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Full Length Research Paper

Soil chemical properties under a no-tillage system: Forage grass seeding modes of gender *urochloa* intercropped with maize

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Different farming practices and soil management can make changes in the chemical properties of the soil. In this sense, the monitoring of these possible changes is essential for proper soil correction and/or using systems that are more sustainable. The objective of this work was to verify the behavior of the chemical attributes of a Latosol (Oxisol) under a no-tillage system of plantation, by intercropping maize with two species of forage grasses in different methods of sowing. The experiment was conducted in an area of the Laboratory of Agricultural Equipment and Mechanization of Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP, São Paulo State University), in Jaboticabal, Brazil, and the treatments were maize intercropped with two species of grass, of the *Urochloa* gender, namely *Urochloa brizantha* cv and *Urochloa ruziziensis cv*, sown in four modes: maize with *Urochloa* in row seeding (MFL); maize with *Urochloa* between rows, sown on the same day of the sowing of maize (MFE); maize with *Urochloa* sown in rows, covered by fertilizer at the V4 stage (MFC); maize with *Urochloa*, sown by casting, along with fertilizer cover at the V4 stage of maize (MFLA) and maize alone, without any intercropping (control). Composite samples were taken for chemical analysis (P, MO, Ca, Mg, K, H + Al, SB, T, and V) in layers from depths 0.00 to 0.10 m; 0.10 to 0.20 m; and 0.20 to 0.30 m. The experimental design was that of randomized blocks, with nine treatments, in a factorial design (2×4) +1, with four replications. Major changes were observed in the soil chemical attributes in layer 0.10-0.20 m, within modes MFE and MFLA. In the no-till system, *Urochloa brizantha* cv has greater cycling of Ca^{2+} and Mg^{2+} in the layer from 0.10 to 0.20 m.

Key words: Fertilization, soil fertility, sowing mode, crop rotation, nutrient cycling.

INTRODUCTION

The intensive use of areas for agricultural production, coupled with inadequate techniques of soil management and the uneven distribution of rains hamper the implementation of autumn/winter crops for both the

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formation of straw and the production of grain, a fact observed almost wherever in the state of São Paulo as well as in the Brazilian Midwest (Barducci et al., 2009). In tropical regions, the decomposition of organic material is faster and this fact is worthy of greater attention by producers (Liu et al., 2010). In recent decades, with the advancement in research and the use of new technologies, a change is happening in the agricultural sector, which is explained by the incorporation of more intensive processes in production systems (Barcellos et al., 2008). Among planting systems used by producers, the no-till is considered the most conservationist, for recommending the supply of coverage over the soil, rotation and intercropping. In the no-till system, intercropping is an alternative aimed at increasing the sustainability of the agricultural production model, because the consortium of crops changes the physical, chemical and biological soil properties over time, may favor the improvement of the sustainability of agricultural systems as a result of yield diversity (Garcia et al., 2008; Calonego et al., 2011).

The maize crop has excelled in integration with forage grass for providing increased straw provision for the maintenance of tillage, in addition to allowing the use of the dry mass after harvest, used in animal feed during periods of lower supply of pasture. Barducci et al. (2009), claim that the implanted forage grass species in the consortium is crucial for obtaining good yields of both maize grains and accumulation of forage grass dry matter. Various forage grass species stand out in intercropping with maize, but in the literature are found a few that stand out, such as *B. brizantha cv*, *B. ruziziensis cv*, *Panicum maximum cv. Tanzania* and *P. maximum cv. Mombaça*, (Pereira et al. 2014). Forage grasses provide large amount of mass (dry matter), according to Costa et al. (2014) the *Urochloa brizantha cv.* and *Urochloa ruziziensis cv* are good alternatives in the production of straw under no-tillage. Thus, forage grasses protect the soil for longer against erosion and change the physical and chemical properties of soil through nutrient cycling and aggregate stability (Loss et al, 2011; Seidel et al, 2014). It is extremely valuable to understand the dynamics of soil properties, be they of physical, biological or chemical order, in view of the direct influence of these factors in the success of agricultural production. Thus, the monitoring of soil fertility levels is important not only for the correct nutritional supply of crops, but also to allow that adequate management practices of fertilization and soil preparation are performed efficiently, enabling improvements in management practices in order to improve the production and crop management (Tasso Júnior et al., 2010).

The no-tillage improves soil chemical conditions due to the level of organic matter from straw, contributing to soil cover, while maintaining system stability (Chioderoli et al., 2012a). Also, according to Mateus et al. (2012), the simple fact of maintaining straw in the soil, increases the level of organic matter, phosphorus, potassium, calcium, magnesium, pH, effective CEC and micro-nutrients on the soil surface, as well as there is a decrease of exchangeable Al. Freitas et al. (2014) reported that the main chemical changes in cultivated soils compared to the original conditions, are due to the variation of pH and cation levels.The chemical properties of the soil are affected by the removal of natural vegetation and cultivation, mainly on its surface, due to the addition of lime and fertilizers and agricultural operations. According Zanão Junior et al. (2010), in the no-tillage system (SPD), the management itself, such as the surface application of limestone, fertilization, accumulation of crop residues, can alter the soil chemical fertility. Thus, the adoption of certain management practices, such as surface fertilizer; sowing by casting; sowing in rows; crop residues in succession and/or rotation over the years, contribute in the dynamic behavior of the soil chemical properties. In this sense, the evaluation of soil chemical properties is required due to the heterogeneity of these attributes, especially when associated with methods of sowing and intercropping. Therefore, the objective of this study was to evaluate the behavior of soil chemical properties due to the consortium maize-forage grass of the *Urochloa* species under different methods of sowing.

MATERIALS AND METHODS

The experiment was conducted in the experimental area of the Laboratory of Machines and Agricultural Mechanization of UNESP, in Jaboticabal, São Paulo state, Brazil, located in the following geodetic coordinates: latitude 21°14 'S and longitude 48°16' W, featuring local altitude of 560 m, with a 4% slope. The soil of the experimental area was classified as Latossolo Vermelho eutroférrico típico (according to the Brazilian System of Soil Classification of the Brazilian Agricultural Research Corporation, (Embrapa, 2006), or “Ferralsol”, according to the FAO Soil Classification, aka “Oxisol”), with a particle distribution of 200 g/kg sand, 290 g/kg silt and 510 g/kg clay. The experimental area was being treated in the SPD for over ten years. The climate, according to Koeppen classification is Aw, defined as tropical humid, with a rainy season in summer and a dry winter, with an average annual rainfall of 1,425 mm and an average temperature of 22°C. The precipitation, maximum temperature, minimum and average (°C) during the experiment are shown in Figure 1.

The treatments consisted of two species of *Urochloa* (*U. brizantha cv* and *U. ruzizienses cv*) and four modes of intercropping of urochloas with maize, namely: maize with *Urochloa* at sowing line (MFL); maize with *Urochloa* between rows, sown on the same day of the sowing of maize (MFE); maize with *Urochloa* sown between rows, covered by fertilizer at the V4 stage of maize (MFC); maize with *Urochloa* sown by casting, with surface fertilization at the V4 stage of maize (MFLA), and maize without intercropping (control). Maize received basic fertilization in two growing years, of 300 kg/ha of the commercial formula (08-28-16) with supplementary cover fertilization at the V4 stage, corresponding to 120 kg/ha of potassium chloride and 300 kg/ha urea, while for soy, the basic fertilizer was 250 kg/ha commercial formula (04-20-20), and for *Urochloas*, we used 20 kg/ha commercial formula (08-28 -16) for forage grass seeding between rows (MFE) and at the time of the maize crop at the V4 stage (MFC), being the fertilizer used only as a vehicle for distribution of seeds. The experimental design was a randomized block design, with nine treatments in a factorial scheme (2×4) +1. Two forages of the genus Urochloa (Urochloa brizantha

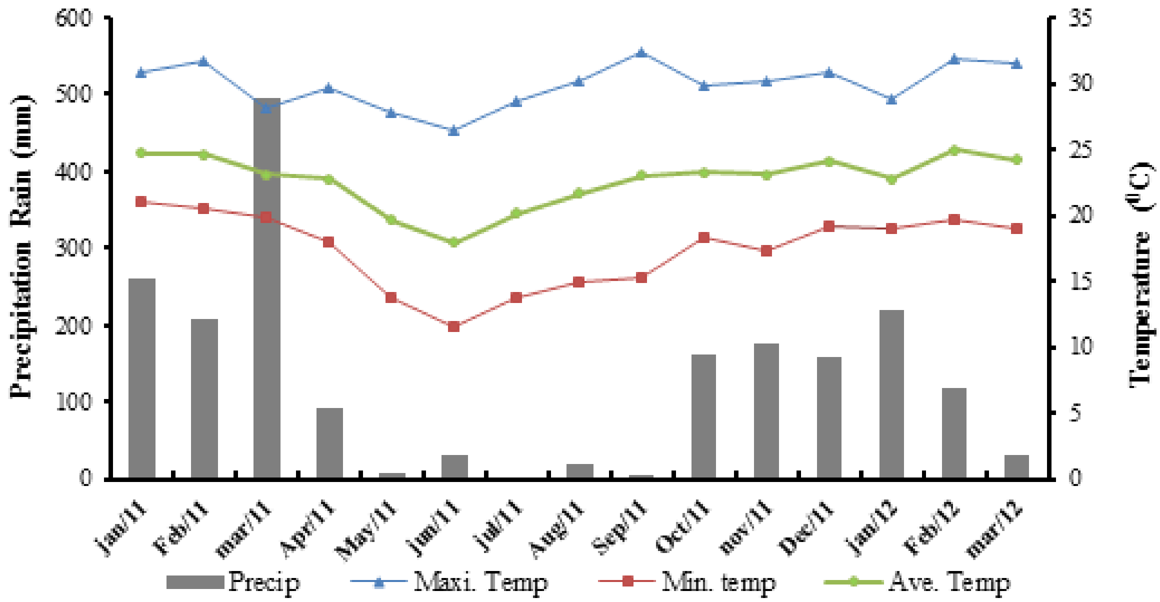

Figure 1. Rainfall (mm), average maximum temperature, minimum and average (° C) during the experiment.

cv and Urochloa ruziziensis cv), sown in four sowing modalities, Maize with urochloa in the sowing line (MFL); Maize with urochloa in the interweave, sown on the same day as maize sowing (MFE); Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage (MFC); Maize with urochloa on the haul along with maize V4 maize mulch (MFLA) and maize without intercropping (control).

Each experimental plot consisted of eight maize lines (DKB 390) for a population of 60 thousand ha^{-1} plants, with 0.90 m row spacing, sowing density of 5.4 m^{-1} seeds and 14 Soybean cultivar Valiosa Roundup Ready, spaced at 0.45 m. The plots were 25 m long, 15 m haulers for machine and equipment maneuvers, useful area corresponding to the two maize lines and three soybean rows with five meters each, discounting the ten meter border in each end. Soil samples were collected from depths of 0.0-0.10; 0.10-0.20; 0.20-0.30 m for subsequent chemical analysis (P, MO, Ca, Mg, K, H + Al, SB, T, and V), following the method proposed by Raij et al. (2001). Data were submitted to analysis of variance by the F test ($p<0.05$) and when significant, factorially compared to the control group (maize, only) and this comparison performed by applying Dunnett's test ($p<0.05$). Statistical analyzes were performed by using a statistical software, *Assistat*, version 7.7 (beta) Silva and Azevedo (2016).

RESULTS AND DISCUSSION

In general, the soil chemical attributes in the layer from 0.0 to 0.10 m do not differ statistically among themselves by Dunnett test ($p<0.05$) presented in Table 1. The non-significant difference among the soil chemical properties in the layer from 0.0 to 0.10 m, may possibly be explained by the short development period of the study, which lasted one agricultural years. However, the phosphorus levels were higher and significantly different within modes, MFLA (Casting V4) and MFL (Rows), and possibly these results may be due to the process of decomposition of the roots of *Brachiaria*, releasing nutrients, together with the colloids present in latosols, as kaolinite clays, Fe and Al oxides, favoring greater fixation of phosphorus.

Another factor that may be contributing to the higher phosphorus values within modes (sown by casting in V4 stage) and (in the maize planting row), is the pH, since as it rose there was an increase in the phosphorus level, and this may be due to competition between the OH^- anions (from the rising pH) and $H_2PO_4^-$ and HPO_4^{2-} from the surface of colloids. With regard to the detailing for the phosphorus level, forage grasses within the modes, as well as modes within forage grasses (Figure 2) , it is noticed that there was an increase in the phosphorus level within modes MFL (Rows) and MFLA (Casting V4) for forage grasses *U. ruzizienses* cv and *U. brizantha cv* respectively. Means followed by same letter do not differ at Tukey test 5% probability. Forages: U .B – (*Urochloa brizantha); U.R – (*Urochloa ruziziensis); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). These results may be due to continuous fertilization in rows and between rows (Ciotta et al., 2002; Costa et al., 2009), in addition to biopores formed by the roots and soil fauna (Adiscott, 1995), promoting redistribution of P in the profile and by its low mobility in the soil. Furthermore, the soils in the tropical regions have clays with high fixation

Table 1. Average values of the chemical soil parameters, evaluated in the layer from 0.0 to 0.10 m, according to seeding mode and forage grass species.

Causes for variation		P	MO	pH	K	Ca	Mg	H +Al	SB	T	V
Forage grass species	Modes	(mg dm^{-3})	(g dm^{-3})	($CaCl_2$)	---mmol$_c$ dm^{-3}---						(%)
U. Brizantha	Rows	38.0^b	21.6	5.4	4.4	33.3	15.0	28.0	52.7	80.7	64,6
U. Brizantha	Between Rows	61.3^b	30.6	5.5	4.7	47.0	18.6	32.3	70.4	102.7	66,3
U. Brizantha	Cover	38.7^b	29.0	5.2	4.0	38.5	16.2	31.7	58.8	90.5	64,4
U. Brizantha	Casting V_4	83.0^a	26.5	5.6	5.2	45.5	21.0	23.5	71.7	95.2	70,8
U. Ruziziensis	Rows	86.3^a	29.3	5.8	5.9	54.3	23.6	18.0	83.9	101.9	81,7
U. Ruziziensis	Between Rows	62.3^b	30.6	6.0	5.3	61.6	27.3	17.3	94.3	111.6	84,3
U. Ruziziensis	Cover	62.6^b	24.6	5.3	4.0	34.0	17.3	31.3	55.4	86.7	61,5
U. Ruziziensis	Casting V_4	52.0^b	26.3	5.3	3.6	33.3	15.6	31.3	52.6	84.0	62,1
Control		33.5^b	26.6	5.6	4.5	40.3	21.0	24.0	65.9	89.9	66.5
FxT		5.6*	0.1^{ns}	0.1^{ns}	0.6^{ns}	0.1^{ns}	0.2^{ns}	1.0^{ns}	0.1^{ns}	0.0^{ns}	0.1^{ns}
DMS		39.7	6.4	0.7	2.8	27.1	10.4	17.2	38.5	25.8	25.0
CV%		34.0	11.7	6.5	30.3	31.4	26.4	32.6	26.9	13.7	18.0

*Significant at 5% probability level (p<0.05); NS (not significant). Averages followed by the same letter and no letters in columns do not differ by the Dunnett test (p <0.05). F - Forage grass; T - Control; U. Brizantha - Urochloa brizantha; U. Ruziziensis - Urochloa ruziziensis; Rows - Maize with Urochoa in planting rows; Between Rows - Maize with Urochoa between rows; Cover - Maize with Urochoa sown in rows cover fertilizer in maize V4 stage; Casting V4 - Maize with Urochoa sown by casting in the V4 stage of maize; Control - maize only.

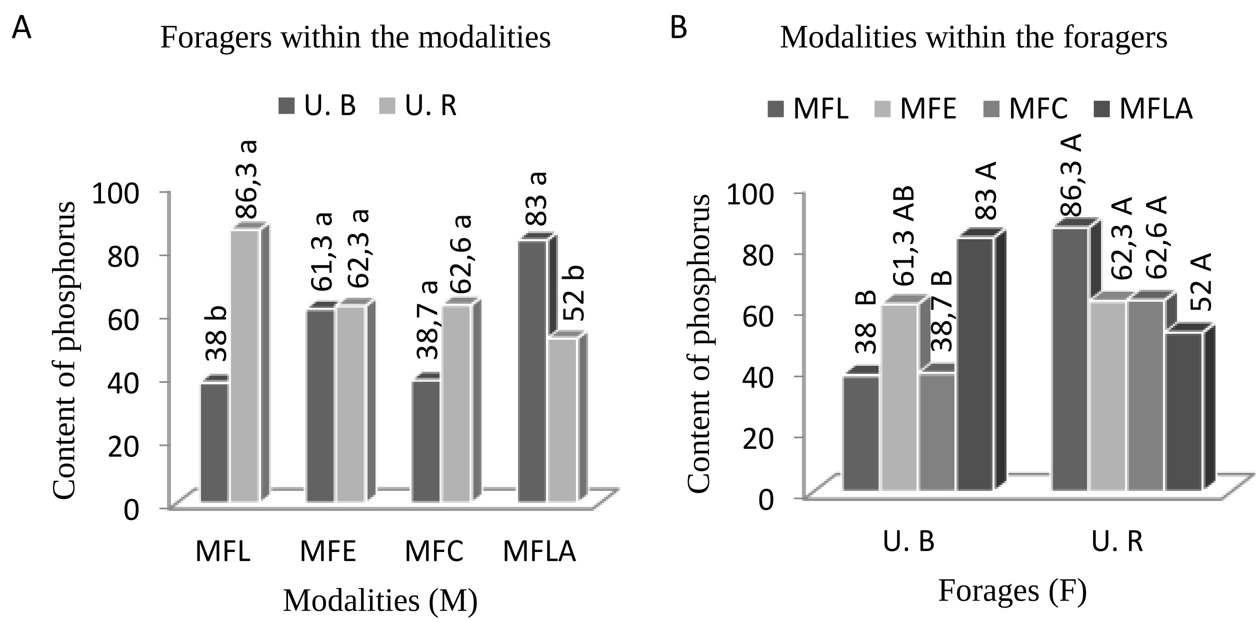

Figure 2. Ramifications of interaction between factors, sowing modalides and fodder for the variable phosphorus.

capacity of phosphorus (Ferreira et al., 2014). For the values of chemical parameters of the soil in depth from 0.10 to 0.20 cm (Table 2), in general, significant differences in the interaction between grasses and modes for the pH, Mg, H + Al, SB and V, as detailed in Figures 3, 4, 5, 6 and 7 respectively.

Nascente et al. (2014), by studying the chemical attributes of an Oxisol under no-tillage affected by soil management and crop rotation, concluded that chemical attributes Ca, Mg, organic matter, P, K, concentrated in the most superficial layer, regardless of rotation used in managements with lesser soil revolving.With the exception of phosphorus, which is found in the between rows mode for *Urochloa ruziziens*, the other attributes (pH, Mg, SB, T and V) had higher values observed in mode Casting V4 for *Urochloa brizantha cv*, (Table 2).

Table 2. Average values of the chemical soil parameters evaluated in the layer from 0.10 to 0.20 m, according to seeding mode and forage grass species.

Causes for variation		**P**	**MO**	**pH**	**K**	**Ca**	**Mg**	**H +Al**	**SB**	**T**	**V**
Forage grass species	**Modes**	**(mg dm^{-3})**	**(g dm^{-3})**	**($CaCl_2$)**	**-------------------------mmol$_c$ dm^{-3} --------------------**						**(%)**
U. Brizantha	Row	29.6^{b}	22.0	5.3^{a}	4.0	27.6^{a}	12.6^{b}	27.0^{b}	44.3^{a}	71.3	62.1^{a}
U. Brizantha	Between Rows	39.3^{b}	21.6	4.9^{b}	3.1	24.3^{a}	11.0^{b}	40.0^{a}	38.5^{a}	78.5	48.7^{b}
U. Brizantha	Cover	57.0^{b}	22.2	5.0^{a}	3.9	24.7^{a}	10.2^{b}	35.5^{b}	38.9^{a}	74.4	52.0^{a}
U. Brizantha	Casting V_4	50.0^{b}	27.5	5.6^{a}	5.5	38.5^{a}	19.6^{a}	23.5^{b}	63.0^{a}	86.7	72.2^{a}
U. Ruziziensis	Row	32.0^{b}	21.6	5.3^{a}	3.7	33.3^{a}	14.6^{a}	29.0^{b}	51.7^{a}	80.7	63.2^{a}
U. Ruziziensis	Between Rows	80.3^{a}	24.6	5.5^{a}	4.2	37.3^{a}	19.0^{a}	24.3^{b}	60.6^{a}	84.9	70.6^{a}
U. Ruziziensis	Cover	29.0^{b}	20.6	4.8^{b}	3.5	16.6^{b}	8.6^{c}	41.6^{a}	28.8^{b}	70.5	40.1^{b}
U. Ruziziensis	Casting V_4	30.0^{b}	26.3	5.3^{a}	3.3	32.0^{a}	15.6^{a}	30.3^{b}	50.9^{a}	81.3	60.6^{a}
Control		27.6^{b}	23.6	5.5^{a}	4.1	38.3a	19.0^{b}	24.3^{b}	62.1^{a}	86.5	69.9^{a}
FxT		2.3^{ns}	0.03^{ns}	4.5*	0.1^{ns}	3.4^{ns}	10.1**	5.3*	4.8*	2.6^{ns}	4.6*
DMS		38.5	6.6	0.5	2.5	18.3	6.8	11.5	25.9	18.4	19.5
CV%		46.0	14.1	5.5	32.1	30.1	23.6	18.8	26.5	11.5	16.2

**Significant at 1% probability level (p <0.01); *Significant at 5% probability level (p<0.05); NS (not significant). Averages followed by the same letter and no letters in columns do not differ by the Dunnett test (p <0.05). F - Forage grass; T - Control; U. Brizantha - Urochloa brizantha; U. Ruziziensis - Urochloa ruziziensis; Rows - Maize with Urochoa in planting rows; Between Rows - Maize with Urochoa between rows; Cover - Maize with Urochoa sown in rows cover fertilizer in maize V4 stage; Casting V4 - Maize with Urochoa sown by casting in the V4 stage of maize; Control - maize only.

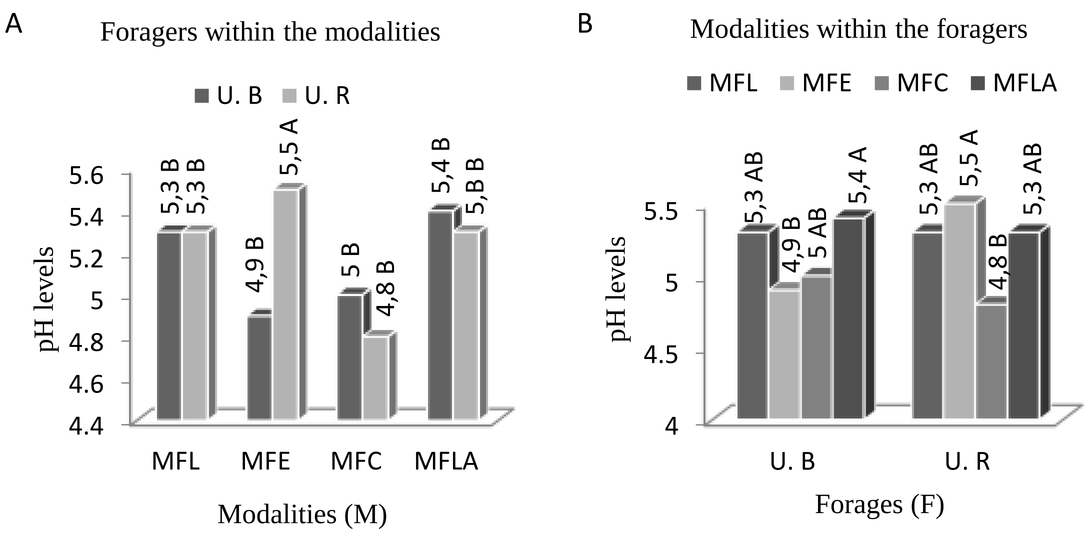

Figure 3. Ramifications of interaction between factors, sowing modalides and fodder for the variable pH.

These results can be explained by the low competition occurring between maize and forage grasses in the vegetative stage V4 of maize, together with supplementary surface fertilization and a higher pH value, where higher pH values contributed directly in the reduction of potential acidity levels (H + Al) and increased levels of Mg, SB and V (Strojaki et al., 2013).

Also with respect to the pH, when it is increased, there is also an increased mineralization of organic matter, and this process is favored in soils of pH values between 5.0 and 6.0, freeing N, P and S, as well as macro and micronutrients in smaller amounts (Cardoso et al., 2014). For the detailing of pH (Figure 3), the *Urochloa ruziziensis cv* showed higher pH levels in the MFE mode differing from the others. For Portugal et al. (2010), the forage grass *Urochloa ruzizienses* cv has a positive effect on the increase of cations in the soil, which can positively affect crop productivity in the short and long run

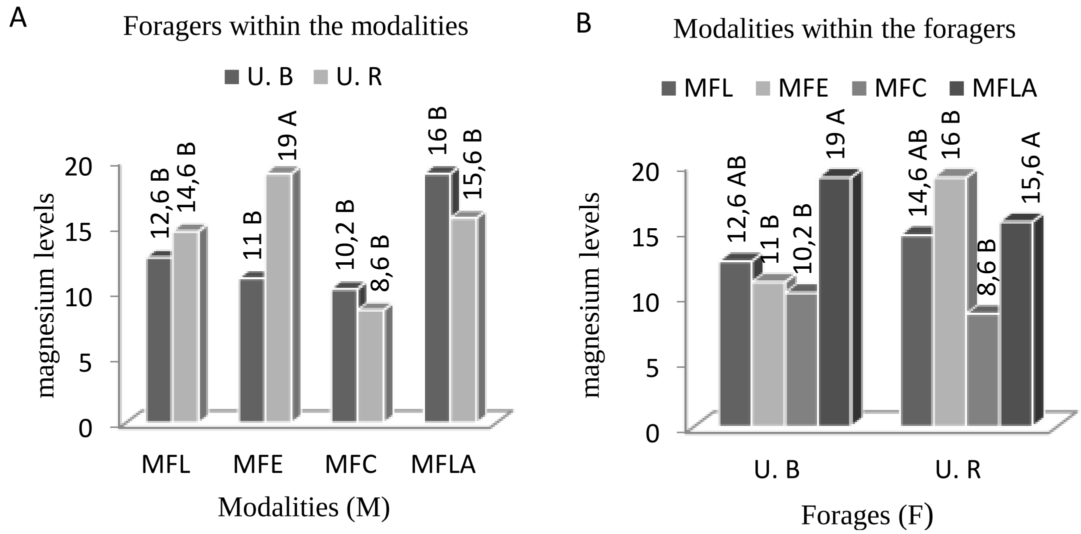

Figure 4. Ramifications of interaction between factors, sowing modalides and fodder for the variable magnesium.

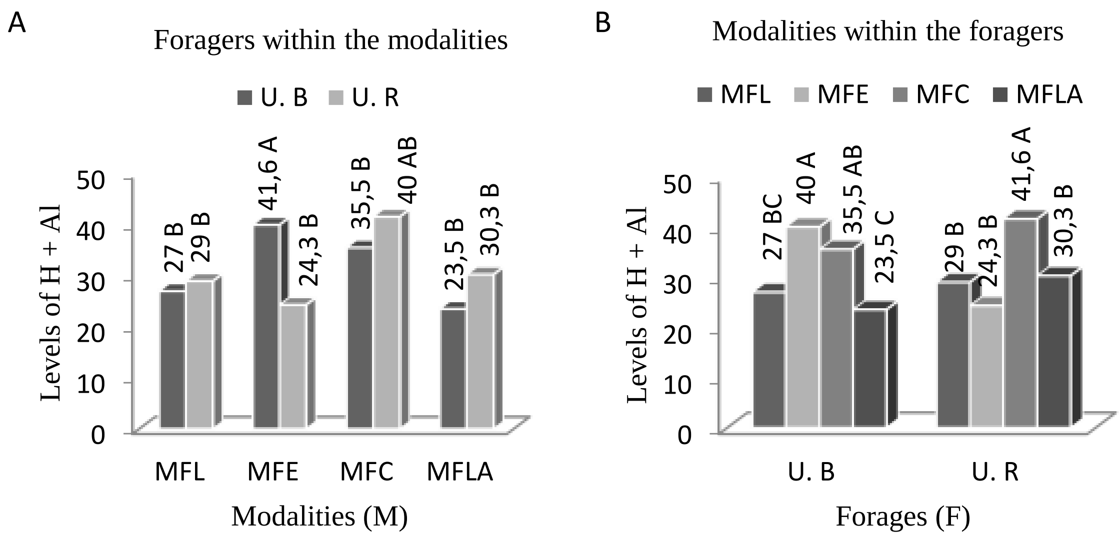

Figure 5 . Ramifications of interaction between factors, sowing modalides and fodder for the variable H + Al.

(Chioderoli et al., 2012b). Means followed by same letter do not differ at Tukey test 5% probability. Forages: U.B – (*Urochloa brizantha); U.R – (*Urochloa ruziziensis); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). As for the magnesium levels (figure 4), the highest values were found in the MFE mode for *Urochloa ruzizienses cv*, differing from the others. This result can be explained by the nutrient cycling capacity that have the *Urochloa ruzizienses cv*, together with the fertilization carried out between rows. Dalchiavon et al. (2012), by evaluating the spatial variability of fertility of an oxisol under no-tillage system, report that high Mg^{2+} levels, layered from 0.10 to 0.20 m, occured by providing considerable amounts of exchangeable bases during liming.

Means followed by same letter do not differ at Tukey test 5% probability. Forages: U .B – (*Urochloa brizantha); U.R – (*Urochloa ruziziensis); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize

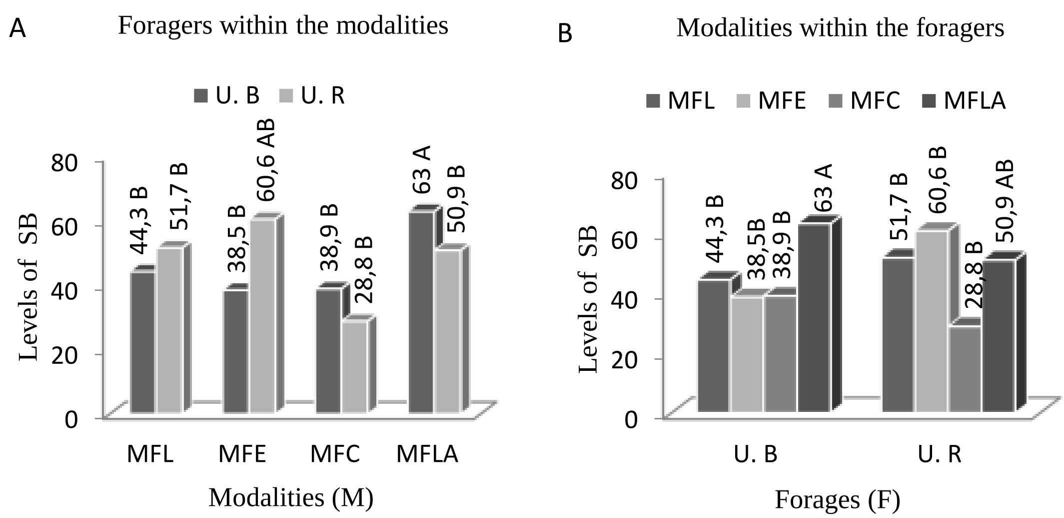

Figure 6. Ramifications of interaction between factors, sowing modalides and fodder for the variable SB.

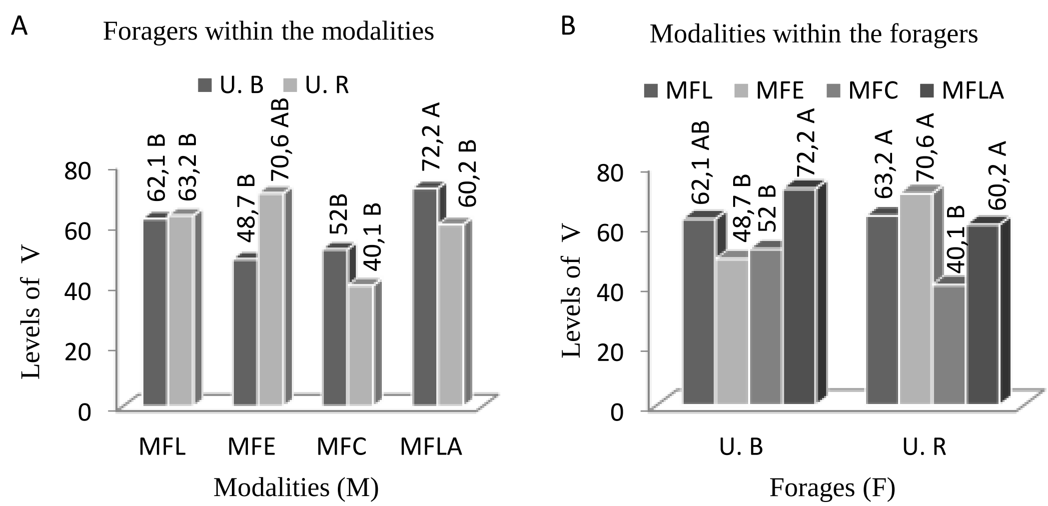

Figure 7. Ramifications of interaction between factors, sowing modalides and fodder for the variable V.

with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). To have the contents of H + Al (figure 5), higher values within the MFE mode were found, as a greater value for *Urochloa brizantha cv*. This result is explained by the pH increase in the same mode (figure 3). According to Steiner et al. (2011), the potential acidity has a behavior opposite to that of the pH, therefore, as the pH is raised, the potential acidity tends to decrease. The author comments that when the pH is increased in depth it is due to the downward movement of Ca^{2+} and Mg^{2+} to deeper soil layers. However, Oliveira et al. (2002) observed that in the no-tillage system the higher pH values are found in the surface layer up to 0.10 m.

Means followed by same letter do not differ at Tukey test 5% probability. Forages: U.B – (*Urochloa brizantha); U.R* – *(*Urochloa ruziziensis); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). For SB levels (figure 6) the highest values were found in the MFLA mode for *Urochloa brizantha cv*, both for forage grasses within the modes and to the modes within the forage grasses. This result can be explained by higher values of Ca^{2+}, Mg^{2+}

Table 3. Average values of the soil chemical parameters, evaluated in the layer from 0.20 to 0.30 m, according to the sowing mode and forage grass species.

Causes for variation		P	MO	pH	K	Ca	Mg	H +Al	SB	T	V
Forage grass species	Modes	(mg dm^{-3})	(g dm^{-3})	($CaCl_2$)	----------------------mmol$_c$ dm^{-3}					-----------------------	(%)
U. Brizantha	Row	66.3	27.0	5.3	4.7	38.0^a	16.0	30.3	58.7	89.0	62,0
U. Brizantha	Between Rows	28.6	20.0	5.1	3.4	22.6^b	10.6	32.0	36.7	68.7	53,3
U. Brizantha	Cover	47.0	22.0	5.1	4.0	26.6^b	12.6	33.6	43.4	77.0	56,2
U. Brizantha	Casting V_4	36.3	21.0	5.1	3.9	21.3^b	12.0	1.0	37.2	68.2	54,2
U. Ruziziensis	Row	32.3	19.6	5.2	4.3	29.3^b	14.3	27.0	48.0	75.0	62,7
U. Ruziziensis	Between Rows	53.3	24.6	5.4	4.3	33.0^b	18.3	23.0	55.6	85.6	64,5
U. Ruziziensis	Cover	37.0	22.0	4.8	3.7	16.3^b	8.3	40.3	28.4	68.7	41,4
U. Ruziziensis	Casting V_4	32.6	21.0	5.1	3.2	23.0^b	14.0	32.3	40.2	72.6	55,4
Control		33,3	21.0	5.1	3.6	21.0^b	12.3	33.0	37.0	70.0	52.2
FxT		0,5^{ns}	0.2^{ns}	0.3^{ns}	0.2^{ns}	1.4^{ns}	0.2^{ns}	0.1^{ns}	0.9^{ns}	0.9^{ns}	0.9^{ns}
DMS		41,5	9.0	0.4	2.2	16.8	7.1	10.7	24.8	21.9	15.8
CV%		50,8	20.3	4.0	28.3	32.7	26.9	16.6	28.9	14.5	14.1

NS (not significant). Averages followed by the same letter and no letters in columns do not differ by the Dunnett test ($p < 0.05$). F - Forage grass; T - Control; U. Brizantha - Urochloa brizantha; U. Ruziziensis - Urochloa ruziziensis; Rows - Maize with Urochoa in planting rows; Between Rows - Maize with Urochoa between rows; Cover - Maize with Urochoa sown in rows cover fertilizer in maize V4 stage; Casting V4 - Maize with Urochoa sown by casting in the V4 stage of maize; Control - maize only.

and K^+ found in Table 2, in mode Casting V4, as well as the base saturation Figure 6.

Means followed by same letter do not differ at Tukey test 5% probability. Forages: U.B – (*Urochloa brizantha); U.R – (Urochloa ruziziensis*); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). These results corroborate Sarto et al. (2014), in which working with soil chemical properties depending on the silicon fertilization, found higher SB levels because of cations Ca^{2+}, Mg^{2+} and K^+. Means followed by same letter do not differ at Tukey test 5% probability. Forages: U.B – (*Urochloa brizantha); U.R – (Urochloa ruziziensis*); sowing modalities: MFL – (Maize with urochloa in the sowing line), MFE – (Maize with urochloa in the interweave, sown on the same day as maize sowing), MFC – (Maize with urochloa in the seeded line together with the cover fertilizer in the V4 stage), MFLA – (Maize with urochloa on the haul along with maize V4 maize mulch). For layer 0.20 to 0.30 m (Table 3), there were no significant differences at the 5% probability level ($p<0.05$). Several studies show that in a no-till system there is a tendency in accumulation of surface nutrients after four to six years of cultivation. It is explained by the lack of tillage, liming and fertilization on the surface, by casting or in rows, which favors the formation of gradient concentration, according to Júnior et al. (2010).

Although this work has been developed in a no-till system, established more than ten years ago, there is a trend towards lower levels of exchangeable bases in soil, with increasing depth, given that the effects of liming in these regions tend to be smaller (Bayer et al., 1997; Souza et al, 2003; Cavalcante et al., 2007).

Conclusions

The intercropping of forage grasses with maize, in different modes, promotes changes in the soil chemical attributes, mainly in the layer from 0.10 to 0.20 m. The intercropping of maize with *Urochloa brizantha cv* presents a higher nutrient recycling. Both modes maize with *Urochoa* between rows (MFE) and maize with *Urochoa* sown by casting in the V4 stage of maize (MFLA) presented higher values of chemical changes.

Conflict of Interests

The authors have not declared any conflict of interests.

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Influence of environmental factors on carpetgrass seed germination

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The aim of this study was to verify the influence of temperature, light, substrate, sowing methods, and salt and water stress on carpetgrass (A*xonopus affinis*, in the Poaceae family) seed germination. All four trials were performed in a germination chamber under controlled conditions of temperature, moisture and photoperiod. Experimental designs were entirely randomized with 100 seeds per plot. For the study of temperature and light (Experiment 1), treatments were arranged in a 6 x 2 factorial scheme, with six temperature regimes (constant at 20, 25, 30, and 35°C, and alternate at 20 to 30 and 20 to 25°C) combined with two light conditions (light: 8 h of light and 16 h of dark, and dark: 24 h without light) and four replications. For the study of substrate and sowing methods (Experiment 2), there were four treatments (on paper, between paper, on sand, and in sand) with five replications. For the study of salt stress (Experiment 3), there were five treatments, composed of NaCl concentrations (0, 25, 50, 75 and 100 mM), with four replications. For the study of water stress, treatments were arranged in a 3 x 2 factorial scheme (Experiment 4), with three substrate water contents (50, 75 and 100% water retention capacity) and two sowing methods (on sand and in sand), and four replications. Germination percentage and rate were evaluated. It was concluded that seed germination was more effective at the alternate temperatures of 20 to 30 and 20 to 35°C, under light, sown on sand, on a paper substrate or between papers. The NaCl concentrations did not affect germination percentage; however, germination was slower as the NaCl concentration increased. Faster and higher germination occurred when seeds were sown on sand at 100% of its water retention capacity.

Key words: A*xonopus affinis*, light, salt stress, substrate, temperature, water stress.

INTRODUCTION

Lawns may usually bring many benefits to the surrounding environment. Besides the aesthetic effects, erosions caused by either water or wind are prevented, and problems related to mud and dust are minimized,

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among others. The increasing demand for quality lawns by the consumer market is the main factor that boots grass producing areas, especially those close to great consumer poles (Godoy et al., 2012).

The grass species A*xonopus affinis* Chase, in the Poaceae family, is commonly known as carpetgrass. It is still little cultivated in Brazil for ornamental purposes; however, it shows expansion potential for its decorative effect, hardiness and resistance to trampling and cold. Travi et al. (2014) reported that *A. affinis* is one of the most important grass species in native pastures of Southern Brazil, besides being aggressive and resistant to trampling and cold.

In Brazil, most lawns are still implemented from vegetatively propagated plants; however, the use of seeds is increasing according to the global trend. Batista et al. (2015) highlighted that the lawn formation from seeds, which is a common practice in the United States and Europe, is currently expanding also in Brazil.

However, seed germination depends on several endogenous and exogenous factors, so water, temperature, oxygen, and light are the most important ones (Baskin and Baskin, 1998) among others, such as substrate and salinity (Batista et al., 2015). The influence of these factors varies according to the species; so, knowledge on specific requirements of each plant contributes to guiding research on seed germination and field requirements.

Water is one of the most important factors affecting germination, as it activates the seed metabolism besides being both directly and indirectly involved in all the remaining germination stages (Marco Filho, 2005).

Temperature affects germination percentage and rate, seed water absorption and, also, biochemical reactions that determine all the germination process (Carvalho and Nakagawa, 2000). Furthermore, the substrate used in germination trials greatly influences the germination process due to some factors that may vary according to the material, such as structure, aeration, water retention capacity and pathogen infestation (Popinigis, 1977).

Some species are tolerant to saline environments, what becomes important for their cultivation in areas that are naturally saline or usage of residual water for irrigation. However, salinity may interfere with seed germination and may even inhibit it because of the osmotic effect, which may cause either the physiological drought or seedling toxicity, which is a result of the ion concentration in the cell protoplasm (Tobe et al., 2000).

In this sense, with the aim to endorse and clarify aspects related to seed germination of *A. affinis* grass species, the objective of this work was to verify the influence of temperature, light, substrate, sowing methods, and saline and water stress on such process.

MATERIALS AND METHODS

The experiments were conducted in the first half of 2015 in the Laboratory of Seed Analysis, Department of Crop Production at the College of Agricultural and Veterinary Sciences of the State University of São Paulo (FCAV/UNESP), located in the municipality of Jaboticabal, Brazil (21°15'22" S and 48°18'58" W, at 590 m altitude).

Seeds of *A. affinis* were bought from a commercial producer, already treated with 0.18% Mayran fungicide [700 g kg^{-1} Thiram + 0.06% Rovral (500 g L^{-1} Iprodione)]. Seed water content was determined applying the oven method, at 105 ± 3°C for 24 h, as described by Brasil (2009); it was 14.86%.

Temperature and light

The experimental design was entirely randomized and treatments were arranged in a 6 x 2 factorial scheme: six temperature conditions (constant at 20, 25, 30, and 35°C, and alternate at 20-30 and 20-35°C) and two light regimes (light: 8 h of light and 16 h of dark; and dark: 24 h without light), and four replications of 100 seeds each.

Experimental plots were composed of plastic boxes (11 x 11 x 3.5 cm) of "gerbox" type, which were placed in tied low-density polyethylene bags to avoid moisture loss. Both transparent (light treatment) and black (dark treatment) plastic boxes were used. Seeds were sown on two sheets of filter paper moistened with distilled water, whose mass applied was 2.5 times the dry paper mass (Brasil, 2009).

The experiment was conducted in BOD germinators set at different temperatures according to the proposed treatments.

Substrate and sowing methods

The experimental design was entirely randomized with four treatments (on paper, between paper, on sand and in sand) and five replications of 100 seeds each. Experimental plots were composed of plastic boxes (11 x 11 x 3.5 cm) of "gerbox" type, which were placed in tied low-density polyethylene bags to avoid moisture loss. Seeds were sown either on filter paper or between two sheets of filter paper moistened with distilled water, and mass applied was 2.5 times the dry paper mass (Brasil, 2009). For the sand treatments, there was 250 g washed sand per plot, which was sterilized at 200°C for 2 h. For the 'in sand' treatment, seeds were sown at 4 mm depth. The experiment was conducted in a BOD germinator at the alternate temperature of 20-35°C and photoperiod of 8 h of light and 16 h of dark.

Saline stress

The experimental design was entirely randomized with five treatments (five NaCl concentrations: 0, 25, 50, 75, and 100 mM) and four replications of 100 seeds each. The electrical conductivity (EC) of the solutions was, respectively, 2.16, 2.90, 5.80, 8.46, and 11.37 µS cm^{-1}. For the 0 mM concentration, only distilled water was used. Experimental plots were composed of plastic boxes (11 x 11 x 3.5 cm) of "gerbox" type, which were placed in tied low-density polyethylene bags to avoid moisture loss. Seeds were sown between two sheets of filter paper, moistened with distilled water, and mass applied was 2.5 times the dry paper mass (Brasil, 2009).
The experiment was conducted in a BOD germinator at the alternate temperature of 20-35°C and photoperiod of 8 h of light and 16 h of dark.

Water stress and sowing methods

The experimental design was entirely randomized and treatments

Table 1. Germination percentage and germination rate (GR) of *Axonopus affinis* seeds subjected to different temperatures, light regimes, substrates and saline stress.

Temperature and light				
Treatments	**Germination (%)[1]**		**GR**	
Temperature (ºC)	**Light[2]**	**Dark[2]**	**Light[2]**	**Dark[2]**
20	0.25[Ad]	0.25[Ac]	0.01[Ac]	0.02[Ac]
25	3.00[Ac]	0.50[Bc]	0.22[Ac]	0.03[Ac]
30	4.25[Ac]	0.00[Bc]	0.29[Ac]	0.00[Ac]
35	24.50[Ab]	0.00[Bc]	1.86[Ab]	0.00[Bc]
20-30	88.00[Aa]	24.00[Bb]	8.97[Aa]	2.62[Bb]
20-35	81.25[Aa]	60.75[Ba]	8.67[Aa]	6.24[Ba]
CV (%)	15.11		16.65	
Substrate				
	Germination (%)[1]		**GR**	
On paper	77.60[a]		8.00[a]	
Between paper	80.40[a]		8.77[a]	
On sand	76.20[a]		8.52[a]	
In sand	25.80[b]		1.64[b]	
CV (%)	5.09		10.27	
Water stress				
Treatments	**Germination (%)[1]**		**GR**	
WRC[3] (%)	**On sand**	**In sand**	**On sand**	**In sand**
50	56.00[Ab]	38.25[Ba]	6.22[Ab]	3.89[Ba]
75	52.25[Ab]	34.75[Ba]	6.01[Ab]	3.23[Ba]
100	75.00[Aa]	25.25[Bb]	8.48[Aa]	1.61[Bb]
CV (%)	6.43		10.22	

[1]Original data; data were transformed into arcsine $(x/100)^{1/2}$ for statistical analysis only. [2]Light: 8 hours of light and 16 hours of dark; Dark: 24 hours without light. [3]WRC: Water retention capacity. Means followed by the same upper case letter in the line and lower case letter in the column do not differ from each other by the Tukey test at 5% significance level.

were arranged in a 3 x 2 factorial scheme: three substrate water contents (50, 75, and 100% substrate water retention capacity) and two sowing methods (on sand and in sand), with four replications of 100 seeds each. Experimental plots were composed of plastic boxes (11 x 11 x 3.5 cm) of "gerbox" type, which were placed in tied low-density polyethylene bags to avoid moisture loss. For the sand treatments, there was 250 g washed sand per plot, which was sterilized at 200°C for 2 h. For the 'in sand' treatment, seeds were sown at 4 mm depth.

The experiment was conducted in a BOD germinator at the alternate temperature of 20-35°C and photoperiod of 8 h light and 16 h dark. Sand maximum water retention capacity was calculated prior to definition of the water volume to be provided to the other treatments. For 100% water retention capacity, 218 mL water kg^{-1} sand was provided. Plastic boxes with sand were daily weighted so water replacement was performed whenever necessary to maintain the calculated water retention capacity for each treatment.

Assessment and statistical analysis

Seed germination was daily observed and noted for 28 days. Seeds were considered germinated when presented normal seedlings of at least 5 mm. Analyzed variables were germination percentage and rate, which were calculated according to Maguire (1962).

For statistical analysis, values of germination percentage were transformed into arcsine $(x/100)^{1/2}$ for normalization. Data of both variables were submitted to variance analysis and means were compared by the Tukey test at 5% significance level. Polynomial regression analysis was also performed to evaluate variable behavior according to increasing salinity.

RESULTS AND DISCUSSION

Temperature and light

The interaction between temperature and light was significant for both germination percentage and rate. Higher percentage and faster germination occurred under light, reaching 88.00 and 81.25% germination at the alternate temperatures of 20-30 and 20-35°C, respectively. Germination under dark was null or very low at the constant temperatures; however, there was 60.75% germination when seeds were submitted to the alternate temperature of 20-35°C (Table 1). It was also this temperature regime, in the dark, that promoted faster germination (Table 1). Therefore, in general, great germination percentages of *A. affinis* were observed at alternate temperatures (Table 1). Similar results with other Poaceae species were obtained by Carmona et al.

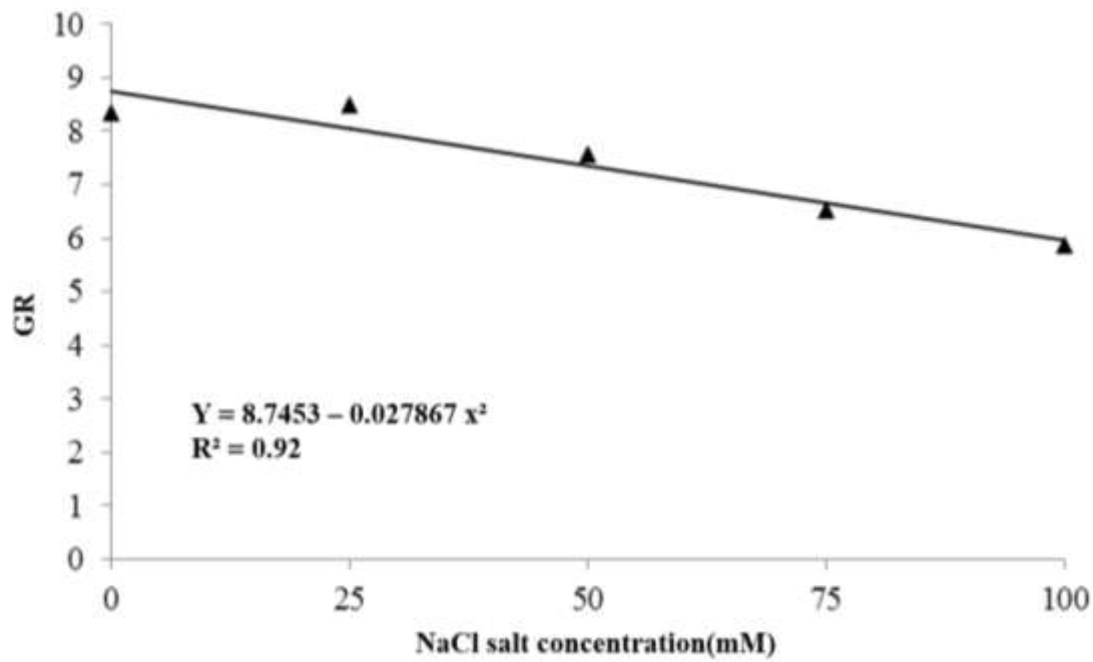

Figure 1. Germination rate (GR) of *Axonopus affinis* seeds subjected to different NaCl concentrations.

(1998), who studied several grass species, and by Ever and Parsons (2009) and Batista et al. (2015) for *Cynodon dactylon*.

Seeds of some species do present higher germination percentage, germination rate, and vigor when subjected to alternate temperatures, which corresponds to natural fluctuations found in the environment (Copeland and McDonald, 1995). Although, germination percentage and rate were significantly higher when *A. affinis* seeds were sown under light, there was also germination in the dark, which indicates that the species was insensitive to light. Therefore, light did stimulate *A. affinis* seed germination, but it did not limit the process. Similar behavior of other grass species have also been reported by Opeña et al. (2014), Bastiani et al. (2015) and Batista et al. (2015), corroborating our results.

Different temperatures, combined with presence or absence of light, are also important environmental factors acting as germination triggering agents (Carvalho and Nakagawa, 2000). According to these results, the combination of alternate temperatures and light stimulated seed germination of *A. affinis*. Such combination also promoted germination of *Melinis minutiflora* grass species (Carmona and Martins, 2010), bermudagrass 'Riviera' (Batista et al., 2015), and other grass species native to the Brazilian cerrado (Carmona et al., 1998).

Substrate and sowing methods

For both germination percentage and rate, there were no significant differences among 'on paper', 'between paper' and 'on sand' treatments, which were superior to sowing in sand (Table 1). These results corroborate recommendations from the Rules for Seed Analysis (Brasil, 2009), which indicates that ideal substrates and sowing methods for germination trials are on either paper or sand, so we may extend it to seed sowing and also between paper.

Lower germination percentage observed for seed sowing in sand may be related to seed size. For instance, Evers and Parsons (2009), when studying bermudagrass seeds, recommended sowing on a lightly compacted surface because of seed minute size.

Saline stress

For germination percentage, there were no significant differences among treatments, resulting in the mean of 84.10%; germination was slower as the saline concentration increased, shown by the adjustment of the negative linear regression (Figure 1).

Even with the slower germination with salt increase, *A. affinis* was considered tolerant to salinity as germination percentage was high regardless the electrical conductivity, which ranged from 2.16 to 11.37 µS cm^{-1}. Therefore, seeds of this species may be selected for lawn implementation in saline soils or when using brackish or residual water for irrigation.

Some plant species; however, do benefit from salinity during germination, which denotes greater adaptation capacity to such condition along their life cycle (Viana et al., 2004). Nevertheless, salt effects depend on factors such as species, cultivar, phenological stage, salt kind, intensity and duration of saline stress, crop management, irrigation, and soil and weather conditions (Tester and Davénport, 2003).

In comparison with other grasses, Myers and Couper (1989) reported that, after *Puccinellia ciliata* and *Lolium perenne* seed germination has started and, also, at seven days after sowing, there were germination losses from substrates irrigated with a saline solution. Also, Coan et al. (2008) observed that irrigation with saline waters of up to 6.0 dS m^{-1} electrical conductivity did not inhibit seed germination of *Lolium perenne* and bermudagrass 'Mirage'. In addition, Batista et al. (2015), when studying the effects of NaCl concentrations on germination of bermudagrass 'Riviera' and 'Princess 77', noted that germination of both cultivars was more effective under NaCl absence.

Water stress and sowing methods

The interaction among substrate water contents and sowing methods was significant for germination percentage and rate. Both variables presented superior results when seeds were sown on sand maintained at 100% water retention capacity (Table 1).

The most favorable substrate water content for seed germination of many species ranges from 40 to 60% substrate water retention capacity (Piana et al., 1994). However, it varies with species and cultivar, substrate composition, and sowing method, as reported by Batista et al. (2015). These authors observed that bermudagrass 'Princess 77' seeds presented an even germination when sown either in or on sand maintained at 50% water retention capacity; for 'Riviera' cultivar, germination was more effective when seeds were sown on sand at 100% water retention capacity, similarly to what was found in this study.

Conclusions

Seed germination of *A. affinis* was more effective at the alternate temperatures of 20-30 and 20-35°C, under light, when sown on paper, between paper and on sand. The NaCl concentrations did not affect the germination percentage; however, its increment gradually decreased the germination rate. Greater germination percentage and rate were obtained when seeds were sown on sand at 100% water retention capacity.

Conflict of Interests

The authors have not declared any conflict of interests.

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White thread blight disease caused by *Marasmiellus scandens* (Massee) Dennis & Reid on cocoa and its control in Ghana

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White thread blight disease (WTBD) is currently emerging as an important foliar disease on cocoa in Ghana. The disease has been known in the country for many years. Yet, the incidence and severity levels on cocoa in the growing regions are not known. Surveys and sampling were conducted between 2011 and 2013 to estimate incidence and severity of WTBD in the six cocoa growing regions (Ashanti, Brong-Ahafo, Central, Eastern, Western and Volta) of Ghana. Diseased samples were assayed for the infecting fungus and its identification. Chi square tests were used to find relationships between age, sanitation practice and the disease severity. Effectiveness of chemical and cultural control methods against the disease were tested. The disease was found in all the cocoa growing regions of Ghana and out of 24,000 trees inspected, 1,281 (5.3%) were infected. The majority of infected trees (74.2%) were moderately affected but 3.2% of the trees were very severely affected and almost dead. A positive correlation (r = 0.889) was found between WTBD incidence and the severity. The most severely affected regions were Ashanti (13.8%), Brong-Ahafo (10.2%) and Western (7.6%) regions. Poor maintenance significantly (p=0.0001) increased the levels of disease occurrence and severity. Older cocoa trees also appeared more susceptible than younger ones. Pruning of affected branches controlled the disease better than fungicides spray. However, Nordox (75% copper (I) oxide) at 5 g/l and Metalm (12% metalaxyl and (60% copper (I) oxide) at 3.3 g/l fungicides were effective in reducing mycelial growth of the *Marasmiellus* fungus. Therefore, fungicide should be used in situations of severe infection to supplement pruning.

Key words: Thread blight, cocoa, *Marasmiellus*, disease severity.

INTRODUCTION

Theobroma cacao L. (cocoa) is the source of chocolate, one of the world's most popular foods. Ghana produces nearly 20% of the world total cocoa output contributing 8.2% to gross domestic product (GDP) and 30% of the total export earnings (Asante-Poku and Angelucci, 2013). Production in the country has recently seen appreciable

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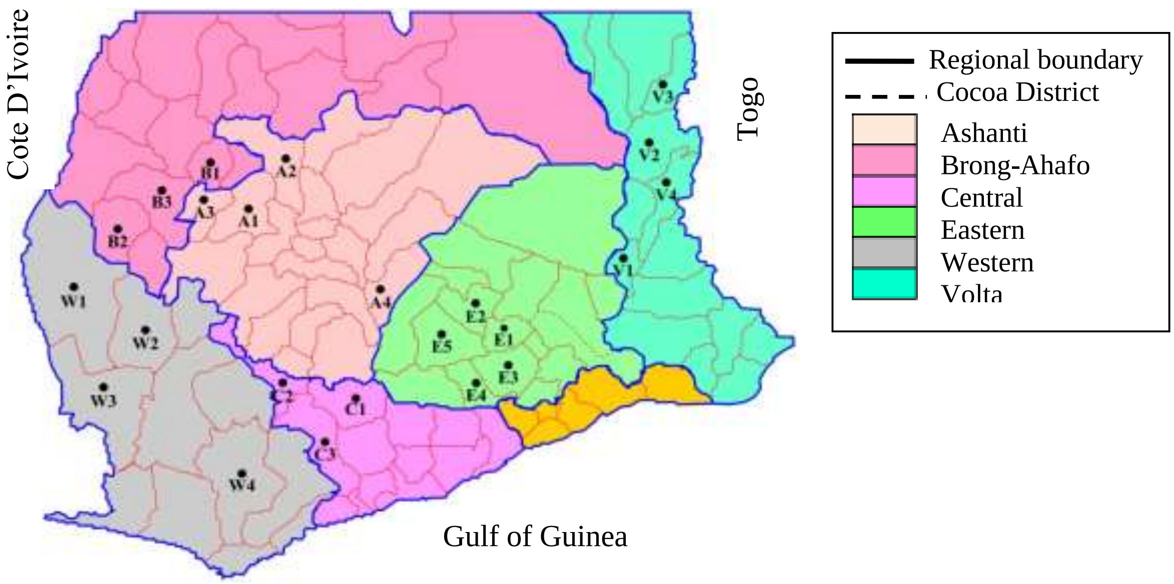

Figure 1. Surveyed districts (in dots) in the different cocoa growing regions of Ghana.

increases from 450 000 tons in the year 2000 to 900,000 tons in 2010 and in excess of 1,000,000 tons in 2011 (FAO), 2012). However, Ghana's average annual cocoa yield of 440 kg/ha as against potential yield of over 2.5 tonnes per hectare, is among the lowest in the world and compares unfavorably to important producers such as Cote d'Ivoire (580 kg/ha) and Indonesia (770 kg/ha). This is attributed largely to fungal diseases among several other important factors (Bailey et al., 2016; Wood and Lass, 1992). Cocoa tree is under constant threat from fungal pathogens where ever it is cultivated. The most important fungal disease of cocoa in Ghana is *Phytophthora* pod rot popularly known as "black pod disease" which can cause 100% yield loss (Dakwa, 1987). There are several other fungal diseases but most of them are considered less important because they apparently cause minor losses or are limited in distribution. One such disease which is increasingly emerging as a serious foliar disease of cocoa in the country is white thread blight disease (WTBD) caused by *Marasmiellus scandens* (Massee) Dennis & Reid [syn: *M. scandens* (Massee) (Opoku et al., 2007). The disease is worldwide in distribution and in the Amazon where cocoa tree originated, it has been reported on several important plant species including 18 native fruit trees (Gasparotto and Silva, 1999). The disease has wide host range and is found on economically important trees crops some of which are also found in cocoa farms as fruit crop or shade trees (Benchimol, et al., 2001).

The white thread blight disease derived its name from mycelial strands (threads) of the fungal pathogen that grow underneath cocoa branches, petioles and leaves causing leaf blight. Blighted leaves show distinctive brown to dark-brown decay followed by defoliation. Typically, defoliated leaves cling to each other and the tree from the mycelial strands of the fungus that can be seen on twigs and petioles (Opoku et al., 2007). Dead leaves with mycelia are major source of inoculum and are spread by wind, rain, insects, nesting birds and human activities (Kusunoki et al., 1997). The disease may be devastating when poor agricultural practices and favourable weather conditions prevail over time. In spite of reports of WTBD on cocoa in Ghana over the years, little advances have been made on the disease. Leston, (1970) reported white thread blight incidence of about 6-48% from four outbreak cocoa growing communities. Asare-Nyako (1987) studied the infection processes of the thread blight fungus on cocoa in the field, gauze house and laboratory. Scientific documentation regarding the disease incidence and severity levels on cocoa farms in Ghana is still lacking. This study was, therefore, carried out to document the occurrence and distribution of white thread blight disease on cocoa in all the growing regions of Ghana. It also assessed the relationships between the farm age, the farm sanitation level and the disease severity.

MATERIALS AND METHODS

Disease assessment

Surveys were conducted between 2011 and 2013 cocoa seasons to assess incidence and severity of WTBD in the six cocoa growing regions (Ashanti, Brong-Ahafo, Central, Eastern, Western and Volta) of Ghana (Figure 1). Twenty farms were selected from each region and in each farm, 200 trees were visually inspected along a diagonal transect. The numbers of cocoa trees with WTBD symptoms were counted and the Disease Incidence (DI) estimated using the equation:

$$DI = \frac{\text{Number of trees showing disease symptoms}}{\text{Total number of trees inspected}} \times 100$$

Disease Severity (DS) was estimated using a rating key similar to Akrofi et al. (2014) where;
None (0): No TBD symptom on tree canopy (values in parentheses are rating score).
Moderate (1): more than 25% but less than 50% of canopy infected.
Severe (3): more than 50% but less than 75% of canopy infected.
Very severe (5): more than 75% of canopy infected.

The DS was then measured based on a modified equation from that proposed by Kranz (1988);

$$DI = \frac{\Sigma (a \times b)}{N} \times 100$$

Where,
Σ (a×b) = Sum of symptomatic trees and their corresponding score rating.
N = Total number of trees sampled

Sanitation levels in the farms were assessed based on visual observations and information from the farmers on their maintenance practices of weeding frequency, shade management, removal of mistletoe/epiphytes, infected fruits and over hanging branches. The farmers' practices were scored on a 1-5 scale (1=none, 2=, incomplete, 3= infrequent, 4= complete, 5 =frequent) and the farms classified as either low in sanitation when there is no or incomplete and infrequent maintenance practice. High sanitation is when there is complete and frequent maintenance. The ages of the farms were recorded through interviewing the farmers.

Fungi isolation, identification and pathogenicity testing

Samples of cocoa leaves, twigs or branches showing signs and symptoms of white thread blight infection were collected from the surveyed farms for fungi isolation. Single strand isolations were made from the mycelial strands onto water agar plates and subsequently onto Potato Dextrose Agar (PDA) media. Pure cultures of the isolates were observed under light microscope (Leica, USA) and identification based on morphological characteristics using standard references (Singer, 1986; Humber, 2005; Kirk et al., 2008; Desjardin et al., 1993). Pathogenicity test was conducted on healthy cocoa leaves (3-months old) from mixed cocoa hybrids. The leaves were surface sterilised with 70% alcohol (Sigma-Aldrich, USA) and rinsed twice in sterile distilled water (SDW). Three, 10-mm mycelia discs of the test isolates were placed on abaxial and similarly on adaxial surface of leaves in completely randomised design with 5 replications. The leaves were incubated in aluminum tray (72 x 62 x 10 cm) lined with moist plastic foam (Latex foam, Ghana Ltd) on laboratory bench and examined after 7 days for infection and re-isolation of test isolates. The entire experiment was repeated thrice.

Evaluation of management practices

Partial systemic and contact fungicides which proved promising in a laboratory assay viz. Nordox (75% copper (I) oxide a.i., Nordox Industries, Oslo, Norway), Metalm (12% metalaxyl and 60% copper (I) oxide a.i., ALM International, South Africa) and Ridomil Gold (6% mefenoxam + 60% copper (I) oxide a.i., Ceiba Geigy Ltd., Basle, Switzerland) were used. The fungicides were also tested in combination with pruning of white thread blight fungus on naturally infected cocoa trees on farmer's farm at Osino (6°20′84.4″N, 0°29′64″W) in the Eastern Region. The farm was selected because of the proximity to Cocoa Research Institute of Ghana to facilitate continuous monitoring and also to demonstrate to farmers in the locality, treatment options available to them. The treatments applied were:

1) Spraying infected branches at 4 weekly intervals.
2) Pruning and spraying of pruned surfaces at 4-weekly intervals
3) Pruning infected branches
4) Untreated (control) trees

Forty replicate trees were selected for each treatment during the peak disease period of August - November, 2014. Test fungicides were applied in 15 L volumes using pneumatic knapsack sprayer (MATABI®, Goizper S. Coop, Spain). Long handled pruning knife were used to prune affected branches. Treatment trees were assessed weekly and the mycelia growth measured with tape measure. Data obtained was expressed as growth rate per week and percent mycelial growth inhibition calculated for each fungicide.

Statistical analysis

Descriptive and inferential statistics were employed to analyse survey data using the Statistical Package for Social Sciences (SPSS) Version 10. For descriptive statistical techniques, frequency distribution and mean were computed to study the data. Chi-square test was used to infer relationships between the farm age, its sanitation and the disease severity. The farms were grouped into old and young for analysis. The productive age of the surveyed farms was taken as 40 years with 1-20 years as the most economic phase (that is, younger farm) and 21-40 as old farm. One way ANOVA with Duncan's Multiple Range Test (SASS Program, version 22) was performed to compare the distribution of Disease Incidence (DI) and Disease Severity (DS) amongst surveyed regions. Test of normality was employed to determine whether DI and DS data should be transformed or not prior to analysis.

RESULTS

White thread blight disease was found in all the cocoa growing regions of Ghana (Figure 1). From the 24,000 trees aged between 12-35 years which were inspected nationwide, 1,281 of them representing 5.3% were infected (Table 1). Majority of the infected trees (951), were moderately affected and these constituted 74.2% infection. However, 40 (3.1%) trees were very severely affected with some of them completely killed (Table 1). Among the surveyed districts, the disease incidence (DI) ranged from 1.9% in Akim West of Eastern region to 10% in Asante Akyem south in the Ashanti region. The most severe forms of the disease were encountered in the Asante Akyem south district (Table 1). In the regions, disease incidence of 7.8% occurred in the Brong-Ahafo and it was significantly ($p<0.05$) higher than the other regions except Ashanti. In the Western region however, incidence levels were significantly ($p<0.05$) higher than Central, Eastern and Volta regions (Table 2). The most severely affected regions were Ashanti, Brong-Ahafo and Western. White thread blight disease incidence was found to correlate well ($r = 0.889$, $y=1.517x$) with the

Table 1. Distribution of white thread blight disease and characteristics of cocoa trees assessed in selected growing districts of Ghana.

Region	District	Av. age of farm (yrs.)	No. of trees inspected	No. of trees infected	% Infected	No. of infected trees identified as		
						Moderate	Severe	Very Severe
ASHANTI								
A1	Ahafo Ano South	24	800	48	6.0	38	10	0
A2	Offinso Municipal	30	800	56	7.0	49	7	0
A3	Ahafo Ano North	20	400	20	5.0	12	8	0
A4	Asante Akyem South	35	2,000	200	10.0	24	156	20
BRONG AHAFO								
B1	Tano North	26	1,200	91	7.6	78	11	2
B2	Asunafo North	26	1,200	94	7.8	92	2	0
B3	Asutifi	33	1,600	128	8.0	112	12	4
CENTRAL								
C1	Assin North	17	1,400	39	2.8	39	0	0
C2	Lower Denkyira	15	1,000	26	2.6	25	1	0
C3	Upper Denkyira East	30	1,600	48	3.0	29	17	2
EASTERN								
E1	Akim- East	14	800	21	2.6	12	9	0
E2	Atiwa	12	400	8	2.0	7	1	0
E3	Suhum	14	1,200	48	4.0	39	7	2
E4	Akim West	14	800	15	1.9	13	2	0
E5	Kwaebibirem	24	800	24	3.0	24	0	0
WESTERN								
W1	Bia	18	600	30	5.0	18	10	2
W2	Wiawso	21	1,200	84	7.0	73	10	1
W3	Akontombra	22	1,400	112	8.0	111	1	0
W4	Tarkwa/Prestea/Huni	29	800	32	4.0	29	3	0
VOLTA								
V1	Kpeve	26	800	32	4.0	29	3	0
V2	Jasikan	24	800	26	3.3	24	2	0
V3	kajebi	25	800	29	3.6	16	11	2
V4	Hohoe	33	1,600	70	4.4	58	7	5
TOTAL			**24,000**	**1,281**		**951**	**290**	**40**

Source: Field survey (2011-2013).

disease severity. From a total of 120 120 farms (20 farms from each of the 6 regions) surveyed, sanitation levels in 86 (71.6%) of them were high while 34 (28.4%) appeared low (Table 3a). There was highly significant (p=0.0001) relationship between the levels of sanitation and TBD occurrence and severity. Farms with high sanitation levels were either free of the disease or had low incidence and severity. The disease did not occur in 31 farms and 28 (90.3%) of them had high sanitation levels (Table 3a). Meanwhile, the sanitisation levels in all the farms which were severely affected were low. It was again observed that older cocoa trees (20^+ years) were more susceptible (p=0.023) than younger ones (Table 3b). In the study, 24 young farms (1-20 years) and 96 older cocoa farms (20-40 years) were assessed for WTBD. Infection was recorded in 73 (76%) of the old farms and 16 (66.6%) of the young farms.

Pathogen isolation and identification

In the field, *M. scandens* on cocoa was seen as a

Table 2. White thread blight disease incidence and severity in cocoa growing regions of Ghana[1].

Region	Incidence (%)	Severity (%)
Ashanti	6.9±0.7[bc]	13.8±2.4[b]
Brong-Ahafo	7.8 ±0.8[c]	10.2±2.8[b]
Central	2.8 ±0.8[a]	4.4±2.8[a]
Eastern	2.6 ±0.6[a]	3.6±2.3[a]
Volta	3.8 ±0.7[a]	5.3±2.4[a]
Western	5.9 ±0.7[b]	7.6±2.4[b]
CV (%)	**6.1**	**14.7**

[1]Means (±standard error) followed by the same letters are not significantly different at p<0.05. CV= coefficient of variation.

Table 3. Effects of sanitation and age of cocoa tree on TBD severity[1].

A) Sanitation	Severity class		
	None (n= 31)	Moderate (n= 82)	Severe (n= 7)
High (n= 86)	28	58	0
Low (n= 34)	3	24	7
X^2=17.086, df=2, P=0.0001			

B) Age of trees	Severity class		
	None (n= 31)	Moderate (n= 82)	Severe (n= 7)
1-20 (n= 24)	8	13	3
20-40 (n= 96)	23	69	4
X^2=6.909, df=2, P=0.032			

[1]Values in parentheses are the total observations.

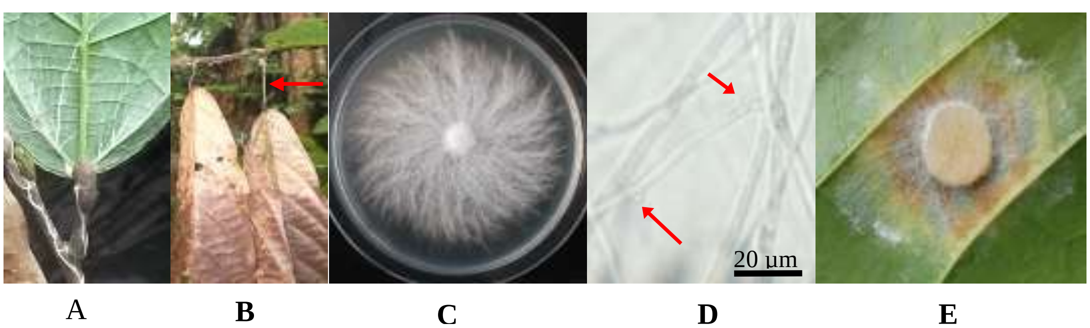

Figure 2. A: mycelia strand growing onto leaf. B: mycelia strand (arrowed) holding detached blighted leaf. C: colony morphology of a 5-day old culture incubated at 28°C ± 2°C on PDA. D: pure culture of *Marasmiellus scandens* under microscope showing hyaline hyphae with clamp connections (arrowed). E: Pathogenic symptom on inoculated leaf test.

network of web-like dried strands, predominantly, on the lower surfaces of leaves and undersides of branches. The strands always branch off from the petioles to leaves and then spread out into numerous fine ones (Figure 2A). The fine strands initiated dark-brown necrosis and as the whole leaf became involved, the leaf separated at the petiole but usually remained hanging from mycelial strand that grew over the petiole from the branch (Figure 2B). On agar plates, the fungus produced characteristic thread-like mycelia with more or less feathery margins (Figure 2C). The colonies were cottony white and produced stroma in abundance

Table 4. Effects of fungicide spray and pruning on growth inhibition of white thread blight fungus.

Treatment	Active ingredient	Fungicide mode of action	Dosage (g/l)	% Inhibition[1]
Nordox	75% copper (I) oxide	Contact	5.0	22.5[b]
Metalm	12% metalaxyl + 60% copper (I) oxide	Partial systemic	3.3	29.5[b]
Ridomil Gold	6% mefenoxam + 60% copper (I) oxide	Partial systemic	3.3	24.9[b]
Pruning				94.0[a]
Pruning + Nordox				100.0[a]
Pruning + Metalm				100.0[a]
Pruning + Ridomil Gold				91.8[a]
Fpr (5%)				**0.001**
Cv (%)				**34.5**

[1]Means followed by the same letter are not significantly different.

when kept under light (either continuous or alternate light/dark) but no fruiting bodies were observed. Hyphae were hyaline and contained numerous clamp connections that were identical to each other (Figure 2D). Pure cultures of the fungus infected cocoa leaves from the lower surface initiating necrosis similar to those observed in the field (Figure 2E). Pathogenicity was confirmed when the fungus was re-isolated from leaves with characteristic symptoms similar to those from the field.

Disease management

All the fungicides performed similarly against the WTBD disease (Table 4). Mycelia growth reduction achieved with pruning was 94% and was higher (p = 0.001) than the fungicides spray (22-30%). Combining pruning with fungicides application resulted in 92-100% growth reduction. Pruning combined well with fungicides, Nordox and Metalm, to completely suppress mycelia growth of the white thread blight fungi (Table 2). Metalm a partially systemic fungicide was effective at slightly lower dosage (3.3 g/L) when compared to the contact fungicide, Nordox (5.0 g/L).

DISCUSSION

White thread blight disease has been known on cocoa in Ghana for many years but few studies have been conducted on it since Leston (1970) drew attention to the potential threat of the disease to production. This study represents the first scientific information on regional distribution of abundance and severity of the disease on cocoa in Ghana. Previous studies noted *M (Marasmius) scandens* as the causal fungus of WTBD on cocoa in Ghana (Opoku et al., 2007; Asare-Nyako, 1987). This was confirmed in the current study where *Marasmiellus* Murill fungi isolates were associated consistently with the WTBD. They produced culture characteristics on PDA similar to those previously found on cocoa in Ghana. Necrotic symptoms development on healthy leaves after inoculation indicated that the isolates were pathogenic and their identities were confirmed as *M. scandens* (Massee) Dennis & Reid according to the original taxonomic work of Dennis and Reid (1957), then Singer (1986) and Desjardin et al. (1993). The disease is widespread on cocoa in Ghana with high incidence rates in the growing districts. Most farmers have limited knowledge about the disease and were therefore indifferent towards its control resulting in incidence levels which increasingly affected the disease severity. The correlation coefficient of relationship between the incidence and severity was close to 1 (r = 0.889). Therefore, it was not surprising that the survey data described the highest disease incidence and the most severe forms of the disease as both occurring in Asante Akyem South district of Ashanti region. Thus, supporting the assumption that disease incidence always create epidemiologically significant concept with its severity (Seem, 1984). The study also found a significant (p= 0.032) relationship between the disease severity and age of cocoa. Prevalence of the disease on older trees (20^+ yrs) and its slow development on young ones suggests *Marasmiellus* fungi as weak pathogen which attacks maturing tissue. Susceptibility of plant tissue to diseases with age has been reported (Reuveni et al., 1986; Rodrigues, 1994). Hence, improved host plant nutrition which delays tissue maturation is important to boost cocoa trees resistance to WTBD. In the long term, replacement of old tree stock is recommended especially when cocoa yield decreases at an increasing rate over time despite the improved host nutrition.

The high WTBD incidence and severity in most cocoa growing regions is attributed largely to low adoption of farm sanitation practices. This is because the farmers hardly remove excess shade trees or prune overhanging and interlocking branches of the cocoa trees which create humid conditions that support fungi infection (David, 2005). The farmers also tend to disregard debris of blighted leaves on their trees which are the main source of inoculum that can initiate epidemics once favorable conditions for dispersal and infection prevail. The recommended cultural practice for efficient management of cocoa in Ghana requires frequent weeding (4 times/year where cocoa canopy is not closed), removal of mistletoes/epiphytes, excess shade or branches, basal chupons, infected and mummified pods (Akrofi et al., 2003). This package of farm sanitisation is an essential part of integrated black pod disease management and has been found effective against white thread blight infection. The disease occurrence was very low in farms maintaining high sanitation standards and the most severe forms were virtually absent from such farms. Pruning of white thread blight affected branches was the most effective way of managing the disease. However, re-growth of fungal strands on pruned branches was observed on some treated trees. This probably occurred when parts of the fungus mycelia remained on the trees. It will therefore require additional experiments to determine the most effective point below which infected branches should be pruned to make the practice efficient. Meanwhile, re-growth of the fungus was prevented when pruning was supplemented with copper-based fungicides spray (Nordox 75 WG and Metalm 72 WP). Therefore, fungicides should necessarily be included in the treatment of severely affected farms for effective control of the disease.

Conclusion

White thread blight disease has been known on cocoa in Ghana but essentially this is a first report of spread of the disease in all the cocoa growing regions in the country. Age of cocoa farm and the cultural maintenance practiced highly influenced the disease distribution and abundance. Integrated method of sanitation and chemical control of the disease has been demonstrated assuring farmers of effective management of *M. scandens*, causal fungus of white thread blight disease on cocoa in Ghana.

Conflict of interests

The authors have not declared any conflict of interests.

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Adaptability and stability parameters for immature seeds and pods and mature dried seeds in cowpea genotypes in Brazil northeast

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The aim of this study was to estimate adaptability and stability parameters in genotypes of cowpea for the yield of immature seeds and pods and mature dried seeds, in order to enable the recommendation of cultivars for the region of São Francisco Submedium valley. We evaluated 30 cowpea genotypes, being fourteen lines of Embrapa Semiarid, six commercial cultivars and 10 landraces in the municipalities of Juazeiro-BA and Petrolina-PE. The experiments were conducted in the second semester, during the years 2013, 2014 and 2015. For adaptability and stability analysis, the methodologies of Eberhart and Russell, Lin and Binns were used, in addition to the multiplicative method, based on principal components analysis (AMMI). A significant difference was observed for the mean squares of treatments in all environments, as well as the pooled ANOVA (P<0.01) for the effects of genotypes (G), environments (E) and G*E interaction. The lines P290, P303, P508 and PC950409D02E showed yield of immature seeds exceeding 2140 kg ha^{-1}, broad stability and good predictability in the series of evaluated environments, and has great potential to be recommended as new cultivars for the region of São Francisco Submedium Valley.

Key words: Vigna unguiculata, G*E interaction, semiarid, additive effects and multiplicative interaction (AMMI).

INTRODUCTION

Cowpea yield (*Vigna unguiculata* (L.) Walp.) in Brazil is concentrated in the Northeast and North, and it has been expanding in the cerrado of the north and center-west regions, where it has been cultivated using large areas and high technology, reaching new markets (Freire Filho et al., 2011). Cowpea is distinguished for its

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socioeconomic importance, being one of the main components of diet in these two regions, especially to the rural populations, generating jobs and income (Freire Filho et al., 2005). Due to its genetic variability, large capacity for adaptation, high yield potential and excellent nutritional value, cowpea is considered a species of great value (Santos et al., 2013).

Despite being traditionally cultivated and marketed as mature dried seeds, the marketing of immature seeds of cowpea gains prominence in some regions of the Northeast, because it presents some advantages in relation to the dried seeds, as the less time to cook (Andrade et al., 2010). The immature seeds correspond to the pods around the maturity, with seeds with approximately 50 to 60% moisture (Freire Filho et al., 2005). Rocha et al. (2006) reported yield of 826 to 2.975 kg ha^{-1} for yield of immature pods and 519 to 2.818 kg ha^{-1} for yield of immature seeds in the evaluation of 14 accessions under irrigated conditions.

Although considered a crop with broad adaptation to the most diverse environments, Leite et al. (2009) point out that the cowpea still has low yield of dried seeds, around 300 kg ha^{-1}, in the Northeast region. According to Silva et al. (2010) the climatic adversities, the use of low agricultural technology and the planting of not improved seeds are the main causes of low yield in this semiarid region. The conditions of cultivation practiced in areas that produce cowpea causes unsimilar performance of genotypes in different environments where they are cultivated (Carvalho et al., 2013).

Among the main objectives of the genetic improvement of cowpea in Brazil, Rocha et al. (2013) emphasize the increased yield and high adaptability and stability for different cultivation environments. According to Santos (2008) the cowpea breeding program to the region of São Francisco Valley considers the development of cultivar, both for the rainfed area as well for irrigated area, once the recommendation for cultivars developed in other regions is not the best option from the agronomic standpoint. This behavior is due to G*E interaction and this is a factor that hinders the selection of genotypes better adapted (Cruz et al., 2012).

Several studies have identified genotypes with broad adaptability and good stability for yield of dried seeds (Nunes et al., 2014; Silva et al., 2016). The most used methods for assessing the adaptability and stability have been the model used by Eberhart and Russell (1966), Lin and Binns (1988) and the model of additive effects and multiplicative interaction (AMMI). However, studies with this purpose involving the cultivation of cowpea genotypes for the yield of immature seeds are unusual (Rocha et al., 2012). Until then, farmers of the region carried out the yield of seeds independently, without any study that indicate what the best cultivar for this activity.

The purpose of this study was to estimate adaptability and stability parameters in cowpea genotypes cowpea for immature seeds and pods yields and mature dried seeds in order to enable the recommendation of cultivars for the São Francisco Submedium Valley.

MATERIALS AND METHODS

Plant material

Thirty cowpea genotypes were evaluated, among then, 14 lines of Embrapa Semiarid, six commercial cultivars and 10 landraces from the municipalities of Juazeiro-BA and Petrolina-PE (Table 1). The experiments were conducted in the second semester, during 2013, 2014 and 2015, in experimental fields of Bebedouro, Petrolina-PE, and Mandacaru, Juazeiro-BA, totaling six environments. The adopted experimental design was a randomized block with three replications in a plot with a total area of 6 m^2, with two rows. The spacing used was 1.0 m × 0.1 m, corresponding to the density of 100,000 plants ha^{-1}. Fertilizations were not used, and the irrigation was done through micro sprinkling. The area was weeded and the pest controlled by using insecticide.

Variable and statistical analysis

Immature seed yield (kg ha^{-1}), mature dried seed yield (kg ha^{-1}) and immature pod yield (kg ha^{-1}) were analyzed taking individuals and pooled environments data. Immature seed and pod harvesting were done two times per week in one row, until 70 days after seed sowing. Mature dried seeds harvesting were done in the opposite row in complete mature pods.

For analysis of adaptability and stability the methodologies of Eberhart and Russell (1966) and Lin and Binns (1988) were used by means of the computational program Genes (Cruz, 2006), and the multiplicative method based on main components (AMMI) using SAS software (1989), as described by Duarte and Vencovsky (1999).

The method Eberhart and Russell (1966) is based on linear regression analysis, providing an estimate of the stability as well as to the adaptability, that is, both the regression coefficients of phenotypic values of each genotype in relation to environmental index, and the deviations of the regression provide estimates of adaptability and stability parameters, respectively (Cruz et al., 2006). The genotypes with index $\beta i = 1$ have broad adaptability, being that deviations from the regression equal to zero ($\sigma^2_{di}=0$) indicate good stability. In the method of Lin and Binns (1988), based on non-parametric analysis, Pi defines the stability of a genotype, being defined as the mean square of the distance between the mean of a genotype and the maximum mean response for all locations, so that genotypes with lower values correspond to those with a better performance.

The methodology AMMI, combines in a single model, components additives for the main effects of genotypes (gi) and environment (aj) and multiplicative components for main effects of interaction G x E (ga)ij (Duarte and Vencovsky, 1999). This

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Table 1. List of cowpea genotypes evaluated for adaptability and stability parameters for immature seeds and pods and mature dried seeds.

Treatments	Genotypes	Origin
1	BRS Acauã	EmbrapaSemiárido
2	BRS Guariba	EmbrapaSemiárido
3	BRS Marataoã	EmbrapaSemiárido
4	BRS Patativa	EmbrapaSemiárido
5	BRS Pujante	EmbrapaSemiárido
6	BRS Rouxinol	EmbrapaSemiárido
7	Lineage PC951015D01E	EmbrapaSemiárido
8	Lineage PC950409D02E	EmbrapaSemiárido
9	Lineage PC951016D01E	EmbrapaSemiárido
10	Lineage CPCR3F6L15	EmbrapaSemiárido
11	Lineage CPCR3F6L17	EmbrapaSemiárido
12	Lineage C1J	EmbrapaSemiárido
13	Lineage C2M	EmbrapaSemiárido
14	Lineage C2S	EmbrapaSemiárido
15	Lineage C3F	EmbrapaSemiárido
16	Lineage C3Q	EmbrapaSemiárido
17	Lineage C3S	EmbrapaSemiárido
18	Lineage P290	EmbrapaSemiárido
19	Lineage P303	EmbrapaSemiárido
20	Lineage P508	EmbrapaSemiárido
21	PJJ21	Nilo Coelho N8-Petrolina, PE
22	PJM22	Nilo Coelho - Petrolina, PE
23	PL23	Mandacaru-Juazeiro, BA
24	PAG24	Maniçoba-Juazeiro, BA
25	PC25	Nilo Coelho N9-Petrolina, PE
26	PD26	Maniçoba-Juazeiro, BA
27	PJ27	Maniçoba-Juazeiro, BA
28	PJJ28	Nilo Coelho N8-Petrolina, PE
29	PJN29	Nilo Coelho - Petrolina, PE
30	PLP30	Mandacaru-Juazeiro,BA

This analysishelps to identify high yield genotypes and broadly adapted, as the location of agronomic zoning, with the purpose of recommendation and selection of test sites (Gauch and Zobel, 1996).

RESULTS

Evaluation of environments for yield of immature seeds and pods and mature dried seeds in lines of cowpea

Significant difference was observed for the mean squares of treatments in all environments for all three variables analyzed. The average yields of immature seeds ranged from 766 to 2.705 kg ha^{-1}, highlighting the environments MAND13 and BEB13, for having presented the highest averages (Table 2). The yield of immature pods ranged from 1.150 to 4.866 kg ha^{-1}, highlighting the environments MAND13 and BEB13 (Table 2). The average yield of dried seeds ranged from 625 to 1,716 kg ha^{-1}, and the environments MAND13 and BEB13 were more productive (Table 2). The relations between the smaller and larger residues mean squares were below or near to seven for all variables, which, according to Cruz and Regazzi (1997) are necessary conditions for the experiments pooled analysis, indicating homogeneity of variances.

In the pooled variance analysis we observed statistically significant differences by F test ($p<0.01$) for environments (E), genotypes (G) and G*E interaction to the three variables (Table 3). This indicates that genotype and environment showed variability, and that the genotypes showed different behavior for yield of immature seeds and pods and dried seeds in different environments evaluated. The significance of G*E interaction justifies the need to conduct a study to identify

Table 2. Treatment mean square (QMT), mean square of the residue (QMR), means and variation coefficient (CV) for yield of immature seeds and pods and mature dried seeds in 30 cowpea genotypes evaluated in six environments.

Environments	Immature seed				Immature pod				Mature dried seed			
	QMT	QMR	Average	CV	QMT	QMR	Average	CV	QMT	QMR	Average	CV
BEB13	656873.5**	218879.2	2203.9	21.22	2787283.3**	727179.9	3912.35	21.79	681285.7*	340725.7	1716.2	34.01
BEB14	1025124.2*	526881.6	1846.7	39.30	6222743.7**	1819366	3505.1	38.48	131880.8*	61898.8	698.20	35.63
BEB15	655582.1**	96334.3	1081.8	28.69	1547479.0*	751098.2	2202.5	39.34	282801.9**	83557.2	806.1	35.86
MAND13	4351374.**	569927.8	2705.1	27.91	13097469.2**	1770712	4866.4	27.34	-	-	-	-
MAND14	561450.1**	106699.4	1743.7	18.73	3054582.7**	817605.4	3772.6	23.97	184097.9**	50210.0	1129.56	19.83
MAND15	235758.3**	61452.8	766.4	32.34	263005.9^{NS}	244941.7	1150.6	43.01	202945.8**	38864.7	625.6	31.51

Table 3. Pooled variance analysis for immature seed and pods (kg/ha^{-1}) and mature dried seed (kg/ha^{-1}) in 30 cowpea genotypes evaluated in six environments.

Variation source	Mean square					
	DF	Immature seeds	DF	Immature Pods	DF	Dried seed
Genotype (G)	29	2682995.0**	29	9828703**	29	421011.6**
Environment (E)	5	41728529.3**	5	294687733**	4	12019400.8**
G x E	145	875044.6**	145	3075365**	116	273778.2**
Residue	305	314418.6	343	1017424.0	208	117719.7
CPI1	33	0.77**	33	2.13**	32	0.26**
CPI1%		54.69		46.16		55.47
CV(%)		31.95		34.66		34.88

**,*, ns significant at 1%, 5% or non-significant by F test, respectively.

the genotypes of greater adaptability and stability.

Adaptability and stability parameters for yield of immature seeds and pods and mature dried seeds in cowpea genotypes

The average yield of immature seeds for the genotypes, in six environments, ranged from 932 kg ha^{-1}, in the cultivar BRS Marataoã, 2.532 kg ha^{-1}, in the line P-508, with an overall average of 1.706 kg ha^{-1} (Table 4). Only six genotypes showed yield above 2000 kg ha^{-1} (BRS Guariba, P290, P303, PC951015D01E, PC950409D02E and CPCR3F6L17). Those genotypes also showed the highest values for yield of immature pods, which ranged from 3.515 to 4.294 kg ha^{-1}, and yield of dried seeds above the overall average (1.002 kg ha^{-1}).

Among the cultivars, BRS Guariba, BRS Acauã and BRS Pujante were the most productive for all three variables (Table 4). As for the genotypes of the producers, only three showed yield above the overall average for yield of immature seeds and pods, and for yield of dried seeds, two presented averages higher than the general average. That was explained by the fact that the farmers perform the dried cowpea cultivation without selection for the yield of immature seeds.

In the analysis of adaptability and stability, the method Eberhart and Russell highlighted the genotypes BRS Guariba, P-290 and PC950409D02E with high yield, broad adaptability and good stability. The analysis with the Lin and Binns method emphasized the genotype P-508 (1) with high immature seed yield and good stability

Table 4. Stability and adaptability for immature seeds and pods and mature dried seeds yields in 30 lines of cowpea, evaluated in six environments, using the method of Eberhart and Russell (1966) and Lin and Binns (1988).

Genotypes	Immature seeds				Immature pods				Mature dried seeds			
	Eberhart and Russell			Lin and Binns	Eberhart and Russell			Lin and Binns	Eberhart and Russell			Lin and Binns
	βo	βi	σ_{dii}	Pi	βo	βi	σ_{dii}	Pi	βo	βi	σ_{dii}	Pi
BRS Acauã	1986	1.29^{NS}	177726.6*	635590[9]	3388	1.24^{NS}	366482.4^{NS}	3720816[10]	1209	1.66**	106569.0*	142904[3]
BRS Guariba	2029	1.20^{NS}	373336.8**	595452[7]	3516	1.09^{NS}	787292.2*	3364203[9]	1082	0.66^{NS}	-5238.8^{NS}	298069[13]
BRS Marataoã	932	0.50*	328831.5**	2807197[30]	2748	1.11^{NS}	539015.7*	5846900[20]	770	0.64^{NS}	223516.8**	723521[29]
BRS Patativa	1616	0.88^{NS}	-8995.6^{NS}	1173306[16]	2641	0.71^{NS}	698906.9*	7352452[23]	1228	1.31^{NS}	-19620.1^{NS}	166151[4]
BRS Pujante	1776	0.99^{NS}	271589.6**	891303[10]	3343	1.00^{NS}	690921.1*	4464629[15]	1045	1.26^{NS}	107474.3*	284312[12]
BRS Rouxinol	1248	0.57*	126292.1^{NS}	2027764[25]	2376	0.70^{NS}	610139.3*	7538238[25]	1117	0.76^{NS}	126995.1**	350370[14]
L. PC951015D01E	2165	1.64**	298108.5**	432024[5]	4128	1.56**	33436.4^{NS}	2120414[4]	1145	1.73**	22831.0^{NS}	199726[7]
L. PC950409D02E	2177	1.34^{NS}	-17675.7^{NS}	421522[4]	4019	1.40*	-2368.9^{NS}	2539971[6]	1051	-0.07**	3410.9^{NS}	524299[23]
L. PC951016D01E	1340	1.08^{NS}	197336.2*	1728546[22]	2565	1.01^{NS}	1012301.5**	7444748[24]	686	1.02^{NS}	-17821.2^{NS}	597324[28]
L. CPCR3F6L15	2400	1.75**	696760.3**	337378[2]	4386	1.89**	1098029.6**	1946225[2]	1261	1.44*	217750.9**	135474[2]
L. CPCR3F6L17	2149	1.81**	446183.8**	611276[8]	3850	1.41*	1517760.2**	3828157[12]	1140	1.50*	119371.6**	200775[8]
L. C1J	1749	1.24^{NS}	118038.9^{NS}	900659[11]	4167	1.67**	1373829.2**	2580664[7]	751	0.72^{NS}	1801.0^{NS}	550515[26]
L. C2M	1716	1.25^{NS}	273688.3**	1147839[15]	4544	0.97^{NS}	2786782.8**	2312880[5]	871	0.75^{NS}	79504.5*	519785[21]
L. C2S	1761	0.98^{NS}	301122.0**	1173646[17]	3101	0.29**	-76491.9^{NS}	5361070[18]	901	1.41^{NS}	4531.1^{NS}	368037[16]
L. C3F	1214	0.89^{NS}	64616.5^{NS}	1765082[23]	2909	1.15^{NS}	-141603.9^{NS}	5393301[19]	895	0.57^{NS}	122759.8**	555746[27]
L. C3Q	1478	1.15^{NS}	-92103.1^{NS}	1323130[19]	2990	1.04^{NS}	-164273.6^{NS}	5075775[17]	1012	1.76**	16228.4^{NS}	267994[11]
L. C3S	1587	1.01^{NS}	-66533.2^{NS}	1181694[18]	3725	1.02^{NS}	569419.4*	3766503[11]	903	1.01^{NS}	140699.0**	515621[20]
L. P290	2141	0.93^{NS}	-23310.1^{NS}	499448[6]	4294	1.18^{NS}	-6469.1^{NS}	2085384[3]	1148	1.29^{NS}	16138.5^{NS}	180601[5]
L. P303	2282	1.71**	112577.4^{NS}	371543[3]	3976	1.68**	61682.6^{NS}	2618724[8]	1089	0.87^{NS}	-26523.5^{NS}	262500[10]
L. P508	2532	1.56**	281022.4**	166379[1]	4716	1.37^{NS}	2039686.5**	1283228[1]	1351	1.22^{NS}	6845.3^{NS}	103249[1]
PJJ21	1291	0.22**	50193.9^{NS}	2060685[26]	2588	0.20**	693802.9*	7873413[26]	957	1.03^{NS}	-36439.6^{NS}	358504[15]
PJM22	1981	1.22^{NS}	201690.0*	904855[12]	3593	1.11^{NS}	366140.8^{NS}	3961597[13]	1263	2.44**	-14613.4^{NS}	186871[6]
PL23	1301	0.52*	12120.0^{NS}	1922153[24]	2163	0.52*	-30147.4^{NS}	8807759[28]	949	0.63^{NS}	32185.9^{NS}	466630[18]
PAG24	1543	0.62^{NS}	168704.8*	1593046[21]	2677	0.76^{NS}	350431.3^{NS}	6604367[22]	979	0.34**	77842.1*	525751[24]
PC25	1123	0.41**	-16916.9^{NS}	2221915[28]	1942	0.42**	-179017.1^{NS}	9652145[29]	777	0.76^{NS}	7454.3^{NS}	521307[22]
PD26	1089	0.49*	53334.1^{NS}	2322003[29]	1783	0.35**	-166658.9^{NS}	10395120[30]	950	1.12^{NS}	62392.7^{NS}	422759[17]
PJ27	1698	0.21**	-31265.4^{NS}	1324245[20]	3115	0.59*	-202460.1^{NS}	5040849[16]	976	-0.04**	73531.3*	547289[25]
PJJ28	1918	0.94	213617.1*	990547[13]	3452	1.06^{NS}	262504.6^{NS}	4154864[14]	881	0.39**	43082.6^{NS}	502961[19]
PJN29	1741	1.04	7834.9^{NS}	1040486[14]	2895	0.88^{NS}	88347.0**	5962821[21]	1137	1.27^{NS}	8105.8^{NS}	201810[9]
PLP30 ereira	1234	0.51*	245556.9*	2167848[27]	2412	0.59*	1063932.7	8222718[27]	557	0.56*	49760.1^{NS}	867983[30]
Geral	1707				3267				1003			

**,*, ^{ns} significant at 1%, 5% or non-significant, respectively, by F test F.(1-30) numbering according to lower and higher P value.

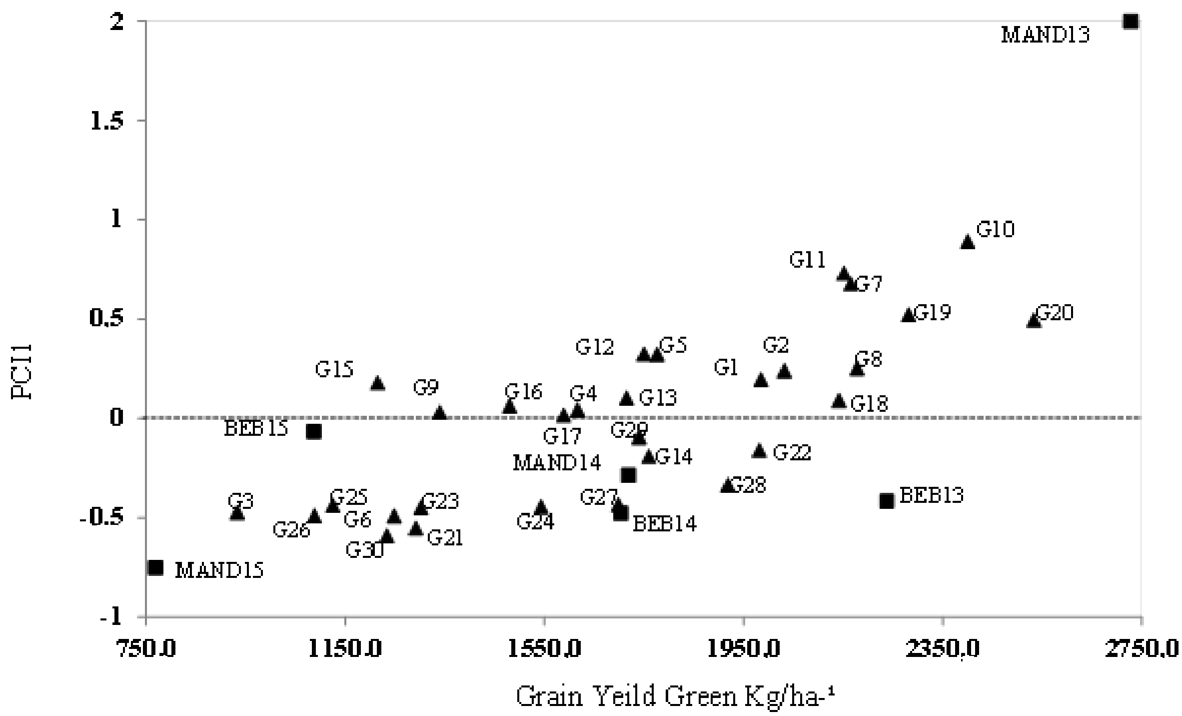

Figure 1. Biplot AMMI for immature seed yield of 30 cowpea genotypes (▲) (*Vigna unguiculata*) evaluated in six environments (■).G1=BRS Acauã; G2=BRS Guariba; G3=BRS Marataoã; G4=BRS Patativa; G5=BRS Pujante; G6=BRS Rouxinol; G7=Lin.PC951015D01E; G8=Lin.PC950409D02E; G9= Lin.PC951016D01E; G10= Lin. PCCR3F6L15; G11= Lin.CPCR3F6L17; G12= Lin.C1J; G13= Lin.C2M; G14= LinC2S; G15= Lin.C3F; G16= Lin.C3Q; G17= Lin.C3S; G18= Lin.P290; G19= Lin.P303; G20= Lin.P-508; G21= PJJ21; G22=PJM22; G23=PJL23; G24=PAG24; G25=PC25; G26=PD26; G27=PJ27; G28=PJJ28; G29=PJN29; G30=PLP30.

(Table 4). For immature pods, only the genotypes PC951015D01E, PC950409D02E, CPCR3F6L15, PJM22 and the BRS Acauã have been highlighted by Eberhart and Russell method, with good yield, broad adaptability and stability. As for Lin and Binns method, the genotypes P-508 (1) and CPCR3F6L15 (2) showed the lowest values of Pi for immature pods, being considered the most stable (Table 4).

For yield of dried seeds, using the Eberhart and Russell method, the genotypes BRS Rouxinol, BRS Guariba, BRS Pujante and BRS Patativa, PC951015D01E, C1J, C2M, C3F, PL23 and PC25 showed broad adaptability and stability. While by the analyses of Linn and Binns, the genotypes BRS Acauã, Patativa, P508 and CPCR3F6L15 were the most stable (Table 4).Using the multivariate method AMMI, the interaction G*E was performed in five main components of interaction (CPI) for yield of immature seeds and pods and four main components of interaction (CPI) for yield of dried seeds. Only the first axis (CPI1) had its significant residue ($p<0.01$), using the model AMMI1 for all the variables evaluated (Table 2).

The first principal component of the interaction explained 54.7% for yield of immature seeds, 46.2% for yield of immature pods and 55.5% for yield of dried seeds (Table 2). These values correspond to the pattern adjacent to the G*E interaction and agronomic importance. The values that represent the noise, that is, random variation resulting from the influence of factors micro environment and without agronomic importance were 55.5, 54.7 and 46.2 for yield of immature seeds and pods and dried seeds, respectively.

Considering the analyses of AMMI, the genotypes CPCR3F6L15, PC951015D01E and PC950409D02E showed high yield of immature seeds and good stability (Figure 1). For yield of immature pods the genotypes P-290, P-508 showed high yield and good stability (Figure 2). As for yield of dried seeds, the genotypes stood out by the AMMI method were BRS Patativa and P-508 (Figure 3). The environments MAND2013 and BEB2013 were the most productive, presenting, the last one, the greater stability for yield of immature seeds and pods (Figures 1 to 3).

DISCUSSION

Cowpea is grown in Brazil, by family farming, predominantly in the Northeastern region, presenting low technological level, without irrigation and unimproved

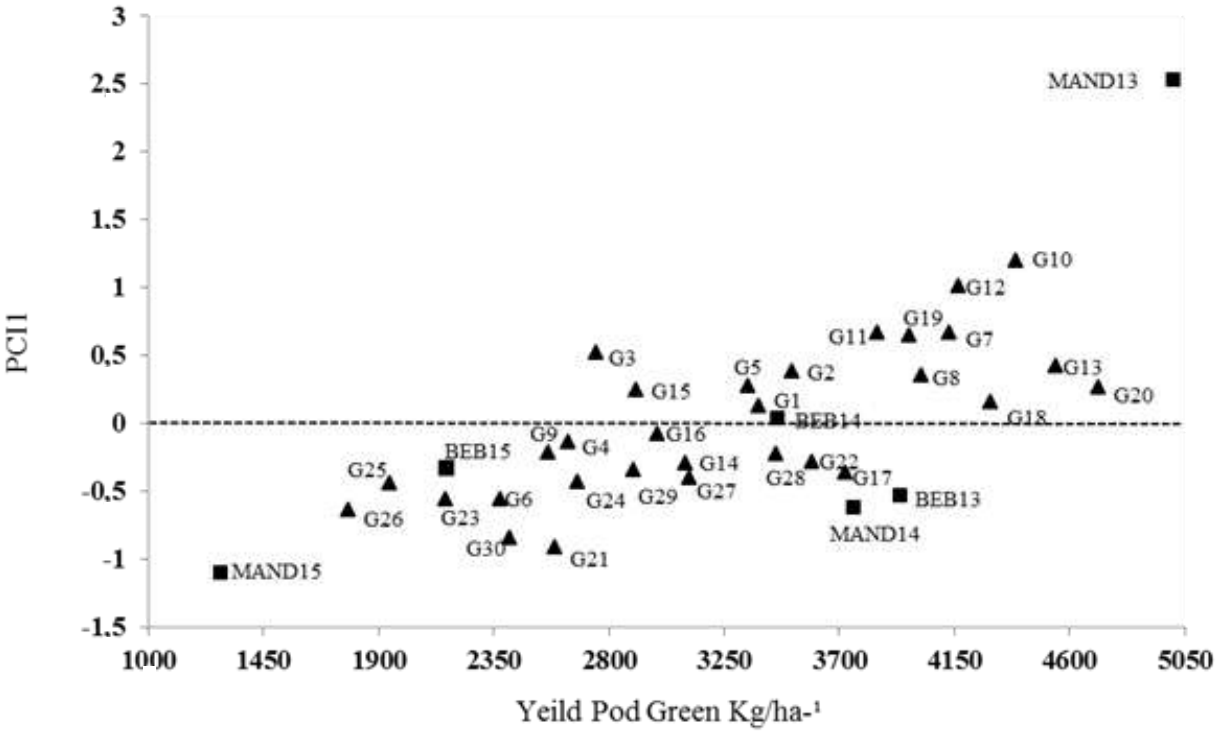

Figure 2. Biplot AMMI for immature pod yield of 30 cowpea genotypes(▲) (*Vigna unguiculata*) evaluated in six environments (■).G1=BRS Acauã; G2=BRS Guariba; G3=BRS Marataoã; G4=BRS Patativa; G5=BRS Pujante; G6=BRS Rouxinol; G7=Lin.PC951015D01E; G8=Lin.PC950409D02E; G9= Lin.PC951016D01E; G10= Lin. PCCR3F6L15; G11= Lin. CPCR3F6L17; G12= Lin.C1J; G13= Lin.C2M; G14= LinC2S; G15= Lin.C3F; G16= Lin.C3Q; G17= Lin.C3S; G18= Lin.P209; G19= Lin.P-303; G20= Lin.P-508; G21= PJJ21; G22=PJM22; G23=PJL23; G24=PAG24; G25=PC25; G26=PD26; G27=PJ27; G28=PJJ28; G29=PJN29; G30=PLP30.

crops, what results low yield. Ratings of lines of cowpea genotypes at different locations, either for yield of dried grain or immature seeds, are of great importance because they can allow the selection of genotypes with good performance in different environments and different technological levels.

The yield of cowpea type immature seeds presents great potential for expansion of consumption, since it is broadly used in cooking from the northeast and the cultivation has been carried out independently by the farmers, without studies that indicate what the best cultivar for this activity. The selection of cultivars with high yield of immature seeds is a great contribution to the populations of semiarid regions.

Several methodologies have been developed to interpret the G*E interaction and identify genotypes which have predicted behavior in various evaluated environments. The methods of linear regression (Eberhart and Russell, 1966) and non-parametric (Lin and Binns, 1988) have been the most broadly used, and according to Silva and Duarte (2006) they presented a low correlation between them, indicating that one method does not replace the other, and they must be applied together (Pereira et al., 2009), as performed in this study.

Silva et al. (2016) evaluated the adaptability and stability for the protein content and grain yield in 44 lines of cowpea genotypes, in seven environments, and observed that the methods of Eberhart and Russell and Lin and Binns showed similar results regarding the selection of superior materials. Using parametric methods and non-parametric tests in 20 cowpea genotypes, Nunes et al. (2014) found that some methodology should not be used simultaneously, and that others must be complementary. Freire Filho et al. (2005) used the AMMI, evaluating the grain yield of 15 lines of cowpea genotypes in 13 environments and observed that the Evx91-2E and Evx63-4E can be cultivated in all environments studied.

The application of these methods is essential for the recommendation of new cultivars. In the state of Piauí, Rocha et al. (2008) evaluated the grain yield of 20 lines of cowpea, and by means of the methodology of Eberhart and Russell (1966) indicated that the cultivars Canapuzinho, Canapu-BA and BRS Xiquexique with broad adaptability and stability. Using this same methodology, Santos et al. (2008) evaluated 64 lines of cowpea genotypes, in a variety of environments in the São Francisco Valley, and the lineage PC 95-05-12-2-2 was released as cultivar BRS Pujante and recommended for cultivation in the semiarid region of Pernambuco and

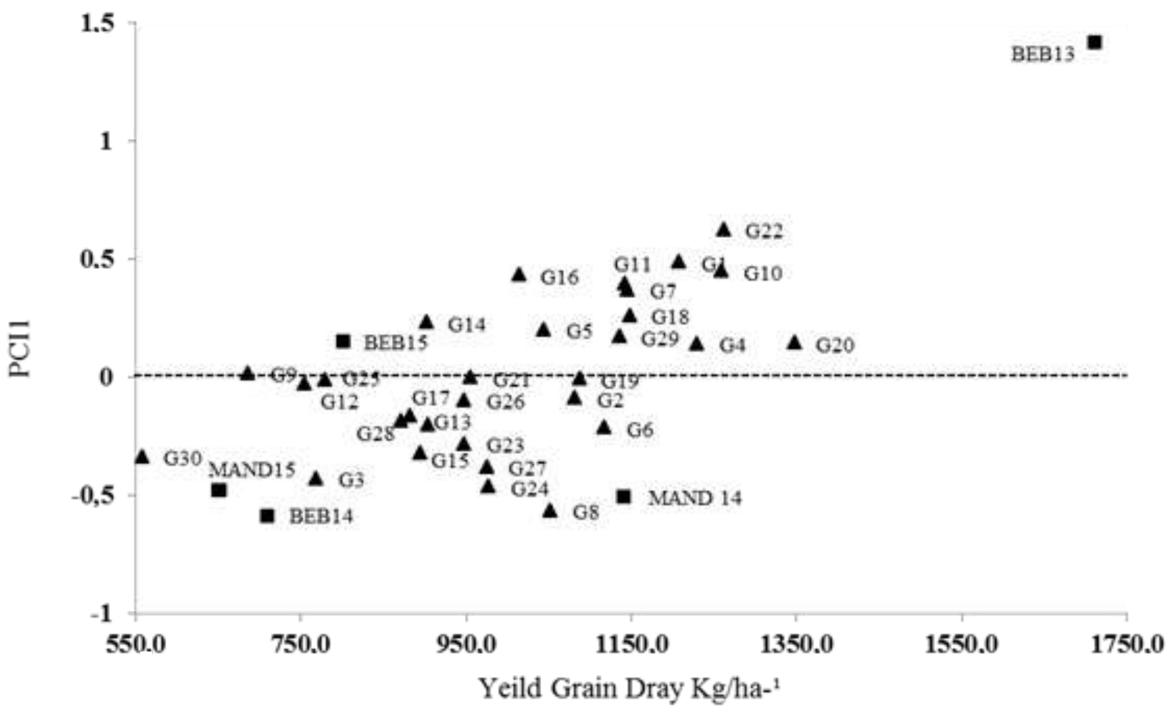

Figura 3. Biplot AMMI for mature dried seed yield of 30 cowpea genotypes (▲) (*Vigna unguiculata*) evaluated in six environments(■).G1=BRS Acauã; G2=BRS Guariba; G3=BRS Marataoã; G4=BRS Patativa; G5=BRS Pujante; G6=BRS Rouxinol; G7=Lin.PC951015D01E; G8=Lin.PC950409D02E; G9= Lin.PC951016D01E; G10= Lin. PCCR3F6L15; G11= Lin. CPCR3F6L17; G12= Lin.C1J; G13= Lin.C2M; G14= LinC2S; G15= Lin.C3F; G16= Lin.C3Q; G17= Lin.C3S; G18= Lin.P209; G19= Lin.P-303; G20= Lin.P-508; G21= PJJ21; G22=PJM22; G23=PJL23; G24=PAG24; G25=PC25; G26=PD26; G27=PJ27; G28=PJJ28; G29=PJN29; G30=PLP30.

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Studies have been conducted with the objective of evaluating cowpea genotypes for cowpea market, especially for traits associated with the yield (Andrade et al., 2010; Oliveira et al., 2003). Ramos et al. (2014) evaluated cowpea genotypes under different irrigation levels and yield of immature seeds was 2.937 kg ha^{-1} (BRS Guariba) and 2.493 kg ha^{-1} (BRS Paraguaçu). However, there are no recommended varieties for immature seeds market, considering adaptability and stability parameters.

The genotypes P290, P303, P508 and PC950409D02E showed immature seeds yield higher than the averages of the experiments. They also showed higher yield in comparison with the evaluated cultivars, stability and good predictability in the evaluated environments, both for the methodology of Eberhart and Russell and Lin and Binns (Table 4), as well as by the multiplicative method, based on main components (Figure 1), having great potential to be recommended as new cultivars for the region of São Francisco Valley.

Conclusion

The lines P290, P303, P508 and PC950409D02E showed yield of immature seeds exceeding 2140 kg ha^{-1}, broad stability and good predictability in the series of evaluated environments, and has great potential to be recommended as new cultivars for the region of São Francisco Valley.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Irrigation history and pruning effect on growth and yield of jatropha on a plantation in southeastern Brazil

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Jatropha is an oilseed culture that has been highlighted due some specific agronomic aspects. This plant produces high oil quantities in its seeds that is used for biodiesel production. One of the major challenges regarding to the Jatropha cultivation is the lack of information on many management techniques, especially the irrigation and pruning management in adult plants in southeastern Brazil. The objective of this study was to study the combined effect of irrigation history and different pruning on Jatropha plant growth and yield. The experiment was conducted during the 4th year of Jatropha growing season at University of Sao Paulo experimental area in Piracicaba, Brazil. The experiment was arranged in randomized block with four replications and treatments were considered the pruning type: No pruning (P1), pruning at 1.5 m high and 2 m canopy diameter (P2), pruning at 2 m high and 2 m canopy diameter (P3). In addition, two water conditions were also evaluated: Irrigated (I) and rainfed conditions (R). Plant growth (height and canopy diameter) and leaf area index (LAI) were evaluated monthly and productive variables were determined at the end of the experiment. Irrigation history influenced canopy diameter, absolute growth rates for plant height and canopy diameter, the relative growth rate for canopy diameter, and all productive variables. Pruning provided differences in all growth variables, where P2 presented the highest average on plant growth rates. The plants under irrigation history conditions showed the highest yield.

Key words: *Jatropha curcas* L., oilseeds, center pivot, water management, biofuels.

INTRODUCTION

The bioenergy exploitation from the biofuels production has become part of the global sustainable development agenda and has received scientific and commercial attention. Bioethanol and biodiesel are considered the

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most important liquid biofuels used and they are derived mainly from agricultural crops such as sugar cane and corn for ethanol, and oilseed for biodiesel (Ye et al., 2009; Jingura, 2011).

One of the alternatives for biodiesel production is Jatropha (*Jatropha curcas* L.), a culture with promising potential of feedstock supply for energy generation. This oilseed belongs to the Euphorbiaceae family and is native from Mexico and Central America, also cultivated in many other countries in Latin America, Asia and Africa (Mishra, 2009). Jatropha is a perennial plant which has attributes such as fast growth, develops in marginal areas and in different rainfall conditions. Its seeds produce viscous oil which can be used as feedstock for cosmetics industry and, more importantly, for biodiesel production (Jongschaap et al., 2007; Kumar and Sharma, 2008; Sujatha et al., 2008).

There is many conflicting information about Jatropha water requirement and the influence of irrigation management on the plant development. According to Behera et al. (2010) and Srivastava et al. (2011), the irrigation frequency does not affect significantly the Jatropha growth, however, Openshaw (2000) reports that this plant increase production with irrigation, also allowing from three to four harvests per year. Despite of Jatropha is considered a water drought tolerant, in some cases water is a limiting factor for an adequate production, which requires regular irrigation during the dry season (Singh et al., 2013). Moreover, the efficient water supply interferes directly in agricultural production process (Evangelista et al., 2011).

According to Rajaona et al. (2011), there are few information about Jatropha development under different management technics such as the fertilizer application, irrigation, and pruning. However, many studies can be found in the literature with Jatropha oil processing technics. The pruning technic can contribute on the plant canopy structure development, reducing disease and insect incidence, improving the air flow within the canopy, and, associated to the irrigation, increasing the number of productive branches (Yarborough, 2006; Oliveira and Beltrão, 2010; Pescie et al., 2011). Moreover, when pruning on Jatropha is not performed, it interferes on the manual harvest efficiency and may decrease the flowering uniformity and increase the fruit maturation time (Alam et al., 2011; Brasileiro et al., 2012; Everson et al., 2013).

Due the atypical events that may occur in a region that usually has a standard climate during the years, mainly by the extended drought period, it is necessary to evaluate the effect of water deficit for crops that were continuously irrigated. In addition to these information, it is still necessary to evaluate the effect of pruning on adult Jatropha trees. The hypothesis of this study were: The irrigation history influences the plant growth and yield even with irrigation interruption and; the differences on pruning levels may change the plant growth index, as well as the Jatropha yield.

The aim of this study was to evaluate the effect of irrigation history on the Jatropha plant growth and yield with four years old, associated with three pruning types under Brazil southeastern climatic conditions.

MATERIALS AND METHODS

Study site

The experiment was performed at University of Sao Paulo research area at Piracicaba city, São Paulo, Brazil (22°41’ S and 47°38’ W, and 511 m altitude) (Figure 1). The local climate is classified as Cfa according to the Köppen-Geiger world soil classification (Peel et al., 2007), with mean annual temperature of 21.6°C and annual precipitation of 1,328 mm (Marin et al., 2011). The soil was classified as loamy (57.1% clay, 20.9% silt and 22% sand), with 1.4% organic matter content, and density around from 1.4 g cm^{-3}. Meteorological data was determined by an automatic weather station located close to the experimental area (Figure 1). Temperature, relative humidity, net radiation, wind speed and direction, and precipitation data were registered at 15 min, hourly and daily frequency by a datalogger.

Plant establishment and management

Jatropha was cultivated in the experimental area in late December 2011, using 4 months old seedlings (cultivated at greenhouse). It was used a 3 m x 4 m between plant and row spacing, respectively, totalizing 833 plants ha^{-1}. Fertilization was performed in accordance with FACT (2010) recommendations, with three applications during the growing season. Fungicides and insecticides were applied continuously to promote adequate plant development.

Irrigation was performed from December 2011 to May 2014 using a center pivot irrigation system. The Christiansen Coefficient of Uniformity was evaluated and presented 82% for the center pivot used, considered great in the Bernardo et al. (2006) scale. The amount water required was determined according to the soil available water that was measured by two large weighing lysimeters (Flumignan, 2011; Lena, 2013).

Experimental design and treatments

The treatments were divided into two water management and three pruning types. The water management was divided into irrigated (or irrigation history treatment) from late-2011 to mid-2013 (I) and rainfed (R), considered the two main experimental areas. In each water management area, it was applied the pruning treatments considering no pruning (P1), pruning at 1.5 m height and 2 m canopy diameter (P2), and pruning at 2 m height and 2 canopy diameter (P3). Pruning was performed at the end of 3rd growing season in early-September 2014, coinciding with leaf senescence growing stage which is considered the most efficient moment for improving new branches production (Behera et al., 2010). In each water management treatment, it was divided into 4 blocks in which there were 1 replication with 16 plants for each pruning treatment. For analysis, it was used only the four internal plants in each replication (Figure 1).

Plant growing parameters

The plant height (cm), canopy diameter (cm) and leaf area index

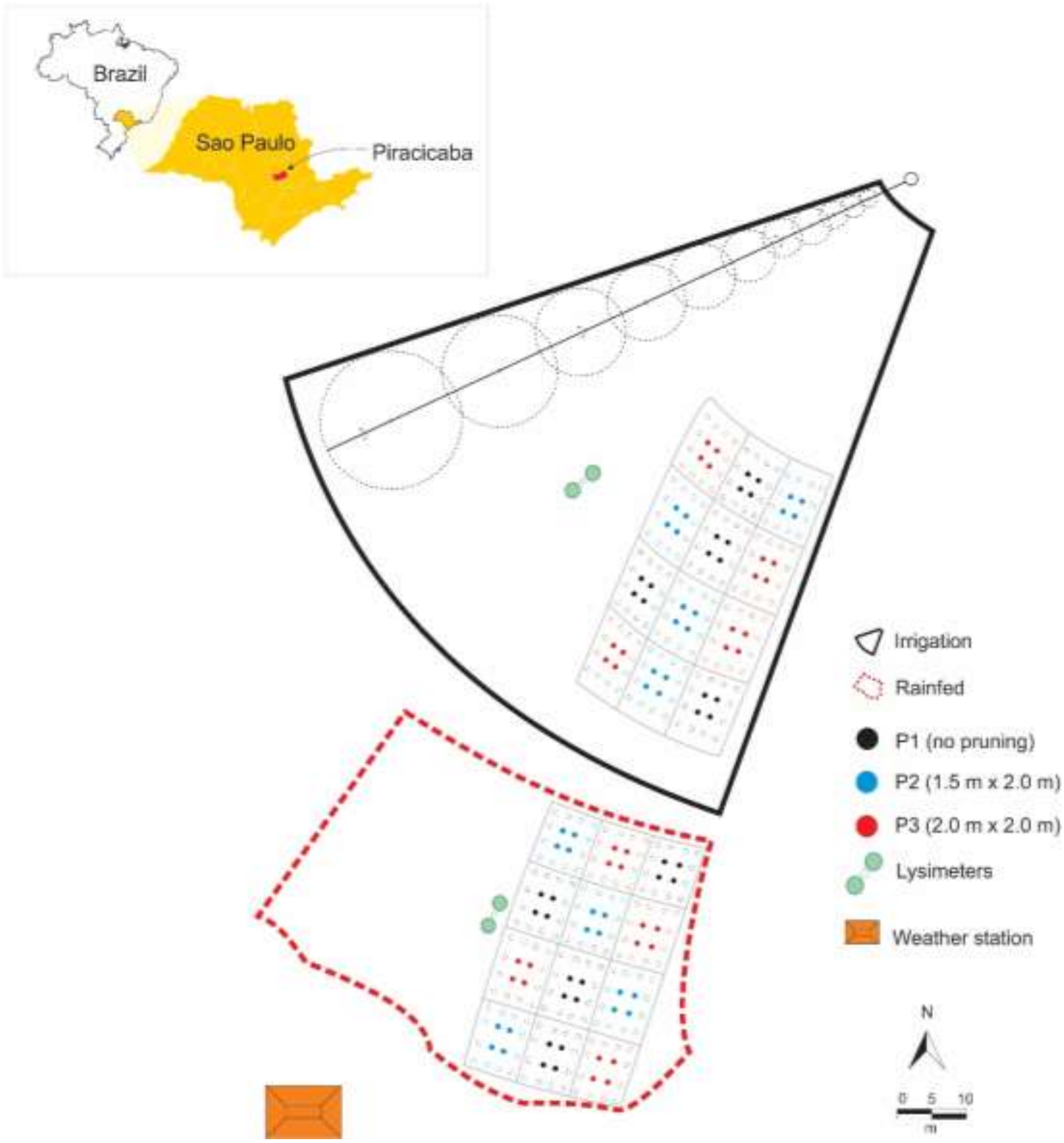

Figure 1. Location of the *Jatropha curcas* L. study site and plot treatments details.

(LAI, dimensionless) were determined from 1 to 210 days after pruning (DAP) every 30 days. Plant height was determined using a scale considering the distance between the soil level and the highest leaf of the plant and canopy diameter by the mean of perpendicular and parallel reading of plant row direction. LAI index was determined using the LAI-2200 plant canopy analyzer. Using the monthly data of plant height and canopy diameter, it was calculated the absolute growing rate for height (AGR_H) and canopy diameter (AGR_D), and relative growing rate for height (RGR_H) and canopy diameter (RGR_D) (Benincasa, 2003).

Harvest and yield

Harvest was performed from January to May 2015. Since Jatropha has different fruit maturation level in the same plant (green, yellow, yellow-brown, and brown) (Kumar and Sharma, 2008), the harvest was performed manually multiples times during the productive period choosing only fruits that were from yellow to brown maturation level (Pessoa et al., 2012). After harvest, all fruits were kept to dry at ambient temperature for 7 days or until all fruit were at brown maturation process (around 8% humidity). The fruits were weighted for each treatment separately, and it was determined the fruits and seed weight (kg tree^{-1} and kg ha^{-1}), and fruits and seed number per tree.

Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using both R 2.15.1 (R Development Core Team, Vienna, Austria) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA) softwares. It was used a 0.05 level of probability as the decision level for the acceptance or rejection of statistical significance in all analysis. If a statistical

Table 1. Analysis of variance for irrigation (I), pruning type (P) and days after pruning (DAP) for tree height (cm), canopy diameter (cm), absolute growth rate in height (AGR_H, cm day^{-1}) and canopy diameter (AGR_D, cm day^{-1}), relative growth rate in height (RGR_H, cm $cm^{-1}day^{-1}$) and canopy diameter (RGR_D, cm $cm^{-1}day^{-1}$) and leaf area index (LAI, dimensionless) for *Jatropha curcas* L..

Source of variation[a]	DF	F values						
		Height	Canopy diameter	AGR_H	AGR_D	RGR_H	RGR_D	LAI
I	1	1.03[ns a]	3.73[ns]	1.04[ns]	3.84[ns]	0.33[ns]	1.23[ns]	1.61[ns]
P	2	21.95***	15.14**	58.79***	8.35**	132.46***	13.48**	5.89*
DAP	6	363.97***	556.56***	194.88***	172.86***	113.42***	147.85***	541.19***
I × P	2	1.06[ns]	0.38[ns]	1.59[ns]	1.26[ns]	2.12[ns]	0.33[ns]	1.30[ns]
I × DAP	6	0.51[ns]	3.85*	3.23**	2.63*	0.79[ns]	2.94*	2.02[ns]
DAP × P	12	16.2***	5.45***	18.66***	8.77***	26.01***	10.04***	3.04***
I × P × DAP	12	1.81[ns]	1.61[ns]	1.45[ns]	1.21[ns]	1.88*	0.94[ns]	2.08*
General mean		262.03	283.36	0.5	0.85	0.002	0.004	1.76
CV (%)		7.67	6.46	39.78	33.43	40.87	36.03	15.87

[a] Level of significance: *0.01<P< 0.05; **0.001<P<0.01; ***P<0.001. ns., no significant difference. DF, degree of freedom; CV, coefficient of variation.

significant effect was found, means were compared using Tukey's multiple comparison test. All plant growing parameters data were analyzed by mixed model where the variance and covariance structures were rejected, selecting those with lowest AIC and BIC information criteria.

RESULTS AND DISCUSSION

Effect of treatments on plant growth parameters

The average of all variables analyzed was different significantly by F test between pruning types and DAP (Table 1). It was observed significantly difference between water management and DAP for canopy diameter, AGR_H and AGR_D and for RGR_D by the ANOVA analysis results.

The increasing of plant height was different between pruning types, observed by an increment of 73, 142.7, and 102 cm for P1, P2, and P3, respectively, representing 29, 95, and 51% in comparison to the beginning of the experiment (1 DAP) (Figure 2A). During all experimental period, plant height varied significantly over time, in which only at 150 DAP the plant height was the same for all pruning types (P>0.05). In each pruning type, plant height differed over time, but plant height difference was not observed from 180 to 210 DAP for P2 and P3.

The canopy diameter followed the same trend observed for plant height with increase of 78, 128, and 125% for P1, P2, and P3, respectively, in comparison to the beginning of experiment (1 DAP) (Figure 2B). From 150 DAP, the canopy diameter did not present statistical difference between pruning types. It was observed that there were significant effect for canopy diameter at 120, 150, and 180 DAP, where plant for these treatments presented the highest averages (Figure 2C). Faria et al. (2011), studying the influence of different water management and fertilizer levels, also found a positive irrigation effect on the Jatropha plant canopy diameter, showing the high influence of water management in the plant development. The fast increase in plant height and canopy diameter over time was explained due the fast Jatropha plant development, in which it can reach easily 5 m height in adequate soil and climate conditions (Openshow, 2000; Arruda et al., 2004).

AGR_H were higher for more drastic pruning (P2), observed by the statistical differences at 60 and 90 DAP in comparison to P1 and P3. It indicates that P2 was more efficient on the vegetative growth (Figure 3A). In the literature it is possible to observe that plants which received more drastic pruning presented higher vegetative growth when it was performed during winter (Scarpere Filho et al., 2011; Rufato et al., 2012), corroborating with the results presented in this study. For the crop management, the fast vegetative development is not considered adequate because reduces yield due the high nutrient demand to produce new branches and leaves as well as making it difficult the disease and past control and the harvest (Laviola and Dias, 2008). However, pruning in high density vegetative canopy may promote better sunlight interception and the plant waste material can be used in other sectors such as for industry energy (material burn) (Prueksakorn et al., 2010).

Between irrigation history and rainfed treatments, it was observed significantly difference for AGR_H only at 30 DAP (Figure 3B). Behera et al. (2010) reported that Jatropha required frequent irrigation to improve canopy development and yield. According to Singh et al. (2013), Jatropha has its development affected in areas with high drought potential, requiring water from irrigation during the driest period of the year.

No statistical differences were observed in almost all periods for all pruning types for AGR_D during experimental period. Statistical differences were observed only at 120 and 30 DAP for P1 and P2 respectively, exhibited the lowest values (Figure 3C). It

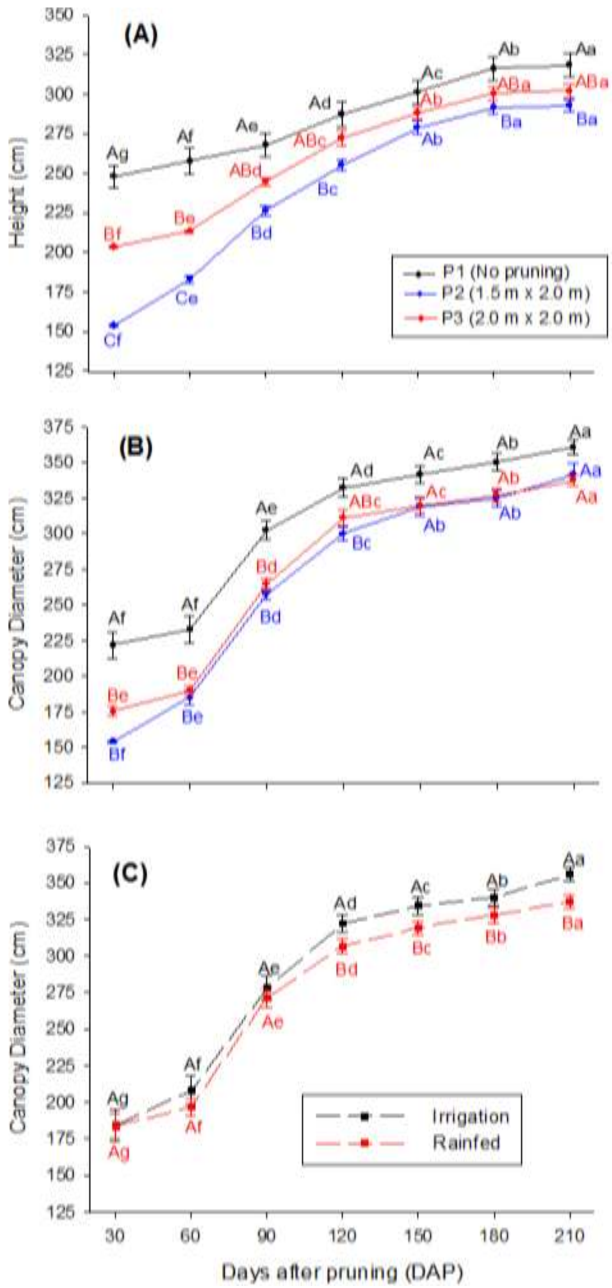

Figure 2. Effect of treatments on (A) tree height and (B, C) canopy diameter at days after pruning. Different letters indicate differences between pruning type or water management (capital letters) and days after pruning (small letters) according to Tukey's test (P<0.05). Vertical bars indicate the mean standard error.

was observed difference between the interaction of I and R x DAP at 60, 120, and 210 DAP, with higher growing rates for I (Figure 3D). In both water management treatments, the higher growing rates was observed at 90 DAP for all pruning types.

Both RGR_H and RGR_D had the same pattern of AGR_H and AGR_D results as described previously, but P2 presented significant differences for RGR_H (Figure 4A) in relationship to other pruning types at 60 and 90 DAP. For RGR_D, differences were observed only at 30 DAP with P3 having higher rates compared to P1 and P2 (Figure 4B). Regarding irrigation, RGR_D values were different from the 120 and 210 DAP, which were higher in irrigated plants (Figure 4C).

RGR is a measurement of production efficiency from new dry matter to those that has already been stablished, also called specific growing rate (Silva et al., 2000). Moreover, this measurement is considered very important indicative of genetics materials under different stress conditions (Benincasa, 2003). The plant height and canopy diameter growing rates had a similar pattern, showing a higher increasing between 60 and 120 DAP, characterized by the leaves emergency and development, as already observed by Ghezehei et al. (2015). This increase was also associated to the climate factors during the experiment, which was observed high amount of precipitation (Figure 5).

The higher growing rates values for I treatment is explained by the irrigation history. Since the plants from this treatments were being adequately irrigated until the end of 3rd growing season, it contributed for a better physiological performance and plant growing rates in comparison to the rainfed treatment, showing that Jatropha has a better development when the water availability is adequate, as already observed by other authors (Nery et al., 2009; Carvalho et al., 2011; Ghezehei et al., 2015). In the same experimental area, Lena (2013) determined evapotranspiration rates (ET_c) for center pivot and rainfed water management for Jatropha nut from 1st to 2nd growing season and observed that center pivot treatment presented 35% higher ET_c rates in comparison with rainfed treatment. During the 4th growing season, ET_c values were around 20% higher for I treatment (not irrigated but with irrigation history) than R treatment (Table 2), which is explained by the higher plant height and LAI index, as well as observed by Lena (2013). Therefore, although Jatropha is considered a drought tolerant plant, adequate water available is a limiting factor for the plant development, especially when associated with temperature and photoperiod (Saturnino et al., 2005; Bianchini et al., 2006).

During the experimental period, it was observed that plants presented a trend to reduce the growing index from 90 DAP which is explained by the interruption of vegetative development due the high productive development, as also observed by Larcher (2000) and Carvalho et al. (2013).

LAI is an important information that can be used to describe the plant photosynthetic activity (Kara and Mujdeci, 2010), as well as to be used in models for growth and yield prediction (Lizaso et al., 2003; Xiao et al., 2006). The LAI fluctuation between P2 and P3 were very similar, with values varying from 0.6 to 3.4 for P2 and from 0.5 to 2.9 for P3 (Figure 6A) and highest values

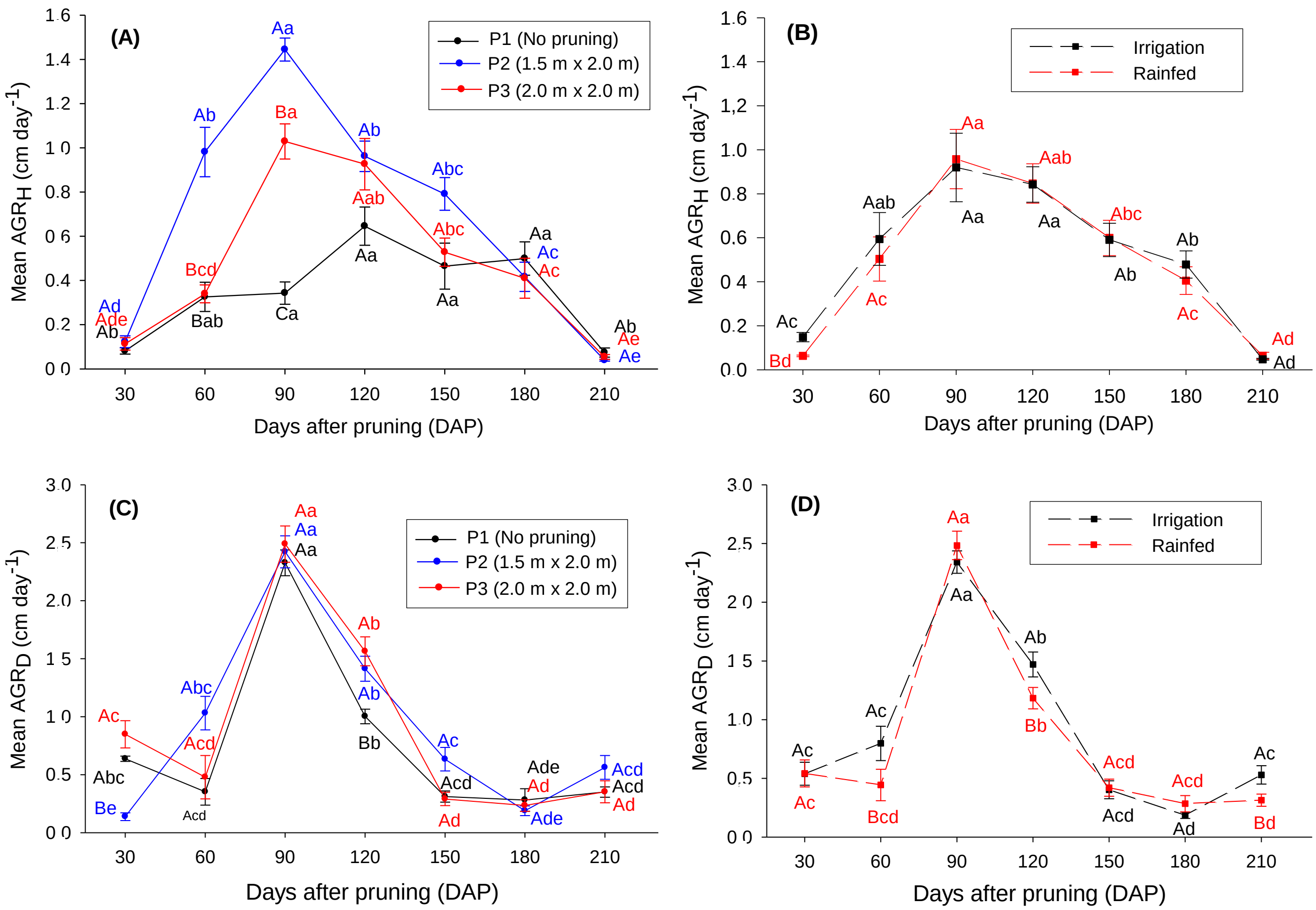

Figure 3. Mean absolute growth rate in height (AGR_H) and canopy diameter (AGR_D) under (A, C) pruning types and (B, D) water management at days after pruning. Different letters indicate differences between pruning type or water management (capital letters) and days after pruning (small letters) according to Tukey's test (P<0.05). Vertical bars indicate the mean standard error.

observed at 120 DAP. There were significantly difference between P2 and P3 only at 90 DAP, period where P2 presented the highest LAI values. Values were higher from 90 to 150 DAP for all pruning treatments, in which P2 was around 1.8 higher than others treatments (P<0.001). LAI behavior during the study period may be explained by high amount of precipitation during this period, mainly from December 2014 (90 DAP) to January 2015 (120 DAP). LAI values presented similar trend in comparison with plant growth rate, indicating that the vegetative growth increased the plant total leaf area. Tjeuw et al. (2015) observed that LAI, as well as the canopy density, decreased around 40% with pruning at 0.75 m height. According to this authors, it is difficult to compare the pruning effect with others findings due some studies did not include the no pruning treatment (Everson et al., 2013; Suriharn et al., 2011).

The LAI reduction due the water drought stress in Jatropha was already observed in the literature (Petropoulos et al., 2008; Ghanbari et al., 2013; Mofokeng et al., 2015), showing that strong influence of water for the plant development. As the leaf area increases, LAI also increases, demonstrating that the reduction of water available promotes reduction of leaf area for Jatropha plant (Silva et al., 2011; Horschutz et al., 2012), castorbean (Barros Júnior et al., 2008) and sugarcane (Farias et al., 2008). However, in the present study, it was not observed LAI differences between I and R treatments, that is, the water management did not affect this parameter (Figure 6B). One of explanation for these findings is supported by the Jatropha water drought stress tolerance. According to Maes et al. (2009), the Jatropha metabolism must receive some attention, since there is the possibility that the plants do not have a full C3 metabolism, with Crassulacean Acid Metabolism (CAM) inside the stem and a change from C3 to CAM in water drought condition (Ting et al., 1983; Luttege, 2008), also observed for *Frerea indica* (Lange and Zuber, 1977). According to Hokmalipour and Darbandi (2011), the leaf area has an important role in the sunlight interception of a crop canopy, as well as the water demand (Liu and Stutzel, 2002).

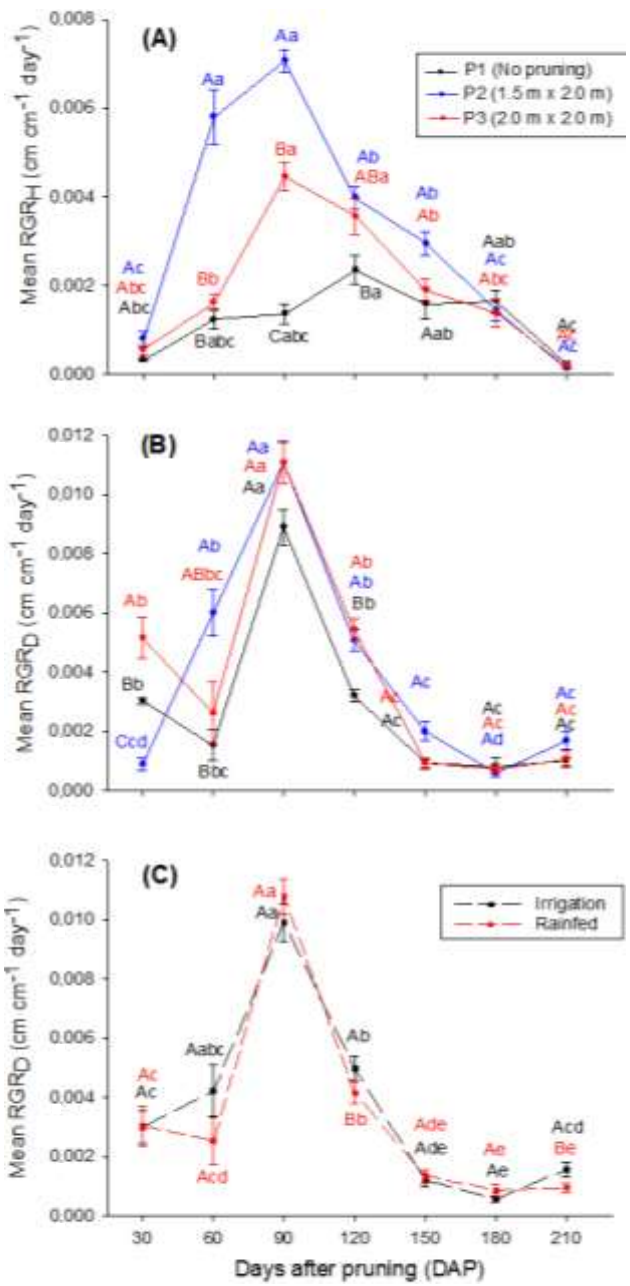

Figure 4. Mean relative growth rate in height (RGR_H) and canopy diameter (RGR_D) under (A, B) pruning types and (C) water management at days after pruning. Different letters indicate differences between pruning type or hydric conditions (capital letters) and days after pruning (small letters) according to Tukey's test (P<0.05). Vertical bars indicate the mean standard error.

Effect of treatments on fruit and seed yield

The statistical analysis results for fruit and seed yield, and number of seeds, presented influences only for the water management at 0.1% significance level (Table 3). However, it was observed significant effect in I and P for seed yield.

By Tukey's mean test, it was observed that I treatment was higher than R in all production characteristics analyzed (Table 4). Plants from I treatment produced 947.1 fruits tree^{-1}, 3.2 kg fruits tree^{-1}, and average yield of 2,651.3 kg fruits ha^{-1}, presenting increase of 45.6, 45.4 and 45.2%, respectively, in comparison to R treatment (Table 4). For seed yield, it was observed 2,638.3 seed tree^{-1}, 2.1 kg seed tree^{-1}, and 1,761.1 kg seed ha^{-1} for I treatment, following the same proportion of fruit production in comparison to R treatment, representing, respectively, 46.3, 50 and 48.2%. In the literature, some studies have described that Jatropha yield can vary from 0.2 to 2 kg seeds tree^{-1} and from 2,000 to 5,000 kg seeds ha^{-1} depending on the climate condition and crop management (Foidl et al., 1996; Heller, 1996; Francis et al., 2005; Tewari, 2007). In India, Jatropha under irrigation conditions presented an increase of harvest fruits (Daniel, 2008), with some reports of two times high yield for irrigated plant in comparison with rainfed (Ariza-Montobbio and Lele, 2010). Kheira and Atta (2009), Singh et al. (2013), and Tikkoo et al. (2013) also found that seed yield was higher with the better water availability to the Jatropha plants. Some studies in Brazil also reported that Jatropha under irrigation presented better plant development in growth and yield (Oliveira et al., 2012; Evangelista et al., 2011; Faria et al., 2011).

The yield results presented in this current study were higher than those presented by Prajapati and Prajapati (2005), and Tomomatsu and Brent (2007), presenting Jatropha yield in rainfed condition (R) of 1,200 kg ha^{-1} and up to 60% higher when irrigated. In India, the highest yield found for three years old Jatropha tree was 750 and 450 kg ha^{-1} for irrigated and rainfed conditions, respectively, with possibility to increase up to 1,200 kg ha^{-1} during the 6th growing season under irrigation (Behera et al., 2010). According to Jongschaap et al. (2007), depending on the crop management, such as seedling density, fertilizer, pruning types, and other, interfere on the understanding of Jatropha yield characterization.

The difference between P2 and P3 was observed only for plants of I treatment (p=0.0365) (Table 4). For R treatments, P2 presented the highest seed production (1.7 kg seeds tree^{-1} and 1,436.6 kg seeds ha^{-1}). The pruning effect observed in this study was very different to those presented by Ghosh et al. (2011), where plants without pruning had better yield than those under pruning. Tjeuw et al. (2015) verified that pruning reduced around 75% on yield in comparison with plants without pruning. On the other hand, Singh et al. (2013), trying to standardize the agronomics technic for Jatropha production, did not observed influence of pruning on yield.

The plant architecture changing due the pruning technic can be used as objective of modifying the source/drain relationship to increase yield (Guimarães et

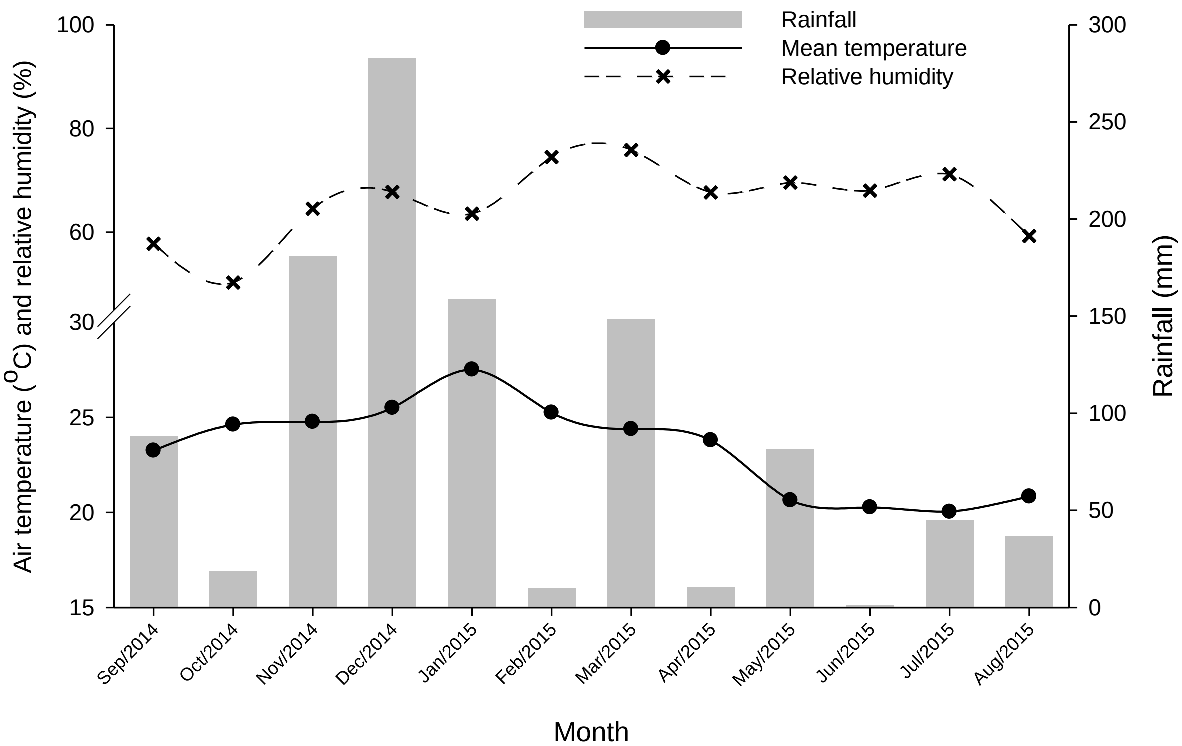

Figure 5. Monthly values of total rainfall and mean temperature at experimental site during the study period.

Table 2. Monthly values of total evapotranspiration (ETc, mm) of *Jatropha curcas* irrigated by center pivot and rainfed.

Month/year	Irrigation	Rainfed
Sep/2014	61.6	64.5
Oct/2014	74.7	65.7
Nov/2014	140.4	99.6
Dec/2014	181.2	120.9
Jan/2015	152.4	135
Feb/2015	206.7	147
Mar/2015	130.6	123
Apr/2015	92.7	74.1
May/2015	29.7	37.2
Jun/2015	30.1	22.8
Jul/2015	29.6	27
Aug/2015	28.5	25.5
General mean	96.5^{ns}	78.5^{ns}

ns, no significant difference.

al., 2007). The pruning effect on yield for P2 and R can be explained due the more intrusive pruning promotes a significant reduction of apical dominance, inducting to new shoots formation on the cut segment (Raven et al., 2001). Moreover, the vertical braches reduction due the pruning technic, the horizontal branches become predominant, improving yield since the horizontal branches are more associated to the reproductive buds (Scarpare Filho et al., 2011). One of the reason of lower yield results for R treatment is due the higher auto canopy shading (Azevedo et al., 2013), decreasing the plant sunlight interception and due the high amount of unproductive branches.

Conclusions

The analysis of pruning effect and irrigation on the Jatropha plant growth and yield is important to understand and characterize some plant management. The results presented in this study indicate that the irrigation history has influenced on a higher plant growth and yield of Jatropha during the 4th growing season. Even with irrigation interruption, the irrigation history was essential to improve the plant ability to increase in growth during some period of the year and to improve yield. This information is important to identify the plant behavior when irrigation is interrupted in region where climate may change and water became scarce. The pruning influenced significantly all variable analyzed, in which the more drastic pruning (1.5 m × 2 m) presented the highest plant height for both water management and best yield for rainfed area. Since Jatropha is a perennial plant, it is necessary to find more reliable information about the pruning effect on the plant growth and production.

However, due the lack of standardized management

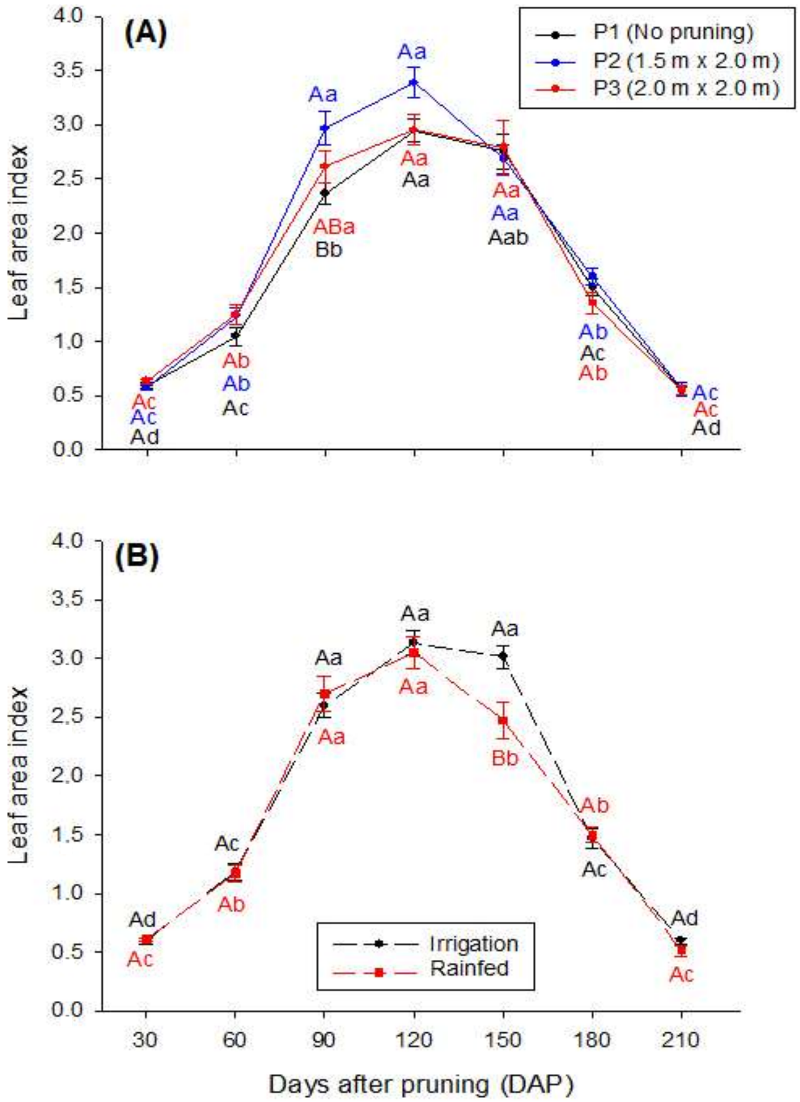

Figure 6. Mean leaf area index (LAI) under (A) pruning types and (B) hydric condition at days after pruning. Different letters indicate differences between pruning type or water management (capital letters) and days after pruning (small letters) according to Tukey's test (P<0.05). Vertical bars indicate the mean standard error.

Table 3. Analysis of variance for irrigation (I), pruning type (P) and days after pruning (DAP) on yield and number (No) of fruits and seeds for *Jatropha curcas* L.

Source of variation [a]	DF	Fruit			Seed		
		Yield		No. tree^{-1}	Yield		No. tree^{-1}
		(kg tree^{-1})	(kg ha^{-1})		(kg tree^{-1})	(kg ha^{-1})	
I	1	30.06***	30.06***	33.11***	39.39***	39.39***	36.62***
P	2	0.85^{ns}	0,85^{ns}	0.90^{ns}	0.92^{ns}	0.92^{ns}	0.84^{ns}
Block [I]	6	3.57*	3.57*	4.11*	3.68*	3.68*	3.25*
I x P	2	3.33^{ns}	3.33^{ns}	3.25^{ns}	4.12*	4.12*	3.53^{ns}
General mean		2.69	2,238.43	3,195.04	1.77	1,474.74	8,883.23
CV (%)		16.60	16.48	15.80	14.90	15.16	15.22

[a] Level of significance: *0.01<P< 0.05; **0.001<P<0.01; ***P<0.001. ns., no significant difference. DF, degree of freedom; CV, coefficient of variation.

Table 4. Number of fruits and seeds per tree, and yield for fruits and seeds for *Jatropha curcas* L.

Treatments[a]	P1 (no pruning)	P2 (pruning at 1.5 m × 2.0 m)	P3 (pruning at 2.0 m × 2.0 m)	Mean
		Number of fruits		
Irrigated	952	907	982.25	947.1[A]
Rainfed	632.5	781.5	537.25	650.4[B]
Mean	792.3[a]	844.3[a]	759.7[a]	
		Fruits yield (kg tree^{-1})		
Irrigated	3.2	3.0	3.3	3.2[A]
Rainfed	2.1	2.6	1.8	2.2[B]
Mean	2.7[a]	2.8[a]	2.5[a]	
		Fruits yield (kg ha^{-1})		
Irrigated	2685.7	2518.3	2,750	2,651.3[A]
Rainfed	1780.9	2203.6	1,492	1,825.5[B]
Mean	2,233.3[a]	2,360.9[a]	2,121[a]	
		Number of seeds		
Irrigated	2,607.5	2,534	2,773.5	2,638.3[A]
Rainfed	1,760.7	2,154.2	1,495	1,803.3[B]
Mean	2,184.1[a]	2,344.1[a]	2,134.2[a]	
		Seeds yield (kg tree^{-1})		
Irrigated	2.1[Aa]	2.0[Aa]	2.2[Aa]	2.1
Rainfed	1.4[Bb]	1.7[Aa]	1.2[Bb]	1.4
Mean	1.7	1.9	1.7	
		Seeds yield (kg ha^{-1})		
Irrigated	1,744.9[Aa]	1,682.1[Aa]	1,856.3[Aa]	1,761.1
Rainfed	1,159.1[Bb]	1,436.5[Aa]	969.5[Bb]	1,188.4
Mean	1,452	1,559.3	1,412.9	

[a]Capital and small letters indicate differences between pruning types and hydric conditions, respectively according to Tukey's test ($P<0.05$).

technics for this culture, it is necessary others studies with the Jatropha behavior under field condition in different regions.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of BAU- biofungicide and Proud on disease severity, seed quality, vigour index and health status of seed against brown spot disease of rice

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The experiment was conducted with three replication in randomized complete block design in field laboratory of Department of Agronomy, Bangaladesh Agricultural University, Mymensingh. Extracts of garlic (*Allium sativum*) and neem (*Azadirachta indica*), BAU-Biofungicide (*Trichoderma* based preparation) and Proud (Propiconazole) and Bavistin (Bavistin DF) were evaluated under field condition in controlling brown spot disease *Bipolaris oryzae* (Breda de Haan) Shoem of rice cv BRRI dhan28. Foliar spray of BAU-Biofungicide (2%) and Proud (0.1%) showed profound effect in marked reduction of disease severity. BAU-Biofungicide (2%) resulted grain yield 5785 kg/ha in the field and reduction in cost of production was found with benefit-cost ratio (BCR). BCR 2.78:1 was achieved in BAU-Biofungicide (2%) which is close to 2.87:1 in Proud (0.1%). Highest (98.00%) germination percentage was observed with BAU-Biofungicide (3%) as well as maximum (35.64%) increase of vigour index was found with Proud (0.1%) over control. BAU-Biofungicide and Proud were found to have in inhibiting seed borne fungi when they were applied as foliar spray. *Trichoderma harzianum* was associated with *B. oryzae* infected seeds and when kept for few days for incubation it overgrew *B. oryzae*.

Key words: BAU-Biofungicide, *Bipolaris oryzae*, benefit-cost ratio (BCR), disease severity, seed borne fungi, vigour index,

INTRODUCTION

Rice is the staple food crop in Bangladesh. The country is occupying 4th position as a rice producer in the world (USDA, 2015). About 76% of the total cultivated land covers about 11.37 million hectares under rice cultivation

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(BBS, 2014). At present, the total amount of rice production is approximately 34.36 million tons (BBS, 2014). The average yield of rice in our country is 3.03 t/ha but the world average yield of rice is 4.42 t/ha (USDA, 2014). Disease plays an important role for low yield of rice in Bangladesh. Out of 32 diseases reported to occur on rice, 10 diseases have the potentiality to cause economic damage to the crop in Bangladesh (Haq et al., 2008) but rice is suffering from brown spot to a great extent and caused "Bengal Famine" in 1943 (Padamanabhan, 1973). Brown spot disease of rice caused by *Bipolaris oryza* impairs grain quality and results in about loss of 4 to 52% yield (Barnwal et al., 2013). Kamal and Mia (2009) reported that 18.75 to 22.50% yield loss was found due to brown spot disease of rice in Banngladesh. Application of fungicides for the control of brown spot is the most effective management option, but under high disease pressure effective control is not achieved (Lore et al., 2007).

Fakir (2004) stated that roughly 10% production loss of rice may be incurred annually due to seed borne diseases in Bangladesh and according to this estimate, 2.5 million tons of rice worth TK 30,000 millions is lost annually in Bangladesh. Quality seed for planting is an important input for successful crop production. Good quality seed possess major characteristics such as high yielding potentiality, viability, purity, free from varietals mixtures and being healthy, that is, free from infection by pathogens or having maximum acceptable tolerance limit of infection by a given pathogen in a given seed lot (Fakir and Mia, 2004). In Bangladesh, 27 of the 43 diseases known to occur on rice are seed-borne (Fakir, 2004). Seed borne diseases caused by fungi such as brown spot (*B. oryzae*), blast (*Pyricularia grisea*), sheath rot (*Sarocladium oryzae*), seed rot and seedling blight (*B. oryzae*, *Sclerotium rolfsii* and *Fusarium* spp.) and grain spots (*B. oryzae, Curvularia lunata, Nigrospora oryzae, Phoma glumarum* and *Cladosporium sp.);* by bacteria such as Bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*) and bacterial leaf streak (*Xanthomonas oryzae pv. oryzicola*); and by nematode like white tip (*Aphelenochoides besseyi*) are harmful to rice seed health for inflicting diseases in seed bed as well as in the field (Fakir, 2004). Rice disease management strategies mainly aim at prevention of outbreak or epidemics through the use of host plant resistance and chemical pesticides. Although some plants have been with antifungal properties (Mia et al., 1990), but recommended dose of plant extracts has not yet been completely formulated. Biocontrol assumes special significance being an eco-friendly and cost effective strategy which can be used in integration with other strategies for a greater level of protection. An antagonist is an organism that exerts a damaging effect on another organism by producing lytic enzymes and antibiotics or by competition. *Trichoderma* spp. elicits biocontrol mainly by being mycoparasites and by being aggressive competitor of the pathogens (Cumagun, 2012). The present study has been designed to control brown spot disease by using plant extracts and biocontrol agent as an alternate means avoiding environmental pollution.

MATERIALS AND METHODS

The experiment was conducted during two Boro seasons of 2012 and 2013 in the field Laboratory of the Department of Agronomy, Bangladesh Agricultural University (BAU), Mymensingh. The field was fertilized as per recommendation of Bangladesh Rice Research Institute, Gazipur (BRRI, 2004). The experiment was carried out in Randomized Complete Block Design having three replications. The individual plot size was 5.0 m × 2.0 m (10 m^2). Block to block and plot to plot distances were 1.5 m and 1.5 m, respectively. Thirty five days old seedlings was uprooted from the seed bed and three seedlings per hill were transplanted in the field on January 21 in two successive 2012 and 2013. Hill to hill and row to row distances were 15 and 25 cm, respectively. The spray schedule was started just after commencement of disease symptom and two sprays were maintained at 15 days interval. Disease severity of each plot was assessed following the procedure of Standard Evaluation System for Rice (IRRI, 1996).

Healthy leaves of neem and garlic cloves were collected and the samples were washed thoroughly under running tap water followed by sterile distilled water (SDW). The extracts were prepared by homogenizing 5 g of plant sample in 50 ml of sterile distilled water (SDW) using a blender and the extracts were then prepared at 1 and 2% concentration by dilution with water and kept in conical flasks separately before use (Hossain, 2012). BAU-Biofungicide was used at 2 and 3% in this experiment. BAU-Biofungicide is a *Trichoderma* based preparation (Hossain, 2011). Bavistin DF (Carbendazim) and Proud 250 EC (Propiconazole) were also used at 0.1 and 0.05% concentration.

70 g seeds were taken randomly from each harvested sample for dry inspection. The seed samples were categorized visually for the presence of any distinct disease symptom or any other physiological abnormalities and were grouped into (a) apparently healthy seeds, (b) spotted and discoloured seeds and (c) chaffy grain. Each category was recorded and expressed in percentage (Chowdhury, 2012).

The experiment (Tray method) was conducted in the nethouse of the Seed Pathology Centre, Bangladesh Agricultural University Mymensingh. Sand was collected from Brahmaputra River, Mymensingh. The collected sand was sterilized with formalin (40%) at the rate of 5 ml formalin Diluted with 20 ml of water for 4 kg sand (Dasgupta, 1988). The formalin treated soil was covered with polythene sheet for 48 h and then exposed for 48 h for aeration before setting experiment. The plastic trays (12″ × 8″) were filled with the sand. The experiment was carried out in Complete Randomized Design with three replications. Three hundred harvested seeds of each treatment including control were sown in plastic trays (100 seeds/tray) maintaining equal distances among the seeds. Plants were watered as when necessary for maintaining proper moisture. Randomly selected 10 seedlings were uprooted carefully from each tray and washed thoroughly with running tap water. Data was recorded for each treatment at 14 days after sowing (DAS) on different parameters. Vigour Index (VI) was computed using the following formula of Baki and Anderson (1973): Vigor index = (Mean shoot length + Mean root length) x % Germination

Blotter method for seed health test was carried out following blotter method to detect seed borne pathogens associated with seed sample (ISTA, 1996). Three layers of blotting paper (Whatman filter No.1) soaked in water and were kept at the bottom of a 9.0 cm dia. plastic Petri dish and there after 25 seeds were kept on filter

Table 1. Effect of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Proud on disease severity of rice cv. BRRI dhan28 in 2012 and 2013.

Treatment (dose)	Percent disease severity					
	Brown spot					
	At 65 DAT		At 80 DAT		At 95 DAT	
	2012	2013	2012	2013	2012	2013
Garlic (1%)	3.37[a](8.17)	3.00(14.29)	5.20[bc](39.35)	5.25[bc](34.38)	11.07[c](28.72)	10.75[b](31.75)
Garlic (2%)	3.90[a](-6.27)	-	5.10[c](40.70)	-	9.53[d](38.63)	-
Neem (1%)	3.40[a](7.36)	3.50[a](0.00)	6.50[b](24.42)	6.00[b](25.00)	14.03[ab](9.66)	13.00[a](17.46)
Neem (2%)	3.13[a](14.71)	-	6.00[bc](32.50)	-	12.90[b](16.93)	-
BAU-Biofungicide (2%)	3.13[a](14.71)	3.25[a](7.14)	4.70[c](45.35)	4.50[c](43.75)	2.57[e](83.45)	2.50[d](84.13)
BAU-Biofungicide (3%)	4.00[a](-8.99)	-	4.70[c](45.35)	-	2.37[e](84.74)	-
Bavistin (0.1%)	3.40[a](7.36)	3.00[a](14.29)	5.20[bc](39.53)	5.25[bc](34.38)	9.17[d](40.95)	8.75[c](44.44)
Bavistin (0.05%)	3.53[a](3.81)	3.50[a](0.00)	6.10[bc](29.07)	5.75[bc](28.13)	11.00[c](29.17)	10.50[b](33.33)
Proud 250 EC (0.1%)	3.33[a](9.26)	3.00[a](14.29)	4.80[c](44.19)	4.50[c](43.75)	2.03[e](86.93)	2.00[d](87.30)
Proud 250 EC (0.05%)	3.20[a](12.81)	3.25[a](7.14)	4.70[c](45.35)	4.75[bc](40.63)	2.27[e](85.38)	2.50[d](84.13)
Control (water)	3.67[a]	3.50[a]	8.60[a]	8.00[a]	15.53[a]	15.75[a]

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; DAT = Days after transplanting; data represent the means of three replications; Data in parentheses indicate % disease severity reduction over control
- = Not tested in 2013.

paper. Four hundred harvested seeds of each treatment were taken randomly from each sample. The petri dishes containing seeds were incubated at 20±2°C under alternating cycles of 12 h near Ultra Violet light and darkness for 7 days. Incubated seeds were examined under stereo-binocular microscope to record the incidence of different seed borne fungi. Each seed borne infection was recorded and expressed in percentage (Agarwal et al., 1989).

All recorded data on different parameters were analysed statistically using MSTAT-C computer program and treatment means were evaluated for significance using DMRT following Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Highest (86.93%, 87.30% at 95 DAT) reduction of disease severity of brown spot disease was observed with foliar spray of Proud (0.1%) followed by (85.38%, 84.13%) with Proud (0.05%) and (83.45%, 84.13%) with BAU-Biofungicide (2%) among three counting period in two successive years 2012 and 2013 (Table 1). No statistical significant difference of disease severity was found between Proud and BAU-Biofungicide. These findings of this trial were in accordance with the observation of Razu and Hossain (2015). They reported that BAU-Biofungicide (2%) and Tilt 250EC (0.1%) were applied as foliar spray for 3 times of rice cv. BRRI Dhan49 under field condition. The lowest 13.93, 14.51 and 14.87% disease severity of brown spot were recorded at 70, 80 and 90 DAT as foliar spray of BAU-Biofungicide (2%) as well as 15.39, 15.73 and 16.41% disease severity were found in Proud 0.1%. Gupta et al. (2013) conducted a field experiment with three rice varieties viz., Basmati-370, Jaya and PC-19 during 2011 to 2012 with application of propiconazole and azoxystrobin which significantly reduced the disease severity of brown spot (69, 73 and 70%) of all the varieties as compared to their respective controls (Table 2).

Highest (89.00%, 87.50%) apparently healthy seed was recorded in Proud (0.1%) followed by (88.67%, 87.00%) with Proud (0.05%) and (87.67%, 86.50%) with BAU-Biofungicide (2%). In germination test, Proud (0.1%) showed the highest (99.00%) germination, while maximum (93.67%) normal seedling was recorded in Proud (0.1%). Higher increase (35.75 and 33.81%) of normal seedlings was found with Proud (0.1 and 0.05%) as well as in BAU-Biofungide (2%) having (32.36%) over control in harvested seeds in 2012. Maximum (76.70%) reduction of diseased seedling was found in Proud (0.1%) followed by Proud (0.05%) and BAU-Biofungicide (2%). In case of germination failure, maximum (86.96%) reduction was found with BAU-Biofungicide (2%) and Proud (0.1 and 0.05%) in 2012. Vigour index appeared with the highest increase (35.23%) with Proud (0.1%), while (32.74%) increased in BAU-Biofungicide (2%) compared to control (Table 3). The results were supported by Ora et al. (2011). They showed the better performance in terms of lowest pathogenic incidence, rotten seed, dead seed, seed germination and seedling vigour index. Mahmud and Hossain (2013) alsoevaluated the efficacy of BAU-Biofungicide (2 and 3%), garlic (1 and 2%) and neem extracts (1 and 2%), Bavistin (0.1 and 0.05%) and Proud (0.1 and 0.05%) for controlling brown spot of rice cv. BR11. BAU-Biofungicide (3%) showed the highest germination (98.00%) along with maximum 93.00% normal seedling among the treatments. Highest 78.50% of apparently healthy seed was recorded in

Table 2. Effect of extracts of Garlic and Neem; BAU-Biofungicides, Bavistin and Proud on germination(%) and vigour index at 14 days after sowing of harvested seeds of rice cv BRRI dhan28 following tray method during Boro season in 2012 and 2013.

Treatment (dose)	% Apparently healthy seed		Germination (%)		Normal seedling (%)		Diseased seedling (%)		Germination failure (%)		Vigour index	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Garlic (1%)	82.00^{abc}	81.00^{abc}	95.00^{ab} (+8.78)	94.00^{ab} (+8.46)	84.00^{ab} (+21.74)	84.67^{bc} (+21.53)	6.33^{cd} (-36.70)	5.00^{c} (-44.44)	3.00^{c} (-60.89)	3.00^{d} (-63.99)	2259.67^{cd} (+15.41)	2234.28^{bcd} (+14.24)
Garlic (2%)	84.00^{abc}	-	95.67^{ab} (+9.55)	-	85.67^{ab} (+24.16)	-	5.67^{d} (-43.30)	-	3.00^{c} (-60.89)	-	2382.69^{bc} (+21.69)	-
Neem (1%)	78.00^{bc}	78.75^{bc}	92.00^{bc} (+5.35)	91.33^{bc} (+5.38)	80.00^{bc} (+15.94)	80.33^{c} (+15.30)	6.33^{b} (-36.70)	6.00^{b} (-33.33)	4.67^{b} (-39.11)	4.67^{bc} (-43.94)	2100.58^{de} (+7.28)	2101.33^{cd} (+7.44)
Neem (2%)	80.33^{abc}	-	92.33^{ab} (+5.73)	-	82.33^{ab} (+19.32)	-	5.33^{b} (-46.70)	-	4.67^{b} (-39.11)	-	2191.88^{cd} (+11.94)	-
BAU-Biofungicide (2%)	87.67^{ab}	86.50^{ab}	98.00^{a} (+12.22)	97.00^{a} (+11.92)	91.33^{ab} (+32.36)	90.66^{ab} (+30.13)	3.33^{e}f (-66.70)	2.67^{d} (-70.33)	1.00^{d} (-86.96)	2.00^{e} (-75.99)	2589.46^{ab} (+32.25)	2571.32^{ab} (+31.48)
BAU-Biofungicide (3%)	88.00^{a}	-	98.00^{a} (+12.22)	-	90.67^{ab} (+31.41)	-	3.67^{e} (-63.30)	-	1.00^{d} (-86.96)	-	2599.11^{ab} (+32.74)	-
Bavistin (0.1%)	84.33^{abc}	83.25^{ab}	95.00^{ab} (+8.78)	94.00^{ab} (+8.46)	84.33^{ab} (+22.22)	84.00^{bc} (+20.57)	6.00^{cd} (-40.00)	6.00^{b} (-33.33)	3.00^{c} (-60.89)	4.33^{c} (-48.02)	2432.48^{abc} (+24.23)	2398.30^{abc} (+22.63)
Bavistin (0.05%)	82.67^{abc}	81.00^{abc}	94.33^{ab} (+8.02)	92.00^{ab} (+6.15)	82.00^{ab} (+18.84)	82.00^{c} (+17.70)	7.00^{c} (-30.00)	6.00^{b} (-33.33)	3.00^{c} (-60.89)	5.33^{b} (-36.01)	2312.23^{cd} (+18.09)	2243.25^{bcd} (+14.70)
Proud 250 EC (0.1%)	89.00^{a}	87.50^{a}	99.00^{a} (+13.36)	98.00^{a} (+13.07)	93.67^{a} (+35.75)	92.33^{a} (+32.53)	2.33f (-76.70)	3.00^{d} (-66.67)	1.00^{d} (-86.96)	2.00^{e} (-75.99)	2647.88^{a} (+35.23)	2632.870^{a} (+34.62)
Proud 250 EC (0.05%)	88.67^{a}	87.00^{a}	98.00^{a} (+12.22)	96.67^{a} (+11.54)	92.33^{ab} (+33.81)	91.00^{ab} (+30.62)	2.67^{e}f (-73.30)	2.67^{d} (-70.33)	1.00^{d} (-86.96)	2.00^{e} (-75.99)	2599.93^{ab} (+32.78)	2549.47^{ab} (+30.36)
Control (water)	75.00^{c}	74.00^{c}	87.33^{b}	86.67^{c}	69.00^{c}	69.67^{d}	10.00^{a}	9.00^{a}	7.67^{a}	8.33^{a}	1958.03^{e}	1955.74^{d}

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; data represent the means of three replications; data in parentheses indicate % increased (+) and % decreased (-) over control; DAS = days after sowing; **-** = not tested in 2013.

Potent (0.1%) followed by BAU-Biofungicide (3%) (74.33%), while minimum (47.75%) was in control. Biswas et al. (2008) reported that *Trichoderma* treated rice seeds showed maximum germination (92%) and increased shoot and root length 21 and 25%, respectively. Hossain et al. (2015) also reported that 28.25, 18.31 and 17.79% vigour index were increased over control when wheat seeds of Kanchon variety were treated with BAU-Biofungicide (2.5%), Bavistin (0.1%) and garlic (1%), respectively at 10 days after sowing. This result is in accordance with the findings of Hasan et al. (2005) and Chowdhury et al. (2005).

Plant extracts, BAU-Biofungicide and fungicides on health status of rice seeds were evaluated by standard blotter incubation test and the seeds were found to be associated with 9 different seed borne fungi viz., *Alternaria padwickii*, *Alternaria tenuis, B. oryzae, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Sarocladium oryzae, Trichoderma harzianum* and *Penicillium sp.* in 2012 and 2013 (Tables 3 and 4).

Hundred percent reduction of seed-borne infection of *A. padwickii* was found with BAU-

Table 3. Effect of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Proud on germination (%) and seed borne fungi of rice seeds of cv BRRI dhan28 following Blotter method during Boro season in 2012.

Treatment (dose)	Germination (%)	Germination failure (%)	*Alternaria padwickii*	*Bipolaris oryzae*	*Curvularia lunata*	*Fusarium moniliforme*	*Fusarium oxysporum*	*Alternaria tenuis*	*Sarocladium oryzae*	*Trichoderma harzianum*	*Penicillium sp.*
Garlic (1%)	95.00^{abcd} (+6.15)	5.00^{d} (-52.38)	0.00^{d} (-100.00)	4.00^{cde} (-42.86)	3.75^{e}f (-58.33)	4.50^{b} (-21.74)	12.50^{cd} (-16.67)	2.50^{e} (-70.59)	1.00^{d} (-66.67)	0.00^{c}	2.00^{bc} (-50.00)
Garlic (2%)	96.00^{abcd} (+7.26)	4.00^{d} (-61.90)	0.00^{d} (-100.00)	4.50^{cd} (-35.71)	3.50^{e}fg (-61.11)	4.50^{b} (-21.74)	12.00^{de} (-20.00)	2.75^{e} (-67.65)	1.00^{d} (-66.67)	0.00^{c}	2.00^{bc} (-50.00)
Neem (1%)	92.50^{cde} (+3.35)	7.50^{c} (-28.57)	1.00^{c} (-33.33)	6.50^{a} (-7.14)	4.50^{de} (-50.00)	4.50^{b} (-21.74)	14.00^{abc} (-6.67)	5.50^{c} (-35.29)	2.50^{b} (-16.67)	0.00^{c}	2.50^{b} (-37.50)
Neem (2%)	93.75^{bcde} (+4.75)	6.25^{c} (-40.48)	2.00^{a} (33.33)	6.00^{ab} (-14.29)	3.50^{e}fg (-61.11)	3.50^{c} (-39.13)	12.50^{cd} (-16.67)	4.00^{d} (-52.94)	2.00^{c} (-33.33)	0.00^{c}	2.50^{b} (-37.50)
BAU-Biofungicide(2%)	97.50^{abc} (+8.94)	2.50^{e} (-76.19)	0.00^{d} (-100.00)	2.50f (-64.29)	2.50g (-72.22)	2.00^{e} (-65.22)	12.00^{de} (-20.00)	3.00^{de} (-64.71)	0.00^{e} (-100.00)	6.50^{a}	2.00^{bc} (-50.00)
BAU-Biofungicide(3%)	98.50^{ab} (+10.06)	1.50^{e}f (-85.71)	0.00^{d} (-100.00)	3.00^{e} (-57.14)	2.75fg (-69.44)	2.50^{de} (-56.52)	12.50^{cd} (-16.67)	3.25^{de} (-61.76)	1.00^{d} (-66.67)	6.00^{b}	2.25^{bc} (-43.75)
Bavistin DF (0.1%)	93.75^{bcde} (+4.75)	6.25^{c} (-40.48)	1.00^{c} (-33.33)	5.00^{e}f (-28.57)	6.50^{b} (-27.78)	3.00^{cd} (-47.83)	15.50^{a} (-3.33)	7.00^{b} (-17.65)	2.00^{c} (-33.33)	0.00^{c}	2.00^{bc} (-50.00)
Bavistin DF (0.05%)	91.75^{de} (+2.51)	8.25^{b} (-21.43)	1.00^{c} (-33.33)	5.00^{e}f (-28.57)	8.50^{a} (-5.56)	3.50^{c} (-39.13)	14.50^{ab} (-3.33)	6.50^{b} (-23.53)	2.50^{b} (-16.67)	0.00^{c}	2.00^{bc} (-50.00)
Proud 250 EC (0.1%)	99.00^{a} (+10.61)	1.00f (-90.48)	0.00^{d} (-100.00)	3.50^{de}f (-50.00)	5.25^{cd} (-41.67)	2.50^{de} (-56.52)	14.00^{abc} (-6.67)	3.00^{de} (-64.71)	0.00^{e} (-100.00)	0.00^{c}	1.50^{d} (-62.50)
Proud 250 EC (0.05%)	98.50^{ab} (+10.06)	1.50^{e}f (-85.71)	1.00^{c} (-33.33)	3.75^{de} (-46.43)	6.00^{bc} (-33.33)	2.00^{e} (-65.22)	13.00^{bcd} (-13.33)	5.00^{c} (-41.18)	1.00^{d} (-66.67)	0.00^{c}	1.50^{cd} (-62.50)
Control (water)	89.50^{e}	10.50^{a}	1.50^{b}	7.00^{a}	9.00^{a}	5.75^{a}	15.00^{a}	8.50^{a}	3.00^{a}	0.00^{c}	4.00^{a}

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; Data represent the means of four replications; data in parentheses indicate % increased (+) and % decreased (-) over control.

Biofungicide (2 and 3%) and Proud (0.1%) and garlic (1 and 2%). Highest (64.29%) reduction of seed-borne infection of *B. oryzae* was obtained in harvested seeds by spraying plots with BAU-Biofungicide (2%) over control followed by BAU-Biofungicide (3%) having (57.14%) reduction. Elham et al. (2011) evaluated the efficacy of *T. harzianum* against *B. oryzae. T. harzianum* inhibited the growth of rice brown spot pathogen. Farid et al. (2002) also found that four fungicides *viz.,* Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 were evaluated against *B. oryzae*. Cent percent mycelial growth inhibition was obtained with Dithane-45, while Tilt 250 EC inhibited (95.58%) mycelial growth inhibition at concentration of 500 ppm. Higher reduction (72.41%) of seed-borne infection of *C. lunata* was recorded in BAU-Biofungicide (2%) followed by BAU-Biofungicide (3%) and garlic (2%). *T. harzianum* inhibited pathogenic fungus *C. lunata* to a great extent in rice seed as also reported by Sagar et al. (2005). Jat and Agalave (2013) showed that *Trichoderma* species inhibited the growth of seed borne pathogn *C. lunata*. Maximum (25.93%) reduction of seed-borne infection of *F. oxysporum* was found in BAU-Biofungicide (2%) followed by garlic (2%) and

Table 4. Effect of extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Proud on germination (%) and seed borne fungi of rice seeds of cv BRRI dhan28 following Blotter method during Boro season in 2013.

Treatment (dose)	Germination (%)	Germn. failure(%)	*Alternaria padwickii*	*Bipolaris oryzae*	*Curvularia lunata*	*Fusarium moniliforme*	*Fusarium oxysporum*	*Alternaria tenuis*	*Sarocladium oryzae*	*Trichoderma harzianum*	*Penicillium sp.*
Garlic (1%)	95.50^{ab} (+7.30)	4.50^{d} (-59.09)	0.00^{c} (-100.00)	3.00^{d} (-50.00)	2.50^{e}f (-65.52)	4.00^{cd} (-38.46)	11.25^{c} (-16.67)	2.50^{e} (-67.74)	1.00^{d} (-69.23)	0.00^{b}	1.50^{c} (-50.00)
Garlic (2%)	-	-	-	-	-	-	-	-	-	-	-
Neem (1%)	91.50^{bc} (+2.81)	8.50^{b} (-22.73)	1.00^{b} (-42.86)	5.50^{a} (-8.33)	3.00^{de} (-58.62)	4.75^{b} (-26.92)	12.50^{ab} (-7.41)	4.50^{c} (-41.94)	2.75^{b} (-15.38)	0.00^{b}	2.00^{bc} (-33.33)
Neem (2%)	-	-	-	-	-	-	-	-	-	-	-
BAU-Biofungicide (2%)	99.00^{a} (+11.24)	1.00^{e} (-90.91)	0.00^{c} (-100.00)	3.00^{d} (-50.00)	2.00f (-72.41)	3.00^{e} (-53.85)	10.00^{d} (-25.93)	3.25^{cde} (-58.06)	0.00^{e} (-100.00)	5.50^{a}	1.00^{d} (-66.67)
BAU-Biofungicide (3%)	-	-	-	-	-	-	-	-	-	-	-
Bavistin DF (0.1%)	93.50^{abc} (+5.06)	6.50^{c} (-40.91)	0.00^{c} (-100.00)	4.25^{bc} (-29.17)	6.00^{b} (-17.24)	3.50^{de} (-46.15)	11.50^{bc} (-14.81)	6.00^{b} (-22.58)	2.00^{c} (-38.46)	0.00^{b}	2.50^{ab} (-16.67)
Bavistin DF (0.05%)	92.00^{bc} (+3.37)	8.00^{b} (-27.27)	2.00^{a} (-14.29)	4.50^{b} (-25.00)	6.50^{ab} (-10.34)	4.25^{bc} (-34.62)	12.25^{bc} (-9.26)	7.25^{a} (-6.45)	2.75^{b} (-15.38)	0.00^{b}	3.00^{a} (-0.00)
Proud 250 EC (0.1%)	98.50^{a} (+10.67)	1.50^{e} (-86.36)	0.00^{c} (-100.00)	3.50^{cd} (-41.67)	3.50^{cd} (-51.72)	3.00^{e} (-53.85)	12.00^{bc} (-11.11)	3.50^{cde} (-54.84)	0.00^{e} (-100.00)	0.00^{b}	2.00^{bc} (-33.33)
Proud 250 EC (0.05%)	98.50^{a} (+10.67)	1.50^{e} (-86.36)	1.00^{b} (-42.86)	3.75^{bcd} (-37.50)	4.00^{c} (-44.83)	3.50^{de} (-46.15)	11.25^{c} (-16.67)	4.00^{cd} (-48.39)	1.00^{d} (-69.23)	0.00^{b}	2.25^{b} (-25.00)
Control (water)	89.00^{c}	11.00^{a}	1.75^{a}	6.00^{a}	7.25^{a}	6.50^{a}	13.50^{a}	7.75^{a}	3.25^{a}	0.00^{b}	3.00^{ab}

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT; Data represent the means of four replications; data in parentheses indicate % increased (+) and % decreased (-) over control; **-** = not tested in 2013.

Proud (0.1%) over control as shown in Tables 3 and 4. Patale and Mukadam (2011) reported that *T. harzianum* inhibited the growth of *F. oxysporum* and later overgrew the test fungus. Nisa et al. (2011) also evaluated carbendazim, hexaconzol, bitertanol, myclobutanil, mancozeb, captan and zineb and extracts of *Allium sativum, Allium cepa* and *Mentha arvensis* for their effect on the inhibition of mycelial growth and spore germination of *F. oxysporum.* Highest (65.22%) reduction of *F. moniliforme* was found with BAU-Biofungicide (2%) and Proud (0.05%) over control. These findings were in accordance with the observation of Jat and Agalave (2013). They observed that *Trichoderma* species inhibited the growth of seed borne infection of *F. moniliforme*. Sagar et al. (2005) and Ahmad and Mukesh (2002) reported that *T. harzianum* was effective in reducing seed borne infection of *F. moniliforme* and increased seedling vigour and seed germination. Maximum inhibition in mycelial growth was observed in hexaconozole at 1000 ppm. Hundred percent reduction of seed-borne infection of *S. oryzae* was found with BAU-Biofungicide (2%) and Proud (0.1%), while garlic (1%) showed (69.23%) reduction (Table 3 and 4). Kalaiselvi and Panneerselvam (2015) reported that *T. harzianum* was found to be most effective with 96% inhibition against *S. oryzae* over control after 7th day of incubation by dual culture. Highest (66.67%) reduction of seed-borne infection of *penicillium sp* was recorded in BAU-Biofungicide (2%) followed by Proud (0.1%) in Tables 3 and 4.

Table 5. Benefit-cost ratio (BCR) analysis of foliar spray with two extracts of Garlic and Neem; BAU-Biofungicide, Bavistin and Proud in controlling diseases of rice in cv BRRI dhan28.

Functions	Garlic clove (1%)	Neem leaf (1%)	BAU-Biofungicide (2%)	Bavistin (0.1%)	Proud (0.1%)	Control
Seed (Tk.)	750/-	750/-	750/-	750/-	750/-	750/-
Preparation of land (Tk.)	7200/-	7200/-	7200/-	7200/-	7200/-	7200/-
Seed bed preparation (Tk.)	400/-	400/-	400/-	400/-	400/-	400/-
Fertilizer cost (Tk.)	10985/-	10985/-	10985/-	10985/-	10985/-	10985/-
Lay out and Transplantation	5000/-	5000/-	5000/-	5000/-	5000/-	5000/-
Weeding and irrigation (Tk.)	5000/-	5000/-	5000/-	5000/-	5000/-	5000/-
Cost of treatments (Tk.)	1664/-	1130/-	2100/-	2470/-	1827/-	-
Insecticide cost (Tk.)	500/-	500/-	500/-	500/-	500/-	500/-
Harvest cost (Tk.)	3000/-	3000/-	3000/-	3000/-	3000/-	3000/-
Cost of processing (Tk.)	1000/-	1000/-	1000/-	1000/-	1000/-	1000/-
Transportation cost (Tk.)	500/-	500/-	500/-	500/-	500/-	500/-
Others cost (Tk.)	1000/-	1000/-	1000/-	1000/-	1000/-	1000/-
Total cost of cultivation (Tk.)	36999/-	36465/-	37435/-	37805/-	37162/-	35335/-
Yield (kg/ha)	5345	5110	5785	5635	5930	4905
Sell price (Tk/ha)	96210/-	91980/-	104130/-	101430/-	106740/-	88290/-
Profit (Tk/ha)	59211/-	55515/-	66695/-	63625/-	69578/-	52955/-
(%) return over control	11.81	4.83	25.95	20.15	31.39	-
Benefit-cost ratio	2.60:1	2.52:1	2.78:1	2.68:1	2.87:1	2.50:1

For costs: Labour: Tk. 200/labour; Seed: Tk. 30/kg; ploughing: Tk. 2400/ha (one time); Bavistin: Tk. 2500/kg; Proud : Tk. 1850/litre; BAU Biofungicide: Tk. 50/kg; garlic clove: Tk. 64/kg; neem leaf : Tk. 40/kg; Urea: Tk. 20/kg; TSP: Tk. 24/kg; MP: Tk. 17/kg, Zypsum: Tk. 10/kg, Zinc: Tk. 220/kg; rice sell price: Tk. 18/kg.

Patale and Mukadam (2011) also found that three species of *Trichoderma* showed antagonistic activity against *P. notatum.* These findings were also supported by Jat and Agalave (2013). *T. harzianum* was associated with *B. oryzae* infected seeds and when kept for few days for incubation it overgrew *B. oryzae*. *T. harzianum* was associated even one year preservation of seeds of BAU-Biofungicide sprayed plot.

Highest net profit Tk 69578.00 was achieved in case of foliar spray of Proud (0.1%) followed by Tk 66695.00 with foliar spray of BAU-Biofungicide (2%). The benefit-cost ratio (BCR) 2.87:1 was found in Proud (0.1%), while BAU-Biofungicide (2%) showed 2.78:1 (Table 5). Hasan et al. (2014) reported that BAU-Biofungicide and Bavistin were found to have in controlling tikka disease of groundnut under field condition. They obtained benefit-cost ratios by 2.64:1 and 2.30:1 in application of BAU-Biofungicide (3%) and Bavistin (0.1%), respectively as foliar spray. Hossain (2012) applied BAU-Biofungicide and Tilt for controlling biological control of leaf blight of wheat under field condition. The benefit-cost ratio (2.16:1) was achieved in BAU-Biofungicide (2.5%) (seed treatment plus foliar spray), while by Bavistin 0.1% + Tilt 0.1% was 2.33:1.

Conclusion

Proud (0.1 and 0.05%) showed profound effect in controlling brown spot disease of rice, while BAU-Biofungicide (2%) was found to have in reducing disease severity as well as reduced cost of production with BCR and protecting seed borne pathogens in filed as they were applied as foliar spray. Highest benefit-cost ratio (BCR) 2.87:1 was found in Proud (0.1%) which is close to BAU-Biofungicide (2%) spray (2.78:1). It was evident that BAU-Biofungicide (2%) pronounced significant effect in increasing germination of seeds, seedling growth and vigor index and enhanced grain yield of harvested seeds in BAU-Biofungicide sprayed plot.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Dry bean crop productivity simulation for soil and climatic conditions of Tangará da Serra, MT - Brazil

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The Brazilian national growing of dry bean (*Phaseolus vulgaris*) currently comes in three annual harvests, which are the wet season, sowing between October and December, the Dry Season, sowing between February and May, and finally the Winter Season, sowing in June to August. The objective of this study was to determine the optimal sowing date for each of the three different sowing seasons wet, dry and winter of dry bean to the Tangará da Serrá region using a crop simulation software called Decision Support System for Agrotechnology Transfer (DSSAT). The DSSAT is comprised of crop simulation models, in which the CROPGRO-Drybean model was used to simulate the dry bean growth, development, and yield. The model was calibrated using the dry bean cultivar 'BRS Esplendor', planted on 15 December 2011 in Tangará da Serra, located in the Mato Grosso state of Brazil. The weather variables (maximum and minimum temperature, solar radiation and precipitation), phenological and soil variables were recorded during the season and used in the model calibration to ensure a satisfactory simulation. Following the calibration, simulations were performed for six sowing dates in each of the three seasons. Of the three growing seasons simulated, the wet season had the best grain yields for the dry bean 'BRS Esplendor', the sowing date of December 1st had the highest yields of 3.3 t ha^{-1}. The dry season had the second high simulated yields, and the highest yield into this growing season was 3.0 t ha^{-1}. In the dry season, grain yield decreased as late sowing date occurred, and the lowest simulated yield was 0.1 t ha^{-1}. Finally, the winter season had the lowest simulated yields among the three growing season, with a maximum yield of 0.5 t ha^{-1}. The CROPGRO-Drybean model had a high sensitivity to rainfall events, and drought periods during the reproductive stage of dry bean was the weather parameter that most affected grain yield. The winter season had lower yields than the wet and dry season in consequence of low rainfall events during the simulated crop cycles, the soil moisture was highly affected by precipitation, which directly affected the leaf area index and crop yield in all sowing dates.

Key words: CROPGRO-dry bean, yield, water stress, precipitation, soil moisture

INTRODUCTION

In Brazil, the dry bean (*Phaseolus vulgaris*) is cultivated by small and big farmers around the country, the leguminosae is considerate a subsistence crop that requires low technological development as well as the use of low seeds quality (Leite et al., 2009).

The Brazilian national growing of dry bean currently comes in three annual harvests, which are wet season, sowing between October and December, the dry Season, sowing between February and May, and finally the winter season, sowing in June to August (Vieira et al., 1995).

The Mato Grosso state stands out in 2014, as the third highest national yield of dry beans, with 23.4, 82.5 and 36.2 thousands of tons for the wet season, dry season and winter season, respectively. However, the crop cultivation is undergoing by a significant variation in relation to cultivated area, consequences of weather characteristics and market behavior (CONAB, 2014).

Weather characteristics such as temperature and solar radiation are important environmental factors, which affect the crop development. The dry bean has an ideal temperature of 21°C, which determine the crop development (Fancelli, 2009). While the solar radiation lead processes photosynthetic and photo-morphogenetic (Kunz et al., 2007). On the other hand, the stress hydric in non-irrigated agricultural areas is the main factor that has been affecting the dry bean. The high temporal variability may cause an excess or deficit hydric, and in both cases, it is directly influencing the dry bean development and production (Dallacort et al., 2011b).

Current weather variability has led growers and researchers to make decisions on best management practices based on simulation techniques. In this context, the Decision Support System for Agrotechnology Transfer (DSSAT) is increasingly used, and the CROPGRO-Drybean model (Hoogenboom et al., 1994), which is one of several crop development models present at the DSSAT, have been used extensively to evaluate effects of irrigation requirements (Heinemann and Hoogenboom, 2000), sowing dates (Dallacort et al., 2005; Lima Filho, Coelho Filho and Heinemann, 2013) and yield simulation (Dallacort et al., 2011a; Oliveira et al., 2012; Meireles et al., 2002, 2003).

The difference between the daily water uptake by plants and the crop transpiration is the factor that most penalize crop development and yield in the CROPGRO-Drybean. The water stress will affect the crop development through two different physiological factors, the photosynthesis, a less sensitive factor, and the cell elongation, which is highly affected by drought (Hoogenboom et al., 1994).

Dallacort et al. (2005) reported the use of the CROPGRO-Drybean model to determine the optimum planting dates for south of Brazil. The model strongly penalized the grain yield when the crop was submitted to water stress. Authors concluded that accumulative rainfall had a direct influence on leaf area index (LAI), biomass dry weight and yield.

The dry bean aptitude to Mato Grosso state (Marco et al. 2012) and the performance of the CROPGRO-Drybean model to simulate water stress factors (Heinemann and Hoogenboom, 2000; Dallacort et al., 2005) are already known. However, there is a lack of information on the influence of weather patterns on dry bean planting dates for the Mato Grosso state, therefore, the necessity of crop development stages and yield predictions to the region, which will help growers to better strategy best management practices, is required. The objective of this study was to determine the optimal planting date for each of the three different sowing seasons wet, dry and winter of dry bean to the Tangará da Serrá region, located in the Mato Grosso state, using the CROPGRO-Drybean simulation model.

MATERIALS AND METHODS

Model characteristics

The CROPGRO-Dry bean is a cropping system model from the Decision Support System for Agro-technology Transfer (DSSAT) (Jones et al., 2003). The model was developed by (Hoogenboom et al., 1994) and it simulates the common bean crop growth, development, and yield, as well as weather, genotype and soil properties (Meireles et al., 2003).

The minimum data set required to run the model are the plant genetic coefficients, soil characteristics, weather data and crop management data. The genetic coefficients are comprised by three files: .ECO, which characterize the ecotype, genetic coefficients that differ cultivars of determinate and indeterminate growth, .SPE, which characterize the species, genetic coefficients that determine the photosynthesis, nitrogen uptake capacity, phenology, growth, and senescence, and finally that file. CUL, which characterize the cultivar, such as photoperiod, photosynthetic rate, leaf area index (LAI), grain mass, trefoil maximum area, mean of grain per pod, period between emergence and first flower, first flower and first pod, first flower and first grain, first grain and maturation and first flower and end of leaves expansion.

The CROPGRO-Drybean uses physical soil characteristics as field capacity, permanent wilting point and saturation to calculate the soil water balance for the soil layer based on the water from irrigation, precipitation, and drainage. Furthermore, the model estimates the soil water evaporation (Es), plant transpiration (Ep) and crop evapotranspiration (ETc) in mm day^{-1}, using the orientated-model of the soil water balance developed by Ritchie (1985).

The weather data required is maximum and minimum temperature, rainfall and solar radiation, which are stored in two files: Station, WTH and station CLI. While the soil characteristics data, such as chemical analysis and physical-hydric analysis stored in the file SOIL.SOL. Finally, the crop management data for fertilizer applications, irrigation events, tillage and sowing dates are separately stored in the X file.

Experimental procedures

The field trial was carried out during 2011/2012 dry bean season in the experimental field of UNEMAT (Mato Grosso State University) at Tangará da Serrá, Mato Grosso state, latitude 14º 39' 55'' S, longitude 57º 25' 05'' W and altitude of 321.5 m. The research area has the soil classified as Oxisol, with 1 % of slope. The soil has a

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Table 1. Soil chemical analyses for 0-30 cm soil depth at the experimental area.

Soil depth (cm)	pH		-------------------- $cmol_c/dm^3$ --------------------					mg/dm³
	H_2O	$CaCl_2$	Al	H + Al	Ca + Mg	Ca	K	P
0-30	5.90	5.10	0.00	2.88	2.34	1.42	0.12	1.50

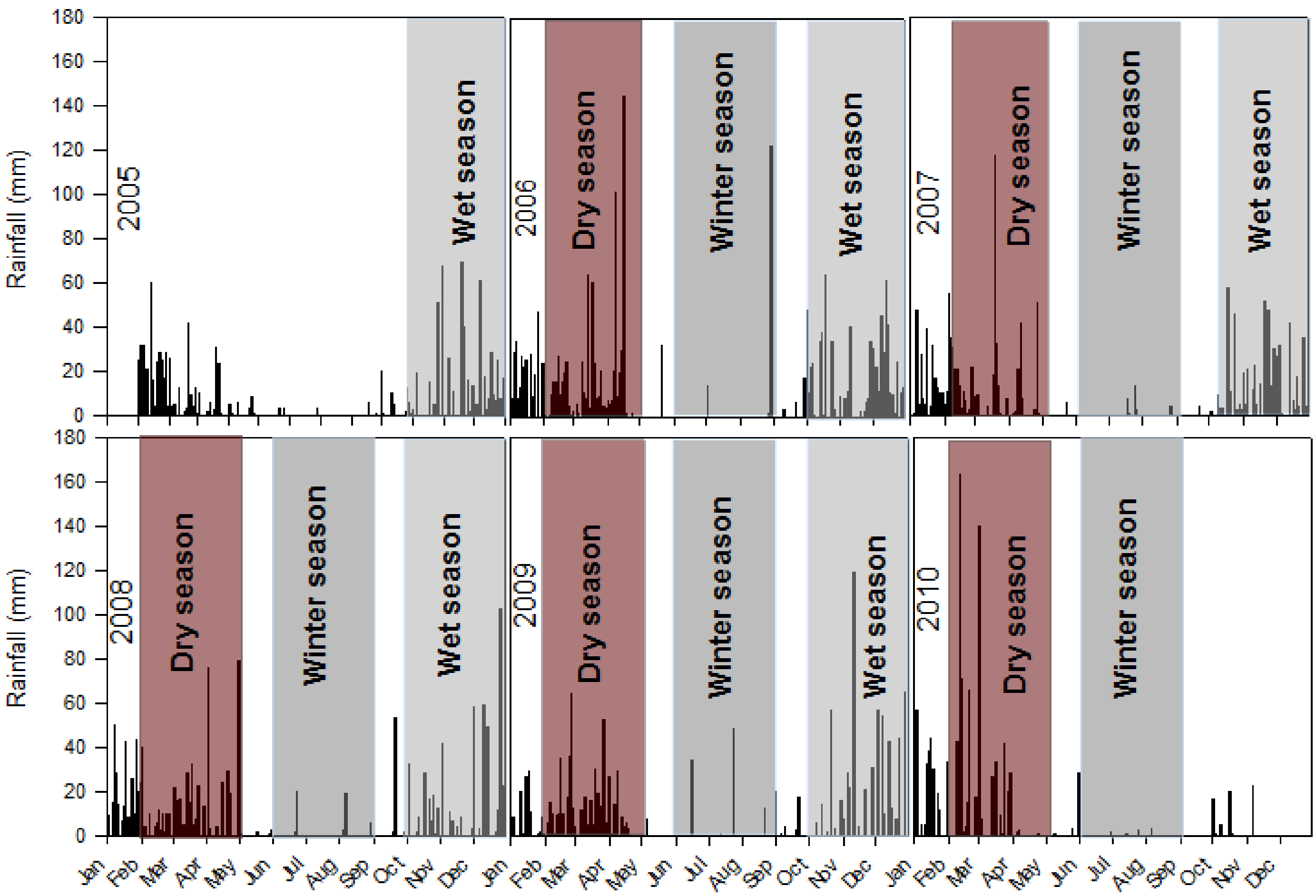

Figure 1. Rainfall events during wet, dry and winter sowing seasons in the period of 2005 and 2010.

clay texture, with the proportion of particle fractions of 67%, 7 and 26% of clay, silt and sand, respectively. The soil physical parameters, such as field capacity (0.301 cm^3cm^{-3}), permanent wilting point (0.239 cm^3cm^{-3}) and bulk density (1.09 $g.cm^{-3}$), and the soil chemical analyses (Table 1) were determined by soil samples.

The studied region has two well-defined weather seasons, which is a dry season from May to September and a wet season October to April (Dallacort et al., 2011b). The minimum weather data required by the model was collect in a meteorological station from UNEMAT located in site, and six years data set, from 2005 to 2010 used to model simulation. The 2011 data set was used to model calibration. During the experimental periods and simulations, rainfall events were the only source of water (Figure 1) for all dry bean harvest seasons (wet, dry and winter season).

The experimental purpose was to collect data to calibrate the CROPGRO-Drybean model; therefore, the crop management practices followed growers activities and local crop recommendation. No irrigation events occurred at any period of crop development. The dry bean seeds (var. 'BRS Esplendor') were planted on December 15th, 2011. Seeds were planted in-row spacing of 0.45 m and 12 plants per linear meter. The total experimental area was four replications of 6 m of length by 6 dry bean rows.

The fertilizer application was split into three applications: Initially, 240 kg ha^{-1} of the N, P, K formulated 5-25-15 was applied before planting at 20 cm of soil depth. The second application of 50 kg ha^{-1} of urea occurs 15 days after plant emergence, and the last application was 28 days after plant emergence with 50 kg ha^{-1} of urea. The grain harvest occurs in the four center rows of each replication, the average yield used to model calibration.

The data of genetic coefficients (Table 2) required by the model were collected through frequent field inspections. The genetic coefficients collected were critical day length (CSDL), response inclination regarding development for the photophase with time (1/h) (PPSEN), period between plant emergence and the appearance of the first flower in photothermal days (EM-FL), period

Table 2. Calibrated genetic coefficients for cultivar BRS Esplendor.

Cultivar	CSDL	PPSEN	EM-FL	FL-SH	FL-SD	SD-PM	FL-LF
BRS Esplendor	12	0	28.7	3	9.5	28.5	16.84
LFMAX	SLAVR	SIZLF	XFRT	WTPSD	SFDUR	SDPDV	PODUR
1	308	133	1	0.251	13	5.2	9.1

between the appearance of the first flower and the first pod in photothermal days (FL-SH), period between the appearance of the first flower and the start of seed formation in photothermal days (FL-SD), period between the start of seed formation and physiological maturity in photothermal days (SD-PM), period between the appearance of the first flower and the end of leaf expansion (FL-LF), maximum leaf photosynthesis rate at an optimal temperature rate of 30°C (LFMAX), specific leaf area under standard growth conditions in cm^2 (SLAVARN), maximum size of completely expanded leaf in cm^2 (SIZLF), maximum fraction of the daily growth that is partitioned between the seed plots the pod (XFRT), maximum weight per seed in g (WTPSD), duration of the grain swelling period in the pods, under standard growth conditions in photothermal days (SFDUR); mean seeds per pod (SDPDV), and time necessary for the cultivar to reach ideal pod conditions in photothermal days (PODUR). During the model calibration the genetic coefficients were adjusted following the methodology proposed by Boote (1999).

Simulations

The simulations were performed to Tangará da Serrá region for six different planting dates in each of the three harvest seasons during the six years of weather data set, from 2005 to 2010. The planting date was determined to occur every 15 days for all harvest season. The wet season was comprised by October 1st (10/01) and 15th (10/15), November 1st (11/01) and 15th (11/15), and December 1st (12/01) and 15th (12/15). The dry season sowing dates were January 15th (01/15), February 1st (02/01) and 15th (02/15), March 1st (03/01) and 15th (03/15), and April 1st (04/01). Finally, the winter season sowing dates were April 15th (04/15), May 1st (05/01) and 15th (05/15) June 1st (06/01) and 15th (06/15), and July 1st (07/01).

The simulated grain yields in response to the planting dates were analyzed in a cumulative probability distribution for each studied growing season to determine the best planting dates for each season (wet, dry and winter season).

RESULTS

Highest average yield (2.3 t ha^{-1}) were simulated for the planting dates of the wet season in the six years studied, followed by the dry season (1.3 $t.ha^{-1}$). Lowest grain yields were simulated for the winter season (average of 0.2 $t.ha^{-1}$) (Table 3). The average rainfall for each harvest season studied; during the 6 years were 678, 742 and 60 mm for the wet season, dry season and winter season, respectively. The lower average of rainfall presented by the wet season compared to the dry season is explained by an atypical wet season in 2010, in which the total rainfall amount was 212.9 mm during all season.

The wet season had in 2007 the highest total rain accumulation during the full season, with 1,131 mm well distributed during all season (Figure 2), the average dry bean yield from all planting dates was 3.0 $t.ha^{-1}$. The winter season had a highest average yield of 0.2 $t.ha^{-1}$ in 2009, when rain accumulation was only 183 mm.

The wet season planting date of 01/12/2010 showed the highest grain yield (3.3 $t.ha^{-1}$). The maximum LAI simulated for the planting date was 2.8 $m^2.m^{-2}$ (Figure 3), however, this LAI was considerate smaller than the average of other planting dates from the wet season (4.0 $m^2.m^{-2}$). The soil depths of 5, 15 and 30 cm had an average soil moisture content of 0.16, 0.29 and 0.32 cm^3 cm^{-3} (Figure 4), respectively, during the vegetative stage. Between flowering and harvest (a period of 40 days), the average soil moisture in the same soil depths were 0.3, 0.34 and 0.34 cm^3cm^{-3} (Figure 3), respectively.

In contrast, the winter season had the highest grain yield for the planting date of 06/01/2009; the yield was 0.5 $t.ha^{-1}$. The maximum simulated LAI for this planting date was 1.2 m^2m^{-2} (Figure 3). Soil moisture content (Figure 4) was below the wilting point (0.239 cm^3 cm^{-3}) most of the crop development at the first soil layer (5 cm). At 15 and 30 cm of soil depth, the soil moisture was higher than the 5 cm during the vegetative stage; the averages were 0.31 and 0.33 cm^3 cm^{-3}, respectively. After the flowering stage, the soil moisture decreased because of the reduction in rainfall events, the averages were 0.26 and 0.28 cm^3 cm^{-3}, respectively.

The comparison between planting dates within the wet season, simulations indicated that when dry bean was planted in 01/12 yields increased, the average yield for this planting date was 2.9 $t.ha^{-1}$ (Figure 5). The planting date of 01/10 presented the lowest simulated yields, and the average was 1.6 $t.ha^{-1}$ (Figure 5). The cumulative probability analysis for the wet season (Figure 6) indicated that higher grain yield could be achieved with the planting date of December 1st (01/12), and yield decreases as early as planting occurs in the season. Lowest yields will most likely occur with the planting date of October 1st. Simulated data of 2010, when the cumulative rainfall was 593 mm concentrated during the late season, had grain yields of 0.2, 0.1, 0.4, 1.0, 3.3 and 2.6 $t.ha^{-1}$ for 01/10, 15/10, 01/11, 15/11, 01, 12 and 15/12, respectively.

The most appropriate simulated planting date for the dry season was 15/01, followed by 01/02, which showed highest yields (Figure 5). The lowest yields of dry season were obtained by the planting date of 01/04. The planting date of 01/15 showed higher yield than the other planting

Table 3. Simulated grain yield of each planting date during all six year studied for all dry beans growing season in Brazil.

Planting date (Year)	Wet season					
	10/01	10/15	11/01	11/15	12/01	12/15
	Grain yield t ha^{-1}					
2005	2.5	2.9	3.2	2.5	3.2	2.7
2006	0.9	1.5	2.7	3.1	2.8	2.9
2007	3.0	3.2	3.2	3.2	3.0	2.9
2008	1.1	1.5	2.7	2.5	2.1	1.5
2009	2.1	3.1	3.1	2.7	3.1	2.5
2010	0.2	0.1	0.4	1.0	3.3	2.6
Year	Dry season					
	01/15	02/02	02/15	03/01	03/15	04/01
	Grain yield t ha^{-1}					
2005	1.4	1.1	0.4	0.1	0.2	0.1
2006	1.9	2.5	2.6	1.4	0.8	0.3
2007	2.1	1.7	1.4	1.3	0.7	0.2
2008	2.5	2.9	3.0	3.0	1.5	0.3
2009	2.9	2.8	3.0	0.9	0.3	0.2
2010	2.4	2.5	1.2	0.2	0.2	0.3
Year	Winter season					
	04/15	05/01	05/15	06/01	06/15	07/01
	Grain yield t ha^{-1}					
2005	0.2	0.2	0.2	0.1	0.1	0.1
2006	0.3	0.2	0.2	0.1	0.1	0.4
2007	0.1	0.1	0.2	0.2	0.2	0.1
2008	0.3	0.3	0.2	0.1	0.1	0.1
2009	0.3	0.4	0.4	0.5	0.4	0.2
2010	0.2	0.2	0.2	0.1	0.1	0.1

dates, under the cumulative probability of 100% (Figure 6). When planting occurs at 02/01 yields were higher for all other cumulative probabilities (75, 50, 25 and 0%). Under 75% of cumulative probability, grain yields were 1.7, 1.8, 1.1, 0.3, 0.1 and 0.1 t.ha^{-1} for planting dates of 15/01, 01/02, 15/02, 01/03, 15/03 and 01/04, respectively (Figure 6).

The winter season is unfeasible (Figure 5), because of the low precipitation during this year period (Figure 1). Simulated yields were not higher than 0.5 tha^{-1}, and the planting date that showed highest yield at the cumulative probability of 75% was 15/04 (Figure 6).

DISCUSSION

The main weather variable that influenced plant development and grain yield was the rainfall. The high yields of the wet season (Table 3) can be explained by the rainfall events typically occurring during the season period (Figure 1). The crop development variables affected by water stress are reported in the comparison between the highest precipitation year for the wet season (planting date of 01/12/2010) and the typical year of the winter season (planting date of 06/01/2009).

The planting dates of wet season had plenty rainfall events associated with a good rain distribution, which increased soil moisture and dry bean yield. However, the low and non-uniform rain distribution observed in the winter season resulted in a soil drought during crop flowering and maturation (Figure 3). Nascimento (2004) reported that a reduction in soil available water of 40% during the reproductive stage could reduce pod number, pod size and number of grain per pods. In addition, Guimaraes et al. (2011) and Bastos et al. (2011) showed an average yields loss of 58% when irrigated dry bean plants were compared to non-irrigated plants.

The water stress in the first soil layer presented during the vegetative stage, when leaf number is produced, may reduce LAI and biomass accumulation (Nascimento,

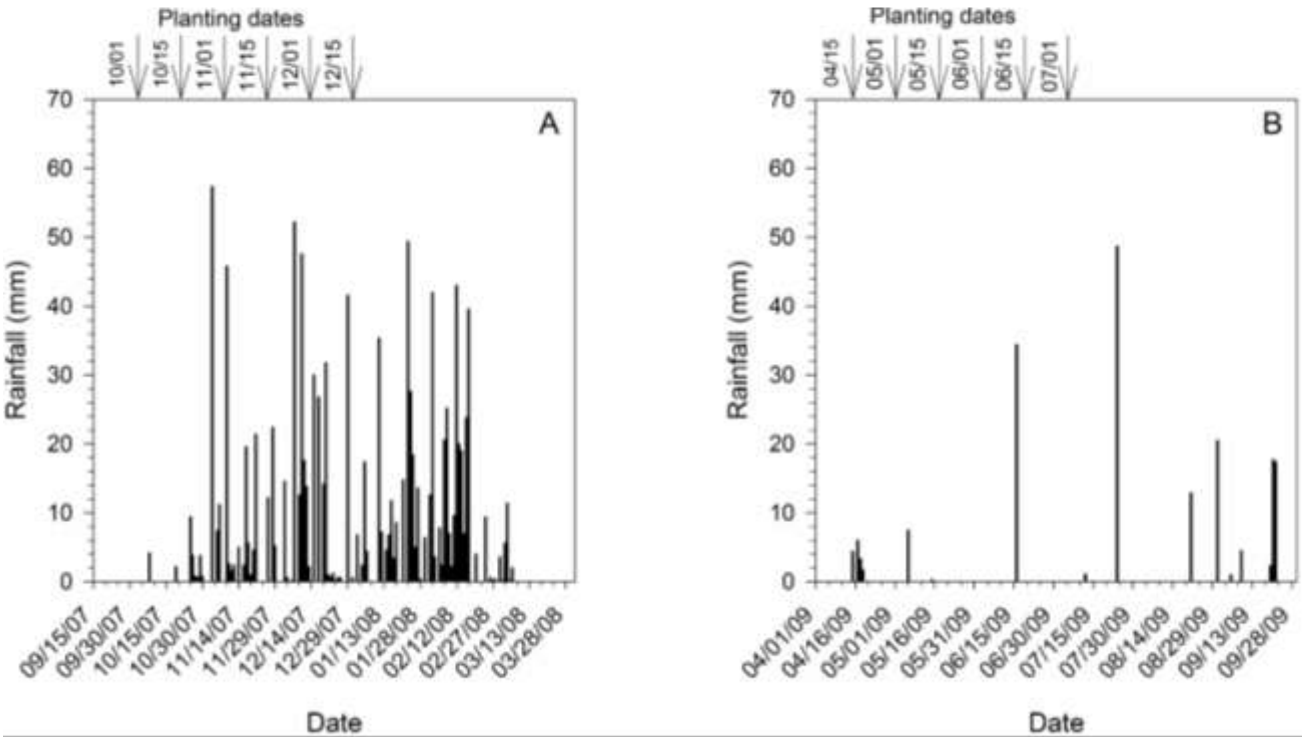

Figure 2. Rainfall events during the wet season of 2007 for all planting dates (graph A), and rainfall events during the winter season of 2009 for all planting dates (Graph B).

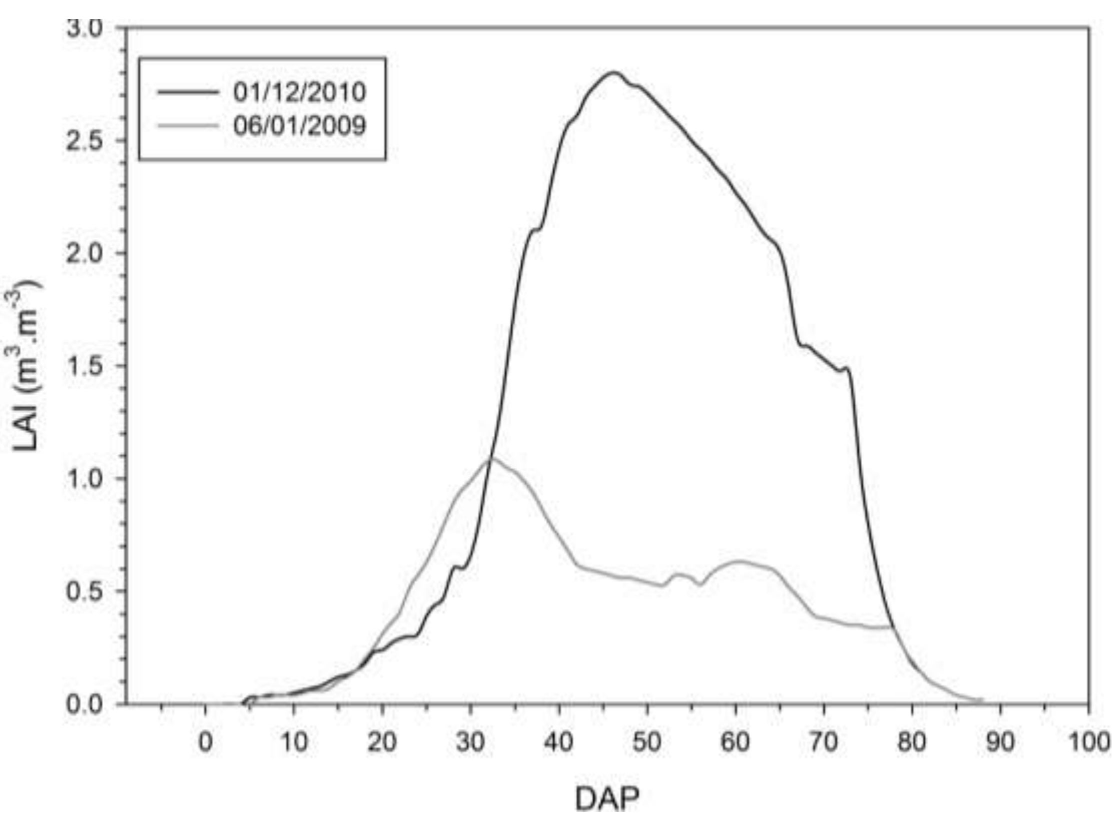

Figure 3. Simulated LAI in days after planting (DAP) for the lowest and highest simulated grain yield planting date, 01/12/201 and 06/01/2009, respectively.

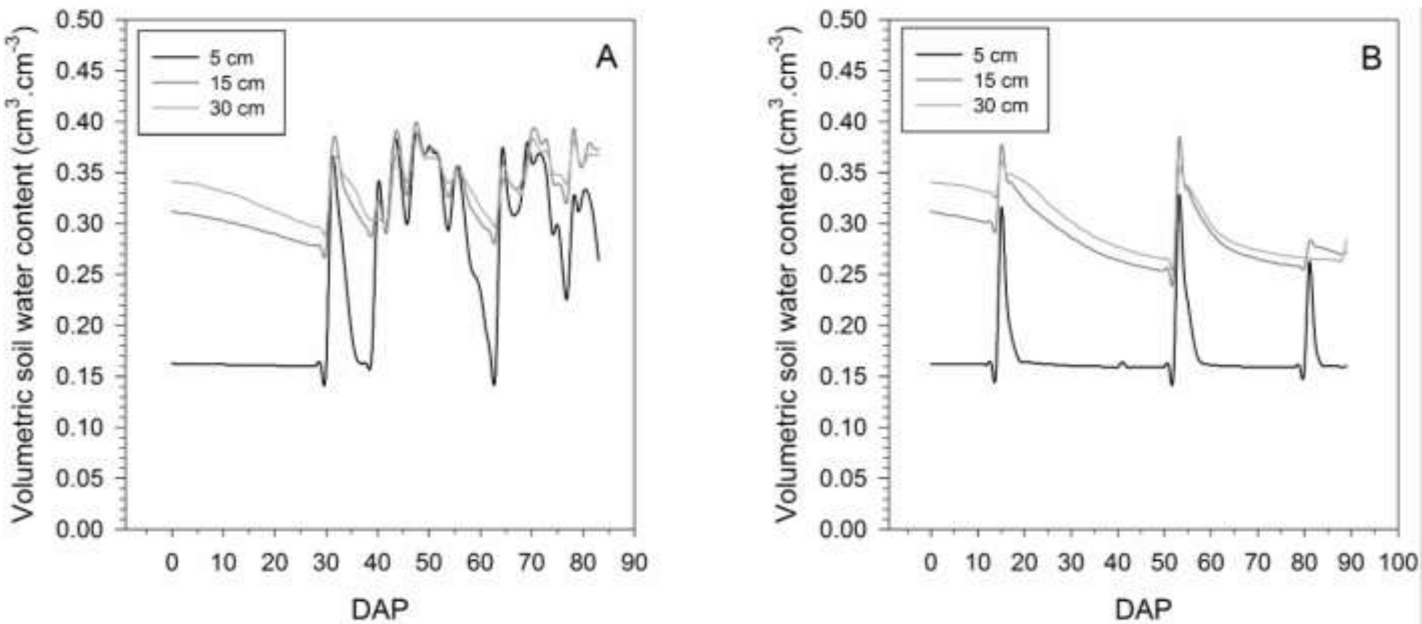

Figure 4. Simulated soil moisture for 3 depths (5, 15 and 30 cm) in days after planting (DAP) for the highest simulated grain yield planting date and year (01/12/2010) (graph A), and lowest simulated yield production planting date and year (06/01/2009) (Graph B).

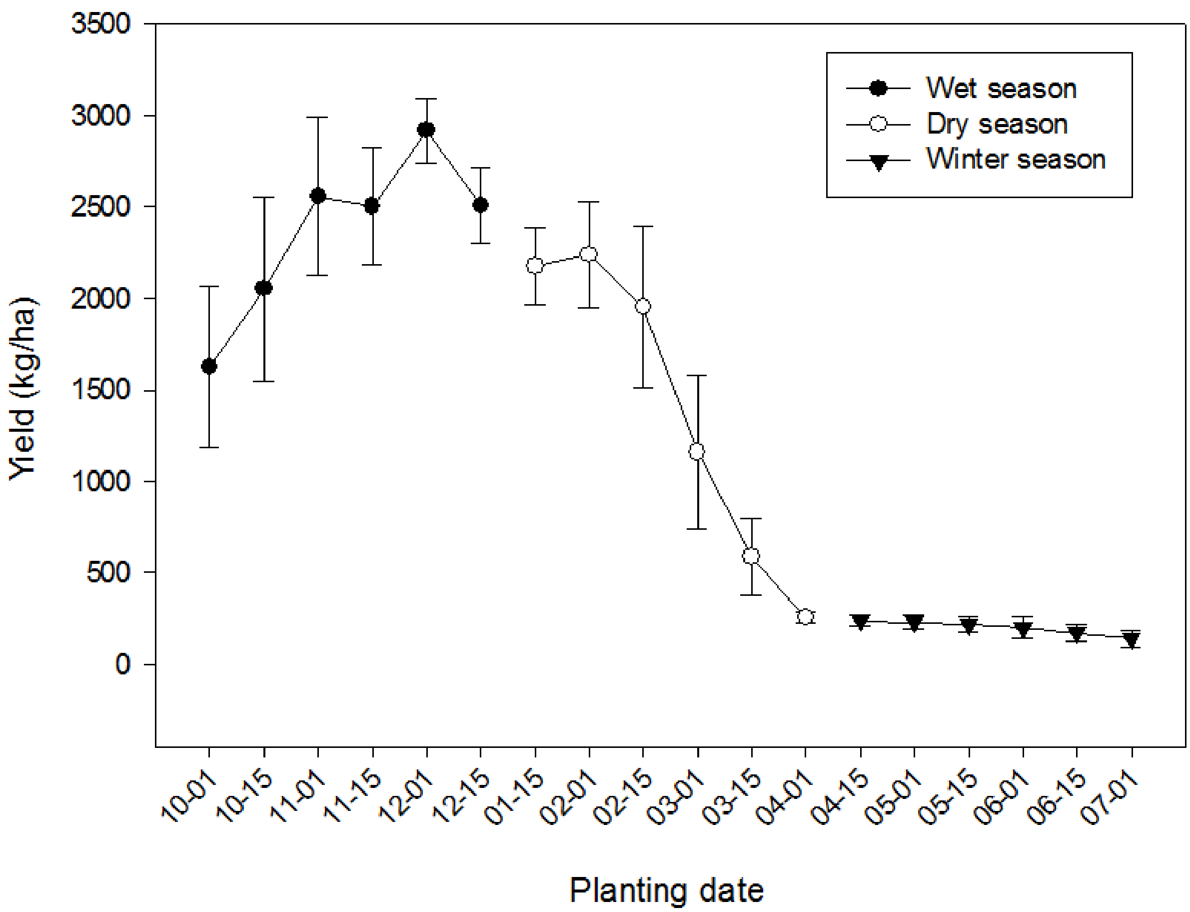

Figure 5. Average simulated grain yield of studied years (2005 to 2010) for each planting date of all dry bean growing season in Tangará da Serrá - MT. Error bars represent ± standard errors from the mean, n=6.

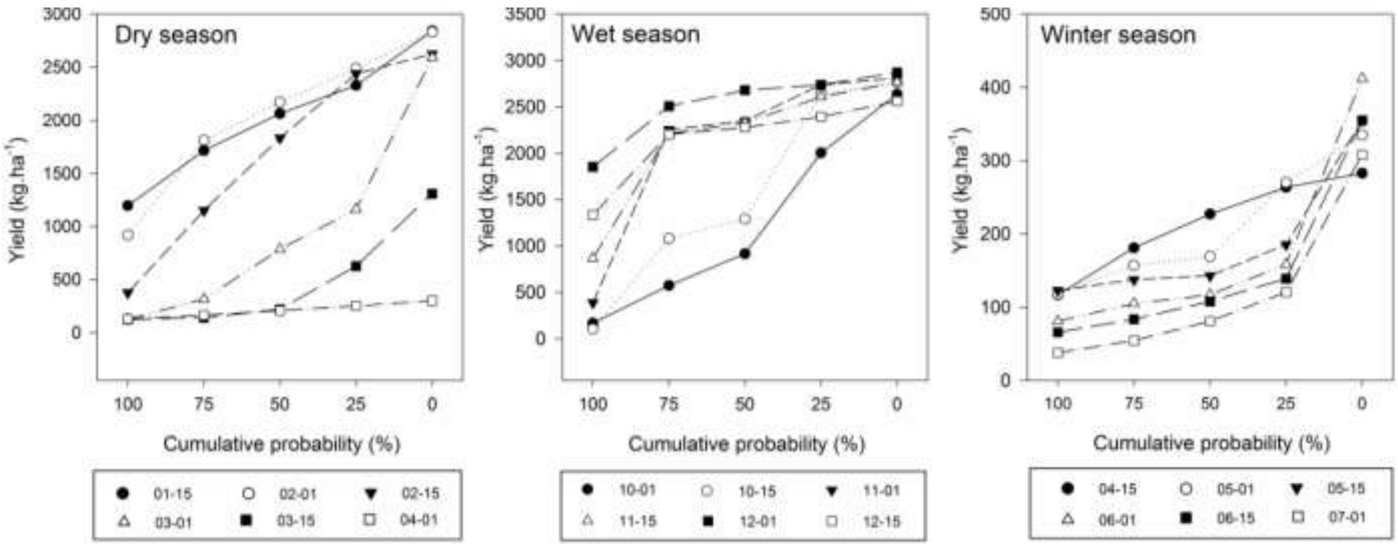

Figure 6. Cumulative probability distribution of grain yield production in each planting date for all three dry bean growing season in Tangará da Serrá -MT.

2004; Miorini et al., 2011). In the present study, wet and winter seasons had a water stress in the initial stages of crop development for the planting dates of 01/12/2010 and 06/01/2009, respectively, which decreased LAI (Figure 4). However, the planting date of the wet season (01/12/2010) had the highest grain yield, explained by the increasing in rainfall events during the reproductive stages (Guimaraes et al., 1996). The planting date of 06/01/2009 had a low precipitation during all crop development, therefore lower yield.

Overall, most of the low simulated yields were consequence of soil water stress between flowering and pod maturation, growth stages that water shortage can strongly reduce grain yield (Araujo, 1996; Dallacort et al., 2010). In addition, planting dates of the winter season received rainfall amounts smaller than the dry bean water demand recommendation of 300 to 600 mm during all crop development. The dry bean daily water consumption is from 3 to 4 mm per day, requiring 100 mm monthly (Fancelli, 2009).

In the wet season, the grain yield increased as late as planting occurs, which is consequence of a well-distributed rainfall events in the late of the season, according to the six years studied. The planting date of December 1st had the highest average yield for all cumulative probabilities. At 75% of cumulative probability, the grain yield was 2.5 t ha^{-1}. The regular rainfall distribution during the crop season of the planting date of wet season supply the dry bean water demand (Fancelli, 2009) mainly at the reproductive stages, when water stress most penalize grain yield (Guimaraes et al., 2011).

The dry season has an opposite weather pattern than the wet season, high precipitation and better rain distribution is presented in the early moment of the dry season. However, plentiful soil moisture content only during the early season can reduce grain yield. The soil water stress at reproductive stages will decrease nutrients uptake and grain yield (Nascimento, 2004). Therefore, late planting dates were most affected by a water stress after flowering. The grain yield of 1.7 and 1.8 t ha^{-1} at 75% of cumulative probability was simulated for the early planting dates of January 15th and February 1st, respectively, planting dates that had rainfall events well distributed over all crop development.

The drought periods during the plant development of all planting dates from the winter season affected the nutrient uptake and biomass accumulation (Santos and Carlesso, 1998, Fiegenbaum et al. 1991). The effects of drought start when plants evapotranspiration is higher than water absorption by the root system (Vieira et al., 2006). Irrigation practices are an option to supply plants water requirement and consequently increase grain yields for the winter season.

Conclusion

Despite several available models, like the DEMANDAsis, which determine best management practices through the soil water balance, the CLIGEN, which simulate agricultural managements based on weather parameters, and several other crop models. The CROPGRO-Drybean model demonstrated to be an excellent tool to help research and growers to increase dry bean yields. The CROPGRO-Drybean model showed high sensitivity to precipitation events, in which high rainfall events well distributed over the crop development increased the dry bean grain yield. The drought stress during the reproductive stages for all seasons was the environmental variable that most affect dry bean productivity simulation.

The wet season had the highest simulated grain yields, consequence of high rainfall events well distributed over the season. Furthermore, as late planting occurs in the wet season higher were the probability to achieve high yields. The planting date of December 1st provided the highest simulated grain yield within the wet season. In the dry season, planting dates of January 15th and February 1st are the best planting date for growers achieve higher yields, those planting dates had the highest likelihood to attend the crop water demand through rainfall events. Finally, the winter season requires irrigation practices for all simulated planting dates to increment dry bean grain yield

Conflicts of Interests

The authors have not declared any conflict of interests.

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