African Journal of Agricultural Research

Volume 11 Number 28 14 July 2016
ISSN 1991-637X

Academic Journals

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Vol. 11(28), pp. 2433-2442, 14 July, 2016
DOI: 10.5897/AJAR2016.10964
Article Number: 9C205CF59383
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Improving production of aromatic compounds by indigenous yeasts in Chenin Blanc grape must

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Received 4 March, 2016; Accepted 2 June, 2016

The aim of this study was to evaluate the effect of indigenous yeasts on the aroma of fermented Chenin Blanc grape must by determining the volatile compounds, the odor activity value (OAV) and carrying out sensory analyses. The must, fermented by *Hanseniaspora opuntiae*/*Saccharomyces cerevisiae* combinations, presented higher concentrations of compounds such as ethyl acetate, ethyl hexanoate, 2-methyl-1-propanol and 2-phenylethanol, and a lower production of acids and acetaldehyde. This fermented must presented higher OAV values for compounds such as 2-methyl-1-propanol and ethyl acetate, 687.06 and 1264.16, respectively. 2-phenylethanol was produced by *H. opuntiae* and *Hanseniaspora guilliermondii* in combination with *S. cerevisiae* in amounts that resulted in OAV values of 5.63 and 4.62, respectively. In appropriate concentrations, these volatile compounds contribute positively to the aromatic quality of the fermented must. The highest mean acceptability and purchasing intention scores were obtained by the must fermented by *H. opuntiae/S. cerevisiae.* In the must fermented by *H. guilliermondii/ S. cerevisiae*, the absence of ethyl hexanoate and high concentrations of octanoic acid and acetaldehyde probably contributed to its low acceptability. Thus, it was suggested that the yeast genus *Hanseniaspora* in combination with *S. cerevisiae*, showed the potential to positively impact the wine aroma.

Key words: Non-*Saccharomyces* yeasts, volatile compounds, odor activity value, acceptability test, *Hanseniaspora*, “São Francisco Valley region”.

INTRODUCTION

Grape juice in natural or spontaneous alcoholic fermentation, is routinely dominated by strains of the yeast *Saccharomyces cerevisiae*, but non-*Saccharomyces* yeasts such as *Candida*, *Hanseniaspora*,

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Pichia, *Torulaspora*, *Hansenula*, *Issatchenkia*, *Metschnikowia*, *Kluyveromyces* and *Zygosaccharomyces* initiate the process (Fleet, 2003; González et al., 2006; Lopandic et al., 2008). Non-*Saccharomyces* yeasts develop in the early stages, but do not survive to the end of fermentation. Nevertheless, they produce sufficient biomass to impact the chemical composition of the wine, and the contribution of these yeasts to the overall wine character is important (Swiegers and Pretorius, 2005).

Some studies have suggested that different yeasts (Torrens et al., 2008; King et al., 2011; Robinson et al., 2011) due to their genetic differences (Bisson and Karpel, 2010) can influence the volatile composition, and consequently the aroma and type of the wine. For example, some *Hanseniaspora*/*Kloeckera* species may produce more appealing mixtures of flavor volatiles than *Saccharomyces* species (Mendoza et al., 2009; Moreira et al., 2008; Assis et al., 2014), *Hanseniaspora osmophila* produces more 2-phenylethyl acetate than a pure culture of *S. cerevisiae* (Viana et al., 2009). Thus carrying out wine fermentations by co-inoculating with mixtures of different starter cultures, is a strategy which could harness the unique activity of such yeasts (Fleet, 2008).

The influence of the volatile compounds on the final aroma depends on their concentration in the wine, and on the perception threshold of the specific compound. A volatile compound contributes to the aroma when its concentration in the wine is above the perception threshold, therefore odorants with odor activity value (OAV) > 1 can be perceived (Guth, 1997; Jiang and Zhang, 2010; Juan et al., 2012; Welke et al., 2014). Capone et al. (2013) identified 51 volatile compounds in Negroamaro red wines, and of these, only 18 components were perceived as active odorants (OAV > 1).

A combination of analytical and sensory methodologies has been particularly important in resolving the effects of interactions between aroma compounds and the nonvolatile matrix, as well as with other volatile compounds (Mamede et al., 2005; Pineau et al., 2009; Aleixandre-Tudo et al., 2015). According to Robinson et al. (2014) these interactions may result in variations in the sensory character of the mixture, due to perceptual enhancement and suppression effects as well as to physicochemical effects on the volatility and release of aroma compounds.

The São Francisco Valley is the second most important wine production region in Brazil. This region stands out for the volume of grapes produced and the geochemical characteristics that favor the production of white wine and sparkling wine. Wine making demands innovation, so the aim of this study was to investigate the contribution of using a combined inoculation of indigenous non-*Saccharomyces* yeasts (*Hanseniaspora opuntiae*, *Hanseniaspora guilliermondii*, *Issatchenkia terricola* and *Cryptococcus flavescens*), obtained from the natural ecosystem in the “São Francisco Valley region”, together with the commercial *Saccharomyces cerevisiae* var. *bayanus,* on the aroma of the fermented Chenin Blanc grape must. The results are discussed based on the data obtained in the quantification of the volatile compounds/OAV and a sensory analysis.

MATERIALS AND METHODS

Yeast strains

Hanseniaspora opuntiae, *Hanseniaspora guilliermondii*, *Issatchenkia terricola* and *Cryptococcus flavescens* were obtained from grapes cultivated in the municipality of Lagoa Grande (PE-Brazil) at 361 meters, 8°59'47" south latitude and longitude 40°16'18", and isolated using the method of Assis et al. (2012). The non-*Saccharomyces* yeasts were identified by sequencing the D1/D2 region of the 26S subunit of the ribosomal RNA (Kurtzman et al., 2011). The pure colonies were maintained on Yeast Malt Agar (YMA) (1% glucose, 2% agar, 0.5% peptone, 0.3% malt extract and 0.3% yeast extract) slopes with the addition of sterile glycerol (Vetec), and kept under refrigeration (4°C).

Physicochemical analyses

The pH was determined using a digital pH meter (pHtek/P100, USA). The total soluble solids were determined using a refractometer (Atago/2313- USA), and the results expressed as °Brix. The SO_2, titratable acidity and nitrogen concentrations present in the grape musts were determined according to Brazil (2005). These analyses were carried out on the unfermented must. The alcohol content, residual sugar, volatile acidity and titratable acidity were determined in the fermented grape must, using the method described in Brazil (2005). The alcohol content was determined by hydrostatic balance Densi -Mat (Gibertini ®, Italia) at 20°C, after a previous distillation into Automatic distiller of drinks Super D.E.E.R (Gibertini ®, Italia) and the dried extract using the AlcoMat-2 reading module of the same hydrostatic balance. The total residual sugars of musts fermented was determined by the Lane-Eynon method in which the products of residual sugars, in alkaline medium, are oxidized by copper of the Fehling's, in which it is forming a complex cupro-tartaric-sodium-Potassium. The total acidity was analyzed by the neutralization of titratable acid with 0.1 N NaOH solution. The titratable acidity was determined with the aid of the distiller Super D.E.E.R, and the distillate titrated with 0.1N NaOH, using phenolphthalein as the indicator, through the titrator Quick Analyzer (Gibertini ®, Italia). All analyses were carried out in triplicate.

Fermentation conditions

Chenin Blanc grape must, donated by Vinibrasil S/A, located in the state of Pernambuco (Brazil), was used for fermentation. The four non-*Saccharomyces* yeasts were inoculated individually and in combination with *Saccharomyces cerevisiae* var. *bayanus*, according to the method described by Assis et al. (2014). The inoculums of *S. cerevisiae* var. *bayanus*, *H. opuntiae*, *H. guilliermondii*, *I. terricola* and *C. flavescens* were previously cultured in YMA (1% glucose, 2% agar, 0.5% peptone, 0.3 malt extract e 0.3 yeast extract) medium, and incubated in a BOD incubator (Alfakit) for 48 h at 28°C. A suspension of each yeast was obtained in Milli-Q water. They were then standardized at 1×10^6 yeast cells.mL^{-1} according to the McFarland scale, and readings made in a spectrophotometer (Femto 800 XI) with an optical density range of

0.08 to 0.1, and wavelength of 625 nm. An aliquot of 1 ml of the standardized suspension was removed and inoculated into 250 ml conical flasks containing 83.3 ml of Chenin Blanc grape must. The samples were placed in a model TE-424 temperature controlled orbital shaker (Tecnal) at 100 rpm for 168 hours (7 days) in triplicate, at a temperature of 15°C. After fermentation, the musts were filtered through a Millipore membrane (0.22 µM pore) to carry out the analyses. The commercial yeast *S. cerevisiae* var. *bayanus* (Maurivin) was used as the control in the fermentation process.

Volatile compound analysis

The determinations of higher alcohols, esters and carboxylic acids were carried out according to Webber et al. (2014), as reported in the Reference Laboratory of Enology – LAREN (Caxias do Sul-RS, Brazil). A HP 6890 Agilent Technologies gas chromatograph (Palo Alto, USA) was used with a flame ionization detector (GC-FID). The compounds were identified by comparison with authentic standards from Sigma–Aldrich under the same chromatographic conditions.

Determination of the esters, alcohols and volatile acids

The samples of the fermented cell free must were subjected to three liquid/liquid extractions (4:2:2) with a mixture of diethyl ether/n-hexane (1:1). Aliquots of 2 ml of 3-octanol (40 mg.L^{-1}) and 2 ml of heptanoic acid (50 mg L^{-1}) were added to 50 ml of fermented Chenin Blanc grape must as internal standards, plus 0.3 ml of phosphoric acid (1:3). After decantation in a separation funnel, the organic phases were pooled, constituting the extract. A CP Inowax (30 m, 250 µm and 0.25 µm) capillary column was used and the sample injected (2.0 µL) in the splitless mode at 60 ml min^{-1} and 240°C. The carrier gas was hydrogen 5.0 at 2.0 ml min^{-1}, and the nitrogen as auxiliary gas at 37 ml min1. The oven temperature was 40°C for 5 min; followed by 40 to 230°C at 3°C $min.^{-1}$; and finally 230°C for 20 min. Combustion maintained with a synthetic air was used at an airflow of 350 mL/min and nitrogen at 35 mL.min^{-1}. The detector temperature was 230°C.

Determination of higher alcohols, acetaldehyde, ethyl acetate and methanol

After a simple distillation, 70 µL of a 4-methyl-2-pentanol solution (5 g L^{-1}, internal standard) were added to 5 ml samples of the fermented cell free must. A CPWax 57CB capillary column (60 m, 250 µm and 0.25 µm) was used, and the injection (1.0 µL) done in the split mode at 60 ml min^{-1} and 220°C. The carrier gas was hydrogen 5.0 at 2.0 ml min^{-1} and the nitrogen as auxiliary gas at 37 ml min1. The oven temperature was 40°C for 5 min, followed by 40 to 90°C at 3°C min^{-1}, 90 to 200°C at 10°C min^{-1}, and finally 200°C for 5 min. Combustion maintained with synthetic air was used at an airflow of 350 mL/min and nitrogen at 35 mL min^{-1} and the detector temperature was 230°C.

Odor activity value

The odor activity value (OAV) was calculated by dividing the mean concentration (n=3) of a compound by its odor threshold value (Guth, 1997; Peinado et al., 2004; Escudero et al., 2007; Li et al., 2008; King et al., 2008).

Sensory analyses

The typical aroma of the Chenin Blanc wine was evaluated, after fermentation using an acceptability test and purchasing intention. A total of 50 wine consumers were recruited based on their consumption frequency (at least once a week). The evaluations were carried out in individual booths under artificial daylight, a temperature between 22 and 24°C and air circulation. The samples were evaluated in a monadic way. Each consumer evaluated the nine beverage samples (A, B, C, D, E, F, G, H and I) in three sessions, according to an experimental design of complete balanced and randomized blocks. The same consumer panel took part in the three sessions.

The samples (50 ml) were served in tulip-shaped glasses covered with watch glasses, coded with random 3-digit numbers. A 9-cm non-structured hedonic scale was used in the acceptability test with extremes of "(1) disliked extremely" and "(9) liked extremely" (Stone and Sidel, 2004). The consumers also registered their purchasing intentions for each sample on the same score sheet, using a five-point attitude scale, with extremes of "(1) would definitely not buy" and "(5) would definitely buy" (Meilgaard et al., 2007).

The study plan was evaluated by the Ethics in Research Committee of the Federal University of Bahia-UFBA, resolution number 01 of 13/06/1988-CNS, presenting a favorable opinion for the development of the study (number 094/2009 and CEP record 102/2009).

Data analysis

The acceptance data were evaluated using a main effects ANOVA procedure, and the physicochemical data evaluated by a one-way ANOVA and Tukey test (5% significance level) using the STATISTICA software (version 8.0, StatSoft, Inc., Tulsa, OK, USA). The variability of the samples in relation to the volatile compounds was analyzed by Principal Component Analysis (PCA), carried out using the Pirouette 4.0 software (Infometrix, Washington, USA). The results for purchasing intention were presented as a percentage.

RESULTS AND DISCUSSION

Physicochemical characterization of the grape must and fermented grape musts

The Chenin Blanc grape must presented concentrations of SO_2, N_2, pH value, titratable acidity and °Brix of 60.0 mg L^{-1}, 150 mg L^{-1}, 3.1, 71.64 mEq L^{-1} (5,4 g L^{-1}) and 19.8, respectively. According to Ribéreua-Gayon et al. (2006) the Chenin Blanc grape must was in appropriate conditions to start fermentation.

The ethanol content of the fermented musts reached an average of 11.53% for *S. cerevisiae*, 10.56% for the yeast combination of *H. opuntiae*/*S. cerevisiae*, 7.30% for *H. guilliermondii*/*S. cerevisiae*, 8.53% for *I. terricola*/*S. cerevisiae* and 8.04% for *C. flavescens*/*S. cerevisiae*. No ethanol was produced in the grape musts fermented by the non-*Saccharomyces* yeast cultures (Table 1). The sugar consumed may have been used in a different metabolic routes giving rise to volatile compounds such as alcohols and acetaldehyde. In mixed culture fermentations, Moreira et al. (2008) observed antagonistic competition between the inoculated microorganisms, making the production of ethanol less efficient.

Table 1. Physicochemical characterization of Chenin Blanc must fermented at 15°C.

Fermented musts	Ethanol (%, v/v)	Reducing sugars (g L^{-1})	Titratable acidity (g L^{-1})	Volatile acidity (g L^{-1})
S. cerevisiae	11.53±0.01	1.88±0.02	5.99±0.02	0.21±0.01
H. opuntiae	-	160.02±0.02	3.58±0.01	0.14±0.03
H. guilliermondii	-	136.51±0.01	3.08±0.01	0.12±0.02
I. terricola	-	135.50±0.00	4.89±0.00	0.26±0.00
C. flavescens	-	154.02±0.00	5.97±0.00	0.27±0.00
H. opuntiae/*S. cerevisiae*	10.56±0.01	1.91±0.01	7.29±0.00	0.23±0.01
H.guilliermondii/*S. cerevisiae*	7.30±0.00	2.45±0.00	5.64±0.01	0.16±0.00
I. terricola/*S. cerevisiae*	8.53±0.01	3.13±0.02	5.56±0.01	0.12±0.02
C. flavescens/*S. cerevisiae*	8.04±0.00	1.72±0.01	6.29±0.01	0.30±0.00

All parameters are listed with their standard deviations (*n*=3).

The sugar concentrations were derived from the sugars present in the grapes, which were not metabolized during fermentation of the wine. As expected, the sugar was not completely consumed in the samples fermented by pure cultures of non-*Saccharomyces* yeasts, resulting in elevated residual sugar values of 160.02 g L^{-1} for fermentation by *H. opuntiae*, 136.51 g L^{-1} by *H. guilliermondii*, 135.50 g L^{-1} by *I. terricola* and 154.02 g L^{-1} by *C. flavescens* (Table 1). This demonstrates the higher efficiency in the use of the sugar present in the must in the fermentations carried out by mixed yeast cultures.

In relation to the titratable acidity, only the values found in the grape musts fermented by *H. opuntiae* and *H. guilliermondii* individually were below the minimum value (55 mEq L^{1}) set by Ribéreua-Gayon et al. (2006). Thus one can deduce that these yeasts (*H. opuntiae* and *H. guilliermondii*) were not good acid producers. In the present study the volatile acidity levels were between 0.12 g L^{-1} and 0.30 g L^{-1}, all conforming with Brazilian legislation (Brazil, 2005) which permits a maximum of 20 mEq L^{-1} volatile acidity (corrected) or 1.2 g L^{-1} acetic acid.

Analysis of volatile compounds

Table 2 shows the results obtained for the volatile components, expressed in mg L^{-1}, present in the Chenin Blanc grape musts fermented by different non-*Saccharomyces* yeasts, inoculated alone and in combination with *S. cerevisiae*.

The odor activity value (OAV) can be used to establish which compounds contributed to the aroma, since the OAV calculation depends on both the concentration determined and the odor threshold of a specific compound in a determined matrix. In order to be perceived by the human nose, the compound must have an odor activity value > 1 (Welke et al., 2014). The values obtained in this study are presented in Table 4.

Ethyl acetate was detected in higher concentrations, varying between 2.76 and 15.17 mg L^{-1}, in the grape musts fermented by *S. cerevisiae* and by *H. opuntiae*/*S. cerevisiae*, respectively (Table 2). At low levels (<50 mg L^{-1}) this compound may be adequate and add complexity to the flavor, whereas at high concentrations, it tends to produce an unpleasant aroma in the wine (Swiegers and Pretorius, 2005). Remarkably, the OAV value for this compound in grape musts fermented by *H. opuntiae*/*S. cerevisiae* was 1264.16 (Table 3).

Esters confer characteristic aromas on wines, even when present in low concentrations. Isoamyl acetate (banana and fruit aroma), ethyl hexanoate (apple aroma) and hexyl acetate (apple, pear, floral) are fairly influential in the aromatic composition of the majority of wines (Peinado et al., 2004). However, the OAV value for ethyl hexanoate was only significant for the aroma of the must fermented by *H. opuntiae*/*S. cerevisiae* (Table 4). Hexyl acetate, ethyl octanoate and ethyl decanoate were detected in low concentrations and did not contribute to the aromatic composition of the fermented grape must, with OAV values < 1 (Tables 3 and 4).

2-Phenylethyl acetate, described as an aroma with "rose" and "flowers" nuances in wines, is very important for the wine aroma composition, providing elegance to the beverage (Swiegers and Pretorius, 2005). This compound was not detected in the must fermented by *S. cerevisiae* alone, but it was detected in the other fermented musts at very low concentrations (Table 3), without influencing the aroma (Table 4). In their studies Viana et al. (2008) found that non-*Saccharomyces* yeasts showed high potential for the production of 2-phenylethyl acetate, and stressed that the co-inoculation of *Saccharomyces*/non-*Saccharomyces* yeast could be of interest.

High levels of volatile acids such as butyric acid and isobutyric acid are undesirable in wines, since they can decrease beverage acceptance (Nikolaou et al., 2006). High levels of these acids were detected in musts fermented by *C. flavescens* and *I. terrícola* (Table 3), negatively impacting the aroma. Thus it is understood that the use of these yeasts in wine production brings no

Table 2. Volatile composition of Chenin Blanc grape must fermented at 15°C.

Volatile compounds (mg L^{-1})	*S. cerevisiae*	*H. opuntiae*	*H. guilliermondii*	*I. terricola*	*C. flavescens*	*H. opuntiae*/*S. cerevisiae*	*H. guilliermondii*/*S. cerevisiae*	*I. terrícola*/*S. cerevisiae*	*C. flavescens*/*S. cerevisiae*
Esters									
Ethyl acetate	2.760^{d}	3.430^{d}	2.850^{d}	3.030^{d}	nd	15.170^{a}	6.900^{b}	5.670^{c}	7.550^{b}
Isoamyl acetate	nd	0.013^{c}	0.014^{c}	nd	nd	0.071^{a}	0.041^{b}	0.014^{c}	nd
Ethyl hexanoate	nd	nd	nd	nd	nd	0.040^{a}	nd	nd	nd
Hexyl acetate	0.064^{a}	0.063^{a}	0.062^{a}	0.056^{a}	0.057^{a}	0.061^{a}	0.061^{a}	0.062^{a}	0.053^{a}
Ethyl octanoate	0.088^{fe}	0.094^{de}	0.100^{dc}	0.084^{f}	0.091^{fe}	0.163^{a}	0.103^{c}	0.114^{b}	0.094^{de}
Ethyl decanoate	0.070^{b}	0.071^{b}	0.082^{a}	0.077^{ba}	0.074^{ba}	0.077^{ba}	0.071^{b}	0.068^{b}	0.074^{ba}
Phenylethyl acetate	nd	0.027^{d}	0.121^{a}	0.001^{e}	0.006^{ed}	0.005^{ed}	0.091^{b}	0.029^{d}	0.001^{e}
Total esters (C6-C10)	0.158	0.165	0.182	0.161	0.165	0.280	0.174	0.182	0.168
Acids									
Isobutyric acid	7.269^{d}	0.131^{g}	2.964^{e}	7.272^{d}	11.465^{b}	0.063^{g}	1.417^{f}	9.513^{c}	14.46^{a}
Butyric acid	0.188^{e}	1.917^{c}	0.836^{d}	1.932^{c}	2.931^{b}	0.157^{e}	0.443^{ed}	3.436^{a}	2.140^{c}
Isovaleric acid	0.067^{g}	2.580^{d}	1.390^{e}	3.476^{c}	5.427^{a}	0.066^{g}	0.681^{f}	5.647^{a}	4.414^{b}
Total acids (C4-C5)	7.524	4.628	5.190	12.680	19.823	0.286	2.541	18.596	21.014
Hexanoic acid	1.319^{b}	0.342^{g}	0.480^{f}	0.776^{e}	1.122^{c}	0.337^{g}	0.496^{f}	0.963^{d}	1.743^{a}
Octanoic acid	0.201^{e}	0.232^{d}	0.110^{g}	0.289^{c}	0.713^{a}	0.028^{h}	0.190^{e}	0.136^{f}	0.683^{b}
Decanoic acid	0.006^{b}	nd	nd	nd	0.012^{b}	nd	0.046^{b}	nd	0.295^{a}
Dodecanoic acid	0.119^{a}	0.116^{ba}	0.111^{bc}	0.109^{dc}	0.109^{dc}	0.106^{dc}	0.106^{dc}	0.106^{dc}	0.104^{d}
Total acids (C6-C12)	1.645	0.690	0.701	1.174	1.956	0.471	0.838	1.205	2.825
Alcohols									
Methanol	27.720^{c}	15.680^{d}	34.560^{a}	25.360^{c}	28.470^{bc}	17.470^{d}	34.440^{a}	23.340^{c}	33.370^{ba}
Hexanol	0.055^{e}	0.130^{d}	0.187^{c}	0.038^{e}	0.061^{e}	0.250^{b}	0.313^{a}	0.066^{e}	0.038^{e}
Cis-3-Hexen-1-ol	0.001^{ed}	0.022^{c}	0.042^{b}	nd	0.001^{ed}	0.042^{b}	0.086^{a}	0.007^{d}	nd
Trans-3-Hexen-1-ol	0.056^{bac}	0.056^{bac}	0.059^{ba}	0.017^{dc}	0.028^{bdc}	0.053^{bac}	0.080^{a}	0.005^{d}	nd
2-phenylethanol	0.247^{e}	0.967^{ba}	0.742^{c}	0.415^{d}	0.374^{ed}	1.126^{a}	0.924^{b}	0.611^{c}	0.333^{ed}
Higher alcohols									
1-propanol	3.070^{f}	14.600^{d}	7.440^{e}	5.280^{fe}	2.780^{f}	37.310^{b}	23.910^{c}	23.040^{c}	52.930^{a}
2-methyl-1-propanol	4.060^{dc}	24.900^{b}	26.160^{b}	3.710^{d}	7.330^{dc}	51.530^{a}	46.810^{a}	47.930^{a}	10.250^{c}
2-methyl-1-butanol	4.510^{f}	14.760^{d}	8.070^{e}	19.010^{c}	28.940^{ba}	4.430^{f}	5.920^{fe}	27.540^{b}	32.010^{a}
3-methyl-1-butanol	6.330^{h}	70.030^{d}	24.130^{f}	12.950^{g}	6.300^{h}	115.100^{b}	131.440^{a}	106.430^{c}	51.480^{e}
Total higher alcohols	17.97	124.29	65.80	40.95	45.35	208.37	208.08	204.94	146.67
Aldehydes									
Acetaldehyde	60.56^{a}	12.62^{d}	8.95^{e}	45.74^{b}	46.79^{b}	12.60^{d}	21.67^{c}	8.59^{e}	9.56^{ed}

Means followed by different letters in the same line are significantly different according to Tukey's test ($p \leq 0.05$). nd= not determined.

benefit or improvement in the aroma of the wine. The methanol levels in the fermented musts were within the limits established by Brazilian

Table 3. Volatile compounds identified with their respective odor thresholds and odor activity values (OAV*).

Volatile compounds	Odor threshold+ (mg L^{-1})	*S. cerevisiae*	*H. opuntiae*	*H. guilliermondii*	*I. terricola*	*C. flavescens*	*H. opuntiae*/*S. cerevisiae*	*H. guilliermondii*/*S. cerevisiae*	*I. terrícola*/*S. cerevisiae*	*C. flavescens*/*S. cerevisiae*
Esters										
Ethyl acetate	0.012[a]	230.0	285.83	237.50	252.50	nd	1264.16	575.0	472.50	629.16
Isoamyl acetate	0.160[a]	nd	<1	<1	nd	nd	<1	<1	<1	nd
Ethyl hexanoate	0.008[a]	nd	nd	nd	nd	nd	5	nd	nd	nd
Hexyl acetate	0.670[a]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Ethyl octanoate	0.580[a]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Ethyl decanoate	0.500[a]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Phenylethyl acetate	0.250[b]	nd	<1	<1	<1	<1	<1	<1	<1	<1
Acids										
Isobutyric acid	0.200[b]	36.345	<1	14.82	36.36	57.325	0.315	7.085	47.565	72.30
Butyric acid	3.0[b]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Isovaleric acid	3.0[b]	<1	<1	<1	1.16	1.81	<1	<1	1.88	1.47
Hexanoic acid	3.0[a]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Octanoic acid	0.010[a]	20.1	23,20	11.0	28.90	71.30	2.80	19.0	13.60	68.30
Decanoic acid	0.006[a]	1	nd	nd	nd	2	nd	7.66	nd	49.17
Dodecanoic acid	1.0[c]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Alcohols										
Methanol	Nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd
Hexanol	1.1[a]	<1	<1	<1	<1	<1	<1	<1	<1	<1
Cis-3-Hexen-1-ol	0.400[d]	<1	<1	<1	nd	<1	<1	<1	<1	Nd
Trans-3-Hexen-1-ol	0.400[b]	<1	<1	<1	<1	<1	<1	<1	<1	Nd
1-propanol	0.306[a]	10.03	47.71	24.31	17.25	9.08	121.93	78.14	75.29	172.97
2-methyl-1-propanol	0.075[a]	54.13	332.0	348.80	49.47	97.73	687.06	624.13	639.06	136.66
2-methyl-1-butanol	75.0[e]	<1	<1	<1	<1	<1	<1	<1	<1	<1
3-methyl-1-butanol	0.060[a]	105.5	1167.16	402.16	215.83	105.0	1918.33	2190.66	1773.83	858.0
2-phenylethanol	0.200[a]	1.23	4.83	3.71	2.07	1.87	5.63	4.62	3.055	1.665
Aldehydes										
Acetaldehyde	0.500[b]	121.12	25.24	17.9	91.48	93.58	25.20	43.34	17.18	19.12

*OAV= odor activity values; +Odor threshold determined as 9.5-12% (v/v) ethanol obtained from the literature: [a]Peinado et al. (2004); [b]Guth, 1997; [c] Li et al. (2008); [d]Escudero et al. (2007); [e]King et al. (2008); nd= not determined.

law, which establishes a maximum of 300 mg L^{-1} (Brazil, 2005). The methanol content in the fermented grape musts ranged from 17.47 mg L^{-1} to 34.56 (Table 3). Higher alcohols such as 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol belong to the group of active aroma compounds which form the aromatic basis of the wine.

The majority of higher alcohols present in wine are secondary products of the alcoholic fermentation, formed from the fermentation of sugars, or via the Ehrlich reaction or from amino

Table 4. Consumer acceptability and purchasing intention for the aroma of fermented Chenin Blanc grape must.

Variable		*S. cerevisiae*	*H. opuntiae*	*H. guilliermondii*	*I. terricola*	*C. flavescens*	*H. opuntiae*/ *S. cerevisiae*	*H. guilliermondii*/ *S. cerevisiae*	*I. terrícola*/ *S. cerevisiae*	*C. flavescens*/ *S. cerevisiae*
Acceptance mean	Hedonic score (consumers)	4.30^{ced}	7.30^{a}	5.38^{cb}	4.02^{ed}	2.74^{f}	7.22^{a}	5.86^{b}	5.16^{cbd}	3.34^{fe}
Consumer purchasing intention (%)	Definitely would buy	6	34	18	4	2	34	38	16	0
	Probably would buy	6	42	8	8	2	36	20	20	4
	Maybe buy/maybe not	26	16	42	22	8	20	6	28	14
	Probably would not buy	36	8	18	36	22	2	12	16	46
	Definitely would not buy	26	0	14	30	66	8	24	20	36

Means followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$).

acids present in the grapes (Ribéreua-Gayon et al., 2006).

1-Propanol occurred in all the musts, but in higher concentrations in the co-inoculated musts,where the concentrations were 37.31, 23.91 and 23.04 mg L^{-1} respectively for musts fermented by *H. opuntiae*/*S. cerevisiae*, *H. guilliermondii*/ *S. cerevisiae* and *I. terrícola*, respectively. The OAV values calculated were > 1 for all the fermented grape musts. According to Ribéreau-Gayon et al. (2006), the concentration of this compound should be from 10 to 50 mg L^{-1}, and values above this amount may contribute negatively to the aroma. This compound is described as having a ripe fruit aroma (Peinado et al., 2004). The must fermented by *C. flavescens*/*S. cerevisiae* presented a concentration slightly higher than the others, negatively influencing the aroma.

The production of 2-methyl-1-propanol and 3-methyl-1-butanol was high enough to positively increase the aroma in all the fermented grape musts, with OAV values > 1, especially in the co-inoculated musts, but the concentration of 2-methyl-1-butanol was not high enough, and thus its impact on the aroma was limited (Tables 3 and 4). Notably the must fermented by *H. guilliermondii*/*S. cerevisiae* showed the highest concentrations and OAV values for 2-methyl-1-propanol and 3-methyl-1-butanol. Total higher alcohols below 300 mg L^{-1}indicate quality and positively contribute to the wine aroma, since at higher concentrations they can mask the aroma and compromise all the sensory properties of the beverage (Escudero et al., 2007).

The musts fermented by *S. cerevisiae* in combination with non-*Saccharomyces* yeasts showed the highest total higher alcohols, and the musts fermented by *H. opuntiae*, *H. guilliermondii*, *I. terricola* and *C. flavescens* produced more total higher alcohols than *S. cerevisiae*. Thus it can be considered that the high levels found in the co-fermented musts referred specifically to the ability of the non-*Saccharomyces* yeasts to produce this class of alcohols (Table 3).

2-Phenylethyl alcohol plays an important role in the aroma of white wines when present in concentrations above the threshold of 0.200 mg L^{-1} (Peinado et al., 2004). In all the fermentations, the values found for 2-phenylethanol were above the threshold, and the odor activity values were > 1 (Table 4). Thus it effectively contributed to the aromatic composition of the musts analyzed.

The highest acetaldehyde concentrations (60.56 mg L^{-1}, Table 3) were found in the musts fermented by *S. cerevisiae*. In co-inoculation the production of acetaldehyde decreased, the metabolic pathway producing this compound possibly being diverted to balance the aroma of the fermented must. When present in high concentrations, acetaldehyde results in a "pungent" aroma, negatively affecting the wine aroma (Gurbuz et al., 2006).

The variability of the samples with respect to the total esters, total acids (C4-C5), total acids (C6-C12), total alcohols and acetaldehyde (Figure 1A and 1B) was determined. The first two PC accounted for 96% of the variability of the experimental data. As shown in Figure 1A, the total esters and total acids (C4-C5) were located high to the left side of PC1 and the total acids (C6-C12) to the right side of the same PC. The total alcohols and acetaldehyde projected into PC2, on the positive and negative sides, respectively. In Figure 1B, the samples F, H and C were located to the left of PC1 and the samples I, G and B to the right side. The samples E, D and A were located to the right of PC1, but also on the negative side of PC2. The total esters were the most important variable of PC1 with respect to explaining the variability of the samples. The total

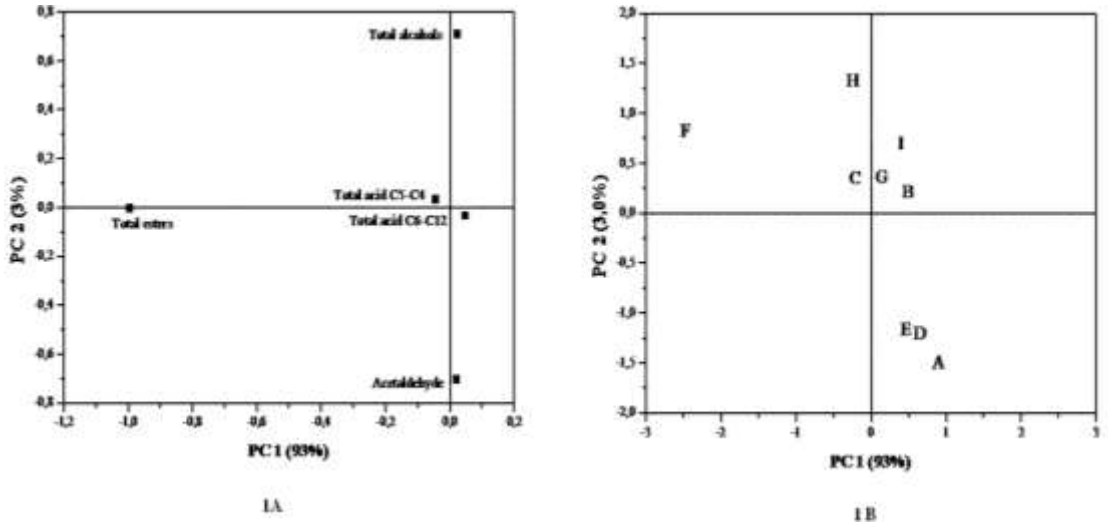

Figure 1. Variability of samples.

alcohols and acetaldehyde were important in discriminating the samples in groups H, I, G, B and C and the samples in groups E, D and A, respectively. Sample F was on the same side of the PC as the total esters, indicating that this volatile group wasimportant in distinguishing this sample over the others.

Consumer acceptance and purchasing intention

The Chenin Blanc grape musts fermented by *H. opuntiae* and combined with *S. cerevisiae* presented the highest acceptability means of 7.30 and 7.22, respectively, statistically different from the other fermented musts. The must fermented by *H. guilliermondii*/*S. cerevisiae* presented an acceptability mean of 5.86 and did not differ statistically from the must fermented by *H. guilliermondii* (5.38) (Table 4). The musts fermented by *H. opuntiae* and by the *H. opuntiae*/*S. cerevisiae* combination showed the highest percentages of purchasing intention, corresponding to the concepts “definitely would buy” and “probably would buy”, with values of 76 and 70%, respectively.

The must fermented by *S. cerevisiae* presented a lower acceptability than those fermented by *H. opuntiae*/*S. cerevisiae* and *H. guilliermondii/S. cerevisiae,* and also lower percentages for the concepts of “definitely would buy” and “probably would buy” in the purchasing intention test (12%). Similar results were found by Mamede et al. (2005) when evaluating the acceptability and purchasing intention of the aroma of Pinot noir and Chardonnay musts fermented at 15°C by *Pichia membranifaciens*, *Kloeckera apiculata*, *Candida valida* and *S. cerevisiae.* They observed that the Chardonnay must fermented by *S. cerevisiae* showed the lowest purchasing intention.

The fermentation carried out by *C. flavescens* presented the lowest acceptability mean (2.74), and did not differ statistically from the mean obtained by the combined fermentation with *C. flavescens*/*S. cerevisiae* (3.34). When associations are made between the sensory data and the quantified data/OAV for the volatile compounds, it is easier to understand the acceptability scores and determine the contribution of each of the yeasts to the aroma of the fermented must.

It can also be seen that the must fermented by *H. opuntiae*/*S. cerevisiae* presented the highest OAV values for compounds such as 2-methyl-1-propanol and ethyl acetate, which, in appropriate concentrations, contribute positively to the aromatic quality of the must.

The concentration of the total higher alcohols was within the limits which contribute positively to the aroma. 2-phenylethanol is an important volatile compound in wine quality, and was produced by *H. opuntiae* and *H. guilliermondii* in combination with *S. cerevisiae* in amounts that resulted in OAV values of 5.63 and 4.62, respectively. Under the same fermentation conditions, a low production of the volatile acids (C4-C5 and C6-C12) and acetaldehyde was observed, but the must fermented by *H. opuntiae/S. cerevisiae* achieved a greater acceptability score. Probably the absence of ethyl hexanoate in the must fermented by *H. guilliermondii/ S. cerevisiae* and the high concentrations of octanoic acid and acetaldehyde contributed negatively to the acceptability of this fermented must.

A statistical analysis of pearson’s correlation between the acceptability of the fermented musts and the five classes of volatile compounds was carried out. The results showed a strong negative correlation between the acceptability of the must fermented by *C. flavescens*/*S. cerevisiae* and the total acids (C4-C5) (r = - 0.81, p = 0.08) and total acids (C6-C12) (r = - 0.83, p = 0.05). Thus, the low mean for acceptability found in the must fermented by *C. flavescens*/*S. cerevisiae* may be associated with the presence of high concentrations of

volatile acids negatively affecting the wine aroma.

Conclusions

The combined use of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* var. *bayanus* in the fermentation of grape musts showed that not all the combinations resulted in good production of volatiles with a consequent positive contribution to the aroma, with the exception of *H. opuntiae* and *H. guilliermondii*. The must fermented by *H. opuntiae*/*Saccharomyces cerevisiae* showed greater acceptability and purchasing intention, probably due to the production of volatile compounds in amounts sufficient to positively enhance the wine aroma, highlighting total esters (ethyl acetate, ethyl hexanoate), total alcohols and 2-phenylethanol.

The low acceptability of the must fermented by *C. flavescens*/*S. cerevisiae* could have been due to increments of undesirable components that contributed negatively to the wine aroma. It was therefore suggested that the yeast species *H. opuntiae and H. guilliermondii* in combination with *Saccharomyces cerevisiae* showed potential to positively impact the wine aroma. A more comprehensive survey of the use of non-*Saccharomyces* yeasts in wine production is feasible, considering their contribution to the aroma, seeking innovations in winemaking and in the types of wine.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to Vinibrasil S/A for their assistance, and for donating the Chenin Blanc grape must. This study was financially supported by fellowships received from FAPESB and CNPq.

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Vol. 11(28), pp. 2443-2449, 14 July, 2016
DOI: 10.5897/AJAR2016.11193
Article Number: DCBD49B59381
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Drippers used in vinasse application: Uniformity and obstruction

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Received 5 May, 2016; Accepted 8 June, 2016

In sugar cane crop fertigation management systems, about 500 m^3 ha^{-1} of diluted vinasse is applied annually by cycle, promoting nutrient recycling and supply to the soil, especially that of potassium (K). With the increase of sugar cane areas with subsurface drip irrigation, hydraulic characterization related to vinasse intermittent application is important, in order to verify uniformity flow in drippers. The aim of this study was to hydraulically characterize performance and orifice position of different dripline models subjected to intermittent vinasse application, in order to verify flow disorders. The experiment conducted was in a test bench in the Biosystem Engineering Department - ESALQ/USP. The experimental design was completely randomized, with 10 repetitions. Four dripline models (Hydrolite HY 0.65, Hydrolite HY 1.0, Drip Net PC DN 0.6 and Drip Net PC DN 1.6) and 13 consecutive weekly assessment periods were used as treatments. At the same time, dripline orifice position influence (upward and downward) was assessed in two (Hydrolite HY 0.65 and Drip Net PC DN 0.6) of the four dripline models in the respective periods (2x2x13 factorial = Two models, two orifice positions and 13 periods/weeks). Ten drippers from each model were randomly selected, in order to monitor flow. Vinasse application was conducted daily during four hours, with dripline product rest for 20 h, and flow reading at every 168 test h (weekly). HY models (0.65 L h^{-1}) with drippers positioned upwards and downwards, and upwards HY (1.0 L h^{-1}) and DN (1.6 L h^{-1}) showed flow variation coefficient (VC) lower than 5%, which is rated as excellent. The other models showed VC lower than 10%, which was classified as good, enabling vinasse use in the 2:1 concentration in driplines.

Key words: Flow variation coefficient. clogging. fertigation. dripping. potassium. vinasse.

INTRODUCTION

Brazil produced about 27 million cubic meters of ethanol from sugarcane (*Saccharun* spp) in the 2010/2011 season, generating approximately 337 billion cubic meters of vinasse as a byproduct (Unica, 2012). Sugar cane fertigation with vinasse is used by most plants, as it partly or totally replaces potassium fertilization for crops,

providing increases of this nutrient in the soil and in the plant shoot (Brito et al., 2005; Silva et al., 2014). Vinasse fertigation is an economically viable alternative to the producer and a strategic alternative for the country, since 90% of potassium fertilizers are imported (Anda, 2012).

Vinasse is an ethanol distillation byproduct with approximately 97% water. The solid fraction consists mainly of organic matter and minerals, in which potassium (K) is the most abundant, accounting for about 20% of the elements. This element is taken as the limiting factor to define the vinasse dose to be applied per hectare in soils cultivated with sugar cane (Marques, 2006).

Vinasse can replace some of mineral fertilizer nutrients. Its *in natura* use through fertigation has positive effects on plantation yield and longevity due to sugar production increase per hectare, which is more pronounced as the number of cuts increases (Có Júnior et al., 2008; Barbosa et al., 2013).

Vinasse composition knowledge is important for guidance regarding the dose to be applied in the soil, in order to meet legislative requirements through proper application in agricultural areas. Application cost particularly depends on the system, which should be related to characteristics and needs of each production unit.

In a survey conducted in plants from the state of São Paulo, it was observed that vinasse has large chemical composition variability. However, in general, organic matter amounts were high, followed by potassium, sulfur, calcium, nitrogen, magnesium and phosphorus. In addition, high oxygen chemical and biochemical demand was also observed (Elias and Nakahodo, 1995).

Glycerol, lactic acid, ethanol, acetic acid, fructose, glucose, sucrose, galactose, acetate, oxalate, citrate and others are the organic components found in the highest ratios in sugar cane vinasse (Decloux and Bories, 2002; Parnaudeau, 2008; Doelsch et al., 2009). Vinasse may also contain phenolic compounds, cellulose and hemicellulose (Benke et al., 1998).

In sugar cane fertigation management systems, around 500 m^3 ha^{-1} vinasse is applied per crop cycle (annual). Application is carried out via self-propelled irrigation systems equipped with a large sprinkler, which has a flow rate of m^3 h^{-1} (Silva, 2006).

Tasso et al. (2007), while studying different residue types, sewage sludge + KCl and vinasse + urea doses regarding ratoon cane total recoverable sugar (TRS) changes, found higher TRS amounts in the treatments that received two residues that were not associated, compared to treatments that received the two residues in association.

From an environmental point of view, residue application in the soil, in a total area, causes its dilution, suggesting this application form. On the other hand, when considering residue use in agriculture, nutrient use and the beneficial effects that such products could eventually promote in the soil are of primary interest. Thus, through these principles, it can be stated that the application has advantages regarding residue agricultural efficiency as fertilizer (Có Júnior et al., 2008).

However, although studies show vinasse benefits in sugar cane and other crops (Barbosa et al., 2013; Silva et al., 2014), vinasse distribution uniformity in the area is necessary to avoid soil saturation, especially of K, as well as to provide nutrients in the product in appropriate quantities and under the law (CETESB, 2005).

Thus, this study aimed to hydraulically characterize four dripper models in relation to vinasse intermittent application, in order to check flow disorders.

MATERIALS AND METHODS

The study was conducted in a hydraulic test bench installed in the greenhouse from the Biosystem Engineering Department of ESALQ/University of São Paulo, located in Piracicaba, SP. Test benches had dimensions of 13 m x 1.5 m (Figure 1A).

The experimental design was completely randomized, with 10 repetitions. On a sideline, 10 drippers were randomly selected for repetition composition. Four dripline models (Hydrolite HY 0.65, Hydrolite HY 1.0, Drip Net PC DN 0.6 and Drip Net PC DN 1.6) and 13 consecutive weekly assessment periods were used as treatments. At the same time, drip line orifice position influence (upward and downward) was assessed in two (Hydrolite HY 0.65 and Drip Net PC DN 0.6) of the four drip line models in the respective periods (2x2x13 factorial = two models, two orifice positions and 13 periods/weeks). The flow of all drippers was collected in a graduated container (Figure 1B). Vinasse application was performed daily for 4 h, with the dripline product rest for 20 h and flow reading at every 168 test h (weekly).

Bench pressurization was conducted by a KSB, Hydrobloc P1000 model pump. The system was connected to a PVC tank with 500 L capacity equipped with a mechanical agitator, in order to homogenize vinasse. Therefore, organic matter decantation from the bottom of the reservoir was avoided. Bench operation regarding the start and stop time was conducted with the aid of a digital controller, which strictly obeyed application schedules.

A pressure plug was installed before the input that connects driplines, allowing for pressure adjustment and, if necessary, adjustment to the pre-established pressure of 150 kPa. In order to measure working pressure, a digital manometer with 0-700 kPa reading range was used. The list of drip lines that were used in the study, and their technical features, is found in Table 1.

Emitter spacing in the drip line, as well as flow rates and pipe diameters, were adopted according to technical recommendation. However, dripper separation for bench testing was not conducted, avoiding differences related to amendments and emitter proximity influence by providing similar conditions to the field. The vinasse used in the study was obtained from Costa Pinto plant, COSAN Group, located in Piracicaba, SP. Vinasse was applied in the 2:1 concentration. Previously, vinasse chemical analysis was

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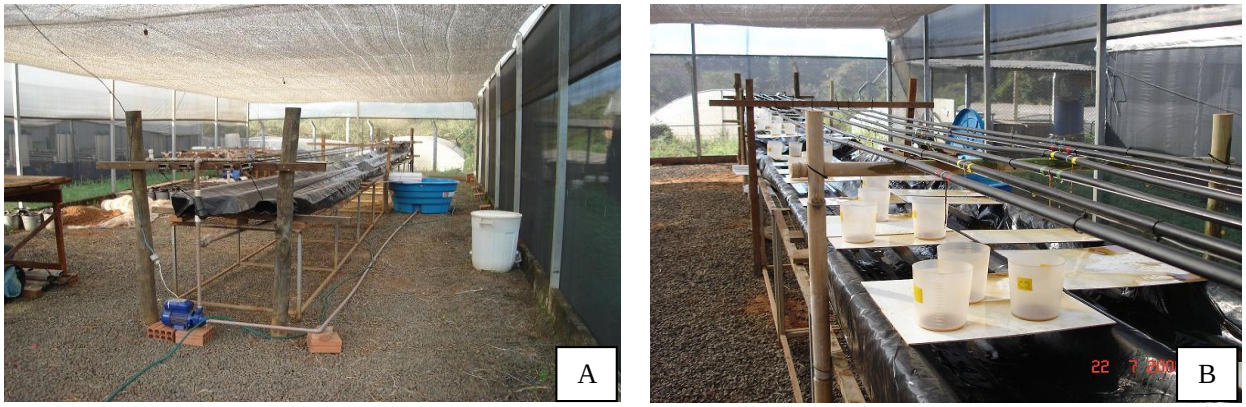

Figure 1. Test bench (A), reservoir and mechanical stirrer (B), flow measurement collectors (C) and vinasse application residues held in the test bench trough.

Table 1. Technical characteristics of drip lines models used in the study, as follows: flow (Q), nominal diameter (Ø N) and operating pressure (PS).

Manufacturer	Model	Identification	Q	Ø N	PS
			L h^{-1}	mm	kPa
Plastro	Hydrolite	HY 0.65	0.65	16	100-350
Plastro	Hydrolite	HY 1.0	1.0	16	100-400
Netafim	Drip Net PC	DN 0.6	0.6	17	50-400
Netafim	Drip Net PC	DN 1.6	1.6	16	100-350

Source: Manufacturer's catalogs.

conducted according to the methodology proposed by Malavolta et al. (1989), resulting in the following values: pH=4.60; Electric conductivity (EC)=8.27 dS m^{-1}; K=3.195 mg L^{-1}; Ca=523 mg L^{-1}; Na=59 mg L^{-1}; NO_3^-=426 mg L^{-1} and S=890.37 mg L^{-1}.

The vinasse was obtained at the plant fortnightly and applied to the test bench using a trough system that captured the product after it passed the driplines. Afterwards, the product was routed back to the collection tank, thus, keeping a closed circuit. A 100 mesh disc filter was used and a ¾' shut-off valve was coupled in the main line to control the application. Driplines were joined at the beginning and at the end by ¾' shut-off valves and PVC connections supported by devices that allowed for maintaining 0.15 m spacing between horizontal lines and 0.50 m spacing above the trough system.

Firstly, 36 h irrigation was conducted with the four dripper models under study, where two dripper models (Hydrolite HY 0.65 and Drip Net PC DN 0.6) had drip lines with orifices downwards and upwards. The other two models (Hydrolite HY 1.0 and Drip Net PC DN 1.6) were installed on the test bench with orifices faced only upwards, thus, consisting of an irrigation system with six driplines. Application was conducted daily at an interval of 4 h with the product resting on the line for 20 h, and pressure was measured during each application. A certified precision scale (OHAUS) with an accuracy of 0.01 g was used to weigh each container with the applied solution. Flow reading procedure for each dripper consisted of system pressurization (150 kPa), plastic container positioning (1 L) under their respective drippers with five-second delays, sequential recipient withdrawal after 5 min with 5 s delays, weighing (gravimetric method) and data tabulation, with flow values expressed in L h^{-1} (Figure 1).

After the data was tabulated, mean flow, flow variation coefficient and water distribution uniformity were calculated using equations 1 to 3.

$$q = \frac{P}{1000t}\ 60 \quad (1)$$

$$CV_q = \frac{s}{q}100 \quad (2)$$

$$UD = \frac{q_{25\%}}{q}\ 100 \quad (3)$$

where: P - collected solution weight (vinasse), g; t - collection time, min; q - mean flow, L h^{-1}; CV_q – flow variation coefficient,%; *s* - dripper mean flow standard deviation, L h^{-1}; UD - water distribution uniformity coefficient,%; and - mean flow of the 25% lowest values; L h^{-1}.

In order to calculate the maximum vinasse dosage to be applied to agricultural soils under sugar cane crops, information described in the P4.231 standard (Vinasse - Criteria and Procedures for Agricultural Soil Application) by the Environmental Sanitation Technology Company (CETESB, 2005) should be observed. Among recommendations, this standard determines that the vinasse dose to be applied should be based on K^+ content, as

shown in Equation 4.

$$\text{Vinasse } (m^3\ ha^{-1}) = \frac{[(0.05\,x\,CTC - KS)\,x\,3744 + 185]}{Kvi} \quad (4)$$

where: 0.05 = 5% CEC; CTC = cation exchange capacity, expressed in $cmol_c\ dm^{-3}$ at pH 7.0; Ks = potassium concentration in the soil, expressed in $cmol_c\ dm^{-3}$ (0.8 m depth); 3744 = Constant to transform fertility analysis results, expressed in $cmol_c\ dm^{-3}$, for K^+ kg in the volume of one hectare per 0.80 m depth; 185 = K^+ kg extracted from the crop through cutting; Kvi = vinasse K^+ concentration, expressed in K_2O kg m^{-3}.

For potassium application rate analysis related to its availability in vinasse, the recommendation proposed in the P 4.230 standard was used (CETESB, 1999). In order to check the recommended amount of nitrogen or potassium for the crop, recommendations by Embrapa Cerrados for sugar cane fertilization should be consulted (Sousa and Lobato, 2004).

Data were submitted to variance analysis by "F" test and, when significant to the variables, means were compared by Tukey's test at 5% probability. For vinasse application periods, regression analysis was carried out. In order to conduct analysis, SAS program (1999) was used by GLM (Generalized Linear Model) procedure.

RESULTS AND DISCUSSION

Variance analysis showed significant effects ($p < 0.01$) for dripper model, assessment time and interaction between the two factors (Table 2). The variation coefficient was 3.60%, which is considered low (Pimentel-Gomes, 1990), showing good data accuracy.

From a practical point of view, emitter mean flow can be considered a good parameter to assess changes regarding emitter proper functioning, which may be caused both by clogging problems (Cararo et al., 2006; Ribeiro and Paterniani, 2008) or by damage to the emitter internal structure caused by chemical action (Souza et al., 2006; Coelho and Teixeira, 2009), hindering flow uniformity (Frizzone et al., 1998).

It was observed in Table 3 that the different models under study, regardless of dripper orifice positioning, showed no significant flow reduction due to vinasse application. This was probably due to vinasse acid constitution and its fluidity (particulate matter), which prevented its sedimentation in the dripper pre-filter chamber, maze and

orifice, as was also observed by Lelis Neto (2012).

Therefore, observing other aspects, such as the chemical characteristic of the applied product and the solution volume to which the emitter was exposed become important, as they may contribute to clogging (Lelis Neto, 2012) and application non-uniformity (Frizzone et at., 1998) along with the water passage orifice diameter.

The Hydrolite model, with the exceptions of the second (168 h) and seventh (1008 h) assessment weeks, showed higher flows than the Drip Net model until the ninth assessment week. In the last three weeks, the opposite behavior occurred, that is, the Drip Net model showed higher flow than Hydrolite.

Table 2. Variance analysis of dripline models recommended for vinasse subsurface application in sugar cane related to time.

Variation source	DF	Mean square
Model (M)	3	26.976**
Assessment (A)	12	0.031**
M x A interaction	36	0.014**
Residue	468	0.001**
CV (%)	-	3.60

Regarding orifice position, downwards orifice flow only occurred in the seventh and the ninth weeks. Meanwhile, the upwards orifice had higher flow in the tenth assessment week. However, in periods with significant differences, except for the first week, in which the Hydrolite model showed a 14.8% higher flow than Drip Net, flows did not differ much, with total flow values lower than 8.5%.

It was observed in Table 4 that, in the HY model, the applied vinasse volume was the highest among all dripper models tested models. However, this value was lower than the volume generally used in the field, which is about 500 $m^3\ ha^{-1}$ (Lelis Neto, 2012), contributing to clogging non-occurrence for this drip line during the study period.

There are different emitter classifications regarding flow uniformity, of which the Abnt (1986) standard may be cited, which classifies flow uniformity with CVF up to 10% as good. It was observed in Figure 2 that, for the different dripline models under study, regardless of dripper orifice position (downwards or upwards), there were no CV values (%) higher than 10%, allowing for classifying them as well, according to Abnt (1986), and as adequate, according to Asae (1999).

Except for the DN model (0.6 L h^{-1}) with drippers positioned downwards, all others showed water distribution uniformity values higher than 90% throughout the experiment (Figure 3).

Conclusion

All drippers evaluated did not undergo significant flow reduction, and were classified as little sensitive to clogging by vinasse application. Flow values low variation indicated that, regardless of dripper arrangement (downwards or upwards), they were classified as good (Abnt, 1986) and appropriate (Asae, 1999).

Conflict of interests

The authors have not declared any conflict of interests.

Table 3. Mean flow (q_m) of drippers assessed during the test period, expressed in L h^{-1}, and mean tests.

Time	Dripline models					
	DN 0.6	DN 0.6	HY 0.65	HY 0.65	HY 1.0	DN 1.6
Total (h)	Dripper orifice positioning					
	Downwards	Upwards	Downwards	Upwards	Downwards	Upwards
0	0.69abcA	0.70bcA	0.82aA	0.80aA	1.27a	1.65b
168	0.67bcA	0.66cdA	0.67cdeA	0.65deA	1.07bcd	1.62bc
336	0.65bcA	0.66cdA	0.68cdeA	0.67cdeA	1.08bcd	1.64bc
504	0.68abcA	0.66cdA	0.68cdeA	0.68cdA	1.08bcd	1.62bc
672	0.66bcA	0.65cdA	0.72bA	0.72bA	1.11b	1.56c
840	0.64cA	0.64dA	0.69bcdA	0.70bcA	1.08bcd	1.64bc
1008	0.70abcA	0.66cdA	0.70bcdA	0.68cdA	1.08bcd	1.65b
1176	0.68abcA	0.66cdA	0.70bcdA	0.69bcdA	1.10b	1.66ab
1344	0.67bcA	0.66cdA	0.71bcA	0.66cdeB	1.09bc	1.67ab
1512	0.65bcA	0.68bcdA	0.66deB	0.69bcdA	1.05cd	1.60bc
1680	0.72abA	0.71abA	0.65eB	0.68cdA	1.03d	1.68ab
1848	0.74aA	0.75aA	0.68cdeA	0.67cdeA	1.08bcd	1.74a
2016	0.69abcA	0.69bcdA	0.67cdeA	0.64eA	1.05cd	1.66ab

*Means with the same lowercase letter in the column were not significant by Tukey's test at 5% probability. ** Means with the same capital letter in the line (Downwards versus upwards), within their respective model, were not significant by Tukey's test at 5% probability.

Table 4. Vinasse volume applied per hectare for the different dripper models under study.

Model	Spacing between drippers (m)	Total no. of drippers ha^{-1}	Dripper flow $^{-1}$ (L h^{-1})	Vinasse volume m^3 ha^{-1}
DN	0.5	11111	0.60	26.67
DN	0.5	11111	1.60	71.11
HY	0.15	37037	0.65	96.30
HY	0.15	37037	1.00	148.15

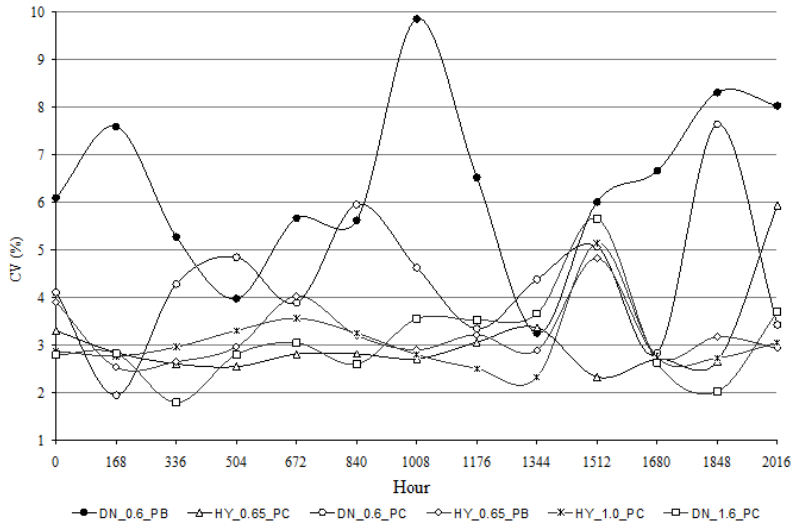

Figure 2. Flow variation coefficient for dripper models under study.

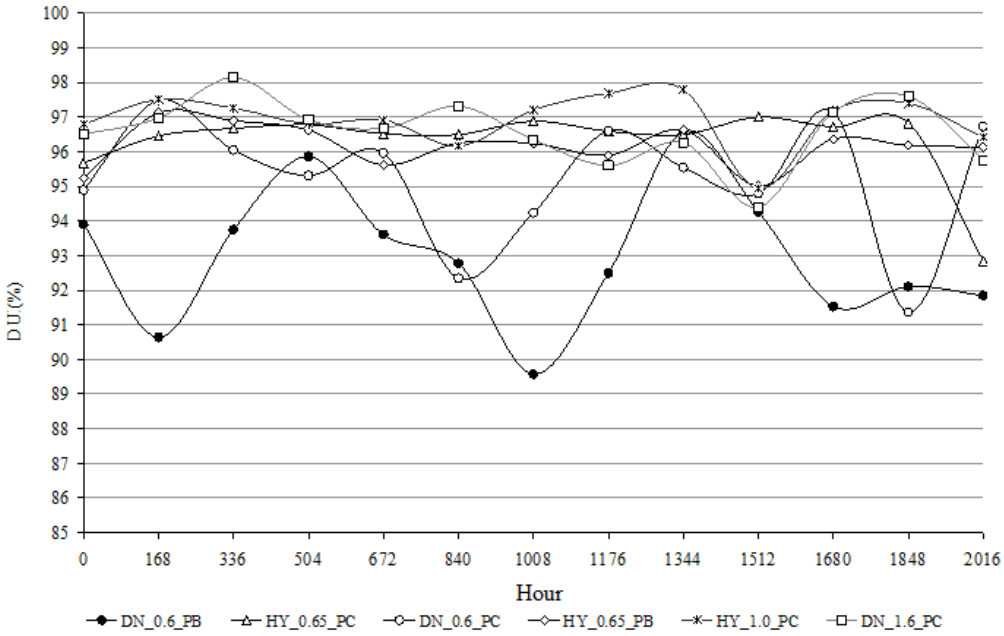

Figure 3. Water distribution uniformity for dripper models under study.

ACKNOWLEDGMENT

The authors sincere thanks go to the Ministry of Science and Technology (MCT), to the National Council for Scientific and Technological Development (CNPq), to the Higher Education Personnel Improvement Coordination (CAPES), to the São Paulo State Research Support Foundation (FAPESP), to the Goiás State Research Support Foundation (FAPEG) and to FINEP for the financial support to this research.

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Vol. 11(28), pp. 2450-2455, 14 July, 2016
DOI: 10.5897/AJAR2016.10883
Article Number: 633891A59387
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Growth of *Cordia trichotoma* seedlings in different sizes of recipients and doses of fertilizer

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Received 9 February, 2016; Accepted 23 April, 2016

This aim of this work was to evaluate the initial growth of *Cordia trichotoma* seedlings, submitted to different sizes of recipients and doses of controlled-release fertilizer (CRF). This experiment was performed in a greenhouse, and evaluation was done in factorial scheme using two volumes of recipients (110 and 180 cm^3) combined with five doses of CRF (0 (controlled); 2.5; 5.0 and 7.5 g L^{-1} de substrate). The experimental design was completely random with four repetitions each evaluated in factorial scheme. Significant interaction was observed between the size of the recipient and the doses of CRF for most analyzed variables. It indicated that, the quantity of fertilizer to be used depends on the size of the recipient, emphasizing that in the conditions in which the seedlings were produced; the minor volume of substrate does not cause restrictions on growth. It is perceived that most evaluated parameters need higher doses than the maximum provided, in at least one of the recipients, demonstrating elevated nutritional requirement of the species during the nursery phase. The recipient of 110 cm³, combined with the dose of 7.5 g L^{-1} of CRF is recommended because of the economy of substrate, nursery space and practicality of transportation.

Key words: Native species, controlled-release fertilizer, louro-pardo, seedling production.

INTRODUCTION

Studies related to the performance of tropical tree species with ecological and commercial potential are incipient, mainly regarding the inherent characteristics of the processes of seedling production. Among these tree species is *Cordia trichotoma* (Vell.) Arrab. Ex Steud. (louro-pardo), recognized for its timber quality, easy workability and multiplicity of use, which gives it high aggregated commercial value, it has wide natural occurrence in tropical and subtropical forests in Argentina, Bolivia, Brazil, Paraguay and Uruguay (Carvalho, 2003).

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The tree presents high survival rate and growth in planting (Salvadori et al., 2013), and it can be used in reforestation geared towards the reestablishment of altered areas (Carvalho, 2003) and for commercial purposes (Scheeren et al., 2002).

In this sense, considering the potential of *C. trichotoma* in environmental recovery, as well as its economic importance due to wood quality and volume growth, it becomes essential to carry out studies, focusing on initial process of seedling production which will provide high survival and growth after planting.

In the seedlings production of arboreal species in nursery, the growth characteristics of the various species which may be partially controlled through the use of appropriate inputs should take into account. In the production of forest seedlings, the main factors that influence the final quality of seedlings, are based on the kind and the size of recipient (South et al., 2005; José et al., 2005) and the basis fertilization (Jacobs and Landis, 2009; Rossa et al., 2015). The dimensions of the recipient can influence several aspects, because they control the quantity of water and nutrients available for the seedling growth (Lana et al., 2010; Brachtvogel and Malavasi, 2010). If the substrate does not provide sufficient quantities of the mineral elements demanded by the plants, the fertilization in turn has to serve this nutritional demand, allowing the adequate growth and development (Landis, 1990; Rossa et al., 2011).

Among the fertilizers used currently are the ones with controlled- release (CRF), mainly, because of its peculiarity of providing the nutrients slowly to the plants, in a synchronized way, balancing the demand with the availability in the substrate, and maintaining constantly, the levels of the essential elements to the seedlings during the growth period (Landis and Dumroese, 2009; José et al., 2009). Besides, the release rates are adjusted by the manufacturer, according to the thickness and the nature of the material (coating), and the variable duration is 3 to 18 months (Valeri and Corradini, 2005; Landis, 2009; Landis and Dumroese, 2009). However, because of the high cost the FLC, adequacy of doses to production systems becomes necessary to optimize the cost-benefit ratio (Rossa et al., 2013).

Thus, knowledge of substrate volume associated with mineral nutrition used in the production of seedlings in the nursery represents a strategy to achieve seedlings in less time and cost with minimum standard of quality capable of providing adequate growth of *C. trichotoma*. Therefore, the aim of this work is to evaluate the seedling growth of the species, submitted to different sizes of recipient and doses of controlled-release fertilizer.

MATERIALS AND METHODS

The study was conducted in a greenhouse located at (29°43'14,3" S and 53°43'17,5" O) in Universidade Federal de Santa Maria (UFSM), Santa Maria, Brazil. The climate of the region, according to the Köppen classification, is of Cfa fundamental type, humid subtropical, characterized by average temperature of between -3 and 18°C in the coldest month, and higher than 22°C in the hottest month, with an annual average precipitation of 1,769 mm (Alvares et al., 2013).

The seeds used in the work came from diaspores of *Cordia trichotoma*, collected from 14 seed trees located in fragments of the Decidual Seasonal Forest (29º45'30,3" S and 53º34'43,9" O), in the municipality of Santa Maria, Brazil. After the collection, the next stage was extraction and processing, in which the remaining petals were removed to form a seed lot.

The treatments consisted the following: two sizes of recipients, containers of 110 cm^3 (6 splines, internal diameter of 35 mm and height of 13.5 cm) and of 180 cm^3 (8 splines, internal diameter of 52 mm and height of 13 cm); and five doses of controlled-release fertilizer - CRF (Osmocote® of NPK formulation (15-09-12), in the doses of 0 (controlled); 2.5; 5.0 and 7.5 g of CRF L^{-1} of substrate. For seedling production, the commercial substrate peat based on *Sphagnum* and vermiculite, plus 20% of carbonized rice husk (CRH) was used.

The sowing was performed directly in the recipients (containers) with five seeds in each; the containers were arranged in trays, which were taken to the greenhouse. The seedling first emerged on the 60^{th} day after sowing, then, thinning was performed to eliminate the excess seedlings, leaving just one seedling per recipient. On the 210^{th} day after sowing, the seedlings were evaluated using the following morphological variables: height (H), stem diameter (SD), relation H/SD, shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM).

The height of the seedling was measured from the stem to the apical bud, using a graduated scale (millimeters). The stem diameter was measured with a digital caliper (precision of 0.001 mm). To evaluate SDM, RDM and TDM, the seedlings were cut and separated in shoot and root part. The root part containing the substrate was washed in running water in sieves with mesh size of 1mm. Samples of both the roots and the shoot part were then placed in Kraft paper bags, identified and submitted for drying in kiln with a temperature of 70°C until they had constant weight. After these, they were weighed on an analytical balance (0.001 g) in order to obtain dry mass.

The experiment was a completely randomized (DCR) design of 2x5 factorial scheme (two sizes of recipients and five doses of controlled-release fertilizer), consisting of 10 treatments. Each treatment was represented by four repetitions of 24 plants.

After the normality of the residues has been confirmed, and the variance homogeneity done with the Shapiro-Wilk and Bartlett tests, respectively, through the Action software (TEAM ESTATCAMP, 2014), variance analysis (ANOVA) was performed. When interactions were significant, they were divided and the averages were compared by the Tukey test and/or the polynomial regression (α 0.05). The statistical analysis of the variables used was made with the following statistical model for the factor analysis:

$$Y_{ijk} = \mu + a_i + d_j + (ad)_{ij} + e_{ijk}.$$

Where Yik = observation of the i factor in the level j, k in the repetition; μ = general average; ai = size of the container; dj = dose of the controlled release fertilizer; (D) ij = effect of interaction and eijk. = Error associated to each observation.

Statistical analysis

For the analysis, the SISVAR statistical package was used (FERREIRA, 2011). Pearson correlation was performed to

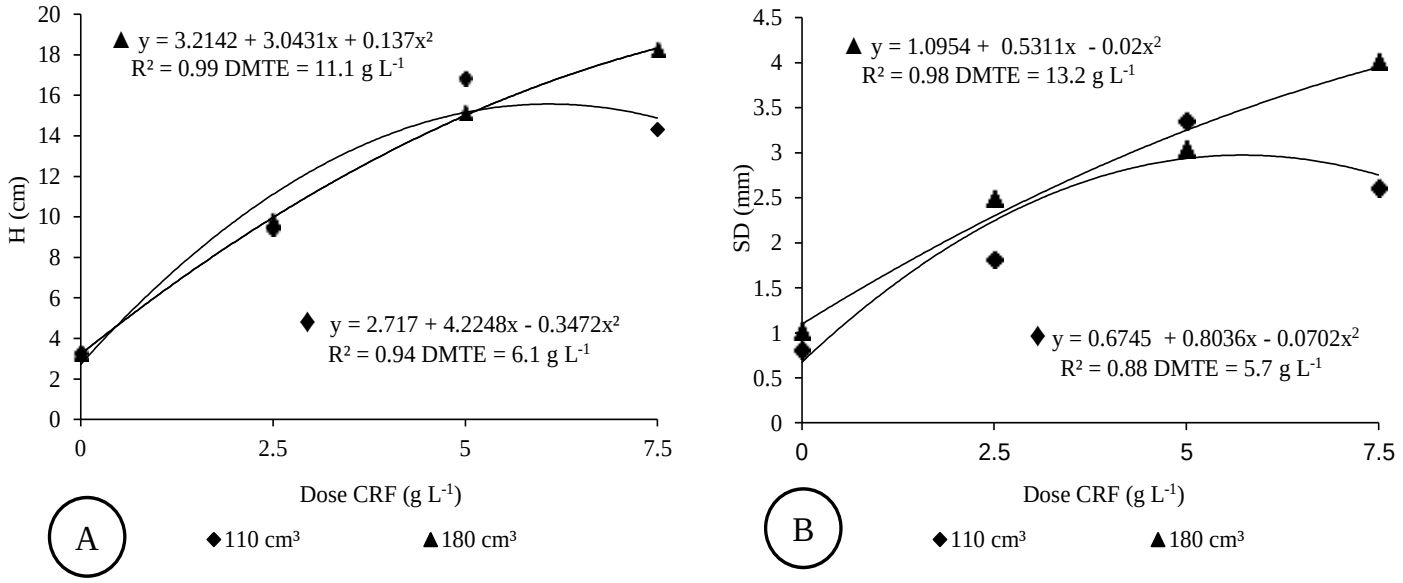

Figure 1. A: Height (H) and B: Stem diameter (SD) of seedlings of *Cordia trichotoma* as a function of different sizes of recipients and doses of controlled-release fertilizer (CRF) on the 210th day after sowing. DMTE - Dose of maximum technical efficiency.

complement the interaction among traits.

RESULTS AND DISCUSSION

By two hundred and ten days after sowing, it was observed that the height (H) of the *Cordia trichotoma* seedlings was influenced ($p<0, 05$) by the size of the recipient and the dosage of controlled-release fertilizer (CRF) used. (Figure 1A).

When the doses of maximum technical efficiency (DMTE) was calculated, it was observed that the height of the recipient in 110 cm^3 with 6.1 g L^{-1} of CRF was 15.6 cm, while the height of the recipient in 180 cm^3 with 7.5 g L^{-1} of CRF was 18.3 cm, which was the maximum height observed. This shows that the H variable responds positively when submitted to higher doses of fertilization (Figure 1A). Similar results were obtained by Rossa et al. (2013) in seedlings of *Schinus terebinthifolius* Raddi (aroeira-vermelha), with recipient of the same capacity. This result can be justified by the fact that the volume capacity of the recipient is greater and thus allows appropriate development of the root system before theapplication of the substrate and fertilizer to the seedlings (Lopes et al., 2005; Abreu et al., 2015).

The seedlings produced in the recipients of 110 cm^3, demonstrated a greater growth in stem diameter (SD) when 5.7 g L^{-1} of CRF (DMTE) was used (Figure 1B). This suggests that with this dosage, the seedlings will have greater survivability in the field, since according to Brisset et al. (1991); SD is an appropriate parameter, to evaluate the quality of the seedlings, due to the fact that it positively correlates with the root growth.

In the recipient of 180 cm^3 the dosage of 7.5 g L^{-1} presented result higher than that of other doses, (Figure 1B). The SD of the seedlings with the recipient of 180 cm^3 indicates that this variable responds to the addition of fertilization, demonstrating the same tendency observed in the H variable, when this recipient was used. This fact displays the evidences of the high nutritional demand of *Cordia trichotoma* in the nursery, which responds positively to significant doses of CRF, increasing its growth.

For the relation between H and SD, the seedlings of *C. trichotoma* presented significant difference ($p<0.05$) only in recipient size, and the greatest average (5.59 $cm.mm^{-1}$) was obtained in the recipient of 110 cm^3. The lower value (4.33$mcm.mm^{-1}$) of the relation between the H and SD of the seedlings produced in the container of 180 cm^3, occurred due to the greater increase in SD, this was observed when compared with the seedlings of the 110 cm^3 recipient. It is worthy to note that the seedlings did not present etiolation, or unbalanced growth, according to the description of José et al. (2005). This demonstrates that there was balanced allocation of assimilates for air growth.

According to Carneiro (1995), the relationship between H and SD demonstrates that the height of the seedlings has to be compatible with the stem diameter; and the lower the relation, the greater the capacity of the seedlings to survive and to establish themselves in the field, considering that the ideal values for this relation in

seedling of *Pinus taeda* have to be between 5.4 and 8.1. However, according to Birchler et al. (1998), this value can be as high as 10, which is what is considered in seedlings with adequate quality, high growth rate and survival after planting

We infer that for *C. trichotoma* seedlings, values between 5 and 6 cm mm^{-1} can be used with reference to the H/DC relation, representing balanced growth in height and stem diameter, which might provide faster establishment post planting. The analysis of the values of dry mass (SDM, RDM and TDM), indicated interaction between the factors doses and recipient for RDM and TDM; for SDM only the factor dose of fertilizer presented significant difference (p <0.05), and the responses of growth were quadratic, estimated by DMTE in 7.0 g L^{-1} (Figure 2A).

According to Navroski (2013), the SDM is considered the structure of the seedling responsible for performing photosynthesis and allocate carbon for the different parts of the plant. With this, we can infer that seedlings with greater value of SDM will be able to adapt to the conditions of planting, because they present greater reserves of photo-assimilation, which are essential to the supply of the vital necessities to the plants (Bellote and Silva, 2000).

The root dry mass (RDM) of the seedlings produced in the recipient of 110 cm^3 presented a tendency to increase with a dose of 5.4 g L^{-1} and decrease with a dose of 7.5 g L^{-1}.

Those produced in the recipients of 180 cm^3, when used the dose of 7.5 g L^{-1}, presented an average production of dry mass superior to recipient of lower volume, however the same was not sufficient to express its maximum technical efficiency (DMTE = 8.3 g L^{-1}) (Figure 2B). We believe that the use of 110 cm^3 container provides greater cost benefit, given that at a dose of 5.4 g L-1 of CRF it results in approximately 3.5 g of RDM per plant, while the plastic tube with 180 cm^3 requires larger volume of substrate and reaches about 7.2 g L^{-1} RDM of CRF.

Because seedlings with greater root biomass tend to grow more and survive better in field than the plants with smaller root biomass (Haase, 2008). When the accumulation of total dry mass (TDM) was analyzed, it was observed that the DMTE of the recipient with greater capacity, was (8.2 g L^{-1}), and this is higher than the DMTE of the 110 cm^3 recipient which was 5.9 g L^{-1} (Figure 2C).

In the absence of fertilization (Controlled) and with 2.5 g L^{-1}, independent of the recipient used, it was observed that the contribution of RDM and TDM was lower when compared to the other dosages (Figure 2B and C). This behavior is associated with the limitation of growth and development due to the insufficient supply of nutrients that can result in metabolic alterations in the plant (Taiz and Zeiger, 2013).

Some authors verified that the reduction in the volume of the recipient resulted in decrease of dry mass production of seedlings, as it was reported for *E. grandis* (GOMES et al., 2003), *S. terebinthifolius* (José et al., 2005) and *P. rigida* (Gasparin et al., 2015). Nevertheless in the study, we verified that in recipient of smaller size, the seedlings of *C. trichotoma* showed satisfactory results when lower doses of CRF were applied, while in recipient of greater volume the seedlings required higher quantity of the same fertilizer. Thus, seedlings of *C. trichotoma* when produced in recipient of lower volume (110 cm^3), presented greater efficiency in the use of nutrients.

From the analysis, it can be observed that most of the studied variables presented significant correlation (Table 1). According to Filho et al. (2010) the intensity of the correlation is represented by a numerical value ranging from -1 to 1. Callegari-Jacques (2003), classifies the correlation rate in relation to intensity, where; r = 0 (there is no correlation), 0 < r < 0.3 = (weak); 0.3 ≤ r < 0.6= (regular); 0.6 ≤ r < 0.9= (strong); 0.9 ≤ r < 1 = (stronger) and r = 1 = (perfect). The H variable is strongly correlated to the SD variable, demonstrating the balance existing in growth, in which the increasing of H corresponds to the increment of SD. The H also correlates strongly with SDM, RDM and TDM (Table 1). On the other hand, the relation H and SD was the parameter that was weakly correlated with the other variables under evaluation (Table 1).

The RDM positively correlates with the H and the SD (Table 1), demonstrating that the seedlings which have a well-developed root system can develop better in field. According to Almeida et al., (2005) this is possible due to the greater area of water and nutrients absorption and the greater capacity of sustainability of seedlings. As most of the variables are strongly correlated and bearing in mind that the dry mass represents the net photosynthesis, seedlings of *C. trichotoma* produced in container of 110 cm^3 demonstrated better quality, however it becomes relevant care with the maintenance in post planting, especially, with the control of invasive herbaceous species capable of competing with the seedlings for water and nutrients.

In this study, when the seedlings were planted in recipient with greater dimension (180 cm^3), the DMTE of most of the evaluated parameters were above the maximum tested dose, while in seedlings planted in recipient of 110 cm^3, all the evaluated parameters achieved the DMTE. Thus, it can be concluded that the nutritional demand of *C. trichotoma* seedlings depends on the size of the recipient used, thus, smaller recipients have lower nutritional demand

Conclusions

The application of controlled-release fertilizer (CRF) in

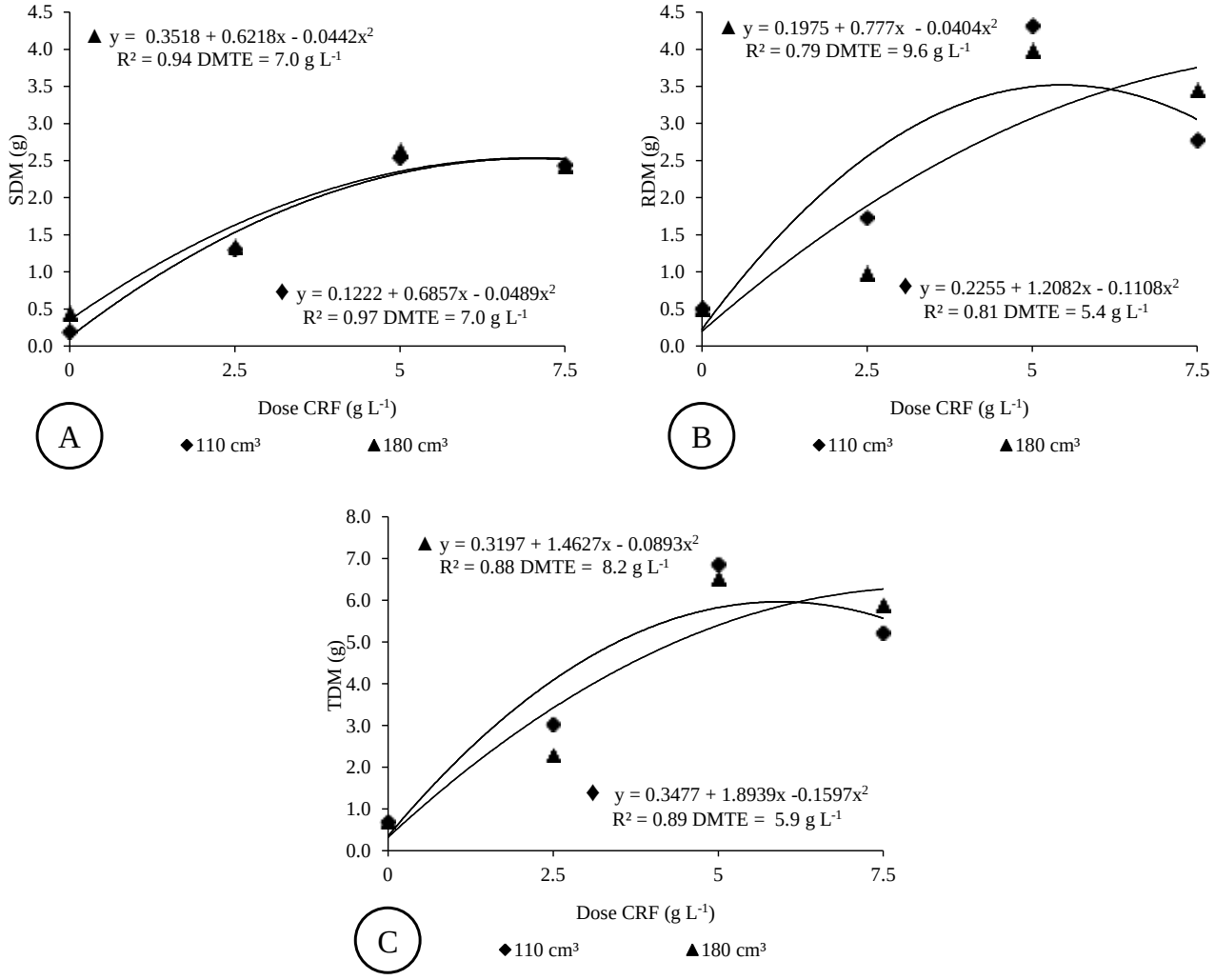

Figure 2. A: Shoot dry mass (SDM), B: Root dry mass (RDM) and C: Total dry mass (TDM) of seedlings of *Cordia trichotoma* in function of the use of different sizes of recipients and doses of controlled-release fertilizer on the 210th day after sowing. DMTE - Dose of maximum technical efficiency.

Table 1. Coefficient of Pearson correlation among the variables height (H), stem diameter (SD), relation H/SD, shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) in seedlings of Cordia trichotoma in function of the use of different sizes of recipients and doses of controlled-release fertilizer on the 210th day after sowing.

Variables	H	SD	H/SD	SDM	RDM	TDM
H		0.97*	0.09^{ns}	0.97*	0.91*	0.94*
SD			-0.11^{ns}	0.90*	0.83*	0.86*
H/SD				0.24^{ns}	0.15^{ns}	0.19^{ns}
SDM					0.96*	0.98*
RDM						0.99*
TDM						

*Coefficients of Pearson correlation significant at 5% of probability of error; ^{ns} coefficients of Pearson correlation not significant at 5% of probability of error

the production of *C. trichotoma* seedlings had positive effect on the growth of plants. Thus, for the production of seedlings in nursery, the following is recommended: recipient of 110 cm³, combined with the dose of 7.5 g L^{-1} of CRF (15-09-12 NPK).

Other factors to be considered are: the economy of substrate, nursery space, practicality of production, transportation and the quality of seedlings. It is also recommended that other studies be carried out, which will test doses of CRF higher than the ones tested in this study; in order to elucidate the best dose required for the species.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The first author gratefully acknowledges the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (Capes) for funding this research and granting scholarship.

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academicJournals

Vol. 11(28), pp. 2456-2461, 14 July, 2016
DOI: 10.5897/AJAR2016.11005
Article Number: 4B6484F59388
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effects of ethylene on the postharvest quality of inflorescences of *Oncidium varicosum* 'Samurai'

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Received 16 March, 2016; Accepted 20 May, 2016

Oncidium varicosum **is an orchid largely distributed in South America, whose inflorescences with bright yellow flowers are of rare beauty. One of the objectives of the postharvest physiology of flowers is the study of the factors related with the quality loss of cut flowers. The ethylene performs a very important function on the senescence of flowers because it induces abscission of flowers buds and open flowers causing precocious wilting and loss of quality. The sensitivity to ethylene has been reported as variable in function of floral species, exposition period and regulator concentration. The present research evaluated the postharvest of cut inflorescences of *O. varicosum* 'Samurai', after application of ethylene (Ethrel: 1, 10 and 100 μL L^{-1}). The experiment was performed as complete randomized design with factorial scheme composed of two factors: four postharvest treatments and four dates of evaluation. The inflorescences were maintained in the refrigerated ambient. The results demonstrated that the exposition to ethylene caused physiological alterations such as reduction of relative water content of the flowers, decrease of the soluble carbohydrates contents of the petals, increase of the respiration rate and flowers abscission. The concentration of 100 μL L^{-1} of Ethrel reduced the decorative life of flowers in seven days in relation to the control without product application, while at 1 μL L^{-1}, the flowers presented lower sensitivity to product, with results similar to the control.**

Key words: Postharvest physiology, orchids, carbohydrates, respiratory rate.

INTRODUCTION

The orchids of genus *Oncidium* are economically important and contains more than 750 species. Most of these species grow in South America, as 105 species of the orchid genera are found in Brazil, being the genus *Oncidium varicosum* native to the Atlantic Forest of Rio de Janeiro (Miller et al., 1996). Presently, new orchid

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hybrids for cutting are commercialized.

Ethylene is a simple molecule composed of two carbon atoms symmetrically linked by a double bond and it naturally occurs in gaseous form. It is, furthermore, a plant growth regulator involved in the regulation of a wide range of different physiological processes, including germination, growth, floral initiation and opening, leaf and floral senescence as well as organ abscission and fruit ripening (Yoo et al., 2009).

There are significant differences in ethylene sensitivity between species and even cultivars of the same species (Serek et al., 2006b; Scariot et al., 2008). The response and sensitiveness to ethylene are different according to the growth stage, variety and the plant organ perception (Ciardi and Klee, 2001; Jones et al., 2001). For example, petals of orchids (*Phalaenopsis*) and *Hibiscus* (Çelikel and Reid, 2002), wilt in response to ethylene, in other species, such as wax flower (*Chamelaucium uncinatum*) (Macnish et al., 2000), ethylene induces petal or flower abscission. Huang et al., (2007) reported through the gene expression standard analysis of ethylene receptor in *Oncidium* 'Gower Ransey' inflorescences, pointing out that their senescence is associated with the increase of ethylene receptors.

Exposure to exogenous or endogenously produced ethylene can be controlled in several ways. These include the use of ethylene biosynthesis inhibitors or ethylene action inhibitors, and ethylene removal technologies (Scariot et al., 2014). Among the main biochemical and metabolic alterations described during the orchid flower senescence are related the anthocyanin synthesis and breakdown; ethylene production; increase of leaf respiration; RNA synthesis; synthesis and activation of various enzymes; transport of organic and inorganic substances; hydrolysis of structural and reserve molecules (Serek et al., 2006a).

Lin (1999) observed that the shelf life of *Oncidium* gender is reduced after harvest, and this is more pronounced due to its branched-inflorescence, which exposes the flowers to damages. Huang and Paull (2009) observed that for *Oncidium* 'Gouver Ramsey' orchid, the floral buds were more sensitive to the exogenous ethylene than the proper flower. The present study aimed to evaluate the ethylene application effect over the postharvest conservation of *O. varicosum* 'Samurai' orchid inflorescences.

MATERIALS AND METHODS

O. varicosum ('Samurai') inflorescences were taken in the morning when 30% of the orchids were open, in a commercial production area in Atibaia (SP). Subsequently, each stalk was placed in a closed plastic tube with 10 ml of distilled water and was wrapped in micro-perforated plastic. The transport of the flowers to the Vegetal Physiology Laboratory at FCAV-UNESP (Jaboticabal, São Paulo) was completed using a refrigerated vehicle. Once in the laboratory, the floral stalks were standardized, labeled, weighed and sprayed with Ethrel (2-chloroethylphosphonic) at concentrations of 0 µL L^{-1} (control treatment), 1, 10 and 100 µL L^{-1}. Two applications were done in 30 min interval.

Experimental delineation was entirely random, in a factorial scheme formed by two factors including the following: four postharvest treatments and four evaluation dates. Three repetitions for each treatment were used, with three inflorescences in each repetition. The inflorescences were kept in a refrigerated environment at 20±1.1°C (88% HR), and lighted for 12 h. Every four days it was evaluated:

i) Relative water content: obtained from 10 flowers in each repetition. Flowers were weighed and immersed in distilled water (to be hydrated) during 4 h. Subsequently, the orchids were weighed for a second time and were taken to a drying stove at 70°C (Kramer, 1983).
ii) Color evaluation of the inflorescences: completed using a colorimeter (Minolta CR-400b), which determined the following values: L (100 = white; 0 = black), a* (positive = red; negative = green), and b* (positive = yellow; negative = blue). Chromaticity was calculated using equations according to the Konica (2007).
iii) Soluble carbohydrates of flowers from *Oncidium* inflorescences: extracted in ethanol using a phenol-sulfuric method (Robyt and White, 1987).
iv) The amount of CO_2 (respiratory activity) produced: quantified by a gas analyzer (model PBI-DANSENSOR 9900) when the samples were removed from the atmosphere inside the containers.

The vase life was evaluated in a separate batch of inflorescences and it was considered finished when 50% or more flowers in a specific inflorescence lost their ornamental quality. The obtained data were submitted to variance analysis through an F test, and the averages were compared using a Tukey test ($P \leq 0.05$), in which the differences between the two treatments were considered significant when they were higher than the sum of two standard deviations (Shamaila et al., 1992).

RESULTS AND DISCUSSION

After 8 days of vase life (DVL) the Ethrel at the concentration of 100 µL L^{-1} proportioned the greater reduction of relative water content (RWC) (77.57%) (Figure 1). At 11 DVL there was an intense and significant RWC reduction (48.93%) in the inflorescences maintained at the concentration of 10 µL $L^{-1}$of Ethrel , what points out that treatments even with concentrations 10 times lower can reduce the *O. varicosum 'Samurai'* orchid shelf life. The water balance alterations after exogenous ethylene application are related with the first signs for flower senescence (Goliás and Kobza, 2003).

In the Figure 2 is observedthat a reduction of the inflorescence brightness during the evaluation period, especially in the treatment at 100 µL L^{-1}. The Ethrel spray in the 100 µL L^{-1} made the inflorescences browning what was statistically significant at 4 and 8 DVL. The flower browning is a senescence indicative and may be related to water stress and low carbohydrate levels, caused by the phenol oxidation, mainly the leuco-anthocyanin, which react with other cell compounds producing brown precipitate (Reid, 2002). In hybrids of *Dendrobium* the discoloration of the flowers it was also related to a possible increase in the pH of the vacuole of the cells or to the effect of the polyphenol oxidase and peroxidase

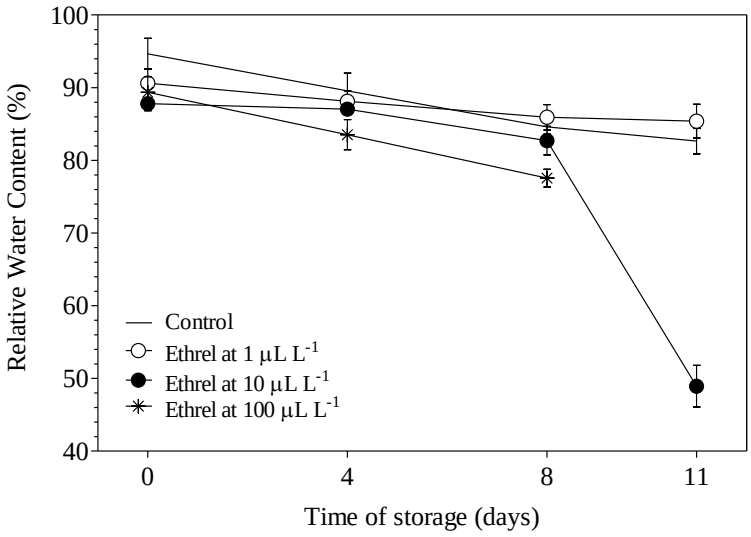

Figure 1. Relative water content (RWC), in percentage, of cut inflorescences of *O. varicosum* 'Samurai' treated with Ethrel. Data shown are mean ± standard deviation.

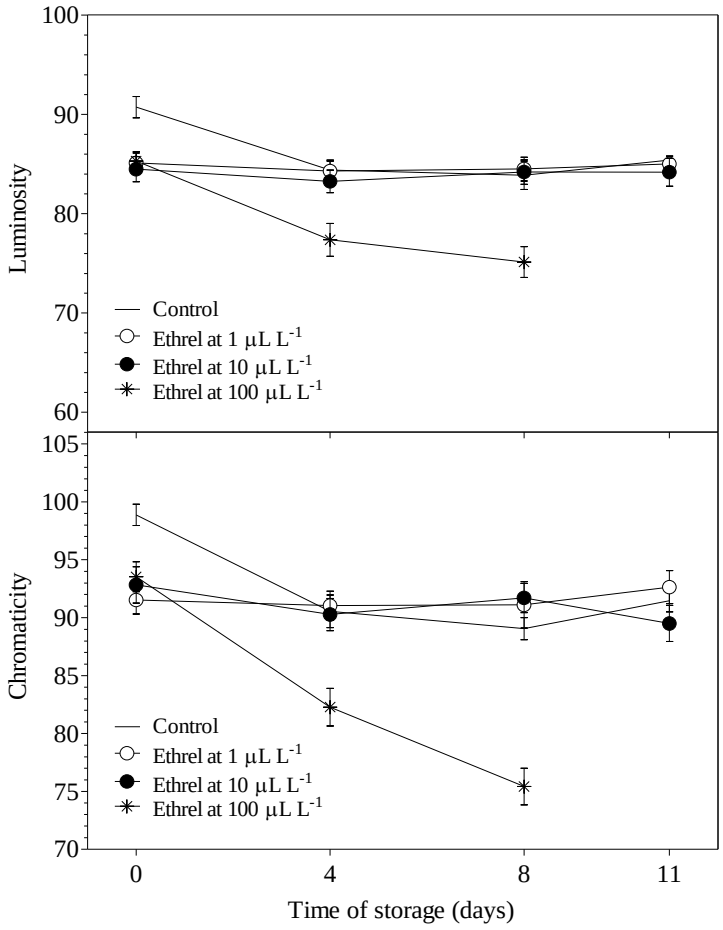

Figure 2. Luminosity and chromaticity of cut inflorescences of *O. varicosum* 'Samurai' treated with Ethrel. Data shown are mean ± standard deviation.

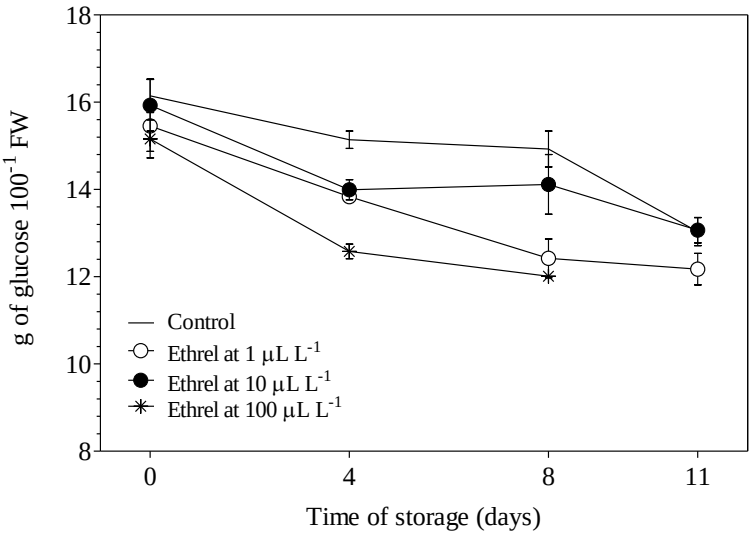

Figure 3. Contents of soluble carbohydrates (g of glucose 100 g^{-1} FW) of cut inflorescences of *O. varicosum* 'Samurai' treated with Ethrel. Data shown are mean ± standard deviation.

enzymes, both caused by the senescence of the flowers after exposure to ethylene (10 µL L^{-1}, 24 h) (Almasi et al., 2012). The obtained results for the chromaticity parameter showed that the color intensity was drastically reduced in the highest Ethrel concentration treatment (100 µL L^{-1}) at 8 DVL, which differed from the others.

It is observed in the Figure 3 a significant reduction of the soluble carbohydrate content in the inflorescence along the days of vase life. The reduction of soluble carbohydrate content in the inflorescences is an indicative of vegetal senescence, when using its carbon reserves to maintain the respiration to repair the degrading processes of the metabolism. At 4 DVL, the inflorescences sprayed with Ethrel in the 100 µL L^{-1} concentration presented soluble carbohydrate content in the petals of 12.58 g of glucose per 100 g^{-1} of fresh mass, and more than 50% of the flowers old, culminating in a shelf life end. Orchid flowers are particularly sensitive to ethylene (Halevy and Mayak, 1981), as the ethylene production is an autocatalytic process, greater quantities of this substance can be produced once they are put together with other senescent flowers (Hew, 1994). The Ethrel concentrations of 1 and 10 µL L^{-1} and control presented soluble carbohydrate content similar to those from 11 DVL, what can indicate that the exposition to ethylene concentrations of 1 and 10 µL L^{-1}, for the experimental conditions, were less damaging.

Arditti (1992) related that the carbohydrate content is variable among the different genders and species of orchid flowers. *Aranda* orchid generally maintain a high level of sugars in the sepals, while in *Cymbidium*, the sugar content decreases with age. Huang and Paull (2009) found that flower buds of *Oncidium* 'Gouver Ramsey' are extremely sensitive to ethylene. The vase live of flower buds was significantly reduced by their exposure to 0.03 nL L^{-1} ethylene concentration. The buds were more sensitive than the flowers, since it was necessary five days to the ethylene treatment result in any effect on buds, and seven days to the flowers.

It is verified in the Figure 4 that the inflorescences sprayed with Ethrel in the concentration of 100 µL L^{-1} presented an intense respiration peak (265 mg of CO_2 kg^{-1} h^{-1}) at 4 DVL, sharp decline at 8 DVL which coincided with the end of the flower longevity. These results agree with those published by Chadwick et al. (1980) and Harkema and Woltering (1982), saying that the exposure to high ethylene concentration may cause early flower senescence in orchids, and also flower buds, flowers and leaves abscission and early wilting. The application of the exogenous ethylene (10 µL L^{-1}, 24 h) in *Dendrobium* hybrids showed distinct variances in ethylene sensitivities and degrees of deterioration. The hybrids in the sensitive group exhibited the utmost hyponasty, weight loss and degradation of anthocyanin content in sepals and petals (Almasi et al., 2012).

For *Oncidium* 'Goldiana' flowers, the ethylene production started after a latent period of 100 hours post harvesting and an increase in the climacteric period that formed a peak at 11 days (Hew and Yong, 2004). Ketsa and Thampitakorn (1995) working with *Dendrobium* 'Caesar' and Uthaichay et al. (2007) working with *Dendrobium* 'Karen' observed that the production of ethylene for open individual flowers presented low rates, during the first ten days after the cut of inflorescence while the flower buds produced high rates.

Bunya-Atichart et al. (2006) found that in *Dendrobium* cv. 'Miss Teen' the response to the exogenous ethylene application started in few days and resulted on the drop

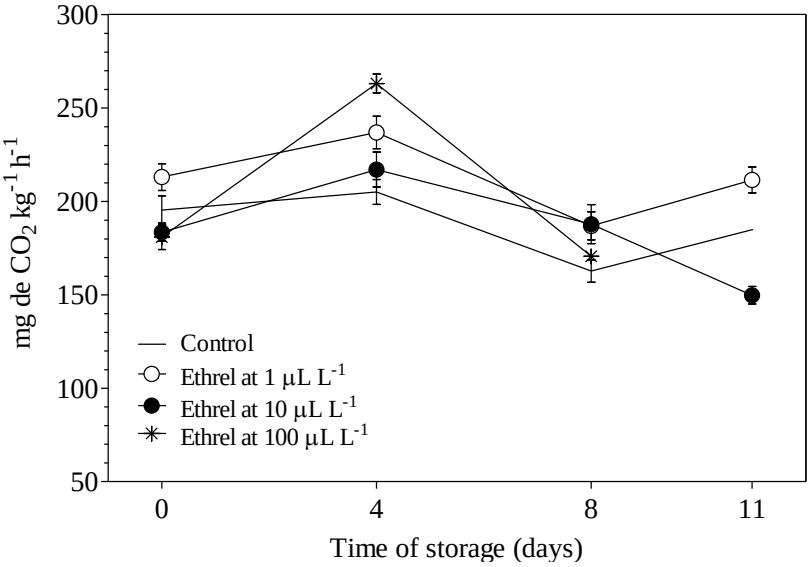

Figure 4. Respiratory activity of cut inflorescences of *O. varicosum* 'Samurai' treated with Ethrel. Data shown are mean ± standard deviation.

of 70% of flower buds.

The concentration of 100 µL L^{-1} led to a greater physiological response of the flowers, causing a pronounced reduction of the relative water content, respiratory rate increase, soluble carbohydrate content reduction, flower browning, intense flower drops and longevity reduction.

Conclusion

Oncidium varicosum 'Samurai' presented a varied sensitivity to different exogenous ethylene concentrations (1, 10 and 100 µL L^{-1}). The concentration of 100 µL L^{-1} of Ethrel reduced the decorative life of flowers in seven days in relation to the control without product application, while at 1 µL L^{-1}, the flowers presented lower sensitivity to product, with results similar to the control.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) provided resources (Proc. 05/51186-0) for this research.

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academicJournals

Vol. 11(28), pp. 2462-2468, 14 July, 2016
DOI: 10.5897/AJAR2016.11014
Article Number: 6100C7D59390
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Impact of harvesting with burning and management of straw on the industrial quality and productivity of sugarcane

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Received 17 March, 2016; Accepted 16 June, 2016

Change from the traditional harvesting system with burns to mechanized harvesting of sugarcane, as well as the amount of straw needed to remain in the field for sustainability of the production system and how much could be removed for sectors such as energy cogeneration and bioethanol production, are not clarified issues. The objective of this study was to evaluate the impact of harvest management with burning (traditional) and cultivatation with different amounts of straw on the industrial quality and productivity of sugarcane. The effect of six treatments were evaluated: cane burning, 0, 25, 50, 75 and100% (20 Mg ha^{-1}) of straw on the industrial quality (soluble solids (°Brix), Pol, apparent purity, total sugars (TS), reducing sugars (RS) and fiber) and productivity (Mg Pol ha^{-1}) of sugarcane. At the end of the cycle, the straw decomposition rate for each treatment was also verified. The higher the percentage of straw, the higher the degradation rate. The change of burned cane harvesting system for sugarcane under straw results in improved productivity of sugarcane and favors the production of sugar. The straw and harvest system change do not affect the industrial quality of sugarcane. The harvest with burning, the total withdrawal or of 75% of the straw of the field result in lower productivity. The maintenance of 50% of the straw on the soil surface is sufficient to improve the productivity of sugarcane in dry period, and the remaining 50% can be used for second generation of ethanol production or electricity without damaging the crop productivity.

Key words: *Saccharum* spp, soil cover technological quality, biomass, sucrose.

INTRODUCTION

The major sugarcane producing areas of world have recently adopted the practice of mechanical harvesting and this practice tends to increase both in current areas and in expansion (Braunbeck; Magalhães, 2010).

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In this system, large amount of straw is produced. It is estimated that each year, more than 300 million Mg of straw are produced (UNICA, 2015). In the field, values are found to be from 10 to 30 Mg ha^{-1} of dry straw, oscillating because of the variety and age of the cane field (Christoffoleti et al., 2007).

This amount of plant material that remains in the soil, causes changes in the chemical, physical and biological conditions of the agricultural environment, such as increased soil moisture (especially important in areas with water deficit), elevated levels of organic matter, changes fertility and temperature in the surface layers of the soil, greater efficiency in the control of erosion, changes in incidence of light on the soil surface, budbreak in irregularity under straw with a possible decrease in productivity of varieties susceptible to straw (Silva et al., 2003; Christoffoleti et al., 2007; Cavenaghi et al., 2007; Guimarães et al., 2008).

The industrial quality and productivity of sugarcane are strongly influenced by changes in the production environment. Marques and Pinto (2013) reported that some soil characteristics such as porosity, storage capacity of water and evaporation, can be altered to promote positive change in factors of production, and the use land cover is a technique to promote these changes.

The benefits obtained with the straw surface have been reported by several authors, although not accosted what quantity would be sufficient to achieve these improvements. Quantification of straw needed to promote these benefits is essential information for the sustainability of the sugarcane production system and to optimize the energy generation sector, enabling the excess of straw can be used for the production of bioethanol and/or bioelectricity, which play an important role in the global energy grid. It is estimated that the use of straw can triplicate the ethanol production without the need to increase the planting area, once one ton of straw results in 270 liters of ethanol, and one ton of sugarcane results in 80 L of ethanol (Santos et al., 2012). Thus there is a concern to determine the required amount of straw that should remain in the field, in order to provide greater crop protection and soil.

Importantly, in addition to contributions on soil fertility already described (Franchini et al., 2001 Resende et al., 2006), the straw as cover also plays an important role in environmental protection, from the point of view of soil conservation. However, several producing countries still use as a traditional method, the harvesting system of sugarcane with straw burning. Soil degradation is currently considered one of the most serious environmental problems. Erosion is a form of most harmful degradation, since it reduces irreversibly the productive capacity of the cultures, besides causing sedimentation and pollution of water supplies. According to Braunbeck and Magalhães (2010), the straw cover protects the soil in all phases of the erosion process because it absorbs the kinetic energy of the rain drops, decreases the speed of runoff and hinders displacement of the particles. Thus, maintenance of stubble on the surface is a management of great importance when seeking the sustainability of the sugarcane production system.

Considering these aspects, the aim of this study was to evaluate the effect of harvest management with burning (traditional) and cultivation with different amounts of straw in industrial quality and productivity of sugarcane.

MATERIALS AND METHODS

The experiment was implemented at the Bandeirantes Sugar and Alcohol Processing Plant, located in the municipality of Bandeirantes, Parana State, Brazil, at 23° 06' S latitude and 50° 21' W longitude, and at 440 m above the sea level. The annual average precipitation is 1.300 mm and the annual average insolation is 7.14 $h.day^{-1}$. The soil is classified as Rhodic Eutrudox (Embrapa, 2013) of clay texture, with 61% clay; 2% silt and 37% sand.

The installation of the experimental area was in August of 2010 when chemical analysis was performed on soil (Embrapa, 1997) layers ranging from 0–0.10; 0.10–0.20; 0.20–0.40; and 0.40–0.60 m in profile depth (Table 1). There was no need for chemical fertilization, but 70 Mg ha^{-1} of sugarcane filter cake was spread over the entire area previously for the implementation of the trial. The soil was prepared by using heavy and then light disc harrow.

The climatological hydric balance of the area (Figure 1) was calculated based on Thornthwaite and Mather (1955). Normal average monthly temperatures and total monthly rain data were provided by the meteorological station of the Parana State Agronomical Institute (IAPAR), located also in Bandeirantes, PR, 3 km from the experimental location. As available water capacity value (AWC) in the soil, 100 mm was used for hydric balance calculation. In the experimental area, sugarcane had been grown for 65 years, using manual harvesting with straw removal by burning. In 2010, the sugar mill plant adopted the mechanized harvesting system without straw burning, which was also used at the experimental site.

The experiment was conducted during the course of two sugarcane crop cycles, with the SP801816 cultivar (first and second ratoon) in a randomized block design with four replications. Each plot "consisted of" 10 rows of sugarcane, 10 m in length (10 rows x 10 m) and 1.50 m between rows. For evaluations, 6 central rows of 9 linear meters each, with a total of 54 linear meters were considered.

The variety of sugarcane used was SP 80-1816, a more widespread in the South Central of Brazil, due to its good tillering and regular closing lines, high agricultural productivity, good budding ratoons, early maturing, high content sucrose, low fiber content, tipping and flowering absence (Fernandes, 1991).

The following treatments were evaluated: 0, 25 (5 Mg ha^{-1}), 50 (10 Mg ha^{-1}), 75 (15 Mg ha^{-1}) and 100% (20 Mg ha^{-1}) of straw on the soil, and burned sugarcane (where 100% of the straw was burned). The industrial quality components evaluated were: juice soluble solids (°Brix), Pol (%), total sugars (TS), apparent purity (%), reducing sugars (RS) and fiber.

In August 2010, immediately after planting, quantities of straw of each treatment were added to the soil. The straw deposition in the plots was obtained in another area, after the mechanical harvesting of cane plant of the same variety. In this area, plots of the same size of the experimental area were demarcated and they contained straw wrapped in bags, weighed and redistributed on the plots in the respective amounts of each treatment. To evaluate the dry matter of straw produced by the variety, 1 m^2 was measured immediately after the harvest, and all the straw contained in this

Table 1. Results of the chemical analysis of soil Rhodic Eutrudox at depths of 0 to 0.60 m, city Bandeirantes - PR, 2010.

Depht (m)	M.O	pH	P	K	Ca	Mg	H+Al	SB	CTC	Ca	Mg	K
	g kg^{-1}	$CaCl_2$	mg dm^{-3}	———	———	———	$Cmol_c$ dm^{-3}	———	———	——% Saturation———		
0 – 0.10	26.8	5.4	8.6	2.50	7.8	1.7	3.1	12.0	15.1	51.6	11.2	16.5
0.10 – 0.20	41.6	5.9	71.3	3.60	7.9	1.9	2.9	13.4	16.3	48.5	11.7	22.1
0.20 – 0.30	34.9	6.1	31.0	3.70	8.0	2.1	3.0	13.8	16.8	47.6	12.5	22.0
0.30 – 0.40	30.9	6.2	5.1	4.60	8.1	2.1	2.2	14.8	17.0	47.6	12.3	27.0
0.40 – 0.50	37.6	6.3	9.0	4.20	7.3	2.0	2.4	13.5	15.9	45.8	12.6	26.4
0.50 – 0.60	28.2	6.3	5.3	3.20	6.1	2.1	2.4	11.4	13.8	44.2	15.2	23.1

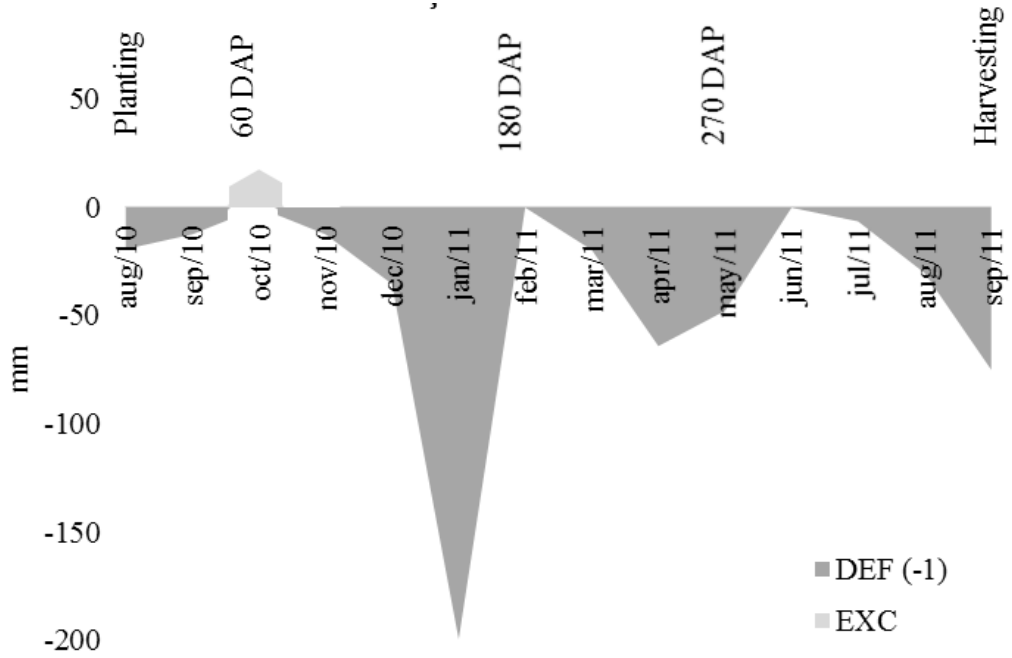

Figure 1. Extract of the monthly water balance during the experimental period.

area were removed, kiln dried and weighed. This procedure was performed in 48 repetitions. The percentage of decomposition of straw was evaluated at the end of the cycle, the DAC 360, in 12 replicates per treatment, by weighing the straw contained in 1 m^2. These samples were collected randomly in the plot and kiln dried until constant weight.

At the end of the cycle, at 360 DAP to determine the industrial quality, ten stems of sugarcane were harvested

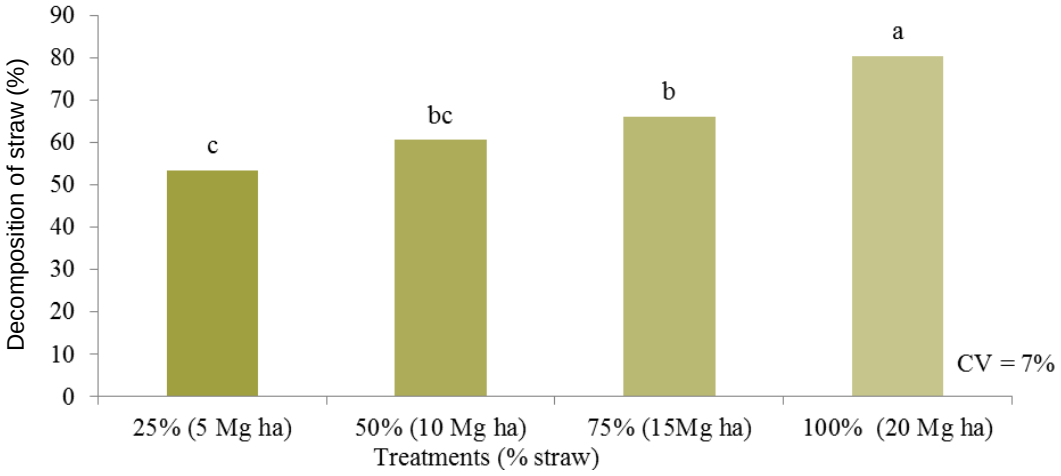

Figure 2. Decomposition rate of the straw for treatments 25 (5 Mg ha^{-1}), 50 (10 Mg ha^{-1}), 75 (15 Mg ha^{-1}) and 100% (20 Mg ha^{-1}) of straw, at the end of the crop cycle in August of 2011. Means followed by the same letter are not significantly different by Duncan test at 1% significance level.

from each plot after the green and dried leaves had been removed, and then the topping was performed. The samples were immediately sent to the quality control laboratory of the sugarcane mill and they were analyzed according to the methodology of CONSECANA (2006).

The productivity evaluation (Mg Pol ha^{-1}) was performed at 400 DAP (September 2010) and obtained by the formula $Productivity = (Mg\ sugarcane\ ha^{-1} \times Pol_{cane})/100$ (Silveira et al., 2012). The production of sugarcane in Mg per hectare was obtained through the harvesting and weighing of all stalks contained in the plots.

The data were analyzed by variance analysis (ANOVA) and the averages were compared by the Duncan test (P<0.05 and 0.01). The software Sisvar 5.3 (Ferreira, 2010) was used for the analyses.

RESULTS AND DISCUSSION

The average production of straw for SP80-1816 cultivar was 20 Mg ha^{-1}. Figure 2 is the percentage of decomposition of the straw at the end of the crop cycle (360 days). It can be seen that higher values were obtained with 75 treatments and 100% (15 to 20 Mg ha^{-1}, respectively) of straw, reaching respectively, decomposition rate of 75 to 80%. Note also, that smaller amounts of straw (25 and 50%) showed the lowest rates of decomposition, 53 and 61%, respectively. This can be explained by the fact that the presence of higher amount of straw on the surface, provides maintenance of higher moisture and lower thermal fluctuation (Tavares et al., 2010) particularly in the superficial layers, favoring the water cycle and nutrients (Freitas et al., 2004). Thus, the greater the amount of straw on the soil, the more moisture retained therein, creating a microclimate that favors the proliferation of fungi and more rapid decomposition of the material (Glória et al., 2000). Oliveira et al. (1999) in system under irrigation, also reported 80% reduction in the mass of dry straw after weighing at the beginning and after 11 months in the field, corroborating results obtained in this study. In relation to industrial quality of juice components, the following averages were obtained: Soluble solids: 21 °Brix, Pol: 17%, pureza aparente: 84% and TS: 145 kg Mg^{-1} (Figure 3).

Fernandes (2000) considered 14.4 °Brix as an “adequate” amount of soluble solids for the beginning of the harvest. The purity of the juice must be over 80% in the beginning, and 85% throughout the rest of the harvest. It was observed that, no matter how treatments were employed, the cultivar showed values above those cited as good, and “it is considered as a rich cultivar of sucrose”. The average value of RS was 0.9%, according to the results found by Souza et al. (2005), evaluating the management of cane harvested with and without burning and found that AR values were below 1.0%. The average fiber content was 13% (Figure 3), a value slightly above the average standard considered, that is 12.5% (Fernandes, 2000), probably due to the long period of low water availability faced during the cycle.

The change of sugarcane harvesting system with burning for 65 years for the management system with different amounts of straw did not interfere with industrial quality components juice (soluble solids (°Brix), Pol, TS, purity, RS and fiber) indicating good adaptation of the plant.

These results confirm those obtained by Resende et al. (2006) evaluatingthe effect of straw, after 16 years of cultivation on industrial quality of sugarcane and observed that the maintenance of straw for system did not affect the soluble solids values (Brix), Pol, fiber, purity and percentage of sugar juice. However, Souza et al. (2005) found a reduction of ST and apparent sucrose of ratoon sugarcane harvested without burning, 18 varieties of sugarcane, when incorporated up to 70% of the straw

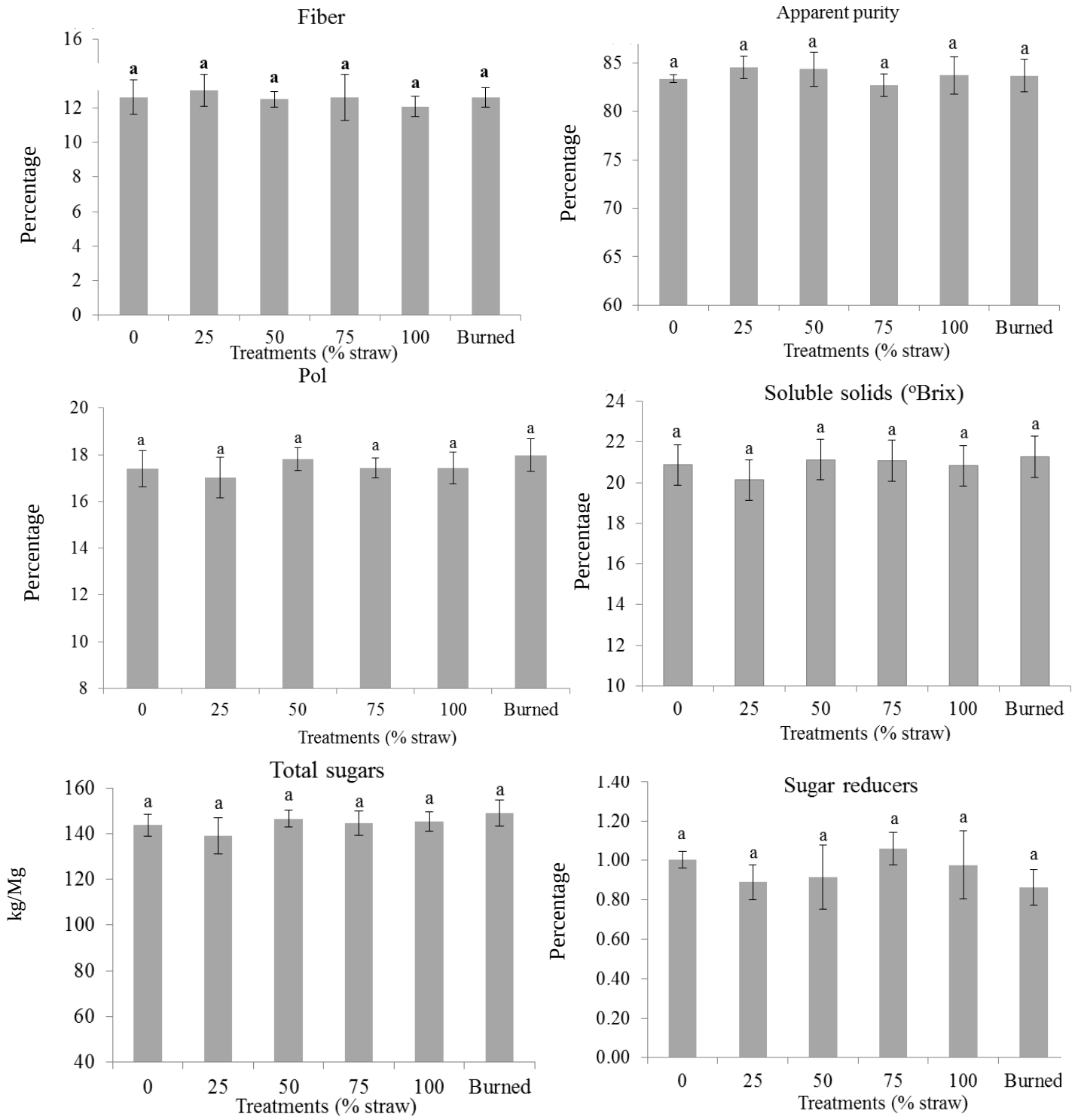

Figure 3. Fiber (%), apparent purity of juice (%), Pol (%), soluble solids (°Brix), total sugars (kg Mg^{-1} of sugarcane) and sugar reducers (SR%) of sugarcane in relation to the amount of straw surface. Means followed by the same letter are not significantly different by Duncan test at 5% significance level.

at a depth of 0 to 0.30 m, emphasizing the importance of knowledge of the effects of different managements of straw to maintain a high crop yield with satisfactory quality.

There was a significant effect of management of straw on productivity (Mg Pol ha^{-1}). The harvest with burned

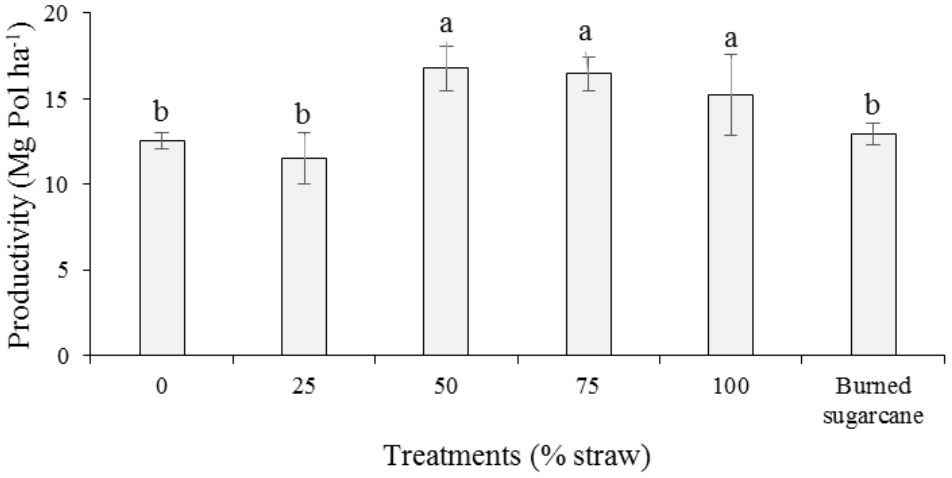

Figure 4. Production of sugarcane (Mg Pol ha^{-1}) in relation to the amount of straw surface (%). Means followed by the same letter are not significantly different by Duncan test at 1% significance level.

cane has resulted in 20% lower productivity (12.95 Mg Pol ha^{-1}) than the treatments of 50, 75 and 100% straw (average of 16.16 Mg Pol ha^{-1}) (Figure 4). Comparing between treatments without burning, the quantities of 50, 75 and 100% of straw did not differ (average of 16.16 Mg Pol ha^{-1}) and provided average 25% increase in productivity when compared with soil treatment discovered (0%) and 25% of straw (12.56 and 11.53 Mg Pol ha^{-1}, respectively). Thus, it is observed that although there was no influence of treatments on components of industrial quality, production of sugar was favored by higher crop productivity in quantities above 50% straw.

The significant result of straw in the first year of cultivation should probably prolong drought in this period, with rainfall below the historical average causing water deficit of up to 200 mm (Figure 1), which resulted in low productivity of sugarcane plantations all over the south-central Brazil, down 11.20% (CONAB, 2015). It can be seen that only in October, at 60 DAP were greater water available (Figure 1), thus affecting the initial growth stages, essential period that cause adequate supply of water for the development of crops. The damage to the plant and stem productivity due to periods of water stress are greater when it occurs in the early stages of culture, it can hinder or delay the development of aerial part. When it occurs in the other phases, the sugarcane productivity is rarely affected (Inman-Bamber and Smith, 2005). Water deficit reduces gas exchange and its conduction to the leaves. When the hydric deficit is interrupted, the gaseous exchanges tend to go back to normal, however, at a slower rate, which can compromise the crop production during the entire cycle (Silva and Pincelli, 2010).Braunbeck and Magalhães (2010) emphasized that the straw provides reduction in soil water loss of approximately 70%, and reduction in the average temperature of the surface soil layers, and an increase of organic matter, favoring not only the largest structure of soil microbiota, but also increasing the root system (Aquino et al., 2016) crop yield.

It was observed in this study, that there are different answers to culture in accordance with the amount of straw that remains in the field. This is particularly important if one considers that, recently, the use of this waste for second generation ethanol and bioelectricity production has been one of the main alternatives to supply of the increasing global demand for this type of energy, causing doubts about what amount required to be maintained the field to guarantee the sustainability of the sugarcane production system.

It can be seen in this study that 50% of straw was sufficient to provide increased crop productivity in drought cycle and above that amount there was no statistically significant difference. It was also observed that the removal of 75% of soil straw resulted in decreased productivity (Mg Pol ha^{-1}) not differing from the treatments where the soil was discovered (0% straw and burned cane). Thus, it appears that the maintenance of straw in the system is essential for the productivity of sugarcane, the system sustainability and sugar production.

Conclusions

1. The higher the percentage of straw, the greater the degradation rate.
2. The change of burned cane harvesting system for sugarcane under straw results in improved productivity of sugarcane favors the production of sugar.
3. The industrial quality of sugarcane is not affected by the straw and harvest system change.

4. The harvest with burning, the total withdrawal or of 75% of the straw of the field result in lower productivity.
5. The maintenance of 50% of the straw surface is sufficient to improve productivity.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank PETROBRAS for financing this work, the Coordination of Higher Education Personnel (CAPES) (Scholarship Grants 6104 and 0704932), and the Bandeirantes Plant for the assignment of the experimental area and technical support in the ratings.

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academicJournals

Vol. 11(28), pp. 2469-2474, 14 July, 2016
DOI: 10.5897/AJAR2016.11235
Article Number: D17DB7959393
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Agronomic characteristics of corn in function of the application of K, S and Mo by foliar via with and without plaster in red Latosol

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Received 18 May, 2016; Accepted 2 June, 2016

The study aimed to verify the response of the application of foliar fertilizers based on potash, sulfur and molybdenum, with and without plaster in the soil, on the agronomic traits of corn crop. The experimental design was a randomized block in a factorial 5 x 2 with four replications. The sources of variation showed no significant effect. These results indicate that the plaster efficiency in the agronomic characteristics can be possibly found with more reaction time of this conditioner into the soil, this justifies the non-significant effect of plaster on this cycle. The application of foliar fertilizers provided no significant increase in any of the variables, with no nutrients, probably due to availability and sufficient quantity in the soil where the study was conducted.

Keywords: Yield, calcium sulphate dyhidrate, *Zea mays*.

INTRODUCTION

Among the cereals grown in Brazil, corn (Zea mays L.) is the most significant, with an expectation of 83 million tons of grain produced in an area of approximately 15 million hectares, referring to two seasons, summer and winter. For its physiological characteristics, the corn crop has high yield potential, having already been obtained a productivity of more than 16,000 kg ha^{-1}. However, Brazilian productivity is very low, about 5469 kg ha^{-1}, demonstrating that the different corn production systems should be greatly improved to achieve the productivity and potential of this culture (conab, 2016).

The application of conditioners as the agricultural plaster in the soil, can improve the chemical characteristics, favoring the development of the roots in depth, contributing to the correction of calcium deficiency, neutralization of Al^{3+} toxic and supply of S, in the deeper layers, decisive factors for crop productivity. As a result, and with the greatest amount of roots underground, there is a better use of water and nutrients by plants especially in water deficit periods (Zandoná et al., 2015).

Foliar fertilization is among the several ways to provide nutrients for plants, and when well managed it can be an

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effective alternative for the solution of specific problems and/or to complement a rational fertilization (Faquin, 2005), but there is in the literature a certain shortage related to application of fertilizers based on K, S and Mo by foliar application in corn.

According to Büll and Cantarella (1993), potassium has a great impact on crop quality influencing positively on the individual grain weight and number of grains per ear; however there is little publication and research material in Brazil on the implementation of this element by foliar via, stressing the need for further study. Karlen et al. (1998) concluded in their work that S is accumulated throughout the crop cycle, and almost all S accumulated by the ear and peduncle is absorbed by the plant during the stage of reproductive development. According to Rezende et al. (2009), more expressive works with the application of S through the leaves can be seen in soy with increase in grain yield of up to 32% compared to the control.

According to Teixeira (2006), the application of molybdenum in corn crop provides no additions to plant growth or grain yield. However, Ferreira et al. (2001) and Silva et al. (2011) obtained increases of corn productivity with different doses of molybdenum fertilization, occurring variation between years and it can be influenced probably by the variation of rainfall and by other environmental factors such as temperature and light.

The application of plaster in the soil and mineral sources by foliar via on corn leads to improvements in the nutritional conditions of the plants and consequently favors the expression of genetic potential of corn, expressed in agronomic characteristics.

In this context, the aim of this study is to evaluate the agronomic characteristics of maize depending on the application or not of the plaster in the soil and foliar fertilizers on corn, based on potash (K), sulfur (S) and molybdenum (Mo).

MATERIALS AND METHODS

The experiment was conducted under field conditions in Toledo - Paraná - Brazil, situated (24°32'30") of South latitude and (53°54'32") West longitude, with an altitude of approximately 386 m of the sea level. The soil of the experimental area was classified as eutrophic Red Latosol (eRL) and by chemical analysis the following values was observed:

to layer of 0-0,20 m: Ca= 4.19 $cmol_c\ dm^{-3}$; Mg= 0.99 $cmol_c\ dm^{-3}$; K= 0.12 $cmol_c\ dm^{-3}$; Al = 0.35 $cmol_c\ dm^{-3}$, H + Al = 5.88 $cmol_c\ dm^{-3}$, SB= 5.30 $cmol_c\ dm^{-3}$; CTC= 11.18 $cmol_c\ dm^{-3}$; MO = 27.34 g dm^{-3}, V = 47.41%; Al = 6.19%; P = 4.11 mg dm^{-3}; CaCl pH = 4 62 mg dm^{-3}; Cu = 16.50 $cmol_c\ dm^{-3}$; Zn = 4.00 $cmol_c\ dm^{-3}$; Mn = 121.00 $cmol_c\ dm^{-3}$; Fe = 27,50 $cmol_c\ dm^{-3}$ to layer. 0,21-0,40 cm: Ca = 4.92 $cmol_c\ dm^{-3}$; Mg = 1.44 $cmol_c\ dm^{-3}$; K = 0.06 $cmol_c\ dm^{-3}$; Al = 0.05 $cmol_c\ dm^{-3}$, H + Al = 4 30 $cmol_c\ dm^{-3}$, SB = 6.42 $cmol_c\ dm^{-3}$; CTC = 10.72 $cmol_c\ dm^{-3}$; OM = 13.67 g dm^{-3}, V = 59.89%; Al = 0.77% ; P = 3.67 mg dm^{-3}, pH CaCl = 5.10 mg dm^{-3}; Cu = 16,90 $cmol_c\ dm^{-3}$; Zn = 2.50 $cmol_c\ dm^{-3}$; Mn = 57.00 $cmol_c\ dm^{-3}$; Fe = 34.40 $cmol_c\ dm^{-3}$.

During the experiment conduction period, the study collected weather information, as presented in Figure 1, being the minimum temperature of 20°C and maximum 34°C. The experimental design was in randomized blocks (R.B.D.) with four replications, in a factorial 5 x 2. The study evaluated foliar fertilizers and a witness along with the application or not of agricultural plaster, totaling 40 experimental plots.

The foliar fertilizers were applied in their commercial doses, recommended by the manufacturers, with and without the presence of plaster, defining the treatments as it follows:

For the treatments in which it was added agricultural plaster in superficial application in the soil, the treatments were: T_1- treatment with product 0-0-50+S (2 kg ha^{-1}) with plaster (1065 kg ha^{-1}); T_2-treatment with L-S product (2.5 L ha^{-1}) with plaster (1065 kg ha^{-1}); T3-treatment with Potamol-Plus (0.4 L ha^{-1}) with plaster (1065 kg ha^{-1}); T4 treatment with Ammonium Molybdate (0.030 kg ha^{-1}) with plaster (1065 kg ha^{-1}); T_5-Witness with plaster (1065 kg ha^{-1}). With regard to the treatments in which plaster was not added, the treatments were: T_1-treatment with product 0-0-50 + S (2 kg ha^{-1}) without plaster (0 kg ha^{-1}); T_2-treatment with L-S product (2.5 L ha^{-1}) without plaster (0 kg ha^{-1}); T_3-treatment with Potamol-Plus (0.4 L ha^{-1}) without plaster (0 kg ha^{-1}); T_4 treatment with Ammonium Molybdate (0.030 kg ha^{-1}) without plaster (0 kg ha^{-1}); T_5-control without plaster (0 kg ha^{-1}).

The total area of the experiment was 675 m^2, composed of four blocks with 10 treatments each. Each experimental parcel was composed of six planting lines with 5 m length and spacing between lines of 0.45 m, totaling 13.5 m^2 per parcel and its useful area was of 3.6 m^2.

As for the seeding, using the P30F53YH corn material, it was made on October 11, 2013 (agricultural year 2013/2014) using a tractor and seeder with 9 lines of planting and spacing of 0.45 m, set to distribute 3.3 seeds per meter, totaling 73,332 h^{-1} of corn seed and for the basis fertilization it was used 400 kg ha^{-1} of fertilizer formulated 06-30-22 (N-P-K), equally distributed the seeds and the fertilizer for all parcels.

Seven days after the planting, the demarcation of the main plots was carried out along with the application of dolomitic lime on the soil surface based on the chemical analysis of soil and under recommended criteria of 2000 kg ha^{-1}. Limestone was applied to the total area of the experiment. The agricultural plaster was applied on the 19 October, 2013 (8 DAP). Its application was on the soil surface at a dose of 1065 kg ha^{-1}, applying it only in the experimental plots relevant to their use.

The coverage fertilization was carried out according to Embrapa (2008), based on the results of the soil analysis and expected productivity for corn destined to grain production. The fertilizer used was ammonium sulphate composed of 21% of nitrogen and 24% of Sulphur, and the applications were divided into two times. The first application was held on October 30 (12 DAE) when the corn plants showed four expanded leaves (V_4 stage) in the dosage of 70 kg ha^{-1} of N and 80 kg ha^{-1} of S. The second application occurred on October 27, 2013 (40 DAE) when the corn plants showed eight expanded leaves (V_8 stage) at a dose of 70 kg ha^{-1} of N and 80 kg ha^{-1} of S.

The application of foliar fertilizers occurred on 25 November, 2013 (138 DAE) (V_7 stage) when the corn plants showed seven expanded leaves. The products were applied on this stage in their commercial doses following the manufacturer's recommendation. The application was terrestrial with costal spray with capacity for 20 L, properly calibrated, showing a flow of 200 L ha^{-1}.

For the final population, the manual counting on 18 February 2014 (133 DAE) of all plants in the useful area represented by the agronomic characteristics, in all experimental plots was carried out. The harvest of the plots was carried out manually, and the ears were placed in plastic bags properly identified, and subsequently it was made the threshing with the aid of a stationary manual threshing.

All the plants of the useful area of the portion of each treatment

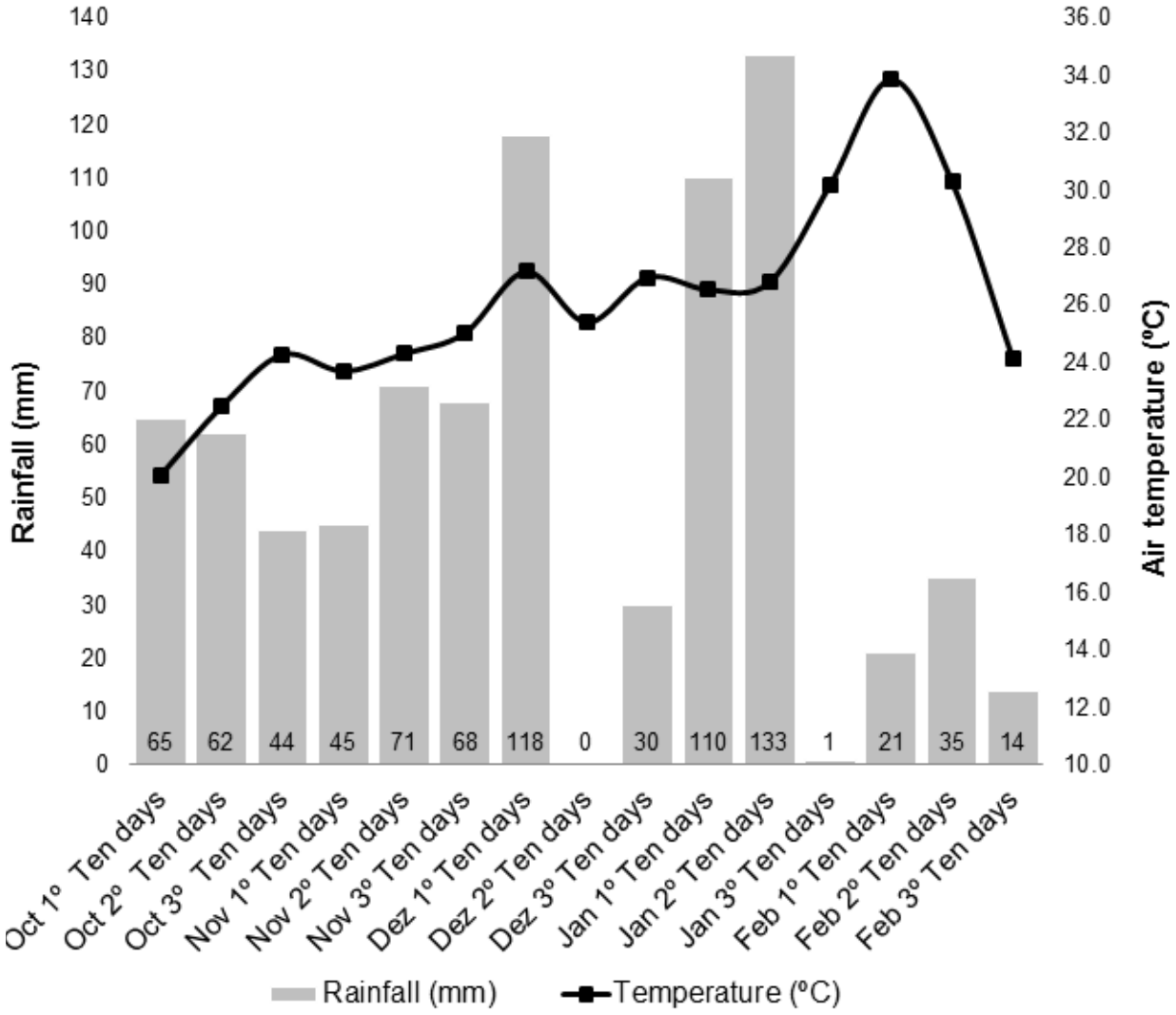

Figure 1. Ten-day cumulative rainfall data in millimeters (mm) and average temperature in degrees Celsius (°C) for the conduction period of the experiment.

and repetition were counted, and thus it was obtained the number of plants in each experimental area. They have been converted by simple rule of three to 10.000 m² thus obtaining the final population of plants per hectare. The stem diameter (STE) was measured at random, within the useful area of each treatment and repetition, the stem of ten plants in full female flowering (visible style-stigma). This measurement was carried out using a caliper (mm) in the first half expanded internode, getting the average basal diameter of the stem portion. The plant height was also made at random, using 10 plants within the useful area of each treatment and repetition, measuring from the plant lap to the curvature of the flag leaf in full female flowering (visible style-stigma), using a tape measure in cm and obtaining the average height of the plants.

After the harvest, the study sampled all the ears inside the useful area of the plot of each treatment and repetition, thus obtaining the average number of rows of grains per ear (RGE). The number of grains of all rows (GPR) of the ears in the useful area of the portion of each treatment and repetition was also counted, thus obtaining the average number of grains per row of each parcel ear.

The number of grains of all ears in the useful area of the portion of each treatment and repetition was counted, thus obtaining the average number of grains per ear (AGE). The mass of 1000 grains (MAM) was evaluated according to the Rules for Seed Analysis (Brazil, 2009). Eight repetitions of 100 seeds per plot were used. The samples were identified and weighed in a precision balance, and then placed to dry in a greenhouse at 105°C for 24 h. With the samples being withdrawn, they were weighed again and made subsequent conversion of grain moisture to 14% (wet basis). To obtain the mass of 1000 grains, a mass transformation of 800 grains to 1000 grains as simple rule of three was carried out.

The mass of the ear (MAE) was defined from the data of mass of 1,000 grains (MAM), and the average number of grains per ear (AGE) as simple rule of three. The productivity calculation in kilograms per hectare (PKH) was based on the analysis of population (POP), on the average number of grains per ear (AGE) and on the 1000 grain mass (MAM), expressing the results in productivity in kilograms per hectare (PKH). Data were tabulated and submitted to analysis of variance by F test at 5% probability of error. When the F test indicated differences between treatments, the averages were compared by Tukey test at 5% error probability

Table 1. Summary of the analyses of variance with the medium squares (M.S.) of the variables population (POP), stem diameter (SD), plants height (PH), number of grains row per ear (NRE), number of grains per row (NGR), average number of grains per ear (AGE), mass of 1000 grains (MAM), mass of the ear (MAE) and productivity kg per hectare (PKH).

Variation sources	LD	Medium square		
		POP	STE	PH
Block	3	0.2694187^{ns}	0.8888867^{ns}	0.9500556^{ns}
Plaster (with and without)	1	1736111.0^{ns}	0.5760000^{ns}	0.9712103^{ns}
Foliar Fertilization	4	4147377.0^{ns}	0.5349000^{ns}	0.1566608^{ns}
Plaster x Foliar Fertilization	4	0.1089892^{ns}	0.5508850^{ns}	0.9891615^{ns}
Residue	27	7508859.0^{ns}	0.2771570^{ns}	0.8539767^{ns}
Total	39	-	-	-
CV (%)	-	4.1668	2.3015	1.1219

Variation sources	LD	Medium square		
		NRE	GPR	AGE
Block	3	0.3166159^{ns}	28.39233^{ns}	10525.40^{ns}
Plaster (with and without)	1	0.3939477**	2.605517^{ns}	2264.419^{ns}
Foliar Fertilization	4	0.1581997*	4.662933^{ns}	1500.706^{ns}
Plaster x Foliar Fertilization	4	0.2216681^{ns}	2.280914^{ns}	720.8473^{ns}
Residue	27	0.5866546^{ns}	3.369881^{ns}	1046.463^{ns}
Total	39	-	-	-
CV (%)	-	1.5514	5.3073	5.9865

Variation sources	LD	Medium Square		
		MAM	MAE	PKH
Block	3	155.6890^{ns}	1937.924^{ns}	3666012.0^{ns}
Plaster (with and without)	1	0.5574966^{ns}	298.4334^{ns}	763854.5^{ns}
Foliar Fertilization	4	127.1457^{ns}	298.1885^{ns}	1599380.0^{ns}
Plaster x Foliar Fertilization	4	66.23129^{ns}	121.2257^{ns}	563945.8^{ns}
Residue	27	61.05655^{ns}	154.1975^{ns}	685258.5^{ns}
Total	39	-	-	-
CV (%)	-	2.1658	6.3659	6.4659

*Significant (P ≤ 0,05) by F test; ** significant (P ≤ 0,01) by F test; ns: non-significant**.**

(Table 1). The statistical program used was Sistema De Análises Estatísticas e Genéticas – SAEG (SAEG, 2007).

RESULTS AND DISCUSSION

The weather conditions in this experiment, shown in Figure 1, demonstrate satisfactory effect for the development of the crop with well-distributed rainfall (473 mm) and average minimum temperature of 20 to 27°C since germination to the phenological stage V12. Nonetheless, the drought faced in the 2nd and 3rd ten days of December (days 10 to 27), during which the corn was in growth stage R1 (silking and pollination), can be directly related to the lack of results of this work. According to Cruz et al (2008), the lack of water in this period causes poor pollination and low graining tang, that once under drought, both the "hair" and the grains tend to dissection. Water stress was extended for R2 stages which may have caused deficiency in dry matter accumulation, and R3 which although has been less critical than in the previous phase, can affect production.

According to Fancelli and Dourado-Neto (2000), the process of transpiration and evapotranspiration are responsible for the movement of water and nutrients absorbed by corn. Thus, in the absence of water, nutrient assimilation process can be affected by decreasing the accumulation of dry matter. In the 1st and 2nd periods of ten days of January, due to rainfall occurrence, the crop can continue the process of maturation, however, facing again a water deficit in the 3rd ten days of January and 1st ten days of February, along with the rise in temperature, causing an early death of plants.

Table 1 shows the results of analysis of variance with the mean squares of the set of the agronomic characteristics analyzed both in the field and in the

Table 2. Number of rows of grains per ear of corn P30F53YH in function of the foliar application in the 2013/2014 harvest, in Toledo-PR.

Foliar fertilization	Number of rows
0-0-50+S with plaster	15.7a
L-S with plaster	15.5a
Potamol-Plus with plaster	15.6a
Ammonium molybdate with plaster	15.7a
Witness with plaster	15.4a
Average	15.6
CV (%)	1.6
DMS	0.5

Lowercase letters above do not differ statistically among each other by Tukey test at 5% probability.

laboratory, based on the F significance test. The coefficients of variation (CV%) were of low magnitude, below 7%, which indicates high accuracy and demonstrates credibility in the conduction of the experimental procedures.

Analysis of variance showed that there was no significant effect on the F test in the following variables: POP, STE, PH, GPR, AGE, MAM, MAE and PKH. For the NRE variable, there was significant effect in F test ($P \leq 0.05$) in the source of foliar fertilizer variation requiring the application of Tukey's test for multiple comparison between the averages (Table 2).

When the averages were compared by Tukey test, it was concluded that there was no significant effect between foliar fertilizer treatments and NRE variable. Analyzing Plaster environments (presence or absence), it is concluded that there was no significant difference between any of the variables. Conte et al. (2013) in a similar experiment to evaluate the plaster in the development of corn grown in a Yellow Red Latosol dystrophic with plaster application 30 days prior to planting in dosages of 1000, 2000, 4000, 6000, 8000 kg ha^{-1}, obtained the same results, in other words, plaster did not significantly influenced the height and stem diameter of corn plants. This same author assessed in another experiment the plaster reaction time, applying the 75, 60, 45, 30, 15 and 0 days before planting, at a dose of 3300 kg ha^{-1}, the variables plant height and stem diameter had no significant difference, showing that even with a longer reaction time, plaster application did not lead to positive results.

According to Oliveira et al. (2007), in an experiment with plaster in the same day of seeding, the mass of 100 grains of corn had no significant difference, however, productivity increased significantly. This can be associated with an increased number of grains per ear, because as the weight of 100 grains was the same, there was a greater average in grain crop with the plaster applied, expressing this results in productivity. More promising resultson the efficiency of plaster on agronomical characteristics can be more expressive with more reaction time of this conditioner in the soil, since according to Caires et al. (2003), the effect of plaster can last for several years, justifying no significant effect of plaster application at planting time.

For the purposes of foliar fertilizers based on Mo, K and S and their impacts on the agronomic characters of all treatments, they had no effect. Similar results were also obtained by Ferreira et al. (2001), for the number of plants per plot, number of ears per plant and plant height with the application of Mo. The final stand and grain yield were not significantly affected by the application of molybdenum doses, as well as the 1000 seeds and productivity. Better results were obtained by Araújo et al. (1996), with an increase of 14.3% in production with application of 90 g ha^{-1} of Mo, and by Coelho (1997), with an increase of 39.5% in production, using a dose of 50 g ha^{-1} of Mo.

With regard to the application of potassium, the variables did not obtain positive results before their application, probably due to the large amount of K from the NPK formulation (06-30-22 to 400 kg ha^{-1}) which was added to the soil, made based on the chemical analysis of the soil and applied the formulation at the time of sowing.

As for the response to foliar application of S, the components of the production, and also Mo and K, they did not obtain significant responses. This nutrient can be supplemented to the culture in order to complete the N need of corn in the form of ammonium sulfate, (N 21%) and (S 24%) twice, stage (V4) and stage (V8) totaling 160 kg ha^{-1} of S, a factor that may be crucial to the insignificant response to the application of the element S by foliar via.

Considering the application of foliar fertilizers, the study did not obtained significant results in any of the variables with any of the nutrients, probably due to sufficient acquisition of these, by the crop, through the ground, concluding that the a correct fertilization at sowing and supplement the soil in peaks of need, are sufficient for the result of productivity.

Bearing in mind the application of agricultural plaster at the time of sowing, the plaster also had no effect on the assessed agronomic variables, probably due to the short response time of this conditioner in the soil, suggesting more visible results over the years.

Conclusion

Based on study carried out in the period of the experiment, it was not observed significant effect with increase in the agronomical characters of the corn crop in function of the plaster. With regard to the foliar fertilizer, there was no significant difference on the agronomical

variables assessed in function of its application by foliar via.

Conflict of interests

The authors have not declared any conflict of interest.

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Vol. 11(28), pp. 2475 -2480, 14 July, 2016
DOI: 10.5897/AJAR2016.11146
Article Number: 1A1937859397
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Termiticidal activities of few plant extracts against *Macrotermes subhyalinus* smeathman and *Trinervitermes geminatus* wasmann (Isoptera: Termitidae) survival

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Received 21 April, 2016; Accepted 16 June, 2016

Termites are most pestiferous insects causing damage to crop and buildings. Their control still relies mainly on harmful chemical pesticides to the detriment of eco-friendly pesticides. The aim of this study was to evaluate the effect of three plant extracts on the survival rate of termite species, *Macrotermes subhyalinus* Smeathman and *Trinervitermes geminatus* Wasmann, known to cause damage to crops, vegetation and buildings in Togo. *Cissus quadrangularis* (Vitaceae), *Pennisetum purpureum* Schumach (Poaceae) and *Vetiveria zizanoides* Nash (Poaceae) extracts were examined for their termiticidal activity against these termites. Three formulations including acetone and hexane extracts and powder were prepared for each plant species at five different concentrations. Six replicas were made for each tested concentration with 30 workers per replica in the laboratory conditions. All the tested plants showed termiticidal activities by reducing the rate of survival duration of tested termites. The powder of *V. zizanoides* was very effective on *M. subhyalinus*; its lowest concentration (5 mg/cm^2) reduced the survival rate of this termite by up to 87%. Among the extracts, acetone extract of *P. purpureum* caused the highest reduction of *M. subhyalinus* survival rate at higher concentration. Both acetone and hexane extracts of *V. zizanoides* were very effective on *T. geminatus*, reducing by more than 90% survival rate of this termite. Although these plants extract seem to bear potential termiticidal activity, however, further studies need to be carried out in order to determine their respective components.

Key words: Botanical insecticides, *Cissus quadrangularis*, *Pennisetum purpureum*, *Vetivera zizanoides*, termite species.

INTRODUCTION

Environmental problems caused by the persistent pesticides have gradually led to an increasing interest in the development of alternative pest control methods. With the prohibition of the chemicals, which were toxic to humans, termite control is currently centered on the use of non-chemical methods including the use of biopesticides (Verma et al., 2009; Rust, 2014).

Thus, plants with insecticidal properties could be regarded as potential alternatives to chemical pesticides. Indeed, various plants or plant extracts are used (under

various formulations) to control termites or other insect pests (Isman, 2006; Dhang and Sanjayan, 2014). A broad range of plants are toxic, repellent, or have some antifeeding properties several of which were regarded as insecticides (Bläske and Hertel, 2001; Ganapaty et al., 2004, Boulogne et al. 2012; Raina et al. 2012; Addisu et al. 2014).

Osipitan and Oseyemi (2012) have evaluated the insecticidal effect of three plant extracts including *Citrus sinensis*, *Theobroma cacao*, *Tithonia diversifolia* and *Anacardium occidentalis*, against *Macrotermes bellicosus*. They found that the acqueous extract of these plants not only caused the mortality of tested termites, but also showed an important repellency to them. The repellent effect of extracts from few tropical plants on termite has also been reported by Maistrello et al. (2011), Osipitan et al. (2013) and Acda (2014).

The termiticidal proprieties of plants lie mainly on their secondary compounds or organic extractives such as waxes, alkaloids, fat, gums, resins, terpenes and essential oils (Pettersen, 1984). Thus, some flavonoids extracted from the Japanese larch and *Larix leptolepis* (Lamb) showed strong feeding deterrent activities against *Coptotermes formosanus* (Shiraki) (Ohmura et al., 2000; Chen et al., 2004). Recently Chowański et al. (2016) reported the toxicity caused by alkaloids from few Solanaceae plants. According to these authors, both formulations (metabolites and water/alcohol extracts) showed lethal and sublethal effects on tested termites and other pestiferous insects.

Locally, plants or plant products are used by farmers to control termites. Some species of living plants are usually left inside the farms in order to deter termites from crops (Sileshi et al., 2008; Mugerwa et al., 2014). In Togo, some farmers believe that keeping plants such as *Vetivera zizanoides*, *Cissus quandrangularis* and *Pennisetum purpureum* within the crop field deter pestiferous termites (especially *Macrotermes* and *Trinervitermes* species). However no scientific study has yet been carried regarding this assertion. Hence the aim of this study is to check the termiticidal activity of extracts from these plants through the evaluation of their effect on the survival rate of *Macrotermes subhyalinus* and *Trinervitermes geminatus*, termite species known to cause damage to crops, vegetation and buildings in Togo.

MATERIALS AND METHODS

Plant materials

The plants used in these experiments include *C. quadrangularis* (Vitaceae) *P. pupureum* (Poaceae) and *V. zizanoides* (Poaceae). The leaves of *V. zizanoides* and the stems with leaves of *C. quadrangularis* were collected in the botanical garden of the Faculty of Science of the University of Lomé (Togo). The leaves of *P. pupureum* were bought in the market at Lomé. In the laboratory, these vegetable materials were cut into small pieces and dried in an air-conditioned room. After drying, they were finely crushed using a blender (OPTIBLEND 2000 TRIO) and sieved. The fine powder obtained was stored at -20°C for further use. The non-fine portion was macerated in acetone and hexane for a week then filtered and evaporated with the rotavapor in cold conditions. The extracts obtained were also stored at - 20°C.

Termites

Macrotermes subhyalinus and *Trinervitermes geminatus* were used in the biological tests. These termites were collected on the campus of the University of Lomé and acclimatized to laboratory conditions (28°C, Relative Humidity 70%) in total darkness (12:12H DD). During acclimatization, they were fed on fungus comb and filter paper for *M. subhyainus* and on straw for *T. geminatus*.

Biological tests

The biological tests were carried out by contact following Raina et al. (2012). Three formulations, powder, acetone and hexane extracts were used. Concentrations were expressed in mg/cm². Each concentration of powder was introduced into a glass Petri dish of 9 cm diameter.

The powder was then distributed uniformly over the surface of Petri dish. The plant extracts (acetone and hexane extracts) were initially dissolved in 5 ml of acetone or hexane respectively. The solution obtained from each concentration was put in a glass Petri dish (9 cm diameter) whose bottom was covered with filter paper. The soaked paper was air dried for 24 h. The different concentrations used are: 0.5, 1, 2, 4 and 6 mg/cm^2.

Thirty workers of each termite species were exposed to each concentration and six replications were made. The Petri dishes containing the tested termites were then placed in controlled conditions of 28°C temperature and 78% relative humidity in total darkness.

After each hour and during six hours, all the Petri dishes were checked for dead termites. After the sixth hour, the checking was done at the eighteenth hour then after each 24 h until the death of all termites. The controls were done with filter paper only for tests with plant powder, and filter paper previously soaked with acetone or hexane and air dried.

Determination of survival duration

The survival duration of termites was determined according to the formula below (adapted from Krebs, 1999).

$$Sd = \frac{\Sigma \text{Nli x ti}}{\Sigma \text{ti}}$$

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Sd, survival duration
Nli, Number of living termites at each checking time
ti, checking time

Survival rate was determined using the formula,

$$Sr = \frac{\text{Sdi}}{\text{Sdc}} \text{ x } 100$$

Sr, Survival rate
Sdi, mean survival duration obtained with each tested concentration and 6 different replicas.
Sdc, mean survival duration obtained in control

The rate of survival reduction was also determined with the survival rate:

$$Rr = 100 - \text{Sr}$$

Rr, survival reduction rate

Statistical analysis

The data obtained from calculation of survival duration and the rate of survival reduction were subjected to an analysis of variance (ANOVA) at 5%, and the means were discriminated with the Student-Newman-Keuls (SNK) test using STATISTICA software, version 5.1 (1998).

RESULTS

Effect of plant powders on *M. subhyalinus*

The powders of *P*. *purpureum*, *C*. *quadrangularis* and *V*. *zizanoides* reduced the survival duration of *M*. *subhyalinus* at all the tested concentrations (Table 1). At the highest concentration (6 mg/cm^2), the reduction rates were 80, 78 and 90% respectively with *P*. *purpureum*, *C*. *quadrangularis*, and *V*. *zizanoides*. At 0.5 mg/cm^2 the powder of *V*. *zizanoides* significantly reduced the survival rate of *M*. *subhyalinus* (F = 83; df = 5; P < 0.001). Among the plant powders, *V*. *zizanoides* caused the highest reduction of survival rate. On the other hand, *C*. *quadrangularis* (F = 44.11; df = 5; P < 0.001) was more effective than *P. purpureum* (F = 33.65; ddf = 5; P < 0.001) at low concentration but they had the same effect at 4 and 6 mg/cm^2.

Effect of plant extracts on *M. subhyalinus*

Like the plant powders, acetone and hexane extracts also affected the survival rate of *M*. *subhyalinus*. At the lowest concentrations (0.5 and 1 mg/cm^2), the acetone extract of *V*. *zizanoides* (F = 20.4; df = 6; P < 0.0001) caused more survival reduction (34 and 48% respectively) than *C*. *quadrangularis* (16 and 30% respectively) and *P*. *purpureum* (3 and 8% respectively) at the same concentrations (Table 1). At these concentrations (for acetone extracts), *P*. *purpureum* was less effective than the other two plants. Nevertheless, at the higher concentrations (4 and 6 mg/cm^2) *P*. *purpureum* (F = 40.26; df = 6; P < 0.0001) and *C*. *quadrangularis* (F = 42.23; df = 6; P < 0.0001) became more effective than *V*. *zizanoides* with 95% (both two plants) reduction rate against 73% for the former at 6 mg/cm^2 (Table 1).

With the hexane extracts, the threshold of 50% reduction rate was observed at 4 mg/cm^2 contrary to the acetone extract (Table 1). Nevertheless, like the acetone extract, the hexane extract of *V*. *zizanoides* was more effective from 0.5 mg/cm^2 to 2 mg/cm^2 than the other two plants (F = 12.3; df = 6; P < 0.0001). It was followed respectively by *C*. *quadrangularis* (F = 30.1; df = 6; P < 0.0001) and *P*. *purpureum* (F = 16.5; df = 6; P < 0.0001). At 6 mg/cm^2 *C*. *quadrangularis* was the most effective with a reduction rate of 95% while *V*. *zizanoides* reduced only 56% of the survival rate (Table 1). But from 1 to 6 mg/cm^2 both acetone and hexane extracts of *C*. *quadrangularis* were pasty and this considerably reduced the movement of termites.

Effet of plant powders on *T. geminatus*

The results showed that plant powders caused a significant reduction of *T. geminatus*' survival duration (F = 8.48; df = 5; P < 0.0001; F = 40; df = 5; P < 0.0001; and F = 51.4; df = 5; P < 0.0001, respectively for *P*. *purpureum*, *C*. *quadrangularis* and *V*. *zizanoides*). At the lowest concentration (0.5 mg/cm^2), all plant powders reduced the survival rate to more than 50% (Table 1). The effect of these powders seemed not to be influenced by concentration (within each plant species) and was also comparable from one plant to another

Effect of plant extracts on *T. geminatus*

All the tested extracts reduced the survival duration of *T*. *geminatus* (P < 0.0001). At 0.5 mg/cm^2 the acetone extract of *C*. *quadrangularis* (F = 129; df = 6; P < 0.0001) affected termite survival with 53% reduction, and at 6 mg/cm^2 it caused the highest reduction rate (97%) (Table 1). *V*. *zizanoides* acetone extract was the second most effective on *T*. *geminatus* especially from 1 to 6 mg/cm^2 (F = 96.6; df = 6; P < 0.0001). The dose 4 mg/cm^2 of *P*. *purpureum* was more effective (F = 44.5; df = 6; P < 0.0001) than that of 6 mg/cm^2 of the same plant. In the latter concentration, the extract was highly stuck to the filter paper. The lower concentration (0.5 mg/cm^2) of hexane extracts of these plants reduced the survival rate to more than 50% (Table 1). It was respectively 63, 54 and 59% for *P*. *purpureum*, *C*. *quadrangularis* and *V*. *zizanoides* (Table 1). The highest reduction was recorded

Table 1. Rate of the survival reduction caused by the tested plants on termites.

Formulation	Concn (mg/cm^2)	*Macrotermes subhyalinus*			*Trinervitermes geminatus*		
		Rate of survival reduction(%)					
		Pp	***Cq***	***Vz***	***Pp***	***Cq***	***Vz***
Powder	0	0*±0.27^{a}	0*±0.27^{a}	0*±0.27^{a}	0±0.26^{a}	0±0.26^{a}	0±0.26^{a}
	0.5	50±0.19^{b}	58±0.05^{b}	87±0.01^{b}	54±0.09^{b}	64±0.25^{b}	54±0.2^{b}
	1	66±0.08^{bc}	75±0.06^{c}	88±0.01^{b}	64±0.21^{b}	73±0.25^{b}	66±0.15^{b}
	2	68±0.04^{cb}	76±0.05^{c}	88±0.009^{b}	78±0.04^{c}	77±0.13^{b}	66±0.08^{b}
	4	77±0.07^{c}	76±0.03^{c}	90±0.04^{b}	79±0.11^{c}	77±0.1^{b}	69±0.13^{b}
	6	80±0.05^{c}	78±0.07^{c}	90±0.02^{b}	82±0.03^{c}	82±0.12^{b}	84±0.16^{c}
Acetone extract	0+	0±0.31^{a}	0±0.31^{a}	0±0.31^{a}	0±0.3^{a}	0±0.3^{a}	0±0.3^{a}
	0-	2±0.27^{a}	2±0.27^{a}	2±0.27^{a}	1±0.26^{a}	1±0.26^{a}	1±0.26^{a}
	0.5	3±0.27^{a}	16±0.21^{ab}	34±0.19^{b}	48±0.16^{b}	53±0.07^{b}	42±0.14^{b}
	1	8±0.15^{a}	30±0.13^{b}	48±0.15^{bc}	56±0.13^{b}	76±0.08^{c}	78±0.1^{c}
	2	52±0.1^{b}	74±0.03^{c}	58±0.13^{cd}	62±0.16^{b}	94±0.01^{d}	84±0.08^{c}
	4	84±0.09^{cd}	86±0.03^{cd}	68±0.08^{cd}	85±0.05^{c}	94±0.02^{de}	88±0.07^{c}
	6	95±0.01^{d}	95±0.02^{d}	73±0.09^{d}	61±0.21^{b}	97±0.005^{df}	91±0.05^{c}
Hexane extract	0+	0±0.31^{a}	0±0.31^{a}	0±0.31^{a}	0±0.26^{a}	0±0.26^{a}	0±0.26^{a}
	0-	7±0.27^{a}	7±0.27^{a}	7±0.27^{a}	9±0.26^{a}	9±0.26^{a}	9±0.26^{a}
	0.5	9±0.31^{b}	19±0.11^{b}	29±0.21^{b}	63±0.07^{b}	54±0.11^{b}	59±0.12^{b}
	1	19±0.12^{b}	28±0.19^{b}	45±0.1^{bd}	64±0.05^{b}	58±0.11^{b}	66±0.09^{b}
	2	32±0.19^{b}	41±0.18^{c}	47±0.07^{bd}	65±0.2^{b}	73±0.09^{c}	83±0.13^{c}
	4	66±0.11^{c}	68±0.03^{d}	54±0.2^{cd}	86±0.05^{c}	80±0.07^{c}	87±0.05^{c}
	6	74±0.12^{c}	95±0.01^{e}	56±0.05^{d}	89±0.05^{c}	96±0.009^{d}	93±0.03^{d}

at 6 mg/cm^2 with *C*. *quadrangularis* (96%) followed by *V*. *zizanoides* (93%) and *P*. *purpureum* (89%). Here also, from 1 to 6 mg/cm^2 both acetone and hexane extracts of *C*. *quadrangularis* were pasty.

DISCUSSION

The powders of these tested plants were effective against *M*. *subhyalinus*. The effect of *V*. *zizanoides* was very spectacular even at the lower concentration. This is likely due to the higher amount of silica (SiO_2) present in the leaves of Vetiver grass.

According to Methacanon et al. (2003), silica content of *V. zizanoides* leaves is about 50%. This inorganic component not only affects positively plants growth but also confers to them a certain resistance against phytophagous insects and other wood eating microorganisms (Djamin and Pathak, 1967; Massey et al., 2007). It is also known that the essential oil extracted from the root of Vetiver grass contains more than 300 compounds belonging to the sesquiterpene group of natural products including α and β vetivones (Jain et al., 1982; Zhu et al., 2001a), and nootktanone (Zhu et al., 2001b) which were all reported as insect repellents.

However, leaves but not roots were used in our study. So we could not sustain the presence of these repellent components within the leaves, since there is no reliable data on the essential oil extracted from leaves of Veriter grass. Acetone and hexane extracts also affected the survival rate of *M*. *subhyalinus*. Among the plant species, *V*. *zizanoides* extracts (acetone and hexane) were not effective as was the case with other plant species on the survival rate of *M*. *subhyalinus* at higher concentrations (contrary to the two lowest concentrations).

This suggested that the most active compounds which had an insecticidal property in *V*. *zizanoides* against *M*. *subhyalinus* may be volatiles. These volatiles are abundantly present in *V*. *zizanoides* essential oil extracted from roots (Zhu et al., 2001a, b). The survival reduction rate observed in acetone and hexane extracts of *C*. *quadrangularis* was likely due essentially to the physical aspect of the extracts than their toxicity. Indeed all the extracts were pasty (except at the lower concentration) which considerably reduced the movement of termites. On the other hand, the acetone and hexane extracts of *P*. *purpureum* were not pasty. These extracts were very effective especially at the highest concentration of

acetone extract.

T. *geminatus*' survival rate was also affected by plant powders and plant extracts. Like the test with *M*. *subhyalinus*, the threshold of 50% survival rate reduction was observed with all the plant powders. But here, *V*. *zizanoides* (like the other plants) powder seemed to be less effective on *T*. *geminatus* than on *M*. *subhyalinus*. The lowest concentration of *V*. *zizanoides* caused more survival rate reduction of *M*. *subhyalinus* than did the highest concentration on *T*. *geminatus*. This could be explained by the feeding habit of *T*. *geminatus*. Indeed *T*. *geminatus* like other *Trinervitermes* feed essentially on herbaceous plants (Ohiagu and Wood, 1976; Wood, 1978). They may have developed a certain resistance to some defensive extracts of these grasses (Harborne, 1982) than did *M*. *subhyalinus*. Thus *T*. *geminatus* was less affected by plant powders than *M*. *subhyalinus* which seemed to be polyphagous, preferentially eating bark, wood or living plants (Anani Kotoklo et al., 2009) rather than grasses. Nevertheless, *T*. *geminatus* could not overcome the defensive extracts of these plants as there was a significant difference between the controls and all concentrations of plant powders.

Acetone and hexane extracts of *P*. *purpureum* seemed to have almost the same effect on *T*. *geminatus* at the highest concentrations. In the test with acetone extract, more reduction of survival rate occurred with 4 mg/cm^2 than 6 mg/cm^2 (actually the highest). Because, extract with 6 mg/cm^2 concentration was highly stuck to the filter paper, and could not be removed by the leg of *T*. *geminatus* (contrary to *M*. *subhyalinus* which is stronger). Thus there was less contact between the extract and the termite. The acetone and hexane extracts of *V*. *zizanoides* were very effective on *T*. *geminatus* especially at higher concentration unlike *M*. *subhyalinus* (more susceptible to powder than extracts). Therefore the active components of *V*. *zizanoides* against *T*. *geminatus* might be less volatile.

CONCLUSION

All the three tested plants showed antitermite activity by reducing the survival rate of *M*. *subhyalinus* and *T*. *geminatus*. The powder of *V*. *zizanoides* was very effective against *M*. *subhyalinus* at lower concentration. It was also very vulnerable to the higher concentration of the acetone extract of *P*. *purpureum*. Both acetone and hexane extracts of *V*. *zizanoides* were highly effective against *T*. *geminatus*. All the extracts of *C*. *quadrangularis* were pasty, thus unsuitable for these experiments.

Conflict of interests

The authors have not declared any conflict of interests.

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Vol. 11(28), pp. 2481-2485, 14 July, 2016
DOI: 10.5897/AJAR2016.11250
Article Number: AE3FA4C59398
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Emergence and early growth of baru seedlings on different substrates

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Received 23 May, 2016; Accepted 16 June, 2016

This study aimed to evaluate the effect of substrates in the emergence and early development of Baru seedlings. The experiment was performed in a greenhouse in a completely randomized design with five treatments (substrates) and four replications. The substrates were: S_1) Oxisol; S_2) Oxisol + washed sand (1: 1 v: v); S_3) washed sand; S_4) Oxisol + washed sand + manure (1: 1: 1 v: v: v); S_5) Oxisol + sand washed + poultry litter (1: 1: 1 v: v: v). The emergence, emergence speed index, seedling height (SH), stem diameter (SD), root length, shoot dry mass (SDM), root dry mass (RDM), SH/SD and SDM/RDM ratios and the Dickson quality index were evaluated. The emergence and early development of seedlings were influenced by the type of substrate. The Oxisol substrate is the most efficient for the early development of Baru seedlings.

Key words: *Dipteryx alata* Vog, emergence, germination, reforestation.

INTRODUCTION

Baru (*Dipteryx alata* Vog., Fabaceae) is a native forest species in Brazil that is preferably propagated via seeds. The species is important for reforestation, wood production and human consumption (Sano et al., 2006). Baru nuts have a high nutritional value, being a source of minerals such as iron, zinc, calcium, protein, unsaturated fatty acids and tannins (Takemoto et al., 2001; Marin et al., 2009) and the seeds are commercialized. Baru fruits and seeds show great variations (Corrêa et al., 2000; Corrêa et al., 2008; Zuffo et al. 2014a).

Due to the great importance of Baru in food and for being a reforestation species, the recovery of areas with this species becomes timely, being important to produce seedlings with high quality and low cost (Zuffo et al., 2014b), so that the production of seedlings becomes feasible. In the production of seedlings, the substrate to be used is one of the most important factors because it provides ideal conditions for the seedling germination and the root system development (Negreiro et al., 2004; Ajalla et al., 2012). There are several types of substrates, such

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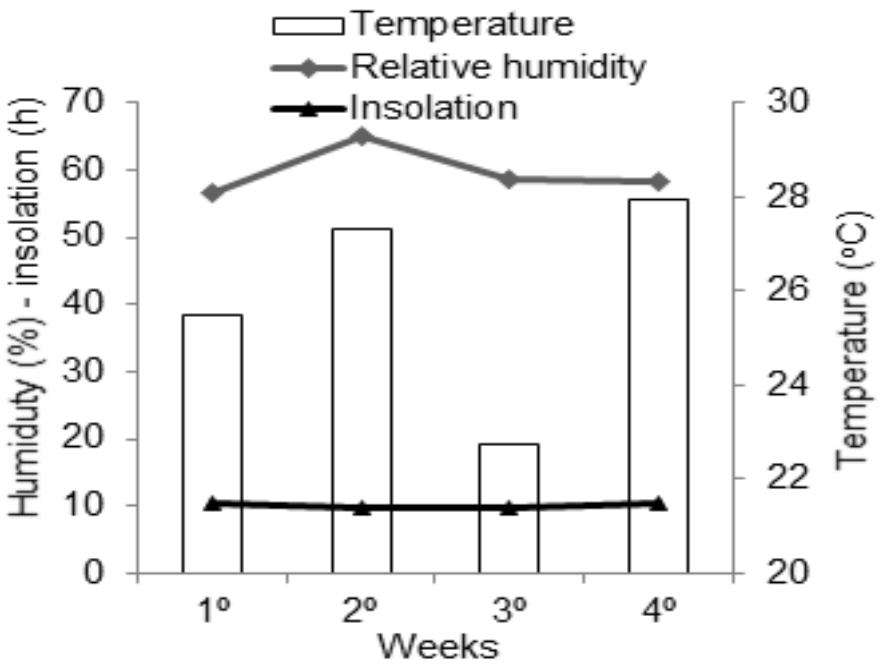

Figure 1. Average temperature, relative humidity and insolation in the period of 08/24/2013 to 09/21/2013 (Source: INMET - Nova Xavantina-MT station).

as subsoil, organic compound, vermiculite, sand, animal manure, sawdust and decomposed trees bark (Wendling et al., 2006).

According to Silva et al. (2011) substrates should have good structure, aeration, water retention capacity and low degree of contamination by pathogens, and these characteristics vary according to the material used in the substrate composition and may affect the germination and establishment of seedling, demonstrating the importance of choosing the ideal substrate composition.

Regarding the production of forest seedlings, each species has a suitable substrate for the formation of seedlings. For example, Albuquerque et al. (2013) observed that the growth of seedlings of sucupira (*Bowdichia virgilioides* Kunth) is favored with the use of a substrate made of Cerrado soil and vermiculite + black soil + carbonized rice husk in a proportion of 1: 1: 1. However, to Cavalcante et al. (2011), the most suitable substrate for the production of Gurguéia nut seedlings (*Dypteryx lacunifera* Ducke) is the washed sand.

Therefore it is demonstrated that there are different responses in the production of forest seedlings depending on the substrate type. Thus, this study aimed to evaluate the effect of substrates on the emergence and early development of Baru seedlings.

MATERIALS AND METHODS

The experiment was performed at União farm, municipality of Nova Xavantina-MT (14°50'41"S, 52°22' 49"W, with average altitude of 290 m) during the period of 08/24/2013 to 09/21/2013 in a protected environment at 50% of brightness. The region climate is Aw according to the Koppen global climate classification, with two well defined seasons, a dry season that lasts from May to September and a rainy one that lasts from October to April. The climatic data were collected at the meteorological station of the National Institute of Meteorology - INMET and are shown on Figure 1.

The seeds for the experiment were collected from fruits coming from native trees in the municipality of Nova Xavantina-MT. After harvest, the fruits were taken to the laboratory for selection and standardization, and it was promoted the fruit cut and the seeds were taken for sowing. The experiment was performed in a completely randomized design, with five treatments (substrates) and four replications, each plot consisted of 20 seeds. The evaluated substrates were: S1) Oxisol; S2) Oxisol + washed sand (1: 1 v: v); S3) washed sand; S4) Oxisol + washed sand + manure (1: 1: 1 v: v: v); S5) Oxisol + sand washed + poultry litter (1: 1: 1 v: v: v).

The homogenization of the substrate mixture was performed manually and then placed in perforated plastic trays with 7 L capacity (46 cm x 29 cm x 6.5 cm). The Oxisol was removed from a layer of 0 to 20 cm deep in a Cerrado area and the following physical characteristics were found in the soil analysis: 500, 100 and 400 g kg^{-1} of sand, silt and clay, respectively. Each replication was consisted of a tray. For the sowing, the seeds were placed in the 'hilum down' position, according to the recommendation of Zuffo et al. (2014b).

At 15 days after sowing, a daily assessment of the number of emerged seedlings up to 28 days started, the percentage of emergence and emergence speed index (ESI) of each treatment were determined. To calculate the ESI, the formula of Maguire (1962): ESI=[N1/1+(N2-N1)/2+(N3-N2)/3+....(Nn-Nn-1)/n], where N1, N2, N3...Nn was adopted, which corresponds to the number of emerged plantlets and 1, 2, 3...n, being the number of days after sowing (DAS).

At 28 days after sowing: seedling height (cm) - determined from

the soil surface to the insertion of the last leave with the aid of a millimeter ruler; stem diameter (cm) - measured at the height of the surface stem plant by reading in Clarke® digital caliper; root length (cm) - taproot, with the aid of a millimeter ruler were evaluated. Seedlings were separated into shoot and root system, placed in paper bags and taken into a forced air circulation oven for 72 h at 60°C until constant weight, in order to determine the shoot and root dry mass (g).

From these evaluations, the total dry mass (TDM) were determined, and the morphological indices were calculated: height ratio (cm) / stem diameter (mm) (SH/SD); shoot dry mass /root dry mass (SDM / RDM) and Dickson quality index (DQI) (Dickson et al., 1960), by the equation:

DQI = total dry mass/(height/diameter) + (shoot dry mass/root dry mass).

The obtained values were submitted to analysis of variance, and, when significant, the means were grouped by Scott-Knott criteria at 5% of significance using the statistical program SISVAR® (Ferreira, 2011).

RESULTS AND DISCUSSION

All the parameters were significantly influenced by the type of substrate, except the stem diameter and the SDM/RDM ratio (Table 2). The S_1 substrate (Oxisol) provided highest values of emergence and emergence speed index (81.25% and 1.78, respectively); which is statistically different from the others (Table 3).

These results are similar to those obtained by Rosa et al. (2006), which also found a higher emergence speed index in baru seedlings using only the soil as substrate. It is important to emphasize that the seedlings that emerge first, tend to grow more and produce more biomass due to the photosynthesis in the early stages of growth, except in stressful conditions. The S1 substrate (Oxisol) has lower sand content in its composition. According to Silva et al. (2011), this component has a high drainage capacity, and does not hold the required amount of water that would start the germination process, which may have adversely affected the emergence and emergence speed index.

There was no significant statistical difference between the substrates for the stem diameter (Table 3). The absence of significant effect of substrates in stem diameter was verified by Cavalcante et al. (2011), studying the Gurguéia nut (*Dypterix lacunifera* Ducke), species of the same family of baru. By analyzing the height of seedlings, the S_1 substrates (oxisol), S_2 (oxisol + washed sand) and S_3 (washed sand) have provided higher means, being statistically different from the others (Table 3). These results are partially similar to those obtained by Sobrinho et al. (2010) that evaluated different substrates for the production of baru seedlings. The authors found that the substrates that contained only soil (Alfissol eutrophic), or soil + cattle manure or soil + carbonized rice husk showed greater plant height.

The S_1 substrate (oxisol) also promoted the root growth and root dry mass (Table 3). Such findings corroborate to those observed by Sobrinho et al. (2010), in which the authors also obtained better root growth and root dry mass with the exclusive use of soil. Probably, with a larger root growth and root dry mass, seedlings tend to absorb a greater amount of water and nutrients by the roots, which may promote a better seedlings growth. Moreover, the substrate has smaller sand content mixed to its composition, presenting no water loss due to the drainage. Regarding the shoot dry mass, it was verified that the S_1 substrate (oxisol) and S_2 (oxisol + washed sand) had higher means. Similar results were obtained by Rosa et al. (2006) for this parameter.

For the SH/SD ratio, it is observed that the substrates S_1 (oxisol), S_2 (oxisol + washed sand) and S_3 (washed sand) had higher means, being statistically different from the others (Table 3). For the SDM/RDM ratio, no statistical difference between the substrates was verified. However, for the Dickson quality index, the S_1 substrate (oxisol) obtained the greater mean, differing statiscally from the others. It should be noted that this index is a good indicator of seedlings quality (Fonseca et al., 2002), however, to obtain this index, the destruction of the seedlings is required (Andrade et al., 2012).

Overall, it was observed that the substrate S_1 (oxisol) was superior to the others, because it provides suitable conditions of moisture and aeration, favoring the emergence and early seedling growth (Table 3). Additionally, Neto et al. (2000) reported that species with larger seed size and weight, have greater availability of stocks in order to ensure the initial growth. Therefore, asbaru has large cotyledons, it probably have been responsible for the supply of nutrients for the seedling.

Moreover, Sousa et al. (2015) evaluated nitrogen and potassium in baru seedlings and concluded that this species is undemanding to those macronutrients. Besides the low demand in the early stage and the contents in the S_1 substrate (oxisol) may be sufficient for the baru seedlings.

The highest values of emergence, emergence speed index, root length, shoot dry mass, root dry mass, SH/SD ratio and the Dickson quality index showed by the use of S_1 (oxisol), may indicate that the baru species, as a native species from naturally poor and acid pH soils, shows adaptability to this type of soil conditions, and therefore, does not respond well to the increase of organic matter and fertility, caused by the substrate that had the addition of manure and poultry litter (S_4 and S_5).

In Table 1, it is verified that the S_1 (oxisol) has the lowest pH and low fertility. For Melo et al. (1998) the native species of the Cerrado, which has low fertility, do not respond to fertility improvement of the substrate, and this increase in fertility, and can even harm the seedlings development. Moreover, as native species and due to their evolutionary characteristics such as hardiness, it is well suited to harsh conditions (Zuffo et al., 2014c). Those authors also verified such hardiness in cajuí (*Anacardium microcarpum* Ducke).

Table 1. Chemical composition of the substrates before the experiment installation at União Farm, Nova Xavantina, MT.

Substrate	pH	OM[1]	P (Mehlich)	K	Ca	Mg	Al	H + Al
	$CaCl_2$	g dm^{-3}	mg dm^{-3}		$cmol_c$ dm^3			g dm^{-3}
S_1	4.4	1.99	10.31	96.00	1.80	0.80	0.50	4.52
S_2	4.9	1.18	18.04	74.00	1.70	0.50	0.10	1.66
S_3	6.1	2.23	84.55	36.00	2.20	0.60	0.00	2.59
S_4	5.8	4.29	76.96	112.00	5.20	4.20	0.10	1.86
S_5	5.4	4.14	393.55	496.00	5.60	3.20	0.10	2.52

Substrate	V[2]	CTC[3]	Fe	B	Mn	Zn	Cu	S
	%	$cmol_c dm^{-3}$			mg dm^{-3}			
S_1	38.62	7.37	182.13	0.04	42.15	2.22	0.51	13.08
S_2	59.01	4.05	133.79	0.10	28.42	1.57	0.40	11.68
S_3	54.44	5.69	72.04	0.06	26.70	2.29	0.16	11.68
S_4	83.87	11.55	76.96	112.00	5.20	4.20	0.10	1.86
S_5	69.03	14.59	393.55	496.00	5.60	3.20	0.20	4.52

S_1) Oxysol; S_2) Oxysol + washed sand (1:1 v:v); S_3) washed sand; S_4) Oxysol+ washed sand + manure (1:1:1 v:v:v); S_5) Oxysol + washed sand + poultry litter (1:1:1 v:v:v).[1]). O.M: Organic Matter; [2]V: Base Saturation; [3]CEC: Cation Exchange Capacity at pH 7.0.

Table 2. Variance analysis on the emergence (E), emergency speed index (ESI), seedling height (SH), stem diameter (SD), root length (RL), shoot dry mass (SDM), root dry mass (RDM), SH/SD ratio, SDM/RDM and Dickson quality index (DQI), obtained in the experiment of substrates types for the formation of baru seedlings [*Dipteryx alata* Vog], Nova Xavantina - MT, 2013.

Variation sources	DF	Mean squares				
		E	ESI	SH	SD	RL
Substrate	4	630.0000**	0.6201**	1.1435*	0.1053^{ns}	31.6123**
Replication	3	27.9166	0.0156	0.1714	0.0879	3.0263
Residues	12	63.3333	0.0197	0.2882	0.1113	2.3129
CV (%)	-	12.89	11.75	14.31	10.25	14.24

Variation sources	DF	Quadrados médios				
		SDM	RDM	SH/SD	SDM/RDM	DQI
Substrate	4	0.0142**	0.0177**	0.0722**	0.2755^{ns}	0.0125**
Replication	3	0.0001	0.0011	0.0025	0.0909	0.0006
Residues	12	0.0016	0.0014	0.0114	0.1046	0.0014
CV (%)	-	15.07	20.16	9.32	21.03	21.34

** and * significant at 1 and 5% respectively, by F test.

Table 3. Emergence (E), emergence speed index (ESI), seedling height (SH), stem diameter (SD), root length (RL), shoot dry mass (SDM), root dry mass (RDM), SH/SD ratio, SDM/RDM ratio and Dickson quality index (DQI), obtained in the experimente of substrates types for the formation of baru seedlings (*Dipteryx alata* Vog), Nova Xavantina - MT, 2013.

Substrate	E (%)	ESI	SD (mm)	SH (cm)	RL (cm)
S_1	81.25 ^{A}	1.78 ^{A}	3.41 ^{A}	4.31 ^{A}	15.27 ^{A}
S_2	67.50 ^{B}	1.41 ^{B}	3.29 ^{A}	4.22 ^{A}	11.25 ^{B}
S_3	55.00 ^{C}	1.03 ^{C}	3.22 ^{A}	3.81 ^{A}	8.12 ^{B}
S_4	52.50 ^{C}	0.82 ^{C}	3.34 ^{A}	3.26 ^{B}	9.75 ^{B}
S_5	52.50 ^{C}	0.93 ^{C}	2.99 ^{A}	3.15 ^{B}	9.01 ^{B}

Substrate	RDM (g)	SDM (g)	SH/SD	SDM/RDM	DQI
S_1	0.29 ^{A}	0.33 ^{A}	1.26 ^{A}	1.14 ^{A}	0.26 ^{A}

Table 3. Contd.

S_2	0.19 ^{B}	0.32 ^{A}	1.28 ^{A}	1.71 ^{A}	0.18 ^{B}
S_3	0.15 ^{B}	0.27 ^{B}	1.18 ^{A}	1.78 ^{A}	0.14 ^{B}
S_4	0.18 ^{B}	0.25 ^{B}	0.97 ^{B}	1.39 ^{A}	0.18 ^{B}
S_5	0.11 ^{C}	0.18 ^{C}	1.05 ^{B}	1.65 ^{A}	0.11 ^{B}

Means followed by the same letter in the columns do not differ significantly by the Scott-Knott test at 5%. S1) Oxisol; S2) Oxisol + washed sand (1: 1 v: v); S3) washed sand; S4) Oxisol + washed sand + manure (1: 1: 1 v: v: v); S5) Oxisol + sand washed + poultry litter (1: 1: 1 v: v: v).

Conclusion

The S_1 substrate (oxisol) is the most efficient for early growth of baru seedlings for providing better emergence, emergence speed index, seedling height, root length, shoot dry mass, root dry mass, SH/SD ratio and Dickson quality.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(28), pp. 2486-2498, 14 July, 2016
DOI: 10.5897/AJAR2016.11156
Article Number: 191C44359403
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Contribution of previous legumes to soil fertility and millet yields in West African Sahel

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Received 26 April, 2016; Accepted 8 June, 2016

Studies on combined effects of 4 legume crops residues and rock phosphate application on pearl millet yield were undertaken on sandy acid soil field from 2012 to 2015 at ICRISAT Sahelian center (ISC)-Sadore, Niger. The objective of the experiment was to assess the best combination of legume species x rate of crop residue x rock phosphate doses that can sustainably improve pearl millet yield in cereal monoculture system with a low input cost and minimum soil tillage. Over 3 years, the residual effect of previous legume crop residue significantly improved not only the grain yield (P<0.001) and dry residue yields (P<0.001) but also the growth parameters of pearl millet than millet mono-cropping. Treatments with or without natural rock phosphate did not show any statistical differences on millet yield while adding a micro dose of urea improved significantly the yield (P<0.001). The interaction effects of preceding legume crops in rotation with millet and restitution of dry residue on the earlier mentioned parameters across 3 years mono-cropping were studied in this experiment.

Key words: Millet, legume, cropping system, soil fertility.

INTRODUCTION

The practice of mono-cropping drastically reduces soil nutrients (Beninga, 2014). In Sub Saharan Africa, pearl millet yield is constantly very low because of monoculture and mining of nutrients by the cropping pattern followed in the previous rainy season crop. Many other causes of low productivity can be cited but the difficult availability and high cost of fertilizers have contributed greatly to the reduction of agricultural soils productivity in the Sahelian Western Africa (Bado and Kumar, 2002, Bado, 2012; Adamou et al., 2007).

The use and good management of locally available legume crop residues, and rock phosphate fertilizer can be an alternative for improving the soil fertility at lower cost. Many studies have been carried over the last two decades and have proved the efficiency of crop rotation combined with chemical and organic fertilizers on increasing crop yield (Bationo et al., 2004 Saidou et al., 2009). Soil content in Nitrogen and soluble phosphorus are two limiting factors that are determinant in cereal grain and dry residue yields (Bationo and Ntare 2006).

As such, all soil fertility management system intended to increase cereal production seeks to induce, develop

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and maintain the availability of these two limiting factors (Wopereis et al., 2008). The use of legumes crops in rotation with cereals have a lot of advantages not only on soil physical and chemical properties but also on soil biological efficiency in terms of nutrients balance regulation through the symbiotic links that are established between crops and soil bio fertilizers (Bationo et al., 2011; Bado et al., 2012). So, the crop rotation is a very important aspect and part of West African farming system (Traore et al., 2004).

Many studies have been carried over the last two decades and have proved the efficiency of crop rotation, the restitution of crop residues on soil surface, and being combined with chemical and organic fertilizers (Bationo et al., 2004, 2007; Saidou et al., 2009) on millet yield. But yet Sahelian farmers in Niger are still practicing millet mono-cropping and removal of crop residues while they do not systematically do soil amendment. Therefore, there is crucial need to help farmers with cheaper cropping techniques that will help them rise their millet yield performance, and also to identify the appropriate legumes crop species and dry residue as well the rate of rock phosphate that will be more effective in soil fertility improvement.

This research was undertaken to study the effects of crop rotation and the restitution of previous dry crop residues on pearl millet yield in mono-cropping system. Therefore, our hypothesis for this study is that in the legume based rotation, the restitution to the above-ground dry residue of previous crop has durable impacts on the yield of the millet crop. The main objective is to investigate the effects of legumes crops (Cowpea, Voandzou, Dolichos, Sesbania), and pearl millet, as well as the restitution of their dry residue on the performance of millet. The specific objectives include:

1. To assess the impact of the return of the dry residue of the previous crop on the yield of millet.
2. Identify the legume species, the most effective as a previous crop for durably improving the performance of pearl millet in monoculture system.

MATERIALS AND METHODS

Description of the area

The experiment was carried out in the experimental field at ICRISAT Sahelian Centre of Sadoré (13°14'N, 2°16'E) which is located about 45 km south of Niamey, Niger. Plant development conditions are affected by yearly weather conditions, mostly rainfall pattern and other soil properties.

Weather conditions

The climate of this region is typically Sahelian, with the raining season being between June and September. The pick rainfall is in August and has fluctuated between Mean annual rainfalls at Sadore for the period 2012 to 2015 which was 593.±64.55 mm (Figure 1).

The mean rainfall of the 33 years period from 1983 through 2015 is 556.92mm (ICRISAT unpublished). Air temperatures during the dry season tend to be high, with monthly mean daily maximum temperatures (Figure 2 and 3) ranging from 33 to 40°C between October and May (Sivakumar et al., 1993). Prevailing winds blow from the southwest during the rainy season, disturbed by mostly easterly storms. During the dry season, easterly winds dominate.

Vegetation

The vegetation is essentially made of shrubs of Combretaceae, such as *Combretum glutinosum, Combretum micranthum,* and *Guiera senegalensis*, Mimosaceae such as *Acacia Senegal* and *Ziziphus mauritiana*. The tree species include *Prosopis africa, Ferdherbia albida, Sclerocarya birrea, Balanites aegyptiaca, Cassia sieberiana, Pilostigma reticulatum and Hyphaena tebaica,* etc (ICRISAT, 1991). The grass stratus, deeply affected by the overgrazing of cattle and small ruminants, is very poor and is dominated by species such as *Sida cordifolia* and *Sesbania pachycarpa* that are less appetited by cattle. On the other hand, the inventory made on the experiment plots showed a very rich weeds species that include gramineous grass species such as *Eragrostis tremula, Digitaria gayana, Cenchrus biflorus, Cleome vicosa, Pennisetum sp and Andropogon gayanus,* legumes such as *Tepphrosia, gracilice, Zornia glochidiata and legumes species including Alysicarpus ovalifolius, Fimbristylis hispidula, Ipomea pes-tigridis, Ipomea vagans Merremea pinnata, Mitracarpus scaber, Pandiaka involucrata, Sesamum alatum, Commelina forskalaei, Indigofera pilosa* and *Phyllanthus pentandrus*.

Soils

The trial was conducted from 2012 to 2015 on a sandy soil of the ICRISAT Sahelian Center at Sadore station in the main rainy cropping season from July to October. The soil of the experiment belongs to the family of the paleustalf (ICRISAT, 1990). It is a tropical, reddish, friable, sandy ferruginous soil strongly acid (pH = 4-5.2), low in fertility and poor in organic matter. Between 0 to 17 cm, water holding at field capacity is 16.5 mm and permanent wilting point is 1.7 mm while between 17 and 32 cm, the field capacity reaches 7.4 mm and the permanent wilting point is of 2.5mm (ICRISAT, 1990). Particle size analysis, according to Saminou (2003), showed an enrichment of 90 to 95% of wind moved sand to a depth of 20 cm, 3% of silt, 2.9% of clays and 0.22% of organic matter.

Plant material

Seed material for this study is a high yielding, genetically improved millet variety ICMV IS 89305 developed by ICRISAT in 1989. The average plant height is 250 cm and its ability to produce tillers averages between 5 to 6 productive tillers per plant. The length of the panicle is intermediate and the yield potential is 2 t/ha. Natural phosphate of Tahoua (PNT), Urea, and dry residue of previous cropping have been used as fertilizing materials.

The dry residue applied in 2013 trial comes from 4 legumes ((*Sesbania pachycarpa, Vigna unguiculata* (L.)Walpers*; Voandzea subterranean (*L.) Verdc)*; Dolichos lablab* (L.)), and 1 pearl millet ((*Pennisetum glaucum* (L.) R.Br) crops cultivated in 2012 in 2 densities each and restituted in the form of mulch in May 2013, two months before planting of the trial on 15 July 2013. Then, the 2014 trial received the dry residue (dry matter) of previous trial. No dry residue was applied in rainy season (RS) 2015 trial.

It is worth to mention that the dry residue returned to the soil are those obtained on their respective plots. Crops that have produced

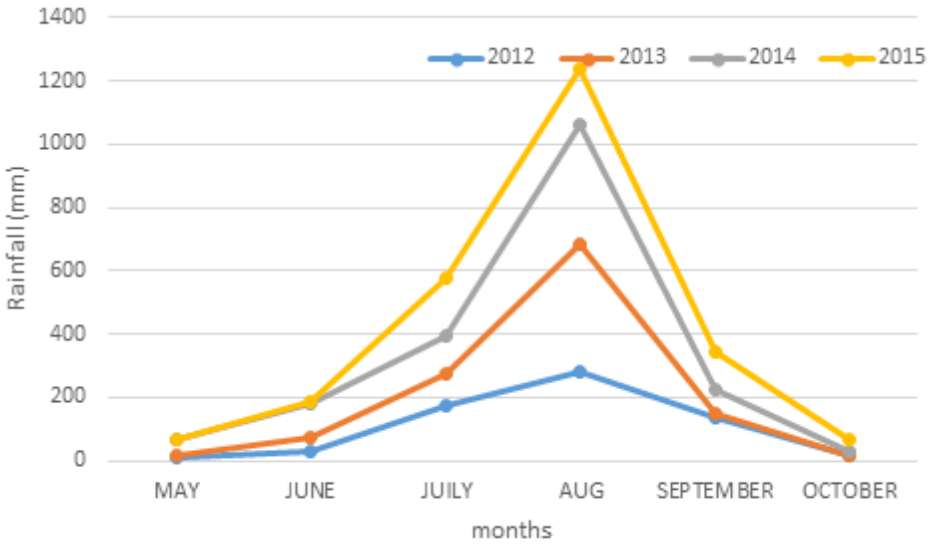

Figure 1. Monthly rainfall during the periods of cropping from 2012 to 2015 as recorded on the Sadore weather station.

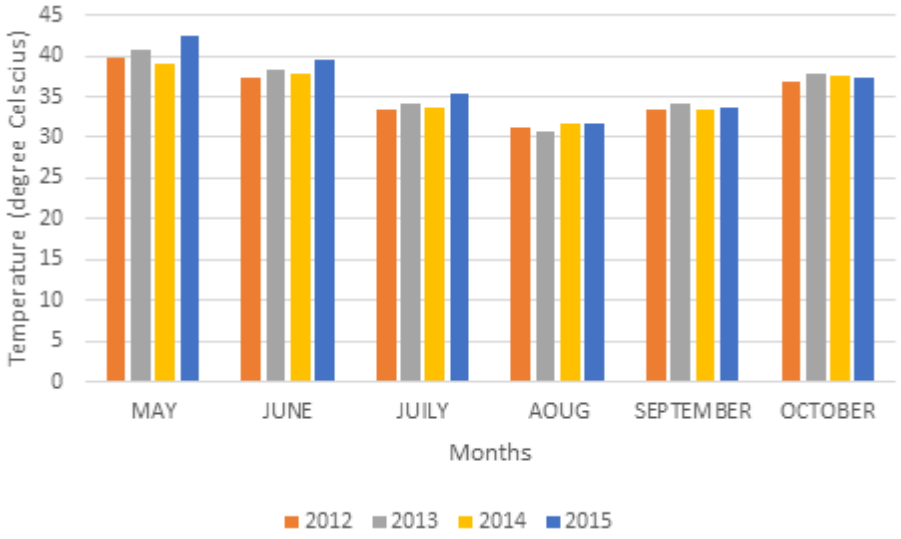

Figure 2. Monthly maxi temperature during crop growing periods for 2012 thru 2015 (degree Celcius) at ICISAT Sadore.

dry residue the most have proved well suited to the soil conditions (Sesbania, millet, cowpeas) while others having produced low productions have had difficulties to withstand the environmental conditions. This is the case of Voandzou (Bambara groundnut) which has suffered attacks by rodents (squirrels, rats, hedgehogs). Dolichos although having had very good germination, did not withstand well because of the texture of the sandy of soil.

Agronomic experiment

The objective of the experiment was to study the effects of crop dry residue on soil fertility, the experiment was initiated in rainy season (RS) 2012 in order to produce the required dry residue. To achieve the objectives, a preliminary test has first been implemented in 2012 to produce the crop dry residue necessary for the test itself (2013 through 2015), from 4 legumes species and a cereal crops sown at two densities each. This preliminary test permitted to characterize the soil in terms of initial physical, chemical and biological properties as a starting situation. The second test has been implemented in 2013 with a sole millet crop and a unique plant spacing of 0.8m x0.8 m. Just before planting the millet, 3 levels of Tahoua Rock Phosphate (PNT) were applied accordingly to the trial protocol. The aim was to study the rear effects of 2012 legumes and cereal crops and their returned dry residue to their respective plot, on the soil fertility, the mycorrhizae colonization and the performance of millet yield. The subsequent 2014 and 2015 trials were laid out using the same variety of millet crop and

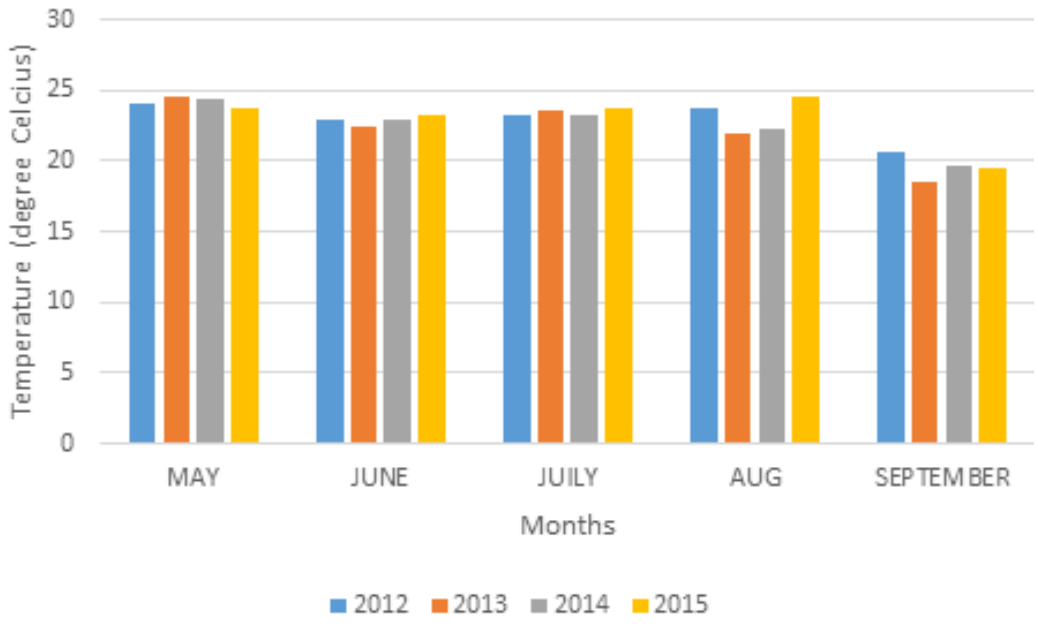

Figure 3. Monthly mini temperature during the growing periods for 2012 thru 2015 (degree Celcius) at Sadore at ICRISAT Sadore.

spacing. No other basal fertilizer was applied except Urea applied in micro-dosing as a top dressing fertilizer. The aim was to investigate on the effects of the previous crops and restituted dry stalks on a monoculture of millet on the one hand, and the contribution of 2013 applied rock phosphate levels on soil fertility level mostly available Phosphate-Bray1 on the other hand. So, the 2013 trial seeks the effects of previous legume crop and incorporation of their stalks on millet yield while the 2014 and 2015 seek for the durability of a system of mono-cropping of millet on an underlying legume crop.

Experimental design

The experiment was layout in split plot design with three replications and five main treatments that represent the five previous cropping including four legumes and one cereal. Two secondary treatments are the two doses of buried dry residue of previous cropping, characterized by the clearances of seedlings, and the three tertiary treatments that are 3 doses of applied phosphate. The additional check plots are the natural fallow (J) and bare soil fallow parcel (Pnue) (So either: 5x2x3x3 + (2 x 3 x 3) = 108 experimental units).

Prior to the initiation of the experiment, the site was fallow until 2011. During cropping season of 2011, all plots were cropped with mucuna. The composition of the site soil has been evaluated before and after 2012 before and during each growing season after up to 2015 season harvesting. Soil samples were taken from the plot before any soil perturbation.

After the delimitation of repetitions, soil sampling has been done before seedlings at depths: 0 to 10 cm, 10 to 20 cm and 20 to 30 cm at the level of each repetition on the diagonals and the etc. Samples were mixed by depth to have three composite samples by repetition, to end up with a total of 9 composites samples which were analyzed pour initial physical and chemical properties such as a texture in 5 fractions and the content elements undermined mostly for P total, P Olsen, P Bray1, N total, C org, pH eau et pH Kcl, Al, Fe, Mg, Ca, CEC etc.

Soil analysis

For the assessment of the effect of the different treatments applied, the evaluation focused on some physical and chemical properties of the soil, prior to the layout of the trial: pH Kcl, Al, C org., Total P, P Olsen, Na, K, Ca, Mg, CEC, N; the results are shown in Table 1. The analysis of particle size in 5 fractions concerned the percentages of coarse sand, fine sand, coarse silt, fine silt, and clay that are expressed in Table 2.

Plants culture

Each crop specie was planted at two densities including farmer usual density (D1) and research recommended density (D2) at spacing as indicated in Table 3. The number of row per plot depended on the crop species and row spacing. The number of seed per hill also was depending on crop species. For millet, a pinch of two fingers was put in each hill with an average of 8 to 10 seed per hill. For cowpea and Dolichos, 3 seeds per hill. For Sesbania, a pinch of 2 fingers was seeded. The seeds were presoaked 12 h before planting. The seed of cowpea and millet were treated with Apron Star at a rate of 50 g of chemical for 5 kg seed.

Measurement of yield attributing traits

Plant germination was monitored 7 days and 14 days after sowing in order to get the percentage of germination of all the crops. Plant stand was recorded at harvesting. SPAD meter was used to measure the chlorophyll content in leaves. For millet, the measurement was taken on the leaves located at the third position below the flag leaf. Harvesting was done accordingly to the duration of each crop (Table 4). For cowpea, this was done twice starting when 50% of the pods were maturing. Millet was harvested when the grain attained physiological maturity. Voandzou (Bambara

Table 1. Initial soil chemical properties of the trial field in 2012.

Variable	pH-H_2O	pH-KCl	H^+	Al^{3+}	C. O	Total-P	P-Bray1	P-Olsen	Na^+	K^+	Ca^{2+}	Mg^{2+}	CEC
	1:2.5	1:2.5	cmol+/kg	cmol+/kg	%	mg-P/kg	mg-P/kg	mg-P/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg
Depth : 0-10 cm													
SD	0.06	0.23	0.01	0.00	0.02	9.88	1.47	1.78	0.04	0.03	0.22	0.01	0.37
Mean	5.39	4.59	0.06	0.04	0.23	75.08	7.30	9.05	0.13	0.19	1.30	0.19	1.85
Depth : 10-20 cm													
SD	1.89	1.50	0.03	0.10	0.07	26.79	2.23	2.81	0.04	0.06	0.39	0.06	0.58
Mean	5.39	4.16	0.08	0.23	0.15	84.02	5.21	6.18	0.12	0.13	1.15	0.14	1.88
Depth : 20-30 cm													
SD	1.69	1.33	0.03	0.19	0.06	26.73	2.18	2.51	0.04	0.05	0.38	0.06	0.65
Mean	5.27	4.00	0.11	0.49	0.13	53.64	3.34	5.26	0.09	0.14	1.13	0.10	2.20

Table 2. Initial soil physical properties at 5 fractions (%) in 2012.

Depth	Coarse sand %	Fine sand %	Coarse silt %	Fine silt %	Clay %
0-10	43.73	52.42	1.89	1.23	0.74
10-20	41.18	53.82	1.59	1.13	2.28
20-30	43.42	48.60	1.48	1.13	5.36
Standard deviation	1.39	2.70	0.21	0.06	2.35

Table 3. Effect of previous crop species on millet grain yield (kg/ha).

Crop specie	Mean grain yield (kg/ha)	Duncan's multiple range test	Mean stalk yield (kg/ha)	Duncan's multiple range test	Mean plant height (cm)	Duncan's multiple range test
M	69.90	a	357.00	a	62.28	a
J	74.60	ab	459.90	abc	65.22	a
Pnue	99.70	abc	427.60	ab	76.18	ab
V	127.90	bcd	398.60	ab	74.37	a
N	145.80	cde	499.90	bc	86.46	ab
D	163.70	de	437.00	ab	88.90	ab
S	185.00	e	603.80	c	103.73	B

Lsd grain yield: 53.7 cv%: 89.1 Lsd plant height: 67.888 cv%: 83.70; Lsd stalk yield: 145.48 cv%: 68.70; Lsd (least significant differences) ; cv% (coefficient of variation percent) M (pearl millet); J (natural fallows); Pnue (Bare soil); V (Voandzou); N (Cowpea); D (Dolichos); S (Sesbania); Letter a, b, c, d, and e indicate difference between means; same letter in the same column means that mean are equivalent that is have the same level of impact.

Table 4. Impact of previous crop specie and year x crop specie on millet stalk weight (kg/ha).

Crop specie	D	J	Pnue	M	N	S
-	437.02[a]	459.86[a]	427.55[a]	357.04[a]	499.89[a]	603.82[b]
Year* crop specie						
2013	545.84[a]	77.50[b]	286.46[cb]	183.02[d]	520.01[a]	658.69[a]
2014	213.40[a]	252.15[a]	376.16[a]	328.00[a]	407.16[a]	478.98[a]
2015	551.84[a]	1049.93[b]	620.04[a]	560.10[a]	572.50[a]	673.78[a]

lsd *Crop Specie* =142.621 lsd Year x Crop Specie =247.026; cv%: 58.3.

groundnut) harvesting was undertaken after checking that the underground pods contained matured seeds.

Only 40 to 50% of Sesbania pods were harvested because the senescence of leaves occurred earlier due to drought spell which took in September. The harvesting of fallows plot consisted in cutting the above ground bulk vegetation cover. On the bare soil, no harvesting was done as no vegetation was allowed to grow during the season. The harvesting of plant stalks was done also accordingly to the specific maturation state of each crop. After harvesting the stalks, these were weighed then put to dry under the sun for a month. The final dry weight was recorded when, after, two consecutive measurements, the weight remained the same. This later weight was taken to calculate the yield in terms of total dry matter production of each treatment.

The interest in this 2012 trial was in priority axed on dry residue production of the crops and secondarily on pearl millet grain yield. The reason is that this is an initiating experiment with the objective of producing initial soil conditions for the following 2013 through 2015 trials. Before harvesting, soil samples were taken in each treatment plot in order to assess the status of chemical properties. In addition, the initial soil chemical and physical properties, and the fungal biodiversity of the soil as well were assessed.

From year 2013 to 2015, the experiments were axed on studying the rear effects of previous 2012 legumes crop and their residue on mono-cropped pear millet yield. A factorial split-splot design was used to lay out the trials. The 2013 experiment consisted of the following factors: 3 replications, 5 preceding crops including Dolichos (D), Cowpea (N), Sesbania (S), Voandzou (V), and Pearl millet (M), as main factors, additional 2 check factors such as Natural Fallows (J) and Bare soil (Pnue); two levels of returned dry residue of previous crops as minimum dose of dry residue restituted to the soil (D1) and maximum dose previous crop dry residue restituted to the soil (D2), as secondary factors, 3 levels of phosphorus into the form of rock phosphate (P0, P1, and P2) and they were applied on the 4th of July, 2013 for five days before the planting on July 9, 2013. Only the Pearl millet variety used in 2012 experiment was planted with a spacing of 0.8m x 0.8 m on each treatment plot. It is important to notice that in the subsequent following experiments, D1 and D2 refer to the minimum and maximum doses of dry residue of preceding crop applied to the respective plots. Natural fallows and bare soil did have only D1.

The 2014 experiment was a follow up of 2013's and comprised the following factors: 3 replications, 5 preceding crops (Dolichos, cowpea, Sesbania, Voandzou, and pearl millet), as main factors, two levels of returned dry residue of previous crops (D1 and D2), as secondary factors, 3 levels of phosphorus into the form of Tahoua rock phosphate (PNT) with P0 (no PNT), P1 (60kg/ha of PNT), and P2 (120kg /ha of PNT. Only the same Pearl millet variety used in 2012 experiment was planted with 0.8 m x 0.8 m spacing on each treatment plot.

No fertilizer was applied but the above ground dry residue of 2013 pearl millet was restituted on their originating previous plot. The stalks were not incorporated but dispatched over the plots as a mulch. The rainy season (RS) 2015 experiment was the same as that of the previous year experiment except that each plot was split in two subplots corresponding to 2 levels of nitrogen (U0 and U1) under the form of Urea in micro-dosing. No previous millet dry residue from 2014 RS was returned on to the plot of 2015 RS.

Plot preparation

The dry stalks of the legumes and millet of 2012 trial were manually cut into pieces using machetes, then thoroughly dispatched all over their respective originating plot during the month of February, 2013. To avoid deep disturbing of the soil (protection of mycorrhiza arbuscular fungi) a superficial mixing with soil was done using hoes. Then each plot was subdivided into 3 subplots corresponding to the 3 levels of phosphate. The rock phosphate was in the form of powder. The method of application of rock phosphate was as follows: for each plot, on the basis of treatment the required quantity of rock phosphate was weighed then thoroughly mixed in a 10 L plastic bag manually with soil taken inside the respective treatment plot. The mixture was dispatched all over the plot then incorporated into the soil. To apply the Urea fertilizer, a 3 fingers pinch of urea (ICRISAT, 2009) that is about 3.31 g, was put in each planting whole at panicle initiation stage and at panicle emergence stage. In 2012, along with experiments plots, two blocks of check plots were laid out above each block of replications. One block of natural fallows (J) on which weeds were allowed to grow naturally, and one block with bare soil (Pnue) which was kept cleaned from weeds and crop. Each of the check blocks consisted of plot (5mx10m) replicated 6 times. There was no planting density for them, however they were treated as having density D1. In 2013 thru 2015, these were planted with millet as the other plots.

Data analysis

For the statistical analysis of collected data, an unbalanced ANOVA analysis was used with GENSAT14.1 program. Whenever a significant difference was found between treatments, couples of means were tested using the least significant difference (LSD) and DUNCAN test for multivariate test of multi-annual data.

Calculation of legume dry biomass efficacy value vs millet dry biomass (Bado et al., 2012)

The efficacy of millet dry biomass on millet yield was estimated based on the following formula:

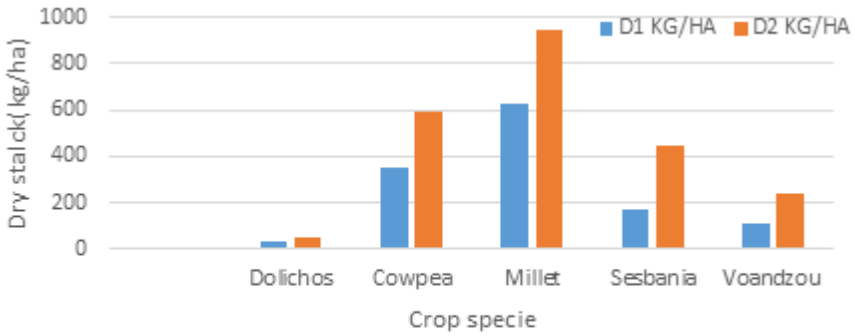

Figure 4. Crop residue yield as affected by planting density in 2012.

Eff Leg = (B- A)/X

Where A is yield of millet after mono-cropping, B is yield of millet after legume, X is quantity of legume biomass returned to the soil.

RESULTS

Millet yield

The influence of planting density on millet yield is indicated in Figure 4. Millet grain yield in 2012 experiment was significantly influenced by the farmer planting density D1 (6666.66 plant pocket/ha with 1.5m x 1m) recommended planting density D2 (10000 plant pocket/ha with 1m x1m); the effect of planting density D2 (459.54kg/ha) was greater than that of D1 (358.44 kg/ha). The dry residue production was influenced by the planting density (P = 0.011) and planting density D2 gave more dry residue than D1. The analysis of variation indicated that the dry residue production of crop species was highly depending on the interaction Culture x Density (P<0.001).

The impact of crop planting density on dry residue yield was significantly depending on the specie. All the species produced lower quantity of dry residue with farmer usual planting density D1 compared with D2. For instance, Sesbania produced the rate of dry residue with 671.84kg/ha with farmer usual planting density D1, while1795.95 kg/ha were produced with recommended planting density D2; at the recommended planting density D2, Dolichos produced the maximum of 199.37 kg/ha while only a minimum of 140.19 kg was obtained at density D1 (Figure1). In the same way, Voandzou produced 243.67 kg/ha of dry residue with recommended planting density but produced 106.65 kg/ha with farmer usual planting density.

Effects of preceding crop on millet yields

Millet grain yield varied from year 2012 to 2015 as indicated in Table 3. The pearl millet yields in 2012 trial are considered as the initial yields. As such, it was observed that the millet yield decreased significantly in all 3 years from 2013 through 2015 (Table 3). The effect of year on grain yield was highly significant (P <0.001) with the following ranking: 2012>2013>2015>2014. Millet yield was highly influenced by preceding crop species (P <0.001); as indicated in Table 3, the preceding Sesbania, Dolichos and Cowpea had higher impact on 2013, 2014, and 2015 millet grain yield compared with preceding Voandzou, natural Fallow, Bare plot and millet. The output of ANOVA table indicated that millet grain yield is also highly affected (P <0.001) by the dose of dry residue of previous crops; the Duncan's multiple range test showed the following ranking in Table 3. On the other hand, the interaction of the Year x Dose dry residue had some positive impact on millet grain yield at P = 0.07 as shown by the result of the analysis of variance; and the impact on plot having received the maximum dose D2 of dry residue of preceding crop which was greater than those having received the minimum dose D1 of dry residue of preceding crop. The dry residue of previous RS 2012 cultures was restituted to the soil in 2013 (kg /ha).

Effect of phosphorus doses on millet grain yield

The impact of phosphorus application on millet mean grain yield was not significant for no statistical difference was found (Prob. 5%) while comparing means of millet grain yield with the DUNCAN test over the 3 years under the rate P0 (no application), the study obtained 123 kg/ha of millet grain, P1 (60 kg/ha of PNT), 130 kg/ha of grain, and with P2 (120 kg/ PNT) 136.5 kg/ha of grain.

Effect of nitrogen

The application of Urea in micro dosing at the rate of 3.31 g/hill, had highly influenced the yield of millet in 2015, (P<0.001) as indicated by the analysis of variance. The

Table 5. Impact of previous crop on millet yield over rainy seasons 2013-2015.

Preceding crops	RS 2013				RS 2014				RS 2015			
	D1		D2		D1		D2		D1		D2	
S	192	a	192	ab	113	b	123	ns	125	ns	653.1	b
D	188	a	169	ab	77	a	96	ns	140	ns	657.2	b
N	161	b	175	ab	96	ab	84	ns	124	ns	655.2	b
V	136	c	150	bc	80	a	78	ns	119	ns	549.8	b
Pnue	128	c	128	cd	99	ab	99	ns	139	ns	620	b
J	88	d	80	e	114	b	114	ns	140	ns	1049.9	a
M	80	d	101	de	84	a	98	ns	144	ns	618	b
Mean	139	-	142	-	95	-	99	-	133	-	686	-
P0	138	ns	138	ns	95	ns	42	ns	134	ns	141	ns
P1	138	ns	146	ns	99	ns	46	ns	135	ns	142	ns
P2	141	ns	143	ns	90	ns	48	ns	130	ns	134.7	ns
Mean	139	-	142	-	95	-	45	-	133	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-

Treatment values with alphabetical letters a, b, c, d, have significant differences; Treatment value with same letters in the same columns have equivalent impact while those with different letters are different; ns: no significance; PNT: natural phosphate of Tahoua; D1: minimum dose of dry residue; D2: maximum dose of dry residue; S (Sesbania); D (Dolichos); N (Cowpea); V (Voandzou); M (pearl millet), J (natural fallows) Pnue (Bare soil).

very limited contribution of returned crop stalk to the plot had been observed at Prob. 0.082. The Phosphorus x Nitrogen interaction did not have any impact on the millet grain yield over the 3 years of mono-cropping. This might mean that the treatments had impacted on millet grain yield with the same efficiency.

Effects of previous legumes

The mean dry residue production significantly (P<0.001) varied over the three years of trial with the maximum obtained in 2015 (621.1kg/ha), 2013 (422.2kg/ha), and the minimum in 2014 (326.7 kg/ha). Among the preceding crop, Sesbania had the highest impact on millet dry residue production (Table 5). .

The interaction Year x Preceding crop specie had a significant influence on the production of millet dry residue (P<0.001) as shown in Table 6 and Sesbania was ranked first with 603.82 kg/ha of millet dry residue; this represents 16.9, 14.1, and 13.1% respectively of the impact obtained with the mono-cropping of millet preceding Pnue and J. On the other hand, no statistical difference was observed among the remaining four crops meaning that each of these later did not bring to the millet better suitable growth condition than the others.

The impact of the dose of previous crop straw on millet dry residue production was highly significant (P <0.001). The most important effect was observed for the maximum dry residue dose D2 (527.34 kg/ha of dry residue) against D1 (406kg/ha). In the condition of 2013 experiment, the legume dry residue use efficiency is the best for Cowpea (0.49) followed by Sesbania (1.01) and Voandzou (1.02) while for Dolichos it is very low (6.16). This means that for producing 1 kg of millet grain it is necessary to apply only 0.49 kg of Cowpea dry residue while 6.16 kg of Dolichos dry residue will be requested.

Use efficiency of crops residues

The residual effect of the dry residue of preceding crop has a much reduced use efficiency on 2014 millet grain yield as shown in Figure 5. In the 2014 experimental conditions previous legumes, their residual residues plus the previous millet residues have lower contribution to millet grain yield buildup as compared with 2013 millet grain production; however, the millet grain yields have globally decreased, we can notice that preceding legumes have requested more of dry residue for each kg of grain produced. For instance, while for millet on preceding Sesbania and Dolichos, 0.09 kg of dry residue was necessary to produce 1 kg of millet grain, 0.05 kg of dry residue from preceding natural fallows were requested to produce 1 kg of millet grain.

Effect of preceding crop and subsequent cumulative preceding crop residues on millet plant height

In 2013, significant differences on millet height increases were found among preceding crops; the predominance of the effects of preceding Sesbania, Dolichos and cowpea over the others precedents for both minimum and maximum doses of dry residues D1 and D2 could be noted. Millet and natural fallow had reducing impact on

Table 6. Impact of preceding crop and residue on millet plant height (cm).

Preceding crop	RS 2013				RS 2014				RS2015			
	D1		D2		D1		D2		D1		D2	
S	192	a	192	a	113	b	123	ns	125	ns	125.7	b
D	188	a	169	ab	77	a	96	ns	140	ns	144.9	b
N	161	b	175	ab	96	ab	84	ns	124	ns	148.6	b
V	136	c	150	bc	80	a	78	ns	119	ns	144.9	b
Pnue	128	c	128	cd	99	ab	99	ns	139	ns	139.1	b
J	88	d	80	e	114	b	114	ns	140	ns	140.2	a
M	80	d	101	de	84	a	98	ns	144	ns	131.1	b
Mean	139	-	142	-	95		99		133		139	-

Notes: Treatment values with alphabetical letters a, b, c, d, have significant differences; Treatment value with same letters in the same columns have equivalent impact while those with different letters are different; ns: no significance; PNT: natural phosphate of Tahoua; D1: minimum dose of dry residue; D2: maximum dose of dry residue; S (Sesbania); D (Dolichos); N (Cowpea); V (Voandzou); M (pearl millet), J (natural fallows) Pnue (Bare soil).

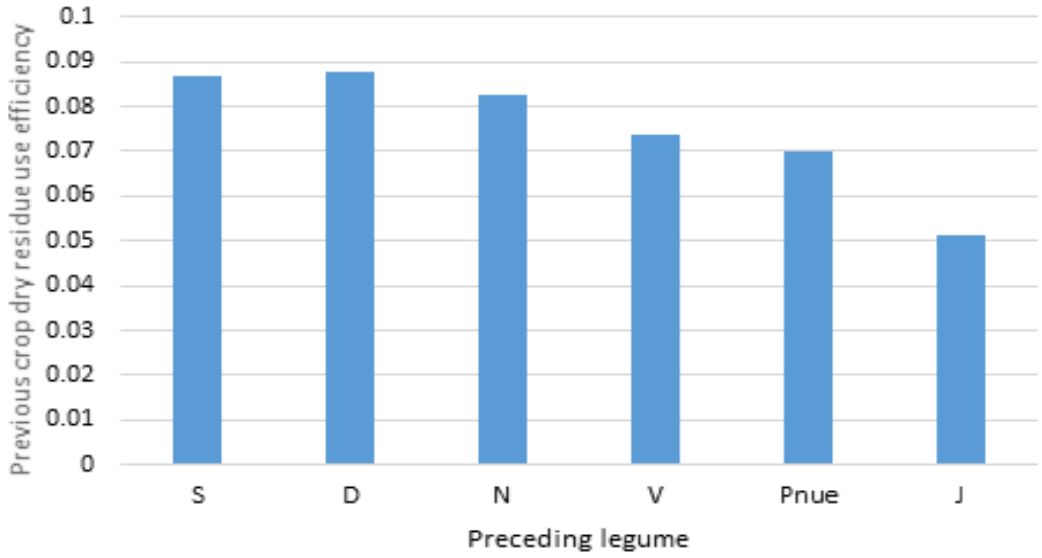

Figure 5. Legume biomasse use efficiency on millet grain yield in RS 2014 (Notes: S (Sesbania); D (Dolichos); N (Cowpea); V (Voandzou); M (pearl millet), J (natural fallows) Pnue (Bare soil).

plants height. The year 2014 brought a general shortening of plant height for both crop residues doses D1 and D2 while in 2015, the overall increase of millet height was observed no matter the preceding crops.

The effects of preceding crop on soil contain in minerals

The results, as shown in Table 7, indicated the available nutrients in the soil for each preceding crop as per year 2014. The ANOVA analysis showed that the rate of the different elements organic carbon, P-Bray1, and total nitrogen are the highest in the soil having had J and Pnue with respectively 0.2219% , 7.605 and 196 mg/kg for organic carbon, phosphorus Bray1, and total nitrogen for J and 0.1927%, 6.602 and 152.5 mg/kg for pour Pnue.

DISCUSSIONS

In this study, we wanted to firstly find out if millet grain yield and dry matter yield were influenced by its planting density, secondly, to investigate on the effects of previous crop and the restitution of its dry above ground residue would increase or not the yield of the following millet grain and dry biomass (dry residue). The residual effect of rock phosphate applied on mono cropped millet yield is

Table 7. Available nutrient in the soil for preceding crop in 2013.

Preceding crops	C. orga (%)	P_Bray1 (mg /kg) soil	N_Total (mg /kg)
S	0.1845**a**	5.752**a**	150.8**a**
D	0.1681**a**	6.902**b**	139.7**a**
N	0.1671**a**	6.113**ab**	142.2**a**
V	0.1626**a**	6.999**cb**	140.6**a**
Pnue	0.1927**c**	6.602**ab**	152.5**a**
J	0.2219**b**	7.605**db**	196**b**
M	0.1713**a**	6.53**ab**	145.7**a**
Dose of dry residue			
D1	0.1673ns	6.791ns	140.4a
D2	0.1748ns	6.191ns	149.9b

Notes: Treatment values with alphabetical letters a, b, c, d, have significant differences; Treatment value with same letters in the same columns have equivalent impact while those with different letters are different; ns: no significance; PNT: natural phosphate of Tahoua; D1: minimum dose of dry residue; D2: maximum dose of dry residue; S (Sesbania); D (Dolichos); N (Cowpea); V (Voandzou); M (pearl millet), J (natural fallows) Pnue (Bare soil).

also investigated.

Effect of planting density on millet grain yield and dry residue yield

The effect of recommended planting density D2 on millet grain (459.54kg/ha) was greater than that of farmer usual planting density D1 (358.44 kg/ha). This is supported by the results of Bationo and Mokwunye (1991) who obtained a millet grain yield increase by 400% when the number of planting hills increased from 2000 to 7000 per ha millet, planting with no fertilizer application. An increase in dry biomass resulting from the effect of lower planting density was obtained while comparing the effect of planting density D1 and D2.

These results are showing that high density of planting reduces the quantity of dry residue produced while lower planting density will increase the dry residue production. The main reason is that with a high number of plants per unit area, individual plants compete for light, nutrients and water more than with a reduced number of plants per unit area.

This goes in the same direction with the results of Maobe et al. (2014). The study indicated that for each crop there exists an optimum planting density below or above the yield (Maobe et al., 2014). Depending on the production objectives, crop planting density must be decided. The competition for nutrients, water, and sun light takes place among reduced spacing planted crop which is observable through erected plant stand, less leave biomass, and reduced number of fruit or grain compared with increased spacing planted crops which would be laterally extended branching, more leave biomass and adequate fruit and grain number (personal observations).

Effects of preceding crop on millet yield performance

The interaction of the Year x Dose dry residue had some positive impact on millet grain yield at P = 0.07 as shown in Figure 2, and the impact on plot having received the maximum dose D2 of dry residue of preceding crop was greater than those having received the minimum dose D1 of dry residue of preceding crop. Similar results were obtained by Power et al. (1998) who found, in a 10 years study, significant impact of preceding crop residue returned to soil on corn grain yield (of about 16% gain from the return of 150% of previous crop residue) and soil properties with an increase of N being available in the soil; grain yield increases were function of the quantity of previous crop residues returned to soil.

The depreciating effect of millet mono-cropping on soil fertility and the yield of millet or sorghum has been similarly demonstrated in many studies in Niger, Burkina, and Mali, in similar condition, by many scientists (Bationo et al., 2011; Bado et al., 2012). Other studies are also in the same direction as that of this study which showed that the cowpea cropping can generate about 40 to 80 kg of N/ha (Quin, 1977), and the millet grain yield increase can reach between 149 to 252 kg/ha, and still provide an increase of 40 to 65 kg/ha of the dried stalk (Bationo and Kumar, 2002).

The legume/cereal rotation is the commonly used practices in sub Saharan Africa and with usually the legume crop as first crop although cereal/legume and cereal/cereal rotations might be performed somewhere else (Bationo et al., 1989; Traore et al., 2003; Bado et al., 2012). The advantages of crop rotation include improving soil fertility mostly with nitrogen fixing legumes as previous crops as these improve soil contain in nitrogen

fixed in the nodes that can be available for succeeding cereals (Bationo and Ntare, 2006). The preceding legume crop roots can help to solubilize adsorbed phosphorus by their root exudates and also made it available to the subsequent cereal crop the Nitrogen content in dead nodules and leaves (Odendo et al., 2011).

The use of legumes crops in rotation with cereals have a lot of advantages not only on soil physical and chemical properties but also on soil biological efficiency in terms of nutrients balance regulation through the symbiotic links that are established between crops and soil bio fertilizers. For instance, while the rhizobium bacteria improves soil nitrogen status via nodulation of host legumes roots, the arbuscular mycorrhiza fungi (AMF) develop symbiotic relationships with the roots of both legume and cereals plants (Jean-Pascal, 2005).

The impact of natural rock phosphate on millet yield

The effect of Tahoua natural rock phosphate (PNT) application at 3 different doses did not show any statistical difference on millet yields. It implies that the effects of 60 kg/ha of PNT and that of 120 kg/ha of PNT on the millet yield were not better than no application of PNT. This is in contradiction with some studies done in the sub-region that demonstrated that the agronomic effectiveness of natural phosphates increased the crop yields of 30 to 80% in the first year of application depending on pH and soil moisture conditions (Bationo et al., 1986; Sef Van et al., 2001; Gbadamassi, 2008).

This might be due to the missing of soluble Nitrogen in the soil as we did not apply any Nitrogen until the last year of the trial. Our expectation in this experiment was that the nitrogen resulting from the synthesis of biological nitrogen via the symbiotic fixation in the legume roots, would be enough to enhance the efficiency of the partially soluble PNT applied in the following season. This argument is supported by the results of Wopereis et al. (2008) that advised that Nitrogen has to be applied along with rock phosphorus to support its efficiency. The lack of Nitrogen in the soil after the legumes crop is imputable to the fact that no nitrogen was applied to these crops in 2012. Similar responses were obtain by Bado et al. (2012) in a trial where cowpea yield and performance were lower if no fertilizer applied at the young stage of growth. In fact, legume crops request a minimum basal fertilizer in order to boost nodulation and dry matter production of Bado et al. (2012).

Effect of nitrogen application on millet grain yield

The application of nitrogen significantly increased the millet grain yield performance. The application of nitrogen (N) was determinant in uprising the level of millet growth and yield as the dry residue and grain yield were increased after applying 45 kg of urea/ha. In fact, there was a sink of millet global production from 2012 to 2014 meaning that the residual effects of previous 2012 legumes crop residues and that of 2013 millet, in addition to the rate of PNT added, were not enough to rise up or at least to keep the magnitude reached in 2012.

The lack of interaction Nitrogen x Phosphorus might indicates that phosphorus was not certainly a missing nutrient but rather nitrogen was the limiting factor. This could be explained by the fact that there was not enough stock of available nitrogen in soil solution deriving from the previous legumes crop microorganism's symbiotic activities. The millet straw contain in nitrogen is usually very low (Ganry et al., 1978). The nitrogen might be used by the bacteria involved in the decomposition process of applied previous crop residue.

This is contradicting the findings of Prasad and Power (1991) and Power et al. (1998) in Nebraska, who found that the soil physical properties (water retention) and biochemical (activities of microorganisms, organic matter contains, nutrient availability), and crop (corn /sorghum) yield were improved when increased previous crop residues were added to the soil. But the difference with our trial is that in theirs, nitrogen (46N) was regularly added every year while we did not apply nitrogen until the last year (46N); in addition, the experimental conditions are different in terms of soil texture and climatic conditions. This shows the importance of nitrogen as limiting factor to enhance and sustain the residual impact on soil productivity in mono-cropping.

This result corroborates with the findings of Traore et al. (2003) in Mali who found that after a preceding legume crop with phosphate fertilizer applied, the millet yield increased when nitrogen with 23N was added and increases of 20% grain and 30% dry residue could be yielded. Adamou et al. (2007) found 210 kg increase of millet yield with a treatment of nitrogen fertilizer added to crop residue and up to 1012 kg/ha of yield with a combination of nitrogen fertilizer added to crop residue and phosphorus fertilizer. The rate of residue decomposition depends not only the crop but also on the climate. Consequently, the amount of resulting nutrients, organic matter, and impact on erosion protection vary (Soil Quality, National Technology Development Team, 2006)

Millet dry residue production as a function of preceding crop specie and restitution of dry residue

Except for Sesbania as preceding crop, no statistical difference was observed among the effects of four crops on millet dry residue production; this means that effects each of these preceding crops did not bring to the millet better suitable growth condition than the others. The good performance of Sesbania as preceding crop in terms of high impact on soil fertility and following crop production was also demonstrated by Becker and Johnson (1998) in

Cote d'Ivoire. The restitution of crop residues to the soil, allows to return parts of nutrients exported by previous crop (Bationo et al., 2011; Wopereis et al., 2008). It improves soil organic matter and maintains the durability of soil biological activities, termites channeling in the soil profile (Badjissaga, 2007; Bationo, 2008). Crop residues as mulch, protect soil surface against wind, and water erosion while improving soil water conservation (Bado et al., 2012). It reduces the impact of sun rays on the soil surface and maintains a soil temperature that is favorable to the development of microorganisms.

Effect of preceding crops millet plant height

In 2013, significant differences on millet height increases were found among preceding crops; the predominance of the effects of preceding Sesbania, Dolichos and Cowpea over the others precedents for both of dry residues doses D1 and D2 could be noted. Millet and natural fallow had reducing impact on plants height. This is in line with the results of Kouyate et al. (2000). The main reason of 2014 bad performance of millet can be attributed to the combined effect of nitrogen deficiency and of climatic conditions linked with the rainfall which exceeded 740 mm. But, in 2015 rainy season, while the plants were in the stage of panicle exertion, the whole crop suffered serious damaging attacks of spiders (unspecified) from August through September. Consequently, the plants dried and the yield was compromised.

The effects of preceding crop on soil contain in minerals

The overpassing of the effects of preceding legume crops by the effects of natural fallows in terms of available carbon, phosphorus, and nitrogen, can be explained by the fact that on fallow soil, weeds are dense in population, rich in species diversification and consequently, develop very abundant network of root systems that explore the maximum soil volume and produce all kind of exudates that solubilize fixed form of phosphorus and others nutrients (Kolawole and Tian, 2010). On the other hand, these roots accumulate important amount of carbon in the soil (Bado et al., 2012). In addition, the weed population comprise legumes species that can fix nitrogen via symbiotic link with rhizobium bacteria. The decomposition of the dense root system increases the stock of organic matter and organic carbon of the soil (Fonte et al., 2009). This explains the accumulation of these elements in soil having had previously natural fallow compare with soil having had legume crops.

Conclusion

The effects of the restitution to the soil of aerial dry residue of the previous crop on the yield performance of the millet vary depending not only on the quantity of dry residue returned to soil but also species of previous cropping. The effects of the doses of dry residue on the yield of millet for the previous crops Sesbania, natural fallows, and cowpea surpassed those of Voandzou (Bambara groundnut) and the millet. With these preceding crops, the yields of millet were higher with the maximum dose of dry residue than the minimum dose of dry residue. The residual effects of Sesbania culture on the yield of millet have been the highest during the year 2013 and 2015 (with about 4 to 5 times) but could not still reach the level of the 2012 millet yield.

The expected effects of Tahoua rock phosphate to gradually supply the necessary phosphate to the mono-cropped millet could not be seen over the year 2013, 2014 to 2015 of mono-cropping because the first year grown legumes failed to produce enough nitrogen stock to sustain the efficiency of the system. These two nutrients are limiting factors and must be in appropriate proportion in order to fill crop needs.

Applying natural rock phosphate, previous crop residues after only a first year legume crop, and successive millet stalk in a system of mono-cropping cannot be profitable if a source of nitrogen is not applied. It is necessary to strengthen the sustainability of the residual effects of the preceding legumes and subsequent additional previous millet stalk by providing a source of nitrogen in micro-dosing on a yearly basis.

The natural fallow and bare soil as preceding have increased the overall available soil nutrients level more than other legume crops and millet.

RECOMMENDATION

Further researches need to be done using more indigenous wild species legumes to assess their ability to improved soil organic matter level and phosphorus, nitrogen and other nutrients as well. More investigation should be done on improved fallows using weeds that can trap more carbon while their aptitude to develop symbiotic mycorhization should be studied in order to have targeted species to be included in improved fallows.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors would like to thank the ICRISAT Regional programme Director for West and Central Africa for allowing to facilitate this research. They acknowledge the special assistance and support of the following ICRISAT Niamey scientists Dr Bado B. Vincent, Dr Prakash

Gangashetty, Dr Savadogo Patrice Dr Falalou and Dr Jupiter Ndjeunga. They also thank Amadou Wankoye Lab Technician of ENS/UAM, Niamey.

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Vol. 11(28), pp. 2499-2507, 14 July, 2016
DOI: 10.5897/AJAR2016.11059
Article Number: 3D52A3F59404
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Biometric evaluation of monthly growth rate as a criterion to study the genetic diversity in *Coffea canephora*

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Received 28 March, 2016; Accepted 16 June, 2016

The variability of growth patterns and capacity to resist to environmental stresses that exists in populations of *Coffea canephora* Pierre ex Froehner makes it possible to select genotypes for different types of cultivation conditions. The objective of this study was to evaluate the monthly growth rate as a criterion to measure the genetic diversity of genotypes and to estimate the direct and indirect effects of the monthly growth rate, by path analysis, over the length of orthotropic stems. The experiment followed a randomized complete block design, studying 10 genotypes of *C. canephora* Pierre ex A. Froehner, with four replications and six plants per experimental plot. The magnitudes of the direct and indirect effects observed in the path analysis are consistent indicatives that the growth rate during recovery months, in the peaks after periods of slower growth, is highly important to determine the length of the stems during the final of the season. There is a considerable level of similarity between the growth of genotypes from the same group regarding ripening cycle; however, the high variability makes possible to identify genotypes from different behaviors regardless of the group.

Key words: Conilon coffee, orthotropic stems, genetic parameters, variability.

INTRODUCTION

Coffee is one the most valuable commodities traded in the world, and Brazil is the country with the highest production of coffee if considered the amount produced by both of the main cultivated species: *Coffea arabica* Lineu and *Coffea canephora* Pierre ex A. Froehner (Conab, 2015). Due to the importance of this agricultural product, Brazil keep advancing in the genetic improvement of both coffee species, being considered

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the world leader in the development of improved coffee cultivars for the renovation of the coffee scenery (Borém and Miranda, 2005). Specifically, for *C. canephora*, the breeding programs have been seeking to improve several agronomic traits, such as crop yield, crop stability, resistance for the main phytosanitary problems, beverage quality, and drought tolerance (Ferrão et al., 2004; Carvalho, 2008).

Regarding drought tolerance, studies aiming to evaluate the capacity of the plants to tolerate water deficit are extremely important, especially considering the actual scenario of climate change. To enhance the crop yield regardless of the occurrence of more frequent dry periods, the breeding programs need to understand the behavior of plants grown under water deficit to be able to identify genotypes capable of expressing mechanisms of drought tolerance (Cattivelli et al., 2008; Lawn and Likoswe, 2008; Blum, 2005).

For coffee, several studies have been developed to understand the behavior of genotypes cultivated with reduced water supply (Dias et al., 2007; Rezende et al., 2009; Fialho et al., 2010; Miranda et al., 2011; Cavatte et al., 2012).

These studies justified the need to evaluate and select genotypes with higher level of resistance to water deficit, in order to characterize their traits and better understand the expression of drought tolerance mechanisms. The efforts of the breeding program of *C. canephora* in the Espírito Santo State, Brazil, to seek to improve the drought tolerance in the species resulted in the development of the clonal cultivar named “Emcapa 8141 - Robustão Capixaba”, which group genotypes able to grow and yield satisfactorily without irrigation (Ferrão et al., 2000).

The seasonal variation in the growth rate of coffee is highly related to the environmental conditions, the change in photoperiod, temperatures, light intensity and water availability triggers the metabolic changes, which leads the plant to start different new phenological stages of its life cycle (Ronchi and DaMatta, 2007). The variability of growth patterns and capacity to resist to environmental stresses that exists in populations of *C. canephora* makes it possible to select genotypes with higher vigor and crop yield for different types of cultivation conditions (Bonomo, 2002).

It is possible to identify coffee genotypes that are more adequate to cultivation in different crop systems and in different regions (Fonseca et al., 2006; Ferrão et al., 2008, Rodrigues et al., 2014a, b), as long as enough genetic variability is expressed in the specific conditions. Therefore, it is possible to justify the recommendation of certain genotypes of coffee that are better choices for each farmer, based on the region and system adopted for the plantation.

C. canephora still suffer from a smaller library of scientific data when compared to the other major cultivated specie of coffee regarding the growth rate, which makes it necessary to intensify field studies that allow the exploration of growth variables. These variables can be used to optimize management practices and improve the recommendation of fertilization, pruning and irrigation. It is known that the growth rate of the aerial part of coffee trees suffer seasonal variations due to environmental conditions (Amaral et al., 2006; Ronchi and DaMatta, 2007).

Growth analyses allow the study of the performance of species, and genotypes of the same species, subjected to different kinds of environmental stimulus, making possible to identify vigorous individuals in populations that present better responses to specific conditions (Hunt, 1990; Benincasa, 2003). Larcher (2000) and Dardengo et al. (2010) describe that several intrinsic and extrinsic factors may influence the metabolic performance of plants, causing modifications in the magnitude or pattern of their growth and development.

Therefore, the objective of this study was to evaluate the monthly growth rate as a criterion to measure the genetic diversity of genotypes of *C. canephora* Pierre ex A. Froehner and to estimate the direct and indirect effects of the monthly growth rate on the length of orthotropic stems using path analysis.

MATERIALS AND METHODS

Experimental setup

The experiment was conducted in a region where Conilon coffee is typically cultivated, located in the countryside of the municipality of Castelo, Espírito Santo State, Southeast Region of Brazil (20º34'19” S and 41º18'51” W). The area has elevation of 123 m over sea level and presented average temperature of 24ºC and accumulated rainfall of 1,080 mm along the year, with the rainy season from October to April and the dry season from May to September.

The experiment followed a randomized complete block design, studying 10 genotypes of *C. canephora* Pierre ex A. Froehner, with four replications and six plants per experimental plot. Each genotype was propagated vegetatively using cuttings and the plants were spaced 3.00 × 1.00 m, being cultivated with 4 stems per plant, keeping a total population of near 13,000 stems per hectare, following the recommendation of Ferrão et al. (2007).

The agricultural practices were applied in accordance with those normally employed in the region, according to their need and following the current recommendations for the cultivation of Conilon coffee in Brazil (Prezotti et al., 2007; Ferrão et al., 2007; Fonseca et al., 2015).

Genotypes evaluated

Ten genotypes of *C. canephora* Pierre ex A. Froehner, which were originated from the breeding program of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), were used for the study. These genotypes have desirable agronomic traits, high crop yield, average drought tolerance and different durations of ripening cycle. The genotypes are components of the clonal cultivar “Emcapa 8141 to Robustão Capixaba”. The genotypes are: RC01, RC02, RC03, RC04, RC05, RC06, RC07, RC08, RC09, and RC10.

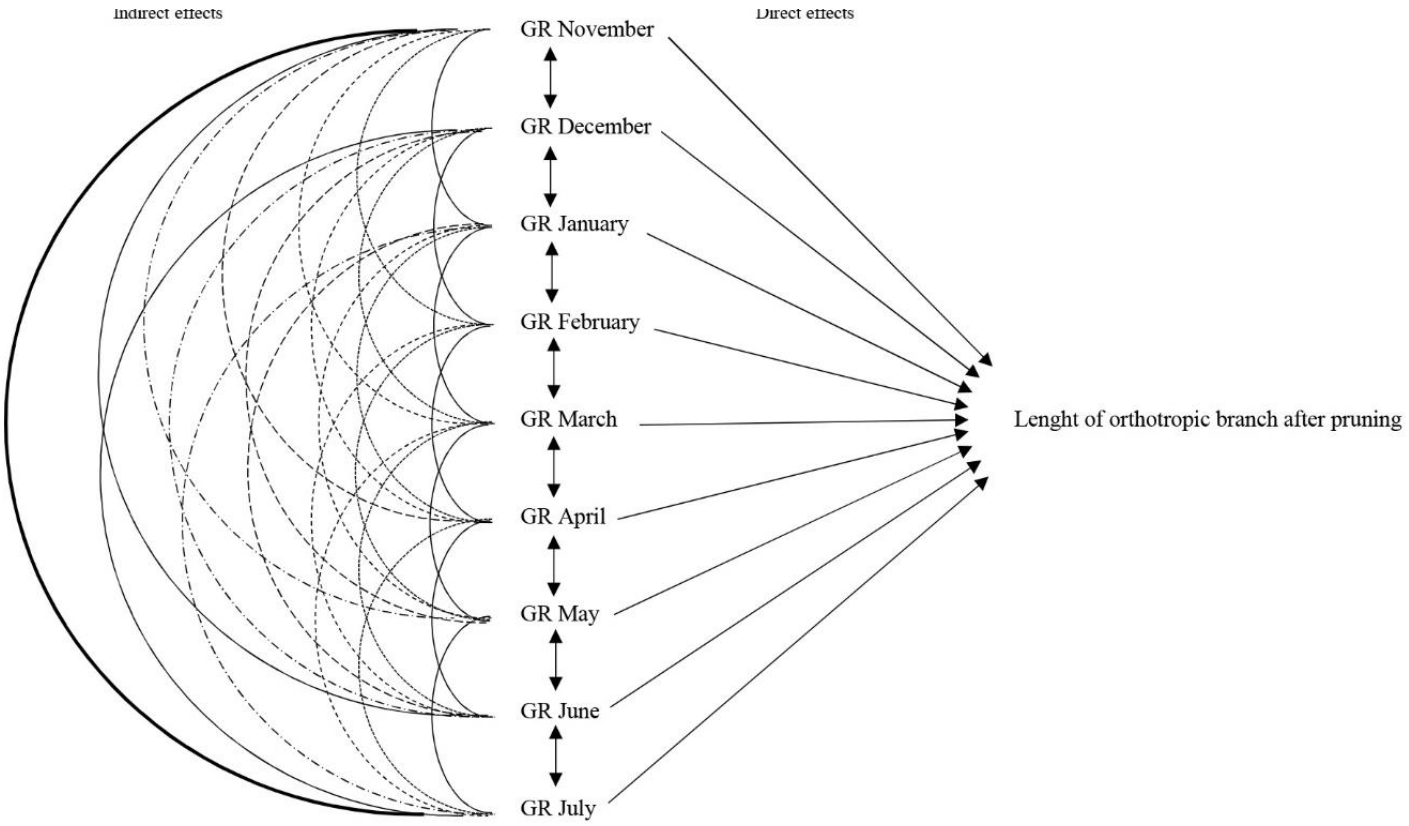

Figure 1. Chain diagram for the interrelationship of the direct and indirect effects for the explicative variables (monthly growth rate) and the length of the new orthotropic stems at the flowering (Castelo, Espírito Santo, Brazil, 2014-2015).

Data collected

The plants were cultivated and pruned for renovation of the canopy in 2014. After the process, the growth of the new stems was evaluated along the vegetative cycle, until the plants entered the phenological stage of vegetative rest of the next cycle (2015).

The monthly growth rate, estimated in mm day^{-1} for each month from November 2014 (selection of renewed stems) to July 2015 (induction and maturation of the new flower buds in the plant), was calculated based on the temporal variation of the vertical length of the new stem (from insertion to the apex), using the methodology described by Embrapa (2000).

Data analyses

The collected data were subjected to an analysis of variance using the F test in order to identify the existence of differences between the growth rates of the genotypes. The genetic parameters were estimated based on the model $Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij}$, where Y_{ijk} represents the phenotypic value of the ijth observation, μ is the general mean, G_i is the fixed effect of the ith genotype, B_j represents the effect of the jth block, and ε_{ij} is the random error related to the ijth observation.

The estimated values of phenotypic variance ($\hat{\sigma}_p^2 = \text{mean square of genotypes} / \text{number of blocks}$), environmental variance ($\hat{\sigma}_e^2 = \text{mean square of residue} / \text{number of blocks}$), and genotypic variance ($\hat{\Phi}_g = [\text{mean square of genotypes} - \text{mean square of residue}] / \text{number of blocks}$), coefficient of genetic variation (CV_g), variation index (CV/CV_g), and coefficient of genotypic determination ($H^2 = \hat{\Phi}_g / \hat{\sigma}_p^2$) were calculated according to the methodology described by Cruz and Carneiro (2006). The correlations were unfolded between direct and indirect effects over the length of orthotropic length, through path analysis (Figure 1), following the methodology described by these same authors.

The diagonal elements of the matrix and the component of residual variance were used to establish the multicollinearity of the matrix. To reduce the effect of high variances, the system of normal equations was modified by the implementation of a constant *k*, multiplied by the diagonal elements of the matrix (Hoerl and Kennard, 1970). The value of *k* was established following the methodology described by Cruz and Carneiro (2006), using graphics to choose values to which most of the path analysis coefficients were stabilized (Carvalho et al., 2002). The Mahalanobis distance was used as dissimilarity measure to delineate groups using an optimization technique based on a proposal by Tocher applied as described by Cruz and Carneiro (2006). The analyses were performed using the statistical software GENES (Cruz, 2013).

RESULTS

Genetic diversity

The results revealed that significant variation was observed for growth rate in all the months. This indicate the existence of variation for growth rate among of the

Table 1. Estimative of phenotypic and genetic parameters of the monthly growth rate of genotypes of *C. canephora* Pierre ex A. Froehner (Castelo, Espírito Santo, Brazil, 2014-2015).

Parameter	Growth rate (mm dia^{-1})								
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
MS_g[(1)]	4.83*	5.59*	1.86*	2.57*	2.14*	1.50*	0.82*	0.81*	0.71*
CV(%)[(2)]	10.77	11.92	13.91	17.78	10.82	11.06	14.59	16.37	15.39
Minimum	4.03	2.55	1.64	0.80	2.18	1.80	0.92	0.73	1.29
Mean	6.39	4.71	2.80	1.84	3.85	3.24	2.02	1.82	2.04
Maximum	9.06	7.41	4.49	4.28	5.07	4.26	3.02	2.86	3.53
$\hat{\sigma}_p^2$[(3)]	1.20	1.39	0.46	0.64	0.53	0.37	0.20	0.20	0.17
$\hat{\sigma}_e^2$[(4)]	0.11	0.07	0.03	0.02	0.04	0.03	0.02	0.02	0.02
$\hat{\Phi}_g$ [(5)]	1.09	1.31	0.42	0.61	0.49	0.34	0.18	0.18	0.15
CV_g(%)[(6)]	16.33	24.36	23.32	42.60	18.23	18.1	21.20	23.39	19.23
CV_g/CV[(7)]	1.52	2.04	1.68	2.40	1.69	1.64	1.45	1.43	1.25
H^2[(8)]	90.20	94.35	91.84	95.83	91.91	91.46	89.41	89.09	86.21

*Significant by the F test; [(1)]genotypic mean squares (MS_g); [(2)]coefficient of variation; [(3)]phenotypic variance; [(4)]environmental variance; [(5)]genotypic variance; [(6)]coefficient of genetic variation; [(7)]variation index; [(8)]coefficient of genotypic determination (%).

tested genotypes. Therefore, the MS_g was significant for all variables at 5% of probability (Table 1).

The study of the growth rate during the periods of higher vegetative growth, which happened between the months of November to December, and the period of slower growth, from June to July, helped to identify divergences between the genotypes in higher magnitude. Being especially helpful to evaluate and identify the differences between the genotypes regarding how stable is their seasonal growth patterns. The growth rate during these months returned higher values of estimated relative contribution to the variability between there genotypes.

The use of the growth rate during the period of active growth is especially helpful in this case, since the high estimated values of genetic parameters between November and December favors the identification of differences that are highly related to the genotypic variance (Table 1).

The estimated values of genotypic variances ($\hat{\Phi}_g$) were higher than the values for the environmental variance ($\hat{\sigma}_e^2$) for all monthly rates. Considering this results, it is possible to associate the greater proportion of the phenotypic variance ($\hat{\sigma}_p^2$) to the genotypic differences between the plants (Table 1).

The coefficient of genotypic determination was higher than 86% for all variables, showing that the growth rate was more influenced by genotypic factors than by the environmental conditions during the period of evaluation of this experiment (Ramalho et al., 2004).

The estimated CV_g was superior to the CV, resulting in a variation index (CV_g/CV) greater than 1.00 for the growth rate occurred in all months; therefore, genetic factors predominated over environmental factors to determinate this variables (Table 1).

The cluster analysis for the 10 genotypes through the Tocher's method is presented at Table 2. It was possible to identify four groups of genotypes: Group I clustered 40% of the genotypes; Group II grouped 30% of the genotypes; Group III clustered 20% and Group I was formed by only one genotype (RC06).

Table 2. Clustering by the Torcher's method, based on the standardized Euclidian mean distance of the monthly growth rate of 10 genotypes of *C. canephora* Pierre ex A. Froehner (Castelo, Espírito Santo, Brazil, 2014-2015).

Groups	Proportion (%)	Genotype
I	40	RC01, RC02, RC05 and RC07
II	30	RC03, RC04 and RC08
III	20	RC09 and RC10
IV	10	RC06

Effects of monthly growth rates over the length of the stems

The correction of the distortions was done with the coefficient *k* being equal to 5.07×10^{-2} to obtain the multicollinearity diagnostic. The direct effects of the monthly growth over the length of the orthotropic stems after the whole season from pruning to flowering of next cycle followed the decreasing order of magnitude: $|GR_{December}| > |GR_{January}| > |GR_{November}| > |GR_{March}| > |GR_{April}| = |GR_{July}| > |GR_{February}| > |GR_{May}| > |GR_{June}|$.

Considering the positive direct effects, the growth rates in December and January presented the higher coefficients (Table 3). During these months, the indirect effects were higher for the month right before, that is, the length of the stems was highly influenced by the growth

Table 3. Estimative of direct and indirect effects of nine monthly growth rates of 10 genotypes of *C. canephora* Pierre ex A. Froehner over the length of the new orthotropic stems after pruning, obtained by path analysis, with diagnosis of multicollinearity (Castelo, Espírito Santo, Brazil, 2014-2015).

Secondary component	Association	Length of the stems
Growth rate in November	Direct effect	0.20
	Indirect effect through growth rate in December	0.24
	Indirect effect through growth rate in January	0.18
	Indirect effect through growth rate in February	0.05
	Indirect effect through growth rate in March	0.11
	Indirect effect through growth rate in April	0.10
	Indirect effect through growth rate in May	0.03
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.01
Growth rate in December	Direct effect	0.31
	Indirect effect through growth rate in November	0.15
	Indirect effect through growth rate in January	0.14
	Indirect effect through growth rate in February	0.09
	Indirect effect through growth rate in March	0.10
	Indirect effect through growth rate in April	0.09
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.01
Growth rate in January	Direct effect	0.24
	Indirect effect through growth rate in November	0.15
	Indirect effect through growth rate in December	0.18
	Indirect effect through growth rate in February	-0.01
	Indirect effect through growth rate in March	0.13
	Indirect effect through growth rate in April	0.12
	Indirect effect through growth rate in May	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.05
Growth rate in February	Direct effect	0.13
	Indirect effect through growth rate in November	0.07
	Indirect effect through growth rate in December	0.20
	Indirect effect through growth rate in January	-0.02
	Indirect effect through growth rate in March	0.00
	Indirect effect through growth rate in April	0.00
	Indirect effect through growth rate in May	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.08
Growth rate in March	Direct effect	0.15
	Indirect effect through growth rate in November	0.14
	Indirect effect through growth rate in December	0.19
	Indirect effect through growth rate in January	0.20
	Indirect effect through growth rate in February	0.00
	Indirect effect through growth rate in April	0.15
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.06

Table 3. Contd.

	Direct effect	0.14
	Indirect effect through growth rate in November	0.14
	Indirect effect through growth rate in December	0.19
	Indirect effect through growth rate in January	0.19
Growth rate in April	Indirect effect through growth rate in February	0.00
	Indirect effect through growth rate in March	0.15
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.06
	Direct effect	0.06
	Indirect effect through growth rate in November	0.09
	Indirect effect through growth rate in December	0.02
	Indirect effect through growth rate in January	0.03
Growth rate in May	Indirect effect through growth rate in February	0.03
	Indirect effect through growth rate in March	0.01
	Indirect effect through growth rate in April	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.10
	Direct effect	0.00
	Indirect effect through growth rate in November	0.07
	Indirect effect through growth rate in December	-0.01
	Indirect effect through growth rate in January	-0.01
Growth rate in June	Indirect effect through growth rate in February	0.04
	Indirect effect through growth rate in March	-0.01
	Indirect effect through growth rate in April	-0.01
	Indirect effect through growth rate in May	0.06
	Indirect effect through growth rate in July	0.11
	Direct effect	-0.14
	Indirect effect through growth rate in November	-0.02
	Indirect effect through growth rate in December	0.02
	Indirect effect through growth rate in January	0.09
Growth rate in July	Indirect effect through growth rate in February	-0.08
	Indirect effect through growth rate in March	0.07
	Indirect effect through growth rate in April	0.06
	Indirect effect through growth rate in May	-0.04
	Indirect effect through growth rate in June	0.00

Determination coefficient = 0.97.

rate of December, with a high indirect effect of the growth rate of November; and directly influenced by the growth rate of January with higher indirect effect of December. In addition, the determination of the length was highly dependent of the growth rate in November, with strong indirect effect of the growth in both December and January.

DISCUSSION

Between November and December, the plants were in conditions of longer days and abundant rainfall, achieving higher growth rates (Table 1). In this period, the new stems are in the vegetative stage of their phenological cycle, characterized by the formation of new leaf buds (similar for the coffee species, as described by Camargo and Camargo (2001), a highly active stage for growth and development of vegetative structures, therefore, high growth rates were observed in this period.

A lower GR was observed in February, which is related to the occurrence of a dry period, the water deficit caused by the lack of rainfall for 14 consecutive days slowed the growth of the stems, causing the smaller gain of extension

in this period.

Another period of smaller growth is observed after June, which is related to the phenological cycle of the new stems, which are ending the vegetative stage and starting the rest stage (Camargo and Camargo, 2001; Ronchi and DaMatta, 2007). This moment of the year is characterized by a slow growth of the coffee plants as a whole due to the low metabolism during the start of the dry and cold season.

Due to its gametophytic self-incompatibility (GSI) mechanism, with monogenic heritage ruled by a set of three alleles of the gen *S* (Lashermes et al., 1996; Berthaud, 1980), populations of *C. canephora* commonly present high phenotypic and genotypic variability (Fonseca et al., 2006; Ferrão et al., 2008; Rodrigues et al., 2012). For many agronomic traits, the existence of high genetic diversity had been reported, e. g., Colodetti et al. (2014) and Martins et al. (2013c), studying improved genotypes of *C. canephora* in environments with different levels of nutritional stresses, reported the expression of different growth behaviors and nutritional efficiencies.

The existence of variability for crop yield and bienniality between genotypes from different ripening cycles have been studied by Rodrigues et al. (2013), which concluded that there is high diversity intrinsic to each of the groups of genotypes: From early, intermediate and late ripening cycle. This fact is also described by the study of genetic parameters of several traits used in the breeding program of *C. canephora*, which show high variability for genotypes from all of these ripening groups (Rodrigues et al., 2012).

Regarding the growth rate, several authors found different behaviors regarding the increase of leafiness and biomass of improved genotypes of *C. canephora* during the early stages of development, indicating the existence of variability for this trait (Rodrigues et al., 2012; 2013; Silva et al., 2013; Martins et al., 2013a, b, c; 2015; Colodetti et al., 2014, 2015; Menezes-Silva et al., 2015).

Considering the grouping presented at Table 2, most genotypes from the Group I presented early ripening cycle, which normally present length from 34 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). The genotypes from this group also presented higher growth in November and December, lower growth rate on February and a promptly growth retake after May.

Most genotypes from the Group II presented intermediate ripening cycle, which are classified as such for having ripening length near 41 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). The genotypes from this group had slower growth on November and in the period between February and April.

The Group III was formed by genotypes with ripening cycle between intermediate and late, that is, presenting 41 to 45 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). This group was formed by genotypes with typical slow growth on February and from May to June, with rapid increase in growth rate in July.

The Group IV was singly formed by the genotype RC06, which presented higher growth from November to February. This contrasting genotype has been studied by DaMatta et al. (2003), which concluded that this genotype may present similar physiological traits to others genotypes of *C. canephora* when cultivated with irrigation, but respond differently in environments where it is subjected to periods of drought, being classified as a drought tolerant genotype.

The magnitudes of the direct and indirect effects observed in the patch analysis are consistent indicatives that the growth rate during the highly active months and in the moments of recovery after periods of slower growth, due to low rainfall, may be extremely important to determine the length of the stems during the final of the season. Therefore, genotypes capable of restarting the growth earlier or these able to achieve higher growth rates in the rain season may end up with larger and more vigorous stems.

The growth and therefore the extension of the stems are especially important for fruit bearing species, such as coffee, since these stems are going to support all the plagiotropic branches that will be responsible for the fruit yield. A slow growth may reduce the recovery capacity of the plants, which may not be able to produce properly in the next cycle; and an excessively fast growth of the stem may compromise the growth of the primary plagiotropic stems, which also can limit the crop yield in the next cycle. The pruning management must be adequate to renew the plantation without intensifying such effects, and an interesting recent alternative is the programmed pruning cycle for Conilon coffee (Verdin Filho et al., 2014), which alternate the production and keep the plantation being constantly renewed along the years.

New studies involving growth rates of genotypes of *C. canephora* are important since the specie has high genetic variability for several growth traits. Studies involving other environmental conditions that may regulate the expression of growth traits are especially important to help validating the results, such as studies in other regions and other crop systems with different technological levels, different water and nutritional management and even different pruning managements.

Conclusion

The monthly growth rate can be a useful tool to identify and to study the variability among genotypes of *C. canephora* Pierre ex Froehner, and the evaluation of the growth rate during recovery months, in the peaks after periods of slower growth, is especially advantageous to study the diversity and the growth rate in these months is highly important to determine the length of the stems during the final of the season.

There is a considerable level of similarity between the growth of genotypes from the same group regarding ripening cycle; however, the high variability makes possible to identify genotypes from different behaviors regardless of the group.

Conflicts of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are grateful to Centro de Ciencias Agrárias of the Universidade Federal do Espírito Santo (CCAUFES) for providing access to the necessary facilities and laboratories. Moreover, the first author would like to thank the Fundação de Amparo à Pesquisa do Espírito Santo (FAPES) for awarding postdoctoral scholarships and financially supporting this research.

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academicJournals

Vol. 11(28), pp. 2508-2518, 14 July, 2016
DOI: 10.5897/AJAR2016.11163
Article Number: 5E66C9B59408
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Hydrology and water quality of a underground dam in a semiarid watershed

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Received 29 April, 2016; Accepted 20 May, 2016

In the Brazilian semiarid regions, underground dams can efficiently store water under possible scenarios of climate change. However, the annual humidity vector in these regions is vertical, and annual evapotranspiration exceeds annual rainfall, resulting in the accumulation of salts in groundwater reservoirs. This study investigated over the course of two agricultural years the hydrologic behavior, soil moisture and the seasonal behavior of electrical conductivity (EC) of the irrigation water from an underground dam constructed in the Jacu watershed in a Brazilian semiarid region. The underground dam retained more soil moisture than other nearby areas during the rainy season; however, during dry periods, its storage capacity was reduced by evapotranspiration occurring inside and outside of the groundwater dam and after rainfall the same level of evaporation occurred from soil as from the dam. During the dry season the underground dam raised the concentration of salts in the irrigation water which was categorized as C4, corresponding to the far too saline irrigation water; however, in subsequent rainy seasons, the electrical conductivity of irrigation water decreased to be included in a group of low salinity of 0.95 dS m^{-1} (C1). The irrigation water sodicity changed from the risk of sodium accumulation to without sodium risk category.

Key words: Evapotranspiration, soil moisture, electrical conductivity, sustainability of semiarid.

INTRODUCTION

Groundwater is the only available water resource in arid regions where surface water resources are scarce or even non-existent (Alley et al., 1999). Onder and Yilmax (2005) noted that in incoming decades sustainability of

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water resources would be a key issue and the dependence on groundwater would increase. Sand-storage dams are a successful water harvesting technology in Kenya and a promising solution to ensure water and food security in other semi-arid regions. Assessment of the suitability of sand-storage dams for other semi-arid regions requires both an understanding of the hydrological factors for the success of a single dam and the regional effects of a network of dams (Quilis et al., 2009). Moon et al. (2012) reported that six underground dams in alluvial aquifers were built to retain flood flows and thus to acquire additional water supply for many uses as drinking water and agricultural irrigation.

An underground or groundwater dam is a facility that stores groundwater in the pores of strata for sustainable use. Groundwater dams have many advantages, e.g., unlike a surface dam, land is not submerged to store water and there is no danger of breaching due to natural or manmade disasters. The surface area can be used in the same way as before after the construction of the dam. An underground dam allows for the development of water resources in regions where the construction of surface dams is difficult due to geological conditions, and where groundwater cannot be used in its current state (low water table, etc.). Underground dams are composed of a cut-off wall to dam the groundwater flow and prevent the intrusion of seawater, as well as facilities (wells, intake shaft, and pumps) that draw up the stored groundwater (Ishida et al., 2011).

An underground dam significantly changes the groundwater level and groundwater flow from natural conditions and this change influences the quality of groundwater. It is important to evaluate the impact of groundwater dam construction on groundwater quality. Soil salinity is a serious problem in arid and semiarid areas that can lead to significant levels of groundwater salinity, which can be further aggravated by direct evaporation when impoundment results in a shallow water table (Ishida et al., 2011).

Evapotranspiration (ET), the process by which water in its liquid state evaporates from soils and due to plants to the atmosphere, is an important hydrological process. Referring to agricultural production, measurement of evapotranspiration is essential for determining crop water demand (Bakhtiari et al., 2001). Over the entire land surface of the globe, rainfall averages around 750 mm $year^{-1}$, of which about two thirds is returned to the atmosphere as evapotranspiration, making evapotranspiration the largest single component of the terrestrial hydrological cycle (Fisher et al., 2005), which is important to arid and semiarid regions.

The variability of evapotranspiration (ET) on shorter timescales is poorly constrained, but is critical for the coupled cycling of water, energy, and carbon in arid and semi-arid environments. In particular, the variability of evapotranspiration affects (1) the amount of precipitation partitioned into runoff and recharge; (2) how land-atmosphere interactions influence weather and climate; and (3) processes, such as plant productivity, soil respiration, and biogeochemical cycling. Considering the broad importance of evapotranspiration, ecosystem-level observations of evapotranspiration from semiarid environments are surprisingly limited. The limited field studies that have been completed in semiarid environments demonstrate that evapotranspiration varies greatly with time (Fernandez-Illescas et al., 2001).

Soil moisture is the key link between climate fluctuations and vegetation dynamics in space and time. Meteorological and vegetation conditions control evapotranspiration when soil moisture is not limiting, however, because precipitation is much less than potential evapotranspiration in arid and semiarid environments, evapotranspiration is believed to be limited by soil moisture most of the time in dry land ecosystems (Rodriguez-Iturbe, 2000). Unfortunately, field observations required to test these relationships are often lacking, particularly in arid and semiarid environments where limitations from soil moisture are believed to be the most important (Kurc and Small, 2004).

In water-limited ecosystems soil moisture and vegetation have a coupled relationship that is basic to ecosystem dynamics. The soil moisture plays a central role in the dynamic interaction between climate, soil, and vegetation and makes explicit the coupled dependence of water balance and water stress processes on soil moisture. Through transpiration, plants have an active role in soil water use that heavily conditions the water balance. The water balance, in turn, impacts plant growth, reproduction, and germination through the onset of water stress (Fernandez-Illescas et al., 2001).

Groundwater is a preferred source of water supply. Due to increasing population growth and a higher demand for potable water, the exploitation of groundwater resources is increasing (Verplanck et al., 2008), and it is estimated that approximately one third of the world's population consumes groundwater (UNEP, 2000). For the majority of small rural communities, groundwater remains the only source of potable water (Sharma et al., 2012).

In the Brazilian semiarid region, rural watersheds are exploited only during the rainy season, when local residents engage in shifting cultivation, largely because there is no technological process that involves the planting of subsistence crops, such as "macassar" bean, corn, sorghum, cassava and others with very low aggregate economic value, and extensive ranching. A technological alternative to increase the availability of water in the semiarid region of Northeast Brazil is the installation of underground dams, which can increase agricultural productivity of small and medium-sized farms, particularly the farms that lack the water required for conventional irrigation (Brito et al., 1999).

In arid regions, groundwater plays the critical economic role of facilitating rural subsistence farming, which is the basis of human survival; thus, groundwater enables

human settlement and supports individual livelihoods (Giordano, 2006). However, groundwater may have a higher soluble salt content than surface water due to its slower flow and longer period of contact with mineral and sediment-rich rocks. Additionally, groundwater quality varies due to changes in the chemical composition of sediments composing and overlying the aquifers (Jameel, 2002). In this sense Burger and Celkova (2003) reported that processes in that groundwater is drawn by evapotranspiration, and soluble salts coagulate on the surface of soil particles and sodium ions are adsorbed into the soil colloidal system. The water motion processes are determined by hydro-physical characteristics and hydraulic parameters of the porous subsurface environment and by water flow from and to the groundwater level. These processes result in the emergence and expansion of saline and alkali soils. Salt accumulation is a phenomenon that is not unique to any particular soil type in many soil classifications, but it is typically associated with flow and quantity of water, especially Solonetz (alkaline) and Solonchak (salt enrichment upon evaporation) (Bui, 2013). It occurs where evaporation is high relative to precipitation due to seasonal water deficit and leaching is insufficient to move salts out of the soil. Ben-Gala et al. (2009) said that studies of the whole-plant or crop responses to salinity often focus on yield or growth reduction in terms of solution ion concentration or electrical conductivity.

García-Garizábal and Causape (2010) observed that the electric conductivity (EC) and nitrate concentration increased in irrigated areas of Spain in the medium Ebro River basin; however, simple irrigation management was able to decrease the salt and nitrate export by 20 and 24%, respectively, and hence improved water quality. In this sense, acid mine drainage (also sometimes referred to as acid rock drainage) is a well-understood process and arises primarily when the mineral pyrite (‘fool’s gold’ or iron disulphide) comes into contact with oxygenated water (McCarthy, 2011). This process that occurs in underground mining can decrease the water quality in the watersheds.

The Komesu underground dam is the first full-scale underground dam constructed to prevent saltwater intrusion in Japan. Although the cutoff wall of the dam effectively reduces the movement of saltwater into the reservoir area, saltwater masses remained behind the dam at the time of its completion, and saltwater can intrude beneath and diffuse through the wall, particularly when the reservoir level is below the sea level because of high pumping levels during the drought years. Therefore, it is necessary to estimate in advance whether the saltwater concentration in the pumped water is likely to exceed the permissible salinity level or not because of an increase in the residual saltwater mass as a result of saltwater intrusion and to take necessary measures to suitably manage the saltwater level behind the dam (Nawa and Miyazaki, 2009).

The objective of this study was to investigate over the course of two agricultural years, the hydrologic behavior, soil moisture and the seasonal behavior of the quality of irrigation water from an underground dam in the Jacu watershed in a Brazilian semiarid region.

MATERIALS AND METHODS

Location and characterization of experimental area

An experiment was conducted in a 2.1 km^2 area watershed of the Jacu River, located in the upper Pajeu region, a semiarid environment in the Brazilian state of Pernambuco. This region, near the Serra of Lagartixa, is found in the municipal boundary between the cities of Serra Talhada and Floresta, with the geographical coordinates of 8°07'07" South latitude and 38°23'55" West longitude. The climate is categorized as type BWh by the Köppen classification system, as the environmental conditions are semiarid, hot and dry, with summer-autumn rains, an average annual rainfall of 647 mm $year^{-1}$ between 1912 and 1991(SUDENE, 1990) and an annual temperature above 29°C. Entisols predominate in the region of the semiarid watershed of the Jacu River, particularly in the area that includes the riverbed banks in the lower parts of the Watershed, in accordance with EMBRAPA (2006). In the Jacu watershed, 82.76% of the area is occupied by native hyperxerophilous shrub-arboreal and 17.24% by shifting cultivation. Figures 1 and 2 show the location and soil cover maps of Jacu watershed, respectively.

Installation of underground dam

The area for the installation of an underground dam was selected in August 2009 using a topographic survey conducted in accordance with Silva et al. 2007). This survey defined the best location for the placement of the dam’s components, that is, its catchment area, planting area and dam wall, given the morphology of the alluvial deposit. At the beginning of the experiment, the selected area contained remnants of dry matter that were left after the corn harvest.

For underground dam scaling, a trench perpendicular to the direction of the water flow was initially dug to an average depth of 1.90 m by excavating the trench with a mechanical backhoe until the impediment layer was reached. The stabilization of the dam wall was not required. A brick-and-mortar wall was constructed on the upstream side of the top part of the trench to standardize the slope cutoff and avoid perforation of the plastic by rock fragments, roots and other debris.

A 200 µm thick polyethylene tarp was fixed at both ends of the trench to create a functional dam, as the tarp formed a barrier that was impermeable to the subsurface water flow. Subsequently, the trench was closed with the aid of a backhoe, using the material excavated during the opening of the trench. This process left soil accumulation along the trench that covered the plastic tarp. At the time of dam construction, an existing well was used for the drainage of excess salts during high flows of the Jacu River by a water pump.

Water sample collection

To monitor water quality, a total of seven samples were collected in 500 mL containers over three years, with the first sample obtained from the well during the installation of the underground dam in December 2009. As the highest rainfall in 2010 occurred only during the month of October, the second and third samples were

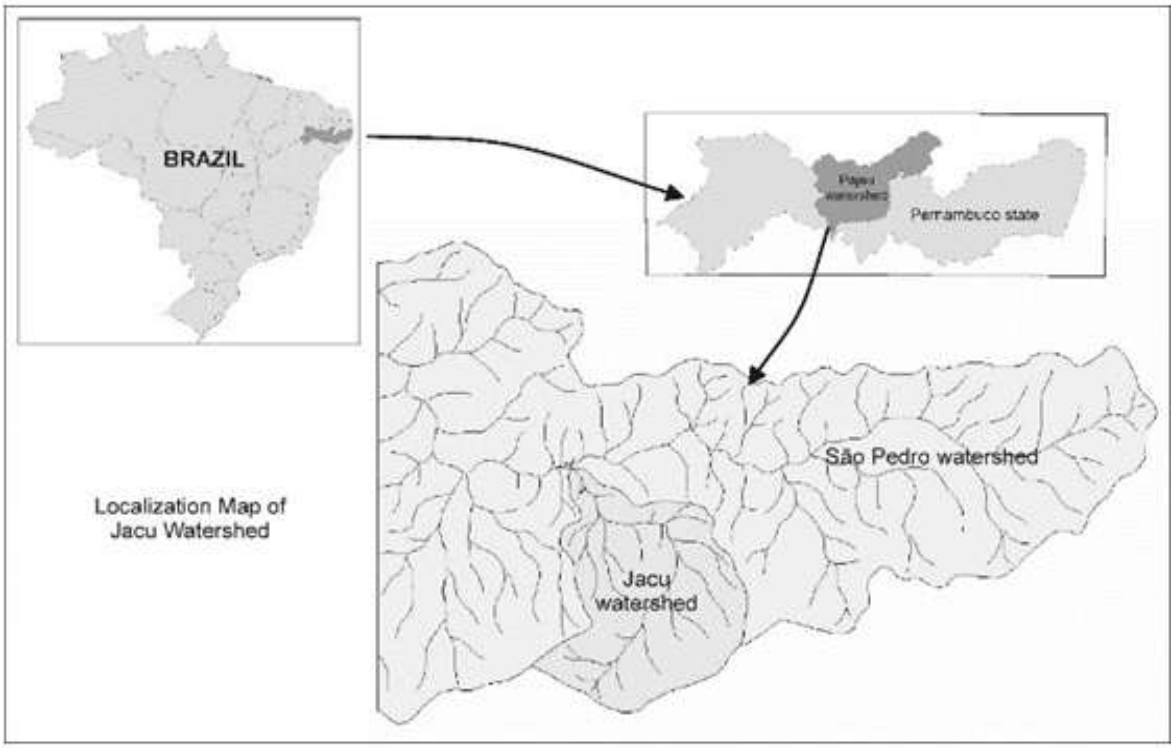

Figure 1. Location map of Jacu River watershed.

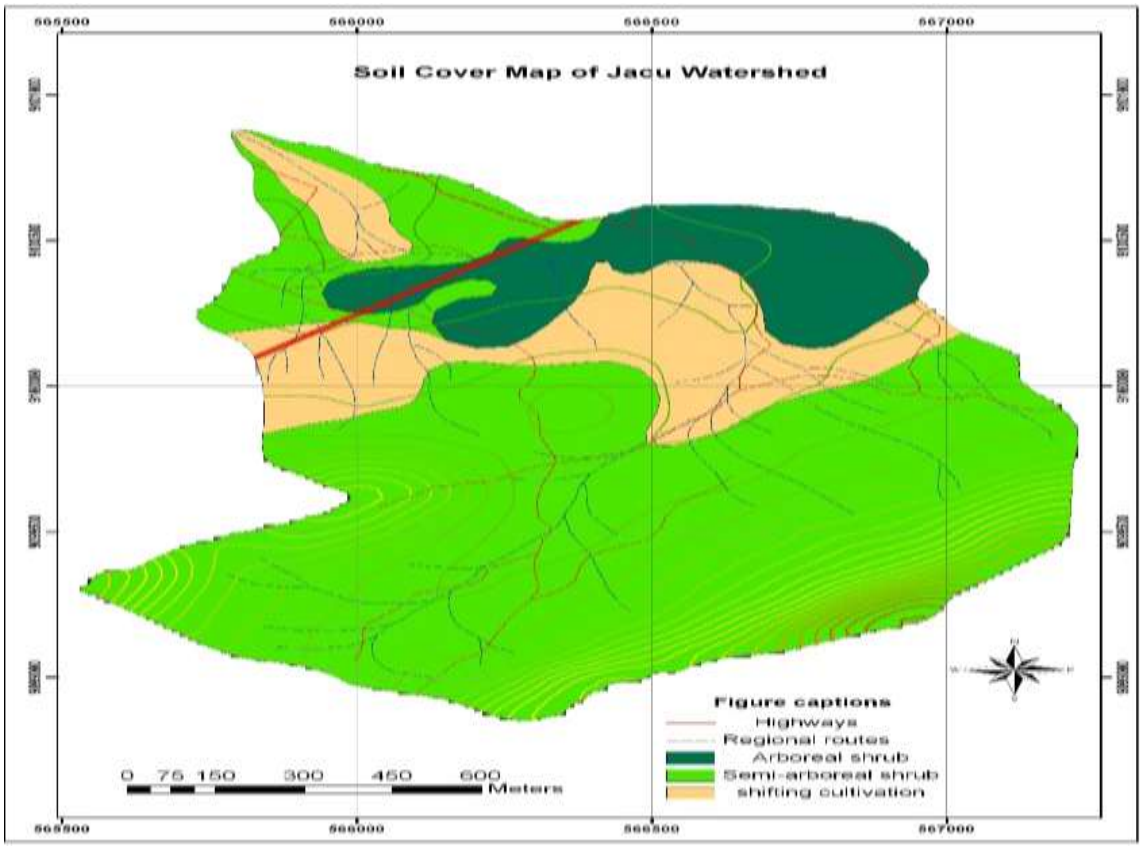

Figure 2. Soil cover map of Jacu River watershed.

Table 1. Soil physical characteristics of semiarid Jacu watershed in semiarid of Pernambuco State, Brazil.

Soil depth	D_s	D_p	U	θ	P
cm	g cm^{-3}	g cm^{-3}	g g^{-1}	cm^3 cm^{-3}	
0-20	1.14	2.59	0.040	0.046	0.559
20-40	1.20	2.50	0.072	0.086	0.520
40-60	1.13	2.66	0.107	0.120	0.575
60-80	1.17	2.63	0.120	0.140	0.555
> 80	1.45	2.63	0.146	0.212	0.448

D_s, Soil density; D_p, particle density; U, soil moisture; θ, volumetric soil moisture; P, total porosity.

collected in January and December 2010. In the year 2011, which included a period when rainfall was more evenly distributed, samples were collected in March, April and May 2011. The final sampling was performed in February 2012.

The following parameters were assessed: Water pH (1:2.5); electrical conductivity; the concentrations of soluble calcium and magnesium ions, measured by atomic absorption spectrophotometry; the concentrations of sodium and potassium ions, measured by flame photometry; the concentrations of soluble carbonate and bicarbonate ions, measured by titration with 0.005 mol L^{-1} H_2SO_4; and the concentration of chloride ions, measured by titration with 0.1 mol L^{-1} $AgNO_3$. The sodium adsorption ratio (SAR) values were also calculated in accordance with the method outlined by FAO (1999) and Richards (1954).

Soil sample collection

To monitor soil moisture, a total of 10 samplings at a soil depth (0-5 cm) were performed inside and outside of the dam one day after the rainfall. Each measurement was performed in triplicate. During the physical characterization of the soil (Table 1), the following parameters were determined: Soil density, assessed using the volumetric ring method; particle density, assessed using the volumetric flask method; particle size distribution, assessed using the densimeter method; moisture content, measured by mass and volume; and total porosity, calculated from the soil and particle density (EMBRAPA, 2006).

Hydrologic data

Rainfall (mm) and flow (m^3 s^{-1}) data were obtained using an automatic stage and a rain gage station linked with data logger, containing a level and flow sensor placed in the control section of the riverbed, and a pluviograph that recorded rainfall amounts and duration. The collected data in 10 minute time intervals were automatically recorded in a Data Logger (model SL2000MIM) of SOLAR Instrumentation, which was also part of the station and was powered by a photoelectric cell and a 12-volt auxiliary battery.

The water balance in the underground dam was used to calculate evapotranspiration considering the processes that affect the temporal dynamics of the water storage in the control volume S of dam (t):

$$\frac{d_S(t)}{d_t} = P_{(t)} - ET_{(t)} - Q_{(t)} \quad (1)$$

Where d_s/d_t = is store water in time, P is the precipitation, ET is the evapotranspiration, and Q is the total runoff. Considering the equation 1 integrated over a 24 month time interval of the experiment, evapotranspiration was obtained by:

$$0 = P_{(t)} - ET_{(t)} - Q_{(t)} \quad (2)$$

The evaporative fraction (EF), the water content in soil after rainfall, decreases in time and was calculated using the yield-density model:

$$EF(\theta)_t = (a + b\Delta_\theta)^{-1/C} \quad (3)$$

Where EF $(\theta)_t$ is the soil moisture at time t after the rain; Δ_θ is the difference between the water content observed on the first day following rainfall (θ_1) and the water content observed at the end of interval without rainfall (θ_f); and **a**, **b** and **c** are the coefficients. The evaporative fraction (EF) was calculated for the inside and outside areas of the groundwater dam, and the time series between rainfall events varied from 30 to 107 days due to long dry periods. Other approaches have been used by Kurc and Small (2004) and Hunt et al. (2002) to calculate EF.

Statistical analysis

Regression analysis with a 95% confidence interval was used to determine the best fits between water quality, soil moisture and hydrologic parameters. The Curve Expert 1.5 professional software program was used to obtain the best regression models, based on the coefficient of determination and standard error.

RESULTS AND DISCUSSION

Watershed hydrology: rainfall, soil moisture and evaporation

The distribution of rainfall in the watershed of the Jacu River between 2008 and February 2012 is depicted in Figure 3. It is evident that the semiarid region has a distinct hydrological rainfall pattern characterized by variability and intense rainfall, as rainfall volumes are concentrated into short periods of time (January to April 2008; February to May 2009; October 2010; and November and December 2011) with widely varying spatial and temporal occurrences of rainfall events (March 2008; May 2009 and October 2010); consequently,

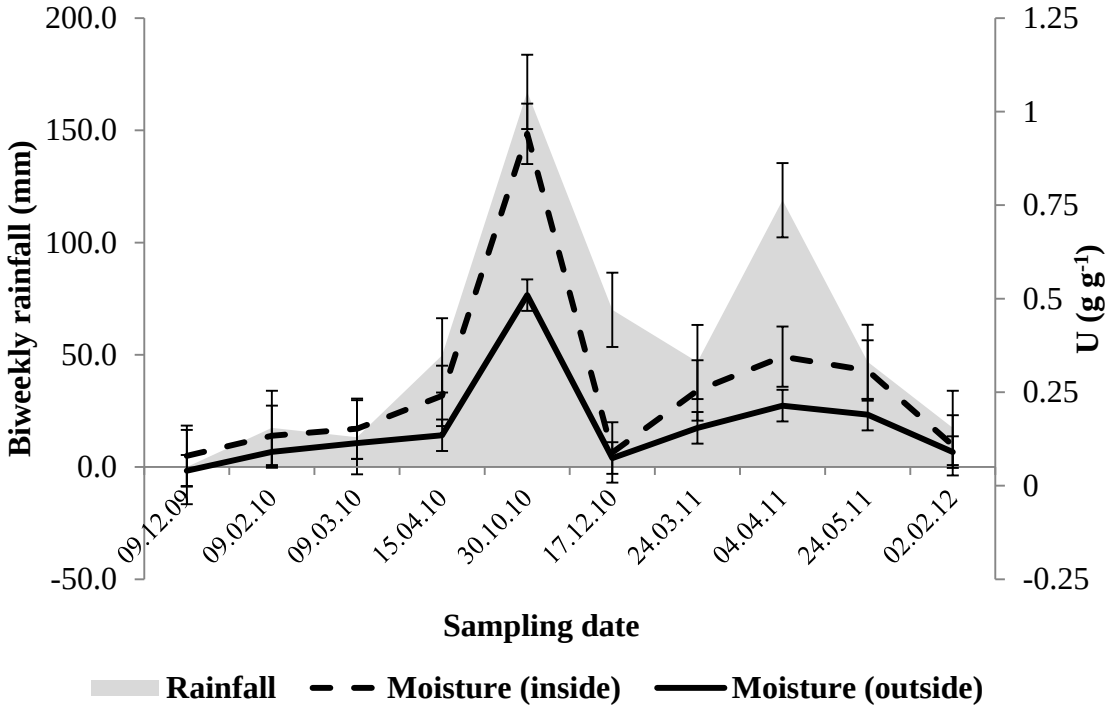

Figure 3. Biweekly distribution rainfall from Jacu River watershed from 2008 to February 2012, semiarid of Brazil.

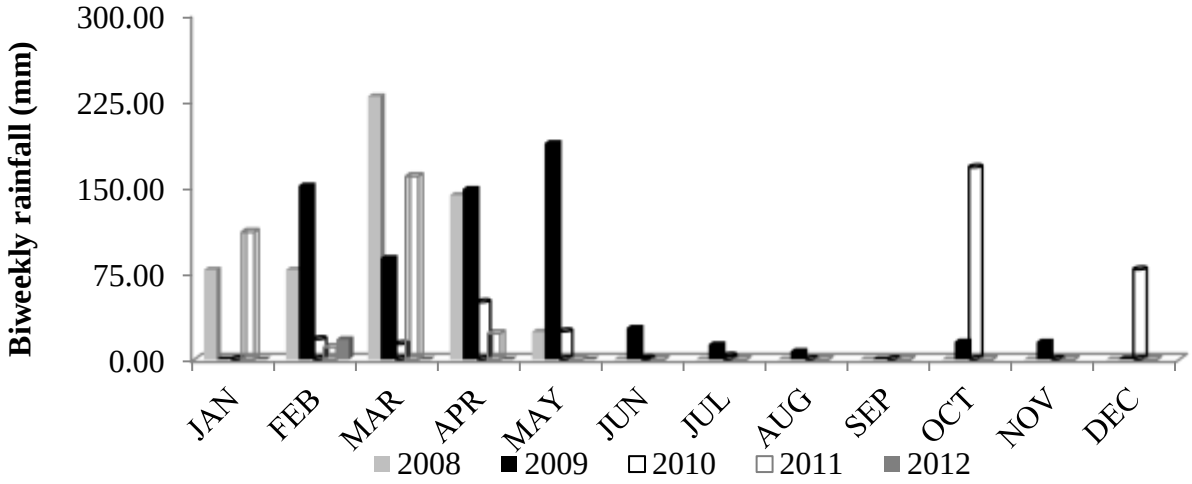

Figure 4. Soil water behavior inside and outside of the underground dam at 0 to 20 cm depth, and biweekly rainfall from Jacu river watershed from 2008 to February 2012.

extreme surface and subsurface flows occur in October/December 2010 and March/April 2011, as shown in Figure 4, that illustrates the behavior of soil moisture from the groundwater dam from the inside and outside areas of the groundwater dam, that is, the regions adjacent to the dam in both the upstream and downstream directions. The standard error bars of this figure demonstrate that there are differences in soil moisture between these two areas in the time period of rainfall, which indicates that water storage in the control volume increased during this period; the water storage did not reduce because surface water did not flow fast from the dam area and did not cause water losses from the watershed that were observed in the area outside the dam.

However, during the periods of no or low rainfall, there

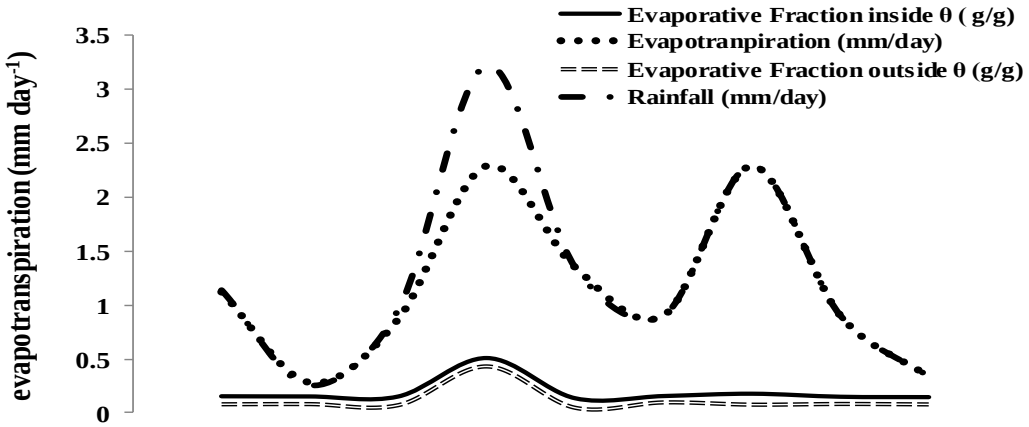

Figure 5. Daily rainfall and evapotranspiration, and gravimetric water content (0-5 cm) (evaporative fraction) inside and outside of the groundwater dam for days sampled from 09/12/2009 to 02/02/2012.

Table 2. Coefficients of "Equation 3" used to calculate the evaporative fraction of soil water content in inside and outside areas of the groundwater dam, on Jacu watershed, Brazil.

Coeff.	Inside groundwater dam	Outside groundwater dam
a	846997	38.93
b	2.2613×10^6	109.03
c	7.22	1.46
r^2	0,97	0.96

was less use of the retained moisture due to the occurrence of evapotranspiration, which acted similarly inside and outside of the dam. In other words, the occurrence of evapotranspiration in the dam during dry periods led to soil moisture losses, which reduced the efficiency of the underground dam. This result is consistent with the findings of Quilis et al. (2009), who reported losses to evaporation in underground dams, albeit smaller losses than were observed in the regions outside of the dams in question. According to the data presented in Figure 4, the time period of soil moisture retention in the groundwater dam of the Jacu River varied from two to six months.

Figure 5 shows evapotranspiration rates obtained by water balance of the Jacu watershed and underground dam as well as evaporative fractions inside and outside of the dam. The coefficients of the yield-density function (Equation 3) for calculating the evaporative fraction of surface soil inside and outside of the underground dam in years 2010 and 2011 are shown in Table 2. Figure 5 shows that evapotranspiration values approached rainfall values, typical of water limited semiarid regions (Potter and Zhang, 2009; Sankarasubramanian and Vogel, 2002) which means the evapotranspiration ratio of Jacu watershed, that is, the ratio of mean annual evapotranspiration to mean annual precipitation (E/P) calculated was 0.99945, close to 1, and leaving the dryness index in Budyko's curve to water-limited regions, confirming the local semiarid feature.

Evapotranspiration rates observed in the Jacu watershed, which has 82.76% of its area covered by shrub-arboreal and 17.24% of its area used for shifting cultivation, are of the same order of magnitude as those observed in other semi-arid areas of the world (Kurc and Small, 2004; Potter and Zhang, 2009; Sankarasubramanian and Voge, 2002; Zhang et al., 2008; Wang and Alimohammadi, 2012; Raz-Yaseef et al., 2012). The evapotranspiration occurred alike in the inside and outside areas of the underground dam, which had the same levels of evaporation from soil water content as validated by the close performance of the model to quantify the evaporative fraction (EF) inside and outside of the dam (Table 2). The simultaneous evapotranspiration inside and outside of the underground dam and the same levels of evaporation from soil from both these areas after rainfall, mainly in dry period attest to the reduction in the efficiency of the underground dam in the Jacu watershed, which was able to maintain the soil moisture during four to six months, while the dry periods can last up to 10 months.

Quality of irrigation water

Relationships among salinity, soil moisture and flow of the Jacu watershed

Figure 6 illustrates the behavior of the Jacu River groundwater electrical conductivity (EC) as a function of rainfall, which produced surface water and the resulting soil moisture values that were observed between September 2009 and February 2012. Initially, there was a peak in the electrical conductivity caused by the

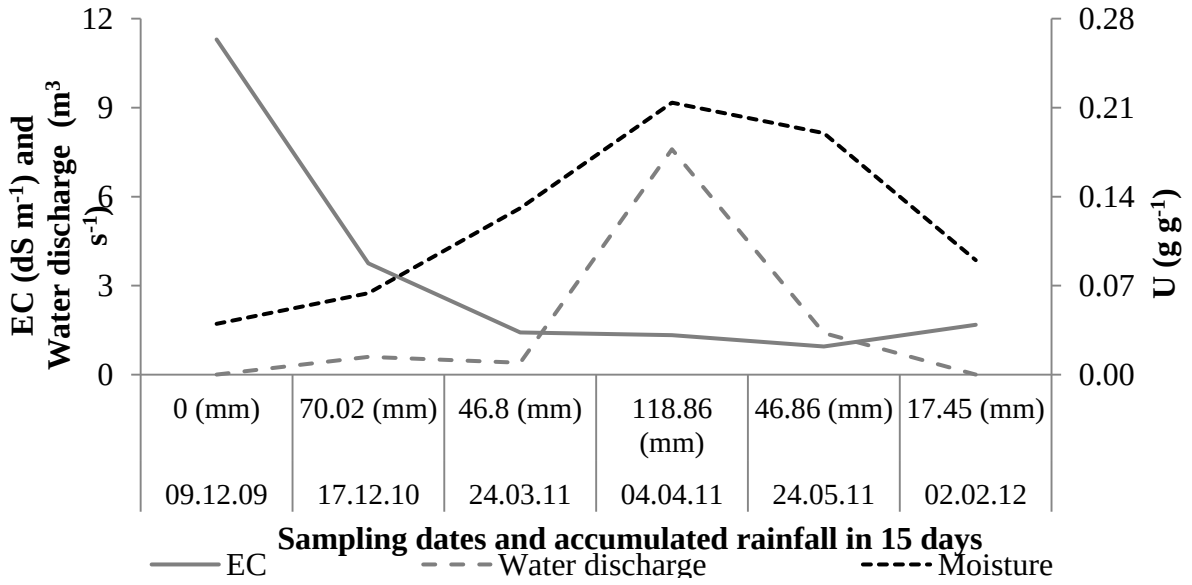

Figure 6. Behavior of the Jacu River underground electrical conductivity (EC), soil moisture, rainfall and water discharge from underground Dam in Jacu River watershed, semiarid of Brazil.

Table 3. Chemical characteristics of water from groundwater dam in Jacu River watershed.

Day	pH	EC	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	SAR
		dS m^{-1}	$mmol_c$ L^{-1}							$(mmol\ L^{-1})^{1/2}$
Dry season										
09.12.2009	8.2	11.30	28.94	16.30	79.16	2.05	0.00	7.16	118.48	16.63
20.01.2010	7.1	1.19	2.99	1.48	7.39	0.77	0.00	2.47	7.00	4.94
17.12.2010	8.8	3.75	3.72	3.00	29.11	0.38	0.00	0.85	34.20	15.91
02.02.2012	7.5	1.68	4.75	2.78	8.69	0.90	0.00	0.12	16.60	4.48
Rainy season										
24.03.2011	7.6	1.42	3.08	2.40	9.14	0.21	0.00	0.95	12.00	5.51
04.04.2011	6.8	1.33	2.76	1.92	8.41	0.20	0.00	0.95	9.30	5.50
24.05.2011	7.6	0.95	2.25	1.81	5.12	0.17	0.00	1.00	6.60	3.61

pH, hydrogenionic potential; EC, Eletrical condutivity; Ca^{2+}, calcium; Mg^{2+}: magnesium; Na^{+}, sodium; K^{+}, potassium; $CO3^{=}$, carbonate; HCO_{3-}, bicarbonate; CL^{-}, chloride; SAR, sodium adsorption ratio. Date, day/month/year.

installation of the dam; however, the electrical conductivity began to decrease as the flow rate effectively increased, declining markedly in October 2010, when more intense rainfalls occurred, and subsequently remaining at these levels until the end of experimental observations. The flow levels were low from the time of installation of the dam until October 2010, after which they increased, first due to an extreme rainfall event and then due to the more regular rainfall in 2011, which also caused an increase in the soil moisture and a decrease in electrical conductivity (EC).

The observed electrical conductivity values in arid and semiarid regions, which are frequently between 1 and 5 dS m^{-1}, but can be higher than 5 dS m^{-1} at times, are still satisfactory for livestock. The Australian standards recommend water salinity levels below 6.6 dS m^{-1} for cattle and 10 dS m^{-1} for sheep. These standards also establish a water salinity limit of 5.2 dS m^{-1} for other animal species (Ayers and Westcot, 1999), and also the pH values between 6.5 and 8.4 that were observed in this study were in accordance with established irrigation water quality guidelines.

The electrical conductivity of 11.30 dS m^{-1} observed in September 2009 (Figure 6 and Table 3) is categorized as C4 according to the classification diagram for irrigation water that was created by (FAO, 1999; Richards, 1954), as this EC is above 2.250 dS m^{-1}.

The high electrical conductivity observed in 2009 was due to the installation of the dam, as the excavation, the mechanical disruption of the soil profile and the

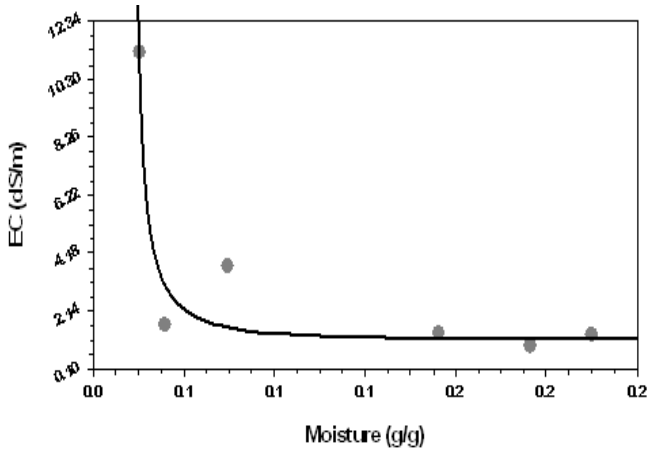

Figure 7. Behavior water electrical conductivity in functions of soil water content in underground Dam in Jacu River watershed, semiarid of Brazil.

installation of the physical barrier that makes up the dam forced the translocation and concentration of water and salt from groundwater into the thalweg of the river, which is between the impediment layer of the septum bottom and the dam during the dry period. The observed salt concentration was also augmented by the natural conditions in the semiarid region, which include low rainfall that is surpassed in quantity by evapotranspiration and therefore favor salt accumulation in the soil profile and surface of the region (Hanson et al., 1993).

The levels of electrical conductivity were related to the soil moisture in accordance with the Hoerl regression model, which clearly predicts reductions in electrical conductivity when soil moisture levels increase as a function of flow rate increase (Figure 7). Sharma et al. (2012) evaluated water quality at 40 groundwater sites of Northeast India and found most of the groundwater samples fell in the field of C2S1 and C3S1 indicating medium to high salinity and low sodium water, which can be used for irrigation on almost all types of soil.

The assessment of the dam's irrigation water, Table 3 shows the pH ranged from 6.5 to 8.4, considered adequate according to water quality guidelines for irrigation (Ayers and Westcot, 1999). These values are coherent with the values observed for carbonate ions. The values of pH higher than 8.4 indicate the presence of soluble carbonates, and Richards (1954) suggested that the presence of carbonate ions indicates pH higher than 8.5. The electrical conductivity (CE), analyzed before and after dam's construction, respectively, ranged from 11.30 to 0.95 dS m^{-1}.

Among the cations, sodium was the dominant ions, followed by calcium, magnesium and potassium in small proportions, whilst the dominant anion was the ions chloride. The dominant ions in irrigation water in the study region are sodium and chlorides. Leaching is the basic requirement tool for controlling the level of salts in root zones, chiefly because not only the sodium but also the chlorides in excess levels are toxic for animals and plants. Sodium saturation will replace calcium and magnesium ions on the clay surface, providing reduction in soil infiltration, surface crusting and reduced hydraulic conductivity. According Fernandes et al. (2009) the reason is its relatively large size, single electrical charge and hydration status. Excess ions levels (leading to toxicity) provoke morphological and physiological changes in the plants, due to high ionic concentration, especially sodium, that reduces the absorption of other nutrients. The sodium adsorption ratio (SAR) classified the water as S1 and S2, once that the values ranged from 3.61 to 16.63, respectively, according to the guidelines proposed by Richards (1954). In the period between 2009 and 2012, the water showed risk of sodicity, mainly for soils with high amount of clay particles and high cations exchange capacity.

Chloride toxicity and resulting sodium adsorption ratio (SAR) based classification of irrigation water

Concentrations of chloride in groundwater after the establishment of underground dam was 118.48 mmol L^{-1} and among 35.45 and 34.20 mmol L^{-1} during the dry season in 2009 and 2010, respectively (Figure 8). During the three rainy seasons observed in this study, there was a marked decrease in the chloride concentrations to 12.00, 9.30 and 6.60 mmol L^{-1}. These results indicate a positive effect of rainfall on the dilution of chloride concentration. In the water samples collected in December 2009, December 2010, March 2011 and April 2011, the chloride concentration was above 7.05 mmol L^{-1}, limit for human supply, which corresponds to 250 ppm Cl^-. In contrast, the chloride concentration in the samples collected in May 2011 was 6.60 mmol L^{-1}, which is below the threshold indicated by WHO. This decrease occurred because, starting in April 2011, rainfall provided increased soil moisture that increased the water volume of the dam, diluting the chloride that was present in the water and thereby reducing the chloride concentration.

Using the classification system proposed by Richards (1954), the irrigation water was categorized as either S1 or S2 because its SAR values ranged from 3.61 to 16.63; these classification levels imply that between the years 2009 and 2012, the water would present a danger of sodicity in soils of fine texture and high cation-exchange capacity.

This danger was not observed in the water of the Entisol of the Jacu River, as the irrigation water from this source demonstrated a without-sodium risk (Table 3). Thus, the monitoring of the electrical conductivity (EC) and SAR of the water from the dam is essential for accurately assessing the risk that this water may pose with respect to increasing the salinity and the levels of

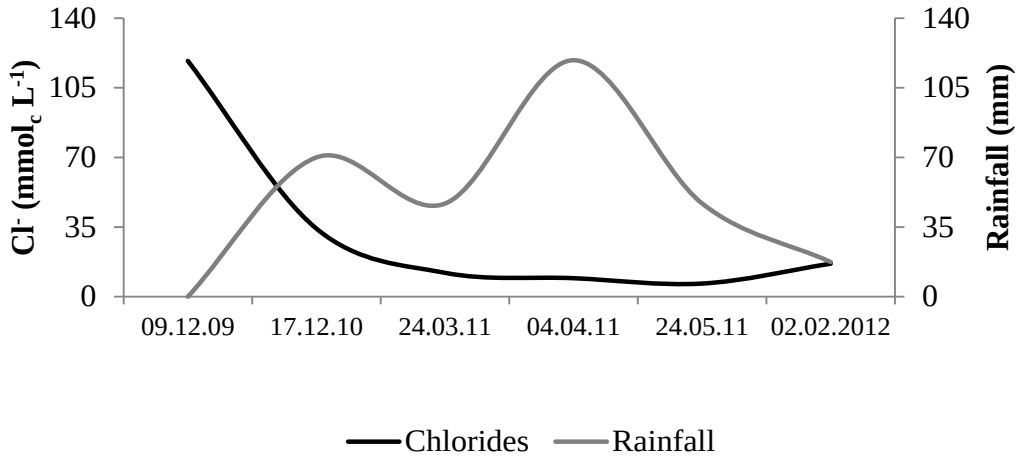

Figure 8. Chlorides variations and rainfall biweekly rainfall from Jacu River watershed from 2008 to February 2012.

exchangeable sodium in the soil.

Conclusions

The underground dam retains more soil moisture, surface water and subsurface water than other nearby areas during the rainy season; however, during the dry period, its efficiency may be reduced by evapotranspiration inside and outside of the underground dam area and the same level of evaporation from soil from both these areas after rainfall. During the dry season when the underground dam was built, salts were concentrated in the irrigation water due to the forced convergence of groundwater in the dam, and thus, the water was classified as C4, corresponding to the far too saline irrigation water; however, in the subsequent rainy seasons, the electrical conductivity (EC) of irrigation water decreased being included in a group of low salinity 0.95 dS m^{-1}(C1). The construction of a underground dam in the Jacu River watershed does not present problems related to the sodicity of the irrigation water, which shifts from the S2 category (with risk of sodium accumulation) to the S1 category (without sodium risk) by the end of the evaluation period.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(28), pp. 2519-2523, 14 July, 2016
DOI: 10.5897/AJAR2016.11167
Article Number: D8EEA9259407
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Camu-Camu super fruit (*Myrciaria dubia* (H.B.K) Mc Vaugh) at different maturity stages

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Received 29 April, 2016; Accepted 2 June, 2016

Camu-camu (*Myrciaria dubia*) H.B.K.) Mc Vaugh) is a shrub native to the Amazon region and its fruits are promising sources of various bioactive compounds such as vitamin C, phenolic compounds and carotenoids. Camu-camu fruit is considered the greatest natural source of vitamin C worldwide and also good source of dietary fiber, potassium, iron, calcium, and various kinds of amino acids such as serine, valine and leucine. Therefore, the presence of different bioactive compounds in camu-camu fruits could be used to retard or prevent various chronic non-communicable diseases such as dyslipidemia, obesity, cardiovascular and cancer. The objective of this study was to analyze pulp green and ripe camu-camu to see differences in its attributes vs. maturation stage. Ripe camu-camu pulp had a great antioxidant capacity, 2671 µmol TE/g and unripe 2563 µmol TE/g fresh weight. It is also very rich in vitamin C (1230 mg/100 g in unripe, and 1150 mg/100 g in ripened fruits), calcium (13.2 mg/100 g to ripe and 12.1 mg/100 g to unripe fresh weight), dietary fiber (2.50 g/100 g to unripe and 2.40 mg/100 g to ripe). Camu-camu is also an excellent source of other bioactive compounds, such as minerals and different phenolic compounds. In conclusion, camu-camu fruit can be used to introduce bioactive compounds into food products and to delay or prevent many human diseases.

Key words: Camu-camu, Vitamin C, phenolic compounds, carotenoids, antioxidant capacity.

INTRODUCTION

Camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh) is an exotic tropical fruit native to the Amazon region and it is one of the few Amazon fruits that have been explored for commercial purposes. The camu-camu fruit belongs to the group of the so-called super fruits and it is known by its outstanding content of ascorbic acid and health-relevant flavonoids (Borges et al., 2014; Akter et al., 2011). Fruits are essential for good health. Consumption of fruits has been increased because of their high content of bioactive compounds. The most common bioactive compounds are vitamin C, polyphenols, β-carotene and lycopene. In recent years, there has been a global trend toward the use of natural phytochemicals as antioxidants and functional ingredients, which are present in natural resources such as vegetables, fruits, oil seeds and herbs (Kaur and Kapoor, 2001; Aguiar and Souza, 2014). Natural antioxidants from plant extracts have attracted considerable attention due to their safety.

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(a) (b)

Figure 1. Camu-camu fruits at different maturity stages (a); ripe fruits (b); and unripe fruits.

Polyphenol compounds such as anthocyanins, flavonoids and phenolic acids are responsible to reduce oxidative stress (Inoue et al., 2008; Liu et al., 2008). Because of its natural acidity, the camu-camu fruit is mainly consumed after processed into juices, concentrates, and for the production of vitamin C capsules. As a result, great volumes of residue, consisting of seeds, peels and residual pulp that represent around 40% of the fruit in weight, are generated (Rodrigues et al., 2001). Despite this, while several studies have focused in investigating the properties of camu-camu pulp (Gonçalves et al., 2014; Fujita et al., 2013), the bioactive potential and biological effects of the residue remain understudied (Azevêdo et al., 2013). Camu-camu fruits are rich in polyphenols which could be used to retard or prevent various human diseases. However, there are few evidence in *in vivo* studies on the polyphenol of camu-camu fruits. Camu-camu (*Myrciaria dubia*) H.B.K.) Mc Vaughis, a member of the Myrtaceae family is native to the Amazon region. Camu-camu is an important source of nutritional antioxidants, vitamins C, β-carotene, and phenolic compounds (Chirinos et al., 2010). Camu-camu fruits are considered the richest natural source of vitamin C worldwide (Aguiar and Souza, 2015). Bioactive compounds in camu-camu fruits are not only responsible of vitamin C but also responsible of other compounds such as phenolic compounds and β-carotene contents (Chirinos et al., 2010). Camu-camu fruits are also good source of potassium, iron, calcium and phosphorous and various kinds of amino acids such as serine, valine and leucine (Aguiar and Souza, 2014). Japan and the European Union are the main export markets for products such as cellulose, extract and juice. Camu pulps are used as ice cream and puree. Camu whole fruit and slices can be used to make dried products. It appears that the fruits are promising sources of bioactive compounds that could be used as functional food not only in the Amazon, but also throughout the world. Therefore, the objective of this study is to provide information of the chemical composition and phytochemicals in promoting health, especially vitamin C, polyphenols and β-carotene in the fruit at two maturity stages, of the camu-camu.

MATERIALS AND METHODS

The study camu-camu was collected manually during the ripening stage in Rio Branco, Roraima (RR), in a region called Santa Izabel de Boiaçú, municipality of Rorainópolis, RR, with the following geographic coordinates: 0°23'27.3"S to 61°48'22.5"W. The fruits were placed in sterile plastic bags and transported to the Physical and Chemical Food Laboratory (LFQA) of the Society, Environment, and Health Coordination (CSAS) of the National Research Institute of Amazônia (INPA).

The shape of the camu-camu fruits is round (Figure 1) and the diameter and length are 1.0 to 3.2 cm and 1.2 to 2.5 cm, respectively. All camu-camu samples were homogenized in a blender before the physical and chemical analyses, to quantify fiber, protein, cholesterol, sugar, moisture, ash, polyphenols, minerals, fatty acids, lipids, some vitamins, and antioxidants. The moisture, ash, protein, lipid and cholesterol, contents of the camu-camu were analyzed three replicates as recommended by AOAC (2005) and sugar also three replicates as recommended by Mason and Slover (1971). Soluble and insoluble fiber contents were determined by the method proposed by Asp et al. (1983). Vitamin C, carotenes content was measured three times by high-performance liquid chromatography (HPLC) following the method proposed by AOAC (2005). Calcium, sodium, potassium, magnesium, manganese, iron, zinc, and copper contents were determined by digesting the sample (CEM Coorporation, model MD-2591) and reading the solution with an atomic absorption spectrometer (Variam Spectra AA, model 220 FS). Antioxidant capacity was determined as recommended by Brand-Williams et al. (1995) using 2,2-dyphenyl-picrylhydrazil (DPPH). Ten grams were extracted with 100 ml of 60% ethanol under constant stirring at 30°C for 24 h. The extracts were filtered by filter paper number one and the fluid portions were analyzed for antioxidant activity.

Table 1. Nutritional composition of camu-camu fresh fruits at different maturity stages.

Camu-Camu (Fresh weight)	Unripe	Ripe
Carbohydrates (g/100 g)	7.9	8.6
Ash(g/100 g)	0.258	0.288
Moisture (g/100 g)	91.2	90.3
Fiber (g/100 g)	2.50	2.40
Ferro (mg/100 g)	0.247	0.211
Sodium (mg/100 g)	2.66	3.45
Calcium(mg/100 g)	12.1	13.2
Protein (g/100 g)	0.53	0.71
β-Carotene (mg/100 g)	0.113	0.147
Cholesterol (mg/100 g)	Tr	Tr
Fructose (g/100 g)	0.3	0.7
Glucose (g/100 g)	0.2	0.5
Tiamin (mg/100 g)	Tr	Tr
Riboflavin (mg/100 g)	0.09	0.09
Niacin (µg/100 g)	470	680
Polyphenols(mg/100 g)	1380	1280
Vitamin C (mg/100 g)	1230	1150
Saturated Fatty Acids (g/100g)	0.032	0.039
Monounsaturated Fatty Acids(g/100 g)	0.040	0.083
Polyunsaturated Fatty Acids (g/100 g)	0.015	0.013
Total Fatty Acids (g/100 g)	0.087	0.135
Antioxidant Capacity(µmol TE/g	2563	2671

The absorbance was read three times at 515 nm, and the antioxidant capacity was calculated as µmol of Trolox equivalents (TE) per gram fresh weight.

RESULTS AND DISCUSSION

The contents antioxidante capacity in camu-camu fruit at two maturity stages are presented in Table 1 and extremely high antioxidant activity, 2671 µmol TE/g for ripe and 2563 for unripe. The ripening process is a critical variable in camu-camu bioactive properties, especially with respect to its reduction potential. These results agree with the antioxidant activity measured during ripening. The antioxidant potential may be related to the phenolic composition of the extracts, but other components may also make an important contribution. Camu-camu fruits are considered the richest natural source of vitamin C in Brazil (Justi et al., 2000). Due to their high level of this vitamin, the Camu-camu derivatives such as pulp, extract and juice are extensively exported to Japan and European Union markets (Akter et al., 2011 and Chirinos et al., 2010). The content of vitamin C is 20 times higher than Acerola and 100 times greater than lemon (Vidigal et al, 2011). Due to it high nutritional value, the Amazon Research National Institute (INPA) introduced the seed of Camu-camu in the interior of Brazil, in Minas Gerais, São Paulo and Paraná states (Yuyama, 2011). Nevertheless, Justi et al. (2000) observed that the fruit grown in Paraná presented lower content of vitamin C (1400 mg/100 g in the pulp) than the one from the Amazon region (2400 to 3000 mg/100 g in the pulp). This suggests that different conditions influencing the development of this plant might modulate the levels of bioactive compounds. Furthermore, Chirinos et al. (2010) reported that total phenolic contents in Camu-camu depend on the maturity stages. Different types of polyphenols such as anthocyanins (cyanidin-3-glucoside and delphinidin-3-glucoside), quercetin, quercitrin, rutin, myricetin, naringenin, catechin, kaempferol, ellagic acid and eriodictyol are found in Camu-camu fruits (Akter et al., 2011; Chirinos et al., 2010; Reynertson et al., 2008; Rufino et al., 2010). The total phenolic content of dried Camu-camu is 1161 mg GAE/ 100 g DM (Akter et al., 2011) (Table 2). The ellagic acid and flavan-3-ols groups represent the main phenolic compounds in this berry (Chirinos et al., 2010). According to Rufino et al. (2010) the total polyphenols in aqueous-organic extracts is higher in Camu-camu fruits (11.615 mg GAE/100 g DM) when comparing with Acerola and Jaboticaba (10.280 and 3584 mg GAE/100 g DM, respectively). On the other hand, the total anthocyanins (42.2 mg/100 g FW).

Different methods such as DPPH, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid radical (ABTS) and ferric reducing antioxidant potential (FRAP) are used to determine the antioxidant capacity of this fruit. According to Rufino et al. (2010), Camu-camu exhibited higher antioxidant capacity than Açaí, Acerola, Jaboticaba and Jambolão when used ABTS (153 µmol Trolox/g of fresh matter) and FRAP (279 µmol Fe_2SO_4/g FW) assays. In addition, Chirinos et al. (2010) reported a positive correlation between total phenolic content and DPPH antioxidant capacity (r^2 = 0.931) but not between ascorbic acid levels and DPPH antioxidant capacity (r^2 = 0.190), suggesting that the antioxidant capacity of the fruit is derived mainly from phenolic compounds. These results demonstrated that the vitamin C-rich fraction was the major contributor to the total antioxidant capacity of camu-camu fruit despite the high losses incurred. Nutritional compositions of camu-camu fruits shown in Table 1 are good source of minerals such as sodium, potassium, calcium, zinc, magnesium, manganese and copper. It also contains small amount of glucose and fructose the major sugar for camu-camu fruit. In addition, camu-camu pulps also contain different kinds of fatty acids monousaturated, saturated and polyunsaturated are presented into ripe and unripe. Kaneshima et al. (2016) this study exhibited stronger antioxidant activities measured by both single electron transfer assays and a hydrogen atom transfer assay than gallic acid and ascorbic acid. The peel and seeds of camu-camu are industrial waste products from the production of camu-camu juice, thus, applications of the seeds and peel as functional foods and food additives may be beneficial for the camu-camu industry. The extract of camu-camu seeds and peel showed potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC50 ¼ 32.2 mg/ mL), and C-glycosidic ellagitannins, vescalagin (2) and castalagin (3) were shown to be responsible for the DPPH radical scavenging activity (Kaneshima et al., 2013).

Many researchers have already considered that camu-camu fruits are a good source of vitamin C, and what we find in the mature was 1150 mg/100 g and green 1230 mg/100 g fresh matter of Vitamin C. (Table 1). According to Chirinos et al. (2010) the vitamin C of camu-camu fruits depends on the maturity stages. Vitamin C content in camu-camu was higher than other traditional Brazilian fruits such as acerola (1053 mg/100 g fresh matter), acai (84.0 mg/100 g fresh matter) (Rufino et al., 2010). Fiber contents (Table 1) are also very high, making camu-camu a good natural source of these nutrientes, 2.50 g/100 g for unripe and 2.40 g/100 g for ripe. Studies have shown that high-fiber diets have great therapeutic potential against dyslipidemia, cardiovascular diseases, and some types of cancer. Fibers also decrease intestinal transit time and glucose absorption, with consequent lowering of glycemia and blood cholesterol. Further studies are necessary to elucidate the overall potential of this fruit.

Conclusion

Camu-camu fruits are excellent sources of different bioactive compounds, such as vitamin C, fibers, minerals, and phenolic compounds. Camu-camu fruits show high antioxidant capacity as compared to other fruits. In conclusion, camu-camu fruits can be used to increase the amount of bioactive compounds in food products and to delay or prevent many human diseases.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Amazon Research Foundation (FAPEAM – Fundação de Amparo à Pesquisa do Estado do Amazonas), for financial support, process 062.01725/2014-PAPAC and 062.00682/2015 Universal.

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academicJournals

Vol. 11(28), pp. 2524-2527, 14 July, 2016
DOI: 10.5897/AJAR2015.10367
Article Number: 5FE862859413
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Lignin content in seed coats of Glyphosate-resistant soybean and their respective parents

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Received 31 August, 2015; Accepted 20 November, 2015

The large-scale cultivation of Roundup Ready (RR) has recently increased among Brazilian farmers. However, few studies have compared the intrinsic characteristics of the seeds of RR soybean cultivars and their respective conventional parental. Thus, the purpose of this study is to verify if the genetic modification of the RR soybean affects the lignin content and its monomeric composition in the seed coats, in comparison with the parental cultivar. To do that, five groups, each one with a RR cultivar and its respective parental were selected. After the physiological analysis of the seeds, the coats were separated and dried in an incubator at 80°C for 24 h. Next, the contents of lignin and its monomers *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) were determined. In order to compare the RR cultivars and their respective parents, a statistical evaluation of contrasts was performed. The results revealed that only the group BRS133 *vs* BRS245RR was different; the transgenic cultivar showed significant increases in the lignin contents and their monomers. In conclusion, the introduction of the sequence CP4-EPSPS in the genome of soybean cultivars, had no any influence on seed coat lignification.

Key words: Monolignols, seeds, *Glycine max* L. Merrill, Roundup®Ready.

INTRODUCTION

The development of the transgenic soybean, resistant to the Roundup Ready© (RR) herbicide, changed the international soybean market forever. Its approval for cultivation was obtained in Brazil in 2005. According to the CONAB (2013), the total acreage for the cultivation of soybean at the 2012/2013 crop reached 27.65 million hectares, with the RR soybean planted in 90% of this area, which is similar to the numbers in the United States and Argentina (Céleres, 2014).

The RR soybean encodes a variation of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSP synthase), which shows a low affinity for glyphosate, creating resistance against this herbicide in this plant. Thus, the enzyme activity remains independent from the presence, or absence, of the glyphosate (Shan et al., 1986; Padgette et al., 1995; Harrison et al., 1996).

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The 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase is an important enzyme of the shikimate pathway, which is responsible for the synthesis of aromatic amino acids, such as phenylalanine, tyrosine and tryptophan. Besides being important for the synthesis of proteins in plants, these amino acids participate in the phenylpropanoid pathway, which is the main route for the synthesis of phenolics, including the monolignols, which are precursors of the lignin polymer. Thus, an inhibition of EPSP synthase by glyphosate can affect not only the production of soybean proteins, but also the phenylpropanoid pathway and, by consequence, the lignin production. Lignin is associated with many different specialized cells to fulfil specific physiological functions, but exhibits distinct properties for each cell type, which may explain why no general mechanism for lignification has been yet defined (Barros et al., 2015).

At the cytosol, the pathway begins with the phenylalanine ammonia lyase (PAL), which deaminates the L-phenylalanine in *t*-cinnamic acid. The second step is the hydroxylation of the *t*-cinnamic acid by the cinnamate 4-hydroxylase (C4H), generating *p*-coumaric acid (first phenylpropanoid in the free acid form). Next, a hydroxylation occurs on the position 3 of the *p*-coumaric acid by the *p*-coumarate 3-hydroxylase (C3H), producing the caffeic, ferulic, 5-hydroxyferulic and sinapic acids, respectively. The free acid forms of the phenylpropanoids are connected to a coenzyme A through the action of the 4-coumarate: coenzyme A ligase (4-CL), reduced to the aldehydic forms by the cinnamoyl-CoA reductase (CCR) and, next, reduced to the alcoholic forms through the action of the cinnamyl alcohol dehydrogenase (CAD) (Boerjan et al., 2003). Finally, the *p*-coumaryl, coniferyl and sinapyl alcohols (or monolignols) are transported to the apoplast (Escamilla-Trevino et al., 2006). In the cell wall, these monolignols are converted into the respective monomers *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), which are polymerized into lignin by action of peroxidases and laccases (Bray et al., 2000).

It is well known that the content of lignin may vary depending on plant species, development stage and tissue type. For example, the high lignin content in the stalk of eucalyptus (*Eucalyptus* sp) is damaging to the extraction of paper and cellulose pulp (Endt et al., 2000). The high content of lignin at the upper part of a fodder causes low digestibility and, consequently, bad pastures (Lacerda et al, 2003).

Lignin deposition depends on the cell type, the developmental stage and the species. This spatial distribution is characterized by differences in time, amount, size and monomeric composition of the lignin polymer (Terashima et al., 2012). Some studies have revealed different lignin contents between RR and conventional plants, which can be due to the intense lignification in transgenic cultivars. An enhanced production of lignin of up to 20% more in transgenic cultivars has been related (Coghlan, 1999; Kuiper et al., 2001). Under water deficit and high temperatures, the overproduction of lignin causes fissures and breakages in the soybean stems, as noted in United States and Brazil (Nodari and Destro, 2002). Based on these reports, the hypothesis that claims that RR soybean cultivars contain higher contents of lignin, when compared to the conventional cultivars, has gained strength. It is due to the fact that the sequence CP4-EPDPS inserted in the genome of soybean to produce a glyphosate-resistant plant may cause a pleiotropic effect and, thus, modify the synthesis of lignin. In this context, the present study intends to determine the content of lignin and its monomeric composition in the seed coats of five RR soybean and their respective conventional cultivars.

MATERIALS AND METHODS

Five soybean cultivars were selected RR (BRS 245RR, BRS 255RR, BRS 242RR, Emgopa 33RR and Emgopa 316RR) and their respective conventional parental cultivars (BRS 133, BRS 137, Embrapa 48, Emgopa 313 and Emgopa 316). Initially, the seeds were submitted to germination tests (Brasil, 2009) in order to evaluate its quality. The soybean cultivars used in the experiment were grown in the city of Rio Verde, GO (17°47'24'' S; 50°56'31'' W; a 740 m altitude). The predominant climate is Cwa, according to the Köppen classification and the soil used was a typic dystrophic Red Oxisol, medium texture.

A sample of 200 seeds of each cultivar was submerged in water for 12 h. After this period, the coats were manually separated from the seeds, dried in an incubator (80°C, 16 h) and grinded. To determine the lignin content, dry coat (0.3 g), was homogenized in 50 mM phosphate buffer pH 7.0 (7 ml) and transferred to a centrifuge tube (Ferrarese et al., 2002). The precipitate was centrifuged (1.400*g*, 6 min.), washed and successively centrifuged, as follows: twice with 50 mM phosphate buffer pH 7.0 (7 ml); 3 times with 1% (v/v) Triton® X-100 in buffer pH 7,0 (7 ml); twice with 1 M NaCl in buffer pH 7.0 (7 ml); twice with distilled water (7 ml) and twice with acetone (5 ml). The material was dried in an incubator (80°C, 24 h) and the resulting sample was defined as a fraction of the cell wall free of proteins. Next, the sample was used to determine the total content of lignin through the acetyl bromide method (Morrison, 1972). A portion (20 mg) of the sample was placed in a centrifuge tube with 500 µl of acetyl bromide at 25%. The samples were heated (70°C, 30 min.), transferred to an ice bath and the reaction was interrupted by the addition of 0.9 ml of NaOH 2 M. Then, 0.1 ml of hydroxylamine HCl 7.5 M and 2 ml of cold acetic acid were added. The samples were centrifuged (1.000 g, 5 min.), and the supernatant was diluted and the reading were taken at 280 nm. The lignin content was determined according to a standard curve and recorded in mg lignin g^{-1} of the cell wall.

In order to determine the monomeric composition of the lignin, the nitrobenzene oxidation method was performed. A protein-free fraction of the wall cell (50 mg) was placed in a Pyrex® ampoule containing 1 ml of NaOH 2 M and 100 µl of nitrobenzene. The ampoule was sealed and heated (170°C, 150 min.), while shaking the sample occasionally during the reaction. After oxidation, the sample was cooled, washed twice with chloroform, acidified with 350 µl of HCl 5 M, and extracted twice with chloroform. The organic extracts were combined, dried and re-suspended in methanol. All samples were filtered through a 0.45-µm disposable syringe filter and analyzed (20 µl) with a Shimadzu® Liquid Chromatograph equipped with an LC-10AD pump, a Rheodyne® injector, an SPD-10A UV detector, a CBM-101 Communications Bus Module, and a Class-CR10 workstation system. A reversed-phase Shimpack®

Table 1. Lignin contents and its monomeric composition in glyphosate-resistant soybean seeds coats and their respective conventional cultivars.

Lignin monomer					
Cultivar	**Lignin**	**H**	**G**	**S**	**H + G + S**
BRS 133	54.12±0.00[a]	0.02±0.003[a]	0.10±0.014[a]	0.01±0.003[a]	0.13±0.018[a]
BRS 245RR	71.80±0.00[b]	0.06±0.004[b]	0.17±0.004[b]	0.02±0.002[a]	0.26±0.010[b]
BRS 137	67.46±0.00[a]	0.08±0.004[a]	0.18±0.001[a]	0.04±0.003[a]	0.32±0.019[a]
BRS 255RR	63.85±0.00[a]	0.06±0.005[a]	0.22±0.010[a]	0.03±0.004[a]	0.31±0.017[a]
Embrapa 48	67.72±0.00[a]	0.08±0.006[a]	0.15±0.002[a]	0.02±0.002[a]	0.26±0.009[a]
BRS 242RR	66.74±0.00[a]	0.07±0.005[a]	0.17±0.001[a]	0.03±0.007[a]	0.28±0.020[a]
Emgopa 313	67.65±0.00[a]	0.06±0.004[a]	0.23±0.014[a]	0.06±0.005[a]	0.34±0.023[a]
Emgopa 313RR	71.13±0.00[a]	0.08±0.004[b]	0.30±0.025[b]	0.08±0.008[b]	0.46±0.038[b]
Emgopa 316	53.22±0.00[a]	0.06±0.004[a]	0.13±0.007[a]	0.03±0.003[a]	0.22±0.014[a]
Emgopa 316RR	53.61±0.00[a]	0.07±0.004[a]	0.14±0.006[a]	0.04±0.002[a]	0.25±0.015[a]

The results are expressed in mg g^{-1} of cell wall (for lignin) and µg mg^{-1} of cell wall (for the monomers). Means followed by the same letter, within contrasts, do not differ significantly through the F test, respectively, at 5% probability. H, monomer *p*-hydroxyphenyl; G, monomer guaiacyl and S, monomer syringyl.

CLC-ODS (M) column (150×4.6 mm, 5 µm) was used at room temperature together with the same type of pre-column (10×4.6 mm). The mobile phase was methanol/acetic acid 4% in water (20/80, v/v), with a flow of 1.2 ml min^{-1} for isocratic race of 20 min. The quantification of *p*-hydroxybenzaldehyde, vanillin and syringaldehyde occurred at 290 nm using the corresponding standards. The results were expressed as µg monomer mg^{-1} of wall cell.

The experimental design used was entirely randomized, with 4 repetitions performed for each type of evaluation. Then, the data were submitted to the variance analysis, and compared the means of the transgenic cultivars and their respective parents with the F test ($p > 0.05$) and the option "contrast" of the SAS software.

RESULTS AND DISCUSSION

The comparison between RR cultivars and their respective parents indicated a significant difference (F test at 5% P) only for the contrast formed by the cultivars BRS 133 and BRS 245RR (Table 1). An increase of 33% in the lignin content was noted in the RR cultivar, when compared with the conventional cultivar.

Some studies suggest that RR soybean plants, which have suffered a physiological stress during certain period of times are more lignified. Nodari and Destro (2002) evaluated 9 Brazilian soybean crops during a period of drought and high temperatures, and they observed that RR cultivars contained fissures, bents or breaks (about 50 to 70% of the plants), which were probably due to an enhanced lignin production. According to Coghlan (1999) under high temperature (45°C, for example), the high lignin content hardens and breaks the soybean stems. A similar behavior occurred in United States crops, with great loss of productivity (Nodari and Destro, 2002). Investigating the lignin contents in seed coats of 5 different contrasts formed by conventional and RR soybean, Gris et al. (2010), noted significant difference ($p<0.05$) only in the Jataí *vs* Silvânia RR contrast. The transgenic cultivar contained 27% more lignin than the conventional cultivar.

As noted herein, except for one contrast, the lignin contents were not found in others cultivars (Table 1). It can be due to the possible interferences in the quantification of this polymer, or still, the fact that the RR parentage was attained through backcrossing and, therefore, is not completely isogenic to its parent strains. Another factor refers to the structural complexity of the lignin molecule; a challenge for its quantification in different tissues. This occurs because the most of the techniques used for this purpose are carried out at 280 nm. It is well known that proteins and aromatic compounds absorb energy in this spectral range and, therefore, the presence of these substances may interfere in the quantification of lignin (Vilar et al., 2014). It is important to point out that several authors have quantified lignin by gravimetric analysis (Ferreira, 2003). However, the applied methodology in this work to determine lignin removes the interference of proteins, and allows a more precise analysis (Capeleti et al., 2005).

The obtained results herein suggest a different behavior only for the BRS 133 *vs* BRS 245RR contrast referring to the content of lignin in the soybean's seed coats. However, this finding is not enough to state that the insertion of the CP4-EPSPS sequence in the soybean

genome increases the seed coats lignification. Nevertheless, possible structural changes may occur in this polymer. It can be due to the fact that the insertion of the gene that causes resistance against glyphosate in the RR soybean takes place in the same pathway of the lignin. Thus, further experiments were conducted to quantify the lignin monomers: H, G and S (Table 1).

Similarly to the contents of lignin, the BRS 133 *vs* BRS 245RR contrast showed also significant differences in the composition of H and G monomers (Table 1). In the transgenic cultivar (BRS 245RR), the contents of the H and S monomers were, respectively, 200 and 70% higher than the conventional cultivar (BRS 133). This findings corresponds to an increase of 100% when the lignin content is referred as the sum H+G+S.

Although the lignin contents were similar in the Emgopa 313 *vs* Emgopa 313RR contrast, the monomeric composition was different (Table 1). In fact, the contents of H, G and S monomers were higher in the transgenic cultivar (on average 32%) in comparison with the conventional cultivar. This result corresponds to an increase of 35% of lignin content, referred to as the sum H+G+S.

Conclusion

The obtained results indicated a different behavior in certain RR soybean cultivars in regards to the lignin content and its monomeric composition in the seed coats. However, the differences were significant only for one of the evaluated contrasts. Thus, it is possible to conclude that the CP4 EPSPS sequence, introduced in the genome of soybean cultivars, had no influence on the lignification process of the seed coats.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(28), pp. 2528-2535, 14 July, 2016
DOI: 10.5897/AJAR2016.11172
Article Number: BB46FB659417
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Agronomic performance of common bean (*Phaseolus vulgaris* L.) according to foliar application of potassium silicate in two sowing times

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Received 1 May, 2016; Accepted 16 June, 2016

Common bean (*Phaseolus vulgaris* L.) cultivation is of relevant economic importance for Brazil, since this legume is one of the main Brazilian staple foods. The objective of this study was to evaluate, in two sowing times, the agronomic performance of two bean cultivars according to foliar application of different doses of potassium silicate. Experiments were conducted under field conditions, in the rural area of the city of Assis Chateaubriand, Paraná, Brazil. The first experiment was implanted in August, 2014 (rainy season crop) and the second one was implanted in February, 2015 (dry season crop). A randomized block design was used in both experiments, in a 2 × 5 factorial design, with four replications. The first factor refers to common bean cultivars (IPR Campos Gerais and IPR Tuiuiú) and the second factor refers to the different doses of potassium silicate (0.0, 250, 500, 750 and 1000 ml ha^{-1}). The product used contained 0.9 w/v of SiO_2 (90 g L^{-1} of water) and 18% K_2O in its formulation. The agronomic characteristics evaluated were plant height, dry matter weight of the aerial parts, amount of pods per plant, thousand grain weight, and grain yield. Foliar fertilization with potassium silicate did not influence the agronomic characteristics of common bean cultivars. Regardless of foliar application with potassium silicate, IPR Campos Gerais cultivar presented greater plant height, thousand grain weight and grain yield for rainy season crop when compared with IPR Tuiuiú cultivar, which in turn presented higher productivity in dry weather crop.

Key words: IPR Campos gerais, IPR Tuiuiú, silicon.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is cultivated in nearly all regions of Brazil by small and large producers, in different production systems, due to its edaphoclimatic adaptation (Moura et al., 2015). Thus, there is the importance of having a previous knowledge about soil and weather of the region in which common bean will be cultivated, as well as about the cultivation requirements and limitations, in order to choose a proper environment

for plants to grow, develop and produce evenly, and to take full advantage of inputs and of benefits from other practices or technologies applied (Andrade et al., 2015).

Being a day-neutral plant, in Brazil, common bean is cultivated in three different sowing times: the first time is called "Safra das águas" (rainy season crop, or Southern and Southeastern crop); the second time is called "Safra das Secas (dry season crop, also called Second crop or Northeastern and Southeastern crop); the third time is called "Safra de outono-inverno" (fall-winter crop, also called Southeastern crop or irrigated crop) (Moura et al., 2015).

An alternative management for this leguminous plant is the adoption of foliar fertilization with some micronutrients, such as silicon (Si). The main feature of Si is to act as a plant resistance inducer, making plants more tolerant to climatic stresses and even to pest attacks and diseases. The way by which Si exerts a protective effect against pathogens and insects is still not defined (Ghanmi et al., 2004; Goussain et al., 2005). However, protection conferred to plants by Si is considered to be due to the accumulation and polymerization of this element in plant cells, creating a mechanical barrier which hinders insect pest attacks and pathogens (Yoshida et al., 1962). Silicon's role as a mechanical resistance enhancer was questioned by Menzies et al. (1991) and Samuels et al. (1991). According to Chérif et al. (1992), Si is related to specific defense reactions of plants. According to Gomes et al. (2005), this element acts as an elicitor of induced resistance mechanism in plants.

According to Marschner (1995) and Malavolta (2006), Si is characterized as a beneficial element for plants, as it confers increased resistance against pest attacks and diseases, improved photosynthetic capacity, increased number of leaves, larger stem diameter and plant size.

The use of Si in agriculture has presented a reduction of insect pest and disease incidence in host plants, since that element, when absorbed, promotes deposition of silica on cell wall, making plants more resistant to fungi and insect attack (Gomes et al., 2009). This is only possible because silica associates with cell wall constituents, making it less accessible to degrading enzymes (mechanical resistance) of invaders. Si also acts against some fungal diseases in Si non-accumulating plants, as in the case of common bean. In this case, the action of this element is believed to occur not exclusively by mechanical barrier formation, but also by induction of phenol (phytoalexins) production (Yamada and Abdalla, 2006).

The importance of common bean in Brazil makes research of alternative means to provide increased productivity with decreased production cost necessary. However, studies concerning management methods that employ Si are still incipient and inconclusive (Franzote et al., 2005), especially those seeking to clarify the relation between nutrition and problems caused by pests, as well as the relation of this element to agronomic aspects of the cultivation.

Thus, use of foliar fertilization with Si is believed to provide better conditions for common bean regarding the evaluated agronomic characteristics.

Therefore, the objective of this study was to evaluate, in two sowing times, the agronomic performance of two common bean cultivars according to foliar application of different doses of potassium silicate.

MATERIALS AND METHODS

Experiments were conducted under field conditions, in the rural area of the city of Assis Chateaubriand, Paraná, Brazil. The first experiment was established in August 2014 (rainy season crop) and the second one was established in February 2015 (dry season crop), both in eutroferric Red Latosol. The area is located at coordinates: Latitude 24°17´27.40´´ S and Longitude 53°35´03.99´´ W, at an altitude of 321 m.

Climate data referring to the experimental management period were collected and provided by COAMO (Agro industrial Cooperative), Brasilândia do Sul branch, Paraná, located 10 km from the experimental area. These data were correlated with phenological stages of the crop, as shown in Figures 1 and 2.

A randomized block design was used in both experiments, in a 2 × 5 factorial design, with four replications. The first factor refers to common bean cultivars (IPR Campos gerais and IPR Tuiuiú) belonging to pinto and black bean groups, respectively.

The second factor refers to doses of a commercial potassium silicate product (0.0, 250, 500, 750 and 1000 ml ha^{-1}) reported to contain 0.9 w/v of SiO_2 (90 g/L water) and 18% K_2O in its formulation, according to manufacturer's information. The product was diluted in humic acid. Experimental plots were 10 m long by 7.74 m wide, totaling 18 lines and 77.4 m^2 total area.

Prior to the first experiment installation, soil was sampled to a depth of 0 to 20 cm, presenting the following results: P = 6.30 mg dm^{-3} (Mehlish-1); pH ($CaCl_2$) = 4.80; H + Al = 3.18 $cmol_c$ dm^{-3}; Al^{3+} = 0.00 $cmol_c$ dm^{-3}; Mg^{2+} = 1.25 $cmol_c$ dm^{-3}; Ca^{2+} = 4.05 $cmol_c$ dm^{-3}; K^+ = 0.16 $cmol_c$ dm^{-3}; Mn = 146.49 mg dm^{-3}; Fe = 55.72 mg dm^{-3}; Cu = 7.62 mg dm^{-3}; Zn = 3.28 mg dm^{-3}; Si = 19.6 mg dm^{-3}; V% = 63.19; Clay = 73.5%; Coarse sand = 2.3%; Fine sand = 3.2%; Gravel = 0.0%; Silt = 21.0%; Textural class = Clayey soil.

Liming was not performed in both experiments conducted in order to avoid favoring of one experiment over another. This is because when this procedure is performed to increase soil pH, hydroxyl formation occurs in the corrected soil profile (however, a time interval is needed for such effect to occur). Thus, it could lead to an advantage for the second experiment conducted over the first one, with regard to productivity increase and other agronomic characteristics evaluated in this study.

Fertilization employed was based on soil analysis, abiding by recommendations of IAPAR (2003) for common bean culture. At the time of planting, 300 kg ha^{-1} of 16-16-16 fertilizer was

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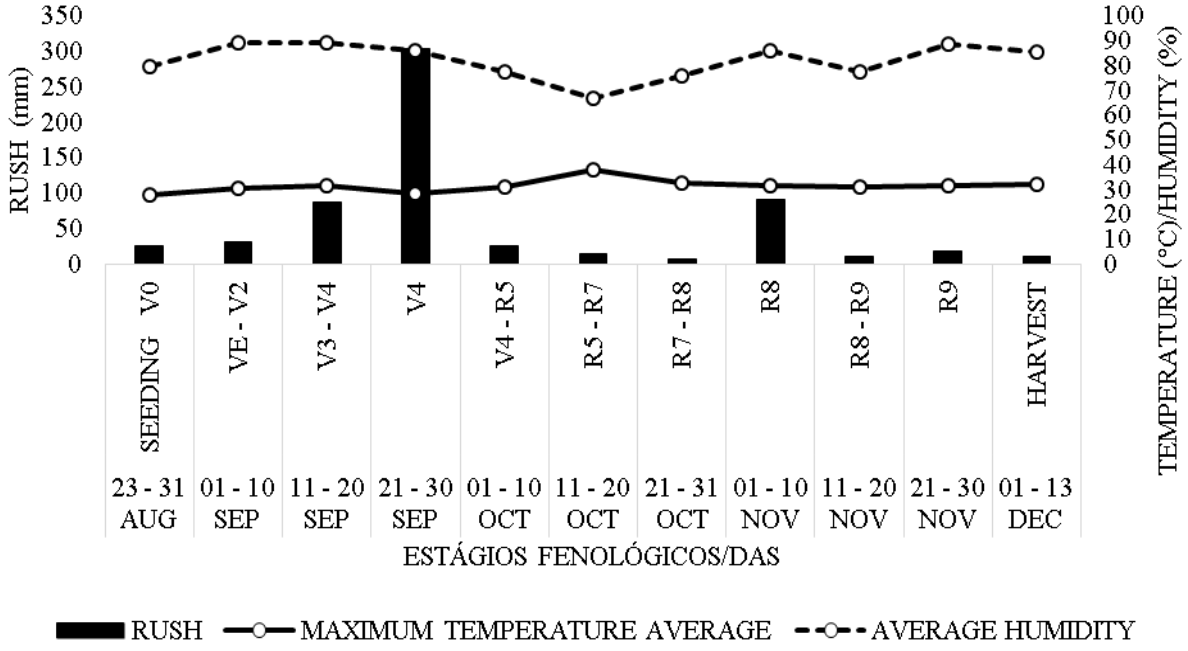

Figure 1. Maximum temperature, precipitation, relative humidity during the experiment, rainy season crop - 2014. Assis Chateaubriand, Paraná, Brazil. Source: COAMO, Brasilândia do sul.

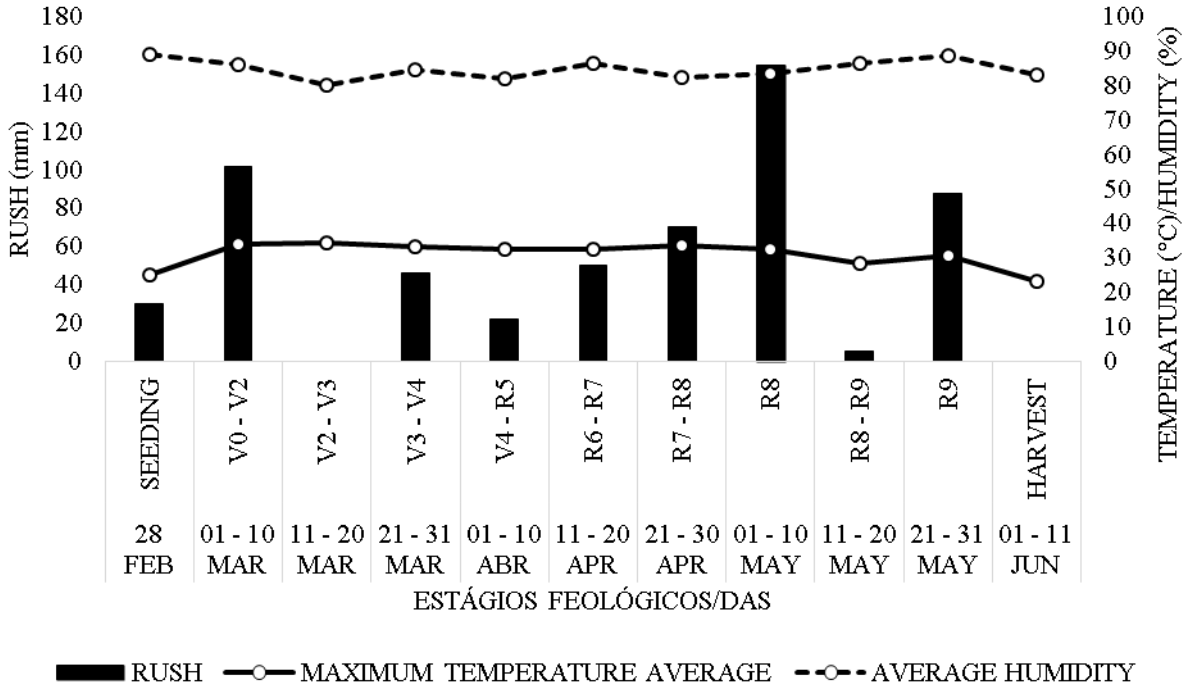

Figure 2 Maximum temperature, precipitation, relative humidity during the experiment, dry season crop - 2014. Assis Chateaubriand, Paraná, Brazil. Source: COAMO, Brasilândia do sul.

incorporated in the sowing groove. At the V4/R5 phenological stage, nitrogen top dressing was performed by using urea (45% N) as N source at a dose of 64 kg ha^{-1}.

Row spacing used was 0.43 m, with 12 seeds per linear meter. Seeds were treated with fungicide (Carbendazim) and insecticide (Imidacloprid + Tiodicarb) according to the dose recommended by

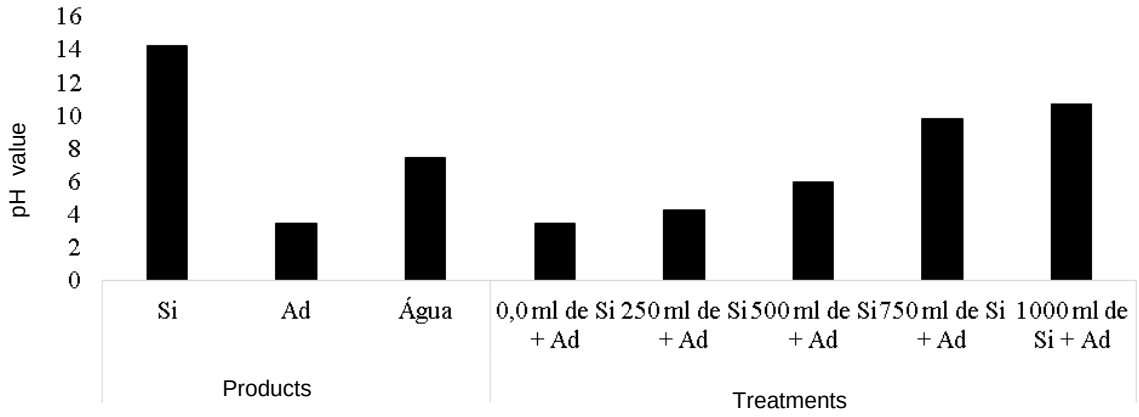

Figure 3. The pH of the products and doses used: potassium silicate (Si), spray adjuvant (Ad), water and spray mixes (treatments).

the manufacturer.

Sowings were performed on August 23 (rainy season crop) and on February 28 (dry season crop). Harvests were performed on December 13 and on June 11, respectively. During the crop development, weed control through manual weed and/or through herbicide Fomesafem + Fluazifop-p-butyl use (according to the dose recommended by the manufacturer) were adopted in both experiments when necessary.

For insect pest control, insecticides Imidacloprid + beta-Cyfluthrin and Teflubenzuron were used in the experiment conducted during rainy season crop, whereas Imidacloprid + beta-Cyfluthrin, Teflubenzuron, Spiromesifen and Methomyl were used in the experiment conducted during dry season crop. Regarding disease control, fungicides Carbendazim; pyraclostrobin + metconazole and copper hydroxide were used in the experiment conducted during rainy season crop, whereas only copper hydroxide was used in the experiment conducted during dry season crop. Doses were used according to recommendation of each manufacturer.

An adjuvant was added to the spray mix preparation, in order to provide better abrasive effect between the mix of different treatments and potassium silicate solution on bean leaves. The pH of the products used and of the treatments can be checked in Figure 3. The adjuvant added to the spray mix is composed of a blend of Phosphatidylcholine (Soy lecithin) and Acid Propionic, which improves foliar absorption of nutrients by plants.

Potassium silicate applications were divided into fortnightly applications, from phenological stage V3 to R8. The same quantity of potassium silicate established for each treatment was used at the different application times. In both experiments, the treatments evaluated were applied 4 times.

For application, an electric knapsack sprayer was used with fixed working pressure at 45 Psi, aided by a spray bar with 4 flat fan nozzles spaced 50 cm apart, with spray volume of 186 L ha^{-1}. A digital pH meter was used to determine the spray mix pH of each treatment. The device was calibrated with pH 4 and 7 buffers for subsequent measurements. The pH values of the products used (potassium silicate and spray adjuvant) were provided by the manufacturers.

Two central 8-m long planting rows from plots were considered as floor area (that is, 1 m between plots of the same block was disregarded), totaling 6.88 m^2, from which 10 plants were chosen randomly to determine agronomic characteristics (plant height, number of pods per plant and dry matter weight of the aerial part).

Ten central 8-m long planting rows from plots were considered as A graduated tape measure was used to determine plant height (PH) by measuring plant length from its base (stem above soil surface) to the end of the branch.

As for dry matter weight of the aerial part (DWAP), plants from floor area were placed in Kraft paper bag and dried to constant weight in a forced air circulation oven (62°C). The material was removed from oven. Its weight was immediately determined by using an analytical balance and subsequently extrapolated in kg per hectare. Plants from floor area were collected to determine grain yield and humidity was corrected to 13%.

Data were submitted to variance analysis by using Sisvar 5.1. statistical software.

RESULTS AND DISCUSSION

Variance analysis results regarding agronomic characteristics evaluated for the experiments conducted (rainy season crop and dry season crop) are shown in Tables 1 and 3. Mean values obtained for the evaluated characteristics are shown in Tables 2 and 4, for both crops.

As observed in Table 1 regarding the experiment conducted in rainy season crop (2014), there are statistical differences between cultivars evaluated by F-test ($p<0,05$) for agronomic characteristics: plant height (PH), thousand seed weight (TSW) and grain yield (GY).

IPR Campos Gerais was found to reach 73.95 cm average plant height (PH), whereas IPR Tuiuiú reached 67.88 cm average plant height. Regarding dry matter weight of the aerial part (DWAP), there was no significant difference ($P>0.05$) between cultivars. The mean values obtained for IPR Campos Gerais and IPR Tuiuiú cultivars were 13820.41 and 13056.85 kg ha^{-1}, respectively. Regarding thousand seed weight (TSW), there was statistical difference ($P<0.05$) between the means compared. IPR Campos Gerais cultivar reached an average thousand seed weight of 247.30 g, whereas IPR Tuiuiú cultivar reached an average of 167.85 g. As for grain yield, there was statistical difference ($P<0.05$) between the means obtained. IPR Campos Gerais

Table 1. Variance analysis of agronomic characteristics summary: plant height (PH); dry matter weight of the aerial part (DWAP); number of pods per plant (NPP); thousand seed weight (TSW) and grain yield (GY). Rainy season crop – 2014. Assis Chateaubriand, Paraná, Brazil.

Sources of variation	Rainy season crop – 2014					
	GL	PH	DWAP	NPP	TSW	GY
Cultivar	1	368.45*	349.22^{Ns}	0.064^{Ns}	63123.03*	222689.96*
Block	3	217.38	3086.74	16.35	2350.76	110912.91
Doses	4	29.36^{Ns}	176.94^{Ns}	0.11^{Ns}	173.35^{Ns}	10316.07^{Ns}
Cultivar x Doses	4	33.10^{Ns}	415.38^{Ns}	1.90^{Ns}	71.15^{Ns}	747.21^{Ns}
Error	27	67.28	489.26	2.20	511.57	11859.10
C.V. (%)	-	11.12	21.27	20.21	10.90	25.01

^{Ns}Not significant, *Significant at the 0.05 significance level (F-test).

Table 2. Mean values obtained for the evaluated characteristics: plant height (PH); dry matter weight of the aerial part (DWAP); number of pods per plant; (NPP); thousand seed weight (TSW) and grain yield (GY). Rainy season crop – 2014. Assis Chateaubriand, Paraná, Brazil.

Cultivar	Mean values				
	pH (cm)	DWAP (kg ha^{-1})	NPP (Unit)	TSW (g)	GY (kg ha^{-1})
IPR Campos Gerais	73.95^{a}	13820.41^{a}	7.30^{a}	247.30^{a}	510.02^{a}
IPR Tuiuiú	67.88^{b}	13056.85^{a}	7.38^{a}	167.85^{b}	360.79^{b}
CV (%)	11.12	21.27	20.21	10.90	25.01

Means followed by the same letter are not significantly different by Tukey's test at the 5% probability level.

Table 3. Variance analysis of agronomic characteristics summary: plant height (PH); dry matter weight of the aerial part (DWAP); number of pods per plant; (NPP); thousand seed weight (TSW) and grain yield (GY). Dry season crop – 2015. Assis Chateaubriand, Paraná, Brazil.

Sources of variation	GL	PH	DWAP	NPP	TSW	GY
Cultivar	1	1550.03*	6630.63*	0.63^{Ns}	140.63^{Ns}	916393.98*
Block	3	16.03	5662.29	12.29	803.16	375252.51
Doses	4	103.54^{Ns}	1478.75^{Ns}	8.59^{Ns}	223.63^{Ns}	20765.57^{Ns}
Cultivar x Doses	4	24.71^{Ns}	1080.63^{Ns}	4.44^{Ns}	255.75^{Ns}	34571.33^{Ns}
Error	27	39.84	1251.64	3.87	130.10	49199.21
C.V. (%)	-	7.92	17.90	13.77	6.01	19.44

^{Ns}Not significant, *Significant at the 0.05 significance level (F-test).

Table 4. Mean values obtained for the evaluated characteristics: plant height (PH); dry matter weight of the aerial part (DWAP); number of pods per plant; (NPP); thousand seed weight (TSW) and grain yield (GY). Dry season crop- 2015. Assis Chateaubriand, Paraná, Brazil.

Cultivar	Mean values				
	pH (cm)	DWAP (kg ha^{-1})	NPP (Unit)	TSW (g)	GY (kg ha^{-1})
IPR Campos Gerais	85.90^{a}	23.869.51^{b}	14.40^{a}	191.75^{a}	989.43^{b}
IPR Tuiuiú	73.45^{b}	27.196.38^{a}	14.15^{a}	188.00^{a}	1.292.15^{a}
CV (%)	7.92	17.90	13.77	6.01	19.44

Means followed by the same letter are not significantly different by Tukey's test at the 5% probability.

produced 510.02 kg ha^{-1}, whereas IPR Tuiuiú cultivar produced 360.79 kg ha^{-1} (Table 2).

For the experiment conducted in dry season crop, significant differences were observed in cultivars

evaluated by F-test (P<0.05) regarding plant height (PH), dry matter weight of the aerial part (DWAP) and grain yield (GY) (Table 3).

The average plant height (PH) presented was 85.90 for IPR Campos Gerais cultivar, and 73.45 for IPR Tuiuiú. Regarding dry matter weight of the aerial part (DWAP), there was statistical difference between cultivars (P<0.05). IPR Tuiuiú presented 23869.51 kg ha^{-1}, whereas IPR Campos Gerais cultivar presented 27196.38 kg ha^{-1}. Moreover, there was statistical difference (P<0.05) regarding grain yield, as IPR Tuiuiú reached 1292.15 kg ha^{-1} and IPR Campos Gerais reached 989.43 kg ha^{-1} (Table 4).

Differences observed at the same sowing time can probably be assigned to genetic basis of the cultivars studied, since different common bean groups are concerned. IPR Campos Gerais belongs to Pinto bean group, whereas IPR Tuiuiú cultivar belongs to Black turtle bean group. Consequently, genotypes behaved differently due to their characteristics, as observed in other studies (Coelho et al., 2010; Teixeira et al., 2010).

Thousand seed weight (TSW) is one of the main characteristics that differentiate common bean genotypes, being little influenced by environment (Ramalho et al., 1993). Thus, even under different environment conditions, it was observed that TSW of each genotype evaluated may undergo slight alterations only (Coelho et al., 2007).

However, in a study performed by Hoffmann Junior et al. (2007), the authors observed a general negative impact on thousand seed weight when common bean are exposed to high temperatures during reproductive stage, as genotypes behaved differently, presenting tolerant materials according to climatic conditions under which the study was conducted, keeping constant TSW for some evaluated materials. Similar results are observed in this study (Tables 1 and 3).

According to Coimbra et al. (1999), TSW is highly associated with grain yield. Consequently, a reduced thousand seed weight in genotype will cause significant losses in final grain yield.

According to an informative technical bulletin provided by IAPAR (2015), cultivars have an indeterminate growth habit type II, and its inflorescences arise from axillary buds. Apical bud continues to grow even in reproductive stage, forming a branch that does not exceed a few centimeters; total plant height reaches approximately 70 cm. Lateral buds are short and cultivars present a flowering period ranging from15 to 20 days, with pods maturing evenly. Plants have a life cycle of 80 to 90 days, with about 3897 kg ha^{-1} of yield potential for IPR Campos Gerais cultivar, and 3950 kg ha^{-1} for IPR Tuiuiú cultivar.

By analyzing the climatic behavior and phenological development of materials in the cultivation environment of both crops (Tables 1 and 2), differences were observed in each crop, demonstrating that environmentally adverse conditions can negatively affect common bean development, especially precipitation and temperature.

Values corresponding to productivity of both crops (Tables 2 and 4) are considered low when compared with productive potential of the materials studied. Such low productivity may be assigned to unfavorable climatic conditions for crop development, occurred during experiments conduction. During rainy season crop and dry season crop, climatic conditions presented mean values of maximum air temperature over 30°C along the entire experiment period, as observed in Tables 1 and 2, reaching nearly 40°C during rainy season crop when it was at the reproductive stage of development R7 (pod formation).

Common bean plant is susceptible to abrupt and/or extreme climatic factors, mainly temperature (below 15°C or over 27°C) and uneven precipitation. In such cases, the crop cannot complete its cycle optimally, undergoing productivity losses mainly due to flower/pod abortion, grain malformation, small size or lodging.

Still in relation to productivity loss, very high temperatures are known to cause the most harmful adverse effect for common bean flowering and fruiting, as observed in this study, and are one of the main influential factors on flower abortion, fruit setting, and final pod retention in common bean (Dickson and Boettger, 1984; Portes, 1988). High temperature is also responsible for a fewer number of seeds per pod. Air temperature adopted as optimal for a proper physiological development of plant ranges from 15 to 27°C (Bulisani et al., 1987).

According to Dickson and Petzoldt (1989), common bean crop can be harmed by the occurrence of high air temperatures at the different phenological stages of plant development. It is also known that the greatest damages caused by high temperatures occur at the reproductive stage of development (R5 and R6). Air temperature conditions ranging between 30 and 40°C are the cause for higher flower and floral bud abortion rate, reducing bean plant yield. In this study, these relations were observed more clearly for rainy season crop (Dickson and Petzoldt, 1989).

In this context, according to Gonçalves et al. (1997), temperatures over 30°C can promote sterilization of pollen grain and increase ethylene production in the plant, factors related to blossom drop and graining deficiency.

For Maluf and Caiaffo (1999), several common bean reproductive stages are susceptible to high temperature, including floral bud formation (R5), pollen formation, fertilization, and pods and seeds formation (R7) The author reports damages after anthesis, as flower abscission and low fruit setting of pods and seeds (due to lack of pollination or fertilization) resulting from exposure to 38°C average temperature during the first days of flowering, are responsible for productivity losses of about 67%.

Another yield limiting factor is precipitation, which, as well as air temperature, was unfavorable for plant development during the experiments conducted, affecting

grain yield (Tables 1 and 2). During rainy season crop rainfall distribution was irregular and insufficient, hampering crop development.

Despite a better rainfall distribution during dry season crop, from emergence to filling of pods, the quantity of precipitation was also insufficient to meet crop needs. According to studies conducted by Back (2001), common bean requires about 100 mm evenly distributed rainfalls monthly to fulfill its cycle with no restrictions. Though, according to studies conducted by Maluf and Caiaffo (1999), common bean requires 300 to 400 mm evenly distributed precipitation between sowing time and physiological maturity, to fulfill its cycle with no restrictions. That is because this species is not very tolerant to water deficiency mainly due to its shallow root system, which results in low recovery capacity after severe water deficit in the soil (Guimarães et al., 1996).

During reproductive stages of growth R5 to R9, mainly between R5 and R7, common bean is highly susceptible to water deficiency in the soil (Fageria et al., 1991), because in such stages plant is at its maximum metabolical potential to form flower buds and, after anthesis, to develop pods and grains. The same phenomenon was observed in a study conducted by Matzenauer et al. (1991). According to the authors, the critical period for common bean regarding water deficiency is the sub-period from beginning of flowering (R5) to beginning of grain filling (R8).

When water stress occurs in the reproductive stage, yield reduction is associated with decreased leaf area and number of pods per plant (Acosta-Gallegos and Shibata, 1989). According to Gomes et al. (2000), yield decrease is more than 50% when water stress occurs between the 5 and 10th day before anthesis. Productivity reductions are proportional to the number of days common bean is subjected to drought (Stone et al., 1988), as observed in this study.

No significant effect was observed regarding potassium silicate application for the evaluated characteristics (Tables 1 and 3). It probably happened because the experiment was conducted in a clayey soil (73.5% clay). According to Camargo (2007), soluble Si content is higher in that type of soil. Eutroferric Red Latosol is a very weathered type of soil, with higher quantity of clay. In the experimental area, the quantity of soluble Si measured in the soil was 19.6 mg dm^3, value considered to be very high. This is the reason why Si would hardly demonstrate its effects with foliar fertilization of crop, even more by the fact that common bean is a silicon non-accumulating plant.

When Si is found in the liquid phase of the soil as a monocyclic acid, it is absorbed by plant roots through passive transport, which occurs when soil nutrient moves to the root surface with the concentration gradient (nutrient moves from the higher concentration area, rhizosphere, to a lower concentration area, root), requiring no expenditure of metabolic energy by the plant (Taiz and Zeiger, 2013).

Plants can absorb mineral more easily through root system, since they have a greater quantity of membrane transport proteins by which molecules and ions can diffuse through membrane, making a lower expenditure of metabolic energy possible (Taiz and Zeiger, 2013).

This way, as plants absorb Si through roots in order to meet their needs for this element, foliar absorption is not justifiable. Furthermore, since root system presents larger specific surface area (contact of the element with the plant tissue) and larger quantity of transport proteins, the amount of Si it absorbs will always be greater than the quantity absorbed by the aerial part. However, the precise way Si is absorbed is still unknown (Takahashi et al., 1990).

Nonetheless, as soluble Si concentration in this soil is very high, this element was probably absorbed by the roots and met plant needs. Therefore, regarding the potassium silicate doses studied and the interaction between these doses and the cultivars, foliar fertilization with potassium silicate did not provide significant results ($P>0.05$) for such evaluated characteristics. It is important to emphasize that Latosols contain a large quantity of kaolinite, which still undergoes weathering action, and consequently releases soluble Si to soil by the action of the weather (Lima, 2001).

Moreover, common bean is a dicotyledon plant considered to be silicon non-accumulating. This legume is classified as a plant able to accumulate under 2% SiO_2 in dry matter, quantity considered insignificant to improve and 3 formerly presented (Takahashi, 2002; Hodson et al., 2005).

For future studies, it is important to explore results for potassium silicate application in plants grown in soils with different clay percentages and soluble Si, and in Si-accumulating plants.

Conclusions

Foliar fertilization with potassium silicate did not influence the agronomic characteristics of common bean cultivars, in both evaluated crops.

IPR Campos Gerais cultivar presented greater plant height, thousand grain weight and grain yield for rainy season crop when compared with IPR Tuiuiú cultivar, which in turn presented higher grain yield in dry weather crop.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordination for the

Improvement of Higher Education Personnel (CAPES) and Fundação araucária (State of Parana Research Foundation) for financial support.

They would also like to thank Mr. Valdir José Alves, the owner of the area where the experiments were conducted, for all the support and consideration along the development of this study.

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