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Full Length Research Paper

Effects of drought on yield and yield components of two contrasting growth habits of common beans (*Phaseolus vulgaris* L.)

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Common bean is grown by smallholder farmers in Malawi who produce low yield due to drought constrains. Two experiments comprising of two different bean growth habits (IIa and IVa) were conducted at Chitedze Research Station in Malawi, to assess eleven common bean genotypes for yield and yield components under drought conditions. Drought reduced significantly number of pods plant^{-1}, number of seeds pod^{-1} and consequently seed yield in both growth habits. Within growth habit IIa, the most productive genotype was BCB 2, that gave highest yield in both irrigated and drought stress conditions, also presented a higher yield percentage reduction (51.13%) after Sugar 131 (53.11%) implying highest drought susceptibility. On the other hand, VTTT 923/10-3 (growth habit IIa) had the highest drought tolerance (32.40%). Under growth habit IVa, genotype MAC 109 was found to be the most drought tolerant (31.43%) under drought and 12D/2 highest drought susceptibility (50.92%). The results suggest that selection for genotypes with higher number of pods plant^{-1}, number of seeds pod^{-1} and low yield percentage reduction might improve grain yield under drought stressed condition.

Key words: *Phaseolus vulgaris* L., drought, growth habits, yield components.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop with several uses; increasing food and reducing poverty, providing health and nutritional security, stability and provides a stable and lucrative source of income for many rural households (FAO, 2014) and also enhancing ecosystem resilience (Beebe, 2001). It is estimated that two-thirds of common bean production in the world occur under drought conditions (Beebe, 2008). Drought is the

second most important factor in yield reduction after diseases (Rao, 2014). Malawi depends on agriculture where in every four years; farmers are experiencing crop losses due to drought (Kambewa, 2003). Beebe et al. (2011) reported that occasional severe droughts affecting Malawi are often associated with El Niño weather events. Farmers relying on rain fed agriculture are no more producing the required quantities from their fields even providing all the necessary crop inputs. While are challenges with production and productivity, the demand for food continues to increase year by year. There is need to produce more due to the continued population increase and the demand for food.

According to World Bank (2014), the Malawian population was at around 17 million and the World population was pegged at around 7.2 billion in the same year from around 6.1 billion in the year 2000, which means that the growth is significant year by year.

Climate change has resulted in change in food production patterns due to higher temperatures, increase carbon dioxide in the atmosphere, change of precipitation patterns, and increased vulnerability of the landless and the poor. A number of indirect techniques have been used for the evaluation of drought tolerance; however, seed yield is the most consistent indicator because it represents the harvestable product White et al. (1992) reported that the understanding of the relationship between yield and its components is important for making the best use of these relationships in breeding and selection.

The need for climate change adaptation measures has become more necessary to ensure farmers continue to produce for their own food security and that of the growing population (Chirwa, 2007). One such adaptation measure is to produce tolerant genotypes which can survive, yield better and adapt in different environments and to develop strategies to cope with cost-effective drought management techniques particularly for the poor smallholdes farmers who cannot afford to irrigate (Cavalieri, 2011).

Thus, a selected number of bean genotypes which were developed at International Center of Tropical Agriculture (CIAT) and at Lilongwe University of Agriculture and Natural Resources (LUANAR), with different growth habits were studied in a translucent plastic screening house at Chitedze Research Station. The main objective was to evaluate yield and yield components performance under drought condition.

MATERIALS AND METHODS

The study was conducted at Chitedze Research Station which lies 1100 masl, latitude 13°85′S and longitude 33°38′E. Chitedze has a mean annual temperature of 20^0C. Maximum temperatures are more than 24°C in November and lowest below 16°C in July. The station receives a mean annual rainfall of 892 mm, 85% of which falls between November and March. A pot experiment was used because it is easy to have uniform management of treatments in

terms of soil fertility and distribution of water.

Germplasm description

Eleven common beans genotypes with two different growth habits (types IIa and IVa) were used in the experiment. Types IIa have determinate growth with stem and branches ending in a reproductive guide. Types IVa are indeterminate with excessively long stems and branches making them weak and ending in vegetative long stems (Singh, 1995).

Experimental design and treatments

The experimental design was split-splot, laid out on completely randomized design (CRD). Two levels of water conditions (irrigated and drought) were the main plots and 11 genotypes namely : BCB 2 (Bunda 2), DRK 57, Sugar 131, VTTT 923/10-3, VTTT 924/4-4, VTTT 925/9-1-2, 12D/2, CIM-Climb 01-03-40, DC 86-263, Kanzama and MAC 109 were the sub-plots, making a total of 22 treatments. Each treatment (experimental unit) was composed of four pots, making a total of 88 pots per replicate.

Experimental management

Plants were grown from March to July in 5 litters plastic pots (17.7cm high × 21.2 cm diameter) carefully packed with 4.8 kg of sand clay loam soil. The whole trial was irrigated to field capacity one day before sowing. Four uniform seeds were sown 5cm deep in each pot and then thinned to three plants at two leaf stage. The pots were watered at two-day intervals to replace the water lost by evapotranspiration, keeping the pot soil moisture at 80% of field capacity. Watermark model 200SS-15 soil moisture sensor with cable was used for soil moisture measurement. A compound fertilizer (23% N: 21% P_2O_5: 0% K_2O +4% S) was applied at a rate of 30 kgha[-1] during the trifoliate leaf of the crop growth stage. Each pot was supplied with 0.64 g for fertilizer as a basal dressing, aiming to improve plant vigor. Hand weeding was done when necessary. Drought was imposed when 50% of the genotypes in each growth habit had flowered.

Data collection

Parameters such as: soil data, days to 50% flowering, days to 90% physiological maturity, number of pods plant [-1], number of seed plant [-1], pod length and seed yield were recorded. Percent reduction (change) in parameters measured under water-stress was derived from the difference in parameters' values between non- stress and stress conditions (Basal et al. 2006) as follows:

$$\text{Percent reduction}(\%) = \frac{(\text{Nonstress water regime} - \text{water stressed regime}) \times 100}{\text{Non-stress water regime}}$$

Soil data

Soil was analyzed for physical and chemical properties in a soil laboratory at Lilongwe University and Natural Resources (LUANAR) for nitrogen, phosphorus, pH, soil texture and organic matter, and at Agriculture extension Research (ARET) soil laboratory for Potassium, Calcium, iron and zinc. The methods used to analyze the soil were: Dispersal and hydrometric readings, Anderson and Ingram (1993), pH Extractable method Maclean (1982) and total N with Micro-Kjedahl digestion distillation Bremner and Mulvanay (1982). Potassium and phosphorus were determined using Mehlich

three extraction methods (Chilimba et al., 2011). The soil had the following characteristics: pH, 5.35; OM%, 3.8; N%, 0.14; P (ppm), 55.4; K (meq100 g^{-1}), 0.35; Mg (meq100 g^{-1}), 0.017; Fe (ppm), 8.2; Cu (ppm), 2.0, Zn (ppm), 4.0; and Mn (ppm), 8.47.

Statistical data analysis

The statistic model used in the experiment was:

$$Y_{ijk} = \mu + G_i + W_j + (GW)_i + (GW)_j + E_{ij}$$

Where: Y_{ijk} = the response of ith genotype within j^{th} water regime in the kth replicate (k= 1, 2), μ = the overall mean, G_i = the fixed effect of ith genotype (i = 1, 2, 3….11), W_j= the fixed effect of jth water regime (j= 1, 2), Gw_{ij} = fixed effect of interaction of genotype x water regime, E_{ij} = random deviation of the kth replicate from the average of genotype x water regime.

Analysis of variance (ANOVA) was conducted using General statistics (GenStat 17th edition) to test for differences among genotypes, water conditions and interactions between genotypes and water conditions effects (Montgomery, 2001). Treatment means were separated using Fisher's protect Least Significant Difference (LSD) test at P<0.05.

RESULTS AND DISCUSSION

Phenological data within growth habit IIa

There was no significant difference (P>0.05) on days to 50% flowering due to water stress but there was significant differences among genotypes (Table 1). The earliest flowering genotype was noted in Sugar 131 (25.00 days) followed by VTTT 925/9-1-2 (26.00 days) and delayed flowering (27.00) was found in the genotype BCB2, DRK 57, VTTT 923/10-3, and VTTT 924/4-4. According to Schmalenbach et al. (2014) early flowering genotype is a common drought escape strategy that ensures plant survival under severe water deficit; still, early flowering shortens the time available for carbon assimilation during vegetative development and, thus, possibly results in yield reduction. The effect of water condition and genotypes interaction at 50% flowering were not significant (P>0.05) among the genotypes. The results from growth habit IIa showed significant differences between number of days to 90% physiological maturity in different bean genotypes. BCB 2, DRK 57 and VTTT 924/4-4 were the earliest maturing bean genotypes in 70.0, 74.0 and 74.0 days, respectively. Genotypes which have traits of shorter maturity normally escape terminal drought compared to longer days to maturing.

Effect of drought on yield components of common bean genotypes within growth habit IIa

The effect of drought on number of pods plant[-1] and pod length was significant (P<0.01) different for genotypes and water conditions effect (Table 2). Results indicated that production of pods was less under drought condition as compared to irrigated condition in all genotypes. However, BCB2 had higher number of pods (18.00) followed by Sugar 131 (13.50) and the lowest number of pods were observed on DRK 57 (8.00). A similar result was also reported by Beebe et al. (2012) in common bean genotypes. This finding is also in agreement with Nielsen and Nelson (1998) who observed a reduction in the number of pods in plants subjected to drought; that effect could have been due to ovule or pollen abortion (Kokubun et al. 2001) and also due to increase of ethylene (ABA) production when plants are subjected to drought causing flowers abortion. The mean number of pods plant[-1] was reduced by 30% and number of seeds plant[-1] by 21%, these suggest that reduction of seed yield in drought conditions is mainly due to number of pods plant[-1]. The reduction of number of pods under drought in this study was probably due to limited assimilate supply under drought condition, as reported by Leport et al. (2006) in legumes, including common bean varieties.

The overall means of pod length under drought was (6.42 cm) compared with (8.08 cm) under irrigated condition. Higher pod length was observed in BCB 2 (9.00 cm), followed by VTTT 925/9-1-2 (8.50 cm). Sugar 131 and DRK 57 had lowest pod length (7.00 cm). Acquaah (2007), report that when the genetic materials are different, there is also huge variability within them. Hence, the results in pod length on this study are in accordance to (Acquaah 2007).

Analysis of variance indicate that number of seeds pod[-1] and seed yield (g plant[-1]) were significantly (P<0.01) affected by the water condition. The interaction between genotypes and water condition on number of seeds pod[-1] and seed yield (g plant[-1]) were also significant (P<0.05), suggesting a great amount of variability for drought tolerance in bean genotypes under study. Results are presented in Table 3.

The overall means of number of seed pod[-1] under drought was (6.17) compared with (7.83) under irrigated condition resulting in a 21.58% reduction. Average seed yields were 9.44 and 17.39 g plant[-1] under drought and irrigated conditions, respectively. The percentage reduction was 45.72%. However, genotype VTTT923/10-3 had the highest drought tolerance (32.40%), followed by VTTT 925/9-1-2 (40.55%), VTTT 924/4-4 (46.15%), DRK 57 (46.70%). Both BCB2 (51.13%) and Sugar 131 (53.11%), had more than 50% reduction. Genotype BCB 2 showed second highest drought susceptibility level, after Sugar 131, though it produced the highest seed yield under drought condition. The highest seed yield of BCB2 may be attributed to high number of pods plant[-1] and number of seeds pod[-1]. Furthermore, Rosales-Serna et al. (2004), also reported that drought resistant genotypes that display high yield under stress are more efficient in photoassimilate remobilization and this difference in seed yield among common bean cultivars under drought can be associated with physiological and

Table 1. Days to 50% flowering and days to 90% physiological maturity within growth habit IIa, recorded in a translucent plastic screen house at Chitedze during March to June 2015

Genotypes	Days to 50% flowering			Days to 90% physiological maturity		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
BCB 2	27.00	25.50	26.25^a	78.50	70.00	74.25^c
DRK 57	27.00	25.00	26.00^a	75.50	74.00	74.75^c
Sugar 131	25.00	24.00	24.50^b	77.50	75.50	76.50^b
VTTT 923/10-3	27.00	25.00	26.00^a	80.00	76.00	78.00^a
VTTT 924/4-4	27.00	26.00	26.50^a	76.00	74.00	75.00^c
VTTT 925/9-1-2	26.50	24.50	25.50^{ab}	78.50	74.50	76.50^b
Grand Mean	26.58	25.00	25.79	77.67^A	74.00^B	75.83
CV (%)	2.9			0.70		
G	*			**		
W	NS			*		
G*W	NS			**		

CV= Coefficient of variation, G = Genotype, W= Water conditions, G*W= Genotype versus water conditions interaction, NS = Not significant, *= significant at P<0.05. Means followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

Table 2. Effect of two water conditions on number of pods plant $^{-1}$ and pods length (cm) of six bean genotypes (growth habit IIa) in a translucent plastic screen house at Chitedze Research Station during March to June 2015

Genotypes	Number of pods plant $^{-1}$			Pod length (cm)		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
BCB 2	18.00	11.00	14.50^a	9.00	7.50	8.25^a
DRK 57	10.00	8.00	9.00^d	7.00	5.00	6.00^d
Sugar 131	13.50	9.50	11.50^b	7.00	5.00	6.00^d
VTTT 923/10-3	11.00	8.50	9.75^{cd}	8.00	7.00	7.50^c
VTTT 924/4-4	13.00	9.00	11.00^{bc}	9.00	7.00	8.00^{ab}
VTTT 925/9-1-2	12.50	8.50	10.50^{bcd}	8.50	7.00	7.50^{bc}
Grand mean	13.00^A	9.08^B	11.04	8.08	6.42	7.21
CV (%)	9.8			4.0		
G	**			**		
W	*			NS		
G*W	NS			NS		

CV= Coefficient of variation, G = Genotype, W= Water condition, G*W= Genotype versus water conditions interaction, NS = Not significant, *= significant at P<0.05. Means followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

biochemical responses, such as tissue water retention, osmotic adjustment, integrity of membrane system, protease activity and stomata adjustment (Lizana et al.,2006). Genotypes and water conditions interaction had showed significant differences (P<0.05), implying that drought had decreased seed yield in all genotypes.

Phenological data within growth habit IVa

There was no significant difference (P>0.05) in days to 50

% flowering and 90% physiological maturity due to water stress condition (Table 4).

The earliest flowering genotype was noted in DC 86-263 (30.00 days) followed by MAC 109 (31.50 days) and delay flowering (38.00 days) was found in the genotype Kanzama. Early flowering indicates short life cycle and is considered a positive character for improvement of genotypes. Results under this study are in agreement with Oladosu et al. (2014) in a similar work on rice. The same scenario was observed on days to physiology maturity (Table 4).

Table 3. Effect of two water conditions on number of seed pod [-1] and seed yield (g plant [-1]) of growth habit IIa in a translucent plastic screen house at Chitedze Research Station during March to June 2015

Genotypes	Number of seeds pod [-1]			Seed yield (g plant [-1]) at 14% moisture		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
BCB 2	10.00	8.00	9.00[a]	30.00	14.66	22.33[a]
DRK 57	6.00	4.00	5.00[c]	10.00	5.33	7.67[c]
Sugar 131	6.00	4.00	5.00[c]	13.50	6.33	9.92[bc]
VTTT 923/10-3	8.00	7.00	7.50[b]	14.66	9.91	12.29[b]
VTTT 924/4-4	9.00	7.00	8.00[b]	19.50	10.50	15.00[ab]
VTTT 925/9-1-2	8.00	7.00	7.50[b]	16.67	9.91	13.29[b]
Grand Mean	7.83[A]	6.17[B]	7.00	17.39[A]	9.44[B]	13.41
CV (%)	7.8			9.7		
G	**			**		
W	*			*		
G*W	*			*		

CV= Coefficient of variation, G = Genotype, W= Water condition, G*W= Genotype versus water condition interaction, NS = Not significant, *= significant at P<0.05. Means followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

Table 4. Days to 50% flowering and days to physiological maturity within growth habit IVa, recorded in a translucent plastic screen house at Chitedze during March to June 2015

Genotypes	Days to 50% flowering			Days to 90% physiological maturity		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
12D/2	36.00	34.00	35.00[c]	87.00	85.00	86.00[c]
CIM-Climb 01-03-40	38.00	36.00	37.00[b]	89.00	87.00	88.00[b]
DC 86-263	32.00	30.00	31.00[e]	83.00	81.00	82.00[e]
Kanzama	40.00	38.00	39.00[a]	91.00	89.00	90.00[a]
MAC 109	34.00	31.50	32.75[d]	85.00	82.50	83.75[d]
Grand Mean	36.00	33.90	34.95	87.00	84.90	85.95
CV (%)	3.5			1.5		
G	*			*		
W	NS			NS		
G*W	NS			NS		

CV= Coefficient of variation, G = Genotype, W= Water condition, G*W= Genotype versus water conditions interaction, NS = Not significant, *= significant at P<0.05. Means followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

Effect of drought on yield components of common beans genotypes within growth habit IVa

Analyses of variance had shown that number of pods plant[-1] and pod length (cm) were significantly affected water condition. Genotypes and water condition interaction did not affect pod length (Table 5). However, interaction between genotypes and watering regimes was significant (P<0.05). Genotype 12D/2 had more number of pods plant[-1] (11.00), followed by CIM-Climb 01-03-40 (10.50), Kanzama and DC 86-263 (10.00), respectively. MAC 109 genotype had less number of pods plant[-]

[-1] (9.00).

Graham and Ranalli (1997) found that drought results in many phenotypic changes in the plant development including reducing number of pods[-1], number of seed plant[-1] and pod lenght. Under drought conditions, Kanzama, 12D/2 and CIM-Climb 01-03-40 had higher pod length with means of 8.50, 8.00 and 7.50 cm, respectively. DC 86-263 had lowest pod length (7.00 cm).

There were significant differences (P<0.05) for number of seeds pod[-1] and seed yield (g plant[-1]) Results are presented in Table 6, suggesting that indirect selection for this trait is possible. Number of seeds pod[-1] were

Table 5. Effect of two water conditions on number of pods plant $^{-1}$ and pods length (cm) of six bean genotypes (growth habit IVa) in a translucent plastic screen house at Chitedze Research Station during March to June

Genotypes	Number of pods plant $^{-1}$			Pod length (cm)		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
12D/2	18.50	11.00	14.75[a]	9.50	8.00	8.75[ab]
CIM-Climb 01-03-40	15.50	10.50	13.00[b]	9.00	7.50	8.25[bc]
DC 86-263	12.50	10.00	11.25[c]	8.50	7.00	7.75[c]
Kanzama	11.00	10.00	10.50[c]	10.00	8.50	9.25[a]
MAC 109	10.50	9.00	9.75[c]	9.50	7.50	8.50[abc]
Grand Mean	13.60	10.10	11.85	9.30	7.70	8.50
CV (%)	7.3			7.0		
G	**			**		
W	NS			NS		
G*W	*			NS		

CV= Coefficient of variation, LSD= Least significance difference, ns = not significant, *= significant at P<0.05, G*W= Genotype versus water conditions interaction. Means and standard errors followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

Table 6. Effect of two water conditions on number of seed pod $^{-1}$ and seed yield (g plant $^{-1}$) of growth habit IVa in a translucent plastic screen house at Chitedze Research Station during March to June 2015

Genotypes	Number of seeds pod $^{-1}$			Seed yield (g plant) at 14% moisture		
	Irrigation	Drought	Mean	Irrigation	Drought	Mean
12D/2	11.50	9.50	10.50[a]	35.50	17.42	26.46[a]
CIM-Climb 01-03-40	9.50	9.00	9.25[a]	24.42	15.75	20.09[b]
DC 86-263	9.50	8.00	8.75[a]	19.83	13.33	16.58[ab]
Kanzama	7.00	5.00	6.00[b]	12.83	8.33	10.58[c]
MAC 109	10.00	8.00	9.00[a]	17.50	12.00	14.75[b]
Grand Mean	9.50	7.90	8.70	22.02	13.37	17.69
CV (%)	7.0			7.7		
G	**			**		
W	NS			NS		
G*W	NS			**		

CV= Coefficient of variation, G = Genotype, W= Water condition, G*W= Genotype versus water conditions interaction, NS = Not significant, *= significant at P<0.05. Means followed by the same letter are not statistically significantly different at 5% level of significance. Lowercase letters compare genotypes and capital letters compare water conditions levels.

different under irrigation condition, but not under drought, meaning that drought did not affect the number of seeds pod^{-1}. However, genotypes 12D/2 (9.50), CIM-Climb 01-03-40 (9.00) had more seeds. Kanzama had least seeds pod^{-1} (5.00).

Drought had reduced yield by 39.29% (Table 7) under growth habit IVa. However, genotype MAC 109 had the highest drought tolerance (31.43%), DC 86-263 (32.78%), Kanzama (35.07%) and CIM-Climb 01-03-40 (35.50%). The genotype 12D/2 has been found to be most drought susceptible (50.93%).

Comparison between group habit IIa and IVa

In general, growth habit IIa had produced less yield and yield components compared with IVa. Yield and yield components are effective traits used in breeding programs. From this study, the lowest number of pods plant^{-1} was DRK 57 (10.00 and 8.00) under irrigated and drought conditions, respectively. DRK 57 and Sugar 131 had least number of seeds pod^{-1} (6.00 and 4.00) under both conditions, respectively. The same genotypes had least pod length (7.00 and 5.00 cm). In terms of seed

Table 7. Percentage reduction within all growth habits in a translucent plastic screen house at Chitedze Research Station during March to June 2015

Genotypes	Growth habit	Irrigated	Drought	% Reduction
BCB 2	IIa	30.00	14.66	51.13
DRK 57	IIa	10.00	5.33	46.70
Sugar 131	IIa	13.50	6.33	53.11
VTTT 923/10-3	IIa	14.66	9.91	32.40
VTTT 924/4-4	IIa	19.50	10.50	46.15
VTTT 925/9-1-2	IIa	16.67	9.91	40.55
Mean		17.39	9.44	45.71%
12D/2	IVa	35.50	17.42	50.93
CIM-Climb 01-03-40	IVa	24.42	15.75	35.50
DC 86-263	IVa	19.83	13.33	32.78
Kanzama	IVa	12.83	8.33	35.07
MAC 109	IVa	17.50	12.00	31.43
Mean		22.02	13.37	39.29%

yield (g plant -1) DRK 57 had least yield (10.00 and 5.33g plant -1) under irrigated and drought condition, respectively. The highest on this group was BCB2 genotype with the follow values: number of pods (18.00 and 11.00) under irrigated and drought condition; number of seeds pod -1 (10.00 and 8.00); pod length (9.00 and 7.50 cm) and seed yield of (30.00 and 14.00 g plant -1) under irrigated and drought conditions, respectively. On other hand, the lowest number of pods plant -1 in growth habit IVa was MAC 109 (10.50 and 9.00) under irrigated and drought conditions, respectively. Lowest number of seeds pod -1 was recorded on Kanzama (7.00 and 5.00) under irrigated and drought, respectively. Lowest pod length was from DC 86-263 (8.50 and 7.00 cm), under irrigated and drought conditions. Lowest seed yield was from Kanzama (12.83 and 8.33 g plant-1), under irrigated and drought conditions. Genotype 12D/2 had highest number of pods plant -1 (18.50 and 11.00) and number of seeds pod -1 (11.50 and 9.50) under irrigated and drought conditions. The highest pod length was Kanzama (10.00 and 8.50 cm), under irrigated and drought conditions, respectively. The highest yield was 12D/2 (35.50 and 17.42 g plant-1) under both water conditions. VTTT 923/10-3 (growth habit IIa), MAC 109 and DC 86-263 (growth habit IVa) were more tolerant to drought than the rest. The genotype that was most susceptible to drought was BCB2 under growth habit IIa and 12D/2 under growth habit IVa.

CONCLUSION

Common bean is an important crop for food and reducing poverty for most of Malawian population. Development of drought tolerant genotypes is crucial important to produce crops which can yield better and adapt in different environments in Malawi. Drought has been found to,

considerably, reduce the number of pods plant -1, number of seeds pod -1 and final yield. Yield performance under growth habit IVa was greater than in growth habit IIa. The findings indicate that selection for growth habit IVa would be useful to improving yield under drought condition.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Forage yield and nutritive value of naturally growing *Brachiaria decumbens* as undergrowth to an aroeira tree stand in a silvopasture system

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This study evaluated the forage yield and feed chemical composition of a *Brachiaria decumbens* natural pasture as undergrowth to a *Myracrodruon urundeuva* (aroeira) tree stand in a silvopasture system at UNESP/Ilha Solteira, Selvíria, Mato Grosso do Sul, Brazil. Sampling was conducted in a completely randomized block design with a factorial scheme of two plots and two sub-plots, with plots as the light regimen (full sunlight or shade from aroeira trees, spaced 3 × 3 m) and sub-plots as the grazing rotation scheme (pre-grazing and post-grazing). Sampling was performed along the four seasons of a year in four replicates. Canopy height, forage mass, morphological composition, nutritive value, and forage digestibility (whole-plant sample) were evaluated. Forage mass was significantly higher in the full sun area (1,306 kg DM/ha) than in the shaded site (727 kg DM/ha). Forage yield was low during the experimental period (1,529 and 58 kg/ha in the full sun and shaded sites, respectively). The nutritive value of *B. decumbens* was not significantly different between light regimens. Growing *B. decumbens* as undergrowth in aroeira stands may be an option in areas where the trees still occur, but other planting densities should be examined.

Key words: Forage yield, *Myracrodruon urundeuva*, rising plate meter, shading, sward height.

INTRODUCTION

Aroeira, *Myracrodruon urundeuva* Fr. All. (Anacardiaceae) is an endemic species that has suffered from direct human action and is at an increased risk of irreversible loss of populations and reduction in genetic variability in extensive areas (Kageyama and Gandara, 1993). Among the approaches evaluated to ensure the survival of the species and reduce its risk of extinction, the use of silvopasture systems seems to have great

Table 1. Monthly rainfall, net solar radiation, day light hours and average mean, minimum and maximum air temperatures on the experimental site from October 2006 to January 2008.

Month	Rainfall(mm)	Net solar radiation (MJ. m^{-2}.dia)	Day light hours (h)	Temp mean (˚C)	Temp minimum (˚C)	Temp maximum (˚C)
October/2006	80.8	21.9	7.4	26.1	32.0	21.4
November/2006	152.6	22.8	8.0	26.4	32.5	21.8
December/2006	325.9	18.0	5.0	26.3	31.6	22.4
January/2007	540.4	14.8	2.7	25.6	30.5	22.5
February/2007	104.9	21.7	6.9	26.6	32.2	22.4
March/2007	58.7	21.8	8.6	27.3	33.3	22.8
April/2007	28.7	18.4	7.9	26.5	32.5	21.5
May/2007	90.4	15.8	7.5	22.7	29.1	17.1
June/2007	0.0	14.8	8.3	22.5	29.4	16.0
July/2007	44.2	15.5	9.1	20.9	27.9	14.5
August/2007	0.0	20.4	10.8	23.2	30.6	16.5
September/2007	7.6	23.7	11.0	27.7	35.2	20.3
October/2007	66.7	21.9	8.2	28.0	34.8	21.9
November/2007	167.4	21.0	7.0	25.5	31.0	20.9
December/2007	199.6	25.0	9.0	26.5	32.6	21.7
January/2008	596.1	19.8	5.4	25.3	30.7	21.5

potential. However, for silvopasture systems to be viable, helping preserve aroeira, the forage plant must yield forage in great enough amount and with sufficient nutritional value to enable its use for animal production.

Choosing the right components of the system is crucial for the success of sustainable silvopasture systems. In the case of forage plants, the species selected must have tolerance to shade, high yield, and be adapted to the management, soil and weather conditions of the area where it will be planted. This is particularly important for Cerrado ecosystems because of their poor and acidic soils and prolonged and well-defined dry season (Andrade et al., 2003). Forage plants in the genus *Brachiaria* are well-adapted to Cerrado conditions, especially *B. decumbens*, because of its low nutrient requirements. This species belongs to Group III, according to the classification of Werner et al. (1996), which comprises the species with the lowest nutrient requirements among tropical grasses. In addition, *B. decumbens* exhibits high phenotypic plasticity to changes in the degree of shading and climate conditions, and thus is a good option for silvopasture systems (Paciullo et al., 2011).

Shading reduces dry matter production (forage yield) in tropical grasses (Carvalho et al., 2002; Andrade et al., 2004; Sousa et al., 2007; Martuscello et al., 2009), but moderate shading may sometimes boost forage yield (Paciullo et al., 2008). Shading may also affect the nutritional quality of forage (Sousa et al., 2007), sometimes improving it: For instance, shading may raise the nitrogen content of tropical forage grasses and, consequently, crude protein, by up to 49% compared to

full sun (Carvalho et al., 1994; Meirelles et al., 2013). Additionally, better digestibility of grasses under moderate shade has been reported (Carvalho et al., 2002).

This study evaluated the forage production and morphological and feed chemical composition of *B. decumbens* (Poaceae) naturally grown as undergrowth to a *Myracrodruon urundeuva* (aroeira) tree thicket with 3×3 m spacing, in a silvopasture system.

MATERIALS AND METHODS

The study was conducted at the Teaching Research and Extension Farm of Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista (FEIS/UNESP), in Selvíria, Mato Grosso do Sul, Brazil (20° 19' S and 51° 26' W, elevation 372 m). Rainfall, net solar radiation and day light hours, average mean, minimum and maximum air temperatures are described in Table 1.

The soil is a clayey typical dystrophic Red Latosol (Santos et al., 2006). Average soil chemical characteristics for the 0-15 cm layer were: pH H$_2$O: 5,1; OM = 17 dg/ dm^3; P (ion-exchange resin extraction method) = 1,0 mg/ dm^3; Ca = 17 mmol$_c$/ dm^3; Mg = 15 mmol$_c$/ dm^3; K = 0,8 mmolc/ dm^3; H + Al = 41 mmol$_c$/ dm^3; sum of bases = 33 mmol$_c$/ dm^3; cation exchange capacity = 74,0 mmol$_c$/ dm^3; base saturation = 45%. This soil is considered low fertility (Werner et al., 1996).

Two sites were used in the study. The first was shaded under a 2.42 ha aroeira (*M. urundeuva* Fr. All) thicket, planted in 1992 at a spacing of 3 × 3 m (1.111 trees/hectare) with average height 6.0 m, diameter at breast height (DBH) 6.7 cm, and average crown diameter (ACD) 3.0 m (Figure 1). The second site had approximately the same area and received full sunlight. Both areas contained *B. decumbens* that had developed naturally without any type of agricultural practice or management.

A completely randomized block design with a factorial scheme of

Figure 1. Shaded area under aroeira.

Figure 2. Rising plate meter.

two plots and two sub-plots was used, in which the plots consisted of the light regimen (full sunlight and shade) and the sub-plots of the grazing rotation (pre-grazing and post-grazing). The different seasons of the year during which the evaluations were performed (spring 2006 - summer 2007; summer 2007 - autumn 2007; autumn 2007 - winter 2007; winter, 2007 - summer 2008) formed four blocks.

The experiment was conducted between 31 October 2006 and 11 January 2008 for a total of 391 days. During the experimental period, pre-grazing samplings were performed in all seasons. Specifically, a head of 40 cattle (stocking rate 14,5 animal units per hectare in each area - sun and shaded) was placed in the experimental area for grazing, where they remained for eight to 10 days (except for winter grazing when the animals remained for only three days because of the low quantity of forage). After this grazing period, which is sufficient to minimize fire hazards on the experimental farm, the animals were removed and post-grazing assessments were performed.

Forage mass in the plots was estimated from measurements taken with a pasture ruler or with a rising plate meter (Figure 2). A

calibration equation for canopy height measurements was created to estimate the forage mass of the plots, minimizing the need for destructive sampling. The equation was obtained for the experimental area and represented the relationship between canopy height measured with a ruler and rising plate meter and forage mass, as described by Bransby et al. (1977). Table 2 shows the equations for the determination of forage mass. The equation developed for the rising plate meter was used for the calculation of pre- and post-grazing forage mass to estimate forage yield, because the measurements obtained with the rising plate meter are affected by vegetation height and density, which, combined, are more strongly associated with forage mass than canopy height alone (Mannetje, 2000; Pedreira, 2002).

Pre- and post-grazing forage samples were collected with a six-month interval, between spring and summer (the two seasons with highest growth), for morphological separation and feed chemical analysis. The forage was cut manually with a scythe at ground level in six 0.25 m^2 areas within each experimental unit. Fresh samples were weighed. A sub-sample was separated, weighed, and dried in a forced-air furnace at 65°C until constant weight was obtained. The dry weight of samples was converted into forage mass values (kg DM/ha). Next, the sub-sample was ground in a Wiley mill and used for feed chemical analysis.

The remaining forage sample was separated into leaves (leaf blades), stems (stems + sheaths), and dead material. These components were weighed, stored in paper bags, and dried in a forced-air furnace at 65°C until constant weight, and weighed again. The morphological components are expressed as percentage (%) of total forage mass. These forage mass values were not used for the calculation of forage yield because they were obtained only in two seasons.

Forage yield was calculated as the difference between current pre-grazing forage mass and previous post-grazing forage mass of each experimental unit determined by the nondestructive dual sampling method. The rate of forage yield (kg DM/ha.day) was calculated by dividing forage yield by the number of days of regrowth shown in Table 3. For the calculation of forage yield in the last regrowth period, forage mass was determined in the summer of 2008. The average bulk density of the forage (kg DM.cm/ha) was calculated by dividing pre-grazing forage mass by the forage canopy height measured with a ruler.

With role plant samples, crude protein (CP) content was determined by the micro-Kjeldahl method according to the A.O.A.C. (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were measured using the method described by Van Soest et al. (1991). Lignin was determined by sulfuric acid hydrolysis of NDF residue (Van Soest et al., 1991). Cellulose and hemicellulose contents were determined by the difference between NDF and ADF content between ADF and lignin, respectively. In vitro dry matter digestibility (IVDMD) was determined by the two-stage method of Tilley and Terry (1963).

The PROC MIXED procedure (mixed models) of SAS® (Statistical Analysis System) package was used for the analysis of variance (ANOVA). Means were compared by the Tukey's test using the LSMEAN procedure at P<0.05. Linear regression analysis was used to evaluate the relationships between canopy height and forage mass (calibration equations) using the PROC REG procedure of SAS® package.

RESULTS AND DISCUSSION

Canopy height, measured with a rising plate meter, was significantly greater in the full sun than in the shade, whereas canopy height measured with a ruler was not significantly different between full sun and shade

Table 2. Calibration equations for the determination of forage mass.

Instrument	Equation	Significance	Coefficient of determination (r^2)
Rising plate meter	FM = 384.9 + 72.12h	0.0003	0.8363
Ruler	FM = -340.91 + 44.697h	0.0162	0.464

FM, Forage mass; h, height.

Table 3. Regrowth periods of *B. decumbens* under two light regimens and two grazing rotation schemes from October 2006 to January 2008 in Selvíria, Mato Grosso do Sul, Brazil.

Period	Duration (days)
Spring 2006 - Summer 2007	94
Summer 2007 - Autumn 2007	76
Autumn 2007 - Winter 2007	115
Winter 2007 - Summer 2008	106

Table 4. Forage canopy height, measured with a rising plate meter (cm, compressed height) and a ruler (cm), of *B. decumbens* pastures grown under two light regimens and two grazing rotation schemes from October 2006 to January 2008 in Selvíria, Mato Grosso do Sul, Brazil.

Forage height	Full sun	Shade	Mean
Rising plate meter, compressed height, cm			
Pre-grazing	16.45 (2.25)	5.11 (2.25)	10.77[A] (1.59)
Post-grazing	9.10 (2.25)	4.35 (2.25)	6.72[A] (1.59)
Mean	12.77 [a] (1.59)	4.72 [b] (1.59)	
Ruler, cm (S.E.M)			
Pre-grazing	38.57 (4.38)	27.42 (4.38)	33.0[A] (3.10)
Post-grazing	25.1 (4.38)	19.17 (4.38)	22.13[B] (3.10)
Mean	31.83 (3.10)	23.3 (3.10)	

Means (standard error of the mean) followed by different lowercase letters in rows and uppercase letters in columns are significantly different ($p < 0.05$).

treatments (Table 4). This difference may reflect the fact that, whereas the ruler measures only height, the rising plate meter combines information on forage height with forage density, which was probably greater in the fullsunlight treatment. Indeed, in various plots in the shaded area we observed low density of grass. Further, the ruler method does not differentiate between sites with low and high tiller population densities. On the other hand, ruler measurements recorded significant differences between pre- and post-grazing forage height (33.0 and 22.2 cm, respectively (Table 4).

Pastures grown under shade had significantly less forage mass (726 kg/ha) than pastures under full sun (1,306 kg/ha), as estimated by pre- and post-grazing sampling ($p<0.05$). Similar results, forage mass significantly higher under full sun conditions than in a shaded area, were reported by Carvalho et al. (2002), Andrade et al. (2004), Paciullo et al. (2007), Sousa et al.

(2007), and Martuscello et al. (2009). Martuscello et al. (2009) observed a linear reduction in forage mass, forage yield, and number of tillers/plant, and a linear increase in forage canopy height and leaf area index of *B. decumbens* with increasing shade level (0, 50 and 70% shade). In contrast, in shaded pastures an increase in leaf elongation, and particularly in stem elongation, can compensate for low tiller density, resulting in increased forage yield at higher shade levels (Paciullo et al., 2008; Meirelles et al., 2013). Paciullo et al. (2008) found reduction in tiller density and forage yield and increase in final leaf length and in the elongation rate of leaves and stems of *B. decumbens* as shade level increased (from 0%, to 50%). However, the increased proportion of stems in forage mass may reduce the nutritive value of forage. While the above-mentioned studies tested partial shading, in our experiment we believe the close-planted trees provided too much shading: with a 3×3 m spacing

Table 5. Dry mass of leaves, stems, and dead material (kg/ha) and proportion of leaves, stems, and dead material (%) in forage mass of *B. decumbens* grown under two light regimens and two grazing rotation schemes from October 2006 to January 2008 in in Selvíria, Mato Grosso do Sul, Brazil.

Mean leaf mass, kg/ha (S.E.M)	Full sun	Shade	Mean
Pre-grazing	824.55(159.85)	164.4(159.85)	494.48[A](113.03)
Post-grazing	177.6(159.85)	84.65(159.85)	131.13[A](113.03)
Mean	501.08[a](113.03)	124.53[a](113.03)	
Mean stem mass, kg/ha (S.E.M)			
Pre-grazing	1289.9(305.44)	167.6(305.44)	728.75[A](215.98)
Post-grazing	281.9(305.44)	158.3(305.44)	220.1[A](215.98)
Mean	785.9[a](215.98)	162.95[a](215.98)	
Mean dead material mass, kg/ha (S.E.M)			
Pre-grazing	542.45(118.19)	33.6(118.19)	288.03[A](83.57)
Post-grazing	707.9(118.19)	480.9(118.19)	554.4[A](83.57)
Mean	625.18[a](83.57)	257.25[b](83.57)	
Leaf proportion, % of leaves in FM (S.E.M)			
Pre-grazing	31.95(6.57)	42.43(6.57)	37.19[A](4.64)
Post-grazing	14.89(6.57)	11.13(6.57)	13.01[B](4.64)
Mean	23.42(4.64)	26.78(4.64)	
Stem proportion, % of stems in FM (S.E.M)			
Pre-grazing	47.31(4.72)	46.85(4.72)	47.08[A](3.34)
Post-grazing	23.87(4.72)	21.31(4.72)	22.59[B](3.34)
Mean	35.59(3.34)	34.08(3.34)	
Proportion of dead material, % of dead material in FM (S.E.M)			
Pre-grazing	20.73(8.90)	10.71(8.90)	15.72[B](6.29)
Post-grazing	61.24(8.90)	67.55(8.90)	64.39[A](6.29)
Mean	40.98(6.29)	39.13(6.29)	

FM, Forage mass. Means (standard error of the mean) followed by different lowercase letters in rows, or uppercase letters in columns, are significantly different ($p < 0.05$).

the area allowed for each tree is 2.2 m^2, which, with an average crown diameter of 3.0 m^2, means that the canopies overlapped, allowing light penetration only through the canopies.

Leaf and stem mass were not significantly different between light and grazing conditions (Table 5). Mean forage green dry matter (DMG) (leaf mass + stem mass) in the full sun area was 1,287 kg/ha, similar to the values reported by Paciullo et al. (2007), which ranged from 1,260 to 1,501 kg/ha. However, DMG in the shaded area was only 286 kg/ha, and that result may be due to the low grass cover in the shaded area, whereas in the study of Paciullo et al. (2007), DMG in the shaded area was 658 and 1,158 kg/ha in the first and second year, respectively. The main difference between the current study and that of Paciullo et al. (2007) is that *B. decumbens* had grown naturally in our study, whereas in theirs it was grown under adequate agricultural and

pasture management practices. Furthermore, tree density in this study was over twice that used by Paciullo et al. (2007), which resulted in much greater shading. Dead material mass was significantly higher in the full sun treatment than in the shaded condition, probably because of the greater forage growth in the full sun area and the lack of difference in the regrowth period between treatments (Table 3). Because forage mass was higher in the full sun treatment, more forage became senescent without being harvested when compared to the shaded area, which had lower forage mass. Sousa et al. (2007) evaluated the ratio between live and dead material in *Brachiaria brizantha* cv. Marandu and observed a higher ratio in shaded areas and a higher amount of dead material in areas exposed to full sun.

The proportion of leaves, stems, and dead material was not significantly different between light regimens (Table 5). However, significant differences were observed in the

Table 6. Forage yield (kg.DM/ha) and pasture growth rate (kg.DM/ha.day) of *B. decumbens* grown under two light regimens and two grazing rotation schemes from October 2006 to January 2008 in Selvíria, Mato Grosso do Sul, Brazil.

Period	Forage yield		Pasture growth rate	
	Full sun	Shade	Full sun	Shade
Spring 2006 - Summer 2007	591.4	-180.0	6.3	-1.9
Summer 2007 - Autumn 2007	295.7	93.8	3.9	1.2
Autumn 2007 - Winter 2007	79.6	64.9	0.7	0.6
Winter 2007 - Summer 2008	562.5	79.3	5.3	0.7
Total	1,529.2[a]	58.0[b]	3.91[a]	0.14[b]
Standard error of the mean	241.85	130.34	2.44	1.39

Means in the same row followed by different lowercase letters are significantly different (p < 0.05).

proportion of the three morphological components between grazing conditions, with a higher proportion of leaves and stems at pre-grazing than at post-grazing, and a higher proportion of dead material at post-grazing. This finding is indicative of forage selection by cattle, which consume primarily leaves, followed by stems, and dead material.

There was a significant positive relationship between light exposure vs. forage yield (p=0.0367) and light exposure vs. pasture growth rate (p=0.0367), with the highest values observed in the full sun areas (Table 6). Forage yield values declined considerably during the experimental period, even in the full sun area, due to the low defoliation frequency employed (Table 3), resulting in very long regrowth periods and parts of the forage produced not being harvested before senescence. Other experiments involving *B. decumbens* that used nitrogen fertilization have reported much higher accumulation rates than those observed in the current study. Fagundes et al. (2005) evaluated the growth of *B. decumbens* under full sun and four nitrogen rates (75, 150, 225, and 300 kg N/ha.year) and found mean pasture growth rates of 9.7 and 67.1 kg/ha.day in winter and summer, respectively. Vitor et al. (2014) evaluated forage yield at four nitrogen rates (0, 100, 200, 300, and 400 kg N/ha.year) and reported accumulation rates of 60 to 200 kg/ha.day, indicating that the accumulation potential of the species is much higher when plants are fertilized with nitrogen.

The negative forage yield value observed for the shaded site during the spring 2006 to summer 2007 period may have been caused by changes (increase) in tree foliage that affected the light environment, reducing grass growth, while tiller senescence rates were high, resulting in lower pre-grazing mass in shaded plots compared to the previous post-grazing period. Da Zeferino (2006) also reported negative forage yield values for *B. brizantha* cv. Marandu under rotational stocking, ranging from -13.2 to -18.1 kg/ha.day in winter, characterized by low temperatures and low rainfall. These findings indicate that in winter forage senescence may have been lower than growth and that environmental factors such as low water and light availability and low

Table 7. Contents of crude protein, neutral detergent fiber, acid detergent fiber, lignin, and IVDMD (%) in forage dry mass of *B. decumbens* grown under two grazing rotation schemes from October 2006 to January 2008 in Selvíria, Mato Grosso do Sul, Brazil.

Fraction	Grazing event	
	Pre-grazing	Post-grazing
Crude protein	4.93[a] (0.78)	4.04[b] (0.78)
NDF	76.89[b] (2.86)	81.70[a] (1.89)
ADF	49.18[b] (3.01)	55.97[a] (3.57)
Lignin	07.35[b] (0.72)	10.26[a] (2.33)
IVDMD	51.58[a] (4.26)	39.22[b] (9.23)

Means (standard error) in the same column followed by different uppercase letters are significantly different (p<0.05). IVDMD, *in vitro* dry matter digestibility.

temperatures could negatively affect forage yield.

Forage density was not significantly different between light regimens and grazing schemes, and values of 43.0 and 33.0 kg DM.cm/ha were recorded in the full sun and shade treatments, respectively. This lack of difference may be due to the high heterogeneity in tiller density and soil cover in the pastures, especially in the shaded site.

No significant differences were observed in feed chemical variables between light regimens. This similarity may have been due to the prolonged periods of pasture regrowth in our study (Table 3). Paciullo et al. (2007) evaluated grazing cycles of seven days of occupation and 35 days of rest and found higher leaf CP levels in shaded than in full sun areas (12.4 and 9.6%, respectively). In addition, the authors reported values of 58.0% (leaves) and 42.9% (stems) for in vitro dry matter digestibility (IVDMD) and mean IVDMD values of 47.6% and 53.2% for full sun and shade treatments, respectively, values that are similar to those reported in the current study (Table 7). Similarly, the CP content of *B. decumbens* pastures grazed by heifers was positively affected by shading in Paciullo et al. (2011). In that study, the maximum CP content (9.8%) was recorded under the tree canopy, decreasing with the distance from the

hedgerow (3.0 to 15.0 m tree distance). Meirelles et al. (2013) clipped the forage when the canopy reached a height of 35 cm and reported significant increases in CP concentration of Marandu (*B. brizantha* cv. Marandu) and Piatã (*B. brizantha* cv. Piatã) cultivars grown under varying shading levels (0 to 60%).

Conclusions

The low accumulation rate in both light regimens points to the need for fertilization and management in naturally growing grasses. Growing *B. decumbens* as undergrowth in aroeira stands may be an option in areas where the trees still occur, but the 3×3 m spacing proved inadequate for forage production and other planting densities should be investigated.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physical properties of a latosol eutrophic red under management systems after different winter crops successful by the soybean crop

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The objective was to verify the influence of winter crops under management mechanical (roller knife) and chemical (glyphosate), on soil physical properties and yield of soybeans. The experiment was carried out at the field under randomized block design in tracks scheme. The treatments consisted of four different winter crops (oats IPR 126, wheat BRS Tarumã crambe FMS Bright and forage radish cultivar common) in tracks A and management different (chemical and mechanical) in bands B. The soil properties (macroporosity, microporosity, total porosity and density) were determined by collecting soil core in layers 0-10 and 10-20 cm depth, penetration resistance was determined with the aid of a penetrometer impact to a depth of 30 cm. The soybean harvest was held on 03/12/13, collecting two lines of the floor area of each plot. The evaluations were carried out after the winter crop management and post-harvest of soybeans. There was no significant difference in the interaction of the factors to the values of the porosity in the layer 0-10 cm of soil. As to the values obtained for the penetration resistance of the soil, it was found that the oat (0.91 MPa) and crambe (1.43 MPa) provided significant differences in the layer 0-5 cm depth, after the cycle of winter crops. Winter crops and different managements not affect soybean yield.

Key words: Plantation direct, compaction, conservation systems, soil structure.

INTRODUCTION

The adoption of technologies based on conservationists foundations as the tillage and the use of winter crops are alternatives to increase the sustainability of agricultural systems (Torres et al., 2014; Boer et al., 2007).

The success of the system lies in the fact that the straws accumulated by cover crops and crop residues

from commercial fields create favorable environments for the recovery and the maintenance of the quality of soil and water (Kliemann et al., 2006), beyond allowing favorable conditions for crop development and effective erosion control (Brancalião and Moraes, 2008) Because of the enormous benefits for soil biodiversity, this technology has expanded to various regions of the world, especially in countries such as Argentina, Brazil, Paraguay and Uruguay, which adopt this system in about 70% of the total cultivated area (Derpsch et al., 2010).

In general, the soil when in its natural state, under vegetation present physical characteristics as permeability, structure, soil density and pore space, agronomically desirable. However, as the soils are being worked (Andreolla et al., 2000) and the continuous adoption of soil management systems conventional, considerable physical changes are occurring (Silva et al., 2008).

The structure of the soil is one of the most important properties for the adaptation of the species, and it is by means of physical properties that can be done their monitoring, such as soil bulk density, microporosity, aggregate stability, resistance of soil, permeability, among others. These properties can indicate overgrowth, crusting, susceptibility to productivity loss, environmental degradation and mainly compression (Laurindo et al., 2009).

The process of soil compaction, to increase its density and its mechanical resistance to penetration (PR), as well as to reduce the volume of macropores, the capacity of water infiltration, the aeration and hydraulic conductivity affects the root development, resulting in reduction of crop productivity (Beutler et al., 2005).

For decompressing the ground the use of species of winter crops, especially with the use of crop rotation in species with root system quite aggressive, it is necessary, since this practice protects the soil against erosion, brings benefits to fertility and soil structure due to the elevation of the organic matter content, and improves the thermal amplitude of soil maintaining its moisture, enabling better performance of succeeding crops (Amossé et al., 2013).

According to Campiglia et al. (2010), the benefits of winter crops may still be supplemented, as the maintenance of high rates of infiltration of water through the combined effect of the root system and vegetation cover and promote large and continuous inflow of vegetal mass on the ground.

Among the winter crops that deliver these benefits may be indicated the crambe (*Crambe abyssinica* Hochst) considered a rustic plant widely used as fodder in crop rotation and soil cover (Varisco and Simonetti, 2012), the radish (*Raphanus sativus* L.) that in addition to favoring the inflow of organic matter to the soil has adverse allelochemicals reducing the infestation of weeds (Martins et al., 2016) and oats can also be indicated how to plant cover crops, all these cultures have great development in the southern region of Brazil.

Although there are already research related to direct planting in Paraná State is important to test this factor associated with winter cover and handlings checking and monitoring the physical properties of the soil (macroporosity, microporosity, total porosity and density) under the effects of white oat cultivation IPR 126, crambe, oilseed radish and wheat double purpose BRS Tarumã, in function of mechanical and chemical handlings succeeded by the soybean crop.

MATERIALS AND METHODS

The study was conducted at the Experimental Farm "Professor Antonio Carlos dos Santos Person " (latitude 24º 33 ' 22' ' S and longitude 54º 03' 24 ' ' W , with an altitude of approximately 400 m) at the Universidade Estadual do Oeste do Paraná - *Campus* Marechal Cândido Rondon in Eutrophic Red Latosol (LVe) (Embrapa, 2013). The intercropping antecedents in the area constituted in no-tillage system. In the Table 1 is described the chemical and physical characteristics of the area before the experiment. Due to the low of V% (percentage of saturation of bases) liming was performed 30 days before sowing at a dosage of 2 Mg ha $^{-1}$ (large 80 %) to raise it to 70%.

The area of conducting of the experiment has a history in which for a period of four years, traditionally, the winter corn were grown (for silage production) in the off season and soybeans in the summer crop. These crops were always performed under the no-tillage system.

The local climate, classified according to Koppen, is Cfa, subtropical humid mesothermal dry winter with rainfall well distributed throughout the year and hot summers. The average temperatures of the quarter more cold vary between 17 and 18°C, the quarter more hot between 28 and 29°C, in its turn, the annual temperature ranged between 22 and 23°C. The total average annual precipitation normal pluvial for the region vary from 1600 to 1800 mm. with quarter more humid presenting totals between 400 to 500 mm (IAPAR, 2006). The climate data of the experimental period were obtained in automatic climatological station of the University of Paraná, distant approximately 100 m of the experimental area and are presented in Figure 2.

The experiment was started in autumn-winter of 2012 and the area has been desiccated 30 days before sowing, using glyphosate-isopropylamine salt in the dose of 3.0 L ha $^{-1}$ with a volume of 250 L ha $^{-1}$.

The experimental design used was randomized blocks in schematic of tracks, with three repetitions. On tracks A (5 x 40 m), four winter crops were allocated (IPR 126 oats, crambe Bright FMS, forage radish cultivate common wheat and BRS Tarumã). In ranges B (20 × 23 m), were allocated the managements of winter crops (chemist with isopropylamine and mechanical glyphosate -salt using knife roll). The plots were formed by a combination of bands A and B (5 × 20 m), each block had an area of 920 m² (23 × 40 m). During the development of the cultures was not performed any application of the herbicide. Winter crops were sown in the day 19/04/12, with drill seeder, coupled to the tractor on direct sowing system on maize straw. 60 kg ha $^{-1}$ of oats` seed, 15 kg ha $^{-1}$ of crambe` seed, 15 kg ha $^{-1}$ of radish` seeds of and 90 kg ha $^{-1}$ of wheat` seeds, with 0.17 m between lines were used. The fertilizer for growing oats, f. radish, fodder wheat and radish, was performed according to CQFS -SC (2004). For the correction of soil fertility 200 kgha $^{-1}$ a formulated 8-20-20 (N, P_2O_5 and K_2O, respectively) were used. The fertilization in coverage was carried out using 90 kg ha $^{-1}$ of N as urea.
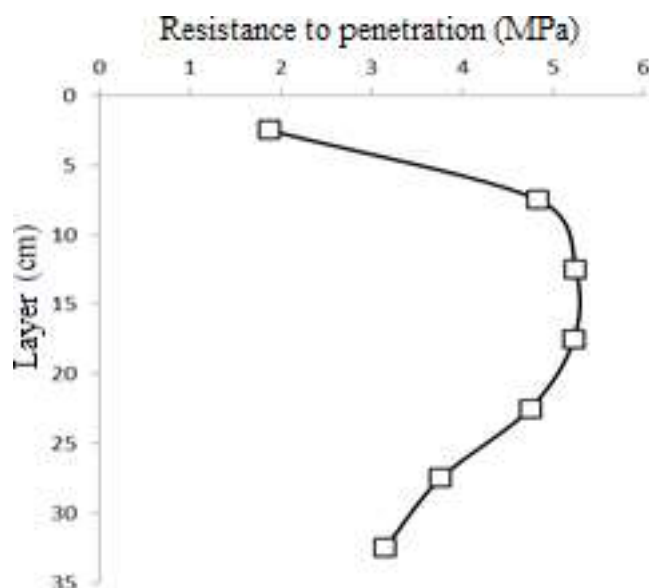
Figure 1. Soil resistance to penetration (MPa), at layer 0 to 35 cm depth, prior to deployment of winter crop, in Marechal Cândido Rondon (in March 2012).

Table 1. Chemical and physical characteristics of the soil (0 - 10; 10 - 20 cm), in the experimental area, prior to deployment of winter crop, in Marechal Cândido Rondon in March 2012.

Chemical characteristics of the soil											
Layer	**P**	**MO**	**pH**	**H+Al**	**Al^{3+}**	**K$^+$**	**Ca^{2+}**	**Mg^{2+}**	**SB**	**CTC**	**V**
cm	**mg dm^{-3}**	**g dm^{-3}**	**CaCl$_2$**				**cmol$_c$ dm^{-3}**				**%**
0-10	24.49	32.64	4.55	9.40	0.46	0.53	4.56	1,54	6,63	16,02	41,66
10-20	25.86	32.64	4.65	8.62	0.34	0.44	5.32	1,67	7,42	16,04	46,32

Physical characteristics of the soil						
Layer	**Macropores**	**Micropores**	**Porosity of soil**	**Density of soil**	**Sand**	**Silt**	**Clay**
cm	**dm^{-3} m^{-3}**			**Mg m^{-3}**	**g kg^{-1}**		
0-10	0.08	0.45	0.54	1.29	52.52	266.48	681.00
10-20	0.10	0.43	0.53	1.29	49.39	199.11	751.50

The values of resistance to penetration in the layer from 0 to 35 cm, before installation of the treatments are presented in Figure 1.

The treatment was performed 90 days after sowing, being the mechanic performed with knife roll and chemical with the application of the herbicide glyphosate-isopropylamine salt 480 g L^{-1} in the dose of 3.0 L ha^{-1}, with a volume of 250 L ha^{-1}.

After 30 days of culture management, the first collection was held for determination of properties physical properties. The density values (Ds-kg dm^{-3}) were evaluated by the method of volumetric ring (Blake, 1965) and the macroporosity (S-cm^3 cm^{-3}), from the relationship S = a - q, where the (cm^3 cm^{-3}) is the total porosity, calculated by the ratio a =1-(Ds/Dr), where Dr (kg dm^{-3}) is the real density and q (cm^3 cm^{-3}) is the water content in soil volume when subjected to a matric potential of -60 cm water column (Vomocil, 1965).

Sowing of soybean using the soybean cultivar BMX Potencia RR was held on 22/11/12. The area was previously desiccated using glyphosate-isopropylamine salt in the dose of 3.0 L ha^{-1} with a

volume of 250 L ha^{-1}. For the base fertilization was used 347 kg ha^{-1} of a commercial formulated 2-20-20 (N, P$_2$O$_5$ and K$_2$O), being performed on the basis of chemical analysis of the soil (SFREDO, 2008). The seeds were treated with fungicide Carbendazim (150 g L^{-1}) + Tiran (350 g L^{-1}) 2 ml kg^{-1} of seed, insecticide Fipronil (250 g L^{-1}) 0.8 ml kg of seed^{-1} and inoculated with Bradyrhizobium. The spacing, as well as the density of sowing, were carried out in accordance with the recommendation for the cultivar (BRASMAX, 2012).

For the sowing was used a seeder fertilizer coupled to a tractor, with the seeds deposited at a depth of average of 4 cm. During the crop development cycle fungicide applications were performed (triazole) at a dose of 0.65 L ha^{-1} with spray volume of 250 L ha^{-1} and (estrobilurina + triazol) in the dose of 0.30 L ha^{-1} with volume of 250 L ha^{-1} of commercial product. The soybean harvest was performed on 03/12/13, collecting two lines of the useful area of
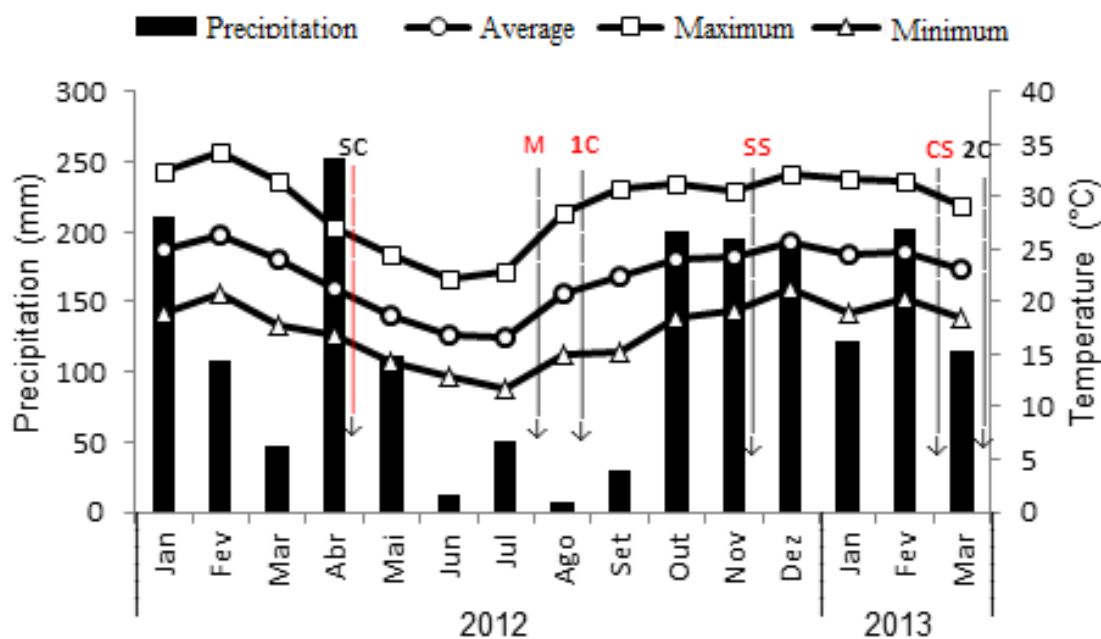
Figure 2. Monthly average temperatures maximum, minimum and average and precipitation accumulated during the months of the experimental period. SC: Seeding of winter crops. M: The realization of the managements of winter crops. 1C: First data collection. SS: Soybean sowing. CS: Soybean harvest. 2C: second data collection. Source: Automatic climatological station of the Nucleus of Experimental Stations of UNIOESTE, Marechal Cândido Rondon-PR.

each plot, which totaled 0.90 m² with this were estimated the quantity produced per hectare. For the determination of the weight of one thousand seeds and yield of soybean was realized the trail of the material with trailed crop beater. After the trail was determined the thousand seed weight according to Brazil (1992), and productivity (kg ha^{-1}), with discounts of impurity and moisture.

To 15 days after soybean harvest volumetric rings were collected for the determination of soil physical parameters as well as, performed the determinations of soil resistance to penetration. The determination of resistance to penetration and other physical properties of the soil was performed according to Embrapa (1997). The determination of soil resistance to penetration was performed with the use of an impact penetrometer model Stolf, with needle tip cone thin (60°), at three points in each plot. To minimize differences in soil moisture between treatments and between the depths, evaluation was performed three days after a precipitation, with humidity next of field capacity. The points were taken randomly, up to 30 cm of depth, and the data obtained in the field in the unit of impacts/decimeter processed in MPa, using the equation described by Stolf (1991).

The data obtained were submitted to statistical analysis using the SISVAR program (Ferreira, 2011), and the averages compared by the Tukey test at 5% level of probability.

RESULTS AND DISCUSSION

There was no difference (p>0.05) for average values of macroporosity, total porosity, microporosity and bulk density in the layer of 0 - 10 and 10 - 20 cm, on the basis of the factors studied after the managements of cover plants (Table 2). With regard to the results obtained after the harvesting of the soybean crop was found significance

between the factors, for the values of macroporosity in the superficial layer of 0 - 10 cm of soil (Table 3). For the other physical characteristics of the soil (microporosity, total porosity and density) average values obtained were similar, not showing influences suffered by the treatments applied. There being thus possible to differentiate the most effective species, as well as more efficient management systems, improvement of soil physical properties.

It was expected that the different winter crops involve changes in the physical characteristics of the soil, because the root system of crops requires an adequate supply of oxygen to maintain its physiological operation once that, its roots perform gaseous exchanges through a system porous that must also ensure an adequate supply of nutrients and water (Torres and Saraiva, 1999). However the results obtained are similar to those found by Sanchez (2012), that evaluated the influence in the physical properties of the soil by the winter crops observed that the use of these plants, in its first cycle of cultivation, not promoted changes in soil bulk density, microporosity, total porosity, however, in the layer from 0.10 to 0.20 m were verified larger values of macroporosity in treatments of oat and ryegrass.

The values found (Table 3) demonstrate that there was little variation between the results. These results corroborate with the study conducted by Bertol et al. (2004), in which the authors have not observed variation in the physical properties of the soil by the use of different

Table 2. F values calculated for the soil properties in the layer 0-10 and 10-20 cm after the managements of winter crops.

Source of variation	DF	Macroporosity		Microporosity		Porosity total		Soil density	
		0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20
Block	2	0.690	0.870	0.360	4.060	0.020	0.080	0.760	0.690
Crops (C)	3	4.130	0.760	0.340	0.500	1.100	0.130	0.370	0.570
Error 1	6	2.969	4.442	2.186	1.675	8.032	6.361	0.006	0.002
Management (M)	1	0.006	5.070	0.330	0.200	0.450	0.450	0.630	0.240
Error 2	2	8.310	1.063	16.681	4.235	9.981	4.114	0.004	0.004
C X M	3	0.280	1.340	0.350	1.320	2.720	8.050	0.860	1.780
Error 3	6	5.926	3.321	2.777	1.621	1.263	0.999	0.004	0.002
CV 1 (%)		20.330	27.920	5.770	3.040	5.410	5.030	6.290	3.650
CV 2 (%)		34.010	13.660	9.300	4.830	6.030	4.050	4.950	4.870
CV 3 (%)		28.720	24.140	3.790	2.990	2.140	1.990	5.000	3.330

CV 1: Coefficient of variation for crops; CV 2: Coefficient of variation for managements; CV 3: Coefficient of variation for crops with managements.

Table 3. F values calculated for the soil properties in Layer 0 to 10 and 10 to 20 cm, after the harvesting of the soybean crop.

Source of variation	DF	Macroporosity		Microporosity		Porosity Total		Soil density	
		0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20
Block	3	1.250	0.840	0.570	0.350	1.560	1.840	4.320	0.500
Crops (C)	2	1.670	0.680	0.410	0.690	0.990	0.880	0.140	0.770
Error 1	6	3.324	11.518	10.483	13.432	8.689	2.765	0.014	0.016
Management (M)	2	3.620	1.350	1.470	1.030	0.930	1.260	3.570	0.450
Error 2	6	0.314	12.860	5.467	5.943	3.264	2.496	0.001	0.005
C X M	4	0.80*	0.340	0.990	1.510	1.880	1.660	2.180	0.550
Error 3	12	3.670	6.850	2.647	15.148	3.513	12.138	0.010	0.004
CV 1 (%)		28.300	49.480	7.190	8.520	5.730	3.340	9.220	9.460
CV 2 (%)		8.690	52.280	5.190	5.670	3.510	3.170	1.940	5.160
CV 3 (%)		29.740	38.160	3.610	9.050	3.640	6.990	7.620	4.810

*Significant at 5% probability by the F test, respectively. CV 1: Coefficient of variation for the crops; VC 2: Coefficient of variation for the managements; VC 3: Coefficient of variation for the crops with the managements.

cultivation systems, understood as rotation and succession with cultures of coverage in a production cycle, concluding that it would be necessary to carry out experiments for longer period of time to be able to check the results of the action of the plants on the physical properties of the soil.

Macroporosity

The values of macroporosity values obtained on the Layer 0 - 10 cm after completion of the managements of winter crops showed no significant difference between the treatments and the same occurred in the layer of 10 - 20 cm (Table 4).

For the found values of macroporosity after soybean harvest, the cultures that stood out were the forage radish in camanda of 0 - 10 cm (0.07 m^3m^{-3}) in the mechanical handling and the crambe in this same layer with the use of chemical management (0.07 m^3 m^{-3}).

These same cultures showed higher values (0.09 m^3m^{-3}) also in the layer of 10 - 20 cm (Table 5). It is believed that regarding the macroporosity wheat presented higher results, because it is long cycle and with the mechanical handling may have suffered a stimulus for regrowth and rooting.

Considering the optimal values for the full development of plants, ranging from 0.07 to 0.17 m^3 m^{-3} (Drewry et al., 2003), in all layers, macroporosity values (Tables 4 and 5) found in this study (average of 0.06 m^3 dm^{-3}) are considered low which increases the risk of deficit of O_2 in the roots and reduces the continuity of pores and the permeability of soil (Lanzanova et al., 2007). The reduction of macroporosity in agricultural production systems tend to reflect negatively, reducing the total porosity and increasing soil density (Reichert et al., 2003).

The lower volume of macropores, with consequent greater volume of pores on the surface of the soil under no-tillage, can reduce the rate of water infiltration in this

Table 4. Macroporosity, microporosity, total porosity and density for the cultures of oats, crambe, radish and wheat, after being submitted to mechanical and chemical managements in the layer of 0 - 10 cm and in the layer of 10 - 20 cm of soil.

Crops	Macroporosity ($m^3 m^{-3}$)		Microporosity ($m^3 m^{-3}$)		Total porosity ($m^3 m^{-3}$)		Density ($mg m^{-3}$)	
Layer 0 - 10 cm								
	Management		Management		Management		Management	
	Mechanic	Chemical	Mechanic	Chemical	Mechanic	Chemical	Mechanic	Chemical
Oat	0.06	0.06	0.43	0.45	0.49	0.52	1.23	1.23
Crambe	0.09	0.08	0.43	0.45	0.53	0.53	1.19	1.19
F. radish	0.08	0.09	0.43	0.43	0.51	0.52	1.18	1.23
Wheat	0.10	0.09	0.43	0.43	0.53	0.53	1.15	1.16
Layer 10 - 20 cm								
	Macroporosity ($m^3 m^{-3}$)		Microporosity ($m^3 m^{-3}$)		Total Porosity ($m^3 m^{-3}$)		Density ($mg m^{-3}$)	
Oat	0.07	0.06	0.42	0.43	0.49	0.49	1.29	1.29
Crambe	0.06	0.07	0.42	0.43	0.48	0.51	1.31	1.26
F. radish	0.09	0.06	0.42	0.41	0.52	0.48	1.24	1.28
Wheat	0.09	0.07	0.41	0.43	0.50	0.50	1.25	1.31

Averages followed by the same capital letter in line and tiny in column in each characteristic do not differ by the Tukey test at 5% of probability of error.

Table 5. Macroporosity, microporosity, total porosity and density to the cultures of oats, crambe, forage radish and wheat at layer 0 to 10 cm and in the layer of 10 to 20 cm of the soil after the implementation of the collection of soybean.

Crops	Macroporosity ($m^3 m^{-3}$)		Microporosity ($m^3 m^{-3}$)		Total Porosity ($m^3 m^{-3}$)		Density ($m^3 m^{-3}$)	
Layer 0 – 10 cm								
	Management		Management		Management		Management	
	Mechanic	Chemical	Mechanic	Chemical	Mechanic	Chemical	Mechanic	Chemical
Oats	0.05	0.04	0.43	0.46	0.48	0.51	1.26	1.3
Crambe	0.06	0.07	0.43	0.44	0.49	0.51	1.38	1.21
F. radish	0.07	0.06	0.45	0.45	0.53	0.52	1.20	1.30
Wheat	0.07	0.05	0.45	0.46	0.52	0.51	1.30	1.25
Layer 10 - 20 cm								
	Macroporosity ($m^3 m^{-3}$)		Microporosity ($m^3 m^{-3}$)		Total Porosity ($m^3 m^{-3}$)		Density ($m^3 m^{-3}$)	
Oats	0.05	0.07	0.44	0.43	0.5	0.51	1.31	1.3
Crambe	0.09	0.05	0.38	0.44	0.47	0.5	1.34	1.35
F. radish	0.09	0.05	0.43	0.41	0.52	0.47	1.25	1.34
Wheat	0.06	0.04	0.43	0.44	0.49	0.49	1.36	1.36

Average followed by the same capital letter in line and tiny in column in each characteristic, do not differ by the Tukey test at 5 % of probability of error.

system of management, in relation to conventional tillage (Bertol et al., 2004).

Microporosity

With relation to the microporosity, in general there was no significant difference ($p > 0.05$), as well as the different managements also did not influence the results. In the mechanical control values were established with an average of 0.43 $m^3 m^{-3}$, and the chemical management with an average of 0.44 $m^3 m^{-3}$ in the layer 0 - 10 cm in the evaluation performed after the handling of the cultures. The same occurred for the layer of 10 - 20 cm of

this same evaluation in which the average remained at 0.42 m^3 m^{-3}, for both the mechanical management as for the chemical management, not differentiating among cultures (Table 4). For the evaluation performed after soybean harvest, the layer 0 - 10 and 10 - 20 cm, the values showed no differences. The cultures with larger sized were the crops of oats (0.46 m^3 m^{-3}) and wheat (0.46 m^3 m^{-3}) in the chemical management in the layer of 0 - 10 cm. In the layer 10 - 20 cm excelled the culture of oats in mechanical handling and cultures of crambe and wheat in chemical management with the average of 0.44 m^3 m^{-3} for each of these cultures (Table 5).

It can be inferred that the ideal soil is the one with values of 0.10 to 0.16 m^{3}m^{-3} for macroporosity, up to 0.33 m^{3}m^{-3} for microporosity and approximately 0.50 m^{3}m^{-3} for total soil porosity (Kieh, 1979). Thus, the values of microporosity in this work, in practically all layers studied, are above the ideal conditions. The volume of micropores that are relatively high, present in all the treatments studied indicates the possibility of occurrence of capillarity in soil (Bertol et al., 2004). The microporosity is related to the water storage in the soil, influencing the development of plants especially in critical water availability times (Veiga, 2005). This factor has acted as a supply in the early establishment of winter crops, since this development time of the occurrence of precipitation was reduced over the subsequent months, as can be seen in Figure 2. Bertol et al. (2004) found a greater microporosity under no-tillage compared to conventional soil pre-paration, at layer 0 to 10 cm.

For Albuquerque, Ender and Sangoi (2001), the increase of the microporosity can be considered a reflection of the reduction of structure and assigned to the reduction in the volume of macropore, that makes harmful to the development of the plants. Similar results were obtained by Silva et al. (2008), evaluating soil management systems in crop succession and its influence on soil physical properties, they found that microporosity was not affected, regardless of the studied layer.

Total porosity

As there was no difference in the values of macroporosity and microporosity, total porosity was not affected (Table 4). Changes in soil porosity limit nutrient absorption, infiltration and redistribution of water, gas exchange and root development (Bicki and Siemens, 1991).

Whereas the ideal soil should be roughly 0.50 m^{3}m^{-3} for total soil porosity (Kiehl, 1979), the results found for this factor are considered ideal or very close to the ideal. In the evaluation performed after the management, the average of the different cultures in the layer 0 - 10 cm consisted of 0.51 m^{3}m^{-3} for mechanical handling and chemical management obtained an average of 0.52 m^{3}m^{-3}. In the layer of 10 - 20 cm the averages of winter crops were of 0.50 m^{3}m^{-3} when used the mechanical handling and 0.49 m^{3}m^{-3} when used chemical management (Table 4).

For the evaluation performed after the soybean harvest, the total porosity values established on the average of 0.51 m^{3}m^{-3} in the 0-10 cm both in mechanical handling and for chemical management. In the layer of 10 - 20 cm values were of 0.50 m^{3}m^{-3} and 0.49 m^{3}m^{-3} for the managements mechanical and chemical respectively (Table 5). These results are similar to those obtained by Sanchez (2012), that by checking the physical properties of the soil and yield of soybean in succession to winter crops have been obtained in the layer of 0 - 10 cm of soil, results show that the treatments showed no significant differences in porosity with medium that varied between 0.61 and 0.69 m^{3}m^{-3}, having a cycle of winter cover crops, until the moment of its flowering, not producing any change in this property.

Soil density

The values of density obtained for both layer of 0 - 10 cm as to layer of 10 - 20 cm showed no significant difference between the treatments. For the evaluation performed after the crop management mean values were 1.19 Mg m^{-3} and 1.20 Mg m^{-3} for the mechanical and chemical handlings respectively in the 0 - 10 cm. And in the layer of 10 - 20 cm the averages were of 1.27 Mg m^{-3} for the mechanical handling and 1.28 Mg m^{-3} for the chemical management (Table 4). The same occurred in the evaluation carried out after soybean harvest, there was no difference for soil density (Table 5), in both managements and in different cultures, with an average of 1.27 Mg m^{-3} for the depth of 0 - 10 cm and 1.32 Mg m^{-3} for the layer of 10 - 20 cm.

The density values for all treatments are well below critical levels. For Reinert and Reichert (2001), the values considered ideal for the development of the cultures are approximately 1.45 Mg m^{-3} for clay soils. Reinert et al. (2008) in studies with different species of coverage of winter in Clayey found that the root growth was normal until the limit of density of 1.75 Mg m^{-3}. Soils with high density cause restrictions on root growth of crops being that the root system focuses near the surface (Seidel et al., 2009). However, Argenton et al. (2005) found that in Rhodic Oxisol, the deficiency of aeration begins with soil density close to 1.30 Mg m^{-3}, while Klein (2006), for the same soil class based on limiting water range, noted that the limiting density was 1.33 Mg m^{-3}. In compacted soil, the number of macropores is reduced, the micropores are larger amount and density is also higher (Jimenez et al., 2008).

Resistance to penetration

There was a significant effect (p<0.05) of culture on the resistance to penetration in the layer of 0 - 5 cm depth,

Table 6. F values calculated for the soil resistance to penetration after the managements and after the harvesting of the soybean crop.

		After the management					
Source of variation	**DF**	**Layers (cm)**					
		0-5	**5-10**	**10-15**	**15-20**	**20-25**	**25-30**
Block	2	0.820	0.280	0.570	3.010	2.520	1.970
Crops	3	4.810*	1.360	0.210	0.670	0.710	0.720
Error 1	6	0.058	0.409	1.456	0.654	0.929	0.671
Management	1	0.480	0.610	4.330	9.920	1.980	1.200
Error 2	2	0.052	0.013	0.356	0.286	1.255	1.405
C X M	3	1.550	0.280	0.960	0.750	1.120	0.900
Error 3	6	0.095	0.313	0.468	1.033	0.384	0.398
CV 1 (%)		19.850	20.340	31.890	19.540	24.890	24.990
CV 2 (%)		18.890	3.640	15.780	12.910	28.920	36.150
CV 3 (%)		25.440	17.810	18.700	24.560	16.000	19.240

		After soybean harvest					
Source of variation	**DF**	**Layers (cm)**					
		0-5	**5-10**	**10-15**	**15-20**	**20-25**	**25-30**
Block	2	0.210	0.070	0.430	0.500	1.960	1.430
Crops	3	0.190	0.660	1.720	0.980	1.080	0.990
Error 1	6	0.271	1.377	0.557	0.920	0.779	2.291
Management	1	5.360	0.130	0.350	0.150	0.010	1.260
Error 2	2	0.025	1.024	1.401	2.607	1.598	0.507
C X M	3	0.350	0.060	0.310	1.680	1.180	0.180
Error 3	6	0.497	1.051	2.344	1.156	1.650	3.646
CV 1 (%)		44.170	39.140	18.770	23.820	21.610	36.070
CV 2 (%)		13.310	33.740	29.770	40.090	30.950	16.970
CV 3 (%)		59.790	34.190	38.510	26.690	31.450	45.500

*Significant at 5% probability by the F test, respectively. CV 1: Coefficient of variation for the crops; VC 2: Coefficient of variation for the managements; VC 3: Coefficient of variation for the cultures with the managements.

after the harvest of winter crops (Table 6). In this layer, the values obtained for the soil penetration resistance, demonstrate that the oat and crambe showed significant differences, offering modifications to the ground in this property, with values of 0.91 and 1.43 Mpa respectively after the harvesting of the crops of winter. This positive effect of oat, in decreasing soil resistance, was also ratified by Neiro et al. (2003), that evaluated the soil resistance to penetration in a Oxisol, with rotation and succession of crop under no-tillage verified that the treatment with crop rotation (wheat/oat/corn/soybean) presented lower values of resistance to penetration in the layer of 15 to 20 cm. This result obtained for oats, probably due to the positive effect of the root system of culture of oats, which acts by conducting biological soil scarification, reducing soil compaction in this treatment.

It is important to stand out that crambe has taproot system being eficiente in the unpacking to deeper layers of the soil, however these roots that have large diameter provide greater constrain to development and penetration in compacted soil. Already the oats is a plant with dense and branched roots type that shows efficient penetration and decompression of the upper layers of the soil, thus justifying the results obtained in this research.

According to the USDA (1993), the value considered as limiting factor and causing strong restriction to root growth for many annual crops is 2.0 Mpa, but can vary according to the texture and organic matter content of the soil. De Maria et al. (1999), studied soil preparation systems (heavy harrow and direct seeding) and concluded that there was soil compaction between 10 and 35 cm (2.09 and 1.86 MPa) and 10 and 20 cm (2.52 MPa) respectively, evaluated through the resistance of soil (Figure 3).

Genro Junior et al. (2004) found the resistance to penetration in a clayey Oxisol under no tillage with crop rotation, a great temporal variation and was associated with the variation of soil moisture for each condition of soil density or state of compaction. In this same evaluation the authors obtained the largest state of soil compaction at layer around 10 cm depth and the lowest in the superficial layer, up to 7 cm. Beutler and Centurion
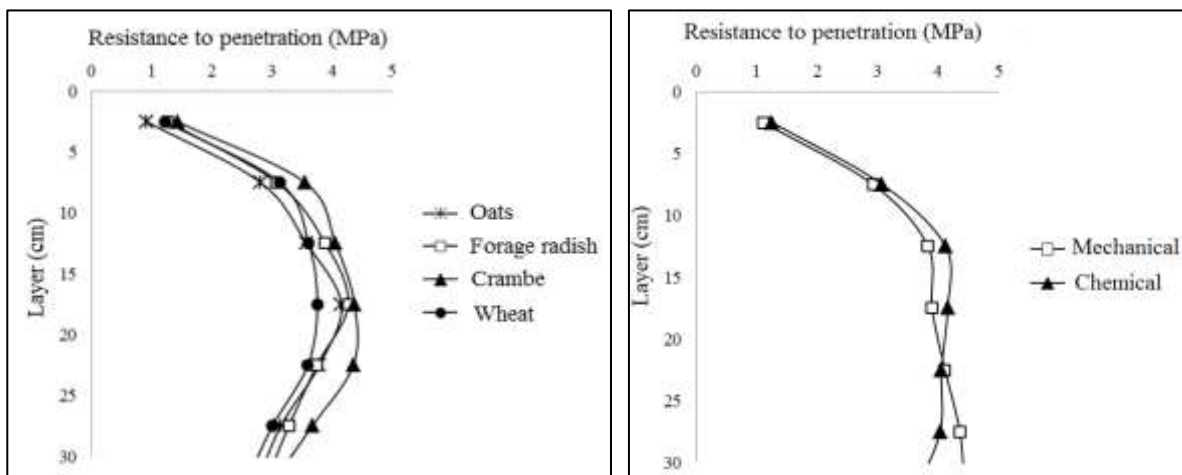
Figure 3. Soil resistance to penetration (MPa), at layer 0 to 30 cm depth, after the harvest of winter crops (A) and after the harvest of soybean (B). Oats, crambe, forage radish and wheat: winter crops. Managements: mechanical and chemical.

Table 7. Values of productivity (kg ha^{-1}) and weight of a thousand grains (g) for the soybean crop under the influence of the different cultures of winter and handlings employees.

Crops	Productivity (kg ha^{-1})			Weight of thousand grain (g)		
	Management		Average	Management		Average
	Mechanic	Chemistry		Mechanic	Chemistry	
Oat	1938.27	1918.00	1928.14[a]	117.02	111.14	114.08[a]
Crambe	2132.23	2007.61	2069.92[a]	108.32	114.28	111.30[a]
F. radish	2391.21	2503.58	2447.40[a]	115.75	127.79	121.77[a]
Wheat	1595.25	2629.38	2112.32[a]	103.50	119.53	111.52[a]
Average	2014.24[A]	2264.64[A]		111.15[A]	118.19[A]	

*Medium followed by the same capital letter in line and tiny in column in each characteristic, do not differ by the Tukey test at 5% of probability of error.

(Table 7), it was found that there was no significant difference between the results, that is, the different cultures of winter and the different managements not influenced in the weight of a thousand grains of soybean, that is, as there was no change to the physical soil, also did not change the absorption of water and nutrients and did not affect soybean.

The small differences in macroporosity were not enough to affect the weight of a thousand grains and soybean yields. The weight of a thousand grains is one of the key factors to achieve good yields, since this variable is directly correlated with the productivity. This variable may be used to estimate if there was a good efficiency during the process of grain filling, besides expressing indirectly the size of these seeds and its good physiological status as covered by Marques et al. (2008).

With respect to productivity all managements provided yields statistically similar, the results did not differ significantly (Table 7). In a study conducted by Debiasi et al. (2010), assessing the productivity of soybean and

corn after winter cover and decompression mechanical soil, the authors verified that the increased soybean yield was obtained in the treatments which had winter crop, fact that was assigned to the best soil aggregation, resulting from higher soil organic matter levels observed in these treatments, as well as to better physical condition of the surface of the soil.

However for this job a single cycle of cultivation of winter crops not promoted changes in soybean yield. Improvements on the soybean crop and even on the physical properties of the soil, by the influence of winter crop, materialize itself an experimental period greater to be observed, and there is the need of more than one crop cycle.

Conclusions

In the studied conditions was found the interaction between the factors (crops × managements) modifies the

macroporosity in camanda of 0 - 10 cm after the harvest of the soybean. The use of cover crops plants in winter with chemical or mechanical handlings and soybean cultivation in succession does not alter the macroporosity, microporosity, total porosity and density, but the oats decreases the resistance to penetration. The soybean yield is not affected by the cultivation of cover plants and managements of winter.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determination of parameters for selection of Eucalyptus clones tolerant to drought

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Climatic changes have induced the spread of aridity in many parts of the globe. *Eucalyptus* plantations show wide variations in productivity due to several factors, including climatic changes. Therefore, *Eucalyptus* genotypes that can adapt to such conditions is very welcome. Thus, an evaluation was done by comparing the physiological responses of two *Eucalyptus* genotypes, tolerant and drought-sensitive. They were assessed in different stages of experimental period and the rate of chlorophyll and chlorophyll fluorescence (minimal fluorescence and maximal fluorescence) were recorded. The potential photosystem II (PSII) quantum efficiency was then calculated. A decline in the rate of total chlorophyll (values below 25) and in the minimal fluorescence (values below 190) in clone sensitive due to the water stress was observed, while an increase was noted in clone tolerant to aridity. A reduction in the maximal fluorescence and photochemical efficiency was recorded in the genotype not being irrigated, with an earlier and more severe effect being verified for the sensitive genotype with values below 300 and 0.40, respectively. The variables studied confirm that clone tolerant exhibits greater drought tolerance, thus indicating that the photochemical efficiency of photosystem II is a reliable tool that enables the nondestructive choice of genotypes naturally tolerant to water scarcity conditions.

Key words: Abiotic stress, arid land plants, chlorophyll, ecophysiology, photochemical efficiency, tree drought tolerance, water deficit, water potential.

INTRODUCTION

Despite the fact that planted forests represent merely 7% of the world's forest cover, they make a vital contribution to forest services (Villar et al., 2011), particularly the plants of the *Eucalyptus* genus. Although endemic to Australia and the nearby isles, *Eucalyptus* is predominantly one of the most popularly cultivated species in the tropics and Mediterranean countries (Cromer et al., 1993; Stape et al., 2001). Brazil, which

boasts one of the world's most progressive technologies with respect to planted forests, has extensive *Eucalyptus* cultivations.

Climatic changes over the recent decades have caused the proliferation of arid conditions across the world (Allen et al., 2010; Kirono et al., 2011), resulting in a growing demand for *Eucalyptus* genotypes adapted to such situations. Scientists believe that the escalating emissions of greenhouse gases is one of the main reasons causing the recent spurt in the global average temperature and alterations in the global hydrological cycle, including predictable sharp surges of aridity (Sterl et al., 2008). The need of the hour, therefore, is to identify parameters which can rapidly and efficiently distinguish between genotypes that are tolerant and susceptible to drought for forest development programs.

Eucalyptus plantations vary widely in productivity due to several factors, primarily water availability (Shvaleva et al., 2006; Villar et al., 2011). Therefore, even a slight drop in soil water availability can produce negative effects on plant growth, development and productivity (Santos and Carlesso, 1998; Chaves et al., 2009).

There are many ways to express plant responses to drought. The ability of a plant to tolerate drought stress is usually determined by a combination of attributes expressed during a season of drought.

Eucalyptus exhibits very complex responses to drought which depend upon the strength and length of the dry conditions, the species, genotype and developmental stage of the plant (Santos and Carlesso, 1998; Taiz and Zeiger, 2009; Rodrigo, 2007). Morphological changes (in the size and biomass of the various plant organs) and the physiological (in the efficiency of water use and light capture) are some of the consequences observed (Li and Wang, 2003; Chaves et al., 2004; Merchant et al., 2007; Coopman et al., 2008; Pereira et al., 2010).

Distinguishing between the genotypes tolerant and susceptible to drought is possible using the values of the variables of gas exchange and also the evaluation of the photochemical efficiency of photosynthesis, obtained by measuring the chlorophyll *a* fluorescence. According to Krause and Weis (1991), the fluorescence emitted is equal to the amount of light energy not used by the photosynthetic apparatus and variations in the emission patterns indicate the presence of lesions (damages) in the plant's photosynthetic apparatus. Therefore, the main parameter used to evaluate these lesions is the ratio of the variable and the maximum chlorophyll fluorescence (Fv∕Fm) which is the measure of the intrinsic or maximum efficiency of photosystem II (PSII). Values ranging from 0.75 to 0.85 reveal that the photosynthetic apparatus of the plant is in good condition; therefore, any decline in this ratio proves to be an excellent indicator of photoinhibitory damage when the plants are subjected to environmental stresses, including water limitation (Björkman and Powles, 1984; Bolhàr-Nordenkampf et al., 1989).

Another physiological parameter sensitive to stress due to water conditions is the chlorophyll content (Dutra et al., 2012; Ebrahimiyan et al., 2013; Huang et al., 2013), which has proven to be as effective as the other techniques used to measure gas exchange in distinguishing between the genotypes tolerant susceptible to drought. Further, it presents equally well as the variables of chlorophyll fluorescence with the extra advantage of being accurate, economical, fast and non-destructive. Therefore, this has been proven to be an important tool in ecophysiological studies (Krause and Weiss, 1991).

In this paper, we did a comparative study of the physiological responses of the chlorophyll content and chlorophyll *a* fluorescence in two contrasting *Eucalyptus* genotypes with respect to their ability to tolerate drought under two conditions of water availability. This study focused on the commercial clone sensitive of the *Eucalyptus* hybrid *urograndis* (*Eucalyptus grandis* vs. *Eucalyptus urophylla*), a genotype inefficient under water stress and clone tolerant of the hybrid *Eucalyptus camaldulensis* vs. *E. grandis* - a genotype model with high tolerance to water shortage. The objective of this study was to identify and understand some of the underlying tolerance of the photosynthetic apparatus deficit mechanisms to water level. There was need also to assess the ability of the variables in the study to distinguish the *Eucalyptus* genotypes of known sensitivity and tolerance. Our hypothesis states that genotype tolerant to aridity possesses a more efficient photosynthetic apparatus against the harmful effects of water shortage.

MATERIALS AND METHODS

Season and experimental environment

The experiment was conducted between December 2012 and January 2013 at the Integrated Center for Propagation of Forest Species, in the greenhouse on the campus of the Federal University of JK (Jequitinhonha and Mucuri), in Diamantina, Minas Gerais, Brazil (18° 12'9.76''S, 43° 34'46.13''E). The average temperature and average relative humidity inside the greenhouse were 21.9°C and 76.9%, respectively.

Plant, containers and substrates

In this study, we used *Eucalyptus* seedlings derived from clonal propagation, namely, commercial *Eucalyptus* hybrid clone sensitive *urograndis* (*E. grandis* vs. *E. urophylla*) and clone tolerant of the hybrid *E. camaldulensis* vs. *E. grandis*, from Aperam Bioenergy Ltd. These genotypes are selected in a breeding program. Growth performance was evaluated based on observations in field plantations subjected to drought on a dry season. First, the 56-day-old seedlings were transplanted to 2.0 and 1.5 L plastic bags, respectively, containing a substrate composed of vermiculite (40%), carbonized rice hull (30%) and coconut fiber (30%) and fertilized in line with the recommendations of Barros and Novais (1999). Initially, the seedlings were subjected to an adjustment period (30 days - period of acclimatization) in the bags, in the shade house, to

ensure the establishment and survival of those seedlings receiving daily irrigation, sufficient to keep the substrate to as close to 60% of the field capacity at the time the treatments were started.

Application of treatments and statistical design

Different water regimes were started after 30 days of acclimatization in the shade house, for a 15-day period. Using the completely randomized 2 × 2 factorial design, the experiment was conducted with 25 seedlings per treatment: the water regime factor had two levels, irrigated (control) and not irrigated, while the genotype factor had two types, tolerant and sensitive. The irrigated treatments were maintained to as close to 60% of the field capacity. Every day, each bag to be irrigated with seedlings was weighed individually, and the mass plus water loss or used was replenished for each experimental unit.

Characteristics evaluated and statistical analysis

The chlorophyll and the variables of chlorophyll fluorescence were recorded from 16 plants in each treatment, just after their installation in the greenhouse and just before the treatment was started (time zero) and after that on days 6, 11 and 15.

The first fully expanded leaf (towards the apex from the base of the plant) was used to take the measurements and it was correctly identified with white wool. In the non-destructive method the total chlorophyll content was quantified indirectly using the chlorophyll ClorofiLOG brand, model CFL in 1030, according to the manufacturer's instructions. It was expressed as a dimensionless unit called Falker Chlorophyll Index (FCI) (Falker Agricultural Automation, 2008). Three measurements were recorded, avoiding the rib region and any portions damaged by pests or pathogens. These values were used to analyze the mean value.

The variables of chlorophyll fluorescence were measured using a portable PAM (JUNIOR-PAM, Heinz Walz GmbH) fluorometer. Taking the values of Fo (minimum fluorescence intensity), when the PSII reaction centers were open and at Fm (maximum fluorescence intensity), when the PSII reaction centers were closed, we calculated the variable fluorescence (Fv), obtained by Fm-Fo. Then we determined the potential quantum efficiency of PSII (Fv/Fm) (Maxwell and Johnson, 2000). Readings were taken using magnetic leaf clips attached to the fluorometer, placed in the middle region on the adaxial side of the leaf blade, avoiding the midrib. The measurements were recorded between 20 and 22 h, with the provision of a saturating light pulse of 0.3 s, at a frequency below 0.6 Khz.

The evaluations were done with the same experimental units and the same treatments four successive times. Statistical analysis was done using the nlme package in R software (R Core Team, 2013), allowing the adjustment of mixed linear models for data repeated measures (Pinheiro et al., 2013).

RESULTS

Index of total chlorophyll

The sensitive *Eucalyptus* clone sensitive showed no significant response in the total chlorophyll rate until the day 14. However, on day 15, without water supply, this rate dropped by 33.9% (Figure 1). The tolerant *Eucalyptus* clone tolerant, on the other hand, responded to the water restriction stress by increasing its chlorophyll rate from

day 6. Emphasis was also placed on day 11 of the stress, clear differences were noted in the chlorophyll content between the genotypes under the irrigated water regime, highlighting the clone tolerant. On analysis of the behavior of the genotypes under the non-irrigated water regime, on all the evaluation days except for the first day of stress application, a statistical difference in the chlorophyll content of clone tolerant compared with that of clone sensitive was observed, with higher total chlorophyll concentrations recorded for clone tolerant.

Variables of chlorophyll a fluorescence

Minimal fluorescence (Fo), maximum (Fm) and the quantum efficiency of PSII Fv/Fm revealed significant interactions between the treatments tested (Table 1). The analysis of the opening up of the genotype vs. water regime vs. time shows that water stress caused only significant effects on the values of minimal fluorescence in both clones on day 15 of stress application (Figure 2) purposes only. On day 15, the Fo value of clone sensitive decreased by 37.76%, due to lack of irrigation, whereas in clone tolerant, the Fo increased to 39.52%.

On analyzing the maximum fluorescence upon unfolding of the genotype vs. water regime vs. time, it appears that, contrary to what was reported for the variable discussed earlier, since day 6 of stress, the water restriction induced a 36.90% reduction in the Fm in plants of clone sensitive, becoming clearer day 15 (85.36%) of the stress (Figure 3). However, in the case of clone tolerant, the effects of the non-irrigated water regime were only observed on the Fm on day 15 of the drought, which decreased by 64.20%.

According to the analysis of the interaction between water regime vs. time, from day 6 onwards effect statistically significant due to drought was seen on the Fv/Fm ratio. During the last days of the experiment (11 and 15 days), limiting the water caused the Fv/Fm ratio to plummet to near zero values. A decline in the potential PSII (Figure 4) quantum efficiency was observed, beginning from day 6 of the stress, which can be related to the damage of the photosynthetic apparatus in the plants. Plants with their photosynthetic apparatus in perfect condition express typical optimum conditions for development, showing the quantum efficiency of PSII, expressed as the Fv/Fm ratio, with values between 0.75 and 0.85 (Bolhàr-Nordenkampf et al., 1989).

The expression of genotype vs. water regime reveals that the altered quantum of efficiency of PSII occurred due to water limitation in both clones, to less than 0.75 (Figure 5). Clone sensitive revealed the most substantial drop in the Fv/Fm ratio in which the quantum efficiency decreased to 56.27% for values below 0.40. On analysis of the genotypes under each water regime, no differences in the quantum efficiency were observed between the two clones subjected to the irrigated water regime, with
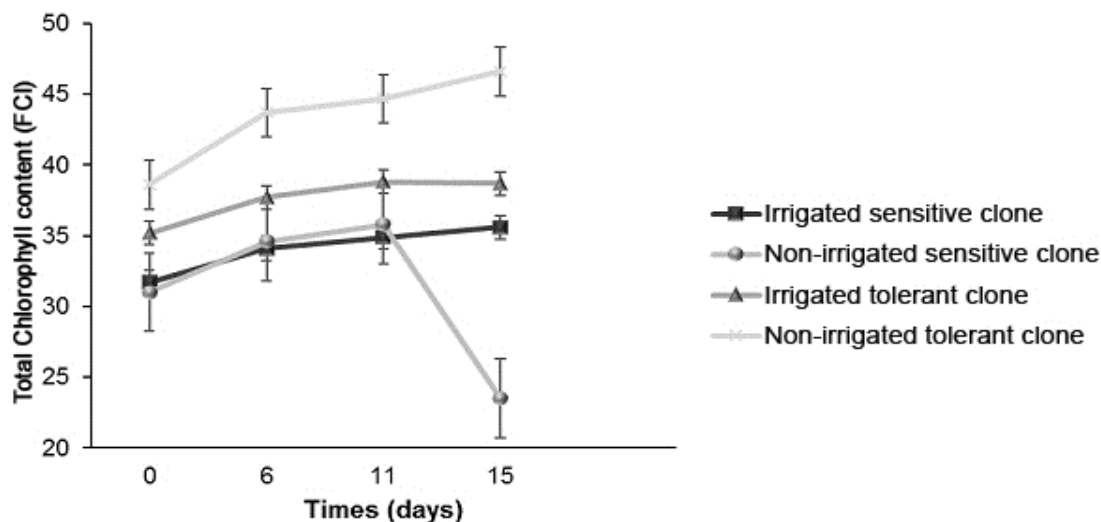
Figure 1. Total chlorophyll content of the *Eucalyptus* clones under two water regimes at different evaluation times.

Table 1. *F* statistic and *P*-value for minimal fluorescence (Fo), maximal fluorescence (Fm) and quantum efficiency of PSII (Fv∕Fm) of the seedlings of two *Eucalyptus* genotypes under two water regimes.

Sources of variation	D.F.	Fo		Fm		Fv/Fm	
		F statistic	*P*-value	*F* statistic	*P*-value	*F* statistic	*P*-value
Genotypes (G)	1	35.1959	<0.0001	0.001	0.9746	54.362	<0.0001
Water regime (Wr)	1	0.9322	0.3359	210.4869	<0.0001	754.99	<0.0001
Time (T)	3	80.6771	<0.0001	121.2374	<0.0001	171.694	<0.0001
G×Wr	1	21.9493	<0.0001	14.311	0.0002	12.922	**0.0004***
G×T	3	12.2007	<0.0001	5.0721	0.0023	5.76	0.0009
Wr×T	3	0.1668	0.9186	47.0605	<0.0001	181.375	**<0.0001***
G×Wr×T	3	9.7293	**<0.0001***	5.426	**0.0015***	2.634	0.0522

For interactions of interest, significance levels (P-values) are presented in bold and with * when significant (<0.01). D.F.: Degrees of freedom.
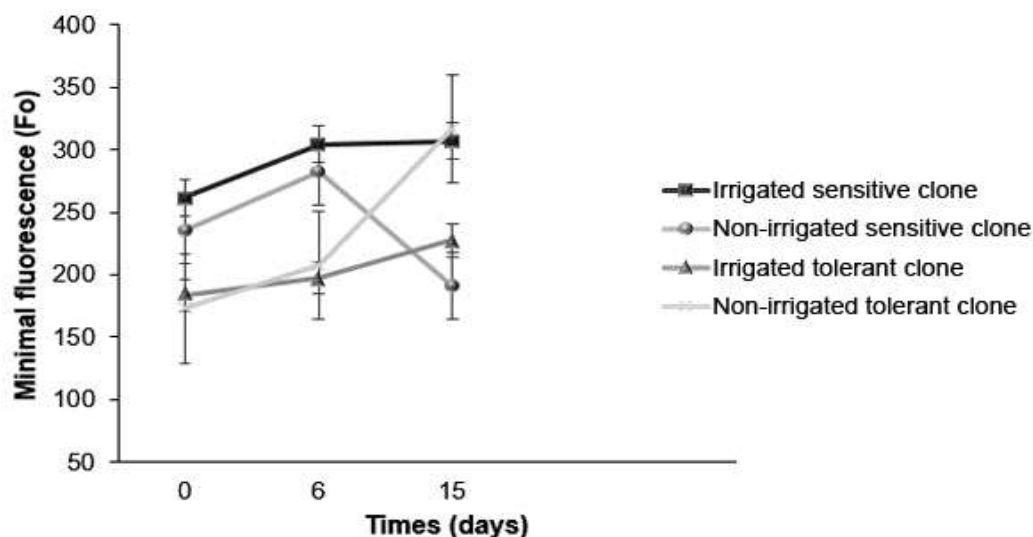
Figure 2. Minimal fluorescence (Fo) of *Eucalyptus* plants under two water regimes at different evaluation times.
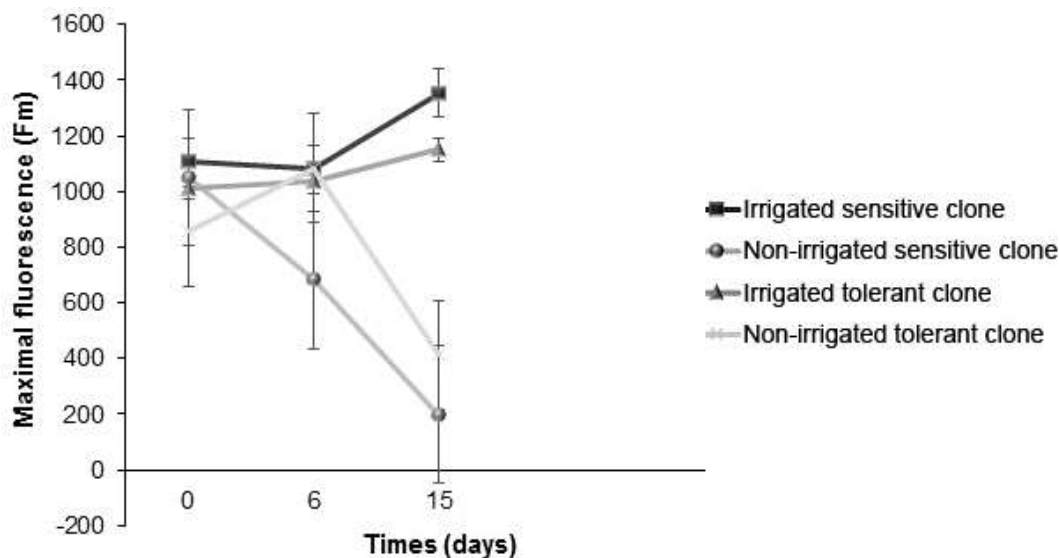
Figure 3. Maximal fluorescence (Fm) of the *Eucalyptus* plants under two water regimes at different evaluation times.

recorded values greater than 0.75. However, under the water restriction regime, clone sensitive showed the Fv∕Fm ratio at 31.13%, a lesser value when compared with clone tolerant, implying the greater effect of stress and its greater sensitivity to water deficit; however, clone tolerant has also expressed measurements below ideal values.

Although, the genotype vs. water regime vs. time showed no significance at the 5% level of significance; it is noted, as shown in Figure 6 that on day 6 of the water stress regime clone sensitive alone was negatively affected, probably due to the decrease in the maximum fluorescence (Figure 3). It was on this day, that the plants of clone tolerant recorded values above 0.75 for the Fv∕Fm ratio, as well manifested visible symptoms in response to the water deficit.

DISCUSSION

Index of total chlorophyll

The responses of plants to drought can be expressed in different ways and the ability of a plant to tolerate a drought stress is generally determined by the combination of attributes expressed during the development of a drought. In this study, the two contrasting genotypes showed different behaviors as the studied variables.

As seen in Figure 1, the subjection of the clones to up to 11 days of water stress did not induce any changes in the chlorophyll content, although in the sensitive genotype reductions began to be observed only from day 15 of the stress. Nautiyal et al. (1996) in their study had a drop in the chlorophyll content in the *Pongamia pinnata*

plants only under the more severe water conditions. These authors therefore suggest that the species in question is capable of survival and can remain photosynthetically active under moderate drought conditions. However, the more severe conditions exert an adverse effect on the chlorophyll content. Huang et al. (2013) stated that the substantial decrease or change in the chlorophyll content during water deficit periods varies with the intensity, duration and severity of the deficit.

On day 15 of the evaluation the effect of the severity of the stress was observed, confirmed by the visible symptoms and dryness of the substrate used. This state probably favored the appearance and accumulation of the reactive forms of oxygen, which damage the plant tissues by oxidizing the photosynthetic pigments, membrane lipids, proteins and nucleic acids (Raoudha et al., 2007; Xue et al., 2011). It is these reactive forms of oxygen that actually degrade the thylakoid membranes of the chloroplasts, by peroxidation of their lipids (Marenco and Lopes, 2005). The same phenomenon was observed in wheat plants subjected to water limitation during which the peroxidation rates were seen to rise significantly depending upon the severity of the stress (Tatar and Gevrek, 2008) they were experiencing. The drop in the chlorophyll content under the stress due to drought has therefore been suggested as a characteristic symptom of oxidative stress and could be caused by pigment photo-oxidation and chlorophyll degradation (Xue et al., 2011).

Clone tolerant, however, expressed an increase in the chlorophyll content when subjected to drought conditions. A similar result was observed by Silva et al. (2004) in *E. grandis* and by Yanqiong et al. (2007) in four shrub species under conditions of water limitation. A few other authors recorded an increase in the chlorophyll content

under moderate stress conditions and explained it as likely being due to a slowing down of cell growth in relation to the chlorophyll synthesis. Ebrahimiyan et al. (2013) for instance, observed a relationship between the chlorophyll content and dry matter production during moderate stress conditions. This implied that the loss of leaf weight after moderate stress could produce a relative rise in the chlorophyll content. On the other hand, some authors did not encounter any significant difference in the total chlorophyll content of certain plant species under varying water regimes, as did Egert and Tevini (2002) in the plants of *Allium schoenoprasum* and Shvaleva et al. (2006) in *Eucalyptus globulus*. Although plants exposed to severe water stress usually undergo degradation of their photosynthetic pigments due to oxidative damage, plants can, according to Egert and Tevini (2002), protect themselves by synthesizing antioxidant molecules (carotenoids, ascorbate, a-tocopherol, flavonoids and glutathione) or by increasing the antioxidant enzymes synthesis (peroxidase, catalase and superoxide dismutase).

From these reports, the rise in the chlorophyll content of clone tolerant observed from the first day of water limitation until the last day of the stress may be attributed to a slowing down of plant growth in relation to chlorophyll synthesis in the early stages of the stress and associated with the activity of an efficient antioxidant mechanism under more severe water restriction.

Variables of chlorophyll a fluorescence

Drought affects the Fo causing light emission from the excited chlorophyll molecules, prior to the energy being dissipated to the PSII reaction center (Krause and Weiss, 1991; Baker and Rosenqvist, 2004). This has been the focus of controversy in the literature. Some authors are of the opinion that the decrease observed for this variable can be associated with the impairments in the reaction center of PSII or due to the faulty transfer of excitation energy from the antenna complex to the reaction centers, indicating an even greater sensitivity to the water-restriction condition (Silva et al., 2006; Tatagiba and Pezzopone, 2007; Michelozzi et al., 2011). Tatagiba and Pezzopone (2007) reported an increase in the minimal fluorescence of the *Eucalyptus* plants during the dry season, the phenomenon being credited to the ability of these clones to tolerate water restriction conditions in the soil.

Authors Zlatev and Yordanov (2004) view the increase in the Fo as a negative effect of drought from their study in bean plants, as did Calatayud et al. (2006) on rosebushes. These authors hold that the highest observed Fo level can be attributed to an increase in the fraction of the PSII reaction centers in the photo-inactivated state, resulting in a drop in the photochemical capacity of the PSII. Efeoğlu et al. (2009) too, in their

work on corn plants, recorded an increase in the Fo under water restriction conditions. They cited the dissociation of the complex light collection of the photosystem II reaction centers as the possible cause. Photoinhibition of the reaction centers or even the reduced pool of plastoquinone (PQ) in leaves that have endured dark adaptation could be another reason. Matos et al. (2010) credited the increase in the minimal fluorescence in *Cicer arietinum* under water restriction to the reaction center, showing that the greatest losses occur during the energy transfer from the antenna after excitation.

Silva et al. (2006) showed that the reduction in the Fm was observed in the non-irrigated water regime in both genotypes. They indicated in general the faulty photoreduction of quinone A (QA), the primary electron acceptor of photosystem II, which may be associated with PSII inactivation in the thylakoid membranes, directly affecting the electron flow between the photosystems. The same authors reported similar behavior in different forage species when subjected to water restriction. The later and less intense effect of water deficiency observed on clone tolerant implies a lower sensitivity to the higher intensity and duration of the water deficits applied (Silva et al., 2006) and indicates a greater drought tolerance compared with clone sensitive. The results concur also with the report of Zlatev and Yordanov (2004) in bean plants and that of Bączek-Kwinta et al. (2011) in *Chamomilla recutita*. Tatagiba and Pezzopone (2007) however, found this variable to increase in the *Eucalyptus* plants during the dry season, which according to these authors implies that the restricted water supply caused no decrease in the photoreduction of quinone A (QA) and none in the electron flow between the photosystems in the clones studied.

In both genotypes, the decrease in the maximal fluorescence noted could be correlated with the increase in the degradation rates of the D1 protein with increasing water limitation, as a result of the action of the reactive oxygen (Zlatev and Yordanov, 2004; Rivero et al., 2010). The D1 proteins are significant in the functioning of photosystem II as it is through these that the electron flow from the reaction center to the quinone occurs, which to achieve complete reduction emits fluorescence to the maximum level (Araújo and Deminicius, 2009; Rivero et al., 2010). Thus, with the increase in the degradation rates of these proteins due to water deficit stress, less energy is transferred from the photosystem II reaction center to the quinone, producing low maximal fluorescence levels.

A decrease in the quantum yield of PSII, as seen in Figures 4, 5 and 6, implying a reversible photoprotective regulation or an irreversible inactivation of PSII, have been recorded in many plant species due to soil water deficit (Ditmarová et al., 2010; Araújo et al., 2010; Michelozzi et al., 2011; Wang et al., 2012).

The drop in the Fv∕Fm ratio in the *Eucalyptus* clones
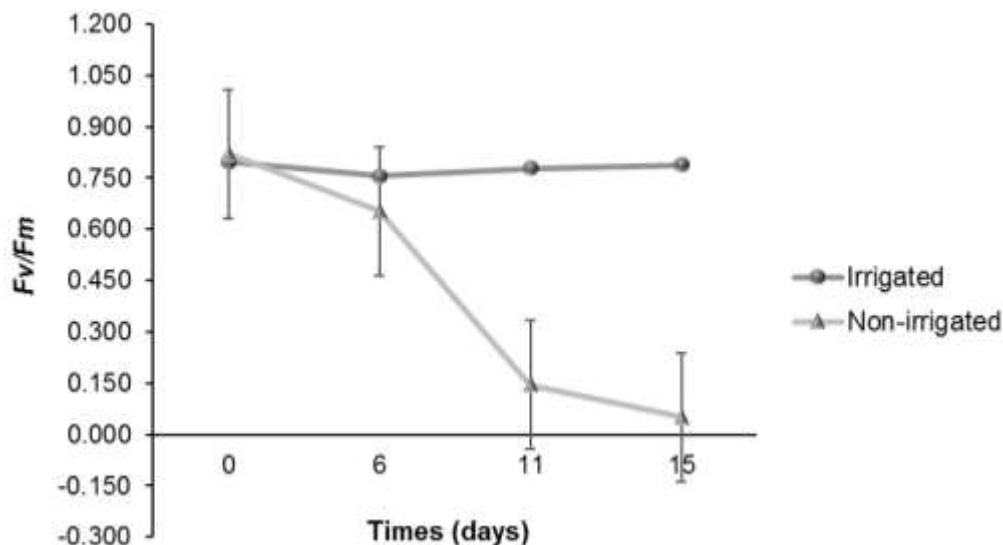
Figure 4. Quantum efficiency of PSII, expressed as Fv∕Fm ratio, in *Eucalyptus* plants under two water regimes at different evaluation times.
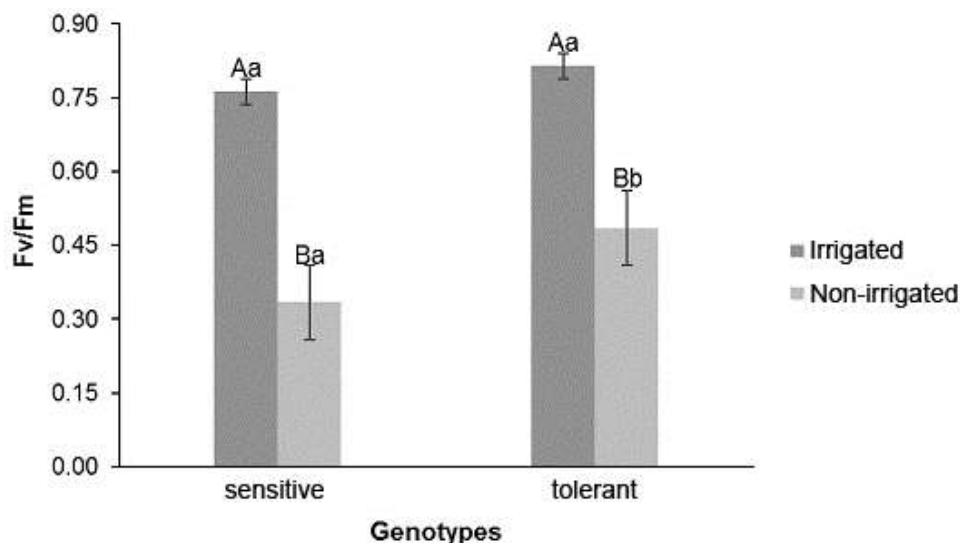
Figure 5. Quantum efficiency of PSII, expressed as the ratio Fv∕Fm of plants of two *Eucalyptus* genotypes subjected to two water regimes. Different lowercase letters indicate significant differences between the genotypes in each water regime while the different capital letters indicate significant differences between the water regimes within each genotype (** $P \leq 0.01$).

due to water deficit is similar to observations of Rolando and Little (2003) in *E. grandis* and by Lima et al. (2003) in five species of *Eucalyptus* (*E. grandis*, *E. urophylla*, *E. camaldulensis*, *Eucalyptus torelliana* and *Eucalyptus phaeotrica*). Although, this ratio normally decreases in plants experiencing some type of stress, Rolando and Little (2008) observed no changes in the chlorophyll fluorescence when the *E. grandis* seedlings were subjected to water restriction conditions. These authors

propose that this unexpected absence of perceptible changes in the fluorescence parameters could be the result of using plant species possessing a higher tolerance to drought or due to the short experimental time. In contrast, Susiluoto and Berninger (2007) studied the response of *Eucalyptus microtheca* to drought at the home of a greenhouse reported an increase in the Fv∕Fm ratio under stressful water conditions. However, in a study by Susiluoto and Berninger (2007), the response of
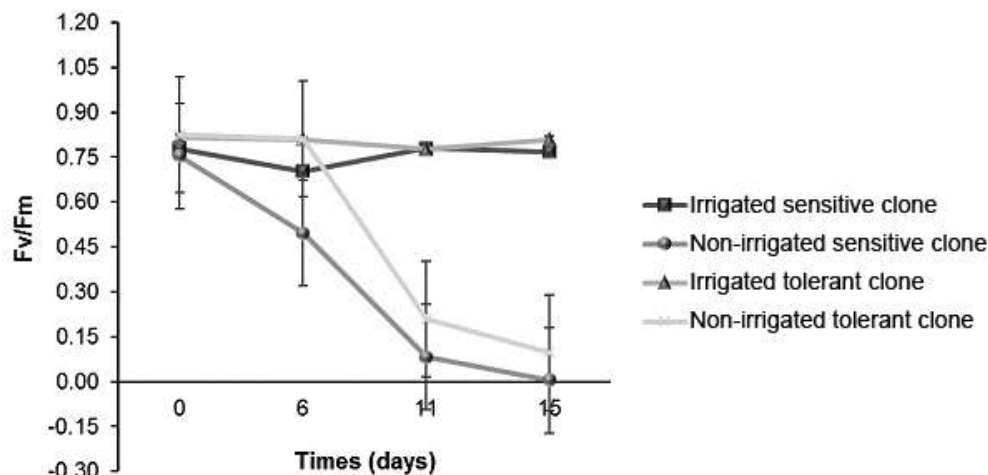
Figure 6. Quantum efficiency of PSII, expressed as the Fv∕Fm ratio in *Eucalyptus* plants subjected to two water regimes at different evaluation times.

E. microtheca to drought conditions in a greenhouse revealed an increase in the Fv∕Fm ratio.

The negative effects of drought on the functional integrity of PSII, which were observed in this experiment too, were certainly the result of the degradation of the PSII components other than the chlorophylls. From day 6 of the experiment, the chlorophyll content of clone sensitive showed no change due to water limitation, although, this day revealed a reduced Fv∕Fm (Figure 6) relationship. This same clone revealed a drop in the chlorophyll content, which coincides with the decline of the quantum efficiency of PSII on day 15 of the stress. Clone tolerant, however, experienced an increase in the chlorophyll content throughout the experiment, under the water restriction conditions, as observed.

As interpretation in Figure 6, the later and less intense damage to PSII seen from the appearance of clone tolerant may be indicative of a photosynthetic apparatus more efficient than that of clone sensitive with respect to tolerance of the photo-inhibitory conditions resulting from the stress of water restriction.

Conclusions

The gradual rise in the chlorophyll rate as well as the reported behavior of the variables of fluorescence confirm the hypothesis that the features of genotype tolerant reveal a more efficient photosynthetic apparatus for tolerance to the photo-inhibitory conditions arising from low water availability. The potential use of such variables in the forest breeding programs whose objective is to selecting drought tolerant genetic material, emphasizing the quantum efficiency of PSII, expressed as the Fv∕Fm ratio was also highlighted. The latter was found to be a reliable tool facilitating the selection for plants with drought tolerance, having the additional advantage of

enabling a precise, economic, rapid and non-destructive evaluation. This is very important for forest farmers and breeders, since they need to select donor trees and *Eucalyptus* clones tolerant to water stress in the young stage when they are still in the seedling nursery.

Conflict of interests

The authors have not declared any conflict of interests.

Abbreviations

Fo, minimum fluorescence; **Fm,** maximum fluorescence; **Fv/Fm,** potential quantum efficiency of photosystem **II**; **FSII,** photosystem **II**; **FCI,** Falker Chlorophyll Index.

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Full Length Research Paper

An optimization model for the combined planning and harvesting of sugarcane with maturity considerations

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Planting and harvesting are important stages in the sugarcane crop cycle, because well planned planting and harvesting phases promote a series of benefits throughout the cultivation cycle and in the subsequent industrial use of the products. These benefits are operational, economic and environmental such as: improved utilization of the land area and transport logistics; an increase in sugarcane output; better cane reception in the factory; in administrative simplification of the industrial activities; in enhanced response to the demands of the industry; in cost planning; and in the control of pests and weeds. In this work a methodology of optimal cultivation planning to sugarcane planting and harvesting is proposed. The cultivation plan is for 5 years; and key decisions to be made in this period are to determine the planting date, the variety selection and the harvesting date corresponding for each plot such that the global production is optimized. We propose a mathematical model for this optimization task. The model uses computational and mathematical strategies to ensure that date of harvesting is always in period of the maximum maturation of the sugarcane and considers all demand and other operational constraints of the processing mill. The binary nonlinear optimization model was solved by a proposed genetic algorithm, giving an optimum plan with a potential sugarcane production 17.8% above production obtained by conventional means in the mill.

Key words: Genetic algorithm, integer nonlinear optimization model, optimal planning, *Saccharum* spp., sugarcane planting and harvesting.

INTRODUCTION

The growing of sugarcane has been gaining importance in recent years in several countries of the world due its use in the production of sugar, ethanol alcohol and electrical power from its bagasse and residue of

harvesting. Due to expansion of this crop in recent years, an efficient sugarcane production planning system for new and renewed areas becomes essential, because it will help produce optimal economical, social and environmental benefits of the sugar-alcohol sector. According to Scarpari and Beauclair (2010), optimized agricola planning is a fundamental activity for increasing the quantity and quality of the sugarcane crop. This in turn enhances the sugar-alcohol industrial sector as it increase profits and decrease costs. In this context the need for a decision support technique that helps the mill manager to obtain an optimized planning system for sugarcane production is evident. Due to the complexities involved, this system necessarily needs to contain one or more mathematical optimization tools. The literature contains some works focused in planning the cultivation of sugarcane using optimization techniques with the objective of improving the quality and quantity of the raw material in sugar-alcohol mill. These are reviewed below.

Piewthongngam et al. (2009) propose an optimization model for planning and cultivating sugarcane. The model aims to select the period and variety for planting in order to avoid oversupply during the peak harvest time. The plan ensures that the cane is cut properly throughout the harvest period, hence optimizing the global sugar production.

Jena and Poggi (2013) propose a planning system specifically for sugarcane harvesting aiming to improve the production of sugar and ethanol in a specific mill in Brazil. The authors present an optimization model for tactical and operational planning such that the total sugar content in the harvested sugarcane is maximized. They present a case study to illustrate the benefits of the proposed planning. These authors consider the total profit increase of 2.6% as satisfactory, but discuss the difficulties encountered regarding computational time and the need for studies that develop new techniques for this problem.

There are many other works addressed using optimization techniques to improve processes for cultivation and exploitation of sugarcane. But these studies do not necessarily consider the planning for sugarcane planting and harvest over a medium term planning period, nor incorporate the maturity date of the sugarcane into their models. They hence may obtain results with a low exploitation of the quality of the sugarcane, (Buddadee et al., 2008; Florentino et al., 2015; Florentino and Pato, 2014; Higgins et al., 1998; Leboreiro and Hilaly, 2011; Salassi et al., 2002; Stray et al., 2012). In this work, we propose a methodology to optimize the cultivation planning for sugarcane planting and harvesting.

The cultivation plan is for a five years period and determines the planting date, the variety selection and the harvesting date corresponding for each plot such that the global production is optimized. The methodology uses computational and mathematical strategies to ensure that

date of harvesting is always in the period of the maximum maturation of the sugarcane.

MATERIALS AND METHODS

This work proposes a methodology that aims to produce an optimal planting and harvesting plan for the sugarcane crop. Therefore, we present here a mathematical model as a tool for determining this plan. A Genetic Algorithm is developed to solve the resulting mathematical model.

We propose an integer nonlinear optimization model to assist in the planning of sugarcane planting and harvesting during a 5 years (4 cuts) planning period such that the overall sugarcane production is optimized. This integer nonlinear optimization model is difficult to solve with commercial software due to the relatively large number of binary variables and hence a heuristic approach is required. Therefore, we propose a genetic algorithm (GA_PlanHarv) that has a relatively straightforward computational implementation and interpretation of results.

A mathematical model is proposed to choose the sugarcane variety i to be planted in each available plot j, to determine the appropriate period t_o for this planting and to determine the period t_c for harvest in the four years following the year of planting; in order to maximize the total sugarcane production over five years, i.e. four cuts ($c=1,2,3,4$). Where $i=1,\ldots n$; $j=1,\ldots k$; $t_o=t_{o1},\ldots t_{oz}$; $t_c=t_{c1},\ldots t_{cs}$; $c=1,2,3,4$; n is the number of the varieties adaptable to local climate and soil; k is total number of the plots available for sugarcane planting; z and s are the numbers of months appropriate for the planting and harvesting of sugarcane respectively.

According to Rudorff et al. (2010), the sugarcane cycle is semi-perennial and begins with the planting of a stem cutting that grows for about 12 months (called *year sugarcane*) or 18 months (called *year-and-half sugarcane*). The main climate components which control growth, production and quality of the sugarcane are temperature, light and moisture availability, so the most appropriate months for sugarcane planting in Brazil are January, February and March for *year-and-half sugarcane*; September and October for *year sugarcane*; in this way the climate assists the correct development of this culture. The sugarcane cut should be made from April to December, because these are the optimal months for harvesting in Brazil due humidity and temperature. After the first harvest, the ratoons are harvested annually for a period of about 5 years. Successive harvests lead to a gradual yield loss until the crop is no longer economically profitable. At this point, the cycle is interrupted, and the area is renovated with the planting of the new stem cuttings. Should the ripe sugarcane not be harvested, it will keep on growing, but loses a lot of its sucrose and fibre quality. An important sugarcane quality parameter for assessing cane maturity is the sucrose percentage or also referred to as pol percent (pol% cane). Pol% cane is the sucrose percentage present in sugarcane juice.

In the formulation of the mathematical model two possible periods for planting are considered. The first is P1={January, February, March} for *year-and-half sugarcane* and the second is P2={September, October} for *year sugarcane* or referring the month by number: P1={1,2,3} and P2={9,10}. Thus, if the sugarcane is planted in period P1 the first harvest is made 12 months after planting, and if the sugarcane is planted in period P2 the first harvest is made 18 months after. From the second harvest onwards, the cut is always made approximately after each 12-month period. It is very difficult to obey exactly the mentioned periods for harvesting the sugarcane due to the technical capabilities of the mill (machinery, milling, transportation, etc.). Therefore, harvest up to two months before or after these time points is permitted.

Figure 1 illustrates a planning for 5 years (60 months) of a *year-*
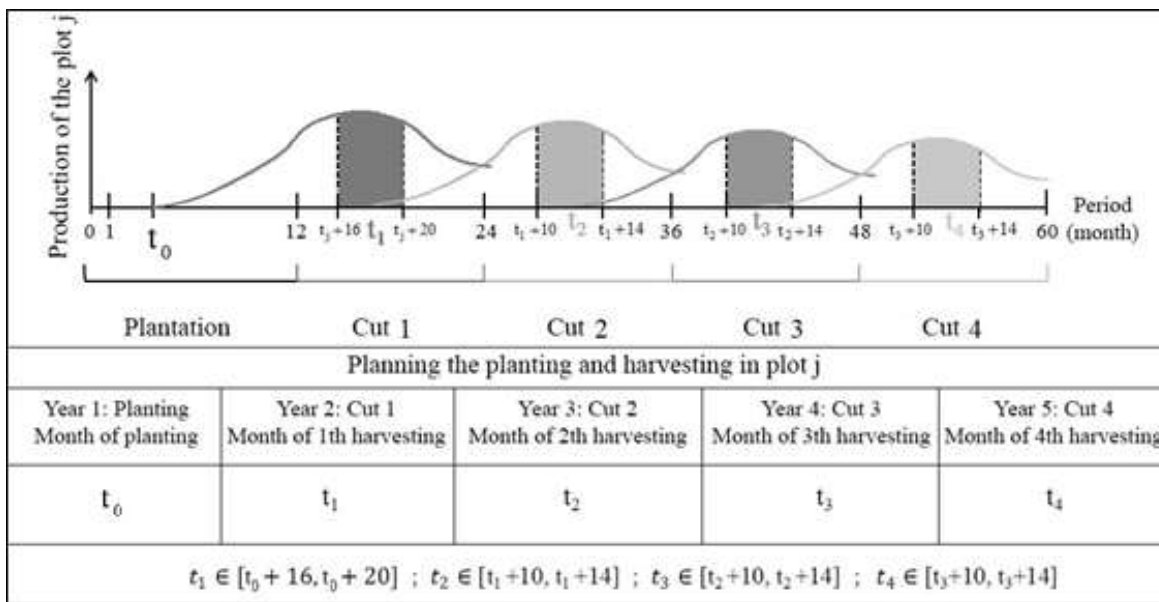
Figure 1. Planning the planting and harvesting in plot j.

(a)   (b)
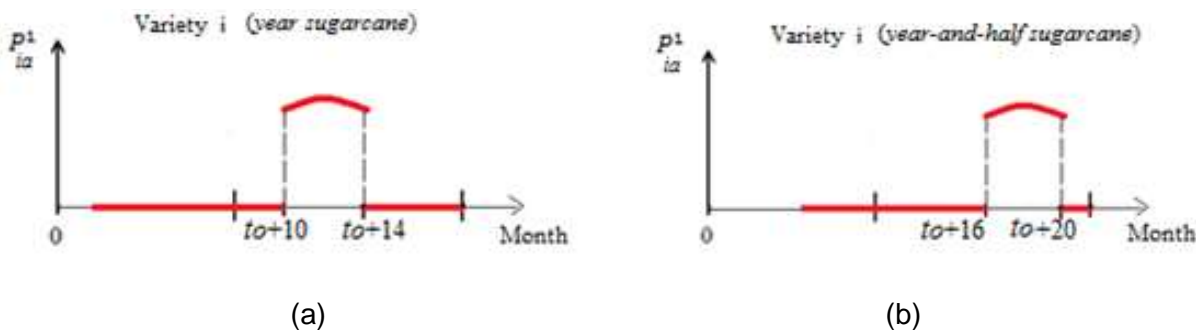
Figure 2. Function P_{ia}^1 for the cases of the first cut of *year sugarcane* and *year-and-half sugarcane*.

and-half sugarcane of variety *i* planted in plot *j*. In the first year of this planning the sugarcane should be planted in month $t_o \in$ P1. The first cut should be done preferably 18 months after planting, and may vary within a two month interval, ie, the first cut should be conducted in month $t_1 \in [t_o +16, t_o+20]$. The second cut should be done in month t_2 preferably 12 months after t_1, belonging to the time interval $[t_1 + 12, t_1 + 14]$, and so on. Thus, during the five year planning horizon, the sugarcane will always be harvested within its high productivity interval.

The optimal time for first cut depends if the sugarcane is *year sugarcane* (12 months) or *year-and-half sugarcane* (18 months) and for the next cuts is 12 months for both. The sugarcane productivity (P_{cia}), pol %cane (A_{ica}) and fibre (F_{ica}) vary with cut number (*c*), sugarcane variety (*i*) and period of time that it remains in field (*a*), then is advisable that harvesting in all plots is undertaken very close to the optimal time (Colin, 2009, Rudorff *et al.* (2010)). To force a plan with a cut of the sugarcane close to the optimal time the following productivity function is proposed.

Let *i* be a index associated with sugarcane variety and P_{ia}^1 the produtivity of the variety *i* when it is harvested *a* months after the planting or most recent cut. This produtivity function is defined as follows.

$$P^C{}_{ia} = \begin{cases} P_{cia} \geq 0, \text{if } a \in [t^o, t^{oo}] \\ 0 \text{ otherwise} \end{cases}$$

Where:

P_{cia} is the known productivity of the sugarcane of variety *i*, in the *c-th* cut and it has *a* months that were planted or cut ($0 < a \leq 18$; *i*=1, 2, …, *n*; *c*=1,2,3,4; n is the total number of the sugarcane varieties adaptable to local climate and soil);
$[t^o, t^{oo}]$ =[10, 14] if (*c*=1 and *i* is *year sugarcane*) or if (*c*>1);
$[t^o, t^{oo}]$ =[16, 20] if (*c*=1 and *i* is *year-and-half sugarcane*);

The graphical representation of the function $P^1{}_{ia}$ for the cases of the first cut of *year sugarcane* and *year-and-half sugarcane* are shown in Figure 2.

Let x_{ijt} and y_{jt} be decision variables, such that: x_{ijt}=1 if variety *i* is planted in plot *j* at time *t* and x_{ijt}=0 in the contrary case, y_{jt}=1 if the sugarcane variety planted in plot *j* is harvested in time *t* and y_{jt}=0 in the contrary case. The proposed model is therefore:

$$max\left(\sum_{j=1}^{k}\sum_{i=1}^{n}\sum_{t_o\in\{P1\ or\ P2\}}\left(\sum_{t_1=t_o+10}^{t_o+20}\left(P1_{i(t_1-t_o)}x_{ijt_o}y_{jt_1}+\sum_{t_2=t_1+10}^{t_1+14}\left(P2_{i(t_2-t_1)}y_{jt_2}L_j+\sum_{t_3=t_2+10}^{t_2+14}\left(P3_{i(t_3-t_2)}y_{jt_3}L_j+\sum_{t_4=t_3+10}^{t_3+14}P4_{i(t_4-t_3)}y_{jt_4}L_j\right)\right)\right)\right)\right)$$
(1)

Subject to:

$$\sum_{i=1}^{n}\sum_{t_o\in\{P1\ or\ P2\}}x_{ijt_o}=1\qquad j=1,\dots,k$$
(2)

$$\bar{\imath}_j=\sum_{i=1}^{n}\sum_{t_o\in\{P1\ or\ P2\}}x_{ijt_o}\,i\qquad j=1,\dots,k$$
(2a)

$$t_{o_j}=\sum_{t_o\in\{P1\ or\ P2\}}x_{\bar{\imath}_jt_o}\,t_o\qquad j=1,\dots,k$$
(2b)

$$\sum_{t=t_o+10}^{t_o+20}y_{jt}=1\qquad j=1,\dots,k$$
(3)

$$t_{1j}=\sum_{t=t_o+10}^{t_o+20}y_{jt}\,t\qquad j=1,\dots,k$$
(3a)

$$\sum_{t=t_c+10}^{t_c+14}y_{jt}=1\qquad j=1,\dots,k\qquad c=1,2,3$$
(4)

$$t_{(c+1)j}=\sum_{t=t_{cj}+10}^{t_{cj}+14}y_{jt}\,t\qquad j=1,\dots,k\qquad c=1,2,3$$
(4a)

$$\sum_{j=1}^{k}A_{\bar{\imath}_jc(t_{cj}-t_{(c-1)j})}y_{jt_{cj}}\geq D_c\qquad c=1,2,3,4$$
(5)

$$FI\leq\sum_{j=1}^{k}F_{\bar{\imath}_jc(t_{cj}-t_{(c-1)j})}y_{jt_{cj}}\leq FS\qquad c=1,2,3,4$$
(6)

$$\sum_{t=1}^{t_j}\sum_{j=1}^{k}L_jx_{ijt}\leq 0.15\qquad i=1,\dots,n$$
(7)

$$MI\leq\sum_{j=1}^{k}L_j\,P^c_{\bar{\imath}_j(t_{cj}-t_{(c-1)j})}y_{jt_{cj}}\leq MS\qquad c=1,2,3,4$$
(8)

$$x_{ijt}=0\ or\ 1\qquad i=1,\dots,n;\quad j=1,\dots,k;\quad t=1,\dots,60$$
(9)

$$y_{jt}=0\ or\ 1\qquad j=1,\dots,k;\qquad t=1,\dots,60$$
(10)

Where:
n is the number of the sugarcane varieties adaptable to the local climate and soil; k is the number of the plots available for planting; i = 1, 2, ..., n is the index associated to sugarcane variety; j = 1, 2, ..., k is the index associated with the plots; t = 1, 2, ..., 60 is the index associated to time in month for planning; L_j is the area of the plot j in ha; P^C_{ia} is the productivity of the variety i when it is harvested a months after planting ($a=t_c-t_{c-1}$); A_{ica} is the productivity of the pol %cane of the sugarcane variety i in t.ha^{-1} when it is harvested a months after the last cut ($a=t_c-t_{c-1}$); D_c is the demand of sugar (in tons) in the year of the cut c; F_{ica} is the productivity of the fibre of the sugarcane variety i in t.ha^{-1} when it is harvested a months after the most recent cut ($a=t_c-t_{c-1}$); FI and FS are the lower and upper bounds for the sugarcane fibre at any time. The mills in the São Paulo state of Brazil use FI = 8% and FS = 14%; MI and MS are the lower and upper bounds for the sugarcane milling capacity of the mill.

The model determines which month t_o and which sugarcane variety i will be planted in each plot j in first year of the planning and during which period t this sugarcane will be harvested in next 4 years of planning, so as to maximize the objective function (1) related with sugarcane production during this 5 year (4 cuts) period. The constraints (2) guarantee that there will be sugarcane planting in all plots j in the first year of the planning, in months belonging to P1 or P2. The Equation (2a) derives $\bar{\imath}_j$ (the index of the sugarcane variety to be planted in plot j) and the equation (2b) derives t_{oj} (the month that the sugarcane variety $\bar{\imath}_j$ must be planted in plot j). The constraint set (3) guarantees that there will be sugarcane harvesting in all plots j in the first year of planning. The equations (3a) calculate t_{1j} (the month that will be the first sugarcane harvesting in plot j). The constraint set (4) guarantees that will be sugarcane harvesting in all plots in years 2, 3, 4 of the planning. The equations (4a) derive t_{cj}, c=2,3,4 (the month that will be sugarcane harvesting in all plots j in years 2, 3, 4 of the plan). The constraint set (5) guarantees the production of the pol %cane demanded by the mill in the planning period. The constraint set (6) guarantees the production of the fibre demanded by the mill in planning period. The constraint set (7) guarantees that each sugarcane variety will be planted in a maximum of 15% of the total area intended for planting. This is a requirement of the Brazilian mills to prevent pests and diseases. The constraint set (8) guarantees that the capacity of the mill for sugarcane grinding will be satisfied in all harvest periods and the constraints (9) and (10) define the decision variables of the problem as binary.

The model (1)-(10) is a binary nonlinear program (INLPP 0-1) which is difficult to solve, especially when it has large numbers of plots and varieties. The number of plots in current mills can make it impossible the solve using classical optimization techniques; this paper therefore investigates heuristics for determining good quality feasible solutions. A Genetic Algorithm is proposed, as follows.

Genetic Algorithm: GA_PlanHarv

The genetic algorithm (GA) was developed by Holland (1975). GA is based upon evolutionary Darwinian principles. An individual that has good fitness in a population has a greater chance of passing its genes to future generations via reproduction or crossover. Species carrying the correct combinations in their genes become dominant in their populations. Sometimes, occur mutations in genes and arise new species. Unsuccessful changes are eliminated by natural selection.

In this technique, a solution is called an *individual* or *chromosome*. A collection of individuals is called a *population*. The

Figure 3. Chromosome structure.

Figure 4. Crossover process.

individual is a plan for planting and harvesting of the farm, and it is composed of the a matrix with k columns representing the plots and 41 rows representing the 5 months of planting belonging to P1 and P2, and 36 months for harvesting for the four cuts (9 possible months for harvest in each year). For the creation of these individuals, firstly two random numbers are chosen for each column of the matrix: an integer number in the interval [1, n] and another integer number in the interval [1, 5]. The first number represents the sugarcane variety to be planted in each plots j (column j), chosen among the sugarcane varieties (listed from 1 to n), and the second number represents the period when this variety will be planted in each plot j. After planning the planting we beginning the harvest planning. For harvest planning, a month is chosen randomly in all years intended for cutting of the sugarcane for each plot, but such that the constraints (3), (4) and (10) are satisfied. This structure of the individual satisfies the constraints (2), (3), (4), (9) and (10), is shown by Figure 3.

The evaluation of the individuals is made by their *fitness* value. The *fitness* for each individual is measured as follows:

$$f_{ind} = FO_{ind} - p_{ind}.$$

Where f_{ind}, FO_{ind} and p_{ind}, are respectively the *fitness*, the objective function value and the penalty of the individual ind. The penalty p_{ind} of individual ind is zero if the individual is related to a feasible solution to the mathematical model and $p_{ind} = 0.8\,FO_{ind}$ for an infeasible solution.

In the first iteration a copy of the best individual (with highest fitness) is made and this is updated in later iterations if a superior individual is found. After all individuals are evaluated, the genetic operators are applied.

The first genetic operator to be applied is the *selection*. In all iterations of the population Pc% individuals are copied into an *intermediate population* to perform *crossover*. In this work the selection of individuals to be copied is made via Roulette Method (Holland, 1992). This method was chosen because empirical tests showed that this approach was more efficient than others. The second genetic operator is the *crossover*. In this process is chosen randomly two individuals among the elements of the *intermediate population* (copied by selection), called *Parent 1* and *Parent 2*, and a cutting place from the columns of the matrices representing those individuals is chosen by sampling a uniform random discrete variable. This process assists the separation of the genes that form two new individuals (*child 1* and *child 2*) while keeping characteristics of the parents, as shown in Figure 4.

The mutation is the third genetic operator. After crossover, individuals from the current generation are randomly selected for the mutation. A draw with low probability ($pm < 0.05$) is realized for each individual, in order to determine whether to change the information contained in these genes. If the number drawn is less

initial population can be randomly started or built. GA uses the operators *selection*, *crossover* and *mutation* to generate new solutions from existing one. In crossover, two solutions are generally combined to form a new individual. The solutions are *selected* among existing solutions in the population using some methods for the selection (e.g. Roulette Wheel Selection, Boltzman Selection, Tournament Selection, Rank Selection and Steady State Selection). These methods in general give a preference for individual with better *fitness*, so that a new individual is expected to inherit good characteristics. By iteratively applying the crossover operator, characteristics from good individuals are expected to appear more frequently in the population, eventually leading to convergence to an overall good solution. The mutation operator introduces random changes in the characteristics of individuals, generally applied at the discrete unit of solution level with the probability of changing the properties of a unit being very small– typically less than 1%.

The individuals in the proposed GA in this work (GA_PlanHarv) are generated using a random/constructive heuristic in order to comply with the periods of planting (P1 and P2) and cuts such that the genetic operators preserve this feasible structure. Each

Table 1. Average data of the varieties in the first year of the cultivation.

Variety	Productivity (t ha⁻¹)	Pol (%)	Fibre (%)	Harvest season
CTC 15	132.80	14.50	12.36	July - December
CTC 9	100.00	15.84	12.34	April - July
RB925211	89.29	14.67	12.30	May - August
CTC 6	136.00	14.98	11.16	August - December
RB855156	117.80	14.50	12.41	April - May
CTC 2	129.10	14.31	12.21	June - October
RB857515	148.20	14.82	11.47	July - December
SP80-1842	112.80	14.90	12.90	June - October
SP83-2847	126.70	13.20	12.74	July - December
SP80-3280	121.70	14.80	11.30	July - December
RB928062	113.00	15.75	12.38	September - December
RB966928	123.10	13.32	11.97	April - July
CTC 20	165.00	13.50	11.50	May - December
CTC 17	112.30	14.98	12.38	April - August
SP81-3250	140.60	15.02	12.91	June - October
CTC 4	130.90	13.54	11.80	June - November
RB92579	142.40	15.70	12.93	July - October
RB855453	133.35	13.90	12.38	April - July

Sources: CTC (2012) and RIDESA (2008).

Table 2. Parameters used for the implementation of the GA_PlanHarv.

G	N	Pc	Pm	n	MI	MS	D	FI	FS
3500	200	0.8	0.05	18	850	3200	114	8%	14%

Where: G is the maximum number of generations; N is the number of individuals in population; Pc is the crossover rate; Pm is the mutation rate; n is the number of sugarcane varieties adaptable to local climate and soil; MI and MS are the lower and upper bounds for the sugarcane milling capacity of the mill (tons month⁻¹); D is the demand for sugar (in tons month⁻¹); FI and FS are the lower and upper bounds for the sugarcane fibre.

than *pm* the change will occur, otherwise it will not occur.

In order to choose the gene to undergo change, as well as the new value to insert in this gene another draw takes place. These individuals are then evaluated (computing their *fitness* values). The *NP* best individuals from the previous population and the new individuals produced form the new population. The entire process is repeated until a pre-specified stopping criterion is achieved. This paper chooses the number of generations as the stopping criterion. The solution that offers the best fitness in the final generation is considered as the recommended solution to the problem.

RESULTS AND DISCUSSION

We validated the proposed methodology on a real case of a Brazilian farm in São Paulo state with the area of agricultural holdings of 183.12 hectares, divided into 21 plots for planting of the sugarcane, which are labeled from 1 to 21. The planning of the culture was conducted by a team composed of members of the agricultural and technical departments and coordinated by an agronomist. The planning was conducted during the period from July to September of the year before planting. The plan was executed and obtained a production total of 61,909.69 tons in four cuts.

For the planning of planting and harvesting of the sugarcane during a 5 years (4 cuts) period using the model (1)-(10), such that overall sugarcane production is optimized we use 18 candidate varieties for planting, which are presented in Table 1. Here we use the parameters presented in Table 2 to solve the model (1)-(10) using the GA_PlanHarv.

The GA_PlanHarv was implemented with MATLAB 7.6.0.324 (R2008a) software MATLAB (Matrix Laboratory, version 2012), and run on a micro-computer Dual Core i5-650 with 4 GB memory and a 400 GB hard drive, for a planning period of 5 years of the planting and harvesting of sugarcane in the cited farm and the results are presented in Table 3. The results can be achieved in an average of 50 min, an acceptable timeframe given the strategic nature of the planning process.

The agronomists typically spend about three months

Table 3. Planning of sugarcane planting.

Variety to be planted	Plot (J)	Area of plot (ha)	Total area (ha)	Month of planting
CTC 15	7	10.06	10.06	m_3
RB925211	4	4.68	4.68	m_2
CTC 6	8, 14	10, 7.39	17.39	m_1, m_2
RB857515	6, 10, 19	4.74, 10, 9.83	24.57	m_1, m_2, m_3
SP80-1842	13	8.1	8.1	m_2
RB966928	1, 9, 12	7.86, 8.19, 6.09	22.14	m_3, m_1, m_1
CTC 20	18, 20	10.03, 16.39	26.42	m_{10}, m_{10}
CTC 17	3	15.81	15.81	m_1
SP81-3250	5, 11, 15, 17	5.45, 8.14, 8.47, 4.75	26.81	m_1, m_3, m_2, m_1
RB855453	2, 16, 21	11.81, 8.31, 7.02	27.14	m_1, m_3, m_3

m_1= January, m_2= February, m_3= March, m_{10}= October

Table 4. Planning of the 4 cuts of the sugarcane harvesting.

Plot	Harvest season
1	m_7, m_6, m_6, m_7
2	m_5, m_6, m_4, m_4
3	m_4, m_5, m_5, m_6
4	m_5, m_7, m_7, m_5
5	m_8, m_8, m_8, m_8
6	m_7, m_9, m_{10}, m_{10}
7	m_{10}, m_{10}, m_{10}, m_{10}
8	m_9, m_9, m_{11}, m_{11}
9	m_4, m_4, m_6, m_5
10	m_{10}, m_{11}, m_{12}, m_{11}
11	m_9, m_{10}, m_{10}, m_{10}
12	m_4, m_5, m_5, m_7
13	m_8, m_9, m_9, m_{10}
14	m_8, m_8, m_{10}, m_9
15	m_6, m_8, m_7, m_8
16	m_6, m_5, m_4, m_5
17	m_6, m_6, m_7, m_9
18	m_{11}, m_{12}, m_{11}, m_{12}
19	m_7, m_7, m_8, m_7
20	m_{12}, m_{11}, m_9, m_9
21	m_5, m_7, m_7, m_7

m_4=April m_5=May m_6=June m_7=July m_8=August m_9=September m_{10}=October m_{11}=November m_{12}=December

planning this culture, thus a mathematical and computational tool like GA_PlanHarv can be an important and quick assistance for to assist them in achieving optimized decision making.

Table 3 shows that was possible to determine the planting plan using 18 sugarcane candidate varieties. The model chooses 10 among these 18 sugarcane varieties for planting. We note that the RB855453 variety has the greatest area of 4.68 ha. Regarding the months of planting we observe that the months intended for planting are well distributed.

The proposed model was able to plan the planting of sugarcane during the correct period, using all the available area and achieved a good distribution of the varieties.

Table 4 presents the results of the sugarcane harvesting planning during the 5 years of the cultivation of the sugarcane (4 cuts).

Table 4 shows that the model was able to plan the harvest for the proposed five years (four cuts) and satisfied all demands and technical capabilities imposed by mill.

The model plans the planting and harvesting together in an optimized manner, considering the mill conditions. In this way, it becomes easier to achieve goals, attend to the required demand and meet the constraints imposed by the mill.

Figure 5 shows the increase of the sugarcane production values during the computational development of the generations in the proposed GA for planning of the sugarcane planting and harvesting and compares these values with the actual value of the production presented by mill manager.

The Figure 5 shows that in 30 iterations of the proposed GA, the value of production estimated by the proposed model exceeded the value of the production given by mill manager. The estimated value for sugarcane production found by model was 75,319.61 tons for the five years of the planning, which corresponds to 13,409.92 tons more than the value presented by mill manager, representing an increase of 17.8% in sugarcane production.

The proposed methodology for the optimized planning of the process of planting and harvesting of sugarcane has a strong potential to assist mill managers, supporting decisions in a quick and safe way.

Conclusion

This paper has developed a binary nonlinear optimization
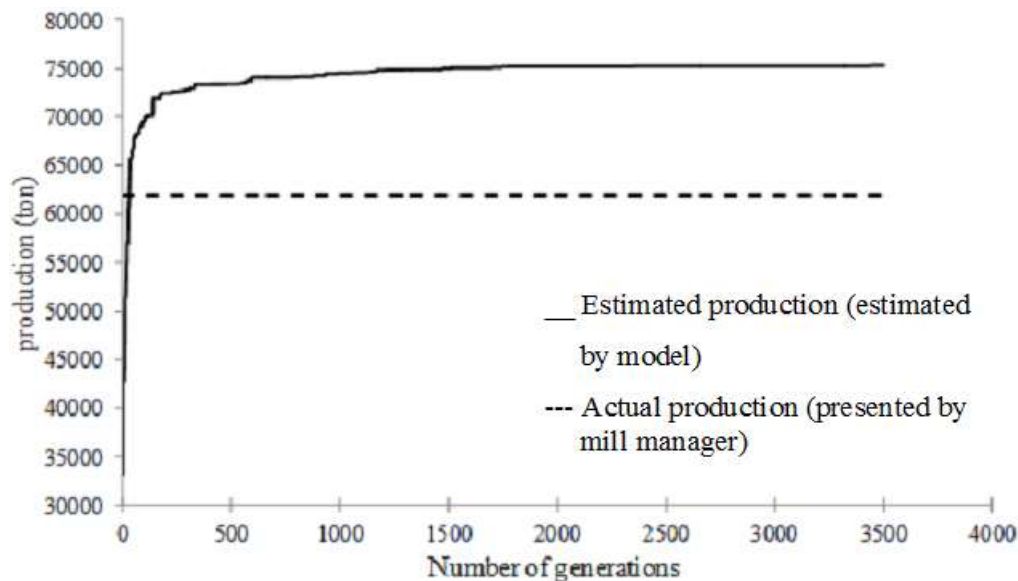
Figure 5. Sugarcane production, in tons, depending on the number of generations for the farm area.

model for decision support in the planning of planting and harvesting of the sugarcane for a period of five years, such that overall sugarcane production is optimized. The model uses mathematical strategies to enforce that the date of harvesting is always in the period of the maximum maturation of the sugarcane and considers all demand and other operational constraints of the mill. A genetic algorithm (GA_PlanHarv) is developed for efficiently solving the full binary nonlinear optimization model, finding good quality feasible solutions that meet the needs of the manager for the complex decisions involved.

The proposed methodology proved to be a good tool for the optimized planning of the planting and harvesting of the sugarcane, increasing by 17.8% the production as compared with that presented by the mill.

Due to the global energy and climate change crisis, sugarcane has become one of the most important crops in tropical and subtropical countries due to its use in bioenergy production, and additionally because the sugarcane can be used in sugar production. It is therefore an important product for the economy of those countries. However, this crop has undergone a recent and rapid expansion, resulting in the need for tools that assist managers of the mills in their decision making and implementation of their planning. Thereby it can be concluded that this research offers a worthwhile contribution by providing an effective mathematical tool with an efficient computational implementation that can offer results potentially better than those traditionally obtained in mills in a reasonable computational time.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of agricultural spray adjuvants in surface tension reduction and spray retention on *Eucalyptus* leaves

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Agricultural spray adjuvants (ASA) are widely used in pesticide applications to enhance the effective control of pest, weed and disease. The aim of this research was to investigate the effects of ASA used in Brazil agriculture on surface tension reducing capacity and foliar spray retention on different Eucalyptus species. Static surface tension of adjuvants at concentrations of 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0% v v^{-1} were determined by the drop weight method. Spray retention on *Eucalyptus* leaves using ASA were performed at concentrations of 0; 0.005; 0.01; 0.05; 0.1; 0.5; 1.0 and 2.0% v v^{-1}. The ASA assessed were: vegetal oil, mineral oil, spreader-sticker and a drift reducing based on soybean lecithin more propionic acid. All ASA adjuvants reduced surface tension of aqueous solutions. Heptomethyltrisiloxane (HT) provided the best performance on decrease of the surface tension reaching values below to 20 mN m^{-1} at concentration of 0.05% v v^{-1}. Spray retention was influenced by *Eucalyptus* species, adjuvants types as well as adjuvants concentrations. The increase of ASA concentration contributed to reduce spray retention. Different characteristic of the adjuvants on spray retention was observed in different *Eucalyptus* leaves species. *Eucalyptus grandis* and *Eucalyptus torelliana* species showed respectively the lower and higher spray retention values. The mineral oil and vegetal oil provide the higher and HT the lower level of spray retention. Application at high spray volume must be carefully performed to avoid losses by run-off when added some ASA.

Key words: Foliar retention, surfactant, application techniques, chemical control.

INTRODUCTION

Despite the negative perception of the society, chemical control using pesticides are still going to be used for many decades to ensure the food supply for the ever growing world population. One of the possible reasons for this is that alternative methods for plant protection are either inefficient or too costly for farmers (Wang and Liu, 2007).

Correct selection of application equipment and spray adjuvants are powerful tools to maximize pesticide efficacy, reduce detrimental environmental effects and improve the economic viability of the farmer (Dorr et al., 2014). Adjuvants are used to modify the physical, biological and chemical properties of spray mixtures to improve chemical performance (Kudsk and Mathiassen, 2007) impacting on viscosity, surface tension, contac angle, droplet retention, and deposits on the target (Gitsopoulos et al., 2014; Lin et al., 2016; Stock and Brings, 2000; Wang and Liu, 2007). The adjuvants may also influence spray atomization and formation, which is important because each type of application requires a certain optimum droplet size for its biological activity (Gimenes et al., 2013).

The spray efficacy depends on the amount of pesticide solution retained on leaves surface. In most cases, the wax on a leaf surface acts as a substantial barrier to wetting for having hydrophobic characteristic, which can make spray applications ineffective and increasing the environmental pollution (Tang et al., 2008). According to Lin et al. (2016) surfactant may not only effectively reduce the surface tension of solution but also dissolve the epicuticular waxes on the leaf surfaces and consequently, the addition of surfactant could strongly enhance the spreading ability of droplets. In order to improve targeting of the spray, it is important to know how formulation/liquid properties interact with the characteristics of the target plant to affect spray deposition (Butler-Ellis et al., 2004).

During disease and pest control applications, surfactant additives are commonly used to improve the efficiency of pesticides (Lin et al., 2016). Several studies showed that surfactants can greatly reduce surface tension and maximize the spread, penetration, and absorption efficacy of pesticides on leaf surfaces (Gimenes et al., 2013; Gitsopoulos et al., 2014; Lin, et al., 2016). Besides foliar uptake and biological efficacy of the active ingredients are improved with the surfactant by overlapping some leaf barriers such as cuticular membrane, trichomes and others features that decrease droplet deposition, spread and uptake of pesticide solutions.

The global planted area of *Eucalyptus* crop has increased significantly in the last decades. The trend in areas where Eucalypt are being grown in plantation is that pest and pathogen problems are increasing (Wingfield et al., 2003). Despite the contamination problems presented by the use of pesticide, this practice is required in various situations to control satisfactory pest and disease. The addition of corrected adjuvants on tank-mixture could enhance the performance of pesticides on pest, weed and disease control. The comprehension of adjuvants behavior is fundamental to prescribe the correct product/concentration maximizing pesticide control, avoiding losses and environmental contamination.

The aims of this research were to identify (1) the most effective and economic adjuvants/concentrations in reduce surface tension of water and (2) the spray retention capacity of adjuvants/concentrations on leaves of different *Eucalyptus* species used as a target surface.

MATERIALS AND METHODS

Surface tension determination

Static surface tension (SST) assessments were performed at the Laboratory of Pesticide Application Technology Laboratory, at the College of Agricultural Sciences UNESP - Botucatu, SP - Brazil. Details of the adjuvants composition selected for evaluations are summarized in Table 1. The adjuvants were tested at 11 concentrations levels (0.001; 0.0025; 0.005; 0.01; 0.025; 0.05; 0.1; 0.25; 0.5; 1.0 and 2.0% v v^{-1}) plus an additional treatment with no adjuvant (distilled water).

The SST of aqueous solution was determined by gravimetric method quantifying the weight of the droplets formed at the tip of the glass capillary burette (50 mL capacity) placed in a vertical direction. The droplets free detached at the tip of glass fall into a 25 mL Becker containing 10 mL of vegetal oil to avoid solution evaporation losses. Becker was allocated within the analytical precision balance with 0.1 mg accuracy (Marte, model AY 220, São Paulo, SP, BR) and the tip of the glass burette was kept 0.10 m above Becker.

The burette was adjusted to formed droplets at the time between 15 to 20 s and the liquid column was kept at 25 mL of graduation scale. The test was carried out at the room temperature of 25±1°C and air relative humidity of 60±10%. Fifteen droplets were measured per treatment and each droplet considered a repetition. All adjuvants solutions were prepared with distilled water assuming a SST of 72 mN m^{-1} at 25°C (Vazquez et al., 1995). Since the weight concentrations of surfactant were low, both the liquid density and viscosity of the surfactant solution were considered similar to the distilled water. The average droplets weight data were converted into surface tension according to Equation 1.

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Table 1. Agricultural adjuvants descriptions.

Adjuvants	Content	Dosage (v v^{-1})	Supplier
Haiten	nonionic spreader sticker surfactant - 200 g L^{-1} polyoxyethylene alkyl phenol ether	0.01-0.015%	Arysta LifeScience
Veget'Oil	70 g L^{-1} emulsifier and 930 g L^{-1} vegetal oil	0.2-1%	Oxiquímica Agrociência
Agral	nonionic spreader sticker surfactant - 200 g L^{-1} nonylphenoxy polyethoxy ethanol	0.03-0.05%	Syngenta Proteção de Cultivos Ltda
Silwet L-77 AG	superspreader surfactant - 1000 g L^{-1} Polyalkyleneoxide modified heptomethyltrisiloxane	Until 0.1%	Momentive Performace Materiais Industrias de Silicone
Li 700	acidifying and penetrating surfactant - 350 g L^{-1} soyal phospholypids and 350 g L^{-1} propionic acid	0.5%	De Sangosse Agrochemical Ltda,
Iharol	760 g L^{-1} mineral oil	0.5%	Iharabras S.A.

$$\gamma = (w_t \times 72)/w_w \qquad (1)$$

Where: γ = Surface Tension (mN m^{-1}); w_t= Droplet weight (g) of treatments and w_w= Droplet weight (g) of distilled water.

Statistical analysis of each adjuvant data was performed using the SAS program (SAS Institute, 1995) and regression analysis was used the Mitscherlich equation model, denoted by $\gamma = a[1-10^{c \cdot (x + b)}]$. Mitscherlich model was modified to get better fit to the data (Silva et al., 2006), expressing relationship between surfactant input and surface tension reducing.

Modified model used: $\gamma = \gamma_{dw} - a[1-10^{-cx}]$

Where γ: surface tension in mN m^{-1}; γ_{dw}: distilled water surface tension (72 mN m^{-1}); a: maximum horizontal asymptote attainable in the original model; c: curve concavity representing the efficiency of the surfactant. Higher value of this parameter represents the most effective the surfactant is to attainable the minimum surface tension in a lower concentration; x: surfactant concentration (%v v^{-1}); γ_{dw} - a: corresponds to the minimum surface tension reached by adding surfactant in aqueous solution. To compare the effects between adjuvants on SST a factorial design 6 adjuvants x 11 concentrations were analyzed.

Spray retention determination

Eucalypti leaf spray retention was evaluated by the same adjuvants used to determine SST. The experimental design was done in a factorial 6 (adjuvants) x 5 (*Eucalyptus* species: *Corymbia citriodora*, *Eucalyptus urophylla*, *E. camaldulensis*, *E. torelliana* and *E. grandis)* x 8 (adjuvants concentrations at 0; 0.005; 0.01; 0.05; 0.1; 0.5; 1.0 and 2.0% v v^{-1}) with seven replicates (each leaf was considered one repetition). *Eucalyptus* mature leaves were classified by Gaskin et al. (2005) as an easy moderate-to-wet. The *Eucalyptus* leaves were collected from a four years old plants grown outdoor located at Plant Protection Department - UNESP – Botucatu, Brazil. *Eucalyptus* branches were removed from the trees and immediately carried to the laboratory. At the time of tests the branches base was kept into a 10 L pot filled with water to avoid leaves humidity losses. *Eucalyptus* mature leaves were removed from the branches and determined the weight by analytical precision balance (Marte, model AY 220, São Paulo, SP, BR). Afterwards the leaves were placed in a vertical position (holding the

petiole leaves by hand) and the solution was sprayed by an adaptation of Potter Spray Tower (Burkard Scientific, Uxbridge, UK) sprayer at 100 kpa pressure, typically producing fine spray droplet diameter. The spraying was performed on both sides of leaves at the distance of 0.3 m until run-off point was reached at temperature of 21 ± 2°C and 50 ± 7% air relative humidity. After spraying the leaves were again weighted and by weight difference the spray solution retained on each leaf was determined. Area of each target was measured using a foliar area meter (Licor Inc., Li 300, Lincoln, NE, US). The weight value was divided by the foliar area of each leaf and the retention results expressed in µg cm^{-2}.

Statistical analysis

Spray retention and surface tension dates were subject to analysis of variance (ANOVA) using SISVAR Statistical Software (Ferreira, 2011). Fisher's least significant difference (LSD) was calculated to identify significant difference between mean treatments at 5% probability.

RESULTS AND DISCUSSION

Surface tension study

The median values of surface tension (mN m^{-1}) of adjuvants on 11 concentrations levels are showed in Table 2. Significantly difference were verify in the interaction adjuvants x concentrations (F=77.4; p<0.001). All aqueous solutions containing the adjuvants reduced the surface tension of destilled water with the increased concentration. The adjuvant heptomethyltrisiloxane (HT) presented the best performance to reduce surface tension of aqueous solution follow by polyoxyethylene alkyl phenol ether (PAPE), mineral oil (MO), nonylphenoxy polyethoxy ethanol (NPE), vegetal oil (VO) and soyal phospholypids and propionic acid (SPPA) (Table 2). Stevens et al. (1993) verify a more rapid and more extensive reduction of surface tension on aqueous solution when organosilicone surfactant is used.

The surface tension value of HT (18-19 mN m^{-1}) for the

Table 2. Influence of adjuvants and concentrations on aqueous solution surface tension.

Concentrations (% v v^{-1})	Surface tension (mN m^{-1})					
	PAPE	VO	NPE	HT	SPPA	MO
0	72.0					
0.001	70.6 (0.3)[1]b	70.3 (0.2)b	70.0 (0.2)b	63.6 (0.4)a	70.7 (0.2)b	69.2 (0.5)b
0.0025	70.1 (0.2)c	69.3 (0.4)bc	68.8 (0.2)bc	60.8 (0.3)a	70.4 (0.3)c	68.4 (0.4)b
0.005	69.1 (0.4)c	68.5 (0.9)bc	67.4 (0.3)b	53.3 (1.0)a	69.0 (0.3)c	68.1 (0.2)bc
0.01	66.2 (0.2)d	67.7 (0.9)de	64.2 (0.3)c	33.9 (0.8)a	67.9 (0.3)e	61.6 (2.6)b
0.025	50.3 (0.5)b	61.2 (4.6)d	52.2 (0.5)c	23.0 (0.4)a	64.2 (0.2)e	52.3 (0.7)c
0.05	38.9 (0.3)c	55.6 (1.4)e	37.3 (0.3)b	19.9 (0.3)a	54.6 (1.3)e	47.1 (0.4)d
0.1	31.8 (0.3)b	55.0 (1.9)f	33.4 (0.3)c	19.2 (0.2)a	49.4 (0.2)e	38.1 (1.0)d
0.25	27.2 (0.7)b	44.8 (1.6)f	30.1 (0.3)c	18.5 (0.2)a	42.4 (0.5)e	32.1 (0.7)d
0.5	27.4 (1.0)b	31.7 (0.7)d	29.3 (0.2)c	18.6 (0.3)a	38.7 (0.4)e	29.7 (0.4)c
1.0	28.2 (0.3)b	31.3 (0.4)c	29.0 (0.2)b	18.5 (0.3)a	37.0 (0.8)d	27.7 (0.4)b
2.0	28.3 (0.2)b	30.5 (0.4)c	29.0 (0.1)bc	18.3 (0.2)a	34.1 (0.9)d	27.4 (0.5)b
LSD[2]	1.61					

[1]Standard deviation/[2]Least significant difference. Different superscript letters indicate significance difference among adjuvants at the same concentration (LSD, p<0.05), PAPE - polyoxyethylene alkyl phenol ether, VO - vegetal oil, NPE - nonylphenoxy polyethoxy ethanol, HT – heptomethyltrisiloxane, SPPA - soyal phospholypids and propionic acid, MO - mineral oil.

aqueous solution is significantly lower compared to the other adjuvants tested. Lower surface tension of trisiloxanes surfactant (approximately 22 mN m^{-1}) measured by a pendent drop technique is reported by Wang et al. (2015). The surface tension of HT was a little lower than reported by those authors, probably due the different technique performed in this research. SPPA is the adjuvant with least ability to lowering surface tension of aqueous solutions at all adjuvants studied. Similar to this research Nairn et al. (2014) observe surface tension values approximately of 19 mN m^{-1} with HT and 31.5 mN m^{-1} with (alcohol ethoxylate both at concentration of 0.1%. Iost and Raetano (2010) report approximately surface tension values of 19 and 33 mN m^{-1} for aqueous solutions of the adjuvants HT and SPPA respectively using the same technique adopted in this research.

The SST became steady state at concentrations up to 0.25% to PAPE, 0.5% to VO, 0.25% to NPE, 0.05% to HT and 1% to MO (Table 2). These minimum concentrations points are considered to be the Critical Micelle Concentration (CMC) (Aliverdi et al., 2009). When a surfactant concentration is above the CMC the surfactants produce aggregates called micelles and generally the minimum equilibrium surface tension is achieved (Hazen, 2000). An increase of concentration above the CMC will not modified the surface tension. The CMC of the adjuvant SPPA, apparently, was not reached by the concentrations tested in this research as illustrated in Table 2.

Variance analysis results of surface tension by Mitscherlich modified model are shown in Table 3. High coefficient of determination (R^2) values and low coefficient of variation (CV) indicate that these equations provided good models profiles and accurate estimate of SST of the aqueous solutions containing adjuvants. According to parameter "*c*" the adjuvant HT is the most efficient adjuvant to reach the minimum surface tension in a lower concentration (46.3) follow by NPE (11.9); PAPE (11.3); MO (7.8); SPPA (4.6) and VO (4.3) (Table 3).

Linking the minimum surface tension values adjusted by the Mitescherlich model can be verify that HT present the lower SST to 18.2 mN m^{-1} follow by PAPE (27.1 mN m^{-1}); NPE and MO with the same value (28.8 mN m^{-1}); VO (30.3 mN m^{-1}) and SPPA (36.5 mN m^{-1}) as show in Table 3. Stevens et al. (1993) demonstrate that the reduction in surface tension of aqueous solutions is the most important factor controlling droplets adhesion and the organosilicone surfactants must have an advantage because they reduce aqueous surface tension more quickly than conventional hydrocarbon-based surfactants. HT showed lower surface tension values (33.9 mN m^{-1}) at concentration of 0.01% v v^{-1} (Table 2). Despite the adjuvants NPE and MO reached the same minimum values of surface tension they presented different values of "*c*" parameter of 11.9 and 7.8 respectively (Table 3).

Spray retention study

A regression analysis among adjuvants concentrations did not fit well in any model with coefficient of determination low than 40% and for this reason a mean comparison was analyzed.

The average retention on leaves provides by all adjuvants and all concentrations on Eucalyptus species

Table 3. Parameters of regression analysis obtained by Mitscherlich modified model equation ($\gamma = \gamma_{dw} - a[1-10^{-cx}]$) to the adjuvants.

Adjuvants	*a*	*c*	MST[a] (mN m^{-1}) γ_{dw} - *a*	F$_{regression}$	R^2	CV[b](%)
Polyoxyethylene alkyl phenol ether	44.9	11.3	27.1	67.166**	0.993	1.77
Vegetal oil	41.7	4.3	30.3	22.236**	0.965	2.29
Nonylphenoxy polyethoxy ethanol	43.2	11.9	28.8	33.022**	0.985	3.79
Heptomethyltrisiloxane	53.8	46.3	18.2	31.898**	0.992	1.25
Soyal phospholypids and propionic acid	35.5	4.6	36.5	41475**	0.977	2.82
Mineral oil	43.2	7.8	28.8	28.178**	0.980	3.33

[a]Minimum surface tension/ [b]Coefficient of variation /**p<0.001/ R^2: Coefficient of determination.
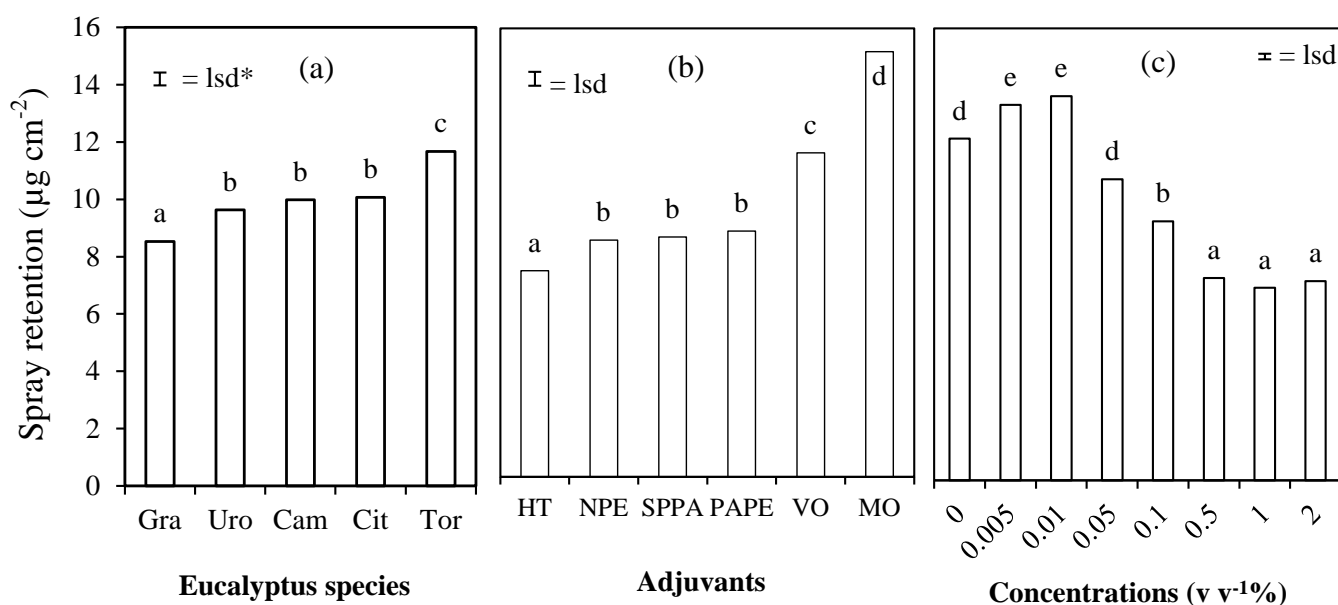
Figure 1. Spray retention on leaves of *Eucalyptus* species using six adjuvants at eight concentrations (a). Spray retention provided by different adjuvants from eight adjuvants concentrations on five *Eucalyptus* leaves (b); Spray retention using eight concentrations of adjuvants from six adjuvants at five *Eucalyptus* leaves. (c). *Eucalyptus* especies: *E. grandis* (Gra), *E. urophylla* (Uro), *E. camaldulensis* (Cam), *C. citriodora* and *E. torelliana* (Tor). Adjuvants: heptomethyltrisiloxane (HT), nonylphenoxy polyethoxy ethanol (NPE), soyal phospholypids and propionic acid (SPPA), polyoxyethylene alkyl phenol ether (PAPE), vegetal oil (VO) and mineral oil (MO), *least significant difference.

are given in Figure 1. Significant difference on spray retention values (F= 47.8; p<0.001) on leaves of *Eucalyptus* species is detected. Leaves of *E. toreliana* had the highest retention with the mean values differing significantly to the other species. Lowest retention value was attributed to leaf of *E. grandis* (Figure 1a). Spray retention difference verified between the species could be attributed to leaf surface structure as for example surface micro-roughness, trichomes and microcrystalline waxes composition which can vary from *Eucalyptus* species. Lin et al. (2016) verify larger wetted area on *E. tereticornis* leaves comparing to leaves of eucalipt hybrid urograndis

(*E. urophylla* × *E. grandis*) at any surfactant concentrations.

The effect of adjuvants on spray retention was significant (F= 265.6; p<0.001). The highest spray retention on leaves was achieved by adjuvant MO, followed by VO. NPE, SPPA and PAPE showed no significant difference of spray retention values. HT was the adjuvant with least ability to increase spray retention (Figure 1b). The greatest spray retention (15.2 µg cm^{-2}) provided by MO was approximately 2.1 times bigger than that provided by HT (7.3 µg cm^{-2}).

A significant effect of adjuvant concentration in spray

retention on *Eucalyptus* leaves (F= 189.2; p<0.001) was observed (Figure 1c). Spraying retention reduction was verified as adjuvant concentrations increased becoming steady at concentration of 0.5%. Concentrations over 0.1% reduced the spray retention on leaves Concentrations of 0.005 and 0.01% increased spray retention differing significantly from treatments applied with only distilled water. The highest spray retention (13.6 µg cm^{-2}) appeared on the adjuvant concentrations of 0.005 and 0.01% increased by 2 times over the concentration of 1% (6.8 µg cm^{-2}).

Gaskin et al. (2000) report that aqueous solution of the insecticide acephate with addition of spreader-sticker adjuvants reduced spray retention on cucumber plants. The effects were attributed to observable spray droplets coalescence and run-off. As this research had the spraying done until run-off point (maximum volume of leaf saturation) the spray retention decreased as the adjuvants concentrations increased. In the previous papers published by Matuo and Baba (1981); Ocampo-Ruiz and Matuo (1994); Oliveira et al. (1997) and Silva et al. (2008) is verified reduction of spray retention liquid capacity on leaves when spraying was made with aqueous solution containing spreader-sticker adjuvants at high spray volume. Pesticide application using adjuvants which reduce surface tension must ensure more security when is done at high spray volumes in order to avoid run-off and consequently pesticide losses.

It is very important attempt that the values of spray retention obtained in this research were realized using only water plus adjuvants. Different spray retention results can be found when used solution with pesticide formulation due to the different characteristics. Lin et al. (2016) studying the effects of surfactant concentration on the spreading properties of pesticides, observe as nonionic surfactant concentration increased continuously from 0.1% to 0.25, 0.5 and 1%, the wetted area of solution (water + surfactant) droplets present a reduction rather than expansion trend on surface of *Eucalyptus* leaves. When the solutions contained pesticide the trend of wetted area is increase as surfactant concentrations also increase. The same authors conclude that spread properties using only water are distinct than those pesticide droplets containing surfactant and a specific spray solution has an optimal spreading efficacy at a specific surfactant concentration.

Conclusions

The adjuvant heptomethyltrisiloxane show the best performance on reducing surface tension of aqueous solution at the lowest concentrations and exhibited the highest efficiency. Spray retention on *Eucalyptus* leaves varies with the species. *E. grandis* leaves had the higher amount of spray retention and *C. citriodora* the lower.

Mineral oil was the adjuvant which provides the best spray retention on *Eucalyptus* leaves. The adjuvants concentrations of 0.005 and 0.01% increased foliar spray retention while 0.5, 1.0 and 2.0% decreased foliar spray retention. Pesticide spray application using high volumes in the presence of adjuvant which reduces drastically the surface tension should be carefully performed to avoid losses by run-off, increasing the cost of production and environmental pollution.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Systems of land use affecting nodulation and growth of tree legumes in different soils of the Brazilian semiarid area

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The growth of tree legumes in degraded areas must be preceded by assessments of nodulation ability of naturally established rhizobia populations since such information contributes to defining the species which can be planted for recovering disturbed areas. The aim of this study was to evaluate the growth and natural nodulation of "sabiá" (*Mimosa caesalpiniifolia* Benth.) and leucaena (*Leucaena leucocephala* (Lam.) de Wit.) in soils of the Brazilian semiarid area under different systems of land use: native vegetation (locally called caatinga) and areas with different agricultural systems (a monocrop system and an intercropping with various species). For each species, a greenhouse experiment in randomized block design was realized, using soils of different types (Luvisol and Ultisol), with 4 replicates. The results evidentiate significant differences in the evaluated growth characteristics (height, leaflet number and shoot diameter) of *M. caesalpiniifolia*, that have displayed lower plant growth when cultivated in the Luvisol under conventional system. Plant growth, nodulation and total N accumulation in both seedling tree legumes increased in Ultisol under the different systems of land use. *L. leucocephala* showed higher potential of biological nitrogen fixation and nodulation effectiveness promoted by indigenous rhizobia.

Key words: Biological nitrogen fixation, indigenous rhizobia, symbiosis, sustainable agriculture.

INTRODUCTION

In tropical regions, the predominant agricultural production systems are based on the conversion of native forests to croplands, with cutting and burning of native vegetation, exploration and subsequent abandonment (fallow), before again clearing and burning cycle. In the semi-arid region of Brazil, the native vegetation (caatinga), which would cover an estimated area between 6.09×10^5 km^2 (Sampaio, 1995), is also a part of the shifting cultivation cycle, besides being the main form of native pasture for the extensive livestock farming in the region. Some of

these uses over the centuries left the native vegetation degraded, with wide stretches in the process of desertification. Presently, there are less than 50% of the original vegetation (Menezes et al., 2012).

The introduction of leguminous trees is considered one of the main practices that can be employed to recover degraded areas (Pereira and Rodrigues, 2012). The cultivation of these species promotes protection against soil erosion (Garba and Dalhatu, 2015) and improves the soil fertility by the addition of organic matter (Wu et al., 2016). However, the main characteristic of some legume species is their ability to establish symbiotic associations with bacteria that fix nitrogen (Moreira and Siqueira, 2006; Sprent, 2007) and the success of the integration of certain species depends on their nodulation with effective rhizobia. Thus, the use of tree legumes must be preceded by assessments of growth and nodulation ability of such species in association with the native rhizobial populations. This information will contribute to the definition of which species can be planted for the recovery of degraded areas.

The nodulation and the efficiency of biological nitrogen fixation process can be restricted by many conditions related to the plant, to microsymbiont and the climate and soil conditions. Of course, in the absence of native rhizobia populations, the symbiosis will not be established. Generally, microsymbionts populations are abundant in soil of the region that the legume species are native (Bala et al., 2003). But it may be that even in the presence of compatible rhizobia populations, the symbiosis is not efficient (Faye et al., 2007). The growth of rhizobia in free life in the soil and its ability to nodulate and fix nitrogen in symbiosis with the legume are sensitive to environmental conditions and can be dependent on soil quality. Different vegetation cover or managements affect the diversity of rhizobia (Jesus et al., 2005; Boakye et al., 2016), and may favor, differently, more or less efficient populations.

"Sabiá" (*Mimosa caesalpiniifolia* Benth.) is a small tree legume native from the Brazilian semiarid region which have great social and economic importance especially in function of the use in the production of fences, firewood and coal, and still be used for animal feed, due to the high nutritional value (Ribeiro et al., 2008; Costa Filho et al., 2013). Leucaena (*Leucaena leucocephala* (Lam.) de Wit) is a perennial tree legume distributed throughout the tropical region (Aquino, 2011), and have multiple uses as application in soil improvement, shading, windbreak and has been widespread in the tropics (Barreto et al., 2010). Leucaena and "sabiá" represent tree legumes with potential to be used in the recovery of degraded areas in

semiarid regions, especially due to their fast growth characteristics (Amaral et al., 2016), high biomass production (Moura et al., 2006) and ability to establish effective symbiotic associations with specific rhizobia strains (Reis Junior et al., 2010).

Before these considerations, the aim of this study was to evaluate the growth and natural nodulation of "sabiá" and leucaena in soils that were originally covered by caatinga and currently are under different systems of land use.

MATERIALS AND METHODS

Soil samples from the 0 to 20 cm superficial layer were collected in areas under diferent systems of land use in two municipalities, with different climate conditions and soil type (Table 1): Belo Jardim, in the Agreste zone, and Serra Talhada, in the Sertão zone. Each municipality area with native vegetation (caatinga) and areas with different agricultural systems (a monocrop system and an intercropping with various species) were selected. In both municipalities, the systems of land use were chosen in areas with the same soil type.

In Belo Jardim, the land use are: 1) agreste caatinga (Subhumid deciduous forest); 2) banana (*Musa* sp) crop and 3) grass and legumes intercropping (sorghum, *Sorghum bicolor* (L.) Moench; cowpea, *Vigna unguiculata* (L.) Walp.; jack bean, *Canavalia ensiformis* L. DC; and sunn hemp, *Crotalaria spectabilis* Roth). In Serra Talhada, the land use are: 1) sertão caatinga (Semiarid deciduous forest); 2) cowpea crop and 3) legumes and fruit trees intercropping (cowpea, sunn hemp and cashew tree, *Anacardium occidentale* L.).

In the six areas, four plots with 10 x 10 m were established. In each plot, five sub-samples of soil were collected. The subsamples from the same plot were mixed to obtain composite samples to represent the treatments with different systems of land use. Physical and chemical analysis of soils samples (Tables 2 and 3) were realized following Embrapa (2011). Each treatment was sampled four times and correspond the four replicates used in the greenhouse experiment.

The experiment was realized using a randomized block design with four replicates. To each of the two tree legume species, the experimental units consisted of three pots (one plant per pot) containing the soil samples collected in the different areas. Seeds of *Mimosa caesalpiniifolia* Benth. and *Leucaena leucocephala* (Lam.) de Wit. were surface disinfected in ethanol (70% v/v- 3 min) and sodium hypochlorite (1% v/v- 3 min), rinsed five times with sterile distilled water, rolled onto YMA plates to test for surface sterility and then were sown in the pots. The pots received destiled water until harvest (90 days after seed germination). Plants were harvested at 90 days after seed germination and determined the nodules number and the dry biomass of shoots and roots, after drying in an oven at 65°C, for 72 h. To determine the shoot biomass, the samples were passed in Willey type mill and subsequetly quantified the total N content, according to Embrapa (2011).

The results of plant height, stem diameter, number of leaflets, dry biomass of shoots, roots and nodules, nodules number and total N accumulation in shoots were submitted to analysis of variance by F

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Table 1. General characteristics of the municipalities of Belo Jardim, in the Agreste mesoregion, and Serra Talhada, in the Sertão mesoregion, semiarid of Pernambuco State, Brazil.

Characteristics	Municipality	
	Belo Jardim	Serra Talhada
Coordinates	08° 20' 08" S	07° 59' 31" S
	36° 25' 27" W	38° 17' 54" W
Altitude (m)	608	429
Annual rainfall (mm)	660	716
Months with water deficit	4-5	6-7
Average temperature (∘C)	24	24
Soil type	Ultisol	Luvisol

Table 2. Physical analyses and textural classification of the used soils submitted to different systems of land use in the Brazilian semiarid region.

Soil/land use	Soil density	Sand	Silt	Clay
	$g\ cm^{-3}$		$g\ kg^{-1}$	
Luvisol				
Caatinga	1.45	730	120	160
Agriculture	1.61	770	70	130
Intercropping	1.54	720	100	180
Ultisol				
Caatinga	1.30	740	90	170
Agriculture	1.55	710	100	190
Intercropping	1.31	750	100	150

Table 3. Chemical analysis of the soils collected in the different land use system in the Brazilian semiarid region.

Soil/land use	pH (H_2O)	C	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	H+Al
		$g\ kg^{-1}$	----$mg\ dm^{-3}$----		----------- $cmol_c\ dm^{-3}$-----------			
Luvisol								
Caatinga	6.3	10.6	114	234	2.97	1.02	0.12	2.48
Agriculture	6.7	7.7	105	292	2.85	0.86	0.11	2.15
Intercropping	6.3	7.7	75	284	2.93	1.37	0.11	2.31
Ultisol								
Caatinga	5.8	14.5	68	241	2.32	0.88	0.07	3.30
Agricultura	6.2	8.4	69	284	2.04	0.89	0.09	2.70
Intercropping	5.7	7.0	89	269	2.14	0.99	0.08	3.52

test and means compared by Tukey test (p<0.05). Nodule numbers were transformed by $(x + 1)^{1/2}$. The statistical analyzes were performed using the computer program Sisvar (Ferreira, 2011).

RESULTS AND DISCUSSION

The occurrence of compatible microsymbionts populations was demonstrated by the natural nodulation of the tree legume species in both soils. The "sabiá" displayed a more abundant nodulation in the Ultisol; however, in the Luvisol, the nodules were greater. The plants did not show difference in the total biomass of nodules when comparing the two soils. The complexity of the different systems of land use did not influence natural nodulation

Table 4. Number of nodules and dry biomass of nodules in seedlings of "sabiá" (*Mimosa caesalpiniifolia*) and leucaena (*Leucaena leucocephala*) in soils colleced in different land use systems, in the Brazilian semiarid region.

Soil/land use	Nodules number		Dry biomass of nodules (mg plant^{-1})	
	"Sabiá"	Leucaena	"Sabiá"	Leucaena
Luvisol				
Caatinga	4.7^b	33.4^{bc}	42.5^a	190.75^{ab}
Agriculture	4.6^b	40.0^{bc}	40.5^a	201.7^{ab}
Intercropping	12.8^b	18.7^c	45.5^a	91.7^b
Ultisol				
Catinga	71.3^a	48.7^b	116.2^a	138.5^{ab}
Agriculture	63.1^a	100.2^a	99.7^a	262.7^a
Intercropping	57.9^a	37.9^{bc}	153.5^a	152.0^{ab}
Means	35.78	46.51	82.95	172.91
F	13.090**	25.655**	1.55^{ns}	0.01
CV (%)	48.67	23.86	92.45	34.46

Means with the same letter are not different by the Tukey test (p < 0.05).

of "sabiá". Leucaena produced more nodules when grown in the Ultisol under the conventional agriculture, and it may be observed that the nodules were small and the total biomass of nodules differed only in plants grown in the Luvisol submitted to the intercropping system (Table 4).

The soil samples collected in different systems of land use did not show limitations to nodulation with regards to soil acidity and the occurrence of exchangeable aluminum (Table 3). In spite of the higher mean annual precipitation in Belo Jardim than in Serra Talhada, the Agreste sites have higher water availability throughout the year than the Sertão site. Rainfall is concentrated mostly in three months (February to April) in the Sertão mesoregion (average of 73% of the annual rainfall) and the month of highest rainfall represents 41% of the annual rainfall. In contrast, in the Agreste mesoregion, rainfall is reasonably well distributed over five months, from March to July (67% of the annual rainfall), and the month of highest precipitation represents only 16% of the annual rainfall. In addition, there is a large interannual variation in total rainfall in the Sertão (Freitas et al., 2010), resulting in an decrease in survival of natural rhizobia populations established in Serra Talhada, which could explain the different nodulation observed for the two legumes grown in the two used soils.

The results of nodulation contrasted with data from other studies that compared the ability of two legume nodulation (Souza et al., 2007), since in the present study, there was a higher nodulation of leucaena. The species showed high specificity to the microsymbionts (Silva et al., 2009) and this fact may be related to native rhizobial populations prevailing in the areas and the

ability of these native rhizobia to establish effective symbiotic associations.

Recent studies have shown that tree legumes have higher affinity for different proteobacteria groups and the "sabiá" is more associated with beta-rhizobia (Reis Junior et al., 2010; Martins et al., 2015), while leucaena tree legume nodulated predominantly with alpha - rhizobia (Wang et al., 1999). It is possible that different legumes and the simbionts partners have influenced the natural nodulation. However, probably is necessary for more refined studies regarding the ecology and diversity of nodulating rhizobia species in the Caatinga biome to explain the specific nodulating behavior among different legumes

The height constituted an important variable in predicting the development of plants, being technically accepted as important measure for assessing the performance potential of the seedlings growth (Dutra et al., 2015). For tree legume species, the seedlings are ready to be planted in the field when they have the height between 15 and 30 cm (Paiva and Gomes, 2000). Thus, in this study, 30 days for all soils tested "sabiá" and leucaena are included in the above range. Therefore, according to this criterion, the seedlings of the two legumes would be suitable for transplantation to the field 30 days after sowing.

Seedlings of "sabiá" and leucaena developed normally with 100% of survival. The legumes seedlings grown in the Ultisol were higher than those grown in the Luvisol, especially when agricultural land use system was used (Figure 2). In soils derived from the land use system, "sabiá" seedlings reached height of 65.9 cm as compared to soil from Caatinga and agriculture (Ultisol) and lower
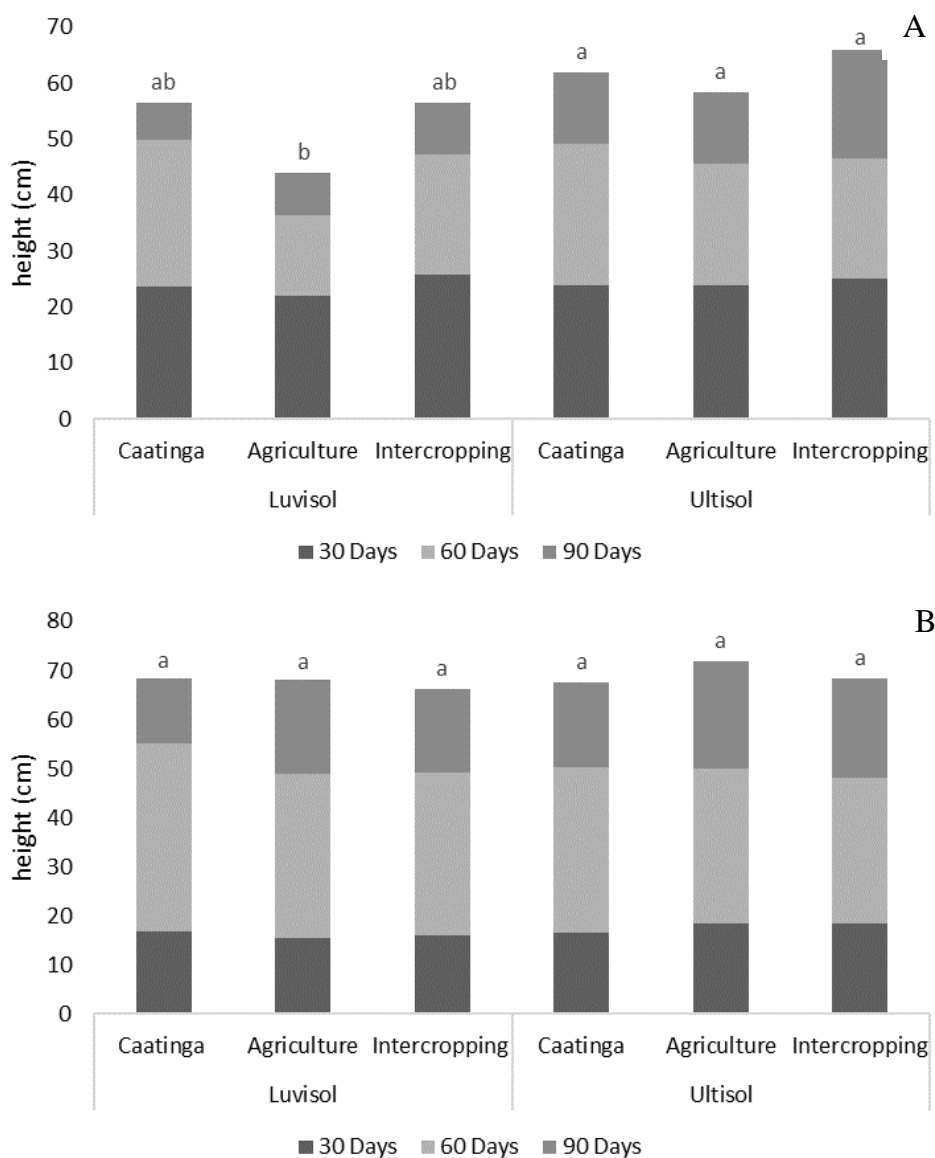
Figure 1. Height of "sabiá" (A, *Mimosa caesalpiniifolia*) and leucaena (B, *Leucaena leucocephala*) tree legumes 90 days after cropping, in soils collected in the different land use systems, in the Brazilian semiarid region. Means with the same letter are not different by the Tukey test (p < 0.05).

height was observed in the Luvisol with the agriculture land use system. In leucaena grown in two soils, influence in height with the different land use system was not observed and varied from 68 to 90 cm (Figure 1).

The stem diameter is a very important variable in survival potential assessment studies and growth after planting (Souza et al., 2006; Cruz et al., 2012). In "sabiá", the stem diameter showed a similar response as obtained in plant height, and also promoted lowest development in the Luvisol submitted for agricultural land use system (Figure 2). In leucaena, the stem diameter

was not influenced by the land use system, displaying an average diameter of 4.7 mm. However, the value of stem diameter reflects the actual standard quality of seedlings for transplanting to field conditions that depends on the species, the location sites and the techniques of production (Gomes et al., 2002).

The number of leaflets of the two species was influenced by land use system. For "sabiá", the lowest leaflets number was observed in the Luvisol under agriculture cultivation and the largest values obtaianed in the Ultisol under Caatinga land use system. For
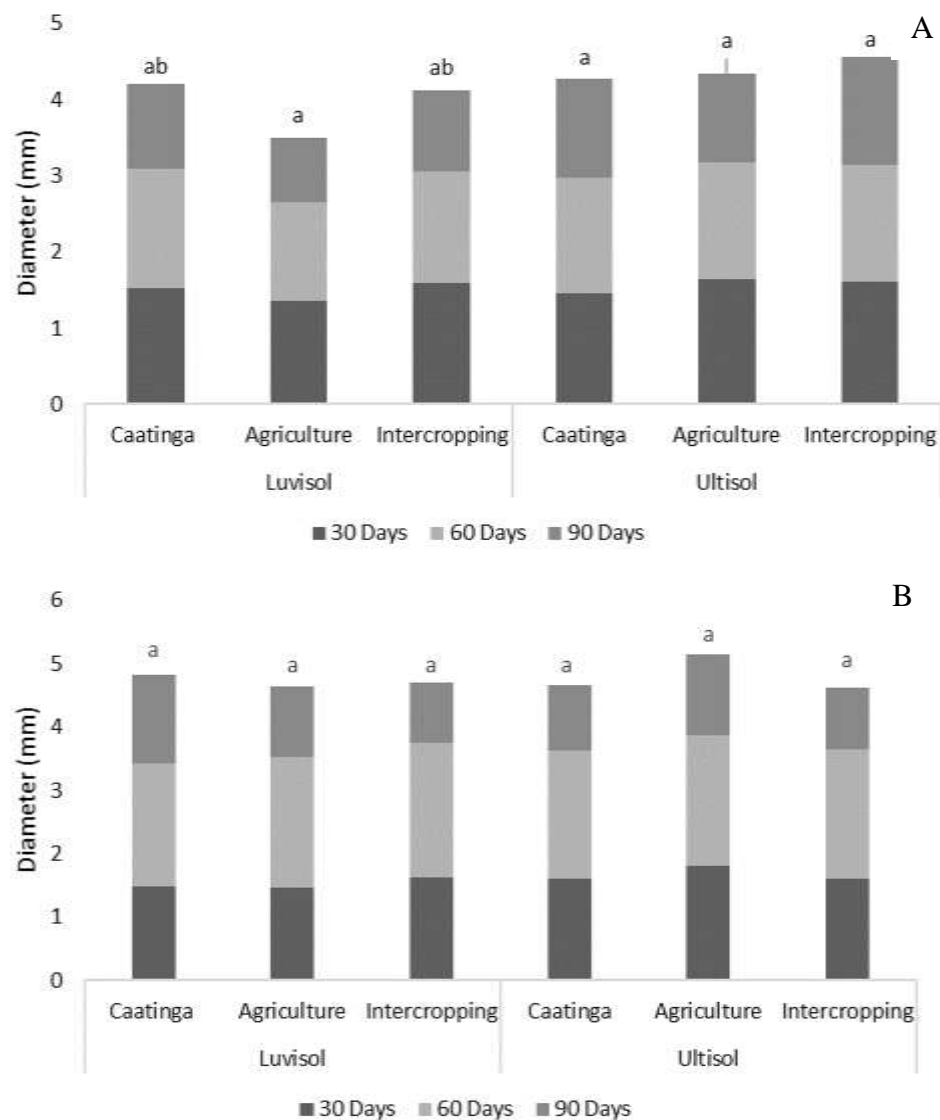
Figure 2. Shoot diameter of "sabiá" (A, *Mimosa caesalpiniifolia*) and leucaena (B, *Leucaena leucocephala*) tree legumes 90 days after cropping, in soils collected with different land use systems in the Brazilian semiarid region. Means with the same letter are not different by the Tukey test ($p < 0.05$).

leucaena, there was a greater number of leaflets in the plants grown in the Luvisol submitted to the Caatinga land use system (Figure 3).

The dry biomass of shoots and roots of leucaena were quite similar in the different land use systems, with an average accumulation of 6.3 and 2.4 g for shoots and roots, respectively. For "sabiá", there was a lower biomass production of shoots in the Luvisol (Table 5). The highest values were obtained in agriculture land use system in plants grown in the Ultisol. When comparing the two legumes, it appears that leucaena showed a shoot biomass and root biomass higher than those found

for "sabiá". These results were different when compared with those obtained by Souza et al. (2007) who found greater accumulation for "sabiá" biomass in shoots and roots when compared with leucaena tre legume.

The seedlings of leucaena accumulated more total N in the plant biomass. For the two legumes, the nutrient accumulation has been in greater quantity when used the Ultisol. In "sabiá", the best total N accumulation was observed in plants grown in soil under agriculture cultivation that present 36 mg plant^{-1}. When grown in soil of Belo Jardim under caatinga, leucaena showed the best total N accumulation that corresponded to 37.2 mg
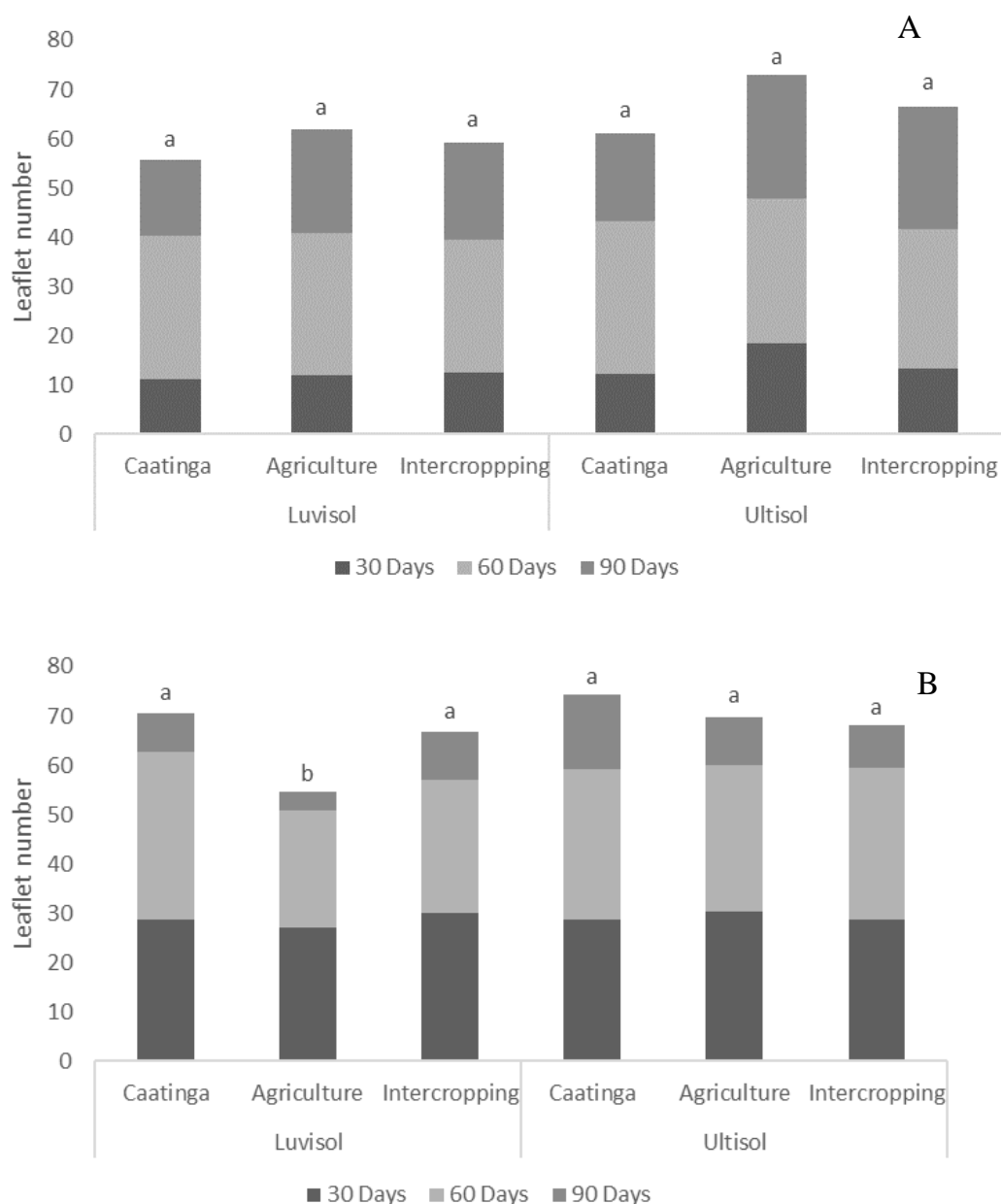
Figure 3. Number of leaflets for "sabiá" (A, *Mimosa caesalpiniifolia*) and leucaena (B, *Leucaena leucocephala*) tree legumes 90 days after cropping, in soils collected in the different land use systems, in the Brazilian semiarid region. Means with the same letter are not different by the Tukey test ($p < 0.05$).

plant^{-1} (Table 5).

The low aboveground biomass and total N accumulation in "sabiá" grown in the Serra Talhada soil were a result of the low nodulation in such areas. Commonly, nitrogen is the nutrient required in high amount by plants and is the nutrient that limit productivity in semiarid regions (Freitas et al., 2015) and is important to promote efficient management to optimize the FBN process.

Conclusions

In the seedling stage, the growth, development, nodulation and accumulation of total N in "sabiá" and leucaena were favoured in the Ultisol.

Leucaena showed efficient nodulation and greater potential for N$_2$ fixation in symbiosis wtih natural rhizobia populations established in the systems of land use

Table 5. Dry biomass of shoots, dry biomass of roots and total N accumulation in shoot biomass in "sabiá" (*Mimosa caesalpiniifolia*) and leucaena (*Leucaena leucocephala*) tree legumes grown in soils collected in the different land use systems in the Brazilian semiarid region.

Soil/Land use system	Dry biomass of shoots		Dry biomass of roots		Total N accumulation	
	"Sabiá"	Leucaena	"Sabiá"	Leucaena	"Sabiá"	Leucaena
	(g pot^{-1})		(g pot^{-1})		(mg plant^{-1})	
Luvisol						
Caatinga	6.0^{ab}	6.3^a	1.7^a	2.6^a	9.6^{bcd}	15.2^{bc}
Agriculture	3.8^b	5.7^a	1.1^a	2.1^a	3.5^d	5.8^c
Intercropping	5.8^{ab}	6.1^a	1.6^a	2.6^a	7.5^{cd}	22.3^{bc}
Ultisol						
Caatinga	6.2^{ab}	6.3^a	1.4^a	2.2^a	28.5^{ab}	37.2^a
Agriculture	7.1^a	7.3^a	1.6^a	2.9^a	24.8^{ab}	32.5^{ab}
Intercropping	7.0^a	6.0^a	1.6^a	2.3^a	36.0^a	23.5^{ab}
Média Geral	5.99	6.3	1.61	2.46	18.34	22.75
F	5.26*	1.671^{ns}	12.080^{ns}	0.36^{ns}	10.10**	5.91**
CV (%)	17.53	13.22	16.44	23.22	45.19	41.17

Means with the same letter are not different by the Tukey test ($p < 0.05$).

studied.

Conflict of interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Simulation of oat grain (*Avena sativa*) using its panicle components and nitrogen fertilizer

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Nitrogen fertilizer management modifies oat (*Avena sativa*) panicle components and its grain yield. The work aims to study the potential of the variables of oat (*A. sativa*) panicle with N-fertilizer, and to simulatate its grain yield using multiple linear regression in succession systems of high and reduced N-residual release. The study was done in 2013 and 2014. The experiment was done in a complete randomized block of 4×2 factorial design, with four replications. The treatments include: nitrogen fertilizer of four doses (0, 30, 60 and 120 kg ha^{-1}), oat (*A. sativa*) cultivars at two levels (Barbarasul and Brisasul) and succession system at two levels (soybean/oat (*A. sativa*) and corn/oat (*A. sativa*). The multiple linear models were efficient in the harvest index of panicle of soybean/oat (*A. sativa*) system, regardless of the dose evaluated. However, at high doses, the number of grain per panicle was included. In the corn/oat (*Avena sativa*) system, the harvest index of panicle, the number of grains and spikelets panicle were adjusted based on the model. The multiple linear regression efficiently simulates N-fertilizer to affect the grain yield of oat (*Avena sativa*) and one or more potential variables of panicle in the succession systems.

Key words: Inflorescence, succession system, stepwise, multiple linear regression.

INTRODUCTION

Oat (*Avena sativa*) is an excellent culture of diverse functions in agriculture: it is used in feed and food as source of protein and fiber (Crestani et al., 2012; Hawerroth et al., 2014). The grain yield of oat (*A. sativa*) is affected by genetic characteristics of the cultivars, environmtal conditions and the management technologies of oat (*A. sativa*) (Benin et al., 2012; Mantai et al., 2015). Flores et al. (2012) claimed that the

management of nitrogen fertilizer in grasses is one of the technologies that can increase grain yield. Study conducted by Frey (1959) and Jat et al. (2015) has shown that the application of nitrogen fertilizer in oat (*Avena sativa*) led to a greater number of panicles and grains per panicle. Kolchinski and Schuch (2003) mention that this nutrient has a strong influence on the number of oat (*A. sativa*) grains that can be produced.

Research by Wang et al. (2009) showed that using different levels of fertilizer in oat (*Avena sativa*) increased the number of its spikelets, length and the panicle mass. Furthermore, nitrogen fertilizer use efficiency in this species was different based on the type of succession system (Mantai et al., 2015). This indicates the need for more efficient cultivars during the uptake and use of N-fertilizer on the conditions of N-residual harnessing by the type of vegetation cover (Nascimento et al., 2012). Silva et al. (2015) found that the amount of nitrogen fertilizer in the corn/wheat system exerted changes in the spike components. These authors observed that in soybean/wheat, the amount of nitrogen fertilizer led to changes in the spike length and number of fertile and sterile spikelets. That way, the relationship between inflorescence oat (*A. sativa*) components with the management of nitrogen fertilizer can lead to the construction of efficient simulation models in expectation of grain yield (Cover et al., 2011).

In the multiple linear regression models, it is possible to combine several factors to estimate grain yield (Chai et al., 2012; Dalchiavon et al., 2014; Godoy et al., 2015). Dalchiavon et al. (2012) estimate the grain yield of rice by multiple regressions, incorporating the total number of spikelets, fertile and infertile panicle. Leilah and Khateeb (2005) also used this model to predict the grain yield of wheat under drought conditions, selecting the variable mass of grains per spike, harvest index, biological yield, number of spikes per square meter and spike length. Godoy et al. (2015) analyzed soil attributes to explain the grain yield in rice with copper nutrients, nitrogen fertilizer, iron and acid phosphatase. Thus, the use of efficient models that integrate components of the plant and its management can contribute to the predictability of agricultural harvest and crop planning, as well as allowing productivity analysis in the survey of agro-livestock activity assurance programs.

The objective of this study is to define potential variables linked to oat (*Avena sativa*) panicle with the N-fertilizer, to simulate grain yield using multiple linear regression in succession systems of high and reduced N-residual release.

MATERIALS AND METHODS

The study was done in a field in 2013 and 2014 in Augusto Pestana City, RS, Brazil (28°26'30' 'South latitude and 54°00'58'' West longitude). The soil of the experimental area is classified as Distrofic Red Latosol, and the climate of the region, according to Köppen classification (Kuinchtner and Buriol, 2001), is Cfa type,

with hot summer without dry season. Ten days before sowing, soil analysis was performed and the following chemical characteristics of the local crops were identified: i) corn/oat (*Avena sativa*) succession systems (pH = 6.5; P = 34.4 mg dm^{-3}; K = 262 mg dm^{-3}; organic matter = 3.5%; Al = 0.0 cmolc dm^{-3}; Ca = 6.6 cmolc dm^{-3} e Mg = 3.4 cmolc dm^{-3}) and ii) soybean/oat (*Avena sativa*) succession systems (pH = 6.2; P = 33.9 mg dm^{-3}; K = 200 mg dm^{-3}; organic matter = 3.4%; Al = 0.0 cmolc dm^{-3}; Ca = 6.5 cmolc dm^{-3} e Mg = 2.5 cmolc dm^{-3}). In both experimental years, the oat (*A. sativa*) was sown at the ideal time, that is, in the first week of June with seeder-fertilizer. Each plot consisted of 5 rows of 5 m length and row spacing was 0.20 m, forming the experimental unit of 5 m^2. During the study, tebuconazole fungicide named commercial FOLICUR® CE at a dose of 0.75 L ha^{-1}. Moreover, the weed control was performed with metsulfuron-methyl herbicide was applied, at a dose of 2.4 g ha^{-1}, and weeding was done when necessary. In the experiments, during sowing, 60 and 50 kg ha^{-1} of P$_2$O$_5$ and K$_2$O were applied, respectively, based on the levels of P and K in the soil on the expected grain yield of about 3 t ha^{-1}, plus nitrogen fertilizer of 10 kg ha^{-1} (except in the standard experimental unit). The remainder was applied based on the contemplation of using N-fertilizer on the phenological stage. This is indicated with the four expanded leaves, using urea (45% N).

The experimental design used was randomized complete block with four replications, based on a factorial scheme of 4 × 2 for the N fertilizer doses (0, 30, 60 and 120 kg ha^{-1}) and oat (*Avena sativa*) cultivars (Barbarasul and Brisasul), respectively. There was a total of 32 experimental units for the succession system of high and reduced condition N-residual in corn/oat (*Avena sativa*) and soybean/oat (*A. sativa*), respectively. The grain yields were obtained by cutting three central rows of each plot during harvest at maturity stage. The grain moisture was 18%. The plants were threshed with a stationary harvester and directed to the laboratory to correct the humidity of grain to 13%, and weighed to estimate grain yield (GY, kg ha^{-1}). In the analysis of the panicle components (Figure 1), there was a random collection of 20 oat (*A. sativa*) panicles per experimental unit. They were directed to the laboratory to correct the grain moisture to 13%, and subsequent decomposition of inflorescence components. The followings were measured: panicle length (PL, cm), number spikelet per panicle (NSP, n), number of grains per panicle (NGP, n), panicle mass (PM, g), grain mass of panicle (GMP, g) and harvest index of panicle (HIP, g g^{-1}) given by the ratio of the mass grain panicle by panicle mass.

To meet the assumptions of normality and homogeneity via *Bartlett* test, analysis of variance was conducted for the detection of the main effects and interaction. Although there was an evidence of interaction (data not presented), regression analysis was obtained by cropping year to confirm differences in nitrogen fertilizer absorption capacity for oat (*Avena sativa*) in favorable and unfavorable cropping years, and jointly (2013 + 2014) to estimate the optimal dose of nutrient independent of the cropping year. It is noteworthy that in the equations, the average effect among the cultivars was considered, because inferences were generalized about the species and not cultivars. Therefore, the data were submitted to variance analysis of regression for the definition of the

polynomial equation $y = b_0 \pm b_1 x \pm b_2 x^2$ and its parameters.

From this, based on the model $x = -b_1 / 2b_2$, the maximum level of technical efficiency of nitrogen fertilizer used by oat (*Avena sativa*) was estimated in the maximum grain yields expected. In addition, analysis of the average of panicle components was done using Scott & Knott's grouping method. The next step is to select the potential variables for the multiple linear regression models via *stepwise* technique. This procedure builds iteratively a regression models sequence for adding and removing variables, selecting the one with highest relationship with the main variable (y). In this
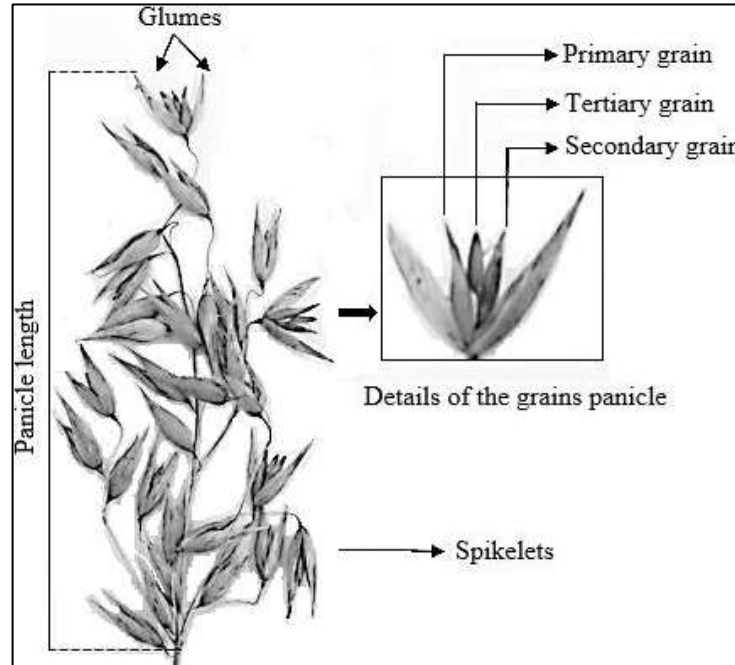
Figure 1. Inflorescence detail of oat (*Avena sativa*) type panicle.

study, it is represented by the grain yield, using the statistical partial F (Nunes et al., 2001), according to the model below:

$$F_j = \frac{SQ_R(\beta_j \mid \beta_1, \beta_o)}{MQ_E(x_j, x_1)} \tag{1}$$

Where SQ_R is the quadratic sum of regression and $MQ_E(x_j, x_1)$ is the average square error for the model containing the variables x_1 and x_j. The variables selected via *stepwise* were used to determine the multiple linear regression equation, which simulates the grain yield of oat (*Avena sativa*) from two or more variables,

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + \cdots + b_n x_n \tag{2}$$

Described in matrix form as,

$$Y = \begin{bmatrix} Y_1 \\ Y_2 \\ \vdots \\ Y_n \end{bmatrix}; \quad X = \begin{bmatrix} 1 & X_{11} & X_{21} & \cdots & X_{p1} \\ 1 & X_{21} & X_{22} & \cdots & X_{p2} \\ \vdots & \vdots & \vdots & \cdots & \vdots \\ 1 & X_{1n} & X_{2n} & \cdots & X_{pn} \end{bmatrix}; \quad \beta = \begin{bmatrix} \beta_0 \\ \beta_1 \\ \vdots \\ \beta_n \end{bmatrix}; \quad e \; \varepsilon = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{bmatrix} \tag{3}$$

From these matrices, one obtains the value of the regression coefficients, being,

$$\hat{\beta} = (X'X)^{-1} X'Y \tag{4}$$

And the variance of the coefficients was obtained by covariance

matrix of vector of the regression coefficients,

$$C\hat{o}v(\hat{\beta}) = (X'X)^{-1} \hat{\sigma}^2 \tag{5}$$

$$\hat{\sigma}^2 = \frac{(Y - X\hat{\beta})'(Y - X\hat{\beta})}{n - p - 1} \tag{6}$$

Being 'n' the number of equations and 'P' the number of parameters. The hypothesis testing is verified $H_0 : \beta_i = 0$ vs. $H_a : \beta_i \neq 0$ expressed by,

$$t = \frac{\hat{\beta}_i - \beta_i}{\sqrt{\hat{V}(\hat{\beta}_i)}} \tag{7}$$

The values of the optimal dose of nitrogen fertilizer were used in the multiple linear regression models. The average values of the oat (*A. sativa*) panicle components were validated by the *stepwise* technique, considering the combined effect of the year and cultivar. All analyses were performed using the Genes software versen 2005.5.0

RESULTS AND DISCUSSION

The analysis of variance, regression equation of grain yield of oat (*A sativa*) using N-fertilizer dose, the mean square of linear and quadratic equations were significant, regardless of the system and year of cultivation (Table 1). However, only the equation of degree two was employed to estimate the maximum technical nutrient use efficiency

Table 1. Summary of variance analysis the regression equation and its parameters with estimation of optimal nitrogen fertilizer dose and grain yield of oat (*A. sativa*).

Year	SV	MS (GY)	Equation: $GY = b_0 \pm b_1 x \pm b_2 x^2$	P (b_i)	R^2	N_{MTE} (Kg ha^{-1})	GY_{MTE} (Kg ha^{-1})
			Soybean/oat (*A. sativa*) system				
	L	508135*	3471.9 + 2.84 x	ns	0.13		
2013	Q	3471885*	3100 + 28.50 x - 0.21 x²	*	0.98	68	4067
	Error	55691	-	-	-		
	L	260018*	2814 + 2.03 x	ns	0.25		
2014	Q	657463*	2652 + 13.20 x - 0.09 x²	*	0.88	73	3136
	Error	21164	-	-	-		
			Corn/oat (*A. sativa*) system				
	L	2431152*	2281 + 6.21 x	*	0.55		
2013	Q	1950169*	2002 + 25.44 x - 0.15 x²	*	0.99	85	3080
	Error	34732	-	-	-		
	L	10162933*	1746 + 12.70 x	*	0.72		
2014	Q	3576696*	1369 + 38.74 x - 0.21 x²	*	0.98	92	3156
	Error	15006	-	-	-		

SV, source of variation; MS, means square; GY, grain yield; P(b_i) parameter what measures the significance of inclination; R², coefficient of determination; N_{MTE}= nitrogen fertilizer dose by the maximum technical efficiency; GY_{MTE}, grain yield estimated by N_{MTE} (kg ha^{-1}); L, equation linear; Q, equation quadratic; *, Significant at 5% error probability by *F* or *t* test; ns, not significant.

for grain yield. In soybean/oat (*Avena sativa*) system, in 2013 (Table 1), the maximum technical efficiency of nitrogen fertilizer was obtained with 68 kg N ha^{-1}, in expectation of superior grain yield (4067 Kg ha$^{-1)}$. In 2014, although the maximum technical efficiency is similar to that of 2013 with 73 kg N ha^{-1}, the maximum grain yield was around 3 t ha^{-1} (Table 1), indicating strong reduction of grain yield between the years of cultivation.

For the corn/oat (*A. sativa*) system (Table 1), regardless of the cropping year, the need for greater amount of nitrogen fertilizer was confirmed, mainly because it is a succession system in which the N-residual is less available. This way, the technical maximum efficiency in 2013 was obtained with 85 kg ha^{-1} in an expectancy of grain yield of around 3 t ha^{-1}. It is noteworthy that the grain yield in 2014 (3156 Kg ha^{-1}) is similar to that of 2013 (3080 Kg ha^{-1}), in this system; however, with greater need for nitrogen fertilizer use in 2014. The results obtained confirm the change of nitrogen fertilizer use efficiency for grain yield of oat (*A. sativa*) by the cropping year. It interacts directly with the succession system.

The changes in nitrogen fertilizer use efficiency in the cropping year are best justified by the analysis of Figure 2, which shows the information of rainfall and air temperature during the growing season. In the cropping year 2013, there was greater rainfall. This led to fertilization with N-fertilizer, resulting in more favorable soil moisture. Furthermore, throughout the cultivation cycle after fertilization, the volume and distribution of rainfall were also appropriate, favoring the development of the grain yield.

In Figure 2, the maximum temperatures observed at

the beginning of the oat (*Avena sativa*) development (read time) proved superior in 2014; the condition stimulates faster elongation and reduces the incentive to produce new tillers, component directly related to the productivity of biomass and grain. From the fertilization, the variations in temperature did not show strong alteration to the point of harming the culture of oat (*A. sativa*). Therefore, the grain yield results (Table 1) together with weather conditions in the crop cycle (Figure 2) allow one to classify 2013 as a favorable year and 2014 as an unfavorable year in the cultivation of oat (*A. sativa*).

Nitrogen fertilizer is a nutrient that is more absorbed and exported by grasses, and has great effect on crop yields (Prando et al., 2013). The optimal dose of nitrogen fertilizer is dependent on the plant species, type soil, preceding crop, meteorological conditions and quality of fertilizer (Fontoura and Bayer, 2009; Prando et al., 2013; Silva et al., 2015). The conditions of cultivation mostly contribute to the variation of the grain yield (Storck et al., 2014). Mantai et al. (2015) observed that the influence of favorable or unfavorable year of cultivation generates instability in the grain yield of oat (*A. sativa*). Research conducted by Benin et al. (2012) observed higher response of the grain yield of wheat to nitrogen fertilizer when the rains were not limited. However, excessive rains in the grain filling stage may contribute to plant lodging, leading to losses in the productivity and quality of grains (Prando et al., 2013). The type of cultural residue determines the mineralization or immobilization of N-residual by modifying the dose adjustment and ideal season of N-fertilizer in the expression of grain yield components (Silva et al., 2015). Silva et al. (2012) have
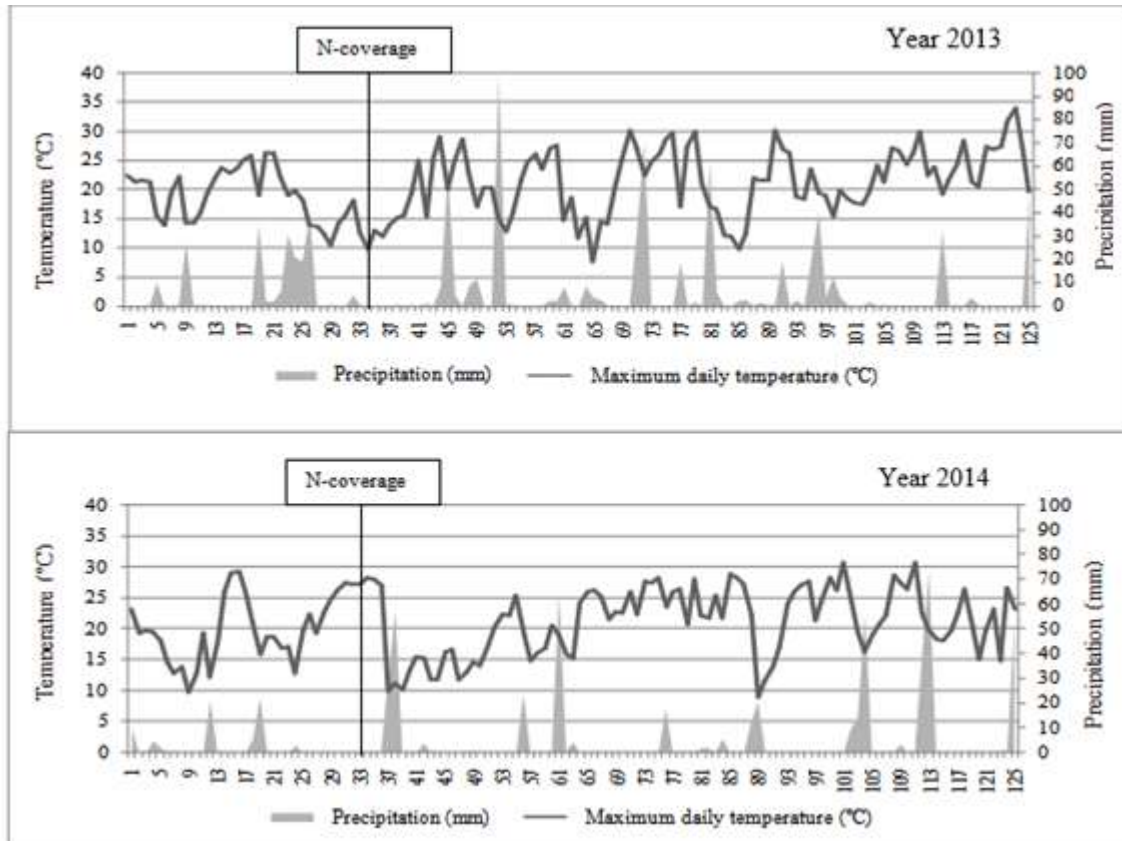
Figure 2. Rainfall and maximum temperature in oat (*A. sativa*) cycle.

shown that biomass yield and grain of oat (*A. sativa*) are favored by the succession system with low C/N ratio, reflecting in economic use of nitrogen fertilizers. Mantai et al. (2015) achieved maximum grain yield of oat (*A. sativa*) by using 70 and 96 kg nitrogen fertilizer ha^{-1} in soybean/oat (*A. sativa*) and corn/oat (*A. sativa*) succession system, respectively; this is similar to the results obtained in this study. Kolchinski and Schuch (2003) also had maximum grain yield of oat (*A. sativa*) in soybean/oat (*A. sativa*) using 70 kg N ha^{-1}.

Simulation of the grain yield per cropping year is not based on efficient models. Strong variation exists in the cultivation year by modifying the capacity of nitrogen fertilizer used for oat (*Avena sativa*). Therefore, the use of multiple linear regressions involving the cumulative effect of the variability between favorable and unfavorable year can ensure the development of coefficients that are more adjusted to the model. Table 2 shows the regression equations that describe the maximum technical efficiency of nitrogen fertilizer, along with the average effects of the components based on the inflorescence of oat (*A. sativa*) and joint analysis of favorable and unfavorable cultivation year.

In Table 2, in the joint analysis (2013 + 2014) of the soybean/oat (*A. sativa*) system, the optimal dose of nitrogen fertilizer (70 kg ha^{-1}) was obtained, with an

estimate grain yield of 3.6 t ha^{-1}. For the corn/oat (*A. sativa*) system, the maximum efficiency (90 kg N ha^{-1}) was obtained with an expectation of 3.1 t of grain yield. The soybean/oat (*Avena sativa*) system afforded an increase grain yield higher than 500 kg ha^{-1} and with reduction of 20 kg ha^{-1} N-fertilizer. These results strengthen the benefits of soybean/oat (*A. sativa*) succession system by using N-residual. Another positive fact is the greater stability provided by soybean/oat (*A. sativa*) system on the panicle components, indicating that increase in N-fertilizer doses does not alter panicle length, grain mass of panicle, panicle mass and number of grain per panicle. The differences in this system were obtained only in the number of spikelets per panicle and the harvest index of panicle. In the corn/oat (*Avena sativa*) system (Table 2), the mean of panicle components showed no change when subjected to N-fertilizer, both at reduced or elevated dose of the nutrient; though the absence of the nutrient significantly reduces the expression of these variables. Overall, in the joint analysis of N-fertilizer doses (0 to 120), there was higher contribution of the averages of panicle components of oat (*Avena sativa*) in soybean/oat (*A. sativa*) and corn/oat (*A. sativa*) system (Table 2).

Silva et al. (2012) observed that the type of vegetable residue interferes in the elaboration of the components

Table 2. Summary of variance analysis the regression equation and its parameters with estimation optimal dose of nitrogen fertilizer and grain yield and average values of the panicle components by N-fertilizer doses in cropping systems.

Year	SV	MS (GY)	Equation $GY=b_0 \pm b_1 x \pm b_2 x^2$	P (b_i)	R^2	N_{MTE} (kg ha^{-1})	GY_{MTE} (kg ha^{-1})
Soybean/oat (*A.sativa*) system							
	L	747566*	3143 + 2.43 x	ns	0.17	-	-
(2013+2014)	Q	3575514*	2876 + 20.84 x - 0.15 x²	*	0.99	70	3600
	Error	52985	-	-	-	-	-
Corn/oat (*A. sativa*) system							
	L	1126772*	2013 + 9.45 x	*	0.67	-	-
(2013+2014)	Q	5404487*	1685 + 32.02 x - 0.18 x²	*	0.99	90	3110
	Error	61043	-	-	-	-	-

Variables panicle	Nitrogen fertilizer doses (2013+2014)				
	0	30	60	120	(0-120)
soybean/oat (*A. sativa*) system					
GMP (g)	2.06[A]	2.04[A]	2.15[A]	2.05[A]	2.07
PL (cm)	19.19[A]	19.46[A]	20.17[A]	19.77[A]	19.65
PM (g)	2.78[A]	2.69[A]	2.83[A]	2.70[A]	2.75
NSP (n)	41[B]	40[B]	45[A]	45[A]	43
NGP (n)	80[A]	74[A]	82[A]	80[A]	79
HIP (g g^{-1})	0.74[B]	0.76[A]	0.76[A]	0.76[A]	0.76
Corn/oat (*A. sativa*) system					
GMP (g)	1.40[B]	1.91[A]	2.14[A]	2.06[A]	1.88
PL (cm)	17.54[B]	19.10[A]	19.02[A]	19.68[A]	18.83
PM (g)	1.99[B]	2.61[A]	2.79[A]	2.72[A]	2.53
NSP (n)	33[B]	41[A]	46[A]	46[A]	41
NGP (n)	59[B]	75[A]	84[A]	83[A]	75
HIP (g g^{-1})	0.70[B]	0.73[A]	0.77[A]	0.76[A]	0.74

SV, source of variation; MS, mean squared; GY, grain yield; P (b_i), parameter what measures the significance of inclination; R^2, coefficient of determination; N_{MTE}, nitrogen fertilizer dose the maximum technical efficiency; GY_{MTE}, grain yield estimated by N_{MTE} (kg ha^{-1}); L, equation linear; Q, equation quadratic; GMP, grain mass of panicle; PL, panicle length; PM, panicle mass ; NSP, number of spikelets per panicle; NGP, number of grain per panicle; HIP= harvest index panicle (MGP/MP); *, Significant at 5% error probability by *F* or *t* test; ns, not significant. Means followed by same letter horizontally do not differ statistically each other at the level of 5% probability of error by Scott & Knott model.

linked to the panicle of oat (*A. sativa*) changing the grain yield. Freitas et al. (2012) state that the use of legumes for straw production is a favorable management; it increases the content and availability of nitrogen fertilizer in the soil for culture succession. Silva et al. (2014) found that the panicle mass of Sudan grass is the component that expresses highest variability in the panicle characters. In oat (*A. sativa*), Hartwig et al. (2006) concluded that the panicle mass increment is based on the increased number of grain per panicle, with little effect of the grain mass of panicle. Silva et al. (2015) observed that the mass of spike and number of grains per spike of wheat are the components that showed the greatest change on the inflorescence.

In the indication of potentials variables for inclusion in the multiple model, Table 3 presents the significance of means square of the variables by *stepwise* technique, in individual condition per dose of N-fertilizer and joint analysis in the cropping systems. In the soybean/oat

(*Avena sativa*) system, the harvest index of the panicle was qualified to compose the multiple linear regression equation in all doses of N-fertilizer tested. using the highest dose (120 kg N ha^{-1}), the inclusion of the number of grains per panicle was also appropriate. In the corn/oat (*A. sativa*) system, the harvest index of panicle proved to be adjusted for simulation by multiple regression in the absence of N-fertilizer use. On the other hand, 60 kg N ha^{-1} dose was used to identify the panicle mass and number of spikelets per panicle as the most adjusted.

The possible combination of the panicle components of oat (*A. sativa*) with N-fertilizer (Table 3) for the simulation of grain yield using multiple linear regression, and joint analysis (0-120) of the soybean/oat (*A. sativa*) system confirmed the harvest index of panicle with the N-fertilizer for multiple model composition. In corn/oat (*A. sativa*) system, the use of harvest index of panicle with the N-fertilizer has also been made viable; however, with the need to include the number of spikelets per panicle and

Table 3. Means square values in identifying of potentials variables by *stepwise* technique for use in the multiple regression model.

Source of variation	Means square/*stepwise* (2013+2014)				
	0	30	60	120	(0-120)
Soybean/oat (*A. sativa*) system					
Regression	557125*	759972*	2352557*	588907*	2630021*
GMP (g)	ns	ns	ns	ns	ns
PL (cm)	ns	ns	ns	ns	ns
PM (g)	ns	ns	ns	ns	ns
NSP (n)	ns	ns	ns	ns	ns
NGP (n)	ns	ns	ns	509250*	ns
HIP (g g^{-1})	298524*	1487783*	3694273*	491476*	3568478*
N (kg ha^{-1})	-	-	-	-	887438*
Error	29662	42351	97355	49113	138872
Corn/oat (*A. sativa*) system					
Regression	995688*	ns	119449*	ns	3179631*
GMP (g)	ns	-	ns	-	ns
PL (cm)	ns	-	ns	-	ns
PM (g)	ns	-	116673*	-	ns
NSP (n)	ns	-	245820*	-	395156*
NGP (n)	ns	-	ns	-	1353118*
HIP (g g^{-1})	1145125*	-	ns	-	2029257*
N (kg ha^{-1})	-	-	-	-	3303315*
Error	28064	-	10070	-	97524

GMP, grain mass of panicle; PL,panicle length; PM, panicle mass; NSP, number of spikelets per panicle; NGP, number of grain per panicle; HIP, harvest index of panicle (MGP/PM); N, dose of nitrogen fertilizer; *,Significant at 5% error probability by *F* test; [ns,]not significant.

number of grain per panicle (Table 3).

The verification of components is essential; it estimates significantly the grain yield of crop by management techniques (Leal et al., 2015). Balbinot et al. (2005) comment that the use of *stepwise* technique allows the selection of potential components for simulation using multiple linear regression. In studying corn, these authors defined the spike mass components, number of grains per row, number of row per spike and number of spikes and plant per area as the most appropriate in the simulation of grain yield. For rice, Dalchiavon et al. (2012) used this technique by selecting the number of panicles, panicle mass, number of spikelets per panicle and thousand grain mass using the multiple model composition for simulation of grain yield.

Table 4 presented the linear multiple regression equations used for simulation of grain yield per nitrogen fertilizer and the combination of the fertilizer. Potential variables of panicle were used; they were selected by the *stepwise* technique (Table 3). The nitrogen fertilizer dose obtained by the technical maximum efficiency was considered and the average values of the panicle components were determined by joint analysis (2013 + 2014) (Table 2).

In both succession systems of soybean/oat (*A. sativa*) and corn/oat (*A. sativa*)), the grain yield estimated was near the grain yield observed, including within the confidence interval set for reliability of the equation (Table 4). In the system with low C/N ratio (soybean/oat (*A. sativa*), the model proposed denoted by $GY = -1075 + 2719.7\,HIP + 2.7\,N$ proved efficient in the estimation of grain yield. It presented a small grain yield difference of 34 kg ha^{-1} between the actual value and that predicted. In the succession system with high C/N ratio (corn/oat (*A. sativa*), the model $GY = -181 + 1859.7\,HIP + 27.2\,NGP - 33.7\,NSP + 6.8\,N$ was also effective, with differences between the value estimated. Grain yield of 45 kg ha^{-1} (Table 4) was obtained. The use of multiple linear equation allows one to compose in the model the panicle components and the management conditions of N-fertilizer, decisive factors for simulation of the main variable. This gives credibility to the simulation of grain yield of oat (*A. sativa*) with practicality and efficiency.

The utilization of multiple linear regression has been widely used in various vegetal species; it provides important information for culture, mainly grain yield allied with the most influential traits for species; examples are oat (*A. sativa*) (Chai et al., 2012), wheat (Leilah and Al-Khateeb, 2005), rice (Dalchiavon et al., 2012; Godoy et al., 2015), corn (Balbinot et al., 2005), soybean (Merchant et al., 2010), beans (Bonfim-Silva et al., 2014),

Table 4. Multiple linear regression to estimative grain yield per panicle components and nitrogen fertilizer doses in cropping systems.

Variable GY/ N	Equation	GY_E	GY_O	CI_{LL}	CI_{UL}
soybean/oat (*A. sativa*) system					
GY/0	GY = 1320 + 2031.8 HIP	2864	2873	2691	3032
GY/30	GY = 1525 + 2454.3 HIP	3366	3377	3163	3564
GY/60	GY = 298 + 4370.2 HIP	3576	3587	3224	3906
GY/120	GY = 3245 + 1429.9 HIP – 13.4 NGP	3242	3245	3018	3446
GY/(0-120)	GY = 1075 + 2719.7HIP + 2.7N	3304	3270	3135	3397
Corn/oat (*A. sativa*) system					
GY/0	GY = 149 + 2117.3 HIP	1673	1665	1437	1865
GY/30	-	-	-	-	-
GY/60	GY = 3774 + 451.4 PM - 45.7 NSP	2917	2915	2812	3005
GY/120	-	-	-	-	-
GY/(0-120)	GY = - 181 + 1859.7 HIP + 27.2 NGP -3 3.7 NSP + 6.8 N	2465	2510	2353	2656

GY, grain yield (g); HIP, harvest index of panicle (g g^{-1}); NGP, number of grains per panicle (n); PM, panicle mass (g); NSP, number of spikelets per panicle (n); N, dose of nitrogen fertilizer (kg ha^{-1}); GY_E, estimated grain yield; GY_O, grain yield observed; CI_{LL}, lower limit of the confidence interval; CI_{UL}, upper limit of the confidence interval.

among others.

Conclusion

In soybean/oat (*Avena sativa*) system, the harvest index of panicle is efficient to compose the multiple regression linear model, regardless of the amount of nitrogen; but in high doses, the number of grain per panicle is included. In the corn/oat (*Avena sativa*) system, the panicle harvest index, the number of grains and spikelets panicle are adjusted to compose the multiple linear model. The use of multiple linear regression is efficient in the simulation of oat (*Avena sativa*) grain yield with the use of N-fertilizer and panicle components selected by stepwise technique.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth and composition of sugarcane and chemical attributes of the soil by fertilizing with different levels of cow manure

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Four months after planting sugarcane variety RB867515, fertilized with 0.0, 4.5, 9.0, 13.5 and 18.0 Mg ha^{-1} of cow manure, there was positive linear effect of the fertilizer on plant height and stalk diameter, quadratic positive effect on width and length of the largest leaf, and no effect on number of plants m^{-1} linear and the number of leaves plant^{-1}. At 10 months, there was quadratic positive effect of cow manure on plant height and no effect on number of plants, stalk diameter, width and length of the largest leaf and number of leaves plant^{-1}. At 12 months, there was quadratic effect on the yield of green mass with the highest value on 18 Mg ha^{-1} of cow manure, without change on chemical composition. During the cutting time, the most pronounced effect of cow manure on sugarcane was in the yield of green mass and, therefore, up to 18 Mg ha^{-1} of cow manure can be used to increase sugarcane performance, without changing chemical composition and maintaining soil fertility.

Key words: Agronomic characteristics, chemical analyses, organic fertilizer, residue, *Saccharum* spp.

INTRODUCTION

The sugarcane (*Saccharum* spp.) is one of the main crops in tropical countries and its cultivation has great

prominence for several purposes, such as in the production of ethanol, sugar, brown sugar, molasses, fodder and by-products for use as fertilizer and power generation. Currently, most of the cane-producing units still use mineral fertilizers, as a source of nutrients. However, there is a concern to obtain a new product, with high benefit, for some units that use the system of organic fertilization or almost entirely organic (Anjos et al., 2007).

The organic fertilizer with cow manure is a millennial practice, having lost prestige with the introduction of mineral fertilizer, in the mid-19th century, and revived the importance, in recent decades. There is growing concern on the questions related to the need for preservation of the environment, with healthy eating and the need of adequate allocation of large quantities of manure produced in some countries (Holanda, 1990; Blaise et al., 2005; Salazar et al., 2005).

The use of organic compounds improves the physical, chemical and biological properties of the soil (Almeida Júnior et al., 2011), where the improvement of pH and nutrient levels in the soil are some benefits that provide increased sugarcane yield (Bulegon et al., 2012). Andrade (1998) stated that the use of manures can even replace the chemical fertilizer of planting. According to Anjos et al. (2007), it is feasible to replace chemical fertilizer by organic (farmyard manure), without loss of quality and yield of sugarcane.

Gana (2009) suggested that combined application of cow manure between 10 and 20 Mg ha^{-1} and 50 kg N ha^{-1} increase yield of successive cane cropping and Teshome et al. (2014) reached highest cane and sugar yields with 15 Mg ha^{-1} of compost applied before furrowing and 46 kg N ha^{-1} at 2.0-2.5 months after planting. However, according to Gana (2011), there are few works on the recommendation of cow manure in sugarcane production, and farmers are using manure without any scientific data on the most appropriate method of application.

The purpose with this work is to evaluate the development and chemical composition of sugarcane in response to increasing levels of cow manure applied in the soil during the planting and the effect on soil fertility.

MATERIALS AND METHODS

The study was conducted in Boa Vista farm, district of Cachoeirinha, Viçosa, MG, Brazil, belonging to Universidade Federal de Viçosa. The city of Viçosa is located in the Zona da Mata region of Minas Gerais, Brazil, and its geographical coordinates has the position 20° 45' 20" South latitude and 45° 52' 40" West longitude of Greenwich and 651 m altitude. The climate is of the Cwa type (mesothermic), according to Köppen classification, with two well-defined seasons, with hot and humid summers and cold and dry winters. The average rainfall is 1341.2 mm per year. The average maximum and minimum temperatures are 26.1 and 14.0°C, respectively (UFV, 2016).

The experiment was conducted from October 2013 to October 2014. The experimental area has small slope topography and, in

order to prevent influence from previous fertilization, an area with no management for over ten years in which predominated the signal grass (*Brachiaria decumbens*) was chosen. Before the implementation of the experiment, chemical analysis of the soil was performed for characterization, collection of samples from different places at random and with the same volume to obtain a composed sample of 0 to 0.2 m layer. The sample composite was placed in plastic bag, identified and forwarded to the laboratory for soil analysis, showing the following results: pH in water (1:2.5) = 6.2, P = 6.2 mg dm^{-3}, P-rem = 33.1 mg L^{-1}, K = 67 mg dm^{-3}, Ca^{+2} = 2.8 cmol$_c$ dm^{-3}, Mg^{+2} = 1.1 cmol$_c$ dm^{-3}, Al^{+3} = 0.0 cmol$_c$ dm^{-3}, H + Al = 4.13 cmol$_c$ dm^{-3}, SB (sum of bases) = 4.07 cmol$_c$ dm^{-3}, CTC$_{(t)}$ (effective cations exchange capacity) = 4.07 cmol$_c$ dm^{-3}, CTC$_{(T)}$ (cations exchange capacity at pH 7.0) = 8.2 cmol$_c$ dm^{-3}, V (percent base saturation) = 50%, m (percent aluminum saturation) = 0,0% and light sandy loam.

The limestone was spread and incorporated by plowing in August 2013 (two months before planting), based on the method of saturation by bases and recommendation for sugarcane, using the equivalent of 4 Mg ha^{-1} of dolomitic limestone (NP = 70.5%, RE = 99.1%, RPTN = 69.9%, Ca^{2+} = 211 g kg^{-1} and Mg^{2+} = 47 g kg^{-1}). Plowing was performed to approximately 0.30 m depth, and harrowing (hoeing start), ensuring the soil unpacking, the reduction of the infestation of pests in the area and the elimination of signal grass (*Brachiaria decumbens*).

The cow manure was kept heaped and covered with plastic for 60 days, and then five subsamples were collected, at random, to obtain a composite sample for chemical characterization. The manure used in the experiment came from the same place where the experiment was implemented and it presented: 2.71% N, 0.66% P, 1.68% K, 0.336% Na, 1.76% Ca, 0.75% Mg, 0.53% S, 21.06% OC, 7.77 C N^{-1}, 139 ppm Zn, 4,484 ppm Fe, 280 ppm Mn, 28 ppm Cu, 12.4 ppm B, pH = 7.9 and 42.8% humidity.

The planting furrow was performed mechanically, by occasion of the planting, in October 2013, approximately 0.30 m depth and spaced 1 m apart, removing the clods of planting furrow. The experiment was conducted in randomized complete block design, with five treatments (fertilizer levels) and four replications, totaling 20 experimental plots. The treatments consisted of applying 0.0 (control), 4.5, 9.0, 13.5 and 18.0 Mg ha^{-1} of cow manure on natural matter. Each experimental unit was 5 m long and 4 m wide, totaling an area of 20.0 m^2, consisting of four lines of plants, considering useful area (8 m^2), the two centerlines, discarding 0.50 m at each end.

The cow manure was placed at the bottom of the planting furrow. The sugarcane was planted manually, using a variety RB867515, on 1 m spacing between rows and two rows of sugarcane per planting furrow, being stung in sizes of 0.20 m inside the planting furrow. The control of spontaneous plants was performed by hand weeding using hoes.

Four and ten months after planting, the number of plants per meter, plant height (measured from the ground to the highest leaf ligule), stalk diameter (near the surface of the soil), number of leaves per plant and length and width of the largest leaf were measured. At 12 months, the yield of whole plant (Mg ha^{-1} of green mass) was measured and samples were chopped, packed in plastic bags (500 g) and frozen for chemical composition. At this time, soil was sampled for chemical evaluation.

After thawing, the plant samples were placed in paper bags, properly identified and taken to an oven with forced ventilation of air at 55°C for 72 h, and weighted in semi analytics scale for the determination of the dry matter in the air. Then, the samples were ground and analyzed for dry matter content at 105°C, neutral detergent fiber corrected to ash and protein (NDFap), non-fibrous carbohydrates (NFC), crude protein (CP), ether extract (EE) and ashes (As), according to Detmann et al. (2012). The Brix was

Table 1. Chemical attributes of the soil after harvest of sugarcane in areas fertilized with increasing levels of cow manure[1]

Manure (Mg ha⁻¹)	pH Water	P	K		Ca	Mg	Al	H+Al
		mg dm⁻³			cmol$_c$ dm⁻³			
0.0	5.6	2.5	60		1.8	1.0	0.0	3.30
4.5	5.4	2.1	67		1.9	1.0	0.0	3.96
9.0	5.6	2.5	48		1.9	1.0	0.0	3.30
13.5	5.7	4.7	63		2.3	1.2	0.0	3.14
18.0	5.8	4.7	64		2.0	1.2	0.0	3.30
Manure (Mg ha⁻¹)	**SB**	**CTC(t)**	**CTC(T)**	**V**	**m**	**MO**	**P-rem**	
	cmol$_c$ dm⁻³			%		g kg⁻¹	mg L⁻¹	
0.0	2.95	2.95	6.25	47	0	33.5	29.3	
4.5	3.07	3.07	7.03	44	0	42.6	27.3	
9.0	3.02	3.02	6.32	48	0	33.5	28.3	
13.5	3.66	3.66	6.80	54	0	37.3	30.5	
18.0	3.36	3.36	6.66	50	0	33.5	29.3	
Manure (Mg ha⁻¹)	**Zn**	**Fe**	**Mn**	**Cu**	**B**			
	mg dm⁻³							
0.0	2.0	97.1	55.7	1.6	0.2			
4.5	2.2	71.2	49.5	1.6	0.4			
9.0	1.5	65.6	47.8	1.4	0.3			
13.5	2.7	100.9	68.2	1.8	0.2			
18.0	2.7	89.7	66.4	1.5	0.2			

[1]Cow manure: 2.71% N, 0.66% P, 1.68% K, 0.336% Na, 1.76% Ca, 0.75% Mg, 0.53% S, 21.06% CO, 7.77 C N⁻¹, 139.00 ppm Zn, 4,484.00 ppm Fe, 280.00 ppm Mn, 28.00 ppm Cu, 12.40 ppm B, pH = 7.90 and 42.80% humidity. Contents determined in acid extract (nitric acid with perchloric acid), in dry matter at 75 ºC. Nitrogen (N) determined by Kjeldahl method. Humidity in stove at 75 ºC. SB = sum of bases, CTC$_{(t)}$ = effective cations exchange capacity, CTC$_{(T)}$ = cations exchange capacity at pH 7.0, V = percent base saturation and m = percent aluminum saturation.

measured in samples of sugarcane juice obtained in electric mill device, using a saccharimeter (densimeter).

To find out the result of the rates of manure on the growth parameters of the sugarcane, the experimental data were subjected to ANOVA and regression analysis through Minitab (Ryan and Joiner, 1994). The chemical attributes of the soil and chemical composition of the sugarcane were obtained in pooled samples, reporting solely the mean per treatment for the chemical attributes of the soil and mean, standard deviation and coefficient of variation for the chemical composition of the sugarcane.

RESULTS AND DISCUSSION

The gradual increase of cow manure allowed smaller reduction in the content of P relative in the initial condition of soil, but it did not change, noticeably, the remaining parameters (Table 1). It is worth mentioning that although it has been used up to level 18 Mg ha⁻¹ of cow manure, dry matter content was low (57.2%), contributing with 10.3 Mg ha⁻¹ of dry matter.

The agricultural use of organic wastes, such as livestock manure, is an advantageous feature. It provides agronomic benefits, such as raising the pH of the soil (Silva et al., 2001), reducing potential acidity and increasing the availability of macronutrients (Berton et al.,

1997), in addition to the final provision with less impact on the environment (Freitas et al., 2012). In this study, the pH showed slight increase with increase in the level of cow manure.

Oliveira et al. (2007) pointed that the sugarcane, producing large amount of mass, extracts from the soil and accumulate large amount of nutrients. In the present study, the reduction of the levels of phosphorus in the soil became more apparent after the first cut of the cane, especially by using 0.0, 4.5 and 9.0 Mg ha⁻¹ of cow manure (Table 1).

According to Malavolta et al. (2002), chemically, the organic fertilizing is important source of nutrients, especially N, P, K and micronutrients, being the only form of N storage, which does not volatilize, and is responsible for 80% of the total phosphorus found in the soil. It is observed that the crops in general present as a rule high nitrogen and potassium requirements in addition to copper and molybdenum; however, the requirement order of other nutrients may vary between cultures and even cultivate/hybrid. In general, the decreasing standard order of crop extraction is as follows: macronutrients: N > K > Ca > Mg > P ↔ S; and micronutrients: Cl > Fe > Mn > Zn > B > Cu > Mo.

Four months after sugarcane planting, there was no

Table 2. Growth parameters of the sugarcane at four months as a function of organic fertilizing with cow manure

Item	Cow manure (Mg ha^{-1})					SE	Significance	RE
	0.0	4.5	9.0	13.5	18.0			
Plants m^{-1} linear	6.73	8.35	8.63	8.80	8.63	0.92	0.198	
Height of plant, m	0.254	0.308	0.381	0.357	0.464	0.022	0.001	1
Stalk diameter, mm	14.9	17.6	19.7	18.5	21.9	0.82	0.001	2
Leaf wide, mm	26.5	31.9	33.9	32.8	36.8	2.16	0.054	3
Leaf length, m	1.01	1.17	1.35	1.31	1.40	0.043	0.001	4
Number of leaves plant^{-1}	6.56	6.69	6.81	6.88	6.56	0.15	0.198	

Means followed by same letters in the same row do not differ among them, by Tukey test, at 5% probability. SE = standard error of mean; Signif. = significance by F test; RE = regression equation: [1] $0.259 + 0.0104x$, $r^2 = 0.88$; [2] $15.6 + 0.33x$, $r^2 = 0.82$; [3] $27.2 + 0.858x - 0.0214x^2$, $R^2 = 0.85$; [4] $1.02 + 0.0429x - 0.00127x^2$, $R^2 = 0.93$.

effect (P > 0.05) of the increasing level of cow manure only on the number of plants m^{-1} linear and the number of leaves plant^{-1} (Table 2). There was increase in linear effect (P < 0.01) of the level of cow manure on the height of the plant and the stalk diameter, in which the level of 18 Mg ha^{-1} (moisture content = 42.8%) provided values 83 and 47% higher, respectively, than the control treatment (Table 2 and Figure 1).

There was quadratic effect (P = 0.05) of the increasing level of cow manure on the width of the largest leaf (Table 2 and Figure 1), with maximum value estimated by the model using 20 Mg ha^{-1} of manure. The highest value was observed in the level of 18 Mg ha^{-1} of cow manure (38.9% higher than the control treatment), while the lowest value for the level 0 Mg ha^{-1}, and levels of 4.5, 9.0 and 13.5 Mg ha^{-1} showed intermediate values.

There was quadratic effect (P < 0.01) of the increasing level of cow manure on the length of the largest leaf (Table 2 and Figure 1), with maximum value estimated by the model using 16.9 Mg ha^{-1} of manure. The highest value was observed to be in the level of 18 Mg ha^{-1} of cow manure (38.6% higher than the control treatment), while the lowest value for the level 0 Mg ha^{-1}, and levels of 4.5, 9.0 and 13.5 Mg ha^{-1} showed intermediate values.

With the results presented at four months after planting, there was benefit of using cow manure for most of the evaluated parameters. These results are in agreement with those obtained by Freitas et al. (2012), on sorghum, who reported that doses of organic fertilizer applied in the furrow planting provided statistical difference only on initial assessments, when the number of leaves issued by plants was greater in treatments receiving higher levels of organic fertilizer.

At ten months, there was quadratic effect (P < 0.01) of increasing level of cow manure on the height of the plants (Table 3 and Figure 2), with maximum value estimated by the model using 16.8 Mg ha^{-1} of manure. The lowest value was observed for the level 0 Mg ha^{-1} of cow manure, while the highest value for the level of 18 Mg ha^{-1} (30.6% higher than the control treatment), and levels of

4.5, 9.0 and 13.5 Mg ha^{-1} showed intermediate values. There was no effect (P > 0.05) of the increasing level of cow manure on the number of plants, stalk diameter, width and length of the largest leaf and number of leaves plant^{-1} (Table 3). This is in agreement with those obtained by Freitas et al. (2012), who observed statistical difference only in initial assessments of organic fertilizer on the culture of sorghum. According to Santos et al. (2011) study on grasses, it was indicated that the number of leaves plant^{-1} is constant for a given species or cultivar, with little influence by environmental factors, which explains the behavior for this variable. Oliveira et al. (2011), in turn, reported that the stalk diameter depends on the genetic characteristics of the variety, the number of tillers, the spacing used, leaf area and the environmental conditions.

There was quadratic effect (P < 0.05) of the increasing level of cow manure on green mass yield at 12 months, in Mg ha^{-1} (Table 3 and Figure 2). The highest value was observed in the level of 18 Mg ha^{-1} of cow manure, while the lowest values in levels of 0 and 4.5 Mg ha^{-1}, and the levels of 9 and 13.5 Mg ha^{-1} showed intermediate values.

In this study, there was 98.8% increase in yield of green mass of sugarcane by using 18 Mg ha^{-1} of cow manure with 42.8% humidity in comparison with control treatment, showing that the cow manure was used efficiently in the production of sugarcane. This result is in agreement with Gana (2009), who found 101% increase in stalk yield at 12 months after planting sugarcane with 10 Mg ha^{-1} of air dried cattle manure when compared with no fertilization. For other side, Parente et al. (2012) found only 38.4% increase in yield of green mass of elephant grass fertilized with 20 Mg ha^{-1} of cow manure in comparison with control treatment.

According to Doorembos and Kassam (1994), the yield of sugarcane in the humid tropics, ranges between 70 and 100 Mg ha^{-1} with no irrigation and, in the dry subtropics, and between 100 and 150 Mg ha^{-1} with irrigation. In this experiment, the yield as the non-irrigated was obtained between 9 and 18 Mg ha^{-1} of cow manure.
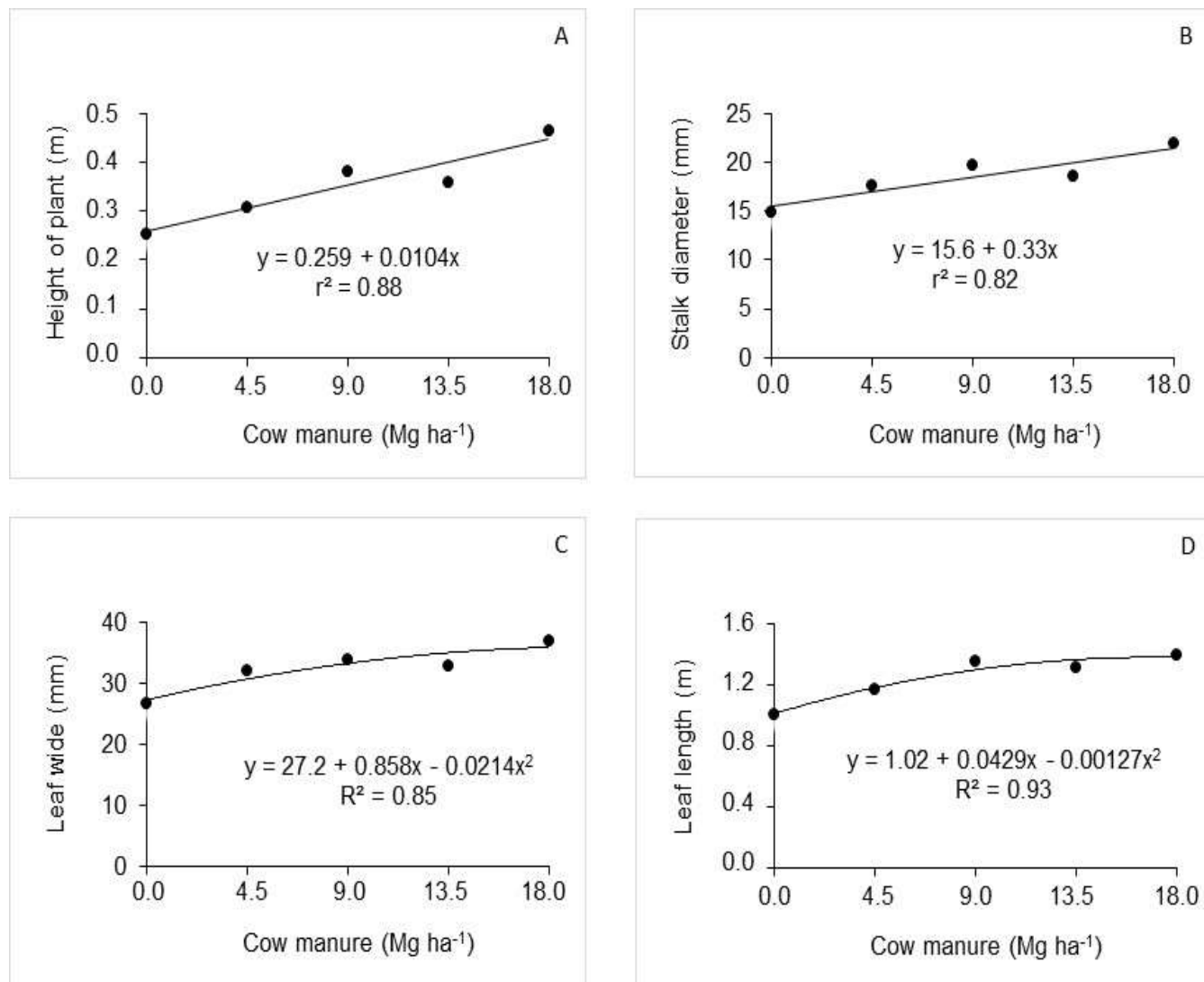
Figure 1. Growth parameters of the sugarcane at four months as a function of organic fertilizing with cow manure (A. Height of plant; B. stalk diameter; C. leaf wide; D. leaf length).

Table 3. Growth parameters of sugarcane at ten months and yield at 12 months as a function of organic fertilizing with cow manure.

Item	Cow manure (Mg ha^{-1})					SE	Significance	RE
	0.0	4.5	9.0	13.5	18.0			
Plants m^{-1} linear	7.55	7.53	8.15	9.13	8.03	0.44	0.103	
Height of plant, m	1.08	1.24	1.37	1.25	1.41	0.044	0.001	1
Stalk diameter, mm	28.3	28.9	28.4	28.2	27.2	0.88	0.230	
Leaf wide, mm	47.8	50.1	51.5	50.3	50.4	2.51	0.240	
Leaf length, m	1.16	1.21	1.23	1.24	1.20	0.033	0.200	
Number of leaves plant^{-1}	6.69	6.81	6.56	6.50	6.56	0.20	0.235	
Yield of green mass, Mg ha^{-1}	57.7	50.8	75.5	83.3	114.7	11.6	0.013	2

Means followed by same letters in the same row do not differ among them, by Tukey test, at 5% probability. SE = standard error of mean; Signif. = significance by F test; RE = regression equation: [1] $1.10 + 0.0318x - 0.000947x^2$, $R^2 = 0.74$; [2] $55.6 - 0.535x + 0.2106x^2$, $r^2 = 0.95$.
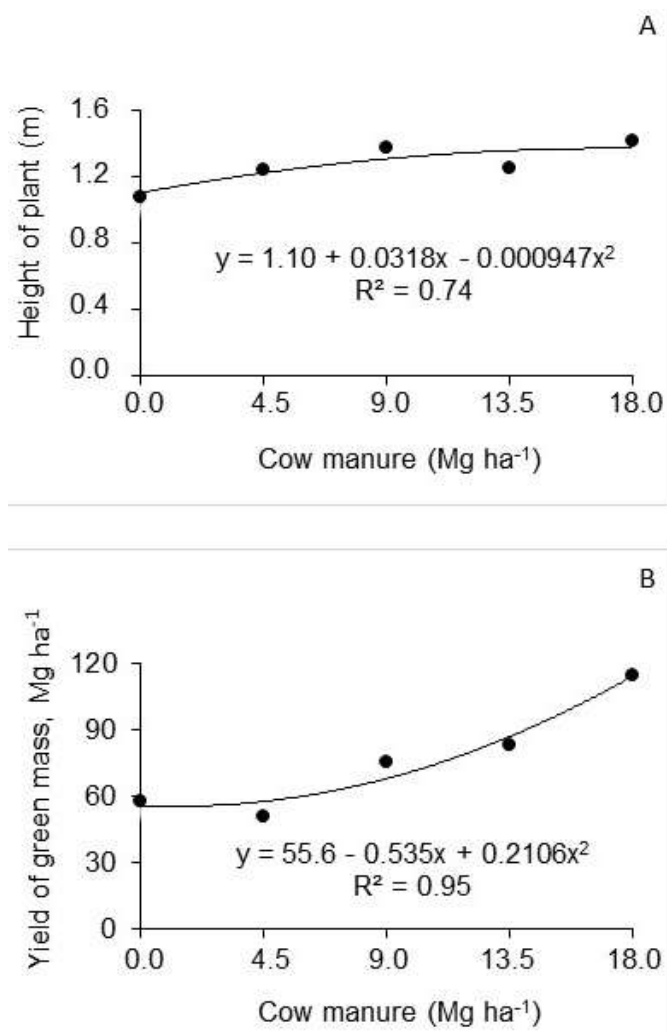
Figure 2. Height of the sugarcane plant at ten months (A) and yield of green mass at 12 months (B) as a function of organic fertilizing with cow manure.

Gava et al. (2011) found average yield of stalks, of three genotypes of sugarcane, of 132 Mg ha-1 for irrigated by drip and 106 Mg ha^{-1} for no irrigation management, in the first production cycle. In this experiment, the yieldsuch as these was obtained only with 18 Mg ha^{-1} of cow manure, with no irrigation. According to Oliveira et al. (2007), it is not likely to get yield up to 150 Mg ha^{-1}, while the P extracted with resin is less than 6 mg dm^{-3}, as was the case of this experiment (Table 1).

The chemical composition of sugarcane at 12 months as a function of the organic fertilizing with cow manure in the planting is presented in Table 4. Change of composition was not observed for different levels of fertilizer, with coefficient of variation less than 10% except for ether extract and ashes, due to the low levels and greater variability normally observed with these analyses.

The average dry matter (DM) and neutral detergentfiber (NDF), obtained in the present study at 365 days, were 31.2 and 46%, respectively (Table 4). Azevedo et al. (2003) showed for sugarcane variety RB867515, at 426 and 549 days, values of 27.1 and 30.6% DM and 50.1 and 47.8% NDF, respectively. The average values obtained in this study for DM and NDF, at 365 days, were close to those reported by those authors, showing that the composition of the sugarcane varies little after the ideal point suitable for cutting.

The NDF Brix^{-1} ratio in sugarcane depends on the environment in which the culture is growing, variety and age of cut, where higher values of NDF are registered under irrigation (Macêdo et al., 2012) or in the rainy season, due to the vegetative growth of plants (Muraro et al., 2009). Highest values of Brix, in turn, occur with increasing age of the plant and the dry season, as it reduces plant growth and sucrose accumulation occurs (Muraro et al., 2009). Macêdo et al. (2012) achieved NDF Brix^{-1} ratio of 2.41 for the variety RB867515 on non-irrigated land, similar to the average value of 2.42 calculated in this study to this same variety, based on the average data of NDFap and Brix as presented in Table 4.

Muraro et al. (2009) verified the age effect of cutting on Brix, with values of 6.3, 10.2 and 16.9 for sugarcane variety RB72454 at 180, 240 and 420 days, respectively, in 0.9 m spacing. The Brix obtained in this study ranged from 18 to 22 for the variety RB867515 with no irrigation (Table 4). According to Amaral and Bernardes (2011), values equal or greater than 18% Brix is recommended for cutting sugarcane for animal feeding, where value of Brix in addition to 40 units characterize the approach to the TDN content of sugarcane, which in this study would be 58 to 62%.

Conclusions

The cow manure improves several growth parameters of the sugarcane crop at four months, being more evident in the height of the plant and the stalk diameter. Level up to 18 Mg ha^{-1} of cow manure increases the yield of green mass of sugarcane variety RB867515 with no irrigation 12 months after planting, without changing chemical composition and maintaining soil fertility.

Conflict of Interests

The authors have not declared any conflict of interests.

Table 4. Composition of the sugarcane at 12 months as a function of organic fertilizing with cow manure.

Cow manure (Mg ha⁻¹)	DM (%)	NDFap in DM (%)	NFC in DM (%)	CP in DM (%)	EE in DM (%)	As in DM (%)	Brix (%)
0.0	30.5	43.6	50.4	1.98	1.23	2.74	19.0
4.5	32.8	48.1	46.8	2.13	0.72	2.25	18.0
9.0	31.6	49.3	45.9	1.76	0.83	2.21	18.0
13.5	30.0	44.2	50.2	1.92	1.01	2.72	18.0
18.0	30.9	44.6	50.9	1.74	0.61	2.19	22.0
Mean	31.2	46.0	48.8	1.90	0.88	2.42	19.0
SD	1.10	2.60	2.30	0.16	0.24	0.28	1.20
CV	3.50	5.60	4.70	8.50	27.7	11.7	6.30

DM = Dry matter, NDFap = neutral detergent fiber corrected for ashes and protein, NFC = non-fiber carbohydrates, CP = crude protein, EE = ether extract, As = ashes, SD = standard deviation and CV = coefficient of variation (%).

publication of this study.

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Full Length Research Paper

Losses of pesticides in runoff from cotton crops under different management systems

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In this study the concentrations of the atrazine, desethylatrazine (DEA), desisopropylatrazine (DIA), azoxistrobin, carbendazim, carbofuran, chlorpyrifos, diuron, endosulfan alpha and beta endosulfan sulfate, malathion, metolachlor, methomyl, monocrotofos, permethrin, profenofos, teflubenzuron, thiacloprid, tiametoxan and triflumuron were assessed in runoff water from cotton plantations under different management systems in Central-Western Brazil. The runoff water was collected from plots delimitated by steel plates, where cotton was sown under no-tillage (direct seeding) and conventional soil management systems in one farm; and no-tillage soil management systems with and without a vegetated filter strip (buffer filter) planted with *Bracchiaria* grass in a second farm. In general terms, there was a 51% reduction in the total mass of off-site transport of pesticides in the no-tillage system compared to the conventional tillage. In addition, when comparing the no-tillage system with conventional tillage, the pesticides: metolachlor and alfa endosulfan as well as endosulfan sulfate, its metabolite, showed the lowest reduction in relation to the other pesticides transported by runoff. Thus, these pesticides showed broad risk of contamination of surface waters. Besides, the buffer filter reduced the off-site transport of pesticides by runoff in 92%, confirming the efficiency of a *Bracchiaria* filter strip in the retention of pesticides carried by runoff.

Key words: Runoff water, buffer filter, direct seeding, conventional plantation system.

INTRODUCTION

The agricultural sector is the primary user of pesticides in the world, consuming more than three billion kilograms of pesticides annually (Barrett and Jaward, 2012). Agricultural production is closely related to the maintaining areas in appropriate conditions for cultivation. The use of the pesticides within a management system of the soil aims to improve the productivity of the crop.

Despite this positive effect in the improvement of productivity, pesticides can be dispersed in the environment. The main mechanisms that determine the distribution of pesticides in the environment are leaching, runoff, volatilization, retention, and transformation (Mariot

et al., 2009).

Kennedy et al. (2001) emphasized that research teams from several universities, government departments of agriculture, land, and water, and the commonwealth scientific, made a cooperative research effort to measure aerial and surface runoff transport of pesticides, and dissipation of their residues from cotton farms. In this context, Dores and De-Lamonica-Freire (2001) made a study in cotton crops in the region of Primavera do Leste, in the state of the Mato Grosso, Brazil. It was observed that the pesticides used in cotton crops in this region are very likely to disperse in water, through the runoff, mainly, organochlorine pesticides (OCPs) such as α-endosulfan, β-endosulfan and endosulfan sulphate. Weaver et al. (2012) reported that the persistence in soil of OCP's applied to cotton crops grown more than two decades ago suggests that they could enter the food chain. Their presence at depths of 1.2 m suggests that they could move into groundwater that may eventually be used for domestic and stock consumption.

Others authors report that in the tropical regions, there is high risk of contamination of the groundwater and surface water by pesticides, therefore, it is necessary to study the influence of the management systems in the dynamic of the pesticides in soil (Carbo et al., 2008; CASARA et al., 2012).

The different systems of the soil tillage significantly influence the dynamics of pesticides in soil, because they provide different physico-hydric behavior. For example, the presence of the crop residues on the surface soil after planting provide the dissipation of impact energy from the rain drops on the soil surface, which consequently minimizes the breakdown of soil particles. Moreover, crop residues in direct contact with the soil surface are effective on the load reduction of sediments in the runoff (Bertol et al., 2007).

Several studies report that in the no-tillage system (direct seeding), in which there is conservation of the crop residues, the losses of water is smaller than the conventional tillage, where there is use of the moldboard plow, which promotes an over spray of top soil, favouring the surface crusting of the soil. In other words, formation of compacted layer, consequently, higher losses of the soil, water, and pesticides (Bernardi et al., 2003; Bertol et al., 2004; Locke et al., 2008).

According to Pinho et al. (2008), the buffer filters, represented by vegetation strips disposed crosswise in the direction of runoff, this management systems, is also a way to mitigate the flow of the pollutants, mainly by runoff. In general, the buffer filters decrease the velocity of runoff, thus, reduce the capacity of the solute transport and provide deposition of particles in suspension; filtering particulate material in suspension by vegetation and increasing of the water infiltration in the soil with consequent reduction capacity of the runoff.

In view of what has been exposed here, the objective of the present study was to assess the water losses and mass of pesticides, atrazine and our metabolites desetilatrazina (DEA) and desisopropilatrazina (DIA), azoxystrobin, carbendazin, carbofuran, chlorpyriphos, diurom, alpha and beta endosulfan and our metabolite endosulfan sulfate, malathion, metolachloro, methomyl, monocrotophos, permetrine, prophenofos, teflubenzuron, thiacloprid, thiamethoxan, and triflumuron by runoff in experimental plots under field conditions, in cotton groups cultivated under different management systems: conventional soil preparation, no-tillage (direct seeding), and no-tillagem with and without a vegetated filter strip (buffer filter) planted with *Bracchiaria* grass, in the micro-region of Primavera do Leste, localized in Mato Grosso State, Central-Western Brazil.

MATERIALS AND METHODS

Experimental sites and water sampling

The experiment consisted of installing four monitoring units on two farms located in the micro-region of Primavera do Leste, localized in Mato Grosso State, Central-Western Brazil, in one farm situated on the banks of the Chico Nunes stream and another on the banks of the Ilha stream, both tributaries of the Mortes River (Deaths Rivers) (Table 1).

On the first farms, two units were installed to monitor runoff in cotton cultivated areas. In one of them, one filter strip was set up planted with *Bracchiaria* grass. On the second farm, two units were also installed monitoring runoff in areas cultivated with cotton. In one, the monitoring units used the conventional tillage and another, the no-tillage system, both with one system collector of the runoff at its end. This collector was formed from collector gutter linked to the pipe of the polyvinyl chloride. The structure of the collector was directed on the lower end in the experimental plot, consisting of one rectangular box, built from galvanized sheet, with a filtering system (geotextile blanket). This box was made with one divisor type Geib, made on the nine openings, and the central opening was linked to a water tank that stored the volume of runoff that passed by fraction of 1/9 on the Geib (Figure 1).

According to data, pesticide applications were selected and analyzed for the following active ingredients: atrazine and our metabolites desethylatrazine (DEA) and desisopropylatrazine (DIA), azoxystrobin, carbendazin, carbofuran, clorpyriphos, diuron, endosulfan alfa and beta and your metabolite endosulfan sulfate, malathion, metolachlor, methomyl, monocrotophos, permetrine, profenophos, teflubenzuron, tiaclopridthiamethoxan, and triflumuron.

Tables 2 and 3 show the application data of pesticides during the monitoring period on the farms with cotton crops, with no-tillage system (direct seeding), conventional plantation, and a system with and without a vegetated filter strip planted with *Bracchiaria decumbens* grass.

Water samples were collected in amber glass bottles, filtered and conserved under ice immediately after collection, and transferred to the laboratory refrigerator. After the arrival of the samples in the laboratory, the analysis was started in the shortest possible time, with a period not exceeding 15 days.

Analysis of pesticides residues by gaseous chromatographic

The residues of the atrazine, DIA, DEA, clorpyriphos, endosulfan alfa and beta and endosulfan sulfate, malation, metolachlor, monocrotophos, permetrine and profenophos in the water, used in the method reported by Laabs et al. (2002), in general consisted of

Table 1. Description of the chemical and physical characteristics of the monitoring units.

Localization	Soil	Soil management systems	S%[1]	DP[2] (m)	Clay	Silt	Sand	OC[3]
					g kg^{-1}			
Chico Nunes small watershed	Yellow latosol	No-tillage with filter strip of the *Brachiaria decumbens* with width of 10 m (MUS).	3.9	10 × 40	372	108	520	20.0
		No-tillage without strip filters *Brachiaria decumbens* (MWS)	4.1	10 × 40	461	107	432	30.0
Ilha small watershed	Yellow redoxisol	Conventional tillage (MUC)	3.4	3.5 × 11	457	65	478	30.0
		No-tillage (MUT)	2.9	3.5 × 11	414	95	491	42.7

[1]Slope of the monitoring units. [2]Dimensions of the monitoring units (width × length) willing toward upright the watercourse. [3]Percentage of the organic carbon in the surface layer of the soil (0 to 20 cm).

the extraction by solid phase extraction with octadecilsylane (C18) (1000 mg) BakerbondTM, Mallinckrodt Baker, USA, previously conditioned with 10 ml of methanol and 10 ml of water, followed by elution with subsequent portions 10 ml of the ethyl, 10 ml de hexane: ethyl acetate (1:1) and 5 ml of the hexane. The extract was concentrated in a rotary evaporator to near dryness and transferred to an autosampler vial with toluene. A gas chromatograph HP-6890 with mass selective detector HP-5973 (Agilent GmbH, Germany), split/splitless injector, automatic sampler and a HP-5MS (5% phenylmethylsiloxane) column (30 m × 250 µm id × 0.25 µm phase thickness) was used for pesticide analysis. Pesticide residues were quantified by GC-MS operated in the selected ion monitoring mode at the following conditions: injector block temperature of 250°C; carrier gas of helium (99,999% pure), gas flow of 1 ml/min; split/splitless injector operated in splitless mode; injection volume of 1 ml; oven temperature program of initial temperature of 92°C held for 2.5 min, heating up to 175°C at 15°C min^{-1}; 175°C held for 13 min, heating up to 280°C at 20°C min^{-1}, 280°C held for 9 min; and transfer-line temperature of 290°C. Pesticides were identified by retention time and by relative abundance of three major ions from mass spectra of each substance (Table 4). Maximum tolerance for confirmation was specified as 20% of relative ion intensity response.

Analysis of pesticides residues by liquid chromatographic

The pesticides residues: azoxystrobin, carbendazin, carbofuran, diuron, methomyl, teflubenzuron, thiacloprid,

thiamethoxan and triflumuron in water, was perfomed according to the method described by Carbo et al. (2008), before pesticides extraction it was concentrated with 500 ml of the samples in a SDVB cartridge (Envi-Chrom P, Supelco) previously conditioned with methanol. The cartridge was dried, leaving the vacuum pump on for 30 min.

The pesticides were eluted with 3 × 5 ml of methanol: acetonitrile 7:3 (v/v) at a flow-rate of about 1 ml min^{-1}. The combined fractions were concentrated in a rotary evaporator (45°C) and the residue was redissolved in 1 ml of acetonitrile, followed by the addition of 50 µl of standard terbuthylazine solution (100 µg ml^{-1}) to the vial and the injection of 10 µl into the HPLC/DAD.

The analysis was performed with a Varian HPLC system equipped with a 410 autosampler, a 240 quaternary pump and 330 UV diode-array detector linked to a personal computer running the software program Varian ProStar, version 5.5 (Varian, USA). The analytical column (250 mm × 4.6 mm I.D.) used here was an Omnisphere 5 µm C[18], and the guard column (20 mm × 4.6 mm I.D.) was also an Omnisphere 5 µm C[18]. For the HPLC analysis, an aliquot (10 µl) was injected into the column and eluted at room temperature at a constant flow-rate of 1 ml min^{-1} under the following conditions. The analytes were eluted with acetonitrile:water with initial composition of 18% acetonitrile, increasing to 40% at 6 min, 80% at 35 min, 90% at 40 min, and 100% acetonitrile at 45 min, where it was kept constant for 3 min and then linearly decreased to the initial analysis conditions in 10 min. The detection and quantification were performed at 230 nm. Analytes were identified by their retention time and identification was confirmed by comparison of their UV spectra to that of standard solutions.

RESULTS

Analysis overview

The runoff coefficients - RC (Table 5) of the monitoring unit with strip filter of the *B. decumbens* - MUS observed values less than the value monitoring units without a buffer filter -MWS. The magnitudes ranged from 0.005 to 0.035 and 0.011 to 0.402 for MUS and MWS, respectively. The runoff coefficients (Table 5) observed on the monitoring unit with no-tillage system -MUT were less than the monitoring unit with a conventional tillage -MUC, the coefficients ranged from 0.00 to 0.528 and 0.00 to 0.94 in the MUT and MUC, respectively.

In general, the increasing or decreasing of the pesticides concentration on the runoff was not observed, due to the presence or absence of the *B. decumbens* strips (Table 6), it can be observed that pesticide concentrations, malathion, metolachlor and atrazine were high with strips of the *B. decumbens* (MUS) compared with strips without the *B. decumbens* (MWS), while for pesticides allfaendosulfan, endosulfan sulfate and diuron the concentrations were the smallest on MWS, already for pesticides methomyl and permetrine the concentrations were very similar between these two monitoring units. Similar
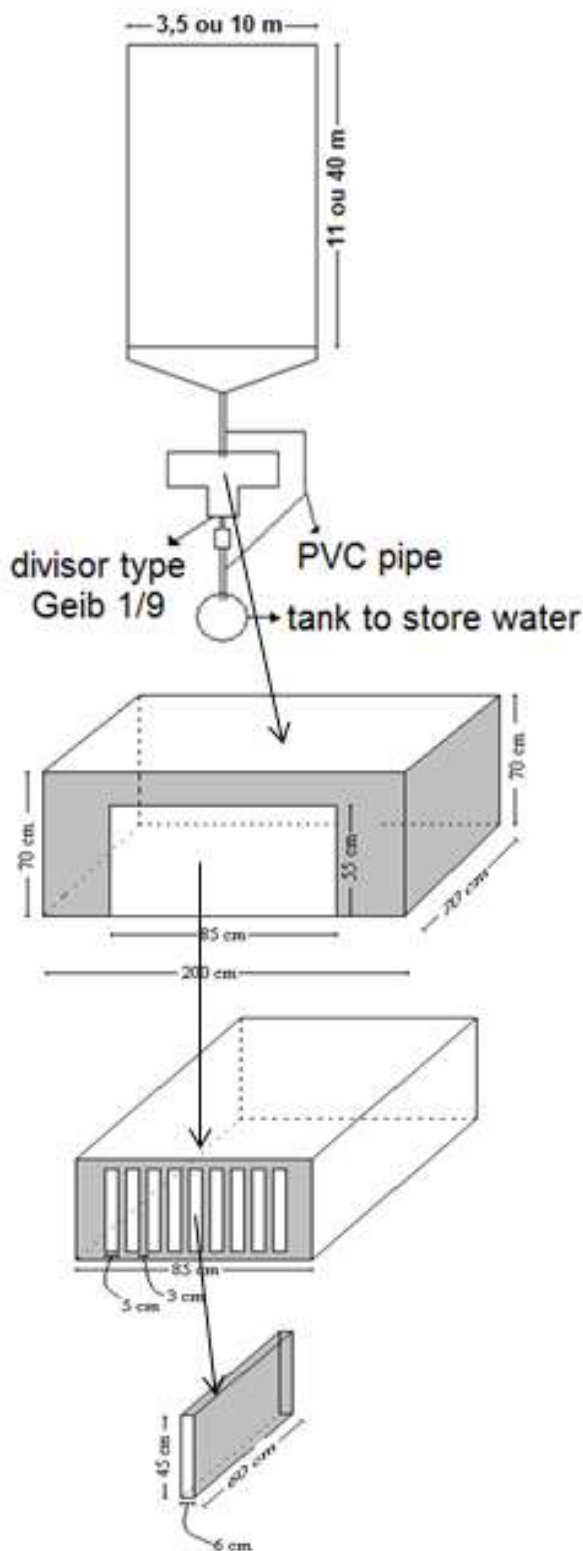
Figure 1. Schematic representation of the experimental plot and collector structure runoff.

behavior can be observed relative to the concentrations

of pesticides on the monitoring with MUT and MUC (Table 6).

Analyzing quantitatively the total mass of the pesticides transported by runoff, it was observed that the total mass of the pesticides detected in the MWS was higher than the mass observed in the MUS (Figure 2). It can also be verified that total mass of pesticides transported by runoff in the MUT was smaller than MUC, the only exception was methomyl pesticide (Figure 2).

Figure 3 shows the percentages of the reduction mass of pesticides carried by runoff in the monitoring units with filter stripes of the *B. decumbens* compared to those without filter stripes of the *B. decumbens* (MUS and MWS, respectively); and no-tillage system compared to the conventional tillage (MUT and MUC, respectively).

DISCUSSION

Runoff water

The smallest runoff in the monitoring unit with no tillage system is probably because this system provides high stable infiltration compared to the conventional tillage (Barcelos et al., 1999; CASTRO, 1995; Sobrinho et al., 2003; Brandão, 2006; Brandão et al., 2007). The high efficiency of the *B. decumbens* strips to reduce the runoff also was observed by Syversen and Bechmann (2004).

Pesticides residues in runoff water

The isomers α and β-endosulfan were broadly detected on the monitoring units studies (Table 6). Pesticides proportion detected increased during the collections after several pesticide applications on the field of study. Already, endosulfansulphate was detected broadly, because endosulfan in soil rapidly forming significant concentrations of endosulfan sulfate, which persists for several months, means that a cotton field can act as a strong source of pesticide residues in runoff water for several months after applications (Kennedy et al., 2001).

The metabolites of the atrazine, DEA and DIA, were detected in increasing concentrations, besides which the frequency of the detection of DEA was larger than DIA. Higher concentrations were detected on the last sampling (Table 6). This fact shows high solubility of DIA and DE, as well as easy transportation by runoff, as well as reported by Correia et al. (2007).

Malathion was detected only once (Table 6). The concentrations observed were lower and similar in the monitoring units with no-tillage and conventional tillages and there was high concentration of this pesticide already in the systems with buffer filters.

Pesticides metolachlor and diuron were detected in almost all samples of the water. Besides, these pesticides were also detected in all monitoring units (Table 6). Metolachlor has been classified as an easily

Table 2. Pesticides applied on the cotton farms where the monitoring units were installed with conventional tillage and no-tillage soil management systems.

Active principle	Average concentration applied (L/ha ou*kg/ha)	Active principle	Average concentration applied (L/ha ou *kg/ha)
Prophenophos	0.51	lambdacialotrine	0.06
Alphaand beta endosulfan	7.50	Cipermetrine	0.30
Azoxystrobin	0.25	Deltametrine	0.10
Carbosulfan	1.92	Thiophanate-methyl	0.58
Teflubenzuron	0.10	Triazophos	1.02
Parathion	1.02	Parathion	1.02
Zeta-cipermetrine	0.30	Acetamiprid	0.13 *
Diafenthiuron	0.33*	Tetroconazol	0.40
Chlormequat chloride	0.20	Gamma-cyhalothrine	0.09
Bifenthrin	0.60	Fenitrothion	0.60
Luphenuron	0.30	Triflumuron	0.06

Table 3. Pesticides applied on the cotton farms with no-tillage soil management systems where the monitoring units were installed with and without a vegetated filter strip planted with *Bracchiaria decumbens* grass.

Active principle	Average concentration (L/ha or*kg/ha)	Active principle	Average concentration (L/ha or*kg/ha)
Metolachlor	0.60	Novalurom	0.15
Diuron	1.0 *	Malathion	1.0
Diuron	0.80	Diafentiuron	0.28*
Carbosulfan	0.80	Tetraconazole	0.4
Acetamiprid	0.23*	Trifloxisulfuron	0.002*
Methomyl	2.9	Propiconazol	0.50
Carbosulfan	2.0	Carbendazim	1.5
α and βendosulfan	4.0	-	-

Table 4. Monitoring ions for identification and quantification of the pesticides by GC/EM.

Pesticides	Target ion	Fisrt ion	Second ion
Atrazine	200	215	202
DIA	173	258	145
DEA	172	174	187
Alphaendosulfan	241	238	195
Betaendosulfam	207	195	237
Endosulfam sulfate	272	274	387
Malathion	173	125	127
Metolachlor	162	238	240
Permetrine	183	163	165
Prophenophos	208	139	339

soluble and mobile pesticide (FAO, 2000), because our solubility is 530 mg ml^{-1} (Rivard, 2003) and adsorption coefficient to organic carbon is 200 g ml^{-1} (Rivard, 2003). Diuron is classified as easily soluble and moderately mobile (FAO, 2000), because our solubility and

adsorption coefficient to organic carbon is 36.4 mg ml^{-1} and 418 to 560 g ml^{-1} (Moncada, 2004), respectively. Moreover, Dores et al. (2005) reported that the half-life of diuron and metolachlor is 15 and 34 days. These properties of diuron and metolachlor explain the high

Table 5. Runoff and runoff coefficients of the monitoring unit with and without buffer filter; unit with system conventional tillage and no-tillage .

Sampling date	[5]CP	MUB[1]		MUW[2]		Sampling date	CP	MUC[3]		MUT[4]	
		[6]RF	[7]RC	RF	RC			RF	RC	RF	RC
		mm						mm			
03/1/2007	60	2.11	0.035	24.1	0.402	05/1/2007	78	19.96	0.260	12.25	0.157
19/01/2007	138	2.11	0.015	1.49	0.011	18/01/2007	59	55.57	0.940	31.13	0.528
01/02/2007	108	1.05	0.010	21.65	0.200	2/2/2007	160	42.23	0.260	17.65	0.110
23/02/2007	396	2.11	0.005	12.56	0.032	22/02/2007	231	109.73	0.480	61.46	0.266
14/03/2007	313	2.11	0.007	30.83	0.098	14/03/2007	104	0.000	0.000	0.000	0.000
5/4/2007	218	2.11	0.010	30.72	0.141	4/4/2007	425	47.79	0.110	35.85	0.112
21/04/2007	122	4.16	0.034	33.8	0.277	20/04/2007	92	44.14	0.480	24.73	0.269
Total	1247	8.38	-	133.5	-		1045	319.42	-	183.07	-
Average	193,571	2.251	0.010	22.164	0.160	-	149.286	45.631	0.422	26.152	0.206
SD[8]	-	-	0.013	-	0.152		-	-	0.292	-	0.170

[1]Monitoring units with strip filter of the *Brachiaria decumbens*; [2]Monitoring units without strip filter of the *Brachiaria decumbens*; [3]Monitoring units with conventional tillage; [4]Monitoring units No-tillage system; [5]Cumulative precipitation, mm; [6]Runoff ; [7]Runoff coefficient; [8]Standard deviation.

Table 6. Pesticides concentrations ($\mu g\ L^{-1}$) in surface waters of the monitoring units with and without stripe filter; and monitoring units with system conventional and no-tillage.

Pesticide	Sampling dates						
	05/01/07	18/01/07	02/02/07	22/02/07	14/03/07	04/04/07	20/04/07
Monitoring units with stripe filter							
α-Endosulfan	n.d.[1]	0.45	0.25	0.61	0.20	0.09	0.11
β-Endosulfan	n.d.	0.27	0.73	0.90	0.48	0.17	0.28
Endosulfan sulfate alpha and beta endosulfam	0.25	0.23	1.93	1.75	3.22	1.05	1.07
Atrazine	n.d.	0.18	0.11	0.18	0.39	0.62	0.16
Metolachlor	0.60	0.62	0.61	0.16	0.13	0.21	0.12
Malathion	n.d.	n.d.	n.d.	3.15	n.d.	n.d.	0.24
Prophenophos	n.d.	0.15	n.d.	n.d.	0.15	n.d.	n.d.
Permetrine	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diuron	0.87	1.17	1.19	0.32	n.d.	n.d.	n.d.
Methomyl	n.d.	n.d.	n.d.	4.65	15.3	11.11	4.22
Monitoring units without stripe filter							
α-Endosulfan	3.85	0.32	0.44	5.0	0.17	0.04	0.05
β-Endosulfan	0.72	0.17	1.06	4.49	0.26	0.10	0.19
Endosulfan Sulfate	0.58	0.22	2.86	3.12	1.93	1.99	1.81
Atrazine	n.d.	n.d.	0.11	0.15	0.65	0.14	0.17
Metolachlor	0.55	0.16	0.31	0.13	0.19	0.11	0.12
Permetrine	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	n.d.
Diuron	0.71	0.91	2.50	n.d.	n.d.	n.d.	n.d.
Methomyl	n.d.	n.d.	n.d.	6.00	4.59	0.10	0.24
Monitoring unit with no-tillage system							
α-Endosulfan	0.39	0.17	0.43	0.53	n.d.	0.04	n.d.
β--Endosulfan	0.07	0.11	1.02	0.83	n.d.	0.11	n.d.
Endosulfan Sulfate	0.46	1.01	2.58	3.32	n.d.	1.93	2.03

Table 6. Contd.

Atrazine	0.05	0.04	0.05	0.16	n.d.	0.32	0.28
DIA	n.d.	n.d.	n.d.	0.06	n.d.	0.1	0.18
DEA	n.d.	0.04	0.04	0.06	n.d.	0.08	0.18
Metolachlor	0.1	0.08	0.08	0.08	n.d.	0.08	0.08
Malathion	n.d.	n.d.	0.12	n.d.	n.d.	n.d.	n.d.
Diuron	20.6	3.98	1.65	1.11	n.d.	n.d.	n.d.
Methomyl	46.34	4.20	n.d.	n.d.	n.d.	n.d.	n.d.
Monitoring unit with conventional tillage							
α-Endosulfan	0.51	0.08	0.31	0.53	n.d.	n.d.	n.d.
β--Endosulfan	0.26	n.d.	0.69	1.04	n.d.	0.21	0.20
Endosulfan Sulfate	0.43	0.45	1.15	2.64	n.d.	1.58	2.02
Atrazine	0.07	0.08	0.06	0.20	n.d.	0.26	0.84
DIA	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	2.56
DEA	0.06	0.04	0.04	0.06	n.d.	0.11	3.46
Metolachlor	0.1	0.1	0.09	0.08	n.d.	0.1	0.1
Malathion	n.d.	n.d.	0.12	n.d.	n.d.	n.d.	n.d.
Diuron	17.37	6.11	2.91	1.61	n.d.	0.20	0.58
Methomyl	18.16	2.11	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.[1]: Not detected; detection limit = 0.02 µg L^{-1}.

detection of these compounds in water samples of the runoff.

Methomyl has solubilty in water of 58 mg L^{-1} (Ferracini et al., 2001) and it is classified as easily soluble (FAO, 2000). Besides, methomyl has absorption coefficient of 72 g ml^{-1} (Ferracini et al., 2001), considered a mobile pesticide. Thus due to these properties of methomyl and also the occurrence of an event of rain after application of methomyl, likely, there was not enough time for it to interact with crop residues in the soil and about the soil, therefore, the straw in the no-tillage system was washed by rainwater, thus one part of the methomyl applied was carried by runoff. In the conventional tillage, due to the absence of the straw, the methomyl entered in direct contact with the soil and, therefore, it was leached from deeper layers of the soil, decreasing the available amount to be carried by runoff. Other authors observed similar behavior for clorimuron-ethyl, that is, also considered to be one pesticide with high solubility in water (Loke et al., 2008).

The MUT has shown an average efficiency of the reduction in pesticides losses ranging from the 24 to 92% in the lost total mass of the pesticide compared to the MUC.

Management system and pesticides residues

The reduction on the concentrations of majority pesticides detected in water samples of the runoff in the no-tillage monitoring unit system compared with conventional tillage (Figure 2) is due to the fact to no-tillage system, there is higher organic carbon percentage in soil than in conventional tillage (Table 1). This fate provides greater pesticide retention and degradation in the soil, this fact is consistent whit results observed by Weaver et al. (2012) that reported strong association between DDE concentrations in soil with organic carbon when compared with the other soil properties.

In addition, Locke et al. (2008) reported that this management system of the soil provides the retention and degradation of the pesticide molecules, as well as better adsorption capacity of soil in relation of the high percentage of the organic matter often observed in this management system which can be observed in Table 1. The reduction ranged for MUS from the 64 to 98% compared to MWS (Figure 3). These observed reductions were due to the smaller capacity of the runoff water, considering that the pesticides concentration was more dependent on the pesticide properties and no soil management. This indicates that conservationist management of soil as well as improving soil properties also provides reduction of the risk of the pesticides being transported by runoff to the water stream.

Conclusion

The monitoring units with filter stripes of the *B. decumbens*
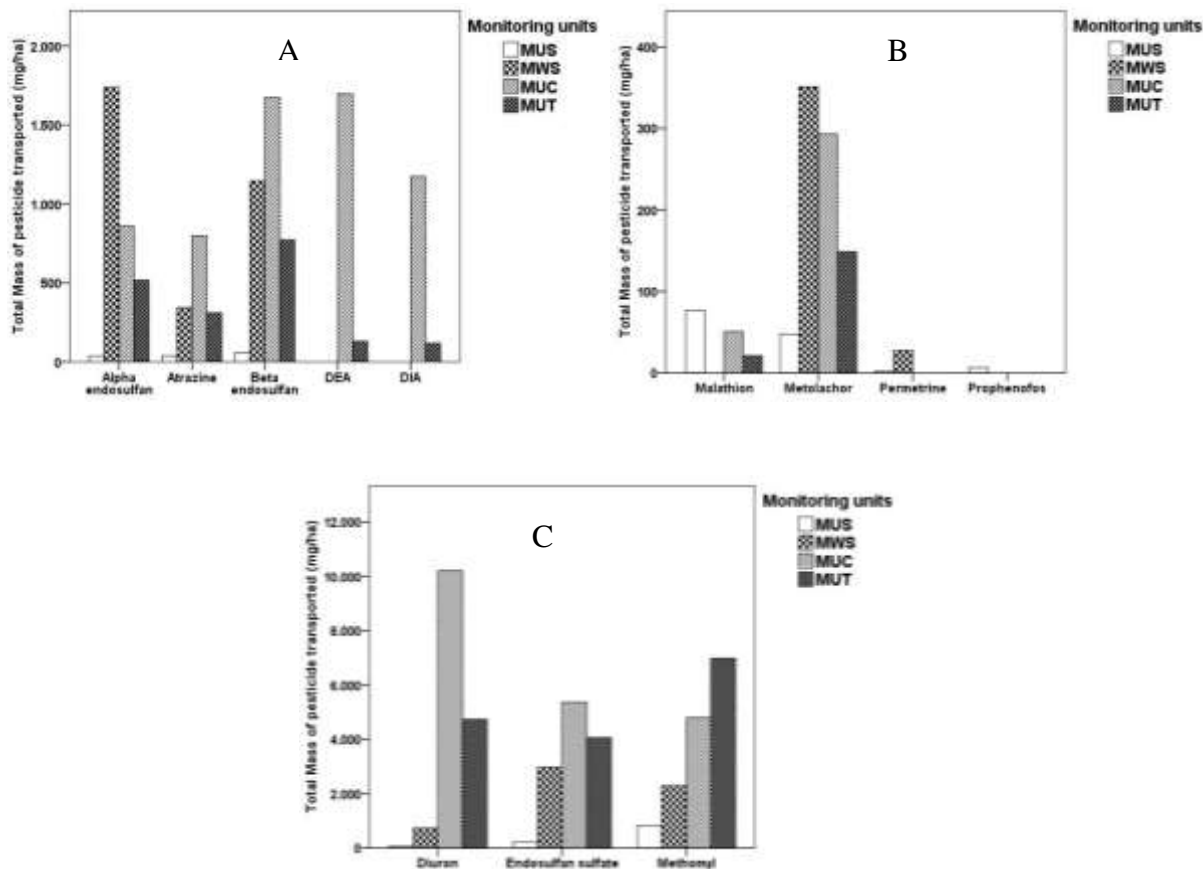
Figure 2. (A, B, C) Total losses of the pesticides (mg ha^{-1}) by runoff in the monitoring units: MUS: Monitoring unit with strip filter of the *Brachiaria decumbens*; MWS: monitoring unit without strip filters *Brachiaria decumbens*; MUT: monitoring unit with no-tillage system; e MUC: monitoring unit with conventional tillage.
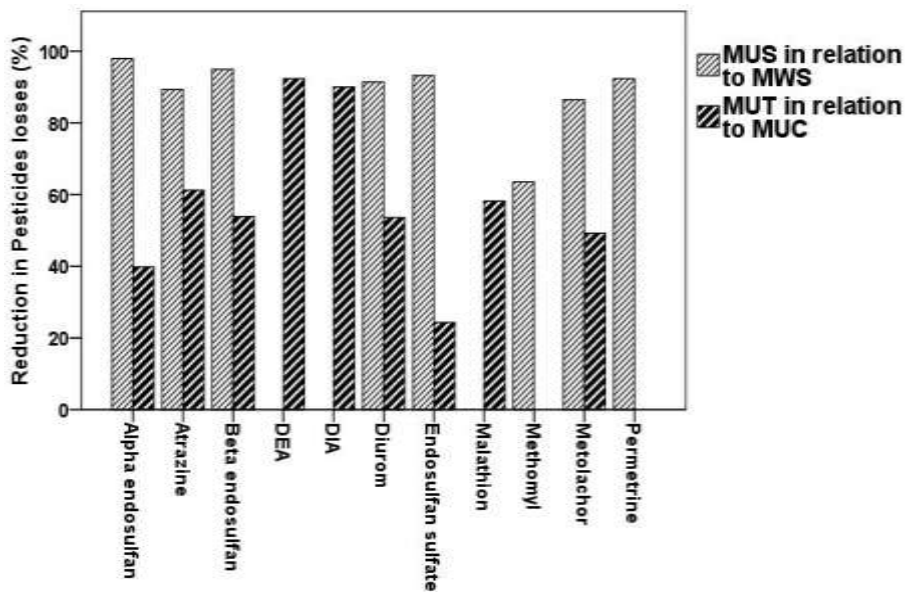

Figure 3. Mass reduction of the pesticides between the monitoring units with strip filter of the *Brachiaria decumbens* (MUS) and without strip filter of the *Brachiaria decumbens* (MWS)*; e*monitoring unit with no-tillage system (MUT) and monitoring unit with Conventional tillage (MUC).

and no-tillage system were the types of the management which reduced more efficiently the total loss pesticides by runoff. The percentage of the decrease was between 24 and 92% in the no-tillage system compared with the conventional tillage. Already, in the monitoring units with strip filters compared with monitoring units without strip filters, the percentage of the decrease was between 64 and 98%.

Furthermore, the type of soil management observed was evaluated in this paper, the risk of the pesticide contamination of surface waters by runoff in tropical regions was reduced more efficiently where there are cotton plantations, because the types of management evaluated decreased the volume of the surface water transported in the soil.

When comparing the no-tillage system with conventional tillage, pesticides, metolachlor and alfa endosulfan as well as endosulfan sulfate, and its metabolite, showed the lowest reduction in relation to other pesticides transported by runoff. Thus, these pesticides can be potentially transported by runoff, therefore, these chemical compounds showed broad risk of surface waters contamination. It is noteworthy that in this paper, only the pesticides transported by runoff were evaluated, thus, it is necessary to evaluate also the pesticides transported by adsorption by sediments.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Growth analysis, nitrogen accumulation, and yield of sugarcane varieties for the pre-amazon region of Brazil

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The great expansion of sugar cane production to non-traditional regions in Brazil has demonstrated the importance of knowledge on the growth characteristics to maintain the productivity and sustainability of the sector. Among the alternatives available to evaluate different varieties of sugar cane, the growth analysis has been one of the most used tools. The objective of this study was to evaluate the growth and development of three varieties of sugar cane for the Pre-Amazon region of Brazil. The varieties used were RB 867515, RB 863129 and RB 92579, and the following parameters of growth were evaluated: accumulation of biomass on the part area, leaf area, number of plants, height of stems, the nitrogen content in different parts of the plant (stem and leaf[+3]), brix and productivity. For that, samples were collected at 60, 120, 180, 240 and 300 days after planting. For all collecting dates and at 300 days after planting the variety RB 863129 presented better results (P<0.05) for plant height, stem dry weight, N content in leaf[+3] and final yield compared to the varieties RB92579 and RB 867515. There was no difference for number of plants (P>0.05) for the varieties RB 863129 and RB 92579. Leaf area was greater (P<0.05) for the variety RB 867515; and nitrogen content of stems were greater for the variety RB 92579 at 300 days after planting. Overall, the RB 863129 variety was the most promising for cultivation in this region during the sugarcane-plant season.

Key words: Biomass, crop development analysis, selection of varieties.

INTRODUCTION

Sugarcane cultivation is one of the main agricultural activities in Brazil and comprises the oldest agroindustrial sector, occupying a prominent position in the Brazilian national and international economy. Brazil is the top sugarcane producer worldwide, harvesting approximately 9 million hectares (CONAB, 2016).

In Brazil, as in other sugarcane-producing countries, sugarcane varieties have been continuously developed

and tested to increase yield, obtain higher resistance to pests and diseases, and seek better adaptation to variations in climate, soil, and cutting or management techniques (Abranches and Bolonhezi, 2011). Particularly considering the vast expansion of the sugar-ethanol sector into regions of non-traditional cane production, that is, within the pre-Amazon region of Brazil, there is still a gap in knowledge regarding the crop growth and

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development characteristics for maintaining yield and more sustainable methods in the sector of the region.

Among the alternatives available for studying sugarcane varieties, one of the most used tools has been growth analysis, which is considered a standard method for measuring crop biological productivity, serving as a very important tool to evaluate crop growth under different cultivation conditions (Batista et al., 2013). This method allows identification of the best crop develop-mental stages by evaluating morphological variables of the plants, such as height, stem diameter, tillering, leaf area, and yield, making it possible to determine the productive capacity of different varieties. These data help in understanding the climatic effect on yield, intervention in agricultural planning, and determining the magnitude of physiological stress and final yield (Simões et al., 2005). Thus, identifying variation during sugarcane development is essential to model and quantify growth in different production environments. For example, Oliveira et al. (2010), evaluating growth and accumulated dry matter in eleven sugarcane varieties (SP 791011, RB 813804, RB 863129, RB 872552, RB 943365, RB 72454, RB 763710, SP 784764, SP 813250, RB 867515, and RB 92579) farmed under full irrigation, found that accumulated stem dry matter was characterized by the following three developmental stages: a first stage characterized by lower accumulated stem dry weight than accumulated leaf+top dry weight (on average 8 Mg ha^{-1} at 120 days after planting - DAP); a second stage where accumulated stem dry weight was higher (on average 48 Mg ha^{-1} at 240 DAP); and a third stage starting at 240 DAP with higher accumulated stem dry weight (85 and 72 Mg ha^{-1}) for the 92579 and SP81 3250 varieties, respectively.

Within the context of growth and yield, sugarcane has a high nitrogen requirement. Nitrogen is considered as one of the elements most absorbed by the crop: it is very important in plant nutrition and physiology because it is a constituent of amino acids, proteins, enzymes, and nucleic acids (Keshavaiah et al., 2012). Thus, N transport to the sugarcane plants and within them is important for plant growth and development, making knowledge of these aspects for better crop management necessary (Toppa et al., 2010).

In view of what has been exposed here, with the expectation of expanding the sector in regions of Brazil that are still untapped, that is, within the pre-Amazon region comprising the Low Parnaíba of Maranhão microregion, this study aimed to evaluate the growth and development of three sugarcane varieties (RB 867515, RB863129, RB92579) for the region's edaphoclimatic conditions.

MATERIALS AND METHODS

Description of the experimental site

This study was performed within an area provided by the Várzea farm, in the municipality of Brejo in the Low Parnaíba of Maranhão

region, located at 03°44'33"S latitude, 43°21'21"W longitude. The region's climate is Aw according to the Köppen classification (hot and wet with rainy season in the Brazilian summer and dry in the Brazilian winter). The soil of the experimental site was classified as yellow Oxisol. Soil samples from 0 to 20 cm depth were collected before planting sugar cane and analyzed for pH (0.01 M water suspension, 1:2.5 soil/solution, v/v), organic carbon (Walkley-Black), P and exchangeable Ca, Mg, K, and H+Al according to standard methods used by Embrapa (1999). Particle size analysis was performed using the pipette method (Robinson, 1967). The soil presented the following characteristics: pH in water = 5.1, Ca = 2.2 cmol$_c$ dm^{-3}, Mg = 2.5 cmol$_c$ dm^{-3}, Na = 0.02 cmol$_c$ dm^{-3}, K = 0.11 cmol$_c$ dm^{-3}; Al = 0.6 cmol$_c$ dm^{-3}, H+Al = 9.4 cmol$_c$ dm^{-3}, P = 2.5 mg L^{-1}; sand = 54%, silt = 22%, and clay = 25%.

The highest rainfall is concentrated in December through May, which represents 70 to 80% of the total rainfall. The meteorological data on monthly rainfall (mm) and mean temperature (°C) during the experimental period were collected at the closest Brazilian National Institute of Meteorology station to the experimental site, located in the municipality of Chapadinha (Figure 1). The following sugar cane varieties were used: RB 867515, RB 863129, and RB 92579, and the soil of the experimental site was subjected to conventional tillage in December 2009. The seed pieces were distributed in 30 cm-deep furrows spaced 1.5 m apart, placing the seed pieces continuously, with the basal end in contact with the apical end of the subsequent seed piece. After distributing the seed pieces in the furrows, the stems were cut into billets of approximately three to four buds. The seeds were covered by 5 to 10 cm of soil. The experiment was laid out in randomized complete block design with three replicates, totaling nine plots, each plot having an area of 880 m^2. The total experimental area was 7920 m^2. The following five harvests were performed to obtain the data: at 60, 120, 180, 240, and 300 days after planting (DAP). The following growth parameters were determined at every harvest period: accumulated shoot biomass, total leaf area (TLA), number of plants, and stem heights. Nutrient content in the different portions of the plants (leaf^{+3} and stem) was determined at the last harvest (at 300 DAP), and the brix and yield parameters were also determined.

The accumulated dry weight of stem and top+leaves was quantified by collecting three plants of each plot at random. After harvest, each plant was separated into stem and top+leaves, which were labeled and placed in an oven at 60°C to obtain the dry weight (Oliveira, 2008). Total leaf area (TLA m^2) was determined by measuring from the first to the sixth leaf of eight plants in two linear meters of sampling area in each experimental plot, measuring leaf length and width in the middle portion with a graduated ruler, using the equation TLA=L.W.c proposed by Buso et al. (2009), where L= length; W= width; c=correction factor.

Biometric data were collected to determine parameters related to plant production following the method proposed by Barbosa (2005), where the number of plants was obtained by counting the plants sampled in two linear meters of each plot, and mean stem height was measured with a graduated ruler, measuring plant height from ground level to the top. Eight plants within the two linear meters of the sampling of each plot were measured. Brix content (%) of the sugarcane was measured using a field refractometer, where three plants were randomly removed per plot, and samples were collected from the stem water. Drops of stem water were extracted from the 4th internode from the soil and from the top of the last internode of the sheath that detaches easily. During the five harvest periods, samples were collected from the plant shoots to evaluate the accumulated nutrient contents. The dry matter of the plant fractions (stem and leaf^{+3}) was ground and subjected to digestion to determine the macronutrient levels using the method proposed by Vaccaro et al. (2004). Yield of the sugarcane varieties was obtained by harvesting plants at 300 DAP in an area of 3 m^2 of each plot, considering the sum of the stem, top, and straw weights.
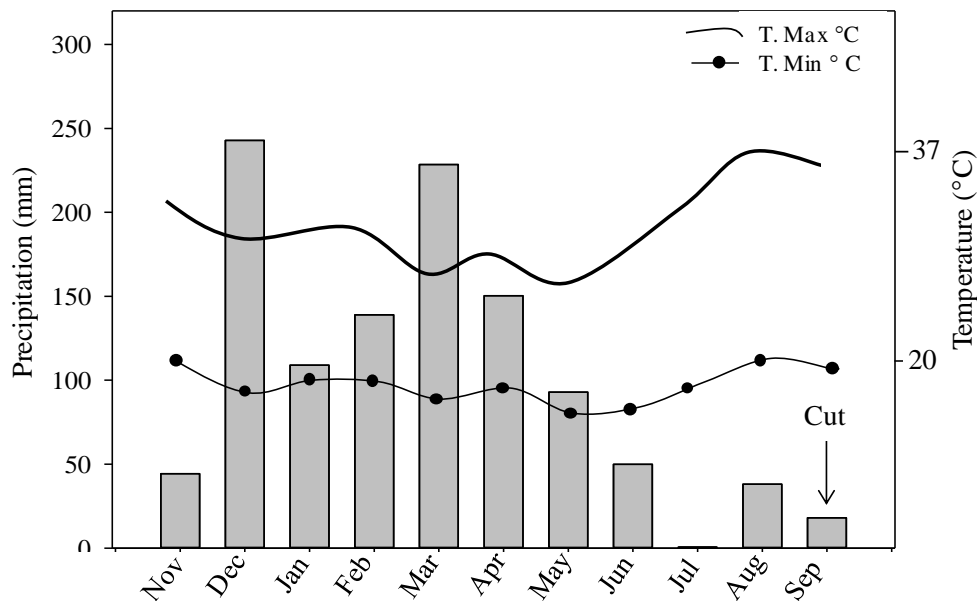
Figure 1. Rainfall (mm) and maximum and minimum temperature (ºC) during the experimental period from December 2009 to October 2010.

Figure 2. (A) Number of plants of three sugarcane varieties in the sugarcane-plant cycle at different harvest periods; (B)- Mean height of three sugarcane varieties in the sugarcane-plant cycle at different harvest periods.

RESULTS AND DISCUSSION

Plant growth analysis

There were no differences in the number of plants at 60 DAP (P>0.05) for this parameter among the varieties evaluated (Figure 2a). At 120 DAP, maximum tillering was recorded for RB 863129 and RB 92579, exhibiting

mean values of 14 and 13 plants per linear meter, respectively. There was a small increase in this parameter from 60 to 120 DAP for RB 867515. At 180 DAP, there was a slight decrease in this parameter for the RB 863129 variety, whereas number of plants remained constant for RB 92579 and increased for the RB 867515 variety (ten plants per linear meter).

During the last harvest, at 240 DAP, the RB 92579 and

RB 863129 varieties remained at the same level, both with twelve plants per linear meter, while the RB 867515 variety again exhibited a small increase at 240 DAP, with eleven plants per linear meter. In general, the number of plants was higher for the RB 863129 and RB 92579 varieties than for RB 867515(P>0.05).

According to the results obtained, there was maximum tillering at the early stages of plant development (120 DAP). This intense tillering was attributed to the high availability of water, light, and space to be exploited by the plants at the onset of the cycle, and there was a small natural decline in number of plants after this period (at 180 and 240 DAP), especially because the first tillers were developing and occupying more space in the soil and air during this period, so their leaves were shading out the younger plants that sprout, which exhibit lower chances of developing, some dying before becoming adult plants. Thus, the first tillers are more efficient in competing for water and light.

Costa et al. (2011) evaluating the growth and development of four varieties in four crop cycles, (RB 92579, RB 931530, SP 79 1011, and RB 93509), found a higher increase in tillering at 90 DAP for the varieties under study, and among the varieties studied, RB 92579 exhibited the highest tillering (27 tillers per linear meter), accompanied by a 63% decrease in number of stems at harvest. Silva et al., (2008), found for the IAC 862480 genotype and RB 72454 variety maximum tillering was obtained at 90 days after implementing the treatments, the IAC 86-2480 genotype having a higher number of tillers than RB 72454, with mean tillers of 29 and 18 per linear meter, respectively.

Overall, stem height exhibited a linear growth curve as a function of time in all of the varieties (Figure 2b); and the same trend was observed in the study by Abreu et al. (2013). Between 60 and 120 DAP, the varieties evaluated exhibit essentially the same plant height. At 180 DAP, the RB 863129 variety exhibited a mean plant height of 165 cm, higher (P<0.05) than the RB 867515 and RB 92579 varieties, which exhibited plant height of 146 and 124 cm, respectively (Figure 2b). The three varieties evaluated were characterized by the following very similar mean plant height values at 240 DAP: RB 867515 (270 cm), RB 863129 (271 cm), and RB 92579 (267 cm). The RB 867515 variety exhibited a higher mean plant height at 300 DAP, 292 cm, compared to the RB 863129 and RB 92579 varieties with 266 and 263 cm, respectively, at this harvest period.

According to the results obtained regarding stem height, three developmental stages can be observed for this parameter: a first stage where there was a small increase in stem height (from 60 to 120 DAP). Growth is slow at this early developmental stage, as intense tillering of the crop is still occurring at this stage. The second stage can be described by a rapid growth in stem height, which coincides with the periods of good meteorological conditions of temperature and rainfall (Figure 1). The final

stage from 240 to 300 DAP is characterized by slow growth because photoassimilates are directed to sucrose accumulation during this period. Oliveira et al. (2004) evaluating growth and development of three sugarcane varieties in the sugarcane-plant cycle (RB 72454, RB 855113, and RB 855536), found an early stage characterized by lower stem height due to intense tillering during this same stage and a second stage (from 279 DAP to 377 DAP), characterized by higher stem height growth as a result of good meteorological conditions and reduced tillering rate. Oliveira et al. (2010), studying growth and accumulated dry weight in eleven sugarcane varieties, including the RB 863129, RB 92579, and RB 867515, found variable stem height with slow early growth until 60 DAP, with higher stem height growth rates from 60 to 240 DAP; the RB 863129, RB 867515, and RB 92579 varieties exhibited the following stem heights at this stage, respectively: 291, 304, and 311 cm.

The leaf area parameter remained practically constant for the three varieties studied until 120 DAP (Figure 3a). The leaf area was increased in the three varieties from 120 DAP to 240 DAP, with RB 863129 and RB 867515 having the highest increases of 21 and 22%, respectively. RB 867515 exhibited increased leaf area from 180 DAP to 240, remaining at the same level as RB 863129, whereas the RB 92579 variety had the lowest mean for this parameter. Higher leaf area was observed for the RB 867515 variety at 300 DAP (0.29 m^2), which was higher than the RB 863129 and RB 92579 varieties, which reached values of 0.26 and 0.24 m^2, respectively. The values significantly differed (P<0.05) for the RB 863129 and RB 867515 varieties compared to RB 92579.

In general terms, from 120 to 240 DAP in this experiment, there was a higher increase in leaf area, as the plants were investing in the production of leaf apparatuses during this developmental stage, also supported by the good rainfall and temperature conditions during this period (December to June) (Figure 1). The reduction in leaf area was observed from 240 to 300 DAP may be explained by lower rainfall and increased temperature during this period and the senescence of older leaves that occurs during this period, with higher plant investment in accumulating sucrose. Santos et al. (2009), in a study performed on the RB 75126 variety, found similar behavior for the leaf area index to the observations in this study, where there was a slow growth period until 60 DAP, followed by a rapid growth period (from 60 to 120 DAP) and then a decrease starting from 300 DAP, where the final period was affected by the maturation process and sucrose concentration. These results also corroborate Vieira et al. (2013), found that water stress causes reduced leaf area, as it accelerates the senescence process of the green leaves.

Accumulated stem dry weight did not differ among the varieties evaluated for the first harvest at 60 DAP, and this parameter only increased significantly for the three

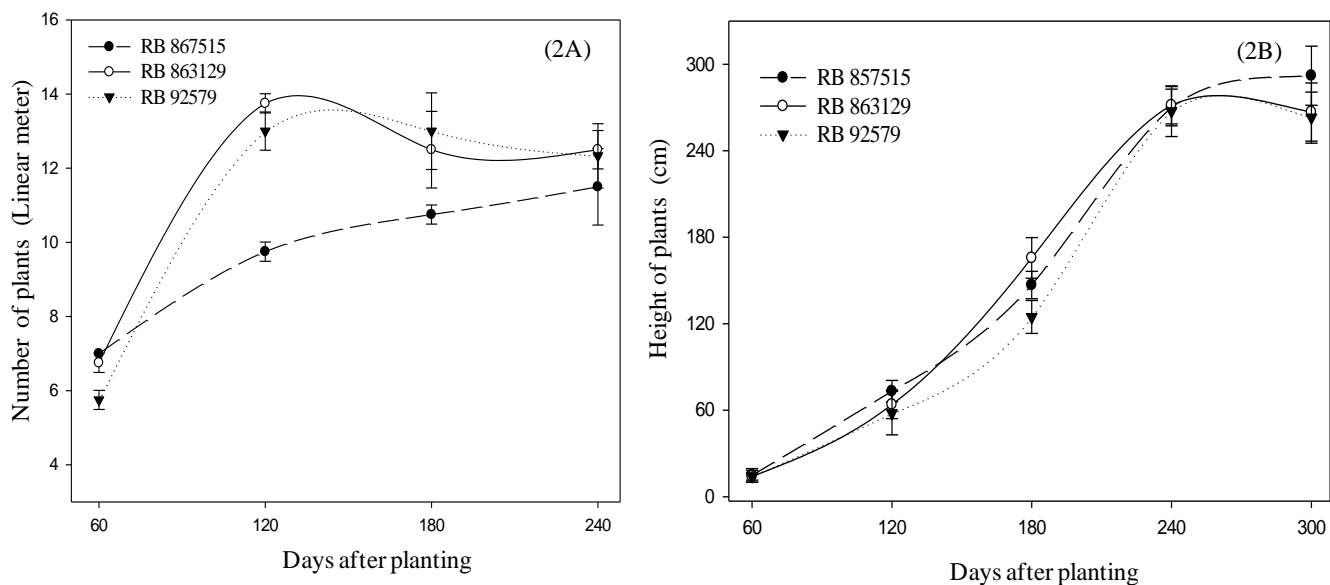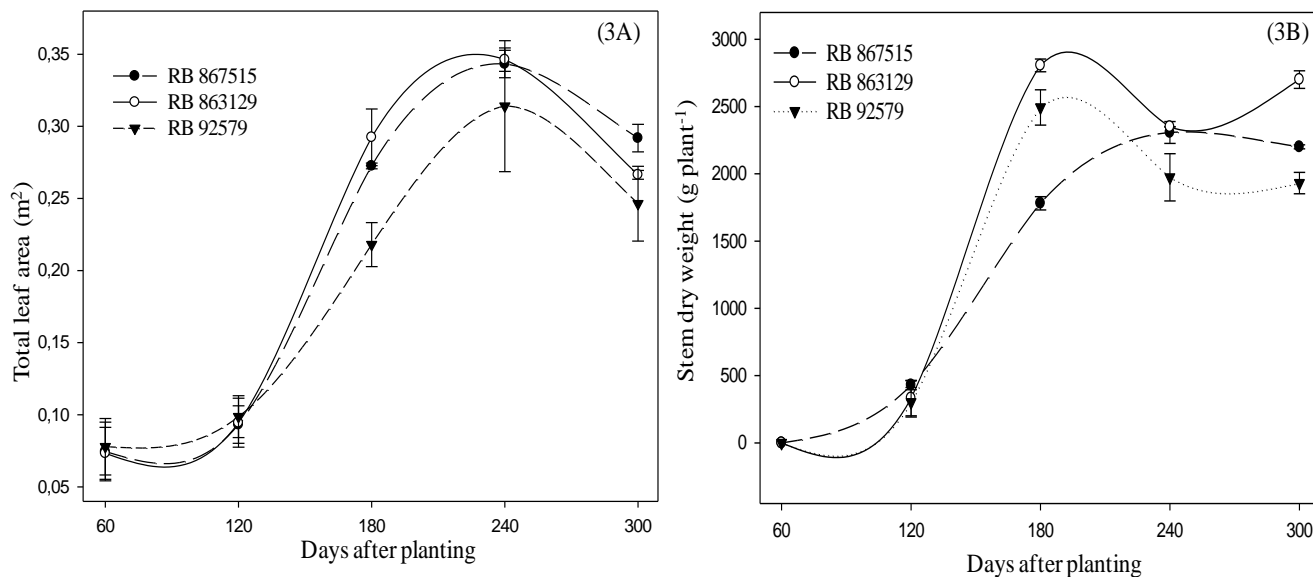**Figure 3.** (A) Total leaf area (TLA) of three sugarcane varieties in the sugarcane-plant cycle at different harvest periods; (B) Stem dry weight of three sugarcane varieties in the sugarcane-plant cycle at different harvest periods.

varieties at 120 DAP. However, it did not significantly differ among them ($P > 0.05$), with values of 429, 332, and 302 g plant^{-1} for the RB 867515, RB 863129, and RB 92579 varieties, respectively (Figure 3b). There was a higher increase ($P < 0.05$) in stem dry weight at 180 DAP (2805 g plant$^{-1)}$) for the RB 863129 variety compared to the other varieties. The RB 867515 variety exhibited a large increase in stem dry weight from 180 DAP to 240 DAP, with values close to the ones obtained for the RB 863129 variety, followed by the RB 92579 variety (2349 and 1974 g plant^{-1}, respectively). During this harvest period, the stem dry weight decreased from a value of 2493 at 180 DAP to a value of 1974 g plant^{-1} at 240 DAP for the RB 92579 variety.

During the last harvest, at 300 DAP, the RB 863129 variety exhibited higher ($P < 0.05$) stem dry weight (2700 g plant^{-1}) than the other varieties (Figure 3b). The results obtained for accumulated stem dry weight show two distinct stages: a first stage that occurs from 60 to 120 DAP, where there was slow accumulation of stem dry weight, because during this early stage of plant development, there is a prevalence of phytomass allocated to leaves+green tops; and a second stage from 120 to 180 DAP characterized by rapid growth, where there was a prevalence of phytomass allocated to stems. In fact, Alvarez and Castro (1999), evaluating raw and burned sugarcane shoot growth for the SP 701143 variety, also found these two distinct stages for accumulated stem dry weight, where one stage was characterized by a slow increase from 30 at 120 DAP, and a second stage was characterized by a higher accumulation starting at 120 DAP. The first stage was characterized by intense tillering, and the final stage had

a predominance of phytomass in the stems. Oliveira et al. (2007), evaluating biomass production in three sugarcane varieties (RB 72454, RB 855113, and RB 855536), observed the same trend.

The results obtained also showed a proportional relationship between leaf area and accumulated stem dry matter, where the two varieties that exhibited higher accumulation (RB 863129) also exhibited higher leaf area, showing that leaf area is directly associated with the quantity of light absorbed, affecting the total photosynthesis rate, thus providing a higher accumulated biomass in the plants. These results corroborate the findings of Abranche and Bolonhezi (2011), who evaluated the vegetative development of five clones and two varieties of sugarcane and found that the best results obtained for accumulated stem dry weight had a proportional relationship between leaf area (AF/m^2) and accumulated biomass and dry matter.

Nitrogen accumulation

Regarding nitrogen content in the leaves[+3], there was higher accumulated N during the early stage of crop development (at 60 DAP) and a decline during the following stages (at 180, 240, and 300 DAP) for all varieties (Figure 4a). Among the three varieties studied, the RB 863129 variety exhibited higher nitrogen content in the leaves[+3] ($P < 0.05$) at 120 and 180 DAP compared with the other varieties evaluated.

Higher accumulated N during the early stages of plant development is due to the metabolic activity of the leaves during this period, and this decline during the final stages
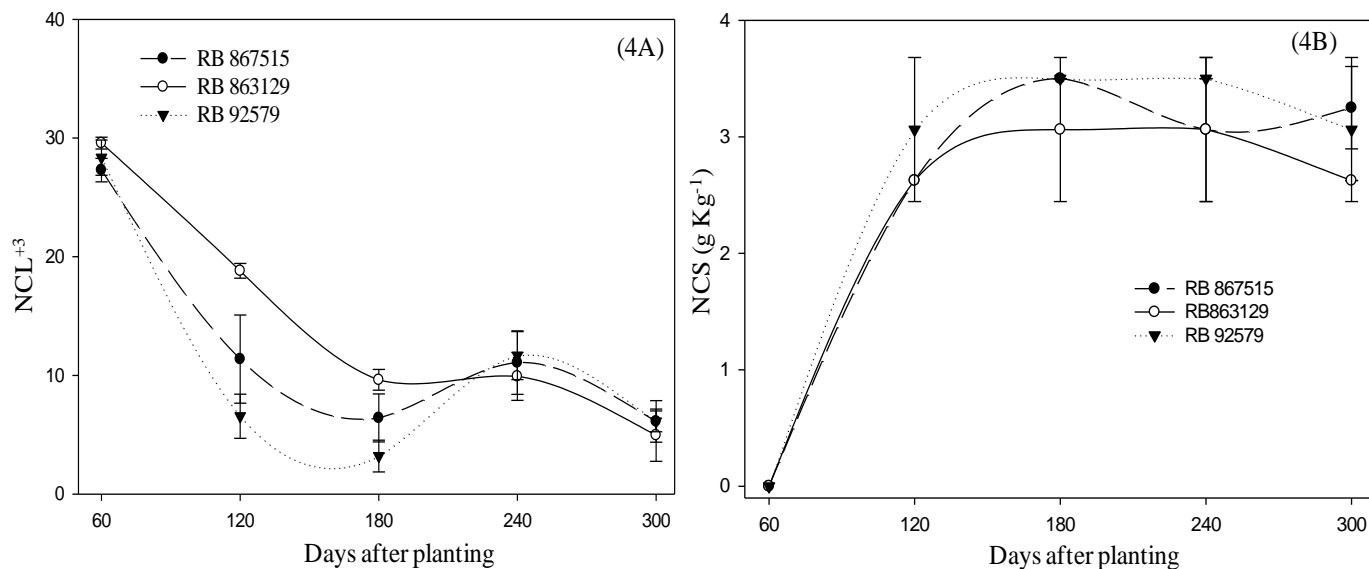
Figure 4. (A) N content in the leaves[+3] of three sugarcane varieties in the sugarcane-plant cycle at different harvest periods; (B) Stem N content of three sugarcane varieties at different harvest periods.

of plant development can be predominantly explained by the effect of diluting nitrogen on plant biomass, imposed by the crop's growth, and by the effect of leaf senescence during the plants' physiological maturity. Leite (2011) evaluating the accumulation of phytomass and nutrients in sugarcane, observed a significant decrease (of 40 kg ha^{-1}) in accumulated nitrogen in leaves+top at 237 DAP, linking this result to nitrogen mobility within the plants and leaf senescence during the period of sugarcane plant physiological maturity.

Oliveira et al. (2011) evaluating nutrient accumulation and allocation in sugarcane for the RB 92579, RB 867515, RB 943365, and SP 81-3250 varieties, observed higher accumulated N at 120 DAP in the leaves compared to that in the stems because the quantity of dry matter produced by 120 DAP was higher for stems, whereas the highest N accumulation occurred in the leaves for the RB 867515 variety, with mean extraction ranging between 62 and 78 kg ha^{-1} of N. For stem nitrogen content, the RB 92579 variety exhibited higher nitrogen content in the stems (P<0.05) compared with the RB 867515 and RB 863129 varieties at 120 DAP. However, the RB 867515 variety exhibited an increase in this parameter at 180 DAP, not differing from the RB 92579 (P>0.05) variety, demonstrating that these two varieties are higher in this parameter at 180 DAP compared to RB 863129 (Figure 4b). The RB 92579 variety continued, accumulating higher N levels in the stems compared to the other varieties at 240 DAP, whereas there was a decrease in N levels in the stems for RB 867515 from 180 to 240 DAP (Figure 4b)

Overall, during the last harvest, at 300 DAP, the RB 92579 variety accumulated higher N levels (P<0.05) than

the other varieties. Stem-accumulated N is low at 60 and 120 DAP and high at 180, 240 and 300 DAP. This pattern most likely occurred because during the early stages of development, the crop has higher metabolic activity in the leaves, which consequently exhibit the highest N levels, whereas N accumulation decreases during the later stages of crop development.

Brix content and final yield

The brix value observed at 300 DAP was within the expected range of maturation, given that the environmental conditions during the crop cycle were favorable (Figure 5), and there were no significant differences among the varieties (P>0.05).

Marques and Silva (2008) evaluating plant growth and maturation in three sugarcane varieties, including RB 867515, found that this variety exhibited a brix value of only 15% after ten months of plant growth. Martins and Munhoz (2009) evaluating qualitative traits using different maturers in three sugarcane varieties (RB 72454, RB 835486, and RB 855113), observed brix values ranging between 13 and 15%, which were lower than the values found in this study, as observed in Figure 5a.

In evaluating the yield of the three varieties regarding productivity, the RB 863129 variety shown better performance (P<0.05) (Figure 5b), with a total mean yield of 144 t ha^{-1} (120 and 24 for stems and tops+leaves, respectively), whereas the RB 867515 and RB 92579 varieties produced 112 and 111 t ha^{-1} (with 93 and 19, 85 and 26 for stems and tops+leaves, respectively). The RB 863129 variety exhibited higher yield, which was directly
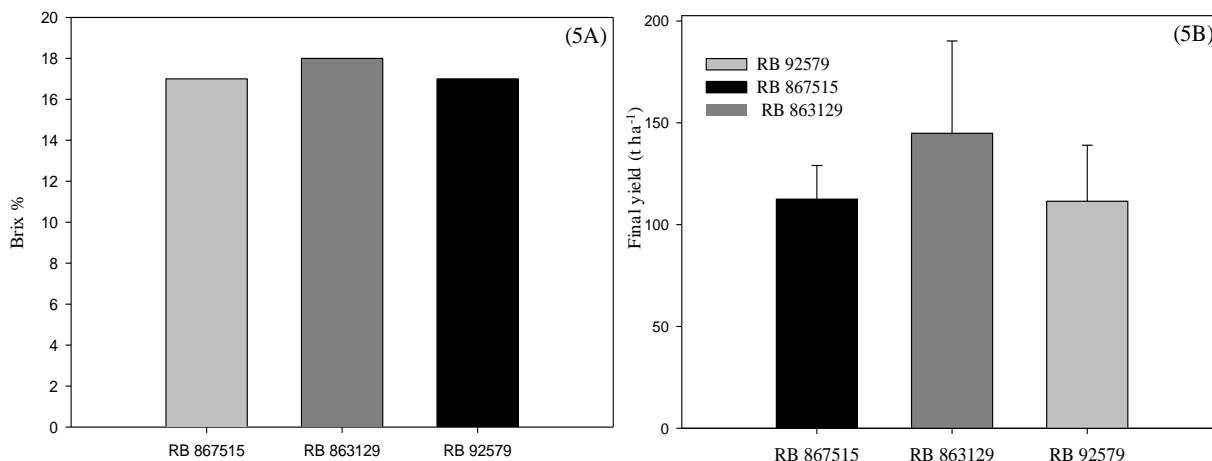
Figure 5. (A) Brix percentage in three sugarcane varieties in the sugarcane-plant cycle at 300 DAP; (B) Yield of three sugarcane varieties in the sugarcane-plant cycle at 300 DAP.

correlated with its good performance in the parameters observed in the growth analysis, such as number of plants, stem dry weight, and leaf area, as shown in Figures 2a, 3a, and b. Capone et al. (2011) studying the behavior of 15 sugarcane cultivars, found that the cultivars that exhibited the best yields also exhibited the best traits for number of plants per hectare and plant height, demonstrating a direct correlation between these traits and yield. Nassif et al. (2012), observing the parameterization and evaluating the DSSAT/Canegro model for Brazilian sugarcane varieties, also found a direct proportional relationship between accumulated stem dry weight, leaf area index, and yield. The RB 863129 variety exhibited a higher yield than the regional and Brazilian national means (57 and 77 t ha^{-1}, respectively) (Conab, 2012).

Conclusions

According to evaluation of the parameters in growth and yield analysis, the RB 863129 variety was the most promising for cultivation in this region during the sugarcane-plant season. The good performance observed for this variety must also be attributed to the good local meteorological conditions, with high rainfall and temperatures (during the rainy season). Within the Brazilian pre-Amazon region, there still exists a gap in the information on varieties that would be best for the region's edaphoclimatic conditions. The findings of this study can be considered for decisions when implementing new producing areas.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Productivity and physical properties of corn grains treated with different gypsum doses

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The productivity of corn grains is directly associated with factors related to soil fertility, which are relevant to the application of gypsum (calcium sulphate), in addition to variables inherent to the grain. Thus, the objective of this study was to evaluate the variability of the physical properties of grains (circularity, roundness, bulk density, real density, volume and porosity) and production components (productivity and weight of 1,000 grains) of corn irrigated with increasing doses of gypsum, and evaluate the effects of direct and indirect associations of physical properties of grains and weight of 1,000 grains on grain productivity. The soil of the experiment was a Dystrophic Red Latosol. The experiment was conducted using a random blocks design. An analysis of variance by F test and a univariate regression analysis were performed regarding the relations among culture variables with different doses of gypsum (0.0, 2.5, 5.0, 7.5 and 10 t ha^{-1}). An analysis of variance by t test was also performed together with a multivariate analysis using a path analysis with multicollinearity in order to analyze the direct and indirect effects of the physical properties of grains and weight of 1,000 grains on productivity. The bulk density of the grains had a direct effect on productivity of corn grains. In addition, the decrease in bulk density in function of the increase of gypsum doses is attributed to a greater increase in porosity in relation to weight of 1,000 grains.

Key words: Zea mays L., calcium sulphate, path analysis, bulk density.

INTRODUCTION

Corn (*Zea mays* L.) is regarded as a basic component of the human diet. It is highly demanded in the production of animal feed because of its high energy content (Coradi et al., 2011). In the 2015/2016 harvest, however, the productivity in Brazil was 4% below average with respect to last season (Conab, 2016). Therefore, it is necessary that production systems be improved considering various factors aiming to an increased productivity. In view of the

various factors that negatively affect the productivity of corn, agricultural gypsum is used to overcome problems related to low productivity because gypsum is considered a soil conditioner providing a greater absorption of water and nutrients by plants mainly at deeper soil layers (Sousa et al., 1996).

Productivity is a complex parameter and depends directly or indirectly on the association between different biotic and abiotic factors and different components of the plant structure itself (Carvalho et al., 1999). Thus, the degree of such associations obtained by correlation studies allows identifying variables that affect productivity. Some physical properties of the grains are important in the cause and effect mechanism related to productivity. Yet, their determination is relevant at various stages of beneficiation, equipment sizing, handling, transportation, drying and storage processes (Gürsoy and Güzel, 2010). Specifically, the shape and size of grains are extremely important for the control and automation of processing equipment and post-harvest equipment (Nunes et al., 2014; Pereira et al., 2014).The unfolding of correlations developed by Wright (1921), called path analysis, allows a better understanding of the causes involved in associations of parameters, where correlations are estimated as direct and indirect effects of explanatory variables on the dependent variable.

For path coefficients to be reliable and generate a biologically correct interpretation, a multicollinearity diagnostic should be made (Cruz and Carneiro, 2003). In the presence of moderate to severe multicollinearity, path coefficients may reach values too high. Therefore, in order to correct this problem, an alternative approach to the least squares, that is, ridge path analysis, should be used (Carvalho, 1995).The objective of this study was to evaluate the variability of the physical properties of grains (circularity, roundness, bulk density, real density, volume and porosity) and production components (productivity and weight of 1,000 grains) of corn irrigated with increasing doses of gypsum, and evaluate the effects of direct and indirect associations of physical properties of grains and weight of 1,000 grains on grain productivity.

MATERIALS AND METHODS

Data were obtained from an experimental research conducted at Brazil, at an experimental area of the Goiano Federal Institute, campus Rio Verde (17°48' S, 50°54' W; altitude 744 m), during the agricultural year 2014/2015.The climate is classified as Aw, tropical with an average annual temperature of 21°C, 1,500-1,800 mm rainfall and relative humidity at 30 to 85% (Sectec, Rio Verde City Hall).The soil of the experimental area is classified as a dystroferric Red Latosol, with a dense, medium texture (Santos et al., 2013). Soil preparation was performed with a disc plow and a leveling tractor. The main chemical characteristics analyzed, according to the methodology described by EMBRAPA (2006), and the physical characteristics are shown in Table 1. Sowing and coverage fertilizations were performed according to soil analysis and the recommendation by Sousa and Lobato (2004). A drip irrigation system was adopted, which was managed by a digital puncture

tensiometer with a sensitivity of 1 kPa. Tensiometric rods were installed 20 cm deep and spaced 15 cm from the drip line in three series. Thus, a voltage limit of 50 kPa was considered, keeping 100% of the available water capacity in the soil (AWC).

The experimental plots were distributed in a randomized blocks design with five doses of gypsum (0, 2.5, 5.0, 7.5 and 10 t ha^{-1}) in five blocks (replications). Each plot was composed of 8 lines 4.0 meters (m) long with a 0.45 m spacing between rows. The use area of the plot consisted of 4 central rows 2.0 m long, totaling 3.6 m^2. The gypsum was manually applied to the surface, keeping a most uniform application, at 45 days after plant emergence. To determine the proper harvest time, the water content was determined using an electric capacitance determiner until the level was suitable for harvesting (the proper level was 14.5% on a wet basis). After the manual harvest of hybrid corn (P 4285 YH) realized in July 6, 2016 totaling 120 days of cultivation, the physical properties of the grains (volume, circularity, roundness, bulk density, real density and porosity), weight of 1,000 grains and grain productivity were analyzed.15 corn grains per treatment were used (Oliveira et al., 2014) to determine the volume in m^3 (Equation 1), circularity in % (Equation 2) and roundness in % (Equation 3) according to Mohsenin (1986). The measurements were taken using a digital caliper with a 0.01 mm resolution, where a = bigger axis of the grain, in mm; b - average axis of the grain, in mm; c - smaller axis of the grain, in mm.

$$V = \frac{\pi(a * b * c)}{6} \tag{1}$$

$$Cir = \frac{b}{a} * 100 \tag{2}$$

$$Esf = \frac{\sqrt[3]{a * b * c}}{6} \tag{3}$$

The bulk density was determined using a Hectoliter Weight (PH) BK 4001 scale, and was expressed in kg m^{-3}. The real density (ρ_r) was determined by the ratio between the mass (kg) and the volume (m^3) of the grain according to Mohsenin (1986).The porosity of the granular corn mass was determined indirectly using an air comparison pycnometer built by the Department of Postharvest of the Goiano Federal Institute, campus Rio Verde, using the average of five replications per gypsum treatment according to the procedure described by Mohsenin (1986).The productivity was obtained from the manual harvest of the four 2 m central rows. After threshed, corn grains (Kg), in known area units, were extrapolated to kg ha^{-1}. At the same time, 1,000 grains were separated per experimental unit and the weight of 1,000 grains was determined. Initially, an analysis of variance (P < 0.05) was performed. When there was a significant effect, the polynomial regression linear of the culture variables was adjusted according to gypsum doses. A diagnosis of multicollinearity and the multicollinearity of the singular matrix X'X, based on the condition number (CN), which is the ratio between the highest and lowest eigenvalue of the matrix, were performed. A NC < 100 means a weak multicollinearity and does not constitute a problem for the analysis; 100<NC<1,000 means an average to strong multicollinearity, and NC>1,000 means a severe multicollinearity (Cruz and Carneiro, 2003).Subsequently, a path analysis with multicollinearity was performed according to Wright (1921) in order to adjust the k value and unfold the phenotypic correlations into direct and indirect effects of variables of the physical properties of the grains on productivity. The variance inflation factor (VIF) was also determined. All statistical analyses

Table 1. Chemical and physical characteristics of dystrophic Red Latosol at the layers 0-20 and 20-40 cm, Rio Verde, March 2016.

					Macronutrients						
Depth	pH	OM	P$_{mehlich}$	K	Ca	Mg	H+Al	BS	CEC	Al	V
cm	H$_2$O	g dm^{-3}	mg dm^{-3}	----------------- mmol$_c$ dm^{-3} --------------------							%
0 - 20	5.2	16	25.3	8.7	9.0	4.3	33	22	55	0.0	40
20 - 40	5.5	17	1.2	6.4	1.2	0.4	24	8.0	41.7	0.0	42.5

	Texture		
Depth	Clay	Silt	Sand
cm	------------------------------------- g kg^{-1} -------------------------------------		
0 - 20	348.6	143.8	507.6
20 - 40	425.1	183.4	391.5

pH = potential hydrogen; OM = organic matter; P$_{mehlich}$= phosphorus determined by Mehlich; K = potassium; Ca = calcium; Mg = magnesium; H+Al = hydrogen + aluminum; SB = base saturation; CEC = cation exchange capacity.Source: Authors.

Table 2. Summary of the analysis of variance of the variables roundness (R), circularity (Cir), bulk density (ρBD), real density (ρRD), volume (Vol) and grain productivity (Prod). Rio Verde, March 2016.

Source of variation	DF	Mean Square							
		R	Cir	ρBD	ρRD	Vol	P	W1000	Prod
Gypsum dose	4	23.3**	14**	1,927.8**	2,285.1**	6,176.3**	2.25**	15,821,861.2**	15,821,861.2**
Blocks	4	0.37	0.67	0.51	24.03	11.98	0.03	44,340.8	44,340.82
Redisues	16	0.61	1.14	3.36	118.01	7.99	0.108	784.5	784.57
CV (%)		1.23	1.35	0.24	0.85	1.19	0.81	0.28	0.28

[**]Significant at 1% probability by the F test; CV - Coefficient of variation. Source: Authors.

were performed using the software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

There was a significant effect of the variables roundness (R), circularity (Cir), bulk density (ρBD), real density (ρRD), volume (Vol), porosity (P), weigh of 1,000 grains (W1000) and productivity (Prod), which shows a variability among gypsum doses. The coefficients of variation were low: 0.24 to 1.35% (Table 2). Figure 1 shows the linear adjustment of culture variables evaluated in function of gypsum doses. Thus, the positive linear regression was adjusted for roundness, circularity, volume, productivity, porosity and weight of 1,000 grains (Figure 1A, B, E, F, G and H) with an estimated increase of 0.79, 0.46, 3.09, 3.39, 0.41 and 0.29%, respectively, by one-unit dose increase of gypsum. The negative linear regression was adjusted for bulk density and real density (Figure 1C and D), resulting in a reduction of 0.59 and 0.38%, respectively, by unit increase in the gypsum dose. Several studies found a positive effect of the application of gypsum on corn productivity. Some particularities were highlighted by Blum et al. (2013), who found that the positive effects of such application on corn productivity are associated with Ca^{2+} and SO$_4^{2-}$ contents in the soil.

Caires et al. (2011) found a significant relation between the productivity of corn and exchangeable Ca^{2+} contents in the soil even after nine years, and concluded that the observed differences in productivity, with respect to gypsum, may be related to the absorption of Ca^{2+} by plant roots due to cation exchange.

Caires et al. (2004), studying soil chemical changes and corn response to liming and the application of gypsum, found that gypsum improved the chemical characteristics of the soil in depth. An increase in Ca^{2+} and SO$_4^{2-}$ in the soil and in N, K and Ca in the leaves of corn was observed, thereby increasing productivity. Importantly, the biological fixation of air nitrogen by *Azospirillum* depends on the concentration of S. Even in the absence of S, there is no N$_2$ fixation in corn. According to the authors, this is because the source of H$_2$ results from water vapor from root respiration by the action of ferredoxin containing sulfur (Vitti et al., 2015).Since gypsum provides a greater absorption of nutrients and water by the plant (Souza and Lobato, 2004), the increase in the physical properties of the grains in function of the increase in gypsum doses is attributed to a greater increase in water content in relation to photoassimilates. This is proven by porosity showing an increase (0.41%) higher than the weight of 1,000 grains (0.29%), which is linked to a decrease in the
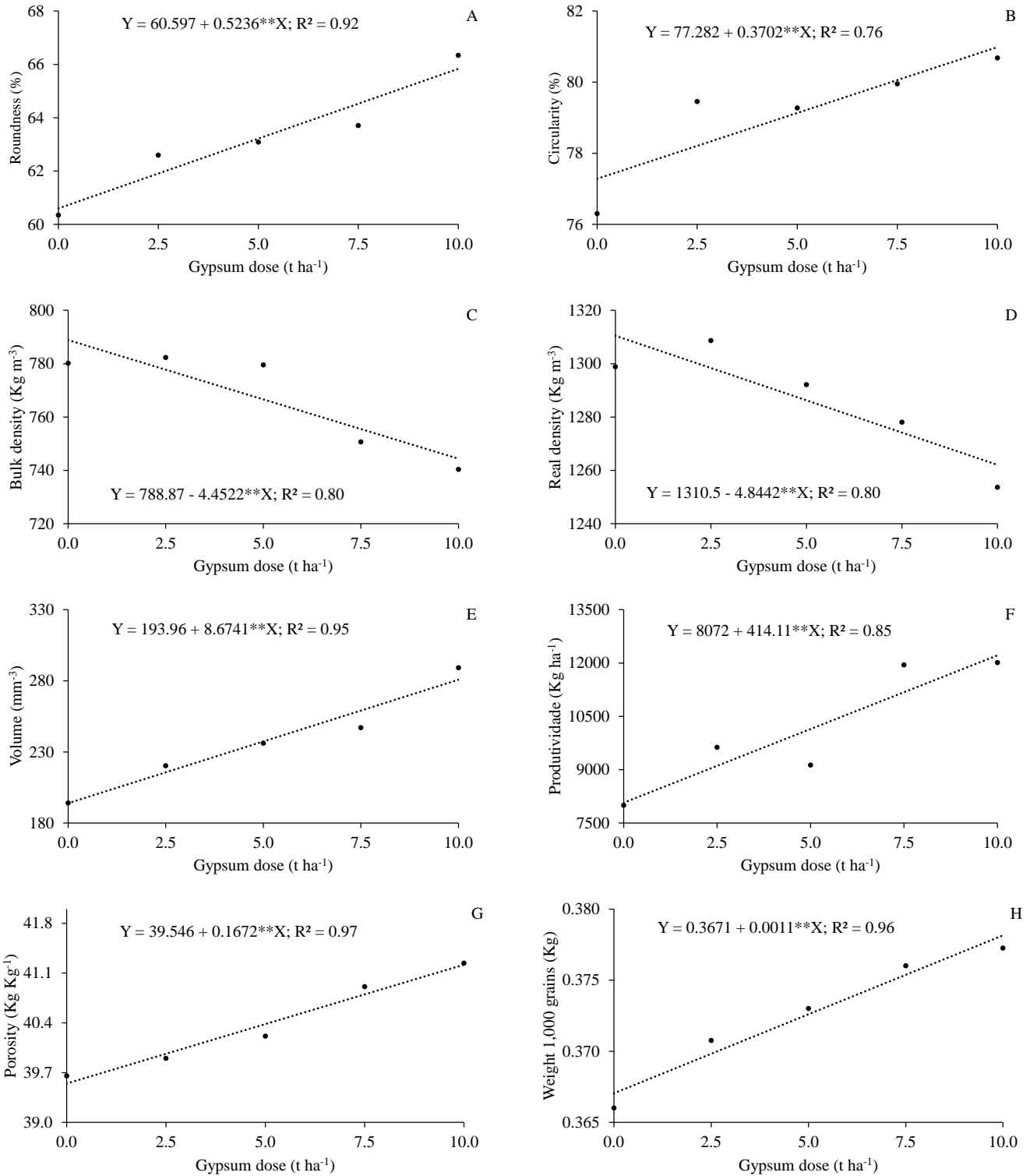
Figure 1. Experimental values and regression analysis for the variables roundness (A), circularity (B), bulk density (C), real density (D), volume (E), productivity (F), porosity (G) and weight 1,000 grains (H) of corn according to gypsum doses. Rio Verde, March 2016. Source: Authors.

bulk density of grains due to an increase in gypsum doses. The increases in drying air temperatures,
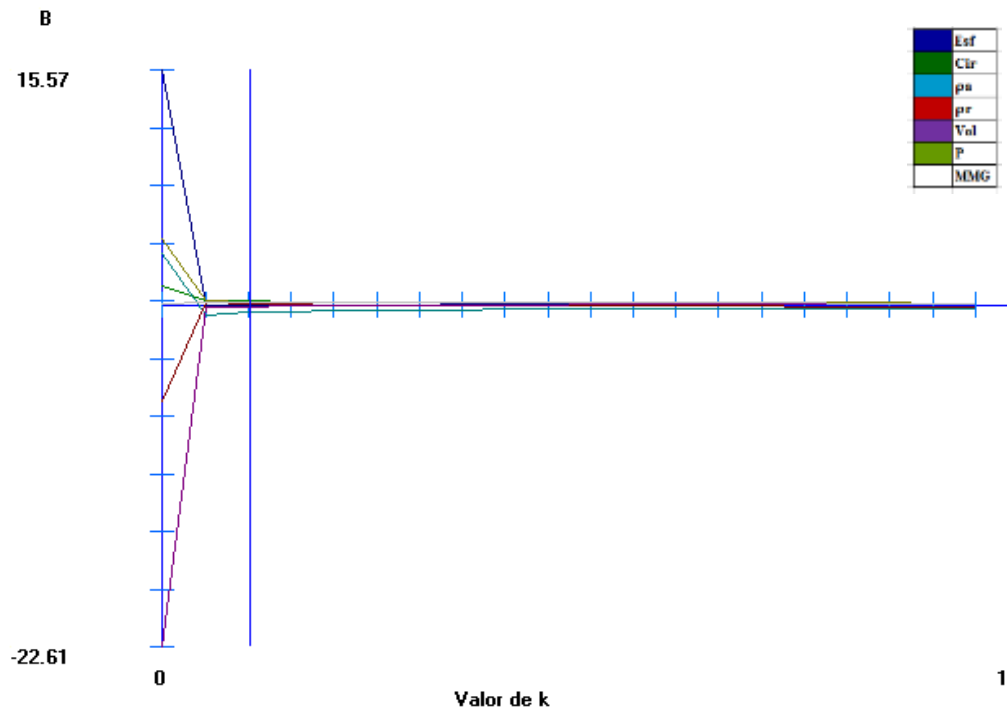
Figure 2. Estimates of path coefficients (θ*) in function of k values obtained from the analysis using the logarithm of the productivity of corn as an independent variable. Rio Verde, March 2016. Source: Authors.

associated with the decrease of water levels, lead to reductions in length, width, thickness, volume, circularity and roundness of corn grains (Coradi et al., 2016).

Since there was variability for gypsum doses, the path analysis revealed a severe multicollinearity for the inverse matrix of independent variables (determinant of the matrix X'X = 1.36 x 10^{-4}, and number of condition (NC) = 184,399.26). According to Montgomery and Peck (1981), the multicollinearity is more intense as the determinant of the correlation matrix between the variables approaches zero. To circumvent the effects of multicollinearity, the path of the K ridge was adjusted in the inverse diagonal matrix and the value (0.1021) was used to decrease the variable inflation factor (VIF) (Figure 2).The estimator in ridge (θ*) is a biased estimator, according to Hoerl and Kennard (1970). For Kalil (1977), the K-value may vary depending on the choice of the researcher being based on subjective criteria. The K-value, according to Carvalho (1995), should also be able to stabilize most path coefficients estimators, and this choice should be based on the lowest value.

The unfolding of estimated correlations into direct and indirect effects of the variables of grains (physical properties) on grain productivity is shown in Table 3.The direct correlation of bulk density with grain productivity is well associated because the simple correlation coefficient (r) (-0.92) and the direct effect (-0.47) are similar in

magnitude and equal in signal considering any other combination (Table 3), indicating that the bulk density has the highest direct effect on productivity.

Because bulk density has a greater importance in the association with productivity, it is attributed to smaller increases of weight of 1,000 grains and to larger increases in porosity due to the increase of gypsum doses, as shown by the univariate analysis.For the variables roundness and volume, it was found that the correlation is caused by indirect effects because the RD is positive and the direct effect is near zero or negative. Thus, these variables associate indirectly with a greater grain productivity by bulk density. These are the situations that presented the highest correlation estimates (Table 3). In all correlations, VIFs presented values below 10, which indicates that the equations of the diagonal of the inverse matrix are well estimated by multicollinearity. All regression models in the multivariate analysis expressed a high reliability because the coefficient of determination for the variables was 92.2%.

Conclusions

The bulk density of the grains had a direct effect on the productivity of corn grains. In addition, the decrease in bulk density in function of the increase in gypsum doses is attributed to a greater increase in porosity in relation to

Table 3. Estimates of the direct and indirect effects and variance inflation factor (VIF) that were related to the main independent variable grain productivity (Prod) and related dependent variables roundness (R), circularity (Cir), bulk density (ρBD), real density (ρRD) and volume (Vol) obtained by path analysis with a diagnosis of multicollinearity.

Variable	Effects of Association	Estimate	VIF
R	Direct on Prod	-0.02	8.02
	Indirect by Cir	0.25	4.21
	Indirect by ρBD	0.39	3.30
	Indirect by ρRD	-0.10	3.93
	Indirect by Vol	-0.09	7.05
	Indirect by P	0.23	6.40
	Indirect by W1000	0.20	5.96
	Total	0.87	
Cir	Direct on Prod	0.27	6.18
	Indirect by R	-0.02	5.46
	Indirect by ρBD	0.31	2.03
	Indirect by ρRD	-0.07	1.91
	Indirect by Vol	-0.08	5.41
	Indirect by P	0.21	4.93
	Indirect by W1000	0.20	6.01
	Total	0.85	
ρBD	Direct on Prod	-0.47	5.86
	Indirect by R	0.02	4.52
	Indirect by Cir	-0.18	2.14
	Indirect by ρRD	0.11	4.86
	Indirect by Vol	0.08	5.34
	Indirect by P	-0.24	6.90
	Indirect by W1000	-0.18	4.59
	Total	-0.92	
ρRD	Direct on Prod	0.11	6.61
	Indirect by R	0.02	4.77
	Indirect by Cir	-0.16	1.79
	Indirect by ρBD	-0.45	4.31
	Indirect by Vol	0.08	5.79
	Indirect by P	-0.24	6.47
	Indirect by W1000	-0.17	4.19
	Total	-0.79	
Vol	Direct on Prod	-0.09	8.68
	Indirect by R	-0.02	6.52
	Indirect by Cir	0.24	3.85
	Indirect by ρBD	0.41	3.60
	Indirect by ρRD	-0.10	4.41
	Indirect by P	0.24	6.75
	Indirect by W1000	0.20	5.96
	Total	0.87	
P	Direct on Prod	0.25	9.13
	Indirect by R	-0.02	5.62
	Indirect by Cir	0.22	3.34

Table 3. Contd.

	Indirect by ρBD	0.45	4.43
	Indirect by ρRD	-0.10	4.69
	Indirect by Vol	-0.08	6.42
	Indirect by W1000	0.20	6.10
	Total	0.95	
	Direct on Prod	0.21	8.15
	Indirect by R	-0.02	5.87
	Indirect by Cir	0.26	4.56
W1000	Indirect by ρBD	0.39	3.30
	Indirect by ρRD	-0.09	3.40
	Indirect by Vol	-0.08	6.35
	Indirect by P	0.24	6.84
	Total	0.93	
Coefficient of determination		0.922	
k value used in the analysis		0.102	
Effect of the residual variable		0.278	

Source: Authors.

the weight of 1,000 grains.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The influence of cover crops on erva de touro (*Tridax procumbens*)

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***Tridax procumbens* is an herbaceous plant belonging to the Asteraceæ family and popularly known as erva-de-touro or margaridinha. The objective of this study is to investigate the effects of cover crops when incorporated or maintained on the soil surface at different levels of straw, on the emergence and initial development of erva de touro. The experiment was conducted in a greenhouse during the period of May to August 2014, in a (5 x 4 + 1) factorial scheme. Factor A consists of five species of cover crops: millet cv. ADR 300 (*Pennisetum glaucum*), Brachiaria (*Urochola brizantha*), sorghum (*Sorghum bicolor* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and sunn hemp (*Crotalaria ocroleuca*), and factor B had four levels of dry mass (dry matter) of these plants (3, 6, 9 and 12 t ha^{-1}) on the soil surface. One treatment had no cover crops (control). The experiment involves a randomized block design with four replications. The analyzed variables were total number of emerged plants, shoot dry mass, leaf area, root dry mass and root volume. The cover crops at different levels of straw were efficient, giving greater prominence to the species, *P. glaucum* and *V. unguiculata* in suppressing the erva do touro (*T. procumbens*).**

Key words: Allelopathy, Brachiaria, millet, weed.

INTRODUCTION

Tridax procumbens L. is a herbaceous plant belonging to the Asteraceæ family and popularly known as erva de touro or margaridinha. It originates from Central America and spread to other regions such as South America and Africa (Kissmann and Groth, 1999), North America (Zimdahl, 1983) and Asia (Shetty et al., 1982). In Brazil, it has high incidence in the southeast and midwest, infesting pastures, roadside areas, vacant lands and

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urban areas (Lorenzi, 1991; Kissmann and Groth, 1999). This species showed very fast dissemination in the last 10 years in annual crops areas in the Cerrado of Central Brazil (Guimarães et al., 2000).

This species is reported to be one of the first to occur in field areas and Cerrado. Under favorable conditions such as good moisture and abundant lighting, it develops and spreads quickly (Kissmann and Groth, 1999; Lorenzi, 2000) and has been found in cotton crops (Albertoni and Almeida Neto, 1981), rice and soybeans (Albertoni and Almeida Neto, 1981). Nonetheless, weeds can reduce the quality and yield of a crop, make it difficult to harvest and, in extreme cases, make it unfeasible (Guglieri-Caporal et al., 2011). Also, weeds can enable the increase of grain moisture and drying costs, can encourage fermentation and increase the incidence of pests at storage (Vargas and Roman, 2005).

The use of cover crops in weed suppression is optimized on identification of more adaptable species to the region. To optimize the use of cover crops to suppress weeds, it is necessary to identify the most adaptable species to the region and adapt them to the best way of implementation and management (Ceretta et al., 2002). The production of dry mass and ground cover are factors that can assist in the control of weeds through chemical (allelopathy) and physical processes. The greater presence of microorganisms in the soil under no-tillage, as noted by Costa and Lovato (2004), which are capable of degrading the bank of the soil seed is important in integrated weed control in no-tillage.

Allelopathy is a process that occurs widely in plant communities, whereby certain plants interfere with the development of others. This behavior can therefore become important factor management cultures, in use of plants that exert control over certain undesired species, obtaining therefore, more productive culture systems (Goldfarb et al., 2009). The allelopathic compounds derived from the secondary metabolism of the plant are found distributed in different concentrations in different parts of the plant and during its life cycle. However, the main forms of release of these compounds into the environment are volatilization of the leaves, decomposition of plant residues, exuding of the roots and leaching through rain, fog and dew (Souza, 1988; Macias et al., 2007).

The allelochemicals in activities may suffer changes when designated under natural conditions, or when the substrate in the soil depends on the coverage to be maintained or incorporated into the soil surface (Ferreira and Áquila, 2000). Thus, the action of allelochemicals depends on the concentration, and incorporation leads to allelochemicals dilution. It is expected that residues placed on the soil surface are the most appropriate way to manage allelopathic action of cultures (Rezende et al., 2003).

T. procumbens is tolerant to diffused lighting, although the species prefer sunny areas (Kissmann and Groth,

1999). In contrast, there is no evidence about the effect of alternating temperatures on dormancy surpassing this species, provided that the studies have focused on constant temperatures. However, *T. procumbens* reaches more than 90% of germination at temperatures between 25 to 35°C (Guimarães et al., 2000). Through aggressive and difficult weed management in agricultural systems, its control has been sustained in the use of herbicides (Pacheco et al., 2013). However invasive, via an evolutionary phenomenon may develop herbicide resistance (Rizzardi et al., 2008). In analyzing this context, the adoption of cultivation methods, in the presence of biomass on the soil surface is critical to minimize the negative effects of chemicals on the environment and reduce the selection pressure caused by the intensive use of the same active ingredient.

In this sense, the objective of this study is to evaluate the effect of cover crops when kept on the soil surface at different levels of straw on the emergence and early development of bull's wort (*T. procumbens*).

MATERIALS AND METHODS

The experiment was conducted in a greenhouse from May to August 2014, on the campus of the Federal University of Piauí (UFPI/CPCE), Bom Jesus (Latitude 9° 16' 78"S, Longitude 44° 44' 25"W and altitude of 300 m), Piaui, Brazil.

The experiment was carried in a randomized block design with four replications, in a (5 x 4) + 1 factorial scheme, with factor A consisting of five species of cover crops: millet cv. ADR 300 (*Pennisetum glaucum*), Brachiaria (*Urochola brizantha*), sorghum (*Sorghum bicolor* L.), cowpea (*Vigna unguiculata* (L.) Walp.) and sunn hemp (*Crotalaria ocroleuca*); factor B having four levels of dry mass (dry matter) of those plants (3, 6, 9 and 12 t ha^{-1}) on the soil surface, and another treatment having no cover plants (control); giving a total of 64 experimental units.

The composition of each experimental unit was distributed in pots with capacity of 8 dm^3 of soil and diameter of 35 cm. The used substrates were soil samples taken from the layer of the Dystrophic Yellow. This depth was adopted in order to avoid larger weeds seed bank in the upper layers of the soil.

Twenty-five seeds from a single invasive species (*T. procumbens*) were randomly sown per pot in each experiment, and all residues were added to the surface. Fresh vegetation cover was added to the soil surface in amounts corresponding to different treatments (3, 6, 9 and 12 t ha^{-1}) in dry weight. The plant material was collected and fractionated on the day of the experiment installation to avoid possible allelochemicals loss.

To obtain the dry mass, the seeds of cover crops were sown by hand and grown in 5 m² beds and their shoots were collected when they were in the reproductive phase (beginning of the flowering stage ± 60 days), considering the culture cycle. The plant residues cover crops were segmented into sections of about 2 to 3 cm, weighed and fixed by a dry basis reference. Later, the plant samples were left in an oven at 65°C for 72 h and/or until constant weight was obtained. The wet material was adjusted according to the required dry matter per hectare, which was subsequently homogenized and kept on soil surface (pot) in accordance to the treatments. Irrigation was performed daily based on the plants' needs.

The variables were: total number of emerged plants (NEP), emergency speed index (ESI), leaf area (LA), shoot dry mass (SDM), root volume (RV) and root dry mass (RDM). The ESI was

Table 1. Variance analysis (F values) for number of emerged plants (NEP), leaf area (LA), shoot dry mass of aerial parts (SDM), root dry mass (RDM) and root volume (RV) for erva de touro.

Source of variation	Mean square				
	NEP	LA	SDM	RDM	RV
Cover crops (CC)	2.95**	163279.50**	13.22**	0.71**	16.49**
Residue level (RL)	121.11**	3349163.76**	151.03**	6.13**	447.30**
CC x RL	0.95*	53223.79*	4.66**	0.19*	10.29**
CV (%)	30.81	45.89	49.51	43.78	45.41

**Significant at 1%; *significant at 5%, CV – coefficient of variation .

calculated using the formula described by Maguire et al. (1962): ESI = [N1/1 + (N1-N2)/2 + (N3-N2) / 3 + ... (Nn-Nn-1)/n], where N1, N2, N3 ... Nn, correspond to the number of emerged seedlings and 1, 2, 3 ... n, are the number of days after sowing (DAS).

The leaf area (LA) was determined when weeds reached the stage of pre-flowering, with the assistance of LI-3100 equipment Area Meter (LI-COR, Inc., Lincoln, NE, USA), in which leaves were separated from the stem to make the measurement, expressed in cm^2 vaso^{-1}. Moreover, the roots were separated from the shoot, washed with water and removed from the soil and then subjected to root volume (RV) measurement expressed in cm^3 vaso^{-1} using the method of the tubes (Basso, 1999). Both the shoot and root parts were subjected to drying in oven at 65°C until they had constant weight to obtain their dry mass.

Data were subjected to analysis of variance by test "F" (p <0.05) with the help of Sisvar 4.2 software, and when significant, the treatment means were adjusted by regression equations with the help of the Sigma Plot 10.1 software.

RESULTS AND DISCUSSION

For the variables of number of emerged plants (NEP), leaf area (LA), shoot dry mass (SDM), root dry mass (RDM) and root volum (RV), a significant interaction was observed (P<0.05) between the management and the residue level only for NEP and RV (Table 1). At the same time, all the evaluated variables were different (P<0.05) for residue amount and cover crops.

All tested cover crops were efficient to reduce the NEP in erva de touro, with *P. glaucum* and *V. unguiculata* presenting higher reductions, mainly for the initial residue amount on the soil (Figure 1). The suppression of bull weed emergence in this study can be justified by the significant content of phenols group substances and flavonoids present in these kinds of toppings (Lisboa, 2009). In a survey conducted by Pacheco et al. (2013), it was observed that the soil cover reduced the number of emerged plants of Bidenspilosa. According to these authors, the reduction of seedling emergency is due to allelopathic action of cover crops.

The cover crops presented exponential decreasing behavior for the NEP of erva de touro (Figure 1). These results indicate that 3 t ha^{-1} of residue of *P. glaucum* and *V. unguculata* reduced the NEP in 78.99 and 76.30%, respectively, when compared to the control (0 t ha^{-1} of residue). The species *P. glaucum*, *C. ochroleuca*, *S.*

bicolore and *U. ruziziensis* were more efficient with the residue amount above 6 t ha^{-1}, with more than 78% of reduction, compared to control (0 t ha^{-1} of residue). Gimenes et al. (2011), analyzing the effect of *U. decumbens* on weed infestation, verified that the plant reduced the amount of *C. echinatus* from 30 to 2 plants m^{-2}, when compared to control.

For the variables LA and SDM of erva detouro, the lowest means of these variables were observed in pots seeded with *P. glaucum*, *S. bicolor* and *V. unguicuata* residue (Figure 1). These results can be explained due to the higher exponential decrease of the number of emerged plants caused by the cover crops. In this way, it is possible to observe that the reduction in SDM and LA became the weed plants less competitive with crops with economical potential. Correia et al. (2006) observed a potential use in the forage millet from 3 t ha^{-1} for the *B. pilosa* control, in a no-till system.

For the variables LA and SDM, the cover crops presented decreasing exponential behavior according to the residue amount, except for *U. ruziziensis* with a linear reduction for SDM (Figure 1). The highest reduction of LA was with 3 t ha^{-1} of *P. glaucum, S. bicolor* and *V. unguiculata* plants, with reductions of 80.78, 62.40 and 80.78%, respectively, compared to control (0 t ha^{-1} of residue). At the same time, the same cover crops reduced the SDM up to 80.22, 56.73 and 75.82%. Gimenes et al. (2011) demonstrated that 10 t ha^{-1} of dry mass from *Brachiaria decumbens* at 60 days after germination reduced more than 80% of the *Digitaria horizontalis* and *Cenchrus echinatus* weeds leaf area.

For root system variables, all tested cover crops were efficient to reduce RDM and RV (Figure 2), according to the exponential decrease of the emerged plants number (Figure 1). Thus, the lower development of the root system can result in a reduction of the competitive capacity of the weeds, because of the reduction and absorption capacity of water and nutrients, mainly in water stress conditions (Pacheco et al., 2013).

The cover crops *P. glaucum* and *V. unguiculata* were adjusted in the exponential regression model for RDM and RV of erva de touro, with higher reductions (more than 75%) when compared to control (0 t ha^{-1} of residue) (Figure 2). Fortes et al. (2009) verified that the use of the
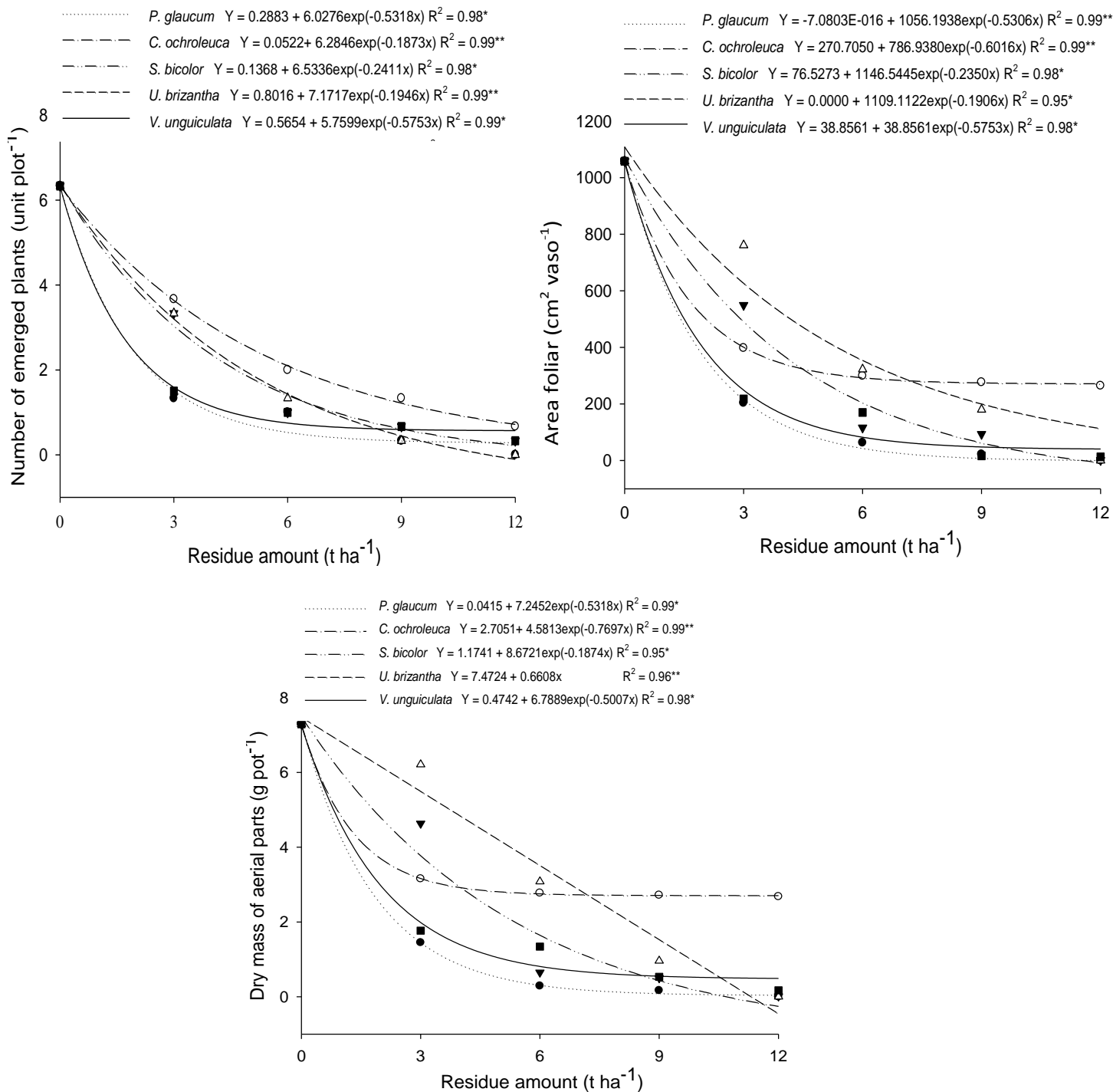
Figure 1. Number of emerged plants, leaf area and shoot dry mass of erva de touro according to crop residue amount of cover crops. ** and *: significant at 1 and 5%, respectively.

Sambucus australis and *Cymbopogon citratus* hot water extracts did interfere in the picão-preto (*Bidens subalternans*) average root length, with a higher effect when higher concentrations were used.

In conclusion, according to the results, all cover crops evaluated had potential to reduce erva de touro infestation. The species *P. glaucum* and *V. unguiculata* presented higher efficiency in erva de touro control, being the residue amount of 3 t ha⁻¹ sufficient to promote a reduction in erva de touro germination. The cover crops *P. glaucum* and *V. unguiculata* presented higher reduction in root system parameters when 3 t ha⁻¹ of

P. glaucum	Y= 0.5910 + 11.5570exp(-0.6167x)	R^2 = 0.98*
C. ochroleuca	Y= 2.7517 + 9.4141exp(-1.0549x)	R^2 = 0.98*
S. bicolor	Y= -1.3624 + 13.7770exp(-0.2293x)	R^2 = 0.97*
U. brizantha	Y= -9.9498 + 22.4915exp(-0.0699x)	R^2 = 0.97*
V. unguiculata	Y= 0.7258 + 11.4021exp(-0.5088x)	R^2 = 0.98*

P. glaucum	Y= 0.1199 + 1.4691exp(-0.5402x)	R^2 = 0.98*
C. ochroleuca	Y= 0.3621 + 1.1914exp(-0.1715x)	R^2 = 0.89*
S. bicolor	Y= 1.5480 - 0.1279x	R^2 = 0.97**
U. brizantha	Y= 1.6284 - 0.1300x	R^2 = 0.99**
V. unguiculata	Y= 0.2345 + 1.3568exp(-0.5195x)	R^2 = 0.99**

Figure 2. Root dry mass and root volume for erva de touro according to residue amount and cover crops. ** and *: significant at 1 and 5%, respectively.

residue was used.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Influence of *Ilex paraguariensis* aqueous extract on safflower growth and germination *in vitro*

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Allelopathy is a natural botanical effect in which certain crops release inhibitory substances which affect growth and development of other crops to the environment. The ability of plants to exhibit allelopathy vary among species. In Brazil, the allelopathic studies are restricted to common plants, such as corn, soy, wheat, and safflower. Given these factors, there are still many cultures as *Ilex paraguariensis* that are forgotten, even though, they play an important role in the regional cultivation. The aim of this study was to evaluate the effect of cold water extract of *I. paraguensis* leaves on germination rates, seeds early growth and speed of germination of *Carthamus tinctorius* L. sprayed with aqueous extract of mate. The extract production process consists in grinding mate leaves along with cold water and then filtering it to remove the particles. Concentrations of 0 (control), 25, 50, 75 and 100% of the extract were used to induce the treatment, considering 200 g of extracted adult fresh leaves per liter of cold water. The seeds were planted in individual tubes containing specific soil for cultivation and they were all sprayed manually every 3 days using the volume of 500 ml in appropriate concentrations. The experimental design was completely randomized as it follows: 5 treatments, 4 repetitions, and 1 safflower genotype. To carry out the analysis, the emergency data were collected until the thirtieth day. Considering the statistical analysis, there was no change resulting in allelopathic effect in the following rates: germination rate, first germination counting, average time of germination and percentage of germination, stem diameter, root length, and leaf dry mass, but it was observed that in the concentration T3 (50%), there was a significant increase in safflower growth in the following characteristics: leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass.

Key words: Allelopathy, germination, influence, safflower, mate.

INTRODUCTION

Safflower, *Carthamus tinctorius,* is an annual crop that belongs to the family Astaraceae and it is native to Africa and Asia (Abud et al., 2010). Its flower has a red dye called cartamina, which was widely used in antiquity for

the manufacture of pigments for fabrics. It was widely used in cooking because of the yellow pigment of some species (Arslan, 2007). Currently, in Brazil, it is a high economic value crop, because of its versatility and use for production of high quality vegetable oil. The seed contains about 30% of oil which can be extracted, thus it can contribute significantly to the production of biodiesel and silage (Bradley, 1999; Siddiqui et al., 2006).

It is widespread throughout the world medical and industrial importance (Velasco et al., 2005). Petals, leaves, and seeds have medicinal values, but the spread of this crop is due to its high oil content, which serves for the biodiesel industry (Dwivedi, 2005; Carneiro, 2012). It is considered as an energy crop or as a source of renewable energy (Kafra, 1998; Feizi et al., 2010).

The allelopathic effect can be set by the process of allelochemicals production due to the presence of one plant and it is produced by exudation, which may have improved the process with the presence of microorganisms in the soil. This effect becomes important in the suppression of unwanted crops, thus, it becomes a tool for future studied for application in several crops (Jabran et al., 2015).

In the plant, allelochemicals are derived from the secondary metabolism of the plant and are released into the environment via root in soil or volatile substances in the air via leaves (Kaffka, 1998; Rickli et al., 2011).

Allelochemicals are substances released by plant metabolism and they can affect the local environment. These toxins that are emitted around the crop decrease the intraspecific and interspecific competition among species, creating advantages for the plant which produces toxins. The inhibition on the growth and development of plants is an interesting knowledge for agriculture, which seeks ways to control pests and inhibit the growth of unwanted species (Brass, 2009).

Mate (*Ilex paraguariensis*) is a conducive crop in subtropical climates and it is widely used in Southern Brazil, Argentina, Paraguay and Chile. It is present as a cultural link between these countries and its production is concentrated in Argentina, which produces 750 thousand tons per year, and in Brazil, the production reaches 80 thousand tons per year (Fiedler et al., 2008). Mate substrate extraction process produces a series of components such as theobromine, caffeine, and derivatives and these substances can have biological, pharmaceutical and industrial effects (Isolabella et al., 2010).

The planted herbal area has increased in recent years and the system that has been most widely adopted is the consortium with other crops, particularly annual crops, such as mate harvest, require a wide spacing between plants and smaller crops to adapt well when intercropped planting of mate (Medrado et al., 2000).

The yerba mate intercropping system with other crops such as safflower, enables increased income of farmers and environmental point of view can contribute to the recovery of degraded marginal land. This type of consortium can also reduce the number of pests because the increased diversity of the local flora will increase natural enemies (Pasinato and Arthur, 2012).

Factors such as climate, soil and mate cultivation period can influence the production of chemical components, which consequently have adverse effects. It can be observed that *I. paraguariensis* contains large amounts of chlorogenic acid (derived from caffeine), and this by-product is used in the industry because of its properties that affect metabolism (Marques et al., 2009).

In Brazil, the allelopathic studies are restricted to common plants such as corn, soy, wheat and safflower. Given these factors, there are still many cultures as *I. paraguariensis* that are forgotten, even though, they play an important role in the regional cultivation. Mate itself has several chemical compounds that are used in medicine, but their adverse effects are not studied or are still being discovered. The allelopathic effect of mate has to be studied in the way that may be harmful to commonly planted crops like safflower, since between them there is an intercrop system. Thus, the aim of this study is to assess the growing and development in safflower germination submitted to mate leaves extract, in order to verify the allelopathic effects.

MATERIALS AND METHODS

Source of plant materials and study location

Mate adult and fresh leaves were used to assess the allelopathic effect on *C. tinctorius* L. seeds. Mate leaves were collected in the city of Cascavel, Paraná, Brazil, with the geographic coordinates of 24° 57'21"S and 53° 27'19"W and average altitude of 781 m. The safflower seeds were obtained from Instituto Agronômico do Paraná (IAPAR). The experiments took place in April and May 2016 (30 days) at the campus of Universidade Estadual do Oeste do Paraná (UNIOESTE) in a greenhouse made of polyethylene (free from any weather phenomenon) in a humid subtropical climate.

Preparation of extract

The extract was manufactured in concentrations of 0 (control), 25, 50, 75 and 100% of extract to induce the treatment, considering 200 g of leaf per each liter of cold water. For the irrigation, 500 ml of water was used and divided into the concentrations. The extract production process consists of grinding mate leaves along with water and then filtered to remove the particles.

Evaluation of allelopathic effects

The germinating of safflower seeds occurred in tubes of polyvinyl chloride (PVC) with diameter of 200 mm and height of 15 cm, housed in trays with sufficient diameter heights to accommodate the PVC pipes. The tubes were filled with the same type of soil up to a height of 14 cm. For the crop planting, 10 seeds per tube were organized, dispersed so that the seeds could germinate and develop without any interference.

The samples were placed in a greenhouse at the campus of Universidade Estadual do Oeste do Paraná (UNIOESTE), in such a

way that they were exposed to sunlight intermittently during the daytime. The experiment was performed in quadruplicate for each concentration and to maintain soil moisture, it was carried out the maintenance of water and extract manually, by means of a surface irrigation since the installation of the experiment. Surface irrigation was performed every three days and started on the first day of the experiment, all containing 500 ml per tube following the considered concentrations.

The experimental design was completely randomized as it follows: 5 treatments, 4 repetitions and 1 safflower genotype (IAPAR). To carry out the analysis, the emergency data were collected until the thirtieth day. The data analyzed were: germination speed rate, first germination count, germination average time, germination percentage, as well as fresh and dry mass of plants, root and plant length, stem diameter and leaf area.

Germination speed rate (GSI): Which was proposed by Maguire(1962) where E1, E2 ... En: number of normal seedlings recorded in the first, second and final counting. N1, N2 ... Nn: number of days after sowing, the first, second and last one.

$$GSI = \frac{E1}{N1} + \frac{E2}{N2} + \cdots \frac{En}{Nn}$$

First germination count (FGC): Counting the number of seeds with leaf protrusion and their respective days.

Average germination time (AGT): According to Labouriau et al. (1976), where ni is the number of seeds germinated in each counting interval; ti is the time elapsed between the start of germination and the i-th counting.

$$AGT = \frac{\sum ni\, ti}{\sum ni}$$

Germination percentage (G): Proposed by Siddiqet et al. (2007).

$$G = \frac{n^{\underline{o}}\ of\ germinated\ seeds}{total\ of\ seeds} \times 100$$

Fresh mass: Weighing of the plant newly collected in precision scale; in this case, we split the top part (plant) and bottom part (root). Dry mass: Plant weighing, top part (plant) and bottom part (root) after drying in an oven at 65°C after 72 h. Plant and root length: Measurement in cm from the plant apex to the base (plant) and subsequent measurement from the root beginning to the longest part using scalimeter. Stem diameter: Measurement of plant stem diameter with a digital caliper, considering the extent slightly below the first leaf protuberance. Leaf area: Holding of the measurement of each leaf in greater length and greater width to calculate the area.

Statistical analysis

The data obtained from the collection of information were analyzed with analysis of variance (ANOVA) to evaluate whether there is any evidence that the sample of the populations of plants differ. This analysis of variance leads to a conclusion that there is evidence that the group of the concentration differ, indicating whether there is a need in investigating which of them is different. This is where the Tukey multiple comparison test is used. The Tukey test compares the difference between each pair of samples with appropriate adjustment for the multiple testing. The test uses tables and comparative letters in columns, meaning equal letters do not differ themselves and columns with different letters have differences in level of 95% confidence.

The results are presented as a matrix showing the result for each pair, either as a P-value (p <0.05), which shows the confidence interval of 95%. The Tukey multiple comparison test and the analysis of variance assumes that the data from different groups come from populations where the observations have a normal distribution and the standard deviation is the same for each group.

Data were statistically analyzed and were carrried out with the Action Stat 3.1 software, using analysis of variance (p < 0.05) and Tukey test.

RESULTS AND DISCUSSION

The different aqueous extract concentrations of *I. paraguariensis* did not cause any effects on the safflower samples in relation to the Germination Rate (1A), First Germination Counting (1B), Germination Average Time (1C) and Percentage of Germination (1D), because all the letters in the same column of concentrations are the same, therefore when considering a significance level at 5%, it can be said that the hypothesis of equality between the average levels is the sameas shown in Table 1.

Evaluating the germination of maize by the mate fruit extract in different concentrations, it was observed that the germination and emergence of the maize seedlings were not affected, and it was also found that the analysis was not performed only with the crop leaf (Miro et al., 1998).

The concentrations of *I. paraguariensis* extract did not cause allelopathic effects on safflower growth. The stem diameter, root length and leaf dry mass showed no effect; in other words, after the analysis of variance, it was found out that p>0.05, thus, the samples were all similar, not rejecting the null hypothesis (equality between samples).

A sample variance was observed between the control sample T1 (0% of extract) and T3 (50% of extract) in leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass, thus noticing that there is rejection of the null hypothesis with p < 0.05. By Tukey test (Figure 2), it was observed that the comparison between the T1 and T3 samples were outside the ranges, with an average higher than the control.

Mate extract may contain many different chemical compounds, among them there are caffeine, chlorogenic acid and rutin, products which can cause effects when applied to different metabolisms (Blum-Silva et al., 2016).

There was a considerable increase in relation to the safflower growth comparing the averages of T1 samples (control) with samples of T3 in Table 2, as follows: 97.92% for leaf fresh mass (2A); 82,31% of root fresh mass (2B); 29.27% leaf length (2C); 97.92% leaf area (2D); and 154.49% to root dry mass (2E), proving the positive variance by Tukey test, where mate extract concentrations strengthened the growth in these analysed characteristics.

All other mate extract concentrations (25, 50, 75 and

Table 1. Tukey confidence interval: Germination Index (A), First Germination Count (B), Average Germination Time (C) Percentage of Germination (D).

GSI (A)			FGC (B)		
Factor	Averages	Group	Factor	Averages	Group
T1	1.70(0.13)	a	T1	6.5 (1.0)	a
T2	1.38 (0.61)	a	T2	6.75 (2.06)	a
T3	1.41 (0.52)	a	T3	6.25 (2.22)	a
T4	1.14 (0.32)	a	T4	5.25 (1.71)	a
T5	1.42(0.52)	a	T5	6 (1.63)	a
AGT (C)			**G (D)**		
Factor	Averages	Group	Factor	Averages	Group
T1	16.66(1.73)	a	T1	65	a
T2	19.43 (2.21)	a	T2	67.5	a
T3	18.34 (1.1)	a	T3	62.5	a
T4	17.71786 (1.61)	a	T4	52.5	a
T5	17.67(2.21)	a	T5	60	a

Values followed by the same letter in the column do not differ itself by Tukey test at 5% significance; Values in parentheses represent standard deviation.

Table 2. Leaf fresh mass (A), root fresh mass (B), leaf length (C), leaf area (D), and root dry mass (E).

Leaf fresh mass (A)			Root fresh mass (B)			Leaf length (C)		
Factor	Averages	Group	Factor	Averages	Group	Factor	Averages	Group
T1	1.85 (0.70)	b	T1	0.049 (0.013)	b	T1	15.98 (1.58)	b
T2	2.04 (0.91)	ab	T2	0.064 (0.02)	ab	T2	17.53 (2.67)	ab
T3	3.67 (1.31)	a	T3	0.09 (0.02)	a	T3	20.66 (2.27)	a
T4	1.58 (0.20)	b	T4	0.087 (0.02)	a	T4	17.08 (1.63)	ab
T5	2.67 (1.28)	ab	T5	0.08 (0.016)	ab	T5	18.38 (2.70)	ab
Leaf area (D)			**Root dry mass (E)**					
Factor	Averages	Group	Factor	Averages	Group			
T1	39.33 (15.04)	b	T1	0.0067 (0.002)	b			
T2	43.43 (19.17)	ab	T2	0.0107(0.003)	b			
T3	77.85 (27.87)	a	T3	0.017 (0.002)	a			
T4	33.50447 (4.27)	b	T4	0.00968 (0.002)	b			
T5	56.66014 (27.1)	ab	T5	0.0112 (0.004)	ab			

Values followed by the same letter in the column do not differ itself by Tukey test at 5% significance; Values in parentheses represent standard deviation.

100%) showed no significant difference from the control sample (0%) in Table 2, because when considering a significance level at 5%, it can be stated that the hypothesis of equality between the average levels is the same.

It can be seen that the confidence intervals in Figure 1 has only the concentration of 50% extract different from all others. The observation is made in the confidence intervals, where there is a comparison with the control sample T1. It is observed that the confidence interval T1-T3 (comparison sample T1 to T3) does not pass within the point of origin (zero), so it can be stated that

the hypothesis of equality between the averages is not the same. Although, it is observed that the confidence interval obtained values higher than zero, so there was a positive development of safflower to extract.

Conclusion

It can be concluded that the different mate extract concentrations (25, 50, 75 and 100%), which were used to induce the treatment, did not cause any allelopathic effects on safflower development and germination.
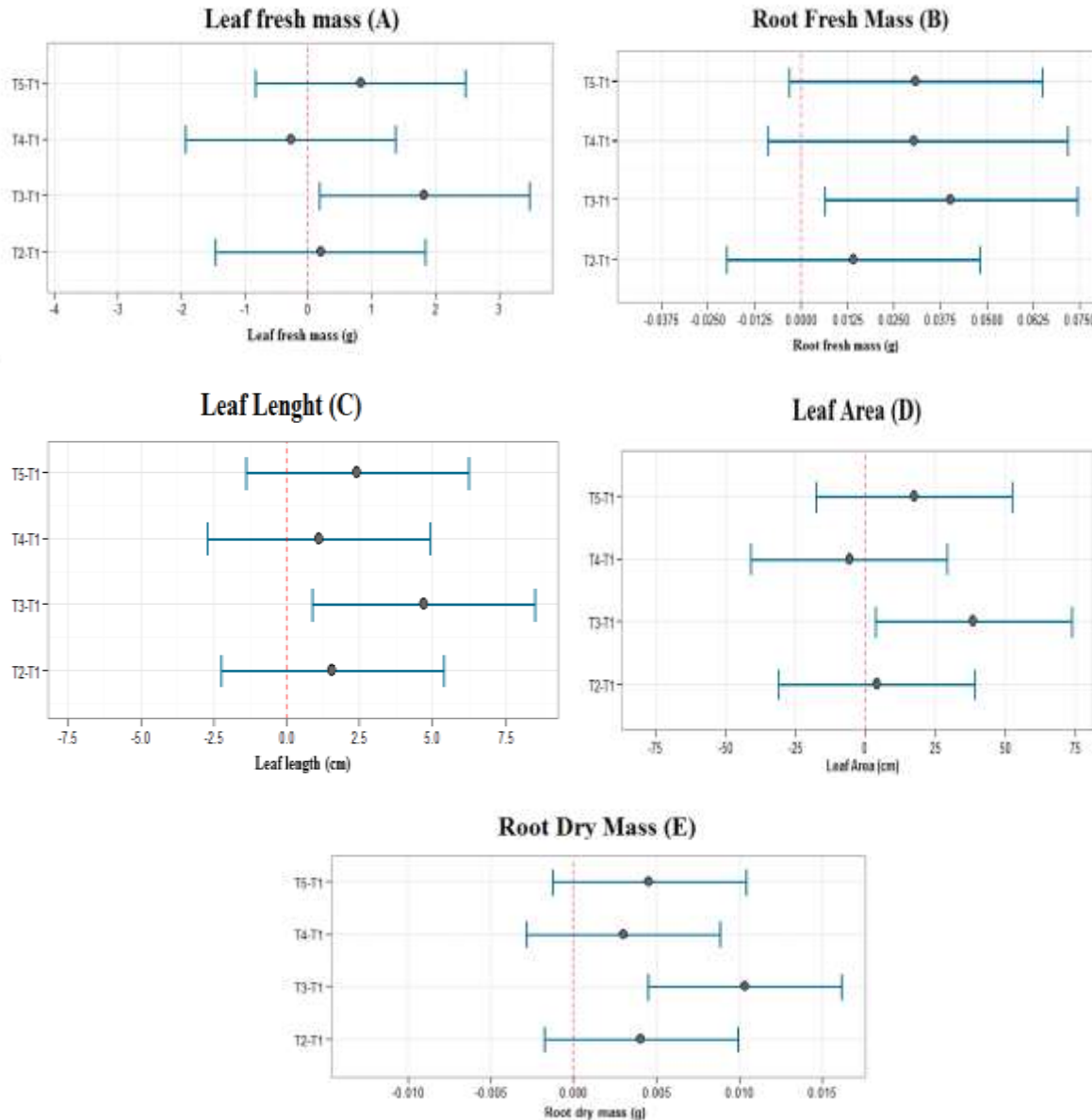
Figure 1. Leaf fresh mass (A), root fresh mass (B), leaf length (C), leaf area (D), and root dry mass (E).

However, given the 50% concentration of the extract, there was a potentiation in the samples average compared to the control sample T1, observing this variance in the following features: leaf fresh mass, root fresh mass, leaf length, leaf area, and root dry mass.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of pre-germination treatments on *Copaifera langsdorffii* seeds

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Copaifera langsdorffii has been intensely exploited, since it attracts several interests, and is now on the list of endangered species. Due to this risk of extinction, seedling production of this species has important economic and social value. The aim of this study was to evaluate seed germination of *C. langsdorffii* subjected to different pre-germination treatments for breaking seeddormancy: i) control; ii) immersion in sulfuric acid (98%) for 2 min; iii) immersion in sulfuric acid for 10 min; iv) removal of the seed coat; v) immersion in distilled water for 24 h; and vi) immersion in boiling water at 80°C for 2 minutes. In the period of 34 days after setting up the experiment, there was 70% germination of the seeds subjected to the method of immersion in sulfuric acid (98%) for 10 minutes, 63% of seeds in which the seed coats were removed, and below 27% in the rest. In this context, it may be concluded that the method of immersion in sulfuric acid for 10 min proved to be more effective and advantageous for overcoming dormancy.

Key words: Arboreal species, Copaiba, germination, dormancy.

INTRODUCTION

*Copaifera langsdorffii (*Copaíba) is a species of the *Fabaceae* family, which reaches from 5 to 15 m height and 20 to 60 cm diameter at breast height (Lorenzi, 1992). This species attracts great commercial interest due to the potential production of wood and of resin oil, with a bitter flavor and fragrance and brown coloring, which is extracted from the trunk and may be used as a fuel for diesel engines and also in home remedies as an antiseptic, healing ointment, expectorant, diuretic, laxative, stimulant, emollient and tonic (Veiga Júnior and

Pinto, 2002). This species may also be used for urban forestry, as well as reforestation for environmental recovery, and is thus highly valued internationally.

Copaiba occurs naturally throughout Brazil and is found from the South to the Northeast. It is also observed in other South American countries, such as Argentina, Bolivia and Paraguay. Since it attracts such diverse interests, Copaiba has been intensely exploited and is on the endangered species list (Carvalho, 2003). Due to this risk of extinction, seedling production of this species has important economic and social value (Toledo and Parente, 1988).

Seed dormancy is a physiological and/or physical state for survival present in forest species that allows longer duration of seed quality and germination and emergence potential in environments unfavorable to the growth and establishment of the seedlings. Although dormancy is advantageous for survival of species in natural conditions, it is not a desirable characteristic for seedling production in a nursery (Fowler and Bianchetti, 2000). Copaiba seeds have dark coloring and an ellipsoid shape, partially covered by a yellowish aril, and they have seed coat dormancy caused by substances present in the seed and also in other structures (Crestana and Beltrati, 1988; Almeida, 1998; Veiga Junior and Pinto, 2002). In the face of this barrier, there is the need for seeking effective pre-germination treatments for breaking seed dormancy. Among the treatments successfully used for breaking seed coat dormancy of forest species, manual and chemical scarification stand out (Fowler and Bianchetti, 2000).

The aim of this study was to evaluate the efficiency of pre-germination treatments for breaking dormancy in Copaiba seeds.

MATERIALS AND METHODS

The experiment was performed in the Seed Analysis Laboratory of the Faculdade de Rondônia/FARO in the months of July and August 2012. To carry out this study, seeds collected in July 2012 were used in a natural state of dehiscence. Seeds were collected from a population of 3 seed trees. The seed trees exhibited stem quality 1 and 2 (1 = straight; 2 = branched) as characteristics. Soil in the region of collection was characterized as Hapludox, which is predominant in the state of Rondonia and equatorial climate.

The collected seeds exhibited medium size, shape and homogeneous morphological characteristics. The experiment was composed of six pre-germination treatments: T1) control (without treatment); T2) sulfuric acid (98%) for 2 min and subsequent washing with distilled water three times; T3) sulfuric acid (98%) for 10 min and subsequent washing with distilled water three times; T4) removal of the seed coat; T5) distilled water at rest for 24 h and subsequent washing with distilled water three times; and T6) hot water at 80°C for 2 min and subsequent washing with distilled water once.

To set up the experiments, the seeds were disinfected with a sodium hypochlorite solution at 2.5% for 10 min and washed with distilled water to remove excess hypochlorite. The substrate used for seeding was germitest paper, which were moistened with distilled water (2.5 times the weight of dry paper) and stored under the form of rolls of paper in a seed germinator at a constant temperature of 25°C (Brasil, 2009). Each treatment was placed in a plastic bag for the purpose of maintaining substrate moisture. Twenty seeds were used in each treatment, with four replications, for a total of 80 seeds per treatment.

Evaluations were performed considering the following germination parameters: 1) first germination count (GC), corresponding to the percentage of seeds germinated in the 15th day after setting up the experiment; 2) total germination percentage (G), corresponding to the total percentage of seeds germinated up to the 34th day after setting up the experiment; 3) germination speed index (GSI), determined according to the formula presented by Maguire (1962), $GSI = G_1/N_1 + G_2/N_2 + ... + G_n/N_n$, where: G_1, G_2, and G_n are the numbers of germinated seeds on the first, second, and last count; and N_1, N_2, and N_n are the numbers of days between the count and n are the numbers of days between the count and n the beginning of the experiment; 4) primary root length (RL), corresponding to theroot length, in centimeters, on the last day of the experiment.

A completely randomized experimental design was used, with the treatments distributed in four replications of 20 seeds each. Data normality was analyzed by means of the Lilliefors Test. Percent values were transformed by arcsine-square root prior to analysis. Analysis of variance (ANOVA) was applied to test the effect of treatments. A Tukey test was applied to compare the above effects between homogeneous groups. Tests of significance were made at a $p \leq 0.05$ confidence level. Analyses were processed by using the GENES statistical software (Cruz, 2006).

RESULTS

Significant differences were detected among the treatments for all the parameters evaluated, which may be seen through the results of the analyses of variance, in which the mean squares of the treatments were significant (P<0.01). As of this result, mean value tests were performed to determine these differences among the treatments (Table 1).

For the parameters of first germination count (GC), total germination (G) and germination speed index (GSI), it was seen that the seeds subjected to the pre-germination treatments of immersion in sulfuric acid for 10 min (T3) and those in which the seed coats were removed (T4) did not differ statistically from each other. Thus, it was verified that the two pre-germination treatments were efficient in breaking dormancy in Copaiba seeds.

Seeds treated with sulfuric acid for 10 min showed higher root length, reaching six centimeters length on average, while there was no significant difference among the other treatments.

As for treatment 4, in which the seed coat was removed, the result was not the same, possibly due to the presence of pathogens, which reduced their vigor. Damages on the cotyledon during seed coat removal can be the most probable cause. Loss of nutrients during imbibitions might have caused low vigor. In relation to seed handling, care wasgiven to cleaning the material used for removing the seed coat for each seed.

It is interesting to observe that both the control (T1) and treatment 2 (H_2SO_4 2'), did not lead to germination at 15

Table 1. Mean values of first germination count (GC), total germination (G), germination speed index (GSI) and primary root length (RL) of Copaiba.

Treatment	GC (15days) (%)	G (34 days) (%)	GSI Number germination/week	RL (35 days) (cm)
ANOVA	**	**	**	**
T1 (control)	0.0[b]	21.2[b]	1.0[b]	10.8[b]
T2 (H_2SO_4 2')	0.0[b]	1.3[b]	0.1[b]	0.3[b]
T3 (H_2SO_4 10')	50.0[a]	70.0[a]	6.1[a]	60.8[a]
T4 (without seedcoat)	61.2[a]	63.8[a]	6.4[a]	16.3[b]
T5 (H_2O 24h)	1.3[b]	27.5[b]	1.4[b]	7.8[b]
T6 (H_2O 80°C 2')	5.0[b]	26.3[b]	2.0[b]	16.8[b]

When treatment factor is significant (ANOVA - * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = p > 0.05 not significant), different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=4).

days after setting up the experiment. In relation to the control, this was already expected because no pre-germination treatment was applied. Nevertheless, for the treatment in which seeds were subjected to immersion in sulfuric acid for a period of two minutes, it was observed that most of the seeds died, for they exhibited a whitish coloring and softening. This probably occurred due to some type of activation of substance(s) that impede germination. However, reports were not found in the literature in regards to this occurrence.

DISCUSSION

The results obtained in this study agree with those presented by Bezerra et al. (2002), who, evaluating diverse pre-germination treatments, found that chemical scarification with sulfuric acid in Copaiba seeds speeded and increased the percentage of germination. This fact may also be verified by means of the results of the germination speed indices (GSI) in which, for seeds subjected to chemical scarification for 10 minutes, Durigan et al. (1997) and Almeida et al. (1998) attribute slow and uneven germination of this species, extending up to 70 days, to an occasional dormancy. Thus, it is fitting to mention that in this study, the treatments with sulfuric acid for ten minutes (T3) and the treatment with removal of the seed coats (T4) led to a high germination index (70 and 63.8%, respectively), in a time period of 34 days, confirming that seed coat dormancy was overcome by these treatments (Table 1).

It is also important to highlight that the control did not differ from T5 treatment (seeds immersed in water for 24 h) and T6 treatment (seeds immersed in boiling water for 2 min), implying that these treatments were not sufficient to promote and accelerate germination of Copaiba seeds. These results are in contrast to those presented by Prado and Perez (1993), in which the authors found good germination in these treatments for the same species. Borges et al. (1982), for their part, tested some methods

for overcoming dormancy in copaiba seeds and, among the most efficient, immersion in water at rest for 24 h at ambient temperature stands out, also in opposition to the results found in this study.

Although the treatment of seeds with mechanical scarification by removal of the seed coat (T4) showed a visual result inferior to chemical scarification with sulfuric acid for 10 min (T3), it showed a greater mean value for primary root length. Machado et al. (1992) state that scarified Copaiba seeds exhibit high germination power, in agreement with this study.

The control (T1) exhibited the first germinated seed on the 18th day after setting up the experiment and, on the last day of the experiment, had only 21.2% of the seeds germinated. Carvalho (1994) cites that Copaiba seeds not treated to overcome dormancy germinate from 12 to 59%, in agreement with the results of study.

On the 19th day, most of the seeds in which the seed coat was removed (T4) had already germinated, exhibiting lengthening of the primary root. In treatment 3, the peak of germination occurred on the 25th day after setting up the experiment. At the end of the experiment (34th day after setting up the experiment), some seedlings already had primary foliage in the treatments in which the seeds were immersed in sulfuric acid for ten minutes (T3) and in the treatment in which the seed coats had been removed (T4) and, in greater number, in the treatment with sulfuric acid for ten minutes (T3).

The present study is in disagreement with the results found by Cruz et al. (2005) in that which refers to the best treatment for overcoming seed coat dormancy in Copaiba, in which mechanical scarification exhibited the best result for overcoming dormancy, exceeding chemical scarification with sulfuric acid. In this study, it was observed that T3 (immersion in H_2SO_4 for 10 min) obtained better results than mechanical scarification through removal of seed coats (T4).

From the results obtained in this study for Copaiba seeds, the pre-germination methods of mechanical scarification (seed coat removal) and chemical scarification

(sulfuric acid for 10 min) increased the permeability of the seed coat, allowing water absorption and acceleration of the germination process. However, the seed coat removal method is difficult to perform and makes for more susceptible to contamination by pathogens.

Conclusion

For Copaiba seeds, the pre-germination method of chemical scarification (sulfuric acid for 10 min) most efficiently overcomes seed coat dormancy, accelerating germination speed and providing greater uniformity in seedling development.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Residual effect of herbicides 2,4-D and Glyphosate on soybeans in a Brazilian Cerrado Ultisol

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The use of herbicides for weed desiccation is what enables the no-tillage system, which increasingly stimulates the growth of the agrochemical market, in particular the class of herbicides. This study aimed to assess 2,4-dichlorophenoxyacetic acid and Glyphosate (N-phosphonomethyl-glycine) herbicides mixture persistence in soybeans of Ultisol in the Brazilian Cerrado. The study was conducted in a greenhouse with a randomized blocks design, in a factorial 6×5, being six application times (0, 3, 5, 7, 10 and 14 days before sowing) and five herbicide doses (0, 750, 1500, 2250 and 3000 g a.e. ha^{-1}) in four replications. Herbicides were sprayed with a manual knapsack sprayer. The residual effect was assessed through emergence speed index (ESI), plant height and dry weight of shoots (DWS) and roots (DWR). The residual effect of mixtures of herbicides 2,4-D and Glyphosate on soybean in a Brazilian Cerrado Ultisol showed greater damage to plants when applied at sowing. The doses 2250 and 3000 g a.e. ha^{-1} were the most severe for the development of soybean plants.

Key words: Bioindicator, *Glycine max*, Herbicide, Persistence.

INTRODUCTION

In 2013, the world population was 7.2 billion people, and studies show that it may reach 9.6 billion by 2050 (UN REPORT, 2013). With this estimate, being able to meet the food demand with the least possible impact on the environment will be a major challenge.There are different methods for weed control. In soybean, chemical control has been most widely used for various reasons, including the extensive cultivated areas. When it comes to no-tillage, weed control depends on the use of herbicides, as crops and hoeing are incompatible with the technology used in the system. Thus, a more sustainable and optimized production becomes necessary to produce more in already explored areas, minimizing at most the opening of new areas. Although it is necessary to adopt techniques aimed at soil recuperation and conservation, many producers opted for the No-Tillage System (NTS), in which there is no soil disturbance, vegetation cover is maintained and crop rotation takes place (Heckler, 2002). This system is one of the sustainable agricultural technologies covered by the agriculture plan for low

Table 1. Chemical and granulometric characterization of the Eutrophic Ultisol in the layer of 0.0 - 0.20 m.

pH	P	K	Ca	Mg	H	Al	SB	CTC	V	O.M.	Sand	Silte	Clay
$CaCl_2$	mg dm^{-3}			Cmol$_c$ dm^{-3}					%	g dm^{-3}	g kg^{-1}		
5.5	13.3	53.0	2.9	1.1	2.9	0.0	0.6	7.0	59.0	28.7	640	83	277

carbon emission (Cordeiro et al., 2012), being justified by the application of herbicides to eliminate existing vegetation in the area before sowing, a practice known as desiccation (Carvalho, 2009). Vegetation cover desiccation and weed management are conducted in such a way that eliminates the damage caused by them, as competition for water, light, nutrients and space (Vasconcelos et al., 2012).Brazil is the country that produces more grains, with 58.5 million hectares in planted area, and the Brazilian production was 210.3 million tons in the 2015/16 crop. This increase amounts to 1.3% or 2.6 million tons compared to the 2014/15 crop, which was 207.7 million tons (Conab, 2016). Because of its extent and availability of arable lands, it has great potential to help meet this demand (Casarin, 2012).Soybean is the most produced grain in Brazil. According to Conab (2016), soybean has the highest absolute growth, with estimated increase of 4.9 million tons, being estimated at 101.2 million tons, 5 million more than in the previous harvest.

The increase in production has been accompanied by the use of herbicides (Peres, 2009), and the use of pesticides increases the risk of poisoning, phytotoxicity and environmental pollution if not used correctly, regardless of product class. Thus, spraying these products requires proper application of technology because, when used correctly, there is minimization of risks and reduction of economic losses, improving field efficiency (Peres-oliveira and Antuniassi, 2012; Gazziero, 1984). In weed control, persistent herbicides are commonly used. These herbicides may cause toxic effects in sensitive species such as soybeans, beans, cotton and other dicotyledonous plants, when grown in sequence (Silva, 1999; Gonçalves, 2001; Silva, 2006).2,4-D was the first organic compound industrially synthesized to be used as a selective herbicide (Barbera, 1976). According to Silva et al. (2007), it has a short to average persistence in soils and at normal doses, the residual activity does not exceed four weeks in clay soils and hot weather. Notwithstanding, even though its degradation in soil is considered fast, its residual effect will depend on the edaphoclimatic conditions, with the possibility of lasting longer and intoxicating the successor culture. Glyphosate is one of the most widely used herbicides in the world (Ghisi, 2013). It is commonly associated with 2.4-D, targeting more species to be controlled; the greatest risks of poisoning have been linked to the first due to the effect at extremely low doses (Constantin, 2007; Oliveira, 2007). The residual effect of

mixing 2,4-D with glyphosate will also depend on the soil type used, the chosen dose and the period between application and implementation of the crop.

This study aimed to assess the persistence of herbicides 2,4-D and Glyphosate in an Eutrophic Ultisol, and their effects on soybean.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at 16° 28' South latitude, 50° 34' West longitude and altitude of 284 m. The experimental design was a randomized block with a factorial 6×5, with six application times before sowing (0, 3, 5, 7, 10 and 14 days before sowing), five doses of herbicide 2,4-D (0, 750, 1500, 2250 and 3000 g a.e. ha^{-1}) and a constant dose of Glyphosate (4000 g a.e. ha^{-1}), in four replications. Each experimental unit consisted of pots with 5 dm^3 soil capacity with eight soybean plants, cv. TMG 4182 (seeded at 5 cm depth). The soil used was Red-Yellow Ultisol (Embrapa, 2013), collected in the region of Rondonópolis, MT, in the depth of 0 to 0.20 m. The soil was sieved on a 4 mm mesh for insertion in the experimental units, characterized by chemical and granulometric analyses according to the methodology of Embrapa (1997) (Table 1). The soil was maintained at 80% field capacity moisture content throughout the test, according to the methodology of Bonfim-Silva et al. (2011).The spraying of herbicides was carried out with a manual knapsack sprayer equipped with XR 11002 and syrup consumption corresponding to 200 L ha^{-1}. The persistence of these herbicides in the soil was evaluated through emergence percentage (%), emergence speed index (ESI), plant height (cm), dry weight of shoots – DWS (g) and dry weight of roots – DWR (g) 26 days after sowing, at the end of the work. The statistical analysis was performed in accordance with the polynomial regression model.

RESULTS AND DISCUSSION

The variables that showed a significant difference were adjusted to linear and quadratic regression models.The emergence speed index (ESI) was influenced at all doses and times studied (Figure 1). The only situation when the emergence speed index was not affected was under dose 0, due to the absence of herbicides (Figure 1a). Under the other doses (750, 1500, 2250 and 3000 g a.e. ha^{-1}) plant number gradually reduced as spraying period approached the sowing date with doses 2250 and 3000 g a.e. ha^{-1} being the most severely affected. In some studies with sorghum, according to Petter et al. (2011), regardless of application time, all studied doses of 2,4-D caused phytotoxic effect to the crop, the effect being progressive as the herbicide doses increased.Regarding application time (0, 3, 5, 7, 10 and 14 days before sowing) and doses applied, the higher the dose, the lower were the
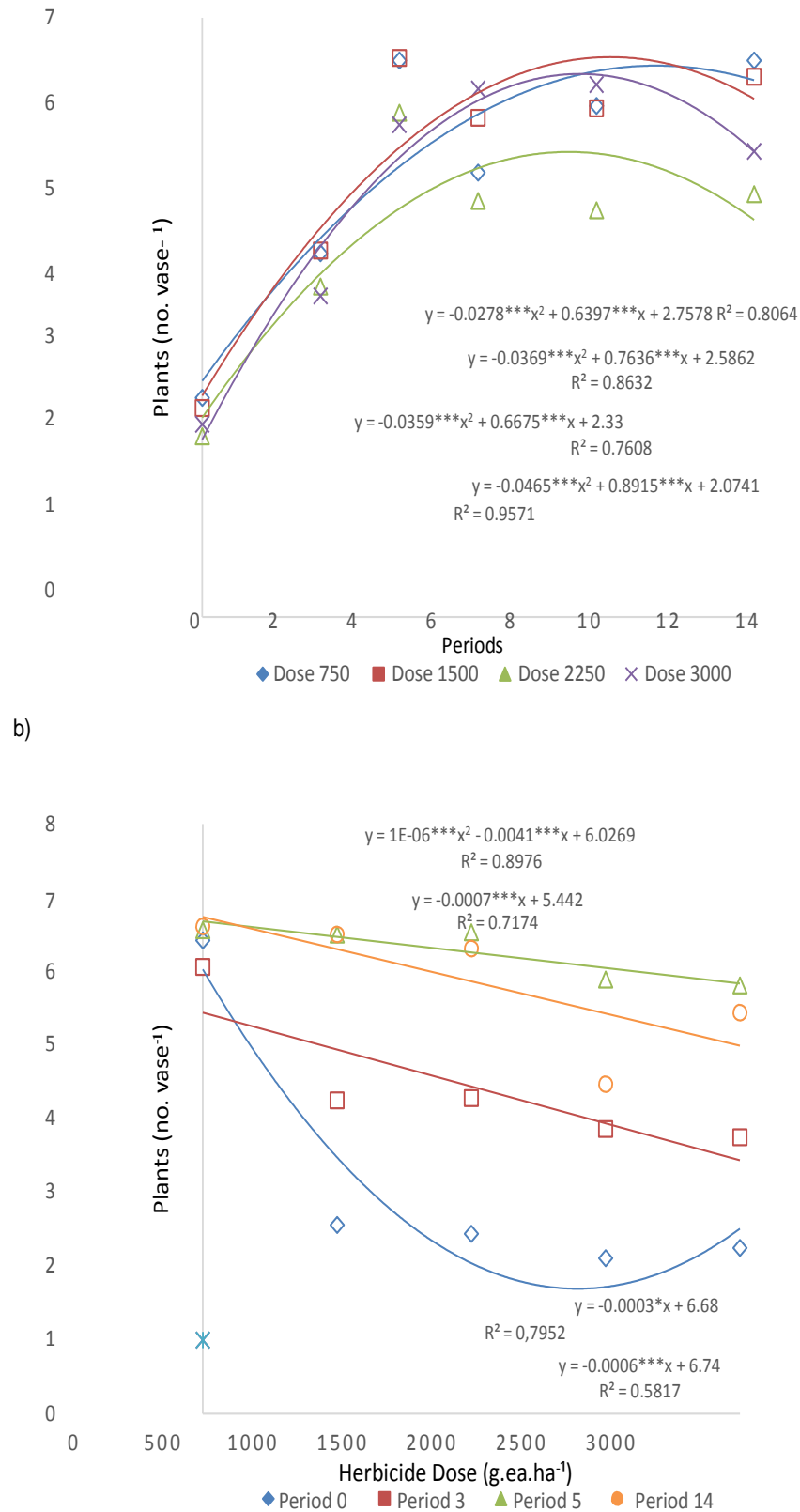
b)

Figure 1. Emergence speed index of soybean seedlings in Red-Yellow Ultisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 01, 1 and 5%, respectively.

number of plants. Generally the most critical time occurred when the sowing and spraying took place on the same day (0 days before sowing) which all doses of the herbicide reduced the number of plants per pot (Figure 1b).The application times five and fourteen days before sowing were the ones, which provided the highest emergence speed indexes, results also obtained by Peres-oliveira et al. (2016). According to Reis et al. (2010), herbicide 2,4-D is a growth regulator which has a similar effect to the hormone auxin, therefore, it can be used as a plant growth regulator.

For soybean plant height (Figure 2), with increasing doses over periods (Figure 2), the 2,4-D doses 750, 1500, 2250 and 3000 g a. e. ha^{-1} were significant and, in general, caused a reduction in plant height as the spraying approached the sowing date. The dose 3000 g a.e. ha^{-1} was the one which reduced plant height the most in the application time where spraying and sowing took place on the same day; however, in the interim period, it was the dose which provided the greatest plant height. A fact confirmed by Farinelli et al. (2005), where the higher the dose of herbicide 2,4-D, the larger the increase in plant height of millet (*Pennisetum americanum* (L.)). Yamashita et al. (2009) worked with 2,4-D alone at a dose of 335 g a.e. ha^{-1} in kapok (*Ceiba Pentandra*) obtained increase in plant height. This increase is caused by auxin concentration in younger branches, and is a characteristic effect of the auxinic herbicides (Deuber, 1992). Pacheco et al. (2007) using higher doses of 2,4-D in millet (*Pennisetum americanum* (L.)) obtained a reduction in plant height.

In the analysis of application times as a function of increasing doses (Figure 2b), the only significant time to plant height was 0 days before sowing, linearly reducing height as doses were increased. Similar results were found by Petter et al. (2011), in which increasing doses of 2,4-D linearly decreased the height of sorghum plants (*Sorghum bicolor* (L.)). This reduction in plant height caused by mixing the herbicides 2,4-D and glyphosate was also observed in kapok (*Ceiba Pentandra*) until 21 days after application, with subsequent stabilization (Yamashita et al., 2009).

There were significant differences between the dry weight of shoots of soybean plants (Figure 3) between the interaction of doses over periods and for the periods within each dose. Except for the dose 0, all other doses (750, 1500, 2250 and 3000 g a.e. ha^{-1}) severely reduced the dry weight of shoots of plants especially when application was close to the sowing date. Reduction in the dry weight of shoots was linearly correlated to the increase in dosages applied (Figure 3a). At 7 and 10 days before sowing, 1500 g a.e. ha^{-1} dose provided greater dry matter production of shoots, results that were similar to those obtained by Reis et al. (2010), who using doses of 1.5 and 2 L a.e. ha^{-1}, found a greater increasing trend of the dry weight of shoots of corn (*Zea mays*); yet Pacheco et al. (2007) noted that the increase in 2,4-D

doses resulted in lower production of dry matter of shoots of millet (*Pennisetum glaucum*), reduction from 13% to 33% between the doses of 335 g.ha^{-1} and 1005 g.ha^{-1}. Farinelli et al. (2005) found increased dry weight of shoots of millet (*Pennisetum glaucum*), with increasing doses of 2,4-D; and Yamashita et al. (2009) obtained a reduction of at least 26% compared to the control treatment for the dry weight of plants of the forest species *Schizolobium amazonicum* at a dose of 335 g a.e. ha^{-1}. According to Mortensen et al. (2012), 2,4-D acts as a herbicide for the control of dicotyledonous weeds, and yet it also has a beneficial effect, depending on the time and dose used. In the evaluation of the application times, with increasing doses applied over the days, the lowest dry weight index was verified at 0 days before sowing. Thus, the closer the spraying and sowing periods are, the lower the crop development also with the increase in application of doses. The application times 3 and 5 days before sowing had no significant impact on the dry weight of shoots. The highest dry weight of shoots was observed at 7 days before sowing. At 14 days before sowing, the dry weight of shoots underwent a gradual decrease with increasing doses (Figure 3b).

The dry weight of roots of soybean plants (Figure 4) was significant in the interaction of doses over periods and for the periods within each dose.In the analysis of doses over the days (Figure 4a), the dose 0 g a.e. ha^{-1} had no significant impact. All other doses (750, 1500, 2250 and 3000 g a.e. ha^{-1}) severely reduced the dry weight of roots on the days near the sowing date, reduction occurring linearly according to the increase in dosage.The dose of 1500 g a.e ha^{-1} provided the highest increase in dry weight of roots, i.e. it most impacted this variable in all evaluated times. The gradual increase of doses caused a significant reduction in the dry weight of roots on the dates near sowing, a fact that was repeated at 14 days. The trend was similar to that reported by Reis et al. (2010), where there was increase in the dry weight of roots between the control treatment and the dose 2 L a.e. ha^{-1}.

Nonetheless, there was a significant difference between the doses of 2 and 3 L a.e. ha^{-1}, with a 38% reduction in the dry weight of roots. Yet the results of Farinelli et al. (2005) showed greater dry weight of the root system with increasing doses (402, 536, 670 g a.e. ha^{-1}) compared to the control. In the analysis of application times as a function of increasing doses (Figure 4b), dry weight of roots was least at 0 days before sowing, decreasing gradually, as a function of increasing doses. For the application times 3, 5 and 14 days before sowing, the higher the dose used, the lower the dry weight of roots were also. The application time 10 days before sowing showed no significant differences among the doses. Application at 7 days before sowing, the 1500g a.e. ha^{-1} dose provided higher dry weight of roots, showing the hormonal capacity of herbicide 2,4-D in a given dose and application time.

$y = -0.0795*x^2 + 1.469*x + 10.744$
$R^2 = 0.4621$

$y = -0.1044**x^2 + 1.9699**x + 10.399$
$R^2 = 0.6878$

$y = -0.1766**x^2 + 3.3674**x + 4.5658$ $R^2 = 0.9206$

$y = -0.2249*x^2 + 4.0275*x + 3.0523$
$R^2 = 0.77$

Plant height (cm)

Periods

◆ Dose 750   ■ Dose 1500   ▲ Dose 2250   ✕ Dose 3000

b)

$y = -0,0058***x + 16.456$
$R^2 = 0.9065$

Plant height (cm)

Herbicide Dose (g.ea ha. $^{-1}$)

◆ Period 0

Figure 2. Height of soybean plants in Red-Yellow Ultisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 01, 1 e 5% respectively.

Figure 3. Dry weight of shoots of soybean in Red-Yellow Ultisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 01, 1 and 5%, respectively.

$y = -0.0095^{**}x^2 + 0.1366^{**}x + 0.4111$
$R^2 = 0.3851$

$y = -0.0113^{**}x^2 + 0.178^{**}x + 0.4123$
$R^2 = 0.3123$

$y = -0.0153^{***}x^2 + 0.232^{***}x + 0.0363$
$R^2 = 0.8891$

$y = -0.0112^{***}x^2 + 0.1627^{***}x + 0.1016$
$R^2 = 0.8233$

b)

$y = 2E-07^{*}x^2 - 0.0009^{*}x + 0.9574$
$R^2 = 0.9517$

$y = -0.0001^{*}x + 1.09$
$R^2 = 0.2087$

$y = -0.0001^{*}x + 1.076$
$R^2 = 0.8514$

$y = -2E-07^{*}x^2 + 0.0005^{*}x + 0.9617$
$R^2 = 0.8494$

$y = -0.0002^{***}x + 1.004$
$R^2 = 0.7465$

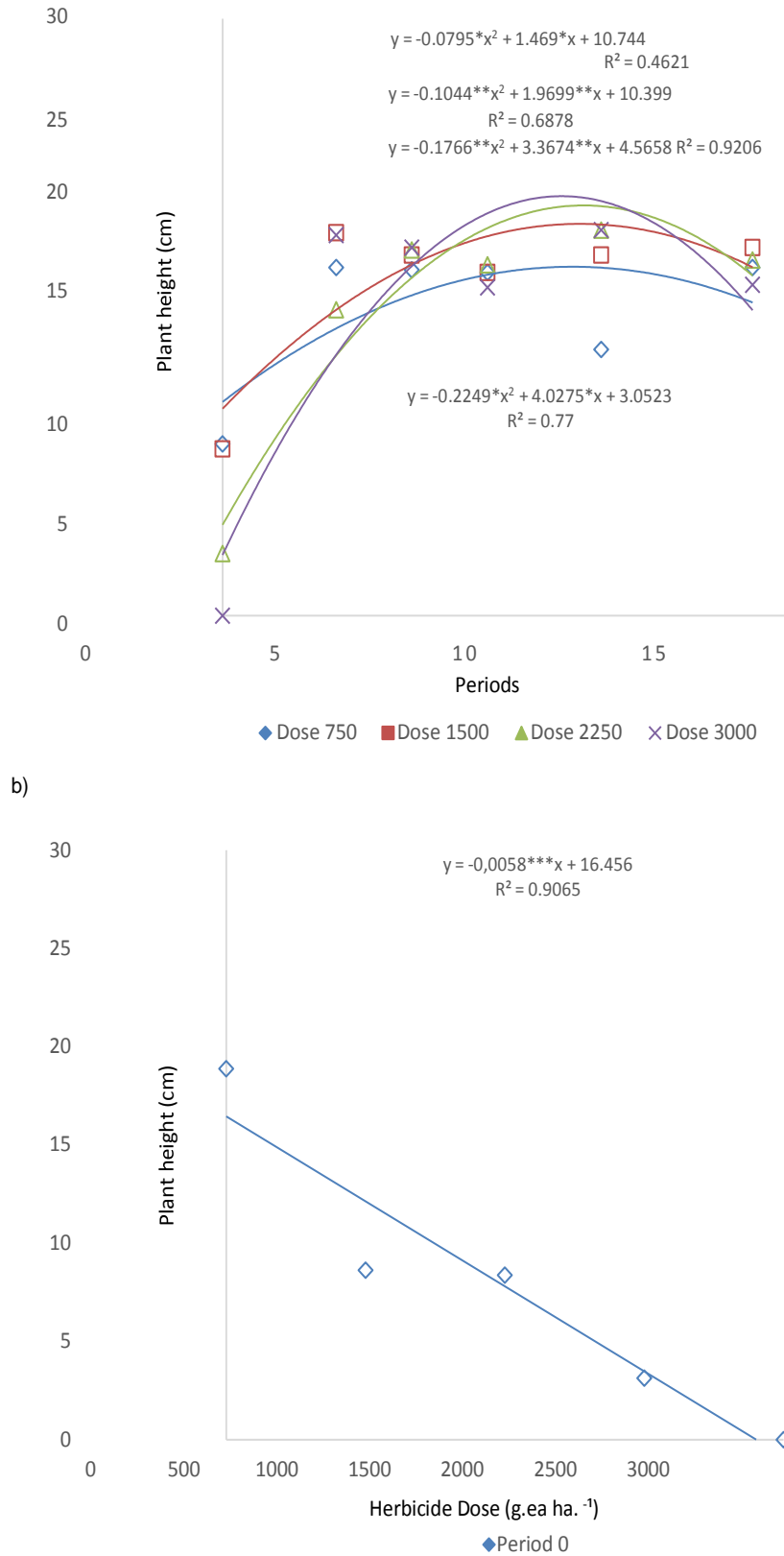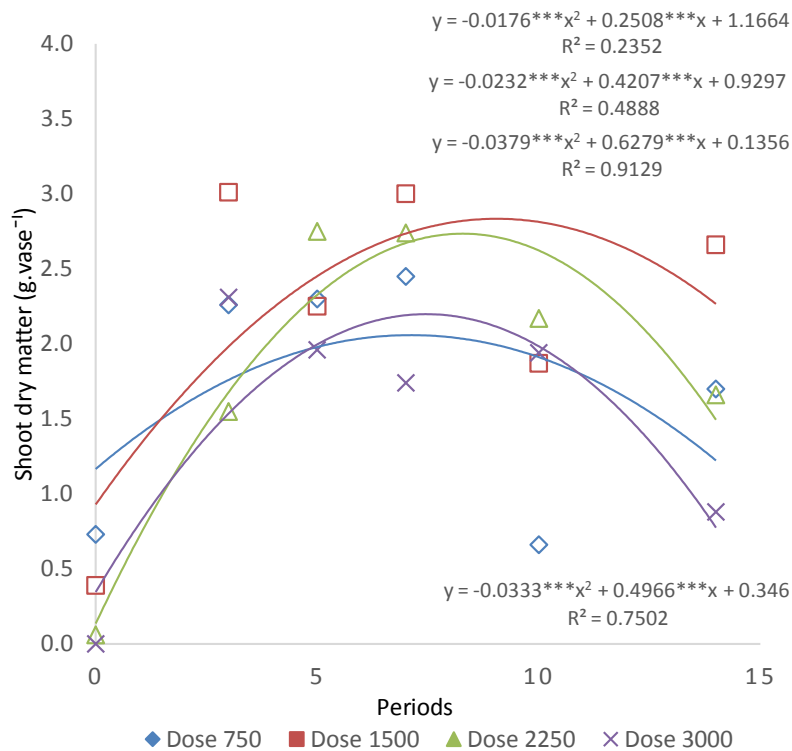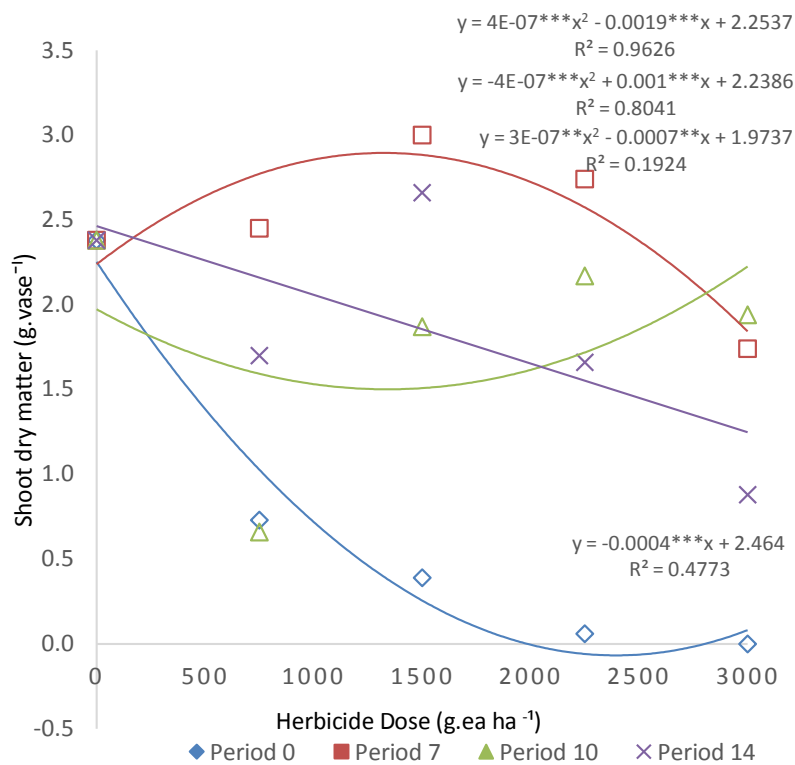**Figure 4.** Dry weight of roots of soybean in Red-Yellow Ultisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 01, 1 and 5%, respectively.
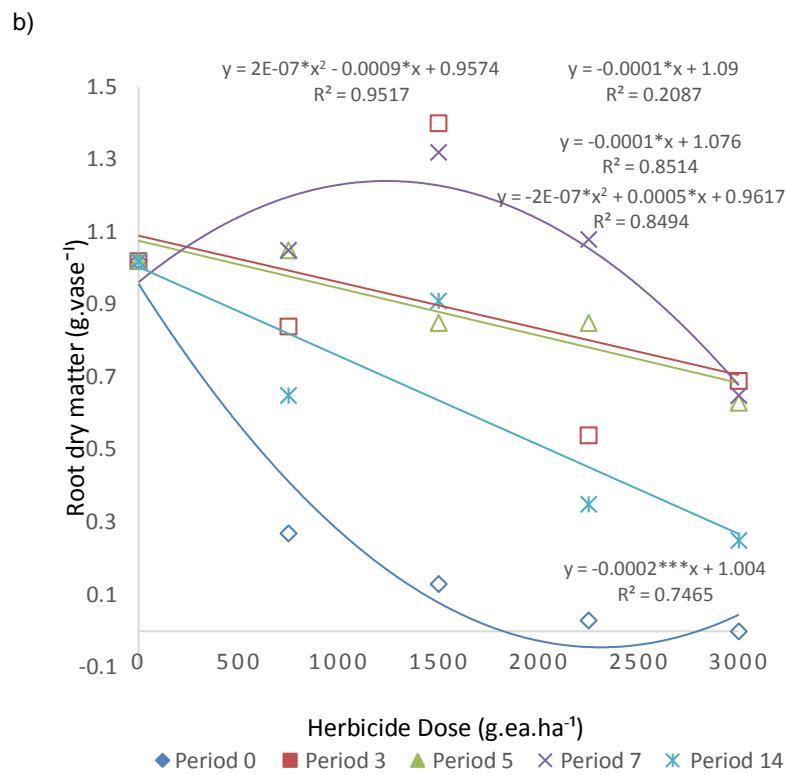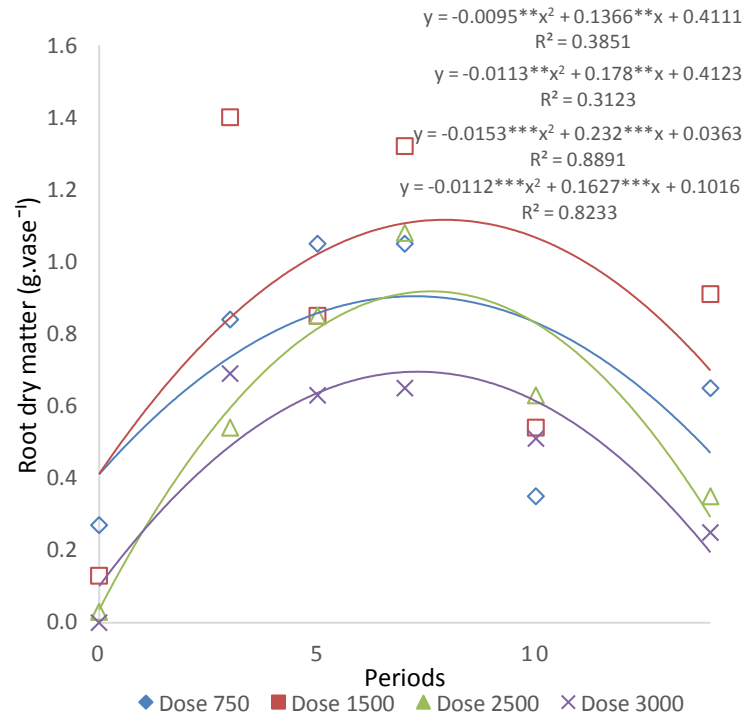
Conclusions

The residual effect of mixtures of herbicides 2,4-D and Glyphosate on soybean in a Brazilian Cerrado Ultisol showed greater damage to plants when applied at sowing.The doses 2250 and 3000 g a.e. ha^{-1} were the most severe for the development of soybean plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agro-morphological diversity within field pea (*Pisum sativum* L.) genotypes

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The objective of this study was to analyze the genetic diversity present in twelve Algerian pea genotypes using 24 agro-morphological traits. The experiment was carried out during three growing seasons (2013 to 2014, 2014 to 2015 and 2015 to 2016). ANOVA analysis revealed the presence of a great genetic variability for all characters studied. This diversity might be used in breeding programs. Also, expression of characteristics is highly influenced by the environment. For quantitative traits, correlation studies showed that weight of 100 seeds was significantly and positively correlated with leaflet length. Number of pods per 1 m² has a positive significant correlation with leaflet width. Weight of pods per 1 m² was correlated with three characters: Stipule length, leaflet length and leaflet width. The principal component analysis revealed that three components explained 85.92% of variation. Two groups were noted by dendrogram. The first group (demchi 1, p069, bouch1, p539, p593, p595 and p596) was characterized by a high pod yield; the other group comprises the less productive genotypes (p071, sefrou, p072, p073 and p350). Otherwise, the genotype p593 produced the best results for pods yield.

Key words: Genetic diversity, agro-morphological traits, field pea, *Pisum sativum* L.

INTRODUCTION

Pea (*Pisum sativum* L) is one of the oldest culture in the world with cereals and lens (Zohary et al., 2012). Field pea primarily is used for human consumption or as livestock feed. It is an important source of proteins (21 to 25%) and potential alternative to soybean in Europe (Barac et al., 2010). It contains high levels of carbohydrates and total digestible nutrients (86 to 87%), which makes it an excellent livestock feed (Enderes et

al., 2016).

According to Janzen et al. (2014), through symbiosis, pea can fix atmospheric nitrogen and therefore does not need nitrogen fertilizer especially since it provide nitrogen for the crop following it. Also, Pea tolerates drier growing season conditions and limited rainfall (Janzen et al., 2014).

Pea is a widely cultivated crop species and the second

Table 1. List of genotypes and their origins.

N°	Code/name	Country
1	Demchi 1	Algeria
2	Bouch1	Algeria
3	P539	Algeria
4	p593	Algeria
5	p595	Algeria
6	p596	Algeria
7	p069	Algeria
8	p071	Algeria
9	p072	Algeria
10	p073	Algeria
11	p350	Algeria
12	Sefrou	Morroco

most important food legume worldwide after common bean (Esposito et al., 2007). Its global production reached 11 332 772 tons with an area of 6 868 131 ha (FAOSTAT, 2014). Canada is the leading producer with approximately 3 million metric tons in 2012 (Jansen et al., 2014) followed by France, Russian federation, China mainland and Ukraine. In 2014, approximately 45000 tons of pea were harvested in North Africa on an area of 63127 ha (FAOSTAT, 2014).

In Algeria, pea exists for a long time (INRAA, 2006). However, this heritage was largely lost. Indeed, in the past, Algeria introduced new performing varieties (Arbouche et al., 2011) and the landraces which presented a greater tolerance to the biotic and abiotic conditions were replaced by new varieties (Cupic et al., 2009). Thus, the local germplasm suffered great genetic erosion (Arbouche et al., 2011). Fortunately, many pea cultivars were preserved in gene banks (Hagenblad et al., 2014) or among farmers occupying marginal lands (FAO, 2011) and practicing family farming. The studies clearly demonstrate the importance of this crop diversity in counteracting the effects of droughts and other environmental hazards and in ensuring family food security (FAO, 2004).

Furthermore, Landraces can play a very important role in improvement works and selection, offering interesting characteristics for farming. However, the description and knowledge of these genotypes is a prerequisite for their use (Marchenay and Lagarde, 1987). So, several studies of pea germplasm using different approaches have been published in the world (Ali et al., 2007, Sarikamis et al., 2010, Ghixari et al., 2014) and in Tunisia and Morocco where interest was brought to the development of local varieties (Mani et al., 2007; Benbrahim and Gaboun, 2008). In Algeria, landraces are still neglected in favor of imported varieties of peas and then the number of local genotypes is much reduced.

Traditionally, germplasm diversity is assessed by morphological descriptors, which remain the only

legitimate marker type accepted by the International Union for protection of New varieties of plants (UPOV, 2009) (Ghixari et al., 2014).

The objective of this research was to determine genetic diversity among pea genotypes using morphological and agronomic traits in a goal of their valorization.

MATERIALS AND METHODS

Search pea landraces from farmers was very difficult to do because of the priority given to the introduced varieties and thus, the number of cultivars collected was limited to twelve (Table 1).

Nine genotypes were obtained from ICARDA (International Center for Agricultural Research in Dry Areas), two genotypes (demchi1 and bouch1) were collected as part of this work, the first being cultivated in Adrar in southern Algeria and the second is harvested in Algiers (Bouchaoui). Sefrou is an introduced genotype (from Morocco) but has been long cultivated in Algeria, at the Technical Institute of Field Crops (TIFC-Sidibellabes).

The study was carried out during the winter seasons of 2013 - 2014, 2014 - 2015 and 2015 - 2016. The first experiment was conducted at the experimental station of ENSA (National Higher school of Agronomy) at El Harrach-Algiers. The second and the third experiments were carried out in the central farm of ENSA. The field trials were in a randomized complete block design with three replications. Seeding was done in a plot of 1.5×1.5 m. The seeds were sown in rows spaced 35 cm.

Observations were made for 24 agro-morphological characters described by UPOV Guidelines for the conduct of tests for distinctness, uniformity and stability. The qualitative traits are presented in Table 2. The quantitative characters were resumed in Table 3. The stem length; the number of pods per 1 m² and the weight of pods per 1 m² were measured only during the last season.

Statistical analysis was performed using the software StatView. The analysis of variance (ANOVA) was performed by Fisher's least significant difference (LSD) method to test the significance difference between means. Correlations were performed based on fifteen quantitative characters (AFF, SL, NNFFN, STL, STW, LL, LW, PL, MNFN, PLE, PWI, NGP, WTS, NPM2, WPM2). The principal component analysis and the cluster analysis were done using eleven characters (AFF, STL, STW, LL, LW, PLE, PWI, NGP, WTS, NPM2, WPM2).The cluster analysis was adopted with the Ward's method as a clustering algorithm (Ward, 1963).

RESULTS AND DISCUSSION

Qualitative traits

According to Solberg et al. (2015), a combination of morphological and genetic characterization can identify if the material is unique or just duplicates of gene bank material. In the other hand, Yirga and Tsegay (2013) characterized pea genotypes using only qualitative traits related to the color of flower and seed shape.

The results of qualitative traits are presented in Table 4. A polymorphism was found within the different genotypes. The presence of anthocyanin coloration of axil was evident in 58.33% of genotypes. Only three genotypes presented strong dentations on leaflets. Four genotypes (p593, p596, p072 and sefrou) have very

Table 2. Qualitative traits.

Qualitative trait	Polymorphism
Anthocyanin coloration of axil (ACA).	1-Present ; 2-absent
Dentation of leaflet (DL).	1-Absent or very weak, 2-weak, 3-medium, 4-srong, 5-very strong
Flecking on stipule (FS)	1-Present ; 2-absent
Stipule: density of flecking (DFS).	1-Very sparse, 2-sparse, 3-medium, 4-dense, 5-very dense
Flower : color of wing (CWI)	1-White with pink blush, 2-pink, 3-reddish purple
Flower : color of standard (CST)	1-White, 2-whitish cream, 3-cream, 4-pink, 5- light purple.
Shape of seed (SS).	1-Ellipsoid, 2-cylindric, 3- rhomboid, 4- irregular.
Seed : spot on testa (SST)	1-Absent, 2-faint, 3-intense
Seed : color of testa (CT)	1-Redish brown, 2-brown, 3-brownish green

Table 3. Quantitative traits.

Character	Descriptors	Code
Phenological character.	Appearance of the first flower	AFF
	Stem length	SL
	Number of nodes including first fertile node	NNFFN
	Stipule length	STL
	Stipule width	STW
Morphological characters.	Leaflet length	LL
	Leaflet width	LW
	Peduncle length from stem to first pod	PL
	Maximum number of flowers per node	MNFN
	Pod length.	PLE
	Pod width	PWI
	Number of grain per pod	NGP
Yield characters.	Weight of 100 seeds	WTS
	Number of pods per 1 m²	NPM2
	Weight of pods per 1m²	WPM2

Table 4. Qualitative characters of the 12 genotypes.

N°	Genotype	ACA	DL	FS	DFS	CWI	CST	SS	SST	CT
1	Demchi 1	1	2	2	-	3	5	2	2	3
2	Bouch1	1	1	2	-	3	5	3	3	3
3	P539	2	2	2	-	2	4	2	2	3
4	p593	2	1	1	1	2	4	2	2	3
5	p595	1	4	2	-	2	4	1	1	1
6	p596	2	2	1	1	3	4	2	1	3
7	p069	1	2	2	-	3	4	4	2	3
8	p071	1	1	2	-	3	5	4	1	3
9	p072	1	2	1	1	3	5	2	2	3
10	p073	1	2	2	-	3	5	2	2	3
11	p350	2	4	2	-	2	5	1	2	2
12	Sefrou	2	4	1	1	3	5	1	1	3

sparse flecking in stipule. For the color of wing (CWI), two types were observed: Reddish purple (66.66%) and pink (33.33%). Light purple color was observed in standard of 58.33% of genotypes. Five genotypes presented a

Table 5. ANOVA.

Code	Mean±SD	Min.	Max.	CV	P (genotype)	P (year)	P (G xY)
SL	90.05±16.96	50.00	118.80	18.80	0.0003**	-	-
AFF	109.25±18.13	78.00	155.00	16.60	<0.0001***	<0.0001***	<0.0001***
NNFFN	16.57±3.14	10.20	21.75	19.00	<0.0001***	0.0040*	<0.0001***
STL	5.51±0.82	4.25	7.80	15.0	<0.0001***	<0.0001***	0.0422*
STW	2.81±0.54	2.00	4.32	19.50	<0.0001***	0.0004**	0.0111*
LL	3.81±0.67	2.35	5.73	17.60	<0.0001***	<0.0001***	0.0078*
LW	1.98±0.49	0.85	3.30	25.10	<0.0001***	<0.0001***	0.0307*
PL	6,05±1.57	3.10	9.75	25.90	0.0004**	0.6377[NS]	0.0008**
PLE	5.19±0.64	4.00	7.03	12.50	<0.0001***	0.0116*	0.4965[NS]
PWI	0.78±0.11	0.50	1.09	14.70	<0.0001***	<0.0001***	0.8168[NS]
NGP	6.91±1.12	4.60	10.33	16.20	<0.0001***	0.2568 NS	0.0022 *
MNFN	1.91±0.27	1	2	14.50	<0.0001***	-	-
WTS	12.27±3.37	6.12	20.27	27.5	<0.0001***	-	-
NPM2	153.05±104.61	2	360	68.40	<0.0001***	-	-
WPM2	193.44±140.13	32	554.67	72.40	0.0002**	-	-

SD, Standard deviation/ Min, observed minimal value; Max, observed maximal value; CV: coefficient of variation, NS, not significant, *significant at p<0.05, **significant at p < 0.001, *** significant at p<0.0001.

cylindrical form of seed; however one genotype (bouch1) had rhomboid shape of seed. Bouch1 is the only genotype that had intense spots on testa. 83% of genotypes have a brownish green color of testa. According to Cupic et al. (2009), use of morphological traits is unavoidable for DUS (Distinctness, Uniformity and stability) testing and in the procedures for protection of varieties.

Quantitative traits

ANOVA analysis

ANOVA analysis revealed the presence of a great genetic variability for all characters studied (Table 5) in concordance with the works of Gixhari et al. (2014), Wani et al. (2013), Khan et al. (2013) and Gatti et al. (2011) who analyzed genetic diversity among different accessions of pea using the same traits and found significant differences .

This diversity might be used in breeding programs (Cupic et al., 2009) by selecting parental lines among accessions (Gatti et al., 2011). Also, the differences were significant for the factor year (except for two parameters which are peduncle length and number of seeds per pod in which non-significant differences were observed) (Table 4). This can be explained by a difference in environment between the three years of experimentation. Indeed, in the first test the soil was silty texture and rich in organic matter (5.53%) as against in the last two test soil (clay loam) was poor in organic matter. Also, monthly cumulative rainfall was an average of 46.3 mm in the first test and 49.89 mm in the second. This result is confirmed by the work of Habtamu and Million (2013) who found

that Ethiopian field pea genotypes were highly influenced by environment. Interaction genotype × year revealed significant differences for parameters AFF and NNFFN, STL, STW, LL, LW, PL and NGP. While the differences were not significant for both parameters PLE and PWI.

Phenological character

Phenology was represented by a single character which is the appearance of the first flower. This character is dependent on the environment. Flowering is considered very late when the number of days between sowing and appearance of the first flower exceed 60 days (Solberg et al., 2015). Thus, all genotypes which were the subject of our study are very late, with an average of 109.25 days. Similar results were obtained by Gatti et al. (2011) who observed the first flower after an average of 105.64 days.

Morphological characters

The highest plant height was taken from genotype p593 (111.33 cm), while p350 showed the lowest (63.66 cm) plant height (Table 6). Researchers obtained lengths varying between 65.67 and 132 cm (Ceyhan and Avci, 2015), 51.20 and 111.30 cm (Georgieva et al., 2016), 65.67 and 126 cm (Khan et al., 2013). On the other hand, the average (63.64 cm) reported by Habtamu and Million (2013) is lower than that obtained in the present work (90.05 cm). Difference in plant height might be due to genetic characteristic of genotypes and adaptability to a particular environment (Khan et al., 2013), especially that this character is dependent on the environment (Solberg et al., 2015).

Table 6. Means of different genotypes.

Code	Demchi1	Bouch1	p539	p593	p595	p596	p069	p071	p072	p073	p350	Sefrou
SL	95.85[cde]	80.15[abc]	109.68[e]	111.33[e]	96.93[cde]	72.95[ab]	89.81[bcd]	87.48[bc]	79.35[abc]	87.6[bcd]	63.66[a]	105.8[de]
AFF	102.66[c]	130.16[e]	102.66[c]	94.66[a]	118.5[d]	94.66[a]	91.33[a]	106[c]	118[d]	101.16[b]	122.16[d]	120[d]
NNFFN	11.49[a]	18.75[gh]	17.46[fg]	18.56[gh]	18.37[gh]	14.52[bc]	15.38[cde]	13.5[b]	16.52[ef]	18.46[gh]	15.22[b]	19.25[h]
STL	5.61[cde]	5.36[bcd]	6.07[e]	6[de]	5.27[bc]	6.2[e]	5.94[de]	5.79[cde]	4.55[a]	4.76[ab]	5.39[bcd]	5.17[abc]
STW	3[cd]	2.55[ab]	3.15[de]	2.96[bcd]	2.62[abc]	3.57[e]	3.07[d]	2.84[abcd]	2.4[a]	2.47[ab]	2.55[ab]	2.51[a]
LL	4.2[def]	3.53[abc]	4.21[def]	3.92[cde]	3.76[bcd]	4.09[def]	4.47[f]	4.33[ef]	3.11[a]	3.4[ab]	3.27[ab]	3.46[abc]
LW	2.37[ef]	1.96[cd]	2.2[def]	1.98[ce]	1.89[cd]	2.4[f]	2.52[e]	2.22[def]	1.45[ab]	1.64[abc]	1.35[a]	1.74[bc]
PL	5.91[bcd]	5.25[ab]	6.92[de]	5.98[bcde]	7.15[de]	6.59[cde]	5.95[bcde]	7.26[e]	5.4[abc]	6.86[de]	4.19[a]	5.17[ab]
NGP	6.84[c]	6.65[c]	7.42[de]	7.46[e]	7.18[cde]	7.09[cde]	6.48[b]	5.77[a]	6.86[cd]	6.66[c]	9.94[f]	6.90[cde]
PLE	5.39[cd]	4.71[ab]	5.19[bcd]	5.35[cd]	5.04[bcd]	4.79[abc]	4.88[abc]	5.49[d]	5.25[cd]	4.43[a]	6.62[e]	5.19[bcd]
PWI	0.81[bc]	0.85[bc]	0.79[b]	0.81[bc]	0.76[b]	0.77[b]	0.82[bc]	0.86[c]	0.78[b]	0.68[a]	0.65[a]	0.79[b]
WTS	13.81[g]	13.81[g]	13.20[fg]	11.61[e]	11.35[de]	10.63[cd]	15.51[h]	18.64i	12.88[f]	8.99[b]	8.04[a]	10.48[c]
NPM2	223.66[de]	247.00[de]	223.33[de]	291.66[e]	171.33[cd]	156.66[bcd]	253.33[de]	86.33[abc]	58.66[ab]	67.00[abc]	2.00[a]	87.00[abc]
WPM2	288.22[def]	266.66[def]	339.55[ef]	408.89[f]	181.33[bcd]	220.44[cde]	261.33[def]	149.33[abcd]	45.33[ab]	58.66[ab]	32.00[a]	96.00[abc]

To get an idea of the variability of stipule and leaflet, we studied traits related to their sizes (length and width of stipule, length and width of leaflet). The results obtained showed a high level of variation. For example, we find that width of leaflet which showed an important CV (25.10%) exhibit averages ranging from 1.35 to 2.52 cm (Table 6).

As regards the size of the pod, genotype p350 had the longest pod (6.62 cm), the lowest pod length was found in genotype p073 (4.43 cm). In general, pod size is a varietal character, but it is also affected by vigor of plant (Khan et al., 2013).

A wide range of variation was noticed for peduncle length (3.10 - 9.75 cm) (Table 5).

Yield characters

In order to estimate yields, we measured the number of grain per pod, weight of 100 seeds, number of pods per 1 m² and weight of pods per 1 m².

For the parameter number of grain per pod, the genotype p350 differed significantly from the other genotypes with an average of 9.94 grain per pod (Table 6) which represented the high value. P071 showed the minimum value (5.77 grain per pod). The results we have obtained are higher than those (2.87 and 5.73 grain per pod) obtained by Ceyhan and Avci (2015). This character can be used in breeding programs to improve yield.

For the weight of 100 seeds, the means varied between 8.04 g (p350) and 18.64 g (p071). The most productive genotype was p593 (Table 6) (408.89 g per 1 m² for weight pods per 1 m² and 291.66 per 1 m² for number of pods per 1 m²). This value is high compared to that obtained by Wozniak (2013) who studied the yielding of pea under different tillage conditions and had results varying between 243 and 320 pods per 1 m².

Correlation matrix

Table 7 represents the correlation coefficients among all the quantitative traits. The appearance of the first flower was significantly and negatively correlated with three characters which are: Stipule width, leaflet length and leaflet width. Characters of yield were significantly and positively correlated with the traits related to size of stipule and leaflet, for example a significant correlation was found between leaflet length and weight of 100 seeds. Number of pods per 1 m² has a positive significant correlation with leaflet width. Weight of pods per 1 m² was correlated with three characters: Stipule length, leaflet length and width. This can be explained by photosynthesis which is more important when the size of stipules and leaflets are large, therefore the yields are higher. Basaran et al. (2012) and Basaran et al. (2013) noted a strong correlation between leaflet length and weight of 100 seeds in grass pea. Number of seeds per pod was negatively correlated to weight of 100 seeds. A negative significant correlation between these two characters was found by Gati

Table 7. Correlation matrix on fifteen traits.

	SL	AFF	NNFFNN	STL	STW	LL	LW	PL	MNFP	PLE	PWI	NGP	WTS	NPM2	WPM2
SL	1.00														
AFF	-0.29[NS]	1.00													
NNFFNN	0.35 [NS]	0.34[NS]	1.00												
STL	0.23 [NS]	-0.59[NS]	-0.33[NS]	1.00											
STW	0.11 [NS]	-0.73*	-0.45[NS]	0.89**	1.00										
LL	0.35 [NS]	-0.70*	-0.49[NS]	0.83**	0.79*	1.00									
LW	0.29 [NS]	-0.65*	-0.45[NS]	0.78*	0.82*	0.94***	1.00								
PL	0.38[NS]	-0.50[NS]	-0.06[NS]	0.28[NS]	0.39[NS]	0.56[NS]	0.50[NS]	1.00							
MNFP	-0.12[NS]	0.14[NS]	0.64	-0.06[NS]	-0.17[NS]	-0.27[NS]	-0.33[NS]	0.05[NS]	1.00						
PLE	-0.25[NS]	0.27[NS]	-0.35	0.07[NS]	-0.14[NS]	-0.16[NS]	-0.37[NS]	-0.52[NS]	-0.11[NS]	1.00					
PWI	0.40[NS]	-0.12[NS]	-0.13	0.39[NS]	0.29[NS]	0.56[NS]	0.64[NS]	0.26[NS]	-0.15[NS]	-0.31[NS]	1.00				
NGP	-0.33[NS]	0.27[NS]	0.04	0.00[NS]	-0.11[NS]	-0.41[NS]	-0.51[NS]	-0.59[NS]	0.08[NS]	0.73*	-0.71*	1.00			
WTS	0.30[NS]	-0.26[NS]	-0.26	0.35[NS]	0.20[NS]	0.69*	0.64[NS]	0.34[NS]	-0.07[NS]	-0.21[NS]	0.82**	-0.69*	1.00		
NPM2	0.57[NS]	-0.44[NS]	0.05	0.61[NS]	0.53[NS]	0.63[NS]	0.70*	0.26[NS]	-0.24[NS]	-0.38[NS]	0.60[NS]	-0.30[NS]	0.45[NS]	1.00	
WPM2	0,59[NS]	-0.49[NS]	-0.03	0.75*	0.64[NS]	0.68*	0.70*	0.29[NS]	-0.24[NS]	-0.22[NS]	0.57[NS]	-0.19[NS]	0.38[NS]	0.95***	1.00

et al. (2011). Stipule length and width, leaflet length and width were correlated between themselves. The same result was obtained by Gatti and al (2011). Number of grain per pod was correlated positively and significantly with pod length. Ali et al. (2007) and Tofiq et al. (2015) found also a significant positive correlation between these two characters. Pod length and width were correlated between themselves. Avci and Ceylan (2006) and Pal and Singh (2012) showed a high positive correlation between these traits.

Principal component analysis

Principal component analysis was performed based on eleven characters (Table 8). The first three principal components (PC) accounted for 85.92% of the variation (56.86, 19.08 and 9.95% for PC1, PC2 and PC3 respectively). The first component was negatively related to STL, STW, LL, LW, PWI, WTS, NPM2 and WPM2, while AFF showed positive correlation.

The second component was associated with NGP. The PLE contributed to the third component. These results are confirmed by Gixhari et al. (2014) who studied PCA on pea and noted that some of these characters as leaflet length and width, number of seed per pod, weight of 1000 seeds and yield per genotype contributed to a great part of variability. In the work of Esposito et al. (2007) on pea genotypes, the two first components explained 67.7% of variability in the first season of experiment and 69.8% in the second one. According to the same author, length and width of stipule, length and width of leaflet, length and width of pod, number of days to flowering explained most of the variability. The study conducted by Umar et al. (2014) on pea

genotypes from different origins showed that the two parameters: Pod length and width are related to the first component which explained 40.29% of variation.

Cluster analysis

As was the case for the principal component analysis (Figure 1), cluster analysis ranged pea genotypes into two different groups (Figure 2). The first cluster which is characterized by a greater yielding (NPM2 and WPM2) includes demchi 1, p069, bouch1, p539, p593, p595 and p596. The second cluster which could be defined as the low-yielding group contains p071, sefrou, p072, p073 and p350.These results were in concordance with the work of Esposito et al. (2007) who identified two groups, the first one contained genotypes with low yielding, the second

Table 8. Principal component analysis (PC) of 12 pea genotypes based on eleven traits.

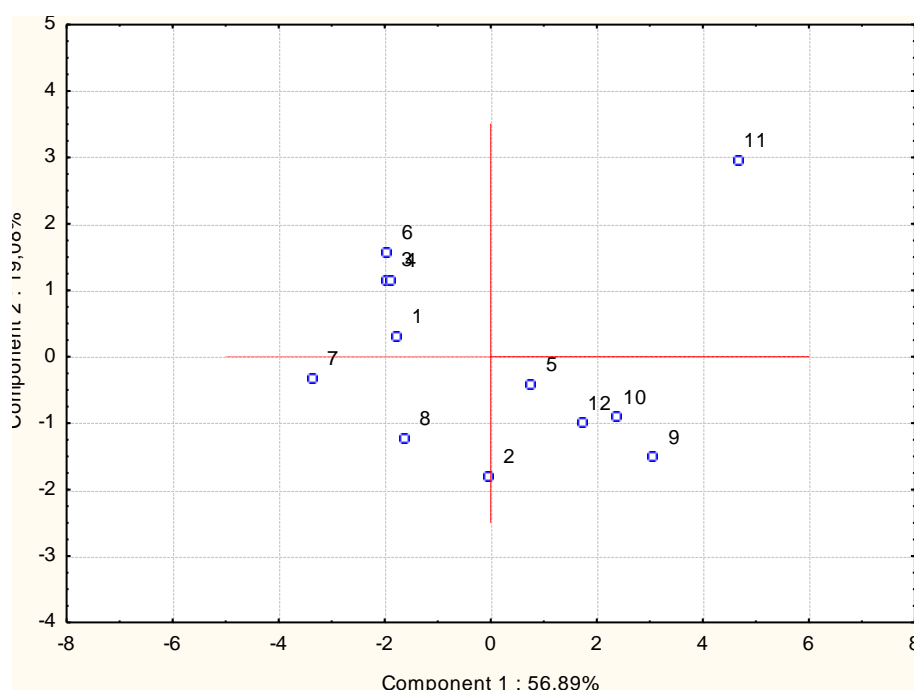| Parameter	PC 1	PC 2	PC 3
Eigen values	6.26	2.09	1.09
% of variance	56.89	19.08	9.95
Cumulative %	56.89	75.97	85.92
Characters	Eigenvector		
AFF	0.681	-0.294	0.477
STL	-0.801	0.521	0.143
STW	-0.787	0.482	0.212
LL	-0.927	0.139	0.072
LW	-0.958	0.021	-0.052
PLE	-0.374	0.584	0.646
PWI	-0.717	-0.498	0.407
NGP	0.531	0.793	0.189
WTS	-0.679	-0.481	0.396
NPM2	-0.817	0.015	0.054
WPM2	-0.828	0.196	0.119

Figure 1. Distribution of pea landraces based on the first two components.

group comprises high yielding genotypes. Two clusters were revealed in the study of Georgieva et al. (2016), one of the two groups includes the low grain yield genotypes, the other group contains high yield genotypes.

Conclusion

The genetic diversity of Algerian pea genotypes was studied using different agro-morphological traits. The results showed the existence of a great variability within the studied genotypes of pea. This variability can be used in the work of selection and improvement is observed on the level of precocity to flowering but also for other qualitative and quantitative traits. On the other hand, expression of characteristics is highly influenced by the environment. Two groups were noted by dendrogram. The first group (demchi 1, p069, bouch1, p539, p593,
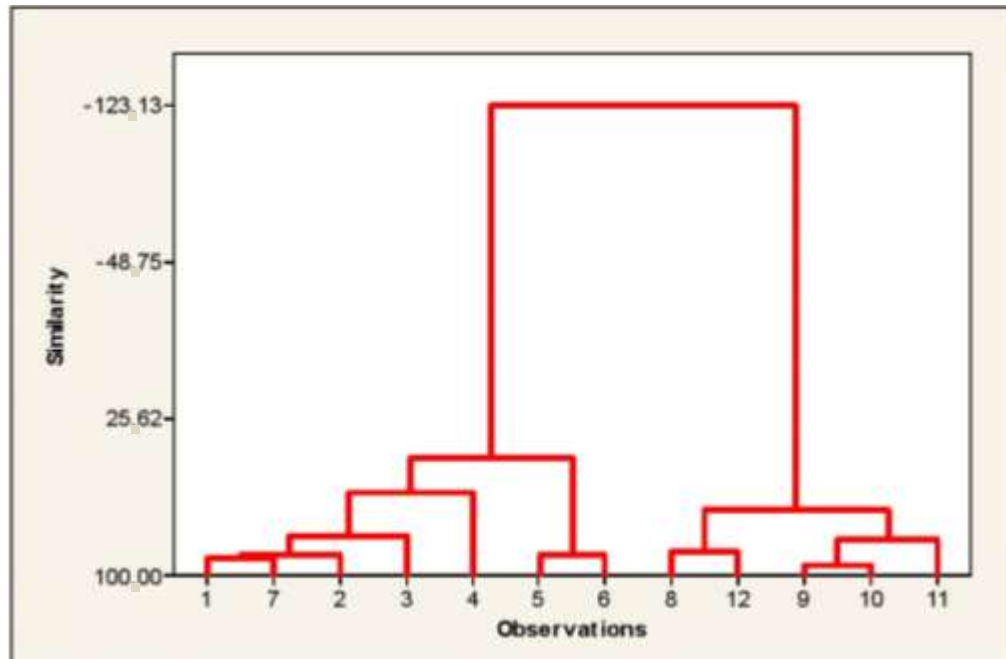
Figure 2. Dendrogram of twelve genotypes based on morphological data.

p595 and p596) was characterized by a high pod yield; the other group comprised less productive genotypes (p071, sefrou, p072, p073 and p350).Otherwise, the genotype p593 produced the best results for pods yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of type of fuel and speed of engine on the performance of agricultural tractor

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Given the need for new alternatives to finite source of energy from fossil fuels, testing of alternative fuels has become quite important. Studies have been carried out using sets of tractor with equipment to evaluate the operational performance together with supplying diesel and biodiesel. Biodiesel is a feasible alternative as it waives adjustments in diesel cycle engines, unlike other clean fuels such as natural gas or biogas, for example. This study aimed at to assess the operational performance and smoke density of a tractor running on diesel and biodiesel, through the parameters of fuel type and engine speed. The assessed engine speeds were 1800, 1900, 2000, 2100, 2200, 2400 and 2600 rpm and the fuel types were diesel B S1800, diesel B S500, soyabean biodiesel and murumuru biodiesel. The results showed that there was an increase in the specific consumption for all fuel types with increasing engine speed, and 1900 and 2000 rpm, mainly for the use of biodiesel speed range that least interferes with the performance. The smoke density was reduced when using soyabean and murumuru biodiesels.

Key words: Biofuel, specific consumption, smoke density, operational performance, engine speeds.

INTRODUCTION

Diesel cycle engines are widely used in agriculture, transport and industry due to their combustion efficiency, reliability, adaptability and cost-effectiveness; however, increasing vehicle fleets have promoted a significant raise in carbon dioxide (CO_2) emissions (Dawody and Bhatti, 2014; Labeckas et al., 2014; Rashedul et al., 2014). Air quality detriment, especially in urban centers, has attracted scientists' attention with a view to proposing solutions and taking mitigation actions against

atmospheric impacts. Brazilian researches on biofuels as energy sources have been assuming major proportions in the recent years (Schirmer and Gauer, 2012). However, most of these investigations are restricted to replacing gasoline and diesel in terms of production and energy equivalence. Thus, studies on biofuels and on greenhouse emission reductions have utter importance as preventive measure for environmental issues. Chemically, biodiesel is an oxygenated fuel consisting of

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long-chain fatty acid which contain 10 to 15% oxygen by weight, deriving from renewable biomass for use in internal combustion engines with ignition by compression, thus being able to replace, partially or completely, petroleum-derived diesel fuel (Can, 2014; Sorate and Bhale, 2015).

A diverse number of raw materials are to be used in biodiesel production, such as vegetable oils and animal fats (Bunce et al., 2010; Lim and Teong, 2010). The oil extracted from peanut, corn, soyabean, palm, cotton, babassu, sunflower seed, castor bean, among many other seeds, almonds or pulps are likely to be considered suitable raw materials for biodiesel production (Tapanes et al., 2013).

It is noteworthy that a molecular oxygen donated to the biodiesel constituting molecules confers the improved burning thereof. With a more efficient burning, lower levels of harmful pollutants are released into the environment, which may include particulate matter (PM), CO, CO_2, volatile organic compounds and total unburned hydrocarbons (Özener et al., 2014).

In addition to low sulphur contents, this fuel has a steadier density range (0.82 to 0.85 gcm^3) and higher cetane number (CN_{min} = 46). As vehicle benefits, one can mention improved cold starting, reduced engine deposits and less lubricant contamination, aside from lower environmental emissions of sulphur (up to 90%) and particulate matter (Silveira, 2013).

Performance of engine when running on biodiesel or blends with diesel largely depends on the combustion air turbulence, air-fuel mixture quality, injector pressure, actual start-of-combustion, among others. Furthermore, it may vary with biodiesel source quality and conditions, as well as engine operating parameters as speed, load, etc. Biodiesel use in agricultural tractors can be assessed by determining engine power, torque, fuel consumption and gas emissions (Harch et al., 2014). Nagi and Nagi (2008) tested a model of four-stroke diesel engine (DWE–47–50–HS–AV) fueled with palm biodiesel at a maximum torque of 3200 rpm. Testing was monitored at 650, 1000, 1350, 1700 and 2050 rpm measuring time spent to consume each 100 mL fuel. Based on that, the authors asserted that palm biodiesel consumption takes longer time and provided lower specific consumption if compared to diesel (16.8%). Therefore, it may be assumed that varied engine working speeds and types of fuel (diesel or biodiesel) can affect tractor operational performance and smoke opacity. Considering this scenario, this study aimed to assess the tractor performance, fuel consumption and smoke opacity of different engine speeds and fuel types.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Biofuel and Machines Tests (BIOEM) of the Bioenergy Research Institute (IPBEN, FCAV/UNESP), Jaboticabal, SP, Brazil. The area is located laterally to the road path *Via de Acesso Prof. Paulo Donato*

Castellane, km 5, at the geographical coordinates of 21° 15' S and 48° 18'W, with an average altitude of 570 m. The area has an average annual temperature of 22.2°C, average annual rainfall of 1425 mm, average relative humidity of 71% and atmospheric pressure of 94.3 kPa. According to Köppen, local climate is classified as *Aw* type, which stands for tropical humid with rainy summers and dry winters (UNESP, 2015).

Local soil was classified as a typical eutro ferric Red Latosol (Oxisol) on a flat to gently wavy relief (3% slope), according to the Brazilian System of Soil Classification (Nagi and Nagi, 2008). Soil moisture contents during chisel-plow pilot testing, measured by standard gravimetric method, at the depth ranges of 0 to 15 cm and 15 to 30 cm were 11.2 and 13.4%, respectively. The particle size analysis of soil samples taken from 0 to 20 cm depth range showed rates of 51, 29, 10 and 10% for clay, silt, fine sand and coarse sand, respectively, that is, a clayey textured soil.

Two types of biodiesel were used: refined soybean (*Glicinemax* L.) and refined murumuru (*Astrocaryum murumuru* MART). Biofuels were produced and supplied by our partner LADETEL (Laboratory of Clean Technology Development), São Paulo University (USP), campus in Ribeirão Preto - SP, Brazil. The fossil diesels used were B S1800 and B S500, being respectively purchased in Jaboticabal - SP and São Paulo - SP, Brazil, with maximum total sulphur of 1800 and 500 mg kg^{-1}, and specific masses of 860 and 840 kg m^{-3}, respectively, according to ANP resolution n° 42/ 2009 (ANP, 2009).

The testing tractor was a Valtra, model BM 125i, 4×2 with front wheel assist (FWA), maximum engine power of 91.9 kW (125 hp) at 2300 rpm (ISO1585). The tractor was equipped with turbo charger and intercooler, total mass of 7000 kg, distributed 40% and 60% in the front and rear axles, respectively, mass/power ratio of 76 $kgkW^{-1}$ (56 $kghp^{-1}$), and 14.9–26 front tires and 23.1–30 rear tires, calibrated according to the manufacturer's recommendation. The braking tractor was a Valmet, model 118–4, 4×2 with front wheel assist (FWA), engine power of 82.43 kW (112 hp) at 2400 rpm, total mass of 7310 kg, distributed 40 and 60% respectively in the front and rear axles, and equipped with 14.9–28 front tires and 23.1–30 rear tires.

Performance was analyzed with a testing tractor Valtra BM 125i instrumented with load cell, slippage meter, radar unit, data acquisition system and a prototype meter of fuel consumption containing three auxiliary tanks for biodiesel, as described by Lopes (2006). From test to another, unconsumed biodiesel was drained from tanks, filters and pipes, in order to avoid contamination of the next test.

The study was divided into two stages. The first one consisted of a dynamic test carried out under field conditions to assess tractor performance, allotted in a completely randomized design, arranged in a 4 × 7 factorial scheme and with three replications, totaling 84 observations. The second composed a static test with the vehicle at rest, aiming to assess its engine smoke opacity, which was carried in a completely randomized design, with 4 types of fuels and 12 replications, totaling 48 observations. The fuels used were soybean and murumuru biodiesel (B100) and diesel B S1800 and B S500 (B0), in addition to seven engine speeds (1800, 1900, 2000, 2100, 2200, 2400 and 2600 rpm). For the performance test, each plot had 40 m in length and from one to another plot, at the longitudinal direction, there was a space of 15 m for conducting maneuvers, machinery traffic and stabilization of the moto-mechanization set in each treatment.

The braking tractor was coupled to the test tractor by means of a steel wire, forming a train. Preliminary testing, so-called pilot, was developed to set the maximum loading technically feasible to be pulled by the test tractor. To achieve that, gear combinations were tried in the braking tractor, thus reaching a workforce of nearly 25 kN. This tractor remained powered off and geared since its only function was to provide a uniform load to the tractor drawbar. The working speed was achieved with a gear combination in fourth L.

In all plots, test tractor started moving 15 m before the first pole

marking the beginning of measurements, aiming at assessment standardization. Data acquisition system was activated at the time when the rear wheel center (referential) overlapped the first pole, being switched off as tractor went through 40 m along the experimental plot, at which rear wheel center overlapped the second pole.

Fuel consumption was measured in each plot in terms of volume spent (mL), obtaining the total volume supplied to the inlet pump injection and the total volume returned. The fuel consumed was measured by the difference between these two measurements.

Based on consumed volume and driving time in each plot, hourly consumption was determined according to Equation 1:

$$HC = \left(\frac{Sv - Rv}{t}\right) \times 3.6 \qquad (1)$$

Wherein: HC is the hourly consumption (L h^{-1}), Sv is the supplied volume (mL), Rv is the returned volume (mL), t is the driving time in the plot (s) and 3.6 is a conversion factor.

The time-weighted hourly consumption was calculated considering the supplied and the return fuel densities at the testing time, according to Equation 2:

$$HC_w = \left(\frac{Sv \times Dsf - Rv \times Drf}{t}\right) \times 0.0036 \qquad (2)$$

Wherein: HC$_w$ is the time-weighted hourly consumption (kg h^{-1}), Sv is the fuel supply volume (mL), Dsf is the supply fuel density (kg m^{-3}), Rv is the fuel returned volume (mL), Drf is the returned fuel density (kg m^{-3}), t is the driving time in the plot (s) and 0.0036 is a conversion factor.

The specific consumption, which is the fuel consumption expressed in mass unit per power unit required in the drawbar, was calculated according to Equation 3:

$$SC = \frac{WHC}{PD} \times 1000 \qquad (3)$$

Wherein: SC is the specific consumption (g kW h^{-1}), HC$_w$ is the weighted hourly consumption (kg h^{-1}), PD is the power on drawbar (kW) and 1000 is a conversion factor.

The smoke opacity test was performed by applying a snap idle test, in which engine rotation speed reaches a full-throttle acceleration, and developed power is absorbed only by the inertia of the mechanical engine components (clutch, gearbox primary shaft), since vehicle is parked (SAE, 1996). Measurements were determined in the BM125i Valtra testing tractor, and results were given in K, which is the light absorption coefficient in m^{-1}, as the manufacturer's manual (Tecnomotor). At the end of each determination, supply system was fully drained out to avoid contamination of incoming tests. Moreover, after refueling, engine had operated for ten minutes prior to each test started.

The data underwent variance analysis and means were compared by the Tukey's test at 5% probability, as recommended by Banzatto and Kronka (2006). A most suitable regression adjustment model was set for fuel specific consumption. Moreover, a response surface model was adjusted to explain fuel density as a function of temperature and fuel type. The variance analysis (F-test) was applied to select an equation model with higher significant exponent.

RESULTS AND DISCUSSION

There was no interaction between fuel type and engine speed for volumetric fuel consumption (Table 1). However, for fuel type, the soybean biodiesel presented a higher consumption, which increased 15.4% when compared to the diesel B S500. This increase is due to the lower calorific value of biodiesel compared to diesel, i.e., it is necessary a higher fuel supply to accomplish the same amount of work. These above-cited results are similar to those found by Lima et al. (2012), who evaluated a tractor engine (Valtra BM110) equipped with turbo charger, running with diesel at total sulphur level of 1800 mg kg^{-1}, and with palm and tucuman biodiesels. They observed an HC increase of 23.0% from biodiesel B100 to B0 that was related to the lower calorific power of palm- and tucuman-produced biodiesels against diesel, which could require more petrol to accomplish the same amount of work. According to Uzun (2010) and Neves et al. (2013), a turbocharger intercooler engine helped diminishing diesel consumption from reduction rates of 3 to 12%, which could also be used for biodiesel owning to their chemical and physical similarities. Analysis of Table 1 highlights that weighted consumption and specific consumption interaction was significant; therefore, these variables were further assessed using two complementary tables of breakdown of interactions (Tables 2 and 3). It is noted that, for the factor fuel type (in the line), the weighted consumption was lower at 1800 rpm for diesel B S1800 and B S500, but did not differ from results at 1900 rpm. On the other hand, soybean and murumuru biodiesel consumptions had no difference at 1900 and 2000 rpm (Table 2), although the lowest weighted consumption was observed at 1800 rpm.

Regarding engine speed in the column of Table 2 it was shown that weighted consumption was lower for B S500 diesel in comparison to soybean biodiesel at all assessed speeds, with the lowest consumption observed at 1800 rpm (21.1%). According to Murugesan et al. (2009) and Tabile et al. (2009), such an outcome can be explained by the lower calorific power and increased biodiesel density compared to the diesel. This measure is relevant for workers when performing fuel distribution, because the amount of mass leaving origin should be the same reaching its destination. Table 3 displays an increasing specific consumption for all fuel types (in the line) as engine speed increased; however, the lowest one was reached at 1800 rpm, using B S1800 and B S500 diesels (30.7 and 35.6%, respectively) whether compared to 2600 rpm speed. Concerning biodiesel use, consumptions were least at 2000, 1900 and 1800 rpm for soybean biodiesel, not differing from each other, and at 1900 and 1800 rpm for murumuru, not differing from each other (Table 3). By observing engine speed in the same Table (in the column), one can verify low specific consumption for B S500 diesel at all studied speeds; emphasizing 1800 rpm, which had a reduction of 27.8% compared to soybean biodiesel. Conversely, at 2100, 2000 and 1900 rpm, this diesel type consumption did not differ from the murumuru biodiesel (Table 3).

Table 1. Means of volumetric hourly consumption (HC), weighted hourly consumption (HC_w) and specific consumption (SC) for four types of fuel and seven engine speed rates.

Factors	HC $L\ h^{-1}$	HC_w $kg\ h^{-1}$	SC $g\ kWh^{-1}$
Fuel type (FT)			
Diesel B S1800	14.4 [a]	12.4	311
Diesel B S500	12.6 [c]	10.9	259
Soybean biodiesel	14.9 [a]	13.2	332
Murumuru biodiesel	13.4 [b]	11.8	272
Engine speed (ES)			
1800 rpm	9.7 [a]	8.5	250
1900 rpm	10.7 [ab]	9.4	262
2000 rpm	11.7 [bc]	10.2	271
2100 rpm	12.7 [c]	11.1	281
2200 rpm	14.0 [d]	12.2	240
2400 rpm	17.1 [e]	14.7	329
2600 rpm	21.0 [f]	18.4	366
F-TEST			
FT	24.9**	43.3**	827.2**
ES	222.4**	302.4**	698.2**
FT × ES	1.6[NS]	2.5**	11.5**
CV (%)	6.7	5.7	1.8

Means followed by the same letter in the columns do not differ from each other by the Tukey's test at 5% probability. **: Significant at 1% ($p < 0.01$); *: Significant at 5% ($p < 0.05$); NS: non-significant; CV: coefficient of variation.

Table 2. Breakdown of the interactions between fuel type and engine speed for weighted hourly consumption ($kg\ h^{-1}$).

Fuel type	Engine speed (rpm)						
	1800	**1900**	**2000**	**2100**	**2200**	**2400**	**2600**
Diesel B S1800	8.5 [ABa]	9.5 [ABab]	10.8 [ABbc]	11.8 [Acd]	13.2 [Ad]	15.7 [Ae]	17.3 [Be]
Diesel B S500	7.1 [Ba]	8.7 [Bab]	9.3 [Bbc]	10.1 [Bbc]	10.9 [Bc]	13.1 [Bd]	16.9 [Be]
Soybean	9.0 [Aa]	10.3 [Aab]	11.0 [Aab]	12.0 [Abc]	13.2 [Ac]	16.0 [Ad]	20.2 [Ae]
Murumuru	8.5 [ABa]	9.0 [ABab]	9.7 [ABab]	10.6 [ABbc]	11.6 [Bc]	13.9 [Bd]	19.4 [Ae]

Means followed by the same uppercase letter in the columns and lowercase letter in the rows do not differ from each other by the Tukey's test at 5% probability.

Table 3. Breakdown of the interactions between fuel type and engine speed for specific hourly consumption ($g\ kWh^{-1}$).

Fuel type	Engine speed (rpm)						
	1800	**1900**	**2000**	**2100**	**2200**	**2400**	**2600**
Diesel B S1800	255 [Ba]	271 [Ab]	286 [Bc]	307 [Ad]	320 [Ad]	368 [Ae]	367 [Be]
Diesel B S500	213 [Da]	234 [Cb]	242 [Cc]	250 [Bcd]	258 [Cd]	285 [Ce]	331 [Df]
Soybean	295 [Aa]	304 [Aab]	304 [Aab]	310 [Ab]	324 [Ac]	366 [Ad]	418 [Ae]
Murumuru	237 [Ca]	243 [Cab]	252 [Cb]	256 [Bb]	273 [Bc]	297 [Bd]	347 [Ce]

Means followed by the same uppercase letter in the columns and lowercase letter in the rows do not differ from each other by the Tukey's test at 5% probability.

Interestingly soybean biodiesel had an increased   specific consumption at all assessed speeds, except for
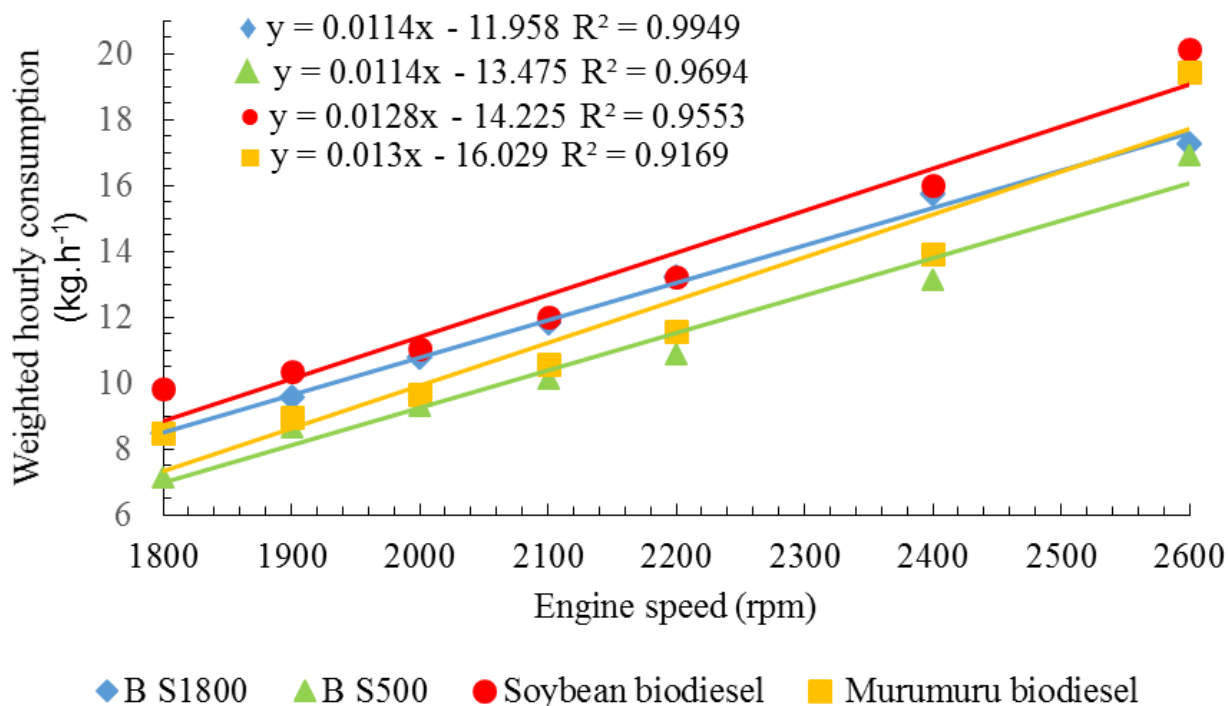
Figure 1. Weighted hourly consumption as a function of engine speeds and fuel types.

2400, 2200 and 2100 rpm, not differing from B S1800 diesel. It might have been due to both higher density and lower calorific power of biodiesel. The outcomes evidenced a rising specific consumption as engine speed was increased, for all fuel types; nonetheless, less performance interference was noted at 1900 and 2000 rpm, mainly when using biodiesel. Almeida et al. (2010), studying energy performance of a tractor-precision seeder system under different gears and engine speeds, also found similar results of fuel consumption as the ones presented here. These authors concluded fuel consumption is lower at low engine and driving speeds, and a maximum specific consumption was achieved at 2200 rpm using fourth gear.

Studying a tractor-seeder-fertilizer energy demand in no-till system as a function of driving and engine speeds (2100, 1800 and 1500 rpm), Silveira et al. (2013) concluded that the lowest specific fuel consumption was obtained at higher operating speeds and at a low engine speed (1500 rpm).

In contrast, Correia et al. (2015), assessing the operational performance of a tractor harrowing a clayey soil at diverse engine working speeds, observed that the highest working speed (2100 rpm) provided lower fuel consumptions and higher field capacity.

Science community has widely used the specific consumption as a measure to compare treatments, once it takes into account the amount of fuel consumed, developed power and product density. Figures 1 and 2

clearly demonstrates that time-weighted hourly consumption and specific consumption means had a linear behavior with regards to the four fuel types and at all engine speeds. It is noteworthy mention that soybean and murumuru biodiesels provided a smoke opacity reduction of 37 and 60%, respectively, if compared to B S1800 and B S500 diesels (which did not differ from each other) (Table 4). Smoke opacity reduction is representative and friendly to the use of biodiesel, which is partially explained by the absence of sulphur in its composition. Moreover, the presence of free oxygen in biodiesel molecule (reduced fuel-rich zones inside combustion chamber and increased yield during diffusive combustion), increasing combustion efficiency and reducing considerably the production of particulate matter (Sahoo et al., 2009; Chauhan et al., 2012). Biodiesel burning in diesel engines significantly reduces the emissions of particulate matter compared to diesel (Bora and Baruah, 2012).

Conclusions

1) Biodiesel from soybean and murumuru oils had no effect on engine performance during the tests.
2) Soybean biodiesel showed an increased volumetric fuel consumption of 15.4% if compared to B S500 diesel.
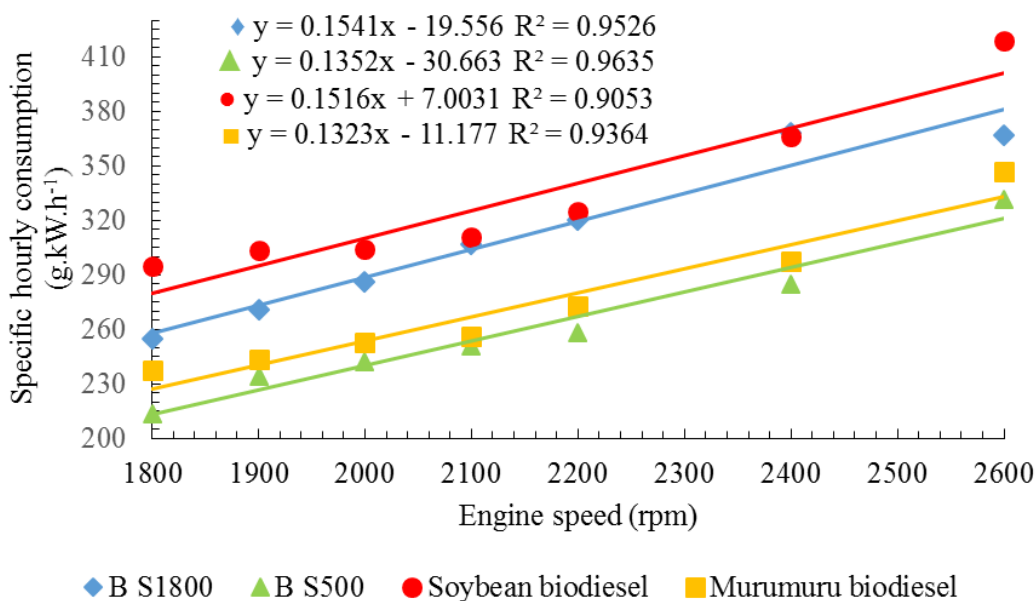3) Weighted consumption for B S500 diesel was lower than that observed for soybean biodiesel, at all the

Figure 2. Specific consumption as a function of engine speeds and fuel types.

Table 4. Summary of the analysis of variance and mean test for smoke opacity.

Fuel type (FT)	Opacity (m^{-1})
Diesel B S1800	2.65 [a]
Diesel B S500	2.56 [a]
Soybean biodiesel	1.67 [b]
Murumuru biodiesel	1.05 [c]
F-TEST	
FT	975.74 **
CV (%)	2.13

Means followed by the same letter do not differ from each other by the Tukey's test at 5% probability. **: Significant at 1% ($p < 0.01$); *: Significant at 5% ($p < 0.05$); NS: not significant; CV: coefficient of variation.

assessed speeds, with the lowest value reached at 1800 rpm (21.1%).

4) Growing specific consumption was observed, for all fuel types, as engine speed was increased, especially for B S500 diesel at 1800 rpm, which had a 27.8% reduction whether compared to soybean biodiesel.

5) Smoke opacity was reduced by 37 and 60% using soybean and murumuru biodiesels, respectively, when contrasted with B S1800 and B S500 diesels.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Smoke opacity of ethyl biodiesel from babassu and two types of diesel at different daytimes

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Renewable energy benefits and disadvantages have been subject of major discussions and studies. Thus, this study aimed at assessing the smoke opacity from a farming tractor running two types of diesel, B S1800 (B0) and B S10 (B0), and ethyl biodiesel from babassu oil at two rates (B50 and B100), at six day periods (2, 6, 10 am, 2, 6 and 10 pm). The study was conducted at the Department of Agricultural Engineering of the Faculty of Agricultural and Veterinary Sciences, UNESP, in Jaboticabal - SP, Brazil. The results showed a reduced smoke opacity during daytimes of lower ambient temperature and higher air relative humidity. In addition, smoke opacity was reduced as higher rates of babassu biodiesel were added to B S1800 diesel and B S10; thus, consisting of an efficient procedure of reducing smoke opacity in farming tractor engines.

Key words: Biofuel, emissions, pollutants, renewable energy, diesel engine cycle, *Orbinya martiana.*

INTRODUCTION

Biodiesel use in compression ignition engines has increased substantially in the recent decades, mainly because it is a sulphur-free fuel in contrast to fossil diesel. Moreover, it is a renewable vegetal source that may be a carbon cycle contributor (Silitonga et al., 2011; Mofijur et al., 2012; Zhou et al., 2012). It is worth mentioning, biodiesel production importance in raising farm income, enhancing environment by reducing greenhouse gas emissions, developing economy, optimizing and decentralizing investments, as well as promoting social development through job and income generations in rural areas (De Gorter and Just, 2010).

Fossil fuel is a scarce resource and, when burnt, releases harmful gases to the atmosphere. Thus, it is important to search for alternative energy sources replacing, even partially, such fossil fuels and face the

challenge of growing demands for sustainable energy, thus reducing environmental impacts (Caland et al., 2009).

In Brazil, fossil fuels have been on agenda of government leaders, mainly because of environmental issues resulting from their increased demand. For the transportation industry of goods and people, three types of diesel are available, serving up to 46.4% of this sector. These products are B S500 (500 mg kg^{-1} of sulphur) and B S50 (50 mg kg^{-1} of sulphur), which were launched by Petrobras in 2009. Apart from these, B S10 diesel (10 mg kg^{-1} of sulphur) was introduced and regulated into domestic market in 2012, motivated by the implementation of increasingly stringent limits on pollutant emissions from circulating vehicles (ANP, 2013). Because of its great similarity with diesel, in terms of chemical structure and energy content, biodiesel is compatible with engines running on diesel and, therefore, no modifications are required (Lam et al., 2009). This fuel is featured by owning a mixture of alkyl esters of long chain fatty derived from different raw materials, including vegetable oils, animal fats and algae lipids by transesterification reaction with alcohols. The properties of biodiesel derivatives from different raw materials can vary according to the composition of fatty acids (Pehan et al., 2009; Hoekman et al., 2012; Oliveira et al., 2015). To ensure biodiesel quality, it is necessary to follow the national regulatory standards of biodiesel.

Among the sources of raw material for biodiesel production, it can be mentioned that the babassu (*Orbinya martiana*), a Brazilian native palm is widely distributed in the northeastern (largest producer), northern, central and western of the country (About 196.000 km² at Brazilian territory), although it can also be found in Mexico and Bolivia (Silva et al., 2014). The crude oil, babassu is easily obtained by way of crushing the fruit kernel, which constitute about 65% by almond weight, been used for biodiesel production since its composition increase the lauric fatty acid (about 44%)(Oliveira et al., 2013).

Many studies, such as those assessing smoke opacity in farm tractors running several raw materials, have enabled biodiesel use in diesel engines. These studies have shown decrease in smoke opacity levels as the rate of biofuels increases (Zhu et al., 2010). Comparing B0 with B100, Chauhan et al. (2013) tested a diesel engine cycle with karanja-oil biodiesel and found lower smoke opacity as amounts of biodiesel for the diesel increased. Likewise, Tabile et al. (2009) evaluated rates of castor-oil biodiesel versus B S2000 and B S500 diesels, and observed that by increasing the amount of biodiesel up to a B75 rate, opacity was reduced; yet when they compared B0 and B75, reduction reached 22.0 and 10.6% for B S2000 and B S500, respectively.

It is assumed that air relative humidity and ambient temperature changes, as well as varied rates of biodiesel blends in diesel, may influence smoke opacity response in the tractor engine. Given the above-mentioned, this study aimed to assess the smoke opacity emitted from a farm tractor running B S1800 diesel (B0) and B S10 (B0) mixed with babassu biodiesel at two distinct rates, 50 and 100% (B50 and B100, respectively) at six periods of the day (2, 6, 10 am, 2, 6 and 10 pm).

MATERIALS AND METHODS

This study was conducted at the Laboratory of Biofuel and Machines Tests (BIOEM) of the Bioenergy Research Institute (IPBEN, FCAV/UNESP), Jaboticabal, SP, Brazil. This laboratory is located at the geographic coordinates of 21° 14' 26.25" S and 48° 17' 13.22" W, and at average altitude of 570 m. The region has an average annual temperature of 22.2°C, average air relative humidity of 71% and atmospheric pressure of 94.3 kPa. According to the Köppen's classification, local climate is rated as an *Aw* type, which stands for a tropical humid with rainy summers and dry winters.

Diesel types used in this experiment were B S1800 (B0) and B S10 (B0), which had been regulated in the domestic market by the Program of Air Pollution Control by Motor Vehicles (PROCONVE). According to the ANP resolution no. 42/ 2009 (ANP, 2009) and ANP resolution no. 46/ 2012 (ANP, 2012), these fuels have total sulphur amounts of 1800 and 10 mg kg^{-1}, respectively. Both of them were purchased in Jaboticabal City, SP, Brazil.

In addition to these diesels, ethyl biodiesel distilled from babassu (B100) was used, being manufactured by our partner, the Laboratory of Clean Technology Development (LADEL), which is in Ribeirão Preto city, SP, Brazil. As blend rate B50, 50% of B S10 diesel and 50% of ethyl babassu biodiesel were used, being mixture by measuring cylinders of 500 and 250 mL, funnel and containers.

Measurements were carried out in a Valtra tractor, model BM 125i, 4 × 2 with front wheel assist (FWA), working at a maximum engine power of 91.9 kW (125 hp) at 2300 rpm (ISO1585). The machine was equipped with turbocharger and intercooler, reaching a total mass of 7000 kg distributed as 40 and 60% in the front and rear axles, respectively, and a mass/power ratio of 76 kg kW^{-1} (56 kg hp^{-1}).

Two experiments were conducted in a completely randomized design, arranged in a 6 × 3 factorial scheme with 18 treatments and 3 repetitions, totaling 54 results for each experiment. It is noteworthy that, in compliant with the smoke opacity testing, each repetition consisted of seven replications, which is based on a principle that the difference between the highest and lowest readings of each replication could not exceed 0.25 m^{-1}.

In this test, the number of samples may vary between seven and ten, being set at the testing time, since the equipment itself manage the process in order to achieve result homogeneity. Such uniformity might be influenced by engine and fuel conservation state, besides environmental conditions (temperature, pressure and air humidity), among others.

The three biodiesel/diesel blends were assessed at six distinct daytimes (2, 6, 10, 2, 6 and 10 pm). The first experiment was composed of B S1800 diesel (B0), 50% B S1800 diesel + 50% biodiesel (B50) and 100% babassu biodiesel (B100). Yet, the second was composed of B S10 diesel (B0), 50% B S10 diesel + 50% biodiesel (B50) and 100% babassu biodiesel (B100).

Local temperature and air relative humidity were obtained from a meteorological station located at UNESP (Jaboticabal, SP), which is near the experimental area. The records of these variables for all daytimes assessed are shown in Table 1.

Table 1. The records of these variables for all daytimes assessed.

Times of trial (h)	Room temperature (ºC)	Relative humidity (%)
2	14,7	72
6	13,9	95
10	22,4	60
14	28,4	27
18	25,2	42
22	18,0	63
Standart deviation	10.20	23.63

Engine smoke opacity was measured by a partial flow opacimeter (Tecnomotor, model TM 133), which measures light absorption and follows the standards proposed by the NBR 13037 of INMETRO and CEE 72/306. A serial controller was also used for the communication of the vehicle inspection equipment through a serial port to the microcomputer and a software of vehicle inspection called IGOR®.

Tests were performed according to the snap idle test, which consists of submit engine to a full-throttle acceleration for 3 to 5 s, with the power developed absorbed only by the inertia of the mechanical engine components (clutch, gearbox primary shaft), since the tractor was static, as described in the NBR 13037 (ABNT, 2001). Opacity measurement results were given in K, which is the light absorption coefficient in m^{-1} (TECNOMOTOR, 2012).

Biodiesel was mixed to the diesel by the time of the test. From one test to the other, all unconsumed fuel was removed out of tanks, filters and pipes avoiding next test contamination. For test standardization, after refueling, engine was operated for ten minutes prior to each test start.

Data underwent variance analysis and means were compared by the Tukey's test at 5% probability, and the Kolmogorov normality test - Smirnov and ANOVA.

RESULTS

According to the Tables 2 and 3, concerning smoke opacity, there was a significant interaction between fuel type and daytime. As a result, those variables underwent an interaction breakdown analysis, which are shown in Tables 4 and 5.

It can be observed that B S10 diesel (B0) showed higher opacity values when compared with B50 and B100 mixture for all times (Table 4), differing from the other fuel types. Smoke opacity decreased as the amount of biodiesel in the mixture increased, within which babassu biodiesel (B100) stood out, presenting a significant reduction of 51.82 and 54.21%, as compared to B S10 at 6 am and 2 pm, respectively.

It can be stated that running biodiesel in diesel engines instead promotes significant reduction in particulate emissions (Ong et al., 2011; Xue et al., 2011; Bora and Baruah, 2012). Moreover, Table 4 shows that opacity levels would be within the limit of 2.5 m^{-1} defined by the CONAMA resolution number 251/1999 (CONAMA, 1999). This outcome can be explained by the fact that biodiesel

Table 2. Summary of the values of analysis of variance and mean test for smoke opacity.

Factors	Smoke opacity (m^{-1})
Fuel type (FT)	
B S10 diesel B0	2.12
Biodiesel B50	1.62
Biodiesel B100	1.11
Time (T)	
2:00 am	1.57
6:00 am	1.47
10:00 am	1.73
02:00 pm	1.85
06:00 pm	1.59
10:00 pm	1.49
F-test	
FT	3964.0309 **
T	162.9397 **
FT x T	31.0043 **
CV (%)	4.18
Standart deviation	0.068

*Significant at 5% (P<0.05); **significant at 1% (P<0.01); CV: coefficient of variation.

have no sulphur within its composition, aside from presenting free oxygen in its molecule (reduced fuel-rich zones inside combustion chamber and increased yield during diffusive combustion). Therefore, combustion efficiency is increased and particulate matter production is reduced substantially (Sahoo et al., 2009; Chauhan et al., 2012).

Investigating emissions from diesel engine running on rapeseed oil (B100) and mixtures B5, B20 and B70 and comparing them with those from diesel-run ones, Buyukkaya (2010) emphasized that biodiesel has less opacity (up to 60%) as compared to the diesel. When biodiesel is added to diesel, fuel oxygen contents are increased, thus, less oxygen is demanded for combustion.

Table 3. Summary of the values of analysis of variance and mean test for smoke opacity.

Factors	Smoke opacity (m^{-1})
Fuel type (FT)	
B S1800 diesel	2.21
Biodiesel B50	1.79
Biodiesel B100	1.11
Time (T)	
2:00 am	1.66
6:00 am	1.51
10:00 am	1.82
02:00 pm	1.93
06:00 pm	1.68
10:00 pm	1.64
F-test	
FT	6630.2308 **
T	208.4222 **
FT x T	27.3399 **
CV (%)	3.38
Standart deviation	0.058

*Significant at 5% (P<0.05); **significant at 1% (P<0.01); CV: coefficient of variation.

Table 4. Summary of interaction breakdown analysis of fuel type (B0 = 100% diesel B S10 + 0% babassu biodiesel; B50 = 50% diesel B S10 + 50% babassu biodiesel; B100 = 0% diesel B S10 + 100% babassu biodiesel) against daytime for smoke opacity (m^{-1}).

Fuel type	Daytime (h)					
	2 am	6 am	10 am	2 pm	6 pm	10 pm
B S10 diesel (B0)	2.00^{Aa}	1.93^{Aa}	2.23^{Ac}	2.49^{Ad}	2.08^{Ab}	1.99^{Aa}
Biodiesel B50	1.54^{Bb}	1.56^{Bb}	1.74^{Bc}	1.91^{Bd}	1.56^{Bb}	1.41^{Ba}
Biodiesel B100	1.19^{Ccd}	0.93^{Ca}	1.21^{Cd}	1.14^{Cbcd}	1.13^{Cbc}	1.08^{Cb}

Means followed by the same uppercase letter in the columns and lowercase letter in the lines do not differ from each other by the Tukey's test at 5% probability.

Table 5. Summary of interaction breakdown analysis of fuel type (B0 = 100% diesel B S1800 + 0% babassu biodiesel; B50 = 50% diesel B S1800 + 50% babassu biodiesel; B100 = 0% diesel B S1800 + 100% babassu biodiesel) against daytime for smoke opacity (m^{-1}).

Fuel type	Daytime (h)					
	2 am	6 am	10 am	2 pm	6 pm	10 pm
B S1800 diesel	2.10^{Aab}	2.04^{Aa}	2.31^{Ad}	2.48^{Ae}	2.17^{Ac}	2.15^{Abc}
Biodiesel B50	1.71^{Bb}	1.59^{Ba}	1.89^{Bc}	2.12^{Bd}	1.75^{Bb}	1.69^{Bb}
Biodiesel B100	1.19^{Ccd}	0.91^{Ca}	1.23^{Cd}	1.14^{Cbc}	1.13^{Cbc}	1.08^{Cb}

Means followed by the same uppercase letter in the columns and lowercase letter in the lines do not differ from each other by the Tukey's test at 5% probability.

However, the ratio oxygen/fuel is the main reason for a fullest combustion and, therefore, resulting in reduced amounts of pollutant emitted (Ghobadian et al., 2009; Reis et al., 2013). Biodiesel properties are influenced

by inherent characteristics of fatty esters composed of the fuel (Lôbo et al., 2009).

Table 4 shows smoke opacity increases of 29.01 and 22.43% for B0 and B50 between the time at which was observed the lowest value (6 am, 13.9°C and 95%) and the time at which the highest one (2 pm, 28.4°C and 27%) was registered. Data recorded at 2 pm showed difference when compared with the other times. For B100, opacity increased (22.58%) at a different time (10 am, 22.4°C and 60%) from that recorded for B0 and B50, differing from 6 am, 6 and 10 pm records. Furthermore, it was observed that the opacity values of B0, B50 and B100 were low at low temperature and high air humidity. According to Lopes et al. (2009), milder ambient temperature and high humidity may improve engine combustion.

The higher opacity registered for babassu biodiesel (B100), at a different time from that recorded for B0 and B50, might have been related to physicochemical properties, which are influenced by inherent characteristics of fatty esters composed of the fuel (Lôbo et al., 2009; Dabdoub et al., 2009).

Through a daytime assessment in Table 5, values of smoke opacity were higher for B0 related to B50 and B100 for all assessed hours, standing out from all the other fuel types. Additionally, smoke opacity decreased as the amount of biodiesel increased, especially for B100, which presented a significant reduction mainly at 6 am and 2 pm, displaying reduction rates of 55.4 and 54.0%, respectively, as compared to B S1800.

DISCUSSION

These results reinforce those reported by Louzeiro (2012), who observed a decrease in CO, NOx and opacity using micro-emulsion formed by oxygenated substances (babassu oil), brandy and isobutyl alcohol, comparing them with the diesel. This behavior was also observed by Lima et al. (2013), who obtained a reduction of 36.25 and 60% in smoke opacity for a farm tractor engine running on blends of palm and tucuman biodiesels, respectively.

Similar results were also obtained by Neves et al. (2013), who observed a reduction of 26.1 and 53.5% when useing increasing proportions of soybean and murumuru biodiesel, respectively, in an agricultural tractor equipped with turbo intercooler.

Concerning the fuel type, B S1800 diesel (B0) and the mixture (B50) promoted the highest smoke opacity values at 2 pm (28.4 °C and 27%), which increased by 17.7 and 25%, respectively, when compared with the lowest values registered at 6 am (13.9°C and 95%). On the other hand, when evaluating biodiesel (B100), opacity increased (26%) under different conditions, that is, at 10 am(22.4°C and 60%) as compared to those recorded for B0 and B50, differing from 6 am, 6 and 10 pm readings.

Furthermore, the values of smoke opacity emitted from engines were reduced as temperatures decreased and humidity increased. According to Janaun and Ellis (2010), the use of biodiesel as fuel in diesel engine cycle reduces the emissions of particulate matter as compared to the diesel use.

Yoon et al. (2014) observed that particulate emissions diminished on average (about 33%) changing diesel for B30. These results are similar to those reported by Gonçalves et al. (2013), in which opacity index was lower in the early hours of the day (6:30, 9:30 and 10:30 am), at a maximum ambient temperature of 25.4°C and air relative humidity of above 50%. From 12 pm, and under opposite conditions (temperature higher than 25°C and humidity lower than 50%), higher opacity values was obtained. Liotti et al. (2010), when assessing diesel (B0), as a function of weather conditions (humidity from 91.4 to 69% and temperature from 20 to 25°C), observed higher opacity indexes at 12 and 3 pm and lower at 6 and 12 am.

The literature tends to corroborate that in internal-combustion engine compression, combustion thermodynamics has most influence from air excess, calculated as the ratio between actual air mass/fuel and the stoichiometric air mass (kg)/fuel (kg); mixture richness or equivalence ratio, which indicates the arbitrary ratio of oxidant and fuel relatively to a stoichiometric mixture; lower fuel calorific power; lower mixture calorific power; and temperature variation. For lower temperatures (about 1250 K) and lean mixtures (high air/ fuel ratio), stable chemical species (mainly CO_2, H_2O, N_2 and O_2) are produced by exothermic combustion; while for higher temperatures (above 1500 K), stable chemical species are dissociated, forming many others, such as CO, H_2, OH, H, O, NO and unburned hydrocarbons, among others (Coelho and Costa, 2007).

Figures 1 and 2 show smoke opacity behavior as a function of testing and weather conditions.

Conclusions

The use of babassu biodiesel and daytime influence smoke opacity emissions from a farming tractor engine. Reduced values of smoke opacity were recorded at 6 am, when a lower ambient temperature (13.92°C) and higher air relative humidity (95%) were measured, decreasing by 55.4 and 51.82% when compared with B S1800 and B S10 with B100, among which babassu biodiesel stood out. The blends of babassu biodiesel with B S1800 diesel and B S10 proved to be efficient in reducing smoke opacity from a farming tractor engine.

Conflict of Interests

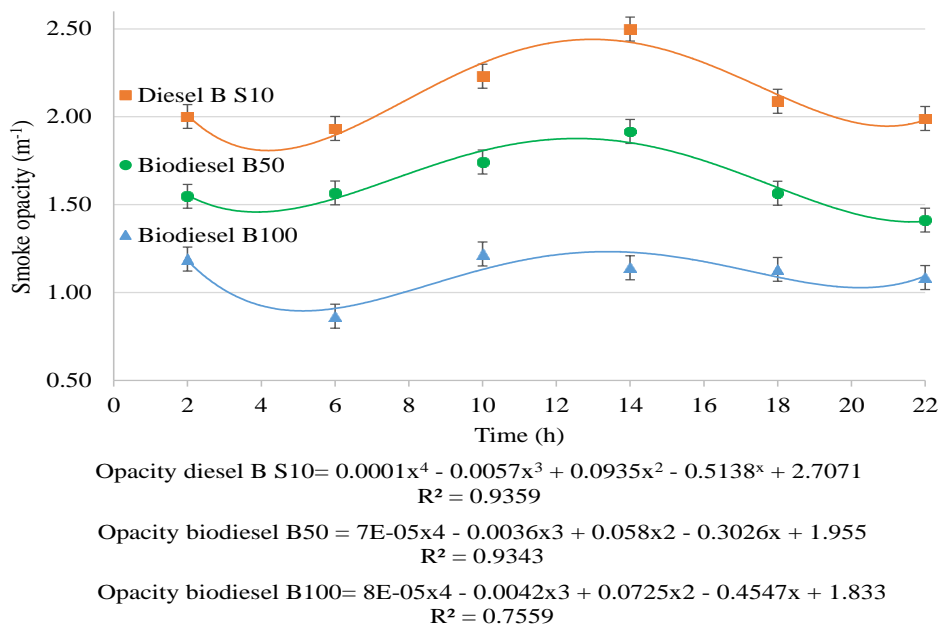The authors have not declared any conflict of interests.

Opacity diesel B S10= $0.0001x^4 - 0.0057x^3 + 0.0935x^2 - 0.5138^x + 2.7071$
$R^2 = 0.9359$

Opacity biodiesel B50 = $7E\text{-}05x^4 - 0.0036x^3 + 0.058x^2 - 0.3026x + 1.955$
$R^2 = 0.9343$

Opacity biodiesel B100= $8E\text{-}05x^4 - 0.0042x^3 + 0.0725x^2 - 0.4547x + 1.833$
$R^2 = 0.7559$

Figure 1. Graphical representation of smoke opacity as a function of testing time for B0, B50 and B100.

Opacity diesel B S1800 = $1E\text{-}04x^4 - 0.0049x^3 + 0.0784x^2 - 0.426x + 2.6804$
$R^2 = 0.9628$

Opacity biodiesel B50 = $0.0001x^4 - 0.0058x^3 + 0.0946x^2 - 0.5313x + 2.4446$
$R^2 = 0.9509$

Opacity biodiesel B100 = $7E\text{-}05x^4 - 0.0037x^3 + 0.0635x^2 - 0.3951x + 1.7463$
$R^2 = 0.7193$
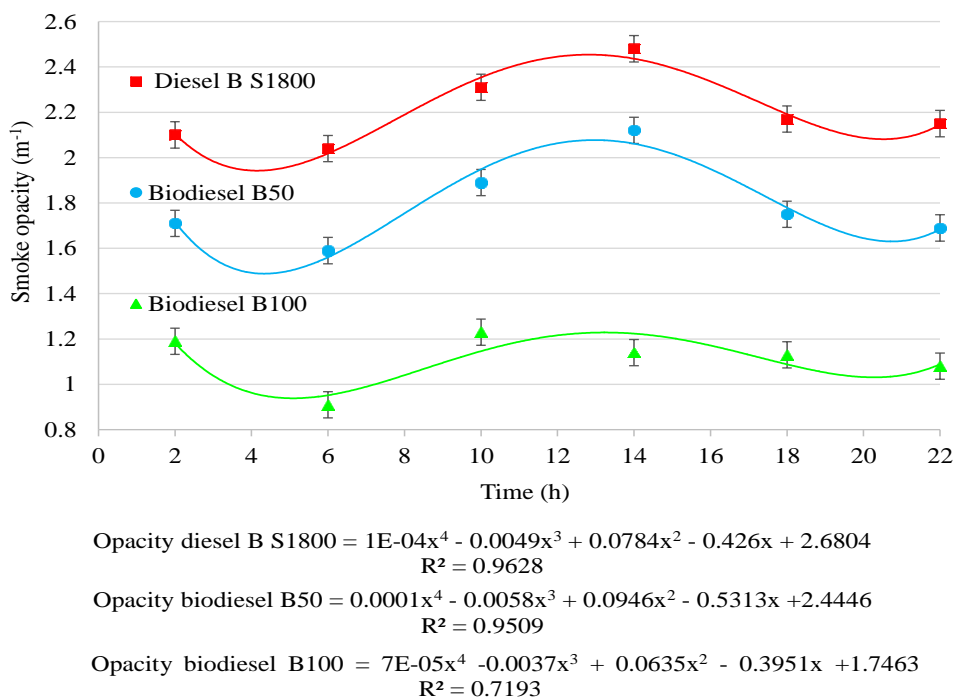
Figure 2. Graphical representation of smoke opacity as a function of testing time for B0, B50 and B100.

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Full Length Research Paper

Occurrence of Cucumber mosaic virus, Zucchini yellow mosaic virus and Watermelon mosaic virus in cultivated and wild cucurbits in the coastal areas of Tanzania

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Cucurbit vegetables play important role as sources of vitamins, micronutrients and income. However, their production is constrained, inter alia, by the virus diseases. *Cucumber mosaic virus* (CMV; *Cucumovirus*), *Zucchini yellow mosaic virus* (ZYMV; *Potyvirus*) and *Watermelon mosaic virus* (WMV; *Potyvirus*) are the major causal agents of devastating virus diseases of the cucurbits. Disease symptoms similar to those caused by these viruses were observed in cucurbits in the coastal lowland of Tanzania. To determine what caused these symptoms, leaf samples were collected from 223 cultivated and wild cucurbit plants and virus infections detected using Double Antibody Sandwich Enzyme-linked immunosorbent Assay (DAS-ELISA). Visual incidence of virus disease symptoms ranged from 0.0 to 90.0% but ELISA test for CMV, ZYMV and WMV revealed the range of 0 to 80%. The highest incidence of the virus infections was that of WMV (33.0%) in *Cucumis sativus*. The highest incidence of ZYMV and CMV were 10.4 and 13.4% in *Citrullus lanatus* and *Cucurbita pepo*, respectively. These viruses were found infecting cucurbits in single, co- and triple infections. Moreover, these viruses were also detected in the wild plants, *Cucumis hystrix* and *Luffa aegyptiaca* and in cultivated *Vigna unguiculata*. ZYMV was the commonest virus in wild plants. Yield losses caused by virus diseases remain undetermined for cucurbits in Tanzania but co- and triple virus infections have implication on disease severity and evolution of these viruses and may pose challenge for breeding for resistance.

Key words: Cucurbit virus diseases, DAS-ELISA, virus mixed infections.

INTRODUCTION

In Tanzania, vegetables play an important role as sources of vitamins, micronutrients and income (Weinberger and

Msuya, 2004). Cultivation of vegetables, including cucumber, pumpkins and watermelons is common in

areas with reliable water sources in the outskirts of Dar es Salaam city and other places in the country. It is a promising sub-sector in creation of jobs for the youths and women. Despite the importance of cucurbit crops, their production, worldwide, is constrained by many factors including virus diseases.

Cucurbits are infected by over 35 viruses (Provvidenti, 1996). *Cucumber mosaic virus* (CMV; *Cucumovirus; Bromoviridae*), *Watermelon mosaic virus* (WMV; *Potyvirus; Potyviridae*) and *Zucchin yellow mosaic virus* (ZYMV; *Potyvirus; Potyviridae*) are among viruses that cause important diseases in cultivated cucurbits (Lisa et al., 1981; Zitter and Banik, 1984). These viruses are transmitted by many aphid species in a non-persistent manner (Lisa et al., 1981; Gal-On, 2007; Gildow et al., 2008). They have a wide range of hosts but some strains, for example of CMV, may be confined to hosts in a certain family (Zitter and Murphy, 2009). CMV is reported to infect or be transmitted to over 1200 species from more than 100 plant families (Dobhal et al., 2015).

Virus diseases of cucurbits caused by CMV, ZYMV and WMV are characterized by such symptoms as mosaic, yellowing, stunted growth, fruit and leaf malformation and rugosity (Lisa et al., 1981; Zitter and Murphy, 2009). These diseases may lead to significant yield losses under field conditions. For instance, information published on the website of the Department of Agriculture and Food, Government of Western Australia, shows that when ZYMV infects cucurbits before flowering stage yield losses can be up to 100%. Rugosity and malformation of fruits, if they occur, render the fruits unmarketable.

Information on occurrence, distribution, and incidence of the viruses is required in order to develop strategies for management of diseases they cause. Likewise, one of the important aspects in management of virus diseases is to explore and understand existence of alternative hosts of the causal viruses. The viruses in alternative hosts may serve as the source inoculum for new crops even when established using virus-free seeds. Studies on specific viruses in alternative hosts in East Africa have been conducted for some crops (Tugume et al., 2008). Information on alternative hosts is useful in the management of diseases through management of these hosts. Moreover, understanding of the molecular evolution of viruses as driven by natural and other hosts is important as breeders strive to develop resistant genotypes.

While there are reports of occurrence of virus diseases of cucurbits from Africa (Lapido, 1988; Ibaba et al., 2015; Desbiez et al., 2016), such information is scanty for major virus diseases of cucurbits in Tanzania. This might be attributed to too much focus on the virus diseases of cassava and sweet potato, which are considered as the main food security crops. The virologists in the Great Lakes region have in recent years focused research in cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and sweet potato virus disease complex (SPVD) at the expense of other crops such as the cucurbits which not only have nutritional values but are also depended on by many for employment and household income. To contribute to the body of knowledge on occurrence and incidence of virus diseases of cucurbit crops, cultivated and wild cucurbit plant leaf samples were collected and subjected to double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) to detect viruses in the same. To the best of the authors' knowledge, this is the first report of occurrence of CMV, ZYMV and WMV in cucurbits and related wild plant species in Tanzania.

MATERIALS AND METHODS

Study area

Cultivated and wild cucurbit leaf samples used in this study were collected from coastal areas in Dar es Salaam and Coast administrative regions in Eastern Tanzania in March 2016 (Figure 1). The collection was made in river valleys or in areas where irrigation allows for all time cultivation of cucurbit crops.

Selection of gardens

The gardens in the river valleys or areas where water is available for irrigation were identified near main and feeder roads. In each of the selected locations, one to two separate gardens were randomly selected from among the many small gardens that were managed by small scale gardeners. Commonly, tens of small scale gardeners cultivate vegetable crops in hired small land plots in areas that are open near water sources. These gardens are found in tandem in many valleys in the outskirts of Dar es Salaam but they may be distantly located in the Coast region. The locations of the gardens were recorded using the GARMIN global positioning system (GPS, GPS72H) and mapped using google tools (Figure 1).

Collection of leaf samples

In each of the randomly selected gardens, leaves were collected from ten plants that were sampled along the garden diagonals following an '**X**' pattern. Five plants were collected as surveyor moved from one corner of the garden to the other. The distance from one plant to another was dictated by the size of the garden but was mostly less than 2 m. Leaf samples were collected from both symptomatic and asymptomatic plants. For each leaf sample, disease symptoms observed were recorded. The leaf samples, after detachment from the mother plants, were immediately placed in the sampling bags (ordered from DSMZ, Germany). The samples were then placed in a cool box with ice blocks. These samples were on the same day moved to a 4°C fridge at Mikocheni Agricultural Research Institute and used in enzyme-linked immunosorbent assays (ELISA) the following day.

Virus disease incidence and prevalence

Observations of disease incidence and prevalence were made on ten randomly selected plants. Incidence was estimated by dividing the number of plants with symptoms by the total number of plants on which observations were made per field. Prevalence was considered as the percentage of the fields with at least one diseased plant as assessed visually or determined by ELISA

Figure 1. Locations of cucurbit gardens from which samples were taken. The exact points are shown as stars on the map.

method. However, incidence of viruses computed after obtaining laboratory results are either shown per crop or plant for a given or all viruses and was computed as the number of plants confirmed by ELISA test to be infected divided by the total number of plants for the crop or wild plant under consideration.

Detection of viruses by DAS-ELISA

Enzyme-linked immunosorbent assay (ELISA) was used to confirm virus infections in the leaf samples. The antibodies used in this study were ordered from Leibniz-Institut (DSMZ, Germany). The double antibody sandwich ELISA (DAS-ELISA) was conducted following the procedures recommended by the manufacturer of the antibodies. Approximately 0.2 g of the leaf samples were put in the ELISA bags that were partitioned with filters. The samples were extracted using 3 mL of the extraction buffer that was prepared following the protocol provided by manufacturer of the antibodies. The coating antibodies (IgG; codes AS-0234, AS-0929, AS-0203 for ZYMV, CMV and WMV, respectively) were used at a dilution of 1:1000 as recommended by manufacturer. Then 200 μL of the diluted antibodies were added to the costar 96-wells flat-bottom EIA plates (Bio-Rad Laboratories (Pty) Ltd.). The plates with coating antibodies (IgG) were covered with clean aluminum foil and incubated at 37°C for 2 h. After washing as recommended, 200 μL of the extracted buffer were added to the wells in duplicate (avoiding edge wells). Appropriate positive samples (provided in the kit) were added along with healthy samples. The samples in plates were covered appropriately and placed in a 4°C fridge overnight. The conjugate antibodies used were diluted as recommended except once when dilutions were changed from 1:1000 for IgG-AP

(AS-0929; CMV) to 1:1250 and from 1:500 for IgG-AP (AS-0203; WMV) to 1:600. This was done in order to optimize for the rate of colour development. Then, 200 μL of the 1 mg/ml para-nitrophenyl-phosphate in substrate buffer or a ready to use solution, alkaline phosphatase yellow liquid substrate system for ELISA (SIGMA-ALDRICH, St. Louis, USA) were used in ELISA assay final step. Where the former was used, substrate buffer was prepared as recommended by DSMZ. Results were both visually assessed and then spectrophotometric (GDMS ELISA plate Analyser; RT00300115GDM; Global Diagnostic and Medical solutions) measurement of absorbance at 405 nm was done after 30 and 120 min. In one of the tests, a problem of rapid development of colour was encountered when detecting WMV and results for that test are not reported. The sample were considered positive if there was clear development of colour and then the absorbance readings were at least three times higher than that of healthy samples.

RESULTS

Symptoms

A variety of symptoms were recorded on three cultivated cucurbits, namely pumpkin (*Cucurbita pepo*), cucumber (*Cucumis sativus*) and watermelons (*Citrullus lanatus*). Similarly, symptoms were recorded for wild cucurbits and non-cucurbit cultivated crops, which were wild cucumber (*Cucumis hystrix*), luffa (*Luffa aegyptiaca*) and cowpea (*Vigna unguiculata*). The symptoms observed on these
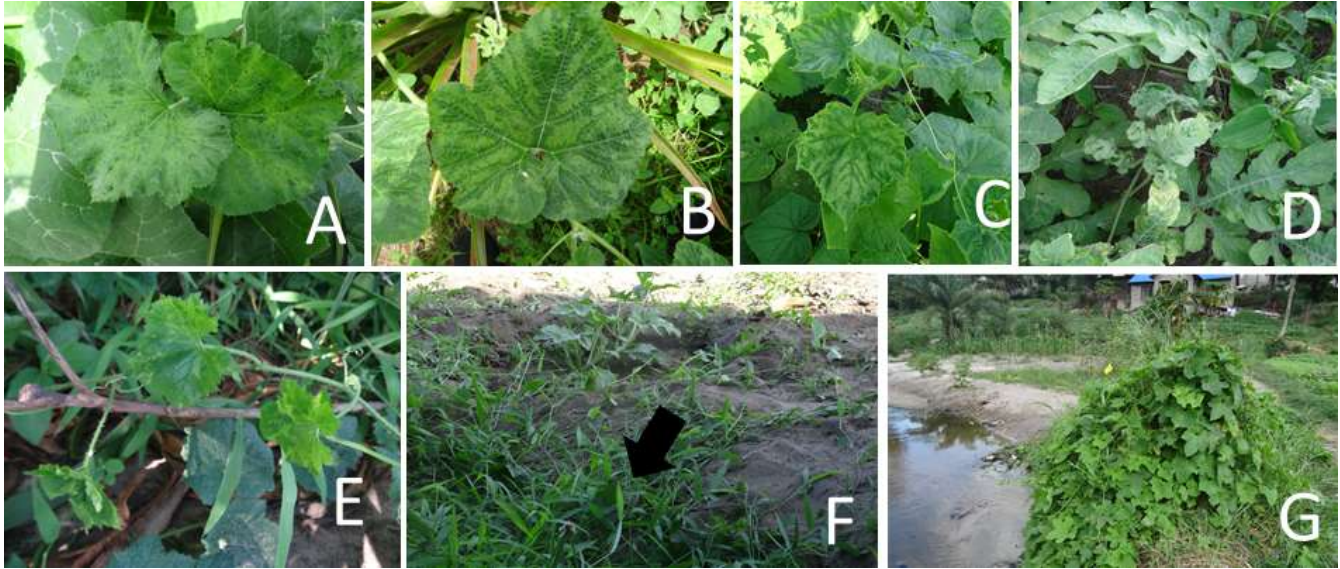
Figure 2. A variety of symptoms observed on cultivated and wild cucurbits: (A) Mosaic and rugosity on pumpkins; (B) green vein banding on pumpkins; (C) green vein banding on cucumber (unidentified local variety); (D) leaf deformation on watermelon; (E) yellowing and mosaic on wild cucumber plant; (F) wild cucumber plant on the edge of the field cultivated with watermelon in Bagamoyo district; and (G) a wild cucurbit plant in a river valley used for vegetable crops cultivation in the outskirt of Dar es Salaam city.

plants were mosaic, leaf curling, wrinkled leaves, stunted growth, green vein banding, yellow spots, and yellow mottling (Figure 2). The commonest symptoms were yellow mottling, mosaic, rugosity or wrinkled leaves and green vein banding. Generally, disease symptoms on wild plants were not as severe as they were on cultivated cucurbits. The symptoms sometimes varied between plants but similar symptoms were observed for plants in different species (Figure 2B and C). Both symptomatic and asymptomatic wild plants from the family *Cucurbitaceae* were found growing near or on the edges of cultivated vegetable cucurbits (Figure 2F and E).

Visual assessment of virus disease incidence

The virus disease symptoms described above were observed in both administrative regions suggesting that virus diseases of cucurbit are widely spread in the coastal areas, particularly in Dar es Salaam and Coast regions. Based on visual observations, the virus-like disease symptoms were seen on cucurbit vegetable crops in 18 out of 21 fields that were surveyed. This represented a disease prevalence of 85.7% when the same is assessed as the number of fields with at least a diseased plant over the total number of fields. The incidence of virus diseases ranged from 0.0 to 90.0% (Table 1). In five fields, the incidence of virus disease symptoms was greater than 50.0%. Visually assessed disease incidences were generally higher in cucumber and watermelon plants than in pumpkins. It was also observed that 33.0% of the wild

plants exhibited conspicuous symptoms similar to those known to be caused by viruses.

Disease incidence based on the ELISA assay

Results on the general disease incidence are presented in Table 1. The same table also shows the viruses detected in the collected leaf samples. The ELISA assay was done on 223 cultivated and wild cucurbit plants for ZYMV and WMV but on only 114 plant leaf samples for CMV.

ELISA based detection of the virus infections revealed incidence levels that ranged from 0 to 80%. Similar to incidence observed under visual assessment, in five gardens, the disease incidence was higher than 50%. However, the gardens recorded as having higher incidence are not the same ones observed to have high level of incidence under visual assessment. Generally, fields that were visually assessed as being free from virus diseases were indeed confirmed by ELISA assays to be having plants that were not infected except in one farm where one plant was found to be infected (Table 1). Forty-one plants were symptomatic but tested negative for the three viruses studied.

On the contrary, 33 plant samples that were symptomless tested positive for the viruses assayed for in this study. Viruses were detected in 15, 9 and 4 *C. pepo*, *C. sativus* and *C. lanatus* symptomless samples, respectively. Moreover, four symptomless wild cucurbits tested positive for viruses.

Table 1. Virus diseases incidence as determined visually and by ELISA methods in cucurbits leaf samples.

Field no.	Number of samples	Incidence based on visual assessment (%)*	Incidence based ELISA assay (%)*	DAS- ELISA detection
1	10	2 (20.0)	5 (50)	ZYMV (1); WMV (2); CMV (4)
2	10	2 (20.0)	8 (80)	ZYMV(1); WMV (4); CMV (7)
3	10	1 (10.0)	1 (10)	ZYMV (1)
4	10	4 (40.0)	5 (50)	ZYMV (1), WMV (3), CMV (1)
5	9	0 (0.0)	0 (0)	None
6	10	4 (40.0)	4 (40)	ZYMV (1); WMV (3)
7	10	0 (0.0)	1 (10)	WMV (1)
8	10	8 (80.0)	3 (30)	WMV (2); CMV (1)
9	11	8 (72.7)	4 (36.4)	ZYMV (3); WMV (2)
10	10	2 (20.0)	1 (10)	CMV (1)
11	12	1 (8.3)	3 (25)	WMV (3)
12[b]	11	4 (36.4)	2 (18)	ZYMV (2); WMV[b]
13[b]	10	4 (40.0)	0 (0)	None
14[b]	10	0 (0.0)	0 (0)	None
15[b]	10	0 (0.0)	1 (10)	CMV (1)
16[b]	11	2 (18.2)	2 (18)	ZYMV (2)
17[b]	12	2 (16.7)	5 (42)	ZYMV (4); CMV (2)
18[b]	16	7 (43.8)	2 (13)	ZYMV (2)
19[b]	11	5 (45.4)	6 (55)	CMV (6)
20[b]	10	8 (80.0)	5 (50)	ZYMV (5); CMV (1)
21[b]	10	9 (90.0)	1 (10)	ZYMV (1)

[b]EIISA results for WMV detection for these fields are not reported. *The number shown in brackets are disease incidence (%) that were calculated based on the number of diseased plants (outside of the brackets) and total number of observations (column 2).

Table 2. Status of single and mixed virus infections in different species of cultivated and wild cucurbits in surveyed areas in 2016.

Crop/wild plant	ZYMV	WMV	CMV	ZYMV+ WMV	ZYMV + CMV	WMV+ CMV	ZYMV+WMV +CMV
C. pepo	7/82(8.5)	8/40(20.0)	11/82(13.4)	0/40(0.0)	0/82(0.0)	2/40(5.0)	0/40(0.0)
C. sativus	4/53(7.5)	7/21(33.3)	7/53 (13.2)	0/21(0.0)	0/53(0.0)	3/21(14.3)	1/21(4.8)
C. lanatus	7/67(10.4)	4/47(8.5)	2/67 (3.0)	1/47(2.1)	1/67(1.5)	0/47(0.0)	0/47(0.0)
C. hystrix	5/16(31.2)	2/3(66.7)	1/16 (6.3)	0/3(0.0)	1/16(6.3)	0/3(0.0)	0/3(0.0)
L. aegyptiaca	0/4(0.0)	ND	1/4 (25.0)	ND	0/4(0.0)	ND	ND
V. unguiculata	0/1(0.0)	ND	1/1 (100.0)	ND	0/1(0.0)	ND	ND

'ND' symbol means at least one virus in the shown combination was not assayed for. The number in fraction refers to the number of infected plants (numerator) for a given number of assayed samples (denominator). The figures shown in brackets represent percentage of single or mixed infections for a given virus/viruses.

Infections by plants and mixed infections

Single and mixed infections results were summarized and shown in Table 2. For plants commonly infected by a particular virus, ZYMV infections were more common in watermelons (10.4%) than in pumpkins (8.5%) and cucumber (7.5%) (Table 2). CMV was detected more in pumpkin plants (13.4%) than in cucumber (13.2%) and watermelons (3.0%).

For WMV, more infections were detected in cucumber (33.3%). The infections of WMV in pumpkins were 20.0% while in watermelon, the crop after which its name was derived, was 8.5%. Co-infections were observed in five plants (2 events in pumpkins and 3 events in cucumber) for CMV and WMV and only once for all other combinations. Infections with all three viruses were observed in only one plant (*C. sativus*) that was collected from Dar es Salaam region.

Infections in wild plants

The total number of wild cucurbits on which ELISA assay was done were 21. Virus infections were detected in 8 plants (38.1%). One sample of cowpea (*V. unguiculata*) was found growing with cucurbit crops and had symptoms of virus disease; it was treated as a weed (wild) plant and detection revealed it was infected with CMV (Table 2). Therefore, in these studies, infections with CMV, WMV and ZYMV in wild plants were about 40.0%. ZYMV was detected in 6 wild plants (28.5%). Singly, WMV was detected in one wild cucumber plant while CMV was detected in *L. eagyptiaca* (1 plant) and wild cucumber (1 plant). Therefore, all types of wild plants that were collected were hosts of at least one of the three viruses studied. One wild cucumber plant was found doubly infected by ZYMV and CMV.

DISCUSSION

Plant viruses are known to cause enormous crop yield losses all over the world. The damage caused by these viruses affects both the quantity and quality of the desired parts of the crops. Virus diseases of cucurbits, for instance, cause rugosity on leaves and fruits thereby making them unfit for human consumption and thus completely or to some degree reducing their marketability. *Cucumber mosaic virus*, ZYMV and WMV are some of the viruses that cause severe symptoms on cucurbits globally (Ibaba et al., 2015; Desbiez et al., 2016). Their occurrence has been reported from many places in the world (Massumi et al., 2007; Lisa et al., 1981; Herrera-Vásquez et al., 2013; Ibaba et al., 2015). Symptoms similar to those caused by these three viruses were observed in plants in Tanzania. The results of this study have shown that most of the symptoms observed on pumpkin, cucumber and watermelon plants in the coastal lowland areas are caused by these viruses. There were, however, some symptomatic plants that tested negative for all three viruses suggesting that there could be strains of these viruses or even other distinct viruses that infect cucurbit crops in Tanzania. This is due to the fact that distinct strains or viruses could differ serologically (Zitter and Murphy, 2009).

Both visual observations and ELISA test revealed high incidence of virus diseases in cucurbits fields in the coastal lowlands of Tanzania. There has not been any assessment of the yield losses associated with the three viruses in Tanzania but it is reasonable to surmise that the losses could be as high as in other regions of the world where such observations have been made. The incidence of virus infections may differ from location to location. In Iran and Brazil, the incidence of diseases caused by these viruses in cultivated cucurbits were found to be higher for CMV and ZYMV as compared to watermelon mosaic virus-2 (WMV-2 (Yuki et al., 2000;

Mussumi et al., 2007). In this study, albeit a few number of samples, the chance of encountering a plant infected with WMV was higher than that of encountering a cucurbit plant infected with ZYMV and CMV. On the other hand, ZYMV appears to infect wild cucurbits at higher rates than CMV and WMV. Generally, the incidence levels observed in Tanzanian coastal areas were comparable to those reported from South Africa (Ibaba et al., 2015). None of the collected wild cucurbit plants was infected with CMV. According to Zitter and Murphy (2009), some CMV strains are host specific, infecting certain hosts in the same family like the legume strain of CMV. However, given the relatively small sample size used in this study, conclusion on this matter cannot be made with regard to the lack of infections in wild cucurbits by CMV.

Most of the plants that were symptomatic tested positive for at least one of the three viruses. Interestingly, ELISA detection revealed virus infections in a considerably large number of leaf samples collected from asymptomatic plants. The absence of symptoms on plants that were found to be infected by at least one of the viruses may be explained partly by the possible genetic variation in the genotypes of the plants as farmers have different sources of planting material and also by the time at which the plants were infected. When plants are infected at their early development stage the symptoms are normally severe and may translate into high yield losses (Zitter and Murphy, 2009). The detection of viruses in asymptomatic plants is in agreement with a common understanding that conclusion on whether the plants are or are not infected with viruses can only be based on results obtained by using such means as ELISA, polymerase chain reaction, next generation sequencing and so forth.

ZYMV and WMV, but not CMV, were detected in wild plants. Although CMV was not detected in any of the wild plants, it is known to infect over 1200 plant species (Zitter and Murphy, 2009). It has been shown that the same isolate of CMV may cause severe symptoms on plants belonging to different families (Eni et al., 2013). The detection of ZYMV and WMV viruses in cultivated cucurbits and their closely related wild plants has implication on the management of these viruses. Both symptomatic and asymptomatic wild plants from the family *Cucurbitaceae* were observed to grow in close vicinity of cucurbit gardens (Figure 1). Since all three viruses are transmitted by aphids, there is a high chance of single or simultaneous transmission of the viruses between these hosts. Not only will the virus transmission between different hosts have implication on crop yields but may also drive the evolution of these RNA viruses, which are famously known to be prone to errors during replication (Domingo and Holland, 1994; Garcia-Arenal et al., 2001). Adaptation to different hosts may result into selection pressure (Chare and Holmes, 2004) on the virus and thus emergence of new variants, which will

make it more difficult to manage the diseases through plant breeding.

Cases of co- and triple infections between a cucumovirus, CMV and the two potyviruses (ZYMV and WMV) were observed in both cultivated and wild plants. Occurrence of mixed infections of both related and unrelated viruses is common in plants (Mukasa et al., 2006; Massumi et al., 2007; Mbanzibwa et al., 2011). The interest on investigating occurrence of mixed infections might have existed for so long but it gained importance in the 1990's following the discovery that unrelated viruses may synergize thereby causing severe symptoms on plants (Pruss et al., 1997). It was also demonstrated that co-infections between CMV (*Cucumovirus*) and ZYMV (*Potyvirus*) in cucumber cv. Delila resulted into synergism and thus breakage of resistance to CMV (Wang et al., 2004). In East Africa, studies on synergism became important following the observation that co-infection between *Sweet potato feathery mottle virus* (SPFMV; *Potyvirus; Potyviridae*) and *Sweet potato chlorotic stunt virus* (SPCSV; *Crinivirus; Closteroviridae*) results into the devastating disease of sweet potato called SPVD (Mukasa et al., 2006). Furthermore, co-infections may result into exchange of genetic materials between the co-infecting viruses resulting into a recombinant strain or virus. Indeed, a recombinant begomovirus that caused severe symptoms on cassava plants in East Africa followed co-infections and exchange of genetic material between *African cassava mosaic virus* (ACMV*; Begomovirus; Geminiviridae*) and *East African cassava mosaic virus* (EACMV; *Begomovirus; Geminiviridae*) (Deng et al., 1997; Zhou et al., 1997). In this study, the sequences of the viruses were not determined, which makes it impossible to explore recombination events in the viruses infecting cucurbits in Tanzania.

Conclusions

Three of the most important viruses known to cause diseases on cucurbits were detected in both symptomatic and asymptomatic cultivated and wild plants. Moreover, they were found to be widely distributed in surveyed locations. As the battle to eradicate cassava mosaic and cassava brown streak diseases continues, the virologists and plant breeders are hereby urged not to neglect the damages on cucurbits that are caused by the viruses reported herein. The cucurbits are undoubtedly among the crops whose cultivation results into creation of jobs, generation of income and improvement of health of the resources constrained persons and probably contributing to reduction of the budget for treatment of malnutrition related disorders.

Future studies should focus on unravelling genetic diversity of the isolates of these viruses in Tanzania. Information on genetic diversity is needed in order to develop management strategies for the same. There

were symptomatic plants that tested negative suggesting there are other viruses which infect cucurbits. There is a need to investigate further the cause for these symptoms on plants in the coast as well as other parts of the country. This work has shown that ZYMV and WMV are infecting wild cucurbits. Therefore, any management strategies that will be developed should take this fact into consideration.

Conflict of Interests

The authors have not declared any conflict of interests.

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