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Review

Green manure in fruticulture: Aspects on soil quality and use in agriculture

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The farming practices have a direct impact in ecosystems and cause changes in the basic structures involved in the environment. Cultivated soils, after years of use, show changes in the physical, chemical and biological attributes, requiring improve production processes, with the intention of management alternatives that minimize environmental degradation and, consequently increasing the sustainability of agricultural environment. In the search for sustainable agricultural practices, it indicates the green manure, which is the use of plants in rotation or intercropped with crops of economic interest. The practice of green manure provides many improvements in chemical, physical and biological characteristics, through increases in organic matter content and microbial activity, nutrient cycling, disruptions of compacted layers, decreased erosion, incidence of pests and diseases, and suppression of weed plants. Thus, appearing to be an agricultural practice efficiently for sustainable fruit production system. In this sense, the present study aims to conduct a literature review on the benefits of green manure in fruticulture in order to improve soil quality and use in agriculture.

Key words: Cover crop, root system, sustainable management.

INTRODUCTION

The farming practices have a direct impact in ecosystems and cause changes in the basic structures involved in the environment. Cultivated soils, after years of use, show changes in the physical, chemical and biological aspects (Soares et al., 2005; Carneiro et al., 2009), in which the organic matter in the soil is an important factor in detecting imbalances in the system due be related to the cation exchange capacity, aggregate stability, and nutrient availability (Ciotta et al., 2003; Conceição et al., 2005; Pavinato and Rosolem, 2008), and negatively

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influenced by human action (Pereira et al., 2010).

Intense soil disturbance provided by the introduction of modern agriculture modifies the balance of pre-existing organic matter in the system, reflecting the decline in organic matter in the soil (Silva et al., 1994), in which it is maximized by erosion, compaction and decreased soil fertility (Llanillo et al., 2006). Thus, requiring improve production processes, aiming management alternatives that minimize environmental degradation, and thereby increasing the sustainability of agricultural environment.

In the search for sustainable agricultural practices, it indicates the green manure, which is the use of plants in rotation or intercropped with crops of economic interest (Calegari, 1995; Silva et al., 1999; Carvalho et al., 2005), resulting in improvements in the characteristics of cultivated soil, and indirectly increased agricultural production (Singh et al., 1991; Pikul et al., 1997; Fageria, 2002, 2007). The used species can be native and/or introduced (Calegari, 1995; Silva et al., 1999), which may be of annual cycle, semi-perennial, and perennial plants (Carvalho et al., 2005), and therefore present in the field for several months or during the year. After its cutting, it can be incorporated in the soil or maintained in covering the soil surface (Espindola et al., 2004).

The main benefits from the use of green manures are: increase in soil organic matter, decreased evapotranspiration and erosion produced by plant residue, nutrient cycling and disruption of compacted soil layers (Calegari, 1995). Other important benefits are the increased microbial activity, decreased incidence of pests and diseases, and suppression of weed plants (Fageria et al., 2005).

In this sense, the present study aims to conduct a literature review on the benefits of green manure in fruticulture in order to improve soil quality and use in agriculture.

HISTORY OF GREEN MANURE

There are reports of the practice of green manure in the various civilizations that inhabited the Earth (Singh et al., 1991; Pikul et al., 1997; Fageria, 2007). The Greek and Roman civilizations cultivated the *Vicia faba* L. and some species of *Lupinos* sp. at 300 years BC in order to improve soil fertility and provide some of the nutrients required by crops used in food. The pioneer settlers in North America used *Fagopyrum esculentum*, *Avena sativa* and *Oryza sativa* to incorporate organic matter to the soil (Fageria, 2007). In China, India and Japan, some legumes were recognized as an important source of nitrogen for irrigated rice, previous use of agricultural inputs (Singh et al., 1991). In the XVIII century and the first half of the XIX century, agricultural productivity was totally dependent on natural resources (Pikul et al., 1997). However, this agricultural trend was modified from the 60's, due to the reduction in the amounts of chemical fertilizers, the adoption of intensive cropping systems and the high demand for nutrients by the modern cultivars (Fageria, 2007). In Brazil, the first studies were conducted by the Instituto Agronômico de Campinas, which aimed to determine the best legumes for the production of green manure and concomitantly, the effects of organic matter incorporated into the soil in crop production economically (Miyasaka, 1984).

ROOT SYSTEM OF GREEN MANURE

Despite the great importance in the ecosystem and agricultural activity, the roots are poorly studied compared to the shoots of plants. This is mainly due to the difficulties imposed by methodological techniques, by their three-dimensional complexity and their temporal and spatial variability (Zonta et al., 2006). The importance of studying the root system of the plant species used in agriculture, discussing their distribution, extent and activity, is indisputable and fundamental to the scientific understanding of agricultural production, since it provides location information of fertilizers, spacing, intercropping and soil management and irrigation. The rooting varies with the species and cultivars, plant age, chemical and physical characteristics of the soil, cultivation, and phytosanitary conditions (Fracaro and Pereira, 2004).

The development of the root system has a direct influence on some plant characteristics, such as: drought resistance, efficient absorption of nutrients from the soil, tolerance to pest attack, germination capacity, and tolerance to handling farm machinery (Vasconcelos and Garcia, 2005). However, according to the authors, it is not the amount of roots that is the determining factor of these benefits, but their distribution in the soil profile during the year, since high amount of roots in the surface layers mean an excessive expenditure of synthesized metabolites in the shoot translocated to the roots, as well as higher risk of water stress during drought periods in non-irrigated areas.

The roots of green manures show important role in the maintenance and improvement of characteristics of the soil structure, by providing the breaking of the compacted layer, which contributes to improving soil aggregation (Camargo and Alleoni, 1997).

EFFECTS OF GREEN MANURE ON SOIL CHARACTERISTICS

The green manure used in the plant cover provides numerous beneficial effects in soil (Ladha et al., 1988; Ganry et al., 2001; Mandal et al., 2003), in chemical, physical and biological attributes of soils (Silva et al., 1998; Mueller and Thorup-Kristensen, 2001; Moraes et al., 2006).

Effects on the chemical attributes

In order to improve the characteristics of soil, the use of legume species is widespread in agriculture to be able to provide nitrogen to the production system (Seganfredo, 1995; Rufato et al., 2006), through the symbiotic relationship involving legumes and *Rhizobium* bacteria (Faria et al., 2004; Pinto et al., 2007), the associations between plants and cyanobacteria (Pinotti and Segato, 2004), or non-symbiotic association between free-living diazotrophs bacteria with the roots of the plant (Peoples and Craswell, 1992; Weber et al., 1999, 2000). The symbiosis result in fixation, mainly, atmospheric N_2 is available in large quantities for the production environment (Kanmegne et al., 1999; Moreira et al., 2010; Ribeiro et al., 2011).

Ramos et al. (2001) studied the amounts of nitrogen fixed by *Crotalaria juncea*, *Mucuna aterrima*, and *Canavalia ensiformis* in different soils, which verified that the quantities of nitrogen fixed by the *C. juncea* were on average 40% higher than those of other species. Mueller and Thorup-Kristensen (2001) assessed the potential for nitrogen fixation in two species of *Vicia* and three species of *Trifolium* in an organic system of crop rotation for two years, founded that all species contributed with more than 100 kg ha^{-1} of N, wherein the species *Vicia villosa* had the most fixed atmospheric N_2 (149 kg ha^{-1} of N).

The biomass produced by green manures positively influences the chemical characteristics of the soil, enabling increase in organic matter over the years, which increases the cation exchange capacity and therefore, the retention of nutrients in the soil particles (Ciotta et al., 2003). Ganry et al. (2001) report the importance of green manure in the management of soil organic matter in African semi-arid regions. In those conditions, with annual rainfall of less than 800 mm, the *Mucuna pruriens* intercropping of maize with a positive impact on productivity, reached values of 3.5 Mg ha^{-1}. Another beneficial effect of green manure on soil chemical properties is the cycling of nutrients. The branched and deep root system of legumes results in an increase in the efficiency of utilization of fertilizers, since there was increase in topsoil due to nutrients lost by leaching, especially potassium, calcium, magnesium, and nitrate (Silva et al., 2002). In addition, green manure is characterized as being a "miner agent" of the limited availability of nutrients as phosphorus and molybdenum (Alvarenga et al., 1995).

Borkert et al. (2003) estimated the quantity of recycled nutrients for five plant species (*Avena strigosa* Schreb; *V. sativa* L.; *M. aterrima*; *Cajanus cajan* Millsp; *Lupinus albus* L.) used as ground cover in a crop rotation system. After four years, it was found that all species evaluated were effective in accumulating macro and micronutrients, especially the *A. strigosa* and *V. sativa* in potassium accumulation, while the *M. aterrima*, *C. cajan*, *V. sativa* and *L. albus* in nitrogen accumulation. The calcium, magnesium, and micronutrients were satisfactorily accumulated for all species evaluated.

Effects on biological attributes

The presence of organic material provided by green manure promotes the activity of soil organisms, while their wastes act as a source of energy and nutrients (Lavelle and Spain, 2001). Besides the presence of vegetation cover influence on soil temperature. The amount and type of plant residue correlate with the thermal properties of the soil, determining the levels of moisture and organic matter, influencing the temperature fluctuations, especially in surface horizons, wherein the variation amplitudes are more intense due to direct incidence of sunlight (Eltz and Rovedder, 2005). In addition to reducing the temperature fluctuations and humidity, plant residue provides conditions that enhance the development of organisms (Espindola et al., 1997) increasing the population of native mycorrhizal fungi (Espindola et al., 1998), in some cases, inhibit harmful effects of nematodes (Moraes et al., 2006). Eltz and Rovedder (2005) observed that *Lupinus albescens* and *A. strigosa* were effective in softening temperature variations, when compared to treatment without cover. Derpsch et al. (1985) to evaluate the residual effects of *L. albus*, *V. villosa*, *Lathyrus sativus*, *Secale cereale*, *A. strigosa*, *Triticum aestivum*, *Raphanus sativus*, *Brassica napus*, *Helianthus annuus,* and the control treatment (without cover) in the water content and soil temperature, observed a lower temperature in the soils covered with *A. strigosa*, and higher in treatments corresponding to without cover.

Effects on physical attributes

The occurrence of compacted layers promoted by intensive agricultural implements reduces water infiltration into the soil. However, this negative effect can be decreased by the cultivation of green manures that show well-developed root system, enabling the breaking of compacted layers (Rosolem et al., 2002; Foloni et al., 2006). The intensity in occurring disruption varies according to the type of soil, as they present different clay classes, have different values of critical density (Reichert et al., 2003), as well as the specifics of each species of green manure (Rosolem et al., 2002). When executed, the breakdown in the soil provides the so-called biopores that are used in subsequent cultures for root depth (Dexter, 1991; Silva and Rosolem, 2002).

Some plants used for green manure, *C. juncea*, *M. aterrima*, and *C. cajan,* provide large amounts of dry matter to the productive system, reaching average values

of 6, 10, and 15 Mg ha^{-1} $year^{-1}$, respectively (Calegari, 1995). After the senescence of the plant, this material produced increases the organic matter content of cultivated soil. This process improves soil physical attributes by decreasing the density and improves aggregate stability, porosity, water infiltration rate, and moisture retention (De Polli et al., 1996). The adoption of management to maintain soil protection systems through organic residue inputs continuum is critical to maintaining good physical soil structure (Alcântara et al., 2000; Boer et al., 2007). Systems commonly known as "conventional", resulting in soil disturbance, generally exhibit reduced aggregation indexes (Silva et al., 2000; Wendling et al., 2005).

Silva et al. (1998) evaluated the stability and aggregates of a Dark-Red Clayey Oxisol in different systems of succession corn and green manure under the conditions of the Cerrado biome. Cover crops grown in the off-season period were: *C. juncea*, *C. cajan*, *M. aterrima*, *Brachiaria ruziziensis*, and *Canavalia ensiformis*. Results were that *B. ruziziensis* as cover provided greater soil aggregation.

The water infiltration is also improved in soil covered by plant mass. Derpsch et al. (1991) evaluated the infiltration of water into a soil subjected to precipitation of 60 mm h^{-1}. When the coverage rate was 100%, the soil showed complete infiltration of water, whereas in the treatment which did not have coverage, only a quarter of the precipitated water infiltrated, the remainder being lost by surface runoff.

The organic matter content is able to positively influence the retention of plant nutrients and reduce losses by leaching (Hernani et al., 1995). According to the authors, the most important effect of the decomposition of plant residues is the improvement of soil attributes, which consequently improves the water/air relation, allowing increased infiltration and retention of water and nutrients in the soil and better root development, resulting in improved physical soil structure and control of water erosion.

Silva and Rosolem (2001) evaluated the effect of subsurface compaction on root growth of six species of green manure (*A. strigosa*; *C. cajan*; *Pennisetum americanum*; *Stilozobium aterrimum*; *Sorghum bicolor*; and *Lupinus angustifolius*). The authors found that density of up to 1.6 Mg m^{-3} at a depth of 0.15 m did not restrict root growth of *A. strigosa*, *C. cajan*, *P. americanum*, *S. aterrimum*, *S. bicolor* and *L. angustifolius*.

RESULTS OF GREEN MANURE IN FRUTICULTURE

In the area of fruit culture, studies that have been conducted aimed at assessing the effect of green manure on soil chemical properties, vegetative growth and fruit yield.

Results in *Citrus sinensis*

Marrero et al. (2009) evaluated the effect of intercropping between *Teramnus labialis* and 'Valência' orange (*Citrus sinensis* L. Osbeck) on the physical properties of a loamy soil in Cuba country. After four years, the intercropping increased soil water content, the total specific pore volume and the percentage of stable aggregates, when compared to treatments with natural vegetation and soil without vegetation cover. Bordin et al. (2008) evaluated the lines of an 'Folha Murcha' orange (*C. sinensis* (L) Osbeck) grafted with Cravo' limon (*Citrus limonia* Osbeck) with a history of soil compaction on the influence of the scarification on growing green manures, in the performance of trees and the physical properties of an Oxisol. The vegetation covers in the soil were: spontaneous vegetation, *C. cajan*, and *P. americanum*. The authors observed that the mass of the aerial part of *C. cajan* and *P. americanum* increased with scarification of the soil and the growth of the root system of *P. americanum* was higher than that of *C. cajan* in the management scarified and similar to this in the non-scarified management.

Results in *Prunus persica*

Rufato et al. (2007) evaluated the effect of cultivation of *A. strigosa*, *R. sativus*, *L. sativus*, *Pisum sativum*, *L. angustifolius*, and control treatment (spontaneous vegetation) on the vegetative development of *Prunus persica* after two agricultural cycles. The variables measured were: stem diameter, length of branches, fertility rate and canopy volume. Except the *R. sativus*, all green manure increased the development of *P. persica* compared to the control treatment.

Results in *Vitis vinifera*

Faria et al. (2004) studied the influence of intercropping of *C. juncea* and *C. ensiformis* in chemical soil characteristics, productivity and quality of fruit of *Vitis vinifera*, and observed that green manure provided improvement in soil chemical characteristics, increasing the organic matter content, exchangeable calcium and the value of the cation exchange capacity of the layer from 0 to 0.10 m depth, compared to the control treatment without cover crops. However, there was no significant effect of green manure on yield and fruit quality.

CONCLUSION

The practice of green manure provides improvements in

chemical, physical, and biological attributes, through increases in organic matter content and microbial activity, nutrient cycling, disruptions of compacted layers, decreased erosion, incidence of pests and diseases, and suppression of weed plants, showing to be a sustainable agricultural practice, with proven efficiency for fruit production system.

Conflict of Interests

The authors have not declared any conflict of interests.

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Response of sugar cane to limitation hydric and nitrogen dose

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The great social and economic importance of sugarcane makes it critical to understand the responses of the crop to adverse stress. This study was carried out to characterize the morpho-physiological index of sugarcane cultivated under different levels of water replacement with four replicates, corresponding to five levels of water replacement (100, 75, 50, 25 and 0% of soil humidity at field capacity) and either associated or not with a nitrogen source (100 kg ha^{-1} de N). Leaf area, leaf water potential, gas exchange, and chlorophyll fluorescence were determined at different stages of plant development. Suboptimal maintenance of turgor pressure by water potential reduced the photosynthetically active area of sugarcane submitted to hydric deficit. A water replacement of 75% in association with nitrogen promoted optimal maintenance of the photosynthetic process.

Key words: Photosynthetic efficiency, nitrogen supply, water potential.

INTRODUCTION

Sugarcane is a major crop grown in Brazil due to its great socioeconomic importance. According to the FAO (2013), the cultivated area with sugarcane was greater than 9.4 million hectares, from which 670.76 million tons were harvested in 2012. In Brazil, the Midwest Central region is responsible for around 60% of national production (UNICA, 2013). The amount produced by area, however, varies with water availability.

Hydric stress is the major limiting abiotic factor in world agricultural production. Responses to production are dependent on the development stage at the time of stress (Çakir, 2004) as well as the frequency and intensity of the hydric stress (Cattivelli et al., 2008). In the case of sugarcane, hydric and nutritional deficits affect productivity by reducing photosynthetic capacity; however, the interaction between these stresses is still

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not well understood (Gava et al., 2010) and in most cases they can easily be confounded.

The physiological response mechanisms to hydric stress in plants suggest a direct perception of hydric stress by roots that then produce root signals. These promote reductions in the aerial portion, roots, absorption of water and nutrients, and capture of radiation and CO_2 by the leaves. These changes can reduce resource use such as transpiration and radiation (Sadras, 2009). According to Lopes et al. (2011), hydric deficiency induces a low water state in tissues and loss of cell turgidity, besides interfering with nutrient absorption and osmotic stress and reducing transpiration by thermal stress.

Nitrogen deficiency interferes with the activity of phosphoenolpyruvate carboxylase (PEPcase) and ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO) (Ranjith et al., 1995; Meinzer and Zhu, 1998). Hydric deficit also affects many physiological processes, such as stomatal closing, consequently reducing the supply of CO_2 for photosynthesis, increasing the diffusive resistance to water vapour, reducing transpiration, and affecting thermal energy dissipation and the transport of nutrients by mass flow, among others (Wu and Campbell 2007). Many of these effects represent mechanisms of adaptation of plants to their environment (Heschel and Riginos, 2005).

Thus, irrigation and fertirrigation systems have been used to maximize water usage and minimize the effects of deficiencies in water and nutrients during crop development (Barbosa et al., 2012). Drip irrigation systems are used to apply water with high frequency and low intensity at specific points over a cultivated area, allowing maintenance of a constant soil humidity in the plant root zone (Vekariya et al., 2011). Supplying water and nutrients directly to the root system characterizes subsurface drip irrigation (SDI) for the high use of resources by plants (Oliveira et al., 2014).

Fertirrigation practices allow splitting the manure during crop development, avoiding loss of nutrients by leaching. Souza et al. (2009) revealed that modern fertirrigation practices should consider environmental sustainability and rigorous management of hydric resources. In addition, the nitrogen content of plant is improved by a SDI supply, assigned the major availability of nutrients in soil or capacity of root absorption (Kraiser et al., 2011).

Knowledge about sugarcane responses is essential to crop management, making it important to obtain information about the efficiency of water use by plants and the viability of sugarcane cultivation (Azevedo et al., 2011). In this way, the objective of this study was to understand the physiological characteristics and development of leaf in response to water stress and nitrogen in sugarcane cultivated under different levels of water replacement (irrigation) by subsurface drip with or without a nitrogen supply.

MATERIALS AND METHODS

This study was conducted to complement the survey conducted by Oliveira et al. (2014) in order to achieve understanding of the physiological behavior of sugarcane plants subjected to different levels of water replacement (irrigation) subsurface drip with and without nitrogen supply. Sugarcane, RB 85-5453 variety, was cultivated from March 2011 until April 2012 in an experimental area at Institute Federal Goiano – Campus Rio Verde, Goias state, 720 m in altitude and soft-wavy relief (6% declivity). The climate in the region is classified by Köppen and Geiger (1928) as Aw (tropical), with the rainy season occurring from October to May, and June to September corresponding to the dry season. The average annual temperature ranges from 20 to 35°C and annual rainfall varies from 1500 to 1800 mm.

The soil was classified as dystroferric Red Latosoil (dRL) of medium texture (458, 150 and 391 g kg^{-1} of sandy, silt and clay, respectively), soil density = 1.27 g cm^{-3}, total porosity = 0.53 cm^3 cm^{-3}, θ_{FC} = 53.4 m^3 m^{-3}, θ_{PWP} = 30.9 m^3 m^{-3}, as chemical characteristics: pH (in water) = 6.4, organic matter = 53.9 g dm^{-3}, P = 4.85 mg d^{-3}, K = 61.42 mg dm^{-3}, Ca = 1.74 $cmol_c$ dm^{-3}, Mg = 1.5 $cmol_c$ dm^{-3}, Al = 0.0 $cmol_c$ dm^{-3}. The experimental area was chemically treated with fertilizer according to the results of soil analysis, with 30 kg ha^{-1} of nitrogen (urea), 120 kg ha^{-1} of P_2O_5 (simple superphosphate) and 80 kg ha^{-1} of KCl (potassium chloride).

The experiment was carried out in randomized complete blocks in a 5 x 2 factorial scheme, with four replicates. The treatments consisted of five levels of water replacement (100, 75, 50, 25 and 0% of soil humidity at field capacity) and two conditions of fertilizer supply (0 and 100 kg ha^{-1} of N) in the form of urea. The experimental plots were set up in a double row composed of three grooves (W-shaped) 8 m long, with a spacing of 1.8 m by 0.4 m between the lines, totalling 35.2 m^2 per plot.

A subsurface drip irrigation system was used for the water replacement treatments. The drip tube, (Dripnet 16150 model) with a thin wall, 1 bar service pressure and 1.0 L h^{-1} nominal flow, was buried at a depth of 0.20 m below the soil surface in the middle of the double row with a spacing of 0.50 m between drippers.

The water volume applied in the 100% water replacement treatment was based on increasing soil humidity until field capacity was reached. With the other treatments, a water blade was applied considering the water replacement of the 0, 25, 50 and 75%. At the end of the experiment, the total volume of water was 0, 126, 252, 378 and 504 mm accounting for 0, 25, 50, 75 and 100% water replacement, respectively. A nitrogen supply was added to the irrigation water (fertirrigation) and divided into ten applications during crop development, while one portion of potassium manure was added to planting grooves, representing 30% of the total, and the remainder was applied via the irrigation water. In the treatment with 0% water replacement, fertilizers were comprised by broadcast application.

The irrigation was carried out with a tensiometry puncture digital base at 0.1 kPa of sensibility, being a tensiometric metal rod installed at depths of 0.20, 0.40, 0.60 and 0.80 m and a distance of 0.15, 0.30, 0.45 and 0.60 m from the drip tube. The soil matrix potential (Ψ_m) was collected daily. A critical tension of 50 kPa was used to determine the need for irrigation. The physical-hydric characteristics of the soil were determined from a water retention

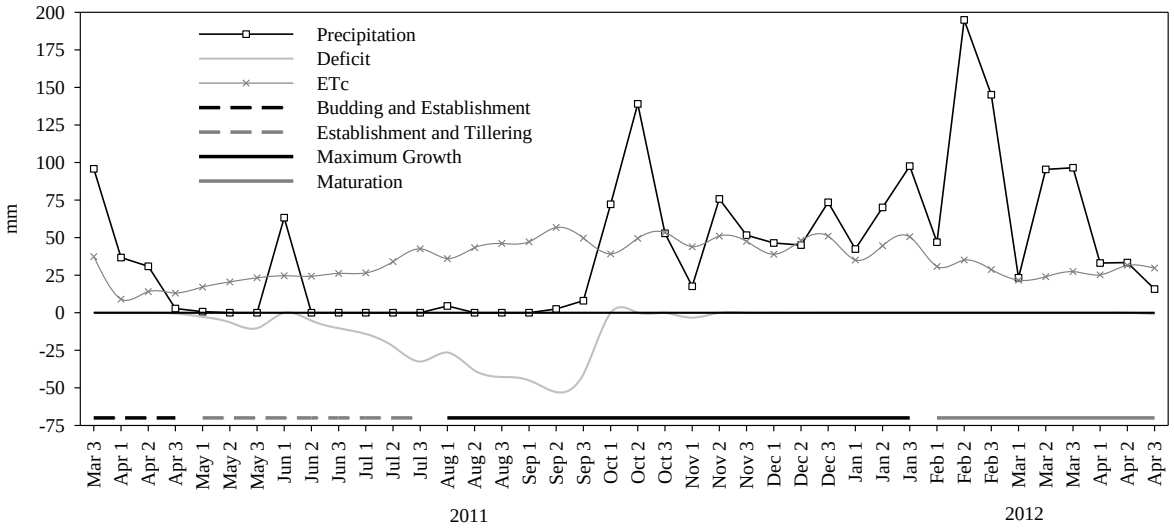

Figure 1. Water balance of sugarcane (0% water replacement) during the experiment. DEF – water deficit; ETc – Evaporation-transpiration of the culture; culture phases (adapted from Doorenbos and Kassam, 1979): budding and establishment (Kc = 0.6); establishment and tillering (Kc = from 0.9 to 1.1); maximum growth (Kc = 1.3); maturation (Kc = from 0.7 a 0.9). Source: INMET – Rio Verde, GO, Brazil.

curve. An equation was developed according to Van Genuchten (1980) to convert the Ψ_m to water content in the soil (θ), minimizing the square sum of the deviation using SWRC software (Dourado-Neto et al., 2000), and so obtaining the empirical parameters of adjustment used in the equation 1:

$$\theta = \frac{0{,}5643}{\left[1+(0{,}2933|\Psi_m|)^{1{,}4937}\right]^{0{,}330522}} \quad (1)$$

Where: θ – water content of soil, g g^{-3}; Ψ_m – matrix potential, mca.

The pluviometric rainfall was used to estimate the decendial water balance, calculating the hydric deficit (HD) during the experiment (March 2011 until April 2012) using the method of Thornthwaite and Mather (1957), in which the Reference Evapotranspiration (ET_0) was calculated according to the Penman-Monteith (Monteith, 1973) equation. These results are shown in Figure 1. Total evapotranspiration during crop development was 1,549 mm and rainfall was 1,618 mm In the useful area (one square meter) around the main line three plants were randomly chosen to determine gas exchange, chlorophyll fluorescence, leaf water potential (Ψ_w), leaf area and number of leaves.

The gas exchange rates of the plants were evaluated at 300 and 410 days after planting (DAP) to obtain the photosynthetic rate (*A*, μmol m^{-2} s^{-1}), transpiration rate (*E*, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and ratio of internal to external CO_2 concentration (C_i/C_a). The water use efficiency (WUE, mmol CO_2 mol H_2O^{-1}) was calculated from the A/E ratio. Measurements were performed on completely expanded leafs using a portable photosynthesis system with a LCi Light Systems (ADC Bioscientific, Herts, England) using a light source emitting a photon flux density of 1000 μmol m^{-2} s^{-1}.

Chlorophyll fluorescence was evaluated at 380 and 410 DAP using a MINI-PAM (Walz, Effeltrich, Germany) modulated fluorometer. Analyses were performed using the methods of Bilger et al. (1995) and Rascher et al. (2000) to determine potential quantum yield (*Fv/Fm* is variable fluorescence/ maximum fluorescence), effective quantum yield (*ΔF/Fm'*), non-photochemical dissipation (NPQ) (Krause and Weis 1991) and electron transport rate (ETR).

Predawn leaf water potential (Ψ_w) was evaluated with a Scholander pressure chamber (model 3005-1412, Soil moisture Equipment Crop, Goleta, USA) at 410 DAP. Leaf area (cm^2) was calculated using the equation: LA = L x W x 0.7, where L (cm): leaf length + 1, W (cm): leaf width + 1, and 0.7 the correction factor.

Statistical analysis

Result were evaluated with variance using an F-test at a probability of 0.05. Where significance was detected, a linear and quadratic polynomial regression to water replacement and nitrogen supply were compared by Tukey test at a probability of 0.05.

RESULTS AND DISCUSSION

Analysis of variance showed that water replacement (WR) significantly altered all variables analysed except transpiration rate, stomatal conductance and water use efficiency (WUE) at 410, and 300 days after planting(DAP), respectively (Table 1), whereas the nitrogen (N) supply promoted significant differences in photosynthetic rate (*A*), stomatal conductance (g_s) and

Table 1. Analysis of variance summary of leaf water potential (Ψ_w), leaf area, number of leaves, photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), ratio of internal to external CO_2 concentration (*Ci/Ca*) and water use efficiency (WUE) of sugarcane submitted to different levels of water replacement (WR), with or without a nitrogen (N) supply, to 300 and 410 days post-application.

Variables[1]	Mean square of source variation				
	Water replacement (WR)	Nitrogen (N)	WR x N Interaction	Blocks	Residual
			300 DAP		
A	45.49**	229.08**	23.82^{ns}	6.65^{ns}	9.47
E	1.83*	1.35^{ns}	0.48^{ns}	1.23^{ns}	0.49
g_s	0.016*	0.079**	0.009^{ns}	0.007^{ns}	0.005
Ci/Ca	0.0096*	0.0031^{ns}	0.0003^{ns}	0.0117*	0.0022
WUE	0.19^{ns}	4.66*	0.58^{ns}	0.69^{ns}	0.58
			410 DAP		
A	146.91**	3.36^{ns}	88.80**	23.90^{ns}	18.18
E	0.56^{ns}	2.07*	0.84^{ns}	1.39*	0.37
g_s	0.003^{ns}	0.005^{ns}	0.001^{ns}	0.002^{ns}	0.002
Ci/Ca	0.028*	0.064*	0.006^{ns}	0.051**	0.008
WUE	1.58**	0.81^{ns}	1.48**	0.40^{ns}	0.30
Ψ_w	0.01087**	0.00004^{ns}	0.00005^{ns}	0.00044^{ns}	0.00087
Leaf area	0.04159**	0.00729*	0.00319^{ns}	0.00134^{ns}	0.0034
Number of leaves	2.69**	0.09^{ns}	0.23^{ns}	0.66^{ns}	0.49

[1]Significance: **p < 0.01, * p < 0.05, ^{ns} not significant, according to F test.

WUE at 300 DAP and the ratio of internal to external CO_2 concentration (C_i/C_a) at 410 DAP. For the interaction WR x N, differences were verified at 410 DAP in *A* and WUE. Sugarcane plants demonstrated a quadratic polynomial regression for photosynthetic rate at 300 DAP. Plants reach 27.97 µmol m^{-2} s^{-1} with 43.19% water replacement. Beyond that point the photosynthetic rate decreased with increasing volume, reaching 21.2 µmol m^{-2} s^{-1} at 100% water replacement (Figure 2A). However, studies on two genotypes of sugarcane showed accelerated reductions in CO_2 assimilation and transpiration of plants submitted to hydric deficit during sucrose accumulation as a response to decreasing stomatal conductance (Machado et al., 2009).

Photosynthetic rate (*A*) decreased by 8.53 µmol m^{-2} s^{-1} with 54,12% of water replacement, followed by increases of 2.83 µmol m^{-2} s^{-1} and 13,68 µmol m^{-2} s^{-1} for 75 and 100% WR, respectively, when we consider the water replacement inside each nitrogen doses at 410 DAP. However in the absence of nitrogen no differences among levels of water replacement were verified (Figure 2B).

When we considered the nitrogen dose inside each level of water replacement for *A* (Table 2), there were no statistical differences among the doses at 25% water replacement. At WR levels of 0 and 100%, plants that received nitrogen fertilizer showed a major *A.* However, at 50 and 75% WR levels the plants without nitrogen supply showed greater photosynthetic rate. The transpiration rate (*E*) evaluated at 300 DAP showed a quadratic response to water replacement levels. The maximum *E* of 5.72 mmol m^{-2} s^{-1} was obtained. At 44% of WR, from which reductions of 0.29, 7.74 and 25.56% in response to increases in water replacement to 50, 75 and 100%, respectively (Figure 2C). The *E* at 410 DAP increased by 25.09% with N application (Table 3). Increases in leaf transpiration favour absorption of solute from soil leading to improved nutrient assimilation, in this case supplied in abundance via the irrigation system. Biomass production is strongly linked to transpiration; maximizing absorption of humidity from the soil is the main point to improve crop yield under conditions of water stress conditions (Blum, 2009).

Plants fertilized with N showed high photosynthetic rates (*A*) and WUE at 300 DAP (Table 3). The nitrogen supply induced an increase of 14.75% WUE when compared to plants that did not receive N (Table 3).

Considering water replacement inside each nitrogen level was verified responses of WUE at 410 DAP, as N application, adjusting to quadratic equation (Figure 2D).

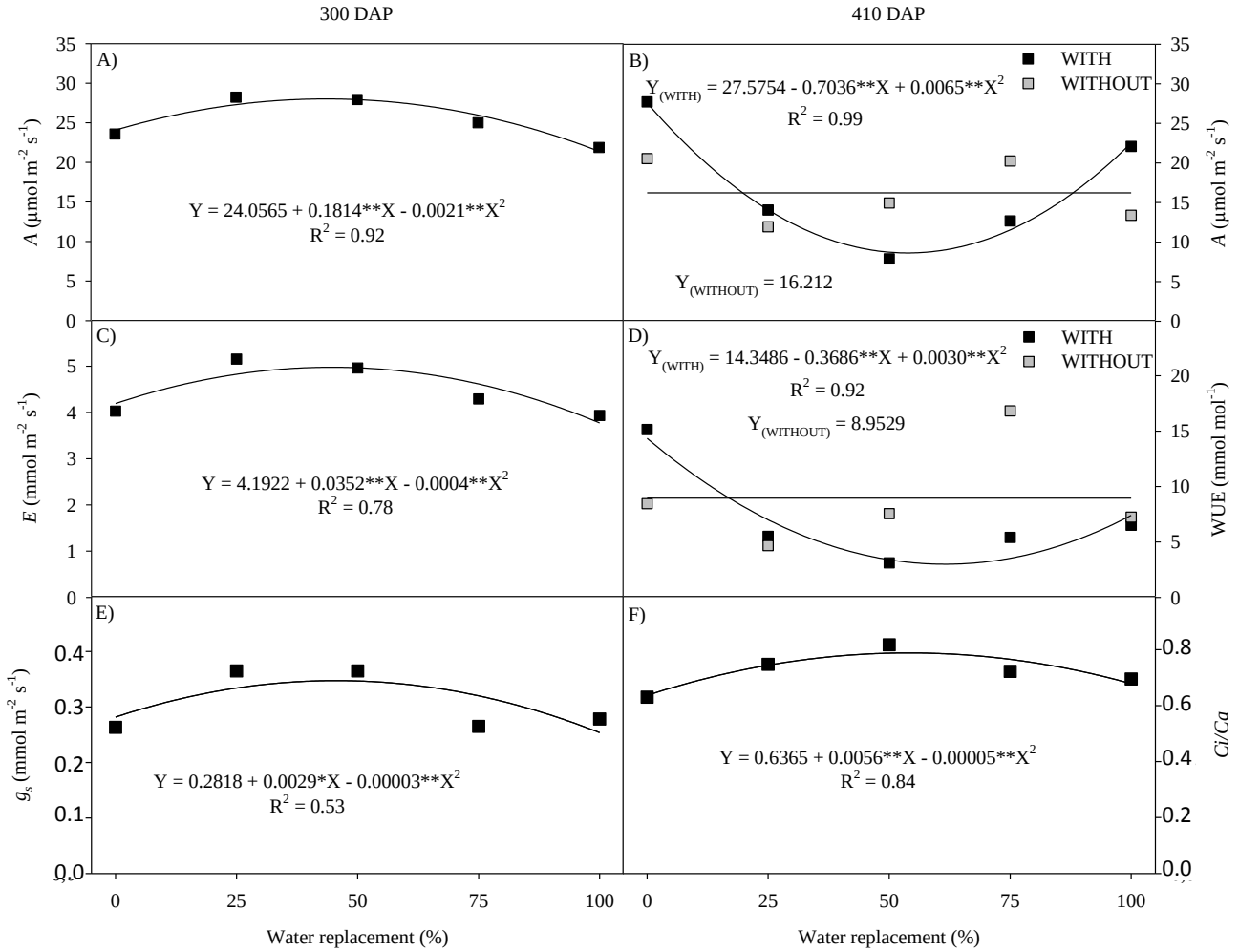

Figure 2. Photosynthetic rate (*A*) at 300 DAP (A) and 410 DAP (B); transpiration rate (*E*) at 300 DAP (C); water use efficiency (WUE) at 410 DAP (D); stomatal conductance (g_s) at 300 DAP (E); and ratio of internal to external CO_2 concentration (*Ci/Ca*) at 410 DAP (F) of sugarcane submitted to different levels of water replacement, with or without a nitrogen supply.

Thereby, reductions in WUE of up to 61.43% water replacement were observed, as 3.03 mmol CO_2 mol H_2O^{-1}, and an increase of 2.47 fold at 100% water replacement. Studies verifying the relationship of sugarcane cultivated in different environments and at different stages of development to water found 4.4 µmol of CO_2 fixed to each water molecule released by transpiration (Endres et al., 2010), as observed in the present study (Figure 2D).

On the other hand, splitting nitrogen doses in each water replacement level at 410 DAP produced significant differences only at 75% water replacement, as a decrease of 11.43% in WUE with nutrient supply (Table 2). The efficiency of water use to crop yield has been used to indicate to increasing plant productivity per unit of water when compared to dry cultivation (Blum, 2009). In this way, is possible to demonstrate that water supply during crop development maintains sufficient humidity for the photosynthetic process. However, plants growing with 0% water replacement presented high resistance to drought, reaching 14.35 mmol CO_2 mol H_2O^{-1} of WUE at 410 DAP (Figure 2D)

Maintaining the photosynthetic process is functionally linked to stomatal conductance (g_s), directly linked to the transpiration and photosynthetic rates of sugarcane plants. Based on the quadratic behaviour of g_s as a response to water replacement at 300 DAP, reaching a maximum response with 48.33% water replacement at a

Table 2. Photosynthetic rate (*A*), water use efficiency (WUE) and electron transport rate (ETR) in sugarcane plants as a response to different levels of water replacement, with or without a nitrogen supply. Data are mean of evaluation realized at 380 and 410 DAP.

Variables	*A* (µmol m^{-2} s^{-1})	WUE (mmol mol^{-1})			ETR		ETR	
	410 DAP	410 DAP			380 DAP		410 DAP	
WR[1]	WITH	W-OUT	WITH	W-OUT	WITH	W-OUT	WITH	W-OUT
0	27.72[a]	20.54[b]	15.14[a]	8.46[a]	113.15[a]	138.05[a]	131.37[a]	159.8[a]
25	14.06[a]	11.93[a]	5.50[a]	4.67[a]	146.85[a]	159.7[a]	210.85[a]	176.7[b]
50	7.87[b]	14.95[a]	3.11[a]	7.56[a]	160.87[a]	173.15[a]	215.35[a]	128.7[b]
75	12.67[b]	20.24[a]	5.41[b]	16.83[a]	126.6[b]	187.15[a]	168.8[a]	181.97[a]
100	22.08[a]	13.39[b]	6.51[a]	7.24[a]	128[b]	180.57[a]	118.3[a]	121.15[a]

[1]Means followed by the same letter in the row, as a function of nitrogen supply, are not significantly different according to Tukey test (* $p < 0.05$).

Table 3. Photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), ratio of internal to external CO_2 concentration (*Ci/Ca*), water use efficiency (WUE) and non-photochemical dissipation (NPQ) in sugarcane plants as a response to nitrogen supply.

Variables	*A* (µmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	*Ci/Ca*	WUE (mmol mol^{-1})	NPQ
DAP[1]	300	410	300	410	300	380
N				-		-
WITH	28.09[a]	2.62[a]	0.36[a]	0.77[a]	6.10[a]	0.34[b]
W-OUT	22.57[b]	2.10[b]	0.26[b]	0.67[b]	5.31[b]	0.52[a]

[1]Means followed by the same letter in a row, as a function of nitrogen supply, are not significantly different according to Tukey test (0.05).

g_s of 0.35 mmol m^{-2} s^{-1} followed by a 22.75% decrease compared to 100% water replacement (Figure 2E). These results reinforce the idea of the stomata being associated with morphological characteristics as tolerance mechanisms against hydric deficit (Endres et al., 2010). When N was applied, a 27.8% reduction in g_s was verified at 300 DAP (Table 3).

Neither regression model linear or quadratic was adjusted to the ratio of internal to external CO_2 concentration (*Ci/Ca*) in sugarcane leaves at 300 DAP, showing analogous efficiencies on stomatic absorption. However, the results observed at different water replacement levels showed that *Ci/Ca* in the stomatal chamber at 410 DAP presented a quadratic behaviour (Figure 2 F). *Ci/Ca* reached its maximum value, 0.793, with 56.0% water replacement and decreased by 2.28 and 12.20% when water replacement was raised to 75 and 100%, respectively.

Significant decreases in internal concentration (*Ci*) should result in reduced photosynthetic rates in response to reductions in CO_2 concentration needed for the activity of the enzymatic complex of PEPC and RUBISCO (Parry et al., 2011). Whereas low values of *Ci* can stimulate stomatal opening, allowing a major influx of CO_2 into the substomatal cavity (Raschke, 1979) and permitting an equilibrium between consumption and entry of CO_2, keeping *Ci* constant. In this way, adequate levels of water replacement could promote excitation of stomata in order to maintain CO_2 at a high concentration in the stomatic chamber.

A significant effect of the nitrogen supply was observed on *Ci/Ca* at 410 DAP, representing an increase of 13.75% compared to no nitrogen treatment (Table 3).

Reductions in transpiration and the photosynthetic rate of sugarcane at high levels of water replacement when compared to other treatments can be associated with rainfall during the experimental period (Figure 1).

According to Ghannoum (2009), increasing the CO_2 concentration after hydric stress can partially recover photosynthesis limits under pre-stress conditions in plants with C4 metabolism. So, the related g_s and *E* responses to plants were not impeded by water replacement at 410 DAP (Table 1), verifying the high capacity for restoration of photosynthetic machinery and

Table 4. Analysis of variance summary of potential quantum yield (*Fv/Fm*), effective quantum yield (*ΔF/Fm'*), non-photochemical dissipation (NPQ) and electron transport rate (ETR) of sugarcane submitted to different levels of water replacement (WR), with or without a nitrogen (N) supply.

Variables[1]	Mean square of variation source				
	Water replacement (WR)	Nitrogen (N)	WR x N interaction	Blocks	Residual
			380 DAP		
Fv/Fm	0.00021^{ns}	0.00018^{ns}	0.00017^{ns}	0.00011^{ns}	0.00019
ΔF/Fm	0.004^{ns}	0.013^{ns}	0.003^{ns}	0.003^{ns}	0.008
NPQ	0.0910**	0.2378**	0.0105^{ns}	0.0009^{ns}	0.0113
ETR	1426.59**	7985.37**	765.74**	409.50^{ns}	128.63
			410 DAP		
Fv/Fm	0.00033^{ns}	0.00029^{ns}	0.00013^{ns}	0.00004^{ns}	0.00024
ΔF/Fm'	0.0065^{ns}	0.0007^{ns}	0.0148^{ns}	0.0060^{ns}	0.0062
NPQ	0.18**	0.05^{ns}	0.07^{ns}	0.09^{ns}	0.03
ETR	5015.22**	1748.79*	3186.94**	369.09^{ns}	244.39

[1]Significance: $^{**}p < 0.01$, $^{*}p < 0.05$, ^{ns} not significant, according to F test.

consequently the photosynthetic activity of plants submitted to hydric deficit (Figure 2B).

Water replacement significantly interfered with NPQ at 380 and 410 DAP. The statistical difference in NPQ was verified at 380 DAP as a response to nitrogen supply (Table 3). Evaluating the WR x N interaction, significant differences in ETR were observed at 380 and 410 DAP (Table 4). However, water replacement and nitrogen supply did not interfere with the potential quantum yield (*Fv/Fm*) or effective quantum yield (*ΔF/Fm'*) of sugarcane in either evaluation (Table 4). This shows the plant's ability to maintain similar values of Fv/Fm under hydric stress, indicating the efficiency of energy use in the photochemical process (Silva et al., 2007) and corroborating Gonçalves et al. (2009), where sugarcane growing under moderate or severe hydric deficit produced little reduction in potential photochemical efficiency.

Productivity responses of sugarcane to water replacement in association with nitrogen manure are well known (Wiedenfeld and Enciso, 2008). Considering the physiological responses to combination management, it is important to emphasize the efficiency of the photosynthetic machinery with regard to nitrogen use when directly available in the soil solution.

The electron transport rate (ETR) of sugarcane showed a quadratic behaviour when splitting WR in each nitrogen dose at 380 DAP (Figure 3A). According to the regression equation, when water replacement associated with nitrogen supply was at 51.47% of maximum, values of ETR were 22.58% higher than with dry treatment (0% water replacement). However, in treatments without a nitrogen supply the ETR response was more efficient but required 85.08% water replacement to reach a maximum value. Usually, plants submitted to hydric deficit shown inadequate dissipation of electrons produced by the electron transport chain, due a small carbon assimilation rate conducive to overproduction of reactive oxygen species (ROS) (Edreva, 2005). Table 2 verifies the negative interference of nitrogen application on the ETR of sugarcane at 380 DAP with 75 and 100% water replacement levels, with reductions of 32.35 and 29.11%, respectively. The ETR of sugarcane at 410 DAP adjusted to a quadratic equation when splitting water replacement levels in each nitrogen dose. The nitrogen supply showed increasing development at 46.22% water replacement, followed by a decrease with further increases in water replacement. Treatment without a supply of N did not present significant differences among water replacement levels (Figure 3B). Splitting the nitrogen in each water replacement level, the ETR of sugarcane was significantly interfered with at 410 DAP only at 25 and 50% water replacement, triggering increases of 19.33 and 67.33%, respectively, when nitrogen was supplied (Table 2).

Non-photochemical dissipation (NPQ) is dependent on high energy and is related to the proton concentration inside thylakoids, which induce thermal energy dissipation by the xanthophyl cycle (Taiz and Zeiger, 2013). Responses of sugarcane to water replacement showed a linear rise at 380 DAP, triggering an increase of 0.9% in NPQ with a 1% increase in water replacement

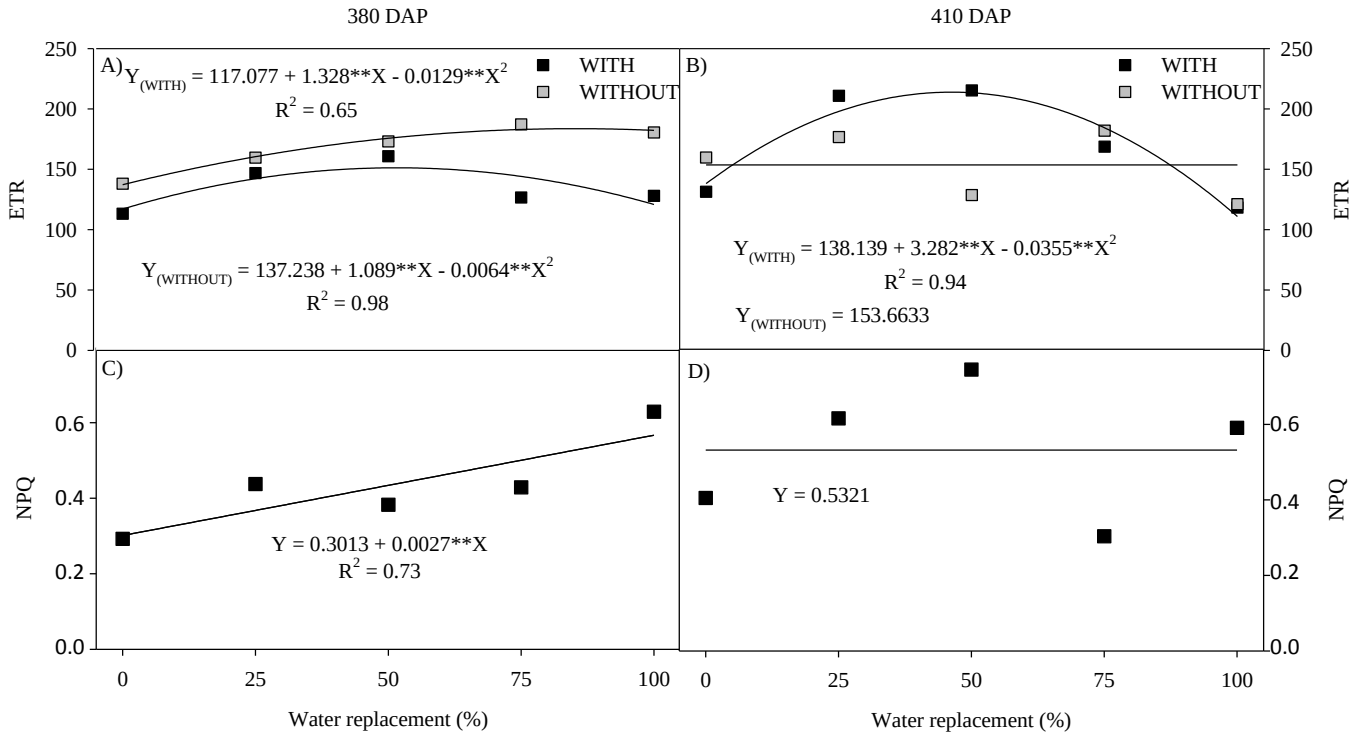

Figure 3. Electron transport rate (ETR) at 380 DAP (A) and 410 DAP (B); non-photochemical dissipation (NPQ) at 380 DAP (C) and 410 DAP (D) of sugarcane submitted to different levels of water replacement, with or without a nitrogen supply.

(Figure 3C). At 410 DAP a variable behaviour was observed in NPQ in response to the water replacement level (Figure 3D).

Excess photons should be a main factor in photo inhibition, altering the quantum efficiency of photosystem II (Taiz and Zeiger, 2013). The NPQ data showed a slight increase in response to water replacement, indicating their capacity to activate protection mechanisms against light or photochemical damage. The nitrogen supply promoted a 34.04% reduction in NPQ at 380 DAP in plants supplied with nitrogen (Table 3).

The responses in terms of leaf area (LA) and leaf number (LN) of sugarcane to water replacement showed a linear response. The leaf area increased 13.96 cm^2 for each interval of water replacement (Figure 4A) and rose 3.21% in terms of number of leaves (Figure 4B). Hydric deficiencies caused levels of leaf senescence and restricted the emergence of new leaves. The extent of these alterations is a direct consequence of the degree of hydric stress (Smit and Singels, 2006).

The development of leaf area, that is, photosynthetic active structure, is directly correlated to a high capacity for photoassimilation. Oliveira et al. (2014) observed that with the makeup water and nitrogen there was an increase in the productivity of stems, as increased total biomass and aerial part. According to Inman-Bamber and Smith (2005) and Ghannoum (2009), the morphological characteristics modified by hydric stress are of great importance to increasing plant productivity.

The leaf water potential (Ψ_w) results indicated a linear reduction when water replacement was reduced, reaching its lowest point in plants that did not receive irrigation (-0.18 MPa) (Figure 5).

Water potential provides a relative index of hydric stress (Taiz and Zeiger, 2013). According to Inman-Bamber and Jager (1986), cellular extension is practically insignificant as Ψ_w approaches -1.3 MPa. Factors promoting reductions in water potential when plants are submitted to hydric deficit (Figure 5) could be related to a lower pressure of water turgor inside cells due to solute accumulation in the cytoplasm as a way to sustain the turgor pressure, elevating the tolerance of the plant to water deficiency (Inman-Bamber and Smith, 2005).

Conclusion

The low maintenance of turgor pressure in cells as

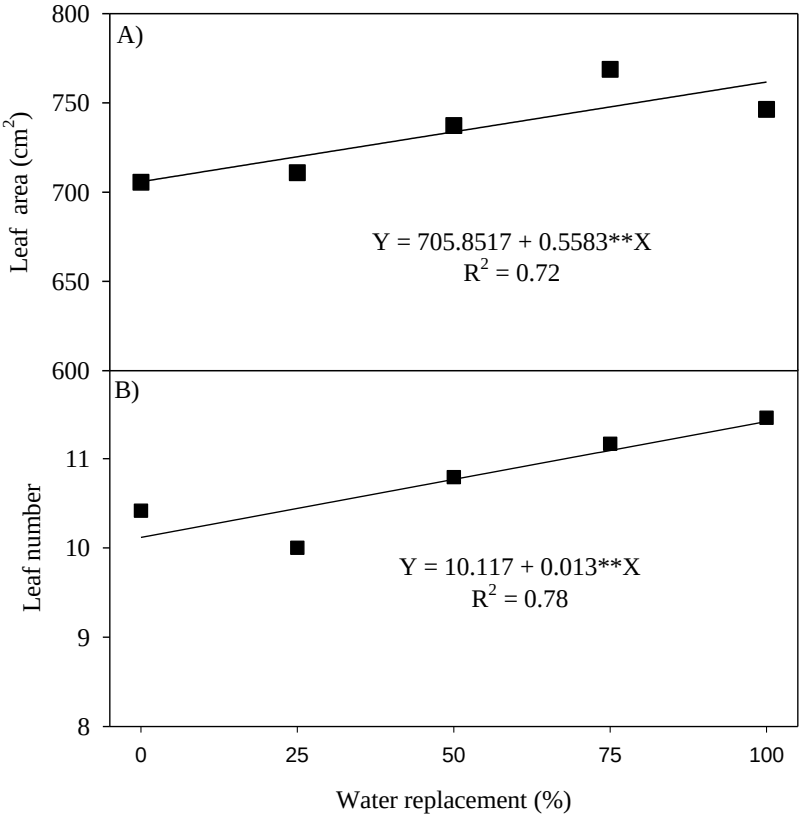

Figure 4. Leaf area (A) and leaf number (B) of sugarcane submitted to different levels of water replacement.

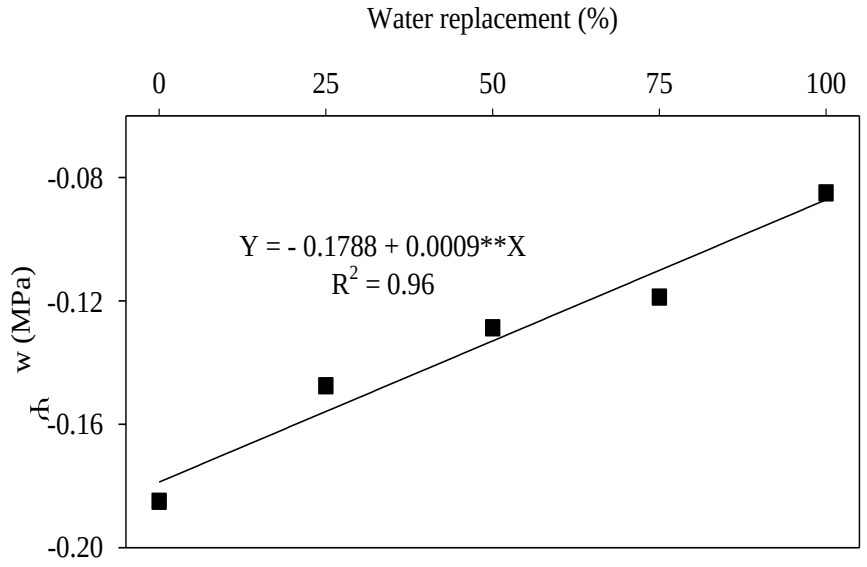

Figure 5. Leaf water potential (Ψ_w) of sugarcane submitted to different levels of water replacement.

evidenced by reductions in leaf water potential promoted severe reductions in the photosynthetically active area of sugarcane when submitted to hydric deficit.

Association of the gas exchange rate and chlorophyll a fluorescence of sugarcane plants submitted to water replacement correlated with major development of leaf area, as shown by the high capacity to photoassimilate production.

A water replacement of 75% associated with nitrogen supply via an irrigation system positively influenced maintenance of the photosynthetic process. In addition, it promoted efficient control of leaf transpiration, permitting a high photosynthetic rate that induces efficient use of water.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Production of sunflower under saline water irrigation and nitrogen fertilization

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The aim of the present study was to evaluate the production components of sunflower Embrapa 122-V2000, as a function of irrigation water salinity and nitrogen doses. The experiment was conducted in plastic pots with a capacity of 100 L under greenhouse conditions in Federal University of Campina Grande, during the period of April-July 2012, in an experimental design adopting factorial arrangement of 5 x 5, with five levels of irrigation water salinity, expressed by electrical conductivity of 0.7, 1.7, 2.7, 3.7 and 4.7 dS m^{-1} (25°C) and five nitrogen rates of 50, 75, 100, 125 and 150 mg N kg^{-1} soil, with three repetitions, totaling 75 experimental units. The evaluated parameters were: total number of achenes, percentage of viable achenes, percentage of biomass of viable achenes, the biomass and diameter of chapter, dry weight of 1000 seeds and production of achenes. Although, the interaction of water salinity and nitrogen levels did not show significant effects on the components of production, increase in the salt content of the water inhibited all the variables while elevation of nitrogen rates stimulated emission of achenes, chapter biomass and production of achenes.

Key words: Marginal quality water, productivity, *Hellianthus annus* L.

INTRODUCTION

The continuous population growth in the world requires the availability of food in the same proportions, which leads to the need for producing more food to meet the demand of human consumption. This demand has been met by the production of food through rainfed agriculture and the use of irrigation systems in arid and semiarid

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regions, which has allowed the expansion of agricultural areas worldwide (Machado et al., 2007).

Sunflower (*Helianthus annuus* L., Asteraceae family) is a plant native to Mexico and currently a viable option among oilseed crops, due to the quality of its oil, in polysaturated fatty acids and vitamin E, besides high protein digestibility (Grompone, 2005). According to Machado and Carvalho (2006), sunflower has the advantage of having tolerance to adverse weather conditions and wider thermal range of exploration than other oilseed crops of commercial importance. Thus, it can be an alternative in the diversification of family farming activities, because, besides grain production for oil extraction, it can be used in apiculture for the good quality of pollen and nectar.

The advance in sunflower cultivation in the last years has shown that this crop may be an alternative to various agribusiness segments, evidencing a sustainable option for the composition of production systems in the many producing regions of Brazil (Vieira, 2005). Among the factors justifying sunflower cultivation, there is an increasing demand for edible oil and biofuels, besides the possibility of using the bran as a complement in animal ration (Ferrari, 2004).

Nitrogen (N) is the second most required nutrient by sunflower and the one that most limits its production, causing yield reduction of up to 60% due to its deficiency (Castro and Farias, 2005). N plays an important role in sunflower metabolism and nutrition, and its deficiency causes nutritional disorders. This nutrient in excess causes a reduction in the percentage of oil and can increase the incidence of pests and diseases, compromising grain production (Biscaro et al., 2008). Nobre et al. (2011) evaluating sunflower cv. 122 Embrapa 122-V2000, under irrigation with waters of different salinities (0.5 to 4.9 dS m^{-1}) and doses (50 to 125% of the N recommendation), concluded that the duration of crop cycle and the time interval between the formation and physiologic maturity of seeds reduces its mass and increases with the dose of N. Ribeiro et al. (2015) studying the effects of irrigation with saline water and nitrogen on growth and production components of sunflower cv. Embrapa 122-V2000, found no siginificant influence on biometric indices and production.

The negative effects of irrigation water salinity in oilseed crops have been observed by many researchers (Silva et al., 2008; Correia et al., 2009), including the sunflower crop (Silva et al., 2009; Nobre et al., 2010; Guedes Filho et al., 2015).

The irregularity of rains, the lack of good quality water for agriculture, inadequate irrigation management and the low tolerance of economically important plants to salinity has compromised food production worldwide (Hans et al., 2015). These problems are more frequent in arid and semiarid areas of the world and in Brazil, where more than 30% of the soils in irrigated lands are affected by salts (Lopes et al., 2008; Smith et al., 2009; Leite et al., 2010) and require the adoption of practices that economically justify the use of high salinity water in agriculture in order to overcome the lack of food for the increasing global population (Rhoades et al., 2000). Thus, several studies have pointed out that there is evidence of competition in uptake of nitrate and chloride, so that an increase in the nitrate concentration in the root zone may inhibit greater absorption of chloride by the plant (Flores et al., 2002).

The knowledge of the effects of salts on this crop and on the soil, as well as the involved phenomena, are extremely important for an adequate management of irrigation and cultivation and the use of saline water (Dias and Blanco, 2010). Therefore, the sunflower crop has stood out in researches with low quality water (Rodrigues et al., 2010; Nobre et al., 2011; Travassos et al., 2011; Guedes Filho et al., 2015), due to the possibility of the production of edible oil, biodiesel and as an ornamental plant and material.

In this context, this study aimed to evaluate the productive behavior of Embrapa 122-V2000 sunflower under irrigation with water of increasing salinity levels and N fertilization.

MATERIALS AND METHODS

The experiment was conducted in a screened shelter at the Center of Technology and Natural Resources of the Federal University of Campina Grande (UFCG), from April to July 2012, located at the geographic coordinates of 07° 13′ 11′′ S and 35° 53′ 31′′ W, and an altitude of 550 m. The climate in the region, according to Köppen's classification, adapted to Brazil, is Csa (humid temperate climate with dry and hot summer), which represents a semi-humid mesothermal climate, with hot and dry summer (4 to 5 months) and rains from autumn to winter; the months of June and July are the coldest ones, with mean temperatures less than 20°C.

The experimental design was completely randomized using a 5 x 5 factorial scheme with three replicates, totaling 75 experimental units. The treatments corresponded to five levels of irrigation water electrical conductivity – ECw (0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1}), prepared through the addition of non-iodized NaCl to the water from the local supply system (ECw = 0.7 dS m^{-1}), combined with five N doses, corresponding to 75, 100, 125 and 150 mg of N kg^{-1} of soil.

The experiment was conducted using the Embrapa 122-V2000 sunflower cultivar, which stands out for its precocity as compared to the hybrids currently cultivated in Brazil. It can reach mean yield of 1,700 kg ha^{-1} and mean oil content of 43.5%. It has a cycle of approximately 100 days and its flowering starts at 53 days (Embrapa , 2002).

Seventy five plastic pots with capacity of 100 L of soil were used and distributed at spacing of 1.5 m between rows and 1.0 m between plants. The bottom of the pots was connected to a 5-mm-diameter plastic tube, for the drainage of the leachate, and had a nylon screen, on which 1 kg of crushed stone (no. zero) was placed. Two plastic containers were placed below the pots for the collection of drained water.

The substrate consisted of material from a soil classified as non-saline Yellow Argisol, according to the Brazilian Soil Classification System - SiBCS (Embrapa, 2013), collected in the district of São José da Mata, in the municipality of Campina Grande-PB, in the layer of 0-20 cm, which was pounded to break up clods, sieved and placed in the plastic pots. During the period of September 2011 to

Table 1. Characteristics of soii in the layer of 0-20 cm, in relation to the chemical and salinity properties before application of treatments.

Soil properties	Values	Saturation extract	Values
pH (H_2O)	6.17	pH_{sp}	5.97
OM (g kg^{-1})	19.1	ECse (dS m^{-1})	2.03
P (mg dm^{-3})	56.2	Chloride ($mmol_c$ L^{-1})	12.5
Ca^{2+} ($cmol_c$ kg^{-1})	3.88	Bicarbonate ($mmol_c$ L^{-1})	5.00
Mg^{2+} ($cmol_c$ kg^{-1})	2.86	Calcium ($mmol_c$ L^{-1})	10.37
K^+ ($cmol_c$ kg^{-1})	0.30	Magnesium ($mmol_c$ L^{-1})	9.63
Na^+ ($cmol_c$ kg^{-1})	0.47	Potassium ($mmol_c$ L^{-1})	0.38
Al^{3+} ($cmol_c$ kg^{-1})	0.00	Sodium ($mmol_c$ L^{-1})	4.86
H^+ ($cmol_c$ kg^{-1})	1.62	SAR (mmol $L^{-1})^{1/2}$	1.54
SB ($cmol_c$ kg^{-1})	7.51	ESP (%)	5.15
CEC ($cmol_c$ kg^{-1})	9.13	Degree of salinity	Slight
V (%)	82.25	Soil of classification	Non saline non sodic

OM- Organic matter; CEC- cation exchange capacity [SB + (H^+ + Al^{3+})]; SB- sum of bases (Ca^{2+} + Mg^{2+} + K^++ Na^+); V- base saturation% = (SB/CEC) x 100; pH_{sp} – pH of the saturation paste; ECse- electrical conductivity in the saturation extract; ESP- exchangeable sodium percentage (Na^+/CEC x 100).

February 2012 castor bean crop was cultivated in the pots under same treatments of water salinity and N rates (Alves et al., 2012). The chemical characteristics (Table 1) refer to the mean results of the soil analysis after leaching with local supply water (ECw = 0.7 dS m^{-1}) until the draining water in each pot had an electrical conductivity around 0.9 dS m^{-1}. Chemical parameters related to fertility were determined according to the methodologies proposed by Donagema et al. (2011) and salinity according to Richards (1954), at the Laboratory of Irrigation and Salinity of the UFCG.

Before sowing, soil water content was increased to field capacity through irrigation. Irrigation management during the cultivation was performed based on water balance, following an irrigation frequency of two days. Thus, the water depth in each treatment was calculated as a function of the applied water volume and the volume of water drained in the previous irrigation, adding a leaching fraction of 10%.

Fertilization followed the recommendations of Novais et al. (1991) for experiments in protected environments. Basal fertilization was performed using 300 mg of P_2O_5 and 4 mg of boron kg^{-1} of soil, as single superphosphate and boric acid, respectively. Potassium fertilization consisted of 150 mg of K_2O kg^{-1} of soil, as potassium chloride. Nitrogen fertilization, as urea, was applied according to the treatments. Both potassium and nitrogen, were applied one third at sowing (basal dose) and two thirds as top-dressing in equal doses at 20 and 40 days after sowing (DAS). Sowing was performed at a depth of 2 cm, using ten seeds per pot. Seedlings emergence started on the fourth day and continued until the 13th day; at 15 DAS, the first thinning was performed, leaving three plants with the best vigor per pot and, at 20 DAS, the second thinning was performed, leaving one plant per pot.

As plants reached the state of physiological maturation of achenes, that is, when they showed a hard mass, referring to the "R9" phenological stage (Silva et al., 2007), irrigation was suspended. At this stage, the capitula inclined downward, with their back and bracts showing yellow and brown color. In this period, 75 DAS, plants were at the end of the cycle, when the evaluation of sunflower production components started. At 85 DAS, harvest was performed by cutting the stem of each plant close to the soil and then separating the different parts (stem, leaf, capitulum and root) and placing them in kraft paper bags. Every part of plant was dried in a forced-air oven at 65°C until constant weight, to determine the following parameters: total number of achenes (TNA), percentage of viable achenes (VA%), percentage of viable achene weight (VAW%), phytomass of capitulum (PCAP), diameter of capitulum (DCAP), weight of 1000 achenes (M1000A) and production of achenes (PROD). The achenes of each capitulum were manually threshed and then separated into viable and non-viable achenes. Non-viable achenes were considered as those that did not develop or were empty. The TNA refer to manual count of all achenes in the chapter; the percentage of viable achenes (AV%) was calculated by relating number of viable achenes - NVA to TNA - NVA/TNA x 100; the VAW was cauculated by the relationship VAW/PROD x 100; the PCAP was obtained by manual threshing of the achenes from receptacle of sepals and bracts and subsequently placed (receptacle, sepals and bracts) for drying in an oven with forced air ventilation at temperature of 65°C, for a period 48 h. The diameter of the chapter (DCAP) was measured with a millimetric ruler; the M1000A was determined by weighing 1000 viable achenes and the production of achenes was calculated based on the weight of achenes of each plot (one plant), considering the moisture content of the achenes of 13%.

The obtained data were subjected to the analysis of variance by F test at 0.01 and 0.05 probability levels; when significant, polynomial regression analysis was performed using the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

According to the results of the F test (Table 2), the interaction between water salinity levels and N doses did not affect significantly any of the evaluated parameters, but salinity had significant effects on all the production components, and N doses on the total number of achenes, phytomass of capitulum and production of achenes of sunflower. Similar results were reported by several authors (Nobre et al., 2010, 2011; Oliveira et al., 2010; Freitas et al., 2012; Guedes Filho et al., 2015), who observed isolated effects of irrigation water salinity and N

Table 2. Summary of the F test and regression for the total number of achenes (TNA), percentage of viable achenes (%VA), percentage of viable achene phytomass (VAP%), phytomass of capitulum (PCAP), diameter of capitulum (DCAP), mass of 1000 achenes (M1000A) and production of achenes (PROD) of sunflower as a function of irrigation water salinity and nitrogen fertilization, evaluated at harvest, 85 days after sowing.

Variation source	F TEST						
	TNA	VA%	VAP%	PCAP	DCAP	M1000A	PROD
Salinity (S)	**	**	**	**	**	**	**
Linear regression	**	**	**	**	**	**	**
Quadratic regression	*	ns	*	**	ns	**	*
Nitrogen (N)	**	ns	ns	**	ns	ns	*
Linear regression	**	ns	ns	**	ns	ns	**
Quadratic regression	ns	ns	ns	ns	ns	ns	ns
Interaction (S x N)	ns	ns	ns	ns	ns	ns	ns
CV (%)	15.46	6.66	3.55	13.31	20.50	8.75	15.21

CV = coefficient of variation; ns = not significant; * = significant ($p < 0.05$); ** = significant ($p < 0.01$).

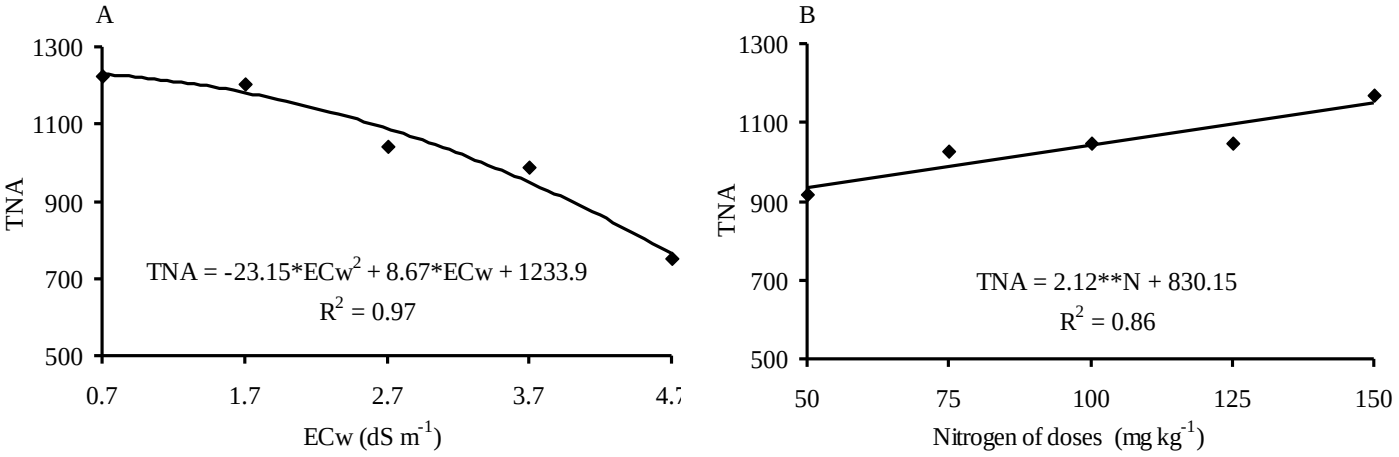

Figure 1. Total number of achenes– TNA of sunflower as a function of irrigation water salinity (A) and nitrogen fertilization (B) evaluated at harvest, 85 days after sowing.

on sunflower growth and production, but, in general, without significant effects of the interaction between both on plant vegetative and productive behaviors. The lack of significant effect of the interaction can be a response of the adequate N supply to plants, which contributed to the attenuation of the effects of increasing water salinity, as observed for the total number of achenes, phytomass of capitulum and production of achenes, for which the increase in salinity was inhibited and the increase in N stimulated each studied variable.

The increase in water salinity compromised the emergence of sunflower achenes and, according to the regression (Figure 1A), the data adjusted to a quadratic model ($p<0.05$). TNA obtained by irrigation waters were: 1217, 1182, 1089, 949 and 682 for the salinity levels of 0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1}, respectively, comparison of TNA obtained in due to lower and higher salinity level, point that there was 44% reduction in the total number of achenes. According to Assis et al. (2007), the reduction in the production in plants cultivated under saline water irrigation can be due to the delay in net carbon assimilation, associated with osmotic effects and accumulation of potentially toxic ions (Na^+ and Cl^-) in leaf tissues. Likewise, despite evidencing the harmful effect of salinity on the number of achenes, the decrease also indicated that the variable is important for the evaluation of the crop under saline stress.

Nobre et al. (2010), evaluating production of sunflower cultivar Embrapa 122-V2000 under different levels of irrigation water salinity (ECw from 0.5 to 4.9 dS m^{-1}), in experiment conducted in a greenhouse, concluded that the production of achenes decreased linearly from the irrigation water salinity of 0.5 dS m^{-1}.

For N fertilization, the increase in the doses stimulated the emergence of achenes (Figure 1B), the number of achenes obtained under different N doses were: 936,

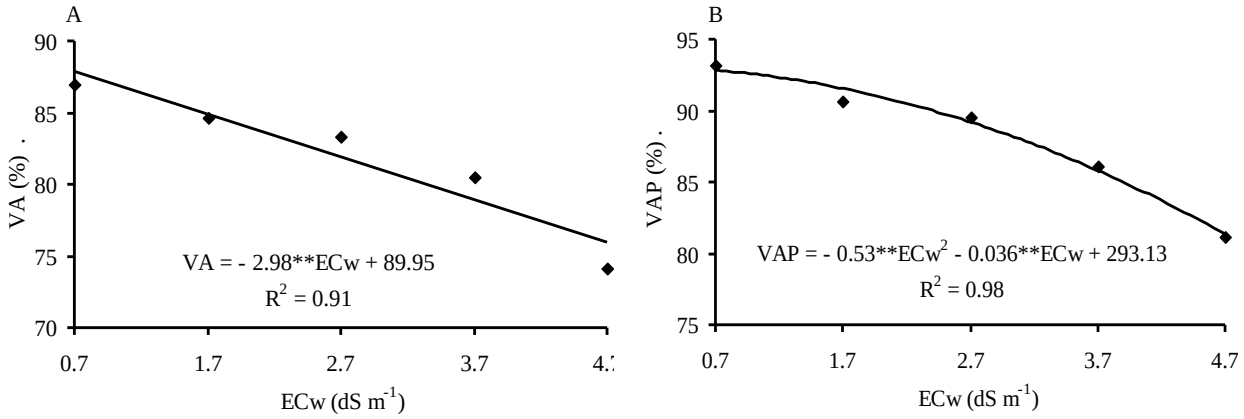

Figure 2. Percentages of viable achenes– %VA (A) and viable achene phytomass– %VAP (B) of sunflower as a function of irrigation water salinity, evaluated at harvest, 85 days after sowing.

989, 1042, 1095 and 1148 achenes for the levels of 50, 75, 100, 125 and 150 mg N kg^{-1} of soil, respectively, the number of achenes obtained under highest N level (150 mg N kg^{-1} of soil) were 22.64% more than that of lowest level (50 mg kg^{-1} of soil). The increment in TNA can be related to the functions performed by this nutrient in plant metabolism, since it is a constituent of chlorophyll molecule, nucleic acids and proteins, besides being an activator of various enzymes. Furthermore, it participates in vital processes in the plant, such as synthesis of protein, ionic absorption, photosynthesis, respiration, multiplication and differentiation of cells (Malavolta, 2006). Abbadi et al. (2008), studying the effects of N on growth, yield and production components of safflower and sunflower, observed increases in the number of achenes per capitulum and concluded that the direct effects are smaller for safflower than sunflower.

According to the regression equation (Figure 2A), the effect of irrigation water salinity on the percentage of viable achenes was linear and decreasing, with reductions of about 3.31% in percentage of VA per unit increase in ECw. For water salinity levels of 0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1}, the obtained percentage of viable achenes were 87.86; 84.88; 81.90; 78.92 and 75.94%, respectively, indicating that there was a reduction of 11.92% in the percentage of viable achenes between the lower and higher levels of irrigation water salinity. As for nitrogen levels, no significant effect was observed. The decrease in VA can be attributed to the reduction in water availability to plants, because of the osmotic effects resulting from the accumulation of salts in the root zone under saline stress, which require higher energy to absorb water (Leonardo et al., 2007) with consequent negative effects on yield. Travassos et al. (2011), evaluating production components and achene production of sunflower under irrigation with differrent water salinity levels (0.5 to 5.0 dS m^{-1}) in protected environment, concluded that the number of viable achenes decreased by 9.64% per unit increase in ECw. Chen et al. (2009) in a drip irrigation system with saline water of electrical conductivity ranging from 1.6 to 10.9 dS m^{-1}, reported linear reduction of 5.5% in viable achenes of sunflower per unit increase in irrigation water salinity.

Viable achene phytomass (VAP) (Figure 2B) adjusted to a quadratic regression model. Considering the equation, the biomass obtained for viable achenes in function of the salinity levels of irrigation water were: 92.85; 91.54; 89.17; 85.74 and 81.25% for levels 0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1}, respectively, causing decrease of 12.49% in biomass of viable achenes between the lowest and highest level of salinity. These results indicated that salinity levels in the irrigation water from 4.7 dS m^{-1} on cause low production of viable achenes, making the production less profitable in salinized areas. Santos Júnior et al. (2011), studying the sunflower cv. Embrapa 122-V2000 in semi-hydroponic system, using coconut fiber as a substrate, irrigated with nutrient solutions of EC from 1.7 to 11.5 dS m^{-1}, observed a decrease of 11.3% in the phytomass of achenes with unit increase in salinity. Studies conducted by Escalante and Rodríguez (2010), on sunflower in Mexico, reported that the number and phytomass of achenes were affected by irrigation water salinity.

As for the phytomass of capitulum (Figure 3A), the regression equation fitted to a quadratic model. It is observed that when plants were exposed to water salinities 0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1}, the biomass of the chapter was 124.8; 113.2; 96.2; 73.7 and 45.8 g, respectively, indicating a reduction of 63.3%, between the lowest and the highest level of the irrigation water salinity. Such reduction is strongly related to the effects of salinity on plants, which lead to loss of yield and/or quality.

For the factor N doses, the data of phytomass of capitulum adjusted best to a quadratic model (Figure 3B); where it is noted that the values increased with

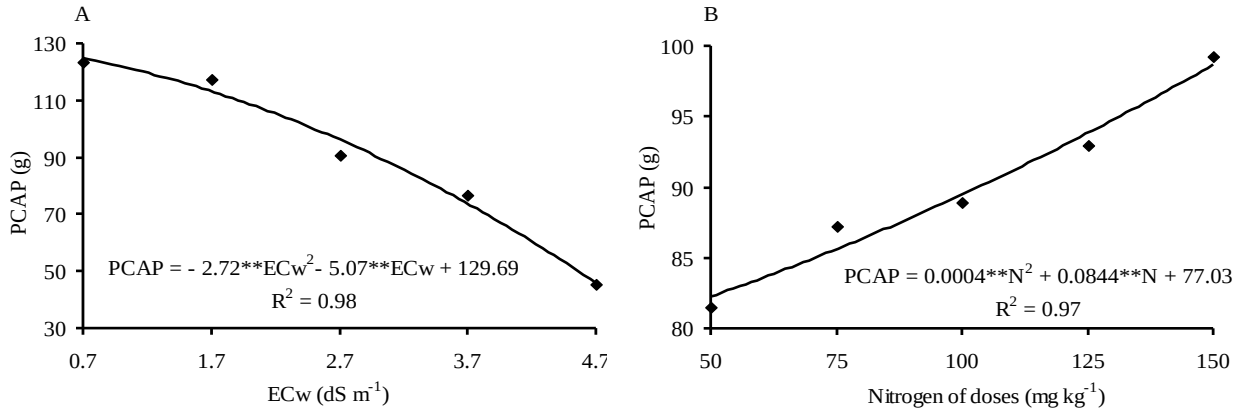

Figure 3. Phytomass of capitulum– PCAP of sunflower as a function of irrigation water salinity (A) and nitrogen fertilization (B), evaluated at harvest, 85 days after sowing.

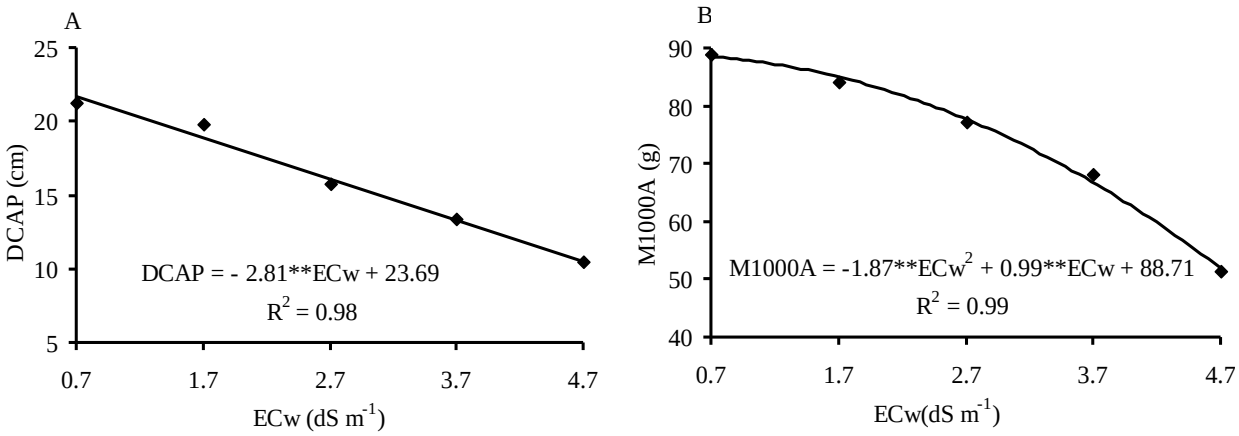

Figure 4. Diameter of capitulum– DCAP (A) and weight of 1000 achenes (M1000A) (B) of sunflower as a function of irrigation water salinity, evaluated at harvest, 85 days after sowing.

increasing doses of nitrogen, the values being 82.3; 85.6; 89.5; 93.8 and 98.7 g of dry matter, depending on levels of nitrogen, respectively, 50, 75, 100, 125 N and 150 mg kg^{-1} of soil. By regression equation, it is also observed that the plants fertilized with the highest dose N obtained an increase of 19.99% in FCAP, in relation to that fertilized with the lower dose. According to Biscaro et al. (2008), N plays an important role in sunflower metabolism and nutrition, and is the nutrient that most limits its production. Excess of N may cause a decrease in oil percentage and high doses N can increase the incidence of pests and diseases, affecting grain production.

Sunflower capitulum diameter was also negatively affected by the different levels of irrigation water salinity and, according to the regression equation (Figure 4A), there was a linear negative effect, with estimated reduction of about 11.86% per unit increase in ECw. The plants subjected to salt stress with water 0.7; 1.7; 2.7; 3.7 and 4.7 dS m^{-1} have had their head diameter: 21.72; 18.91; 16:10; 13:29 and 10:48 cm, indicating a reduction in the order of 51.75% in chapter diameter, between the highest and lowest salinity treatment. This reduction in DCAP as a function of water salinity can also be explained by the osmotic stress caused by the decrease in the external water potential and by the ionic effect, resulting from the accumulation of ions in plant tissues (Munns and Tester, 2008). Silva et al. (2009), evaluating ornamental sunflower cultivated under different levels of electrical conductivity and fertigation, observed that the increase in EC of the nutrient solution significantly reduced capitulum diameter between the salinity levels of 3.5 and 6.5 dS m^{-1}. Travassos et al. (2011), working with salinity levels from 0.5 to 5 dS m^{-1}, observed diameters much lower (ranging from 5.47 to 7.41 cm) than those obtained in the present study.

For weight of 1000 achenes, according to the regression equation (Figure 4B), the increasing irrigation water salinity promoted a quadratic response. For water salinity levels of 0.7, 1.7; 2.7, 3.7 and 4.7 dS m^{-1}, the

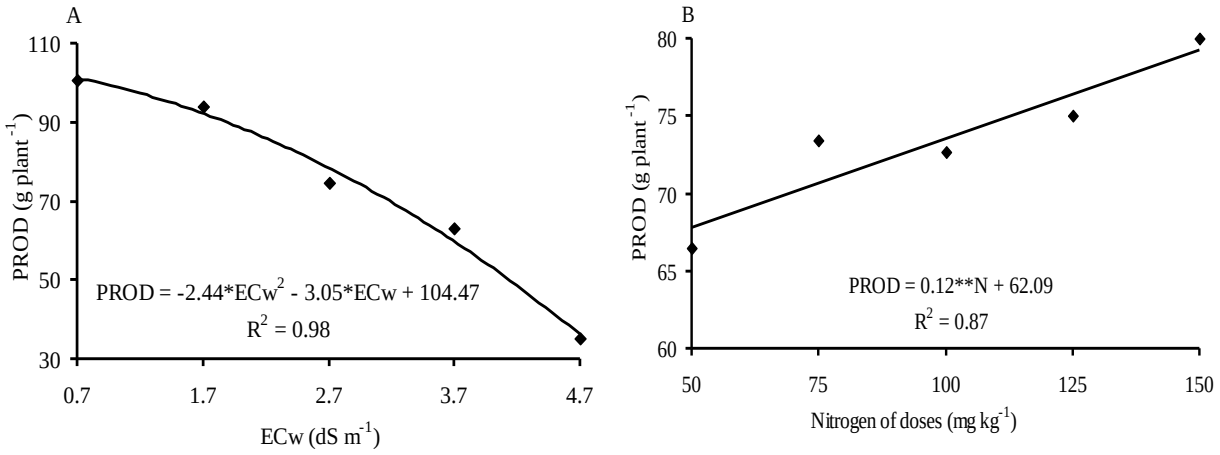

Figure 5. Production of achenes (PROD) of sunflower as a function of irrigation water salinity (A) and nitrogen fertilization (B), evaluated at harvest, 85 days after sowing.

mass of 1000 achene obtained was 88.5, 84.9, 77.6, 66.8 and 52.1 g, respectively, when comparing the plants irrigated with water dS 4.7 m^{-1} with those that were under ECw 0.7 dS m^{-1}, a reduction of approximately 41.13% in weight of 1000 achenes was observed. The negative effect of salinity on the weight of 1000 achenes was expressive, which shows that the production is affected by the presence of salts in the water. Nobre et al. (2010), studying sunflower, cultivar Embrapa 122-V2000, irrigated with saline water (ECw of 0.5 to 4.9 dS m^{-1}), concluded that the weight of 1000 achenes decreased linearly from the salinity level of 0.5 dS m^{-1} in the irrigation water.

As for the total number of achenes and capitulum phytomass, the increases in water salinity and N doses, respectively, inhibited and promoted sunflower production (Figure 5). According to the regression equation, the production of achenes (Figure 5A) fitted to a quadratic model, wherein, when the plants were irrigated with water of salinities 0.7, 1.7, 2.7, 3.7 and 4.7 dS m^{-1}, the production of achenes was 101.14, 92.23, 78.44, 59.78 and 36.24 g $plant^{-1}$. These results indicate a decrease of 64.17% in the production of achenes between the lower and higher levels of irrigation water salinity. Saline stress tends to affect plant physiology, causing metabolic disorders, especially due to the reduction in the absorption of water and nutrients in the soil, which leads to lower development and, consequently, lower crop production. Nobre et al. (2011), in a study with sunflower, cultivar Embrapa 122-V2000, irrigated with saline water, observed that the production decreased by approximately 14.55% per unit increase in the electrical conductivity of the irrigation water from the 0.5 dS m^{-1} upwards. Travassos et al. (2011), working with sunflower in greenhouse irrigated with water of EC levels from 0.5 to 5.0 dS m^{-1}, observed that the production of achenes decreased by 10.6% per unit increase in irrigation water EC.

As for the N fertilization, the effect on the production of achenes was linear and increasing (Figure 5B), with production of 68.09; 71.09; 74.09; 77.09 and 80.09 g $plant^{-1}$ at doses of 50, 75, 100, 125 and 150 mg N kg^{-1} of soil. respectively, which is equivalent to increase of 4.83% for every increase of 25 mg of N kg^{-1} of soil. According to Khalil et al. (2008) and Babaiy et al. (2009), increasing N levels for many crops, including sunflower, significantly increased the production characteristics of the crop, such as the production of achenes and oil.

Conclusions

The interaction between irrigation water salinity and nitrogen doses did not have significant effects on any of the studied variables, while the increases in water salinity and nitrogen doses, respectively, inhibited and stimulated the emergence of achenes, capitulum phytomass and the production of achenes.

The total number of achenes, capitulum phytomass and production of achenes were more compromised by water salinity than the number and phytomass of viable achenes, capitulum diameter and mass of a thousand viable achenes. Nitrogen promoted the linear increase in the production of achenes, total number of achenes and capitulum phytomass, attenuating the harmful effects of water salinity on sunflower.

Conflict of Interests

The author have not declared any conflict of interests.

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Full Length Research Paper

Organic-matter effects on populations of dry rot of yam nematodes

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The objective of this study was to evaluate the effect of organic-matter incorporated into the soil on population densities of the causal agents of the dry rot disease of yam, under field conditions. The experiment was performed in a natural infested area with a mixed population of *Pratylenchus coffeae* and *Scutellonema bradys*, in Quebrangulo county (Alagoas state, Brazil) in a randomized block design with five treatments and five replicates. The sources of organic matter used as soil amendments were: coconut husk powder, castor bean cake, cattle manure and chicken manure. Non amended soil was used as a control. Nine months after planting, the tubers were harvested. No statistical differences were found among disease incidence, yam production and nematode population densities in the soil. However, the application of chicken manure reduced *P. coffeae* population in tubers.

Key words: *Dioscorea* spp., *Scutellonema bradys*, *Pratylenchus coffeae*, nematode management.

INTRODUCTION

Yam (*Dioscorea* spp.) is a monocotyledonous plant of the family Dioscoreaceae, comprising more than 600 species in the genus, mostly grown in Africa (*Dioscorea cayenensis*), the Caribbean, Mexico and Southeast Asia (*Dioscorea alata*, *Dioscorea esculenta*, *Dioscorea composita*, *Dioscorea dumetorum* and *Dioscorea rotundata*) and South America (*D. cayenensis*) (Cazé, 2002).

In 2014, African countries produced 65.7 million tons of tubers equivalent to 96.4% of the world production (68.2 million tons). Among South American countries, Brazil ranks second, with 25.5 thousand hectares and an estimated production of 247 thousand tons (FAO, 2015). According to Santos et al. (2011), the Northeastern region is the largest producer of yams in Brazil with approximately 15 thousand ha and production of 200 thousand tons (average yield of 10.5 t ha^{-1}), mainly cultivated in the states of Paraíba, Pernambuco, Bahia and Alagoas. Among the constraints to yam production in Brazil, dry rot disease caused by *Scutellonema bradys*, *Pratylenchus coffeae* and *Pratylenchus brachyurus* causes the greatest damage to this crop (Moura, 2006).

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In Alagoas, mixed populations are found in most yam-growing areas with an incidence ranging between 0.2 and 85% (Muniz et al., 2012).

The first symptoms of the disease are light yellow lesions below the outer skin of the tubers, turning to a dark brown to black color as the disease progresses. External cracks arise in the tubers's skin and complete deterioration may occur during storage. The damage caused by the nematode is confined to sub-epidermal, peridermal and parenchymatous tissues extending to 1-2 cm into the tuber, although sometimes deeper (Kwoseh et al., 2002; Bridge and Starr, 2007). Above-ground symptoms are not apparent (Bridge and Starr, 2007).

The most successful method for preventing nematodes' damages remains in the use of nematode-free seed tubers in nematode-free land (Bridge and Starr, 2007), but the difficulty to obtaining healthy propagative material turn this technique unfeasible (Moura, 2006).

According to Gowen et al. (2005) and McSorley (2011), an extensive range of organic materials have shown efficiency in reducing nematode populations in a number of pathosystems. Organic materials as agro-industrial and animal wastes may act as nutrient sources and improve water-holding capacity of the soil, increasing plant growth. Higher organic content in soils also stimulates the activity of plant-parasitic nematodes' antagonistic organisms. Furthermore, decomposition of residues results in the accumulation of specific compounds in the soils which may be nematicidal (Bridge, 1996). However, information on the use of organic materials in the management of plant-parasitic nematodes that affect yam crops is limited (Santos et al., 2009; Osei et al., 2013).

Thus, the aim of this work was to evaluate the effect of some animal and agro-industrial wastes that are available in the Northeast region of Brazil, to manage nematodes under field conditions.

MATERIALS AND METHODS

The experiment was conducted on February 2013, on a farm located in Quebrangulo county, AL (9° 15' 50.9" S; 36° 26' 11.7" W), previously grown with yam and naturally infested with dry rot disease nematodes.

The experiment was performed in a randomized block design with five treatments and five replicates, in plots consisting of four ridges 3.50 m long, considering the two central ridges as useful area. Seed tubers weighing approximately 250 to 350 g each were selected from a field known to be free of the dry rot disease (based on the absence of symptoms and after randomly sampling tubers to test presence of plant-parasitic nematodes), and planted at a 1.20 x 0.35 m spacing.

Previous to planting, a composite soil sample of four sub-samples in each plot was collected, using a zig-zag pattern, in the two central rows to evaluate the initial nematode populations. The nematodes were extracted, according to the centrifugal-flotation technique (Jenkins, 1964), in aliquots of 100 cm^3 of soil and the nematodes were quantified with the aid of Peter's counting slides (Astel®, Botucatu-SP, Brazil), under a light microscope. The identification of *Pratylenchus* species was done according to Gonzaga et al. (2012). In addition, the chemical and physical properties of soil were determined (Table 1), as well as the chemical analysis of the sources of organic matter (Table 2).

Soil treatments included: 1) untreated soil (control), 2) coconut husk powder (from coconut epicarp) - 37 t ha^{-1}, 3) castor bean cake – 2.5 t ha^{-1}, 4) cattle manure - 10 t ha^{-1} and 5) chicken manure - 4 t ha^{-1}. These dosages were determined based on the results of the chemical analysis of soil and the nutrient content of the sources of organic materials, using a reference of 150 kg ha^{-1} nitrogen. Due to the high C/N ratio of coconut husk powder, a supplemental fertilizer with ammonium sulfate (10 g/plant) was applied to this treatment. Mineral fertilization with NPK 16-00-20 (20 g/plant) was made 70 days after planting, according to soil testing analysis.

Nine months after planting, the tubers were harvested and assessed for dry rot incidence (percentage of tubers exhibiting symptoms of the disease in relation to the total tubers evaluated) and tuber weight per plot, in a total of 18 plants. Mixed soil and tubers samples were taken to determine the final nematode populations, and were processed according to Jenkins (1964) and Coolen and D´Herde (1972), respectively. The nematode population densities were determined from duplicated 1 ml aliquots, in Peter's counting slide, under a light microscope, using a taxonomic key, as described early. The reproduction factor (RF) of the nematodes [RF = final population (tubers+soil)/initial population], in the different treatments was calculated according to Oostenbrink (1966). For statistical analyses, the data were transformed in log x or $\sqrt{x}$ and means separated by Scott-Knott test at 5% probability, using the software Assistat 7.7 beta (Silva, 2014).

RESULTS AND DISCUSSION

The evaluation of the initial nematode populations showed the presence of *P. coffeae* and *S. bradys* with average of 12.0 to 25.0 specimens/100 cm^3 of soil among plots. There were no statistical differences, indicating the uniformity of the nematode populations in the field (Table 3). These low nematode population levels could be attributed to the stressful conditions due to the dry season. At the end of the experimental period, there was no significant difference among disease incidence, tuber production and nematode population densities in soil due to the use of different sources of organic matter. However, the application of chicken manure reduced *P. coffeae* population in tubers (Table 3).

Despite the efficiency of chicken manure in controlling nematode populations (Ferraz et al., 2010; Abdel-Dayem et al., 2012), negative results have also been observed. Examples can be found in Brazil, with *Meloidogyne javanica* and *Meloidogyne incognita* on bananas (Vilas Boas et al., 2004a; Vilas Boas et al., 2004b), and in the United States with *Heterodera glycines* in soybean crop (Donald et al., 2013). However, no published report on this source of organic matter was found regarding *P. coffeae* or *S. bradys* on yam plants.

In Nigeria, Adesiyan and Adeniji (1976) observed that application of cattle manure to the soil at a rate of 1.89 t ha^{-1}, increased tuber yield of *D. alata*, and significantly reduced the population density of *S. bradys*. The differences in the current results may possibly be related

Table 1. Chemical and physical properties of soil collected in Quebrangulo county, AL, naturally infested with a mixed population of *Pratylenchus coffeae* and *Scutellonema bradys*.

Chemical analysis										
pH	P	K^+	H+Al	Al^{+2}	Ca^{2+}	Mg^{2+}	CEC	V	M	SOM
(H_2O)	(mg dm^{-3})				($cmol_c$ dm^{-3})			%		g kg^{-1}
5.4	5	54	5.3	0.17	2.2	1.1	8.8	40.0	4.6	23.1

Granulometric analysis (g kg^{-1})				
Clay	Silt	Fine sand	Coarse sand	Textural class
123	126	299	452	Sandy loam

Analyses performed at Central Analítica Laboratory, Maceió, AL, 2013. Ca, Mg, Al (KCl 1 mol L^{-1}); P, K (Mehlich extractor); H+Al (calcium acetate at pH 7.0); CEC = cation exchange capacity at pH 7.0; V = base saturation; m = Aluminum saturation; SOM = soil organic matter.

Table 2. Chemical characteristics of the organic materials used in the experiment.

Source of organic matter	Chemical analysis											
	N	P	K	Fe	Cu	Mn	Zn	Ca	Mg	Total OM	C	C/N
	%			mg L^{-1}						%		
Cattle manure	1.54	1.12	0.95	6,746	43	371	803	4,258	3,504	46.41	25.78	16.74
Chicken manure	3.74	1.54	1.20	960	127	273	307	10,500	5,928	74.94	41.63	11.13
Castor bean cake	6.09	1.53	0.90	1,100	31	84	386	6,542	5,344	72.30	40.17	6.60
Coconut husk powder	0.41	0.11	0.16	1,140	11	20	314	7,882	1,940	56.56	31.42	76.63

Analysis performed at Central Analítica Laboratory. Maceió, AL, 2013.

to the yam species and the implementation conditions on each experiment. Although, the mentioned work had been done under field conditions, the soil was artificially infested with the nematode. In addition, the authors reported that the average nematode population per 50 g of tuber peelings at harvest was 1,410 specimens which correspond to approximately 28 individuals per gram of tissue. Although, the application of the treatment significantly reduced the nematode population, according to Bridge et al. (2005), populations of *S. bradys* in excess of 20 nematodes/g of tuber peelings are necessary to produce external symptoms of damage. In the present work, up to 2,727 specimens of *P. coffeae* per gram of tuber peelings were recorded.

In Brazil, Santos et al. (2009) assessed the effect of antagonistic plants used as green manure and organic wastes to control nematodes in yam (*D. cayenensis*), under field conditions. The authors observed incidence of dry rot disease of 36.35% in the first year of cropping and 21.88% in the second, with the use of cattle manure. However, data on the initial nematode populations in the area, the rate of manure application, and the disease incidence from the control plants, which prevent comparisons between results were not shown.

With respect to by-products from the processing of coconut fruits, the data on its use in the management of plant-parasitic nematodes are scarce, and when assessed for controlling *M. javanica* in banana (Vilas Boas et al., 2004a) and tomato crops (Dallemole-Giaretta et al., 2010), only in the second case was observed a favorable result.

Silveira et al. (2002) detected high quantity of microorganisms in coconut coir fiber (from coconut mesocarp) reported as agents of biocontrol for several pathogens, among these are *Trichoderma* species. According to Meyer et al. (2000), culture filtrate from *T. virens* contained extracellular factors that inhibited egg hatch and second-stage juvenile mobility of *M. incognita*.

Concerning the use of castor bean cake to control plant-parasitic nematodes, some researches have been already published. For example, this organic material has been applied to the soil for management of *Meloidogyne* species in sugarcane (Dinardo-Miranda and Fracasso, 2010), and tomato crops (Lopes et al., 2009; Roldi et al., 2013). According to Rich et al. (1989) the nematicidal activity of this product was attributable to the chemical compound, ricin, a natural occurring lectin capable of inhibiting protein synthesis (Audi et al., 2005). The results obtained in the present work are not in accordance with this.

The difference between the reports shown in the literature and the current work could be due to variations in nematode species, host plant, chemical composition of the organic amendments as well as the rate and time of

Table 3. Initial population of the causal agents of dry rot disease in 100 cm^3 of soil in naturally infested field where different organic materials were incorporated to the soil; dry rot incidence; yam production nine months after planting and number of nematodes in 100 cm^3 of soil and 20 g of tubers. Quebrangulo, AL, 2013.

Treatments	Initial nematode populations	Dry rot incidence (%)	Fresh weight of tubers/plot (kg)	Nematode population densities				Total nematode population densities
	S. bradys + *P. coffeae*			*S. bradys*		*P. coffeae*		*S. bradys* + *P. coffeae*
	Soil[a]			Soil[b]	Tuber[a]	Soil[a]	Tuber[a]	Soil + Tuber[b]
Coconut husk powder (37 t ha^{-1})	15.5[a]	100	9.94[a]	0.0[a]	0.0	56.0[a]	35,640[a]	35,696[a]
Castor bean cake (2.5 t ha^{-1})	12.0[a]	100	12.46[a]	0.0[a]	0.0	28.0[a]	54,540[a]	54,568[a]
Cattle manure (10 t ha^{-1})	25.0[a]	100	11.22[a]	10.0[a]	0.0	60.0[a]	42,720[a]	42,790[a]
Chicken manure (4 t ha^{-1})	17.0[a]	100	12.92[a]	10.0[a]	0.0	78.0[a]	19,200[b]	19,288[b]
Control	15.0[a]	100	9.36[a]	4.0[a]	0.0	110.0[a]	41,200[a]	41,314[a]
MSR	0.1323[ns]	-	0.0107[ns]	2.1164[ns]	-	0.5502[ns]	0.3445*	0.0345*
C.V. (%)	31.7	-	10.1	77.4	-	49.8	4.1	4.1

Averages of five replicates; data followed by the same letter within a column do not differ at 5% probability level by Scott-Knott test. [a], [b]analyses of variance with the data converted into log x and $\sqrt{x}$, respectively. Mean-square residue (MSR); *significant at 5% probability by F test; [ns]not significant to probability higher than 5% by F test. CV = coefficient of variation.

application, and the environmental factors like temperature, microbial community and soil type. In addition, the present work involved a mixed nematode population.

Conclusion

The use of organic materials as cattle manure, chicken manure, castor bean cake and coconut husk powder did not reduce the incidence of dry rot of yams under field conditions. However, the application of chicken manure reduced *P. coffeae* population in tubers.

Conflict of interests

The authors have not declared any conflict of interests.

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Fumigant and repellent activity of Rutaceae and Lamiaceae essential oils against *Acanthoscelides obtectus* Say

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***Acanthoscelides obtectus* Say, a principal pest of kidney beans *Phaseolus vulgaris* L. in the Mediterranean area, is a multivoltine and oligophagous bruchid that damages not only host plant (kidney bean) but also other *Leguminosae* species. Essential oils of Rutaceae: *Citrus reticulata* L. and *Citrus limonum* L. and of Lamiaceae *Mentha piperita* L. and *Lavandula angustifolia* L. were evaluated by fumigation upon *A. obtectus* adults using concentrations of 13.33, 40, 80 and 106.66 µl/L air. Their vapours have a toxic increasing effect depending upon concentrations and time exposure. Lamiaceae essential oils exhibited the higher fumigant activity inducing mortality of about 37.5 to 100% and 45.25 to 95% for, respectively lavender and peppermint essential oils after 24 h only. Mortality percentages of 85 and 72.5%, respectively for *C. limonum* and *C. reticulata* were recorded after exposure time of 96 h at the highest dose. Repellency activity studied at 0.105, 0.315, 0.631 and 0.842 µl/cm^2 concentrations showed a higher repellency percentage of 71.25% for peppermint essential oil on *A. obtectus* adults. Our results suggest that Lamiaceae essential oils more than Rutaceae ones may be useful as a seed protecting tool with fumigation effects against *A. obtectus* and could replace synthetic insecticides which are harmful to the environment.**

Key words: *Acanthoscelides obtectus, Phaseolus vulgaris,* essential oils, repellency, fumigant toxicity.

INTRODUCTION

The bean beetle *Acanthoscelides obtectus* Say (Coleoptera, Chrysomelidae, Bruchinae) is a cosmopolitan and polyvoltin insect which attacks its host plant *Phaseolus vulgaris* L. and other Fabaceae such as those in the genus *Vigna* (Leroi and Jarry, 1981; Dobie et al., 1984). As in several other bruchids, *A. obtectus* can complete its development both in maturing seeds and in stored ones (Thiery et al., 1994). Its life cycle on stored beans can occur without returning to the field (Labeyrie, 1962) causing crop losses of about 80% after six to

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seven months of storage (Idi, 1994). Control of this stored product pest is of great importance especially in developing countries where Leguminosae seeds represent a fundamental food resource (Abate and Ampofo, 1996). The most frequently used method and economical tool for managing stored grain insect pests is a chemical one often through fumigation (Mueller, 1990). Since this method pose human health risks, there is actually claim to natural products (Isman, 2000; Huignard et al., 2011). Essential oils are produced in 17500 aromatic species of higher plants belonging mostly to a few families, including the Myrtaceae, Lauraceae, Lamiaceae and Asteraceae which hide a real insecticide arsenal capable of crop protection (Regnault-Roger et al., 2012).

Essential oils of several aromatic plants have been studied in Algeria, demonstrating their efficiency upon stored product pests *Callosobruchus maculatus* (F.) (Kellouche and Soltani, 2004; Kellouche, 2005), *Bruchus rufimanus* (Boh.) (Medjdoub-Bensaad et al., 2007) and *A. obtectus* (Bouchikhi et al., 2008, 2010).

The present study aims to evaluate fumigant and repellent insecticidal activity of essential oils of lemon (*Citrus reticulata* L.), tangerine (*Citrus limonum* L.) (Rutaceae); peppermint (*Mentha piperita* L.) and lavender (*Lavandula angustifolia* L.) (Lamiaceae) as control alternatives against a stored product beetle *A. obtectus.*

MATERIALS AND METHODS

Insects

Acanthoscelides obtectus strain came from a sample collected in Tlemcen (34° 52' 41"N 1° 18' 53"W) (Algeria) and was reared in a dark incubator under controlled conditions (30 ± 1°C and 70 ± 5% relative humidity) on *P. vulgaris* dry beans (Rognon blanc cultivar). Insects were maintained into glass flasks (1 L capacity) closed at the top with a fine mesh nylon screen to allow aeration. Every two months, unsexed adults, new emerged, were transferred on fresh beans for rearing. Adult insects, of 0 to 24 h old, were removed daily from the livestock insect and used in each bioassay.

Essential oils

The plants from which essential oils were extracted were lemon (*C. reticulata* L.), tangerine (*C. limonum* L.), peppermint (*M. piperita* L.) and lavender (*L. angustifolia* L.) collected from Tizi Ouzou (36° 42' 42"N 4° 02' 45''E) (Algeria). Essential oils were obtained by hydrodistillation of fresh peel for Rutaceae and from peppermint leaves and lavender flowered tops for Lamiaceae.

Fumigation bioassay

Fumigation bioassays were carried out with 10 adults (5 males and 5 females of 0 to 24 h old) exposed in Plexiglas bottles (750 ml capacity). Filter papers (Whatman number 1) were attached to inner side of the bottle screw cap and impregnated with different oil doses: 10, 30, 60 and 80 µL, giving calculated fumigant concentrations corresponding, respectively to 13.33, 40, 80 and 106.66 µl/L air. Four replications were made for each concentration and control. Mortality was recorded after 24, 48, 72 and 96 h until total death of individuals.

Repellency bioassay

Repellent effects of essential oils on *A. obtectus* adults were assessed using preferential zone method on filter paper described by Mc Donalds et al. (1970). Whatman filter papers (diameter 11 cm) were cut in equal halves. Then 10, 30, 60 and 80 µL corresponding to 0.105, 0.315, 0.631 and 0.842 µl/cm^2 of essential oils were dissolved in 0.5 ml acetone and applied uniformly to a half filter disc; while the other half received 0.5 ml acetone alone as control. After complete evaporation of a solvent (15 mn), discs were reconstituted then placed in Petri dishes (diameter 11 cm). Ten adults beetles (5 males and 5 females of 0 to 24 h old) were introduced in the filter paper center of each Petri dish sealed immediately using four replications for each treatment. After 30 min of treatment at 25°C, number of insects present on the treated (Nt) and non treated (Nc) halves was recorded. Repellency percentage (RP) was calculated as follows: RP (%) = [(Nc-Nt) / (Nc+Nt)] ×100. Mean repellency percentages were calculated and compared with Mc Donald et al. (1970) classification which proposed five classes: class 0 (RP<0.1%), class I (0.1% ≥RP≤20%), class II (20.1%≥RP≤40%), class III (40.1%≥RP≤60%), class IV (60.1% ≥RP≤80%) and class V (RP≥80.1%).

Data analysis

The data obtained were submitted to one way analysis of variance using Statbox version 6.3 (Grimmersoft, 2004). Newman and Keuls test was performed to compare means ($P < 0.05$) (Dagnelie, 1975).

RESULTS

Fumigant toxicity

Fumigant activity of essential oils is shown in Table 1. It was highly dependent upon oil concentration and exposure time. Essential oils from Lamiaceae compared to Rutaceae showed the highest fumigant effect against *A. obtectus.* At the lowest concentration, 13.33 µl/L air, lavender and peppermint essential oils showed comparable percentage mortality of 37.5 ± 28.7% and 45 ± 25.16 after 24 h, 90 ± 14.14% and 95 ± 5.77% after 48 h and 100% after 72 h. The maximum activity (100% mortality) was recorded after only 24 h of time exposure to a highest concentration of 106.66 µl/L air. Rutaceae essential oils vapours were less toxic to *A. obtectus*. Percentage mortalities of 85 ± 5.77% and 72.5 ± 5% were observed, respectively for lemon and tangerine essential oils after time exposure of 96 h while only 17.5 ± 9.57% and 12.5 ± 5% of beetles died at the lowest concentration (13.33 µl/L air).

Repellent activity

Repellent activity percentages evaluated for the 4 essential oils are given in Table 2. One way variance analysis showed highly significant differences for all

Table 1. Mortality percentage (Mean±SD) of *Acanthoscelides obtectus* adults exposed to *C. limonum*, *C. reticulata, L. angustifolia* and *M. piperita* essential oils vapours.

Essential oils	Doses µl/L air	24 h	48 h	72 h	96 h
C. limonum	0	0±0^{c}	0±0^{d}	0±0^{d}	0±0^{d}
	13.33	10±0^{b}	10±0^{c}	10±0^{c}	17.5±9.57^{c}
	40	7.5±5^{b}	15±5.77^{bc}	17.5±9.5^{bc}	17.5±9.5^{c}
	80	12.5±5^{b}	20±8.16^{b}	25±5.77^{b}	35±5.77^{b}
	106.66	70±0^{a}	72.5±5^{a}	85±5.77^{a}	85±5.77^{a}
C. reticulata	0	0±0^{b}	0±0^{d}	0±0^{d}	0±0^{d}
	13.33	7.5±5^{b}	12.5±5^{c}	12.5±5^{c}	12.5±5^{c}
	40	10±0^{b}	17.5±9.5^{c}	22.5±5^{b}	27.5±5^{b}
	80	10±8.16^{b}	27.5±5^{b}	30±8.16^{b}	32.5±9.57^{b}
	106.66	35±5.77^{a}	45±5.7^{a}	57.5±5^{a}	72.5±5^{a}
L. angustifolia	0	0±0^{c}	0±0^{b}	0±0^{b}	0±0^{b}
	13.33	37.5±28.7^{b}	90±14.14^{a}	100±0^{a}	100±0^{a}
	40	80±24.49^{a}	95±5.77^{a}	100±0^{a}	100±0^{a}
	80	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
	106.66	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}
M. piperita	0	0±0^{c}	0±0^{b}	0±0^{b}	0±0^{b}
	13.33	45±25.16^{b}	95±5.77^{a}	100±0^{a}	100± 0^{a}
	40	90±8.16^{a}	100±0^{a}	100±0^{a}	100±0^{a}
	80	92.5±9.57^{a}	100±0^{a}	100±0^{a}	100±0^{a}
	106.66	95±5.77^{a}	100±0^{a}	100±0^{a}	100±0^{a}

All experiments were replicated four times. P-value: significant differences determined by a one way ANOVA. Numbers followed by the same letter in each table row are not significantly different at 0.05 level (Newman and Keuls test).

Table 2. Repulsion means percentages of *M. piperita, L. angustifolia, C. reticulata* and *C. limonum* essential oils against *Acanthoscelides obtectus* adults classified according to Mc Donalds et al. (1970).

Doses µl/cm^2 essential oils	0.105	0.315	0.631	0.842	Repulsion Mean %	Class
M. piperita	50±16.32	70±19.14	80±18.3	85±16.34	71.25	Repulsive (IV)
L. angustifolia	30±11.5^{c}	60±16.3^{b}	75±10^{ab}	90±11.5^{a}	63.75	Repulsive (IV)
C. reticulata	40±8.16^{c}	35±5.77^{c}	80±8.1^{b}	100±00^{a}	63.75	Repulsive (IV)
C. limonum	10±4.08^{d}	45±5.77^{c}	55±5.7^{b}	65±5.77^{a}	43.75	Moderately repulsive (III)

All experiments were replicated four times. Significant differences determined by a one way ANOVA. Numbers followed by the same letter in each table row are not significantly different at 0.05 level (Newman and Keuls test). Classes are given according to Mc Donalds et al. (1970).

essential oils except peppermint essential oil. The latter is the more strongly repulsive since at the lowest dose (0.105 µl/cm^2), it induced repulsion rate of 50 ± 16.32% showing consequently the highest mean repulsion rate of 71.25% calculated according to Mc Donalds et al. (1970). Repulsion activity evaluated after a time exposure of 30 min is doses dependent. Significant differences were observed between treatments as the doses increase with a maximum of repulsion recorded at the highest concentration (0.842 µl/cm^2); it was of about 85 ± 16.34, 90 ± 11.54, 100 ± 00 and 65 ± 5.77%, respectively for peppermint, lavender, tangerine and lemon essential oils.

DISCUSSION

The four essential oils screened for fumigant activity demonstrated toxicity against *A. obtectus* adults. The

insecticidal activity varied with oil concentrations and time exposure. Results showed higher efficiency of Lamiaceae essential oils compared to Rutaceae.

Fumigant toxicity of essential oils and their major compounds, volatile monoterpenoids was largely described especially for Mediterranean species (Regnault-Roger et al., 1993; Regnault-Roger and Hamraoui, 1995; Regnault-Roger and Hamraoui, 1994). According to Regnault-Roger and Hamraoui (1993), essential oils extracted by hydrodistillation from Lamiaceae, Myrtaceae, Lauraceae and Gramineae present fumigant toxicity against *A. obtectus* adults especially, *Thymus serpyllum*, *Thymus vulgaris* and *Lavandula angustifolia* essential oils which induce a mortality of 95 to 100% after 24 and 48 h when exposed, respectively to 160, 136.1 and 145 mg/dm^3.

Essential oils from Rutaceae seem to exert a low activity against *A. obtectus* adults. Our results corroborate with those obtained by Papachristos and Stamopoulos (2002) who showed that essential oils of *Citrus sinensis* have a low action on *A. obtectus* with LC_{50} of 11.4 µl/L air for males and 19.54 µl/L air for females while *Lavandula hybrida* LC_{50} was 1.64 µl/L air for males and 2.34 µl/L air for females in comparison to Lamiaceae (basil, peppermint and lavender) and Myrtaceae (eucalyptus) essential oils. The same authors screened 13 essential oils for their fumigant activity against *A. obtectus* adults and reported that Lamiaceae essential oils were more strongly toxic to males than females and noted LC_{50} of about 1.1, 1.2, 1.6 and 2.1 µl/L air for *Mentha microphylla*, *Mentha viridis*, *L. hybrida* and *Rosmarinus officinalis* essential oils, respectively. Ayvaz et al. (2010) investigated insecticidal activity of oregano (*Origanum onites* L.), savory (*Satureja thymbra* L.) (Lamiaceae) and myrtle (*Myrtus communis* L.) (Myrtaceae) against three stored product insects. They reported that *A. obtectus* was the most tolerant to essential oils with the most toxic action attributed to *M. communis* (linalool) while oregano and savory essential oils were highly efficient against the Mediterranean flour moth (*Ephestia kuehniella* Zeller) and the Indian meal moth (*Plodia interpunctella* Hûbner) (Lepidoptera: Pyralidae) inducing 100% mortality after 24 h exposure at 9 and 25 µl/L air for *P. interpunctella* and *E. kuehniella,* respectively. Raja et al. (2001) reported that oils derived from *Mentha arvensis*, *M. piperita*, *M. spicata* and *Cymbopogon nardus* influenced significantly adult mortality, oviposition and adult emergence of cowpea beetle (*Callosobruchus maculatus* (F.)).

Similar effects of volatile components of essential oils of laurel, rosemary and lavender were described by Shaaya et al. (1997) for *A. obtectus* and for other stored products pests like *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* (F.) and *Rhyzoperta dominica* (L.). These authors recorded a mortality of 85 to 100% after 4 days exposure to a dose of 70 µl/L air. However, LC_{50} values were higher than those recorded on *A. obtectus* adults. Rossi et al. (2012) studied insecticidal properties of *Citrus bergamia* L. essential oil vapours on *Sitophilus oryzae* adults and showed significant mortality after 96 h at 10 µl cm^{-2} dose; they also observed that a dose of 0.75 µl insect^{-1} induced a relatively high mortality of about 65 and 83% after 24 and 96 h, respectively showing LD_{50} of 0.36 µL insect^{-1} after 96 h.

In addition to the fumigant toxicity, a studied essential oils exhibited repulsion activity against *A. obtectus* adults. Peppermint, lavender and tangerine essential oils were classed in the repulsive class according to Mc Donalds et al. (1970) and the Lamiaceae essential oils again were highly effective against *A. obtectus* adults. Essential oils showed significant variations in their repulsion activity depending upon several factors especially their chemical composition. Although the chemical analysis was not performed for the studied essential oils, the superiority of the insecticidal potential of peppermint and lavender can be attributed to their major components, menthol and menthone (Kumar et al., 2011) for the first and linalool and linalyle acetate (Rossi et al., 2012) for the second. Papachristos and Stamopoulos (2002) reported that *M. viridis*, *E. globulus*, *M. microphylla*, *R. officinalis* and *L. hybrida* essential oils showed high repulsive effects compared to *Thuja orientalis*, *Citrus sinensis* and *Pistacia terebinthus* essential oils concluding, as in our study, that the most toxic essential oils exhibited in the same time high repulsive effects and inhibited beetle reproduction. Aggarwal et al. (2001) evaluated L-menthol and seven of its acyl derivates for repellent activity against *C. maculatus*, *T. castaneum*, *S. oryzae* and *R. dominica* and found the repellent activity of 100, 82, 78 and 72%, respectively at the concentration of 20 µg in 1 ml acetone.

Conclusion

The results of fumigant and repellent activity of peppermint, lavender, tangerine and lemon essential oils against *A. obtectus*, a serious pest in the stocks, permitted to propose natural products of plants from Mediterranean area as alternative to chemical control. As it was studied against several stored grain pests and vectors for its fumigant and repellent activity (Kumar et al., 2011), *Mentha piperita* essential oil seem to be the most strongly effective. More studies should be undertaken using other doses and other indigenous plants so that a range of organic insecticides could be produced in order to preserve nutritional quality of kidney beans in the stocks, especially in developing countries where this commodity constitute a fundamental food resource.

Conflict of interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Wood ash on the fertilization of marandu grass in Brazilian cerrado soils

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Wood ash can be an alternative fertilizer and soil corrective measure, directly contributing to the production of pastures. The objective of this research was to evaluate the growth and production of marandu grass fertilized with wood ash and grown in two soil classes of the Brazilian Cerrado. The experiment was conducted in a greenhouse during the period from December, 2012 to May, 2013. The experimental design was completely randomized in a factorial 2 × 6, corresponding to two soil classes (Ultisol and Oxisol) and six doses of wood ash (0, 3, 6, 9, 12 and 15 g dm^{-3}), with six replications. Pots with soil volume of 5 dm^3 were used with five plants. Three cuts were performed in the shoot of plants in a 30-day interval and at each cut was the plant height, dry mass of leaves and stems and the leaf/stem ratio evaluated. The results were submitted to analysis of variance and, when significant, the qualitative factors (soil classes) were submitted to the Tukey test and the quantitative factors (wood ash doses) subjected to regression analysis, both at 5% probability. The wood ash as fertilizer provides greater growth and dry matter production of marandu grass grown in Ultisol and Oxisol. The use of wood ash as fertilizer may be an alternative for the destination of this waste, and it can provide a reduction of mineral fertilizer and consequently of pasture production costs in the Brazilian Cerrado.

Key words: Solid waste, alternative fertilizer, *Brachiaria brizantha*, ultisol, oxisol.

INTRODUCTION

Wood ash is a residue from the burning of wood in a boiler for energy production and, depending on its origin, it can show high levels of potassium, phosphorus, calcium and magnesium, and it can also be used as fertilizer and corrective, depending on the soil fertility and the culture requirements (Voundinkana et al., 1998). In addition to the macronutrients, it may also contain essential micronutrients for plant growth, such as copper, zinc, iron and boron (Darolt and Osaki, 1991).

The large-scale generation of such solid waste by the industrial sector and the destination of this byproduct become a problem, being often disposed untreated on the environment. An alternative would be to use wood ash as a supplement in fertilizing cover crops and green manures in soils with high acidity, restoring part of the nutrients removed in the harvests, reducing the use of

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Table 1. Chemical and granulometric analyses of Oxisol (0-20 cm layer) in area under Cerrado vegetation, Rondonópolis-MT, 2012.

pH	P	K	Ca	Mg	Al	H	CTC	OM	V	m	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}		.....$cmol_c$ dm^{-3}.....					g kg^{-1}	.....%.....		.....g kg^{-1}.....		
4.1	1.1	47	0.2	0.1	1.0	4.7	6.1	19.7	6.9	70.4	575	50	375

P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Al = Aluminium; H = Hydrogen; CTC = Cation exchange capacity; OM = Organic matter; V = Base saturation; m = Aluminum saturation

Table 2. Chemical and granulometric analyses of Ultisol (0-20 cm layer) in area under Cerrado vegetation, Rondonópolis-MT, 2012.

pH	P	K	Ca	Mg	Al	H	CTC	OM	V	m	Sand	Silt	Clay
$CaCl_2$	mg dm^{-3}		.....$cmol_c$ dm^{-3}.....					g kg^{-1}	.....%.....		.....g kg^{-1}.....		
4.9	4.8	25	1.0	0.6	0.1	1.5	3.3	6.2	50.9	5.7	830	50	120

P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; Al = Aluminium; H = Hydrogen; CTC = Cation exchange capacity; OM = Organic matter; V = Base saturation; m = Aluminum saturation

Table 3. Chemical characterization of the wood ash.

pH	P_2O_5	K_2O	Zn	Cu	MnCNA+Water	BWater	Ca	S
	----------g kg^{-1}----------							
7.85	14.2	3.2	0.0	0.1	0.0	0.0	9.0	16

P_2O_5 = Phosphorus; K_2O = Potassium; Zn = Zinc; Cu = Copper; Mn NAC+Water = Manganese in neutral ammonium citrate and water; Ca = Calcium; S = Sulphur

correctives and commercial fertilizers, and consequently the production costs. Studies prove positive effect of wood ash regarding the structural and productive characteristics of marandu grass (Bonfim-Silva et al., 2013; Santos et al., 2014; Bonfim-Silva et al., 2014). There is still no information of the influence of this waste in the production of forage grasses in different soil classes.

The Cerrado soils have low fertility because they contain low levels of available nutrients, depending on the source material. Wood ash application may be a low-cost alternative to re-establish fertility. Despite the tolerance of pastures to acid and low fertility soils, the absence of correction and fertilization of the soil and the intensive use can lead to their degradation, such management practices are needed because the soil fertility severely limits the ability of crops to achieve maximum productivity (Bezerra et al., 2014).

In this context, the experiment aimed to evaluate the growth and production of marandu grass fertilized with wood ash and grown in two soil classes of the Brazilian Cerrado.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Mato Grosso, Rondonópolis Campus, during the period from December, 2012 to May, 2013. The forage grass used was *Brachiaria brizantha* cv. Marandu, grown in pots with soil volume of 5 dm^3, representing the experimental units. The experimental design was completely randomized in a factorial 2 × 6, corresponding to two soil classes (Oxisol and Ultisol) and six wood ash doses (0, 3, 6, 9, 12 and 15 g dm^{-3}), with six replications. Soil samples were collected in the 0 to 20 cm layer, in area under Cerrado vegetation, both in the region of Rondonópolis-MT. They were passed through a sieve of 2 mm aperture, homogenized, and then chemical and granulometric characterization was done (Tables 1 and 2), according to the methodology proposed by EMBRAPA (1997). The soil for the composition of the pots was sieved on a 4 mm aperture mesh. The wood ash was derived from the ceramic sector activity, analyzed as fertilizer (Table 3), according to Darolt et al. (1993), and showed pH of 7.85. The soil correction was not performed by liming because the wood ash raises the soil pH. The soil was incubated with wood ash for 30 days.

Wood ash does not have nitrogen in its composition because in the wood burning process occurs the loss of this nutrient by volatilization (Obernberger et al., 2006). Therefore, the nitrogen fertilizer was performed for crop establishment and after each cut, at a dose of 200 mg dm^{-3}, using urea as source. The maximum capacity of the soil water retention was determined in the laboratory using the gravimetric method (Bonfim-Silva et al., 2011). Soil moisture was maintained at 80% of the maximum soil water retention through the daily weighing of all experimental plots. The interval between the marandu grass cuts was of 30 days. At each cut, the evaluation of plant height, dry mass of leaves, dry mass of stems, and the leaf/stem ratio was carried out. The first two cuts of the forage were performed at 5 cm from the soil and the third cut near the soil (Bonfim-Silva et al., 2007).

The determination of the height of the plants was obtained with a

graduated scale, from the soil to the curvature of the forage canopy. The plant material was collected at each cut, dried in an oven with forced air at 65°C for 72 h until a constant mass (Silva and Queiroz, 2002) and after drying, weighed in semi-analytical scale for determining the dry mass. The leaf/stem ratio represents the ratio between the dry mass of leaves and the dry mass of stems in each plot. The results were submitted to analysis of variance by F test and, when significant, the qualitative factors (soil classes) were submitted to Tukey test and the quantitative factors (wood ash doses) submitted to regression analysis, both at 5% probability, through the statistical program SISVAR of the Federal University of Lavras (Ferreira, 2008).

RESULTS AND DISCUSSION

In the first evaluation, the variable plant height was adjusted to the quadratic regression model when grown in Oxisol, with the largest height of marandu grass being 47.93 cm in the wood ash dose of 9.12 g dm^{-3}. For the Ultisol, the height of plants was adjusted to the linear regression model with an increase of 44.96% comparing the highest dose of the experimental interval (15 g dm^{-3}) with the absence of fertilization with wood ash (Figure 1A). The height of marandu grass plants, in the second evaluation, showed no significant difference between the doses of wood ash and in the last cut of the forage grass cultivated in Ultisol, the plant height was adjusted to the linear regression model, with a decrease of 18.31% relating the absence of the application with the dose of 15 g dm^{-3} wood ash (Figure 1B).

In the first cut, the height of the marandu grass grown in both soils from the dose of 9 g dm^{-3} exceeded 40 cm. In a study aimed at reconciling greater leaf production and lower production of stems with dead material of *B. brizantha* cv. Marandu irrigated in the field; the greatest heights ranged from 35 to 40 cm, thus justifying the fertilization with wood ash to obtain significant growth of the forage (Rodrigues, 2004). On the contrary, in the third cut, the forage grass did not reach this minimum margin of plant height, since there was no reapplication of the residue from the second cut and the nutrients began to run out.

The Ultisol, even in the absence of fertilization with wood ash, provided greater plant height, standing out about the Oxisol, in the first and third cut. This may be due to higher natural fertility through the nutrient concentration in Ultisol, especially phosphorus, an important nutrient for plant growth. Nonetheless, in the second cut, there was no significant difference between the two soils, indicating that the Oxisol fertilized with wood ash raises the height of marandu grass, equating up to the Ultisol. Gonçalves and Moro (1995) observed that the use of forest biomass ash, under laboratory conditions, substantially increased soil fertility, responsible for the rise in productivity of *Eucalyptus grandis*. In the third evaluation, in particular, there was a reduction in plant height with the increase of wood ash doses, in virtue of the ash solubilization in the soil and the availability of nutrients (Demeyer et al., 2001) for the marandu grass in the first cut, and as the nutrients were extracted at the time of the cuts, they were restricted to plants, since there was no re-application of the wood ash.

According to Garay et al. (1997), plant height is an important structural feature for the adoption of appropriate management, indicating the time of entry of the animals in the pasture. The consumption increases with the elevation of the pasture height to a level in which it remains constant, depending on the species and animal category (Hodgson, 1990).

In the first evaluation of the forage grass, the dry mass of leaves was adjusted to the linear regression model, where the highest wood ash dose increased by 78.41 and 66.25% in the dry matter production of marandu grass leaves grown in Ultisol and Oxisol, respectively, when compared to the treatment without fertilization (Figure 2A). In the second evaluation of the plants, the dry mass of leaves of the marandu grass grown in Oxisol was adjusted to the linear regression model increasing by 17.49% in the production when comparing the dose of 15 g dm^{-3} with the absence of application of wood ash. The marandu grass grown in Ultisol showed dry mass of leaves with adjustment to the quadratic regression model, in which the least amount of leaves was 4.4 g pot^{-1} using 7.09 g dm^{-3} of wood ash (Figure 2B).

In the third cut, the dry mass of leaves was adjusted to the quadratic regression model in which the maximum production (2.63 g pot^{-1}) was observed when the marandu grass was grown in Oxisol using the wood ash dose of 10.87 g dm^{-3} (Figure 2C). It was found, as in this study, the positive effect of wood ash doses on *Brachiaria brizantha* grown in Oxisol, in the first two cuts, with higher results for the dry mass of leaves with increasing doses of this waste (Santos, 2012). In this way, it is emphasized the potential of using fertilization with wood ash in forage, since the dry mass of leaves represents the portion of highest nutritional value, once the leaves are more digestible than the stems and the senescent material, and also have higher crude protein levels (Paciullo, 2002), being therefore preferred by the animal.

In the third cut, the significant difference in dry mass of leaves of marandu grass only when grown in Oxisol indicates that despite being a soil with low natural fertility, the application of the residue can exceed the production potential of this soil about that of greater natural fertility. The agricultural production in low fertility soils requires nutritional supplementation, which can be made with organic fertilizers, such as wood ash (Bonfim-Silva et al., 2014). The dry matter yield of stems of marandu grass in the first cut was adjusted to the linear regression model with an increase of 87.9% for Ultisol and of 77.24% for Oxisol, from the higher dose of wood ash when compared to the absence of the same (Figure 3A).

In the second cut of the forage grass, there was a 33.17% increase in dry matter yield of stems of marandu grass in Oxisol comparing the higher wood ash dose with

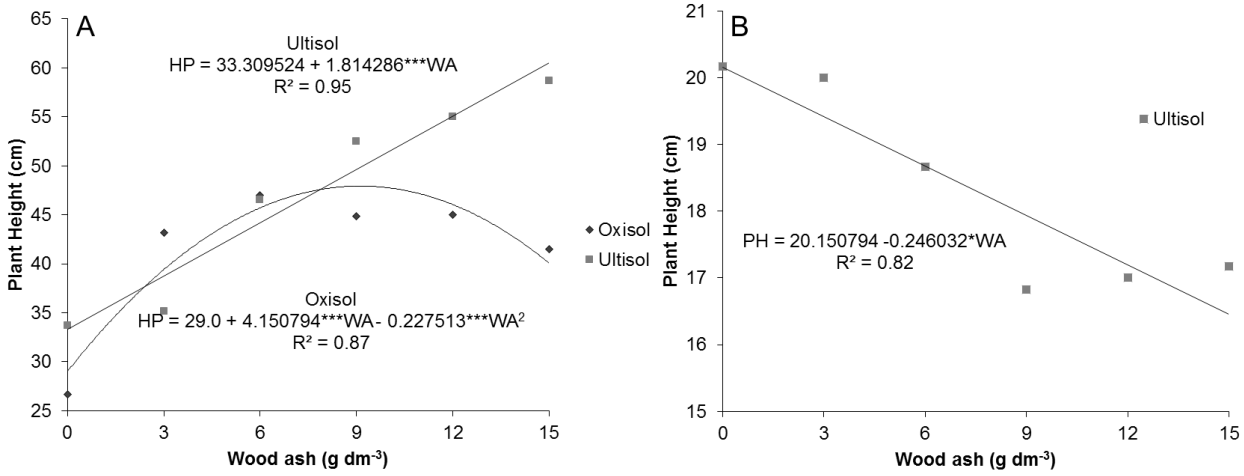

Figure 1. Height of marandu grass plants subjected to wood ash doses in Oxisol and Ultisol in the first cut (A) and in Ultisol in the third cut (B). PH = plant height. WA = wood ash. * and *** significant at 5 and 0.1% probability, respectively.

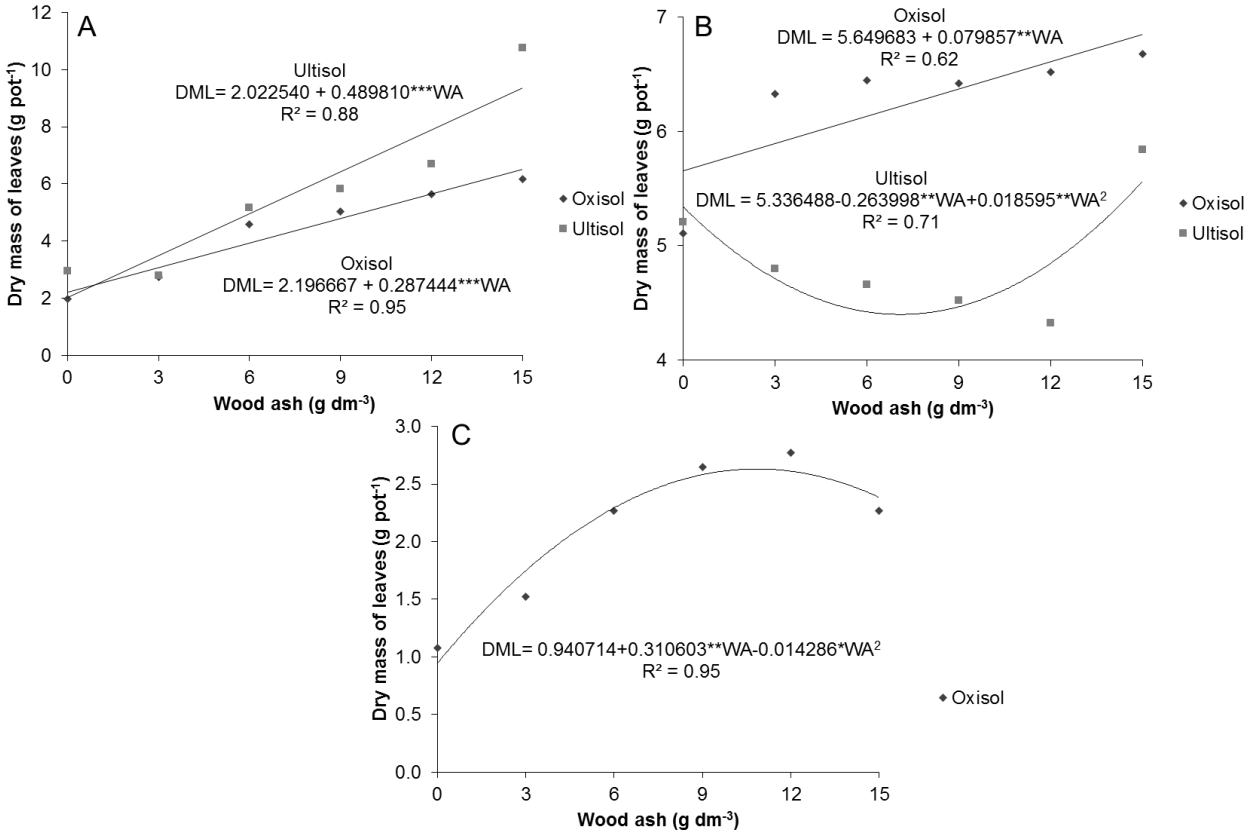

Figure 2. Dry mass of leaves of marandu grass subjected to wood ash doses in and Ultisol in the first (A) and second cuts (B) and in Oxisol in the third cut (C). DML = dry mass of leaves. WA = wood ash. *, ** and *** significant at 5, 1 and 0.1% probability, respectively.

the absence of application of this waste, while the lowest dry matter of stems (1.37 g pot^{-1}) was found at a dose of 6.96 g dm^{-3}, applied in Ultisol (Figure 3B). The dry mass of stems in the last cut was adjusted to the quadratic

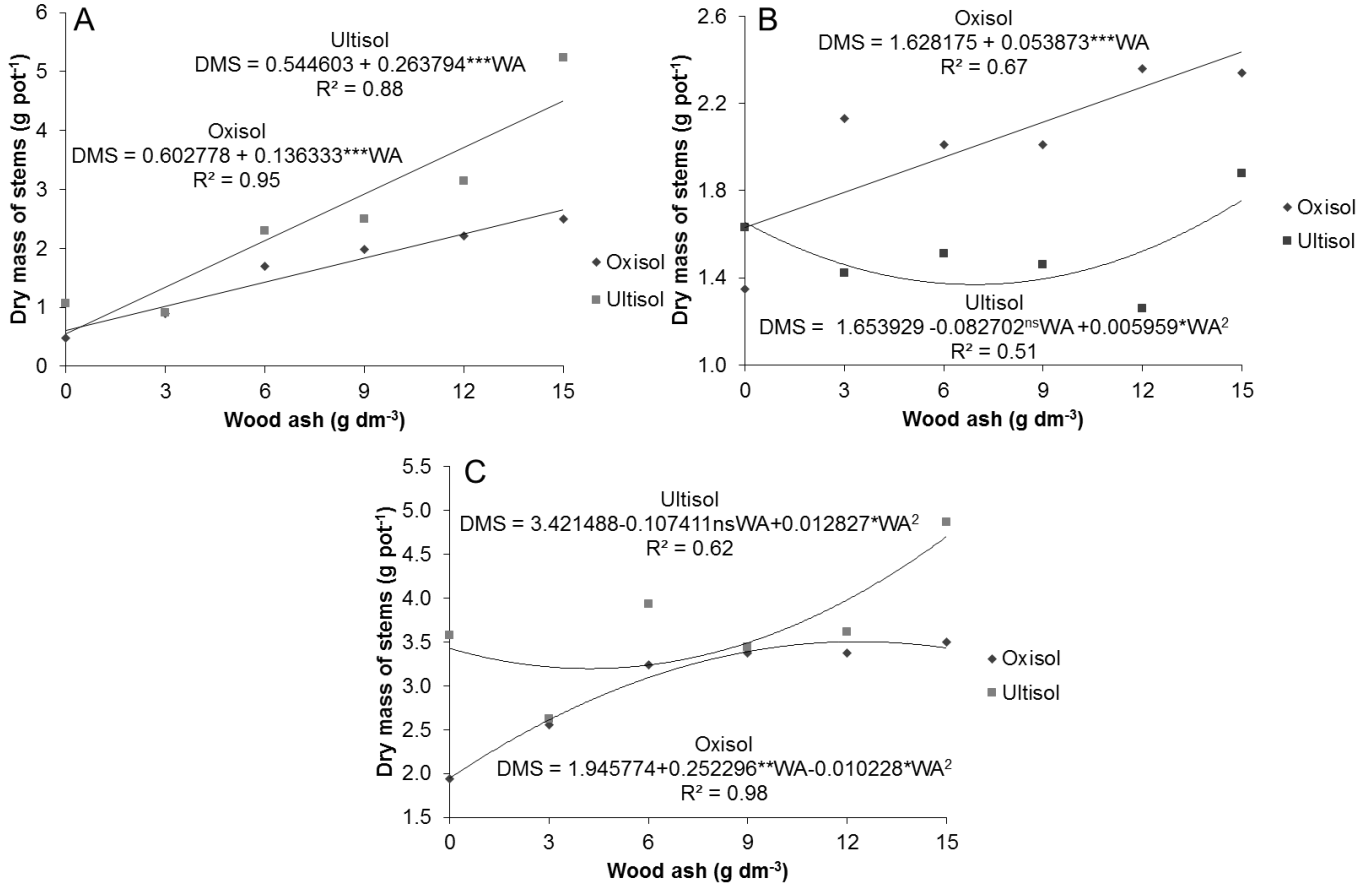

Figure 3. Dry mass of stems of marandu grass subjected to wood ash doses in Oxisol and Ultisol in the first (A), second (B) and third cuts (C). DMS = dry mass of stems. WA = wood ash. ^{ns}, * and *** no significant and significant at 5 and 0.1% probability, respectively.

regression model in which the maximum production was equal to 3.51 g pot^{-1}, observed at a dose of 12.38 g dm^{-3} when the marandu grass was grown in Oxisol. For the Ultisol, the adjustment of the results of this variable was given by the quadratic regression model with minimum point (3.20 g pot^{-1}) in the wood ash dose of 4.22 g dm^{-3} (Figure 3C).

As in the present study, the dry mass of stems of marandu grass in the first cut, in a study conducted by Bonfim-Silva et al. (2013), showed linear effect with increased doses of wood ash. There was a greater increase in dry mass of stems about the dry mass of leaves (Figure 2A) in both soils since forage grasses have a critical time when there is the greater targeting of their photoassimilates to the production of stems (Bianco et al., 2005). From the second cut, the rate of leaf growth slowed the success of the development of stems. This is a strategy of the plant to accumulate mass, since the excess of leaves causes self-shading, reducing the overall plant photosynthesis (Negreiros Neto, 2007). In the first cut of the marandu grass, the leaf/stem ratio were adjusted to the quadratic regression model, and the lowest score, of 2.32, was obtained in the wood ash dose of 10.79 g dm^{-3}, applied in Oxisol. As for the Ultisol, there was a decrease of 41.58% in the leaf/stem ratio when comparing the absence of ash application with the maximum dose of the experiment by 15 g dm^{-3} (Figure 4A).

The wood ash doses decreased the leaf/stem ratio by 21.68% in the second evaluation of the marandu grass when grown in Oxisol, compared with the absence with the maximum dose of this waste (Figure 4B). In the third cut of the marandu grass, there was no significant effect of wood ash doses on both soil classes. The reduction in the leaf/stem ratio of the marandu grass was due to the increase in the production of stems from the application of wood ash doses. As the nutrients have been extracted at the time of the cuts, they have become insufficient because there was no re-application of wood ash, which can interfere with the grass structure, compromising the grazing efficiency due to the decrease in the leaf/stem ratio (Rodrigues et al., 2008). The variable leaves/stems, according to Euclides et al. (2000), is directly related to the performance of grazing animals, important from the point of view of the nutritional value and of the management of forage species (Alden and Whitaker,

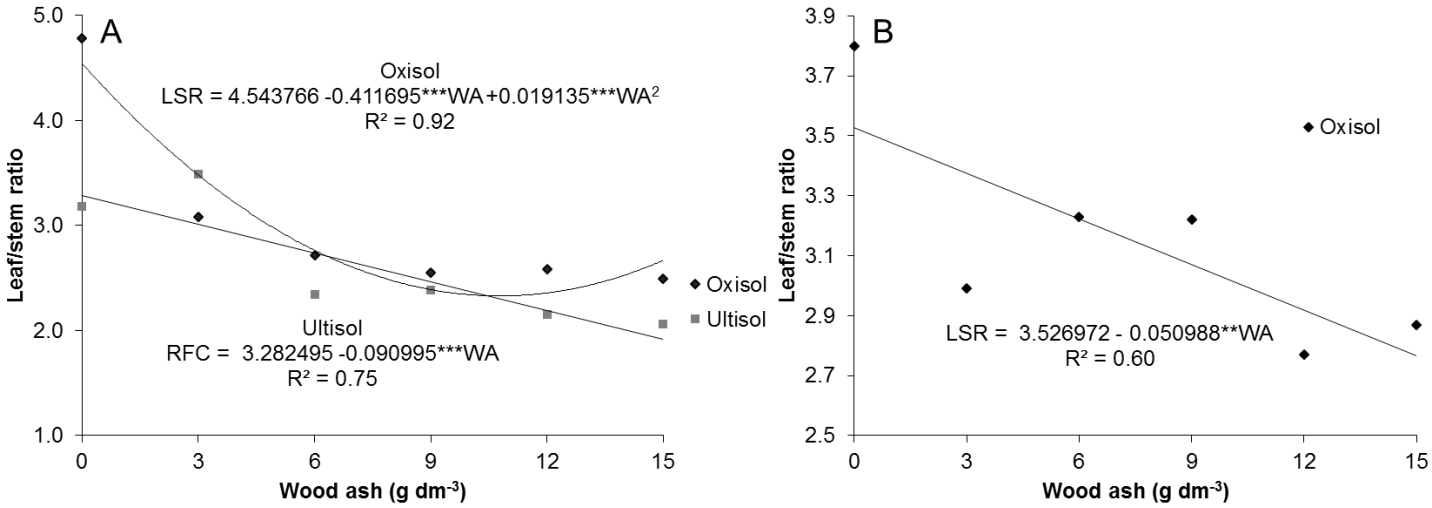

Figure 4. Leaf/stem ratio of marandu grass subjected to wood ash doses in Oxisol and Ultisol in the first cut (A) and in Oxisol in the second cut (B). LSR = leaf/stem ratio. WA = wood ash. ** and *** significant at 1 and 0.1% probability, respectively.

1970; Pinto et al., 1994).

Despite this decrease in the leaf/stem ratio, the results of this study are above the ideal critical threshold, which is equal to 1.00. This critical level regards the quantity and the quality of the produced forage (Pinto et al., 1994; Andrade, 1997). The marandu grass, in general, had higher growth and yield when grown in Ultisol fertilized with the wood ash dose of 15 g dm^{-3}. The maximum yields of marandu grass grown in Oxisol were obtained in the wood ash doses range of 9 to 15 g dm^{-3}.

Conclusion

The wood ash as fertilizer provides greater growth and dry matter production of marandu grass grown in Oxisol and Ultisol. The use of wood ash as fertilizer may be an alternative for the destination of this waste, and it can provide a reduction of mineral fertilizer and consequently of pasture production costs in the Brazilian Cerrado.

Conflict of Interest

The authors have not declared any conflict of interest.

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Bioactive compounds and antioxidant activity of *Tacinga inamoena* (K. Schum.) [NP Taylor & Stuppy] fruit during maturation

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***Tacinga inamoena* (K. Schum.) [N. P. Taylor & Stuppy] is a native Cactaceae from the semiarid region of Northeastern Brazil whose fruit is eaten by the local population. It seems that there is a significant amount of functional compounds such as polyphenols and betalains in this fruit as reported for other well-known cacti. Although there are not enough studies that have been conducted, it has attracted interest regarding nutritional and functional viewpoints. In this sense, changes in bioactive compounds during maturation need to be evaluated as this fruit faces very unstable conditions during its development, which can lead to drastic changes of the constituents in the pulp and peel. Thus, the objective of this study was to evaluate the content of bioactive compounds and antioxidant activity during the maturation of *Tacinga inamoena* fruit from fruit bearing plants grown under Brazilian semiarid conditions. The contents of total chlorophyll declined parallel to an increase of the total carotenoids, yellow flavonoids, and betalains. However, a sharp difference between the content of these pigments in the peel and pulp was observed which characterized the main changes during fruit maturation. This fruit presented considerable carotenoid content, reaching 348 µg/100 g in the peel and 29 µg/100 g in the pulp when fully ripe. Total antioxidant activity (TAA) was higher in the pulp of more mature fruit. TAA was correlated with the bioactive compounds, with the exception of betacyanins, which were betalains present in smaller amounts in this fruit.**

Key words: Cactaceae, quipá, carotenoids, polyphenols, betalains, antioxidant activity.

INTRODUCTION

The Brazilian semiarid region has several species of native fruit with great potential for exploitation, not only due to their peculiar aroma and flavor, but mainly for the presence of compounds with functional appeal, and which are still

unexplored (Silva et al., 2009; Dantas et al., 2013). *Tacinga inamoena*, known as quipá, cumbeba, or gogóia, is a native Cactaceae in Northeastern Brazil that is found throughout almost all of the semiarid region (Lima, 1989), and is one species whose fruit composition needs to be further studied, although it has been recognized as a potential source of functional compounds (Silva et al., 2009).

The differential in quality of cactus fruit is due to the profile of antioxidant compounds, especially those from the *Opuntia* genus, which present betalain pigments and phenolic compounds such as flavonoids and phenolic acids (Stintzing et al., 2005; Kim et al., 2011; Dhaouadi et al., 2013). How these compounds act in reducing oxidative stress may vary according to their physicochemical properties, medium conditions, and inflammatory biomarkers such as COX-2 (cyclooxygenase-2) and iNOS (inducible Nitric Oxide Synthase), whose regulation is dependent upon the extract dose and action time (Tesoriere et al., 2005; Tenore et al., 2012; Kim et al., 2013; Allegra et al., 2014).

Among cactus compounds, betalains stand out as one of the main constituents. Betacyanins are the class that give color ranging from red to pinkish, and betaxanthins correspond to color ranging from yellow to orange (Gandía-Herrero and García-Carmona, 2013). *T. inamoena* fruit presents a significant amount of the betaxanthin group (Dantas et al., 2015). However, due to their simultaneous occurrence in most fruits, their combination results in the development of a very peculiar color, which usually becomes evident during the maturation of the fruit (Castellar et al., 2012). Because of these events, fruits generally increase their antioxidant status by the preponderant accumulation of one of these classes. *Opuntia* fruit with yellow-orange pulp or peel exhibit high amounts of indicaxanthin (Stintzing and Carle, 2007). When isolated, betaxanthins have antioxidant and anti-inflammatory properties, which contribute to the development of products that can reduce the development of oxidative stress (Kim et al., 2013; Naselli et al., 2014), showing antigenotoxic and chemoprotective effects (Brahmi et al., 2011, 2012).

Fruit bearing plants of Brazilian biodiversity have been poorly studied, even though the research in the botany, ethnobotany, and floristic fields has brought valuable information that support further studies aiming to add value through sustainable use (Lucena et al., 2013; Oliveira et al., 2012). Furthermore, they have the potential to go beyond these aspects and bring insights toward health promotion, such as the profile and quantification of bioactive compounds to support further studies by performing *in vivo* evaluation about aspects such as functionality and bioavailability. The Opuntia genus in Brazil presents great exploitation potential, especially its fruits (Silva et al., 2009), which are internationally valued and recognized as important sources of pigments, vitamins, sugars, and gelling materials (pectins). The vast exploitation potential is, above all, due to their antioxidant properties (Cha et al., 2013; Dantas et al., 2015), which can be differentiated according to fruit portion (Osorio-Esquivel et al., 2011) and maturation (Cayupán et al., 2011; Castellar et al., 2012).

Although knowing that maturation is a complex process defined genetically and mainly controlled by hormones and environmental conditions, major compounds in the peel and pulp of cactus fruits drastically change during this process, modifying the antioxidant status in response to the increase or decrease of antioxidant compounds. For *Opuntia megacantha*, the increase of ascorbic acid, phenolic compounds, and betalains was highly correlated with the antioxidant properties (Cayupán et al., 2011). In the same way, *Opuntia stricta* fruit present a remarkable amount of betalains (mainly betacyanin), which are synthetized during fruit development and evolve until completely changing the whole color of the fruit when the highest antioxidant potential is reached (Castellar et al., 2012). Thus, for both cases the screening of non-traditional fruits must start with monitoring major compounds that are mostly responsible for contributing to health promotion.

Considering this, *T. inamoena* fruit represent an increased interest in the search to identify compounds with health promotion appeal, as well as for application in the composition of food products (Saénz et al., 2009; Fernández-López et al., 2010). Overall, these aspects are set as an important tool to add value that would increase and/or diversify the use of Cactaceae fruits in the semiarid region of Northeastern Brazil. In this sense, this study evaluates the content of bioactive compounds and antioxidant activity during the maturation of *Tacinga inamoena* fruit from fruit bearing plants grown under Brazilian semiarid conditions.

MATERIALS AND METHODS

Plant material

Fruit from *T. inamoena* plants were collected in two areas of natural occurrence located in the microregion of Curimataú, State of Paraíba, Brazil, where plants are irregularly distributed in communities. After collection early in the morning, fruit that presented several maturation patterns without any visual defect were submitted to classification based on the evolution of the peel color inherent to the fruit's development. Thus, maturation was classified into six stages: 1 - Entirely green coloration; 2 - Light green coloration; 3 - Predominantly green coloration with yellow nuances;

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4 - Yellowish coloration with pink nuances; 5 - Pink coloration with yellow nuances, and 6 - Pink coloration with orange nuances. Forty fruits were used per replicate (≈ 450 g) for each maturity stage. The fruit was processed, making the separation between peel (epicarp) and pulp (mesocarp + pulp with seeds), and seeds were manually separated. Samples were kept at -18 °C until time of assessment.

Bioactive compounds

The total chlorophyll content was spectrophotometrically determined at 652 nm according to Dantas et al. (2013), and the results were expressed in mg/100 g. For total carotenoids, readings were performed at 450 nm and the results were expressed in µg/100 g of fresh matter according to Higby (1962).

Betalain content was performed spectrophotometrically using Nilson's (1970) equations. Extracts obtained from fruit at each maturity stage were prepared using water as the extractor solution. After the samples were weighed and mixed in distilled water, they were centrifuged at 12,000 rpm for 25 min at 4 °C. The supernatants were stored and the residue was re-extracted twice more. The resulting supernatants were mixed, and the final volume was adjusted to 30 mL with distilled water, and then analyzed immediately afterwards. The absorbance of the extracts was measured at 476, 538 and 600 nm, and the betalain content (mg/100 g) was estimated by the following equations: $x = 1.095(A_{538} - A_{600})$ e $y = -0.258 \times A_{538} + A_{476} - 0.742 \times A_{600}$. Finally, the betacyanin content was obtained by BTC = ($x \times R \times 100$)/1120, and betaxanthin by BTX = ($y \times R \times 100$)/750, where R is the dilution factor, and $E_{1cm}^{1\%} = 1120$ and $E_{1cm}^{1\%} = 750$ the extinction coefficients for betanin and vulgaxantina-I, respectively.

The yellow flavonoid content was spectrophotometrically determined at 374 nm according to Dantas et al. (2013). About 1.0 g of fresh weight was added to 10.0 mL of extract solution composed of 95% ethanol and 1.5 N HCl in the ratio of 85:15 (v/v). The results were expressed as mg/100 g fresh weight.

Total extractable polyphenols and antioxidant activity was performed according to Silva et al. (2012). Samples were then mixed with 4 mL of 50% methanol, and the tubes were shaken for 1 minute, followed by 1-h rest. Afterwards, the extract was centrifuged at 4°C and 15,000 rpm for 15 min. The supernatant was kept, 4 mL of 70% acetone was added to the residue, and then it was subjected to the same procedure. The supernatants were put together and the final volume was adjusted to 10 mL by adding distilled water. The extracts were kept at -20°C until analysis.

The total extractable polyphenol content was determined using a spectrophotometer by Folin-Ciocalteu's method, with modifications (Silva et al., 2012). Based on a previous study, an aliquot of 300 µL of the extract was used for all maturity stages, which was diluted to 1000 µL with distilled water. The oxidation was performed by adding 1 mL of Folin-Ciocalteu's reagent in distilled water (1:3, v:v), followed by neutralization with 2.0 mL of 20% sodium carbonate, and adding 2.0 mL of distilled water. The reading was performed at 700 nm after being kept in the dark for 30 min at room temperature. The estimated content of phenolic compounds was performed using a standard curve of gallic acid (R^2 = 0.99), and the results expressed in mg of gallic acid per 100 g of fresh weight.

Total antioxidant activity

Total antioxidant activity of extracts was determined by α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method (Dantas et al., 2013). Three dilutions (2000, 6000, and 8000 mg/L) were prepared in triplicate by previous tests based on the standard curve of DPPH$^{\bullet}$ (0 - 60 µM DPPH; R^2=0.99). From each dilution, an aliquot of 0.1 mL added to 3.9 mL of DPPH$^{\bullet}$ radical (60 µM) was used. An amount of 100 µL of the control solution composed of 4 mL 50% methanol, 4 mL 70% acetone, and 2 mL distilled water was used. Pure methyl alcohol was set as blank (Genesys^{TM} 10S UV-VIS), and reads were performed at 515 nm. Results were expressed by the EC_{50} value, which aims to provide numerical parameters of how much fresh fruit weight is able to provide antioxidants and verify their effectiveness in scavenging DPPH$^{\bullet}$ free radical (g of fruit/g DPPH$^{\bullet}$).

Statistical analysis

The experiment was designed as completely randomized with factorial arrangement of 2×6 for all variables (two fruit portions and six maturity stages), except for the antioxidant activity and content of phenolic compounds, which were only analyzed in the pulp. All variables were analyzed considering six repetitions. Data were submitted to analysis of variance by F test ($p \leq 0.05$) and Pairwise Correlations using the JMP® software (SAS Institute Inc. 2012). Results were expressed as means and respective standard error values.

RESULTS

The total chlorophyll content in the *T. inamoena* fruit peel decreased as its maturity stages progressed, showing a marked decrease from 11.74 to 0.91 mg/100 g in the peel, and from 4.9 to 0.56 mg/100 g in the pulp (Figure 1A). It was observed that the content of total carotenoids of *T. inamoena* fruit showed significant difference ($p < 0.01$) between the fruit portions studied. During ripening, a rapid quantitative increase from 7.04 to 348.95 µg/100 g in the peel and from 3.40 to 28.67 µg/100 g in the pulp was observed (Figure 1B). All these changes presented a linear behavior as the green color disappeared and carotenoids together with other compounds became more concentrated in both the pulp and peel.

In turn, the amount of betalains, which comprises both betacyanins and betaxathins, varied according to fruit portion and maturation (Figure 2A and B). Betacyanins in the fruit peel were superior ($p < 0.05$) compared to pulp, especially from maturity stage 4, increasing from 0.31 to 0.71 mg/100 g. In the pulp, however, content ranged from 0.08 to 0.17 mg/100 g (Figure 2A). The same behavior was observed for the betaxanthin content, with higher concentration in the peel (Figure 2B). Average betaxanthin content of 0.18 mg/100 g was observed for the pulp and 0.56 mg/100 g for the peel, in which there was a considerable increase until maturity stage 4 (1.14 mg/100 g), where the fruit showed a predominantly yellow color. The following maturity stages presented fruit with less amounts of betaxanthin in the peels.

The content of yellow flavonoids throughout maturation showed a significant difference between the peel and pulp ($p < 0.01$), especially from maturity stage 2. *T. inamoena* fruit showed an increased content from 1.51 to 5.21 mg/100 g in pulp, and from 1.14 to 9.11 mg/100 g in the peel. In both portions, it can be noticed that the content tends to stay nearly the same from maturity stage 4 onwards (Figure 3A). On the other hand, the total

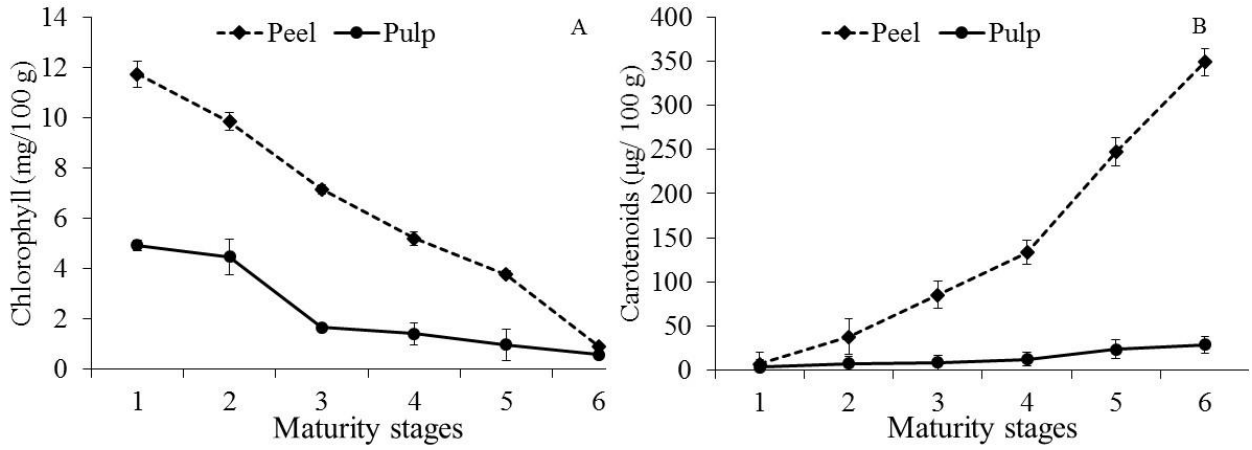

Figure 1. Total chlorophyll content (A) and Total carotenoids (B) contents in the peel and pulp of *T. inamoena* fruit collected in six maturity stages based on peel color in the semiarid region, State of Paraíba, Brazil. (n = 6; Bars mean the standard error).

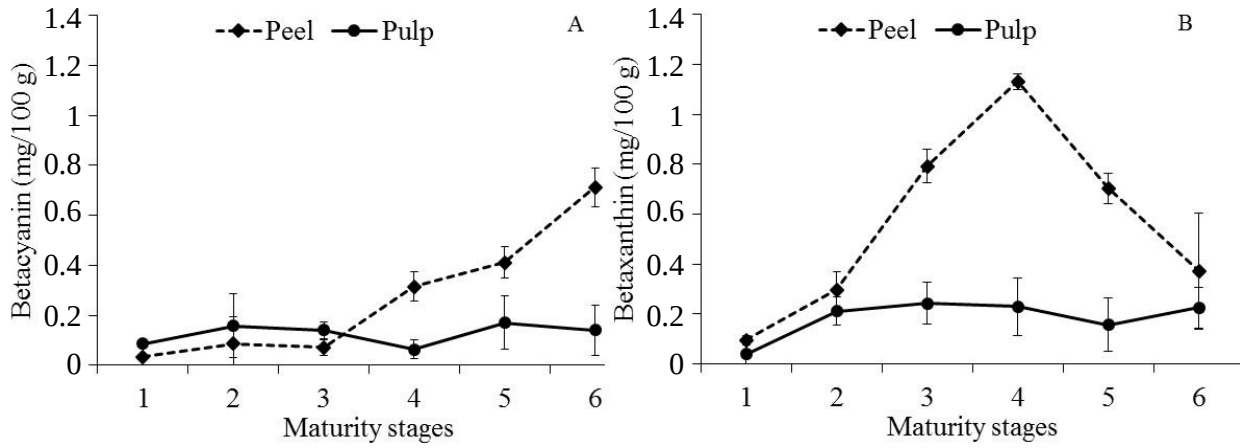

Figure 2. Betalains, betacyanin (A), and betaxanthin (B), in the peel and pulp of *T. inamoena* fruit collected in six maturity stages based on peel color in the semiarid region, State of Paraíba, Brazil. (n = 6; Bars mean the standard error).

extractable polyphenol content of *T. inamoena* was evaluated just in the pulp, in which the amount increased from 11.21 to 29.84 mg GAE/100 g fresh weight (Figure 3B), following the trend of fruit maturation. The correlation with the content of yellow flavonoids in *T. inamoena* pulp was positive (Table 2), showing that this metabolite participates positively in the increase of phenolic compounds during fruit maturation.

Total antioxidant activity of *T. inamoena* pulp showed that the amount of pulp (g) capable of reducing 50% the initial $DPPH^{\bullet}$ concentration continuously decreased over time (Figure 3C). There was a decrease in EC_{50} from 1916.33 to 529.66 g FW/g $DPPH^{\bullet}$, indicating that fully ripe fruit (maturity stage 6) exhibits greater antioxidant activity at the end of maturation. The antioxidant activity of the pulp was highly correlated with total extractable polyphenols (R^2 = -0.87), followed by total chlorophyll (R^2 = 0.95), total carotenoids (R^2 = -0.75), and yellow flavonoids (R^2 = - 0.68). The changes in the betaxanthin content of pulp during maturation of *T. inamoena* fruit was correlated with antioxidant activity (Table 1).

DISCUSSION

The reduction in total chlorophyll content in *T. inamoena* fruit highlights the changes during its maturation, also characterized by increasing flavonoid, carotenoid, and betalain content as the major compounds present in both the pulp and peel, and to which different antioxidant properties are attributed in *Cactaceae* fruits (Felker et al., 2008; Castellar et al., 2012). Because of that, these compounds negatively correlate with flavonoids, carotenoids, and betalains in the peel and pulp (Table 1

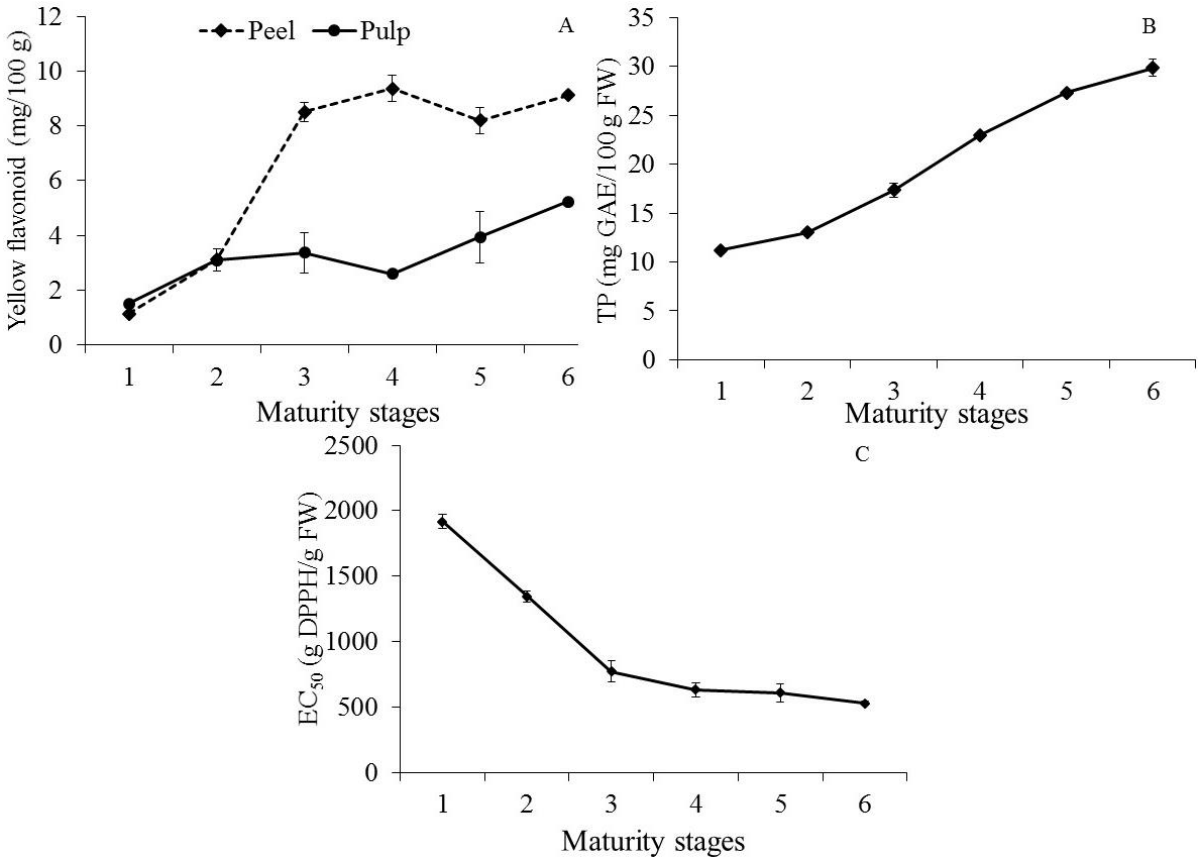

Figure 3. Yellow flavonoids in the peel and pulp (A), Total phenolics-TP (B), and Antioxidant activity (C) in the pulp of *T. inamoena* fruit collected in six maturity stages based on peel color in the semiarid region, State of Paraíba, Brazil. (n = 6; Bars mean the standard error).

and Table 2).

Generally, when chlorophylls decrease, carotenoids take the place of providing an attractive appearance to both the peel and pulp. *T. inamoena* fruit presented this trend probably because mature green fruit contains chloroplasts that differentiate into chromoplasts during maturation, and chlorophylls are degraded by specific enzymes, leading to an accumulation of carotenoids (Tanaka et al., 2008; Paliyath et al., 2009). Cactus fruits are poorly characterized with regard to the biosynthesis of coronoids, as these compounds are not the major constituent in the tissues of these fruits. However, different amounts have been reported for other cacti: 3.23 mg/100 g for pulp and 21.5 mg/100 g for the pericarp of *Opuntia elatior* (Alvarez et al., 2008), and 0.92 mg/100 g for *Opuntia boldinghii* (García-Pantaleón et al., 2009), showing that the values reported herein for *T. inamoena* fruit are quite lower. Souza et al. (2007) reported a content of 0.47 mg/100 g for the pulp and 3.37 mg/100 g for the pericarp of the same species studied herein. In addition to the attractive coloring, carotenoids have high antioxidant capacity with strong health promotion appeal (Cayupán et al., 2011).

Betacyanin is responsible for the reddish coloration and tends to increase with maturation (Castellar et al., 2012; Gandía-Herrero and García-Carmona, 2013). However, the content of this pigment in *T. inamoena* fruit is greatly reduced compared to other species of the *Opuntia* genus due to the prevalence of carotenoids, flavonoids and betalains of the betaxanthin class (Stintzing and Carle, 2007). Stintzing et al. (2005) observed for *Opuntia ficus indica* clones with an orange color that the betaxanthin content for the fully ripe fruit was around 76.3 mg/L, correlating with the antioxidant capacity. In a study performed by Castellanos-Santiago and Yahia (2008) with several species of the *Opuntia* genus, it was found that *O. robusta* showed betaxanthin content of 0.99 mg/100 g, *O. streptacantha* showed 1.04 mg/100 g, *O. ficus-indica* 0.14 mg/100 g, and *O. megacantha* showed 0.16 mg/100 g, thereby setting a wide content variation in the fruit pulp. Additionally, Naselli et al. (2014) have reported that isolated indicaxanthin from *O. ficus-indica* can be useful for cancer treatment. This indicates that *T. inamoena* fruit, as a natural source of this class of compound, can be investigated in this purpose.

Although there is information about the identification of

Table 1. Pairwise Correlations for variables analyzed in the peel of *T. inamoena* fruit collected in six maturity stages based on peel color in the semiarid region, State of Paraíba, Brazil.

Variable	by Variable	Correlation*	Probability	Plotted Correlation -1 0 1
FA	CT	0.7128	0.0009*	
BC	CT	0.7875	0.0001*	
BC	FA	0.5110	0.0302*	
BX	CT	0.1862	0.4595	
BX	FA	0.7371	0.0005*	
BX	BC	0.0648	0.7984	
CH	CT	-0.9626	<.0001*	
CH	FA	-0.8636	<.0001*	
CH	BC	-0.7629	0.0002*	
CH	BX	-0.4069	0.0938	

BC, Betacyanin; BX, betaxanthin; CH, chlorophyll; CT, carotenoids; FA, flavonoids. *n=16.

Table 2. Pairwise Correlations for variables analyzed in the **pulp** of *T. inamoena* fruit collected in six maturity stages based on peel color in the semiarid region, State of Paraíba, Brazil.

Variable	by Variable	Correlation*	Probability	Plotted correlation -1 0 1
FA	CT	0.8272	<.0001*	
BC	CT	0.1514	0.5487	
BC	FA	0.4096	0.0914	
BX	CT	0.2500	0.3171	
BX	FA	0.3614	0.1406	
BX	BC	-0.2592	0.2990	
CH	CT	-0.7988	<.0001*	
CH	FA	-0.6962	0.0013*	
CH	BC	-0.0942	0.7099	
CH	BX	-0.3745	0.1257	
TP	CT	0.9485	<.0001*	
TP	FA	0.7466	0.0004*	
TP	BC	0.0802	0.7517	
TP	BX	0.2839	0.2535	
TP	CH	-0.9208	<.0001*	
AA	CT	-0.7513	0.0003*	
AA	FA	-0.6849	0.0017*	
AA	BC	-0.0784	0.7571	
AA	BX	-0.4758	0.0459*	
AA	CH	0.9599	<.0001*	
AA	TP	-0.8722	<.0001*	

AA, Antioxidant activity; BC, betacyanin; BX, betaxanthin; CH, chlorophyll; CT, carotenoids; FA, flavonoids; TP, total phenolics. *n=16.

betalainic and phenolic compounds and their antioxidant properties, there are few studies on events controlling the ripening in cactus fruits, especially the interrelations with the synthesis of functional compounds. However, some qualitative and quantitative studies have been developed relating the presence of antioxidant substances and maturation. Cayupán et al. (2011) assessed the orange and yellow varieties of *Opuntia megacantha* fruit in 5 maturity stages, finding that the increase in contents of ascorbic acid, polyphenols, and betalains are related to high antioxidant capacity in both the peel and pulp. The evaluation of fruit quality has to expand quality indexes that may be considered as an additional harvest criterion to those traditionally used, such as soluble solids,

titratable acidity, and ratio.

The presence of functional compounds in fruits varies according to several factors. The most distinct pattern is generally observed between the peel and pulp, depending on the group of compounds concerned. With regard to *T. inamoena*, this is the first report regarding the quantification of bioactive compounds and their ability in capturing DPPH$^{\bullet}$ radical as reported for the most world widely consumed *O. ficus-indica* varieties as well as for pitaya groups. Kim et al. (2011) reported differentiated content for both the peel and pulp of red and white pitaya with different antioxidant properties mainly due to the presence of phenolic compound groups such as yellow flavonoids, which comprise a diverse group still in demand of research. The determination of flavonoids and other phenolic groups in cactus fruits, especially those of natural occurrence in the semiarid region of Northeastern Brazil, has an important role as a value-adding tool (Silva et al., 2009). In this sense, it has been reported that quercetin and isorhamnetin are the main flavonoid compounds present in *Opuntia* spp. (Fernández-López et al., 2010; Matias et al., 2014).

Phenolic antioxidants represent most of the profile present in different fruit portions, and the presence of flavonoids and phenolic acids has been reported in flowers and cladodes of *Opuntia* spp. and *Hylocereus* spp. as the most significant components in their composition (Osorio-Esquivel et al., 2011; Tenore et al., 2012). The presence of phenolic acids can be mentioned, with the marked presence of protocatechuic, p-coumaric and ferulic acids, as well as flavonoids, especially taxifolin, myricetin, and isorhamnetin (Tenore et al., 2012; Cha et al., 2013; Dhaouadi et al., 2013). Isorhamnetin and its derivatives are the group of compounds with the highest expression in the composition of *O. ficus-indica* fruit, especially in the form of isorhamnetin 3-O-rutinoside (Matias et al., 2014). Chavez-Santoscoy et al. (2009) studied nine *Opuntia* cultivars and found a content of 22.63 mg GAE/100 g for *Opuntia leucotricha* juice (purple color pulp) and a content of 17.21 mg GAE/100 g for *Opuntia ficus indica* (reddish orange color pulp); values below those found in this study for the pulp of fully ripe *T. inamoena* fruit.

Sumaya-Martinez et al. (2011) reported that the red and purple varieties of *Opuntia* fruit have higher antioxidant activity using the DPPH$^{\bullet}$ method, which is highly correlated with the content of vitamin C and phenolic compounds. This correlation has been reported for *Opuntia* spp. and *Hylocereus* spp. species (Stintzing et al., 2005; Beltrán-Orozco et al., 2009), where very high correlation between total phenolic content and antioxidant activity was verified. Polyphenols together with ascorbic acid were the most active metabolites among the chemical constituents of these fruits. In *O. ficus-indica*, the pulp has low antioxidant activity compared to the peel, and this behavior is also related to the high concentration of phenolic compounds in the peel compared to pulp (Dantas et al., 2015). This also can be seen in *T. inamoena* fruit herein where total extractable polyphenols had a higher correlation with extracts' antioxidant activity by DPPH$^{\bullet}$ method.

Osorio-Esquivel et al. (2011) found that the different portions of xoconostle fruit (*O. joconostle* Web.) have different profiles of phenolic compounds, flavonoids, and betacyanins, with a higher concentration of these metabolites in the fruit pericarp. In addition, the antioxidant activity, particularly the methanol extract, was highly correlated with such compounds, especially phenolic compounds. However, the use of the functional potential of cactus fruits depends on the maturity stage at which they are harvested, and this can be presumed from *T. inamoena* fruit, especially depending on the fruit portion and its stage of maturation.

Conclusion

T. inamoena fruit has a relevant amount of bioactive compounds, mainly phenolic compounds with high antioxidant activity. Changes in the antioxidant activity during maturation of this fruit are correlated to the decrease in pulp chlorophyll content, increased betalains, yellow flavonoids and phenolic compounds. Additionally, more mature fruits present larger amounts of compounds with high antioxidant activity. Finally, further studies are needed to understand the biological behavior of compounds present in *T. inamoena* fruit using *in vivo* models and clinical trials to assess the potential health benefits.

Conflict of Interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

Nitrogen compounds, proteins and amino acids in corn subjected to doses of aluminum

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Corn (*Zea mays* L.) is highly adaptable, but it has difficulties in expressing its productive potential in soils with high aluminum content, since this element is directly related to high acidity in the soil. The objective of this study was to evaluate the nitrogen compounds, proteins and amino acids of two corn cultivars subjected to increasing doses of aluminum. The experiment was carried out in a greenhouse using one of the corn plants from varieties BRS 106 and BRS 4157. The experimental design was entirely randomized, in a factorial design of 5x2, and the factors were composed of five doses of Al^{3+} (0; 50; 100; 150 and 200 mmol L^{-1}), with five repetitions. The variables analyzed were the concentration of nitrate, the activity of the nitrate reductase, concentration of ammonium, amino acids and proteins. There was a decrease in nitrate, activity of the Reductase enzyme of the Nitrate and protein in the highest dose of aluminum (200 mmol L^{-1}) for both cultivars. There was an increase in ammonium and amino acids in the leaves of cultivars BRS 106 and BRS 4157. Cultivars BRS 106 and BRS 4157 were affected by the increasing doses of aluminum, but cultivar BRS 106 showed to be more tolerant.

Key words: Aluminum, toxicity, corn, metabolism.

INTRODUCTION

Maize (*Zea mays* L.) belongs to the botanical family Poaceae and originating in Mexico as one of the most cultivated cereal in the world, and is considered one of the most efficient crops in energy storage. This culture is used as human food and as animal feed, due to its good nutritional qualities. The corn grain has a mean content of 8% protein providing approximately 63 million tons of protein in the world, but these have lower levels of lysine and tryptophan (Vasal, 1994).

Although it is highly adaptable, this crop has some barriers in productive terms in soils with high aluminum content, which is an element directly related to acidity in

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soil. Generally, pH values (in H_2O) of the soil below 5.5 can already cause serious aluminum toxicity problems for the plants. This is one of the problems of the Amazonian soils (Silva et al., 2006). Estimates show that approximately half of the arable land and with great potential for production of food and biomass are acidified, that is, they are subject to toxicity by aluminum (Kochian et al., 2004).

In addition, aluminum can cause harmful effects in the assimilation of nitrogen in the plants (Pal'ove-Balang and Mistrik, 2011). That causes a reduction in the concentration of nitrate in the presence of aluminum (Souza et al., 2014). Thus, high concentrations of this element cause alterations in the biochemical process of nitrogen, which is considered important in the production of the protein precursor (Camargo and Almeida, 1983; Sphear and Souza, 2004).

The aluminum affects in an expressive way both the absorption and the assimilation of the nitrate reductase activity (NRA) in sorghum (Cambraia et al., 1989). Purcino et al. (2003) found a similar result in corn crops. Although there are techniques such as the lining, which reduces the effects of the aluminum, there are still several gaps regarding the behavior of the species and the variety of plants subjected to high concentrations of aluminum. Therefore, it is necessary to have researches about the maximum dose that the corn crop can tolerate in a certain soil, resistant variety, and also to understand and analyze in which physiologic and biochemical mechanism this metal is involved in the plant. That way, it enables techniques, handling and cultivars that can decrease, mitigate or tolerates acid soils, increasing productivity.

It is known that the aluminum affects the plants negatively, specially the more sensitive ones. However, the studies that involve the operation of the nitrogen metabolism subjected to stress by Al^{3+} are incipient. Taking into account the agronomic and economic importance of corn for animal production, this study aims at evaluating the nitrogen compounds, proteins and amino acids of two corn cultivars subjected to increasing doses of aluminum.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at the Rural Federal University of Amazon - Capitão Poço Campus. The plants used were from BRS 106 and BRS 4157 varieties, without control of the environment and with only monitoring of temperature and air relative humidity through a digital thermo hygrometer. 5 seeds per vase were used in the sowing. After 5 days of germination, a thinning was carried out, leaving only one plant per vase. The application of the stress by aluminum started on the 15th day after germination, and the biochemical analyses were done in the 30th day (vegetative stage) after germination. The vases were placed with a space of 0.60 m between the rows and 0.40 m between the plants.

The corn plants were grown in modified Leonard vases containing sand substrate: vermiculite (1-2) and irrigated with modified nutrient solution of Hoagland and Arnon (1950). The plants exposed to light intensity in the greenhouse were 700 lx, and the pH of the nutrient solution was 4.8. The experimental design was entirely randomized, in a factorial design of 5x2, and the factors were composed of five doses of 0; 50; 100; 150 e 200 mmol L^{-1} de Al^{3+} for the both corn cultivars, with five repetitions, totaling 50 experimental units. Each experimental unit was composed of a plant.

The concentration of nitrate was carried out by a method proposed by Cataldo et al. (1975), in which the samples of 50 mg of leaves previously lyophilized were added to test tubes containing 5.0 ml of distilled water, and those were incubated in water bath for 30 min at 100°C. After that, it was centrifuged at 3,000 rpm for 10 min, and removing the supernatant. The reaction was prepared in a test tube containing 100 µL of the extract + 200 µL of salicylic acid solution 5% (p/v), in concentrated sulfuric acid. After agitation, the tubes were added with 4700 µL of NaOH 2 N. After that, the tubes were left at rest until they reached room temperature for about 20 min. The readings were carried out in spectrophotometer at 410 nm. The nitrate reductase activity (NRA) was obtained by the method described by Hageman and Hucklesby (1971). Leaf discs of 0.5 cm^2 in diameter were removed and weighted in approximately 200 mg of the discs. Right after, they were transferred to test tubes containing 5.0 ml of phosphate buffer and, next, were taken to water bath at 30°C for 30 min. The test tubes were added with 2.0 ml of buffer + 1.0 ml of reaction extract +1.0 ml of sulfanilamide 1% + 1.0 ml of NNEDA 0.02%. They were put at rest for 15 min. After that, the reading went to the spectrophotometer at 540.

The concentration of free ammonium was determined by using the method described by Weatherburn (1967). 50 mg of dry mass (DM) of the leaves was weighted and, right after, put on test tubes with the addition of 5 ml of distilled water and taken to water bath for 30 min at 100°C. After the extraction of the samples, they were centrifuged at 1000 rpm to obtain the total extract. There was an addition of 400 µL of total extract + 2.5 ml of A solution (5 g phenol + 0.025 g Sodium nitroprusside/ 500 mL distilled water) and homogenized in vortex, adding another 2.5 ml of B solution (2.5 g of NaOH + 12.6 mL of sodium hypochlorite/ 500 mL of distilled water), and taking them to water bath for 20 min at 37°C. The tubes were removed from water bath and left to rest for 40 min, and then taken to a spectrophotometer reading at 625 nm.

The total soluble amino acids were obtained by the method described by Peoples at al. (1989). 50 mg lyophilized DM was transferred to test tubes, and 5 ml of distilled water was added. Then, they were taken to water bath for 30 min at 100°C. After the extraction, the samples were centrifuged at 1000 rpm to obtain the total extract. Aliquots of 100 100 µL of the extract + 400 µL of distilled water were added. After that, 250µL of the citrate buffer 0.2 M pH 5.0 and 250 µL of Ninhydrin reagent was added. Then, they were taken to water bath for 15 min at 100°C. Next, the reaction was interrupted in ice bath and 1.5 ml of ethanol 50% (v/v) was added.

The tubes remained at room temperature for 20 min, and the readings were done in a spectrophotometer at 570 nm. The total soluble proteins were obtained by using the method described by Bradford (1976). In test tubes, there was an addition of 100 mg lyophilized DM/ 5.0 ml of the extraction buffer (Tris-HCl25 mM pH 7.6). Then, they were agitated for 2 h in the shaker. After the extraction, the tubes were centrifuged at 2000 rpm for 10 min. The test tubes were added with 100 µL of the sample + 2.5 mL of the Bradford reagent. After that, the tubes were manually agitated taking care to not denature the proteins. After 15 min, the readings were carried out at 595 nm.

The results were subjected to the variance analysis, when significant by the F test, and the effect of the nitrogen doses analyzed by regression, adjusting the equations to express the behavior of the variables being studied.

Table 1. Analysis of the variance for nitrate, nitrate reductase activity (NRA), ammonium, amino acids and proteins in corn cultivars in function of Al^{3+}doses.

Variation sources	GL	Nitrate	NRA	Ammonium	Amino acid	Protein
Doses of Al^{3+} (Al^{3+})	4	**	**	**	**	**
Cultivars (C)	1	**	**	**	**	**
Al^{3+} x C	4	**	**	**	**	*
CV (%)	-	5.91	2.39	3.39	2.54	6.81

CV = variation coefficient; * = significant ($p < 0.05$); ** = significant ($p < 0.01$), by the Tukey test.

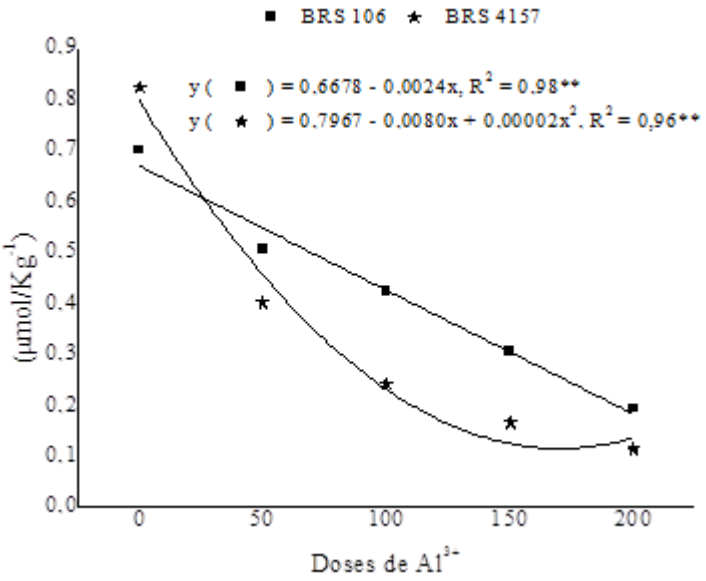

Figure 1. Concentration of nitrate in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant ($p < 0.01$) by the t test.

RESULTS AND DISCUSSION

The aluminum doses (Al^{3+}) influenced ($p < 0.01$) the biochemical variables (Table 1). The cultivars presented a different behavior regarding nitrate, nitrate reductase activity (NRA), ammonium, amino acids and proteins. For biochemical variables, in the aerial part there was a significant effect ($p < 0.01$) of the interaction between doses Al^{3+} cultivars.

Regarding the concentration of nitrate, corns BRS 106 and BRS 4157 presented a polynomial and linear behavior, respectively, in function of the aluminum doses. For the concentration of nitrate, the control treatment presented 0.69 NO^{3-} kg^{-1} DM and the maximum doses of aluminum presented contents of 0.19 NO^{3-} kg^{-1} DM of nitrate for cultivar BRS 106. While cultivar BRS obtained 0.82 NO^{3-} kg^{-1} DM in the control and 0.11 NO^{3-} kg^{-1} DM maximum doses of aluminum (Figure 1). That is, there was a decrease in the nitrate in the highest dose of aluminum (200 mmol L^{-1}). When there is stress by excess of Al^{3+} in the corn crop, the acidification capacity of the roots, as well as the accumulation of nitrate, are reduced (Lidon et al., 1998; Ahn et al., 2001). Al^{3+} promotes the increase in permeability of the roots membrane, which causes an excess of this metal in the root system. The excess of this metal resulting from the increase in the permeability of the membrane limits the nitrate absorption rate due to the inhibition of its carriers (Simon et al., 1994).

This decrease may be related to the lack of nutrients in the plant, because one of the characteristics of the plant subjected to stress by aluminum is the shortening of the root. Thus, the plants will have difficulties in absorbing water and nutrients, and transport them to the leaves. Therefore, the content of substrates (nutrients, among them the Nitrate) will be compromised, causing the reduction of this substrate in the plant, besides contributing to the decrease in the activity of nitrate

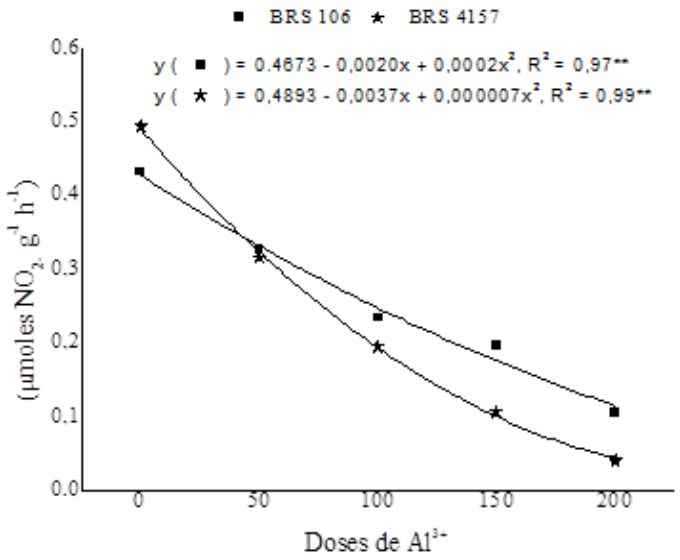

Figure 2. Nitrate reductase activity in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant (p < 0.01) by the t test.

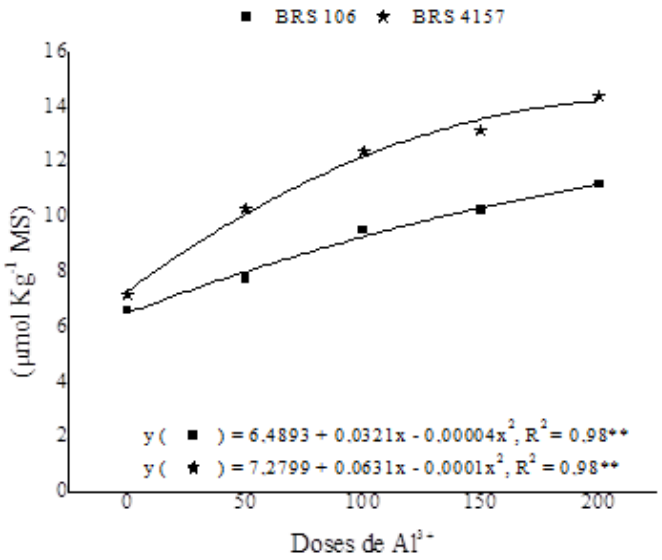

Figure 3. Concentration of free ammonium in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **Significant (p < 0.01) by the t test.

reductase.

Al^{3+} may cause dramatic effects in the assimilation of N in the plants (Pal'Ove-Balang and Mistrik, 2011), which leads to the decline in the concentration of nitrate in the presence of Al^{3+}. In the presence of aluminum, the rice cultivar Fernandes reduced the concentration of nitrate (Justino et al., 2006). Apparently, the effects of Al^{3+} on the absorption of nitrate depend on the species studied, on the concentration of Al^{3+} in the absorption medium, on the duration of the treatment applied and, probably, on its later interference on the process of nitrate reduction and/or assimilation of nitrogen in organic compounds. Al^{3+} has an effect on the absorption of nitrate, and there are no physiologic explanations for the several conflicting results.

The results showed that there was a decrease in the activity of the Nitrate Reductase activity of 0.43 µmols of NO_2^- g MF^{-1} h^{-1} in the control treatment for 0.1 µmoles of NO_2^- g MF^{-1} h^{-1} in the maximum doses of aluminum (200 mmol L^{-1}) for cultivar BRS 106. For cultivar BRS 4157, there was a reduction of 0.49 µmols of NO_2^- g MF^{-1} h^{-1} to 0.04 µmols of NO_2^- g MF^{-1} h^{-1} in the control treatment and in the maximum aluminum doses (200 mmol L^{-1}), respectively (Figure 2). The reduction was probably because the aluminum decreased the root growth, which is shown by the low absorption of nitrate and water by the roots. That can cause the reduction of the transpiration current, leaving the enzyme inactive. In addition, the nitrate reductase suffers a decrease in the plants subjected to acidity (Sharma and Dubey, 2005).

The high acidity in the soil can cause inhibition of nitrate reductase activity (Sharma and Dubey, 2005). The nitrate reductase activity was negatively affected in corn plants when they were grown in conditions of high acidity (Lin-Xianyong et al., 2002). The increase in the doses of aluminum elevated the concentration of ammonium in the leaves of cultivars BRS 106 and BRS 4157, with a polynomial adjustment. Ammonium increased from 6.57 µmols of NO_2^- g^{-1} MF h^{-1} in the control to 11.15 µmols of NO_2^- g^{-1} MF h^{-1} in the highest dose of aluminum (200 mmol L^{-1}) for cultivar BRS 106. For cultivar BRS 4157 there was an increase from 7.15 µmols of NO_2^- g^{-1} MF h^{-1} (control) to 14.37 µmols of NO_2^- g^{-1} MF h^{-1} (200 mmol L^{-1}) (Figure 3).

This result can be explained because the ammonium needs the glutamine synthetase enzyme to transform into Glutamine and, later on, in glutamate, releasing amino acids that will help the good development of the plant. As soon as it notices that the content of free ammonium increases with the increase of the aluminum doses, the toxicity probably inactivates the activity of the Glutamine Synthetase enzyme, preventing ammonium NH_4^+ from incorporating into the Glutamate amino acid to form glutamine, and consequently enabling its accumulation. Purcino et al. (2003) found a similar result in which the assimilation of NH_4^+ was affected by Al^{3+}, once this metal compromises the process by altering the activity of the enzymes capable of incorporating it in amino acids. One of the factors that can contribute to that is the prevalence of NH_4^+ rather than NO^{3-} in the conditions of acidity and toxicity of Al^{3+} experienced by plants of cowpea (Kerbauy, 2008).

Another relevant factor for the accumulation of ammonium in both cultivars is that in the process of photorespiration (mitochondria) occurs the deamination phenomenon, which is a natural procedure of the plant

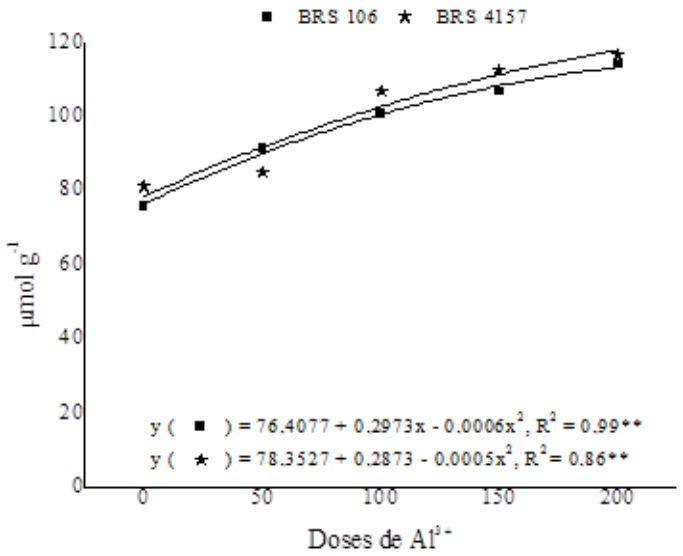

Figure 4. Concentration of amino acids in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. **significant (p < 0.01) by the t test.

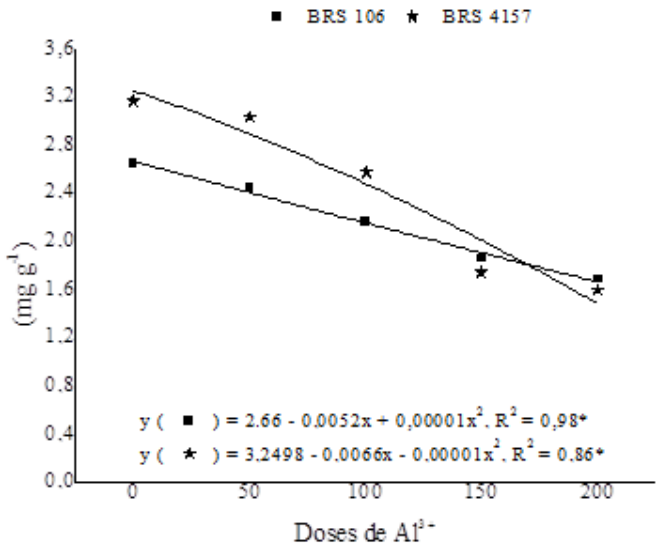

Figure 5. Concentration of protein in the leaves of corn cultivars BRS 106 and BRS 4157 in function of the aluminum doses. *significant (p < 0.05) by the t test

for the release of ammonium. However, with the action of this stress by Al^{3+}, there was possibly a lack of control of this deamination, contributing to the accumulation of ammonium both in BRS 106 and BRS 4157.

As the aluminum dose increased, there was also an increase in the concentration of amino acids of both cultivars (BRS 106 and BRS 4157), with a polynomial adjustment. For cultivars BRS 106 and BS 4157, there was an increase from 75.61 µmol of AA/ g MS (control) to 114.01 µmol of AA/ g MS (200 mmol L^{-1} of Al^{3+}) and from 81.03 µmol of AA/ g MS (control) to 116.43 µmol of AA/ g MS (200 mmol L^{-1} de Al^{3+}) respectively (Figure 4). The growth of total soluble amino acid may have probably been caused by the increase in the activity of proteases enzyme, which break the reserve proteins according to the exposition of a plant to any injury, in this case the effect of aluminum toxicity, contributing with the water deficit.

Those effects are observed as a consequence of the inhibition of root growth (Beutler et al., 2001). This fact is probably due to the increase in the activity of the proteases enzyme, which break the reserve proteins in plants exposed to long periods of water deficit, increasing the content of total soluble amino acids, aiming at adjusting osmotically to the stressing medium (Kerbayy, 2004).

Cruz et al. (2011) obtained results that show the opposite, where they observed that the presence of Al^{3+} caused the decrease in the concentration of amino acids and total soluble proteins in sorghum plants, showing that the presence of this element can actually limit the vegetable growth. Balang and Zelinova (2013) when studying the behavior of both cultivars of *Lotus corniculatus* under stress conditions caused by toxic aluminum, observed that the reduction of free amino acids may be related to the low availability of nitrogen due to the inhibition of absorption of nitrate and ammonium under stress conditions.

As the aluminum dose increased, there was a decrease in the concentration of proteins in both cultivars (BRS 106 and BRS 4157), with a polynomial adjustment. There was a decrease in the concentrations of total soluble proteins from 2.64 mg protein/ g DM (control) to 1.68 mg protein/ g DM (200 mmol L^{-1} of Al^{3+}) in cultivar BRA 106 and from 3.16 mg protein/ g DM (control) to 1.59 mg protein/ g MS (200 mmol L^{-1} of Al^{3+}) (Figure 5). During the stress caused by aluminum, this element acts as a limiting factor for the assimilation of nitrogen, once there is a reduction in the nitrate reductase activity, which is the first enzyme associated to the nitrogen metabolism, and the low supply of nitrogen would cause a reduction in the synthesis of protein (Cruz et al., 2011).

Possibly, the decrease in proteins in both cultivars is related to the breaking of these total soluble proteins by the proteases enzymes, starting to form amino acids and, within those amino acids, there is a deamination, forming ammonium. Therefore, with the protein break, there will be a contribution to the increase of the amino acids and ammonium, respectively. The proteins degrade, forming amino acids that adjust osmotically; among those amino acids you can find proline. It starts to work in order to avoid the loss of water in the leaf tissues. Somers et al. (1996) showed that there was a decrease in the content of total soluble proteins (cytoplasm) in plants subjected to treatments with Al^{3+}, both for plants resistant to metal and sensitive to it. However, in a study made by Souza et al.

(2014) with species of Urochloa subjected to aluminum, it was not observed alterations in the concentration of amino acids and proteins.

The presence of aluminum may inhibit the absorption of other ions, such as Mg (Malavolta et al., 1997). This element plays an important role in the metabolic pathways such as glycolysis, Krebs cycle and pentose phosphate pathway. The addition of aluminum made Mg unavailable and the deficit of this element may have reduced the activity of these metabolic pathways such as the Krebs cycle, and it may also have resulted in an alteration of the proteins biosynthesis, because Mg is necessary for the protein synthesis, as a cofactor to several enzymes (Boutler, 1970).

Conclusions

As the aluminum doses increased, there was a reduction in nitrate, nitrate reductase and total soluble proteins. The increasing doses of aluminum provided an increment in the concentrations of ammonium, total soluble amino acids for both cultivars. Cultivars BRS 106 and BRS 4157 were affected by the increasing doses of aluminum, but cultivar BRS 106 showed to be more tolerant.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Integrated management of termites damaging wooden structures

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Field experiment was set up in November 2011 and continued up to March 2013 on 324 m^2 backyard garden plot of a farmer's residential area. The experiment was laid in 6 m × 6 m Latin square design. The treatments were polyethelene bag covered wooden sticks, chlorpyrifos 48% E.C. treated and polyethelene bag covered wooden sticks, *Masea lanceolata* treated and polyethelene bag covered wooden sticks, chlorpyrifos 48% E.C treated planting hole, *M. lanceolata* treated planting hole and untreated check. Every three months, termite infestation, damage and damage severity were recorded. The chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks (0.2) and the chlorpyrifos 48% E.C. treated planting hole (0.2) protected termite infestation and damage throughout the study period starting from six months after application of treatment. In the rest of the treatments, damage progressed towards the end of the experiment with no significant difference amongst them. The severity of damage was significantly lower in chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks (3 and 1.3) and chlorpyrifos 48% E.C treated planting hole (4.4 and 2.3) than the other treatments starting from twelve to eighteen months after application of treatments. Conclusively, chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks and chlorpyrifos 48% E.C treated planting hole protected the wooden construction materials from both genera of termites. However, the chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks was superior in providing longer duration of protection and reduction of environmental hazards. In the former treatment, no chemical was in contact with soils that saves not only contamination of soils and ground water but also reduced the rate of the biodegradability of the chemical.

Key words: *Masea lanceolata,* polyethelene bag, chlorpyrifos.

INTRODUCTION

With few exceptions, the main food of all termites consists of wood, the chiefly utilized being cellulose (Harris, 1971). Termites cause economic loss by damaging structures such as buildings, bridges, dams, and even roads or by damaging crops, forest trees, or rangelands (Harris, 1971; Pearce, 1997; Gurusubramanian et al., 1999). They are major pests of all kinds of woods and products of wooden origin. They also attack living

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plants (Metcalf, 1967). Non-cellulose materials such as plastic pipes, electric and telegraph cables are also damaged by termites (Harris, 1971; Hickin, 1971). In vast tropical areas no wooden article is safe, especially if termites can gain direct access to it from the earth (Klots and Klots, 1959). Termites attack on field and tree crops and on forest trees especially in the semiarid and sub-humid tropics. They cause significant losses and often a major constraint on reforestation (Logan et al., 1990).

Local houses are constructed using mud and termites can tunnel through these structures and eat wooden roof supports or thatching. Thatching in African houses can be expected to last 5 to 6 years. Wooden or bamboo poles incorporated in the mud or cement walls of traditional houses offer an easy means of entry to termites from floor level to other areas. In all kinds of buildings good design is important but this may still not stop termites invading (Pearce, 1997).

Ethiopia, as one of the tropical countries, suffers from termite damage to buildings, seedling and saplings for reforestation; loss of timber and agricultural crops and forestry trees. Termite damage is particularly serious in Western Ethiopia than other regions (Abdurahman, 1990, 1995).

In Western Ethiopia where it is common practice to build houses and fences on untreated wood and grasses without proper foundation, thatched grass roof huts are destroyed in about five years while corrugated iron sheet houses survived about eight years. About 50% of the houses and fences require maintaining every year. Similar maintaining is required for stores, bridges crossing streams, electric and telephone poles and many others. As a result trees are cut frequently to replace structures destroyed by termites. Such practice would undoubtedly lead to deforestation and ecological disaster (Abdurahman, 1990). One could also imagine the labor force engaged and money spent in maintaining the structures destroyed by termites annually. These losses of labor, money and forest resources would obviously be very striking had it been properly estimated and reported as has been done elsewhere.

Generally, termite damage to buildings, electric and telephone cables, bridges crossing streams, standing trees, transplanted seedlings and so on in Western Ethiopia is apparent to anyone though significant research was not undertaken in the past and recently. Therefore, field experiment was conducted with the objective of evaluating chemical, mechanical, botanical and their combination on termites damaging wooden structures to address the urgent needs of the inhabitants of the Ghimbi district.

MATERIALS AND METHODS

Field experiment was set up in November 2011 on 324 m^2 backyard garden plot of a farmer's residential area. Wooden sticks of 1.25 m long with a diameter of 15 cm cut from six years old *Eucalyptus* tree were prepared for the experiment. The experiment was laid in 6 m × 6 m Latin square design. It required 36 wooden sticks each at 3 m intervals from one another 0.6 cm × 0.6 cm × 0.5 cm hole was dug for each wooden stick. A 19 m × 2 m twenty five micro polyethylene bag was prepared. The polyethylene bag was cut into 0.75 m × 1 m pieces (Figures 1 and 2).

Materials such as two 15 L plastic buckets and a 15 L Knapsack sprayer were prepared to begin the experiment. About 12.5 ml chlorpyrifos 48% E. C. was dissolved in one liter of water. Lower part of the six wooden sticks, each about 0.75 cm was sprayed with 12.5 ml chlorpyrifos 48% E. C. in a bucket and covered with polyethylene bag. The sprayed and covered wooden sticks were planted in their respective holes and any leftover chemical from each treatment was added to the hole and tightly covered with the soil. Similarly, the same amount of the chemical was sprayed in another respective six holes and barren wooden sticks were planted and covered with the soil. Another six untreated wooden sticks but that were covered with polyethelene bag were also planted in their respective holes and covered with soil. The fourth group of six wooden sticks each sprayed with 125 g *M. lanceolata* leaf powder in the same way with the chemical and covered with polyethelene bag and planted in their respective holes and covered with soil as usual. Another six holes each was also sprayed with 125 g *M. lanceolata* leaf powder in a similar manner to the chemicals but uncovered wooden sticks were planted and covered with the soil. Finally six uncovered and untreated wooden sticks were planted in their respective untreated holes for use as a control. Each wooden stake represents one experimental unit. The treatments were:

1. Polyethylene bag covered wooden sticks.
2. chlorpyrifos 48% E. C. treated and polyethelene bag covered wooden sticks.
3. *M. lanceolata* treated and polyethelene bag covered wooden sticks.
4. chlorpyrifos 48 % E. C. treated planting hole.
5. *M. lanceolata* treated planting hole.
6. Untreated check.

Every three months, the sticks were removed for inspection. A visual examination of the sticks was made during each observation period. Termite infestation, damage and damage severity were recorded. Hand lens was employed in the identification of smaller termites and termite damage. Termite specimens were collected and preserved in labeled vials filled with 80% alcohol for later identification in Insect Sciences Laboratory of Addis Ababa University. The collected data were analyzed using NCSS software for analysis of variance.

RESULTS AND DISCUSSION

The wooden sticks were infested with two genera of termites namely *Macrotermes* and *Microtermes* spp. Damage to the wooden sticks did not differ significantly among treatment means until six months after treatment. However, the damage on wooden construction material progressed in time from third month to six month after application of treatment as shown in Table 1 and Figure 1. The chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks (0.2) and the chlorpyrifos 48% E. C. treated planting hole (0.2) protected termite infestation and damage throughout the study period starting from six months after application of treatment. These two

Figure 1. Chlorpyrifos 48% E. C. treated bare planted (A), untreated but polyethylene bag covered (B), chlorpyrifos 48% E. C. treated and polyethelene bag covered (C) and lastly untreated and bare planted (D) wooden sticks.

Figure 2. Wooden construction material experiment layout (A) and termite infested planting hole after removing the wooden sticks (B) and termite damaged wooden sticks (C).

treatments did not differ significantly in protecting the wooden construction material from termite attack. In the rest of the treatments, damage progressed towards the end of the experiment with no significant difference amongst them. But chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks seems to have better protection over extended period of time as compared to the chlorpyrifos 48% E. C. treated planting hole and thus requires extended time of investigation. The polyethelene bag covered wooden sticks and *M. lanceolata* treated polyethelene bag covered wooden sticks did not give any protection from both genera of termite attack.

Polyethelene bag coverage did not protect termite attack. Nevertheless, termites were observed feeding on the botanical and the polyethelene bags themselves which was in accordance with Harris (1971), Hickin (1971) and Pearce (1997) who reported that termites feed on non cellulose materials such as plastic pipes, electric and telegraph cables. It also agreed with Sileshi

Table 1. The effect of different treatments on presence of termite damage on wooden construction material starting from three months after application of treatments (starting from February 2012: Mean± se).

Treatments	Months after Application of Treatment					
	Three	Six	Nine	Twelve	Fifteen	Eighteen
Polyethelene bag covered wooden sticks	0.2±0.1^{a}	0.7±0.1^{a}	0.8±0.1^{a}	0.7±0.1^{a}	0.8±0.1^{a}	0.7±0.1^{a}
Chlorprfos 48% treated polyethelene bag covered wooden sticks	0.0±0.1^{a}	0.0±0.1^{b*}	0.2±0.1^{b*}	0.2±0.1^{b*}	0.2±0.1^{b*}	0.2±0.1^{b*}
M. lanceolata treated polyethelene bag covered wooden sticks	0.3±0.1^{a}	0.8±0.1^{a}	1.0±0.1^{a}	1.0±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}
Chlorpyrifos 48% treated planting hole	0.0±0.1^{a}	0.1±0.1^{b*}	0.2±0.1^{b*}	0.4±0.1^{b*}	0.2±0.1^{b*}	0.2±0.1^{b*}
M. lanceolata treated planting hole	0.2±0.1^{a}	0.7±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}	1.0±0.1^{a}
Untreated check	0.2±0.1^{a}	0.8±0.1^{a}	0.7±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}	0.8±0.1^{a}

Means with in a column followed by the same letter (s) are not significantly different at 5% Tukeys, studentized range test (HSD).

Table 2. The effect of different treatments on termite damage severity in percentage on wooden construction material (starting from May 2012: Mean± se).

Treatments	Six months after application of treatments	Twelve months after application of treatments	Eighteen months after application of treatments
Polyethelene bag covered wooden sticks	5.3 (29.1) ±0.3^{b}	6.8 (46.8)±0.7^{b}	8.0 (66.9)±0.8^{b}
Chlorprfos 48% treated polyethelene bag covered wooden sticks	0.0 ±0.3^{b}	3.0 (13.6)±0.7^{a*}	1.3 (9.1)±0.8^{a*}
M. lanceolata treated polyethelene bag covered wooden sticks	5.3 (29.1) ±0.3^{b}	7.1 (49.7)±0.7^{b}	7.2 (53.5)±0.8^{b}
Chlorpyrifos 48% treated planting hole	0.0 ±0.3^{b}	4.4 (20.9)±0.7^{a*}	2.3 (10.6)±0.8^{a*}
M. lanceolata treated planting hole	5.3 (29.1) ±0.3^{b}	6.9 (48.9)±0.7^{b}	7.7 (60.4)±0.8^{b}
Untreated check	5.7 (33.3) ±0.3^{b}	7.6 (62.1)±0.7^{b}	8.4 (69.1)±0.8^{b}

Means with in a column followed by the same letter (s) are not significantly different at 5% Tukeys, studentized range test (HSD). Data are square root transformed and numbers in parenthesis are actual values in percentage.

et al. (2009) who recommended that management of termites in future should be built on farmers' indigenous knowledge and adequate understanding of the ecology of the local termite species. The current findings is in agreement with James et al. (1990) who recommended the use of appropriate cultural methods combined with minimal modern pesticides in an integrated approach.

The severity of damage done to the wooden construction material was analyzed starting from six months after application of treatments and continued at six months intervals. The severity of damage was significantly lower in chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks (13.6 and 9.1%) and chlorpyrifos 48% E. C treated planting hole (20.9 and 10.6%) than the other treatments starting from twelve to eighteen months after application of treatments as shown in Table 2. The severity of damage increased starting from six months after application of treatments except for the two treatments. The least damage severity (9.1%) was recorded in the chlorpyrifos 48% E.C treated polyethelene bag covered wooden sticks and the highest damage severity was in the control plot (69.1%) toward the end of the investigation period indicating the promising effect of the Chlorpyrifos 48% E.C treated polyethelene bag covered wooden sticks over extended period of time that however requires further investigation.

Conclusively, chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks and chlorpyrifos 48% E.C treated planting hole protected the wooden construction materials from both genera of termites. However, the chlorpyrifos 48% E.C. treated polyethelene bag covered wooden sticks was superior to the chlorpyrifos 48% E.C treated planting hole in two major aspects. These two treatments showed longer duration of protection and reduction of environmental hazards. In the former treatment, no chemical was in contact with soils that saves not only contamination of soils and ground water but also reduced the rate of the biodegradability of the chemical since it was not exposed to moisture and other elements in the soil that may facilitate the biodegradability of the chemical. Nonetheless, the duration of efficiency requires further investigation.

Conflict of Interests

The authors have not declared any conflict of interest.

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Production and reproduction performance of local chicken breeds and their marketing practices in Wolaita Zone, Southern Ethiopia

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A study was conducted in Wolaita Zone, Southern Ethiopia to assess production and reproduction performances of local chickens and their marketing practices. Three districts vize Damot-Gale, Boloso-Sore and Humbo representing, highland, midland and lowland agro-ecologies, respectively, were selected purposively. The data were collected from a total of 135 respondents, 45 from each agro-ecology, using pre-tested structured questionnaires and analyzed using statistical package for social sciences (SPSS) version 20.0. There was a significant (P<0.05) difference in local flock size per household among the three agro-ecologies. The average chicken flock size per household was 6.0 heads. Clutch length, clutch number per year per hen, total number of eggs laid per hen per year, number of eggs hatched, hatchability and chick survival were significantly (P<0.05) different among the agro-ecologies. The overall average age at sexual maturity was 5.6 months (male) and 5.5 months (female) chickens, age at first egg laying was 6.5 months, number of eggs laid per clutch per hen was 14.8, clutch length was 26.0 days, clutch number per year per hen was 4.2 and mean number of eggs laid per year per hen were 59.1. The overall number of eggs placed per brooding hen was 12.8 of which 10.0 were hatched and out of which only 7.1 chickens survived. The overall hatchability was 79.1%. Marketing of live chicken and eggs were common; 69.6% of respondents sold both chicken and egg, 20% sold only eggs, 10.4% sold only chicken, 71.9% used formal market while only 8.9% used informal market to sell their products. Local chicken productivity and re-productivity are low and so, different improvement strategies should be introduced.

Key words: Agro-ecology, chicken product, improvement strategies, marketing practices.

INTRODUCTION

Smallholder societies throughout the world in general and developing countries in particular use poultry for multipurpose uses including: as source of income and means to ensure food security; cultural and social values such as ritual, sacrifice and symbolism; gifts to strength social bonds; and source of economic empowerment for

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women (FAO, 2010). Attributed to their source of income, high quality animal protein, ability to be kept under limited inputs and management and adaptability to different agro-climates, chickens are the most important and widely produced avian species in developing countries by resource limited families (Kondombo, 2005).

In Ethiopia, chickens play crucial roles in the livelihood of resource challenged families. According to Mamo et al. (2013) chicken production has socio-cultural and economic benefits especially in the rural communities. The chicken population of the country was at estimated 56.87 million of which 95.86% are local breeds, 2.79% hybrid breeds and 1.35% exotic breeds (Central Statistical Agency of Ethiopia (CSA), 2013). Traditional/village chicken production system is the dominant system practiced by almost every rural household (Alemayehu et al., 2015) and covers more than 90% of the total chicken meat and egg produced in Ethiopia (Dana et al., 2010).

Research reports from different parts of Ethiopia indicated that, the local chicken breeds have low production and reproduction performances (slow growth rate, late maturity, few egg yields, small sized eggs, high mortality rate, extended reproductive cycle and extended inter-clutch) (Aberra, 2000; Halima, 2007; Dana et al., 2010; Desalew, 2012; Habte et al., 2013; Mamo et al., 2013). On the other hand, local chicken breeds have many desirable traits, including thermo tolerance, disease resistance, good egg and meat flavor, productivity at no or minimal feed supplementation, hard egg shells, high fertility, hatchability and dressing percentage (Aberra, 2000).

Local chicken and eggs are preferred by most consumers because they are tasty and suitable to make traditional sauce (called “Doro wote” in Amharic) and eggs due to their deep yellow coloured yolks (Moges et al., 2010). This shows that there is a market potential for local chicken producers and in fact marketing of chickens and eggs is common by smallholder chicken producers. Despite the importance of local chicken in the farming system of Ethiopia, the marketing structure and overall value chin have not well been studied (Mokennen, 2007), and the marketing channels are informal and poorly developed (Moges et al., 2010). Besides, chicken price is affected by different factors including; chicken’s plumage colour, comb type, size, age, sex, holy days, fasting and non fasting periods, market site and health status of the chicken (Halima, 2007). Therefore, assessing the existing marketing practice in a given locality is necessary.

Wolaita zone is one of the highly populated areas in Ethiopia and mixed crop-livestock farming system, involving the production of cereals, root crops, enset and coffee and different livestock species including chicken are commonly practiced. Traditional chicken production based on local chicken breeds is the most dominant in the zone as elsewhere in different parts of Ethiopia. So far, a study to characterize scavenging chicken production system has been done by Desta and Wakeyo (2010) in Wolaita zone. Desta and Wakeyo (2010) have documented the uses and flock management practices, chicken production constraints and their combating mechanisms practiced by producers and draw backs of scavenging chicken production in the zone. However, information with regard to production and reproduction performances of the local chicken as well as marketing practices of live chicken and egg in Wolaita zone is scanty. Therefore, the objective of the present study was to generate information on some production and reproduction traits of local chicken and their marketing practices in Wolaita zone.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Wolaita zone, located in Southern Ethiopia. Sodo town is the administrative center of the zone and is located at a distance of 383 km far from Addis Ababa. Wolaita zone has an altitude ranging from 1,200 to 2,950 m above sea level (masl); annual rainfall vary between 800 and 1400 mm with two distinct rainy seasons, the main (‘kremt’) occurring in summer (roughly June, July and August) and the small rainy season (‘belg’) occurring in spring (roughly the mid-February to mid-May); and minimum and maximum temperatures of 15 and 20°C, respectively. The study zone has twelve districts distributed in three agro-ecological zones namely; highland (9%; > 2400 masl), midland (56%, 1500 to 2400 masl) and lowland (35%, <1500 masl) (WZFEDD, 2005).

Sampling techniques

Three districts viz. Damot-Galle, Boloso-Sore and Humbo were purposively selected to represent higher, medium and lower altitudes of the zone, respectively. From each selected district, three peasant associations (‘kebeles’) were randomly selected making a total of 9 peasant associations. From each of the randomly selected peasant associations 15 households that had at least one chicken were randomly selected. Thus, a total of 135 households, 45 from each district, were considered for the study.

Data collection

Both primary and secondary sources of data were used for the survey study. Primary data like information about characteristics of the households, local chicken flock size holding, productive and reproductive traits, marketing practices of chicken and eggs, etc. were collected from the respondents via interviewing using pretested structured questionnaire. Secondary data were also collected from the study area Finance and Economic Development Department, journals and books.

Statistical analysis

The data were analyzed by using SPSS version 20.0 (2011) for descriptive statistic and one way analysis of variane (ANOVA). Duncan’s new multiple range tests was used to determine the

Table 1. Sex, marital status and education status of the of the sampled households.

Parameter	Agro-ecology					
	Highland (n=45)		Midland (n=45)		Lowland (n=45)	
	Frequency	%	Frequency	%	Frequency	%
Sex						
Male	42	91.1	37	82.2	38	84.4
Female	4	8.9	8	17.8	7	15.6
Marital status						
Single	3	6.7	2	4.4	15	33.3
Married	41	91.1	40	88.9	29	64.4
Divorced	-	-	3	6.7	1	2.2
Widowed	1	2.2	-	-	4	8.9
Educational status						
Illiterate	11	24.4	15	33.3	6	13.3
Grade 1-4	10	22.2	2	4.4	6	13.3
Grade 5-8	11	24.4	14	31.1	16	35.6
Grade 9-12	7	15.6	10	22.2	14	31.1
Diploma and above	6	13.3	4	8.9	-	-

Table 2. Mean age and family size of the respondents in the study area.

Parameter	Agro-ecology			SEM	P-value
	Highland (n=45)	Midland (n=45)	Lowland (n=45)		
Age	40.8	42.7	40.1	0.90	0.467
Family size					
Male	3.3^{b}	3.2^{b}	4.0^{a}	0.12	0.017
Female	3.4	4.0	3.9	0.16	0.285
Total	6.8^{b}	7.0^{b}	7.9^{a}	0.21	0.064

[a,b]Means in the same row with different superscript letters differ significantly (P<0.05). SEM: Standard error of means

differences between agro-ecologies mean values for the quantitative parameters at 5% level of significance. The statistical model used to analyze the data was: Yij = μ+Ai+eij; where, Yij = response variable, μ = overall mean, Ai = effect of agro-ecology and eij = random error.

RESULTS AND DISCUSSION

Demographic characteristics of respondents

Sex, marital and educational status of the respondents is presented in Table 1. Majority (85.9%) of the respondents were males, while the rest (14.1%) were females and this result is in agreement with Desta and Wakeyo (2010). The percentage of male respondents was higher than that of females in all the three agro-ecologies. Majority (81.5%) of the respondents were also married. With regard to educational status of the respondents, higher proportion (76.3%) was literate, while the rest (23.7%) was illiterate. The presence of high proportion of literate individuals in a farming community could be an opportunity for easily adoption of improved technologies (Asaminew and Eyassu, 2009). The percentage of illiterates in the present study was lower than the values (33.6%) reported by Desta and Wakeyo (2010), but higher than the value (6.9%) reported by Mokennen (2007) in Dale Wosho and Loka Abaya Woredas, southern Ethiopia.

There were no differences in mean age and female family size among the three agro-ecologies (Table 2). However, higher male and total family size was found in the lowland than the highland and midland agro- ecologies.

Table 3. Mean flock size per household, productive and reproductive traits of local chickens in three agro-ecologies.

Parameter	Agro-ecology			SEM	P-value
	Highland (n=45)	Midland (n=45)	Lowland (n=45)		
Local flock size/household	4.4[b]	6.3[ab]	7.2[a]	0.46	0.036
Male age at sexual maturity (months)	5.9	5.5	5.5	0.14	0.360
Female age at sexual maturity (months)	5.9	5.2	5.4	0.14	0.129
Male age at slaughter (months)	8.6	9.4	8.9	0.19	0.236
Age at first egg (months)	7.0	6.2	6.3	0.16	0.060
Number of eggs /clutch/hen	14.4	15.2	14.7	0.26	0.401
Clutch length (days)	24.6[b]	27.2[a]	26.0[ab]	0.46	0.057
Clutch number/year/hen	4.8[a]	4.1[b]	3.6[b]	0.11	<0.001
Total number of eggs/year/hen	66.2[a]	60.0[a]	51.1[b]	1.67	0.001
Number of eggs set/brooding hen	12.9	13.3	12.1	0.22	0.079
Number of eggs hatched	10.7[a]	9.8[b]	9.5[b]	0.19	0.019
Hatchability (%)	83.6[a]	74.1[b]	79.5[ab]	1.29	0.010
Number of chicks survived	7.8[a]	7.1[ab]	6.3[b]	0.19	0.007

[a,b]Means in the same row with different superscript letters differ significantly (P<0.05). SEM: Standard error of means.

The mean age of the respondents in the present study was similar to the 41.9 years reported by Desta and Wakeyo (2010) but higher than the 35.2 years reported by Alemayehu et al. (2015) in Benishangul-Gumuz region, Western Ethiopia. The average total family size per household in the present study was similar to the 7.0 persons per household reported by Desta and Wakeyo (2010); comparable to the 6.95 persons per household reported by Mokennen (2007) in Dale Wosho and Loka Abaya, but higher than the 5.2 (national average) and 6.4 (in Benishangul-Gumuz region, Western Ethiopia) persons per household reported by Central Agricultural Census Commission of Ethiopia (CACC) (2009) and Alemayehu et al. (2015), respectively.

Production and reproduction traits of local chicken

Local chicken flock sizes per household were in the order of lowland ≥ midland ≥ highland agro-ecologies (Table 3). The overall mean local chicken flock size in the present study was 6.0 heads per household. This result was lower than the 9.2, 7.9, 7.7 and 9 to 13.0 heads per household reported by Mokennen (2007), Melesse et al. (2012), Alemayehu et al. (2015) and Moges et al. (2010) in different parts of Ethiopia.

There were no differences in male and female age at sexual maturity and age at slaughter of male chicken among agro-ecologies (Table 3). The overall mean ages at sexual maturity for male and female chickens were 5.6 and 5.5 months, respectively. The findings of the present study were slightly lower than the range values 5.8 to 6.1 and 5.8 to 6.3 months for male and female chickens, respectively (Alemayehu et al., 2015). Meseret (2010) also reported higher age at sexual maturity of 6.47 months for male and 6.33 months for female chicken from Jima zone, Ethiopia. The overall mean age at slaughter of male chicken in the present study (9.0 months) was comparable to the 8.62 months reported by Meseret (2010). Sexual maturity is an important reproductive trait from evolutionary and economic viewpoints (Wright et al., 2012). According to El-Dlebshany (2008), early age at sexual maturity results into high number and mass of eggs. Moges et al. (2010) also reported that late sexual maturity results in low productivity of local chickens.

There were no differences in age at first egg of local chickens among the agro-ecologies (Table 3). This is in agreement with Mokennen (2007) who reported there were no differences in age at first egg of local chickens in highland, midland and lowland agro-ecologies of Dale woreda, Southern Ethiopia. The overall mean age at first egg (6.5 months) in present study was in agreement with the 5.9 to 7.1 months (Moges et al., 2010), 6.6 months (Melesse et al., 2012), but lower than the 7.01 months (Mokennen, 2007) and 7.0 to 7.4 months (Alemayehu et al., 2015) reported from different parts of Ethiopia. Guni et al. (2013) reported higher age at first age (7.48 months) in Tanzania.

Differences were observed in clutch length and clutch number per year per hen among the agro-ecologies; clutch length was higher in the midland and lower in the lowland, but the observed difference was not significant between the lowland and the other two agro-ecologies and clutch length was higher in the highland than the midland and lowland agro-ecologies (Table 3). The

overall mean number of eggs per clutch per hen, clutch length (days) and clutch number per year per hen in the present study were 14.8, 26.0 and 4.2, respectively. The number of eggs per clutch in the present study was comparable to that reported by Mokennen (2007) and Moges et al. (2010), but higher than the reported by Meseret (2010), Addisu et al. (2013), Habte et al. (2013), Yitbarek and Atalel (2013) and Alemayehu et al. (2015). The clutch length found in this study was also higher than that of reported by Mokennen (2007) and Meseret (2010). The variation could be attributed to genetic makeup of the local chickens, environmental factors and management practices provided by the chicken producers (Melesse et al., 2012; Guni et al., 2013).

Total numbers of eggs per year per hen were higher in the highland and midland agro-ecologies as compared to the lowland agro-ecology (Table 3). The lower number of eggs per year per hen in the lowland may be attributed to the lower number of clutches per year recoded compared to the other agro-ecologies. This was in line with the report of Guni et al., (2013). The overall mean eggs per hen per year (59.1) in the current study was higher than the 55.2, 43.8, 55 and 49.5 reported by Mokennen (2007); Meseret (2010); Moges et al. (2010) and Addisu et al. (2013), respectively but lower than the 65 reported by Yitbarek and Zewudu (2013). Eggs per year per hen in the present study were also higher than the 45.2 reported by Guni et al. (2013) in Tanzania. The relatively higher number eggs per hen per year found in current study could indicate that there is potential the local chickens for increased egg production.

There were differences ($P<0.05$) in the number of eggs hatched, percent of hatchability and numbers of chicken survived (Table 3). But the number of eggs set per brooding hen was similar among the agro-ecologies. The numbers of eggs hatched in the highland were higher than the midland and lowland. Percent hatchability was higher in the highland than the lowland. The chicken survival rate was also higher in the highland than the lowland agro-ecology, but the value in the midland was similar with that of the highland and lowland agro-ecologies.

The overall mean number of eggs placed per brooding hen in the present study (12.8 eggs) was within the range (10.3 to 13.2 eggs) reported by Melesse et al. (2012); Meseret (2010); Nigatu and Bezabih (2014) and Alemayehu et al. (2015) but higher than the 11.3 eggs reported by Guni et al. (2013) in Tanzania and 10.3 eggs reported by Hagan et al. (2013) in Ghana. These differences could be attributed to season of incubation, brooding experience, size of the hen, availability of eggs, size of eggs, body size of broody hen and her maternal instinct behavior (Tadelle et al., 2003; Hagan et al., 2013). These traits are known to determine the number of eggs placed per brooding hen (Habte et al., 2013).

According to Meseret (2010) and Habte et al. (2013), hatchability of eggs and the survival rate of the hatched chicken are among the major factors that determine the productivity of chicken. Out of the overall mean number of eggs set for hatching per brooding hen in the present study, on average, 10.0 were hatched. This was similar to the 10.0 reported by Melesse et al. (2012) but higher than the 8.1reported by Meseret (2010) in Ethiopia and 8.7 by Hagan et al. (2013) in Ghana. On average, out of the hatched chicks in the present study only 7.1 chicks survived and this result was higher than the 5.5 chicks that survived as reported by Melesse et al. (2012). The overall percent hatchability (79.1%) in the current study was comparable to the 82.8% reported by Nigatu and Bezabih (2014), and higher than the 72% reported by Yitbarek and Atalel (2013), 59.6% by Yitbarek and Zewudu (2013). However, the percent hatchability was lower than 89.1% reported by Mokennen (2007) in Ethiopia and 84.5% by Hagan et al. (2013) in Ghana. Hatchability depends on instinct maternal behavior of the hen, degree of management of the hen during brooding and prevalence of predators (Habte et al., 2013; Hagan et al., 2013) while survival rate of the hatched chicks depends on prevalence of predation and disease (Hagan et al., 2013).

Chicken and egg marketing practices

Table 4 shows the type of chicken product marketed, type of market used, and customers' preference criteria during purchasing of live chicken and eggs in the study area. Selling of live chicken and eggs were common in the study area. This is in agreement with Mokennen (2007) who reported that selling of live chicken and egg was a common practice in Ethiopia. According to Moges et al. (2010) village chicken producers in Ethiopia sold their live chicken and eggs in their locality and urban markets directly to consumers or traders (collectors) and marketing channels were informal and poorly developed. Meseret (2010) also reported that there was no formal market to sell live chicken and eggs in Gomma Woreda, Ethiopia. However, the results of the present study revealed that majority (71.9%) of the respondents sold their chicken and eggs in formal market while only 8.9% used informal market to sell their products (Table 4). This indicates that chicken producers have better understanding of formal market.

About 35% of respondents reported that customers preferred live chicken based on plumage color, body weight/size, comb type and sex while 20% reported that customers preferred live chicken based on plumage color, body weight and sex (Table 4). This agrees with Halima (2007) who reported that chicken price is affected by different factors such as chicken's plumage color, comb type, size, age, sex, holy days, fasting and non fasting periods, market site and health status of the

Table 4. Type of local chicken product marketed, place of marketing, customers preference criteria during purchasing live chicken and eggs.

Parameter	Agro-ecology					
	Highland (n=45)		Midland (n=45)		Lowland (n=45)	
	Freq	%	Freq	%	Freq	%
Type of chicken product marketed?						
Egg	14	31.1	9	20.0	4	8.9
Live chicken	3	6.7	3	6.7	8	17.8
Both egg and live chicken	28	62.2	33	73.3	33	73.3
Type of market for selling chicken and egg?						
Formal market	31	68.9	34	75.6	32	71.1
Informal market	3	6.7	4	8.9	5	11.1
Both formal and informal markets	11	24.4	7	15.6	8	17.8
Customers' preference during selling chicken?						
Plumage color (1)	2	4.4	2	4.4	5	11.1
Body weight/size (2)	2	4.4	6	13.3	4	8.9
Comb type (3)	9	20.0	2	4.4	2	4.4
Sex (4)	1	2.2	1	2.2	1	2.2
All the above (1-4)	15	33.3	23	51.1	9	20.0
Body weight, comb type and sex	2	4.4	6	13.3	0	0.0
Plumage color, body weight and sex	11	24.4	2	4.4	14	31.1
Comb type and sex	1	2.2	2	4.4	1	2.2
Body weight and sex	0	0.0	1	2.2	3	6.7
Plumage color and body weight	2	4.4	0	0.0	5	11.1
Plumage color and comb type	0	0.0	0	0.0	1	2.2
Customers' preference during selling egg?						
Size	9	20.0	18	40.0	22	48.9
Color	1	2.2	6	13.3	1	2.2
Both size and color	19	42.2	11	24.4	17	37.8
No preference	16	35.6	10	22.2	5	11.1

Freq.: Frequency

chicken.

Respondents (36.3 and 34.8%) reported that the customers purchased eggs based on size and both size and color, respectively. On the other hand, 23% of the respondents reported that customers had no particular preference for egg while only 5.8% reported that customers had color preference of eggs during purchasing. Such information could help chicken producers to produce products which are highly demanded by customers in their localities.

Conclusion

There were differences in productive and reproductive performances of local chickens in the highland, midland and lowland agro-ecologies of the present study zone. The productive and reproductive performances of the local chickens were low and this calls introduction of different improvements strategies. Marketing of live chicken and eggs were common and majority of the chicken producers sold their products in formal market.

Conflict of Interests

The authors have not declared any conflict of interests.

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Energy demand of furrow openers and corn yield according to the soil disturbance in no till system

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The performance of the planter is directly linked to the mechanisms of contact with the soil, more specifically to the furrow openers mechanism. The aim of this study was to evaluate the responses of the soil, machine, and the corn crop, according to furrow openers hoe type and working depths, in an eutroferric Red Latossol and no-tillage system. Soil disturbance, drawbar power and force, speed of operation and corn grains yield was evaluated. The soil disturbance presented difference for the factors; openers and depths. The distinct behavior of the openers, due to their geometry, was a limiting factor for the working depth that determines the area of disturbed soil. Energy demand to the tractor presented difference for openers and depths. The opener with angle of inclination of 17° and width of 21 mm (Opener 1) caused higher soil disturbance and lower force and power. In a Red Latosol with no-tillage system, the working depth of 90 mm required lower draft demand and a soil disturbance suitable to the corn crop development.

Key words: Agricultural mechanization, direct seeding, planter.

INTRODUCTION

One of the factors that contribute to no-tillage system is the selection and proper use of agricultural machinery and equipment. Cepik et al. (2010) debate that farmers should consider using roe type furrow openers for fertilizer application in areas of no-tillage. The furrow openers, according to Siqueira and Casão Junior (2004), are tools whose function is to cut and penetrate the soil, opening the furrow. The action angle and type of rod present different forms of soil penetration and depth variability. The use of furrow openers is conditioned to the use of cutting disks front to prevent the occurrence of bushings associated with the use of double disks for furrowing and seed deposition.

The increasing of the depth performance of the furrow

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openers of the seeders to break in a localized form compacted layers on the surface, is a manner that can also stimulate root development and reduce the effects of soil compaction on corn yield (Conte et al., 2009). The furrow mechanism of rod type, compared to the use of double disk, can favor the increase of grain yield (Kaneko et al., 2010).

The use of furrow openers for the deposition of fertilizer to the detriment of dual disks is growing among farmers. Basically, it summed up to the fact that the chisel type mechanism best break the compacted layers and also work better in clayeyer soils, reducing wasted time and inputs (Conte et al., 2009).

The relationship between the draft and the degree of compaction can be adjusted for use of openers with different geometries, or by changing the operation depth; these are some of the factors that directly influence the draft (Conte et al., 2007). Agreeing with the authors, Cepik et al. (2005) studied the draft in an Argisol under no-tillage and concluded that there was an increase in demand traction with increasing of the depth, and its magnitude depended on the state consistency of the soil.

Furrow opener system for the fertilizer deposition increases the draft requirements, fuel consumption and the slippage index, in relation to the furrow opener of type double disks (Santos et al., 2008). However, Seki et al. (2012) found that the shanks promotes greater soil disturbance. This greater disturbance capacity, when in unfavorable external conditions and heterogeneous areas can stimulate the development of crops and according to Mello et al. (2003) promote an increase of 11.3% in corn yield.

Therefore, the aim of this study was to evaluate the responses of the soil, machine, and the corn crop, according to the furrow openers hoe type and working depths, in an eutroferric Red Latossol and no-tillage system.

MATERIALS AND METHODS

The experiment was conducted in the agricultural year 2011/2012, in the UNESP/FCAV, Jaboticabal City, Brazil, with an average altitude of 560 m, average slope of 4% and Aw climate (subtropical), according to the Köeppen classification. The soil of the experimental area is classified as eutroferric Red Latosol, with 48% of clay (Embrapa, 2006; Andrioli and Centurion, 1999).

A Valtra-AGCO tractor, model BM 125i, 4x2 TDA, was used to pull the planter. It presents power of 91.9 kW (125 cv) engine at 2300 rpm. The planter was a Jumil, JM3060PD model, pantograph, which present mechanical meter for seed distribution, furrow opener hoe type for furrow opening and fertilizer deposition, double disks for seeds with deposition set at 3 cm depth, double compacted wheels in "V" shape, operating with four planting rows and mass of approximately 3,000 kg. The displacement speed of the tractor/planter set was of 4.0 km h^{-1}.

A randomized blocks design was used, with 2 × 3 factorial scheme and four replications. The treatments were two furrow openers hoe type (Figure 1), and three working depths (90, 110, and 150 mm). The area of each plot was 100 m^2 and consisted of four rows of corn, spaced at 0.90 m. The variables analyzed were: Drawbar power and force (average, peak and specific); soil disturbance, planting speed and corn grains yield.

To perform the acquisition and data storage relating to the drawbar power and force was used a system composed by Micrologger CR23X from Campbell Scientific Inc. The required draft was measured using a load cell Shimizu, TF 400 model, with a capacity of 100 kN and accuracy of ± 1 N, installed on the drawbar of the tractor.

The data acquisition was obtained every second, then it was calculated the average power in the plot, with the peak draft represented by the highest obtained value. The calculation of the average power demand and peak in the drawbar was determined by the product of the average force and average displacement speed.

The soil disturbance, width and depth of furrow were evaluated as follow: the furrow was opened manually being possible to model the furrow. Thus, these variables were analyzed as follow:

1. Furrow width (FW) and working effective depth (WED): With the help of a profile meter with 45 rods, spaced of 1 cm and height 30 cm. On the back was nailed a cardstock paper with horizontal lines spaced of 0.5 cm for easy reading and precision on the study, wherein the positioning of the rods upper end copies the geometric form of the furrow. For the reading was used a digital camera, analyzing them in a computer. FW was defined since the first rod that fell on the ground inside the furrow showing different measures. WED was defined by the average of two first rods that showed the higher values.
2. Soil disturbance: with the values from the profile meter, was obtained the transversal section of disturbed soil and the data were analyzed by the integral of the trapezoidal rule (Equation 1), according to Ruggiero and Lopes (1996) and Bertonha et al. (2015).

$$\int f(x) = \frac{h}{2}\left\{f(x_0)+2\left[f(x_1)+f(x_2)+\ldots+f(x_{m-1})\right]+f(x_m)\right\} \quad (1)$$

Where, $\int$ = numerical integral to area of disturbed soil; h = distance between profile meter rods (1 cm), and x = value of the rods readings (cm).

The specific draft and power per soil area disturbed correspond to the relationship between the application of average power demanded by the seeder, and the area of the disturbed soil by the furrow openers.

The real displacement speed was measured by radar, Dick Jonh brand, RVSII model, with an error of ± 3%, installed on the side of the tractor, making a 45° angle to the surface of the soil, as recommended by the manufacturer. The radar works at a frequency of 1 Hz. Considered as real displacement speed, the average obtained by the radar when the tractor was operating in the plot. Speed values were obtained in m h^{-1} and converted to km h^{-1}.

For grain yield was conducted manual harvesting in 5 m of each row, the two central rows of each plot, after the time which the culture reached physiological maturity and water content close to 18%. The corn cobs were threshed in stationary machine, corrected to 13% of water content and grain yield calculated in kg ha^{-1}.

Before planting, there was a data collection of 5 samples for soil mechanical resistance to penetration for the characterization of the

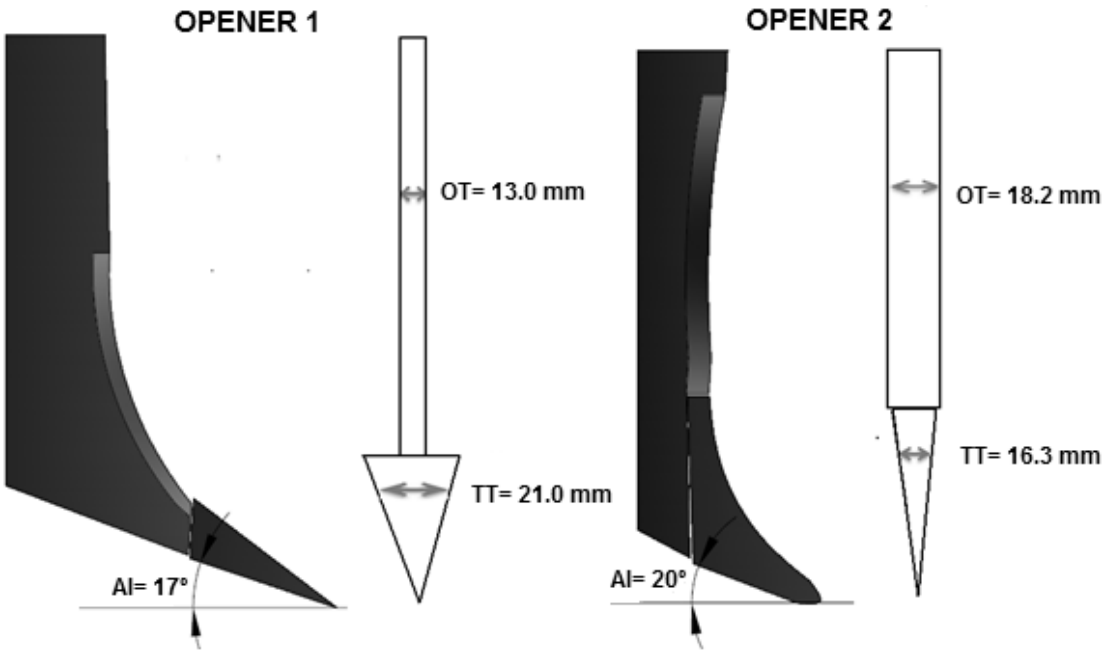

Figure 1. Design and dimensions of the furrow openers. OT, opener thickness, TT, tip thickness: AI, angle of Inclination.

area, in random points and depth of 0.30 m, to determine the cone index (CI).

At the time of planting, the water content of the soil was characterized, presenting 23.6% of water to the layer 0.00-0.10 and 23.8% of water to the layer 0.10-0.20 m.

The investigation of the data normality was performed by the Anderson-Darling test using the program MINITAB 16® (2003), and when asymmetric, Log (x) transformation was applied. Analyses of the results were processed with the SISVAR (Ferreira, 2011) and ASSISTAT (Silva and Azevedo, 2009) statistical program, performing variance analysis and applying the F test, and when there were significant, Tukey test at 5% probability was applied.

RESULTS AND DISCUSSION

The results of (CI) to the 0.00-0.10, 0.10-0.20 and 0.20-0.30 m layers corresponded to 1.47, 2.81 and 3.29 MPa with average levels of water of 25.56, 25.64 and 26.21%, respectively. Opener 1 showed higher soil disturbance than Opener 2, corresponding to 29.8% (Table 1). This increase represents the largest tip width (16.3 to 21 mm). As the working depth is increased, tillage increased by 42 and 119% compared to that of the 90 mm depth. It was noted that the opener width has a direct influence on the vertical soil disturbance. Regarding to the performance of the tractor/planter set the results showed that there was difference to opener as well as in the working depth, with no interaction between them. Regarding the average and peak draft required by the opener, it was observed that the opener 1 required lower draft than 2, and the increase of the working depth also demanded greater average and peak draft (Table 1).

The Opener 2 showed less soil disturbance; however, it demanded higher draft. It can be explained by the fact that Opener 2 has greater rake angle than Opener 1, and it provided forward disturbance, instead of to up disturbance.

Palma et al. (2010) working at four working depths of the furrow openers (0.10, 0.15, 0.20 and 0.25 m) observed when the shoe (tip) of the opener works on the most compacted layers, there is a higher demand for draft than when working with the opener below the layers, which offer higher soil mechanical resistance to penetration. This factor occurs due to the angle of inclination that the furrow opener mechanism have in relation to the soil.

The values of average draft per planting row for opener 1 and 2 were 4.12 and 4.5 kN, and for peak draft was from 4.71 to 5.3 kN respectively, higher than those indicated by the ASAE (2003), reaching 3.5 kN per planting row. Silveira et al. (2011) found less values (2.61 kN) when worked with 3.5 km h^{-1} speed and working depth of 110 mm.

The specific draft was similar to those discussed for the average and peak draft as the behavior of the opener tip.

Table 1. Analysis of variance of the soil disturbance, peak and specific draft at the drawbar power.

Treatments	Soil disturbance (cm^2)	Draft		
		Average (kN)	Peak (kN)	Specific (N cm^{-2})
Furrow Opener (FO)				
1	195.79[a]	16.47[b]	18.84[b]	87.62[b]
2	150.95[b]	17.93[a]	21.29[a]	128.65[a]
Working depths (WD)				
90 mm	113.09[c]	13.32c	16.24c	125.61[a]
110 mm	159.56[b]	16.51[b]	19.36[b]	108.73[ab]
150 mm	247.46[a]	21.76[a]	24.59[a]	90.06[b]
F Test				
FO	14.04*	6.63*	7.45*	32.69*
WD	43.38*	74.95*	29.36*	8.19*
FO x WD	0.03[ns]	0.06[ns]	0.29[ns]	2.42[ns]
CV (%)	16.9	8.1	11.0	16.2

Means followed by the same letter are not different by Tukey test at 5% probability; C.V., coefficient of variation; **significant ($P < 0.01$). *significant ($P < 0.05$); ns, not significant.

The specific draft for Opener 2 was higher than Opener 1 (around 50%). This fact is related to the angle of inclination, and the smallest angle allows that the soil disturbance up, while the largest angle leads it go to the front, requiring greater force.

The specific draft decreased from lower depth to highest working depth. The ideal condition would be that the opener breaks up the soil at 45° angle (Spoor and Godwin, 1978), however, when deepens the opener, the angle of rupture decreased, showing lower specific draft. This fact shows that when working with incorrectly working depth, it cause in the furrow wall a mirroring (compaction), reducing the exploration area of the crop roots.

The planting speed was not influenced by the factors (Table 2), thus, the increasing of the opener did not change the operational field capacity.

A difference for average power and peak drawbar was observed, but there was no interaction between them. The Opener 2 showed higher demand for peak and average power (Table 2). In the highest working depth, there was higher requirements of average power and peak, similar data of the draft; therefore, the drawbar power parameter is a function of the drawbar force and the planting speed (Salvador et al., 2009).

The different behavior of the openers is due to the constructive differences, such as width and tip of the opener. The drawbar power increased 58% when the working depth ranged from 90 to 150 mm. For Opener 2, the specific power had an increase of 47%, and the working depth a decrease of 30%, comparing the depth of 90 to 150 mm.

The corn grains yield did not differ among the treatments and was similar to the data obtained by Debiasi et al. (2010) that worked with two working depths (60 and 120 mm) and three types of soil cover.

Conclusions

The opener with angle of inclination of 17° and width of 21 mm (opener 1) caused higher soil disturbance and lower force and power. In a Red Latosol with no-tillage system, the working depth of 90 mm required lower draft demand and a soil disturbance suitable to the corn crop development.

Conflict of Interests

The authors have not declared any conflict of interest.

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Table 2. Analysis of variance for planting speed, average, peak, and specific power at the drawbar and corn grain yield.

Treatments	Planting speed (km h^{-1})	Power			Grain yield (kg ha^{-1})
		Average (kW)	Peak (kW)	Specific (W cm^{-2})	
Furrow opener (FO)					
1	4.30[a]	19.64[a]	4.30[a]	22.48[b]	6323[a]
2	4.33[a]	21.30[b]	4.33[a]	25.31[a]	6576[a]
Working depth (WD)					
90 mm	4.22[a]	16.08c	4.22[a]	19.58c	6298[a]
110 mm	4.34[a]	19.90[b]	4.34[a]	23.35[b]	6226[a]
150 mm	4.35[a]	25.44[a]	4.35[a]	28.75[a]	6825[a]
F Test					
FO	0.02[NS]	7.17[*]	0.02[NS]	7.45[*]	0.36[NS]
WD	1.67[NS]	76.10[*]	1.67[NS]	26.50[*]	0.81[NS]
FO x WD	2.70[NS]	0.46[NS]	2.70[NS]	0.35[NS]	0.72[NS]
CV (%)	3.79	7.45	3.79	10.60	15.94

Means followed by the same letter are not different by Tukey test at 5% probability; C.V., coefficient of variation; **significant ($P < 0.01$). *significant ($P < 0.05$); ns, not significant.

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Full Length Research Paper

Phytosociological and floristic survey of weeds in Western Paraná

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Weeds can reduce yield, which justify the need for their efficient management. The stage that precedes the management consists of identifying the species present in the area, and then making the decision of the best method of control. Thus, this study aimed to perform a phytosociological survey, and to analyze the composition and floristic similarity of weeds between the end of the corn crop cycle and in the off-season period. The authors analyzed nine areas representing the second crop corn and six in the off-season period, distributed in four municipalities: Francisco Alves, Marechal Cândido Rondon, Palotina and Terra Roxa. For estimation of weed species, the square inventory technique of 1 m^2 was used. The sampling design consisted of three collection ranges, the first starting at 5 m from the edge, followed by 25 and 50 m inside the crop. In the second crop corn, 29 species grouped into 17 families, in the off-season period, and 35 species grouped into 18 families were recorded. *Parietaria* spp. and *Conyza* spp. stood out as main species in areas occupied by second crop corn and *Parietaria* spp. and *Gnaphalium spicatum* Lam. in areas of the off-season period and inside sampling range of this period. The similarity index evidenced a high similarity between the periods sampled.

Key words: Weeds, identification, importance value, density.

INTRODUCTION

Soybean (*Glycine max)*, corn (*Zea mays*) and wheat (*Triticum* spp.) are the main crops in western Paraná. Soybean production is estimated at 14,786,987 tons, corn at 15,779,557 tons and wheat at 3,994,903 tons (IPARDES, 2014). The excellent yield results can be mainly attributed to the favorable climate, topography, soil fertility and structure. In this region, the cultivation of these important crops takes up major market share, thus causing a large development of agribusiness (Belusso and Sierra, 2006). Reflection of that excellent yield results according to Conab (2016) Paraná State is expected to achieve yield of 3944 kg ha^{-1} for the season 15/16, 0.6% higher than the yield of the crop in 14/15.

One of the crops that deserve attention is the corn

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grown in the second growing season, known as second crop corn, which occupies most areas of the region from February to August. According to Conab (2015), second crop corn production in Brazil reached 5254 $kgha^{-1}$, and with this, the production of second crop corn was consolidated in 53.99 million tons in 2015, providing an increase of 5.3% over the previous harvest. In the same period, the area planted with first crop corn was 6109.3 thousand hectares; and with second crop corn, 9599.7 thousand hectares. However, according to Galon et al. (2008), this productivity is lower than those obtained in high tech farming. Among many factors that are responsible for low productivity, one of the most important is the interference by weeds.

A study conducted by Rossi et al. (1996) on the interference by weeds with seven corn cultivars showed reduced first ear height, length and circumference of ears, weight of ears and grains and crop yield, regardless of the cultivar. In agreement with Kozlowski (2002), weed interference throughout the crop cycle can reduce production by 87%.

The off-season is characterized by a period when the cultivation areas remain fallow, soon after second crop corn harvesting until the new sowing of soybean, and may comprise the months of August and September. This rest favors the emergence of invasive species in agricultural areas, which, if not managed efficiently, can become problem at sowing and early development of soybeans.

One of the first steps for a proper weed management in growing areas involves the identification of invasive species and also species with the highest importance. Identification of such species enables a better management decision-making, whether cultural, mechanical, physical, biological or chemical. It also allows establishing an order of priority among the species present to set an effective control program (Oliveira and Freitas, 2008; Kuva et al., 2007; Yanagizawa and Maimoni-Rodella, 1999).

Due to the great importance of identifying weed species, several floristic composition works have been carried out in diverse cultures, such as Albertino et al. (2004) with guarana culture in the Amazon; Marques et al. (2010) with the cultivation of cowpea; Galvão et al. (2011) in pasture. The same is observed with phytosociological surveys in irrigated rice (Erasmo et al., 2004); in sugarcane production (Oliveira and Freitas, 2008); in green sugarcane (Kuva et al., 2007); in sunflower crops (Adegas et al., 2010) and in soybean (Pereira et al., 2000). There are no studies on corn, especially with second crop corn.

Given the importance of second crop corn for the country and regional economy and the losses caused by weed infestation and also the need for drying the areas before planting soybean, an efficient control program becomes essential. In this way, weed identification works are extremely important for the studied regions and crops that may be infested.

In this context, the present study aimed to perform a phytosociological survey, and to analyze the composition and floristic similarity of weeds in the second crop corn during the off-season period in Western Paraná State.

MATERIALS AND METHODS

For this study, two surveys of weeds were carried out, one in the late corn cultivation, and another in the off-season period, considered a short period of about 1 month. At the end of the corn crop cycle, the study was conducted in the municipalities of Palotina, with predominant soil classified as Eutroferric Red Oxisol and Eutroferric Red Nitossoil from very clayey to clayey texture (with areas 1, 2, 7, 8 and 9); Terra Roxa, with soil classified as Distroferric Red Oxisol of medium texture (area 3); Francisco Alves, with soil classified as Distroferric Red Oxisol of medium texture (areas 4 and 5); and Marechal Cândido Rondon, with soil classified as Distroferric Red Oxisol of clayey texture (as area 6). The climate of all municipalities is subtropical (Cfa), according to the Köppen classification, with no defined dry season. A total of nine areas under no-till system with crop succession: Soybean (first crop) - Corn (second crop) were sampled.

In the off-season period, six areas under no-till system with crop succession: Soybean (first crop) - Corn (second crop) were sampled. The survey was conducted in the municipalities of Palotina (areas 1 and 2), Terra Roxa (3), Francisco Alves (4 and 5) and Marechal Cândido Rondon (6), with the same description of soil and climate mentioned above.

For weed survey, the square inventory technique (Braun-Blanquet 1979) was used, with a square of 1.0 x 1.0 m, totaling 1 m^2. The sampling design consisted of three collection range, the first starting at 5 m from the edge, followed by 25 and 50 m (locations 1, 2 and 3, respectively). Five sampling sites of 1 m^2 were set in each collection range, totaling 15 samples (15 m^2) per area. With the sample model, it was possible to measure the weeds by area and the occurrence over the edge or at the time it distanced itself from the same.

Importance value index (IVI) is calculated based on the sum of the values of relative frequency, relative density and relative abundance. For similarity analysis (estimated similarity in species composition) between botanical populations, the Sorensen similarity index (SI) was calculated as follows: $SI = (2a/b + c) \times 100$, where a = number of species common to both areas; b and c = total number of species in both areas compared. SI range from 0 to 100, being maximum when all species are common to the two areas, and minimum when there is no species in common.

After the identification and counting, data were analyzed for density, relative density, frequency, relative frequency, abundance, relative abundance and importance value index, as proposed by Mueller-Dombois and Ellemberg (1974).

RESULTS AND DISCUSSION

In the nine areas of the second crop corn, 28,516 thousand plants were sampled, they divided into 29 species, including seven monocot species (representing 23% total species), 22 dicot species (representing 77% total species). The results confirm the works of Albertino et al. (2004), in guarana culture, which found 80.5% dicots, 15% monocots and 4.5% pteridophytes, Marques et al. (2010), found 74.51% of dicots in cowpea crop.

In accordance with Pitelli (1987), weeds selected usually have botanical characteristics very similar to the culture, which was not verified herein, because the greatest

number of species found was represented by dicots, a fact that can be because the survey has been made at the end of the crop growth cycle, or because the previous crop was soybeans.

The 29 species found are grouped into 17 families, the most representative are Asteraceae and Poaceae, with 6 species each. These families are also the major ones in other crops, as in sunflower (Adegas et al., 2010) and in sugarcane cultivations (Oliveira and Freitas, 2008). Brassicaceae and Euphorbiaceae had 2 species each. The other families had only one species each, as listed in Table 1.

In the six areas of off-season, 13,697 thousand plants were sampled, they divided into 35 species, of which 8 are considered monocots (representing 22.8% of the species), dicots were represented by 27 species (representing 77.2% of the species). The 35 species are grouped into 18 families, standing out Asteraceae and Poaceae with 8 and 7 species, respectively (Table 1).

It was observed that the predominance of some species showed a high value of importance in the phytosociological context because of their great phenotypic adaptability. In second crop corn, some species stood out in relation to their importance value: *Parietaria* spp. (150), *Conyza* spp. (32.2), *Stachys arvensis* L. (18) and *Gnaphalium spicatum* (17) (Figure 1). *Parietaria* spp. presented the highest value of importance value over the other species.

Parietaria spp. is the species with the greatest potential to damage the next crop, or that should receive special attention in the post-harvest management, in order to reduce the seed bank in the soil and negatively affect the dynamics of weed populations in the agro-ecosystem. This is a species little known in Brazilian environments, thus, it is believed that it deserves more attention in future studies. Another weed that deserves attention is *Conyza* spp., which showed IVI of 32.2 (Figure 1). This species is highly competitive with crops and rapidly proliferates, which facilitates its spread across the cultivated areas. In an area without soil management, *Conyza canadensis*, at a density of 10 plants m^2, can produce about 200 thousand seeds per plant (Bhowmik and Bekech, 1993). In turn, *Conyza bonariensis* can produce an average of 110 thousand seeds per plant (Wu and Walker, 2004). Another characteristic that provides high capacity for multiplication is the seed dispersal ability of *Conyza* spp. For dispersal by wind, there were adaptations in achenes, which are formed by structures called papus (Andersen 1993), this characteristic gives the achenes the possibility of being carried on the wind for long periods (Regehr and Bazzaz, 1979). For instance, *C. canadensis* can disperse its seeds in the wind for distances greater than 100 m (Dauer et al., 2006). On the other hand, in a corn crop, distribution of *C. canadensis* seeds was 14,950 m^2 at a distance of up to 6 m from the mother plant; at a distance of 122 m, there was, on average, 149 seeds m^2 (Loux et al., 2006).

Besides the high competition with grown plants, the high multiplication and dispersal rates, *Conyza* spp. may have biotypes resistant to one or more active ingredients, such as the case of *Conyza sumatrensis*, which exhibited biotypes resistant to glyphosate and chlorimuron-ethyl in the state of Paraná (Santos et al., 2012). This resistance makes its control even more difficult, allowing the permanence of this weed species in the field, and the growth of descendants more frequently, if effective control measures are not taken.

In the off-season period, as illustrated in Figure 2, *Parietaria* spp. again stood out from the other species, with IVI of 164.60, followed by *Gnaphalium spicatum* (35.13), *Richardia brasiliensis* G. (13.08) and *Conyza* spp. (11.78). These species are noteworthy in the off-season period, as they will be dried out for planting soybeans, therefore, by knowing the weeds prior to drying, it is possible to choose the best method for control of weeds. Moreover, combining the information of weeds present in the late cycle, with those present at soybean pre-sowing, it is possible to outline the best drying system that precede the first growing season, as well as understand the dynamics of weed populations.

In the drying period, it is essential to select the best active ingredient to control weeds. Among the most widely used active ingredients, glyphosate stands out, which is a post-emergence herbicide, which belongs to the chemical group of substituted glycines, classified as non-selective and of systemic action, with a broad spectrum of action, which enables excellent control of annual and perennial weeds, both narrow and broad-leaved species (Galli and Montesuma, 2005). However, the weed floristic monitoring allows understanding the selection pressure process of herbicides, such as glyphosate, thus serving as indicators to prevent the increase of species resistant to certain molecules.

The high IVI of *Parietaria* spp. and low IVI of other species can be assigned to interspecific competition with *Parietaria* spp. in this period (possibly due to better environmental conditions for their development), which can be dominant over other species, thereby inhibiting their development (Jakelaitis et al., 2003).

In areas planted with second crop corn, 5 m (Figure 3A) inside the crop again, *Parietaria* spp. showed the highest IVI (26.83), in that range of 5 m, the total percentage of plants was 18.89% of the total plants of this species found in the areas. Another species with high IVI was *Conyza* spp. (16.28) and plants concentrated in this first analysis range with 66.88% of plants found in the areas. *Stachys arvensis* L. showed IVI of 14.11 and about 91.22% plants were found in the first five meters into the crop, with little representation inside the crop.

Conyza spp. and *Stachys arvensis* L. were found in the first 5 m (edges) of the winter corn crop, which can be justified by higher traffic of vehicles beside the crop, which can cause compaction, preventing seed germination or resulting in smaller plants as it involves a higher incidence

Table 1. Floristic composition of weeds in nine areas at the end of the second crop corn cycle in four municipalities in Western Paraná State.

Family	Scientific name	Common name
Amaranthaceae	*Amaranthus* spp.	Pigweed
Apiaceae	*Apium leptophyllum*	Marsh Parsley
Asteraceae	*Ambrosia elatior* L.	Annual Ragweed
	Soliva pterosperma	Field Burrweed
	Conyza spp.	Horseweed
	Gnaphalium spicatum	Cudweed
	Bidens spp.	Beggarticks
	Sonchus oleraceus L.	Sowthistle.
	Emilia fosbergii	Florida Tasselflower
	Siegesbeckia orientalis L.	St. Paul's-wort
Brassicaceae	*Raphanus sativus* L.	Cultivated Radish
	Coronopus didymus L.	Lesser Swine-cress
Commelinaceae	*Commelina benghalensis* L.	Tropical spiderwort
Cyperaceae	*Cyperus* spp.	Papyrus Sedges
Euphorbiaceae	Chamaesyce hyssopifolia L.	Small Hyssopleaf Sandmat
	Euphorbia heterophylla	Milkweed
Fabaceae	*Glycine max* L.	Voluntary Soybean
Urticaceae	*Parietaria* sp.	Spreading Pellitory
Malvaceae	*Sida* spp.	Flannel Weed
Poaceae	*Digitaria sanguinales* L.	Hairy Crabgrass
	Avena spp. L.	Oat
	Digitaria horizontalis	Wild Jamaican Crabgrass
	Sorghum arundinaceum S.	Sorghum Bicolor
	Digitaria insularis L.	Sourgrass
	Cenchrus echinatus L.	Southern Sandbur
	Eleusine indica L.	Indian Goosegrass
Rubiaceae	*Richardia brasiliensis* G.	Brazilian calla-lily
Convolvulaceae	*Ipomoea triloba* L.	Littlebell
Caesalpinioideae	*Senna obtusifolia* L.	American Sicklepod
Caryophyllaceae	*Stellaria media* L.	Chickenwort
Phyllanthaceae	*Phyllanthus tenellus*	Leafflower
Solanaceae	*Solanum americanum*	Glossy Nightshade
Lamiaceae	*Stachys arvensis* L.	Field Woundwort

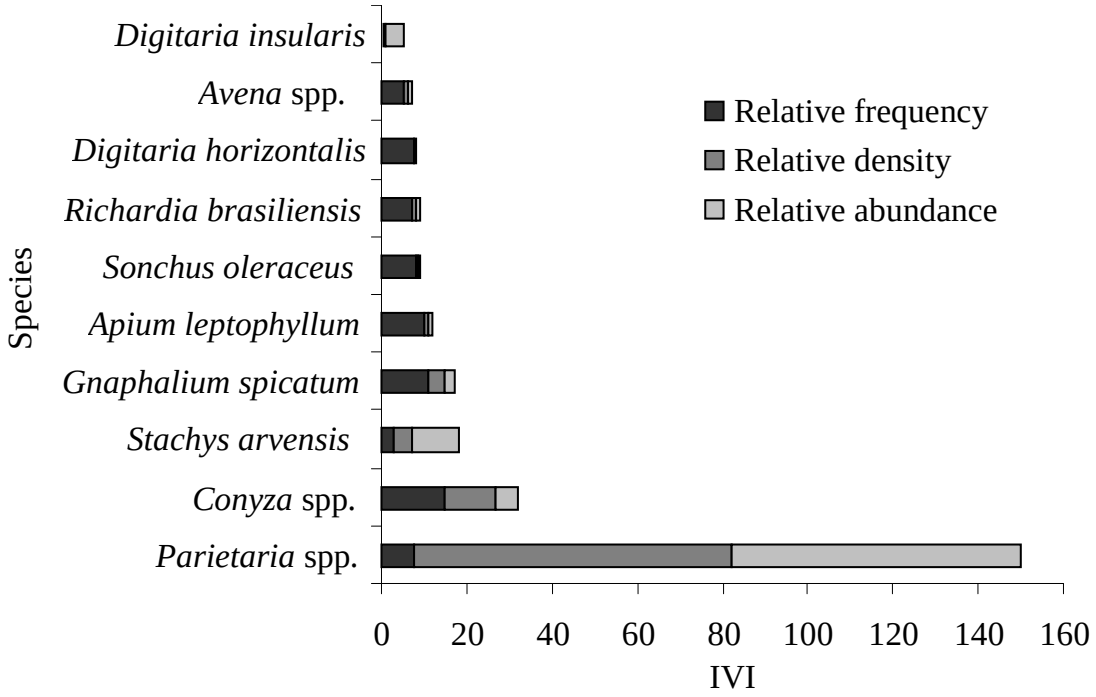

Figure 1. Importance value index of the major weed species in nine areas planted with winter corn in four municipalities of Western Paraná State.

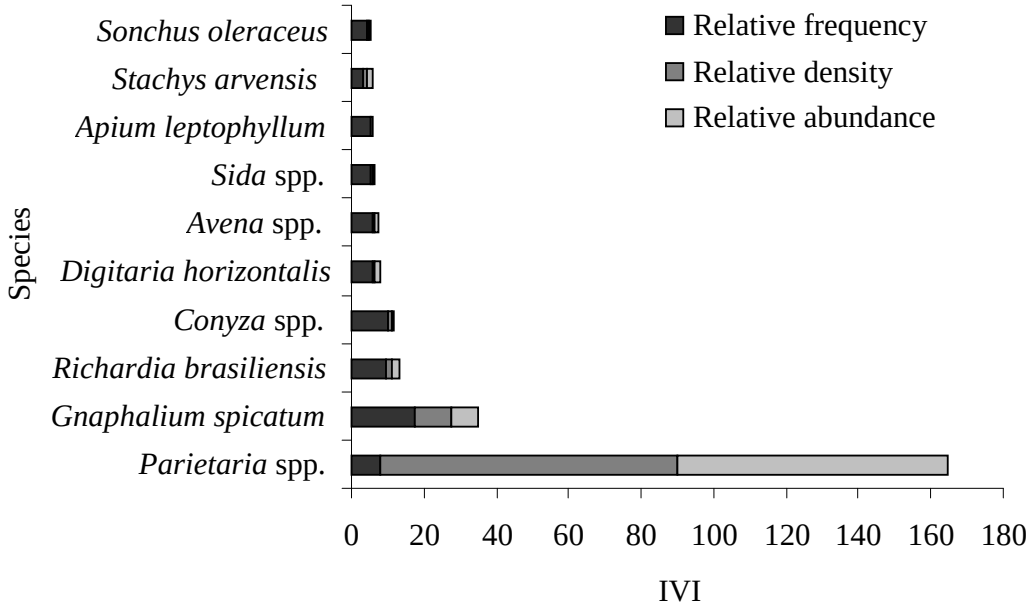

Figure 2. Importance value index of the major weed species in six areas in the off-season period in four municipalities of Western Paraná State.

of light. In addition, the service vehicles of the farmer or even vehicles that travel on the side of the farm, can bring seeds from infected areas, thus favoring their spread in these five meters. In the case of *Conyza* spp., the lightness can result in higher germination of seeds, which can reach 78% germination at 20°C (Vidal et al., 2007). In corn crops, these species can cause economic damage because they compete with the crop for nutrients and light, and these plants can be a source of proliferation and dispersal of seeds throughout the area, so, the proper

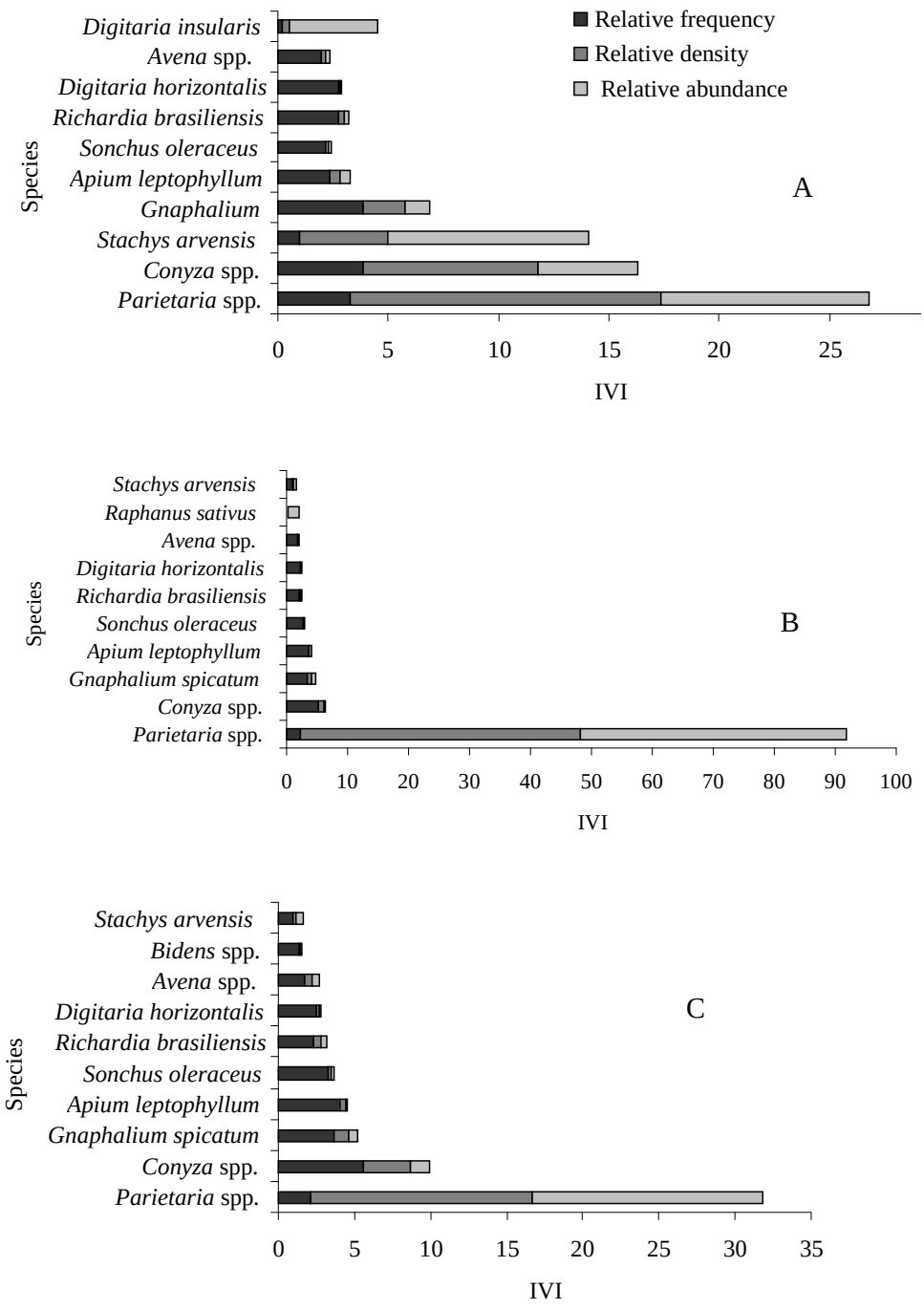

Figure 3. Importance value index of the major weed species located at 5 m (A), 25 m (B) and 50 m (C) inside the crop in nine areas planted with second crop corn in four municipalities of Western Paraná State.

management of weeds in the crop edges is necessary. Thus, monitoring of different distances from the edge allows understanding the process of dispersal and colonization of species within the agro-ecosystem.

At 25 m (Figure 3B), *Parietaria* spp. showed the highest IVI (91.83), representing, in this range, 61.58% of total plants. Field observations indicate that this species prefers areas with greater moisture content and preferably shade. *Conyza* spp. had the second highest IVI in this sampling range (6.44), with 25.96% of plants found in the areas. At 50 m (Figure 3C), some species showed large differences, *Parietaria* spp. presented 61.58% in the 25 m of sampling, demonstrating that this species may prefer areas closer to the edges of the crops; also, for the same species, at 5

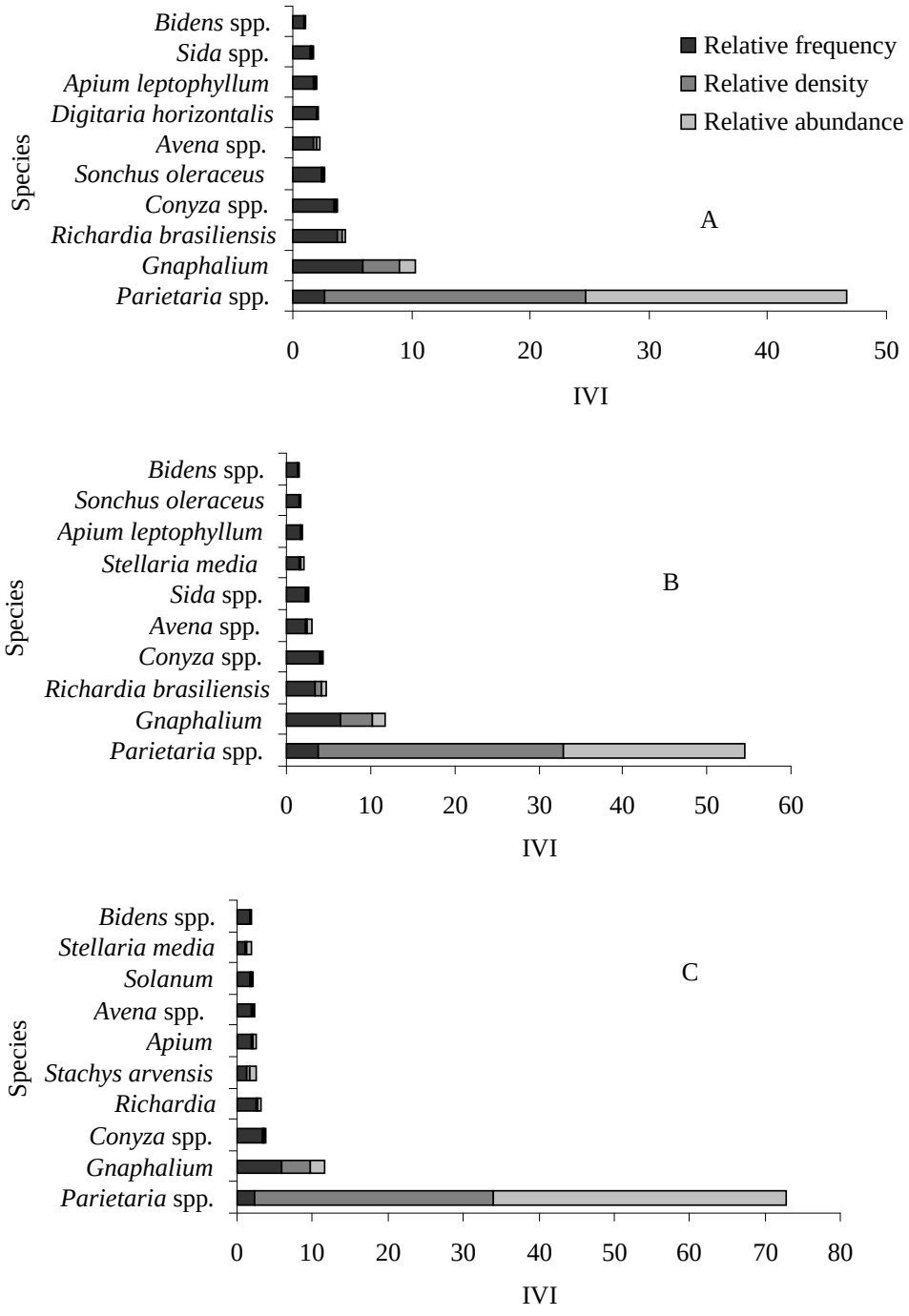

Figure 4. Importance value index of the major weed species located at 5 (A), 25 (B) and 50 m (C) inside the crop in six areas in the off-season period in four municipalities of Western Paraná State.

and 50 m, percentage of individuals is 18.89 and 9.52%, respectively. Of the total of 10 main species sampled at the end of the second crop corn cycle, 5174, 14596 and 8395 were in the range of 5, 25 and 50 meters with a total percentage of 18.4; 51.8 and 29.8, respectively.

In areas that are in the off-season period, at the distance of 5 m inside the crop (Figure 4A), again the species *Parietaria* spp. had the highest IVI (46.71); in this range 21.73% of all plants of this species were found. *Gnaphalium spicatum* Lam. had the second IVI (10.30) and the total in this range was 66.07%. At 25 m (Figure 4B), *Parietaria* spp. Exhibited the highest IVI (54.49),

Table 2. Similarity index between the areas of second crop corn and off-season together with their respective sampling range.

	Comparison criterion	Similarity index (%)
Areas	Second crop corn X off-season	90.6
Range		
5	Second crop corn X off-season	89.6
25	Second crop corn X off-season	81.4
50	Second crop corn X off-season	62.5

followed by *G. spicatum* Lam., both species had the highest total percentage of plants in this range, 68.78 and 20.33%, respectively. Similarly, for the 50 m (Figure 4C), *Parietaria* spp. and *G. spicatum* Lam. presented the highest values of IVI, 72.85 and 11.57, respectively.

There was a high similarity between species found in the second crop corn and in the off-season period (Table 2) given the short time separating the two stages. Among the evaluation range, the 50 m-range had the lowest similarity to one another; this dissimilarity can be attributed to some weeds with uneven distribution in the sampling range, indicating the adaptation of different species to the crop perimeter and also the process of species occupation from the edge to the inside (spread of invasive species).

Conclusions

The main families of weeds found after harvesting the second crop corn were Asteraceae and Poaceae. The species with the highest occurrence were *Parietaria* spp., *Conyza* spp., *G. spicatum* Lam. and *S. arvensis* L.

Differences between families and species, as well as the occurrence of tolerant or resistant weed species, enable studies on the best method for control or the best active principles or modes of action of herbicides available in the market for their control.

From the time the weed community is known, it is possible to choose the best drying method that ensures the best development of the following crop, without interference of the presence of weeds.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

In vitro seed germination and seedling development of *Withania somnifera* (L.) Dunal

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An efficient and improved protocol for *in vitro* seed germination and seedling development technique of *Withania somnifera* (L.) Dunal have been developed. Murashige and Skoog (MS) medium containing 3.0 mg l^{-1} gibberellic acid (GA_3) and 3.0 mg l^{-1} Kinetin (Kn) was found effective for maximum germination percentage (92.67), germination rate (1.83), germination value (56.07) and seedling vigour index (875.73). Whereas minimum days required for germination (8.30), maximum germination speed (6.15), shoot length (7.72 cm), weight of shoot (4.48 g), weight of root (1.83 g), fresh weight of seedlings (5.91 g), dry weight of seedlings (0.78 g), number of leaves per plantlet (5.57) and plant height (8.79 cm) was recorded in MS medium containing 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn. The present protocol clearly describes that *W. somnifera* (L.) Dunal seeds should be germinated first in MS medium containing 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn and after that the completely germinated seeds should be subcultured in MS medium supplemented with growth hormones 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn for seedling development.

Key words: *In vitro*, seed germination, seedling development, *Withania*, medicinal herb.

INTRODUCTION

Withania somnifera (L.) Dunal, is an important herb in the ayurvedic and indigenous medical systems for over 3000 years (Sharma et al., 2010). Both leaves and roots of the plant are used as the drug and steroidal lactones occur in both parts. Roots are prescribed as medicines for hiccups, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammation, and skin diseases. The active pharmacological components of *W. somnifera* are steroidal lactones of the withanolide type. Several chemotypes have been found differing in their withanolide content. The principal withanolide in Indian *W. somnifera* are withaferin A and withanolide D (Ganzera et al., 2003).

According to red list of threatened species, *W. somnifera* proved to be 99.75% of the endangered medicinal plant (Siddique et al., 2005; Rahman, 2001). This medicinally important plant species has been depleted from their natural habitat and is now included in the list of threatened species by The International Union for Conservation of Nature and Natural Resources

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(Kavidra et al., 2000). Usually, *Withania* is propagated commercially by seeds because of the lack of natural ability for vegetative propagation, but the seed viability is limited to one year making the long duration seed storage futile (Sen and Sharma, 1991; Rani and Grover, 1999; Farooqi and Sreeramu, 2004). Seed propagation, however, is not always satisfactory, since percentage of germination is low, due to the presence of certain inhibitory compounds in the fruit and high risk of catching various diseases (De Silva and Senarath, 2009). This resulted in the adulteration of plant materials, making the plant endangered (Antonisamy et al., 2000). Again, multiplication through cuttings give rise to less ramified plants and is consequently less productive than plants obtained from seeds (Supe et al., 2006). However, the conventional propagation method cannot meet the increasing demand of this plant used as raw material for the preparation of pharmaceutical products. Due to poor viability of stored seed and little information regarding seed germination of *W. somnifera* an alternative procedure of propagation through *in vitro* seed germination and seedling development is essential. *In vitro* propagation of *W. somnifera* through sequential procedure of induction of callus, shoot regeneration and rooting take more time and costly comparing to *in vitro* seedling regeneration using single media. The immature seeds obtained from green pods of *W. somnifera* can be germinated asymbiotically *in vitro* for rapid micro propagation (Murashige and Skoog, 1962). The method can be exploited for the rapid propagation and conservation of *W. somnifera.*

Therefore, the present study was carried out to optimize the concentration of gibberellic acid (GA_3) and Kinetin (Kn) in MS media for *in vitro* seed germination and seedling development of *W. somnifera* by using *in vitro* technique.

MATERIALS AND METHODS

The seeds of Indian cultivar *W. somnifera* were collected from the plants grown at Horticultural Research Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India. The health and density of seeds were tested by dipping them in water. Seeds that float were discarded and healthy seeds were selected for sterilization. The viability of seeds were tested by the 2,3,5-triphenyltetrazolium chloride (TTC) test (Hartman et al., 1990). All the chemicals and reagents were purchased from Hi Media (Mumbai, India) and plant growth regulators were procured from Sigma-Aldrich (Bangalore, India).

Seeds were washed thoroughly with running tap water for 5 to 10 min to remove surface dirty particles and then sterilized by immersing in 70% ethanol for 1 min with vigorous shaking followed by 20 min in 4% sodium hypochlorite containing one drop of Tween-20. The seeds were then rinsed three times with sterile distilled water in a laminar air flow cabinet to remove minor amounts of disinfection liquid. The surface-sterilized seeds were used for the treatments of *in vitro* germination trials (Figure 1a).

For germinating, the surface-sterilized seeds were cultured in jam bottle of standard Murashige and Skoog medium containing 3% sucrose and 0.6% agar alone and along with different concentrations of GA_3 (0.5 to 5.0 mg/l) and Kn (0.5 to 5.0 mg/l) in combination for their synergistic action. For induction of culture for seed germination and seedling development, the following media were used: (a) M {MS without growth regulators (control)}; (b) MG_1K_1 {MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}}; (c) MG_2K_2 {MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}}; (d) MG_3K_3 {MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}}; (e) MG_4K_4 {MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}}; (f) MG_5K_5 {MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}}.

The pH of the medium was adjusted to 5.8 before the addition of 0.8% (w/v) agar. All cultures were incubated under controlled condition at 25 ± 2°C temperature, 60 ± 10% relative humidity and 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 20 µmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps (2 × 40 W, Phillips, India).

The cultures were observed daily and the data on daily seed germination was collected until the completion of the germination (maximum up to 30 days). The seeds with 0.5 mm or more radical growth were counted as germinated seeds (Figure 1b). The final germination percentage (Gp) was calculated from the total seeds that germinated on the day of completion. The other germination parameters, such as germination speed (GS), germination rate (Rs) (Rajabi and Poustini, 2005), and germination value (GV) (Djavanshir and Pourbeik, 1976) were calculated. Different growth parameters such as seedling vigour index (SVI) (Abdual Baki and Anderson, 1973) and growth value (GV) (Meredith, 1978) were calculated. Root length and shoot length of the seedlings were recorded (Figure 1e) and root to shoot ratio was calculated. Fresh weight (FW) of seedlings was recorded and dried in hot air oven at 60°C until constant weight and then dry weight (DW) of seedlings were recorded. Moisture content of seedlings was calculated using formula:

(Fresh weight – Dry weight / Fresh weight) × 100

Plantlets with well developed shoots and roots (Figure 1c and d) were transferred to plastic cup containing autoclaved perlite (Figure 1. f) and maintained for four weeks in culture room. The plantlets were then transferred to poly cups containing garden soil and were maintained in a shade net house (Figure 1g).

The experiments were designed in Completely Randomized Design (CRD). In each treatment, 50 seeds were inoculated at 5 seeds per jam bottle and each treatment was replicated four times. The statistical analysis was done by employing the O.P Stat software packages (O.P. Sheoran, 1968) and the mean were compared using Duncan's multiple range test (DMRT) at the 0.05% probability level.

RESULTS AND DISCUSSION

Seed germination and seedling growth are known to be regulated by exogenous hormones. Growth regulators used in pre-sowing seed treatment with growth regulator play an important role in regulating germination and vigour (Raghav and Kasera, 2012). Gibberellins are a family of 136 tetracyclic diterpenes, a small subset of which are active as plant hormones and known to stimulate seed germination in a wide range of plant species, the predominant active GA depends on the species (Thomas et al., 2005)

Seed germination

It is evident from the data presented in Table 1 that days

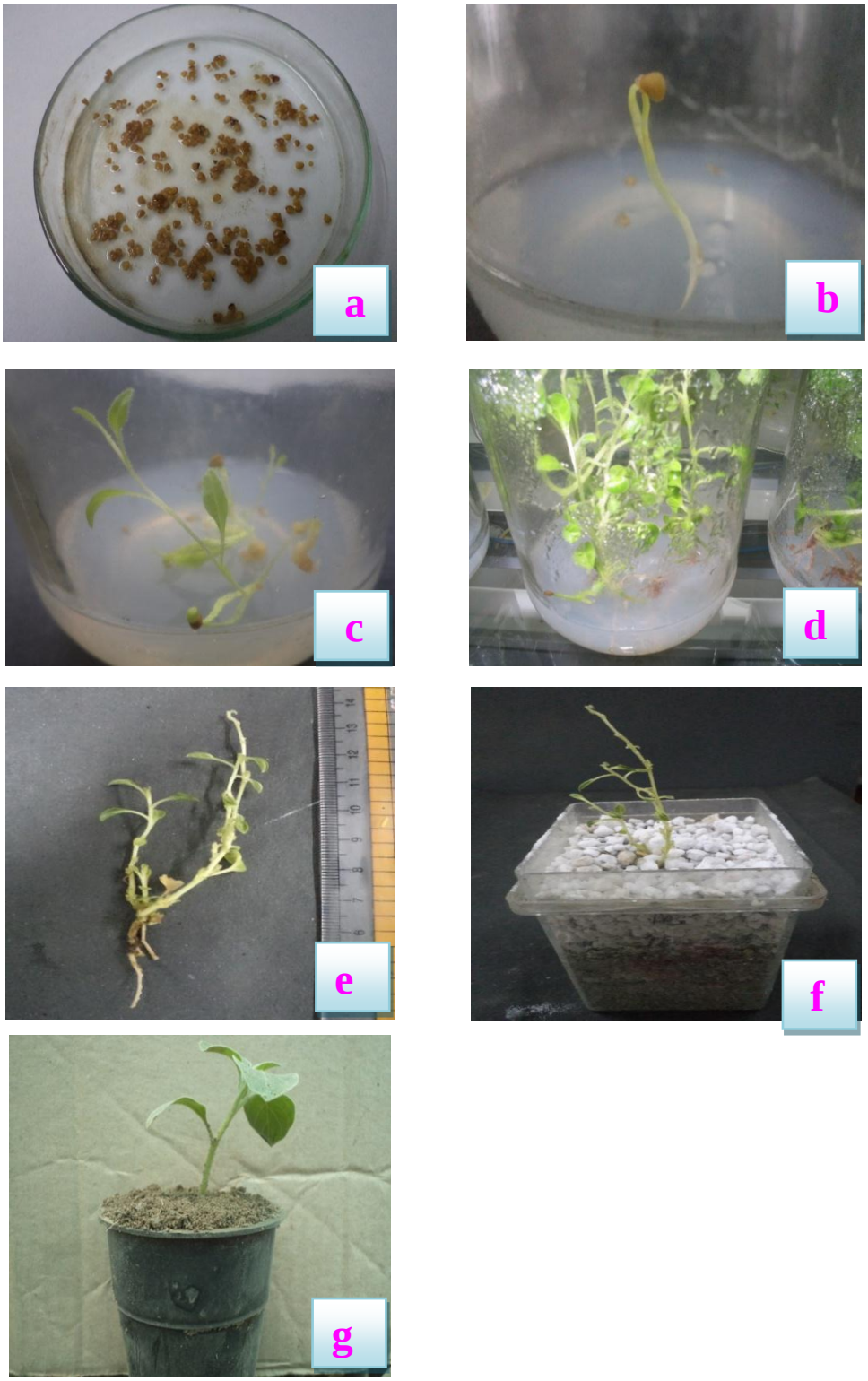

Figure 1. a. Treated seeds of Aswagandha for inoculation, b. Germinated seeds at growing stage, c. *In vitro* seedling at development stage, d. *In vitro* seedlings with profuse rooting, e. *In vitro* raised seedling, f. *In vitro* germinated seedling transferred to hardening media in plastic container, g. Two months old plantlets in hyco pot.

Table 1. Effect of growth regulator concentrations on *in vitro* seed germination characteristics of *Withania*.

Treatment	Treatment combinations	Days to germination	Germination percentage	Germination Speed (GS)	Germination rate (GR)	Germination value (GV)	Seedling vigour index (SVI)
M	MS	14.23	68.53	3.37	1.12	23.09	509.86
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	12.75	75.25	4.02	1.26	30.25	643.39
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	11.32	83.67	5.02	1.56	42.00	754.70
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	10.27	92.67	6.05	1.83	56.07	875.73
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	9.93	85.67	5.84	1.81	50.03	861.84
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	8.30	77.33	6.15	1.81	47.56	827.43
SEM±		0.53	1.285	0.199	0.130	0.904	25.38
CD at 5%		1.66	4.004	0.620	0.405	2.815	8.291

required for germination were decreased by increasing concentration of GA_3 with higher Kn rate. Among the different combinations of GA_3 and Kn, minimum days required for germination (8.3 days) was noted in treatment MS medium supplemented with 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn which was at par with the days required in treatment MG_4K_4. Maximum days (14.23) for germination were recorded on MS medium without growth regulators. The results corroborate the findings of the experiments conducted by Mello (2009). The significantly highest germination percentage (92.67%) was recorded in MS medium supplemented with 3.0 mg l^{-1} GA_3 along with 3.0 mg l^{-1} Kn followed by MG_4K_4 (85.67%). The least germination percentage (68.53%) was observed in MS medium containing no growth regulators. An improvement in seed germination with application of GA_3 was evidenced, but its concentration beyond optimum dose causes reduction in germination percentage (Dhoran and Gudadhe, 2012). The present investigation showed that seed germination percentage increases with increasing rate of GA_3 along with increasing concentration of Kn, but not beyond 3 mg l^{-1} each of GA_3 and Kn. The finding is in line with that of Kaur et al. (1998) which reported the enhanced germination and seedling growth of chick pea when seeds were treated with Kn in combination with GA_3. The maximum germination speed (6.15) was observed in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn, while the least germination speed (3.37) was recorded under control (M). This may be due to the fact that germination speed is greatly enhanced by higher rate of GA_3 application (Dhoran and Gudadhe, 2012). The highest germination rate (1.83) was recorded in MS medium supplemented with 3.0 mg l^{-1} GA_3+ 3.0 mg l^{-1} Kn, while the minimum (1.12) was recorded in MS medium which is devoid of growth regulators. The result is in agreement with the findings of Mello (2009) who reported that GA_3 increases the rate of seed germination. This might be due to effectiveness of GA_3, which at higher concentrations overcome dormancy, causing rapid germination of seed. Higher concentration of GA_3 and Kn proved more effective from their respective lower concentration. Application of GA_3 and Kn at various combinations significantly influenced the germination value over the control (M). The maximum germination value of 56.07 was recorded in MS medium supplemented with $GA_3$3.0 mg l^{-1} + Kn 3.0 mg l^{-1}. The control treatment (M) showed minimum germination value (23.09). The observation supported the report that higher concentrations of GA_3 improve germination value (Naeem et al., 2004).). MS medium when supplemented with 3.0 mg l-1 GA3 + 3.0 mg l-1 Kn produced maximum seedling vigour index (875.73) followed by the treatment MG4K4; while the minimum seedling vigour (509.86) was noticed in control treatment. The result is in agreement with the finding of Mello et al. (2009) which reported that the GA3 treated seedlings of Penstemon digitalis cv Husker Red showed highest vigour index during light period.

Seedling growth

The data presented in Table 2 shows that MS medium when supplemented with 3.0 mg l^{-1} GA_3 + 3.0 mg l^{-1} Kn produced maximum seedling vigour index (875.73) followed by the treatment MG_4K_4; while the minimum seedling vigour (509.86) was noticed in control treatment. The result is in agreement with the finding of Mello et al. (2009)

Table 2. Effect of growth regulator concentrations on seedling development characteristics of *in vitro* raised *Withania*.

Treatment	Treatment combinations	Shoot length (cm)	Root length (cm)	Shoot/root length ratio	Weight of shoot (g)	Weight of root (g)	Shoot/root weight ratio
M	MS	4.21	3.23	1.30	1.72	0.87	1.98
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	4.97	3.58	1.39	2.36	1.04	2.27
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	5.14	3.88	1.32	3.23	1.18	2.74
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	5.27	4.18	1.26	3.84	1.29	2.98
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	5.41	4.65	1.16	4.16	1.65	2.52
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	5.72	4.98	1.15	4.48	1.83	2.45
SEM±		0.116	0.193	0.043	0.061	0.017	0.037
CD at 5%		0.363	0.602	0.134	0.190	0.053	0.116

which reported that the GA_3 treated seedlings of *Penstemon digitalis* cv Husker Red showed highest vigour index during light period. Maximum shoot length (5.72 cm) was recorded to be significantly higher than the rest of the treatment combinations when the germinated seeds were transferred to the MS media supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn which is at par with the treatment MG_4K_4. The minimum shoot length (4.21 cm) was observed in MS containing no growth regulators. Similar result was observed by Tolera et al. (2009) in *Saccharum officinarum* where application of GA3 and Kn showed significant higher value for shoot length as compared to control.

This might be due to the fact that increase in both the concentrations of GA_3 and Kn triggers the cell elongation and faster multiplication of the cells that results in rapid growth and development of the seedlings as compared to the treatments with lower hormonal concentrations. The maximum root length (4.98 cm) was observed in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn which is at par with the treatment MG_4K_4, while the control treatment (M) produced minimum root length (3.23 cm). This finding is in close affirmation with the findings of Naeem et al. (2004). This may be due to the fact that GA_3 is better for inducing root growth and has the tendency of increasing root length with the increase of GA_3 concentration in the MS medium (Ribeirio et al., 2009). Significantly, the least shoot/root ratio (1.15) was recorded in MS medium supplemented with 5.0 mg l^{-1} GA_3+ 5.0 mg l^{-1} Kn (MG_5K_5) whereas the maximum shoot/root ratio was recorded in the treatment MG_1K_1.

The maximum shoot weight (4.48 g) was produced in treatment MG_5K_5, that is, MS supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn followed by the treatment MG_4K_4 (4.16 g). Least shoot weight (1.7 g) was observed in control. The result is in conformity with the findings of Ribeirio et al. (2009) in Lentil; Kaul and Farooq (1994) in Morning Glory; and Chaudhary and Khan (2000) in Okra where they concluded that the hormone Kn show inhibition in shoot length and number of internodes. It may also be associated with a significant expansion in diameter of shoot and an increase in area of leaves as well as their number resulted in overall gain in weight of seedlings (Naeem et al., 2004). The maximum weight of the root (1.83 g) was recorded in the treatment MG_5K_5 followed by the treatment MG_4K_4. While the minimum root weight (0.87 g) was recorded in control (M). This might be due to the addition of biomass per plant with increasing concentration of GA_3 with respect to the root number. Increase in GA_3 concentration might also result in an exponential increase in the number of roots without any phytotoxic effect of GA_3 on root formation even at higher concentration of used GA_3 (Ribeirio et al., 2009). Application of GA_3 and Kn at various combinations significantly influenced the shoot/root weight ratio. The least shoot/root weight ratio of 1.98 was recorded in control treatment (M) where the more shoot/root weight ratio was found in treatment MG_3K_3.

The data presented in Table 3 showed that the maximum fresh weight of seedling (5.91 g) was observed in the treatment MG5K5, that is, MS + 5.0 mg l-1 GA3 + 5.0 mg l-1 Kn followed by the treatment MG4K4 (5.19 g). While the minimum seedling fresh weight (3.13 g) was recorded in control (M). Significantly, the maximum dry weight of the seedling (0.78 g) was recorded in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn followed by MG_4K_4

Table 3. Effect of growth regulator concentrations on seedling development characteristics of *in vitro* raised *Withania*.

Treatment	Treatment combinations	Fresh weight of Seedlings (g)	Dry weight of seedlings (g)	Moisture content of seedling	Number of leaves per seedling	Seedling height (cm)
M	MS	3.13	0.30	90.42	4.12	5.25
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	3.61	0.36	90.03	4.44	5.95
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	4.17	0.41	90.17	4.69	6.71
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	4.84	0.53	89.05	4.84	7.14
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	5.19	0.67	87.09	5.18	8.22
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	5.91	0.78	86.80	5.57	8.79
SEM±		0.015	0.009	0.406	0.080	0.164
CD at 5%		0.048	0.028	1.265	0.251	0.511

(0.67 g) and the least was observed in control (0.30 g). The minimum moisture content in seedling (86.80%) was found in MS medium supplemented with GA_3 5.0 mg l^{-1} and Kn 5.0 mg l^{-1} whereas maximum (90.42%) was observed in control treatment. The result is in line with the finding of Stojicic et al. (2012) where the highest seedling dry weight of *Pinus peuce* was obtained by application of higher concentration of GA_3. The reason might be due to the addition of biomass per plant with increasing concentration of GA_3 with respect to the root number.

The maximum leaf number (5.57) of the seedling prior to transfer to the hardening media was observed in the treatment MG_5K_5 which is followed by the treatment MG_4K_4, while the least (4.12) was observed in control. The finding is in close concurrence with that of Kedia et al. (2012) which reported the significant increase in the number of internodes as well as number of leaves on application of combined of GA_3, IAA and Kn. Moreover, the similar effect of GA_3 application on leaf number was found in *Annona crassiflora* (Ribeirio et al., 2009). The maximum seedling height (8.79 cm) at the stage prior to transfer to hardening media was observed in the treatment MG_4K_4 containing MS medium with 4.0 mg l^{-1} GA_3 + 4.0 mg l^{-1} Kn, while the minimum seedling height (5.25 cm) was observed in the control treatment (M). The present observation is in harmony with the result obtained by various workers and they reported that application of growth regulators enhance plant growth (Hernandez, 1997; Ashraf et al., 1987; Ashraf et al., 1989) and stem length (Lee et al., 1999; Kabar, 1990). Application of GA_3 accelerates bud development and stem elongation, but the best results can be achieved if GA_3 is applied in combination with kn (Hernandez, 1997; Bagatharia and Chanda, 1998).

Conclusion

From the present investigation, it may be concluded that growth hormones gave significantly better response than control both in seed germination and seedling development in *W. somnifera* (L.) Dunal. MS medium when supplemented with 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn was found more effective for maximum germination percentage (92.67), germination rate (1.83), germination value (56.07) and seedling vigour index (875.73). Whereas minimum days required for germination (8.30), maximum germination speed (6.15), shoot length (7.72 cm), weight of shoot (4.48 g), weight of root (1.83 g), fresh weight of seedlings (5.91 g), dry weight of seedlings (0.78 g), number of leaves per plantlet (5.57) and plant height (8.79 cm) were recorded in MS medium supplemented with 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn.

So, the present protocol clearly describes that for *in vitro* seed germination of *W. somnifera* (L.) Dunal, MS basal medium supplemented with 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn is recommended best while subculture of the germinated seedlings in MS basal medium supplemented with 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn is recommended best for *in vitro* seedling development. Since the germination percentage of *Withania* seeds in the natural environment is very poor, the present protocol will be helpful to produce quality seedlings in large quantities and hence, conserve the rare species from natural collection.

Conflict of Interests

The authors have not declared any conflict of

interests.

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Full Length Research Paper

Characterization and evaluation of twenty rice (*Oryza sativa* L.) genotypes under irrigated ecosystems in Malawi and Mozambique

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Field experiments were carried out in Mozambique (Muirrua Rice Research Station) and Malawi (Lifuwo Rice Research Station), during 2010 and 2011 growing season to evaluate twenty rice (*Oryza sativa* L.), genotypes under irrigated ecosystem following randomised complete block design (RCBD) with three (3) replications and 20 treatments. Data on number of days to flowering, number of tillers, number of days to maturity, panicle length, number of grains per panicle, grain length, 1000-grain weight, and grain yield were assessed, and analyzed. Muirrua ecosystem produced the highest mean yield (3.96 t ha^{-1}) followed by Lifuwu ecosystem (3.42 t ha^{-1}). Nine traits, namely number of tillers, 1000-grain weight, panicle length, number of grains per panicle, grain length, number of days to 50% flowering, and number of days to maturity were positively correlated with grain yield. Significant differences (P<0.001) were found in grain length, although 19 genotypes were greater in grain length (>7.5 mm). Faya showed the highest percentage on the whole grain (60%), in comparison with Marista (20.2%).

Key words: Genotypes, ecosystem, environment, characterization, yield.

INTRODUCTION

Rice contains thousands of cultivated varieties belonging to two species, *Oryza sativa* L. grown worldwide and *Oryza glaberrima* grown mainly in West Africa (Khush, 1997). Grown on 154 million hectares worldwide in a wide range of environments (Babu et al., 2012), rice (*O. sativa* L.) is cultivated between 36 ES to 55 ES and grown from sea level to an elevation of 2500 m above the sea level or even higher (Khush and Singh, 1991; Khush, 1997). It constitutes 27% of dietary energy supply and 20% of dietary protein (Kueneman, 2006), and provides food for more than 50% of the world's population (Khush, 2005).

Mozambique is a widespread country naturally endowed with abundant lands appropriate for rice

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production (Agrifood Consulting International, 2005) and has a great diversity of local genotypes of rice, mostly concentrated in the provinces of Zambézia, Sofala and Nampula. However, there is lack of information on their trait characteristics and performance under different agro-ecological conditions including their potential when grown under irrigated environments. The persistence of the rice growers in the preference of their genotypes over the improved ones presents a special challenge for breeders and/or variety developers. Witcombe and Virk (1997) discussed that when choosing some varieties to grow, farmers consider not only the yield but also other traits that may improve their crop, and many breeding programmes do not take into account such traits at the start of breeding varieties. Breeders opt to assess traits like crop yield, plant height, plant type, number of panicles while farms may be interested in grain quality and aroma or other traits which breeders consider in later generations of breeding, often near to release of the varieties. As a result, these varieties do not meet the requirements of farmers (Tripp and Louwaars, 1997) because they show low adoption, or they do not adopt them at all.

In Malawi, rice is the second most important cereal crop after maize, and its production by smallholder farmers in fields is solely less than 1.0 ha on average (Mzengeza, 2010). It is grown under three ecosystems namely irrigated lowlands, rainfed lowlands, and rainfed uplands. Landraces are widespread and popular among farmers which play an important role in local agriculture owing to their genetic diversity (Modi, 2004) that represents wild plant populations as genetic resources (Das and Ashesh, 2014). They possess a large genetic diversity which can be used to complement and broaden the gene pool of advanced genotypes (Ahmed et al., 2012). Landraces play a vital role in the local food security and sustainable development of agriculture (Tang et al., 2009). The major objective in the rice breeding programme is to maintain the desirable traits with an increase in the yield potential of landraces (Kobayashi et al., 2006). Genetic improvement mainly depends on the amount of genetic variability present in the population, and estimation of genetic diversity between different landraces in the crop of interest is the first and foremost process in any plant breeding programme (Charrier et al., 1997).

Based on performance records, varieties and/or crops with reputation for possessing biotic and abiotic resistance can be identified for breeding. Despite this observation, re-creating genotypes with durable resistance and proper agronomic characteristics remains a great challenge in breeding. To improve food security, particularly in Central of Mozambique, where more than 60% of the country's rice is produced, there is need to study and understand farmers' genotypes through characterization and evaluation, as the first step in rice improvement. Genetic material (germplasm) is useful to scientists and plant breeders only when it has been properly characterized and evaluated because it enables scientists to study the diversity of species, search for material caused to direct introduction as cultivars, or provide genetic variability in breeding programs (Perrino et al., 1991).

Since Mozambique depends on imports to meet its rice demand, it is urgent that more productive and stress-tolerant rice varieties be developed. In view of the production constraints and possible benefits of rice, there is a need to develop technologies that are agronomically, ecologically, and economically sustainable with potential of increasing farmers' output. Information on trait characterization and genotypic performance is critical to plant scientists (plant breeders and plant physiologist) and farmers in improvement, production and utilization of rice and it will contribute significantly to improve food security, nutrition, and household cash income in Mozambique and Malawi. The general objective of this research study was to characterize and evaluate twenty rice genotypes under two irrigated ecosystems and two environmental conditions in order to identify traits and genotypes that can be utilized in the genetic improvement of rice or to introduce as cultivars in Mozambique and Malawi, specifically to evaluate yield and yield components of twenty rice genotypes in irrigated ecosystem and determine the correlation between yield and growth parameters of these genotypes.

MATERIALS AND METHODS

Twenty rice genotypes originated from irrigated ecosystems in Mozambique and Malawi were grown in both countries during 2010 and 2011 growing season. 16 genotypes (*Marista, Nassope, Rafik, Djanibwere, Djissa, Gorongosa, Paula, Niwaio, Nene, M'finico, Chibiça, Chencherica, Chupa, Mocuba, Mucandara redondo, Singano*) of the 20 genotypes were sourced from farmers' fields in Mozambique, Zambézia Province, whereas 4 genotypes (*Kilombero, Nunkile, Faya and Mtupatupa*) were from Malawi, Lifuwu Research Station. The work was carried out under field conditions in two sites including Muirrua Rice Research Station and Lifuwu Research Station.

Muirrua Rice Research Station is located in Mozambique, Zambézia Province, and District of Nicoadala about 35 km from Quelimane town, the capital of the Province. The district is in the South part of the Province, between 16° 17' and 17° 32' south latitude and 35° 12' and 37° 35' East longitude. The climate is humid tropical in the strip of the plains, and subtropical in the central plateau and the highlands (PEDD – Nicoadala, 2007). In the humid tropical climate, the relative humidity varies from 90 to 100% in the rainy season and 75 to 90% in subtropical climate. Annual mean temperature varies around 26°C in the plains and plateau, and about 20°C in the highlands (PEDD – Nicoadala, 2007). The climates possess two seasons, namely, hot and rain season from October to April, and the dry and cool season from May to September. Rainfall varies from 1,000 mm in the humid tropical areas to 1,300 mm in the highlands (PEDD – Nicoadala, 2007).

Lifuwu Research Station is situated at the foot of the Lifuwu hill,

which is a part of the expansive and seasonally flooded Katete dambo. The station is at an altitude of 500 m above sea level (masl) in approximate latitude 13° 40' South and longitude 34° 35' East. The soil type of the paddy lowland fields is predominantly vertisol, characterized by low nitrogen and phosphorus content, with pH 7 to 8. The annual average rainfall is 1,200 mm. Mean minimum and maximum temperatures are 19 and 29°C, respectively. Between May and August, absolute air temperatures may drop to 16°C, as low as what occurred during the rainfall period between December, 2010 to June, 2011.

Soil analyses of both sites are shown in Appendix 1. The optimum phosphorus availability to rice occurs at pH lower than 6.5. For upland crops, P availability is usually optimum when the soil pH is between 6.0 and 6.5. In acid soils (pH < 6.0), P is associated with iron and aluminium compounds which are available slowly to most plants. When soil pH is higher than 6.5, P is primarily associated with calcium and magnesium (Cassman et al., 1993).

The experiment was laid out in randomized complete block design with three replications and 20 treatments (genotypes). The genotypes were first established in nurseries and transplanted, one plant per hill, 25 days after sowing (DAS) in net plot of 10 m^2 (5 m long and 2 m width). The plots were kept well-watered at all stages of plant growth, supplemented water according to an irrigation scheme. Weed control was done by hand weed and no pesticide was applied. Rainfall data during the period of the trials was collected at both field sites. Minimum and maximum daily temperatures were also collected at both sites. Data collection on Agronomic parameters (days to flowering, number of tillers, plant height, days to maturity, panicle length, number of grains per panicle, grain length, grain shape, 1000-grain weight, and grain yield) were assessed according to the International Rice Research Institute (IRRI, 1996) procedures and analysed using General statistics (GenStat 14th Edition) computer statistical package. Analysis of variance was used to test the source of variation and test their influence on the genotypes under the study. The statistical model used is described as follows:

Model: $Y_{ipqr} = \mu + ß_r + G_i + E_q + S_p + (GS)_{ip} + (GE)_{iq} + (ES)_{ip} + Є_{ripq,}$

Where; Y_{ipqr} is the observed weight of grain yield within the (p^{th}) ecosystem, (i^{th}) genotypes, (q^{th}) environments, and (r^{th}) block; µ. is the population mean; E_q is the effect of the q^{th} ; B_{r-} is the effects of the r^{th}; S_P is the effect of the p^{th}; G_i is the effect of the i^{th}; GE_{qi} the effect of interaction between the q^{th} and the i^{th}; GS_{pi} is the effect of interaction between the p^{th} and i^{th}; $Є_{ripq,}$ is the random error.

Grain shape (Gsh)

The ratio of the brown rice length and brown rice width define the grain shape as suggested by Saleem et al. (2010). The scale used to describe the grain shape in rice as suggested by IRRI (1996) are: for the scale 1, the shape is slender and the ratio is over 3.0; for scale 3, the shape is medium and the ratio is from 2.1 to 3.0; for scale 5, the shape is bold and the ratio is from 1.1 to 2.0; scale 9 is the last, the shape is round and the ratio is less than 1.1. Additive main effect and multiplicative interaction (AMMI) analysis were carried out to understand whether there was interaction between genotypes and environments. The magnitude of the interactions or the differential genotypic responses to environments was analyzed using a model to determine adequate responses of specific genotypes to specific environments, rank the performance and adaptability of the genotypes in two ecosystems and two environments. The following statistical model was used:

$$Y_{ijkr} = M + G_i + E_j + B_k + \sum I_n V_{in} L_{jn} + Є_{ijkr}$$

Where; Y_{ijkr} is the yield of genotype (i) in ecosystem (j) for replicate ®; M: is grand mean; G_i is mean deviation of the genotype; E_j is mean deviation of the environmental mean; B_k is mean deviation of the block; I_n is the singular value for interaction principal component axis or IPCA (n); V_{IN} is the genotype (i) eigenvector value for IPCA axis (n); L_{jn} is the environment (j) eigenvector value for IPCA axis (n); $Є_{ijkr}$ is a random error.

The data were analysed, combined over locations and location-wise. The genotypic means were separated using Duncan's multiple range test at 5% level of probability (Gomez and Gomez, 1984), while site and environmental means were separated by using the least significant difference (LSD) test at 5% and 1% level of probability. The correlation was used to determine the strength of the relationship between grain yield and other measured variables, which these were calculated using Statistical Package for Social Sciences (SPSS) software version 16.

RESULTS AND DISCUSSION

Grain yields, yield components and growth parameters of twenty rice genotypes across the two environments

Means of yield, yield components and growth parameters of the twenty rice genotypes under the irrigated experiment at Lifuwu (Ecosystem 1) are shown in Table 1. Number of tillers per plant, panicle length, number of filled grains per panicle, grain length (paddy length), 1000-grain weight, grain shape (length/width ratio), days to 50% flowering, days to maturity, and leaf length showed significant differences ($p<0.001$) among genotypes. Osman et al. (2012) reported significant genetic variability for some selected yield and yield components except for number of tillers per plant and panicle length.

Genotype *Chencherica* had the highest yield (4.43 t ha^{-1}) while *Djissa* had the highest number of tillers (14). The lowest yield and number of tillers were found in genotypes *Nassope* (2.3 tha^{-1}) and *Kilombero* (6). Morales (1986) suggested that number of grains per panicle and 1000-grain weight might be considered as important criteria for increasing yield per unit area. The highest mean for panicle length, number of grains per panicle, grain length, 1000-grain weight, grain shape, days to 50% flowering, days to physiological maturity, and leaf length were identified in the genotypes *Nassope* (28.82 cm), *Mucandara* (277), *Nene* (11.20 mm), *Marista* (35 g), *Singano* (4.77), *Faya* (105 d), *Chupa* (150 d), and *Nassope* (55.20 cm), respectively. The lowest mean for panicle length, number of grains per panicle, grain length, 1000-grain weight, grain shape, days to 50% flowering, days to physiological maturity, and leaf length were *Paula* (22.03 cm), *Niwaio* (134), *M'finico* (7.03 mm), *Singano* (19 g), *M'finico* (2.07), *Marista* and *Nene* (79 d), *Nene* (110 d), and *Nunkile* (34 cm), respectively. Results of the present study suggest that five component characters, namely, panicle length, number of filled grains per panicle, grain length, weight of 1000 grains, grains

Table 1. Mean grain yield, yield components and growth parameters of twenty rice genotypes grown under irrigated ecosystem at Lifuwu Research Station, Malawi.

Genotypes	T ha^{-1}	NT	PL	Gr/P	GL	TGW	GS	DFL	DM	LL
Marista	2.73	9	25.4	189	9.70	35.0	2.83	79	117	48.0
Nassope	2.30	7	28.8	236	9.37	25.7	2.60	103	141	55.2
Rafik	3.77	9	27	264	9.77	24.0	3.80	104	144	51.3
Kilombero	3.90	6	26	254	10.70	34.7	3.50	93	137	45.8
Pedra	2.80	9	22.2	161	9.23	24.0	3.37	80	120	48.5
Djissa	2.90	14	22.2	207	10.10	25.3	3.83	90	116	47.2
Gorongosa	3.13	9	26	184	9.60	27.0	2.93	102	137	46.1
Paula	3.30	11	22	190	9.97	31.3	3.23	92	118	44.6
Niwaio	2.70	10	22.2	134	8.50	29.3	2.63	84	120	41.3
Nunkile	3.37	11	22.2	202	9.87	24.7	4.13	85	121	34.4
Nene	3.17	7	23.8	190	11.20	34.0	3.27	79	110	41.4
M'finico	3.90	9	26.1	217	7.03	20.3	2.07	104	141	45.2
Chibiça	4.07	11	25.8	201	9.77	25.3	3.17	95	123	40.8
Chencherica	4.43	12	27.5	198	10.73	32.7	4.23	98	131	48.9
Chibiça	4.07	11	25.8	201	9.77	25.3	3.17	95	123	40.8
Chupa	4.27	7	27.9	261	9.43	32.3	3.23	104	150	46.0
Mocuba	3.37	10	24.7	223	10.13	34.0	3.13	102	137	54.7
Mucandara	4.03	9	25.1	277	9.63	33.0	2.87	99	136	54.7
Singano	2.97	12	24.8	174	10.03	19.0	4.77	95	119	45.2
Mtupatupa	3.70	10	28.4	249	8.87	26.0	3.07	99	140	49.0
Mean	3.42	9.68	25.30	210.90	25.25	28.50	3.39	94.75	129.80	46.99
LSD $_{(5\%)}$	0.72	3.70	1.58	31.70	0.8	5.04	0.43	2.25	7.11	7.15
CV	12.80	23.10	8.00	9.10	5.00	10.70	7.60	11.40	3.30	9.20

Tha^{-1}: Tones per hecter, NT: Number of tillers per plant; PL: Panicle length (cm); Gr/P: grain per panicle; GL: grain length (cm); TGW: Thousand grain weight (g); GS: grain shape; DFL: Days to 50% flowering (d); DM: Days to physiological maturity (d); LL: Leaf length (cm).

shape, days to 50% flowering, days to physiological maturity, and leaf length had high significant differences ($p<0.001$) which implies that the genotypes constitute a pool of germplasm with adequate genetic variability. The number of tillers per plant showed significant differences ($p<0.05$). The genotypes with higher magnitude of these component characters could either be selected from the existing gene pool for on-farm evaluation (participatory selection) in order to introduce them as cultivars or utilize in breeding programs for genetic improvement.

Under Muirrua irrigated environment, the analysis of variance for each character revealed that the twenty rice genotypes differed amongst themselves at $p<0.001$ for yield (t ha^{-1}), panicle length, number of grains per panicle, weight of 1000-grains, grain shape (length-width ratio), days to 50% flowering, and days to maturity, indicating the presence of genetic variation among the genotypes for each quantitative character mentioned. The genotype *Chencherica* had the highest yield (5.63 t ha^{-1}), while *Mucandara* (23) and *Nunkile* (23) had the highest number of tillers. The lowest yield and lowest number of tillers were found in genotypes *Mtupatupa* (2.77 t ha^{-1}) and *Nene* (8), respectively. The highest means for panicle length, number of grains per panicle, grain length, 1000-grain weight, grain shape, days to 50% flowering, days to physiological maturity, and leaf length were found in the genotypes *Mocuba* (31.77 cm), *Mucandara* (313), *Chincherica* (11.47 mm), *Mocuba* (35.60 g), *Singano* (5.43), *Chupa* (114 d), *Chupa* (147 d) and *Mocuba* (147 d), and *Niwaio* (62.10 cm), respectively.

The lowest mean for panicle length, number of filled grains per panicle, grain length, 1000-grain weight, grain shape, days to 50% flowering, days to physiological maturity, and leaf length were in *Kilombero* (23.10 cm), *Niwaio* (152), *M'finico* (6.40 mm), *Singano* (23.6 g), *M'finico* (1.93), *Nene* (66 d), *Nene* (105 d) and *Kilombero* (43.66 cm), respectively (Table 2).

Effect of the ecosystem on grain yield, yield components and growth parameters of the twenty rice genotypes across the environments

The results for grain yield showed significant differences

Table 2. Mean grain yield, yield components and growth parameters of twenty rice genotypes grown under irrigated ecosystem at Muirrua Research Station, Mozambique.

Genotypes	T ha^{-1}	N T	P L	Gr/P	G L	TGW	GS	DFL	DM	LL
Marista	3.50	14	25.9	194	9.6	31.2	3.10	91	129	55.1
Nassope	3.10	16	27.5	267	8.8	25.6	3.50	95	132	56.1
Rafik	4.03	9	25.8	289	9.1	24.7	3.60	94	139	49.7
Kilombero	4.73	15	23.1	287	9.2	35.3	3.40	94	135	43.7
Pedra	3.83	11	24.6	172	9.6	25.0	3.50	97	132	50.7
Djissa	2.93	16	23.4	262	9.1	25.7	3.30	102	135	59.5
Gorongosa	4.50	17	23.8	199	11.2	26.7	3.40	99	144	60.7
Paula	3.64	14	24.3	215	10.7	29.6	3.30	109	141	48.7
Niwaio	3.33	14	23.6	152	9.9	28.6	2.90	95	129	62.1
Nunkile	3.53	23	23.1	241	9.8	27.6	4.50	75	108	49.4
Nene	4.40	8	26.3	259	9.9	35.4	2.80	66	105	52.4
M'finico	3.27	20	27.1	245	6.4	27.1	1.90	95	135	48
Chibica	4.45	19	26.5	220	9.3	25.8	3.30	102	134	47
Chencherica	5.63	15	30.5	257	11.5	33.6	4.20	105	142	45.7
Faya	4.37	19	29.6	257	10	32.5	3.40	97	133	54.7
Chupa	4.70	15	29.7	295	8.8	27.0	3.20	114	147	52.3
Mocuba	5.40	19	31.8	280	10.4	35.6	3.00	104	147	61.3
Mucandara	2.93	23	29.7	313	9.8	35.1	2.90	110	142	59.3
Singano	4.43	19	26.9	197	10.5	23.6	5.40	106	135	51.6
Mtupatupa	2.77	21	29.6	300	9.9	29.9	3.40	102	137	54.0
Mean	3.96	16.00	26.65	245.00	9.70	29.30	3.40	96.00	134.00	53.10
LSD (5%)	0.95	7.52	4.397	54.03	2.51	6.03	1.03	4.09	0.97	10.46
CV	14.60	27.70	10.00	13.30	15.70	12.50	18.20	3.00	0.40	11.90

Tha^{-1}: Tones per hectare, NT: Number of tillers per plant; PL: Panicle length (cm); Gr/P: grain per panicle; GL: grain length (cm); TGW: Thousand grain weight (g); GS: grain shape; DFL: Days to 50% flowering (d); DM: Days to physiological maturity (d); LL: Leaf length (cm).

Table 3. Analysis of variance for grain yield (t ha^{-1}) of twenty rice genotypes across the two sites and two environments (Lifuwu irrigated and Muirrua irrigated).

Source of variation	Degree of freedom	Sum square	Mean square	F-value
Total	239	383.4	-	-
Block	2	0.06	0.03	-
Ecosystem (S)	1	42	42.00***	137
Environment	1	193.98	64.66***	210.87
Genotype (G)	19	45.71	2.41***	7.84
S×G interaction	19	24.09	1.27***	4.13
G×E interaction	57	35.24	0.62***	2.02
Error	138	42.32	0.31	

*, ** and *** mean significant at 0.05, 0.01 and 0.001 probability levels, respectively.

in the interaction between ecosystems and genotypes (S×G), environments and genotypes (G×E), environments (E), genotypes (G) ($p<0.001$), and blocks (B) ($p<0.05$), showing high genetic variability among the twenty genotypes in the environment and site interactions (Table 3). The ecosystems (Lifuwo and Muirrua) and the environments (Lifuwu irrigated and Muirrua irrigated), affected genotypes performance differently and ranked individual genotypes (Table 4).

It is probably that soil and climate features (rainfall, temperature and relative humidity) affected the performance of the twenty genotypes across two

Table 4. Mean grain yield (t ha^{-1}) ranking of the twenty rice genotypes in each ecosystem across the two environments.

Lifuwu ecosystem		Muirrua ecosystem	
Irrigated		Irrigated	
Chincherica	4.40	Chincherica	5.93
Chupa	4.26	Mocuba	5.25
Chibica	4.09	Chupa	4.75
Mucandar	4.01	Nene	4.63
Kilombero	3.94	Faya	4.37
M'finico	3.91	Kilombero	4.37
Rafik	3.75	Singano	4.33
Mtupatupa	3.65	Rafik	4.17
Faya	3.60	Gorongosa	4.06
Mocuba	3.36	Chibica	3.94
Nunkile	3.36	Marista	3.8
Paula	3.31	Nunkile	3.55
Gorongosa	3.18	Niwaio	3.55
Nene	3.14	Paula	3.52
Singano	2.98	Pedra	3.32
Pedra	2.86	Djissa	3.31
Djissa	2.86	Mtupatupa	3.19
Marista	2.70	M'finico	3.15
Niwaio	2.68	Mucandara	3.12
Nassope	2.32	Nassope	2.90

environments. According to Hossain et al. (2013), fluctuations in the temperature and relative humidity can affect rice yield and their component in a considerable manner. Ahmed et al. (2008) reported that extremely low and high temperatures can affect rice production at all growth stages. The relative yield in the irrigated environment can be attributed to supplemented water from the irrigation scheme. Poehlman and Sleper (1995) reported that rice production systems differ widely in cropping intensity and yield, ranging from single-crop rainfed lowland and upland rice with small yields (1 to 3 t ha^{-1}) to triple-crop irrigated systems with an annual grain production of up to 15 to 18 t ha^{-1}. Kush (1997) reported that irrigated rice is the most productive crop system, which covers 75% of the global rice production. In this study, the yield was (3.69 t ha^{-1}) indicating significance in the irrigated system. Muirrua site yielded more with a mean of 3.22 t ha^{-1} while Lifuwu had a mean of 2.39 t ha-1. Atlin et al. (2006) concluded that development of rice varieties would produce acceptable yields under both water limited and favourable environments, while Annicchiarico and Perenzin (1994) and Eberhart and Russell (1966) advised that in situations with unpredictable site to site fluctuations of the environmental parameter, selections of a stable genotype, though not necessarily with the highest yielding, offered the best solution.

The performance of the twenty genotypes could have been influenced by availability of the nutrients depending on environmental factors such as pH, organic matter, and moisture. According to Andriesse et al. (1993), the availability of soil nutrients, however, changes with the moisture regimes of soils. The deficiency of nitrogen is the most common constraint on rice production. Uncertainty exists in abiotic factors affecting soil nitrogen supply in rice production and it has been commonly believed that total soil nitrogen can give adequate information about nitrogen release (Cassman et al., 1993).

Both sites did not meet the requirements of nutrients for rice production, in terms of amount and availability. For example, pH in Muirrua was 5.25 while it was 8.38 in Lifuwu. In terms of locations, it can be stated that geographical factors (latitude, longitude and altitude) may also have played some important roles on performance of the genotypes since most of rice genotypes in high-yielding regions are planted outside the Tropic of Cancer and Tropic of Capricornia. According to Andriesse et al. (1993), latitude is not responsible for high yield alone, although it is possible that latitude may have an effect on yield through resultant differences in temperature, solar radiation and day length.

The study revealed that the number of tillers in Lifuwu irrigated ecosystem was 12 while in Muirrua irrigated ecosystem, it was 14. The high tillering capacity is considered as a desirable trait in rice production, since number of tillers per plant is closely related to the number of panicles per plant. There were no significant differences ($p<0.001$) for panicle length in both ecosystems, Lifuwu and Muirrua, with 26 cm in Lifuwu and 25.92 cm in Muirrua. The mean numbers of filled grains per panicle were 234 for Lifuwu and 222 for Muirrua, with the grain length (paddy length) of 9.59 mm in Lifuwu irrigated and 9.78 mm in Muirrua irrigated.

Relationships between yield, yield components and growth parameters

Correlation analysis for yield, yield components and growth parameters showed that the number of tillers per hill (0.19), panicle length (0.15), the number of grains per panicle (0.07), grain length (0.37), weight of 1000-grains (0.27), grain shape (0.26), days to 50% flowering, and days to maturity are positively correlated with yield. Two traits, 1000-grain weight and grain shape, showed significant correlations at ($p<0.05$), while grain length showed significant correlations ($p<0.01$) (Table 5).

Rice grain yield has been reported to be positively correlated with plant height, panicle length and panicle weight (Samonte et al., 1998; Reddy et al., 1997), the number of grains per panicle and 1000-grain weight

Table 5. Correlation coefficients among grain yield, yield components and growth parameters of twenty rice genotypes at Muirrua irrigated ecosystem.

Parameter	1	2	3	4	5	6	7	8	9	10
Yield	1	0.19	0.15	0.07	0.37**	0.27*	0.26*	0.05	0.19	-0.15
No. tillers		1	0.21	0.14	0.07	0.12	0.14	0.16	0.09	0.03
Panicle length			1	0.36**	0.17	0.25	-0.1	0.31*	0.33**	-0.03
Grains/panicle				1	-0.03	0.26*	-0.06	0.08	0.18	0.03
Grain length					1	0.15	0.68**	0.06	0.07	0.19
1000-grain weight						1	-0.22	-0.12	-0.05	-0.33
Grain shape							1	0.03	-0.08	-0.06
Days to 50% flowering								1	0.89**	0.06
Days to maturity									1	0.11
Leaf length										1

*, ** show that the correlation is significant at 0.05 and 0.01 levels, respectively.

Table 6. Correlation coefficients among grain yield and yield components and growth parameters of twenty rice genotypes at Lifuwu irrigated ecosystem.

Parameter	1	2	3	4	5	6	7	8	9	10
Yield	1	0.14	0.33*	0.42**	0.02	0.16	0.01	0.41**	0.42**	0.06
No. tillers		1	-0.2	-0.34*	0.07	-0.02	0.27*	0.08	-0.23	-0.16
Panicle length			1	0.55**	-0.09	0.05	-0.02	0.66**	0.69**	0.40**
Grains/panicle				1	0.01	0.12	-0.01	0.56**	0.60**	0.42**
Grain length					1	0.31*	0.68*	-0.16	-0.32	-0.08
1000-grain weight						1	-0.23	-0.08	0.02	0.14
Grain shape							1	-0.02	-0.23	-0.14
50% flowering								1	0.83**	0.41**
Days to maturity									1	0.40**
Leaf length										1

*, ** show that the correlation is significant at 0.05 and 0.01 levels respectively.

(Samonte et al., 1998; Geetha et al., 1994), number of days to 50% flowering and number of days to maturity (Geetha et al., 1994). The grain length was highly and positively correlated with grain shape (0.68), while days to 50% flowering were highly correlated with days to physiological maturity.

In Lifuwu irrigated ecosystem, yield showed positive correlation with all nine evaluated traits. Bansal et al. (2000) reported that yield per plant was positively correlated with the number of productive tillers and 1000-grain weight, while Feil (1992) observed positive correlations between grain yield per plant and yield components including total spikelets per panicle, fertile florets per panicle and 1000-grain weight. Significant positive correlations were detected with panicle length (0.33), filled grains per panicle (0.42), days to physiological maturity (0.42) and days to 50% flowering (0.41). The 50% days to flowering showed high correlation with days to physiological maturity (0.83). Effective number of grains per panicle revealed significant positive correlation with days to 50% flowering (0.66) and days to physiological maturity (0.60). Significant positive correlations were found in days to 50% flowering (0.66) and days to maturity (0.69), correlated with panicle length (Table 6).

GENERAL CONCLUSION AND RECOMMENDATIONS

The results, obtained in this study, indicated that the twenty rice genotypes performed significantly different. These results showed that there was genetic variability in the material studied. The yield performance of the genotypes was consistent across the two irrigated sites of Muirrua and Lifuwu, probably suggesting stability of the genotypes. The ecosystems (Muirrua and Lifuwu) and the environments (Muirrua irrigated and Lifuwu irrigated) played an important role in phenotypic expression of

grain yield, yield components and growth parameters (number of tillers per plant, number of filled grains per panicle, 1000-grain weight, days to physiological maturity, panicle length, grain shape, and days to 50% flowering).

The correlation analysis of the study revealed that the number of tillers per plant, panicle length, number of filled grains per panicle, weight of 1000-grains, grain length, and days to maturity were the most important yield components. Therefore, the results suggested that the number of filled grains per panicle, grain length, the number of tillers per hill, and weight of 1000-grain weight are important yield traits which selection based on them would be effective under irrigated ecosystems.

The genotypes *Chencherica*, *Mucandara redondo, Nene, Paula, Kilombero, Mocuba, Nunkile, Pdtra* and *Faya* presented five most important traits that can be probably preferred by farmers both in Mozambique and Malawi, namely yield potential, milling yield percentage, long grains, aroma, and not unsticky grains when cooked. These genotypes could be recommended and introduced to farmers as cultivars; although they need to be more improved in the case of some undesirable traits, for example shattering in *Chencherica.* The genotypes contain adequate genetic variability, which can be used to complement and broaden the gene pool in advanced genotypes. Breeders should therefore use the genotypes in their crop improvement programs not only to incorporate the desirable traits that are present in the genotypes, but also to change some undesirable traits.

Conflict of Interests

The authors have not declared any conflict of interests.

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Appendix

Appendix 1. Soil characteristics of two environments (Lifuwu irrigated and Muirrua irrigated).

Parameter	Ecosystem			
	Lifuwo Irrigated		Muirrua Irrigated	
Deep (cm)	0-15	15-30	0-15	15-30
pH	4.90	4.20	8.50	8.70
P (mg/kg^{-1})	65.20	52.30	166.90	137.30
Ca (mg/kg)	9.60	8.90	651.90	597.80
Mg (mg/kg)	657.80	781.60	113.90	108.00
K (mg/kg)	18.40	19.00	24.10	21.70
Fe (mg/kg)	23.10	12.30	6.70	6.00
Cu (mg/kg)	0.14	0.10	0.10	0.50
Zn (mg/kg)	0.07	0.07	0.10	0.10
O.M (%)	3.20	3.10	4.70	4.60
N (%)	0.15	0.16	0.20	0.20
Sand (%)	27.20	27.70	31.00	29.00
Silt (%)	16.20	17.50	17.00	19.00
Clay (%)	56.50	55.60	52.00	52.00
Texture class	clay	clay	clay	clay

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Standard methods for inoculations of *F. oxysporum* and *F. solani* in Passiflora

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Soil fungi, *Fusarium oxysporum* FO and *F. solani* FS (teleomorph: *Nectria hematococca*), are pathogens of economic importance passion fruit crops. The present work was developed in order to standardize the methodology of inoculation, as an initial step to confirm the etiology of diseases associated with Fusarium wilt and collar rot. Strains of FO for A14, A16, A22, A27, A29, A32, A34, A48, A54, A64 and FS A11, A23, A62, A63 were used; they were obtained from symptomatic crops of *P. edulis*. Inoculations were carried with and without wounds, on seedlings of two and four months of *P. edulis*. To assess incidence and severity, a scale designed for symptoms and growth variables was used. An incubation period of 14 to 19 days for FO, and was found highly virulent strains (A54, A64, A34). The symptoms are characterized by vascular wilt corresponded to a pattern of descending necrosis. Cross sections showed discoloration in vascular vessels and roots showed necrotic processes that lead to delayed development of seedlings. FS causes disease but the evolution in most strains is very low and exceeds 100 days. Wounds are further evidence for the fungus required in the plant tissue. Symptoms are manifested in the collar area with redness, mild canker associated with cracking and dry appearance on the injury.

Key words: Pathogenicity, collar rot, Fusarium wilt, passion flower, *Passiflora edulis*, Koch´s postulates.

INTRODUCTION

Fusarium Link 1809 is a genus that includes important plant pathogens, and some species are mycotoxin producers associated with human and animal health hazards. The fungi can attach to human, animal and plant tissues (Oechsler et al., 2013; Eldridge et al., 2014; Salter et al., 2012; Sarmiento-Ramírez et al., 2014; Kirkpatrick et al., 2013). Exhaustive *Fusarium* studies have been conducted in many fields, such as molecular biology, ecology, phytopathology, medical mycology, toxicology, and others (Torching and Mitchell, 2004;

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Watanabe et al., 2011; Zhang et al., 2006). The genus, *Fusarium*, also known by its teleomorphs, *Nectria* and *Gibberella*, comprises plant pathogenic fungi with a wide variety of hosts and infection strategies (Michielse and Rep, 2009).

Fusarium sp., a plant pathogen of Passifloraceae, *F. oxysporum* f. sp. *passiflorae,* is the agent of *Fusarium* wilt in *Passiflora edulis* (McKnight, 1951), *P. mollissima* (Gardner, 1989), *Passiflora edulis flavicarpa* X *P.edulis* (Ploetz 1991, Ploetz, 2003), *Passiflora* spp (Fischer and Rezende, 2008). Meanwhile *F. solani* is reported as the causal agent of collar rot in *P. edulis* f. *edulis* Sims (Cole et al., 1992), *P. edulis* f. *flavicarpa* (Ponte, 1993; Fischer et al, 2005), *P. ligularis* and *Passiflora* spp. (Ploetz, 2006; Fischer and Rezende, 2008). One of the difficulties in studying interactions of plant-*Fusarium* is that the taxonomy does not determine its pathogenicity; so there is need to conduct pathogenicity tests or Koch's postulates. A common example is *Fusarium oxsyporum* that differs in symptomatology, epidemiology and susceptibility of cultivars and can be distinguished by pathogenicity tests with suitable hosts (Vakalounakis and Fragkiadakis, 1999).

In addition to confirm the etiology of disease, pathogenicity tests also determine the pathogenic variability of a causal agent and assess potential sources of resistance. In *Fusarium* plant pathogens it is possible carry out Koch´s postulates; four steps are adapted to plant pathology in microorganisms that can grow in axenic media: i) The microorganism must be found in large numbers in all diseased plants, but not in healthy ones. ii) The organism must be isolated from a diseased plant and grown outside the body in a pure culture. iii) When the isolated microorganism is “injected” into other healthy plants, it must produce the same disease. iv) The suspected microorganism must be recovered from the experimental hosts v), isolated, compared to the first microorganism, and found to be identical (Kaufmann and Schaible, 2005).

Considering the third step of these principles, the "injection" of the pathogen refers to the way of inoculating the microorganism on its potential host; the route of entry determines the subsequent results, and that is why it is necessary to revise the technique of inoculation into the host tissue. Correct diagnosis of diseases can be reached through determination of specific factor that predominates other causal factors (Wallace, 1978). The ability of a factor to produce disease may depend on the earlier influence of another determinant which itself makes little direct contribution to disease, and inoculation is one of those. Inoculation must be as similar as possible to what occurs in natural inoculations.

With Koch's postulates it is possible to define the infective cycle of a pathogen, through the incubation period defined as time between infection and disease symptom expression in host and latency is the period between infection of host and production of inoculum (De Wolf and Isard, 2007). Fungus in *Fusarium* genus, produces three types of asexual spores: macroconidia produce sporodochia on the surface of infected plants parts; microconidia occur on aerial mycelium. Both macroconidia and microconidia may also be formed in the xylem vessel elements of infected hosts plants, but microconidia are usually the predominant type in infected plant tissue (Nelson, 1981). Those spores can be produced simultaneously to symptom expression in passion fruit plants (Ortiz et al., 2014), so those periods can lead to outlining of the relevance of control measures, and epidemiological tools (Kranz, 2012). Third spore are chlamydospores, formed in axenic culture and dead host plant tissue, in the final stages of wild-disease development. These spores survive for an extended time in plant debris in soil in the absence of a suitable host plant, and chlamydospores are the primary soil borne propagule of *F. oxysporum* (Bennett and Davis, 2013).

This research aims to standardize tests of pathogenicity of *F. oxysporum,* causal agent of *Fusarium* wilt and *F. solani* agent of collar rot on *Passiflora edulis*, which will allow experiments in physiology of host-pathogen interactions, resistant materials testing, pathogen suppression methods, among others.

MATERIALS AND METHODS

Pathogenicity tests on *P. edulis*

We used commercial seedlings of *P. edulis*, analyzed to exclude plant pathogens. Pathogenicity tests were carried out under greenhouse conditions with average temperature of 25 ° C and average relative humidity of 70%. In order to produce inoculum to use in these tests, isolates previously identified as *F. oxysporum* corresponded to A14, A16, A22, A27, A29, A32, A34, A48, A54 and A64 and *F. solani* A11, A23, A62, A63. For all tests a completely randomized design was applied with 10 replicates per treatment, except for the pathogenicity tests on nine month old plants with 5 replicates per treatment. Statistical analyses were performed using Kruskal–Wallis one-way analysis of variance (nonparametric data) SAS software, version 6.1.

F. oxysporum causal agent of Fusarium wilt

The *F. oxysporum* isolates A27, A32 and A32 were grown in liquid medium malt extract, according to the formulation indicated by Pancreac, 2003, with a modification consistent on agar remotion. A 250 mL Erlenmeyer flask was inoculated with 3 discs with young mycelium (5 days), and then prepared a conidial suspension at a concentration of 1.10 6 UFC mL-1. The incubation conditions were temperature of 25 ° C with stirring in shaker at 125 rpm under absence of light.

To simulate natural inoculations were proven two ways to impregnate plant roots with pathogen:

Immersion of roots without wound: Forty five days old seedlings, with two true leaves, were immersed in a conidial suspension for two minutes (Gardner, 1989; Vakalouonakis, 1996). Inoculated volume by plant was 15 mL with the methodology described by Ortiz et al. (2012). Immediately after inoculation, were planted seedlings in sterile peat with nutrients, previously saturated with

Table 1. Ordinal scale used to assess the severity of Fusarium wilt in *P. edulis**.

Description	Class
No symptoms	0
Light to moderate wilting, chlorosis.	1
Severe wilt with stem discoloration, defoliation.	2
Seedling death	3

Modified from Vakalounakis et al. (2005).

Table 2. Treatments for testing pathogenicity of *F. solani/ N. hematococca* isolates in seedling of *P. edulis.*

Treatment	Stage	Strain	Method
T1	*F. solani*	A11	Root immersion in 1.10 6 UFC mL-1
T2	*F. solani*	A23	Root immersion in 1.10 6 UFC mL-1
T3	*F. solani*	A62	Root immersion in 1.10 6 UFC mL-1
T4	*F. solani*	A63	Root immersion in 1.10 6 UFC mL-1
T5	Does not apply	Does not apply	Root immersion of malt extract broth
T6	*N. hematococca*	A11	Direct contact with mycelium plug, in the collar.
T7	*N. hematococca*	A11	Direct contact, with mycelia and perithecia plug, in the collar.
T8	*N. hematococca*	A23	Direct contact with mycelium plug, in the collar.
T9	*N. hematococca*	A62	Direct contact with mycelium plug, in the collar.
T10	*N. hematococca*	A62	Direct contact with mycelia and perithecia plug in the collar.
T11	*N. hematococca*	A63	Direct contact with mycelium plug in the collar.
T12	*N. hematococca*	A63	Direct contact with mycelia and perithecia plug in the collar.
T13	Does not apply	Does not apply	Direct contact with a non-colonized plug in the collar.

water. As a negative control, an equivalent volume of medium malt extract in plants was spread.

***Inmersion of roots with wound*:** The technique is similar to the above; the only difference was that about 0.5 cm of the end portion of the root system was removed (Haglund, 1989, modified). After identifying the most appropriate inoculation methodology, we proceeded to confirm reproducibility through a screening test of more virulent isolates, which is described hereunder.

Screening of more virulent isolates

F. oxysporum isolates A14, A16, A22, A29, A34, A48, A54 and A64, were evaluated in two months old plants. The isolate A54 was used as positive control since it was the most virulent in the standardization of the methodology of inoculation. After inoculation, all plants were kept in a tunnel with plastic cover under greenhouse conditions, with environment temperature and humidity mentioned above. The assessed variables were: incubation period, incidence, number of leaves and plant height (weekly), one month follow-up, and severity, using the scale of Vakalounakis et al. (2005), modified (Table 1). The characterized symptoms and fourth Koch´s postulate were verified.

F. solani causal agent of collar rot

Inoculation without wound: To analyze if a wound is needed to have infection of *F. solani* through the root system and collar in *P. edulis,* two months old plants were evaluated with treatments shown in Table 2. Treatments T1 to T5 were performed by immersion of roots without wound, following the same protocol as described for *F. oxysporum*. Treatments T6 to T12 consisted in direct contact disc 0.7 mm in diameter with fungal growth, located over collar plant, without wound. For anamorphic stages (*F. solani*) 5 days mycelium grown on PDA was inoculated, and for teleomorphic stages (*Nectria haematococca*) mycelium with perithecia grown on agar V-8.

Inoculation with wound: Four months old plants grown in sterile soil were inoculated by direct contact of mycelial disks in the collar area using a modification of the methodology described by Ploetz (1991) and Fischer et al. (2005). Cultures of *F. solani* A11, A23, A62 and A63 grown in PDA medium, incubated for five days at 25 ° C, were cut into discs about 10 mm in diameter. These plugs were located over a small incision on the collar plant, to which previously added 1 mL of sterile water in order to facilitate adhesion. On controls were added clean PDA discs of plants.

In order to verify the reproducibility of the inoculation method with wounds, pathogenicity tests were conducted in four months old plants grown in sterile soil. All plants were kept in a tunnel with plastic cover under greenhouse conditions for 9 months, with environment temperature and humidity mentioned above.

RESULTS

F. oxysporum

Pathogenicity tests indicated an incubation period of 18

Table 3. Incidence and severity at 20 days posterior inoculation (dpi), of *P. edulis* seedlings inoculated with *F. oxysporum* under greenhouse conditions.

Treatment		Incidence (%)	Severity* (index)
1	A27 - immersion without wound in roots	40	0.4
2	A27- immersion + wound in roots	60	0.7
3	A32- immersion without wound in roots	60	0.7
4	A32- immersion + wound in roots	60	0.7
5	A54- immersion without wound in roots	80	0.8
6	A54- immersion + wound in roots	90	0.9
7	Absolute control	0	0
8	Relative Control (non-inoculated + wound in roots)	0	0
	Chi square	66.996	50.839
	Pr > Chi square	0.244	0.4057

Table 4. *P. edulis* seedling inoculated with *F. oxysporum* isolates: Incidence and severity.

Treatment		Incidence	(%)	Severity	Index
		Mean	Group	Mean	group
1	*F. oxysporum* A14	25.5	c	23.5	c
2	*F. oxysporum* A16	52.15	b	61	ab
3	*F. oxysporum* A22	25.5	c	23.5	c
4	*F. oxysporum* A29	56.9	ab	56.5	b
5	*F. oxysporum* A34*	64.05	ab	62.2	ab
6	*F. oxysporum* A48	25.5	c	23.5	c
7	*F. oxysporum* A54*	68.3	a	69.55	a
8	*F. oxysporum* A64*	66.1	a	66.25	ab
9	Control	25.5	c	23.5	c
	Chi square	54.567		62.781	
	p-value	5.36E-06		1.32E-07	

Incidence and severity accumulated during test analyzed by means of Kruskal-Wallis test; (*) the most virulent isolates, early symptoms.

to 19 days, symptoms of mild chlorosis associated with slight to moderate wilt. When comparing methods of inoculation no statistically significant differences were found at 20 dpi; however, the incidence and severity tended to be higher causing wound in the root (Table 3).

Symptoms in infected plants corresponded to *Fusarium* wilt, displaying progression in severity scale used (Figure 1), with an index of severity ranging from 0.1 to 1.0 from 19 to 21 dpi, 1.1 to 2.0 of 22 24 dpi and 2.1 to 3.0 of 25-30 dpi.

Incubation period for two months old plants was 14 days. Isolates of *F. oxysporum* A54, A64 and A34, showed statistically significant differences analyzed by means of Kruskal-Wallis test, with higher incidence values (50 to 80%) and severity (0.5 to 0.8) (Table 4). Additionally, for these isolates the collapse of seedlings was early, at 24 dpi. Meanwhile, the least virulent isolates showed at 14 dpi low incidence values (20-30%) and severity (0.2 to 0.3) and, beginning the collapse of the plant 28 to 30 dpi. At the end of the trial (28 dpi), the incidence was similar for *F. oxysporum* A16, A29, A34, A54 and A64 (90-100%) isolates; however, *F. oxysporum* A34, A54 and A64 isolates reveal an increased severity index (2.2 - 2.7) and lower height of plants (3.2 - 4.0 cm). From ten isolates tested, three were to be non-pathogenic (A14, A22 and A48), showing statistically similar to the control values in variables assessed (Table 4). Regarding number of leaves, analysis showed significant differences ($P<0.00324$) but control was included in two groups formed by Tukey test; therefore, it shows variability in plant species, *P. edulis*, but is not effect of pathogens. Symptoms characterized vascular wilt corresponded to a pattern of descending necrosis, cross sections showed discoloration in vascular vessels and roots showed necrotic processes that led to delayed development of seedlings. From these lesions was obtained *F. oxysporum*, a 60-80% frequency confirmed the fourth Koch´s postulate. In transversal section of

Figure 1. Progression of symptoms caused by *F. oxysporum* in *P. edulis* seedlings, according to the scale of Vakalounakis *et al.,* 2005 modified. A. no presence of symptoms (0-18 dpi), B. mild to moderate wilting and chlorosis (19-21 dpi), C. severe wilt with stem discoloration and defoliation (22-24 dpi), D. death of seedlings (25-30 dpi).

stem, discoloration was observed in the vascular vessels. The results indicated that this pathogen does not require wounds to cause infection.

F. solani

This fungus is less aggressive than *F. oxysporum* in terms of incidence, during the time of evaluation. Two months old plants of *P. edulis* inoculated with *F. solani* strains without wound, showed an incubation period of 108 dpi for two plants: 1 for the treatment 2 (*F. solani* A23) and the other for treatment 12 (*N. hematococca* A63). The symptoms manifested in the collar area were redness, mild canker associated with cracking and dry appearance on the injury. The progress of the lesion showed a non-uniform pattern across the collar with 1.2 cm long x 1.9 cm wide at 120 dpi and 2.2 cm long x 3 cm wide at 128 dpi, for treatments 2 and 12 respectively.

As for the aerial part of plants, severe chlorosis in the lower leaves appeared. At 180 dpi, two additional plants of treatment 12 (N. haematococca A63) showed in the collar zone a slight reddish canker of dry appearance associated to the presence of crazing. The cankers length range was 1.1 to 1.3 cm; plants showed slight chlorosis of lower leaves. In these treatments, at 245 dpi cross sections of the collar revealed chancre with progress towards the pith.

At 210 dpi 2 plants, from treatment 1 (*F. solani* A11) and 4 (*F. solani* A63), exhibit browning color in the collar area, this lesion presented a fast advancing, leading to rot in the collar and necrosis in the stem to 5-6 cm height up, at 240 dpi occur wilting and death of plants. Not teleomorph stages were observed in any treatment. Table 5 summarizes the results of incidence and mortality rate of the test.

Inoculations in fourth month old plants with wound showed symptoms in one plant inoculated with *F. solani* A62 with an incubation period of 47 dpi. Expressed changes included chlorosis primarily in lower leaves, stunted growth, general decay, posteriorly a reddish brown canker in the collar caused constriction and rot to +/- 2 cm of root. At 50 dpi, numerous reddish perithecia on the lesion could be observed (Figure 2) and at 54 dpi started a defoliation. At 90 dpi, cross section of the stem showed discoloration of vascular bundles after verifying the fourth Koch postulate, it was confirmed that *F. solani* is the agent of collar rot in *P. edulis*.

DISCUSSION

External symptoms of wilting consist of an incipient chlorosis of lower leaves, followed by a permanent wilting of these leaves; symptoms gradually move up the plant. Sometimes, they can occur on one side of the plant. Used scale is optimum to assess wilting evolution in *P. edulis* seedlings, because it has few levels, and clearly detailed. Besides in practice test is easy to follow and analyze.

The inoculation of *P. edulis* with *F. oxysporum* shows that the pathogen does not require wounds to cause disease although wounds, injuries or senescence are predisposing factor to *Fusarium* wilting. Some authors state that wounding enhanced *Fusarium* invasion and establishment (Rekah et al., 2000; Kang and Buchenauer, 2000; Sakamoto and Gordon, 2006; Szczechura et al., 2013).

Pathogenicity screening of *F. oxsyporum* allowed detection of the corresponding A54, A64 and A34, as virulent isolates. All strains evaluated showed similar incubation periods 14 days posterior inoculation, but the most virulent isolates showed during the tests higher values of incidence and severity. This suggests that these attributes are reliable and practical for the rapid detection of pathogenic isolates. Number of leaves was

Figure 2. Symptoms of collar rot caused by *F. solani* A 62 in *P. edulis* at 50 dpi. A. canker with perithecia formation on collar plant, B. collar control plant, C. general chlorosis and wilting in aerial organs, D. control plant.

no significant at the beginning of the experiment differences; however at the end of the tests there was noticeable reduction in the number of sheets, which explains defoliation by the process generated by the pathogen.

The occurrence of non-pathogenic isolates (A14, A22 and A48) shows that the presence of *F. oxysporum* does not necessarily imply pathogenicity thereof on the host plant. This behavior may be due to variability pathogenic mechanisms or lack of pathogenicity for the host in question. O'Donnell et al. (2009) mentioned that although there have been non-pathogenic strains, the null hypothesis that some isolates are nonpathogenic is virtually impossible given the large number of potential host plants and no plants as proved in *P. edulis*. Sáenz (2011)'s *personal communication* demonstrated that strains *F. oxysporum* A34 and A54 inoculated in peas (*Pisum sativum*) and beans (*Phaseolus vulgaris*) do not exhibit symptoms, although the fungus can survive and stay in these species without causing disease.

It was proved by indexing, suggesting that they are avirulent fungal hosts. *F. oxysporum* f. sp. *passiflorae* is not mentioned in this paper, since test has demonstrated that *F. oxysporum* A54 is not specific to Passiflora, attacking carnation *Dianthus cariophyllus* (Maldonado *et al*., 2015) and tomato (*Solanum sculentum*) (Rozero et al., 2015).

Pathogenicity tests with *F. solani* revealed that all isolates are pathogenic, causing symptoms ranging in severity depending on the type of inoculation and the age of the plants. But, death occurred in plants inoculated by direct contact of mycelium on injury induced collar (A62) or dipping roots without induced injury (A11, A62), suggesting that presence of wound, in collar tissues or the points of lateral root formation, plays an important role in the development of this disease. In case of *F. solani* A11 and A62, there were no wounds, but Cole et al. (1992) and Fischer et al. (2005) reported that plant transplantation inevitably leads to damage to roots and stem injuries, increased susceptibility to *Fusarium* in plants. Ploetz (1991), who in pathogenicity tests with *N. haematococca* on *P. edulis X P. edulis F. flavicarpa*, establish that only plants inoculated with wound collapsed, made similar observations to those found with *F. solani* A62.

Inoculated plants through direct contact on collar tissue, only display symptoms with A63 teleomorph stage *N. haematococca*, without causing death of the plant. This pathogen can cause infection without the presence of an induced wound; however, under these conditions the plant is able to generate defense mechanisms that counteract pathogen attack. Similar observations were made by Fischer et al. (2005), *P. edulis* f. *flavicarpa*, where plants survive inoculations of the pathogen.

The highest percentage of plants affected by the teleomorph *N. haematococca*, suggesting an important role of this on pathogenicity; nevertheless affected plants were also presented by the anamorphic state *F. solani*, which is in this work referred to as causal agent.

The low incidence observed in these tests can be explained by two factors: i) *N. haematococca* is not considered a particularly aggressive pathogen in passion fruit (Ploetz, 2003). *F. solani* strains compared with *F. oxysporum*, display long incubation periods ii) it could be that oscillations of environmental factors such as soil and weather can modulate the development of disease, which were stables under research conditions.

Finally, standardization of the methodology of inoculation of these pathogens is a tool to consider in future studies aimed at finding sources of resistance, likewise, severity scale developed allows the evaluation of these diseases in a more versatile manner.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Productive, morphological and qualitative characteristics of sugarcane in the understory tree species in agroforestry systems

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This study aimed to evaluate productive, morphological and qualitative characteristics of sugarcane in the understory tree species in two agroforestry systems. The study was conducted in the city of Frederico Westphalen, RS, in a randomized complete block design, characterized by a factorial arrangement of 2x5x3, that is, two agroforestry systems (strip and line), five tree species (angico, bracatinga, canafístula, eucalypt and guapuruvu) and three years of sugarcane cultivation (2009, 2010 and 2011), with three replications. The weight, length and stem diameter, number of nodes, Brix degree, juice volume, amount of sucrose, and how these factors are related to the interception of photosynthetically active solar radiation by the tree components in each system were evaluated. The interception of photosynthetically active solar radiation by tree components is smaller in the strip system, but increased over the years of sugarcane cultivation. Among the tree species, eucalypt is responsible for the highest values of interception. When grown in the understory of angico, bracatinga and canafístula, sugarcane presents greater length, diameter and stem weight, juice volume and amount of sucrose, mainly from the second cultivation year than when under the other tree species. The cultivation of sugarcane in the strip system resulted in an increased stem weight and juice volume from the second year of cultivation.

Key words: *Saccharum officinarum* L., solar radiation, shading, stem weight.

INTRODUCTION

One of the biggest challenges of agriculture in Brazil is managing the balance between crop production and

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environmental preservation. In order to achieve this balance, it is necessary to meet the demand for food and energy without compromising existing agro-ecosystems which can be used in order to achieve these objectives. These systems may consist of integrated use of land for (Godfray et al., 2010). Agroforestry systems deserve highlight in this scenario and are a promising strategy, forestry purposes, crops and livestock. This integration has brought numerous socio-economic, environmental and production benefits, such as the recovery of degraded areas, reduced production costs, and an increased input of organic matter, which has been shown to improve chemical, physical and biological properties of soil (Tracy and Zhang, 2008; Neves et al., 2009; Salton et al., 2013).

The insertion of sugarcane (*Saccharum officinarum* L.) in agroforestry systems can be an interesting alternative, due to its socio-economic importance in Brazil; in addition, the monoculture system is predominant for this culture and because of this predominance, it is becoming increasingly important to consider alternative production systems, which aim to preserve natural resources. These systems can provide an alternative to the cultivation of sugarcane in areas unsuited to mechanized harvesting, and considering ecological, agronomic and socioeconomic aspects, such methods may be more ideal for family farmers who may have small area of cultivation as an alternative source of income.

The growth and development of different species in the same area, such as in agroforestry presupposes the existence of dynamic interactions and change over time especially in areas which include trees; given their continued growth in height, crown projection, and the leaf area index, which can modify the distribution of existing resources. These tree interactions can be a source of a constant change in the productivity of both species system (José et al., 2004).Solar radiation, which is to be intercepted by the canopy of the arboreal components of these agroforestry systems can be absorbed, transmitted and reflected in varying proportions depending on the angle of incident sunlight and structural features of plants. The spatial arrangements of plants can include the arrangement of the leaves, leaf insertion angle, leaf area index and various optical properties of vegetation. The radiation transmitted by the canopy is only available to plants beneath the canopy, and can be propagated in a direct or diffuse way. The interactions of the transmitted solar radiation influence the internal microclimate of intersystem vegetation, which can have an effect on the morphological, physiological and nutritional aspects of this species in the understory, thereby affecting growth (Paciullo et al., 2011; Mendes et al., 2013).

The study aimed to evaluate productive, morphological and qualitative characteristics of sugarcane and relate them to the interception of photosynthetically active solar radiation in the understory of five tree species in two arrays of planting in agroforestry systems.

MATERIALS AND METHODS

The study was conducted in the experimental area belonging to the Agroclimatology Laboratory, linked to the Federal University of Santa Maria campus in Frederico Westphalen– RS, with geographical location at 27°22'S, 53°25'W at 480 m of altitude. According to the Köppen climate classification, the climate is CFA, that is, humid subtropical with average annual temperature of 19.1°C, varying with maximum of 38°C and minimum of 0°C. The soil of the area is classified as typical Entisol Orthents (Cunha, 2011). The values of soil chemical properties were: pH in water = 5.8; available phosphorus (Mehlich^{-I}) = 2.9 mg dm^{-3}; aluminum = 0.0 cmol$_c$ dm^{-3}; potassium = 82.5 mg dm^{-3}; calcium = 8.7 cmol$_c$ dm^{-3} and magnesium = 2.8 cmol$_c$ dm^{-3}. Fertilization was performed according to the recommendations made by the CQFS (2004).

The experimental design was a randomized complete block design, characterized by a factorial arrangement of 2x5x3, that is, two agroforestry systems, strip and line, five tree species, angico (*Parapiptadenia rigida* (Benth.), bracatinga (*Mimosa scabrella* Benth.), canafístula (*Peltophorum dubium* (Spr.) Taubert), eucalypt (*Eucalyptus urophylla* S.T. Blake x *Eucalyptus grandis* Hill ex Maiden) and guapuruvu (*Schizolobium parahybae* (Vell.) Blake) and three years of sugarcane cultivation. The first evaluation of sugarcane occurred in 2009, the second in 2010, and the one third in 2011, cultivar IAC 87-3396. In each repetition, ten experimental units were randomly assigned to the combination between agroforestry system and tree species.

Forest species were planted in the field in September and the sugarcane in November 2007; the process occurred through the manual planting of seedlings and cuttings, after plowing and harrowing. In the strip system (SS), the forest species were divided into separate strips by 12 m, each was composed of three lines, in which the plants were spaced at 3x3 m. The sugarcane was distributed in six lines (between strips, 12 m in space) and two lines in strips (between lines of trees). In the line system (LS), forestry species were distributed at 6x1.5 m spacing, or 6 m between lines and 1.5 m between plants in the line, and the sugarcane distributed in three lines (among lines of trees).

In both systems, the sugarcane had 1.20 m spacing and a density of 18 buds per meter, with both trees and sugarcane oriented in lines towards the East and West. After planting sugarcane, plots were delineated to have two meters in length, and were distributed at different points in the understory of each experimental unit. These plot areas were chosen with the intention to represent existing microclimate conditions in the areas under the canopy of each tree species and agroforestry system. For subsequent analysis of the data, average values of the lines in each system were calculated in order to comply with objectives of the study, which is the recommendation of the best system and species in different years of assessment. The arrangement of trees, sugarcane and plot of evaluation are shown in Figure 1.

The samples were collected in June 2009 (about one and half years after planting), 2010 and 2011, constituting the three years of sugarcane. In each marked line, two medium stalks were collected which were taken to the laboratory for evaluation. For the existing population in the experimental units, values were extrapolated for one hectare (ha).

The stalk weight (SW, t ha^{-1}) was obtained with the aid of a digital scale and stalk length (SL, m) by means of a measuring tape, the length being considered from the basal portion to the intersection of the youngest leaf sheath. The stalk diameter (SD, mm) was determined by measuring three points in the same basal medium and higher, and then the arithmetic means were obtained. The number of nodes (NN) was obtained by the total count of nodes in each stem of evaluation.

The juice volume (JV, m^3 ha^{-1}), was obtained from milling the stalk, and was measured with the aid of a graduated cylinder with a capacity of 1 L. By JV, samples were taken to determine the Brix

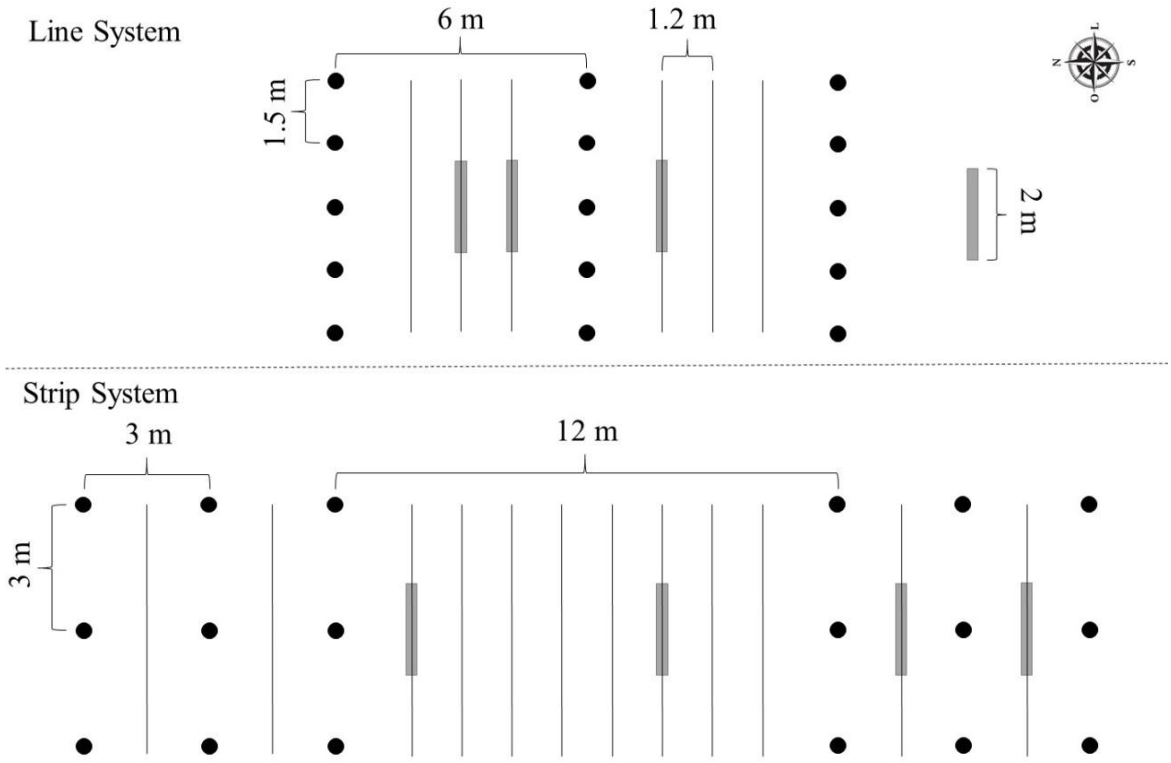

Figure 1. A sketch of an experimental unit of line and strip systems. Black circles represent the trees; continuous lines indicate where the sugarcane was planted, and the rectangles in gray represent the annual evaluation plots of sugarcane.

degree by means of an automatic digital refractometer Acetec RDA 8600. The sucrose concentration (SC, g L^{-1}) was determined using the equation proposed by Torres et al. (2006):

SC = Brix degree x 10.13 + 1.445 (1)

Where, SC = sucrose concentration (g L^{-1}).
The sucrose quantity (S, t ha^{-1}) was determined from the values of SC and JV, by the following expression:

S= SC x JV /1000 (2)

Where, S = sucrose quantity (t ha^{-1}); SC = sucrose concentration (g L^{-1}); JV = juice volume (m^3 ha^{-1}).
Photosynthetically active radiation (PAR) was obtained at harvest over the three years of evaluation, with the aid of a quantum sensor LI-190-1, with spectral strip of 400-700 nm, coupled to a porometer dynamic balance LICOR-LI1600 model. From this, the interception of photosynthetically active radiation was determined (IPAR) by canopy tree species, according to the equation proposed:

IPAR (%) = [100 - (Rn x 100/Rt)] (3)

Where, Rn = photosynthetically active radiation inside the canopy of the tree species; R_t = photosynthetically active radiation inside the canopy of the tree species.

The height values (H), diameter at breast height (DBH) and average crown diameter (MDC) of forest species at harvest of each year of sugarcane cultivation were collected for characterization purposes of the conditions existing in the experimental area (Table 1). They were obtained with the aid of a Hypsometer Vertex III, tape measure and graduated tape, respectively.

The values of meteorological elements during the experiment were obtained from the Climatological Station INMET (National Institute of Meteorology) linked to Agroclimatology Laboratory (UFSM), which is located about 1500 m from the study site at coordinates 27° 39'S and 53° 43'W. The data was statistically analyzed with the software “Statistical Analysis System” (SAS, 2003), and the results were obtained through the analysis of variance, F test and Tukey test (p> 0.05). The Bartlett test was used to verify the homogeneity of variances.

RESULTS AND DISCUSSION

Average monthly maximum, minimum temperature, monthly accumulation of precipitation and solar radiation during the study are shown in Figure 2. The annual average minimum and maximum temperatures were 18.5 and 19.9°C in 2008; 18.7 and 20.1°C in 2009; 18.4 and 19.8°C in 2010; 18.4 and 19.8 °C in 2011. The cumulative annual rainfall for these respective years was 1606.20, 2246.60, 1978.40 and 2229.40 mm. The accumulated global radiation flux averaged 513.4, 501.1, 504.9 and 516.2 MJ m^{-2} $month^{-1}$ in 2008, 2009, 2010 and2011, respectively. These values varied in the same order, from 265.6 to 806.6; 302.7 to 722.1; 275.92 to 717.9 and 258.9 to 808.5 MJ m^{-2} $month^{-1}$.

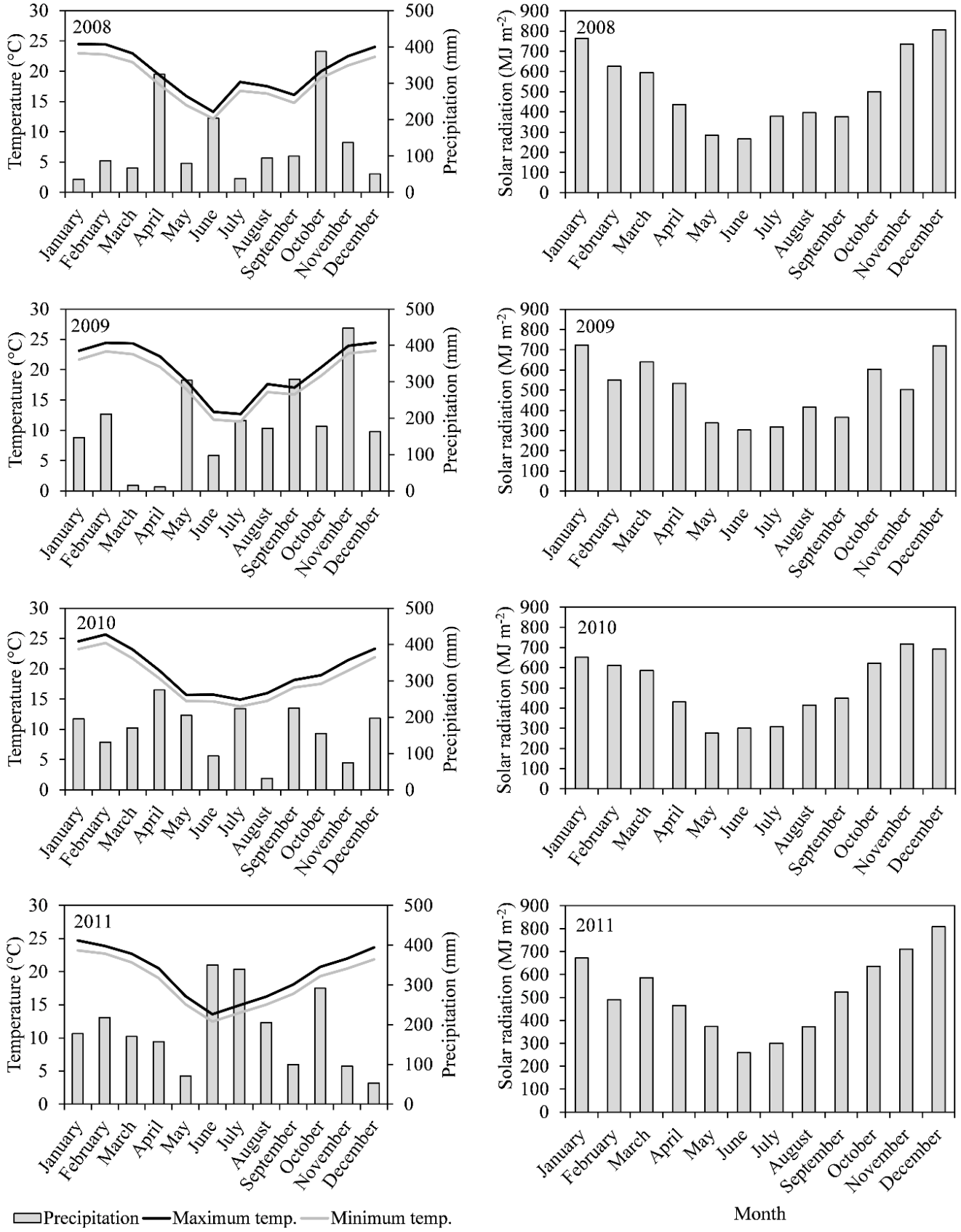

Figure 2. Average monthly maximum and minimum temperature; precipitation and cumulative monthly global solar radiation during the years, 2008, 2009, 2010 and 2011 in the city of Frederico Westphalen for the whole study period.

Table 1. Height (H), diameter at breast height (DBH) and mean diameter of crown (MDC) of forest species at harvest of each year of sugarcane cultivation, in agroforestry systems strip and line.

Variable	Species	Years					
		2009		2010		2011	
		Strip	Line	Strip	Line	Strip	Line
H	Angico	2.42	2.19	3.56	4.01	3.58	3.56
	Bracatinga	3.24	3.00	4.04	4.06	3.78	5.60
	Canafístula	2.40	2.27	3.60	3.14	4.29	4.82
	Eucalypt	6.82	6.24	10.90	10.14	15.34	14.06
	Guapuruvu	1.75	1.96	2.62	4.03	3.18	4.58
DBH	Angico	1.61	1.54	2.80	3.21	3.71	4.58
	Bracatinga	2.53	2.41	4.14	3.30	3.43	4.57
	Canafístula	2.05	1.94	4.35	3.82	4.71	4.88
	Eucalypt	6.83	5.20	12.35	9.31	16.64	12.16
	Guapuruvu	3.00	3.94	6.37	8.91	6.15	8.42
MDC	Angico	0.99	0.65	1.5	1.25	2.34	1.74
	Bracatinga	1.56	1.36	1.58	1.95	1.62	1.98
	Canafístula	1.10	1.00	1.35	1.22	1.86	1.75
	Eucalypt	2.79	2.11	3.08	2.74	4.67	4.16
	Guapuruvu	1.98	1.90	2.48	1.64	2.74	2.95

The analysis of variance revealed differences in the IPAR, SL, SD, SW, JV and S for interactions between tree species x year of sugarcane cultivation and IPAR, SW and JV for agroforestry system x year of sugarcane cultivation. All the tree species showed an increase of IPAR over the growing year of sugarcane (Table 2), except bracatinga, where values between 2010 and 2011 (first and second year, respectively) showed no difference.

Comparing all the species, eucalypt was responsible for the higher values in 2009 and 2011, and did not differ from canafístula in 2010. Angico had the lowest percentage of IPAR in the first two years (2008 and 2009). Bracatinga had the lowest percentages of IPAR in the third year. These variations are related to an increase of the MDC of tree species (Table 1) which increased leaf area and intercept a larger quantity of solar radiation. Similarly, for bracatinga, this small variation in IPAR was influenced by reduced growth in MDC from 2010 to 2011.

The IPAR values are similar to those found by Caron et al. (2012) in between planting lines of from 42.3% in black wattle, 83.2% in bracatinga and 89.1% in eucalypt trees with one year old. In the case of agroforestry systems, the amount of radiation intercepted by the tree component can be considered a determining factor of their deployment in the understory since the radiation transmitted inside the canopy of tree plants should be sufficient for growth and development. The evaluation of dynamic radiation of forestry species and systems is not a widespread practice in scientific circles yet. Another study was carried out by Pezzopane et al. (2015) and Bosi et al. (2014) who reported on high relation between levels of incident solar radiation, and its effect on microclimate, growth characteristics of plants and soil moisture in areas with high rations

During crop cycles, there is an increase in the overall radiation interception, followed by an increase in leaf area index (LAI), but only up to a certain value when full canopy closure is reached, due to leaf self-shadowing (Posada et al., 2012). At this point, the issue of new leaves does not result in an increased amount of light interception. In the case of this study, it can be noted that, maximum IPAR may not be present for up to four years after planting since there was an increase of this variable every year, without stabilization trend (Table 2).
The response of plants to shade varies depending on the species, and the degree of shading. According to Varella et al. (2010), percentages of transmission below 50% can harm the growth and development of fodder of temperate climate. Bosi et al. (2014) found that silvopastoral systems with native trees, indicated shading greater than 39% which affected the productivity of the species, *Urochloa decumbens*. Baruch and Guenni (2007) stated that shading levels above 35 to 40% can affect the growth of most tropical grasses.

The SL of sugarcane when grown in the understory of angico, canafístula and eucalypt remained stable over the cultivation years (Table 2). Under bracatinga, higher means were found in the past two years. The angico understory showed higher SL values in the first year,

Table 2. Interception of photosynthetically active radiation (IPAR), stem length (SL), stem diameter (SD), stem weight (SW), juice volume (JV) and amount of sucrose (S) of sugarcane grown in the understory of various tree species from 2009 to 2011, in the city of Frederico Westphalen - RS.

Variable	Species	Years of sugarcane cultivation		
		2009	2010	2011
IPAR (%)	Angico	27.02^{dC}	40.46^{cB}	46.43^{eA}
	Bracatinga	46.67^{cB}	56.41^{bA}	58.74^{dA}
	Canafístula	50.98^{bC}	68.81^{aB}	73.3^{bA}
	Eucalypt	56.41^{aC}	65.06^{aB}	89.74^{aA}
	Guapuruvú	47.78^{bcC}	55.23^{bB}	67.66^{cA}
SL (m)	Angico	1.51^{aA}	1.74^{aA}	1.52^{bA}
	Bracatinga	1.22^{bB}	1.78^{aA}	1.81^{aA}
	Canafístula	1.19^{bB}	1.63^{aA}	1.56^{abA}
	Eucalypt	1.24^{bA}	1.25^{bA}	1.20^{cA}
	Guapuruvú	1.27^{bB}	1.64^{aA}	1.30^{bcB}
SD (mm)	Angico	21.97^{aA}	20.95^{abA}	20.68^{bA}
	Bracatinga	19.37^{bB}	23.35^{aA}	23.31^{aA}
	Canafístula	19.44^{bA}	20.34^{bA}	21.14^{abA}
	Eucalypt	19.80^{bA}	17.09^{cB}	18.55^{cAB}
	Guapuruvú	20.53^{abA}	19.41^{bcAB}	18.19^{cB}
SW (t ha^{-1})	Angico	61.89^{aA}	53.08^{aA}	41.56^{abB}
	Bracatinga	26.56^{cB}	50.44^{aA}	50.52^{aA}
	Canafístula	30.44^{bcA}	36.19^{bA}	35.26^{bA}
	Eucalypt	30.48^{bcA}	14.81	15.20
	Guapuruvú	36.42^{bA}	36.25	18.04
JV (m^3 ha^{-1})	Angico	30.11^{aA}	25.77	19.68
	Bracatinga	15.34^{bB}	24.97	25.10
	Canafístula	16.59^{bA}	16.60	17.06
	Eucalypt	17.45^{bA}	5.94	6.80
	Guapuruvú	18.74^{bA}	17.11	7.37
S (t ha^{-1})	Angico	5.05^{aA}	4.88	3.79
	Bracatinga	2.32^{bB}	4.75	4.94
	Canafístula	2.76^{bA}	3.28	3.24
	Eucalypt	2.39^{bA}	1.09	1.27
	Guapuruvú	2.86^{bAB}	3.46	1.26

Means followed by the same letter, lowercase in the column compare the species in each year and uppercase letters in each line compare the years for each species, the means do not differ among themselves by Tukey test of probability at 5% of error.

probably due to reduced IPAR (27.0%) as compared to other species. In the second year, this difference was not observed; instead, in the third year, only lower averages were observed in the cultivations located in the understory of eucalypt and guapuruvu. According to Abreu et al. (2007) values of 1.80; 1,.70; 1.88; 1.92; 1.87 e 1.85 m were found for the cultivars IAC 86-2210, IAC 86-2480, IAC 93-6006, SP 81-3250, IAC 87-3396, RB 72-454, respectively, in the city of Barbacena/Minas Gerais, 15 months after planting.

Similarly, one can observe a reduction in SD from 2010 for the understory of Eucalypt and guapuruvu, and similar medians for angico and canafístula, respectively between the years (Table 2). In this context, Guiselini et al. (2013) analyzed the acclimation of sugarcane seedlings in greenhouse under two types of shading screens, found a

limitation of SL and SD of the sugarcane when grown in an environment with less availability of solar radiation. This feature was not observed in bracatinga understory, where levels were higher in the last two years.

Cultivars grown under eucalypt negatively affected SW in the second year of cultivation, as compared with the first, where SW was not strongly effected (Table 2). In addition, for in bracatinga understory, even with increasing solar radiation over the years and an increase in tree components' crown diameter, SW increased in the second and third cultivation year (Table 1). Abreu et al. (2013), working with five varieties of sugarcane, found the following SW averages: 89 t ha^{-1} in first year, 75 t ha^{-1} in second year and 88 t ha^{-1} in the third year in Tabuleiros Costeiros/Alagoas. Torres et al. (2006) found mean values of 64.5 t ha^{-1} for cultivation of SP79-1011 in the first year in the city of Capim/Paraíba. The values recorded in the aforementioned article are higher than those found in this study; however, this may be due to the variety used, the site of cultivation, weather conditions occurring in each cultivation cycle and, in this case, the amount of radiation available for the sugarcane cultures.

This last factor can be observed in this study, where the SW of sugarcane when cultivated under the eucalypt in the third cultivation year (152 t ha^{-1}) was decreased by 63.4% when compared with the angico (41.6t ha^{-1}) and 69.9% as bracatinga (50.5t ha^{-1}); whereas IPAR values in this cultivation year were 89.7% for eucalypt, 46.4 and 58.7% for the angico and bracatinga, respectively. This reduction in the SW of sugarcane may due to the fact that morphophysiological adjustments, as to shade tolerance strategy, were not able to compensate the radiation reduction in Eucalypt understory conditions (Paciullo et al., 2011). By comparing the species, it can be seen that SW values were higher in the cultures under angico in the first cultivation year. In the second and third years, this characteristic was observed to be greater for both angico and bracatinga (Table 2).

The JV of sugarcane grown under the angico and guapuruvu remained stable until the second cultivation year (2010), and were subsequently reduced (Table 2). This decrease was also observed in the second year for eucalypt; however, an opposite behavior was seen in the understory of bracatinga whose JV values increased from the second year and remained similar in the third year. In the analysis between species, higher means was found in cultivation under angico in the first year and, under angico and bracatinga in the second and third year. In third year, intermediate values were observed under canafístula and lower under the eucalypt and guapuruvu.

The average S showed no difference over the cultivation years in the understory of tree species angico, eucalypt and canafístula (Table 2). This result indicates greater stability in the metabolic activity of sugarcane when subjected to shading. In this regard, Caron et al. (2014) found that the production of *Ilex paraguariensis* (leaves + branches) is higher in unshaded cultivations when compared with shaded cultivations. Taking into account the content of some nutrients (calcium, magnesium and phosphorus) in these plant, minor variations were observed in low light conditions (85% shading) for different times of the year which may indicate a more constant level of metabolic activity of the studied plants, and be considered an important factor in the final product quality.

The S of sugarcane in the understory of bracatinga was higher in the last two years, since when grown under guapuruvu, the highest averages were found in the second year, even with the IPAR of 55.2%. In the third year, the S values declined, since the IPAR increased to almost 70%. It can be seen that both the highest values of treatment with guapuruvu were seen in the second year, and for bracatinga in the last two years, the radiation interception remained in the range of 55-60%. In this respect, Paciullo et al. (2011) found that the shading caused a positive effect on crude protein species, *Urochloa decumbens* grown in the understory of *Acacia mangium*, *Acacia angustissima*, *Mimosa artemisiana* and *Eucalyptus grandis.*

Another important aspect to be emphasized is that the lower amounts of solar radiation inside the canopy, due to the interception of it through the canopy, may have been offset by the increase of diffuse radiation in this environment. This fraction of the radiation has the characteristic of being multidirectional and better penetrate inside the canopy (Buriol et al., 1995), promoting more efficient use of solar radiation. However, this increase in efficiency can often not compensate for the reduction in photosynthetic rate, since there is a smaller amount of solar radiation available under the tree species.

IPAR between systems demonstrated the difference of this the first year (Table 3), but this difference did not affect the SW and JV. This variation was observed in the last two years (2010 and 2011), where the strip system was responsible for the greatest values of both productive variables of sugarcane. This system provided higher SW in the second year of cultivation, where the IPAR was 48.9%. Except in this case, the SW and JV did not show difference between the first two years, while the third was reduced.

In the strip system, likely due to the spacing of 3 m between lines and between plants, and 12 m between trees strips, there was lower IPAR as compared to the line system in which the plants were spaced every 1.5 m and the spacing between lines of trees was 6 m. Consequently, the smaller IPAR in strip system led to greater transmissivity of solar radiation into the canopy, which was crucial to the larger SW and MS values.

In addition, the greater proximity to the root systems of trees in the line system may have intensified the competition in the system. Whereas, IRFA values were already higher in the first year, it is assumed that this system demonstrated a closing between lines faster than

Table 3. Photosynthetically active radiation interception (IPAR), stem weight (SW) and juice volume (JV) of sugarcane in agroforestry systems of strip and line for three years of sugarcane cultivation (2009, 2010 and 2011) in the city of Frederico Westphalen - RS.

Variable	System	Years of sugarcane cultivation		
		2009	2010	2011
IPAR (%)	Strip	40.78^{bC}	48.86^{bB}	59.980^{bA}
	Line	50.78^{aC}	65.53^{aB}	74.37^{aA}
SW (t ha^{-1})	Strip	39.304^{aB}	48.891^{aA}	40.180^{aB}
	Line	35.014^{aA}	27.414^{bAB}	24.054^{bB}
JV (m^3 ha^{-1})	Strip	20.888^{aAB}	24.071^{aA}	19.439^{aB}
	Line	18.340^{aA}	12.082^{bAB}	10.969^{bB}

Means followed by the same letter, lowercase in a column compare the system in each year and uppercase in each line compare the years in each system, the means do not differ among themselves by Tukey test of probability at 5% of error.

the strip system, which can be justified by its closer spacing. This may also have not been sufficient to influence the MC and VS sugarcane in his first crop, however, demonstrated influence on subsequent years.

From the results obtained, it can be observed that the productive, morphological and qualitative characteristics, with the passing of sugarcane cultivation years, presented numerous variations, which are strongly influenced by tree species and agroforestry arrangement. The fact of the characteristics, especially productive characteristics (SW, JV, S), are relatively minor as compared to other studies, this does not prevent successful cultivation of sugarcane in these systems. The study sought to provide new sustainable alternatives for farmers, in order to increase the diversification of the rural property and maintain the preservation of existing agro-ecosystems.

Conclusions

The interception of photosynthetically active solar radiation by tree components is lower in the strip system, but increases over the years of sugarcane cultivation. Among the tree species, eucalypt is responsible for the highest values of interception.

When grown in the understory of angico, bracatinga and canafístula, sugarcane presents greater length, diameter and stem weight, juice volume and amount of sucrose, mainly from the second cultivation year than when under the other tree species. The cultivation of sugarcane in the strip system resulted in an increased stem weight and juice volume, from the second year of cultivation.

Conflict of Interests

The authors have not declared any conflict of interests.

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