$ (potassium chloride). The millet seed was sown in between the rows (ADE 300). The treatments with mineral fertilizers were applied on the sowing furrow at 100 kg P_2O_5 ha^{-1} as monoammonium phosphate, 60 kg K_2O ha^{-1} as potassium chloride and 20 kg N ha^{-1} as urea. The fertilization was also conducted with 60 kg ha^{-1} of N (urea) 25 days after sowing.

The treatments corresponding to sewage sludge and cattle manure were conducted as broadcast seeding on the total area, and the applications were made both for the teak plants and on the areas between the rows, however, only after the application of these byproducts the millet was sown. A thick paste application was used with 30 Mg ha^{-1} for sewage sludge and a liquid application of 400 m^{-3} ha^{-1} for cattle manure (Table 1).

The teak monoculture treatments remained on a single culture, only on the agroforestry area the millet was cultivated on an intercrop. The objective was to create some foliage for the posterior soy cultivation (2014 to 2015 crop).

Experimentation in 2014 to 2015 season

In November 2014, the teak plants that corresponded to the treatments with mineral fertilizers received applications of 30 g of N (urea), 30 g of P_2O_5 (monoammonium phosphate) and 15 g of K_2O (potassium chloride). Soybeans were sown in between the rows of the agroforestry system (Nidera Y2123) with 0.5 m of spacing between the plants, and population of 17 seeds per meter.

The treatments that corresponded to mineral fertilizers were conducted with 120 kg ha^{-1} of P_2O_5 (monoammonium phosphate) and 60 kg ha^{-1} of K_2O (potassium chloride), in addition to 40 kg ha^{-1} of K_2O (potassium chloride) for covering. The treatments that corresponded to organic fertilizations with cattle manure and sewage sludge were conducted using the same doses and application technique of the previous crop.

Analyzed variables

In February 2015, a soil sampling was conducted with the help of a screw auger, at depths of 0 to 10 and 10 to 20 cm, and six simple samples were collected at each portion in order to constitute a compound sample. The chemical attributes of the soil were evaluated such as phosphorus and potassium (extracted by Mehlich I), calcium and magnesium (extracted by KCl), determined by EDTA titration, potential acidity ($H+Al^{3+}$) and pH ($CaCl_2$), according to Embrapa (2011). The sums of the bases, the cation exchange capacity and bases saturation were calculated. The organic matter contents of the soil were determined through the oxidation method with dichromate and spectrophotometer reading (Embrapa, 2009). The total micronutrient contents and heavy metals were extracted on a nitro-perchloric acid solution (HNO_3 + $HClO_4$) and then they were determined by atomic absorption (Embrapa, 2011).

The soybean crop was grown up to 105 days, and four linear meters of the soy plants were sampled on two central lines of the useful area. Subsequently, the soybean plants were threshed, and the grains were sent to the laboratory for measurement of humidity, reaching values of 13%. Grain moisture was measured by the oven drying method, under atmospheric pressure. Based on sample mass measurement, moisture content was calculated as a function of water mass reduction during drying. The difference between the mass value after removal from the oven and the mass value before sampling, multiplied by 100, yields the percentage of humidity. The beans were weighted on an analytical scale and the average productivity data were transformed into kilograms per hectare (kg ha^{-1}). At that time, the growth of the teak plants was also evaluated by quantifying the height and diameter values at chest height (established at 1.3 m from the soil), through a hypsometer and a dendrometer, respectively.

Univariate and multivariate statistical analyses

The univariate statistical analysis of the data was conducted through the analysis of variance (F Test) and when significant results were obtained, the means were compared through Tukey's test *(p*<0.05 or 0.01), using the statistical program Sisvar (Statistical Analysis System, version 5.6) (Ferreira, 2011). The data were also analyzed through multivariate methods: hierarchical grouping method (HGM) and Principal Component Analysis method (PCA), at the following soil depths: 0 to 10 cm and 10 to 20 cm.

The objective of the hierarchical grouping method was to simultaneously analyze the variables on each use of the soil. Initially, the data had to be standardized in order to obtain a null mean and constant variance (Sneath and Sokal, 1973). Ward was used as an algorithm to obtain the groupings of similar accesses. On this method, the distance between two groups is defined as the sum of squares for all variables (Hair et al., 2005). The results of the analyses were shown in groups on the PCA biplot, which assisted in the identification and interpretation of access groupings.

PCA sized the set of variables according to the characteristics in order to observe the relations among the variables on the coordinate axes. These new orthogonal axes identified as main components and the values of the new score variables of the main components or main coordinates (Piovesan, 2009). The criterion by

Kaiser (1958) was used to select the components, considering the eigenvalues above 1. These conditions generate components with a relevant amount of information on the original variables. Then, the results were shown on Biplot graphs that related the variables to the cultivation systems and fertilizations.

RESULTS AND DISCUSSION

Macronutrients, organic matter and absorptive complex of the soil

The highest phosphorus contents (P) in the soil were observed at the 0 to 10 cm depth on fertilizations with sewage sludge (SS) (p<0.05), with an addition of 82% when compared to the control treatment (no fertilization) (Table 2). P showed the same behavior on the principal component analysis (PCA) with a high correlation with the SS treatment. The result of this behavior on biplot are close points between P and SS on the 0 to 10 cm layer (Figure 1). P and the other nutrients could not be evaluated on PCA and on the hierarchical grouping method (HGM), since they showed low variance.

These P increments on the 0 to 10 cm layer occurred due to the high supply of this element on the soil solution, since, according to the chemical composition of SS, 238 and 147 kg ha^{-1} of P were applied in the 2013 to 2014 and 2014 to 2015 crops, respectively. Increases on the P contents on the soil with the use of SS were also observed on intercropping cultures with eucalyptus and grass species, and the use of 30 Mg ha^{-1} offered 560 kg ha of P in the crops soils (Bonini et al., 2015). This confirms that, if the practice is ongoing, fertilization with SS may lead to the accumulation of labile P on the soil (Ricci et al., 2010; Bittencourt et al., 2012; Costa et al., 2014).

The monoculture system showed a higher P content (Table 2) at the 0 to 10 cm depth (16.2 mg dm^{-3}) in relation to the agroforestry system (11.22 mg dm^{-3}). This result probably occurred due to the fact that the forest systems on intercrops with annual cultures extract part of the available P on the soil solution, and most of it is exported on the grain crops.

The highest potassium contents (K^+) on the soil occurred on the agroforestry system with the use of mineral fertilizations (p<0.05), both at the 0 to 10 cm depth as at 10 to 20 cm (Table 2). On PCA, it was observed that K^+ showed no specific relationship across the treatments, since the vector related to K^+ showed a distance from all treatments on biplot (Figure 1). In addition, K^+ was the only element whose variability remained on CP2 and with a correlation value of 0.84 (Table 4).

These high K^+ contents on the soil on the agroforestry system might have occurred due to the successive potassic fertilization sin the millet (60 kg ha^{-1} of K_2O) and soybean intercropping (100 kg ha^{-1} of K_2O), for the 2013-2014 and 2014-2015 seasons, respectively, which increased exchangeable K^+ content of soil solution. On the other hand, the reduced K^+ contents observed on the monoculture occurred due to the fact that this system was not fertilized in between the rows of forest species, since there was no intercropped specie with annual cultures.

The fertilizations with sewage sludge showed the lowest K^+ content of the soil, with a variation between 37.0 and 43.7 mg dm^{-3} (Table 2). This inverse relationship was also observed on the multivariate analysis with inversed axes on biplot between K^+ and the SS fertilization (G2, Figure 1).

The SS fertilization was statistically similar to the control treatment (no fertilization), this is probably due to the fact that this byproduct composed of less concentration of K, since they offered only 23 and 32 kg ha^{-1} of K_2O on the first and second year, respectively. The main question is that this fertilization source showed no adequate levels of K^+ for an agricultural production demand on tropical soils. However, it is common for researches not to observe increments on the K^+ contents of the soil with SS fertilizations (Barbosa et al., 2007), usually, recommendations for new mineral fertilizations are necessary when only this organic byproduct is used as a potassic source (Ribeirinho et al., 2012).

The highest Ca^{2+} contents on the soil were observed on fertilization with SS, at the 0 to 10 cm depth (p<0.05). However, the Mg^{2+} contents showed no significant interactions (p>0.05) among the cropping systems and the fertilizations (Table 2). The fertilization with SS (G2) showed a high correlation with the Ca^{2+} and Mg^{2+} contents on biplot, as well as the pH values of the soil (Figure 1).

Increments on the Ca^{2+} contents on the soil occurred because SS goes through a treatment with quicklime (CaO) or hydrated lime [$Ca(OH)_2$] during the chemical stabilization processes, and it becomes a byproduct with a high concentration of this element. These results are in agreement with Zuba Junio et al. (2012), who observed increases on the Ca^{2+} contents on the soil at 0 to 10 cm depths, with the use of SS, reaching contents of 5.49 $cmol_c$ dm^{-3}.

The use of cattle manure (CM) showed low Ca^{2+} contents on the soil when compared to SS, yet, it was statistically similar (p>0.05) to the control treatment (no fertilization). This is probably due to the insufficient Ca^{2+} contents observed on CM (0.34 and 0.42 g kg^{-1}, during the first and second year, respectively). However, the lowest Ca^{2+} increments on the soil occurred with the use of mineral fertilizers, and it was even lower than the control treatment. The lack of Ca^{2+} on the mineral fertilizers N-P-K and the export of exchangeable bases on the crop of millet (2013-2014 crop) and soybean grains (2014-2015 crop) promoted reductions on the contents of this element on the soil solution.

The fertilization with SS promoted increments on the pH values and a reduction on the potential acidity ($H+Al^{3+}$) of the soil at the 0 to10 cm depth (p<0.05) (Table

Table 2. Effects of fertilizations on the chemical properties of the soils and theirs macronutrients contents in cultivation systems in 2015.

Tratament	P	K^+	Ca^{2+}	Mg^{2+}	OM	pH	$H+Al^{3+}$	BS	CEC	V
	--- mg kg^{-1} ---		--- $cmol_c$ dm^{-3} ---		%		--------- $cmol_c$ dm^{-3} ---------			%
Layer	**0-10 cm**									
	Agroforestry systems									
C. manure	2.5 ^{bA}	51.0 ^{bA}	2.7 ^{bA}	0.9	1.8	5.3 ^{bB}	1.3 ^{aA}	3.6 ^{bB}	5.0 ^{bB}	73 ^{bB}
S. sludge	11.2 ^{aB}	41.2 ^{cA}	6.2 ^{aA}	0.6	2.2	6.3 ^{aB}	1.1 ^{aA}	6.9 ^{aA}	8.0^{aA}	83 ^{aA}
M. fertilizer	2.2 ^{bA}	65.2 ^{aA}	2.0 ^{bB}	1.0	1.6	4.7 ^{cB}	2.0 ^{bB}	2.8 ^{cB}	4.8 ^{bB}	58 ^{cB}
Control	2.0 ^{bA}	36.5 ^{cB}	3.0 ^{bA}	0.9	1.6	5.1 ^{bB}	1.4 ^{aA}	3.6 ^{bB}	5.1 ^{bB}	71 ^{bA}
Layer	**Monocultures**									
C. manure	3.2 ^{bA}	52.7 ^{aA}	4.2 ^{bA}	1.1	1.8	5.9 ^{bA}	1.1 ^{aA}	5.6 ^{bA}	6.7 ^{bA}	82 ^{aA}
S. sludge	16.2 ^{aA}	43.7 ^{cA}	6.5 ^{aA}	0.6	2.1	6.7 ^{aA}	1.0 ^{aA}	6.9 ^{aA}	7.9 ^{aA}	87 ^{aA}
M. fertilizer	3.7 ^{bA}	51.7 ^{abB}	3.5 ^{bcA}	0.9	1.6	5.6 ^{bcA}	1.2 ^{aA}	4.6 ^{cA}	6.1^{bcA}	75 ^{bA}
Control	2.1 ^{bA}	45.7 ^{bcA}	3.0 ^{cA}	1.0	1.7	5.5 ^{cA}	1.2 ^{aA}	4.2 ^{cA}	5.7 ^{cA}	74 ^{bA}
F	6.8**	19.8**	4.2*	1.7$^{n.s.}$	1.4$^{n.s.}$	3.4*	3.1*	9.4**	8.1**	11.9*
V.C.	22.0	6.2	14.1	14.6	4.7	3.4	17.8	8.7	6.3	3.8
Layer	**10-20 cm**									
	Agroforestry systems									
C. manure	1.7	32.7 ^{bA}	2.2	0.6	1.7	5.2	1.9	2.7	4.3	63
S. sludge	7.5	37.0 ^{bA}	5.1	0.6	1.5	6.5	1.2	5.7	6.9	82
M. fertilizer	1.5	78.5 ^{aA}	1.5	0.5	1.6	5.5	2.0	2.3	4.2	55
Control	2.0	33.2 ^{bA}	0.6	0.6	1.5	5.0	1.5	2.7	4.1	64
Layer	**Monocultures**									
C. manure	1.8	36.0 ^{aA}	2.9	0.7	1.5	1.3	1.3	3.7	5.0	71
S. sludge	7.5	37.0 ^{aA}	4.5	0.6	1.7	1.2	1.2	5.2	6.4	81
M. fertilizer	2.5	43.0 ^{aB}	2.3	0.6	1.6	1.4	1.4	3.1	4.7	65
Control	1.2	31.7 ^{aA}	2.4	0.7	1.5	1.5	1.5	3.2	4.7	65
F	2.6 $^{n.s.}$	3.3*	0.5 $^{n.s.}$	0.3 $^{n.s.}$	0.6 $^{n.s.}$	1.6 $^{n.s.}$	2.1 $^{n.s.}$	0.7 $^{n.s.}$	0.6 $^{n.s.}$	0.8 $^{n.s.}$
V.C.	20.3	34.1	38.1	15.6	14.8	10.5	26.7	29.9	19.1	11.9

Means followed by different lowercase letters on the same row (comparison between fertilization methods within the same crop system) and uppercase letters on the column (comparison between crop systems) differ from each other according to Tukey's test (p<0.01 or 0.05). V.C: Variation coefficient; n.s., *, ** – not significant at 5%; significant at 5% and significant at 1% of probability according to the F test, respectively.

2). These results occurred due to the fact that, after a chemical stabilization, this byproduct is highly alkaline (Costa et al., 2014), yet, due to the fact that it adds high levels of Ca^{2+} to the soil solution, this promotes changes to the acidity properties of the soils. Corroborating with these results, Bittencourt et al. (2012) reported that SS increased the Ca^{2+} contents and the pH values of the soil solution, in addition to a significant reduction of $H+Al^{3+}$. However, some researchers have observed the opposite, with reductions on the pH values and increases on the $H+Al^{3+}$ content, using sewage sludge, however, they used byproducts without chemical stabilization (Cavalcanti et al., 2015).

The use of mineral fertilizers reduced the pH values and increased the $H+Al^{3+}$ values at the 0 to 10 cm depth on the agroforestry system, as verified on PCA (Figure 1), this is probably due to the removal of bases during the crop, as previously observed. In addition to the use of nitrogenous mineral fertilizers for the millet culture on the 2013 to 2014 crop (80 kg ha^{-1} of N as urea) and on the teak plants (45 g of N $plant^{-1}$, in the form of urea in two years), this promotes a reduction of the pH values on the soil solution due to the reactions that release H^+ ions on the transformation process of ammonium into nitrate (Cantarella, 2007), and it may intensify, mainly when the source of N used is urea (Malavolta, 2006; Almeida et al., 2015).

The agroforestry system showed a reduction on the sum of bases (SB) values and the cation exchange capacity (CEC) on fertilizations with CM, mineral fertilizer and control treatment (p<0.05). However, it maintained similar values to the monoculture when the fertilization

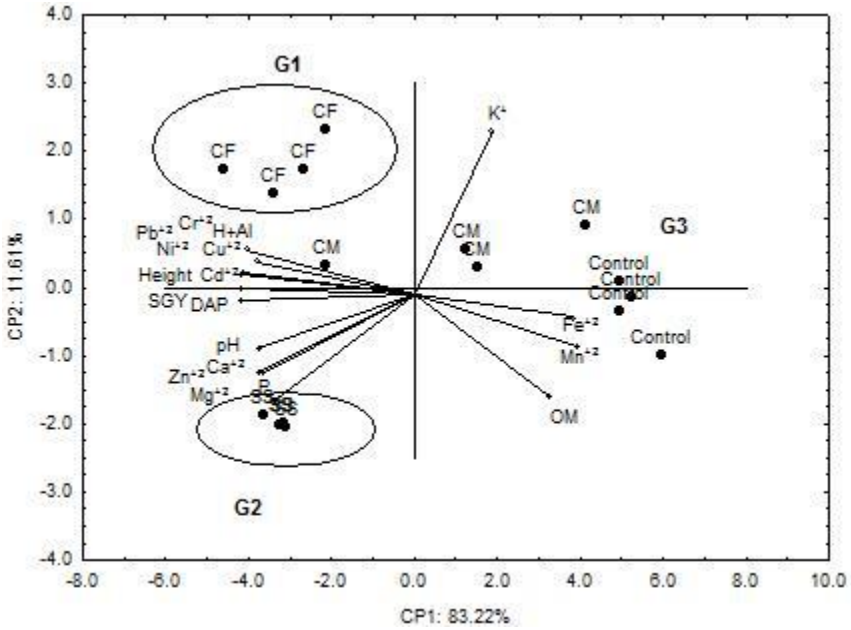

Figure 1. Principal component analysis (PCA) in agroforestry systems and Monocultures subjected to different fertilizations, at the 0-10 cm depth.

occurred with SS, this shows the capacity of this byproduct to maintain high levels of bases on the exchange complex on the soil solution, as also observed by the increments on the saturation of bases (V%). These higher V% values are in accordance with the pH increases of the soil solution with the use of SS, which is explained by the replacement of H^+ and Al^{+3} ions by the exchangeable bases (Ca^{+2}, Mg^{2+} and K^+) on the exchange sites of the soil colloids (Bonini et al., 2015).

The soil organic matter contents (SOM) showed no significant interaction between the fertilizations and the culture systems (p>0.05), both at the 0-10 cm and the 10-20 cm depths (Table 2). The SOM contents were higher for the treatments that used SS, however, the statistical analysis was not able to detect significant changes (p<0.05) for the soil organic matter after two years with organic fertilizations, which, in a way, could explain several changes to the dynamics of the chemical attributes of the soil. These results show that there is not a direct relationship between the source of organic fertilization and the culture system adopted, since both the agroforestry system and the monoculture received the same organic matter loads from CM and SS.

Cation micronutrients and heavy metals in the soil

The zinc contents (Zn^{2+}) showed an increase with high correlation on the SS fertilizations at the 0 to10 cm depth (p<0.05) (Table 3 and Figure 1). The increments on the Zn^{2+} contents on the soil with the use of SS have been observed on several researches on tropical soils (Zuba Junio et al., 2012; Nascimento et al., 2014; Cavalcanti et al., 2015) and, depending on the chemical characteristic of this organic fertilizer, the supply of the micronutrient may reach levels higher than 4 kg ha^{-1} (Albuquerque et al., 2015).

The excessive supply of Zn^{2+} on the soil solution with SS fertilizations did not promote visual toxicity symptoms on the plants, reduced growth on teak plants or grain productivity (Figures 2A, B and 3), since, during the chemical stabilization process of this organic fertilizer large amounts of calcium oxide (CaOH) are added, and when applied on the soil, it promotes an increase of pH, promoting the partial precipitation of Zn^{2+} on the soil solution (Nascimento et al., 2014).

However, when the culture systems are compared, the Zn^{2+} contents were reduced on the CS system, this is probably due to the higher nutritional demand on the previous millet and soybean cultures, on the 2013-2014 and 2014-2015 crops, respectively (Souza and Lobato, 2004).

The copper, iron, manganese, lead, cadmium, chrome and nickel contents did not show significant interactions across the culture systems and fertilizations (p>0.05), both at the 0-10 cm and the 10-20 cm depths (Table 3). In addition, these micronutrients also did not show a significant relationship to none of the evaluated treatments on biplot (Figure 1).

Zn^{2+} contents were reduced on the CS system, this is probably due to the higher nutritional demand on the previous millet and soybean cultures, on the 2013-2014

Table 3. Contents of cation micronutrients and heavy metals in the soil in cultivation systems subjected to different fertilizations, in Goiânia, state of Goiás, Brazil, 2015.

Tratament	Zn^{2+}	Cu^{2+}	Fe^{2+}	Mn^{2+}	Pb^{2+}	Cd^{2+}	Cr^{2+}	Ni^{2+}
	---------- mg dm^{-3} ----------							
Layer	**0-10cm**							
	Agroforestry systems							
C. manure	3.85 ^{bA}	3.87	64.47	33.47	0.012	0.012	0.015	0.010
S. sludge	6.60 ^{aB}	4.45	77.62	33.92	0.017	0.010	0.010	0.012
M. fertilizer	2.72 ^{bA}	4.85	62.62	28.87	0.015	0.012	0.012	0.015
Control	3.17 ^{bA}	3.55	60.72	29.02	0.017	0.017	0.015	0.010
Layer	**Monocultures**							
C. manure	4.17 ^{bA}	4.25	66.20	35.77	0.010	0.012	0.015	0.012
S. sludge	10.8 ^{aA}	4.35	74.55	31.45	0.012	0.012	0.010	0.015
M. fertilizer	3.57 ^{bA}	3.37	66.85	38.00	0.017	0.015	0.012	0.010
Control	3.15 ^{bA}	4.30	63.40	34.05	0.012	0.012	0.015	0.015
F	8.52**	2.86$^{n.s.}$	1.52$^{n.s.}$	2.26$^{n.s.}$	0.97 $^{n.s.}$	1.2 $^{n.s.}$	0.90 $^{n.s.}$	2.10 $^{n.s.}$
V.C.	19.70	19.79	5.38	13.83	35.19	34.78	27.76	33.81
Layer	**10-20cm**							
	Agroforestry systems							
C. manure	2.15	4.07	57.97	31.20	0.012	0.012	0.015	0.012
S. sludge	5.67	4.00	79.55	32.52	0.012	0.012	0.010	0.015
M. fertilizer	2.47	3.87	42.47	22.02	0.015	0.015	0.010	0.015
Control	2.10	3.55	51.05	28.37	0.015	0.012	0.010	0.012
Layer	**Monocultures**							
C. manure	2.17	3.70	54.87	34.80	0.015	0.010	0.015	0.012
S. sludge	5.92	4.02	83.90	37.92	0.015	0.012	0.015	0.015
M. fertilizer	2.45	3.80	58.30	36.10	0.012	0.015	0.010	0.012
Control	1.65	3.85	50.35	32.15	0.010	0.010	0.012	0.010
F	0.89$^{n.s.}$	0.18$^{n.s.}$	1.49$^{n.s.}$	0.62$^{n.s.}$	0.95 $^{n.s.}$	0.20 $^{n.s.}$	0.91 $^{n.s.}$	0.16 $^{n.s.}$
V.C.	14.18	23.56	7.94	28.0	40.29	35.99	29.18	38.55

Means followed by different lowercase letters on the same row (comparison between fertilization methods within the same crop system) and uppercase letters on the column (comparison between crop systems) differ from each other according to Tukey's test (p<0.01 or 0.05). V.C: Variation coefficient; n.s., *, ** – not significant at 5%; significant at 5% and significant at 1% of probability according to the F test, respectively.

and 2014-2015 crops, respectively (Souza and Lobato, 2004).

The copper, iron, manganese, lead, cadmium, chrome and nickel contents did not show significant interactions across the culture systems and fertilizations (p>0.05), both at the 0-10 cm and the 10-20 cm depths (Table 3). In addition, these micronutrients also did not show a significant relationship to none of the evaluated treatments on biplot (Figure 1). Although the copper and zinc contents are above the levels found on agricultural soils of tropical regions, these results were observed even for the control treatment (no fertilization). Basically, the contents of all micronutrients were kept at acceptable levels on the soil solution for agricultural production and environmental preservation, and they are in accordance with the Brazilian specifications by the National Environmental Council (Brasil, 2006).

Phytotechnical variables: Growth and production of plants

The height of the teak plants increased after two years with SS fertilizations (p<0.05), and values that were 15 and 12% higher in relation to the use of CM and mineral fertilizer were observed, respectively (Figure 2). These results probably occurred due to the higher P and Ca^{2+} supply on the soil solution (Table 1).

Table 4. Correlation coefficient of the main components (CP1 and CP2) of the variables.

Variable	CP1 (83.22%)	CP2 (11.61%)
Ca^{2+}	-0.92	-0.35
Mg^{2+}	-0.97	0.19
$H+Al^{3+}$	-0.93	0.31
K^{+}	0.51	0.84
P	-0.84	-0.51
Organic matter	0.72	-0.61
pH	-0.92	-0.20
Zn^{2+}	-0.89	-0.34
Cu^{2+}	-0.89	0.26
Mn^{2+}	0.90	-0.22
Plant height	-0.97	0.19
Diameter of teak plants	-0.99	0.11
Productivity	-0.98	0.03

Phosphorus (P), potassium (K^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), hydrogenionic potential (pH), potential acidity ($H+Al^{3+}$), zinc (Zn^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), lead (Pb^{2+}), cadmium (Cd^{2+}), chrome (Cr^{2+}) and nickel (Ni^{2+}) on the culture systems subjected to different fertilizations, at the 0-10 cm depth.

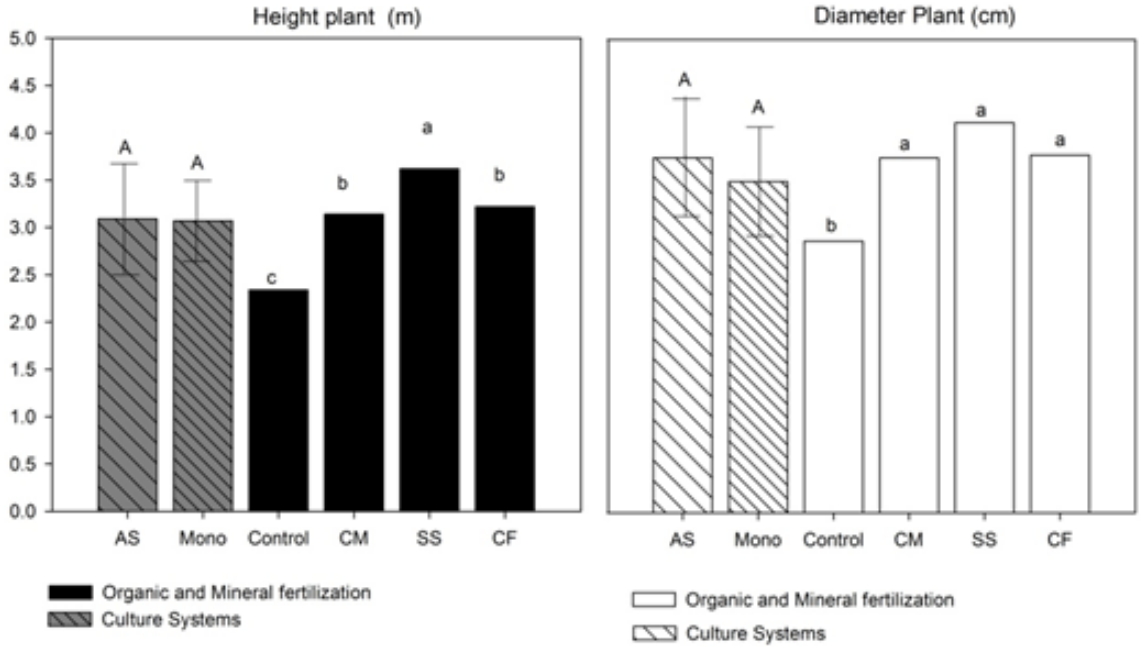

Figure 2. Height (m) and diameter (cm) of teak plants on culture systems with soybean (AS) or monoculture (Mono), subjected to different fertilizations (Control; Cattle manure-CM; Sewage sludge-SS and Mineral fertilizer-CF), *cerrado* region, Goiânia, Goiás, 2015. On the graph: bars identified with different uppercase letters (Plant height: F: 0.03 and Plant diameter: Variation coefficient: 3.23) and lowercase letters (Plant height: F: 56.57 and Plant diameter: Variation coefficient: 14.53) show a significant difference across the treatments, according to the F test (p<0.01).

When absorbed by the radicular system of the plants, P and Ca^{2+} are responsible for the primary growth (Malvolta, 2006). P is extremely important for the initial growth of teak plants, mainly when offered on organic fertilizers (Smitha et al., 2016). In addition, teak is considered as a calcicolous plant, that is, it absorbs high

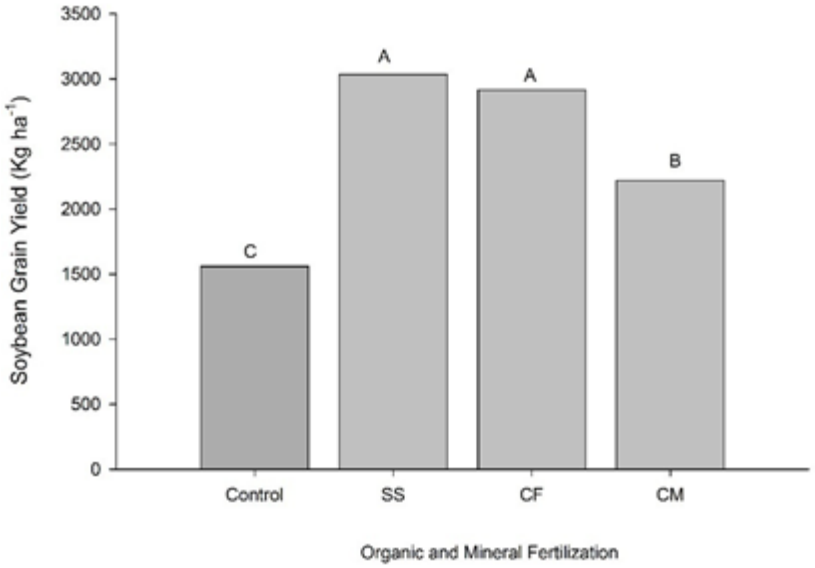

Figure 3. Soybean grain yield (kg ha^{-1}) on a intercropping culture system with and soy (AS) subjected to different fertilizations (Control; Cattle manure-CM; Sewage sludge-SS and Mineral fertilizer-CF), *cerrado* region, Goiânia, Goiás, 2015. On the graph: bars identified with different uppercase letters (F=214.35** Variation coefficient: 3.84)show a significant difference across the treatments, according to the F test (p<0.05).

amounts of Ca^{2+} on the soil solution (Ribeiro et al., 2006). Researchers have reported that the Ca^{2+} supply positively influences the growth parameters of teak (Favare et al., 2012), and this is considered as one of the most limiting elements for the nutrition of this species (Ribeiro et al., 2006).

The diameter of the teak plants showed increments of 23, 30 and 24% with fertilizations with CM, SS and mineral fertilizer, respectively, in relation to the control treatment (p<0.05) (Figure 2). Both the height and diameter of teak plants showed no differences in relation to the culture systems (p>0.05) (Figure 2). This result was also observed on PCA, with the lack of association among the phytotechnical variables (Figure 1). The fact that the agroforestry system showed a growth (height and diameter of the teak plants) of the teak plants that was similar to the monoculture is a positive factor for the sustainable production models. This is because it optimizes the use of areas for a diversified production, both of forest species and bean cultures. In certain agrosystems, intercropping may promote competition and reduce the growth of the tree species and the yield of grain cultures; this fact was not observed on this research.

The quantified soybean grain yield equivalent to single cultures inserted on the agroforestry system showed higher yields with SS and CF fertilizations (p<0.05), showing values of 3.035 and 2.915 kg ha^{-1}, respectively (Figure 3). The productivity results obtained are considered close to the Brazilian means on single production systems with soy beans on the 2014-2015 crop, estimated at 3.016 kg ha^{-1}, according to the National Supply Company (Conab, 2015). The use of SS has also shown an increase on the productivity of sunflower seeds (Albuquerque et al., (2015), in addition to similar productivities when mineral fertilizers are used on cultures for the production of castor beans (Cavalcanti et al., (2015).

Grouping of treatments and variables

After the correlation among all variables and treatments studied on PCA, the same group division on the grouping analysis was observed (results integrated to the biplot). On the biplot, the creation of 3 well defined groups is observed (G1, G2 and G3), as seen on Figure 1. It is noteworthy that, for this response, we used the variables obtained on the 0-10 cm soil layer.

The G1 and G2 groups, represented on SS and mineral fertilizer (CF), respectively, showed well defined, dissimilar and isolated characteristics in relation to each other. However, group G3 showed the control and CM groups with no distinction and high similarity in relation to each other (Figure 1). With these results, we may state that for the chemical attributes of the soil and the phytotechnical characteristics, the use of CM is equivalent to the control treatment (no fertilization) up to the second culture year. This is partially justified by the low content of nutrients on the CM characterization

(Table 1), since this organic byproduct was applied in the liquid state, with high humidity of 97 and 98%, on the first and second year of application, respectively. On PCA, we may observe that he variability of the nutrients and the phytotechnical variables were maintained on CP1 (83.22%), except the K^+ contents. The variance of this nutrient was retained on CP2 (11.61%), according to Table 3.

The Ca^{2+}, Mg^{2+}, P, Cu^{2+} contents and the phytotechnical characteristics related to height, diameter of the teak plants and grain productivity showed values with negative correlations of -0.92, -0.97, -0.84, -0.89 and -0.97, -0.99 and -0.98, respectively. On the other hand, organic matter and Mn^{2+} showed positive correlations of 0.72 and 0.90, respectively (Table 4). The variables that showed negative correlation values showed the same behavior in relation to the axes. The same behavior may be observed with the positive correlation values, however, they remain on the opposite direction of the axis (Figure 1 and Table 4).

Under these conditions, it may be stated that the treatments with SS, CM, mineral fertilizer and control showed different nutrient contents on the soil after two years of culture with different correlations among the nutrients (Figure 1). Considering these results, the advantages of using SS in relation to mineral fertilizers are also noteworthy, and it has a great potential to replace them on agroforestry systems. In addition to preserving the natural resources, since the low levels of heavy metals on the soil are maintained, it would also enable animals to graze on fodder plants, since it is right after the second year that some rural properties change the production systems used, with the option of changing from the agroforestry system (AF) and becoming a crop-farming-forestry integration system (CFFi), however, rather intensified due to the insertion of the new animal component.

Conclusions

Regardless of the culture system, after two years with successive fertilizations, sewage sludge increases the calcium, phosphorus and zinc contents on the soil, as well as the pH, sum of bases and cation exchange capacity values, at the 0 to 10 cm depth. However, the potassium contents are lower in relation to the use of mineral fertilizers, both at the 0 to 10 cm and the 10 to 20 cm depths. Teak plants on agroforestry systems show similar heights to the monoculture plants, and they are higher on fertilizations with sewage sludge. Moreover, the productivity of soy beans on the agroforestry system shows similar yields in relation to the use of sewage sludge and mineral fertilizers. Therefore, farmers are encouraged to adopt agroforestry systems and the practice of organic fertilization with sewage sludge, associating the quality of the chemical attributes of the soil, the growth of forest species and the yield of soy beans.

Conflict of Interests

The authors have not declared any conflict of interests.

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Impact of climate change on the incidences of small ruminant diseases in a pastoral area of Kenya

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Participatory epidemiological methods were used to establish local perceptions and livestock owner's knowledge, attitudes and practices (KAPs) of risk factors that impacts climate variability and the seasonal variations in incidences of livestock diseases, disease vectors, intermediate hosts and rainfall in pastoral Rift valley of Kenya. The interaction of the molecular biology of the pathogen itself; vectors (if any); farming practice, land use; zoological and environmental factors; and the establishment of new microenvironments and microclimates were important in forecasting how contagious caprine pleuronemonia, enterotoxaemia and sheep and goat pox occurred. Thus, the future for traditional pastoralists is depressing if they depend on an environment that may no longer support them. A risk assessment framework was used to examine factors directly affected by climate change or indirectly by human activity, such as land use (e.g. deforestation), transport and movement of animals, intensity of livestock farming and habitat change and their relationship with emergence of unexpected disease events. The present study recommended implementing disease management practices and policy measures to mitigate the impact of climate variability on the spread of livestock diseases.

Key words: Climate change, participatory epidemiology, incidences of small ruminant diseases, pastoral areas.

INTRODUCTION

Climate change, describes a variation in the weather, temperature and environment over a given period of time, be it manifested through changes in mean temperatures or occurrence of extreme weather events (IPCC, 2007). In Africa's pastoral lands, climate variability has manifested through increased temperatures, decreasing rainfall reliability and increased frequency and severity of extreme climate events. Climate change affects the molecular biology of the pathogen itself; vectors (if any); farming practice and land use; zoological and environmental factors; and the establishment of new microenvironments and microclimates, thus influencing the occurrence, distribution and prevalence of livestock diseases under the changing ecological conditions. The implication is that warming means greater than the global annual mean (Alcamo et al., 2007), with drier subtropical

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regions warming than the moister tropics' (Alcamo et al., 2007). Indeed, climate variability by affecting the environmental conditions has the consequence of impacting pasture growth and quality, availability of water resources and thus the distribution of livestock diseases (Gale et al., 2008) and pastoral livelihoods (Ogunsipe and Ayoola, 2012).

Participatory appraisal (PA) methods including interviews, ranking and scoring methods, and visualization methods have been used to analyse the seasonal incidences of livestock diseases, disease vectors and rainfall. They can effectively describe important functional relationships and properties of ecological systems by referring to time, space and resource flow patterns. In addition, they demonstrate visually through the use of decision trees and Venn diagrams, the decision-making processes and power relationships between different stakeholders (Conway, 1985; Conway, 1991). Amongst pastoral communities, it has been applied to establish the seasonal variations in the incidence of livestock disease (Catley et al., 2002; Vallat, 2008; Bett et al., 2009) disease vectors (Catley and Aden, 1996), livestock movements and animal management practices (Elos et al., 1995).

This paper describes the use of participatory epidemiological methods to establish local perceptions of the effect of climate variability on the seasonal variations in incidences of livestock diseases, disease vectors, intermediate hosts and rainfall in pastoral Rift valley of Kenya.

MATERIALS AND METHODS

Location

Kajiado Central District lies between latitude 1050´ 24" South of the Equator and longitude 360 47´23" East. Semi-nomadic pastoralism represents the main spectrum of production systems found in the division classified as a semi arid land (ASAL, zones V and VI) with erratic but bimodal rainfall pattern (average of 300 mm). The long rains fall between March and May while the short rains fall between October and December. Temperatures range between 10 and 34°C increasing East to West. The coolest period is between July and August while the hottest months are from November to April. (Kajiado District Development Plan, 2002-2008). The livestock population in Central Division is estimated at 69,456 cattle, 88859 goats, 83,628 sheep, 7,891 donkeys, 852 camels, 16 pigs and 10,826 poultry (MoLD Central Division Veterinary Annual Report, 2008). Enkaroni Location covers an area of 153.9 km^2. Its characteristics include a population density of 22.7 persons per km^2, average household size of 4.2 persons.

The vegetation types vary from deciduous bush lands to deciduous shrub land consisting mainly of the *Acacia melifera*, *Commiphora*, *Tarconanthus* spp., *Acacia tortilis* and Acacia *xanthopholea* interspaced with star grass and Maasai red oats. In addition, the area has other natural resources such as wildlife.

Data collection

Initial meetings with the Provincial Administration, community based organizations, community leaders large and small scale producers was between August 2008 and April 2009. Qualitative and semi-quantitative data were collected using PA techniques described by Mariner and Paskin (2000). Examples of applications of these methods have been described (Catley and Admassu, 2003; Bedelian et al., 2007). Data was collected from fourteen groups of pastoralists divided into 5 to 8 persons per group. All the scoring exercises utilized 100 beans to represent 100%. Key informants (veterinary personnel, development workers, provincial administration and community opinion leaders and elders) were always interviewed independently either before or after the group sessions.

Focused groups discussions were used to obtain a daily activity calendar, seasonal cropping calendar, matrix scoring, proportional piling and comparison of the control measures of the various livestock diseases. A resource map of the area showing geophysical features, and seasonal migration patterns for livestock, human-wildlife interactions, and livelihood profiles and institutional analysis was developed. Stakeholder analysis was done to establish their presence, interactions, power, influence and control over the flow and access to resources such as pastures, water, charcoal, milk, meat, livestock and firewood both within the community as well as resources at the household level by different gender groups.

Trends lines were developed to elucidate the historical changes in livelihoods, population, food, water, livestock numbers, livestock diseases, rainfall/drought, and pastures over the years. In addition, it indicated important historical events (Table 1). Key-informant including government representative and line ministry personnel, community leaders, development/implementation partners (Red Cross, neighbours Initiative Alliance (NIA)) and other Government officials' from Ministries of Agriculture, Water and Social Services were asked to provide information on livestock diseases, climate (rainfall) data, human population problem, food production and availability addressed in the location, water sources and location, livestock disease control infrastructure and markets that was used to triangulate information from the community. Transect walks were used to elucidate the condition of pastures, vegetation, water sources and availability, climate, land tenure and market support infrastructures. Semi structured interviews (SSI) were used to establish the problem pastoralists faced and their coping mechanisms.

Identification and scoring of livestock species by number and importance to livelihoods

Participants enumerated the list of household livestock species and scored them based upon impact on their livelihood of the number and importance to family's survival, using pair-wise ranking. The exercises were preceded by circles representing the listed livestock species being drawn on the ground. The participants were then given beans and asked to distribute them to the circles (each representing one livestock species) depending on the question asked. A high proportion of beans was always allocated to a species that was either abundant or important in terms of livelihoods. After each exercise, the participants were notified of the outcome and asked if the scores obtained represented their perceptions. They were further asked to give reasons that could support the scores obtained.

Disease ranking

The participants were asked to give a list of diseases acquired by each of the livestock species kept over a 1-year period preceding the time of the interview. The pastoralists often used the local disease names to identify diseases. When the participants provided

Table 1. Trends and changes of events between 1970 and 2007 in Enkaroni Location, Kajiado district.

Variables	1970-1980	1981-1990	1991-2000	2001-2007
Livestock numbers	12	8	6	3
Livestock diseases	1	3	5	11
Annual rainfall received	9	8	4	2
Drought occurrences	2	4	5	10
Pastures	10	7	4	2

syndromes rather than specific names of diseases, probing using open-ended questions were done to characterize the syndrome whilst trying not to guide them. The names of diseases and descriptions given by the pastoralists were later validated at the local veterinary office.

Subsequently, the five diseases perceived to have been most prevalent in the previous year were determined through pair-wise ranking. A total of 100 beans representing the population of each species of livestock were used for scoring. The participants were asked to divide the beans into two, a pile representing the proportion of livestock that became ill during that period and the other representing the proportion that remained healthy over the same period. This gave an overall proportion of goats that became ill over the year (from any of the diseases listed). The participants were then asked to give reasons that could explain the scores given.

The pile representing the proportion of animals that became ill was further sub-divided with three age-categories of small ruminants, that is, Ntare (mature goats/ sheep) and Ilkuo (kids or lambs less than 6 months). The five most-important diseases thus identified by the proportional pile were in a matrix with specific symptoms, season of occurrence and the cost effectiveness disease control measures, ease of application for the treatment method. The final step involved sub-dividing the piles that represented the various diseases into two. These were the proportion representing those animals that recovered and those that died. This enabled a determination of the case fatality rates. The group of ‘‘other diseases’’ was not included in the estimation of case fatality rates.

A seasonal calendar was used to generate associations between the ethological factors and the incidence of disease by mapping the occurrence of rainfall, pastures, livestock diseases, pests, markets and food availability. Additionally, their daily activity log was also drawn showing the daily activities relating to their livelihood.

RESULTS

Impact of climate variability on the occurrence of livestock diseases

A historical profile/trend of the location revealed an increase in the incidence of drought, a corresponding decrease rainfall that became increasingly erratic. The areas experienced devastating droughts accompanied by massive livestock morbidity and mortality in the years 1974, 1981, 1990, 2000, 2004 and most recently in 2008. The droughts cycle appears to be frequent in occurrence (Table 1). Major livestock disease epidemics that occurred during this period include blue tongue in sheep and goats in the year 2000 (Table 2).

Economic impact of small ruminant diseases

The five diseases of sheep and goats in decreasing order of importance were CCPP, footrot, sheep and goat pox, enterotoxemia, orf and helminthosis (Table 3).

Matrix scoring of the severity of their symptoms

The pastoralists ranked the important small ruminant diseases against the severity of their symptoms (Table 4). Tables 5 and 6 show the perceived seasonal occurrence of sheep and goat diseases, respectively. The perceived occurrence of sheep diseases indicate that enterotoxaemia, diarrhoea, sheep pox and FMD occur more in the wet season (Table 6). FMD, goat pox and enterotoxaemia were 100% associated with the wet season while CCPP occurred in both the wet and dry seasons (Table 8).

Estimates of morbidity and mortality

Proportional piling of the five sheep and goats diseases ranked important from disease ranking indicated that CCPP had the highest morbidity of 80% affecting adults (*Ntare*) more at 54% than the young (*ilkuo*) at 26%. Enterotoxaemia had the second highest morbidity at 68% and affected the *Ntare* (44%) more than the *Ilkuo* (24%) (Table 7). Sheep and goat pox was reported with the third highest morbidity at 58%, followed by foot-rot at 33% and Contagious ecthyma (ORF) at 25%. The morbidity for all diseases was higher in the *Ntare* than the *Ilkuo*. Mortality was reported to be highest in CCPP at 49%, followed by Enterotoxaemia at 32%, sheep and goat pox at 23%, Orf at 5% and foot-rot at 4%. The Ntare (adult) age class was affected more than the Ilkuo (young) age class for all the diseases.

Effectiveness of disease control measures

The perceptions by the pastoralists on the effectiveness of control measures of the five most important cattle diseases are given in Table 9. The effectiveness for the control of ECF was considered equally for the three

Table 2. Historical events related by pastoralists in Enkaroni Location, Kajiado district.

Year	Events
1970-1980	1st FMD vaccination Severe Drought, 1974 High livestock deaths *Esilanke* dam constructed 1977-1978 very good weather, ample pastures
1981-1990	Enkaroni Dam constructed Severe drought 1984 Pastoralist migrated to Tanzania Death of livestock *Enkaroni* group ranch started
1991-2000	Severe drought 2000 Migration to Tanzania Migration to Nairobi Outbreak of sheep and goats blue tongue disease
2001-2008	Severe drought 2004 Severe drought 2005/06 Floods, Outbreak of bird flu in world T.D. Jakes drilled a borehole Ol tepesi bore hole drilled Shallow well at Noolera Oloosiyiamalil community borehole drilled Electricity line to Enkaroni 2008/09 drought

Table 3. Pair-wise ranking of diseases of sheep and goats of Enkaroni Location, Kajiado district, 2008.

Pair-wise ranking	FMD	CCPP	BQ	ENT	HEL	POX	ORF	F/ROT	Score	Rank
FMD		CCPP	FMD	ENT	HEL	POX	ORF	ROT	1	7
CCPP			CCPP	CCPP	CCPP	CCPP	CCPP	CCPP	6	1
BQ				ENT	HEL	POX	ORF	ROT	0	8
ENT					ENT	POX	ENT	ROT	4	4
HEL						POX	ORF	ROT	2	6
POX							POX	ROT	5	3
ORF								ROT	3	5
ROT								ROT	6	1

ROT- Foot-rot; FMD- Foot and Mouth disease; POX- Sheep/goat pox; BQ- Black quarter; ENT- Enterotoxaemia; CCPP– Contagious Caprine Pleura Pneumonia; HEL- Helminthiasis; ORF- Contagious ecthyma.

control methods of spraying, fencing and vaccination. However, vaccination was considered slightly expensive than spraying and fencing (Table 9). Individual approach to control of ECF was preferred. Isolation of sick animals and separation of livestock from wildlife were considered the most effective methods for the control of FMD. Treatment for FMD was perceived to be very expensive. The separation of livestock from wildlife required a group approach than an individual approach for effective control of the disease. LSD vaccination of livestock was favoured as a control as compared to spraying, and a group approach was considered the best approach. Methods

Table 4. Matrix scoring for sheep and goat diseases by their indicators of Enkaroni Location, Kajiado district.

Variables	CCPP	SG POX	ENTEROTOXAEMIA	ORF	FOOT ROT
Drought	3(2-5)	3(1-8)	1(0-2)	1(0-2)	0(0-1)
Rainy	6(3-8)	4(3-8)	9(7-10)	6(4-8)	9(8-10)
Coughing	9(7-10)	0(0-4)	-	-	-
Diarrhea	5(3-7)	0(0-1)	6(5-7)	0(0-3)	0(0-1)
Causes death	7(5-10)	3(0-10)	1(0-3)	-	0(0-1)
Lameness	0(0-2)	0(0-3)	-	-	9(6-10)
Mouth swelling	-	3(2-8)	-	6(5-8)	0(0-3)
Skin lesions	0(0-4)	6(4-8)	0(0-2)	5(0-7)	1(0-4)
Tearing	5(0-8)	3(1-6)	1(0-2)	2(1-3)	0(0-1)
Salivation	3(0-4)	2(0-3)	5(3-6)	2(0-4)	0(0-1)
Loss of hair	4(0-8)	6(3-8)	1(0-3)	0(0-2)	0(0-4)

Table 5. Occurrence of sheep diseases and their seasonal distribution in Enkaroni Location, Kajiado district.

Disease	Wet season (%)	Dry season (%)	Both wet and dry season (%)
Enterotoxaemia	76.6	16.9	6.5
Diarrhoea	66.7	33.3	-
Sheep pox	100.0	-	-
Foot and mouth	43.2	21.1	35.8
CCPP	50.0	50.0	-
Trypanosomoses	33.3	33.3	33.3
Anthrax	66.7	33.3	-

– Indicates that no response was given.

Table 6. Goat diseases and their seasonal distribution in Enkaroni Location, Kajiado district.

Diseases	Wet season	Dry season (%)	Both wet and dry season (%)
CCPP	11.8%	38.2	50.0
FMD	100%	-	-
Goat pox	100	.-	-
Enterotoxaemia	100%	-	-
Eye infection	55.6%	22.2	22.2
Anthrax	-	66.7	33.3
Fleas	-	100.0	-

The matrix score indicates the median score with numbers within parenthesis ( ) giving the range.

given for the control of BQ were isolation, treatment and vaccination that were considered to be equally effective. Individual approach to BQ control was the most favoured. Of the three methods given for the control of Anthrax, vaccination and quarantine were perceived to be more effective than treatment of sick animals. Individual treatment was favoured over group management in the control of Anthrax. The pastoralists considered the cost of the control methods to be high in all the methods except for isolation of sick animals and separation from wildlife.

The methods given for the control of CCPP were vaccinations and quarantine whose effectiveness was considered to be equal. Vaccinations, quarantine restriction, spraying, deworming and cleanliness were all considered effective for the control of the various diseases (Table 8). However, the financial implications for the methods were perceived to be high with an exception of quarantine restrictions and hygienic standards in the pens. A rather surprising result was that group approach to the control of most sheep/ goat diseases was favourable than individual approach unlike the case for the control of the cattle diseases (Table 9). Individual approach was preferred for foot-rot and enterotoxaemia.

Table 7. Proportional piling estimates of morbidity (mortality) annual rates (%) of sheep and goat diseases in Enkaroni Location, Kajiado district.

Disease	Annual % morbidity (mortality) by age class		Overall morbidity (mortality)
	<6 months (*Ilkuo*)	adults (*Ntare*)	
Contagious Caprine Pleuropneumonia (CCPP)	26(10)	54(39)	80(49)
Sheep and goat pox	20(7)	38(15)	58(23)
Enterotoxaemia	24(8)	44(24)	68(32)
Contagious ecthyma (ORF)	10(2)	15(3)	25(5)
Foot rot	11(1)	22(3)	33(4)

Table 8. Effectiveness of sheep and goat diseases control measures.

Disease	Control methods	Effectiveness	Financial (cost)	User friendly	Group approach	Individual approach
CCPP (Olkipei)	Vaccinations	8(7-9)	9(6-10)	8(5-10)	8(5-9)	2(1-5)
	Quarantine	7(5-9)	7(5-9)	8(6-10)	7(5-9)	3(1-6)
S/G pox (Erirri)	Spray	6(4-7)	8(5-9)	6(4-7)	7(4-9)	3(2-6)
	Quarantine	7(5-9)	2(1-5)	6(3-9)	8(6-10)	3(1-5)
Enterotoxaemia (Olbus)	Deworming	6(2-8)	7(5-9)	7(5-9)	4(2-5)	7(5-8)
	Vaccinations	7(5-8)	9(7-10)	7(5-9)	7(4-9)	4(2-8)
Foot rot (Elelei)	Clean pens	9(6-10)	3(1-6)	9(7-10)	2(1-3)	9(7-10)

Numbers in brackets are ranges given by 14 groups.

DISCUSSION

This study assessed the perceptions of pastoral communities of the interrelationship between climate change and incidence of livestock diseases. This was done by examining the key climatic factors that affects vectors, pathogen biology, transmission and epidemiology. Bedelian et al. (2007) addressed issues such as the effect of climate change on the vector, host reservoir characteristics and epidemiology of the pathogen to evaluate the risk of emergence and development of infectious diseases in France as a result of global warming.

Pests and parasites are important in either curtailing or proliferating the distribution and spread of diseases, pests and parasites of livestock (Gale et al., 2008). Stem et al. (1989), described the potential of how climate change could lead to changes in spatial and temporal distribution of diseases sensitive to moisture as an etiological factor.

Climate change affects the incidence of livestock diseases transmitted by direct contact due to changes in the frequency and duration of animal contacts. Changes in the degree of mixing of cattle and sheep will affect the prevalence of diseases such as MCF, which is caused by OHV and spread by direct contact. The disease is transmitted through contact between infected and susceptible animals and was attributed to cold weather when animals tend to congregate. The disease reportedly occurred in both the wet and the dry seasons. The probable reason for occurrence in the dry season may be the potential congregation of animals from different herds at watering points. Extreme climatic conditions (e.g. heat stress) induced the cell-free OHV in nasal secretions combined with other farming management responses to climate change (e.g. co-mingling of cattle and sheep in response to flood) that increase direct contact could promote the spread of the disease.

The strategy considered most effective for disease control was vaccination probably because the cost was often borne by NGO's rather than the individual. Livestock movement control and quarantine were not popular methods for disease control as they were considered punitive. However, the pastoralists felt that the methods are effective as they indicated that they normally migrate whenever disease outbreaks occurred. Moreover, animals perceived to be suffering from FMD were always watered after other animals. This indicated

Table 9. Perception of pastoralists on the Effectiveness of Cattle Diseases control measures in Enkaroni Location, Kajiado District, 2008

Disease	Control methods	Effectiveness	Financial Cost	User friendly	Group approach	Individual approach
ECF (oltikana)	Spraying	7(6-9)	7(5-9)	7(6-9)	4(2-5)	6(6-9)
	Fencing	6(4-9)	8(6-9)	6(5-8)	4(1-6)	7(3-9)
	Vaccine	6(3-8)	9(7-10)	7(4-9)	4(1-8)	8(6-9)
FMD (Oloirobi)	vaccine	8(6-9)	7(5-9)	5(3-8)	8(6-9)	3(1-5)
	Isolation	6(3-7)	3(2-6)	5(1-8)	3(1-5)	5(2-7)
	Treatment	3(1-6)	8(5-10)	5(1-9)	3(1-6)	7(5-9)
	Separation from wildlife	6(3-7)	4(1-5)	3(1-5)	8(5-9)	3(1-5)
LSD (Erirri)	Spraying	6(3-7)	7(5-8)	3(1-6)	3(2-5)	4(2-7)
	Vaccine	8(6-9)	7(3-9)	2(1-5)	7(4-9)	3(2-7)
BQ (Empuruo)	vaccination	9(7-10)	7(5-8)	6(5-8)	6(2-8)	4(2-6)
	Isolate	7(5-8)	2(1-4)	7(2-9)	2(1-4)	8(6-10)
	Treatment	8(5-9)	8(6-10)	3(1-5)	2(1-4)	8(6-9)
Anthrax (Entemelua)	Vaccination	9(7-10)	9(8-10)	7(4-9)	2(1-6)	9(7-10)
	Treatment	6(5-7)	9(7-10)	6(4-8)	2(1-4)	8(6-9)
	Quarantine	8(6-9)	4(2-6)	6(5-8)	8(5-10)	6(3-8)

Numbers in brackets are ranges given by 14 groups

that they were aware of the mode of transmission.

Migration was practiced by a large proportion of the surveyed pastoralists in response to drought as well as disease outbreaks. Only a small proportion indicated that they never move and was thus considered sedentary. This latter group had other sources of livelihood that included crop production, quarrying and charcoal burning. A small proportion of pastoralists moved with the whole family as livestock were their only source of livelihood. During migrations, men and young boys moved with the animals while children and women stayed back in the manyattas with recently calved cows and small stock. Similar migration pattern was observed among the pastoral community of Turkana District (Lotira, 2004).

According to the pastoralists of Kajiado District, there have been marked climate changes in the area which have led to changes in their way of live impacting negatively on their livelihoods. There were marked associations between disease occurrence and climate change/variability pertaining to humidity, wind speed and direction as well as temperature. Many infections, especially the arthropod vectors and helminths are known to occur under wet conditions and influenced by climate change. Some diseases were positively associated with certain climate elements (e.g. Helminthiasis with rainfall), while others were negatively associated with other climate elements (e.g. redwater with rainfall). From the results obtained from the study, it can be concluded that; pastoralists of Enkaroni Location recognized livestock diseases as a constraint in their livelihood. Climate change in Kajiado District is positively and significantly related to the occurrence of livestock diseases. This may have led to a significant increase in occurrence of livestock diseases in Kajiado District. Significant variations in certain weather elements may have modified the ecosystems of the diseases causing an increase in pathogens and vectors populations. To respond to this, it is recommended that an early warning system should be developed to predict climate changes in pastoral areas and information network on targeted and strategic disease management interventions that moderate the multiplication of disease-causing pathogens and their vectors as a result of climate variability be developed. In addition, extension education should be used to enlighten the pastoralists on the importance of disease control, stocking density of animals and environmental conservation in order to mitigate against climate variability. Climatic change has adverse impact on pastoral livelihoods. This is through the potentiation of the occurrences of various viral and vector bone diseases by modifying the ecosystems of the diseases causing an increase in pathogens and vectors populations. It is imperative that early warning systems and stronger extension services be used for strategic management of disease control interventions (Speranzaa, 2010).

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

A case study on compensatory growth of emaciated cattle fed on total mixed ration

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A study was conducted in Thailand with the aim of evaluating compensatory growth of emaciated cattle fed on *ad libitum* total mixed rations (TMR) under smallholder feedlot management. Two farms were identified where 121 cattle of various degrees of emaciation were bought for fattening trial. Cattle were grouped according to the degree of emaciation within respective pens and were fed on same type of feed throughout the feeding period. Feed intake was estimated on daily basis and was noted to be low during the start of the feeding trial but gradually increased in an increased rate up to the end of feeding period. The cattle with low weights and body condition scores at the beginning of feeding trial gained more weights than their counterparts which started with high body weights and high body condition scores. There was a significant (P < 0.01) difference between treatments in both weight gains and body condition scores. Final weight gains for bulls in a mixed pen under same feedlot environment were also significantly (P < 0.01) different. A positive linear correlation (r = 0.54) was also observed between weight gains of cattle and feed intake. It was concluded that degree of emaciation, feed intake, breed difference and sex significantly influenced compensatory growth.

Key words: Compensatory growth, *ad libitum,* feedlot, live weight, body condition score.

INTRODUCTION

A number of mechanisms are involved that enable cattle to adapt metabolically to periods of nutrition restriction, and to subsequently exhibit compensatory growth once *ad libitum* access to high energy diets are provided (Carstens et al., 1991). Reduction in energy density for growth and energy requirement for maintenance increases in net efficiency of growth, feed intake and gut fill have been shown to contribute towards compensatory growth in cattle (Abdalla et al., 1988). Knowledge of compensatory growth has assisted some cattle farmers to modify livestock raising systems with the aim of maximizing profits (Tapki, 2012). Beef cattle are generally raised following two systems; the first one is called on-farm production system, where cattle are raised and finished on the same farm (Boxler, 2013). The other system is referred to as speculator system, where cattle are raised and finished on two different farms (Forbes, 2007). In speculator system, a farmer called speculator, goes to different farms and/ or cattle markets to search and buy cattle that are brought to the farm for fattening (Abdalla et al., 1988). The speculators generally buy cattle in poor body condition and put them on good nutritive feeds to finish them within short period of time possible. The speculators take advantage of the concept

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of compensatory growth; a condition of an accelerated growth of cattle following a period of slowed growth particularly as a result of nutrients deprivation.

The speculators buy cattle mainly during dry season when animal feeds (pasture) are in short supply and consequently cattle are in poor body condition (Roche et al., 2004). Compensatory growth is markedly pronounced in semi-arid regions where cattle become bony during dry season when pastures become scarce (Boxler, 2015). In winter, cattle in Thailand are generally in poor body condition and the prices of cattle at cattle markets are generally low but still farmers have to sell their cattle to get enough money to use in their farms. Winters are lean periods to rice farmers in Thailand but this is also a period when money is needed much to use in paddy fields hence selling cattle becomes the only alternative source of cash to farmers with livestock.

Beyond marketing, profitability in cattle feeding business is dependent upon optimizing the utilization of available feed and cattle resources (Abdalla et al., 1988). Since ruminants use microorganisms to derive nutrients from poor quality grass, crop residues are therefore, the best available feed for cattle because they are cheap and are always in plenty during harvest period.

Several studies on compensatory growth have been conducted by many scholars in some universities but such studies were mostly done in animals which were deliberately starved to match with study outcome. The current study realizes that every animal undergoes a period of starvation in its life time so compensatory growth was studied under natural condition. Therefore, the current study was conducted in Thailand on demand by smallholder livestock farmers with the aim of generating and validating locally available information on compensatory growth for domestic use. The results of this study are expected to be utilized by farmers in Phanom Thuan Province in livestock cooperatives for better management decision of their feedlot business.

MATERIALS AND METHODS

This study was conducted in PhanomThuan district, one of the districts of Kanchanaburi Province, in Thailand. The province is regarded as the most significant zone for smallholder beef cattle feedlot business. This Province is located 14°1' North and 99°32' East and is 19,483 Km^2 in area. The province is one of the areas in Thailand where farmers depend on agriculture for living. Farmers grow a lot of baby corns, sugarcane, pineapples and bananas in this area hence beef farmers take advantage of the by-products such as molasses, corn stalks, banana and pineapple peals, from these crops to feed cattle. Farmers who participated in this study used corn stalks and pineapple peals for making silage mainly because these by-products are sold at a relatively cheap price. The study province has agro-industry factories, where farmers buy agro-by-products as raw materials for concentrate feeds.

Study design

Cattle were placed on the two farms after segregating them based on degree of emaciation. Selection of cattle into groups was done in a complete randomized manner since cattle were bought from different farmers and different locations. Body condition score was the main key factor used to determine degree of emaciation when placing the cattle into different groups. All cattle were exposed to same feedlot conditions during the entire period of feeding and data were collected based on the groups or category of cattle within the pens. Data on feed intake were done on daily basis while that on weight gains and body condition scores were done fortnightly.

Feed analysis

Prior to commencement of feeding study, feed samples were collected from the two farms for laboratory analysis in order to know the nutritive values of the feed used mainly energy and protein which are crucial for cattle fattening. The chemical composition of feed was determined by methods described by Association of Official Analytical Chemists (AOAC, 1990) and the fiber fractions of neutral detergent fiber (NDF) and acid detergent fiber (ADF) by method described by Van Soest et al. (1991). The gross energy (GE) was estimated by Ballistic Bomb Calorimeter (Gallen Kamp) as recommended by Harvey (1960).

Animals and animal pens

One hundred twenty one cattle of various ages and various degrees of emaciation were identified from two farms for this study. Most of the cattle were crossbreed of Hindu Brazil and local Thai breeds, which are characterized by big body frame and large ears. The first farm had 19 cattle while the second had 102 cattle. There were three cattle pens in the second farm while the first farm had only one. The cattle pens were open-sided barn constructed from wooden poles and galvanized metal roofs. Feed and water were provided *ad libitum* in well-constructed troughs.

Feed and water Intake

In both farms, cattle were fed twice a day whereby 9 kg per cattle of TMR was given in the morning and 3 kg per cattle of TMR was supplied in the afternoon during the first month. Feed was gradually increased from 10^{th} week, from 12 kg per animal up to 15 kg per animal at the last weeks of the study but no leftovers were collected the following feeding time. The feed was supplied in common feed troughs where all cattle in a kraal came to eat as a group hence feed intake was estimated. Water was also provided in a common water trough and it was replenished once a day during feeding time. It was difficult to estimate water intake since all cattle were drinking from one water trough. Feed was weighed before being supplied to cattle in *ad libitum*. The leftovers were carefully collected and weighed too. Hence, feed intake was roughly estimated per day by dividing the amount of feed taken by the number of cattle in the pen.

Live weight measurement

A single visit per fortnight, multiple-subject survey was carried out using face-to-face farmer interviews during the three months of the study (mid September to beginning of December, 2013). A weigh tape (Giss Marketing Thailand) was used to measure live weights of all cattle at the two farms before and after the period of the study. The exercise was conducted with full participation of the owners of the farms. They were involved in restraining the animals when the weights were being taken. The weights of individual animals were estimated and the data were collected and recorded securely.

Table 1. Composition of experimental diet from Farm 1 and 2.

Parameter	Farm 1		Farm 2	
	Concentrates	Silage	Concentrates	Silage
Moisture (%)	7.7	75.47	8.38	75.47
Crude protein (%)	14.24	8.28	9.32	8.28
Ether extract (%)	4.67	2.84	4.68	2.84
Ash (%)	6.33	6.58	5.70	6.58
NDF (%)		67.72		67.72
ADF (%)		47.75		47.75
Gross energy (kcal)	3910.58	3642.47	3794.85	3602.55

Table 2. Mean (+SD) initial weight, final weight and weight gain (kg) from Farms 1and 2.

ID number of animal	Description	Mean initial weight	Mean final weight	Mean weight gains	P-values
Pen 1 (F1)	Severe emaciation	364.47±22^{a}	580.89±10^{a}	216.42±20^{c}	0.01321
Pen 1 (F2)	Severe emaciation	426.74±21^{b}	634.30±32^{b}	207.57±27^{c}	0.03504
Pen 2 (F2)	Moderate emaciation	367.43±27^{c}	559.54±42^{c}	192.11±31^{c}	0.04194
Pen 3 (F2)	Fairly fat	353.91±43^{c}	544.52±47^{d}	191.61±23^{d}	0.04732

Means within column with different superscripts differ significantly (P<0.05).

Animals were weighed in random manner without segregating them by neither sex nor age.

Body condition scoring

A scale of 1 to 9 adapted from Herd and Sprott (1986) was used to monitor body condition scoring where 4 major segments were classified. The classes were 1 to 3 as thin, 4 as borderline, 5 to 7 as optimum and 8 to 9 as fat. During body condition scoring, brisket and back/flunks of cattle were thoroughly scrutinized for detectable fats while ribs, hooks, pins, and tail heads were observed for visibility and projection for determination of degree of fatness. The exercise involved actual palpation of the body parts of the animal and/ or visual observation.

Typical gross margin for beef cattle fattening in Thailand

Data used to calculate gross margin for cattle in this survey were collected from farm records and farmer interviews in addition to field observations. Data included variables as number of cattle bought, feed cost, veterinary cost, asset, bank interest rate, labor cost and the purchase/selling prices. However, there was no breakdown of how many cattle bought at higher cost or low cost than others instead calculation was based on live weight and the fixed purchase and selling prices. The live weight varied from one animal to another but purchase and selling prices were constant throughout the calculation.

Cattle market transactions for the study beef cattle

The cattle were sold through a bidding cattle market system where buyers and cattle owners came together to decide on the selling price. Cattle were trekked from the farm to a nearby cattle market where different buyers patronized the market transaction. Cattle buyers were pre-identified and terms and conditions for sale were agreed upon by the seller and the buyer before the market day hence, cattle were consigned. The buyers weighed individual cattle and recorded the weight for price calculation.

Data analysis

Data on live weights, body condition score were analyzed by using GLM procedure of SAS for windows program on a personal computer. Initial body weights were used as covariate in data analysis. However, feed intake was not statistically analyzed due to group feeding where it was difficult to measure feed intake by individual animal.

RESULTS AND DISCUSSION

Nutrient composition of cattle feed

The cattle feed was found to have high energy levels of above 3900 kilocalories and low protein content of 14.24% and below (Tables 1 and 2). The feed was ideal for beef cattle fattening program because the high energy levels were required for fast growth and multiplication of rumen microorganisms. As was noted by findings by Eroldogan (2008) protein level for grown cattle is not as important as the energy. It was also stated by Lunn (2006) that growing cattle generally need 13 to 14% protein levels while finishing cattle requirements are closer to 11 to 12%, therefore the feed was within the requirement for fast cattle fattening.

The high moisture content of silage (Table 1) was due

to the fact that the agricultural by-products were freshly bought straight from corn and sugarcane farms and brought to the feedlots for silage making.

Feed intake

The feed intake was low during the first two fortnights of the study as witnessed by amounts of leftovers collected every morning feed time that is, 0.9 and 0.3 kg/head estimated during the first and second fortnights respectively. The study commenced towards the end of rainy season in Thailand so the cattle pens were muddy but the situation improved within the first month of study. During this rainy season feed intake was low and therefore, the study attributed this low feed intake to period of acclimatization of animals in a new environment.

The study also revealed that feed intake increased at an increased rate every subsequent week of feeding as witnessed by the fact that there were no leftovers during the subsequent period of feeding despite increasing amount of feed weekly. The increase in feed intake is deduced to be due to effect of compensatory growth since these cattle experienced nutrient restriction during grazing but were now given good nutritive feed in *ad libitum*. It can therefore be argued that feed intake had a bearing on compensatory growth and feed intake was found to have positive correlation to compensatory growth ($r = 0.54$).

Live weight

Further statistical analysis of the study findings showed a significant difference in weight gains ($P < 0.05$) due to high energy levels of the feed (Table 3). With high levels of energy, rumen microbes multiplied very fast and produced more microbial proteins (Smith et al, 1986) in addition to protein from feeds and the animal added on weight very fast. However, individual cattle registered different weight gains at the end of the study due to differences in levels of gene inheritability levels since these were cross bred cattle. To some extent the variation in weight may be attributed to the degree of emaciation since cattle started feed experiment with different degrees of emaciation. Degree of emaciation was found to have a bearing on compensatory growth in the sense that cattle which entered feeding trial with low live weights gained more weights than those which had good weights at the start of the feeding trial.

Cattle commenced feeding trial with different live weights and degree of emaciation. Findings showed that the cattle gained different live weights at the end of experiment. It was noted that most severely emaciated cattle gained more live weights at the end of the trial than the fairly fat ones (Table 2). The study found out that the extent of emaciation had an effect on the final weight gains of cattle. The rapid weight gains in severely emaciated cattle were attributed to feed intake which was found to be more in severely emaciated cattle than in fairly fat cattle. Hence, it can be argued that live weights had an effect on compensatory growth. The study also found out that degree of emaciation had a positive correlation to live weights ($r = 0.5$).

Body condition

Cattle entered the feeding program with different live-weights and body condition scores (Table 3). The study found that initial and final body condition scores were significantly ($P < 0.001$) different. Most of the cattle managed to attain BSC 8 which was the ideal score for beef cattle but still some could not attain the desired BSC at the end of the study. As noted by Tapki (2012) severe emaciation may not be a result of only nutritional status but also disease condition. However, this survey found that the low body condition scored cattle added more weight than those with good condition scores.

Final BCS and daily weight gains were affected by initial body condition score. It was also observed that individual big-framed cattle of low BCS attained the highest live weight gains as compared to the small framed cattle. The study attributed this difference to the extent of crossbreeding since there was no data to quantify whether the crossbred cattle had different blood levels of crossbreeding.

In both farms steers and bulls had achieved higher body condition scores than cows. The higher initial body condition score seemed to have been affected by feed intake and consequently caused the lesser daily weight gain in some cattle. The high BCS in bulls and steers was mainly due to the fact that bulls and steers commenced the feeding program with higher BCS than cows. Esterhuiizen et al. (2008) in their study with cows and bulls discovered that sex had great impact on daily weight gains and body condition scores of cattle after realimatation period. This was explained with reference to physiological difference between female and male cattle in the sense that many cows lose weight not only because of nutritional problems but also lactation period of nursing calves.

In this study, it was further observed that 80% of cattle achieved desired weight and body scores while 20% did not. This was noted by Carstens et al. (1991) who stated that cattle on same ration gained different amounts of live weights for same period of fattening. Carstens et al. (1991) further observed that not all cattle under their study gained same live weights at the end of the study period. There were some variations in weight gains from individual cattle under same feed treatment. The variations were explained as due to gene differences since the study could not trace the genetic make-up of all

Table 3. Mean initial and final body condition scores from Farm 1 and 2.

Farm ID	Initial BCS	Mean initial BCS	Final BCS	Mean final BCS	P-Value
Farm 1	BCS 3 = 8 BCS 3 = 11	3[a]	BCS 7 = 7 BCS 8 =12	8[A]	0.00754
Farm 2 Pen1	BCS 3 = 5 BCS 4 =18	4[b]	BCS 8 = 1 BCS 8 = 22	8[B]	0.00984
Farm 2 Pen 2	BCS 3 = 21 BCS 3 =25	3[c]	BCS 7 = 20 BCS 8 =26	8[C]	0.00719
Farm 2 Pen 3	BCS 4 = 11 BCS 4=22	4[d]	BCS 7 = 9 BCS 7 =24	7[D]	0.02782

Means with different letter case superscripts differ significantly ($P < 0.001$).

Table 4. Gross Margin results for cattle in Farm 1.

Item description	Unit price/Baht	Amount
Income		
11 cows, 580.89 kg (24 - 36 months)	92	587,860.68
8 bulls, 580.89 kg (20 - 30 months)	92	427,535.04
Total income		**1,015,395.72**
Variable cost		
11 cows, 364.74 kg (24 - 36 months)	84	337,019.76
8 bulls, 364.74 kg (20 - 30 months)	84	245,105.28
Feed cost	6,000	114,000
Veterinary cost	350	6,650
Interest cost	95	1,805
Total variable cost		**704,580.04**
Gross margin	(A) - (B)	**270,815.68**

the cattle under study as was also explained by Sahin et al. (2009). It was therefore concluded that age, sex, breed and extent of emaciation had a bearing on the results of the study. The current study is also attributing the difference in weight gains to gene variability, sex, age and degree of emaciation.

This study also showed few cases of severely emaciated cattle that did not respond positively to feeding in the second farm. It was found out that 3 cattle out of 102 in the second farm were found to have gained below 100 kg for a fattening period of 107 days. Since all cattle in the second farm had the same diet, the study concluded that the cattle were over emaciated and this agrees with what Fox et al. (1988) noted. Therefore, this study has revealed that severely emaciated cattle cannot achieve full potential of compensatory growth even if they were given good nutritive diet. This is in line with what Eroldogan et al. (2008) noted that growth compensation response is species-dependent. It depends on type of cattle, length and severity of feed restriction, being more effective when duration and severity of restriction are short.

Gross margin results of cattle in farm 1

The gross margin calculations for the Farms 1 are shown in Table 4. Since the farms followed the same system of raising cattle, the gross margin calculations represent the scenarios in both farms. Data presented in Table 4 demonstrate that the farmer invested 704,580.04 baht in the business and made a profit of 270.715.68. This profit came about because the farmers used agricultural by-products such as corn stalks, pineapple peals, molasses to feed cattle which reduced feed cost by over a quarter of normal feeding cost. In feedlot business feed cost accounts for 60 to 70% of production costs therefore, by reducing feed costs the farmer were able to realize big profits. The other contributing factor to the high profit was that the farmer used family labor which the farmers said is part of their strategy to build capital and that the farm did not experience any death of cattle during the period of

study, so all these contributed to realization of the high profit as was also noted by Kazito et al. (2011). Furthermore, the final calculations showed that bulls scored high income than cows in all mixed pens hence, it can be concluded that sex had effect on income.

One of the limitations of this study was that the research was hampered by the inability to compare the previous calculated gross margin from both farms with the present calculation because the farms do not usually calculate detailed gross margins. The farms generally calculate the simple profit and loss as noted by Lambertz et al. (2012). However, interviews from the two farms have indicated that the feedlots have been generally profitable over the past five-year period they have been in operational. To get precise estimates of gross margin in feedlot business, additional data are needed although this has implications of additional costs.

Conclusion

The study showed that the lower body condition scored cattle were the best to be used in min-feedlot operation because the expected weight at the end of feeding period was found to be higher in lower body condition scored cattle than that of higher body condition scored ones. Since farmers sell their cattle on live weight basis the final weight is very important because it determines the levels of income farmers get at the end of fattening period. However, the study noted that severely emaciated cattle took very long to fatten and therefore, not ideal for feedlot business. The study also revealed that the knowledge of compensatory growth of cattle was practical and not like other theoretical concepts which had been researched by many scholars.

Use of agricultural farm by-products and agro-industry by-products has shown to be very efficient in reducing production costs, therefore, farmers should always take advantage of them where they are found in proximity to the farm. The study has also shown that knowledge of compensatory growth will really assist feedlot owners in Kanchanaburi Province in deciding projected final weight of finished cattle and determining the best types of cattle for fattening. It has also been revealed that all cattle on pastures undergo compensatory growth in their life cycle so they require compensatory growth if they are to be profitable at the market.

Based on the sample gross margin of cattle fattening in Kanchanaburi Province in Thailand, it is concluded that mini-feedlot operation is one of the lucrative businesses in the area because of availability of agro-industry by-products which are available at low cost and throughout the year.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Harvest time, stem and grain sensibility of maize hybrids with contrasting growth cycles

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The delay of maize harvest may affect the stem and grain sanity. This work was carried out aiming to evaluate the effects of harvest time on the incidence of stem rots and rotten grains of maize hybrids with contrasting growth cycles. The experiment was set in Lages, SC, during the 2012/2013 and 2013/2014 growing seasons. A randomized block design, disposed in split plots was used. Five single-cross hybrids were tested in the main plots: P1630H and P32R22H (hipper early cycle), P2530 (super early cycle) and P30F53YH and P30R50YH (early cycle). Five harvest times were assessed in the split plots: 0 (grain physiological maturity), 10, 20, 30 and 40 days after physiological maturity. The incidence of stem rots increased proportionally to the delay in harvest time, regardless of hybrid growth cycle. More than 60% of the stems presented rot symptoms when harvest was performed 30 and 40 days after physiological maturity. Such behavior enhanced the percentage of lodged and broken stems when harvest was postponed. Harvest time did not affect the percentage of rotten grains, which was higher for hybrid P32R22H due to its poor ear husk coverage. Harvest delay affected more significantly the stem than grain sanity of maize hybrids.

Key words: *Zea mays,* stalk rots, rotten grains, harvest delay.

INTRODUCTION

The concomitant presence of maize and soybean is common in Southern Brazilian farms due to the need of establishing a crop rotation system. This management strategy is important to stimulate nutrient recycling, to increase soil water storage capacity, to enhance weed control efficiency and to prevent disease occurrence in both crops (Olibone et al., 2010; Castro et al., 2011; Franchini et al., 2012). The development of earlier ripening soybean cultivars and the anticipation of its planting date to early Spring (beginning of October) have accelerated soybean harvest to February, a month where maize is also ready to be harvested (Stülp et al., 2009). When this situation occurs, growers harvest soybean first because it is more profitable and more sensitive to harvest delay (Cella et al., 2014). Many times, such decision forces maize to stay in the field for over 30 days after grain physiological maturity (Panison et al., 2016).

The delay on maize harvest is a risky management decision because it brings several negative consequences, such as stem lodging and breaking,

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kernel germination at the ear and insect attack (Panison et al., 2016). These effects are more intense when maize is grown after plants of the same botanical family, such as black oat (*Avena sativa*). This species hosts fungi such as *Fusarium graminearum* and *Colletotrichum gramínicola* that cause stem and grain rot (Casa et al., 2007, 2009).

The intensity of stem lodging and breaking due to harvest delay depends on the hybrid's traits, management practices (fertilization level, plant density and row spacing), meteorological conditions at the end of the crop cycle and damages caused by insects and diseases (Gomes et al., 2010). Soils with high fertility, crowded stands, windy and rainy conditions during grain filling increase the percentage of broken stems at harvest (Casa et al., 2007; Schmitt, 2014). Stem lodging is also enhanced because maize stores more than 50% of its biomass in the grain at harvest (Sangoi et al., 2010). Therefore, the longer maize remains in the field after grain physiological maturity, the higher is the risk of having broken stems before harvesting the crop (Ferreira et al., 2012).

The maintenance of maize kernels in the field for long periods of time after physiological maturity favors ear infection by fungi that decrease grain quality (Kaaya et al., 2005; Lauren et al., 2007). Harvest delay is the major responsible for the increase in rotten grains and mycotoxin content derived from the presence of *Aspergillus*, *Penicillium* and *Fusarium* (Marques et al., 2009). Such fungi use endosperm storage compounds as energy source for their growth and development (Alakonya et al., 2008). Therefore, they reduce kernel mass, changing grain quality and visual appearance (Pinto et al., 2007). Losses due to fungi attack usually range from 7 to 15%, but they can exceed 50% under extreme conditions (Kaaya et al., 2005). Furthermore, these fungi produce toxic chemical compounds, compromising the use of maize kernels to produce oil or feed humans and livestock (Zain, 2011).

The cultivar's choice is a management strategy that can help to mitigate damages caused by late harvests. Maize hybrids present great variability regarding growth cycle duration, kernel type and plant resistance to diseases (Ferreira, 2012). The presence of decumbent ears at the end of grain filling maybe a desirable trait because it prevents water accumulation at the ear tip (Fonseca, 2005). Another favorable feature is adequate ear coverage with well developed husks. This trait avoids the exposition of kernels located at the upper part of the ear to unfavorable weather conditions (Guiscem et al., 2002).

This work was carried out based on two hypotheses: Harvest delay after grain physiological maturity increases the incidence of rotten stems, favoring plant lodging and decreasing kernel quality; the magnitude of damages caused by late harvest depends on the hybrid's traits. The experiment aimed to evaluate the effects of harvest time on stem and kernel sanity of maize hybrids with different growth cycles.

MATERIALS AND METHODS

The experiment was conducted in the city of Lages, Santa Catarina State, in the highlands of southern Brazil, during the growing seasons of 2012/2013 and 2013/2014. The experimental site is located at 27º52' latitude south, 50º18' longitude west and 900 m above sea level. The climate of the region is classified by Köppen-Geiger, mentioned by Kotteck (2006), as Cfb, presenting mild summers, cold winters and adequate rainfall during the whole year.

The soil at the study site was an Oxisol (Hapludox), according to Embrapa (2006), having the following chemical characteristics: Clay content, 560 g kg^{-1}; organic matter content, 60.0 g kg^{-1}; water pH, 5.2; SMP pH, 5.7; phosphorus, 4.4 mg dm^{-3}; potassium, 186 mg dm^{-3}; calcium, 5.79 cmolc dm^{-3}; magnesium, 2.47 cmolc dm^{-3}; aluminium, 0.2 cmolc dm^{-3}; CTC, 8.94 cmolc dm^{-3}.

A randomized block design arranged in split plots was used, with four replicates per treatment. Five single-cross hybrids with contrasting growth cycles were assessed in the main plots: Two hipper early hybrids (P32R22H and P1630H) that require 1282 and 1220 heat units (HU) to reach physiological maturity; one super early hybrid (P2530) that requires 1390 HU to attain physiological maturity; and two early hybrids (P30R50YH and P30F53YH) that achieve physiological maturity with 1493 and 1556 HU. Five harvest times were evaluated in the split plots: 0 (grain physiological maturity), 10, 20, 30 and 40 days after physiological maturity. The first harvest time for each hybrid was carried out when there was a visible black layer in the grain insertion point on the cob (R6 stage of the growth scale proposed by Ritchie et al. (1993). Each split plot comprised four rows, 0.7 m apart and 7 m long. All measurements were taken from the two central rows, leaving borders of 0.5 m at the end of each row.

The experiment was set using a no-tillage system under a dead coverage of black oat (*Avena strigosa*). The experimental area was planted with maize for three consecutive years before installing the trial. The soil fertilization was determined aiming to achieve a grain yield of 18,000 kg ha^{-1}. Fertilization was performed at the sowing date by applying 30 kg ha^{-1} of N, 295 kg ha^{-1} of P_2O_5 and 170 kg ha^{-1} of K_2O. The fertilizers were superficially placed close to the sowing rows. Nitrogen was also side-dressed, applying 250 kg of N divided into three equal parts when the plants were at the V4, V8 and V12 growth stages, according to Ritchie et al. (1993). Urea was used as the N source.

The experiment was hand planted on 12/5/2012 and 10/5/2013. The plots were over-sown, dropping three seeds per hill and thinned to the desired density (80,000 plants ha^{-1}) when the plants had three expanded leaves.

The weeds were controlled with two herbicide applications. The first was carried out immediately after sowing and prior to plant emergence with a combination of atrasine (1,400 g a.i. per hectare) and metolachlor (2,100 g a.i. per hectare). The second application was performed when maize plants were at V4, using tembotriona (100 g ha^{-1} de i.a.). Army worm (*Spodoptera frugiperda*) was controlled by spraying the insecticides lufenuron + lambdacyhalothrin (15 + 7.5 g de i.a. ha^{-1}) when the crop reached the V6 and V12 growth stages, according to Ritchie et al. (1993).

The percentage of lodged and broken stems was determined on the harvest day of each treatment. The plant that presents stem rupture below the ear insertion node was considered broken. The plant that has an angle between the lower stem inter-nodes and the soil smaller than 45º was considered lodged.

On the harvest day of each treatment, ear coverage by husks was also evaluated. A scale with grades ranging from 1 (best husk coverage) to 5 (worst husk coverage) was used to assess this

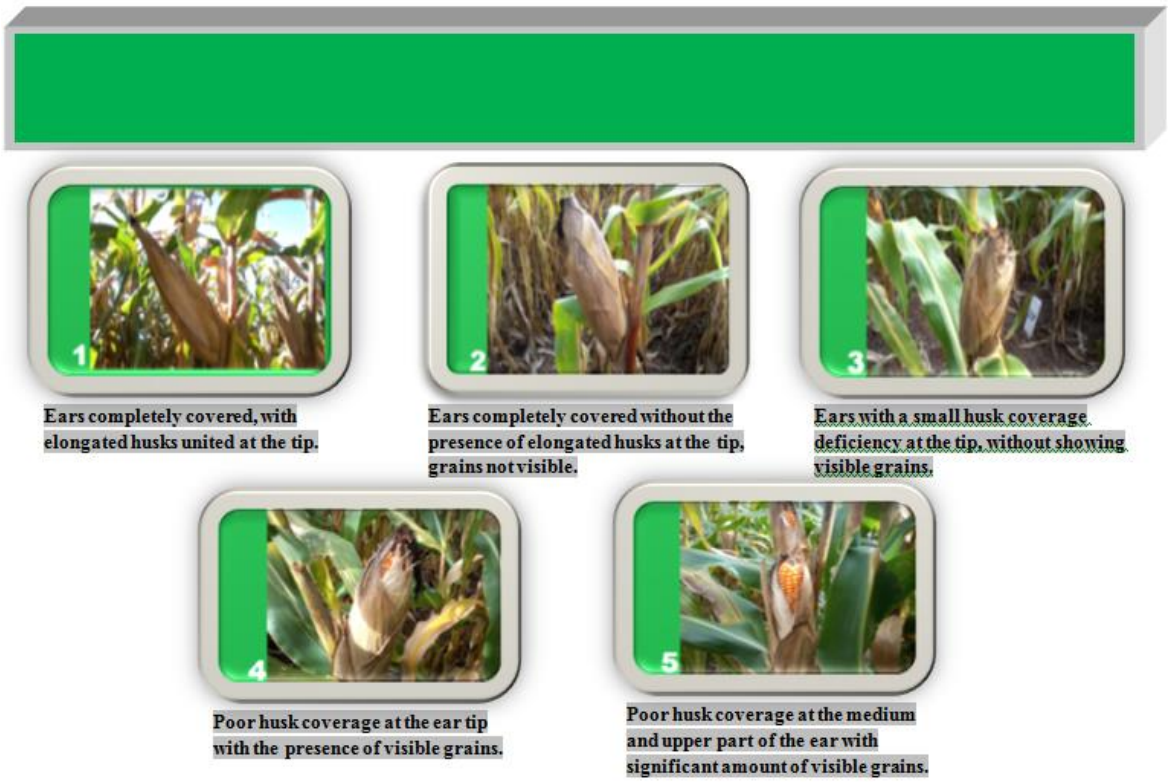

Figure 1. Scale of grades for ear husk coverage of maize hybrids. Lages, SC, 2014.

variable. The evaluation was carried out visually, observing the extension of the modified leaves that protect the ear and identifying the presence of visible kernels not protected by husks at the ear tip. More details about the scale can be seen on Figure 1.

In 2012/2013, harvest time 0 (grain physiological maturity) was carried out on 05/01/2013 and 05/10/2013 for the hipper early and the other hybrids, respectively. In 2013/2014, harvest time 0 was performed on 03/06/2014, 03/16/2014 and 03/26/2014 for the hyper, super and early hybrids, respectively. The other harvests for each hybrid were performed at constant intervals of 10 days from harvest time 0. At all harvest times, the ears were collected manually.

The occurrence of stem rot was evaluated right after ears' harvest. This evaluation was performed following the methodology presented by Reis and Casa (1996). The stems were cut with a knife nearly 30 cm above the soil surface. After that, they were longitudinally opened. The stems that presented internal visual discoloration symptoms were considered sick. The percentage of rotten stems was calculated dividing the number of stems with disease symptoms by the total number of stems of each split plot. A sample of symptomatic stems was taken to the Pathology Lab of Santa Catarina State University to identify the causal agents responsible for the diseases detected in the field.

At all harvest times, ears wee manually collected. Kernels were weighted and have their moisture contend determined. A kernel sample with 500 g was separated and placed in an oven at 65ºC until kernels were entirely dried. Subsequently, another sample of 200 g was taken to determine the incidence of rotten grains. Every kernel that presented more than 25% of its surface area discolored was considered rotten. The discolored kernels were weighted, allowing estimation of the percentage of rotten grains on each treatment.

The data were statistically evaluated by a variance analysis, using the F test, at the significance level of 5%. The data were previously transformed before carrying out the variance analysis using the expression $(x + 1)^{1/2}$. When the F values were significant, the values were compared using Tukey's test. The effect of delaying harvest time was also assessed by polynomial regression analysis, testing the linear and quadratic models. Both evaluations were conducted at the significance level of 5%.

RESULTS AND DISCUSSION

The percentage of lodged and broken stems was affected by harvest time at both growing seasons (Table 1). It ranged from 0 to 33.4% in 2012/2013 and from 0 to 95.4% in 2013/2014 (Table 2). In both growing seasons, the percentage of the lodged and broken plants increased proportionally to the delay in harvest time. The numeric values of the lodged and broken plants were greater in 2013/2014 than in 2012/2013. According to the regression analysis adjusted for the average values of the five hybrids, the percentage of lodged and broken stems presented a linear increase of 4.3 and 10.8% for each 10 days of delay in harvest time for the first and second growing seasons, respectively (Figure 2a).

In 2012/2013, there was no difference in the percentage of the lodged and broken stems among the hybrids (Table 2). Conversely, in 2013/2014 the hipper

Table 1. F values calculated by the Variance Analysis for the main effects of hybrids (A), harvest time (B) and the interaction between hybrids and harvest time (A x B). Lages, SC, Brazil.

Agronomic trait	Hybrid (A)	Harvest time (B)	Interaction (A x B)
		Growing season 2012/2013	
Percentage lodged and broken stems	7.77^{NS}	17.67**	1.53^{NS}
Percentage of rotten stems	27.61**	89.55**	1.36^{NS}
Ear husk coverage grade	15.78**	1.74^{NS}	1.03^{NS}
Percentage ofrotten grains	158.94**	0.77^{NS}	0.42^{NS}
		Growing season 2013/2014	
Percentage lodged and broken stems	10.70*	23.03**	3.20^{NS}
Percentage of rotten stems	11.93**	110.92**	1.97^{NS}
Grain of husk coverage	7.98**	0.83^{NS}	1.18^{NS}
Percentage of rot grains	5.68*	3.83^{NS}	0.43^{NS}

* Significant differences by the F test (P < 0.05). **Significant differences by the F test (P < 0.01). NS - Differences not significant by the F test (P>0.05).

Table 2. Percentage of lodged and broken stems of maize hybrids with contrasting growth cycles as affected by harvest time. Lages, South Brazil.

Days after physiological maturity	Hybrids					Average	CV (%)
	P1630H	P32R22H	P2530	P30F53YH	P30R50YH		
	Lodged and broken stems						
	Growing season 2012/2013						
0	0.0	0.0	0.0	2.1	3.6	1.1**	
10	4.5	5.7	0.4	2.5	8.2	4.3	
20	4.8	4.0	4.8	6.5	5.9	5.2	79.1
30	17.9	25.1	10.4	10.2	10.6	14.9	
40	17.3	33.4	14.5	10.4	12.4	17.6	
Averages	8.9^{NS}	13.6	6.0	6.3	8.1		
CV (%)			96.2				
	Growing season 2013/2014						
0	0.0	3.1	10.8	0.5	0.0	2.9**	
10	2.4	10.7	13.1	0.5	0.0	5.3	
20	4.5	19.9	19.8	6.7	5.3	11.2	80.7
30	8.3	33.7	61.0	3.9	18.6	25.1	
40	58.4	95.4	50.4	15.8	17.0	47.4	
Averages	14.7^{ab}*	32.6^{a}	31^{a}	5,5^{b}	8,2^{b}		
CV (%)			81.7				

Differences among averages not significant in the row (P>0.05); *Differences among averages significant in the row (P < 0.05). Averages succeeded by the same lower case letter do not differ by the Tukey's test. ** Differences among averages significant in the column (P < 0.01). ** Differences among averages in the column significant by the F test (P < 0.01).

and super early hybrids P32R22H, P1630H and P2530 had a higher percentage of lodged and broken stems than the early hybrids P30F53YH and P30R50YH.

The delay of maize harvest time is a risky management strategy because it favours stem lodging due to wind and rain (Gomes et al., 2010). This tendency was confirmed in the present work for both growing seasons. Maize allocates more than 50% of the plant biomass to the grains at physiological maturity (Sangoi et al., 2010). Therefore, when the harvest is postponed, tissue senescence at the stem base and the constant presence of rainfall and temperatures below 17ºC that occur during April, May and June in the highlands of South Brazil (Table 3) increase the ear weight, favouring stem lodging and breaking. This behaviour is accentuated by the crop's early ripening because maize hybrids with shorter growth cycles remobilize greater amounts of the stem-stored carbohydrates to the kernels during grain filling

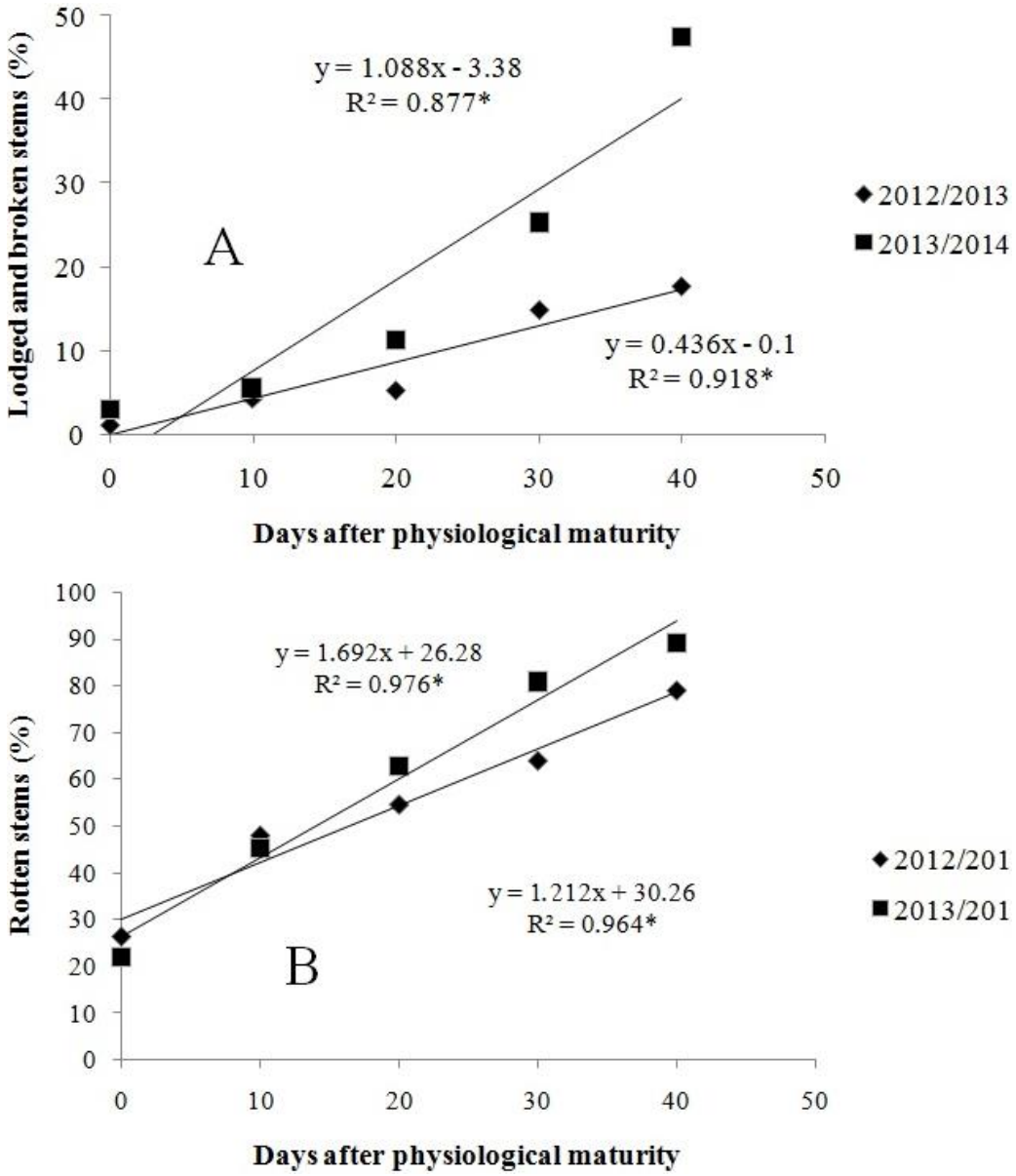

Figure 2. Percentage of lodged/broken stems (a) and percentage of rotten stems of maize hybrids (b), as affected by harvest time. Lages, SC, Brazil.

(Blum et al., 2003).

The percentage of rotten stems was affected by the main effects of hybrid and harvest time at both growing seasons (Table 1). The average value presented by the five hybrids for this variable was more than three times higher at the last harvest date than when maize was harvest at the grain physiological maturity (Table 4). The hipper and super early hybrids (P1630H, P32R22H and P2530) presented greater percentage of rotten stems than the early hybrids (P30F53YH and P30R50YH), at the average of harvest times According to the regression analysis, in 2012/2013 the percentage of rotten stems increased linearly 12.1% for each 10 days of delay in harvest time (Figure 2b). In the second growing season, the rate of increase in rotten stem incidence was 16.9% for each 10 days of harvest delay.

The rotten stems cause direct damage to maize due to the colonization of the vascular tissue, accelerating plant death (Romero Luna and Wise, 2015). Such behavior weakens the stem, favoring plant lodging and increasing grain losses during harvest (Casa et al., 2007). The greater incidence of rotten stems recorded in late harvests (Figure 2b) contributed to the higher values of stem lodging and breaking observed when harvest was performed 40 days after physiological maturity (Figure 2a). The cropping system used in the experimental area,

Table 3. Monthly average temperatures and pluvial precipitation during maize growing seasons in Lages, South Brazil.

Month	Growing season 2012/2013[1/]							
	Dec.	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**Mai.**	**Jun.**	**Average**
Average temperature (°C)	21.4	18.9	19.5	17.4	15.3	12.9	12.0	16.7
Precipitation (mm)	203.5	195.4	214.7	179.2	62.3	86.3	199.3	162.9

	Growing season 2013/2014							
Month	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	
Average temperature (°C)	15.1	17.4	20.2	21.4	21.0	18.5	16.3	18.5
Precipitation (mm)	136.9	146.1	107.7	182.9	210.9	121.3	94.2	142.9

1/ Temperature and precipitation data were collected from a meteorological experimental station located 20 km from the experimental site.

Table 4. Percentage of rotten stems of maize hybrids with contrasting growth cycles as affected by harvest time. Lages, SC, Brazil.

Days after physiological maturity	Hybrids					Averages
	P1630H	**P32R22H**	**P2530**	**P30F53YH**	**P30R50YH**	
	Rotten stems (%)					
	Growing season 2012/2013					
0	50	39.	21	18	16	26 **
10	68	65	45	33	38	48
20	86	71	63	41	38	54
30	83	74	69	49	56	64
40	91	90	88	64	74	79
Averages	76^{a}*	68^{ab}	58^{bc}	41^{d}	45^{cd}	
C.V. (%)			21.7			
	Growing season 2013/2014					
0	26	20	34	21	8	22 **
10	56	51	55	37	27	45
20	69	84	78	39	45	62
30	82	85	85	68	84	81
40	89	98	95	82	81	89
Averages	65^{a}*	68^{a}	70^{a}	49^{b}	49^{b}	
C.V. (%)			18.6			

*Differences among averages significant in the row ($P < 0.05$). Averages succeeded by the same lower case letter do not differ by the Tukey's Test. **Differences among averages significant in the column ($P < 0.01$).

with the succession of black oat and maize for three consecutive years, probably enhanced the percentage of rotten, lodged and broken stems when harvest was delayed. The three main fungi species detected in rotten stems were *Colletotrichum graminicola*, *Stenocarpela macrospora* and *Fusarium graminearum*, which are necotrophic pathogens favored by cropping systems that include plants of the same grass family (Casa et al., 2014).

The most efficient way to mitigate the negative effects of fungi infection to maize kernels is the correct hybrid choice (Carson et al., 2002). Cota et al. (2009) observed different responses of maize hybrids to the infection of *C. graminicola*, indicating that some genotypes presented higher ability to prevent the initial penetration of this pathogen inside the plant. Similar results were reported by Blum et al. (2003). These authors, testing cultivars with contrasting growth cycles, noticed that the hipper and super early ripening hybrids were more prone to rotten stems than the early hybrids. The same trend was detected in the present work. Hybrids with a short growth cycle also present small leaf area. Such trait increases the remobilization of carbohydrates from the stems to the kernels during grain filling, making the stems more susceptible to the infection of pathogens (Sangoi et al., 2010).

Harvest time did not interfere with the ear husk coverage grade probably because this trait is defined

Table 5. Ear husk coverage grade of maize hybrids with contrasting growth cycles as affected by harvest time. Lages, SC.

Days after physiological maturity	Hybrids					Averages	CV (%)
	P1630H	P32R22H	P2530	P30F53YH	P30R50YH		
	Ear husk coverage grade [1/]						
			Safra 2012/2013				
0	4	5	2	2	2	3^{NS}	
10	4	5	2	2	2	3	
20	4	5	2	2	2	3	22,2
30	4	5	2	2	2	3	
40	4	5	2	2	2	3	
Averages	4^{b}	5^{a}*	2^{c}	2^{c}	2^{c}		
CV (%)			43.2				
			Safra 2013/2014				
0	4	5	3	3	2	3.4^{NS}	
10	4	5	3	3	2	3.4	
20	4	5	3	3	2	3.4	17,3
30	4	5	3	3	2	3.4	
40	4	5	3	3	2	3.4	
Média	4^{b}	5^{a}	3^{b}	3^{b}	2^{c}		
CV (%)			34.0				

^{NS}Differences among averages not significant in the column (P>0.05). * Differences among averages significant in the row (P < 0.05). Averages succeeded by the same lower case letter do not differ by the Tukey's Test. [1/] Grade 1: Ears completely covered, with elongated husks united at the tip; Grade 2: Ears completely covered without the presence of elongated husks at the tip, grains not visible; Grade 3: Ears with a small husk coverage deficiency at the tip, without showing visible grains; Grade 4: Poor husk coverage at the ear tip with the presence of visible grains; Grade 5: Poor husk coverage at the medium and upper part of the ear with significant amount of visible grains.

before the kernels achieve their physiological maturity (Tables 1 and 5). On the other hand, there were significant differences among hybrids regarding to this variable at both growing seasons. The hipper early hybrids P1630H e P32R22H showed the worst husk coverage, presenting exposed kernels at the ear tip (Figure 1). The fast cob expansion at the beginning of grain filling that characterizes early ripening maturing hybrids probably contributed to the poor husk coverage presented by P1630H and P32R22H (Guiscem et al., 2002).

There were significant differences among hybrids in the percentage of rotten grains at both growing seasons (Tables 1 and 6). The hyper early hybrid P32R22H presented the higher percentage of rotten grains, on the average of five harvest times. This behavior was probably caused by its worst ear husk coverage (Table 5). Poorly covered ears, with short and loose husks, are more susceptible to fungi infection, due to the easier access of fungi such as *S. macrospora* and *F. graminearum* to the kernels, which increase the occurrence of rotten grains (Costa et al., 2011).

Harvest time did not affect the percentage of rotten grains (Tables 1 and 6). This result differs than the data reported by Santin et al. (2004) and Marques et al. (2009), who observed an increase in the amount of rotten grains when harvest was postponed. In the present work, harvest delay increased the percentage of rotten stems caused by *S. macrospora* and *F. graminearum* (Table 4). After infecting the stem, these fungi can migrate to the ear, enhancing the percentage of rotten grains (Casa et al., 2009). Nonetheless, there was no significant effect of harvest time in the percentage of rotten grains (Table 6). This apparent contradiction can be explained by the fact that the fungi that promote rotten ears and subsequently rotten grains infect the kernels during the early stages of grain filling. Therefore, they hardly colonize maize female inflorescence after the plant physiological maturity (Casa et al., 2014). Such behavior explains the lack of association between the increase in rotten stems and rotten grains incidence when harvest was delayed.

Conclusions

1. The delay of maize harvest time increased the percentage of rotten stems and the percentage of lodged and broken plants, regardless of hybrid's growth cycle.
2. The delay of maize harvest time did not affect the percentage of rotten grains of the five evaluated hybrids.
3. The hipper early ripening hybrid P32R22 presented the worst ear husk coverage and the highest percentage of rotten grains, regardless of harvest time.
4. Harvest delay affected more significantly the stem than

Table 6. Percentage of rotten grains of maize hybrids with contrasting growth cycles as affected by harvest time. Lages, SC.

Days after physiological maturity	Hybrids					Average	CV (%)
	P1630H	P32R22H	P2530	P30F53YH	P30R50YH		
	Rotten grains (%)						
2012/2013 Growing season							
0	8.8	12.7	2.1	3.4	0.7	5.2 NS	
10	7.6	9.3	1.7	4.1	0.9	4.6	
20	10,6	11.2	1.3	4.4	1.8	5.4	51.4
30	9.8	8.5	1.8	4.3	1.6	4.7	
40	9.6	10.2	4.1	3.9	3.1	5.7	
Average	9.3[a]*	10.4[a]	2.2 [c]	4.1[b]	1.6[c]		
CV (%)			23.2				
2013/2014 Growing season							
0	2.4	3.9	1.4	4.0	3.3	3.0 NS	
10	2.7	3.4	2.8	6.9	3.1	3.8	
20	6.8	9.4	3.4	8.2	6.7	6.9	53.5
30	5.0	6.1	5.2	6.2	6.8	5.9	
40	6.3	8.2	3.6	7.1	7.3	6.5	
Average	4,6[ab]	6.2[a]	3.3[b]	6.5[a]	5.4[ab]		
CV (%)			39.8				

[NS]Differences among averages not significant in the column (P>0.05). *Differences among averages significant in the row (P < 0.05). Averages succeeded by the same lower case letter do not differ by the Tukey's Test.

grain sanity of maize hybrids.

Conflict of Interests

The authors have not declared any conflict of interests.

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Interaction of genotype, environment and processing in the chemical composition expression and sensorial quality of Arabica coffee

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The present study was carried out to analyze chemical descriptors present in the raw coffee bean and to establish an association of these descriptors with the sensorial quality of the coffee beverage, based on expressions resulting from the interactions of coffee genotype, environment, and processing. The chemical descriptors caffeine, trigonelline, sucrose, and isomers of chlorogenic acid (3-CQA, 4-CQA, and 5-CQA), were analyzed through the use of high performance liquid chromatography (HPLC). Trained and qualified cuppers, certified as judges of specialty coffees, carried out the sensorial analysis using the methodology proposed by the Specialty Coffee Association of America (SCAA). Based on the cultivation environment, altitude and the genotype, it was possible to associate the chemical composition of the raw coffee bean with the coffee beverage sensorial quality. Yellow Bourbon cultivated above 1,200 m of altitude present higher contents of trigonelline and 3-CQA in the raw beans as well as high sensorial quality in the beverage.

Key words: Altitude, multidimensional scaling, slope exposure, Yellow Bourbon, coffee processing.

INTRODUCTION

Classical genetics theory defines phenotype as a characteristic that describes an organism through its gene expression, the influence of its environment, and through the possible interaction between these two factors. Genotype, in turn, is defined as the hereditary information present in an organism's genome. These

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definitions can be used for a better understanding of the coffee quality phenomenon through the expression of chemical compounds present in coffee beans (Figueiredo et al., 2013; Leroy et al., 2006; Taveira et al., 2014).

The quality of the beverage, represented by flavor and aroma formed in the roasted beans is associated directly with the chemical composition of the raw bean. On the other hand, chemical compounds present in the coffee beans are influenced by various factors throughout the production chain (Sunarharum et al., 2014).

Several researchers throughout the world have investigated the phenomenon of coffee sensorial quality. These studies involve various analyses such as the effect of environmental and genetic factors and of interferences that arise from coffee processing (Avelino et al., 2005; Bertrand et al., 2012, 2006; Borém et al., 2013; Decazy et al., 2003; Figueiredo et al., 2013).

Some environmental factors are strongly associated with the production of coffees of high sensorial quality. This association is especially true for factors that have a greater impact on the origin of chemical compounds present in the raw bean. Examples of these are the altitude and slope exposure of the coffee field as well as climatic factors such as temperature and precipitation (Avelino et al., 2005; Barbosa et al., 2012; Decazy et al., 2003; Guyot et al., 1996; Joët et al., 2010).

The genetic variability between and within coffee species results in beverages with distinct sensorial profiles. One of the explanations for this fact is related to the differences observed in the chemical composition of the beans as a function of the genetic material analyzed (Leroy et al., 2006). These differences become even more significant when the effect of processing is taken into account, because the metabolism of fruits and beans remains active even after they are harvested. Thus, the extension of certain metabolic reactions that occur in the bean depend on stimuli that result from the type of processing used, such as the action of removing or leaving intact parts that constitute the fruit (Bytof et al., 2005, 2007).

Another determining factor in the evaluation of coffee sensorial quality is related to the stage of fruit maturation. If the coffee fruits are harvested still immature, the lot will present beans with several chemical compounds associated with sensorial perceptions disfavored by consumers, such as astringency. Conversely, fruits harvested in a more advanced stage of maturation are subject to microorganism attacks, which accelerate the bean deterioration process. In addition, several toxins are generated as a byproduct of the action of these microorganisms (Borém et al., 2014).

In an attempt to supplement the coffee sensorial description, some chemical compounds present in the raw bean have been associated with coffee beverage quality. Currently, the main compounds being investigated are chlorogenic acid isomers (3-CQA, 4-CQA, and 5-CQA), caffeine, trigonelline, and sucrose (Farah and Donangelo, 2006; Franca et al., 2005; Malta et al., 2003; Silva et al., 2005).

Nevertheless, there are still unanswered questions that require a deeper technical and scientific investigation, especially regarding compound groups resulting from interactions between environmental, genetic, and technological factors that are involved in the processes of coffee production.

The lack of knowledge regarding coffee beverage quality is an obstacle to the development of some strategies, such as launching new varieties through genetic improvement with the aim of producing coffees with exotic flavors and aromas in unsuitable areas. Thus, it is essential to develop a scientific and fundamental base that amply identifies chemical descriptors associated with coffee beverage quality. In order to do that, it is necessary to restrict the studied area to geographical regions that present large environmental variation and express coffee sensorial quality in an evident, well-known, and consistent way (Barbosa et al., 2012).

Therefore, the objective of the present study was to analyze, during consecutive harvests, the effect of genotype, environment, and processing interaction on the contents of trigonelline, caffeine, sucrose, 3-CQA, 4-CQA, and 5-CQA present in the raw bean. More specifically, the present study attempted to verify the relation between the analyzed contents of chemical compounds and the sensorial quality of the coffee beverage.

MATERIALS AND METHODS

Experiment description

Coffee samples of the *C. arabica* L. were collected during three growing seasons (2009/2010, 2010/2011 and 2011/2012) in commercial plantations from properties located in the municipality of Carmo de Minas, Minas Gerais, Brazil (Figure 1).

The experimental design was based on the investigation of the interaction between environmental, genetic, and processing variables.

The coffee field environment was stratified into three altitude categories (more less or equal to 1,000 m, between 1,000 and 1,200 m, more than or equal to 1,200 m) and two slope exposure groups, Sun and Shade, resulting in six environmental variable combinations. The slope exposure groups were defined using cardinal points. The samples collected from crops with the slope aspect facing the W, NW, N, and NE represented the Sun group, and the samples collected from crops with the slope aspect facing the E, SE, S, and SW represented the Shade group.

Fruits of Yellow Bourbon genotype, yellow fruits and Acaiá genotype, red fruits were collected from each environment. Three repetitions were collected and processed using either the dry or the wet method, for all combinations involving environment and genotype, totaling 72 samples per harvest.

Coffee harvesting and processing

In order to evaluate the maximum potential of sensorial quality, harvesting was carried out manually and selectively, ensuring that

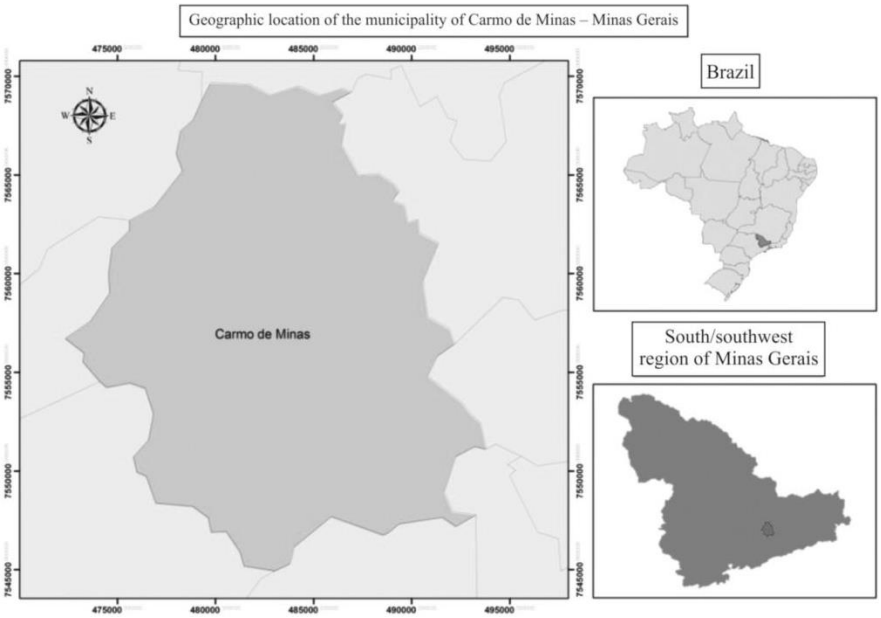

Figure 1. Geographic location of the Carmo de Minas municipality, located in the south/southwest region of the state of Minas Gerais, Brazil.

only mature fruits were picked. Thereafter, fruits were separated by density, with only denser fruits being selected. After this hydraulic separation, another manual selection was carried out to ensure that the samples constituted only dense fruits in their maximum stage of maturation. In addition, all procedures related to processing and drying were performed based on recommendations for coffee post-harvest best practices (Borém et al., 2014).

Sample storage and processing

After drying, the samples were packaged in paper sacks that were then placed in plastic bags, identified, and stored in a chamber under a controlled temperature of 10°C and relative humidity of 60% for 30 days. Thereafter, samples were hulled and the beans were separated by shape and size. Only flat-sided beans of screen size 16/64 to 18/64 inches were used. Flat beans retained in the 19/64 inch screen as well as peaberries retained by a screen with oblong perforations of 11 × 3/4 inches were eliminated. Subsequently, all defective beans were removed from the sample. This procedure guaranteed bean uniformity, thus minimizing interferences nor related to the genetic material, different combinations of environmental factors in field locations, and methods of processing. Subsequently, the coffee samples were prepared for chemical and sensory analysis.

Moisture content

The moisture content of the raw coffee beans was determined by the oven method, at 105±1°C, for 16±0.5 h, based on ISO 6673 (2003). The results were expressed as a percentage in wet basis (%wb).

Chemical analyses

Chemical analyses were carried out in the raw coffee beans. The contents of caffeine, trigonelline and the isomers of chlorogenic acids (3-CQA, 4-CQA, and 5-CQA) were determined simultaneously based on the adapted methodology of Farah et al. (2005). Sucrose determination was carried out through another analysis, according to the adapted methodology of Trugo et al. (1995).

Sample preparation

In preparation for the chemical analyses, raw beans were ground in an IKA brand analytical mill, Model A11 Basic, with liquid nitrogen. For the extraction of the oils and other apolar substances, 1.0 ml of hexane was added to approximately 100 mg of each ground sample of raw coffee beans and thereafter weighed in micro-centrifuge tube, and placed in an ultrasonic bath for 10 min and centrifuged at 6.000 rpm (Casal, 2004).

Extraction and analysis of chlorogenic acids, caffeine, and trigonelline

One hundred milligrams of each ground and degreased sample was placed in a microcentrifuge tube that was then suspended in 1.0 mL of HPLC grade methanol at 60% for the extraction of the chlorogenic acids, caffeine, and trigonelline. Tubes were placed in an ultrasonic bath for 15 min. After centrifuging, at 6.000 rpm, the supernatant solution was diluted at 1:10 in ultrapure water. After filtration in membrane of 0.20 µm, 20 µl of the samples were injected in liquid chromatography.

The system consisted of UV-Vis SPD-20A detector (Shimadzu, Kyoto, Japan). Samples and standardized solutions were analyzed in a Nucleodur 100-5C18 column, 250 mm × 3.0 mm, 5 µm (Macherey-Nagel). Analyses were carried out through the isocratic elution of methanol for HPLC/10 mM of citric acid pH 2.5 (25:75) at room temperature and with a flow of 0.7 $mL.min^{-1}$.

Results were defined by the relation of the peak areas of caffeine, trigonelline and 5-CQA compared to known concentration standards. The quantification of the other isomers, 3-CQA and 4-

CQA, was carried out using the standard area of 5-CQA, combined with coefficients of molar extinction, in accordance with adapted methodology of Farah et al. (2005). The final contents of caffeine, trigonelline, 3-CQA, 4-CQA, and 5-CQA were given in g kg^{-1} (dry basis - db).

Extraction and analyses of sucrose

One hundred milligrams of each ground and degreased sample were suspended in 1.0 mL of ultrapure water. The tubes that contained the samples were placed in an ultrasonic bath. An aliquot of 500 µl of the extract was transferred to another tube and centrifuged at 5.500 rpm. The supernatant was directly injected and a stock solution containing 60.0 mg of sucrose (Sigma ≥ 99%) was prepared in 5 mL of water for the calibration curve. Diluted standard solutions (10% to 100% of stock solution) were used for establishing the calibration curve.

The liquid chromatography used consisted of a ProStar pump (Varian), a RID-410 refraction index detector (Waters) and a Rheodyne injection valve. The samples and standard solutions were analyzed using Dextropak 100 mm × 8 mm columns (Waters) inserted into a RCM-100 compression radial system (Waters) with a line filter (0.22 um) and a C18 pre-column (50 × 4.6 mm) in series. The mobilephase used water filtered through a Milli-Q system at room temperature with a flow of 1.0 mL min^{-1}.

The association between the sucrose peak area in the sample and the respective known concentration standard defined the result (Kuo, et al., 1988). The final sucrose content was given in g kg^{-1} (db).

Sensorial analysis

One hundred grams of each sample were roasted and roasting was done within 24 h prior to the coffee sensory analysis, or “cupping.” The roast point was determined visually, using a color classification system that employs standardized color discs (Lingle, 2011). After roasting, samples were selected one more time and beans with an off coloration that differed from the sample standard coloration were eliminated. This process allowed for the isolation of possible interferences from the roasting process and other interferences not related to the interactions of the factors investigated.

The sensorial analysis was carried out by four trained and qualified cuppers, certified as judges of specialty coffees, using the proposed methodology by the SCAA (Lingle, 2011).

Statistical analyses

Statistical analyses were carried out using the R software, version 3.1.0 (R Core Team, 2014), considering the average values of the three harvests. After the verification of the assumptions of normality and of the homoscedasticity, the results of the contents of trigonelline, caffeine, sucrose, chlorogenic acids, and of the final scores (grade) were subjected to analysis of variance (ANOVA). The Tukey test was applied at 5% significance in order to verify significant differences detected in Test F. However, the results obtained from univariate analyses limit the comprehension of highly complex phenomena. Therefore, the present study chose to investigate the combined interactions from multivariate analysis, using multidimensional scaling (MDS) associated with the Biplots technique as a statistical tool. The investigation of the effect of the interaction among genotype, environment, and processing in the chemical composition of the raw bean and in the sensorial quality of the coffee beverage was carried out using multidimensional scaling (MDS) associated with the Biplots technique. The objective of this type of statistical analyses is to allow a more accessible visual inspection and a less limited exploration of the data. In addition, it allows for the rearranging of the variable distribution in order to detect the smallest significant dimensions to explain their similarities or dissimilarities (Torgerson, 1952).

RESULTS AND DISCUSSION

The average contents in g kg^{-1} (db) of trigonelline, chlorogenic acids (3-CQA, 4-CQA, and 5-CQA), caffeine, sucrose, and the final score of the coffee beverage in relation to the interaction of altitude, processing, genotype, and slope exposure are shown in Table 1.

Average scores found for trigonelline varied from 8.14 to 11.84; from 4.73 to 6.44 for 3-CQA; from 6.92 to 8.43 for 4-CQA; from 56.73 to 68.75 for 5-CQA; from 10.67 to 14.20 for caffeine, and from 53.16 to 89.51 for sucrose, and all were in accordance with scores described in the literature (Duarte et al., 2010; Knopp et al., 2006; Ky et al., 2001; Monteiro and Farah, 2012). Nevertheless, it was verified that significance ($P<0.05$) of fourth-order interaction was not found.

Significant differences were found in the average contents of trigonelline, 3-CQA, and caffeine as a function of altitude as well as in the average levels of sucrose and total beverage score as a function of the isolated effect of processing and genotype. It is important to emphasize that significant differences for the isolated effect of slope exposure type for all variables analyzed were not found (Table 2).

The highest average contents of trigonelline (10.44 g kg^{-1} - db) were found in altitudes higher than 1,200 m. Regarding the chlorogenic acids investigated in the present, 3-CQA was the only isomer that presented a significant difference and its highest average content was found in coffees produced above 1,200 m (6.27 g kg^{-1} - db). However, in regards to caffeine, only the average contents of samples collected above 1,200 m (13.39 g kg^{-1} - db) and below 1,000 m (12.35 g kg^{-1} - db) differed significantly from each other (Table 2).

Environmental factors such as lower temperatures recurring in higher altitudes, associated with physiological events such as longer periods of beans filling, are reported in the literature and provide evidence to explain the differences found in the chemical variables (Fagan et al., 2011; Geromel et al., 2008; Vaast et al., 2006). The temperature effect is especially observed between the stages of coffee fruit endosperm development and maturation, and the extending of these stages, caused by lower temperatures, is directly related to a higher relative accumulation of dry material in coffee beans (Laviola et al., 2007). Nevertheless, even though variations in the coffee quality have already been described as a function of genotype and altitude (Avelino et al., 2005; Decazy et al., 2003), further studies on the correlation of these factors and the combined effect of chemical variables are needed. The highest average levels of sucrose content (74.14 g kg^{-1} – db) were found in coffees processed using

Table 1. Average contents of trigonelline, chlorogenic acids (3-CQA, 4-CQA, and 5-CQA), caffeine, sucrose, and final score of the coffee beverage, as a result of the interaction between altitude, processing, genotype, and slope exposure.

Altitude (m)	Processing	Genotype	Slope exposure	Chemical compound (g kg^{-1})						Coffee beverage
				Trigonelline	3-CQA	4-CQA	5-CQA	Caffeine	Sucrose	Final score
≥1,200	Wet process	Acaiá	Sun	10.05	6.44	7.42	61.44	13.79	66.48	84.75
			Shade	10.56	6.11	8.13	61.10	13.19	58.87	83.15
		Yellow Bourbon	Sun	10.55	5.83	7.34	62.71	14.20	73.09	90.52
			Shade	11.84	6.34	7.22	61.84	13.58	66.28	88.82
	Dry process	Acaiá	Sun	10.47	6.12	7.32	60.43	13.49	74.59	84.81
			Shade	9.55	6.43	7.27	59.73	13.45	68.17	86.11
		Yellow Bourbon	Sun	10.25	6.04	7.73	60.94	13.02	77.20	90.13
			Shade	10.26	6.22	7.31	62.39	12.37	89.51	90.25
1,000-1,200	Wet process	Acaiá	Sun	10.04	6.34	7.48	61.55	11.73	53.16	82.11
			Shade	9.14	5.23	8.43	65.71	13.24	57.67	82.95
		Yellow Bourbon	Sun	9.65	5.78	7.63	59.23	13.09	66.48	84.81
			Shade	10.05	5.45	7.83	64.90	13.29	73.39	85.96
	Dry process	Acaiá	Sun	9.04	6.14	6.92	63.14	12.28	69.69	84.14
			Shade	9.75	5.33	7.63	58.53	13.59	74.19	83.71
		Yellow Bourbon	Sun	10.16	4.73	7.13	58.21	12.88	68.08	86.25
			Shade	9.85	5.69	7.49	59.46	12.89	78.90	86.08
≤1,000	Wet process	Acaiá	Sun	9.14	5.13	7.02	61.72	13.19	56.87	82.00
			Shade	8.63	5.33	7.40	59.75	12.08	61.58	82.68
		Yellow Bourbon	Sun	8.14	4.93	7.73	57.33	12.58	64.68	83.14
			Shade	9.85	5.89	7.43	68.75	11.88	72.99	84.42
	Dry process	Acaiá	Sun	9.35	5.03	6.93	58.33	12.67	70.39	83.56
			Shade	9.55	4.73	7.30	56.73	13.09	74.49	82.72
		Yellow Bourbon	Sun	8.54	4.93	7.48	57.13	10.67	73.69	83.79
			Shade	9.93	5.13	7.22	58.13	12.89	71.89	85.35
P value				0.55	0.54	0.96	0.60	0.82	0.47	0.78

the dry method, independent of genotype, altitude or slope exposure (Table 2).

Among the technological factors involved in the process of coffee production, the processing method alters significantly the sugar content of the raw beans (Duarte et al., 2010; Knopp et al., 2006).

During post-harvest processing, several metabolic processes occur in the interior of coffee beans, altering significantly the chemical composition of the raw bean (Bytof et al., 2005; Selmar et al., 2006). Therefore, the difference

Table 2. Average contents of trigonelline, chlorogenic acids (3-CQA, 4-CQA, and 5-CQA), caffeine, sucrose, and final score of the coffee beverage, as a result of the isolated effect of altitude, processing, genotype, and slope exposure.

Parameter		Chemical compound (g kg^{-1})						Coffee beverage
		Trigonelline	3-CQA	4-CQA	5-CQA	Caffeine	Sucrose	Final score
Altitude (m)	≥1,200	10.44^{a}	6.27^{a}	7.43	61.24	13.39^{a}	71.73	87.32 ^{a}
	1,000-1,200	9.72^{b}	5.60^{b}	7.52	61.22	12.87^{ab}	67.61	84.50 ^{b}
	≤1,000	9.18^{b}	5.19^{b}	7.33	59.67	12.35^{b}	68.21	83.46 ^{b}
*P value**		0.00	0.00	0.53	0.68	0.01	0.28	0.00
Processing	Wet process	9.83	5.78	7.67	62.04	12.96	64.25^{b}	84.61 ^{b}
	Dry process	9.70	5.56	7.34	59.31	12.76	74.14^{a}	85.57 ^{a}
*P value**		0.83	0.23	0.07	0.10	0.52	0.00	0.02
Genotype	Acaiá	9.61	5.72	7.41	60.61	12.92	65.42^{b}	83.56 ^{b}
	Yellow Bourbon	9.99	5.69	7.49	60.87	12.79	72.99^{a}	86.63 ^{a}
*P value**		0.22	0.43	0.95	0.89	0.58	0.00	0.00
Slope exposure	Sun	9.65	5.62	7.32	60.02	12.72	67.87	84.84
	Shade	9.98	5.65	7.52	61.38	12.91	70.63	85.34
*P value**		0.20	0.75	0.21	0,45	0.66	0.22	0.67

*Averages followed by the same letter in the column, do not differ from each other, based on the Tukey test (5% of significance).

found in the present study reinforces these reports, and possibly demonstrates the isolated effect of coffee processing in altering significantly the sucrose content. Coffee quality also presented a significant difference as a function of coffee processing (Table 2). The highest average values of the final score of the coffee beverage were found in coffee processed using the dry method (85.57).

There are reports in the literature that describe coffees produced by the dry process as having a quality comparably inferior to those produced by the wet process (Silva et al., 2004; Vincent 1985; Wilbaux 1963).

However, variations in coffee quality have principally been discussed as a function of the presence or absence of defects in the beverage. If care is not taken in harvesting and drying, naturals coffees have a higher probability of resulting in a beverage with undesirable fermentations and lower quality (Borém et al., 2014).

The present study evaluated the coffee quality from positive sensorial attributes, in the absence of any type of defect in the beverage. Under these conditions, results found in the present study contradict what is traditionally described in the literature, since the total average score of dry process coffees was higher than wet process coffees.

Regarding the factor of genotype, the highest average sucrose contents were found in Yellow Bourbon (72.99 g kg^{-1} - db). The main reports found in the literature that describe the isolated effect of genotype on the chemical composition of coffee are principally in regards to different species of the *Coffea* genus (Campa et al., 2004; Ky et al., 2001). Differences in the sucrose content among distinct varieties of Arabica coffee may be associated with differences in gene expression in both the syntheses and degradation of the compound. Molecular analyses are necessary for a better understanding of the variations in the sucrose content found for the genotype in the present study.

The results obtained from univariate analysis limit the comprehension of highly complex phenomena. For instance, the combined interactions involving environmental factors and different cultivars and forms of processing impact the chemical composition of the raw bean which in turn impacts the expression of the sensorial quality of the resulting coffee beverage. Therefore, the present study chose to investigate the combined interactions from multivariate analysis, using MDS associated with the Biplots technique as a statistical tool.

The determination of the variables that represented the larger contributions for the grouping of samples were not characterized by the weight analysis of the principal components. It was performed basing on the axes of predictive values, for each respective variable. Therefore, we used a minimum square regression for each variable to be calibrated (Graffelman and Eeuwijk, 2005) and mean centered for other variables. The compounds that characterize the axes were obtained as a function of the dissimilarity matrix and the covariance matrix. Therefore, it is feasible to obtain the ellipsis that characterizes the confidence regions. This way, the present ellipses do not represent a confidence region but simply an illustration to facilitate the visualization of the group of samples identified in the figures.

The chemical composition, when analyzed jointly, largely contributed to the formation of groupings as a function of the coffee beverage final score, altitude, genotype, and the post-harvest processing (Figures 2

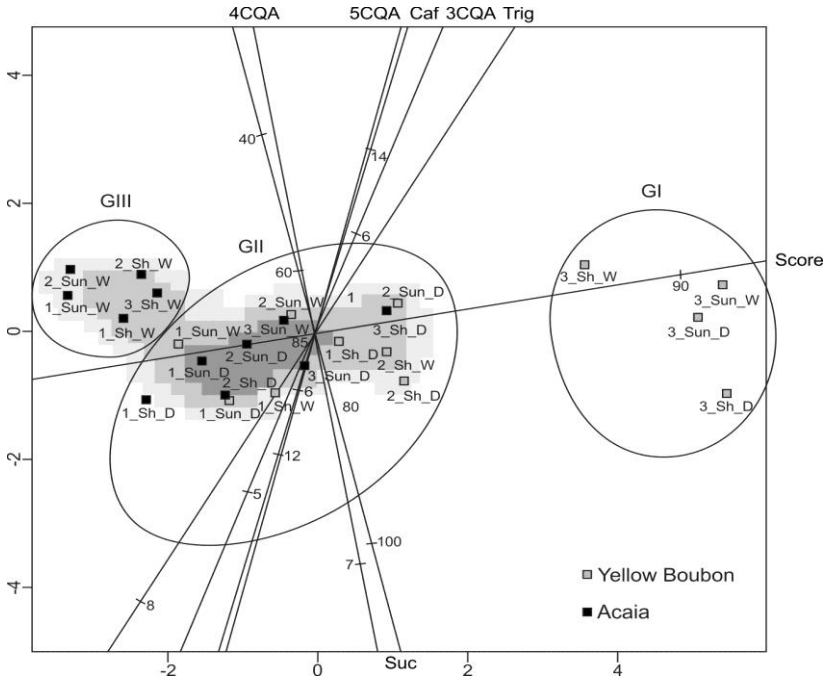

Figure 2. Biplot with MDS Acaiá and Yellow Bourbon genotypes, cultivated in altitudes (1. <1,000 m, 2. 1,000-1,200 m and 3. >1,200 m), in combination with the slopes sun (Sun) and shade (Sh) and processed using dry process (D) and wet process (W), for the variables trigonelline (Trig), 3-CQA (3CQA), 4-CQA (4CQA), 5-CQA (5CQA), caffeine (Caf), sucrose (Suc) and coffee beverage final score (Score), grouped into GI (Group I), GII (Group II), and GIII (Group III).

and 4). The Biplot that was established represented each object (genotype, altitude, slope exposure, and processing) as a dot and each dependent variable (chemical and sensorial) as a vector with representative scales of the average values (Figure 2).

Biplot with MDS of genotypes, altitude categories, slope groups, and processing types for the following variables trigonelline, 3-CQA, 4-CQA, 5-CQA, caffeine, sucrose, and of the coffee beverage final score are shown in Figure 2. Three distinct group formation were observed as follows: Group I (GI) constituted of the Yellow Bourbon genotype cultivated in altitudes higher than 1,200 m; Group III (GIII) consisted of the Acaiá genotype processed using the wet method, and Group II (GII) constituted of objects that represent the interaction of all independent variables investigated, except when the interaction is the combination of Yellow Bourbon above 1,200 m and wet processed Acaiá. This last group represents a confounding zone, since objects occur near the origin with scores around 85 points, objects to the right of the origin and to the left with differing sensorial quality scores.

The variable score, in combination with the mean of the other dependent variables, contributed to this distinction. Analyzing the chemical variables adjustment, it was observed that the higher contents of sucrose and trigonelline largely contributed to the formation of GI. On the other hand, the lowest contents of trigonelline and sucrose and the highest contents of 4-CQA were the main contributors to the formation of GIII. The remaining variables 3-CQA, 5-CQA, and caffeine presented high similarity to each other and contributed little to the formation of GI and GIII.

The Yellow Bourbon genotype cultivated above 1,200 m of altitude, independent of the slope exposure or type of processing applied (GI), presented tendencies towards higher contents of sucrose and trigonelline and the highest potential for the expression of coffee beverage sensorial quality, with a final score of approximately 90 points (Figure 2).

It can also be seen in Figure 2 that the wet processed Acaiá genotype (GIII), except when cultivated above 1,200 m of altitude on a sun-facing slope, presented tendencies of lower contents of sucrose and trigonelline, higher 4-CQA and a beverage sensorial quality with scores below 85 points.

The combined effect of variables characterized by chemical compounds and coffee beverage sensorial quality were found for both interactions, genotype x altitude (GI) and genotype x processing (GIII). Based on this understanding, MDS was applied for wet and dry processes with the purpose of analyzing the interactions between environmental and genetic factors in the chemical composition and sensorial quality, taking into

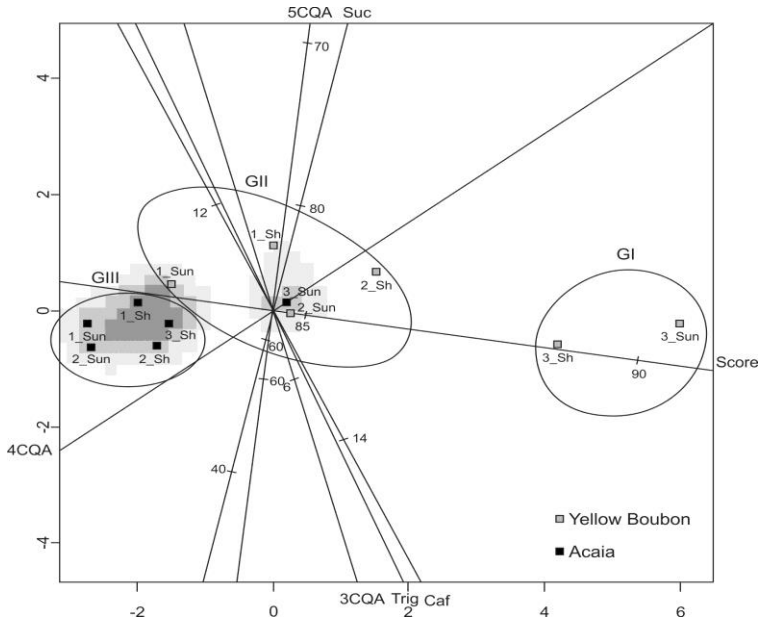

Figure 3. Biplot with MDS of Acaiá and Yellow Bourbon genotypes, cultivated in altitudes (1. <1,000 m, 2. 1,000-1,200 m, and 3. >1,200 m) in combination with slopes sun (Sun) and shade (Sh), and wet processed (W) for the variables trigonelline (Trig), 3-CQA (3CQA), 4-CQA (4CQA), 5-CQA (5CQA), caffeine (Caf), sucrose (Suc), and coffee beverage final score (Score), being GI (Group I), GII (Group II), and GIII (Group III).

account characteristics of coffees obtained from different processes. Therefore, established Biplots presented objects characterized by the combination of genotype, altitude, and slope exposure, in addition to vectors with representative scales of average values found for chemical and sensorial variables (Figures 3 and 4).

Figure 3 represents Biplot with MDS of genotypes, altitude categories, slope groups, and wet processing for the variables trigonelline, 3-CQA, 4-CQA, 5-CQA, caffeine, sucrose, and coffee beverage final score.

The highest scores, highest contents of caffeine, trigonelline, and 3-CQA and the lowest of 4-CQA largely contributed for the formation of GI. On the other hand, the lowest scores along with the lowest contents of sucrose and highest of 4-CQA were also relevant for the formation of GIII. The variable 5-CQA contributed little for the grouping formation.

Yellow Bourbon genotype cultivated above 1,200 m and wet processed (GI) presented a tendency of higher contents of caffeine, trigonelline, and 3-CQA, and lower for 4-CQA. In addition, this genotype exhibited average final score of 90 points, demonstrating a high potential for the expression of beverage sensorial quality.

For wet processing, the genotype Acaiá, except when cultivated above 1,200 m altitude on a sunny slope facing (GIII), present a tendency towards higher levels of 4-CQA, lower levels of sucrose, and a beverage sensorial quality with scores to the left of origin, and therefore below the mean.

The genotype Yellow Bourbon cultivated below 1,200 m altitude, independent of slope exposure, presented a sensorial beverage quality similar to that of Acaiá cultivated above 1,200 m altitude on a sunny slope exposure (GII), when submitted to processing via the wet process (Figure 3).

The Biplot with MDS of the genotypes, altitude classes, slope groups, and processing via the dry process for the variable trigonelline, 3-CQA, 4-CQA, 5-CQA, caffeine, sucrose, and total sensorial beverage score, can be found in Figure 4.

The highest levels of trigonelline, 5-CQA and 3-CQA, together with the highest scores, were principally responsible for the formation of GI. On the other hand, the lower levels of these same compounds and lower scores contributed in an expressive way to the formation of GIII, creating two contrasting groups. The remaining variable 4-CQA, caffeine, and sucrose, presented high similarity between themselves with little contribution to the formation of groups.

The genotype Yellow Bourbon cultivated above 1,200 m and processed using the dry method (GI) presented a tendency towards higher levels of trigonelline, 5-CQA, 3-CQA, and a notable expression of the quality of the coffee beverage, with a final score of around 90 points (Figure 4).

For dry processing, the genotype Yellow Bourbon cultivated below 1,200 m presented a sensorial beverage quality similar that that of Acaiá cultivated above 1,200 on

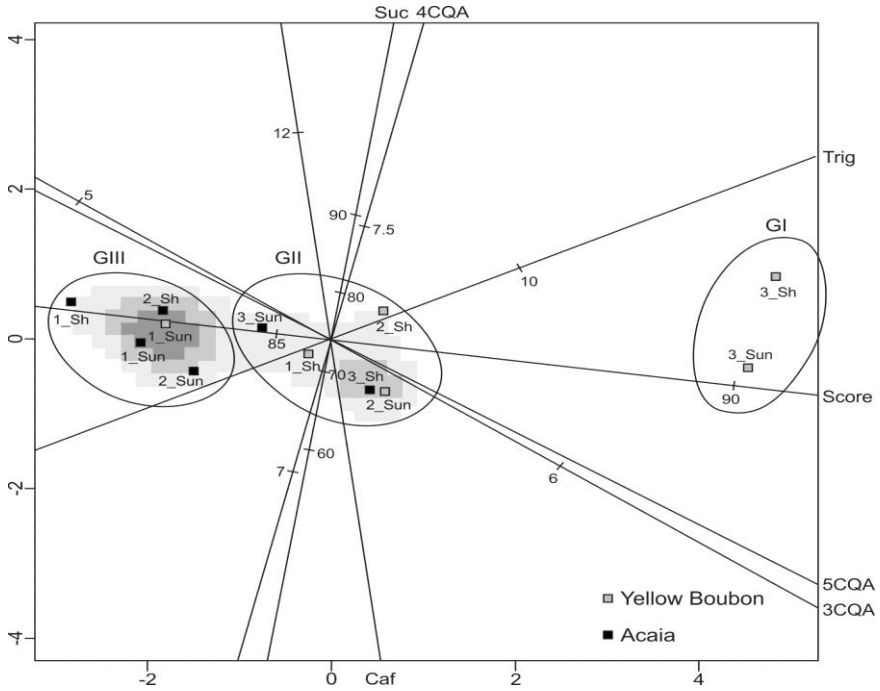

Figure 4. Biplot with MDS of Acaiá and Yellow Bourbon genotypes, cultivated in altitudes (1. <1,000 m, 2. 1,000-1,200 m, and 3. >1,200 m) in combination with slopes sun (Sun) and shade (Sh), and dry processed (D) for the variables trigonelline (Trig), 3-CQA (3CQA), 4-CQA (4CQA), 5-CQA (5CQA), caffeine (Caf), sucrose (Suc), and coffee beverage final score (Score), being GI (Group I), GII (Group II), and GIII (GroupIII).

both sun and shade slopes exposure (GII).

Dry processed coffee of the genotype Acaiá cultivated below 1,200 m, as well as coffee of the Yellow Bourbon genotype processed below 1,000 m, with a sun slope facing (GIII), had a tendency towards lower levels oftrigonelline, 5-CQA, 3-CQA, and sensorial beverage quality with notes below 85 (Figure 4).

It should be noted that the relation between the chemical composition of the raw bean and the sensorial quality of the coffee beverage arising from the interaction genotype x environment was distinct in regards to processing type. The grouping formed by dry process coffees presented the combined effect of the compounds trigonelline, 5-CQA and 3-CQA with the total beverage score. On the other hand, pulped and demucilaged coffees presented distinct groups in function of the combined effect of all of the compounds analyzed with a total beverage score, with the exception of the chlorogenic acid isomer 5-CQA.Their differences reinforce the hypothesis that the metabolism of the bean remains active after the harvesting of the fruits and that the extension of these metabolic processes depends on the type of processing applied (Knopp et al., 2006). Another possible cause is the stimulus promoted in the germination metabolism by the wet process in function of the pulping of the fruit (Bytof et al., 2007).

Conclusions

No interaction of the factors: Coffee cultivar, altitude, slope exposure and processing type were observed by performing univariate analyses. In addition, it is clear that slope exposure does not affect the chemical composition of raw coffee beans and the sensory quality of arabica coffee Yellow Bourbon cv. and Acaiá cv., neither by itself nor interacting with other factors.

Considering all chemical and sensorial variables together, specific trends for each cultivar, altitude range and processing type, were clearly observed by using MDS. Of which was possible to take the following specific conclusions:

a. The genotype Yellow Bourbon cultivated above 1,200 m altitude and processed using the wet processing method present in the raw coffee bean a tendency towards higher levels of caffeine, trigonelline and 3-CQA, lower levels of 4-CQA, and a sensorial beverage quality with a mean score of around 90 points.

b. The genotype Acaiá processed using the wet processing method, except when cultivated above 1,200 m on a sun-facing slope, present in the raw coffee beans a tendency towards higher levels of 4-CQA, lower levels of sucrose, and a sensorial beverage quality with scores

below 85 points.
c. The genotype Yellow Bourbon cultivated above 1,200 m altitude and processed using the dry processing method, present in the raw coffee bean a tendency towards higher levels of trigonelline, 5-CQA, 3-CQA and a sensorial beverage quality with an average score around 90 points.
d. Dry process coffee of the genotype Acaiá cultivated below 1,200 m altitude, as well as Yellow Bourbon cultivated below 1,000 m with a sun-facing slope, present in the raw coffee beans a tendency towards lower levels of trigonelline, 5-CQA, 3-CQA, and a sensorial beverage quality with scores below 85 points.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Irrigation depths in sugarcane crop with drip irrigation system

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This study aims to evaluate the growth and development of sugarcane under different supplemental irrigation depths. Irrigation treatments were 0.30, 0.60, 0.90 and 1.2 rate of crop evapotranspiration (ETc) and the control (no irrigation). The experimental design was a randomized complete block with six replications. The highest yield was ratoon cane with 190 t ha^{-1} in the treatment 0.6 of ETc. The difference between the highest and lowest yield were 11 and 7% in plant cane and ratoon cane, respectively. The rate of maximum technical efficiency of yield was 0.78 of ETc. Irrigation water productivity and water productivity was obtained in 35.8 to 146.0 kg m^{-3} and 18.0 to 70.9 kg m^{-3}, respectively. The total mass and the dry mass showed increase of 20 and 25% in ratoon cane, respectively. The plant height showed no difference between treatments. The highest: number of tillers, leaf area and number of green leaves was treatment 1.2 of ETc. The deficit and excess moisture affect plant development, plant height, leaf area, stem diameter, number of tillers per meter, full mass and dry mass, affects the yield of sugarcane.

Key words: *Saccharum*, drip irrigation, water deficit, strategies for efficient irrigation, sugarcane.

INTRODUCTION

The sugarcane is one of the most significant crop worldwide, cultivated over 100 countries, and it is considered an important source of jobs in rural areas. Brazil, India, China, Mexico, Thailand, Pakistan, Colombia, Australia, Indonesia, and United States of America hold about 80% of sugarcane production (FAO, 2008). In Brazil, sugarcane is the third crop in terms of cultivated area (9.0045 million of hectares), with an

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Table 1. Monthly climatic data of the experimental area, relative humidity and insolation, evaporation cumulative during the 2013 to 2014 and 2014 to 2015 growing seasons.

Months	Relative humidity mean (%)			Insolation (hour)			Evaporation (mm)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Jan	-	73.1	78.3	-	219.2	235.8	-	158.5	148.7
Feb	-	73.8	79.7	-	211.2	207.2	-	138.2	122.1
March	-	76.8	77.6	-	212.6	229.4	-	113.7	118.6
April	-	78.2	77.0	-	219.8	207.2	-	86.5	81.9
May	-	83.4	82.5	-	142.8	146.9	-	49.1	52.5
June	-	86.8	80.9	-	199.8	145.8	-	41.5	34.8
July	-	80.6	86.2	-	176.4	89.6	-	53.2	41.7
Aug	-	75.8	-	-	188.5	-	-	76.5	-
Sept	-	76.4	-	-	134.0	-	-	101.2	-
Oct	-	73.4	-	-	161.0	-	-	146.8	-
Nov	71.6	71.0	-	229.2	173.1	-	144.3	131.6	-
Dec	69.5	76.1	-	286.2	211.0	-	175.2	142.1	-

average of production of 71.31 t ha^{-1}. The Southern region has cultivated sugarcane in 0.636 million of hectares (7% of national sugarcane area), and the Southern of Rio Grande do Sul state grows sugarcane in 0.014 million of hectares (2.2% of Brazil sugarcane area), with a production average of 55.22 t ha^{-1} (CONAB, 2014). Moreover, sugarcane has great socioeconomic importance, provides feedstock to the sugarcane industry for the production of alcohol (hydrous and anhydrous), sugar, brandy, bioplastic, biodiesel, kerosene, fertilizers, paper, animal feeding, and the sugarcane bagasse has been used as an energy source (electricity) (Souza et al., 1999).

The water deficit is the main factor in yield decrease in most crops worldwide (Bray et al., 2000). Strategies for the management and efficient use of irrigation water are the keys for the sustainability and profitability of crops, yet it is the great importance to improve yield and quality, reduce costs, and maximize water use (Padrón et al., 2015a). Thereby, knowing how crops respond to abiotic stresses is a prerequisite to choose the best variety, management strategies, and the use of natural resources (Smit and Singels, 2006).

Sugarcane yield is limited by edaphoclimatic factors such as water and nitrogen deficiency (Gava et al., 2011). Moreover, sugarcane growth and development are directly related to evapotranspiration, and water availability is considered the main factor that causes production variability (Dalri, 2004). According to Inman-Bamber (2004), the duration of water deficit negatively affects leaf production and increases leaf senescence of whole plant, yet it may reduce light interception, water use efficiency, photosynthesis, as well as increase transmitted radiation to the soil surface. Furthermore, the amount of water consumed daily by sugarcane depends on the variety, growth stage, and evapotranspiration demand, which varies according to the region and season of the year (Bernardo, 2006). Although some regions have high precipitation rates, its irregular distribution may sometimes prevent plant growth (Ometto, 1980). Therefore, the management of irrigation water is essential to maximize yield, growth, stem density, leaf area index, increase sugar content, sugarcane life, and the farmer profits (Bernardo, 2006; Neto et al., 2006; Dalri et al., 2008; Farias et al., 2008a).

The evaluation of the phenology behavior provides knowledge and definition of the period that each vegetative phase occurs, and may help in the choice of management strategies, such as the best harvesting and planting time (Larcher, 2004). Sugarcane crop can tolerate some water deficit, however it highly responds to irrigation management (Singh et al., 2007). Effective management of water resources is the key to the sustainability and profitability of the crop, thus encouraging the development of new techniques for the analysis and efficient water management (Padrón el al., 2015b).

Thereby, the aim of this study was to evaluate the growth, development, and sugarcane yield submitted to different irrigation levels as an additional source of water for efficient use of irrigation water.

MATERIALS AND METHODS

This field study was conducted at the experimental area of the Polytechnic School of the Federal University of Santa Maria (altitude of 110 m, and 29°41'25" S, and 53°48'42" W), during the Spring-Summer seasons of 2013 to 2014 and 2014 to 2015. The soil is classified as typical dystrophic yellow argissolo, with a loam texture. The climate of the region, according to the Köppen classification is subtropical humid (Cfa). During the both growing seasons, air relative humidity ranged (69.5 to 86.8%), insolation (134 to 286.2 h), and evapotranspiration (41.5 to 175.2 mm; Table 1). The precipitation, maximum, minimum, and average temperatures are shown in Figure 1. In the growing season 2013 to

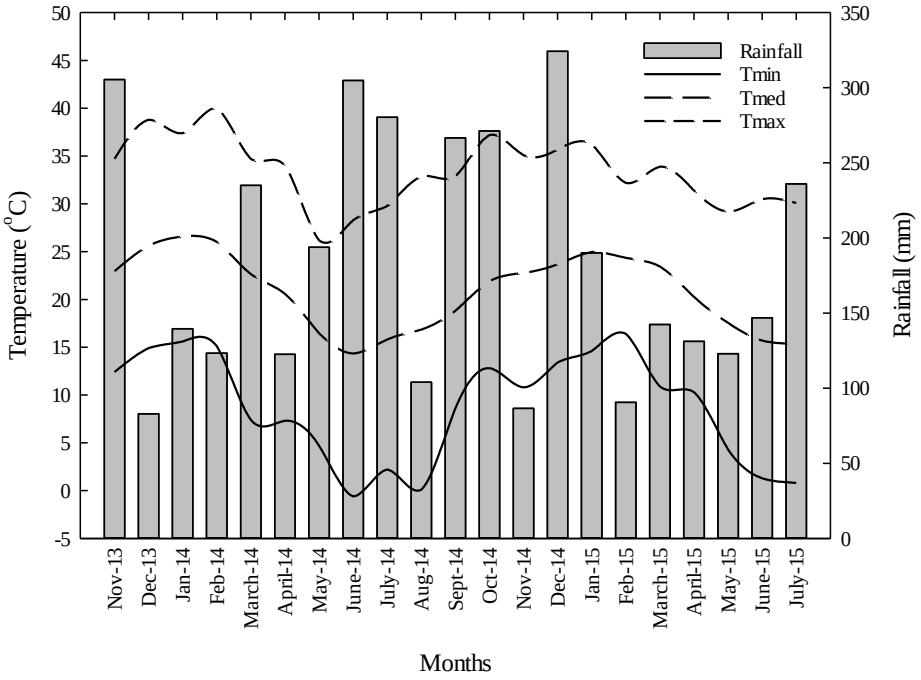

Figure1. Climograph of the experimental area, during the 2013 to 2014 and 2014 to 2015 growing seasons.

2014 and 2014 to 2015, minimum, average, and maximum temperatures were 0.6, 21.2, 40.0, 0.1°C, 20.5 and 37.2°C respectively, with greater variation in the first growing season. Maximum and minimum precipitation occurred in November and June, and December, respectively in the 2013 to 2014 growing season. In the second growing season, maximum and minimum precipitation occurred in December and November, respectively.

Treatments (0.3, 0.6, 0.9 and 1.2 of crop evapotranspiration) and a control treatment (rainfed) were arranged in a randomized complete block design with six repetitions. Each experimental unit had 20 m^2 (4x5m), and 600 m^2 of total experiment area, with plants on the edge. We used the variety RB93-5581, planted on November 14, 2015, row space of 1m, and continuous distributions of stems (3 to 4 buds per stem, totalizing 18 buds per meter) inside the furrow. The harvest occurred on 20 July 2014 and 8 June 2015 in the first and second growing season, respectively. In order to reduce experimental errors, sugarcane stems were divided into top, middle, and bottom parts, and each part was planted in two blocks.

Drip irrigation system was used, with drippers spaced 0.2 m and a flow rate of 0.8 L h^{-1}. In each experimental unit was installed a gate valve and a pressure control valve in order to control irrigation time and obtain regular pressure, respectively. Moreover, irrigation system uniformity and soil wetted volume tests were performed, following the results reported by (Padrón et al., 2015c). From day one up to 29 days after planting, water management was performed based on 1.0 of evapotranspiration for all treatments in order to have uniform emergence of the sugarcane. Subsequently, the irrigation treatments were started, performed every seven days and irrigation was finished 30 days before harvest.

The reference evapotranspiration was calculated on a daily scale, based on this results, different percentages were applied to set the irrigation by the formula of Penman-Monteith/FAO (Equation 1), and the crop evapotranspiration at a standard condition (Equation 2) (Allen et al., 1998). Climate data were obtained from the weather station of the Federal University of Santa Maria, National Institute of Meteorology, localized approximately 2000 m from the experimental area. Precipitation (mm), maximum and minimum temperature (°C), maximum and minimum air relative humidity (%), insolation (hours) and wind speed (m s^{-1}) were collected daily.

$$ETo = \frac{0.408\,\Delta(Rn - G) + \gamma \frac{900}{T + 278} U_2 (e_s - e_a)}{\Delta + \gamma (1 + 0.34\,U_2)} \quad (1)$$

where ETo is the reference evapotranspiration (mm day^{-1}), Rn, G, and T are net radiation value at crop surface (MJ m^{-2} day^{-1}), soil heat flux density (MJ m^{-2} day^{-1}), and daily mean air temperature at 2 m height (°C), respectively. Also, u_2, e_s e_a, (e_s - e_a), Δ and γ represent wind speed at 2 m height (m s^{-1}), saturation vapor pressure (kPa), actual vapor pressure (kPa), saturation vapor pressure deficit (kPa), slope of the saturation vapor pressure curve (kPa/°C) and psychrometric constant (kPa/°C), respectively.

$$ETc = kc \times ETo \quad (2)$$

Where ETc crop evapotranspiration (mm), kc single crop coefficient and ETo reference crop evapotranspiration (mm). Single crop coefficient values used were: Kc_{ini}= 0.40-25 day; Kc_{med}= 1.25-70 day and Kc_{fin}= 0.75-50 day (Allen et al., 1998).

Soils parameters such as chemical analysis, texture, the apparent density of soil, field capacity, and infiltration test were performed (Table 2) (Padrón et al., 2015c). Fertilizers were applied according to the chemical analysis of the soil and crop requirements to obtain a production of 80 to 100 t ha^{-1}. Moreover, 3.5 t ha^{-1} of lime was applied (Broadcast on the soil surface and

Table 2. Average soil attributes of the experimental area.

Soil layers (m)	Bulk density (g cm^{-3})	Field capacity (m^3 m^{-3})	Wilting point (m^3 m^{-3})	Water content (m^3 m^{-3})	Infiltration (mm h^{-1})	Texture
0-0.2	1.42	0.31	0.14	0.18		Loam
0.2-0.4	1.38	0.34	0.17	0.17	15.0	Clay-loam
0.4-0.6	1.36	0.37	0.23	0.13		Clay

disked) to correct soil pH.

The number of tillers was evaluated monthly, by counting in a linear meter. Samplings were performed in the central rows, evaluating 2 meters per row and three rows per experimental unit (six meters per plot). Plant height, number of green leaves, and leaf area were evaluated monthly on six randomized plants per plot, marked along the experiment. Plant height was measured from the base of soil up to leaf A+1, with a measuring tape. The number of green leaves was determined by counting fully expanded leaves, with a minimum of 20% of green area, counted from the leaf A+1. The leaf area was calculated using the methodology determined by Hermann and Câmara (1999) (Equation 3), numbering the leaves of each plant according to Kuijper and Van Dillewijn (1952). Measurements were performed by counting green leaves and with the long and width of the leaf+3, at the mid part. In each plot, leaf area was determined by the multiplication of the number of tillers in a linear meter.

$$LA = L \times W \times 0.75 \times (N + 2) \quad (3)$$

where: LA – Leaf area; C – Length of +3 leaf (m); L – Width of +3 leaf (m); 0.75 - correction factor for crop leaf area; N - number of open leaves with at least 20% of green area; 2 - correction factor. The total mass and dry matter content were determined in both harvests by selecting six randomized plants in each experimental unit. To determine total mass, plants were divided into top, leaves, and stem parts, measured individually using an analytical scale.

Subsequently, each stem was identified; juice extraction performed with a sugarcane mill machine, and the juice volume of each stem was measured using a graduated cylinder of 1000 ml. The bagasse mass, top, and plant leaves were oven dried at 75°C for 72 h, or until they reached a constant mass. Ten randomized plants per plot were used to determine sugarcane yield by measuring the base diameter (between first and second node from bottom) and stem height, with a graduated ruler and a Pocotest micrometer, respectively. The total yield of each experimental unit was determined according to Landell and Silva (2004) (Equation 4)

$$TSH = \frac{D^2 \times C \times H \times (0.007854)}{E} \quad (4)$$

where: TSH – Tons of sugarcane per hectare (t ha^{-1}); D – Base stem diameter (mm); C – Number of stem per linear meter; H – Stem height (m); E – Row space (m); 0.007854 – Correction factor.

Water productivity (WP) and irrigation water productivity (IWP) were calculated with the fresh total yield (kg ha^{-1}) divided by crop evapotranspiration (ETc) Equation 5 and total irrigation water applied Equation 6, respectively (Padrón et al., 2015c).

$$\mathrm{WP} = \frac{\text{Total yield (kg ha}^{-1})}{\text{evapotranspiration (mm)}} \quad (5)$$

$$\mathrm{IWP} = \frac{\text{Total yield (kg ha}^{-1})}{\text{Irrigation water applied (mm)}} \quad (6)$$

Weeds were controlled either by herbicides application or by manual pulling. Moreover, insecticides and fungicides were used to control insect pests and diseases, respectively. Statistical analysis were performed using the SPSS software package (SPSS V17.0).

RESULTS AND DISCUSSION

Evapotranspiration, effective rainfall, irrigation applications and days of irrigation are shown in Table 3. The cycle of sugarcane in the growing season 2013 to 2014 and 2014 to 2015 was 237 and 323 days, respectively, with a difference of 86 days. The evapotranspiration, precipitation, and days of irrigation were greater in the 2014 to 2015 growing season, with a difference of 312 mm, 147 mm, and 7 days, respectively. These differences might be attributed to the climate conditions of the region and crop cycle of the crop, once the irrigation levels were similar.

Dalri and Cruz (2008) studying ratoon cane reported that 26 irrigations were necessary, with a level of 520 mm for the growth period of the crop, performed on an average of 13.1 days. Yet, for the second ratoon cane 37 irrigations (740 mm for the life cycle), performed every 13.1 (average) days were necessary.

The study results are similar to those found by Nogueira et al. (2016), who determined the water demand for sugarcane production for a historic period of 20 years, using supplementary irrigation in Santa Maria-RS, (average of 645 mm and evapotranspiration of 1,310.75 mm). Several studies have demonstrated similar values of water demand in sugarcane: Neto et al. (2006) 1,164.0 mm; Almeida et al. (2008) 1,584.0 mm; Alves et al. (2008), 1,105.7 mm; Gava et al. (2011) for plant cane (1,095.0 mm) and ratoon cane (1,121.0 mm) and Silva et al. (2011), 1,710.0 mm for the whole cycle of production.

The relationship between yield and irrigation rates is shown in Figure 2. No significant results were found for the treatments in the same year; however, a significant difference at 5% of probability level was found between the years, which the second growing season had a greater yield. The difference between greater and lower yield in the first and second growing season was 11 and 7%, respectively. The maximum yields were 177.3 and

Table 3. Evapotranspiration, effective rainfall, irrigation, and days of irrigation during the growing seasons.

Treatment	2013-2014				2014-2015			
	Rainfall[Z] (mm)	ETc (mm)	Irrigation[Y] (mm)	Days irrigation	Rainfall[Z] (mm)	ETc (mm)	Irrigation[Y] (mm)	Days irrigation
0		869.9	-			1129.7	-	
0.3		260.9	126.6			338.9	125.7	
0.6	1362.6	521.9	253.2	17	1508.8	677.8	251.5	24
0.9		782.9	379.8			1016.8	377.2	
1.2		1043.8	506.4			1355.7	503.0	

[Z] Effective rainfall; [Y] Effective irrigation.

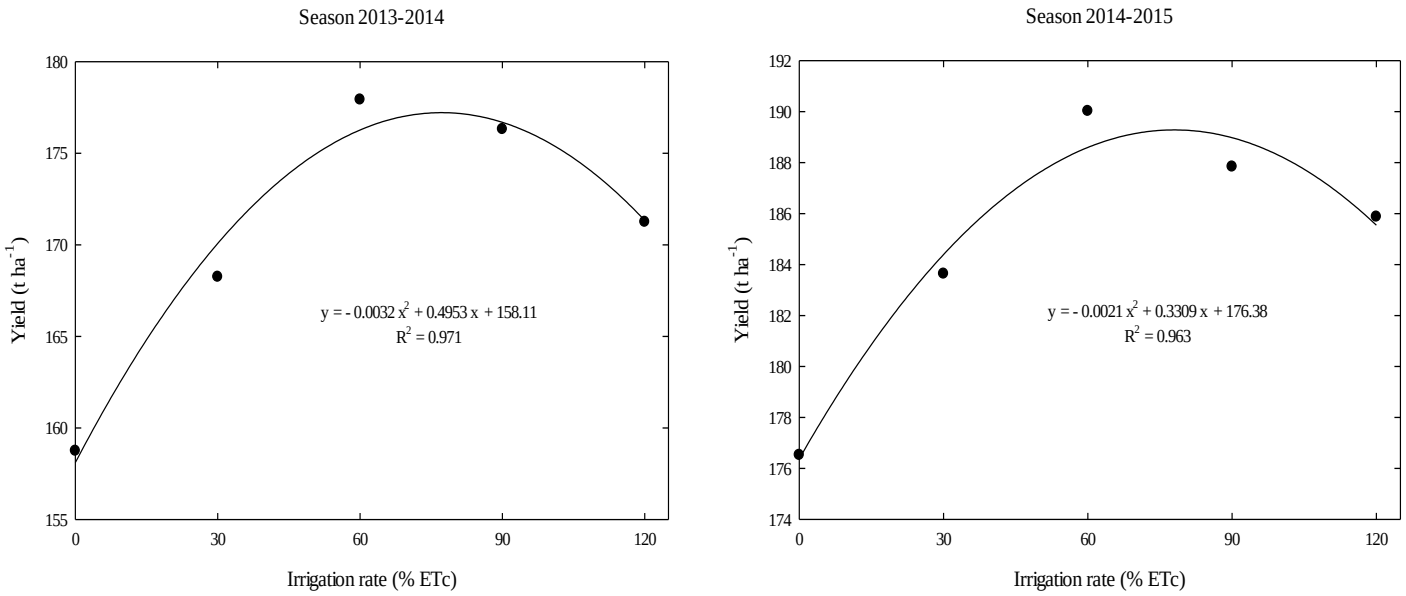

Figure 2. Sugarcane yield with irrigation rate applied as a fraction of crop evapotranspiration with different irrigation depths.

189.4 t h^{-1}, with a 0.77 and 0.79 of crop evapotranspiration in the first and second growing season, respectively. Moreover, the maximum technical efficiency in yield were 177.3 and 189.4 t h^{-1}, with 0.77 and 0.79 of evapotranspiration in the first and second growing season, respectively. The second crop cycle had an increase in yield (6.4%) and evapotranspiration (1.4%) when compared to the first crop cycle, being attributed to a longer life cycle.

Nogueira et al. (2015) studied 11 sugarcane varieties in Santa Maria-RS (dryland cropping system), had a minimum and maximum plant cane yield of 27.22 to66.30 t ha^{-1}, and ratoon cane yield of 35.3 to 149.22 t ha^{-1}, respectively. This small difference may be influenced by the row space (1.4 m) and varieties used. Raskar and Bhoi (2003) studied plant cane and ratoon cane found that yield with 0.9 m row space was significantly greater when compared to 0.3 and 0.6 m row space. Dalri et al. (2008) reported an increase in yield (48.57%) in treatments submitted to irrigation compared to rainfed treatments. Júnior et al. (2012) applying fertigation, reported an increase (49.5 t ha^{-1}; 33%) in irrigated conditions compared to rainfed treatments. Furthermore, Gava et al. (2011) studying three varieties, plant and ratoon cane with drip irrigation system reported an increase in yield of 20 and 28%, respectively. Similar yield results comparing irrigation and rainfed treatments were reported by Neto et al. (2006), Dalri and Cruz (2008) and Farias et al. (2008b).

Sánchez-Román et al. (2015) studying different water depths had highest yields with water replacement of 100% of field capacity. Wiedenfeld and Enciso (2008) studied different water managements and did not increase yield with water depths below the soil water storage capacity. Moreover, Vieira et al. (2014) reported that yield increased when water applied is increase with a maximum value of 112.3 t ha^{-1} (150% of evapotranspiration and of 1,537.2 mm; rainfall and

Table 4. Water productivity and irrigation water productivity the sugarcane with different irrigation depths.

Treatment	Irrigation water use efficiency (kg m^{-3})		Water productivity (kg m^{-3})	
	2013-2014	2014-2015	2013-2014	2014-2015
I_0	-	-	20.1	15.6
$I_{0.3}$	140.8	146.0	70.9	54.2
$I_{0.6}$	74.5	75.6	37.5	28.0
$I_{0.9}$	49.2	49.8	24.8	18.5
$I_{1.2}$	35.8	37.0	18.0	13.7

Table 5. Total mass, stem mass, and dry matter content of sugarcane submitted to different irrigation depths.

Treatment	2013-2014			2014-2015		
	Total mass (kg)	Dry matter (kg)	Stem mass (kg)	Total mass (kg)	Dry matter (kg)	Stem mass (kg)
I_0	1.246^{aB}	0.225^{aB}	0.808	1.523^{aA}	0.283^{aA}	1.143
$I_{0.3}$	1.283^{aB}	0.228^{aB}	0.813	1.628^{aA}	0.312^{aA}	1.238
$I_{0.6}$	1.285^{aB}	0.249^{aB}	0.832	1.656^{aA}	0.332^{aA}	1.251
$I_{0.9}$	1.311^{aB}	0.233^{aB}	0.860	1.617^{aA}	0.320^{aA}	1.249
$I_{1.2}$	1.274^{aB}	0.198^{aB}	0.853	1.613^{aA}	0.312^{aA}	1.247
Sig.	**	**	-	**	**	

irrigation). Yet, Farias et al. (2009) had sugar yield per unit area of less than 12.99 t ha^{-1}, with a total irrigation level of 1.221 mm (precipitation + irrigation), which corresponds to 100% of evapotranspiration. Oliveira et al. (2014) reported that 100% of water replacement increased productivity by 40% when compared with drought-stricken area management (water replacement of 0%) of 178 t ha^{-1}, and maximum curve peak had gross alcohol yield of 25.34 m^3 ha^{-1}, obtained with 79.7% of water replacement.

Carvalho et al. (2014) reported that an irrigation depth of 75% of evapotranspiration in ratoon cane increased (21.34%) when compared to 50% of evapotranspiration. Conversely, irrigation depth of 100% of evapotranspiration reduced yield by 53.4%. Water productivity and irrigation water productivity are shown in Table 4. Water productivity (WP) and irrigation water productivity (IWP) decreased as irrigation rates increased. The IWP was greater in the second growing season due to weather conditions and increase in yield. Conversely, WP was lower in the second growing season affected by the crop cycle. In both growing seasons, the IWP was similar, being attributed to the same irrigations levels, temperature variation, and evapotranspiration. Moreover, the WP was lower (both growing seasons) in the treatment of 1.2 of evapotranspiration, being similar to the rainfed treatment.

Oliveira et al. (2011a) reported an increase in water use efficiency (69.8% on average) when comparing rainfed and irrigated treatments, obtaining values of 70.1 kg m^{-3} and 140.3 kg m^{-3}, respectively. Doorembos and Kassam (1994) reported that sugarcane under irrigated conditions in the dry tropics and subtropics, in soils with 80% of available water, might yield between 5 to 8 kg m^{-3}. Farias et al. (2008b) determined the WP with the total volume of water applied (rainfall + irrigation), and found a difference of 3.23 kg m^{-3} between sugarcane treatments submitted to 100% of evapotranspiration (7.22 kg m^{-3}) and rainfed (3.99 kg m^{-3}) .

The total mass, stem mass, and dry matter content are shown in Table 5. No significant differences were found between the treatments, however, significant results were found between the growing seasons, with highest values for ratoon cane. The greater values for total mass were found in the treatments of 0.9 and 0.6 (cane plant and ratoon cane, respectively), an increase of 20% for ratoon cane. The dry mass content was greater in both growing season for the treatment of 0.6, with an increase of 20% for ratoon cane. The treatment of 0.9 (0.860 kg) and 0.6 (1.251 kg) had greater stem mass in the first and second growing season, respectively.

The study results are similar to those found by Neto et al. (2006), who studied levels of irrigation and nitrogen dose associated with potassium in ratoon cane, and concluded that growth parameters and the quality of ratoon cane were more influenced by fertilization than by irrigation. Yet, the same authors reported an average stem weight for the highest and lowest dose of fertilizer applied of 1,077.50 and 918.42 g, respectively. Moreover, Azevedo (2002) reported a mean weight of 917.00 g per

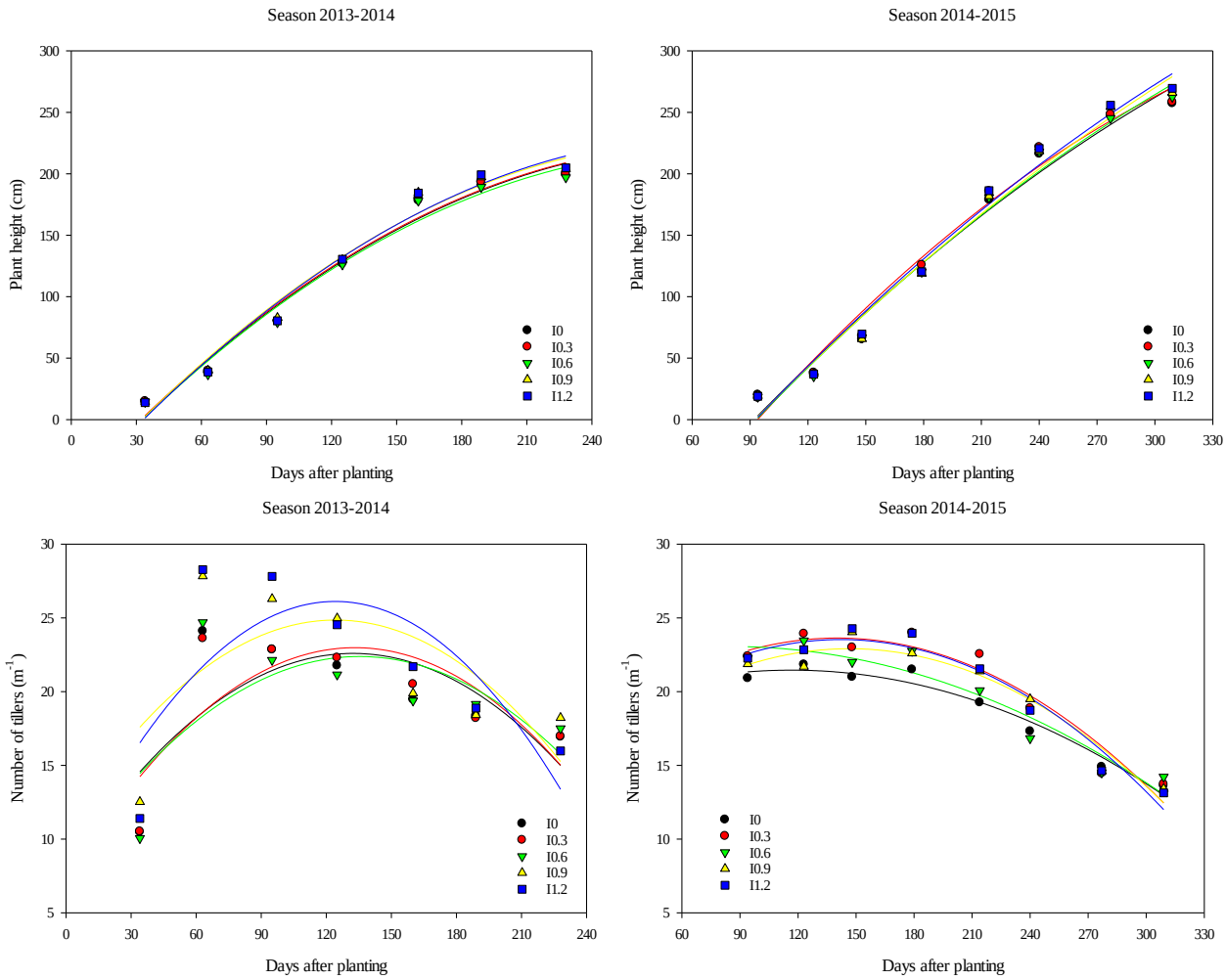

Figure 3. Plant height, and number of tillers with different irrigation depths.

stem in sugarcane plant. Likewise, Silva (2002) studying irrigated sugarcane under different fertilizer levels, reported an average stem weight of 1,384.17 g.

Plant height and number of tillers are shown in Figure 3. These variables did not differ between treatments, but had interaction between the years of study, having the highest value for ratoon cane. Maximum plant height was 227 and 196 cm for ratoon cane submitted to different doses of fertilizer. Moura et al. (2005) studied ratoon cane under different irrigation levels, reported that irrigation increased plant growth (25%) when compared to the rainfed treatments (2.34 and 1.87 m, respectively). Oliveira et al. (2011b) studied different varieties of sugarcane, fertilizer doses, and water deficit found that plant height (average of 3.083 m) did not have a significant difference between treatments. Yet, the average stem height was 359.1 cm (Carmo et al., 2010). Silva et al. (2008) analyzing plant cane growth found values of 280.7 cm, whereas, Farias et al. (2008a) studying the same variety in irrigated and rainfed system, 269.5 and 205 cm in plant cane and ratoon cane, respectively, in the treatment submitted to 0.9 of evapotranspiration. The difference between maximum and minimum height was 3% for the plant cane and 5% for ratoon cane, (increased 24.1% over the seasons), which might be influenced by the crop cycle.

Neto et al. (2006) reported an average plant height of reported that cane plant growth stabilized after 193.85 days (irrigation condition; height of 152. 80 cm), and 236.20 days (rainfed; height of 148.19 cm). Souza et al. (2015) reported that increasing irrigation level to 100% of evapotranspiration increased plant height when compared to the rainfed treatments, yet with total irrigation depths of 1, 177.33 and 568 mm, plant height were 2.66 m and 1.99 m, respectively.

The highest number of tillers per meter was 28 in plant cane, at 63 days after planting and 24 in ratoon cane (148 days after cutting) in the treatment I1.2. The lowest value was in the rainfed treatment, cane plant (23 tillers) and ratoon cane (21 tillers). At harvest of each cycle, the

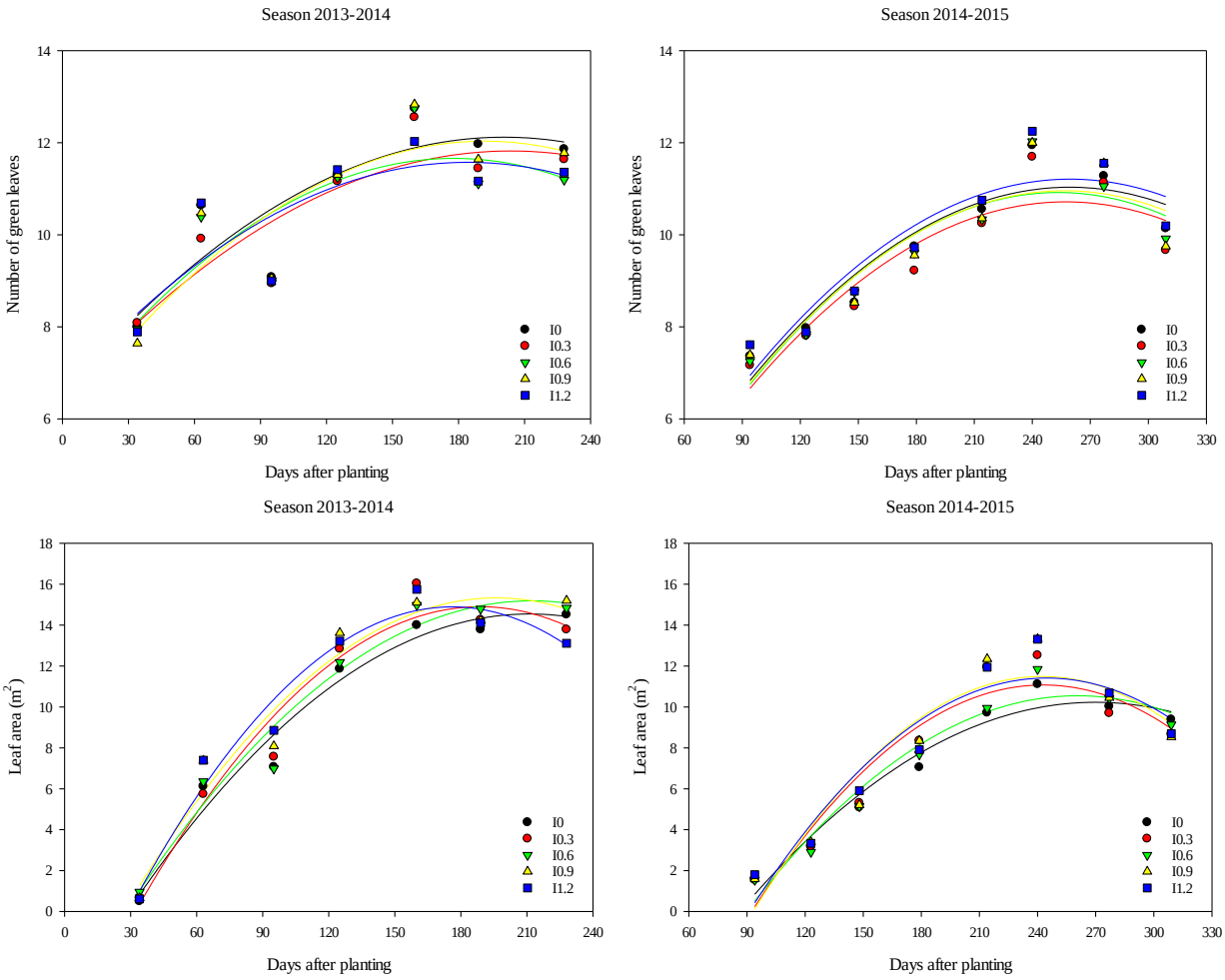

Figure 4. Number of green leaves and leaf area the sugarcane with different irrigation depths.

greatest number of tillers (18) in plant cane (treatment I0.9) and 14 in ratoon cane (treatments I0.6 and I1.2). The lowest were 16 and 13 in the plant cane and ratoon cane, respectively. Watering early in the cycle increased the number of tillers, and at the end of the sugarcane cycle, the excess of moisture affects development. Thereby, Carmo et al. (2010) reported that in early development, under irrigated system and high availability of solar radiation, there is an intense tillering at the beginning of the cycle since there is water, energy, and space for plant growth. Yet, the number of tillers was greater at the beginning of sugarcane cycle, but fell abruptly when the culture demonstrated a tendency toward stabilization in the number of tillers, which occurred about 327 days after cutting.

The number of green leaves and leaf area are shown in Figure 4. The largest number of green leaves (13 in plant cane) was at 160 days after planting (treatment I0.9), and it was 12 green leaves in ratoon cane, at 240 days after cutting (I1.2 treatment). At each harvest cycle, the number of green leaves demonstrated homogeneity in all treatments, with values of 11 to 12 and 10 in plant cane and ratoon, respectively. No significant effect of irrigation was observed for the number of green leaves, yet it was greater for plant cane. Silva et al. (2015) highlight that the mature sugarcane has a number of green leaves per plant around ten, depending on the variety and growing conditions. Pincelli (2010) states that the variable number of green leaves is important because through this we can observe the photosynthetic efficiency of the plant in advance of the stresses (deficit or water excess). In addition, Machado et al. (2009) observed that water deficit causes leaf senescence and restriction to the emergence of new leaves. However, the degree of these changes is due to the intensity of water stress and depends on the genotype (Smit and Singels, 2006).

The irrigation treatments influenced leaf area and leaf development in both growing seasons. The treatment I1.2 had the greatest development at the beginning of the cycle and decrease towards the end. Conversely, rainfed treatments had reduced development at the beginning and increase towards the end. The rate of the plant and

ratoon cane growth were 7.5 dm^2 day^{-1} and 3.6 dm^2 day^{-1}, respectively. There was a decrease in both growing seasons, by 15% in the development of the crop, and 33% at harvest, being the ratoon cane lower. Silva et al. (2015) reported similar results with great values to the plant cane.

Machado et al. (2009) found that for irrigation purposes, the period of greatest susceptibility to water deficit is the rapid development of the crop. At this time, the plants have great leaf area and require more water to make the gas exchange with the atmosphere (Pires et al., 2008). Farias et al. (2008a) reported that leaf area ranged significantly over the growing season for rainfed sugarcane, with a maximum leaf area of 5,168.04 dm^2 (at 166.68 days and growth rate of 31.00 dm^2 d^{-1}).

In addition, the sugarcane cultivated under irrigated conditions reached the point of maximal leaf area (5,359.65 dm^2) at 152.63 days (growth rate of 35.11 dm^2 d^{-1}). Souza et al. (2015), reported the greatest leaf area was obtained in the treatment submitted to 100% of evapotranspiration (LA = 2,461.62 cm^2). According to the climatic conditions of the region, comparing rainfed and irrigated sugarcane, there was an increase in sugarcane yield under irrigation system, thereby justifying investments in irrigation system as a supplementary strategy for the agriculture.

Furthermore, studies on optimizing water resources and maximization of crop yields ensuring economic returns in agriculture are necessary. The deficit and the excess of moisture affect the vegetative development of sugarcane, affecting plant height, leaf area, stem diameter, the number of tillers per meter, total and dry mass content, which might have negative effects on sugarcane yield. Moreover, the experimental design adopted for planting sugarcane, dividing the stem in the tip, mid and base parts, reduced experimental errors and homogenized the blocks. Yet, stem tip had highest emergence and development at initial phase and middle part stem had the greatest development at the end of the cycle in the first growing season, and no differences were observed for ratoon cane.

Conclusions

The irrigation affects positively the vegetative growth of sugarcane, increasing plant height, stem diameter, the number of tillers per meter, and leaf area. The determination of the evapotranspiration rate increases yield, ensuring the efficient use of irrigation water and the profitability of the crop. The irrigation rate of 78% of evapotranspiration as an irrigation strategy for sugarcane production might be adopted to obtain stable yields.

Conflict of Interests

The author has not declared any conflict of interests.

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