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Full Length Research Paper

Soil carbon dioxide flux in a no-tillage winter system

Rafael Pivotto Bortolotto¹, Telmo Jorge Carneiro Amado¹, Douglas Dalla Nora^{1*}, Cristiano Keller¹, Debora Roberti², Jackson Ernani Fiorin³, Klaus Reichardt⁴, João Fernando Zamberlan⁵, Mauricio Paulo Batistella Pasini⁶ and Rodrigo da Silveira Nicoloso⁷

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Soil carbon dioxide flux is a complex process which depends on variations of different factors related to climate and soil. The objective of this study was identifying the abiotic factors that most contributed to this flux during different phenologic stages of the sequence black oat-vetch, cultivated under the no tillage system, in the winter, and find out the most important factors. Soil carbon fluxes were measured every 15 min with a LI-COR “long-term” (stationary) chamber, installed on the no tillage site of the rotation: soybean/black oat/soybean/black oat + vetch/corn/turnip/wheat. The factor that mostly influenced soil carbon fluxes was soil temperature, explaining 57% of the flux variation during the cycles of the crops and 80% from tillering to the begin of the elongation stage of the black oat. The phenologic stages of the black oat in the consortium black oat + vetch that mostly contributed to the carbon soil flux were from the begin of the tillering to the begin of the elongation, and from the elongation to massive grain of the black oat.

Key words: Greenhouse effect, soil temperature, phenologic stages, soil conservations system, *Avena strigosa*, *Vicia sativa*.

INTRODUCTION

Agriculture in the global warming context can be part of the strategy of the greenhouse effect. This fact is associated to the extension of the area managed with no-tillage practices in Brazil and to the flexibility of the

adoption of practices that promote carbon (C) influx and the reduction of greenhouse gas emission (GGE) (Amado et al., 2006). The Intergovernmental Panel on Climate Change (2007) highlights the role of agricultural soils

having in mind that depending on the management practices that are adopted, the soil can become a GGE absorber, mainly in the case of carbon dioxide (CO₂). The production of CO₂ in the soil is related to biological activity (Lou et al., 2004; Iqbal et al., 2008, 2009; Ussiri and Lal, 2009), including root respiration and soil organic matter (SOM) decomposition. The no-tillage management system (NT) can reduce CO₂ emissions, increasing C stocks (Amado et al., 2006).

The NT system has emerged as an effective technique to act as a biological C drain, this process is however a function of the climate (rainfall and temperature), soil (texture, clay type, mineralogy), cultivation systems (annuals, pastures), agricultural practices (soil preparation, fertilization) and conservation practices (erosion control), being therefore very variable (La Scala et al., 2005, 2006; Pes et al., 2011; Marcelo et al., 2012).

Soil temperature is the variable that best has explained the changes in CO₂ emissions (La Scala et al., 2005; Almaraz et al., 2009; Ussiri and Lal, 2009; Wang et al., 2009). In a similar form, the soil water content also had been reported (Franzluebbers et al., 2002; La Scala et al., 2006; Sotta et al., 2006).

Another aspect that has to be mentioned is that variations in soil CO₂ fluxes depend on the phenologic stage of the crop. Few studies were presented to date, most of them related to forest species (Davidson et al., 2006). Studies carried out with soybean (Verma et al., 2005; Hollinger et al., 2005 and Rodrigues et al., 2013) show that the greatest CO₂ fluxes were observed between the phenological stages V5 and V9, that is, during stages of leaf emission and greatest plant vigor. Lower fluxes were found close to the maturation point. These reports, however, take into account the soil CO₂ flux added to the canopy emission.

The objective of this study was the identification of abiotic factors that contribute more to the soil CO₂ flux in the different phenological stages of the black oat + vetch in the NT cultivation system and consequently define the stages that most contribute to this flux.

MATERIALS AND METHODS

The field experiment was carried out in Cruz Alta, RS, Brazil, (28°36'S, 53°40'W), 409 m absl. The climate of the region is of the type Cfa 2a, tropical humid, according to Köppen's classification. The average air temperature is 18.7°C, with an average minimum 9.2°C in July and an average maximum 30.8°C in January (Pes et al., 2011). Annual average rainfall is 1,721 mm, uniformly distributed along the year.

Sowing of the oat (*Avena strigosa* Schreb) (75%) + vetch (*Vicia sativa* L.) (25%) crop was made on 13 May, 2010. The crop was desiccated on 16 September, 2010, with Glyphosate [N-(phosphonomethyl)glycine]. During this period rainfall was well distributed, reaching 643 mm, corresponding to 37.4% of the

annual average (Figure 1). The black oat + vetch were chosen to conduct this study because it is a typical management of southern Brazil, however no studies on these crops.

The soil is classified as a LATOSSOLO VERMELHO Distrófico típico (EMBRAPA, 2006) or a Typic Hapludox (Soil Survey Staff, 2010), with a predominance of caolinite and iron oxides, with a clay content of 570 g kg⁻¹ (Table 1).

The study was performed on an area cultivated by NT for 25 years, using a 40 x 60 m parcel. The area was subjected to crop rotation (summer and winter): black oat/soybean/black oat + common vetch/corn/turnip (*Raphanus sativus* var. *oleiferus*)/wheat/soybean. Seeding was performed directly over the previous crop residuals remaining on soil surface. Soil disturbance was limited to the seeding line using a double disc system to open a narrow furrow for seed deposition.

Measurements of instantaneous CO₂ soil flow were performed at soil surface of the NT plot with a stationary LI-COR "long-term" chamber made by LI-COR (LI-8100, LI-COR, NE, EUA). The chamber monitors changes in CO₂ concentration using an infrared gas analyzer (IRGA) with an internal volume of 991 cm³, and an exposed area to the soil surface of 71.6 cm². The chamber was installed over a PVC ring, previously introduced into the soil. Once closed and ready for measurement, 1.5 min. are necessary for the time interpolation of the CO₂ concentration change in the chamber.

Measurements were performed between 22 May and 16 September, 2010. Due to the lower proportion of vetch in the field and the difficult recognition of its phenologic stages, the study was performed based on the stages of the black oat. Three stages were used: Stage I, seedling emergence to tillering (May 22 to June 29; Stage II, beginning of tillering to beginning of elongation (June 30 to August 13); and Stage III, beginning of elongation to massive grain, when the desiccation of the crop was done (14 August to 16 September). Periodical evaluations of the CO₂ flux were made, with a frequency of 15 min, and CO₂ values were converted into C-CO₂. Soil heat fluxes were also measured in the center of the plot using a Hukseflux sensor, model HFP01SC-L, installed at the depth of 0.02 m; soil temperature with a Campbell Scientific sensor, model TCAV-L, also at 0.02 m depth; and soil water content with a TDR sensor, model CS616-L Campbell Scientific, installed at an angle that allows the measurement of the full 0 to 0.20 m soil layer. All data were stored in a CR 1000 - Campbell Scientific logger.

Air temperature, rainfall and solar radiation data were obtained from an automatic weather station of INMET, located 400 m away from the experimental site. Data were submitted to multiple regression through the "stepwise" method, procedure PROC REG of the program SAS (2009) to verify associations and interdependencies between C-CO₂ emissions and the group of variables related to the environment, as follows: C-CO₂ soil flux = a + b₁Tair + b₂Tsoil + b₃Fg + b₄Usoil + b₅Rg; where a is the intercept; air temperature (Tair); soil temperature (Tsoil); soil heat flux (Fg); soil water content (Usoil); solar radiation (Rg); applied for all three stages separately and also for the whole experimental period. The significance level for the F test was 5% probability for the inclusion of the variables in the model. Regression analysis and the significance level between the C-CO₂ flux and the average daily soil temperature, were also made by the SAS (2009) program.

RESULTS AND DISCUSSION

The average daily C-CO₂ flux observed from 22 May to 16 September was 24.4 kg ha⁻¹ d⁻¹ (Figure 2), totalizing

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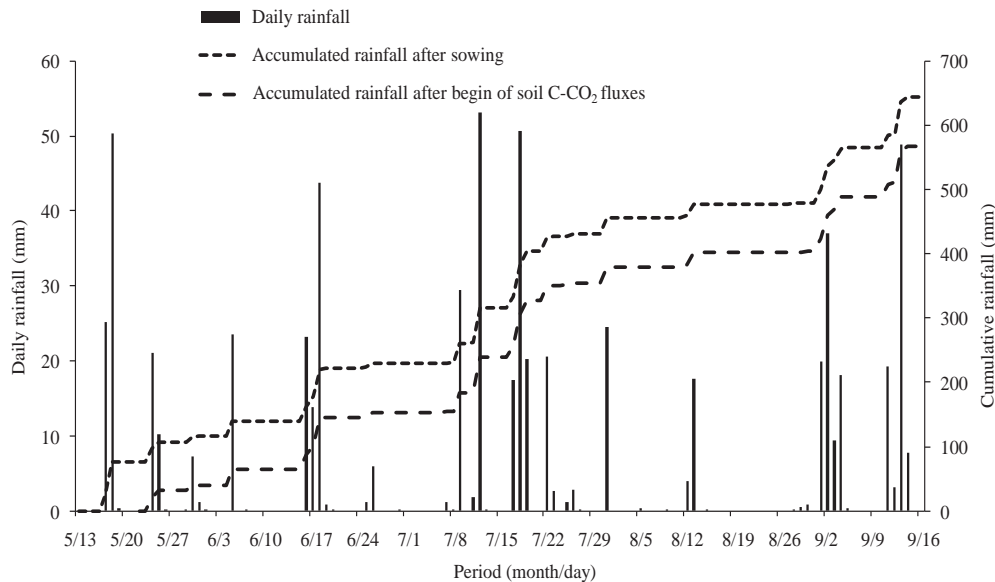


Figure 1. Daily and accumulated rainfall during the experimental period.

2,879.4 kg C-CO₂ per ha, with great variability along the crop cycles. These changes were similarly the variations in soil and air temperatures (Figure 2a) and of the soil heat flux (Figure 2b). This result corroborates the studies performed by La Scala et al. (2005); Almaraz et al. (2009); Ussiri and Lal (2009) and Wang et al. (2009), who report that soil temperature is highlighted as the isolated variable in best explaining the changes in C-CO₂ emissions.

Soil water content apparently did not influence soil C-CO₂ flux (Figure 2c), probably due to the regular rainfall events of the period under study, however, several studies mention the soil water content as one of the main variables affecting soil gas fluxes (Iqbal et al., 2009; La Scala et al., 2006).

Solar radiation (Figure 2d) apparently did also not influence the C-CO₂ soil flux, although some studies propose solar radiation as the control variable of the soil to atmosphere C-CO₂ flux (Ouyang and Zheng, 2000).

In relation to the phenologic stages, during stage I (Table

2) of the black oat, the C-CO₂ emission presented a positive and significant regression coefficient with soil temperature (Table 2). This variable explained 50% of the variation of the C-CO₂ emissions during the development stage of the crops, that is, from emergency to tillering.

In relation to the development stage II (Table 2), in the same way as for stage I, the emission of C-CO₂ presented a significant positive regression coefficient with soil temperature, however the soil heat flux did also adjust to the model in this development stage. In total, the variables explain 82% of the C-CO₂ emissions, 80% being singly attributed to soil temperature. Stage II presented the highlight of being the stage with highest

percentage of explanation of the emissions by the variables.

For stage III (Table 2), corresponding to massive grain - elongation the C-CO₂ emissions presented significant positive coefficients of variation with soil temperature (54%) and soil water content (9%), these two variables explaining 63% of the variation of the C-CO₂ emissions.

Analyzing the three development stages during the 118 days of observation The C-CO₂ emissions presented significant positive coefficients of variation with soil temperature and soil water content, and additionally a negative significant coefficient with soil heat flux. This set of variables explains 62% of the variation of the C-CO₂ emissions during this period. Soil temperature was responsible of 57% of the C-CO₂ flux. Soil heat flux and soil water content also influenced the C-CO₂ flux, however in low intensity, reaching 2 and 3%, respectively. Soil water content was affected by the regular rainfall which amounted to 567.4 mm during the observation period, and 643.4 mm during the whole growth cycle of the crops (Figure 1). The obtained results corroborate to the studies of Xu and Qi (2001), Luo and Zhou (2006), Franzluebbers et al. (2002), La Scala et al. (2006) and Sotta et al. (2006) who cite soil water content as one of the factors that determine the C-CO₂ soil flux. A quick raise of the C-CO₂ emission right after a rainfall or irrigation event, with a consequent increase of soil water content has been described in various reports (Beare et al., 2009; Zhang et al., 2011).

According to Ouyang and Zheng (2000), La Scala et al. (2003) and Iqbal et al. (2008) solar radiation is one of the most important factors that determine soil C-CO₂ fluxes, due to its influence on daily variations of soil temperature and water evaporation. However, in a cropped area

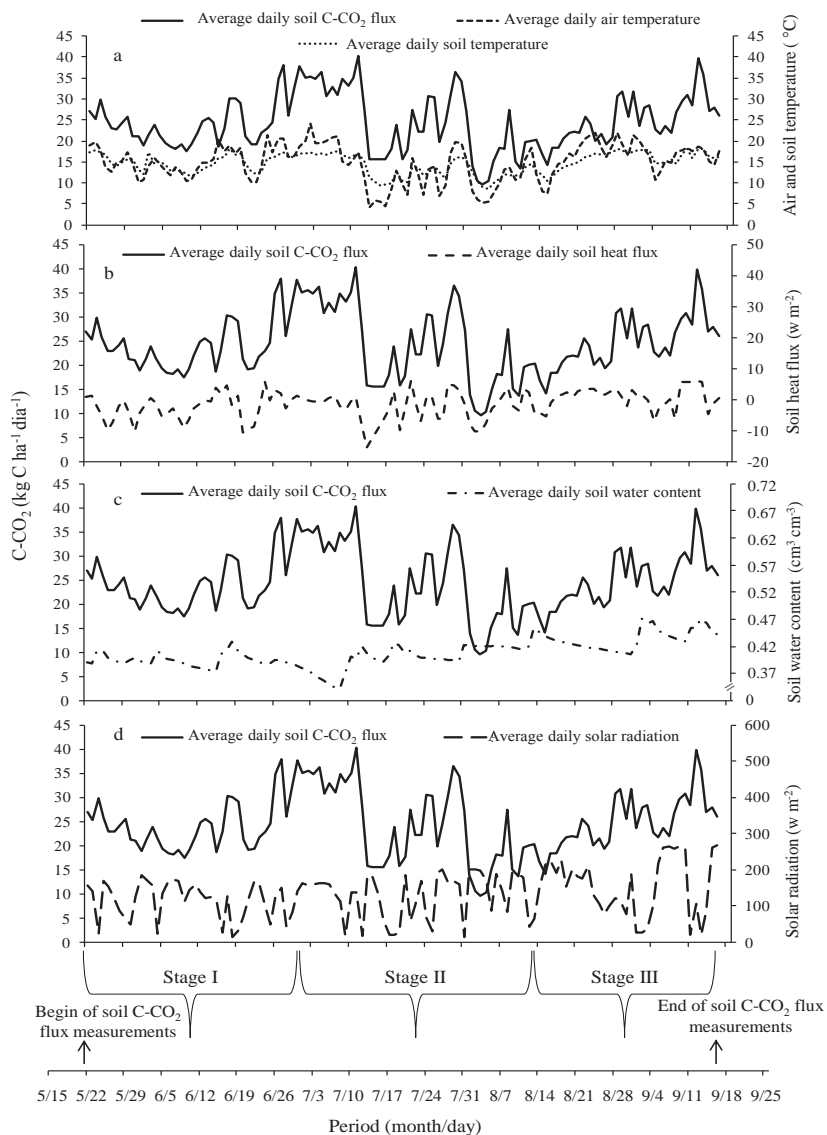


Figure 2. Daily soil carbon dioxide (C-CO₂) flux, soil and air temperatures (a), Soil heat flux (b), Soil water content (c) and solar radiation (d) during the experimental period Stage I: emergency to begin of tillering; stage II: begin of tillering to begin of elongation and stage III: begin of elongation to massive grain.

Table 1. Chemical and textural characteristics of the 0-0.20 m soil layer at the beginning of the experiment.

Period	pH H ₂ O	P ¹	K ²	Ca ³ +Mg ⁴	Al ⁵	Sand	Silt	Clay
		- mg kg ⁻¹ -	----- cmol _c kg ⁻¹ -----	----- g kg ⁻¹ -----				
1985	4.5	19.0	0.21	4.2	1.2	-	-	-
2010	4.9	9.1	0.18	6.5	1.0	310	120	570

¹Phosphorus. ²Potassium. ³Calcium. ⁴Magnesium. ⁵Aluminum. All measured according to Tedesco et al. (1995).

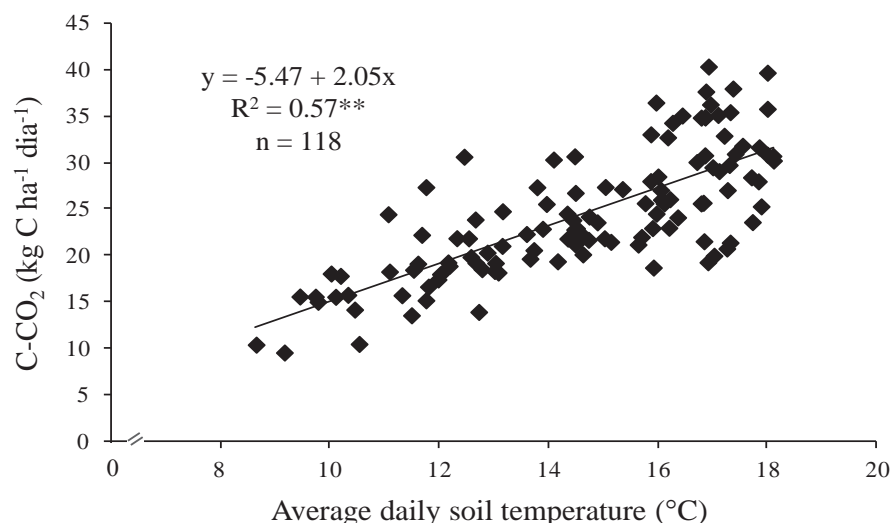
leaves difficult the direct incidence of radiation on the soil surface. Therefore, it is expected that in this study solar radiation is of little effect on soil C-CO₂ fluxes (Table 2).

Soil heat flux is an important component of the energy balance of the Earth-Atmosphere system, representing 5 to 15% of the balance of a cropped area during the day

Table 2. Multiple regression coefficients through the “stepwise” method between the variables and the soil C-CO₂ flux at stages I, II and III of the black oat crop.

State of evaluation of the soil C-CO ₂ flux	Intercept (a)	Tair (b1)	Tsoil (b2)	Fg (b3)	Usoil (b4)	Rg (b5)	Test F	R ² model
Stage I	-2.582	-	1.777	-	-	-	37.4***	0.50
R ² partial		-	0.50	-	-	-		
Stage II	-8.898	-	2.544	0.300	-	-	97.1***	0.82
R ² partial		-	0.80	0.02	-	-		
Stage III	-46.553	-	2.113	-	87.661	-	26.1***	0.63
R ² partial		-	0.54	-	0.09	-		
Whole cycle	18.889	-	1.742	-47.830	0.250	-	61.2***	0.62
R ² total		-	0.57	0.02	0.03	-		

Tair- air temperature; Tsoil – soil temperature; Fg- soil heat flux; Usoil- soil water content; Rg – solar radiation; R²- determination coefficient; ***Significant at 0.5% probability.

**Figure 3.** Relation between the average daily soil C-CO₂ flux and the daily average air temperature, during the experimental period.** Significant at $p < 0.01$.

and about 50% during the night, for different crops (Stull, 1988; Heusinkveld et al., 2004). Vertical variations in soil temperature are determined by physical soil properties and by the soil heat flux. In this paper, however, no significant influence was found for the soil heat flux on the soil C-CO₂ flux, in relation to soil temperature. This result might be due to the analyses made using daily means of the variables.

The low air temperatures 14.7°C which occurred during the study period imposed a low soil temperature (14.5°C) limiting the C-CO₂ flux in the soil, as it can be seen in Figure 3. Under conditions of soil temperature below average, a small dispersion of the C-CO₂ flux is observed, related to these soil temperatures. However, the greatest dispersion occurred with temperatures above 15°C, which might be an indicative that above this temperature biological activity is not restricted. Other

factors like rainfall events and soil water content might define changes of the soil C-CO₂ flux. From emergence to begin of the tillering in stage I (Figure 4a), due to soil temperatures above 15°C, a greater dispersion of the flux data was observed. Although the soil average temperature had been 14.9°C, the determination coefficient was the lowest ($R^2 = 0.50$; $p < 0.0001$), with an average C-CO₂ flux of 23.88 kg C ha⁻¹ d⁻¹. From the begin of tillering to the begin of elongation (stage II), the average soil C-CO₂ flux was 24.45 kg C ha⁻¹ d⁻¹. During this period a lower average soil temperature (13.4°C) was observed, and consequently a better determination coefficient was obtained with C-CO₂ ($R^2 = 0.80$; $p < 0.0001$) fluxes (Figure 4b), a result of the lower dispersion of the data. From the begin of elongation to the stage of massive grain (stage III), the highest soil temperature average was observed (15.8°C) with an average C-CO₂

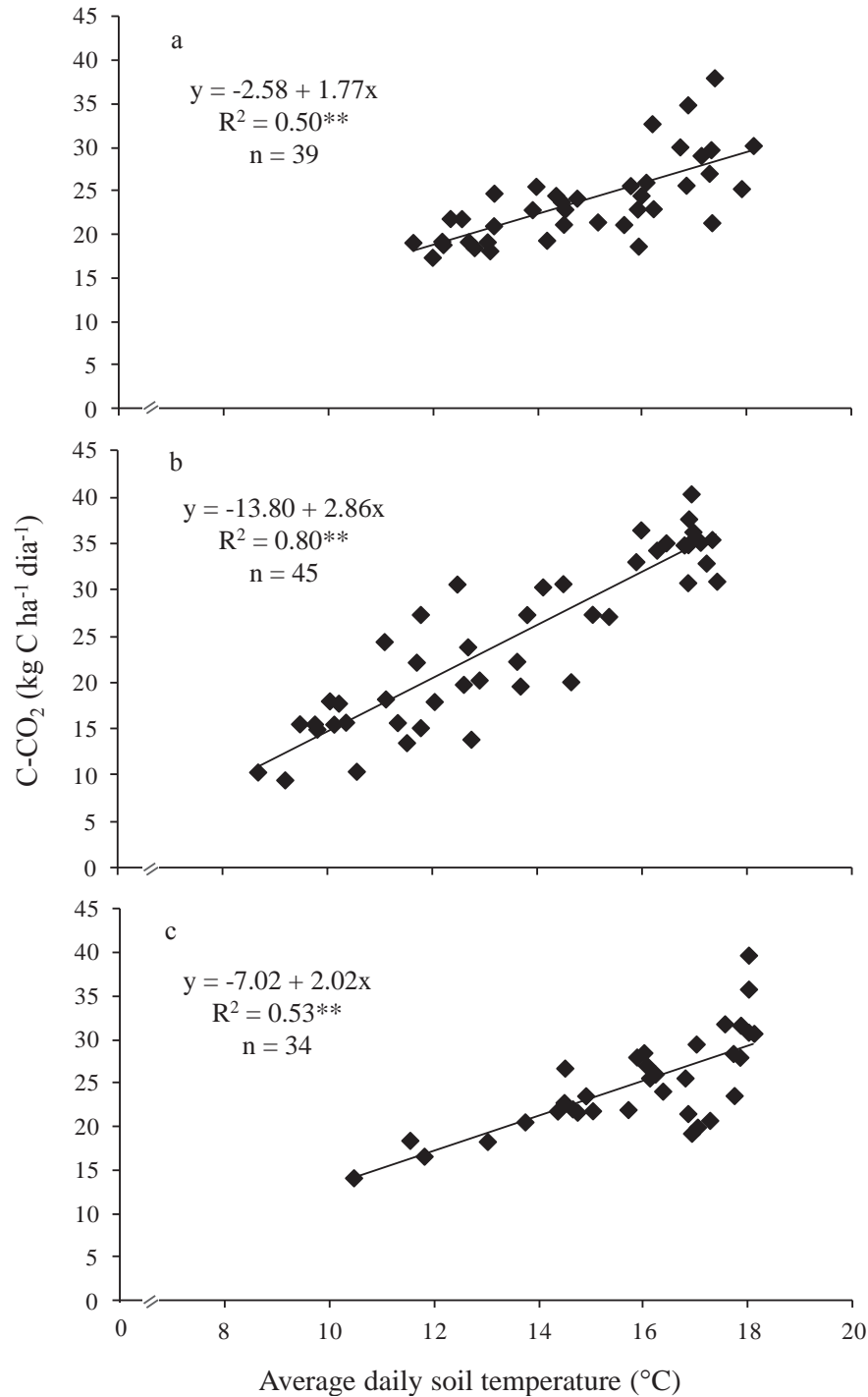


Figure 4. Relation between the daily average soil C-CO₂ flux and average daily air temperature, for stages I (a), 2 (b) and III (c). ** Significant at $p < 0.01$.

flux of $24.92 \text{ kg ha}^{-1} \text{ d}^{-1}$, therefore presenting a lower adjustment of the data due to the larger dispersion for the C-CO₂ ($R^2=0.53$; $p<0.0001$) flux measurements (Figure 4c).

These results corroborate partially with the data of Verma et al. (2005), Hollinger et al. (2005) and Rodrigues

et al. (2013) for the soybean crop, who observed that the greater soil C-CO₂ fluxes and the best adjustments were found for the intermediate stages when air and soil temperatures were not too high.

During days of higher temperatures, associated to adequate soil water content conditions, the CO₂ flux may

be a result of the increase of biological soil activity (Lou et al., 2004; Iqbal et al., 2008, 2009; Ussiri and Lal, 2009). On the other hand, low fluxes observed on cooler days should be related to a decrease in biological activity in the soil (Lou et al., 2004; Al-Kaisi and Yin, 2005). According to this reasoning, one can say that seasonal changes of the soil CO₂ flux are directly associated to soil temperature and other environmental factors (Lou et al., 2004; Iqbal et al., 2008, 2009).

Conclusions

Soil temperature mostly influenced winter C-CO₂ soil fluxes, contributing with 57% during the whole cycle and 80% from the beginning of tillering to the elongation stage of the black oat. In all stages of the crops was checked linear and significant relationship between the soil temperature and the increase in the CO₂ flux. The greatest dispersion of soil C-CO₂ fluxes occurred when the soil temperature were highest. Soil water content and soil heat flux influenced the C-CO₂ soil fluxes to a lesser intensity. The phenologic stages of the black oat in the consortium black oat-vetch, in which the soil C-CO₂ fluxes were mostly affected by the environmental factors were: beginning of tillering to the beginning of elongation, and elongation to massive grain.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Nitrogen and potassium fertilizer influenced nutrient use efficiency and biomass yield of two plantain (*Musa* spp. AAB) genotypes

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Nitrogen and potassium are key nutrients for optimum productivity in *Musa* species. In this study, optimum doses of N and K were determined for two plantain genotypes. The growth and dry matter yield (DMY) of 'PITA 24' (a hybrid plantain of the International Institute of Tropical Agriculture) and a landrace, 'Agbagba' were evaluated on factorial doses of N (0, 200, 400 and 600 kg.ha⁻¹) and K₂O (0, 300, 600 and 900 kg.ha⁻¹). The nutrient use efficiencies of the applied nutrients were also studied. Analysis of variance showed that fertilizer combination significantly ($p < 0.05$) influenced the genotype performance and genotype-by-fertilizer interaction effects. Growth and DMY in both genotypes were superior where both nutrients were applied together. 'PITA 24' maintained a better growth, higher DMY, and greater efficiency of nutrient use than 'Agbagba'. Both genotypes had the best growth where N was applied at 200 or 400 kg.ha⁻¹ in combination with 300, 600 or 900 kg.ha⁻¹ of K₂O. The control plants were the poorest. Agronomic efficiency (AE) of applied K⁺ was high at N₂₀₀K₃₀₀, N₄₀₀K₃₀₀, and N₆₀₀K₃₀₀; similarly, AE of applied N was superior at N₂₀₀K₆₀₀, N₂₀₀K₃₀₀, and N₂₀₀K₉₀₀. The partial factor productivity from the applied nutrients was highest at N₂₀₀K₃₀₀, suggesting that it was most economical to grow plantain with 200 kg N and 300 kg K₂O ha⁻¹. For optimum performance of plantains in the humid tropics of southeastern Nigeria, results from the study suggest the combined application of 200 to 400 kg N and 300 to 600 kg K₂O per hectare, per annum.

Key words: Plantains, dry matter yield, nutrient use efficiency.

INTRODUCTION

The edible bananas (*Musa* AAA) and plantains (*Musa* spp. AAB) belong to the genus *Musa* and family *Musaceae* (Stover and Simmonds, 1987). As principal staple food with rice, cassava and yam, plantains are rich sources of dietary energy, vitamins (A, B6 and C) and

minerals such as calcium, potassium, phosphorus, iron and zinc (Tenkouano et al., 2002). Plantain and banana crops are traditionally grown in heavily manured compound farms where the productivity could be sustained for many years. These crops are now being

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cultivated on large-scale commercial farms under sole cropping where yield decline sets-in after few production cycles (Wilson et al., 1987). The rapid yield decline observed in most plantations has been a major limitation to large scale cultivation of plantain and banana crops in West and Central Africa. Yield decline in *Musa* crops is particularly severe in the landrace genotypes, and has been blamed on poor soil fertility and the associated biotic stresses (Braide and Wilson, 1980) including black Sigatoka (*Mycosphaerella fijiensis* Morelet) disease, and attack by root-knot nematodes (*Meloidogyne incognita*) and corm weevils (*Cosmopolites sordidus*).

In this regard, the International Institute of Tropical Agriculture (IITA), Nigeria, has advanced the use of improved hybrids which are resistant/tolerant to diseases and pests, and are high yielding with good postharvest qualities. However, sustaining the yields of new cultivars in the farmers' fields requires appropriate crop management practices, especially soil fertility management. In the tropics, rapid population growth and continuing land degradation pose a great challenge to soil fertility management (Sanchez et al., 1997). Therefore, external nutrient inputs are needed to sustain crop yields on most tropical soils.

The productivity of determinate fruit species like *Musa* is largely dependent on the prevailing crop environment prior the reproductive growth stage, of which soil fertility is major determinant. Under declining soil fertility, the damage caused by banana weevil and soil nematodes tends to increase (Obiefuna, 1990). Besides, *Musa* plants easily lodge under poor fertility and stressed conditions due to the plant's morphology (Robinson, 1996), which includes superficial rooting, heavy canopy, succulent trunk, and the 'high mat' syndrome (that is, when the plant base grows out of the soil). For optimal yield, bananas require large doses of plant nutrients which cannot be supplied solely by the soil reserve (Lahav, 1995). For instance, Irizarry et al. (1981) calculated that an acceptable harvest of 35 tonnes fruits/ha leaves the soil depleted by 250 kg N, 45 kg P₂O₅, 702 kg K₂O, 100 kg MgO, 252 kg CaO, and 24 kg sulphur. Sound fertilizer recommendations are based on crop requirements for a particular expected yield, corrected for the ability of the soil to meet those requirements (FADINAP, 2000). Fertilizer best practices require that fertilizer nutrients should be applied at the right dose, right time and at the right place (Fixen and Reetz, 2006).

Significant progress has been made in overcoming soil fertility deficit in *Musa* fields. In Nigeria, Obiefuna et al. (1981) recommended 200 g N in 3-4 split applications (at 3 monthly intervals) with a basal dressing of 200 g each of potassium and phosphorus per plant for optimum yield. This recommendation is equivalent to 320 kg.ha⁻¹ of N, P and K each, at a plant population of 1600 per ha. Empirical data have shown that delayed fertilizer application beyond 3 months after planting (MAP) hinders the growth and yield of plantains (Obiefuna, 1984a); thus,

fertilizer application should start not later than 2 MAP to ensure efficient nutrient utilization and optimal performance.

Nitrogen and potassium are the key nutrient elements for optimum growth and yield in *Musa* species (Twyford and Walmsley, 1974; Lahav and Turner 1989; Lahav, 1995). Nitrogen is essentially required in the synthesis of amino acids, proteins and enzymes for metabolic pathways. Among the essential plant nutrients, potassium assumes the greatest significance since it is required in relatively large quantities by plants. The K⁺ requirement for *Musa* crops is often a double-fold that of N, and the high yield of plantains is associated with heavy potassium application (Bekunda and Manzi, 2003). An optimal dose of 300g K per plant ($\approx 500 \text{ kg K.ha}^{-1}$ at 3 × 2 m spacing) applied to plantains at about 19/20th leaf stage (4-5 MAP, when it is most needed for floral initiation) had significant improvements on bunch yield and fruit metric traits compared to the control plants (Obiefuna, 1984b). The efficiency of fruit set is also increased by over 10%, while the maturity period is shortened for three months. K⁺ is connected with the assimilation of CO₂ and the subsequent formation and translocation of sugars within the plant, and also with the utilization efficiency of available water (Ng Kee kwong et al., 1994).

Application of N-fertilizer could sometimes reduce the economic crop yield (Baiyeri, 2002), since nitrogen supports vegetative growth in crops. Baiyeri (2002) evaluated the effect of four rates (0, 224, 448, and 672 kg N/ha) of urea-N with basal application of 200 kg P₂O₅ and 350 kg K₂O per hectare on the growth, yield and harvest index of falsehorn plantain. Harvest index significantly increased up to 448 kg N/ha and then declined; meaning that the application of N-fertilizer beyond 448 kg N/ha had no added advantage in the study area.

Basically, the fertilizer requirements for *Musa* crops range from 200 to 400 kg/ha N, 45 to 60 kg/ha P, and 240 to 480 kg/ha K per year (FAO, 2000). However, Awodoyin (2003) recommended 320 kg N, 160 kg P₂O₅ and 320 kg K₂O per hectare for optimal performance of plantains in the Nigerian rainforest belt. Wilson et al. (1987) suggested split application of mineral fertilizers in combination with organic mulching (3-5 t.ha⁻¹); thus 300 kg N and 550 kg K₂O per hectare annually in six split doses, P₂O₅ at 250 kg/ha (applied at planting) and CaMg(SO₄)₂ at 60 kg/ha biannually.

Despite the inorganic fertilizer nutrient combinations recommended to optimize yield of plantain crops, reports from the major producing countries of West and Central Africa identified that very few farmers use inorganic fertilizers due to poor access predicated on cost and poor distribution (Dauda, 1996; Katungi et al., 2006). Bekunda and Woome (1996), however, identified a paucity of information on the optimal fertilizer recommendations and rates for plantains in most parts of West and Central Africa.

Agriculture in most part of Africa is characterized by

poor yields owing to the severely depleted soils and low use of agricultural inputs including mineral fertilizers and improved seeds (Bationo et al., 2006). Organic sources of plant nutrients are certainly not available in sufficient quantities to optimize yield and feed the sub-Saharan Africa's current population of over 750 million (Vanlauwe, 2004). The use of mineral fertilizers is a swift approach to restore lost nutrients and bring life to the severely depleted soils (Van Keulen and Breman, 1990).

In this study, the growth and dry matter yield of a hybrid plantain, 'PITA 24' (Plantain of the International Institute of Tropical Agriculture) and a landrace, 'Agbagba', and the efficiency of the applied nutrients were evaluated across factorial doses of nitrogen and potassium fertilizers for a growth period of six months. Information obtained thereof would guide in future field trials. Studies (Baiyeri and Mbah, 1994; Ndukwe et al., 2012) have shown that pre-flowering growth parameters in plantains have significant and positive relationship with the final fruit yield.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the screen-house of the high rainfall station of the International Institute of Tropical Agriculture (IITA), Onne (04° 43' N, 07° 01' E, 10m above sea level), Rivers State, southeast Nigeria between March and September, 2007. The station is characterized by an average daily temperature of about 27°C, an annual unimodal rainfall of 2400 mm, solar radiation averaging 14 MJm⁻², and high relative humidity with average values ranging from 78% in February to 89% in July and September (Ortiz et al., 1997). The topsoil used for this study was characterized as a sandy loam (77 sand, 3% silt and 20% clay), and slightly acidic (pH 5.3) with moderate fertility. Organic matter content of 1.53%, total nitrogen of 0.09% and total phosphorus of 0.02% were recorded. The Zn, Fe, Cu and Mn contents of the soil were 8.63, 274, 1.24, and 28 mg/kg of soil, respectively; whereas the exchangeable cations including K⁺, Ca²⁺, Mg²⁺, Na⁺ and H⁺ were 0.07, 1.40, 0.21, 0.38, and 0.84 cmol⁺.kg⁻¹ of soil, respectively. The effective cation exchange capacity (ECEC) of 2.89 cmol⁺.kg⁻¹ was recorded.

Design of experiment

In a screen-house study, a 4 × 4 factorial experiment involving four rates of N (0, 200, 400 and 600 kg.ha⁻¹.yr⁻¹) and K (0, 300, 600 and 900 kg K₂O ha⁻¹.yr⁻¹) were evaluated with a blanket dose of 100 kg P₂O₅ per hectare on growth, dry matter yield and nutrient use efficiency of 'PITA 24' and 'Agbagba' plantains. The sixteen fertilizer nutrient combinations were replicated eight times in split-plots (representing the two plantain genotypes under study) in completely randomized design.

Treatment application

Macro-propagated plants (Baiyeri and Aba, 2005) were grown with topsoil in polypots where fertilizer N, P and K were supplied using urea (46% N), single superphosphate (18% P₂O₅) and Muriate of Potash (60% K₂O), respectively. The varying calculated fertilizer doses were applied top-dressed in 2-split doses at 4 weeks after

planting (WAP), and thereafter at 8 weeks. A systemic insecticide/nematicide Furadan 3G (3% a.i., carbofuran) was applied at 5 g per plant, and watering was regulated to minimize leaching losses.

Data collection

At 3 and 6 months after planting (MAP), data were collected on plant height (from soil level to the V-junction of the topmost opposite petioles), plant base girth (cm), number of live leaves and total leaf area (cm²) per plant following Obiefuna and Ndujizu (1979). Four plants each across the fertilizer treatments were subjected to destructive sampling at 3 and 6 MAP to assess the dry matter accumulation and partitioning to the above-ground and below-ground components. Data were also collected on the number of roots per plant, length of the 5 longest roots and the corm cross-sectional diameter.

Nutrient use efficiencies viz., agronomic efficiency (response ratio) and the partial factor productivity (*Pfp*) from applied nutrients were calculated as weight ratios following Jagadeeswaran et al. (2005); thus:

$$\text{Agronomic Efficiency (AE)} = \frac{(\text{DMYield in fertilized plots}) - (\text{DMYield in control plot})}{\text{Quantity of fertilizer nutrient applied (N or K}_2\text{O)}}$$

Where, DMYield = whole plant dry matter yield.

$$\text{Partial Factor Productivity (Pfp)} = \frac{\text{Total dry matter yield per plant}}{\text{Quantity of fertilizer nutrients received (N + P}_2\text{O}_5 + \text{K}_2\text{O)}}$$

Partial factor productivity from the applied nutrients is a useful measure of nutrient use efficiency since it provides an integrative index that quantifies total yield relative to utilization of all nutrient resources in the system; thus, the decimal fraction of the plant yield to total applied nutrients (Cassman et al., 1996; Jagadeeswaran et al. (2005).

Statistical analysis

Analysis of variance (ANOVA) was performed on the generated data following the procedures outlined for factorial experiments in completely randomized design (CRD) using GenStat Discovery Edition 4 2011. Where the F-test was significant, treatment means were separated using Fisher's Least Significant Difference (F-LSD) at 5% probability level. Principal components analysis was also done to identify the most influenced or responsive parameters following the fertilizer treatment application. The data were further subjected to correlation analysis using the Pearson's multiple correlation analysis of SPSS 17.0 software (SPSS, 2008) to assess the interrelationships among the studied parameters.

RESULTS

Data presented in Table 1 are the plant growth parameters, dry matter yield and the nutrient use efficiencies of 'PITA 24' and 'Agbagba' plantains as influenced by varying combinations of N and K fertilizer doses after a 3-month growth period. Fertilizer application significantly ($p < 0.05$) influenced the clonal performance and clone-by-fertilizer interaction effects in most of the studied parameters. In both clones, the shoot growth (height, pseudostem girth, number of leaves and total leaf

Table 1. Effects of N and K fertilizer doses on plant growth indices, dry matter yield and nutrient use efficiency in Agbagba and PITA 24 plantains measured at 3 months after planting (MAP).

Clone	Fertilizer dose [kg.ha ⁻¹]	Ht [cm]	Gt [cm]	NL [#]	LA [m ²]	NR [#]	ARL [cm]	Dry matter yield			Nutrient use efficiencies		
								per plant [g]		Whole plant	AEK ⁺	AEN	FPF [NPK]
								Above ground	Below ground				
	Control	46.0	8.7	5	0.32	32	71.1	25.4	27.0	52.4	0.0	0.0	18.52
	N ₀ K ₃₀₀	49.0	9.7	4	0.32	28	67.6	24.3	36.2	60.5	1.03	0.0	5.67
	N ₀ K ₆₀₀	58.3	10.8	4	0.43	31	61.9	29.1	31.1	60.2	0.47	0.0	3.09
	N ₀ K ₉₀₀	48.0	9.8	4	0.35	25	63.0	22.5	28.3	50.8	-0.07	0.0	1.86
	N ₂₀₀ K ₀	67.0	11.3	5	0.68	24	52.2	32.1	21.9	54.0	0.0	0.29	6.47
	N ₄₀₀ K ₀	73.3	12.0	4	0.60	28	60.0	39.9	25.5	65.4	0.0	1.18	4.72
	N ₆₀₀ K ₀	61.0	11.3	6	0.73	29	46.4	47.4	22.9	70.3	0.0	1.08	3.63
	N ₂₀₀ K ₃₀₀	78.3	14.0	8	1.05	39	44.3	65.8	35.7	101.5	6.26	8.89	6.26
	N ₂₀₀ K ₆₀₀	91.7	14.7	7	1.22	39	50.4	78.0	51.4	129.4	4.62	13.95	5.17
	N ₂₀₀ K ₉₀₀	95.0	15.2	7	1.34	33	48.6	82.5	45.5	128.0	3.09	13.70	3.89
	N ₄₀₀ K ₃₀₀	83.3	13.6	7	1.15	33	57.3	81.4	37.1	118.5	8.43	5.99	5.46
	N ₄₀₀ K ₆₀₀	90.0	16.0	9	1.26	36	42.1	73.0	39.8	112.8	3.63	5.47	3.69
	N ₄₀₀ K ₉₀₀	88.3	15.4	9	1.43	35	48.8	81.4	48.6	130.0	3.17	7.03	3.39
	N ₆₀₀ K ₃₀₀	76.7	14.4	8	1.08	33	48.5	62.3	31.5	93.8	5.28	2.50	3.44
	N ₆₀₀ K ₆₀₀	80.0	14.4	7	1.05	26	45.2	66.0	37.1	103.1	3.04	3.06	2.86
	N ₆₀₀ K ₉₀₀	85.0	15.4	7	0.98	21	35.5	60.0	28.8	88.8	1.49	2.20	2.02
	Mean	73.2	12.9	6	0.87	31	52.7	54.4	34.3	88.7	2.53	4.08	5.01
	Control	53.3	8.5	5	0.38	38	78.6	28.7	37.1	65.8	0.0	0.0	23.25
	N ₀ K ₃₀₀	61.0	10.3	6	0.68	39	69.8	48.4	49.2	97.6	4.06	0.0	9.15
	N ₀ K ₆₀₀	65.0	11.3	5	0.59	31	62.4	35.8	30.6	66.4	0.04	0.0	3.41
	N ₀ K ₉₀₀	65.0	11.8	6	0.58	31	51.7	29.1	27.1	56.2	-0.39	0.0	2.06
	N ₂₀₀ K ₀	66.7	9.8	4	0.36	36	72.1	25.6	29.6	55.2	0.0	-1.92	6.61
	N ₄₀₀ K ₀	63.3	11.3	5	0.42	33	73.9	29.5	30.6	60.1	0.0	-0.52	4.33
	N ₆₀₀ K ₀	66.7	11.6	6	0.56	31	67.6	37.3	32.8	70.1	0.0	0.26	3.62
	N ₂₀₀ K ₃₀₀	71.7	12.7	9	1.14	50	71.5	46.2	74.5	120.7	7.00	9.95	7.46
	N ₂₀₀ K ₆₀₀	75.0	11.5	8	0.88	41	64.1	46.2	45.0	91.2	1.52	4.60	3.66
	N ₂₀₀ K ₉₀₀	78.3	13.0	8	1.09	40	63.1	60.8	39.1	99.9	1.39	6.18	3.04
	N ₄₀₀ K ₃₀₀	73.3	13.3	10	1.04	29	73.5	65.3	60.8	126.1	7.69	5.46	5.81
	N ₄₀₀ K ₆₀₀	71.7	12.5	9	0.98	36	47.9	56.6	39.9	96.5	1.84	2.78	3.16
	N ₄₀₀ K ₉₀₀	70.0	12.8	8	1.89	35	48.6	53.3	31.1	84.4	0.76	1.68	2.20
	N ₆₀₀ K ₃₀₀	63.3	11.5	9	0.90	30	59.7	51.3	28.0	79.3	1.72	0.82	2.91
	N ₆₀₀ K ₆₀₀	75.0	12.7	10	1.03	28	67.5	63.0	39.4	102.4	2.19	2.21	2.84
	N ₆₀₀ K ₉₀₀	70.0	12.1	9	1.03	23	52.9	52.6	28.3	80.9	0.62	0.91	1.84

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Table 1. Contd.

Mean	68.1	11.7	7	0.85	32	64.4	45.6	38.9	84.6	1.78	2.03	5.33
LSD_{0.05}	3.0	0.4	0.5	ns	ns	6.4	4.9	NS	NS	0.59	0.75	ns
Clone x Fertilizer	12.1	1.8	ns	0.3	ns	ns	19.7	NS	10.3	2.39	3.00	3.59

Ht = plant height; Gt = plant girth; NL = number of live leaves; LA = photosynthetically active leaf area; NR = number of roots; ARL = average length of the 5 longest roots; AE = agronomic efficiency of applied nutrient; PFP = partial factor productivity from applied nutrients; LSD_{0.05} = Least significant difference at 5% probability level.

area per plant) improved significantly ($p < 0.05$) with increasing doses of N and K, and plant growth was superior in all cases where both nutrients were applied together. Both clones maintained the best growth where N was applied at 200 or 400 kg ha⁻¹ in combination with 300 to 900 kg ha⁻¹ of K₂O. At these rates, the plants had the tallest height, largest stem girth and the broadest leaf area, and sustained greater number of leaves. Growth in 'PITA 24' was also fairly good at 600 kg N combined with 600-900 kg of K₂O per hectare.

The number of roots per plant was neither influenced by clone nor clone x fertilizer interaction. The average root length per plant seemingly declined with incremental doses of the fertilizer nutrients. In both clones, the shortest roots were observed at 400 kg N combined with 600 kg K₂O per hectare (N₄₀₀K₆₀₀). At 3 MAP, shoot growth was apparently better in 'Agbagba', but 'PITA 24' had longer roots.

The above-ground dry matter (AGDM) yield was significantly ($p < 0.05$) influenced by clone, and clone x fertilizer interaction effects. In 'Agbagba', the AGDM yield was highest at N₂₀₀K₉₀₀ followed closely by N₄₀₀K₉₀₀. The AGDM yield in 'PITA 24' was highest at N₄₀₀K₃₀₀, but statistically similar in all cases where N and K were applied together. Below-ground dry matter (BGDM) yield at 3 MAP was neither influenced by clone nor clone x fertilizer interaction; rather there was a significant ($p < 0.05$) clone-by-fertilizer interaction effect in the

whole-plant dry matter (WPDM) yield. In 'Agbagba', WPDM yield was highest (130 g) where 400 kg N was combined with 900 kg K₂O (N₄₀₀K₉₀₀) per hectare. This was however not statistically different from the dry matter yield obtained at N₂₀₀K₆₀₀ (129.4 g) and N₂₀₀K₉₀₀ (128 g). At these rates (N₄₀₀K₉₀₀, N₂₀₀K₆₀₀ and N₂₀₀K₉₀₀), there was a somewhat synergistic improvement in WPDM yield (positive nutrient interaction), since the combined doses of both nutrients produced greater dry matter yields than observed from the sole nutrients summed together. In 'PITA 24', dry matter yield was highest at N₄₀₀K₃₀₀ (126.1 g). Dry matter yield in both clones was correspondingly low in all cases where the fertilizer nutrients were applied singly, especially when a high dose of K₂O (900 kg per hectare) was applied. The WPDM yield in both clones increased sequentially with incremental doses of N, where N was applied singly (i.e., N₆₀₀K₀ > N₄₀₀K₀ > N₂₀₀K₀). Dry matter yield was a little higher in 'Agbagba' after the 3-month growth period.

Significant ($p < 0.05$) clone and clone x fertilizer interaction effects were observed in the calculated efficiencies of the applied nutrients. The agronomic efficiency (AE) of applied K⁺ (that is, dry matter yield per unit of K₂O applied) in both clones was highest at N₄₀₀K₃₀₀, followed closely by N₂₀₀K₃₀₀. Nitrogen use efficiency (agronomic efficiency of applied nitrogen, AE-N; that is, the dry matter yield per unit dose of applied N) in 'Agbagba' was highest at N₂₀₀K₆₀₀ (13.95) followed

closely by N₂₀₀K₉₀₀ (13.70). In 'PITA 24', the AE-N was highest at N₂₀₀K₃₀₀ (9.95) followed by N₂₀₀K₉₀₀ (6.18). At 3 MAP, N and K use efficiency values were higher in 'Agbagba'. The partial factor productivity (Pfp) from the applied nutrients (calculated as the plant dry matter yield per unit dose of N + P₂O₅ + K₂O) was not influenced by clone, but by clone x fertilizer interaction. The Pfp value was exceedingly high in the control plants where K and N fertilizers were not applied. Among the plants that received ample doses of N, P and K, the Pfp value from the three fertilizer nutrients was significantly ($p < 0.05$) superior at N₂₀₀K₃₀₀ in both clones.

At 6 MAP (Table 2), plant growth indices (height, stem girth, number of live leaves, leaf area) and dry matter yield in both clones were statistically ($p < 0.05$) superior in plants that received the two fertilizer nutrients (N and K) together. In 'Agbagba', the tallest plants were observed at N₄₀₀K₆₀₀ and N₆₀₀K₉₀₀. In this clone, the pseudostem girth was largest where N was applied at 200 or 400 kg ha⁻¹ in combination with 300 to 900 kg K₂O per hectare. Plants that received 400 or 600 kg of N in combination with 300 to 900 kg of K₂O produced the highest number of leaves and the broadest leaf area. The tallest and widest girth plants in 'PITA 24' were observed where 400 or 600 kg N was combined with 600 to 900 kg K₂O per hectare. In this clone, the number of leaves was high and similar in all the plants that received 400 or 600 kg N

Table 2. Effects of N & K fertilizer doses on plant growth indices, dry matter yield and nutrient use efficiency in Agbagba and PITA 24 plantains measured at 6 months after planting (MAP).

Clone	Fertilizer dose [kg.ha ⁻¹]	Ht [cm]	Gt [cm]	NL [#]	LA [m ²]	NR [#]	ARL [cm]	Dry matter yield per plant [g]			Nutrient use efficiencies		
								Above ground	Below ground	Whole plant	AEK ⁺	AEN	PPF [NPK]
Agbagba	Control	53.3	11.1	4	0.24	38	104.7	37.1	28.7	65.8	0.0	0.0	23.25
	N ₀ K ₃₀₀	56.7	12.9	4	0.31	32	71.7	43.7	55.0	98.7	4.19	0.0	9.25
	N ₀ K ₆₀₀	59.0	14.8	4	0.31	36	79.1	34.7	36.9	71.6	0.35	0.0	3.67
	N ₀ K ₉₀₀	55.0	14.4	4	0.24	30	73.7	32.1	52.6	84.7	0.77	0.0	3.10
	N ₂₀₀ K ₀	71.7	13.8	3	0.32	40	73.5	44.7	29.4	74.1	0.0	1.50	8.87
	N ₄₀₀ K ₀	74.0	14.3	3	0.49	36	69.3	49.2	37.8	87.0	0.0	1.92	6.27
	N ₆₀₀ K ₀	72.5	13.0	5	0.52	32	59.3	55.2	28.5	83.7	0.0	1.08	4.32
	N ₂₀₀ K ₃₀₀	83.0	17.6	6	1.14	43	71.1	88.6	60.5	149.1	10.63	15.09	9.21
	N ₂₀₀ K ₆₀₀	95.0	17.6	6	1.21	42	67.7	83.3	77.5	160.8	5.70	17.21	6.43
	N ₂₀₀ K ₉₀₀	97.0	17.5	6	1.37	45	54.8	106.3	62.1	168.4	4.19	18.58	5.13
	N ₄₀₀ K ₃₀₀	88.0	15.9	6	1.23	45	77.5	86.3	45.4	131.7	8.41	5.97	6.07
	N ₄₀₀ K ₆₀₀	101.0	17.6	7	1.59	37	60.3	116.7	51.3	168.0	6.13	9.26	5.50
	N ₄₀₀ K ₉₀₀	88.0	17.6	7	1.48	37	54.3	109.8	52.4	162.2	3.93	8.73	4.22
	N ₆₀₀ K ₃₀₀	90.0	16.1	8	1.49	36	51.9	100.4	46.4	146.8	10.33	4.89	5.39
	N ₆₀₀ K ₆₀₀	91.0	15.4	7	1.44	33	52.1	104.2	38.3	142.5	4.60	4.63	3.95
	N ₆₀₀ K ₉₀₀	101.0	16.4	8	1.65	27	40.1	122.9	31.6	154.5	3.62	5.35	3.52
	Mean	79.8	15.4	5	0.94	37	66.3	75.9	45.9	121.9	3.93	5.89	6.76
Control	58.0	10.3	4	0.31	44	83.6	29.9	55.8	85.7	0.0	0.0	30.28	
N ₀ K ₃₀₀	66.0	11.4	4	0.55	41	81.5	61.0	112.8	173.8	11.23	0.0	16.28	
N ₀ K ₆₀₀	71.0	12.6	4	0.57	34	66.2	59.4	93.9	153.3	4.05	0.0	7.86	
N ₀ K ₉₀₀	72.0	13.0	4	0.64	38	73.4	63.0	107.8	170.8	3.47	0.0	6.25	
N ₂₀₀ K ₀	80.0	10.9	3	0.48	42	94.0	41.2	73.3	114.5	0.0	5.22	13.71	
N ₄₀₀ K ₀	88.8	12.8	4	0.69	37	75.3	59.2	91.0	150.2	0.0	5.84	10.83	
N ₆₀₀ K ₀	89.0	13.1	4	1.00	53	87.7	65.5	86.2	151.7	0.0	3.98	7.82	
N ₂₀₀ K ₃₀₀	98.0	15.9	6	1.56	62	76.5	111.4	157.9	269.3	23.42	33.26	16.63	
N ₂₀₀ K ₆₀₀	105.0	16.9	8	1.99	61	74.8	110.1	159.7	269.8	11.05	33.35	10.79	
N ₂₀₀ K ₉₀₀	104.0	16.3	7	1.87	53	63.5	110.1	110.3	220.4	5.50	24.40	6.71	
N ₄₀₀ K ₃₀₀	101.0	15.3	10	2.01	37	74.7	112.0	107.0	219.0	17.00	12.07	10.09	
N ₄₀₀ K ₆₀₀	112.0	17.6	11	2.45	54	67.3	129.5	125.3	254.8	10.15	15.31	8.34	
N ₄₀₀ K ₉₀₀	113.0	17.8	11	2.40	59	61.0	151.0	120.7	271.7	7.59	16.85	7.08	
N ₆₀₀ K ₃₀₀	102.0	16.0	11	1.16	52	69.1	115.8	146.5	262.3	22.52	10.66	9.63	
N ₆₀₀ K ₆₀₀	103.0	17.2	11	2.26	56	67.9	131.1	129.5	260.6	10.50	10.56	7.23	
N ₆₀₀ K ₉₀₀	113.0	17.6	11	2.34	51	55.9	140.1	130.7	270.8	7.55	11.18	6.17	

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Table 2. Contd.

Mean	92.2	14.7	7	1.46	48	73.3	93.1	111.1	206.1	8.38	11.41	10.98
LSD_{0.05}	2.9	0.4	1.0	0.08	3.0	ns	5.8	11.5	15.7	1.05	1.50	1.56
Clone x Fertilizer	11.7	1.7	2.0	0.32	11.0	ns	16.7	27.7	Ns	4.20	6.10	ns

Ht = plant height; Gt = plant girth; NL = number of live leaves; LA = photosynthetically active leaf area; NR = number of roots; ARL = average length of the 5 longest roots; AE = agronomic efficiency of applied nutrient; PFP = partial factor productivity from applied nutrients; LSD_{0.05} = Least significant difference at 5% probability level.

in combination with 300 to 900 kg K₂O per hectare. The total leaf area per plant in 'PITA 24' was statistically ($p < 0.05$) superior at N₄₀₀K₆₀₀ (2.45 m²), N₄₀₀K₉₀₀ (2.40 m²), N₆₀₀K₆₀₀ (2.26 m²) and N₆₀₀K₉₀₀ (2.34 m²).

In both clones, the number of roots per plant was superior when 200 kg N was applied with 300 to 900 kg K₂O per hectare, but root number and root length seemingly declined at higher doses of N and K combinations.

In 'Agbagba', the longest roots were observed in the control plants (104.7 cm), whereas N₂₀₀K₀ produced the longest roots (94.0 cm) in 'PITA 24'. The AGDM yield in 'Agbagba' was superior and statistically similar at N₄₀₀K₆₀₀ (116.7 g) and N₆₀₀K₉₀₀ (122.9 g), whereas N₄₀₀K₉₀₀ produced the highest AGDM yield (151.0 g) in 'PITA 24' followed closely by N₆₀₀K₉₀₀ (140.1 g), N₆₀₀K₆₀₀ (131.1 g) and N₄₀₀K₆₀₀ (129.5 g), respectively. Combinations N₂₀₀K₆₀₀ and N₂₀₀K₃₀₀ produced the highest BGDM yield in both clones. In 'Agbagba' the highest WPDM yield was recorded at N₂₀₀K₉₀₀ (168.4 g). A similar whole-plant dry matter (WPDM) yield was produced at N₄₀₀K₆₀₀ (168.0 g), N₄₀₀K₉₀₀ (162.2 g) and N₂₀₀K₆₀₀ (160.8 g). The WPDM yield in 'PITA 24' was highest at N₄₀₀K₉₀₀ (271.7 g), followed by N₆₀₀K₉₀₀ (270.8 g), N₂₀₀K₆₀₀ (269.8 g) and N₂₀₀K₃₀₀ (269.3 g), respectively; and was equally high in all combinations where N and K were applied together.

In both clones, the agronomic efficiency of

applied potassium (AE-K⁺) was highest at N₂₀₀K₃₀₀, followed by N₆₀₀K₃₀₀ and N₄₀₀K₃₀₀, respectively. In 'Agbagba' the agronomic efficiency of applied nitrogen (AE-N) at 6 MAP was highest at N₂₀₀K₉₀₀ (18.58) followed closely by N₂₀₀K₆₀₀ (17.21) and N₂₀₀K₃₀₀ (15.09). The AE-N value in 'PITA 24' was highest and similar for N₂₀₀K₆₀₀ (33.35) and N₂₀₀K₃₀₀ (33.26), followed by N₂₀₀K₉₀₀ (24.40 g).

In all cases where ample doses of N and K were applied together, the partial factor productivity (Pfp) from the applied nutrients (N, P and K) in both clones was highest at N₂₀₀K₃₀₀ followed by N₂₀₀K₆₀₀ and N₄₀₀K₃₀₀, respectively. The Pfp value was exceedingly high in the control plants where N and K were not applied. For each nutrient element, the Pfp value declined progressively as the nutrient dose increased. At 6 MAP, plant height, number of leaves per plant, total leaf area, number of roots and average root length per plant were statistically ($p < 0.05$) superior in 'PITA 24', but pseudostem girth was larger in 'Agbagba'. Dry matter yield and the nutrient use efficiency values were almost double-fold higher in 'PITA 24'.

The main effect of the fertilizer nutrient doses on plantain growth, dry matter yield and nutrient use efficiency (at 3 and 6 MAP) presented in Table 3 were in most cases significant ($p < 0.05$). At 3 MAP, plant height, stem girth and number of leaves were similar and superior in all cases where N and K were applied together, whereas

the poorest growth was observed in the control plants. The largest leaf area was recorded at N₄₀₀K₉₀₀ (1.66 m²), followed by N₂₀₀K₉₀₀ (1.21 m²) and N₄₀₀K₆₀₀ (1.12 m²), respectively. Leaf area was relatively large in all cases where N and K were applied together, whereas root length seemingly declined with incremental doses of applied nutrients. The number of roots per plant was highest at N₂₀₀K₃₀₀ (44), followed by N₂₀₀K₉₀₀ (43) and N₂₀₀K₆₀₀ (40), respectively. Also at 3 MAP, the above-ground and whole-plant dry matter yield values were highest at N₄₀₀K₃₀₀, followed by N₂₀₀K₉₀₀, and significantly ($p < 0.05$) high in all cases where N and K were applied together. The AE-K⁺ was highest at N₄₀₀K₃₀₀ (8.06), followed by N₂₀₀K₃₀₀ (6.63), whereas the efficiency of applied nitrogen (AE-N) was high and statistically similar in N₂₀₀K₉₀₀ (9.93), N₂₀₀K₃₀₀ (9.42) and N₂₀₀K₆₀₀ (9.27). The partial factor productivity (Pfp) value (for the plants that received N, P and K) was highest at N₂₀₀K₃₀₀ (6.86), followed by N₄₀₀K₃₀₀ (5.63), whereas N₆₀₀K₉₀₀ recorded the least value (1.93).

At 6 MAP (Table 3), plant height was tallest at N₆₀₀K₉₀₀ (107.0 cm), followed by N₄₀₀K₆₀₀ (106.5 cm); and correspondingly high in all cases where ample doses of N and K were applied together. Similarly, pseudostem girth, number of leaves per plant, and leaf area values were statistically superior where N and K were applied together, and were highest at N₄₀₀K₉₀₀, N₆₀₀K₉₀₀ and

Table 3. Main effect of combined doses of N and K on growth, dry matter yield and nutrient use efficiency in plantains (*Musa AAB*) measured at 3 and 6 months after planting.

Fertilizer dose [kg.ha ⁻¹]	Ht [cm]	Gt [cm]	NL [#]	LA [m ²]	NR [#]	ARL [cm]	Dry matter yield per plant [g]			Nutrient use efficiencies		
							Above ground	Below ground	Whole Plant	AEK ⁺	AEN	FPF [NPK]
							3 months after planting			6 months after planting		
Control	49.7	8.6	5	0.35	38	77.4	27.0	32.1	59.1	0.0	0.0	20.88
N ₀ K ₃₀₀	55.0	10.0	5	0.50	34	76.6	36.3	42.7	79.0	2.54	0.0	7.41
N ₀ K ₆₀₀	61.7	11.1	5	0.51	33	62.2	32.5	30.8	63.3	0.25	0.0	3.25
N ₀ K ₉₀₀	56.5	10.8	5	0.46	30	62.7	25.8	27.7	53.5	-0.28	0.0	1.96
N ₂₀₀ K ₀	66.9	10.6	4	0.52	38	73.1	28.8	25.8	54.6	0.0	-0.82	6.54
N ₄₀₀ K ₀	68.3	11.7	5	0.51	35	67.7	34.6	28.1	62.7	0.0	0.33	4.52
N ₆₀₀ K ₀	63.9	11.5	6	0.65	30	67.0	42.3	27.9	70.2	0.0	0.67	3.62
N ₂₀₀ K ₃₀₀	75.0	13.4	8	1.09	44	57.9	56.0	55.1	111.1	6.63	9.42	6.86
N ₂₀₀ K ₆₀₀	83.3	13.1	8	1.05	40	57.3	62.1	48.2	110.3	3.07	9.27	4.41
N ₂₀₀ K ₉₀₀	86.7	14.1	8	1.21	43	56.1	71.6	42.3	113.9	2.24	9.93	3.47
N ₄₀₀ K ₃₀₀	78.3	13.5	9	1.10	37	65.4	73.4	49.0	122.4	8.06	5.72	5.63
N ₄₀₀ K ₆₀₀	80.8	14.2	9	1.12	36	45.0	64.8	39.8	104.6	2.73	4.13	3.43
N ₄₀₀ K ₉₀₀	79.2	14.1	9	1.66	36	48.7	67.4	39.9	107.2	1.96	4.36	2.79
N ₆₀₀ K ₃₀₀	70.0	12.9	9	0.99	31	54.1	56.8	29.8	86.6	3.50	1.66	3.18
N ₆₀₀ K ₆₀₀	77.5	13.6	8	1.04	31	56.5	64.5	38.2	102.7	2.62	2.63	2.85
N ₆₀₀ K ₉₀₀	77.5	13.8	8	1.00	22	48.0	56.3	28.5	84.8	1.05	1.55	1.93
LSD _(0.05)	8.6	1.3	1.0	0.4	12	18.2	14.0	ns	29.0	1.69	2.12	2.54
Control	55.6	10.7	4	0.27	38	91.6	33.5	42.2	75.7	0.0	0.0	26.77
N ₀ K ₃₀₀	61.0	12.2	4	0.43	36	68.7	52.4	83.9	136.3	7.71	0.0	12.77
N ₀ K ₆₀₀	65.0	13.7	4	0.44	32	72.7	47.0	65.4	112.4	2.20	0.0	5.77
N ₀ K ₉₀₀	63.5	13.7	4	0.44	31	68.2	47.6	80.2	127.8	2.12	0.0	4.67
N ₂₀₀ K ₀	75.9	12.4	3	0.40	33	72.8	43.0	51.3	94.3	0.0	3.36	11.29
N ₄₀₀ K ₀	81.4	13.6	3	0.59	33	71.6	54.2	64.4	118.6	0.0	3.88	8.55
N ₆₀₀ K ₀	80.8	13.1	4	0.76	43	63.5	60.4	57.3	117.7	0.0	2.53	6.07
N ₂₀₀ K ₃₀₀	90.5	16.8	6	1.35	52	73.8	100.0	109.2	209.2	17.02	24.17	12.92
N ₂₀₀ K ₆₀₀	100.0	17.3	7	1.60	51	71.2	96.7	118.6	215.3	8.38	25.28	8.61

Table 3. Contd.

N ₂₀₀ K ₆₀₀	100.5	16.9	7	1.62	43	59.0	108.2	86.2	194.4	4.84	21.49	5.92
N ₄₀₀ K ₃₀₀	94.5	15.6	8	1.62	35	76.1	99.2	76.2	175.4	12.70	9.02	8.08
N ₄₀₀ K ₆₀₀	106.5	17.6	9	2.02	46	63.8	123.1	88.3	211.4	8.14	12.29	6.92
N ₄₀₀ K ₉₀₀	100.5	17.7	9	1.94	47	57.7	130.4	86.5	216.9	5.76	12.79	5.65
N ₆₀₀ K ₃₀₀	96.0	16.1	9	1.83	44	60.5	108.1	96.5	204.6	16.43	7.77	7.51
N ₆₀₀ K ₆₀₀	97.0	16.3	9	1.85	41	59.8	117.7	83.9	201.6	7.55	7.60	5.59
N ₆₀₀ K ₉₀₀	107.0	17.0	10	1.99	40	44.2	131.5	81.2	212.7	5.59	8.27	4.84
LSD(0.05)	8.3	1.2	1.0	0.22	7	12.3	16.3	32.6	44.4	2.96	4.31	4.40

HT = plant height; Gt = plant girth; NL = number of live leaves; LA = photosynthetically active leaf area; NR = number of roots; ARL = average length of the 5 longest roots; AE = agronomic efficiency of applied nutrient; PFP = partial factor productivity from applied nutrients; LSD(0.05) = Least significant difference at 5% probability level.

N₄₀₀K₆₀₀. Also at 6 MAP, the highest number of roots were produced at N₂₀₀K₃₀₀ (52) and N₂₀₀K₆₀₀ (51), whereas the control plants produced the longest roots. Thus, root length declined with incremental doses of the applied nutrients.

The dry matter yield at 6 MAP was poorest in the control plants, and was generally poor in all cases where the nutrient elements were applied singly. The AGDM yield was high, and statistically similar at N₆₀₀K₉₀₀ (131.5 g), N₄₀₀K₉₀₀ (130.4 g), N₄₀₀K₆₀₀ (123.1 g) and N₆₀₀K₆₀₀ (117.7 g). Below-ground dry matter yield (BGDM) was highest at N₂₀₀K₆₀₀ (118.6 g), followed by N₂₀₀K₃₀₀ (109.2 g). The WPDM yield at 6 MAP was significantly ($p < 0.05$) higher in all cases where N and K were applied together. Whole-plant dry matter yield was highest at N₄₀₀K₉₀₀ (216.9 g), followed by N₂₀₀K₆₀₀ (215.3 g), N₆₀₀K₉₀₀ (212.7 g), N₄₀₀K₆₀₀ (211.4 g), and N₂₀₀K₃₀₀ (209.2 g), respectively. The control plants produced the least dry matter yield (75.7 g) after N₂₀₀K₀ (94.3 g). Potassium use efficiency was significantly ($p < 0.05$) high at N₂₀₀K₃₀₀ (17.02) and N₆₀₀K₃₀₀ (16.43), followed by

N₄₀₀K₃₀₀ (12.70), N₂₀₀K₆₀₀ (8.38) and N₄₀₀K₆₀₀ (8.14), respectively. The AE-N value was high and statistically similar in N₂₀₀K₆₀₀ (25.28), N₂₀₀K₃₀₀ (24.17) and N₂₀₀K₉₀₀ (21.49), followed by N₄₀₀K₉₀₀ (12.79) and N₄₀₀K₆₀₀ (12.29). Among the plants that received the three nutrient elements (NPK), the Pfp value was highest at N₂₀₀K₃₀₀ (12.92), followed by N₂₀₀K₆₀₀ (8.61), N₄₀₀K₃₀₀ (8.08), N₆₀₀K₃₀₀ (7.51) and N₄₀₀K₆₀₀ (6.92), respectively. The poorest value (among the plants that received N, P and K) was recorded in N₆₀₀K₉₀₀ (4.84).

The principal component analysis (PCA) results (Table 4) captured more than 80% of the total variation that existed among the studied parameters following the fertilizer treatments after the 3 and 6 months growth periods. Plant stature (height, stem girth, leaf area, number of live leaves), the above-ground and whole-plant dry matter yield, and the agronomic efficiency of applied nitrogen (AE-N) accounted for more than half (51.44 to 60.70%) of the total variation observed at the end of the 3 and 6 months growth periods. The partial factor productivity

(Pfp) from the applied nutrients (a ratio of dry matter yield to the total applied nutrients) and the length of the plant roots, and the below-ground dry matter yield explained merely 15 to 18% of the total variation, whereas the number of roots per plant explained about 8.34 and 5.48% of the observed variations at 3 and 6 MAP, respectively.

The correlative responses among the studied parameters for the 3 and 6 months growth periods are shown in Table 5. Pseudostem girth had a very strong positive relationship with plant height at both growth periods. Throughout the study period, the number of leaves per plant maintained a moderate positive significant ($p < 0.05$) relationship with plant height, stem girth, whole-plant and below-ground dry matter yield, as well as, the agronomic efficiency of applied nitrogen and potassium.

At 3 MAP, the number of roots per plant had very low positive relationship with plant height ($r = 0.261^*$), girth ($r = 0.270^*$), number of live leaves ($r = 0.029$) and total leaf area ($r = 0.201^*$). The magnitude of these coefficients greatly increased at 6 MAP. The

Table 4. Principal Component Analysis[†] showing the relative contributions of growth traits, dry matter yield and nutrient use efficiencies to the total variation observed following the fertilizer treatment applications after a 3 and 5 months growth periods

Traits	3 months after planting					6 months after planting				
	Prin1 (51.44%)	Prin2 (18.56%)	Prin3 (8.34%)	Prin4 (5.24%)	Prin1 (60.70%)	Prin2 (15.34%)	Prin3 (5.48%)	Prin4 (4.83%)		
AE-K ⁺	-0.26165	-0.29142	0.34909	0.02662	-0.26083	-0.19989	0.06656	0.06644		
AE-N ₂	-0.27881	-0.24057	0.23557	-0.21356	-0.27840	-0.17868	-0.14090	0.36261		
PFP [NPK]	0.04618	-0.44108	-0.36316	0.69081	0.00566	-0.56777	0.03868	-0.65799		
AgDM (g)	-0.35876	0.06509	-0.11315	0.08713	-0.33196	0.13409	0.09943	-0.16919		
AvRL (cm)	0.12476	-0.40335	0.45728	-0.22433	0.06430	-0.49490	0.67057	0.43211		
BgDM (g)	-0.23367	-0.44529	-0.02050	-0.07528	-0.27476	-0.35724	-0.16083	0.03459		
CD (cm)	-0.34381	0.11848	-0.07431	-0.13863	-0.31852	0.06985	-0.13442	0.18455		
PGt (cm)	-0.34523	0.21320	-0.07205	-0.01662	-0.33032	0.17513	0.09402	0.08264		
PHT (cm)	-0.31464	0.21026	-0.16626	-0.17436	-0.31121	0.16485	0.11309	0.09609		
LA (m ²)	-0.30247	0.20608	0.10158	0.20501	-0.33353	0.14681	0.21939	-0.12973		
NLvs (#)	-0.26222	0.13688	0.43159	0.49987	-0.27830	0.19580	0.40072	-0.37845		
Rts (#)	-0.17506	-0.30056	-0.47614	-0.26631	-0.23402	-0.25938	-0.48749	0.02504		
WPDM (g)	-0.35428	-0.20542	-0.08232	0.01303	-0.33723	-0.15916	-0.05237	-0.06171		
Latent Roots	6.687	2.413	1.084	0.681	7.892	1.994	0.712	0.628		

PHT = plant height; PGt = plant girth; NLvs = number of live leaves; LA = photosynthetically active leaf area; CD = corn cross-sectional diameter; Rts = number of roots; AvRL = average length of the 5 longest roots; AE = agronomic efficiency of applied nutrient; PFP [NPK] = partial factor productivity from applied nutrients; AgDM = above-ground dry matter yield; BgDM = below-ground dry matter yield; WPDM = whole-plant dry matter yield ([†]PCA based on correlation matrix).

number of roots per plant also maintained moderate positive significant ($p < 0.05$) relationships with the nutrient use efficiency values (Pfp , $AE-N$ and $AE-K^+$), as well as, the below-ground and whole-plant dry matter yield at both growth periods.

The average root length had very weak relationship with most of the parameters studied at 3 months. At 6 MAP, moderate to high positive significant ($p < 0.01$) relationships were maintained between the root length and most of the other parameters. For instance, the correlation

coefficients between the root length (AvRL) and $AE-N$, $AE-K^+$, WPDM, BgDM yield and number of roots per plant were 0.696**, 0.672**, 0.678**, 0.678** and 0.915**, respectively. Similarly, corn size (diameter) increased significantly ($p < 0.05$) with plant size (height, girth, number of leaves, leaf area and root number), the agronomic efficiencies of applied nitrogen and potassium, as well as, the dry matter yield at both growth periods.

The partial factor productivity (Pfp) from applied nutrients had very poor relationship with most of

the studied parameters at both growth periods. However at 6 MAP, a weak but significant ($p < 0.01$) positive relationship existed between Pfp and root length ($r = 0.329^{**}$), the above-ground dry matter ($r = 0.394^{**}$) and below-ground dry matter yield ($r = 0.203^*$). The agronomic efficiency of applied nutrients (N and K) had moderate positive significant ($p < 0.01$) relationships with the plant growth parameters (including plant height, pseudostem girth, number of leaves, leaf area, number of roots and corn diameter) and dry matter yield at both growth stages. The whole-

Table 5. Linear correlation matrix between the plant growth parameters, dry matter yield and nutrient use efficiency in plantains measured at 3 and 6 MAP as influenced by factorial combinations of N and K fertilizer.

Traits [†]	3 months after planting											6 months after planting														
	PFP _[NPK]	AE-N ₂	AE-K ⁺	WPDM	BgDM	AgDM	AvRL	CD	#Rts	LA	NLvs	PGt	PHt	PFP _[NPK]	AE-N ₂	AE-K ⁺	WPDM	BgDM	AgDM	AvRL	CD	#Rts	LA	NLvs	PGt	PHt
PHt	-0.286**	0.416**	0.330**	0.669**	0.260**	0.836**	-0.434**	0.778**	0.261*	0.674**	0.454**	0.827**	1	-0.165 ^{ns}	0.641**	0.511**	0.799**	0.489**	-0.211*	0.518**	0.836**	0.739**	0.879**	0.685**	0.900**	1
PGt	-0.305**	0.473**	0.396**	0.726**	0.339**	0.859**	-0.487**	0.865**	0.270**	0.791**	0.626**	1	-0.198*	0.697**	0.554**	0.863**	0.500**	-0.259**	0.568**	0.919**	0.812**	0.912**	0.744**	1	1	
NLvs	-0.207*	0.453**	0.521**	0.495**	0.209*	0.606**	-0.239*	0.522**	0.029 ^{ns}	0.676**	1	1	-0.134 ^{ns}	0.407**	0.492**	0.614**	0.388**	-0.243**	0.423**	0.810**	0.667**	0.905**	1	1	1	
LA	-0.249*	0.429**	0.393**	0.562**	0.229*	0.694**	-0.361**	0.704**	0.201*	1	1	1	-0.131 ^{ns}	0.642**	0.599**	0.802**	0.539**	-0.222*	0.584**	0.921**	0.823**	1	1	1	1	
#Rts	0.266**	0.410**	0.387**	0.520**	0.517**	0.374**	-0.059 ^{ns}	0.296**	1	1	1	1	1	0.758**	0.741**	0.837**	0.666**	-0.064 ^{ns}	0.915**	0.857**	1	1	1	1	1	
CD	-0.236*	0.542**	0.450**	0.778**	0.443**	0.849**	-0.375**	1	1	1	1	1	1	0.646**	0.644**	0.830**	0.482**	-0.300**	0.575**	1	1	1	1	1	1	
AvRL	0.238*	0.028 ^{ns}	0.138 ^{ns}	-0.119 ^{ns}	0.201*	-0.366**	1	1	1	1	1	1	1	0.696**	0.672**	0.678**	0.678**	0.134 ^{ns}	1	1	1	1	1	1	1	1
AgDM	-0.077 ^{ns}	0.553**	0.545**	0.867**	0.433**	1	1	1	1	1	1	1	1	0.394**	0.042 ^{ns}	-0.210*	0.002 ^{ns}	1	1	1	1	1	1	1	1	1
BgDM	0.354**	0.666**	0.654**	0.825**	1	1	1	1	1	1	1	1	1	0.585**	0.502**	0.572**	1	1	1	1	1	1	1	1	1	1
WPDM	0.148 ^{ns}	0.715**	0.704**	1	1	1	1	1	1	1	1	1	1	0.649**	0.588**	1	1	1	1	1	1	1	1	1	1	1
AE-K ⁺	0.074 ^{ns}	0.691**	1	1	1	1	1	1	1	1	1	1	1	0.608**	1	1	1	1	1	1	1	1	1	1	1	1
AE-N ₂	-0.001 ^{ns}	1	1	1	1	1	1	1	1	1	1	1	1	0.104 ^{ns}	1	1	1	1	1	1	1	1	1	1	1	1
PFP _[NPK]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

** Correlation is significant at 1% probability level (2-tailed). *Significant at 5% probability level; ns = not significant. [†]Description of studied traits is same as in Table 4.

plant dry matter yield maintained a high positive relationship with plant height, pseudostem girth, leaf area, root number, corm diameter and the efficiency of applied nutrients (N and K), particularly at 6 MAP.

DISCUSSION

The significant variability observed in the growth indices and plant stature (height, stem girth, leaf number, leaf area, etc.), dry matter yield, and the efficiency of nutrient use in this study corroborates the assertion that plant nutrition is the most singular factor controlling growth behavior and crop yields (Akinrinde, 2006). In this study, plants that received ample doses of nitrogen and potassium maintained superior growth and higher dry matter yield than those grown with single doses of the either nutrients (N or K), and the non-fertilized control plants. This observation is in line with the Liebig's law of minimum, that 'the most limiting factor of crop production determines the extent of crop performance'. Thus, an addition of the most limiting element would often cause more efficient utilization of a less limiting element. In addition to nutrient amounts, the balance between different nutrients plays a vital role in the improvement of crop yields (Krauss, 2000). Changing the level of one nutrient element in the soil will often affect the uptake or transport within the plant of another nutrient. In a complete nutrient management program, balanced nutrient supply is very important since 'optimum nutrient ratios' in the soil or plant tissues could be obtained even when nutrient amounts are not in the sufficient range. Two nutrients could both be in the deficiency range or at toxicity levels, yet maintain an optimum balance (Jones, 2002).

The potassium requirement for *Musa* crops is often a double-fold that of nitrogen (Lahav, 1995; Bekunda and Manzi, 2003). Conversely, potassium deficiency diminishes growth and yield potentials in bananas and plantains because of the decreased uptake of other essential elements, particularly N and P (Twyford and Walmsley, 1973; Akinyemi et al., 2004). Although high concentrations of K^+ may be tolerated in plants, a significant yield reduction has been reported in plantain due to the inhibitory action of excessive doses of potassium on the absorption of other plant nutrients (Obiefuna, 1984b). In the present study, the two plantain genotypes maintained their best growth where N was applied at 200 or 400 kg.ha⁻¹ in combination with 300, 600 or 900 kg K₂O per hectare. Thus, fertilizer combinations N₂₀₀K₉₀₀ (that is, 200 kg N + 900 kg K₂O.ha⁻¹), N₄₀₀K₆₀₀, N₄₀₀K₉₀₀, N₂₀₀K₆₀₀, N₆₀₀K₉₀₀ and N₂₀₀K₃₀₀ produced the highest dry matter yield suggesting that besides the essentiality of N and K in the nutrition of

plantain crops, higher doses of potassium are required than nitrogen. The whole-plant dry matter yield was expectedly poorest in the control plants, and was generally poor in all cases where the nutrient elements were applied singly.

It was also observed from this study that the efficiency of nutrients use varied significantly with factorial doses of the applied nutrients. At the end of the 6-month growth period, potassium use efficiency values were high where K₂O was applied at 300 or 600 kg.ha⁻¹ in combination with 200, 400 or 600 kg of nitrogen per hectare. Similarly, the efficiency of applied N was high and statistically similar where nitrogen was applied at 200 or 400 kg in combination with 300 to 900 kg K₂O per hectare. The partial factor productivity value from the applied nutrients (dry matter yield per unit dose of N + P₂O₅ + K₂O) was highest in plants that received a combination of 200 kg N and 300 kg K₂O with the blanket application of 100 kg P₂O₅ per hectare. This suggests that within the limits of the present study, it would be most economical to grow plantain with 200 kg N and 300 kg K₂O per hectare. In soils of poor native fertility, yield potential is expected to be higher at 400 kg N plus 600 kg K₂O.ha⁻¹. As earlier stated, the fertilizer requirements for *Musa* crops range from 200 to 400 kg/ha N, 45 to 60 kg/ha P, and 240 to 480 kg/ha K per year (FAO, 2000). Wilson et al. (1987) suggested split applications of 300 kg N and 550 kg K₂O per hectare annually in combination with organic mulching (at 3-5 t.ha⁻¹) and P₂O₅ at 250 kg/ha (applied at planting) for optimal performance of plantains in the rainforest belt of West and Central Africa. Stover and Simmonds (1987) also recommended 300 to 600 kg nitrogen and 400 to 800 kg potassium per hectare for the dessert bananas.

The principal component analysis results revealed that plant stature (height, stem girth, corm size diameter, number of leaves per plant, and total leaf area), the whole-plant dry matter yield and the agronomic efficiency of applied nitrogen were the most influenced parameters following the fertilizer treatment applications. The correlative responses between these variables were found to be highly positive, meaning that they would increase or decrease correspondingly with each other in response to applied fertilizer treatment. At the end of the 6-month growth period, 'PITA 24', a hybrid genotype, maintained a better growth, produced higher dry matter yield, and had a greater efficiency of nutrient use than the landrace plantain, 'Agbagba'. Variability in the efficiency of resource conversion and biomass yield has been observed in *Musa* species, and could be attributed to differences in genomes (Robinson, 1996; Stover and Simmonds, 1987). In an earlier study (Aba et al., 2009), bunch and fruit yield in 'PITA 24' was found to be higher than that of a landrace plantain, 'Mbi-Egome'. The whole-plant biomass accumulation, just like the size of the

source organs (essentially photosynthetic leaf area) has a direct relationship with the quality and quantity of photo-assimilates partitioned to the harvestable portion (Baiyeri et al., 2005). Results from Ndukwe et al. (2012) showed significant positive relationship between the pre-flowering growth variables of plantains and the final bunch harvest. The higher biomass yield found in 'PITA 24' plantain would eventually translate to higher bunch yield.

Conclusions

(1) At the end of the 6-months growth period, 'PITA 24' hybrid maintained a better growth, produced higher dry matter yield, and had a greater efficiency of nutrient use than the landrace, 'Agbagba'.

(2) Plants that received combined doses of nitrogen and potassium fertilizer maintained superior growth and higher dry matter yield than those grown with single doses of either nutrients (N or K), while the non-fertilized control plants produced the poorest biomass.

(3) The two plantain genotypes maintained the best growth where N was applied at 200 or 400 kg.ha⁻¹ in combination with 300, 600 or 900 kg K₂O per hectare; thus fertilizer combinations N₂₀₀K₉₀₀ (that is, 200 kg N + 900 kg K₂O.ha⁻¹), N₄₀₀K₆₀₀, N₄₀₀K₉₀₀, N₂₀₀K₆₀₀, N₆₀₀K₉₀₀ and N₂₀₀K₃₀₀ produced similar growth and dry matter yield.

(4) The agronomic efficiency (AE) of the applied K⁺ (that is, dry matter yield per unit of K₂O applied) was high and similar at N₂₀₀K₃₀₀, N₄₀₀K₃₀₀ and N₆₀₀K₃₀₀; whereas the agronomic efficiency of applied N (the dry matter yield per unit dose of applied N) was superior at N₂₀₀K₆₀₀, N₂₀₀K₃₀₀ and N₂₀₀K₉₀₀.

(5) For both genotypes, the partial factor productivity (Pfp) values from the applied nutrients (that is, the whole plant dry matter yield per unit dose of N + P₂O₅ + K₂O applied) were highest at N₂₀₀K₃₀₀, suggesting that it was most economical to grow plantains with 200 kg N and 300 kg K₂O.ha⁻¹.yr⁻¹. Absolute yield could be higher at 400 kg N and 600 kg K₂O.ha⁻¹.yr⁻¹ in very poor soils.

(6) For optimum growth of plantains in the humid tropical region of southeastern Nigeria, results from the present study suggest the combined application of 200 to 400 kg N, 300 to 600 kg K₂O and 100 kg P₂O₅ per hectare per annum. This translates to an annual application rate of 261 to 522 g urea, 300 to 600 g MOP and 333 g SSP per plant at the recommended spacing of 3 × 2 m. The exact application rates depend largely on the soil native fertility.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Treatment and post-treatment effects of neem leaves extracts on *Plutella xylostella* (Lepidoptera: Plutellidae)

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The diamond back moth, *Plutella xylostella* Linnaeus, 1758 (Lepidoptera: Plutellidae) is improperly controlled with synthetic insecticides in vegetable crops. This practice, which pollutes the environment, led to human and animal health problems, and *P. xylostella* resistance. Neem (*Azadirachta indica* A. Juss) is reported in the literature as a natural alternative to synthetic insecticides in the control of many insect pests including *P. xylostella*. The aim of our study is to evaluate the toxic and post-treatment effects of neem leaves extracts on *P. xylostella* larvae in comparison to synthetic insecticides namely Conquest plus 388 EC and Cydim Super. Results revealed that exposure of third instars larvae (L₃) of *P. xylostella* on cabbage leaves treated with neem extracts for 24 h induced mortality rates between 1.67 and 6.67% ($p > 0.05$). Larvae consumed between 45.17 ± 4.48 and 210 ± 27.17 mm² of cabbage leaves surfaces after 24 h exposure ($p < 0.05$). The emerging rates of *P. xylostella* adults were lower for extracts of neem and were between 19.44 ± 6.81 and $20.55 \pm 5.38\%$ compared to the controls and insecticide treatments which rates were between 34.07 ± 6.35 and $70.37 \pm 10.25\%$ ($p < 0.05$). The neem leaves extracts were more effective than synthetic insecticides in the control of *P. xylostella*. Therefore they can be considered as a new hope in developing a management program on *P. xylostella*.

Key words: Neem leaves extracts, *Plutella xylostella*, cabbage consumed, mortality.

INTRODUCTION

Cabbage *Brassica oleracea* L. var. F1 KK cross is one of the most important market gardening, heat resistant vegetables grown in the world. It is eaten raw in salads or cooked. Its production generates substantial income for the producers and others stakeholders involved in the marketing system. However, its production limited by

biotic pressure from insect pests particularly from the diamondback moth (DBM) *Plutella xylostella* Linnaeus, 1758 which causes a complete loss of the crop (Kim et al., 2001; Baek et al., 2005). *P. xylostella* also reduces marketability by causing damage to the cabbage through larvae consumption or by their frass. The major method

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to control that pest is the use of synthetic pesticides (Shelton et al., 1997; Améwuamé, 2006; Dovlo, 2007) leading often to serious environmental problems besides affecting the health of producers and consumers. They also reduce natural enemies population, induce insect resistance and increase the need for insecticide and production cost (Rodriguez et al., 2003; Regnault -Roger et al., 2004). To overcome resistance problem, producers in Togo increase frequency and pesticide doses application (Dovlo, 2007). An important alternative to synthetic pesticides is the use of botanical pesticides (Rodriguez et al., 2003; Sharma and Gupta, 2009; Defago et al., 2011) such as those extracted from the neem tree *Azadirachta indica* A. Juss, 1830 (Meliaceae) (Charleston et al., 2005). Different compounds of kernel of this tree have been recognised for their insecticidal properties, i.e. repellent, antifeedant and growth disruptive effects which have been found among several insect species treated with neem extracts (Isman, 2006; Mikami and Ventura, 2008; Mondédji et al., 2014; Shannag et al., 2014). Neem leaves extracts are also recognised for their insecticidal properties (Afshan, 2002; da Cruz et al., 2008). The neem seeds are not available over the year but the leaves are present at any time. Neem products (Agroneem™, Ecozin™, Azatin, Neemix™, Margosan O, Azatrol etc.) have been developed as commercial insecticides and are available in several countries (Liang et al., 2003; Shannag et al., 2014) but not in Togo (Améwuamé, 2006). Adults of Lepidoptera play important role in host plant selection (Schoonhoven, 1987; Bernays and Weiss, 1996) but larvae are responsible of damages (Liang et al., 2003; Charleston et al., 2005). Owing to such potential of neem leaves-based preparations to control insect populations, our hypothesis is that these preparations induce larvae mortality 24 h after ingestion or are antifeedant for them and affect the emergence rate of adults. The aim of this work was to assess treatment effects of neem leaves extracts (lethal and antifeedant effects) and post-treatment effect (emergence) on 3rd instars larvae of cabbage pest *P. xylostella* in laboratory.

MATERIALS AND METHODS

Experimental conditions and cabbage plants production

All experiments were conducted at Laboratoire d'Entomologie Appliquée of the University of Lome in Togo. Cabbage plants (*B. oleracea* L. var. F1 KK cross) were potted in plastic buckets of 22 cm diameter and 20 cm height containing compost and grown outdoors for six to eight weeks. They were produced under natural rainfall and sunlight conditions but not treated. The plants were covered with pieces of untreated net to protect them from insects attack at 28±3°C, 75±5% RH and a photoperiod of 12:12 h LD. At the beginning of the experience, healthy plants with 7-8 fully expanded leaves were used.

Neem leaves extract preparation

Fresh leaves of *A. indica* were collected from trees in the field at the

University of Lome (in southern Togo). These leaves were crushed and mixed with water or ethanolic solution (10%) to obtain aqueous extract of neem leaves called neem foliar water extract (NFW) or ethanolic extract of neem leaves called neem foliar ethanolic extract (NFETOH) respectively. For the mixture, 1 kg of crushed leaves were added to 1.5 L of water or ethanolic solution (10%). The suspension was kept overnight (12 h) at the room temperature. The suspension was then strained through muslin cloth. The extracts effects were compared with controls (C), distilled water (CW), ethanolic solution (CEtOH) and two synthetic pesticides: Conquest plus 388 EC (Acetamiprid: 16 g/L, Cypermethrin : 72 g/L and Triazophos : 300 g/L) and Cydim Super (Cypermethrin : 36 g/L and Dimethoate 400 g/L). Conquest plus (CP), a cotton culture pesticide and Cydim Super (CS) are both used on crops by producers. Conquest plus 388 EC and Cydim Super were diluted in water to obtain doses of 517 and 1017 mg/L respectively.

Rearing of *P. xylostella*

P. xylostella larvae were collected from field cabbages in the Research Farm of the Agronomic School of the University of Lome in Togo. Moth was reared on cabbage plants of six to eight weeks age in bucket. Each bucket containing cabbage, was covered with a section of untreated net stretched with elastic. Ten males and ten females of *P. xylostella* were put on each pot for 24 h. Third instars larvae were obtained after 7 to 8 days.

Treatment effects

Lethal effect

Disks of cabbage leaves (diameter: 9 cm or area: 6358.5 mm²) were cut and soaked into solutions for 20 s except negative untreated control (C) disks. After air-drying for 1 h, each disk was inserted in a Petri dish (diameter: 9 cm) containing agar gel. Ten larvae of third instars were transferred onto treated or untreated leaf disks in Petri dishes. They were then introduced in incubator with a temperature of 25°C on night and 30°C on light, a photoperiod of 12:12 h LD and RH of 35 ± 5%. Dead and survived larvae were counted after 24 h. Each treatment was replicated 6 times and each replicate consisted of 10 larvae.

Antifeedant effect

To study antifeedant effect, the surface eaten by larvae during 24 h, was evaluated through a graph paper. Each cabbage leaf placed in the presence of the larvae was withdrawn after 24 h and deposited on the graph paper. The consumed surfaces were then reproduced and estimated.

Post-treatment effects

The survived larvae were individually transferred onto untreated cabbage leaf disks every 48 h until all larvae died or became nymphs. The nymphs were monitored until they all died or until the adult emergence. In experimental conditions, adults emerged from pupae after 4 days. This experiment aimed to know post-treatment effect on larvae development. The emergence rate (Re) was calculated by the formula:

$$Re = \frac{NAe}{NLs} \times 100$$

NAe is the number of emerged adults and *NLs* is the number of

Table 1. Mortality of 3rd instars larvae after 24 h exposure to cabbage leaf treated with CW, CEtOH, NFW, NFEtOH, CP, CS and untreated (C) ($X \pm SE$).

Treatment	Larvae mortality after 24 h (%)
C	5.00 \pm 2.45
CW	6.67 \pm 2.31
CEtOH	6.67 \pm 2.31
NFW	5.00 \pm 2.45
NFEtOH	1.67 \pm 1.82
CP	6.00 \pm 3.74
CS	5.00 \pm 2.45
Statistics	$F_{(6, 35)} = 0.53$; $df = 6$; $p = 0.78$

C: Control, CW: Distilled water, CEtOH: Ethanolic solution, NFW: Neem Foliar Water extract, NFEtOH: Neem Foliar Ethanolic extract, CP: Conquest Plus and CS: Cydim Super.

survived larvae after 24 h of exposure to the treated and the untreated leaves.

Statistical analysis

Statistical analyses were performed using STATISTICA 5.5 software (StatSoft, Tulsa, OK, USA). The comparisons of the mean numbers were made using analysis of variance (ANOVA-1) followed by Student-Newman-Keuls (SNK) comparison tests when F of analysis of variance was significant at the 5% level.

RESULTS

Treatment effects

Lethal effect

Both neem extracts did not exhibit a significant lethal effect on larvae although the effect varied among the treatments. For instance, 1.67 ± 1.82 to $6.67 \pm 2.31\%$ of larvae died after 24 h on leaf disks. No significant differences in mortality were observed in 3rd instars larvae on treated cabbage foliar disks compared to the controls ($F_{(6, 34)} = 0.53$; $df = 6$; $p = 0.78$) (Table 1).

Antifeedant effect

The antifeedant effects of the different products were indicated by foliar consumption. Both treated and untreated cabbage leaves were consumed by *P. xylostella* larvae. When larvae were introduced on treated leaf disk, they did not cause noticeable foliar damage like in treated and untreated controls or Conquest plus 388 EC treatments. A significant antifeedant effect on *P. xylostella* larvae was observed in Cydim Super treatment. The surface of cabbage leaf disk consumed when leaves were treated with neem extracts was not significantly different from that of water, ethanolic solution and

untreated control. But there was less surface consumed in CS compared to the other treatments ($F_{(6, 35)} = 4.71$; $df = 6$; $p = 0.001$) (Figure 1).

Post-treatment effects on emergence

Figure 2 shows that the emergence rate of adults of *P. xylostella* was significantly lower in NFEtOH and NFW treatments than in the controls and the other treatments ($F_{(6, 35)} = 11.25$; $df = 6$; $p = 0$).

DISCUSSION

The neem tree (*Azadirachta indica* A. Juss) is known to be an important source of triterpenoids (Afshan, 2002; Siddiqui et al., 2004). Effects of various neem extracts or neem-based insecticides on *P. xylostella* larvae have been well documented (Perera et al., 2000; Liang et al., 2003). Generally, neem extracts or neem-based insecticides are effective against *P. xylostella* larvae with significant lethal and antifeedant effects followed by significant reduction in food consumption. But, the 3rd instars larvae were able to cause damage on treated cabbage leaves. Liang et al. (2003) reported that neem based insecticides such as AgroneemTM, EcozinTM and NeemixTM should be applied as early as possible when larvae are neonates, or at the second instars stage to prevent noticeable foliage damage in field. In this study, neem leaves extracts did exhibit lethal or antifeedant effect, 24 h after larvae exposure. But they have a post-treatment effect on larvae resulting in adult emergence regulation. Indeed, consumption of neem extracts-treated leaves affected the development of 3rd *P. xylostella* larvae in reducing adult emergence. Hence, neem leaves extracts showed growth regulating effects. Seeds oil extracts, water and ethanol neem leaves extracts are known to inhibit the growth of various insects species

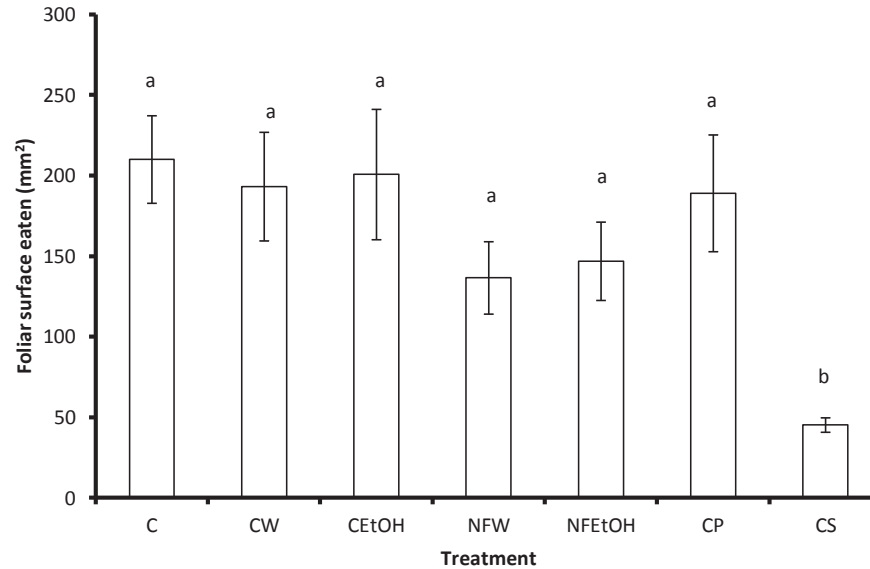


Figure 1. Antifeedant effects of treatments on *P. xylostella* larvae ($X \pm SE$). Different letters over the columns indicate statistically significant differences ($F_{(6, 35)} = 4.71$; $df = 6$; $p = 0.001$) in mm^2 of surface eaten. C: Control, CW: Distilled water, CEtOH: Ethanolic solution, NFW: Neem Foliar Water extract, NFtEtOH: Neem Foliar Ethanolic extract, CP: Conquest Plus and CS: Cydim Super.

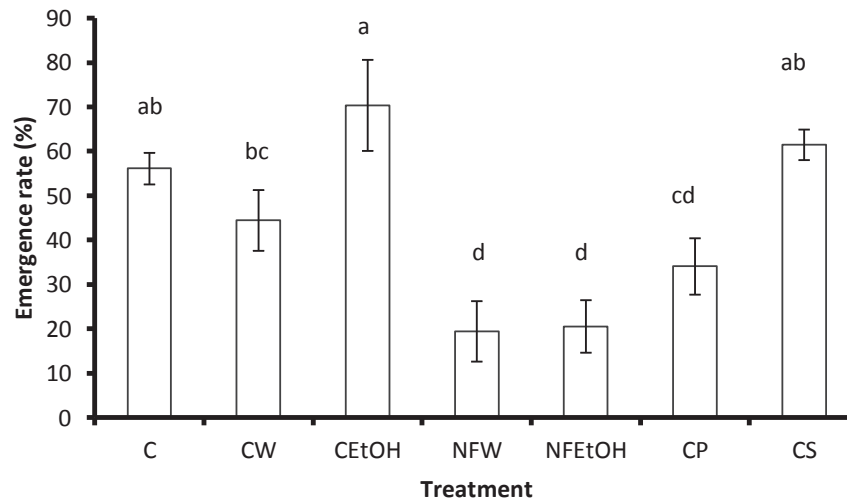


Figure 2. Post-treatment effect on the rate of *P. xylostella* adults emerged from larvae fed on treated leaves ($X \pm SE$). Different letters over the columns of each instars larvae indicate statistically significant differences ($F_{(6, 35)} = 11.25$; $df = 6$; $p = 0$). C: Control, CW: Distilled water, CEtOH: Ethanolic solution, NFW: Neem Foliar Water extract, NFtEtOH: Neem Foliar Ethanolic extract, CP: Conquest Plus and CS: Cydim Super.

(Charleston et al., 2005; Aggarwal and Brar, 2006; Egwurube et al., 2010; Shannag et al., 2014). It was reported that neem leaves contain numerous chemicals with insecticide properties (Afshan, 2002; Siddiqui et al., 2004). Neem triterpenoids showed growth regulating effects towards many species of insects (Schmutterer,

1995; Afshan, 2002). This is probably the synergistic effect of the chemical compounds of neem which regulates the growth of insects. It is not only the action of azadirachtin, main active compound, synthesized and marketed. Gauvin et al. (2003) showed that there was no relationship between the amount of azadirachtin and

insecticidal neem extracts. Thus, the growth regulating effects appear to be due to the effects of all insecticides substances contained in extracts of neem and not just their concentration of azadirachtin. Amtul (2014) found that *A. indica* derived compounds were inhibitors of digestive alpha-amylase in *Tribolium castaneum*. This inhibition can cause digestive trouble or their death. Results of the current study demonstrated that neem leaves extracts regulated the growth of *P. xylostella* 3rd instars larvae after being fed on cabbage treated leaves during 24 h.

Conclusion

This study revealed that the two neem extracts did not exhibit significant lethal and antifeedant effects on *P. xylostella* 3rd instars larvae in laboratory. However, they could enhance the management of *P. xylostella* by regulating its development to adult stage. Thus, extracts of neem leaves can be included in the management strategy of *P. xylostella*.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Rates and sources of zinc applied in sugarcane grown on sandy soil in Brazil

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Productivity and longevity of sugarcane plantations may currently be affected by the expanded cultivation activities in low fertility areas, which can result in micronutrient deficiencies. Zinc is important in this regard because its deficiency in sugarcane can cause reduced tillering, shorter internodes and thinner stalks. Therefore, the agronomic performance of sugarcane was evaluated (plant-sugarcane and first-ratoon-sugarcane), grown on a sandy soil with low zinc content, in response to rates and sources of zinc applications in the planting grooves. The experiment was conducted in an eutrophic Alfisol in Suzanópolis, São Paulo State, Brazil. A randomized blocks design in a factorial scheme (5x3), with five zinc rates (0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹) and three sources of zinc (sulfate, FTE (Zn silicate powder) and EDTA chelated Zinc) applied only in the planting furrow of the plant-sugarcane, with four replications, was used. The zinc sources had a similar effect on the Zn foliar and stalk contents, number of internodes per stalk, number of stalks per meter and sugarcane stalk productivity (cultivar RB867515), in two crops. Increasing zinc rates afforded a linear increase in the levels of foliar Zn and Zn in the stalks of the plant-sugarcane and first-ratoon-sugarcane, independent of the Zn source used. The zinc rates did not affect the production components and productivity of the plant-sugarcane stalks and first-ratoon sugarcane, grown on a sandy and acid soil with low zinc content.

Key words: *Saccharum* spp., fertilization with zinc, micronutrient, stalk productivity.

INTRODUCTION

The sugarcane (*Saccharum* spp.) is an important crop for the global and Brazilian agricultural economy. Brazil stands out as the world's largest producer of sugarcane. Sugarcane production is one of the major contributors of

many countries economy, like the South African (Baiyegunhi and Arnold, 2011).

Historically in Brazil, the alcohol sector has received little attention to the response of sugarcane to the

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application of micronutrients. Consequently, this is not a widespread practice in the agricultural companies producing sugarcane (Franco et al., 2009). However, micronutrient deficiencies can cause serious problems to crop development, especially productivity losses, as the micronutrients play crucial roles in plant metabolism. Among the micronutrients, zinc (Zn) is quite important because it enhances the production of auxin, tryptophan synthase and tryptamine metabolism. Zn is concentrated in growth areas due to high concentrations of auxin (Taiz et al., 2004); thus its main effect is on the development and elongation of plant young parts. The deficiency of this nutrient in sugarcane can result in reduced tillering, shorter internodes, and thinner stalks. According to Malavolta et al. (1997), the Zn requirement for sugarcane production is in average 0.72 kg ha^{-1} , with a relative dispersal to the stalks of $0.50 \text{ kg Zn ha}^{-1}$.

In general, the soils most commonly associated with zinc deficiency problems in plants mainly due to the factors like neutral to alkaline in reaction, especially where the pH is above 7.4, high calcium carbonate content in topsoil or in subsoil exposed by removal of the topsoil during field leveling or by erosion, coarse texture (sandy soil) with a low organic matter status, permanently or intermittently waterlogged soil, high available phosphate status, high bicarbonate or magnesium concentrations in soil or irrigation water and acid soil of low zinc status developed on highly weathered parent material (Arunachalam et al., 2013).

In sugarcane grown in São Paulo State, Brazil, micronutrient deficiencies are not commonly seen. However, according to Orlando Filho et al. (2001), sugarcane frequently exhibits the so-called "hidden hunger", a situation in which the symptoms do not appear visually, yet these levels are insufficient to the point of reducing crop yield. Moreover, this situation may be growing worse due to the expansion of sugarcane cultivation in areas with less favorable production environments (soil and climate), where zinc is generally the most lacking micronutrient due to the poor material source of these soils. In addition, the lack of a tradition of applying zinc-containing fertilizers to the crops of these regions, which are predominantly livestock pasture regions.

Korndörfer et al. (1999) indicate zinc application to sugarcane in nutrient-deficient areas, especially in sandy soils with low organic matter content, which is a common situation in the expanding areas with sugarcane in the Northwest region of São Paulo State, Brazil. Thus, zinc fertilization in sugarcane grown in soils with low content of this nutrient may be important to maintain adequate levels of zinc in the crop, thereby affording higher internodes growth, increasing top growth in the sugarcane and, consequently, increasing stalk length and yield.

Alvarez et al. (1979), in twenty-three experiments conducted in São Paulo State, Brazil, under different

climate and soil conditions, found no effect of zinc application on sugarcane production. However, in a study by Cambria et al. (1989), conducted in a medium-textured red-yellow latosol (oxisol) with low initial levels of Zn in the soil, found that there was a stalk yield increase up to a rate of 10 kg Zn ha^{-1} . However Costa Filho and Prado (2008), while assessing the soil application of Zn rates (0, 5, 10 and 15 kg ha^{-1}) in the form of zinc sulfate, in the third sugarcane ratoon grown in a Red-Yellow Latosol (oxisol) with a low content of this nutrient, found no significant effect on height and stalk productivity in the culture, but found increases in Zn foliar concentrations.

Another important aspect is to determine which source of micronutrient would be more efficient, both in the plant-sugarcane as well as in the ratoon-sugarcane. This is because of the solubility and physical form (powder or granules) of various micronutrient sources and soil conditions may interact, resulting in greater or lesser effect of fertilization for the correction of nutritional deficiencies (Moraes et al., 2004). Furthermore, most studies on zinc fertilization in sugarcane are outdated and were conducted only with zinc sulfate. Therefore, there is need for more studies on this subject, as new sugarcane cultivars continuously emerge, and which can be more responsive to this fertilizer. Within this context, the objective of this study was to evaluate the agronomic performance of sugarcane (plant and first-ratoon) grown on a sandy soil with low zinc content, in response to the application of zinc rates and sources in the planting furrows.

MATERIALS AND METHODS

The experiment was conducted in 2008, in an agricultural area managed by the Usina Vale do Paraná Açúcar e Álcool in Suzanápolis, São Paulo State, Brazil, within the geographical coordinates of $50^{\circ} 58'$ west longitude and $20^{\circ} 32'$ south latitude and 345 m of altitude. The soil is classified as sandy-textured eutrophic Alfisol, according to the classification by Embrapa (2006), with particle-size depth values of 0.0 to 0.20 m of 820, 56 and 124 g kg^{-1} of sand, silt and clay, respectively. At the depth of 0.20 to 0.40 m, the granulometric values were 813, 54 and 133 g kg^{-1} of sand, silt and clay, respectively.

The soil was chemically analyzed before the experiment (Table 1), according to the methodology proposed by Raji et al. (2001), which found low levels of Zn in the soil, as described in Raji et al. (1997). The region's climate classification according to Köppen is Aw, defined as tropical humid, rainy in summer and dry in winter. Figure 1 shows the values of rain precipitation (mm), relative air humidity (%) and maximum, minimum and mean temperatures ($^{\circ}\text{C}$) in the cultivation area during the sugarcane experiment and with a first-ratoon-sugarcane.

The treatments were at a location previously used for grazing (Brachiaria grass) for at least ten years. First, desiccation of the pasture with glyphosate was carried out ($1.44 \text{ kg a.i. ha}^{-1}$). Soil preparation included deep plowing followed by intermediate disking to incorporate lime into the soil (0.20 m deep) and light disking with herbicide trifluralin application (2 L ha^{-1}). Next, soil furrowing was performed at a depth of 0.40 m and the insecticide fipronil was applied ($200 \text{ g a.i. ha}^{-1}$) into the furrow. Manual planting system was adopted (conventional), in which the stalks were distributed and

Table 1. Chemical analysis of the soil from the experimental area. Suzanápolis, SP, Brazil, 2007.

Depth (m)	P (resin)	O.M.	pH	K	Ca	Mg	H+Al	SB	CTC	V
	mg dm ⁻³	g dm ⁻³	CaCl ₂				mmol _c dm ⁻³			%
0.0-0.25	3	24	4.9	3.0	10.0	8.0	20.0	21.4	41.4	52
0.25-0.50	2	12	4.6	0.3	9.0	4.0	21.0	13.3	34.3	39

Depth (m)	Cu*	Fe*	Mn*	Zn*	B**
	mg dm ⁻³				
0.0-0.25	0.8	39.0	4.8	0.4	0.61
0.25-0.50	0.9	12.0	2.5	0.1	0.50

*Determined in DTPA; **Determined in hot water.

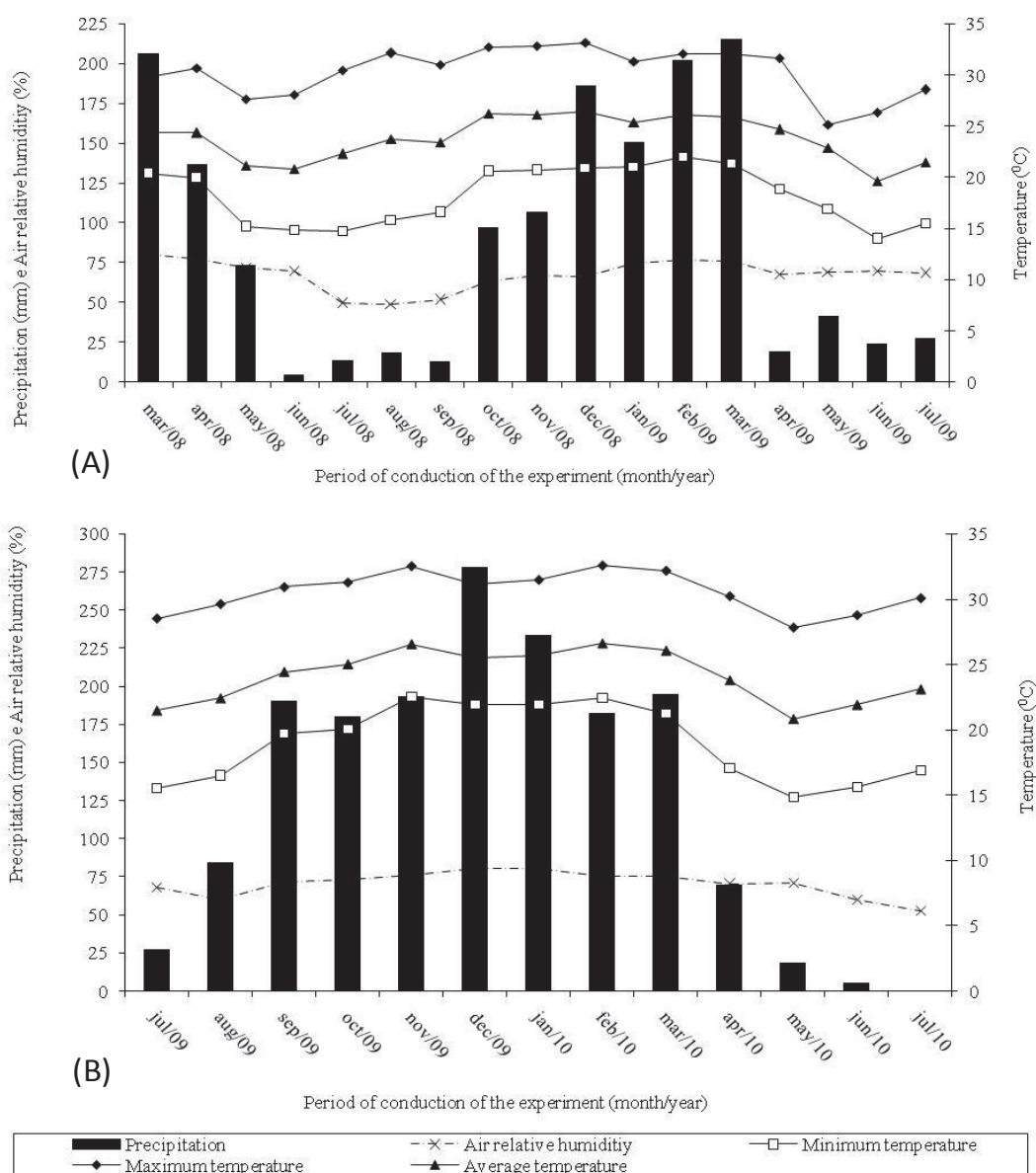


Figure 1. Monthly rainfall (mm) relative air humidity (%), monthly mean maximum, and minimum temperatures (°C) during the plant-sugarcane experiment (A) in the 2008/2009 crop and first-ratoon-sugarcane (B) in the 2009/2010 harvest in Suzanápolis – SP, Brazil.

sectioned within the planting rows, placing six seed pieces with three buds each per row meter.

The sugarcane cultivar used was RB867515, which exhibits a late-maturing cycle and is one of the most widely planted cultivars in São Paulo State. This cultivar is drought tolerant, with good sprouting stumps, even when reaped raw; tall, fast growing and with high productivity and high sucrose content (Hoffmann, 2008).

Thirty days prior to planting the crop, the soil of the experimental area received the application of 2 t ha⁻¹ of lime (302.3 g CaO kg⁻¹, 108.4 g MgO kg⁻¹ and 75% of PRNT) in order to obtain 60% of base saturation, as recommended by Raji et al. (1997) for sugarcane. Also, 30 kg ha⁻¹ of N (urea), 150 kg ha⁻¹ of P₂O₅ (simple super phosphate) and 120 kg ha⁻¹ of K₂O (potassium chloride) were applied into the planting fertilizer, as well as in all treatments, based on soil analysis (Table 1), and according to the fertilizer used by the sugar mill. On 16/10/2009, fertilization in the first-ratoon-sugarcane in between the rows was performed, applying and incorporating into the soil 80 kg ha⁻¹ of N (urea) and 60 kg ha⁻¹ of K₂O (potassium chloride), according to the fertilizer used by the sugar mill.

Weed control was carried out in both crops with the application of diuron + hexazinone herbicides (702+264 g of i.a. ha⁻¹), in post-crop emergence, performed 45 days after planting the plant-sugarcane and 55 days after harvesting the plant-sugarcane. However, there was no need to control diseases and pests. Sugarcane flowering was observed in both crops. The sugarcane harvest (488 day cycle) and the first-ratoon-sugarcane (363 day cycle) was performed manually and individually for each experimental unit, on 7 August, 2009 and 7 July, 2010, respectively. Next, the sugarcane was harvested mechanically without any previous burning of the crop.

The following evaluations were performed: a) Zn foliar content, analyzing the middle third of 15 leaves +1 (highest leaf with top visible leaf collar -"TVP"), excluding the midrib, after collection in the highest development phase (carried out on 1 December, 2008 for the plant-sugarcane and on 21 January, 2010 for the first-ratoon-sugarcane), according to the methodology described by Raji et al. (1997); b) Zn content in the sugarcane stalk, 3 cane stalks were collected and crushed per plot during the sugarcane harvest. Then, the Zn content was determined after drying, as described in Malavolta et al. (1997); c) stalk diameter, determined using a caliper by the average diameter of the base, middle and end of three industrially usable stalks; d) number of internodes per meter was evaluated in three industrially usable stalks, by counting the number of internodes in one meter of the middle third of the stalk, and; e) for stalk height, five stalks per plot were measured during the harvest, using a graduated ruler from ground level to the first visible collar, classified as leaf +1; L) number of stalks per meter by counting the mill's industrially usable stalks, in 5 m of culture rows; g) sugarcane yield, determined based on the average mass of 10 stalks and the number of industrially usable stalks per plot, after being quantified, the data were converted into ha⁻¹ (TCH). The data were subjected to analysis of variance (F test) and the means compared by Tukey test at 5% probability for Zn sources and adjusted to regression equations for the effect of Zn rates. The SISVAR program was used for the Statistical analysis (Ferreira, 2008).

RESULTS AND DISCUSSION

There was more rainfall during the first-ratoon-sugarcane cycle from July 2009 to January 2010 (Figure 1B), as compared to the same sugarcane cycle period (Figure 1A). Was observed that in the early development of the first-ratoon-sugarcane (August and September 2009), above average rainfalls were recorded for the northwest region of São Paulo (Figure 1B).

The experiment shows that there was no significant interaction between Zn sources and rates for any of the evaluations performed (Tables 2 and 3). Zn levels in leaves and stalks of the plant-sugarcane as well as of the first-ratoon-sugarcane, were not affected by Zn sources (sulfate, FTE and chelated Zn) applied to the crop (Table 2). However, it should be noted that foliar zinc contents in the sugarcane plant and in the first-ratoon-sugarcane, independent of the dose applied, are within the range considered adequate (10 to 50 mg of Zn kg⁻¹ of dry matter) for the sugarcane culture, as described in Raji et al. (1997). However, Malavolta et al. (1997) indicate that the adequate range for the Zn foliar content (leaf +3) in the sugarcane is higher in the plant-sugarcane (25 to 50 mg kg⁻¹ of M.S.), when compared to first-ratoon-sugarcane (25 to 30 mg kg⁻¹ of M.S.). Thus, all foliar concentrations of zinc were below the range considered adequate. However, it is important to note that these authors recommend the collection of the leaf +3 (older leaves), four months after sugarcane sprouting. According to Costa Filho and Prado (2008), the fact that the knuckles present lower zinc content in the tissues, when compared to the plant-sugarcane, is indicative of a greater tolerance to reduced availability of zinc in the soil, without productivity loss.

Table 2 shows for Zn stalk contents, that these levels were higher in the stalks of the plant-sugarcane in comparison to the stalks in the first-ratoon-sugarcane. This lower zinc concentration in the sugarcane stalks may be due to the fact that in the cultivation of the first-ratoon-sugarcane there was no fertilization with zinc, but rather the residual effect of this fertilization performed on the plant-sugarcane.

The Zn rates significantly influenced the foliar Zn levels and the stalks of the plant-sugarcane and first-ratoon-sugarcane, always adjusting to the increasing linear function, independent of the Zn source (Table 2). Costa Filho and Prado (2008), evaluating the application of zinc rates in the sugarcane knuckles, also found significant linear increases in the foliar levels of zinc. However, Andrade et al. (1995), working with the application of fritted trace elements and sources of soluble boron, copper and zinc sulfate (Zn sulfate) in the planting furrow, found that the joint application (fritted), as well as the standalone application of micronutrients, did not result in increased foliar concentrations of Zn in the plant-sugarcane and in the first-ratoon-sugarcane, in a medium textured soil with low Zn (0.3 mg dm⁻³).

The sources of Zn provided similar stalk diameters in the plant-sugarcane, but there was a significant difference in this assessment in the first-ratoon-sugarcane, as the chelated Zn yielded higher stalk diameter, when compared to the FTE and Zn sulfate (Table 2). A possible explanation is that as this treatment (chelated Zn) provided smaller stalk heights in this cultivation (Table 3), there was a compensatory effect with the increase in stalk diameter, a common feature in

Table 2. Means, coefficients of variation (CV), Tukey test and regression equations relating to the foliar Zn concentrations and stalk, stalk diameter and number of internodes per meter of plant-sugarcane stalk (2008/2009 crop) and first-ratoon-sugarcane (2009/2010 crop), according to the application of rates and sources of Zn in the planting of sugarcane, in Suzanópolis – SP, Brazil.

Variables	Foliar Zn concentration (mg kg ⁻¹)		Stalk Zn concentration (mg kg ⁻¹)		Stalk diameter (cm)		Number of internodes per meter of stalk	
	Plant-sugar cane	First-ratoon sugar cane	Plant-sugar cane	First-ratoon sugar cane	Plant-sugar cane	First-ratoon sugar cane	Plant-sugar cane	First-ratoonsugar cane
Zn sources								
FTE	15.85 ^a	19.60 ^a	36.87 ^a	15.73 ^a	2.62 ^a	2.74 ^b	9.36 ^a	8.65 ^a
Zn chelated	17.20 ^a	20.53 ^a	40.80 ^a	15.40 ^a	2.59 ^a	3.01 ^a	9.07 ^a	8.94 ^a
Zn sulfate	16.45 ^a	20.33 ^a	41.33 ^a	14.67 ^a	2.57 ^a	2.73 ^b	9.34 ^a	8.55 ^a
D.M.S. (5%)	1.72	1.52	5.72	4.29	0.22	0.26	0.99	0.46
Zn rates (kg ha ⁻¹)								
0	11.75 ⁽¹⁾	19.44 ⁽³⁾	32.78 ⁽²⁾	9.67 ⁽⁴⁾	2.57 ^{ns}	2.77 ^{ns}	9.02 ^{ns}	8.76 ^{ns}
2.5	13.75	19.89	37.11	14.00	2.51	2.90	9.29	8.61
5.0	17.17	20.22	40.56	15.00	2.64	2.74	9.27	8.64
7.5	19.50	20.00	45.67	17.78	2.55	2.84	9.38	8.89
10.0	20.33	21.22	42.22	19.89	2.71	2.82	9.33	8.66
Overall mean	16.50	20.16	39.67	15.27	2.60	2.83	9.26	8.71
C.V. (%)	13.56	8.35	15.96	31.08	11.04	12.12	13.84	6.88

Means followed by same letter in the column do not differ by Tukey test at 5% level of probability. ^{ns}Not significant by regression analysis. ⁽¹⁾, ⁽²⁾, ⁽³⁾ and ⁽⁴⁾ refer to the regression equations: ⁽¹⁾Y = 11.9167+0.9167X (R² = 0.97**); ⁽²⁾Y = 34.1778+1.0978X (R² = 0.77**); ⁽³⁾Y = 19.4222+0.1467X (R² = 0.77*); ⁽⁴⁾Y = 10.4222+0.9689X (R² = 0.97**).

Table 3. Means, coefficients of variation (CV) and Tukey's test related to stalk height, number of stalks per meter and stalk yield (t ha⁻¹) of plant sugarcane (2008/2009 crop) and a first-ratoon sugarcane (2009/2010 crop), according to the application of rates and sources of Zn in the planting of sugarcane, in Suzanópolis – SP, Brazil.

Variables	Stalk height (m)		Number of stalks per meter		Stalk yield (t ha ⁻¹)	
	Plant-sugarcane	First-ratoon sugarcane	Plant-sugarcane	First-ratoon sugarcane	Plant-sugarcane	First-ratoon sugarcane
Zn sources						
FTE	2.90 ^a	2.89 ^{ab}	8.46 ^a	8.74 ^a	87.40 ^a	106.46 ^a
Zn chelated	2.93 ^a	2.85 ^b	8.17 ^a	9.07 ^a	87.90 ^a	114.37 ^a
Zn sulfate	2.92 ^a	2.94 ^a	8.46 ^a	8.68 ^a	86.70 ^a	105.06 ^a
D.M.S. (5%)	0.18	0.08	0.76	0.90	9.50	14.61
Zn Rates (kg ha ⁻¹)						
0	2.91 ^{ns}	2.94 ^{ns}	8.73 ^{ns}	8.77 ^{ns}	79.58 ^{ns}	109.26 ^{ns}
2.5	2.85	2.82	8.37	9.05	89.33	106.72
5.0	2.91	2.89	8.58	8.88	89.42	108.22
7.5	2.96	2.92	7.78	8.60	88.75	103.77
10.0	2.95	2.90	8.35	8.85	89.58	115.19
Overall mean	2.91	2.89	8.36	8.83	87.33	108.63
C.V. (%)	7.91	3.79	11.80	13.19	14.15	17.50

Means followed by same letter in the column do not differ by Tukey test at 5% level of probability. ^{ns}Not significant by regression analysis.

grasses.

Regarding the number of internodes per meter of stalk, no significant difference was observed between the sources of Zn for plant-sugarcane and for the first-ratoon

sugarcane. As for the rates of Zn, these did not affect stalk diameter and number of internodes per meter of stalk, for the plant-sugarcane as well as for the first-ratoon-sugarcane (Table 2). It should be noted that a

typical symptom of Zn deficiency in sugarcane is the shortening of internodes, in other words, greater number of internodes per meter of stalk. Therefore, there was probably no effect of Zn levels on this evaluation, because all foliar concentrations of zinc found in plant-sugarcane and the first-ratoon-sugarcane (Table 2), independent of the dose applied, were within the range considered appropriate for this culture, as previously reported.

Table 3 shows that the height of the plant-sugarcane stalk was not affected by Zn sources. However, in the first-ratoon-sugarcane there was no significant difference between Zn sources, with zinc sulfate affording the maximum stalk height, although this did not differ significantly from the FTE. The stalk heights of the plant-sugarcane and first-ratoon-sugarcane were not influenced by increasing rates of Zn (Table 3), therefore they did not affect stalk growth. Costa Filho and Prado (2008), evaluating the application of zinc in the sugarcane knuckles, also did not see increasing stalk heights.

Regarding the number of stalks per meter, no difference was found among the sources of Zn (sulfate, FTE and chelated Zn) for the plant-sugarcane and first-ratoon-sugarcane. There was also no significant effect for the rates of Zn applied to the sugarcane sowing in both crops (Table 3). This result indicates that zinc did not affect tillering on the sugarcane cultivar RB867515. However, Cambria et al. (1989), applying Zn rates in the soil, in the form of zinc sulphate, found that the tillering of plant-sugarcane was negatively affected when the rates were higher than 15 kg Zn ha^{-1} , a higher dose than that used in this work. According to Landell and Silva (2004), tillering, stalk heights and diameters are important components for the formation of the agricultural potential of sugarcane.

Although it is usually the most productive plant-sugarcane, there was greater stalk productivity (TCH) of the first-ratoon-sugarcane ($108,63 \text{ t ha}^{-1}$) in comparison to the plant-sugarcane ($87,33 \text{ t ha}^{-1}$), independent of the treatment (Table 3). This unusual result occurred mainly because there was more rainfall during the vegetative growth period of the first-ratoon-sugarcane (Figure 1B), in comparison to the same period for the sugarcane plant (Figure 1A).

The Zn sources did not provide significant stalk yield differences (TCH) of the plant-cane and first-ratoon of sugarcane cultivar RB867515 (Table 3), although they have different sulphate and chelate solubilities, when compared to the FTE. According to Volkweiss (1991), it is necessary that the micronutrient sources use solubilization in the soil at least at speeds compatible with the absorption by the roots and applied in a manner they can receive it, since the micronutrients have little mobility in the soil. According to Mortvedt (2001), the FTEs are the more appropriate products for maintenance programs than for correcting severe deficiencies and provide

greater efficiency in sandy soils in regions with higher rainfall rates. However, despite these soil conditions (sandy texture) and favorable climate (Figure 1), the FTE was not significant when compared to other sources of Zn.

On the other hand, based on the indicators of technological quality (sucrose concentration, brix cane and total recoverable sugar) of the first-ratoon-sugarcane, according to Teixeira Filho et al. (2013), would be interesting the application between 4.0 and $5.0 \text{ kg Zn ha}^{-1}$ in the in the planting furrow, in the form of chelate or sulfate of Zn.

Moreover, the Zn rates did not significantly influence sugarcane stalk yields in the cane plant as well as in the first-ratoon-sugarcane (Table 3), despite having been grown on a sandy soil with a low content of the element. These results were also found for the main crop production components (Tables 2 and 3). Therefore, this explains why there was no significant increase in the productivity of sugarcane. But it is important to emphasize that there was a mean increase for plant-sugarcane in the stalk yield of 9.7 t ha^{-1} compared to the control (without zinc fertilization).

Franco et al. (2009), working with rates of Zn (0 , 3 and 6 kg ha^{-1}) applied to the soil about 20 cm from the plants, in the form of zinc sulfate dissolved in water (200 L ha^{-1}) and 90 days after planting the crop, also did not observe increases in the productivity of the plant-sugarcane stalks. Costa Filho and Prado (2008), studying the application of Zn rates in the third-ratoon-sugarcane, also found no significant effect on the productivity of stalks in soils with low Zn content. Similarly, Alvarez et al. (1979), evaluating twenty-three experiments under various climate and soil conditions in the São Paulo State, found no Zn effect on the sugarcane production.

However, significant increases in sugarcane stalk productivity in response to Zn fertilization in soils with low content of this nutrient were verified by Cambria et al. (1989) in a medium-textured Red-Yellow Latosol (Oxisol), up to a dose of 10 kg Zn ha^{-1} , with soil application of zinc sulphate. Moreover, Andrade et al. (1995), studying the application of (fritted trace elements) and soluble Zn sources (zinc sulfate), applied to the planting furrow in medium-textured dystrophic Red Latosol, found that the joint application (fritted), as well as the standalone application of the micronutrient, did not result in increased stalk productivity. Moreover, these authors also observed residual effect of this fertilization on the first-ratoon-sugarcane. Farias et al. (2008), evaluating the efficiency of water use in sugarcane under different irrigation and zinc rates (0 , 1 , 2 , 3 and 4 kg ha^{-1}), on a soil in the coast of Paraíba, Brazil, observed quadratic increase in stalk productivity, estimated at 2.38 kg ha^{-1} , as the dose that maximizes the crop's water use efficiency. Marinho and Albuquerque (1981) also found a significant effect of Zn application on the productivity of sugarcane, in seven experiments performed in soils in Alagoas,

Brazil, when these micronutrient levels were below 5 mg dm⁻³.

Wang et al. (2005) reported that the soil applications of 4.4 and 8.9 kg Zn ha⁻¹ significantly increased sugarcane and sugar yields of LCP 85-384 by an average of more than 23% above the control for acidic and calcareous soils. Madhuri et al. (2013) recommended the application of ZnSO₄ to the soil 50 kg ha⁻¹ for better yield and quality of sugarcane production in sandy loam soils with pH equal to 8.08. In this sense it is important to note that these recommendations are for different soil and climate conditions (such as pH, texture, organic matter content and pluvial precipitation) that was used in our research.

A hypothesis for the non-effect of zinc fertilization on the productivity of sugarcane grown on sandy soils with low zinc content, is that the plant-sugarcane experiment was conducted in an atypical year of rainfall (Figure 1A), that is, much rain during the dry period. This could have then favored a better root development, and therefore this must have explored a greater soil volume and thus absorbed more zinc from the soil. Another hypothesis reported by Tokeshi (1991), indicates that as sugarcane has a deep root system, it enables the absorption of micronutrients in the subsurface layers. However, although no significant response of the sugarcane cultivar RB867515 was found for fertilization with zinc in the first two cuts of the culture, such results may be different for other varieties of sugarcane that are more demanding in this micronutrient and/or in subsequent crops, as these Zn sources may have different residual effects. Casarin et al. (2001) report that in low fertility soils or which are exploited for many years, the occurrence of micronutrient deficiency in sugarcane becomes even more aggravated.

Conclusions

- 1) The sources of zinc (sulphate, FTE and chelated zinc) had a similar effect on the Zn foliar and stalk concentrations, number of internodes per meter of stalk, number of stalks per meter and stalk productivity in sugarcane, in two crops.
- 2) Increasing rates of zinc afforded a linear increase in the foliar Zn levels and Zn in the stalks of the plant-sugarcane and first-ratoon-sugarcane, independent of the Zn source used.
- 3) The zinc rates did not affect the production components and hence the stalk productivity of the plant-sugarcane and first-ratoon-sugarcane, grown on sandy and acid soil with low zinc content.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Studies on direct selection parameters for seed yield and its component traits in pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Twenty three pigeonpea genotypes were evaluated in a randomized block design during *Kharif* 2012-13. The aim of this research was to estimate genetic variability utilizing various quantitative traits for overall improvement in pigeonpea crop. Results showed that sufficient amount of variability was present in entire gene pool for all the characters. Based on mean performance for yield along with some of the component traits the most promising genotypes identified were NDA 5-14, NDA 8-6, NDA 96-6, ICP 2155 and NDACMS 1-6B. Secondary branches per plant showed highest phenotypic as well as genotypic coefficient of variation followed by seed yield per plant and biological yield. High heritability coupled with high genetic advance as per cent of mean were observed by 100-seed weight, pods per plant, seed yield per plant, biological yield per plant; and secondary branches per plant indicate that these traits are highly heritable and governed by additive gene action. While, plant height, primary branches per plant, pods per plant, seeds per pod and harvest index showed high heritability with moderate genetic advance as per cent of mean suggesting greater role of non-additive gene action in their inheritance. It may be concluded that the characters 100-seed weight, pods per plant, seed yield per plant, biological yield per plant and secondary branches per plant, were identified as the most important direct selection criterion intended at developing high yielding pigeonpea cultivars.

Key words: Pigeonpea, genetic variability, yield and yield attributes.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.], also known as red gram, is the second most important pulse crop of India after chickpea. It is a rich source of protein, carbohydrate, vitamins, lipids and certain minerals. Pigeonpea is a hard, widely adapted and drought tolerant crop. It is cultivated in more than 25 tropical and sub-tropical countries, either as a sole crop or intermixed with such cereals as

sorghum, pearl millet or maize or with legumes, e.g., groundnut. Globally, pigeonpea is grown on about 5.54 million ha of land mass, producing 3.22 million tonnes of grain with average yield of 708 kg per hectare. It contributes about 5.6 percent share in global pulse production (Gowda et al., 2009). The Indian sub-continent, Eastern Africa, and Central America, in that

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Table 1. Analysis of variance for 11 quantitative characters in pigeonpea.

Characters	Source of variation		
	Replication [2]	Treatment [22]	Error [44]
Days to 50 % flowering	3.435	45.802**	1.621
Days to maturity	3.522	45.515**	2.082
Plant height (cm)	1.609	411.407**	9.705
Primary branches/ plant	0.339	1.703*	0.646
Secondary branches/ plant	12.192	101.907**	7.530
Pods/ plant	105.33	2245.566**	28.107
Seeds/ pod	0.024	0.345**	0.020
100-seed weight (g)	0.026	9.875**	0.061
Seed yield/ plant (g)	3.399	494.780**	6.784
Biological yield/ plant (g)	80.46	4732.801**	71.474
Harvest-index (%)	0.214	17.090**	0.684

*,** significant at 5% and 1% probability levels, respectively, [] value in parenthesis represents degree of freedom

order, are the world's three main pigeonpea producing regions.

Productivity of pigeonpea worldwide in comparison to cereals is very low and stagnant due to several biotic and abiotic stresses. This low productivity is attributed to its low harvest index because of limited man made selections (Varshney et al., 2010). Compared to other food legumes breeding, pigeonpea has been more challenging due to various crop specific traits and highly sensitive nature. Significant progress has been made over the last few decades through breeding for reducing crop duration, improving seed quality and overcoming the constraints of major diseases like wilt and sterility mosaic.

To accumulate optimum combination of yield contributing traits in single genotype, it is essential to understand the implication of the inter-relationships of various traits using correlation and path coefficients. Being an often cross-pollinated crop, pigeonpea has very high genetic variability but yield potential is very low. Seed yield is a very complex character whose manifestation results from multiplicative interactions of several yield components and environmental factors. Improvement in yield primarily depends on the extent of genetic variability present in the population. The systematic breeding programme involves the steps like creating genetic variability practicing selection and utilization of selected genotypes to evolve promising varieties. It is necessary to find out the relative magnitude of additive and non additive genetic variances, heritability and genetic gain with regard to the characters of concern to the breeder. Estimates of Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), heritability and genetic advance will play an important role in exploiting future research projections of pigeonpea improvement.

MATERIALS AND METHODS

Twenty three pigeonpea genotypes were evaluated in a randomized block design for various agronomical and physiological characters during *Kharif* 2012-13 at Research Farm of Genetics and Plant Breeding, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad. The experimental site is located at 26.47 °N latitude, 82.12 °E longitudes and an altitude of 113 m above mean sea level. Each genotype was raised in single row plots of 4 m length with intra-row and inter-row spacing of 25 cm and 75 cm, respectively.

The recommended agronomic practices followed to raise good crop stand. The observations were recorded on five randomly selected competitive plants of a genotype in a plot in each replication for eleven characters viz., days to 50% flowering, days to maturity, number of primary branches per plant, number of secondary branches per plant, plant height (cm), pods per plant, seeds per pod, 100-seed weight, seed yield per plant (g), biological yield per plant (g) and harvest index (%). Mean values were subjected to analysis of variance to test the significance for each character as per methodology advocated by Panse and Sukhatme, (1967). PCV and GCV were calculated by the formula given by Burton (1952), heritability in broad sense (h^2) by Burton and De Vane (1953) and genetic advance, that is, the expected genetic gain were calculated by using the procedure given by Johnson et al. (1955).

RESULTS AND DISCUSSION

The analysis of variance (Table 1) revealed highly significant difference for all the characters under study among the genotypes, indicating that the parents included in this investigation exhibit sufficient variability for the entire characters studied. The presence of large amount of variability might be due to diverse source of materials taken as well as environmental influence affecting the phenotypes. Similar findings were also reported by Srinivas et al. (1999), Basavarajaiah et al.

Table 2. Mean performances for 11 quantitative characters in pigeonpea.

Genotypes	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches/plant	Secondary branches/plant	Pods/plant	Seeds/pod	100-Seed wt (g)	Seed yield/plant (g)	Biological yield/plant (g)	Harvest index (%)
NDA 2	141.00	250.00	172.33	6.45	27.03	165.05	3.60	13.16	69.46	236.45	29.40
NDA 3	137.33	243.33	170.33	4.97	17.85	167.52	3.80	9.67	54.87	192.40	28.52
NDA 3-3	143.67	250.33	169.33	5.30	15.66	163.57	2.80	13.33	53.87	180.78	29.79
NDA 5-14	139.00	248.67	181.67	5.55	22.56	183.33	3.13	15.14	82.39	292.98	28.14
NDA 8-6	137.67	246.00	187.50	5.80	16.97	219.53	3.43	11.47	78.77	264.37	29.79
NDA 96-1	147.33	252.33	166.93	5.34	16.08	174.01	3.42	10.56	56.40	206.22	27.35
NDA 96-6	142.33	246.00	171.00	6.52	20.36	231.62	3.17	11.41	77.07	250.83	30.75
NDA 98-6	141.33	248.00	181.74	4.92	17.40	158.32	3.25	12.58	58.24	195.34	29.81
NDA 98-7	146.00	252.00	170.97	5.73	15.15	172.02	3.00	7.99	32.24	128.16	25.16
NDA 7-11	135.67	247.33	171.29	4.97	14.84	147.83	3.78	11.38	57.74	200.60	28.80
NDA 7-15	147.00	254.67	155.04	5.40	15.28	153.80	3.53	9.57	43.97	147.85	29.72
NDAGC 31	146.67	254.00	183.85	4.87	19.30	206.04	3.35	10.56	70.88	245.69	28.87
NDAGC 1010	143.67	254.33	190.02	5.21	30.04	245.08	3.10	9.54	64.58	204.71	31.55
ICP 2309	147.00	251.00	166.84	4.63	21.30	196.37	3.00	9.17	52.21	173.75	30.04
ICP 2155	146.33	251.00	167.53	4.18	25.18	241.64	3.00	11.30	74.63	240.91	31.01
ICP 870	137.00	245.00	198.91	6.61	34.63	181.18	3.22	8.76	43.59	199.71	21.82
ICP 7353	145.00	256.67	184.41	3.98	19.36	170.46	3.60	8.83	45.83	183.20	25.04
IPA 208	141.33	256.00	166.07	3.62	24.86	181.08	3.00	12.42	62.78	210.40	29.83
Bahar	147.00	258.00	182.33	5.10	27.48	169.53	3.03	10.26	54.39	171.79	31.66
Amar	143.00	252.67	171.66	4.87	16.83	167.63	2.98	11.67	51.24	164.12	31.22
NDACMS 1-3A/B	146.00	251.00	184.67	5.13	15.56	165.13	2.53	13.72	47.95	181.50	26.43
NDACMS 1-4A/B	148.67	254.67	197.67	5.14	26.51	181.25	3.57	9.60	61.22	228.66	26.77
NDACMS 1-6A/B	147.00	250.00	198.42	5.98	30.45	189.74	3.82	9.87	71.94	250.12	28.77
Mean	143.35	251.00	177.85	5.23	21.33	183.99	3.27	10.96	59.40	206.54	28.71
CV	0.75	0.57	1.75	15.36	12.86	2.88	4.36	2.25	4.38	4.09	2.88
CD 5%	1.77	2.37	5.13	1.32	4.52	8.72	0.23	0.41	4.29	13.91	1.36

(2000), Venkateswarlu (2001), Baskaran and Muthiah (2006), Bhadrhu (2008), Bhadrhu (2011) and Yerimani et al. (2013).

The mean performance of 23 parents of pigeonpea for 11 characters is presented in Table 2. Highest mean performance for seed yield per plant along with some of the component traits was

exhibited by NDA 5-14 (82.39 g) followed by NDA 8-6 (78.77 g), NDA 96-6 (77.07 g), ICP 2155 (74.63 g) and NDACMS 1-6B (71.94 g). The above mentioned genotypes may be used as donor parents in hybridization programme for developing high yielding varieties of respective groups. Some other genotypes exhibiting very

high mean performance for characters other than seed yield per plant are also listed in Table 4. These lines merit consideration as promising parents for hybridization programme for bringing over all improvement in plant architecture in a component breeding approach ultimately leading to high yielding pigeonpea genotypes even if they

Table 3. Genetic variability parameters for 11 quantitative characters in pigeonpea

Characters	General Mean \pm SE	Range		Coefficient of variation		h ² (BS)	GA as % of mean
		Lowest	Highest	GCV	PCV		
Days to 50 % flowering	143.35 \pm 0.62	135.67	148.67	2.69	2.79	92.76	5.34
Days to maturity	251.00 \pm 0.83	243.33	258.00	1.52	1.62	87.43	2.92
Plant height (cm)	177.85 \pm 1.80	155.04	198.91	6.51	6.74	93.24	12.94
Primary branches/ plant	5.23 \pm 0.46	3.62	6.61	11.36	19.11	35.34	13.91
Secondary branches/ plant	21.33 \pm 1.58	14.84	34.63	26.29	29.27	80.69	48.65
Pods/ plant	183.99 \pm 3.06	147.83	245.08	14.78	15.05	96.34	29.88
Seeds/ pod	3.27 \pm 0.08	2.53	3.82	10.07	10.97	84.24	19.04
100-seed weight (g)	10.96 \pm 0.14	7.99	15.14	16.51	16.66	98.18	33.7
Seed yield/ plant (g)	59.40 \pm 1.50	32.24	82.39	21.47	21.91	96.00	43.34
Biological yield/ plant (g)	206.54 \pm 4.88	128.16	292.98	19.08	19.52	95.60	38.44
Harvest-index (%)	28.71 \pm 0.48	21.82	31.66	8.15	8.64	88.88	15.82

*h² (BS) = Heritability broad sense, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, GA = Genetic advance, S.E. = standard error.

have moderate or low seed yield.

In this context, the most desirable lines were NDA 7-11, ICP 870, NDA 3 and NDA 8-6 for days to 50% flowering and days to maturity; ICP 870 and NDACMS 1-6A for plant height, primary and secondary branches per plant; NDAGC 1010 and ICP 2155 for pods per plant and NDACMS1-6A, NDA 3, NDA 7-11, ICP 7353 and NDA 2 for seeds per pod. Similarly, the most promising genotypes were NDA 5-14, NDACMS 1-3A, NDA 3-3 and NDA 2 for 100-seed weight; NDA 5-14, NDA 8-6, NDA 96-6 and NDAGC 31 biological yield per plant and Bahar, NDAGC 1010, Amar, ICP 2155 and NDA 96-6 for harvest-index. An earlier work has also reported wide range of variation for various character and have identified superior pigeonpea genotypes for further use in breeding programmes.

The mean range, GCV, PCV, heritability and genetic advance percentage of mean are given in Table 3. The character possessing high genotypic coefficient of variation value has better scope of

improvement through selection. The influence of environment on each trade could be determined on the basis of difference between phenotypic coefficient of variation and genotypic coefficient of variation. A perusal of coefficient of variation revealed that the highest estimates of genotypic and phenotypic coefficient of variation were observed in case of secondary branches per plant (26.29 and 29.27%), followed by seed yield per plant (21.47 and 21.91%) which can be considered as high because of being very close to 20%.

Moderate estimates were recorded for biological yield per plant (19.08 and 19.52%), 100-seed weight (16.51 and 16.66%), pods per plant (14.78 and 15.05%), primary branches per plant (11.36 and 19.11%) and seeds per pod (10.07 and 10.97%). The remaining four characters exhibited low estimates of genotypic as well as phenotypic coefficient of variation. The lowest estimates of GCV and PCV were observed for days to maturity (1.52 and 1.62%), followed by days to 50% flowering (2.69 and 2.79%), plant height (6.51

and 6.74%) and harvest index (8.15 and 8.64%). The magnitude of phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the characters which may be due to higher degree of interaction of genotypes with the environment. These findings were in close agreement with the findings of earlier works (Bashkarana and Muthiah, 2006; Bhadru, 2008; 2011; Yerimani et al., 2013).

These values alone are not helpful in determining the heritable portion of variation (Falconer, 1960). The proportion of genetic variability which is transmitted from parents to offspring is reflected by heritability (Lush, 1949). In this context, the high estimates of heritability was recorded by 100 seed weight (98.18%), followed by pods per plant (96.34%), seed yield per plant (95.60%), biological yield per plant (88.88%), plant height (93.24), harvest index pod (84.24%) and secondary branches per plant (80.69%); however primary branches per plant

Table 4. The five most desirable genotypes identified for high mean performance for 11 quantitative characters in pigeonpea

Characters	Genotypes
Days to 50 % flowering	NDA 7-11(135.67 days), ICP 870 (137 days), NDA 3 (137.33 days), NDA 8-6 (137.67 days), NDA 5-14 (139 days)
Days to maturity	NDA 7-11 (243.33 days), ICP 870 (245 days), NDA 8-6 (246 days), NDA 96-6 (246 days), NDA 3 (247.33 days)
Plant height (cm)	ICP 870 (198.91 cm), NDACMS 1-6A (198.42 cm), NDACMS 1-4A (197.67 cm), NDAGC 1010 (190.02 cm), NDA 8-6 (187.50 cm)
Primary branches/ plant	ICP 870 (6.61), NDA 96-6 (6.52), NDA 2 (6.45), NDACMS 1-6A (5.98), NDA 8-6 (5.80)
Secondary branches/ plant	ICP 870 (34.63), NDACMS 1-6A (30.45), NDAGC 1010 (30.04), Bahar (27.48), NDA 2(27.03)
Pods/ plant	NDAGC 1010 (245.08), ICP 2155 (241.64), NDA 96-6 (231.62), NDA 8-6 (219.53), NDAGC 31 (206.04)
Seeds/ pod	NDACMS 1-6A (3.82), NDA 3 (3.80), NDA 7-11 (3.78), ICP 7353 (3.60), NDA 2 (3.60)
100-seed weight (g)	NDA 5-14 (15.14 g), NDACMS 1-3B (13.72 g), NDA 3-3 (13.33 g), NDA 2 (13.16 g), NDA 98-6 (12.58 g)
Seed yield/ plant (g)	NDA 5-14 (82.39 g), NDA 8-6 (78.77 g), NDA 96-6 (77.07 g), ICP 2155 (74.63 g), NDACMS 1-6B (71.94 g)
Biological yield/ plant (g)	NDA 5-14 (292.98 g), NDA 8-6 (264.67 g), NDA 96-6 (250.83 g), NDACMS 1-6B (250.12 g), NDAGC 31 (245.69)
Harvest-index (%)	Bahar (31.66%), NDAGC 1010 (31.55%), Amar (31.22%), ICP 2155 (31.01%), NDA 96-6 (30.75%)

(35.34%) had low estimate. The characters with exhibited high heritability, suggests that the selection will be more effective. According to Panse (1957) such characters are governed predominantly by additive gene action and could be improved through individual plant selection. Whereas, low heritability indicated that the characters were highly influenced by environmental factors, genetic improvement through selection will be difficult due to effect of genotypes.

Johnson et al. (1955) have showed that a character exhibiting high heritability may not necessarily give high genetic advance. The highest value of genetic advance in percent of mean was shown by secondary branches per plant (48.65%), while days to maturity (2.92%) had lowest value for this parameter. The characters exhibiting high estimates of genetic advance in percent of mean were seed yield per plant (43.34%), biological yield per plant (38.44%), 100-seed weight (33.70%) and pods per plant (29.04%), along with secondary branches per plant (48.65%). However, the moderate estimates of genetic advance resulted in the case of seeds

per pod (19.04%), harvest-index (15.82%), primary branches per plant (13.91%) and plant height (12.94%). In addition to days to maturity (2.93%), the low estimate of genetic advance was also found for days to 50% flowering (5.34%).

It can be find out with greater degree of accuracy when heritability in conjunction with genetic advance is studied (Dudley and Moll, 1969). Thus a character possessing high heritability along with high genetic advance will be valuable in the selection programme. High heritability coupled with high genetic advance as per cent of mean were recorded for secondary branches per plant, seed yield per plant, biological yield per plant, 100-seed weight and pods per plant; suggesting preponderance of additive gene action in the expression of these characters. Therefore, selection may be effective through these characters in segregating generation. High heritability coupled with moderate genetic advance as per cent of mean were observed for plant height, primary branches per plant, seeds per pod and harvest index. However, days to 50% flowering and days to maturity exhibited high

heritability coupled with low genetic advance suggesting preponderance on non-additive gene action in the inheritance of these traits; hence, in this case selection may not be effective. Most of the above results in respect to heritability and genetic advance are in agreement with earlier reports on pigeonpea by Basavarajiah et al. (2000), Venkateswarlu (2001), Bashkarana and Muthiah (2006), Bhadru (2008), Bhadru (2011) and Yerimani et al. (2013).

Conclusion

The highest mean performance for seed yield per plant along with some of the component traits was exhibited by NDA 5-14, NDA 8-6, NDA 96-6, ICP 2155 and NDACMS 1-6B. The above mentioned genotypes may be used as donor parents in hybridization programme for developing high yielding varieties. The characters 100-seed weight, pods per plant, seed yield per plant, plant and secondary branches per plant showed high heritability coupled with high genetic advance as

percentage of mean, suggesting preponderance of additive gene action in the expression of these characters. Such traits could be improved by mass selection and other breeding methods based on progeny testing; while, plant height, primary branches per plant, pods per plant, seeds per pod and harvest index showed high heritability with moderate genetic advance as percent of mean suggesting greater role of non-additive gene action in their inheritance; to improve these traits heterosis breeding could be used.

Conflict of Interest

The authors have not declared any conflict of interest.

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Short Communication

First report in Southern Brazil of *Alternaria alternata* causing *Alternaria* leaf spot in alfalfa (*Medicago sativa*)

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Alfalfa plants with symptoms of *Alternaria* leaf spot from the Atlantic forest biome and Pampa biome, Brazil, were collected to identify the pathogen associated in this disease. The pathogen was isolated, analyzed morphologically according to literature and later molecularly identified. After that, pathogenicity tests were conducted in a greenhouse to confirm the Koch's postulates. The first symptoms occurred after five days of inoculation. Initially the symptoms were dark formations becoming rounded blotches about 1 mm to 3 mm in diameter that appeared on both edges and in the center of the leaflets. From this data, we concluded that this is the first report of *Alternaria alternata* in Brazil.

Key words: Forage legumes, fungal disease, inoculation, pathogenicity.

INTRODUCTION

Alfalfa stands out from other forage legumes species in terms of its nutritive value, but it is susceptible to attack from more than 70 different pathogens (Thal and Campbell, 1987). In Brazil, there are few studies and only thirteen fungal diseases have been detected (Iamauti and Massola, 2005). When alfalfa plants are attacked by foliar pathogens, losses in quality of hay, reduction in green forage production and limitations on the development of plants occur. Information about diseases occurring in alfalfa in Brazil is restricted and because of the growing interest in this species, it is essential to do more research to better understand these diseases. To our knowledge,

this is the first report in Southern Brazil of *Alternaria alternata* causing *Alternaria* leaf spot (ALF) in alfalfa. These contributions are very important for breeding and selection of productive cultivars that are resistant to pathogens.

MATERIALS AND METHODS

Sampling

Symptoms of ALF were observed in *Medicago sativa* cv. Crioula plants in the highland (Atlantic forest biome) and lowland (Pampa

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biome) regions of Rio Grande do Sul State, Southern Brazil. The symptomatic leaves were collected from six agro-ecological farms located in the highland region and from Embrapa Pecuária Sul, a research institution using conventional tillage in the lowland region. Samples were collected in winter (July and August) and spring (October) of 2013; these are periods of high relative humidity.

Morphological characterization and molecular identification

All isolates were identified as being *A. alternata* based on morphological analysis using literature with a descriptive key (Barnett and Hunter, 1987). This identification was confirmed by the Biological Institute of São Paulo for the isolates from both regions. The isolated DNA was extracted according to the method described by Doyle and Doyle (1987) from the mycelium produced in Potato Dextrose Agar (PDA). The extracted genomic DNA samples were subjected to Polymerase Chain Reaction (PCR) for amplification of the ITS (internal transcribed spacer) rDNA region and part of the RPB2 gene (encoding the second largest subunit of RNA polymerase II). The primers for the ITS region were ITS1 (5' – TCCGTAGGTGAACCTGCGG – 3') and ITS4 (5' – TCCTCCGCTTATTGATATGC – 3') (White et al., 1990) and for gene segment *rpb2* were RPB2-5F2 (5' – GGGWGAYCAGAAGAAGGC – 3') (Sung et al., 2007) and fRPB2-7cR (5' – CCCATRGCTTGYTTRCCCAT – 3') (Liu et al., 1999). Samples of the two isolates were deposited in the fungal collection of the Biological Institute of São Paulo in June 2014 (register number: MMBF 12/14).

Fungus isolation and Koch's postulates

The fungus was isolated from necrotic leaf tissue, grown in PDA and incubated at 25°C for 10 days. Pathogenicity was confirmed based on Koch's postulates using twelve plants from five different cultivars and genotypes (CUF 101, Crioula, ABT 805, E1C4 and Chile). Eight weeks after emergence alfalfa plants were grown in pots containing autoclaved soil. The plants were inoculated with spore suspension (10^6 conidia/ml). The spore suspension of *A. alternata* was prepared by brushing plates containing PDA medium colonized by the pathogen for ten days into distilled water. The concentration of the suspension was determined using a Neubauer chamber. Inoculation was conducted in the greenhouse and plant growth chamber with two repetitions using two types of isolates (highland and lowland). Measurements of length and width of spores (800 for each isolate: highland and lowland) of *Alternaria alternata* were conducted on the stereoscopic microscope Leica MZ-12, with a graduated ocular lens under 80 times magnification.

RESULTS AND DISCUSSION

In 80% of the plants collected, the symptoms occurred in the lower leaves, probably due to higher humidity. Plants with symptomatic leaves died faster than healthy plants and there was senescence only in injured plants. Symptoms began 5 days after inoculation and developed in all inoculated plants with a subsequent increase in the frequency and size of lesions. Leaf symptoms were initially dark formations becoming rounded blotches about 1 mm to 3 mm in diameter that appeared on both edges and in the center of the leaflets (Figure 1).

Symptomatic plants showed reduced vigor, premature senescence and foliar chlorosis. The initial lesions were

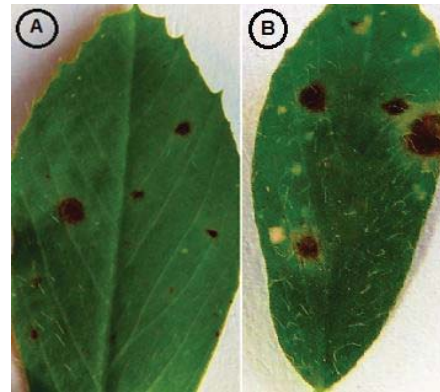


Figure 1. Evolution of the symptoms of foliar injury in random leaves of alfalfa (cv. Crioula). A) 7 days after inoculation; B) 14 days after inoculation.



Figure 2. *Alternaria alternata* spores. A) Spores from the highland area. B) Spore from the lowland area. Mean size for both isolates was 12.6 x 21.2 µm.

small, circular to oval, sunken and medium brown, but later became necrotic and dark brown as the disease progressed, showing concentric zones encircled with a chlorotic halo. Intense dark sporulation was observed on the lesions. Long chains of pale to light brown clavate conidia were observed with up to three longitudinal septa, and one to seven transverse septa which varied in size according to the source of the isolates; with the highland isolates being 5 x 13.7 µm (mean 28 µm) by 5 x 37.5 µm (mean 23.6 µm) and the lowland isolates 5 x 11.5 µm (mean 22.5 µm) by 7.5 x 18.8 µm (mean 32 µm). Conidiophores were elongated, straight, septate, and light to olive golden brown with a conidial scar (Figure 2).

In molecular identification, the sequenced region showed 99% similarity with a reference sequence for *A. alternata*. The RPB2 sequences isolated are 100% identical *A. alternata* (GenBank JQ811952 and JQ911953). These sequences of *A. alternata* were submitted to

mycologist Dr. Barry Pryor, University of Arizona, (Pryor and Gilbertson, 2000).

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENT

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Full Length Research Paper

Effect of temperature and pre-germination treatments on seed germination of juerana branca (*Stryphnodendron pulcherrimum*)

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Aiming to define simple and effective methods to accelerate and standardize the germination of juerana branca (*Stryphnodendron pulcherrimum*) (white juerana), seeds of this species were subjected to four types of pre-germination treatments, testing at temperatures of 25 and 30°C. The treatments were: immersion in sulfuric acid for 15 min, immersion in water at 80°C for 5 min, immersion in water at 80°C for 10 min, and control (no treatment). The experiment was based on weekly observations and analysis of the seed germination process. A completely randomized experimental design with three replications was used. According to the results obtained, treatment with hot water at 80°C for 10 min is not recommended for germination of juerana branca seeds. The pre-germination treatment that could be recommended for pre germination treatment of juerana branca seeds is immersion in sulfuric acid for 15 min at 25°C.

Key words: *Stryphnodendron pulcherrimu*; barbatimão; dormancy.

INTRODUCTION

Stryphnodendron pulcherrimum, which belongs to the *Fabacea Mimooideae* family, has various popular names, such as barbatimão, jubarbatimão, juerana-branca, paricá, paricazinho and caubi. The tree has an average height of 4 to 8 m and a broad, flattened and low canopy. The fruit is a pod which is indehiscent, apiculate, straight or curved and glabrous from 6 to 10 cm length, with 10 to 18 hard seeds (Lorenzi, 1992).

According to Lorenzi (1992), juerana branca is a pioneer species that occurs preferentially in younger or older secondary forests of higher lands or well-drained

sandy or clayey lands with medium fertility. This species annually produces a good quantity of seeds and is also recommended for reforestation. Emergence occurs within two to three weeks and the germination rate is generally low.

The species grows in the Amazon region and the south of Bahia in the Amazon and Atlantic rain forests. It is also present in the Guianas, Venezuela and Colombia. Its wood is moderately heavy, softwood of medium texture not highly resistant and with low natural durability. The tree is quite ornamental when in bloom and may be

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successfully used in tree landscaping. The species is also recommended for ecological reforestation (Lorenzi, 1992).

Seed dormancy is a characteristic process for delayed germination when seeds do not germinate, even under favorable conditions. Around two-thirds of arboreal species have some type of dormancy and this phenomenon is common both in species of a temperate climate and in plants of a tropical climate (Vieira and Fernandes, 1997). Among the most common processes for overcoming seed dormancy are chemical scarification, mechanical scarification, cold and hot-cold stratification, thermal shock, exposure to intense light, immersion in hot water and soaking in cold water (Kramer and Kozłowski, 1972; Fowler and Bianchetti, 2000). For most tropical species, optimum germination temperature is from 15 to 30°C. Maximum temperature ranges from 35 to 40°C and the minimum may arrive at the freezing point. In general, temperatures below the optimal range reduce speed of germination resulting in alteration of uniform emergence perhaps due to increase in time of exposure to pathogen attack. In contrast, temperatures above the optimal range increase the speed of germination, although only the most vigorous seeds manage to germinate (Nassif et al., 1998). Because of the potential usefulness of *Juierana branca*, it is important to identify simpler and more effective means for its germination and development. Therefore, the aim of present study was to define simple and effective methods to accelerate and standardize *Juierana branca* (*S. pulcherrimum*) seed germination.

MATERIALS AND METHODS

The experiments were conducted in the Seed Analysis Laboratory of the Faculdade de Rondônia – FARO, located on highway BR 364 at Km 6.5 in the city of Porto Velho, state of Rondonia, Brazil. The seeds of *Juierana branca* were collected from a species that is found at the Batalhão de Polícia Ambiental – BPA/PM (Environmental Police Battalion), located in the municipality of Candeias do Jamari, RO, Brazil. Collection was made in July 2012. 480 seeds of *Juierana branca* were selected and subjected to four types of pre-germination treatments as follows:

Treatment 1: Control (seeds without application of pre-germination treatment);

Treatment 2: Immersion in concentrated sulfuric acid (H₂SO₄) for 15 min, later washing with running water and drying;

Treatment 3: Immersion in water at 80°C for 5 min, later washing with running water and drying; Treatment 4: Immersion in water at 80°C for 10 min, and later washing with running water and dry.

120 seeds were used for each pre germination treatment. Half of the seeds of each treatment were placed to germinate at a temperature of 25°C, and the other half at the temperature of 30°C for the purpose of evaluating germination at different temperatures (240 seeds for each temperature). Two BOD type germinators were used for that purpose. Each treatment was represented by three replications with 20 seeds each. To accommodate the 20 seeds of each replication, "gerbox" boxes were used and as substrate, "germitest" paper moistened with distilled water. Seeds were immersed in 2% chlorine bleach solution for a period of 5 min for sanitization. The seeds were then washed in distilled water and

dried on a paper towel to subsequently set up the experiments. Evaluations were made up to 28 days after setting up the experiments. Data were collected in the following manner:

- 1) First germination count = Corresponding to the percentage of seeds germinated on the 14th day after setting up the experiment;
- 2) Total germination percentage = Corresponding to the total percentage of seeds germinated up to the 28th day after setting up the experiment;
- 3) Primary root length = Measurement of the root in centimeters on the last day of the experiment.
- 4) Dry matter = At the end of the experiment, the seedlings of each replication within each treatment were subjected to drying in a laboratory oven regulated at 100°C for 24 h, with the results being expressed in dry matter (g) per treatment;

The experimental design used for data analysis was completely randomized design in a 2x4 factorial arrangement, with two temperatures and four pre-germination treatments, with distribution in three replications of 20 seeds each. The GENES statistical software was used for statistical analysis of the data (Cruz, 2006). Mean values were compared by the Tukey test at 5% probability level.

RESULTS AND DISCUSSION

The results of analysis of variance and test of mean values for all the treatments at the two temperatures evaluated are shown in Table 1. By means of significance of the mean square for all the variables evaluated, the occurrence of differences among the treatments tested was verified.

First germination count (1°GC)

According to the information presented in Table 1, the treatment with sulfuric acid at 25°C (25°C T2) obtained an expressive result in its first count (1°GC) which was 45% of seeds germinated. It may also be noted that the same treatment at 30°C had a good result: 23.33% germinated. Nevertheless, this is much less expressive than that at 25°C.

In the two pre germination treatments in which the seeds were subjected to immersion in hot water, both for the temperature of 25°C and for 30°C, there was no emergence up to the first count except for the 30°C T3 treatment which had 8.33% of the seeds germinated. The controls for both temperatures had statistical results similar to those obtained with the hot water treatments; the 25°C T1 treatment resulted in 1.67% germinated seeds.

Figure 1 show the bar graph in relation to the first germination count comparing the four treatments performed at temperatures of 25 and 30°C. It is evident that treatment 2 (with sulfuric acid), for both temperatures showed good performance in relation to the others.

Total germination (G)

After 28 days, we have total germination and the results

Table 1. Mean values in reference to the first germination count (1^aGC), total germination (G), dry matter (DM) and primary root length (RL) of juerana branca (*Stryphnodendron pulcherrimum*).

Interaction	1 ^a GC(%) [14 days]	G (%) [28 days]	DM (g) [28 days]	RL (cm) [28 days]
(25°C T1)	1.67 ^{bc1/}	1.67 ^b	0.00 ^c	0.00 ^c
(25°C T2)	45.00 ^a	65.00 ^a	1.75 ^a	12.20 ^a
(25°C T3)	0.00 ^c	0.00 ^b	0.00 ^c	0.00 ^c
(25°C T4)	0.00 ^c	0.00 ^b	0.00 ^c	0.00 ^c
(30°C T1)	0.00 ^c	3.33 ^b	0.21 ^c	2.73 ^{bc}
(30°C T2)	23.33 ^{ab}	55.00 ^a	0.98 ^b	3.52 ^{bc}
(30°C T3)	8.33 ^{bc}	13.33 ^b	0.41 ^c	5.18 ^b
(30°C T4)	0.00 ^c	0.00 ^b	0.00 ^c	0.00 ^c
Mean Square	803.42 ^{**}	2164.14 ^{**}	1.22 ^{**}	53.89 ^{**}

^{1/}Mean values followed by the same letter in the columns do not differ among themselves by the Tukey test at 5% probability of error. ^{**}Significant by the F test at the level of 1% probability.

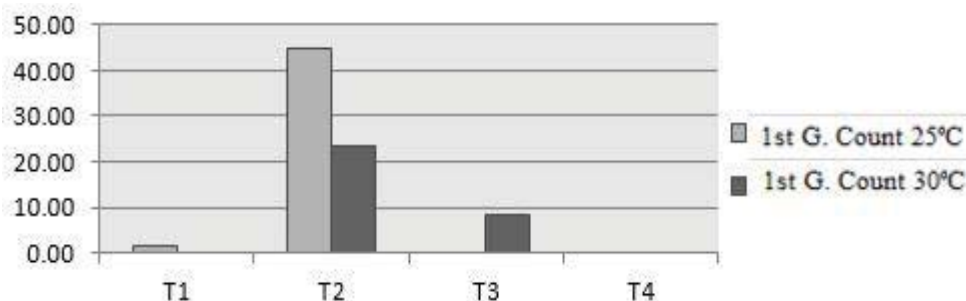


Figure 1. Comparison of the percentages of seeds germinated in the first count at the two temperatures evaluated (1^aGC). T1=immersion in sulfuric acid for 15 min; T2= immersion in water at 80°C for 5 min; T3= immersion in water at 80°C for 10 min, and T4=control (no treatment).

presented in Table 1 show that the treatment with sulfuric acid continued its good performance; the 25°C T2 treatment reached 65% germinated seeds and the 30°C T2 treatment, 55%. In the latter, it may be seen that there was a notable leap in germination in relation to the first count – from 23.33 to 55% (+31.67%). However, the 25°C T2 treatment in its first count showed a more accelerated germination rate, with 45% (1^aGC), therefore passing from 45 to 65% (+20%). The treatments with hot water for a temperature of 25°C and treatment 4 (T4) at 30°C were not successful up to those points. Only treatment T3 at 30°C continued giving results: 13.33% of germinated seeds. Varela et al (1991), tested hot water at a temperature of 90°C for the time of 5, 10 and 15 min and the seeds did not germinate. Those results are thus similar to the results obtained in this study.

In regard to the controls, the germination rate was low. In the 25°C T1 treatment, 1.67% of the seeds germinated after 28 days of the experiment. The 30°C T1 treatment led to 3.33% germinated seeds.

According to Lorenzi (1992), the species *S. pulcherrimum* has a generally low germination rate, which is corroborated by these results. In Figure 2, it may be noted that the treatments with sulfuric acid (T2) for the two temperatures (25° and 30°C) showed good performance after 28 days of the experiment. Treatment 2 at the temperature of 25°C stood out, with 10% more germination as compared to the temperature of 30°C. The controls treatments showed a low germination rate up to the end of the experiment. of the treatments with hot water, the 30°C T3 treatment was the only one in which there was germination.

Dry matter (DM)

In Table 1, in the DM column are the results in regard to weight in grams of the dry matter of the seedlings. The 25°C T2 and 30°C T2 treatments stood out, exhibiting 1.75 and 0.98 g respectively. For the other treatments,

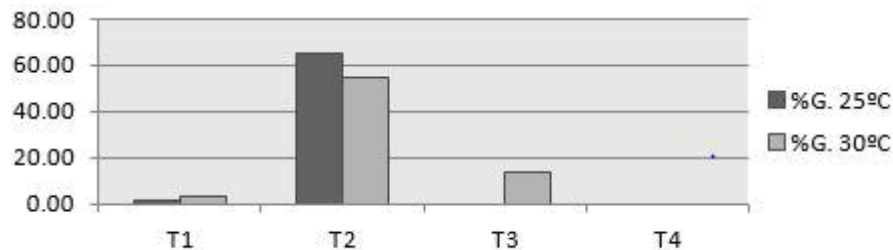


Figure 2. Comparison of the percentages of germinated seeds in the last count at the two temperatures evaluated. T1=immersion in sulfuric acid for 15 min; T2= immersion in water at 80°C for 5 min; T3= immersion in water at 80°C for 10 min, and T4=control (no treatment).

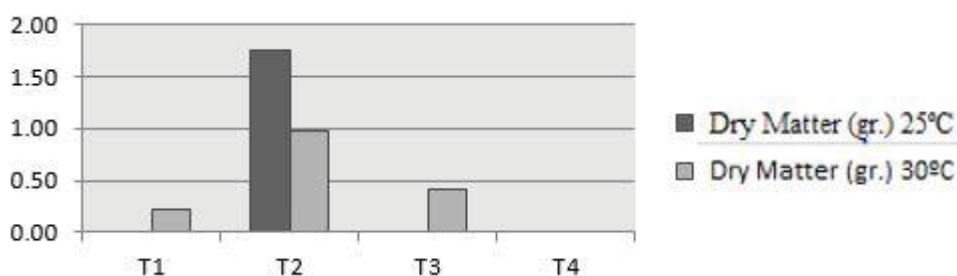


Figure 3. Comparison of the values for dry matter at the two temperatures evaluated. T1=immersion in sulfuric acid for 15 min; T2= immersion in water at 80°C for 5 min; T3= immersion in water at 80°C for 10 min, and T4=control (no treatment).

there was no statistical difference. It was observed that at the temperature of 25°C, the treatment with sulfuric acid provided for greater values of dry matter. Comparing the data for T2 dry matter indicates that there was considerably more development in the experiment at 25°C. It should also be noted that this treatment germinated 10% more than at 30°C (Figure 3).

Primary root length (RL)

In the RL column of Table 1, it may be observed that the 25°C T2 treatment obtained a high mean length in relation to the others, reaching 12.20 cm. Although the 30°C T3 treatment had an apparently higher result than the 30°C T2 treatment. It may not be considered as a better index since the percentages of germination between them are quite different and the quantity of germinated seeds is reflected in the final mean value. Comparing the mean value of primary root growth between the treatments with sulfuric acid at 25 and 30°C, it may be noted that the roots did not develop very much in the 30°C T2 treatment, maintaining a standard short length. Statistically, the 30°C T1 and 30°C T2 treatments obtained similar mean values for primary root growth and

within the treatments at 30°C considering the percentage of germination and dry matter of the 30°C T2 treatment, it may be affirmed that it exhibited better results.

In Figure 4, the big difference among the mean values of primary root length in T2 may be better observed. This is due to the fact that the roots of the treatment at 25°C had better development. From the results presented, it was observed that the treatment with hot water at 80°C for 10 min is not recommended for the two temperatures because it caused death of the seeds. Although, the treatment with hot water at 80°C for 5 min did not show results when subjected to the temperature of 25°C, there was more significant germination for the seeds subjected to 30°C if compared to the results obtained from the controls. However, there was no statistical difference. The pre-germination method that gave the best result was immersion in sulfuric acid for 15 min at 25°C. A good germination rate was observed in this method with considerable seedling development greatly stimulating primary root growth.

Conclusion

The treatment of hot water at 80°C for 10 min is not

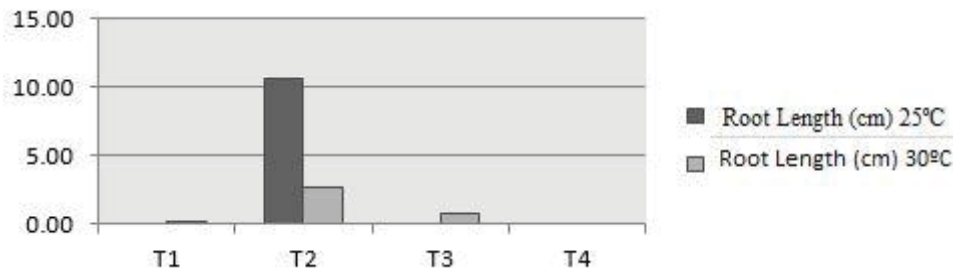


Figure 4. Comparison of the values for primary root length at the two temperatures evaluated. T1=immersion in sulfuric acid for 15 min; T2= immersion in water at 80°C for 5 min; T3= immersion in water at 80°C for 10 min, and T4=control (no treatment).

recommended for germination treatment of juerana branca seeds. The pre-germination treatment most recommended for germination of juerana branca seeds is immersion in sulfuric acid for 15 min at the temperature of 25°C.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Anti-nutritional compounds in fresh and dried lychee fractions (*Litchi chinensis* Sonn.)

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The present study evaluated the anti-nutritional factors of “Bengal” lychee (*Litchi chinensis* Sonn.), in the fresh pulp, peel and seed, both fresh and processed. The samples were analyzed for the levels of phenolic compounds, nitrate, oxalic acid and inhibitory activities of trypsin, lipase and α -amylase. Drying influenced the activity of all enzyme inhibitors, resulting in a reduction in the inhibitory activity of lipase (0.13 and 0.15 lipase inhibitor units for peel and seed, respectively) and an increase in the inhibitory activities of trypsin (10.14 and 10.66 trypsin inhibitor units for peel and seed, respectively) and α -amylase (1.13 and 1.08 amylase inhibitor units for peel and seed, respectively). With drying, it was possible to observe an increase in the levels of phenolic compounds, the low content of nitrate did not change with drying, while oxalic acid was not detected. The antinutrients evaluated in lychee fractions are present in amounts that do not preclude its use; thus, the use of lychee fractions, fresh or dried, is feasible as nutrient sources and add value to the fruits, since industries can use these residues for developing new products, as well as in food enrichment.

Key words: Phenolic compounds, nitrate, oxalic acid, enzyme inhibitors.

INTRODUCTION

Consumer interest for exotic fruits increases every day because of their nutraceutical value and the correlation between healthy eating and the reduction in the risk of diseases and cancer development (Ferguson and Schlothauer, 2012).

Fruits and vegetables have a large amount of substances capable of providing health benefits by preventing or treating diseases, which are called

bioactive compounds. These substances can act in different ways: as antioxidants, activating hepatic detoxification enzymes, inhibiting cholesterol absorption or reducing platelet aggregation. In addition to these compounds, fruits and vegetables may also have anti-nutritional compounds that have deleterious effects on the organism, interfering with digestion and nutrient absorption, or being toxic, depending on the amount in

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which they are consumed (Pennington, 2002; Suneja et al., 2011; Sreerama et al., 2012; Coimbra and Jorge, 2013). These anti-nutrients such as trypsin inhibitors, lectins, oxalic acid, nitrates and phytates, are present in raw or processed foods (Jain et al., 2009); however, in general, heat processing causes a reduction in the content of some of these compounds, such as protease inhibitors, tannins and lectins (Muzquiz et al., 2006).

In the food industry, fruit processing creates a substantial amount of residues and the recovery of these residues can be an important alternative for sustainable development. In this context, lychee (*Litchi chinensis* Sonn) is a fruit natural to China, and has been widely cultivated in warm climates around the world (Zhang et al., 2013). It presents a short postharvest life and can be consumed fresh, canned, dehydrated, and processed into juices, fermented beverages, liqueurs, ice creams, etc. Lychee by-products, such as the peel and the seed, are usually discarded by the industry and consumers, and can be used as an alternative source of nutrients, since they do not present anti-nutritional factors in harmful amounts to the body.

Research has shown that lychee by-products have a high energy and nutritional potential, besides anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepato and cardioprotective activities (Bhoopat et al., 2011; Queiroz et al., 2012; Jiang et al., 2013). Since these by-products have lower storage times, due to water content, drying arises as a tool, as it allows weight reduction, which reduces shipping, packaging and storage costs, besides extending storage time, allowing the application of these farinaceous products as ingredients in the food industry. However, in order to be used, it is necessary to investigate anti-nutritional factors present in lychee fractions and the effect of drying on these factors.

Seeking the discovery of new nutritional sources, as well as the minimization of postharvest losses and the use of agricultural by-products, the fractions peel, pulp and seed of fresh lychee were studied, as well as the peel and the seed dried at 45°C, in order to determine the bioactive compounds with anti-nutritional characteristics.

MATERIALS AND METHODS

Raw material and sample preparation

Lychee fruits from Bengal cultivar were picked in December, at a commercial orchard in Nepomuceno, located in the southern region of the state of Minas Gerais (-21°20'S; 45°23'W), Brazil. The lychees were selected according to uniformity of coloration (intense red peel), average size and absence of defects. After the selection of 280 fruits, they were washed, sanitized with 200 µL⁻¹ sodium dichloroisocyanurate for 15 m, weighed, divided into 2 batches, each with 7 repetitions with 20 fruits each, and separated into fractions (peel, pulp and seed).

The peel, pulp and seed fractions of the first batch (fresh fruit) were frozen in liquid nitrogen and stored in a freezer (-20°C) until the analysis was carried out. The peel and seed fractions of the

second batch (dried) were oven-dried (45°C) until constant weight and stored in an amber flask for approximately 4 days, for the drying of the peel, and 8 days for the seed.

Moisture

The moisture content of lychee peel, pulp and seed was determined by gravimetry, according to the methodology of the Association of Official Analytical Chemists (Horowitz, 2010).

Phenolic compounds

The extraction of phenolic compounds was performed with 50% methanol in reflux for three consecutive times, at 80°C. The extracts were combined, evaporated to 25 ml and subjected to the determination of phenolic compounds, using the Folin-Denis reagent. The results were expressed in tannic acid equivalents (Horowitz, 2010).

Oxalic Acid

The content of oxalic acid was determined by Huang and Tanudjaja (1992), in which the oxalic acid was extracted at 90°C with 6N hydrochloric acid, precipitated and quantified by titration with 0.02 N potassium permanganate.

Nitrates

Nitrates were determined as proposed by Cataldo et al. (1975), by the nitration of salicylic acid under highly acidic conditions dry samples were suspended in deionized water, incubated at 45°C for 1 h, centrifuged at 5,000 x g for 15 m and the supernatant was collected for analysis. The complex formed had its absorbance measured at 410 nm and the results were expressed in mg nitrate 100g⁻¹ dry matter (DM).

Trypsin inhibitory activity

Trypsin inhibitory activity of lychee fraction extracts was determined using the method proposed by Erlanger et al. (1961), using Nα-Benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPNA), prepared in 0.05 mol L⁻¹ TRIS, pH 8.2, as a substrate. The determination of the inhibitory activity was performed from the difference between the activity in the absence (control, without extracts) and in the presence of the extracts. The results were expressed in Trypsin Inhibitor Units (TIU) where 1 TIU is equal to 1µmol min⁻¹ per g of DM that fails to be produced due to the presence of the extract.

α -Amilase inhibitory activity

The inhibitory activity of α-amylase in lychee fraction extracts was determined according to Simão et al. (2012). Aqueous extracts of lychee fractions were prepared at a 1:10 (w/v) ratio under horizontal agitation at 4°C, for 30 m. A 50 µL aliquot of the extracts was pre-incubated with 50 µL α-amylase for 20 m, in a water bath at 37°C; soon afterwards, 100 µL of the substratum was added (1% starch solution prepared in a 0.05 mol L⁻¹ Tris buffer, pH 7.0, added with 38 mmol L⁻¹ NaCl and 0.1 mmol L⁻¹ CaCl₂), at 4 different times. The reaction was stopped with 200 µL dinitrosalicylic acid (DNS) and the reaction product was measured in a spectrophotometer at 540 nm. The results were expressed in α-Amylase Inhibitor Units (AIU), in which 1UIA is equal to 1 µmol min⁻¹ per g of DM that fails to be

Table 1. Levels of phenolic compounds, nitrate and oxalic acid of lychee fractions.

Fractions	Phenolic compounds* (mg 100g ⁻¹)	Nitrate* (mg 100g ⁻¹)	Oxalic acid
Fresh peel	22.04±0.50 ^{C**}	339.89±17.84 ^A	ND ^{***}
Fresh seed	11.45± 0.36 ^D	148.13± 27.54 ^B	ND
Fresh pulp	21.20 ± 0.35 ^C	51.98 ± 1.63 ^C	ND
Dry peel	71.71± 0.29 ^A	351.81± 18.47 ^A	ND
Dry seed	34.72± 0.49 ^B	154.80± 28.77 ^B	ND

* All values are on a dry weight basis. Data represent mean ± SEM (n=7). ** Within the same column, means followed by the same upper case letter in columns do not differ by the Tukey test at 5% probability. *** ND- Not detected

produced due to the presence of the extract.

Lipase inhibitory activity

Lipase activity was determined according to Souza et al. (2011). A sample of 0.5 g lychee fractions was extracted in 20 ml of 80% ethanol, in an ultrasonic bath, at 4°C, for 30 m. Ethanol was removed from the extract by evaporation and the sample residue was suspended in 5 ml water. A 50 µL aliquot of the extract, at a 1:10 (w/v) ratio, was pre-incubated with 100 µL pancreatic lipase, at 37°C, for 10 m. The reaction was started with the addition of 50 µL of the substrate p-nitrophenyl-palmitate, at 8 mmol L⁻¹, in 0.05 mmol L⁻¹ Tris-HCl, pH 8, containing 0.1% Triton-X100, in a bath at 37°C, and was stopped by transferring the reaction mixture to an ice bath and adding 1000 µL of 0.05 mmol L⁻¹ Tris-HCl buffer. The samples were read in a spectrophotometer at 410nm and lipase activity was expressed in Lipase Inhibitor Units (LIU), where 1 LIU is equal to 1µmol min⁻¹ per g of DM that fails to be produced due to the presence of the extract; and inhibition percentage calculated through the difference of the slope of the standard and control curves.

Statistical analysis

The experiment was carried out in a randomized design (CRD), made up of 5 treatments (fresh peel, pulp and seed, and peel and seed dried at 45°C), using 7 repetitions of 20 fruits each. The results were reported as mean ± standard error of mean (SEM), and were submitted to a variance analysis by the SISVAR[®] statistical software, version 5.1 (Ferreira, 2011), and the averages of the treatments were compared by the Tukey test at 5% probability.

RESULTS

Lychee had an average weight of 18.39 g, of which 27.08% were peels, 50.90% pulp and 22.02% seeds. The moisture found in the peel, pulp and seed was 68.93, 83.91 and 47.11 g 100 g⁻¹, respectively. Phenolic compounds, nitrate and oxalic acid contents verified in lychee fractions are shown in Table 1. There was a significant difference in the levels of phenolic compounds and nitrate in the analyzed fractions. Oxalic acid was not detected in any of the analyzed fractions.

Among the fresh fractions, the seed had the lowest content of phenolic compounds (11.45 mg 100 g⁻¹ dry

matter), and no significant difference was found between the content of these compounds in the peel and pulp. The content of phenolic compounds observed in the fractions submitted to drying were significantly higher than those obtained in the fresh ones, especially in the dry peel, which had the highest content of phenolic compounds (71.71 mg 100 g⁻¹).

The contents of nitrate ranged from 51.98 to 351.81 mg 100g⁻¹, and the lowest content was observed in the pulp (Table 1). Although processing resulted in significant changes in food composition, drying did not affect the contents of nitrate in the peel and seed, which were very similar to fresh fractions.

Enzymatic inhibitions verified in lychee fraction extracts are listed in Table 2. The fresh peel presented a higher trypsin inhibitory activity than the fresh seed (14.61 and 3.17 TIU, respectively), and inhibition was not found in the pulp. Drying at 45°C influenced trypsin inhibitory activity, increasing it in 10.14 TIU for the peel and in 10.66 TIU for the seed.

The lychee pulp (Table 2) presented a higher α-amylase inhibitory activity (7.23 AIU). The drying process influenced the inhibitory activity of α-amylase; however, there was no significant difference (p< 0.05) between the inhibitory activity in the peel and in the dry seed (1.13 and 1.08 AIU, respectively).

All of the ethanolic extracts of lychee fractions presented inhibitory activity on pancreatic lipase. The pulp presented the highest lipase inhibition percentage (67%), and although all of the extracts had the same concentration, the result expressed in inhibition percentage is relative to extract concentration and, as such, the results were converted to LIU.

Therefore, as occurred in the inhibition percentage, the fresh pulp presented the most expressive inhibitory activity (0.75 LIU), and differences were not observed (p< 0.05) between the inhibitory activities of the fresh peel and the seed.

Drying negatively influenced lipase inhibitory activity, causing a reduction of 0.13 LIU for the peel and 0.15 LIU for the seed, a result that can be observed by the decreases in the inhibition percentage and the inhibitory activity of the dry fractions in relation to the fresh fractions (Table 2).

Table 2. Trypsin inhibitory activity (TIU), α -amylase inhibitory activity (AIU), lipase inhibitory activity (LIU) and lipase inhibition percentage of lychee fractions.

Fractions	TIU* (TIU g ⁻¹)	AIU (AIU 100g ⁻¹)	LIU (LIU g ⁻¹)	Lipase inhibition (%)
Fresh peel	14.61 ± 0.47 ^{B**}	NS ^{***}	0.19 ± 0.003 ^B	27.44 ± 6.19 ^C
Fresh seed	3.17 ± 0.11 ^C	NS	0.22 ± 0.002 ^B	44.58 ± 3.21 ^B
Fresh pulp	NS	7.23 ± 0.46 ^A	0.75 ± 0.003 ^A	66.64 ± 5.6 ^A
Dry peel	24.75 ± 1.26 ^A	1.13 ± 0.07 ^B	0.06 ± 0.001 ^C	6.4 ± 5.6 ^E
Dry seed	13.83 ± 0.47 ^B	1.08 ± 0.08 ^B	0.07 ± 0.001 ^C	17.30 ± 6.51 ^D

* All values are on a dry weight basis. Data represent mean ± SEM (n=7). ** Within the same column, means followed by the same upper case letter in columns do not differ by the Tukey test at 5% probability. *** NS- Inhibition not significant.

DISCUSSION

Dehydration modifies the nutritional value, the physical and structural properties of fruits and vegetables and, even when this occurs in mild temperatures, there is a destruction of cell wall polymers (Eburn and Santosh, 2011; Harbourne et al., 2009; Kim et al., 2006). Therefore, an increase in the contents of phenolic compounds with drying suggests that heating caused a release of phenolic compounds, possibly bound to cell wall polysaccharides that were not extracted, and consequently, quantified in fresh lychee fractions.

Among phenolic compounds, tannins are considered anti-nutrients since, in diets for humans and species of monogastric animals, they can reduce the digestibility of proteins, carbohydrates and minerals; decrease the activity of digestive enzymes, besides causing damage to the lining of the digestive system or having systemic toxic effects (Benevides et al., 2011).

The maximum phenolic compound intake suggested for humans is approximately 1 g day⁻¹ (Scalbert et al., 2005). It is therefore likely that the use of lychee fractions, fresh or dried, is not sufficient to overcome this daily limit. Although processing causes important modifications in food composition, lychee processing did not cause significant changes in nitrate contents. Drying at 45° C is not capable of degrading nitrates present in lychee fractions, and the highest nitrate levels were observed in the peel, both fresh and dry.

The acceptable nitrate daily intake is 5 mg kg⁻¹ body weight (WHO, 2003). The excessive consumption of this compound can cause cyanosis by the formation of metmyoglobin, and neoplasia from the formation of N-nitroso compounds (Faquin and Andrade, 2004). Taking the example of a person weighing 70 kg, they could ingest 350 mg nitrate; thus, these values will only be achieved if the consumption of dried peel fraction with the highest nitrate content, is close to 100 g day⁻¹, a consumption considered relatively high, which reduces the risk of damage caused by high nitrate doses be acquired with the use of dry lychee peel.

A reduction in the content of nitrate was observed in cruciferous vegetables cooked in boiling water and the

loss of nitrate is due to the loosening of the plant tissue, with consequent mass increase, as a result of water absorption, thus resulting in its dilution. Nitrate loss was also found in fruits and vegetables submitted to boiling; however, nitrate values remained relatively constant during baking and there was a 2- to 3-fold increase in nitrate content after frying products in soy bean oil (Chetty and Prasad, 2009), which did not occur with dry lychee fractions.

The content of nitrate found in all lychee fractions was lower than that found by Kaminishi and Kita (2006) for spinach, whose average content of nitrate ranged from 3.79 to 4.33 g kg⁻¹ fresh matter (FM), which do not preclude the use of lychee fractions, enabling individual use.

Oxalic acid was not detected in any of the lychee fractions, which represents a favorable result, since oxalate, although often found in plants, cannot be metabolized by humans, and is excreted in the urine. It is estimated that about 75% of kidney stones are comprised of calcium oxalate, and hyperoxaluria is a major risk factor for the disease. Thus, the restriction in oxalate intake in the diet has been suggested as a treatment to prevent recurrent nephrolithiasis in some patients (Benevides et al., 2011).

The effect of processing on the trypsin inhibitory activity of dried fractions is similar to that reported by Naves et al. (2010), who observed a lower activity of these inhibitors in the flour of raw pumpkin seeds, than in those steamed and water-boiled, although the process carried out by that author is different from that performed in lychee peel and seed.

Two of the main types of trypsin inhibitors, Kunitz and Bowman-Birk, are proteic and their thermal stability depends on molecular weight and on the degree of stabilization of the active conformation by disulfide bonds. However, although proteic, the use of moderate temperatures, as used in this study, may not be sufficient to denature such inhibitors, keeping their inhibitory activity. The inactivation of the trypsin inhibitor was only possible combining the treatment under pressure with high temperatures (90% inactivation with the treatment, in less than 2 m, at temperatures between 77 and 90°C and

pressures between 750 and 525 MPa) (Vem et al., 2005). This explains the fact that drying at 45°C was not enough to minimize trypsin inhibitor activity of dry lychee fractions.

In spite of the increase due to the drying process, the trypsin inhibitory activity found in all fractions was low in relation to other foods that also present these inhibitors, such as pumpkin seed, white beans, lentil, peanut, chickpea (Naves et al., 2010; Pereira et al., 2010; Pedrosa et al., 2012), and soy, whose values range from 37.73 to 51.68 TIU mg⁻¹ DM (Vem et al., 2005). Many plant families possess inhibitors distributed in various organs, and their expression is constitutive (reproductive organs, reserve organs and vegetative tissue) or induced (response to herbivory, pathogens, mechanical injury and stress) (Chen et al., 2004). The highest trypsin and α -amylase inhibitory activities found in dry lychee fractions are due to the response of the vegetable to the damage due to the process, at the initial drying moments. Although an increase in α -amylase inhibitory activity has occurred with drying, amylase inhibitory activity in the dry fractions was lower than that in the fresh lychee pulp and lower than that found in white bean flour (66.89 AIU) (Pereira et al., 2010).

The highest inhibitory activities of trypsin and α -amylase verified in the dry fractions are probably due to a plant response to the damage caused by the drying process in its early stages (Chen et al. 2004). The reduction in the activity of lipase inhibitors, 0.13 IWU in the peel and 0.15 IWU in the seed (Table 2), suggests that this inhibitor is sensitive to heat treatments. The *in vitro* enzymatic activity can be influenced by the presence of phenolic compounds of fruits and vegetables that, depending on their structure, can react with proteins and alter various properties of these biopolymers, such as their molecular weight, *in vitro* digestibility and solubility, showing a nonspecific inhibition (Rohn et al., 2002; Birari and Bhutan, 2007). Lychee pericarp, pulp and seed contain a great amount of polyphenolic compounds, such as condensed tannins, gallic acid, epicatechin, procyanidin A2, anthocyanin, quercetin 3-rutinoside (rutin), quercetin glucoside and others (Jiang et al., 2013; Zhang et al., 2013), which may exhibit anti-amylase activity, but the distribution of these compounds in lychee fractions varies.

The suppressive effects of this experiment suggest that lychee fractions, especially the pulp, are able to inhibit α -amylase and lipase. Thus, the inhibitory activity of α -amylase found in this study may be caused by the phenolic compounds present in the lychee fraction extract, and additionally, it is possible that protein-phenolic and/or phenolic-phenolic synergies may be involved in the food extract enzyme-inhibition mechanism. Among the compounds that may exhibit potential effects to prevent obesity, polyphenol stands out for inhibiting enzymes related to lipid metabolism, including pancreatic lipase, lipoprotein lipase, and

glycerophosphate dehydrogenase. Suppressive effects of polyphenols on lipase activity are due to their affinity for protein and lipase aggregation (Birari and Bhutan, 2007), which can cause their precipitation.

Research with the objective to determine the inhibition of pancreatic lipase in lychee flower reported that aqueous extracts of lychee flower rich in polyphenols show anti-obesity and anti-inflammatory potentials. The results showed that lychee flower-water extracts have suppressive effects against pancreatic lipase activity, with a 45% inhibition in *in vitro* pancreatic lipase activity and decreased epididymal adipose tissue sizes, as well as decreased serum and *in vivo* liver lipid contents (Wu et al., 2013).

The percentage of inhibition, *in vitro*, against pancreatic lipase activities observed in this study is higher than that reported by Wu et al. (2013), and this is probably due to the solvent used for the preparation of the extract and the lychee fraction analyzed.

Suppressive results of this experiment suggest that lychee fractions, particularly the pulp, are capable of inhibiting lipase and α -amylase, and may be helpful in the treatment of obesity caused by the elevation of fat and carbohydrate levels in the diet, although *in vivo* anti-obesity effects of lychee fractions were not investigated in this study. It should be noted, however, that, despite the high inhibition observed in the pulp, this fraction is rich in carbohydrates, which reinforces the need for future studies on the *in vivo* inhibitory potential of lychee fractions.

Conclusions

The results of this study show that lychee fractions have anti-nutrients such as phenolic compounds, nitrate and trypsin inhibitors, α -amylase and lipase, in amounts that do not preclude the use of these fractions, fresh or subjected to drying at 45° C, as nutrient sources. Therefore, it is possible to add value to lychee, since industries could use residues (peel and seeds) for commercial formulations and food enrichment.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Seed quality of three soybean varieties as influenced by intercropping time and arrangement in maize

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The response of soybean varieties *J499*, *SCS-1* and *La suprema*, with different growth habits, to intercropping conditions when grown in association with maize H614D was evaluated in a study at Bukura Agricultural College (0° 06" N; 34° 50" E), Kenya. The soybean was sown either at the same time as maize or two weeks later in pure stands or intercropped with maize either in the same hill or in alternate rows. The trial was laid out in a randomized complete block design with four replications. Data collected included grain yield, 100 seed weight, percentage germination, and analytical purity among others. Among the three soybean varieties *J499* had significantly higher percentage analytical purity (92%) than *SCS1* (84%) and *La suprema* (82%). In terms of % germination, *J499* had the highest (93.3%), followed by *SCS1* (87.5%) and lastly *La suprema* irrespective of the intercropping pattern and sowing time. Intercropping *La suprema* two weeks later in maize led to a 30% increase in 100-seed weight. Sowing at the same time as maize led to a significant difference in seed weight among the varieties, with *J499* being the highest (15.1 g) and *La suprema* the lowest (12.7 g). However, no significant difference was observed when they were sown two weeks later. Among the three varieties, *J499* had the lowest number of pods per plant and *SCVS-1* the highest. Yields (Kg/ha) of soybean sown in pure stands at the same time as maize were significantly higher (509.9 Kg/ha) than that sown two weeks later (280.2 Kg/ha). The difference in yield between soybean sown at the same time as maize and that sown two weeks later in row intercropping was about 400%. For sowing times pure stands yielded significantly higher soybean yields than intercrops. From the findings it can therefore be concluded that the seed of soybean variety *J499* grown as an intercrop has suitable quality attributes to be used as seed.

Key words: Seed quality, Intercropping patterns, maize H614D, soybean varieties, sowing time.

INTRODUCTION

Farmers in the tropics cultivate their crops through intercropping, which is the most common form of traditional farming (Waddington and Karigwindi, 2001). Intercropping is as old as civilization and is a widespread practice in the warm tropical countries due to its advantages such as optimum utilization of land (Searle et al., 1981), weed

suppression (Haggard-Nelson et al., 2001) and soil fertility improvement through biological nitrogen fixation by Rhizobium bacteria (Li et al., 2014; Cardoso et al., 2006; Li et al., 2007). Intercropping involving non-legume and legume combination have had significant yield advantages (Lithourgidis et al., 2006; Li et al., 2014) compared to

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monocropping system in various crop species. Yield advantages have been reported in: Maize-cowpeas (Dahmardeh et al., 2010) maize-bean (West and Griffin, 1992), sorghum (sorghum bicolor)/soybean (*Glycine max*) (Elmore and Jacobs, 1986), barley (*Hordium vulgare*/Pea (Chen et al., 2004) and Faba/barley (Ghosh et al., 2006). Nitrogen fixed by the legumes is influenced by the component crop morphology, crop density and competitive ability of the legume (Ofori and Stern, 1987). The morphological and physiological differences between non-legumes and legumes benefit their mutual association (Akuda, 2001). The cereal component crop is usually taller and has faster growing or more extensive system of fine roots (Lehman et al., 1998) and very competitive for soil nitrogen than the legumes which usually fix atmospheric N (Jensen, 1996).

Delouche (1980) and Chavez and Mendoza (1986) in their studies indicated that high temperature during the time of maturation reduces the quality of seeds. For instance Chavez and Mendoza (1986) showed that seed subjected to high temperature and high relative humidity conditions in the field declined significantly in germination after reaching physiological maturity. The resulting microclimate under the maize canopies is generally characterized by high relative humidity and temperature. Thus, shading may affect the quality of soybean seed intercropped with maize. However, intercropping has been reported by several other authors not to have any adverse effect on seed germination, seed purity or 1000-grain weight of wheat and various legumes grown in association with cereals (Neupane et al., 1997; Deshpande et al., 1992; Hilli and Kulkarni, 1988).

Seed quality is very essential for optimum stand establishment and maximum yield in soy bean. As a result, it is necessary to have different seed testing parameters that permit rapid, objective and accurate evaluation of seed quality. The quality of the seed lot is judged by the relative percentage of various components. The quality is considered superior, if pure seed percentage (analytical purity) is above 98, and other seeds and inert matter percentage as low as possible (Trivedi and Gunasekaran, 2013). Since germination test are based on pure seed components, it can readily be seen that purity analysis and germination tests complement each other (Joshi et al., 2009). Thus the actual planting value of seed can be determined only when the purity analysis and germination tests are considered together.

In Kenya most farmers use their previous harvest as seed material for subsequent planting. Is it advisable for farmers to use soybean seed produced under maize intercropping. This study was therefore conducted with the objective of evaluating the effect of intercropping time and arrangement on seed quality of three soybean varieties grown as intercrops in maize in Western Kenya.

MATERIALS AND METHODS

The study was carried out during the long rains (March to August) in

2007 at Bukura Agricultural College (0° 06" N; 34° 50" E) in Kakamega County in Western Kenya.

The experimental design was a 3*3*2 factorial in a Randomized Block Design with four replications. There were three factors namely cropping systems at three levels; sole soybean (A1), one row of soybean between two rows of maize (A2), and soybean planted in the same hill with maize (A3); soybean varieties were three; *J499*, *SCS-1* and *La suprema*; and two sowing times; same time as maize (T0) and two weeks after maize (T1) (Table 1)

The gross plot dimensions were 4 m by 3 m. At sowing time, 20 kg of nitrogen and 60-kg ha⁻¹ P₂O₅ were applied along the rows of maize. Forty-five days later 60-kg ha⁻¹ of nitrogen was applied to the maize as topdressing. Two seeds of maize were sown in each hill. Thinning to one plant per hill was done 18 days later.

The soybean in pure stand was drilled in rows 45 cm apart and thinned to 5 cm after four weeks. In addition to initial land preparation, weeds were controlled by hand weeding whenever it was necessary. The crops were sprayed with the insecticide Dimethoate[®] and the fungicides Antracol[®] and Dithane M45[®] to control pests and fungal diseases respectfully, common in the study area (Table 2).

The whole plot was harvested for determination of soybean seed yield and seed quality attributes. The plot yield was determined by weighing the threshed and winnowed grain. The moisture content was measured and the value obtained used to standardize the yields to 15% moisture content using the formula prescribed by FAO (1986):

$$\text{Adjusted yield (kg/ha)} = \frac{\{(100 - M1) Y\}}{\{100 - M2\}}$$

Where, M1 = % Moisture content of seed, M2 = Standardized storage moisture content (for seed soybean = 15%) and Y = Yield in kg/ha before moisture standardization.

A sample soybean seed from each experimental plot was divided in the laboratory to obtain working samples for various laboratory tests. The samples were thoroughly mixed by passing through a seed divider several times and subsequently reconstituting. The sub-samples for various tests were obtained by successively halving the sub-samples until a sample of required weight was obtained for the seed quality tests outlined following.

Seed purity analysis

A working sample of 120 g of seed obtained from the sub-sample was placed on a purity work board. The working sample was then meticulously separated into pure seed, other crop seeds, inert matter and weed seed components in accordance with ISTA purity analysis procedures. Each component was weighed in grams and the percentage of each component calculated (ISTA, 2004).

100-seed weight

The pure seed-fraction was poured on a counting board; from the sample, 10 replicates of 100 random seeds were counted. Each replicate was weighed on a precision balance in grams to two decimal places.

Germination test

Four replicates of 100 random seeds each were obtained using a vacuum seed counter (with counting plates containing 100 holes) and placed in loose, moistened sand media in germination dishes. The seeds were sufficiently spaced on the media and covered with a thin layer of uncompressed sand. The germination dishes were then covered with transparent, moisture proof dishes and placed in a germination room maintained at 20°C. The germination count was carried out at the end of 7 days using the following parameters:

Table 1. Treatment combinations and days of planting.

Treatment code	Description
Control	Sole maize
T0A1V1	Soybean variety <i>J499</i> sown in pure stands on same day as maize
T0A1V2	Soybean variety <i>SCS-1</i> sown in pure stands on same day as maize
T0A1V3	Soybean variety <i>La suprema</i> sown in pure stands on same day as maize
T0A2V1	Soybean variety <i>J499</i> sown in alternate rows with maize on same day
T0A2V2	Soybean variety <i>SCS-1</i> sown in alternate rows with maize on same day
T0A2V3	Soybean variety <i>La suprema</i> sown same day in alternate rows with maize
T0A3V1	Soybean variety <i>J499</i> planted in same hill with maize on same day
T0A3V2	Soybean variety <i>SCS-1</i> planted in same hill with maize on same day
T0A3V3	Soybean variety <i>La suprema</i> planted in same hill with maize on same day
T1A1V1	Soybean variety <i>J499</i> planted in pure stand 14 days after maize
T1A1V2	Soybean variety <i>SCS-1</i> planted in pure stand 14 days after maize
T1A1V3	Soybean variety <i>La suprema</i> planted in pure stand 14 days after maize
T1A2V1	Soybean variety <i>J499</i> planted in alternate rows 14 days after maize
T1A2V2	Soybean variety <i>SCS-1</i> planted in alternate rows 14 days after maize
T1A2V3	Soybean variety <i>La suprema</i> planted in alternate rows 14 days after maize
T1A3V1	Soybean variety <i>J499</i> planted in same hill with maize 14 days after maize
T1A3V2	Soybean variety <i>SCS-1</i> planted in same hill with maize 14 days after maize
T1A3V3	Soybean variety <i>La suprema</i> planted in same hill with maize 14 days later

Table 2. Plant spacing and population density for maize and soybean.

Crop	Spacing	Plant/Hill	Plant population	
			Per m ²	Per Ha
Maize	75 cm x 30 cm	1	4.44	44, 444
Soybean (A1)	45 cm x 5 cm	1	44.44	4444,444
Soybean (A2)	75 cm x 5 cm	1	26	266,667
Soybean (A3)	75 cm x 30 cm	1	4.44	44,444

Number of normal seedlings, abnormal seedlings, hard seeds, fresh ungerminated seeds and dead seeds. Germination capacity of normal seeds was based on total number of seeds planted and expressed as a percentage (ISTA, 2004).

Land equivalent ratio

The production efficiency was based on Land Equivalent Ratio (LER) expressed as:

$$\text{Land equivalent ratio (LER)} = (Y_{ij}/Y_{ii}) + (Y_{ji}/Y_{jj})$$

Where Y is the yield per unit area, Y_{ii} and Y_{jj} are sole crop yields of the component crops i and j and Y_{ij} and Y_{ji} are the intercrop yield (Mead and Willey, 1980). LER is the sum of the two partial land equivalent ratios. Where LER was more than 1.0, this indicates a positive intercropping advantage which shows that interspecific facilitation is higher than interspecific competition (Vandermeer, 1989).

Analyses of variance were carried out on all parameters measured, using computer package GENSTAT 5 ("General Statistic Committee 5"). Treatment means significantly different at 1 or 5% level of significance were separated using the Tukeys Test at the same level of significance. The association among various parameters was

determined using Spearman's Coefficient of Rank Correlation ($P=0.05$) as described by Steel and Torrie (1986).

RESULTS

Effect of intercropping pattern, relative sowing time and soybean variety of % analytical purity

Treatment effects were statistically highly significant ($p=0.001$) for relative sowing times, intercropping patterns, varieties and the interaction between relative sowing time and intercropping patterns. Other effects were not significant.

In general the soybean pure stands gave soybean seed with significantly higher % analytical purity than the intercrops (Table 3). However, no significant difference in % analytical purity was noted on soybean seed from either of the intercrop patterns. Soybean sown simultaneously with maize also gave seed with significantly higher analytical purity than that sown two weeks later.

In general soybean variety *J499* gave significantly higher

Table 3. Effect of intercropping pattern, relative sowing time and variety on the analytical purity (%) of soybean seed.

Intercropping pattern (A)	Soybean variety (A)% analytical purity						Mean(A)
	V1		V2		V3		
	T0	T1	T0	T1	T0	T1	
(A2)	96.9	96.6	95.9	92.0	95.2	87.3	94.0 ^a
(A3)	94.0	89.0	84.1	78.5	87.8	78.4	85.3 ^b
(A4)	94.6	80.5	93.1	63.0	80.6	62.5	79.0 ^b
Mean(V)	92 ^a		84.4 ^b		82.0 ^b		86.1

Mean (T) : % C.V. =7.53; T0=91.3a; T1=80.9b; V1, *J499*, V2, *SCS1*; V3, *La suprema*; A1, sole maize; A2, sole soybean; A3, row between maize; A4, same hill as maize; T1, planted two weeks after maize; C.V., coefficient of variation. Any two means having a common alphabetical letter in the same column are not significantly different at 5% level of significance

Table 4. The effect of the interaction between intercropping pattern and relative sowing time on percentage analytical purity of soybean seeds.

Intercropping pattern (A)	Relative sowing time (T) and% analytical purity		
	T0	T1	Mean
A2	96.0 ^a	92.0 ^a	94 ^a
A3	89.0 ^b	82.0 ^b	86 ^b
A4	89.0 ^b	69.0 ^c	79 ^c
Relative sowing time (T)	Intercropping pattern (A)		
	A2	A3	A4
T0	96.0 ^a	89.0 ^a	89.0 ^a
T1	92.0 ^a	82.0 ^b	69.0 ^b

% C.V.=7.53; S.E.D.=3.25; A1, sole maize; A2, sole soybean; A3, Row between maize; A4,same hill as maize; T1, planted two weeks after maize; S.E.D, standard error of the difference; C.V. , coefficient of variation. Any two means having a common alphabetical letter in the same column are not significantly different at 5% level of significance

% analytical purity than *SCS1* and *La suprema* (Table 3).

As shown in Table 4 whether sown at the same time as maize or two weeks later, soybean pure stands generally gave seed with a significantly higher % analytical purity than intercrops. For all intercrops sowing soybean simultaneously with maize led to a significantly higher % analytical purity than sowing two weeks later. However, no significant differences were recorded in % analytical purity between the two sowing times in soybean pure stands (Table 4).

The later sown soybean spent more time under the maize canopy, which had a modified microclimate with lower temperatures and higher relative humidity. These conditions probably led to development of pests and diseases that affected the analytical purity.

No significant differences were noted in soybean analytical purity between the intercropped varieties indicating the fact that analytical purity may be affected by factors other than varietal differences.

Effect of intercropping pattern, relative sowing time and soybean variety on % germination

As indicated in Table 5, except for soybean variety *SCS1*,

intercropping pattern had no significant effect on the % germination. *SCS1* pure stands and hill intercropping gave soybean seed with significantly higher % germination. In general, soybean variety *J499* gave significantly higher % germination than the other varieties in all cropping patterns. The germination percentage of *J499* was significantly greater than that of *La suprema* and *SCS1* when the three varieties were sown in the same hill as maize. Significantly higher percent germination was observed when *J499* was sown in mixed cropping than in monocropping. The contrast was true for *SCS1*, where monocropping gave significantly higher percentage germination than mixed cropping.

As shown in Table 6, intercrops gave higher % germination than pure stands when sown at the same time as maize. Hill intercropping gave significantly higher % germination than pure stands when sown at the same time as maize. However, between the rows intercrop was not significantly different from pure stands. When sown two weeks later, soybean pure and between the maize rows intercrop gave significantly higher % germination than hill intercrops.

Between the two relative sowing times, a significant difference in percentage germination was observed in pure

Table 5. The effect of the interaction between intercropping pattern and variety on percentage germination of soybean seeds.

Intercropping pattern (A)	Soybean variety (V) and % germination			
	V1	V2	V3	Mean
A2	89.3 ^a	92.0 ^a	84.4 ^a	88.6 ^a
A3	96.4 ^a	84.3 ^b	86.5 ^a	89.1 ^a
A4	94.3 ^a	86.2 ^{ab}	85.3 ^a	88.6 ^a
Soybean variety	Intercropping pattern			Mean
	A2	A3	A4	
V1	89.3 ^{ab}	96.4 ^a	94.3 ^a	93.3 ^a
V2	92.0 ^a	84.3 ^{bc}	86.2 ^{ab}	87.5 ^b
V3	84.4 ^b	86.5 ^c	85.3 ^b	85.4 ^b

% C.V. = 9.6; S.E.D. = 4.2; V1, *J499*; V2, *SCS1*; V3, *La suprema*; A1, sole maize; A2, sole soybean; A3, row between maize; and A4, same hill as maize S.E.D., standard error of the difference; C.V., coefficient of variation. Any two means having a common alphabetical letter in the same column are not significantly different at 5% level of significance

Table 6. The effect of the interaction between relative sowing time and intercropping pattern on percentage germination of soybean seeds.

Intercropping pattern (A)	Relative sowing time (T)/% germination		
	T0	T1	Mean
A2	85.0 ^b	92.1 ^a	88.6 ^a
A3	89.1 ^{ab}	89.4 ^a	89.3 ^a
A4	91.5 ^a	77.8 ^b	85.2 ^a
Relative sowing time (T)	Intercropping pattern (A)		
	A2	A3	A4
T0	85.0 ^b	89.1 ^a	91.5 ^a
T1	92.1 ^a	89.4 ^a	77.8 ^b

% C.V. = 9.6 S.E.D. = 4.2. A1, sole maize; A2, sole soybean; A3, Row between maize; A4, same hill as maize; T1, planted two weeks after maize; S.E.D., standard error of the difference; C.V., coefficient of variation. Any two means having a common alphabetical letter in the same column are not significantly different at 5% level of significance.

stands and same hill intercropping, but not in row between maize intercropping (Table 9). In pure stands later sown soybean gave significantly higher percentage germination than soybean sown two weeks earlier. While for soybean sown in same hill, earlier sown soybean gave significantly higher percentage germination than that sown two weeks later.

Effect of intercropping pattern, relative sowing time and soybean variety on 100-seed weight

There was a significant difference ($p=0.05$) in 100-seed weight between soya bean varieties sown at the same time as maize (Table 7), with 100-seed weight of *La suprema* being significantly lower than the 100-seed weight of *J499* and *SCS1*. However, the 100-seed weight of the three varieties showed no significant difference when they were sown in maize two weeks later. The seed

weight of *J499* and *SCS1* was significantly reduced when sowing was delayed by two weeks, while that of *La suprema* was significantly increased.

Effect of intercropping pattern, relative sowing time and soybean variety on grain yield

Yields of soybean sown in pure stands were significantly higher than in intercrops (Table 8). Further, a marked reduction in soybean yields was recorded when sowing was delayed by two weeks. However, no significant difference in yield was observed between the three soybean varieties.

Whether sown at the same time as maize or two weeks later soybean pure stands yielded higher than the two intercrops (Table 9). Soybean sown in pure stands at the same time as maize yielded on average four times as much grain as mixed intercrops. Yields of soybean pure

Table 7. The effect of the interaction between soya bean variety and relative sowing time on 100 seed weight (g) of soya bean.

Soya bean variety (V)	Relative sowing time (T) and 100-seed weight (g)		
	T0	T1	Mean
V1	16.1 ^a	14.0 ^a	15.0 ^a
V2	15.8 ^a	13.6 ^a	14.2 ^b
V3	11.0 ^b	14.2 ^a	12.6 ^c
Relative sowing time (T)		Soya bean variety	
(T)	V1	V2	V3
T0	16.1 ^a	15.8 ^a	11.0 ^a
T1	14.0 ^b	13.6 ^b	14.2 ^b
		Mean	
			14.3 ^a
			13.9 ^a

%C.V.=20.9 S.E.D.=0.7; V1, *J499*; V2, *SCS1*; V3, *La suprema*; T1, planted two weeks after maize; S.E.D, standard error of the difference; C.V. , coefficient of variation. Any two means having a common alphabetical letter in the same column are not significantly different at 5% level of significance

Table 8. Effect of intercropping pattern, relative sowing time and variety on yield (kg/ha) of soybean.

Intercropping pattern (A)	Soybean variety/grain yield (kg/ha)						Mean (A)
	V1		V2		V3		
	T0	T1	T0	T1	T0	T1	
(A2)	1097	766	1135	750	1058	514	887a
(A3)	158	186	208	94	187	74	151b
(A4)	322	102	225	21	119	15	134b
Mean (V)	439a		406a		328a		391

Mean (T): % C.V. =22.20; T0=501^a; T1=280^b. V1, *J499*; V2, *SCS1*; V3, *La suprema*; A1, Sole maize; A2, sole soybean; A3, Row between maize; A4, same hill as maize; T1, planted two weeks after maize; C.V. , coefficient of variation. Any two means having a common alphabetical letter along a column are not significantly different at 5% level of significance

Table 9. The effect of the interaction between intercropping pattern and relative sowing time on yield (kg/ha) of soybean.

Intercropping pattern (A)	Relative sowing time (T)/grain yield (kg/ha)		
	T0	T1	Mean
A2	1097 ^a	766 ^a	932
A3	184 ^b	118 ^b	151
A4	222 ^b	46 ^b	134
Relative sowing time (T)	Intercropping pattern (A)		Mean
	A2	A3	A4
T0	1097 ^a	184 ^a	222 ^a
T1	766 ^b	118 ^a	46 ^a
			641
			441

% C.V. = 22.20; S.E.D. = 102.14; A1, sole maize; A2, sole soybean; A3, Row between maize; A4, same hill as maize; T1, planted two weeks after maize S.E.D, standard error of the difference; C.V. , coefficient of variation. Any two means having a common alphabetical letter in a column are not significantly different at 5% level of significance.

stands sown two weeks later were about seven times the yield of corresponding mixed intercrops. Only monocrops showed significant difference in yield as a result of delayed sowing, with earlier sown soybean yielding significantly

more than later sown crop. No significant difference in yields was observed in mixed cropping as a result of variation in the sowing time.

The grain yield of soybean was significantly reduced in

Table 10. Seed yield and Land equivalent ratios of maize and soybean in sole crop, sowing time and intercropping systems.

Treatment	Yield Kg/ha		LERs
	Maize	Soybean	
T0A2V1	2960a	158g	1.16a
T0A2V2	2575a	288fg	1.14a
T0A2V3	2898a	187fg	1.17a
T0A3V1	2832a	322fg	1.27a
T0A3V2	2532a	225fg	1.07a
T0A3V3	2563a	119g	1.00a
T1A2V1	2394a	186fg	1.07a
T1A2V2	2348a	94g	0.93a
T1A2V3	2798a	74g	1.11a
T1A3V1	2479a	102g	0.99a
T1A3V2	2790a	21g	0.99a
T1A3V3	2768a	15g	0.98a
C.V%	14.46	12.20	11.66
Mean	2600.00	391.00	1.15
S.E.D	317.92	48.20	0.11

Means followed by the same letter in a column are not significantly different at the 5% level.

both intercropping patterns sown either at same time as maize or two weeks later due to the shading by maize and other interspecific interactions.

Soybean sown in any of the intercropping patterns simultaneously with maize gave significantly higher grain yield than soybean sown two weeks later. The soybean sown at the same time as maize might have escaped the competitive effects of maize. Changes in soil and weather conditions might have also contributed to the lower yields of later sown soybean.

Effect of intercropping pattern, relative sowing time and soybean variety on Land Equivalent ratio (LER)

As shown in Table 10, there were no significant differences in LERs due to treatment effects. However, in general, sowing soybean varieties at the same time as maize in intercrops led to LERs greater than one indicating the advantage of intercropping. Only soybean varieties *J499* and *La suprema* sown two weeks later between the rows gave LERs greater than one. All the other intercrops sown two weeks later in maize had LER values less than one indicating the disadvantage of intercropping due to delay in sowing (Table 10).

Effect of intercropping pattern, relative sowing time and soybean variety on Yield of maize

The treatments had no significant effect on the yield of maize (Table 10).

Correlation of growth and other parameters with yield of soybean

Several parameters measured correlated significantly with the yield of soybean. The number of pods per plant, the yield of maize and the LER were positively correlated to the yield of soybean (Table 11). The height of plants at maturity and 100-seed weight were not significantly correlated to the yield of soybean.

The number of pods per plant and analytical purity were significantly correlated with the yield of soybean indicating that treatments that affected the yield of soybean also affected these parameters. Maize and soybeans use the same environmental resources which they were competing for in mixed cropping. However, the comparison includes pure stands of both crops, which were not in interaction.

There was however no significant correlation between yield of soybean with 100-seed weight and germination percentage. Germination percentage was not significantly correlated with analytical purity. Treatments, which affect the yield of soybean, do not therefore seem to affect these parameters.

DISCUSSION

The results of the study indicated that analytical purity of soybean seed was significantly affected by intercropping with maize, relative sowing time and variety. Pure stands generally gave higher % analytical purity than intercrops probably due competition for resources by maize which affected grain filling leading to underdeveloped and dead seeds. Further the higher humidity and modified

Table 11. Spearman's rank correlation (rs) between soybean seed yields and selected parameters.

Yield correlated with	Rs	t _{0.05}	t-critical
Height	0.28	1.170 ^{Ns}	2.11
Pods per plant	0.62	3.130*	2.11
Yield of maize	0.74	5.503*	2.20
LER	0.79	5.090*	2.11
100-seedweight	0.03	0.120 ^{Ns}	2.11

Rs, Spearman's rank; Ns, not significant; *, significant ($p=0.005$).

temperature might have led to seed pests and diseases. Soybean variety *J499* generally gave significantly higher % analytical purity than the other varieties since it is earlier maturing and was able to escape the competitive effects of maize and produce better seed quality. Further, soybean sown at the same time as maize gave significantly higher % analytical purity than that sown two weeks later because it was also able to escape the competitive effects of maize due to separation in time. However, it is worth noting that all treatments gave a % analytical purity less than 98 which is the below the minimum standard required for quality soybean seed (Trivedi and Gunasekaran, 2013).

Except for soybean variety *SCS1*, intercropping pattern had no significant effect on the % germination. In general, soybean variety *J449* gave significantly higher % germination than the other varieties in all cropping patterns. Significantly higher per cent germination was observed when *J499* was sown in mixed cropping than in monocropping. The contrast was true for *SCS1*, where monocropping gave significantly higher percentage germination than mixed cropping. However all treatments gave a % germination greater than 70 which is the minimum standard required for quality soybean seed (Trivedi and Gunasekaran, 2013).

These finding are consistent with those of Neupane et al. (1997) in a related study, who observed no significant effect on wheat seed germination intercropped with lentils or mustard. However, Deshpande et al. (1992) indicated that the percentage germination of groundnut seeds was higher in pure stands than in 1:1 intercrops. Egbe (2010) observed that seed subjected to high temperatures and high relative humidity in the field declined significantly in germination after reaching physiological maturity. In this study the resulting microclimate under maize canopies was generally characterized by high relative humidity and temperature. This microclimate may have affected the seed quality of the intercropped soybean.

Hill intercropping gave significantly higher % germination than pure stands when sown at the same time as maize. Since the two crops were sown in the hill, the higher percentage would be attributed to the complementarity effect and positive interspecific interactions as explained (Li et al., 2014). When sown two weeks later, soybean pure stands and between the maize rows intercrop gave significantly higher % germination than hill intercrops

probably due to minimal interspecific competition. A delay in sowing in the same apparently intensified the shading effect of maize. The percentage dead seed was higher for *La suprema* sown between the rows at the same time as maize than when the soybean was sown two weeks later as a result of intensive shading by the companion crop.

The larger seed weight of *La suprema* sown two weeks later could be explained by the fact that this variety is late maturing and was probably able to accumulate photosynthates after maize had reached physiological maturity. This is in agreement with observations of Trenbath (1976) that separation of component crops in time may increase the advantages of intercropping by reducing or postponing competition between the component species. On the other hand *La suprema* sown at the same time as maize gave the lowest seed weight due to the fact that the late maturing *La suprema* variety competed for resources at the same time with maize. The competition affected availability of resources grain filling in this variety.

These observations are consistent with those of Yunusa (1989) in maize-soybean mixtures, Narwal and Malik (1982) in sunflower-soybean mixture and Martin and Snaydon (1982) in barley and beans mixtures. Narwal and Malik (1982) for instance observed that sunflower reduced the 100-seed weight of soybean sown in the same hill and between the rows by 12 and 15% respectively. The authors concluded that the reduction in seed weight was due to moisture stress and shading, which reduced availability of photosynthates for grain filling.

The pattern of soybean yields exhibited in this study whereby the pure stand was significantly higher than the intercrops is consistent with observations of; Prasad and Rafey (1996), Edje (1984) and Tetio-Kagho (1988) in maize-soybean intercropping studies. Similar results were reported in related studies: Egbe (2010), Akuda (2001), Olufajo (1995) in sorghum-soybean mixture, and Myaka (1994) in pigeon pea-soybean mixture. Edge for instance suggested that the yield reduction of the intercropped soybean may have been associated with interspecific competition between the intercrop components for growth resources (light, water, nutrients, air, etc.) and the depressive effects of the companion crop. Use of different intercrop patterns led to variation in plant population with soybean sown at near optimum population (pure stand)

yielding the highest. Fast growing and tall crops have an advantage over slower and shorter crops.

Sowing soybean in maize two weeks later led to a significant decrease in the yield of soybean for the three intercropping patterns. Mulatu and Kebede (1993) reported similar results in a related study. They observed a severe reduction in yield of haricot beans under intercropping from delayed sowing. The reduction in the yield of soybean was not significant between the two relative sowing times for the between the rows intercropping pattern indicating possibility of less intercropping competition compared to hill intercropping. As observed by Dahmardeh et al. (2010) and Long Li et al. (2014) in their studies, plant diversity may enhance ecosystem productivity through the ability of some crop species to chemically mobilize otherwise unavailable forms of limiting soil nutrients such as phosphorus and micronutrients such as iron, zinc and manganese. The relative time of sowing a component crop is an important management variable manipulated in cereal-legume intercropping systems.

The LER values of maize-soybean mixtures, based on grain yield, ranged from 0.90 to 1.27 representing an advantage in favour of intercropping. Same hill intercropping sown two weeks later in maize generally gave LER less 1 with very low partial LER contribution by soybean due to severe competition by the companion crop. However, no significant differences were noted in LER values due to treatment effects. Several workers have also obtained LER greater than 1 in maize-soybean intercropping. Dahmardeh et al. (2010) and Muoneke et al. (2007) reported higher production efficiency in various intercropping systems. The higher productivity of the intercrop system compared to the sole crop may have resulted from complementary and efficient use of growth resource by the component crops. Vandermeer (1989) noted that both competition and facilitation take place in many intercropping systems, and that it is possible to obtain the net result of land equivalent ratio (LER) where the complementary facilitation is contributing more to the interaction than the competitive interference. Thus, an LER>1 could result from low interspecific competition or strong facilitation.

The maize component contributed more to the total LERs of the mixture as shown by the partial LER of maize in the results (Table 10). Similar findings have been reported by other researchers. In cereal-legume intercropping, the cereal components usually tend to have greater competitive ability because of their relatively higher growth rate, height advantage, and more excessive root system (Zhang et al., 2007; Ofori and Stern, 1987). In addition, the leaf water potential, stomata conductance, transpiration and photosynthesis have been found to be higher in intercropped maize than the sole crop (Lima, 2000).

Maize alternating with single rows of soybean recorded higher values of LER than maize sown in the same hill with soybean, though this was not statistically significant (Table

10). This may be attributed to the fact that soybean population in between the rows was six times that of the same hill arrangement. This finding is in agreement with those of Chowdhury and Rosario (1993) who observed the highest LER when both intercrop components were at their optimum sole crop populations in maize-mung bean trial.

Results indicated that the yield of maize was not significantly affected by being grown in association with soya bean. This is findings are in close conformity with those of Hayder et al. (2003) who observed a non-significant difference on the yield on intercropped maize.

Conclusions

Sowing maize and a legume (either common beans or soya bean) is the most popular cropping system in the sugar cane growing zone of Western Kenya. The legume may be sown at the same time as maize or later and the legume maybe sown in the same hill with maize or between the rows. The seed harvested from the intercropped legume is saved for use during the next season and/or consumed. From the findings it can therefore concluded that the seed of soybean grown as an intercrop has suitable quality attributes to be used as seed.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Phenological and quantitative plant development changes in soybean cultivars caused by sowing date and their relation to yield

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The study of soybean (*Glycine max*) biometry and phenology can detect characteristics that interfere with yield, and quantitative plant development may vary according to the interaction between the genotype and the environment, and in different phenological development stages of the plant. This study evaluated the quantitative changes and phenological development caused by sowing date and their relation to grain yield of four contrasting soybean cultivars. The experimental design was a split plot with three replications, being the sowing date allocated to plots and the cultivars to subplot. The height of plants, diameter and number of nodes of the main stem, number of the branches, and leaf area index (LAI) were determined at four growth stages: V4, V9, R2 and R5.3, and the number and dry matter (DM) of the nodules at R5.3 growth stage. There was a reduction of growth in all cultivars with delayed sowing dates; however, the cultivar of determined growth habit showed to be less responsive to different sowing dates than the other cultivars. The DM of nodules was higher when plants were sown in October, and the cultivar with larger LAI obtained more DM of nodules than the others cultivars. The definition of the final quantitative characteristic of the plants occurred after the growth stage V9, and the LAI was the character at R5.3 with highest correlation with grain yield.

Key words: Branches, *Glycine max*, leaf area index, nodulation, plants height.

INTRODUCTION

A plant phenological study is a tool that allows us to identify plant quantitative changes at determined growth stages associated with a series of necessities of the plants, which, if attended, will enable normal crop development and consequently high yields (Cruz et al, 2010).

The soybean (*Glycine max*) is a plant that is highly dependent on the interaction between the genotype and the environment; it can change its cycle and its vegetative development depending on this interaction. In the environment, the temperature and the photoperiod are the main factors responsible for the variation of the

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culture development (Sinclair et al., 2005). Thus, the sowing date is probably the most important crop management practice for soybean crop development, because it interferes directly with most of the environmental factors (De Bruin and Pedersen, 2008). Since different soybean genotypes respond differently to environment variation, experiments should be carried out to indicate the best sowing date for each cultivar (Pedersen and Lauer, 2004; Egli and Cornelius, 2009).

In the southern region of Brazil, winter crops like wheat, barley, triticale, and oats are widely cultivated and the soybean crop can be sown late. This late sowing may result in low canopy development and decreasing leaf area index (LAI), translocation of photoassimilates and accumulation of the biomass and consequently low yield (Rao et al., 2002; Setiyono et al., 2008). This was also verified in low south latitude regions (<25°), in research by Calviño et al. (2003) and Sinclair et al. (2005). Unfavorable temperatures out of the range between 24 and 34°C (Egli and Bruening, 2000), low solar radiation (Setiyono et al., 2010) and the short length of the photoperiod, which may affect the flowering (Han et al., 2006), are the abiotic factors commonly found at late sowing dates. These factors may interact with the genotype of the soybean plants, affecting the growth and development of this crop.

Key stages of growth and development of the soybean plants are present both at vegetative and reproductive stages (Setiyono et al., 2008); the duration of the development stages, especially post-flowering, also interfere in the growth and yield of soybean plants (Kantolic et al., 2007).

Therefore, it is important to study the growth of soybean cultivars with contrasting responses to the environment conducted under different environmental conditions. These studies can result in information that leads to an understanding of how the cultivars at different sowing dates result in a lower negative influence of the environment, and how quantitative plant characteristics could interfere with grain yield.

In this context, the present study analyzed the changes caused by different sowing dates on quantitative plant characteristics and phenology during the crop growth, and its relation to final grain yield of four soybean cultivars used in southern Brazil.

MATERIALS AND METHODS

The experiment was carried out in Guarapuava, Parana State, at 25°23'02" S, 51°29'43" W and 1,026 m of altitude. The climate of the region, according to classification of Köppen is temperate of altitude (Cfb) (Kottek et al., 2006).

The soil of the experimental area is classified as Latossolo Bruno (Clayey Oxisol) and the chemical characterization (0 to 20 cm), three months before the first sowing, revealed pH (CaCl₂) of 5.2; 42 g dm⁻³ of organic matter; levels of 3.98 cmol_c dm⁻³ of Ca; 2.04 cmol_c dm⁻³ of Mg; 0.18 cmol_c dm⁻³ of K; 0.0 cmol_c dm⁻³ of Al; and 6 mg dm⁻³ of P, with cation exchange capacity (pH 7.0) of 9.73 cmol_c dm⁻³; and base saturation of 62%. Compound fertilizer 05-25-25 was

given at 310 kg ha⁻¹ prior to sowing totaling 15.5 kg of N, 77.5 kg of P₂O₅ and 77.5 kg of K₂O. The seeds were inoculated with turfs inoculants at recommended dosage. The weeds, pests, and diseases were controlled according to the technical recommendations for growing soybeans under Brazilian conditions when needed.

The experimental design was a split-plot randomized complete block with three replications (that is, blocks). The main plots (environments) were formed by three sowing dates; October 21, November 18, and December 20, 2010. All within agricultural zoning recommendations for soybean crops in the region and commonly utilized by growers. The subplots (genotypes) were formed by four soybean cultivars largely utilized in the region: FPS Urano[®] RR, BMX Apolo[®] RR, BMX Energia[®] RR and BRS 284[®]. The first cultivar has determinate growth habit and the others have indeterminate growth habit; the maturity groups are respectively 6.2, 5.5, 5.0 and 6.5. Each subplot was formed by four rows, spaced by 0.4 m and with 11 m of length totaling 13.2 m².

Twenty seeds for linear meter were sown totaling a population of 500,000 plants ha⁻¹ which at stage VC/V1 according to scale of Fehr and Caviness were thinned to final stand of 260,000 plants ha⁻¹ (10.4 plants m⁻¹) for all cultivars within the recommendation given by the cultivar owners. Plants in 0.4 m of the two central rows of each subplot were sampled at each time totaling on average 8.32 plants per subplot, at four growth stages: V4, V9, R2 and R5.3. For the definition of growth stage, the cultivar FPS Urano was used as reference, that is, when this cultivar reached one of the four growth stages; plants of all four cultivars were sampled on the same day. To determine growth stage, daily observation of seven random plants in each subplot was made, and it was defined when 50% or more (four plants) were in the same stage. Between each sampling, a border was left of 0.4 m.

Plant height (cm), number of nodes (plant⁻¹), main stem diameter (mm), number of branches (plant⁻¹) and LAI was estimated by the software Image-J (Abramoff et al., 2004) through digital photos, and in last sampling, the number (plant⁻¹) and dry matter (DM, g m⁻²) of nodules were determined and recorded at each growth stage. The DM was determined after drying in a forced aeration stove (70°C) until it reached constant mass.

The climatic data were from the meteorological station of Midwest Parana State University (Unicentro) / Agronomic Institute of Parana (Iapar), located approximately 100 m from the experiment. The data of all parameters of the study were submitted to analysis of homogeneity of variance (Box Cox) and if this prerequisite was confirmed, the analysis of variance (F test), was made considering the experimental design of split-plot with the main plots (environments) and the subplots (genotypes). ANOVA was conducted with SAS software. When statistical significance was verified (F ≤ 0.05), the averages of the treatments were submitted to a Tukey test (p ≤ 0.05). The analysis of the Pearson Correlation was performed between the quantitative plant characters and the final grain yield, and the correlation significance was evaluated by Student's t-test.

RESULTS AND DISCUSSION

The number of days in the studied intervals (that is VE-V4, V4-V9, V9-R2 and R2-R5.3) was reduced when plants were sown in December with the exception in the interval V4-V9 (Table 1). This reduction probably occurred because of the shortening of the photoperiod, as the sowing date was delayed, principally from V9 growth stage.

In general, average temperature was more than 2°C lower for plants sown earlier, until V9 growth stage;

Table 1. Climatic data for growth stage intervals and its duration of soybean plants sown on three dates.

Sowing date	Number of days in the interval	Growth stage intervals						
		VE-V4						
		T°Max. ^a	T°Min. ^b	T°Ave. ^c	Ppt. ^d	Rad. ^e	ARad. ^f	Phot. ^g
Oct. 21	30	25.5	13.0	18.5	141	624	20.8	13h19
Nov. 18	25	25.0	16.3	19.8	248	385	15.4	13h38
Dec. 20	24	26.9	17.0	21.0	276	437	18.2	13h37
					V4-V9			
Oct. 21	15	23.9	15.9	19.1	222	201	13.4	13h39
Nov. 18	15	26.7	17.1	21.0	47	270	18.0	13h42
Dec. 20	17	26.8	18.3	21.5	141	260	15.3	13h20
					V9-R2			
Oct. 21	18	26.4	17.0	20.8	52	322	17.9	13h42
Nov. 18	13	26.9	17.3	21.1	231	224	17.2	13h37
Dec. 20	8	24.9	18.2	20.6	73	111	13.9	13h05
					R2-R5.3			
Oct. 21	27	27.3	17.6	21.4	324	464	17.2	13h32
Nov. 18	31	26.5	18.2	21.3	242	471	15.2	13h15
Dec. 20	19	25.0	16.9	20.3	57	295	15.5	12h45

^aMaximum temperature, ^bminimum temperature, ^caverage temperature in °C day⁻¹, ^drainfall in mm interval⁻¹, ^eradiation in MJ m⁻²interval⁻¹, ^faverage radiation in MJ m⁻² day⁻¹ and ^gaverage photoperiod in hours of light day⁻¹, for the respective intervals of soybean growth stages.

however, after R2 growth stage, the average temperature was more than 1°C higher for plants sown in October compared with December. During soybean growth, the precipitation was well distributed with only a reduction in the last growth stage intervals for the plants sown in December however, without occurrence of severe drought. The average radiation was not low during the growth stage intervals with high rainfall evidencing the occurrence of cloudy days without precipitation.

Plant height until V9 growth stage was not an accurate parameter to estimate the final plant height at R5.3 (Table 2) because only after this growth stage, the interference of sowing dates in the final plants height was clear. At R2 growth stage, plants decreased height with a delayed sowing date so when sown in December, the plant height was lower than when sown in October and November for all cultivars.

There was significant interaction in last growth stage (R5.3) when plants from all cultivars sown in December showed a larger reduction in height compared to those in October with the exception of the cultivar Urano, which has a determined growth habit.

Comparing the data of plant height of the cultivars at R5.3 growth stage with the description given by its owners' companies, the cultivars Energia (average of 92 cm) and Urano (average of 73 cm) achieved approximately the height given by their owners when sown in October. For BRS 284 (average of 100 cm) and

Apolo (average of 73 cm) it occurred when sown in November. For cultivars BRS 284 and Apolo in southern Brazil, the early sowing led to taller plants than that reported by the owner companies. This fact is more pronounced for cultivar BRS 284 which among the studied cultivars presented moderate risk to lodging according to the owner company and was the tallest cultivar in the present study. Therefore, for BRS 284 and other tall cultivars with indeterminate growth habit, the early sowing date can be a problem due to lodging.

The cultivar Urano had a reduction of plant height after R2 growth stage; however, the reduction of this cultivar was less than that of others. For Urano, more reduction occurs between V9 to R2 growth stages. This fact occurs due to the reduction in the number of days between V9 and R2 growth stages: 18, 13 and 8 days for sowing in October, November and December respectively. The premature flowering of this cultivar was caused by the sensitivity to a short photoperiod combined with high temperatures with a delaying of the sowing date.

The interaction between treatments in plant height at R5.3 growth stage was probably due to the indeterminate growth habit of cultivar Urano, which at R2 growth stage reached approximately 75% of its final height in all dates of sowing. On the other hand, the others cultivars at R2 achieved 55, 60 and 65% of their final heights when sown in October, November and December, respectively. Bastidas et al. (2008) also verified a reduction in the final

Table 2. Height of plants¹ of four soybean cultivars, at four growth stages sown on three dates².

Cultivar / sowing date	V4				Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	15.8	13.9	14.0	14.6 ^a	30.8	29.7	36.2	32.3 ^b	
Apolo	10.8	8.3	9.8	9.6 ^c	20.2	19.1	26.1	21.8 ^d	
Energia	12.2	9.3	11.0	10.8 ^b	23.2	22.0	29.3	24.9 ^c	
BRS 284	16.0	14.4	14.7	15.0 ^a	33.6	33.5	39.5	35.5 ^a	
Average	13.7 ^A	11.5 ^C	12.4 ^B		27.0 ^B	26.1 ^B	32.8 ^A		

Date / cultivar	R2				Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	52.1	52.0	43.6	49.2 ^b	73.4 ^{Ac}	66.5 ^{Bc}	60.3 ^{Cb}	66.7	
Apolo	46.1	44.8	35.2	42.0 ^c	92.2 ^{Ab}	73.3 ^{Bb}	56.1 ^{Cb}	73.9	
Energia	52.5	46.0	37.6	45.4 ^{bc}	91.7 ^{Ab}	73.1 ^{Bb}	54.9 ^{Cb}	73.2	
BRS 284	65.1	60.8	52.9	59.6 ^a	112.6 ^{Aa}	99.8 ^{Ba}	81.1 ^{Ca}	97.8	
Average	53.9 ^A	50.9 ^B	42.3 ^C		92.5	78.2	63.1		

¹In cm; ²upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

Table 1. Diameter of main stem¹ of four soybean cultivars, at four growth stages sown on three dates².

Date / cultivar	V4				Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	5.5	5.0	5.5	5.3	7.4	7.7	8.2	7.8 ^{ab}	
Apolo	5.4	4.6	5.5	5.2	7.8	7.9	8.0	7.9 ^a	
Energia	5.4	5.1	5.6	5.4	7.9	7.9	8.3	8.0 ^a	
BRS 284	4.8	4.7	5.4	5.0	6.8	7.2	8.1	7.4 ^b	
Average	5.3	4.9	5.5		7.5 ^B	7.7 ^B	8.1 ^A		

Date / cultivar	R2				Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	9.4	9.8	8.9	9.4	11.3	9.8	8.6	9.9 ^a	
Apolo	9.6	10.0	9.3	9.6	10.9	9.9	8.5	9.8 ^{ab}	
Energia	9.8	10.1	8.7	9.5	10.3	9.3	8.4	9.3 ^{bc}	
BRS 284	8.5	9.3	8.9	8.9	10.2	8.9	8.1	9.1 ^c	
Average	9.3 ^{AB}	9.8 ^A	9.0 ^B		10.7 ^A	9.5 ^B	8.4 ^C		

¹In mm; ²upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

growth percentage achieved by soybean plants at a reproductive period when sown late. However, the authors did not observe interaction among the cultivars and the time of sowing.

In this paper, the authors utilized indeterminate growth habit cultivars and one semideterminate. Because of this, the results were probably different from the present study wherein the contrast among cultivars was higher.

There was no statistical difference in the diameter of the main stem caused by the date of sowing for cultivars at V4 growth stage (Table 3). Similar to plant height, the diameter of the plant main stem was greater in plants sown in December than those sown in October and

November at V9 growth stage. However this changed at R2 and R5.3 growth stages, when the delay in sowing resulted in a smaller stem diameter, agreeing with the results of Marchiori et al. (1999).

There was an increase of 0.12, 0.12 and 0.13 mm of the main stem diameter for each 1 cm increase in plant height in the sowing of October, November, and December, respectively, at R5.3 growth stage, showing slight increments of stem diameter in relation to plant height with sowing delay. For cultivars, the increases of stem diameter with increases of plant height were: 0.15, 0.13, 0.13 and 0.09 for Urano, Apolo, Energia and BRS 284, respectively, showing a lower increase of stem

Table 4. Number of branches¹ of four soybean cultivars, at four growth stages sown on three dates².

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	1.3	1.0	1.1	1.1 ^a	2.1 ^{C^a}	2.3 ^{B^{ab}}	4.8 ^{A^a}	3.0
Apolo	1.0	1.0	1.0	1.0 ^b	1.7 ^{B^a}	3.1 ^{A^{ab}}	3.5 ^{A^b}	2.8
Energia	1.0	1.0	1.0	1.0 ^b	2.4 ^{B^a}	3.8 ^{A^a}	3.5 ^{A^b}	3.2
BRS 284	1.0	1.0	1.0	1.0 ^b	1.3 ^{B^a}	2.7 ^{A^b}	2.5 ^{A^b}	2.2
Average	1.1 ^A	1.0 ^B	1.0 ^B		1.9	3.0	3.6	

Date / cultivar	R2			Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	5.8	6.6	5.2	5.9 ^a	6.0	4.8	5.2	5.3 ^a
Apolo	6.1	6.5	6.0	6.2 ^a	6.2	4.6	5.6	5.4 ^a
Energia	6.6	6.7	5.5	6.3 ^a	6.0	4.7	5.7	5.5 ^a
BRS 284	3.5	3.5	3.6	4.5 ^b	3.8	2.0	3.0	3.0 ^b
Average	5.5	5.8	5.1		5.5 ^A	4.0 ^C	4.9 ^B	

¹Plant⁻¹; ²upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

diameter with increase of plant height for BRS 284, making this cultivar more susceptible to plant lodging. It was also noted that the change of sowing date did not result in an increase of stem diameter in relation to an increase of height in the cultivars Urano and BRS 284. However, the cultivars Apolo and Energia presented values of 0.11, 0.13 and 0.15 mm for sowing in October, November, and December, respectively.

In soybean crops the competition among plants decreases the stem diameter (Seiter and Altemose, 2004). These authors observed that at R5.5 growth stage, soybean plants showed the largest stem diameters at lower planting densities resulting in lower competition among plants. Therefore, smaller values on early sowing may be caused by higher competition between plants sown earlier when the plant height and the LAI were larger. Cultivars Urano and BRS 284 maintained the increase of stem diameter in relation to the increase of height for all sowing dates. Thus, we can suppose that for these cultivars, the competition among plants is similar independently of the sowing date. On the other hand, for cultivars Apolo and Energia, the competition among plants decreased in late sowing; it does not mean that late sowing dates are best for these cultivars, since if the competition is too small, the cultivation area can be underexploited and the yield reduced.

The number of branches at V4 growth stage was higher in plants sown in October than in November and December (Table 4). At R2 growth stage, the sowing date did not affect the number of branches; and at R5.3 growth stage, the number of branches was highest in plants sown in October, lowest on November, and intermediary in December. In addition, plants sown in November had a reduction in the number of branches at R5.3 relative to R2 growth stage. Among cultivars, BRS

284 showed a lower number of branches than the others cultivars. At V9 growth stage, there was significant interaction between sowing dates and cultivars. Cultivars increased the number of branches formed as the sowing dates were delayed, and in the cultivar Urano, this increase was higher than in the other cultivars.

The reduction in the number of branches in plants sown in November at R5.3 growth stage compared to at R2 growth stage (Table 4), was probably due to self-shading at the lower third of plant canopy. For the sowing of October, although plants lost formed branches, the largest photoperiod may have induced the new branch formation at the middle third of the plant canopy as cited by Jiang et al. (2011) and Han et al. (2006). This formation of new branches did not occur in plants sown in November due to a short photoperiod. Kantolic and Slafer (2001) explain that there is no difference in the number of branches in plants submitted to an extended two hours of artificial photoperiod. In December, because the self-shading was very low, there was probably less fall of branches at the lower third of the plant canopy.

Kantolic and Slafer (2001) verified differences among cultivars and sowing dates, where late maturing cultivars produced more branches, and the delay of sowing date reduced the number of branches of plants. In this study, cultivars of early and very early maturation group were studied and all had a reduction in the number of branches at R6 with the delay of sowing date.

In our study, the number of branches was also reduced with sowing delay. However, we realized that the late maturing cultivars (BRS 284) had the lowest number of branches. Indeed the number of branches is a characteristic of the cultivar and is not related to the maturity group. The cultivar BRS 284 showed the lowest number of branches, and it was inversely proportional to

Table 5. Number of nodes of the main stem¹ of four soybean cultivars, at four growth stages sown on three dates².

Date / cultivar	V4				Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	6.7 ^{Aa}	6.2 ^{Ba}	6.5 ^{ABa}	6.5	10.2	10.7	11.2	10.7 ^a	
Apolo	6.1 ^{Ab}	5.6 ^{Bb}	6.0 ^{ABb}	5.9	9.4	9.8	10.5	9.9 ^b	
Energia	6.0 ^{Ab}	5.9 ^{Aab}	6.0 ^{Ab}	6.0	9.3	9.6	10.6	9.9 ^b	
BRS 284	6.0 ^{Ab}	6.2 ^{Aa}	6.2 ^{Aab}	6.2	10.2	10.6	11.5	10.8 ^a	
Average	6.2	6.0	6.2		9.8 ^B	10.2 ^B	11.0 ^A		

Date / cultivar	R2				Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20	Oct. 21		Nov. 18	Dec. 20		
Urano	15.1	15.0	14.0	14.7 ^a	17.8 ^{Ac}	17.4 ^{Acb}	16.0 ^{Ba}	17.1	
Apolo	13.9	13.9	12.6	13.4 ^b	20.1 ^{Ab}	18.1 ^{Bb}	14.9 ^{Cb}	17.7	
Energia	14.3	13.4	12.4	13.4 ^b	19.2 ^{Ab}	17.1 ^{Bc}	14.6 ^{Cb}	16.9	
BRS 284	15.1	15.0	13.7	14.6 ^a	21.1 ^{Aa}	19.8 ^{Ba}	16.5 ^{Ca}	19.1	
Average	14.6 ^A	14.3 ^A	13.2 ^B		19.5	18.1	15.5		

¹Plant¹, ²upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

plant height. Heiffig et al. (2005) stated that the intra-specific competition in soybean plants may determine the plant size and its number of branches, these two characteristics being inversely proportional. The number of nodes (Table 5) of the main stem in general showed a behavior similar to the plant height (Table 2). At V9 growth stage, plants formed a higher number of nodes when sown in December, and at R2 growth stage the number of nodes decreased gradually with a delay in the date of sowing.

In the comparison among cultivars, Urano and BRS 284 had a higher number of nodes than cultivars Apolo and Energia except at R5.3 growth stage when the cultivar Urano showed a fewer number of nodes when sown in October compared to the other cultivars. Thus, the cultivar Urano was less affected by a delayed sowing date with a lower reduction in the number of nodes than other cultivars as the sowing date was delayed. This result corroborates with the findings of Setiyono et al. (2007), who verified an increase in the number of nodes with an increase in the interval in days from R1 until R5 growth stages for the indeterminate growth habit cultivars; this explains the larger number of nodes of the other cultivars in relation to the cultivar Urano, at the sowing in October at R5.3.

There is a positive correlation between the number of nodes and the temperature (Sinclair et al., 2005); thus, the higher number of nodes observed until V9 growth stage in plants sown in December may be explained by the increase of temperature in this interval (Table 1). From V9 growth stage, the temperature was lower at sowing in December and the number of days among stages decreased sharply with the delay of sowing partially explaining the higher number of nodes observed in plants on the two first sowing dates in relation to the

last.

In the same way, Bastidas et al. (2008) verified significant interaction between sowing date and cultivar in the number of nodes. The authors explain this result affirming that cultivars with a predisposition to have a lower number of nodes tend to lose fewer nodes than cultivars with a predisposition to have a high number of nodes. Therefore, the present study corroborates these findings and states that the cultivar Urano with determinate growth habit, similar to the cultivar with lower loss in number of nodes in the study of the Bastidas et al. (2008), already achieved most of its growth development until R2 suffering less from the unfavorable environment that occurred after R2 growth stage. The authors also verified that the plants after V9 growth stage, presented a lower increase in the number of nodes with the sowing delay, in agreement with the results of our study.

The sowing date did not influence LAI of soybean plants at V9 growth stage, but at R2 growth stage, the lower LAI was found in the sowing of December (Table 6). At R5.3 growth stage, the highest LAI is clearly noted for plants sown in October middle values in November and the lowest in December. Among cultivars, in the same growth stage, the highest final LAI was for cultivar BRS 284. There was significant interaction between cultivars and sowing date in LAI at V4 growth stage where the cultivar BRS 284 showed slow initial development, but more constant than others cultivars.

The decrease of LAI in plants at R5.3 growth stage with delaying the sowing date may be partially explained by the fewer number of days to plant development of 88, 83 and 67 days after emergence, for sowing in October, November, and December respectively. However, the major influence on this aspect was the photoperiod post-flowering that decreased with delaying the sowing date

Table 6. Leaf area index of four soybean cultivars, at four growth stages sown at three dates¹.

Date / cultivar	V4			Average	V9			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	1.06 ^{Aa}	0.72 ^{Ca}	0.82 ^{Ba}	0.87	3.00	2.66	3.04	2.85 ^a
Apolo	0.80 ^{Abc}	0.49 ^{Cc}	0.63 ^{Bb}	0.64	2.17	2.35	2.13	2.22 ^b
Energia	0.88 ^{Ab}	0.53 ^{Cbc}	0.66 ^{Bb}	0.69	2.81	2.23	2.47	2.50 ^{ab}
BRS 284	0.73 ^{Ac}	0.62 ^{Cb}	0.68 ^{ABb}	0.68	2.47	2.46	2.86	2.60 ^{ab}
Average	0.87	0.59	0.70		2.57	2.42	2.63	

Date / cultivar	R2			Average	R5.3			Average
	Oct. 21	Nov. 18	Dec. 20		Oct. 21	Nov. 18	Dec. 20	
Urano	5.17	5.26	3.45	4.63	6.87	4.70	3.72	5.10 ^{ab}
Apolo	4.41	4.61	3.08	4.03	5.75	3.47	3.25	4.16 ^c
Energia	4.87	4.65	2.94	4.15	5.87	3.50	3.67	4.35 ^{bc}
BRS 284	4.44	5.04	3.65	4.38	7.56	4.88	3.84	5.43 ^a
Average	4.72 ^A	4.89 ^A	3.28 ^B		6.51 ^A	4.14 ^B	3.62 ^C	

¹ Upper case letters compare averages in rows, and lower case in columns. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

according to Han et al. (2006). The plants sown in October were at R2 growth stage in late December to early January when the days in these months have the largest photoperiod of the year.

The cultivar BRS 284 has high leaf production that assures high values of LAI even under unfavorable conditions. This characteristic is favorable because LAI around 5.5 in soybean plants ensures large light interception by the plant canopy both of short and long waves and with this LAI, the light amount incoming to the soil is very low, and thus, the soil water evaporation is also small (Sauer et al., 2007).

The interaction between treatments occurred at V4 growth stage for LAI; the high LAI of plants sown in October was probably due to the large number of days of plant development (30 DAE) and the highest average daily radiation among the sowing dates (Table 1). At this growth stage, plants from cultivars Apolo and Energia sown in November showed the lower LAI, probably caused by low average solar radiation (Table 1) as also explained by Calviño et al. (2003). It may be concluded that for these cultivars, high solar radiation is needed for leaf production.

Models for estimation of soybean plant growth, like that of Setiyono et al. (2010), utilized solar radiation as a parameter that drives vegetative growth. Models suggest a linear increase in biomass and LAI with an increase of radiation. At V4 growth stage, plants from cultivar Urano had the largest LAI in all sowing dates, showing higher early leaf development efficiency than other cultivars. The number of nodules (Figure 1a) was higher in plants sown in October and November, but the DM of nodules (Figure 1b) was higher in plants sown in October, demonstrating that plants sown in October formed larger nodules. Cultivar BRS 284 formed a higher number and DW of

nodules than the other cultivars. The higher number of nodules of cultivar BRS 284 probably occurred because it is more able to nodulate and not because it is the cultivar with more vegetative development with a high amount of nodules to supply nitrogen.

According to Francisco Junior and Harper (1995), in a study with graft and rooting of the leaves and shoot of soybean with normal and mutant cultivars capable of hyper-nodulation, the leaves, in comparison with others organs of the soybean plants, have a greater influence on nodulation by producing a high portion of the physiological signs responsible for infection by *Rhizobium*. However, it was not the amount of leaves that is the most responsible for the control of nodulation but the genotype of the plant.

Environmental conditions, i.e. soil moisture (King and Purcell, 2005) and soil temperatures below 25°C, limited soybean nodulation (Miransari and Smith, 2008); therefore there is risk of reducing soybean nodulation due to lower temperatures in southern Brazil in sowing before October. Zhang et al. (2003), studying yield of different cultivars and strains in Canadian soil, found nodules limitation due to low soil temperatures. The authors verified differences of mass and number of nodules among cultivars, and the greatest differences in the amounts of fixed nitrogen changed according to the strains utilized. Moreover, the authors verified that the bigger the amount of nodules (both number and mass), the higher the amount of nitrogen (kg ha⁻¹) produced by the shoots of the plants.

According to Salvagiotti et al. (2008), there is a positive linear correlation between nitrogen uptake in aboveground and soybean yield. Vollmann et al. (2011) emphasized the importance of nodulation in soybean plants, relating it with chlorophyll content of leaves, yield

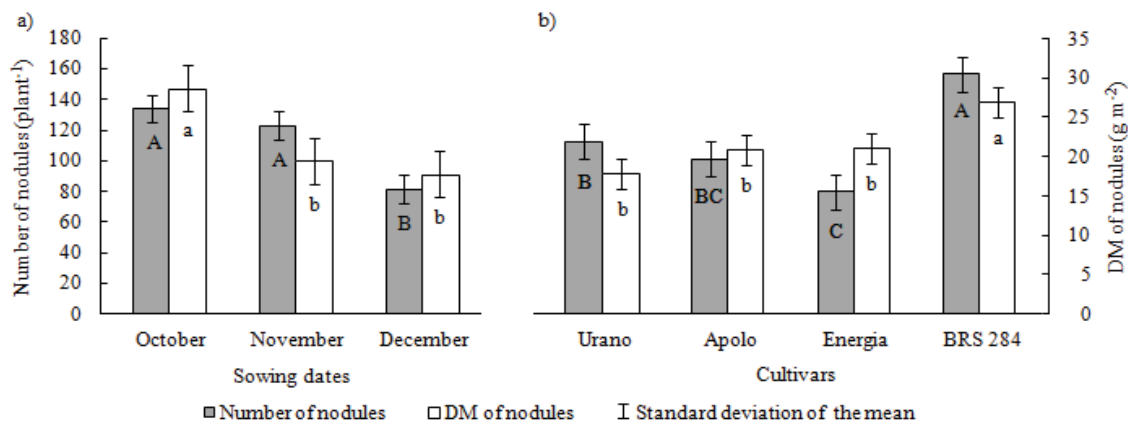


Figure 1. Number of nodules¹ and dry matter of nodules² at a) three sowing dates and b) four cultivars of soybean, at plant growth stage R5.3³.¹Plant⁻¹; ²g m⁻²; ³upper case letters compare averages of number of nodules, and lower case of dry matter (DM) of nodules. Averages followed by same letter do not differ statistically by a Tukey test with 5% of probability.

Table 7. Correlation coefficients between soybean quantitative plant characters analyzed and final grain yield of four cultivars sown on three dates.

Variable	Yield	Plant height	Nº of nodes	Nº of branches	Main stem diameter	Leaf area index	Nº of nodules
Plant height	0.469**						
Nº of nodes	0.486**	0.888**					
Nº of branches	0.242 ^{ns}	-0.427**	-0.203ns				
Main stem diameter	0.489**	0.394*	0.675**	0.428**			
Leaf area index	0.571**	0.680**	0.775**	0.090ns	0.713**		
Nº of nodules	0.072 ^{ns}	0.736**	0.726**	-0.474**	0.367*	0.640**	
DM of nodules	0.483**	0.807**	0.745**	0.067ns	0.472**	0.776**	0.705**

** : Significant at 1%, * : significant at 5%, and ^{ns} : not significant according to t-test.

and oil and protein content of seeds. Thus, it can be concluded that there are genotypes more able to nodulation, and that larger leaves are not responsible for higher nodulation, but the limitation of nodules may reduce the amount of nitrogen produced by the plants. Table 7 shows the correlation coefficients between studied quantitative plant characters and the final grain yield. Leaf area index was the plant character with the greatest influence on final grain yield. Other characters such as plant height and number of nodes, diameter of branches and DM of nodules also had an influence on yield, however lower than LAI. Sauer et al. (2007) verified a high correlation between LAI and plant yield.

In this study, we observed a reduction in LAI of 0.082 per day of delay in the sowing date, from October 21 to November 18. The grain yield in the same period decreased 24 kg ha⁻¹ per day with a delay in the sowing date. Considering the period between November and December, the decrease in LAI was 0.016 day⁻¹, but we did not observe a reduction of grain yield. Therefore, the

time of soybean sowing is essential to achieve elevated values of LAI and obtain high yields. The DM of nodules showed a higher influence on final grain yield than its number. The number of nodules and DM of nodules may change independently, as shown in Figure 1, so it is recommended that it's DM rather than it's number be observed more attentively if the nodulation is a parameter utilized in selection of soybean cultivars with high yield potential in breeding programs.

Conclusion

In southern Brazil, late sowing reduces the quantitative plant characteristics and phenological development of all soybean cultivars studied, independently of growth habit, but the cultivar with determined growth habit had the lowest reductions and is a good option for late sowing. Until the end of the vegetative period, cultivars did not show its final plant characteristics. Dry matter of nodules

has more importance for cultivar selection than the number of nodules. Early sowing of soybean increases the LAI and can increase grain yield. Leaf area index has a high correlation with final grain yield.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Experimental analysis of air flow patterns in performance of flat plate solar collectors

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Solar drying is one of the promising methods of reducing post-harvest losses in rural areas. Studies have shown that, heat transfer mechanisms in a solar collector influences the performance of solar dryers. This study aims at improving heat transfer in flat plate solar collectors by designing different air flow patterns inside the collector. Three flat plate solar collectors were constructed by using *Pterocarpus* timber (Mninga) and tested for their effect in heat transfer at various flow patterns. Three different flow patterns namely: single duct front pass, double duct parallel flow and double duct counter flow were designed and tested. Experimental results show that collector efficiency of single duct front pass, double duct front pass and double duct counter flow were 30.6, 36.1 and 38.2% respectively. It was found that, double duct flow gives improved performance compared to single duct flow due to the increased heat transfer area. In additional, double duct counter flow showed superior performance compared to double duct parallel flow due to extended heat transfer area and the advantage of air preheating at the inlet which reduces heat losses through glazing. Through this study, it was concluded that, solar collector designs with double duct counter flow can improve collector performance for up to 8.3% compared to single duct front pass.

Key words: Single duct front pass, double duct parallel flow, double duct counter flow, energy, solar intensity.

INTRODUCTION

Solar dryer is an enclosed unit that use solar energy to preserve food by removing water from food materials to the level at which microbial spoilage and deterioration reactions are greatly minimized. Main advantages of solar dryer over sun drying are its ability to keep food safe from birds, insects and unexpected rainfall during drying. In addition, solar dryers reduce drying time and maintains the quality of the products in terms of colour,

taste and texture (Tripathy and Kumar, 2008). There are two modes of dryer operation; passive mode and active mode. In passive mode, air is heated and circulated naturally by buoyancy force or as a result of wind pressure or in combination of both. This mode of dryers operation has system efficiency ranging from 10 to 15%. In active mode (forced convection), air is circulated in the system with the help of fans or air blower. The active

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mode improves the efficiency to a range of 20 to 30% which is about twice of that achieved by the passive mode (Fuller et al., 2005). Generally, there are three types of solar dryers namely; direct solar dryer where materials to be dried are placed in transparent enclosure of plastic or glass and receive direct heat from the sun; indirect solar dryer where drying air is heated by the collector and ducted to the drying chamber which is isolated from the direct sun; and mixed mode dryer where material to be dried are heated by direct from solar radiation and by the preheated air coming from the solar collector.

Indirect solar dryers with forced convection flow are one of the best drying technology which can produce high-quality products and eliminate the risk of spoilage during drying (Bennamoun and Belhamri, 2003; Mulokozi et al., 2000). The use of these dryer can reduce drying time by three times compared to other type of dryers and reduce the required collector area by 50. However, several indirect solar dryers have been developed, their applications are still limited due to their unreliable performance and high investment cost relative to production capacity (Janjai et al., 2008). Important criteria dictating the adoption of the indirect solar dryers includes improved quality of products, short drying time and low investment costs. Currently, several indirect solar dryers have been constructed in Tanzania but still they do not meet these adaptation-criteria (Vriens and Van Diesen, 2007). Solar air collectors, although a very important component in the solar drying system, have not received much attention during dryer design (Karim, 2004). In theory, the performance of solar collector depends on several climatic and operating conditions such as collector orientation, thickness of cover materials, wind speed, collector length, collector depth, type of absorber materials and heat transfer mechanisms (Akpınar and Koçyigit, 2010; Benamara et al., 2005; El-khawajah et al., 2011; Kabeel and El-Agouz, 2011; Kundu, 2010). Irrespective of many researches and constructed solar collectors, materials for constructions such as glass size type absorbing materials and heat transfer mechanisms have not been properly considered during design (Mbise, 2008). Therefore, there is a need to study different mechanism of improving heat transfer between air and absorber plate in order to increase collector performance. In this study an attempt to improve heat transfer in the collector by designing different air flow patterns are reported

LITERATURE REVIEW

Flat plate solar collector

Solar collector is a special kind of heat exchangers that transfer heat energy from incident solar radiation to working fluid (Brenndorfer et al., 1985; Ekechukwu and

Norton, 1999; Alta et al., 2010). A solar collector performs three functions which are to absorb solar radiation, converting it to heat energy and transfers the energy to a working fluid passing through it (Kalogirou, 2004). Flat plate collectors have been used to deliver heated air for space heating, crop drying and in similar applications that require hot air (Aldabbagh et al., 2010; Gordon, 2001). Flat plate collectors can heat the working fluid to a temperature range of 10 to 50°C above ambient depending on the construction materials and requirements (Struckmann, 2008). The most important advantage of these type of collectors includes low construction costs and slight effect of pressure drops. However, their main drawback is the low heat transfer coefficient between the absorber plate and air stream due to poor thermal conductivity and low heat capacity of air (El-Sebaili et al., 2011a). The principle parts of flat plate solar collector are cover sheet, absorber plate and insulation.

Cover sheets (Glazing material)

Glazing is a top cover of a solar collector which performs three major functions: to minimize convective and radiant heat loss from absorber, to transmit the incident solar radiation to the absorber plate with minimum loss and to protect the absorber plate from outside environment (Society, 2009).

Thermal insulation

To reduce heat losses to the environment by thermal conduction, the back and edges of the collector must be heat insulated. As the temperature difference between the absorber and the outside air increases the heat losses increases and hence reduces efficiency. It is therefore important to ensure adequate insulation of the thermal solar system (Deutsches, 2005). Thermal insulation must be weather resistant, fireproof, durable and dimensionally stable (Alghoul et al., 2005). Koyuncu (2006) suggest the use of hardwood as collector frame construction which is also used as insulator. Forson et al. (2003) suggest the use of plywood board which serve as absorber materials and insulation.

Absorber materials

Is the main element of a flat-plate collector which covers the full aperture area of the collector and performs three functions: absorb maximum amount of solar irradiance, conduct this heat into the working fluid at a minimum temperature difference, and lose a minimum amount of heat back to the surroundings. Best materials which are suitable for collector absorber plates include copper,

aluminum, steels and various thermoplastics (Alami, 2010; Garg and Prakash, 2006).

Heat transfer from absorber plate to air

To increase collector outlet temperature, heat has to be transferred efficiently from the absorber plate to the flowing air (Ion, 2006). Heat transfer coefficient inside the solar collector is one of the important parameter that affects the efficiency of the collector (Aldabbagh et al., 2010). Researchers have attempted various modifications to enhance heat transfer rate in solar collector by incorporating different modifications between absorber plate and glass plate. Most of these research were focused on; increasing the absorber plate characteristics; optimizing design parameters (Martín et al., 2011); reducing the heat loss from the collector by: insulating the collector box, applying special coats on the absorber plate, inserting trans-parent insulation material between the absorber plate and the glaze and applying antireflective coats on the glaze (Hobbi and Siddiqui, 2009). Also other techniques like using absorber with attached fins, corrugated absorber, absorber with packed bed and baffles have been reported in literature (Akpınar and Koçyiğit, 2010). In this study heat transfer in the collector were enhanced by varying the flow patterns of air.

Influence of air flow patterns

Thermal performance of single and double air passes in the collectors have been investigated experimentally and reported in this study. Suggestions for this settings includes: air passing over absorber plate (single duct front pass-SDFP), air enter over absorber plate and exit below it (double duct counter flow-DDCF) and air passing in both sides of absorber plate (double duct parallel flow). Yeh et al. (2002) reported that a considerable improvement in collector efficiency is obtained by employing a double-flow device instead of using a single-flow. The study of Omojaro and Aldabbagh (2010) suggests passing the air from above and below the absorber plate at the same time in a double-flow solar air heater. According to Yeh et al. (2002), the best thermal efficiency can be achieved in a double-flow solar air heater when the cross-section area of upper and lower channels are constructed equally and at the same fixed mass flow rates. However, the thermal efficiency was found to decrease by increasing the height of the first pass for the double pass solar air collector. The experiment by Omojaro and Aldabbagh (2010) showed that, there was an increase of collector efficiency by 4.7% when reducing the height of first pass from 7 to 3 cm. Ramadan et al. (2007) suggested using double pass solar air collector with air passing above the absorber plate before turning to pass below it. Ramani et al. (2010)

conducted experimental study involving double and single pass air collector and concluded that, the double-pass design had a thermal efficiency of about 10% higher compared to single-pass design. Likewise, Yousef and Adam (2008) conducted similar experiment and found the performance increase of 10 to 12%. Omojaro and Abdabbagh (2010) reports the performance of double pass solar collector as 7 to 19% higher compared to single pass solar collector. According to El-sebaai et al. (2011b), double duct solar collector gave 7 to 9% performance increase when compared to single pass. Moreover, Chamoli et al. (2012) outlines the performance of double pass solar collector as 10 to 15% more compared to single pass. The use of a double-pass resulted in increased pressure drop across the collector. However, the rise in the operating cost due to the increased pressure drop in the collector was reported to be small.

Mathematical energy balance for single and double duct air flow

The basic physical equations used to describe the heat transfer characteristics in single and double duct air flows are developed from the conservation equations of energy.

Energy balance in single duct front air pass

Energy transferred to the working fluid in single duct front pass depends on the temperature difference between glass and absorber plate (galvanized steel plate) to that of exit air.

$$\dot{m} \cdot C_p \cdot (T_o - T_i) = h_{fg}(T_g - T_o) + h_{fp}(T_p - T_o) \quad [1]$$

Where \dot{m} is the mass flow rate of fluid (air), C_p is the specific heat of air, h_{fg} is the heat transfer coefficient between the glass cover and air, and h_{fp} is the heat transfer coefficient between the absorber plate and working fluid.

Energy balance in double duct air pass

It can be noted that for double duct parallel flow, mass flow in each duct is half of the total flow while in double duct counter flow, whole mass flow is passed in each duct. Energy balance in first pass air stream in double duct flow depends on the temperature differences between glass and plate to that of fluid.

$$\dot{m} \cdot C_p \cdot (dT_{f1}) = h_{f1g}(T_g - T_{f1}) + h_{f1p}(T_p - T_{f1}) \quad [2]$$

Energy balance in absorber plate

$$I \cdot \alpha_p \cdot \tau_g = h_{f1p}(T_p - T_{f1}) + h_{f2p}(T_p - T_{f2}) + h_{r,gp}(T_p - T_g) + h_{r,pb}(T_p - T_b) \quad [3]$$

Energy balance in second air flow pass

$$\sqrt{\dot{m} \cdot C_p \cdot (dT_{f2})} = h_{f2p}(T_p - T_{f2}) + h_{f2b}(T_b - T_{f2}) \quad [4]$$

Energy balance in bottom (base) plate

$$h_{f2b}(T_b - T_{f2}) + h_{r,pb}(T_b - T_p) + U_a(T_b - T_a) = 0 \quad [5]$$

General efficiency of flat plate solar collectors

The thermal efficiency of a collector is the ratio of the useful thermal energy to the total incident solar radiation averaged over the same time interval. Mathematically, the efficiency (η) of a collector is expressed by Equation 6 (Struckmann, 2008; Luna et al., 2010).

$$\eta = \frac{\text{useful energy}}{\text{solar energy available}} \quad [6]$$

Useful energy for a solar thermal collector is the rate of thermal energy leaving the collector, usually described in terms of the rate of energy being added to a heat transfer fluid passing through the receiver or absorber (Ekechukwu and Norton, 1999; Farahat et al., 2009).

$$Q_u = \dot{m} \cdot C_p \cdot (T_o - T_i) \quad [7]$$

The area of the collector on which the solar irradiance falls is called the aperture area of the collector. Therefore, total energy received by collector (optical energy captured) can be described by Equation 8.

$$Q_{in} = I \cdot A \quad [8]$$

Accordingly, absorptance and transmittance are multiple effects of optical energy capture and therefore, these factors indicate the percentage of the solar rays penetrating the transparent cover of the collector and the percentage being absorbed (Farahat et al., 2009).

$$Q_{in} = \alpha \cdot \tau \cdot I \cdot A \quad [9]$$

The rate of useful energy of the collector can be expressed by using overall heat loss coefficient and the collector temperature as Equation 10 (Yogi and Jan, 2000).

$$\dot{Q}_{\text{useful}} = \dot{Q}_{in} - \dot{Q}_{\text{loss}} = \alpha \cdot \tau \cdot I \cdot A - U_L \cdot A_C \cdot (T_c - T_a) \quad [10]$$

Since it is difficult to define the collector average

temperature in Equation 5, it is convenient to define a quantity that relates the actual useful energy gain of a collector to the useful gain if the whole collector surface were at the fluid inlet temperature (Struckmann, 2008). This quantity is known as “the collector heat removal factor (F_R)” and is expressed by Equation 11.

$$F_R = \frac{\dot{m} \cdot C_p \cdot (T_o - T_i)}{A \cdot [\alpha \cdot \tau \cdot I - U_L \cdot (T_i - T_a)]} \quad [11]$$

Finally, equation for efficiency of flat plate solar collector can be given by “Hottel- Whillier-Bliss equation” (Karatasou et al., 2006)

$$\eta = F_R \cdot \alpha \cdot \tau - F_R \cdot U_L \cdot \left(\frac{T_i - T_a}{I} \right) \quad [12]$$

If it is assumed that τ and α are constants for a given collector and flow rate, then the collector efficiency is a linear function of the three parameters defining the operating condition: Solar irradiance (I), Fluid inlet temperature (T_i) and collector outlet temperature (T_o). Thus, the performance of a flat-plate collector can be approximated by experimentally measuring these three parameters, and the efficiency can be calculated by using summarized Equation 13 (Ekechukwu and Norton, 1999).

$$\eta = \frac{\dot{m} \cdot C_p}{A} * \left[\frac{(T_o - T_i)}{I} \right] \quad [13]$$

MATERIALS AND METHODS

Three flat plate solar collector models were constructed by using *pterocarpus* timber (hardwood) of thickness 2 inches with black painted marine plywood as bottom heat absorbing plate. Other materials used were galvanized steel plate of 2 mm. The specifications of collectors were: collector length to width ratio 2 (length 1.2 m and width 0.6 m) and depth 15 cm. All collectors were oriented to north-south direction and tilted to an angle of 10° with the ground toward north direction. This experiment was conducted by designing single and double air passes in the collector namely: single duct front pass (SDFP), double duct parallel flow (DDPF) and double duct counter flow (DDCF). With single duct front pass air passes over the surface of the plate as shown in Figure 1, while in double duct parallel flow, air pass from both side of the absorber plate simultaneously (Figure 2a). Similarly, in double duct counter flow, air enters the collector at the top duct and exit at the bottom duct as shown in Figure 2b. In double ducts settings, top to bottom depth ratio were kept at 1:1. Outlet temperatures from collectors were measured by using PT940 thermocouple temperature sensors which were connected to XR5-SE multi-channel data logger. Ambient temperatures were measured by using digital CEM DT-172 temperature and humidity data logger.

On the other hand, solar intensity and air flow rate were respectively measured using PCE-SPM solar radiation meter and Testo 425 Hot Wire Thermal Anemometer. Air flows in each collectors were 1.27 m³/min. Air flow rates were aided by extract fans which were controlled by variable voltage control switch.

Efficiencies of the collector models were established by testing each collector with 5 mm glass thickness which is the common used glass thickness. The aim of testing collector with same glass

Table 1. ANOVA for collector with similar glass thickness.

Collector	Sum of squares	df	Mean square	F	Significance
Between Groups	1.427	3	0.476		
Within Groups	4.428	12	0.369	1.289	0.323
Total	5.854	15			

thickness was to ensure that variation of the efficiencies during other experiments were not due to design variations. The duration for each experiment was 5 days for collector with similar glass thickness and for different flow patterns. Time of experiment was from 7:30 am to 6:00 pm with a 10 min interval for data sampling. The experiments were conducted at the University of Dar es Salaam in College of Engineering and Technology. All collector models were placed on top of block-Q building situated at the Department of Chemical and Mineral Processing.

RESULTS AND DISCUSSION

Collectors performance with similar glass thicknesses

The main objective of this experiment was to find out if there was significant performances difference between designed collector models with similar characteristics. Each collector model was tested for its performance by using 5 mm glass thickness.

Temperature and energy profile of collectors with same glass thickness

Figure 3 show the variation of ambient and outlet temperature of four collector models while Figure 4 shows the rate of outlet flow of energy from collector recorded from 7:30 am to 06:00 pm on 12/09/2011.

From Figure 3 it can be seen that there is no variation in temperature between collectors however, temperature varies according to the fluctuation of solar intensity. Fluctuations of temperature during the morning are high when compared to afternoon due to high clouds coverage which results to low solar intensity reaching the earth. Similar characteristic were observed in energy profile in Figure 4. The efficiencies of the solar collector were evaluated by finding the area under energy curve. Statistical analysis of the thermal efficiency of solar air collector with the same glass thickness were analysed with SPSS program with confidence interval of 95%. Efficiency mean of collector models 1, 2, 3 and 4 were 29.6, 29.8, 30.3, and 30.3% respectively. A one-way between subjects ANOVA (Analysis of Variance) was used to compare the efficiencies of collector models and reported in Table 1. The main objective was to determine if there is a significant difference between collector efficiencies when operated with the same glazing materials.

From Table Table1, the significance value is 0.323 ($p < 0.05$). Therefore, it can be concluded that there were no statistical significant difference between the means of collector efficiency with the same glass thickness, and that, their minor variations are due to changes in environmental conditions and not due to design variations.

Collectors performances with different air flow pattern

Here, the effect of flow pattern on the performance of solar collector by improving the capability of heat capture from absorber plate was discussed. Performance of single pass were studied by designing single duct with air passing over the surface of absorber (Single Duct Front Pass-SDFP) while double pass were studied by designing double duct parallel flow (DDPF) and double duct counter flow systems (DDCF) as elaborated in Figures 1 and 2.

Temperatures profiles for single and double air pass

Figure 5 shows variation of measured collector and ambient temperatures with time for single duct front flow, double duct parallel flow and double duct counter flow on 22/11/2011. Collector temperatures increased with increase in solar intensity and reached maximum between 12:00 am to 13:00 pm. Temperature difference between double and single air pass collectors during the morning were small while at noon and at sunset the differences were significant.

Generally, there was high fluctuation of temperatures during the morning due to high variation of solar intensity. The fluctuations of temperatures were appearing to decrease from noon due to stability of solar intensity and the fact that the system is in equilibrium. However, there was some variation of solar intensity during the afternoon which was not clearly depicted in temperature measured. This was due to the fact that, absorber plate acts as heat storage and therefore at a short time variation in solar intensity the stored energy was transferred to air.

Double duct counter flow solar collector attained the highest maximum temperature of 59.4°C, double duct parallel flow 55.6°C while single duct front pass gave the least maximum temperature of 50.3°C as shown in Figure 6.

Double duct counter flow solar collector produced the



Figure 1. Schematic view of single duct solar collector.

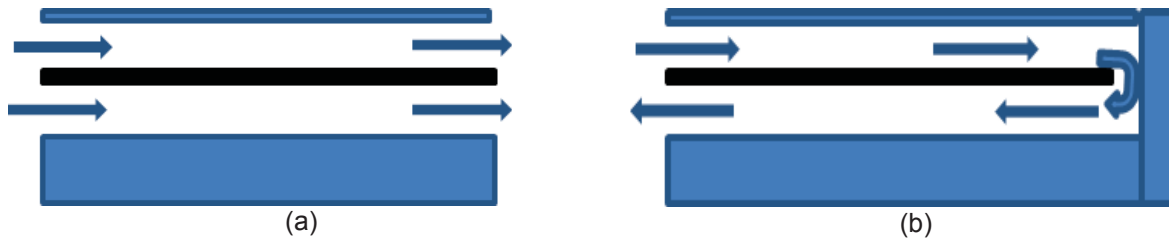


Figure 2. Schematic view of (a) double duct parallel flow (b) double duct counter flow.

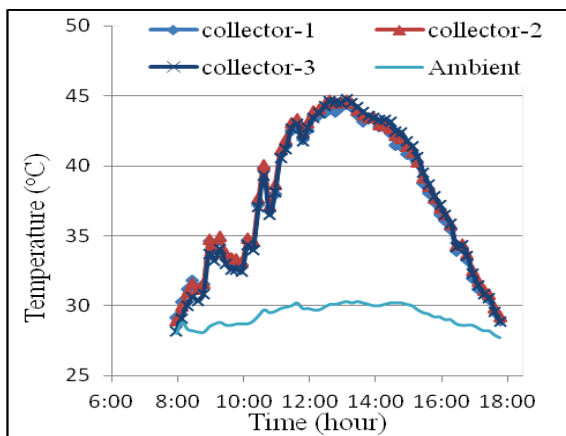


Figure 3. Temperature profile of collector with similar glazing thickness.

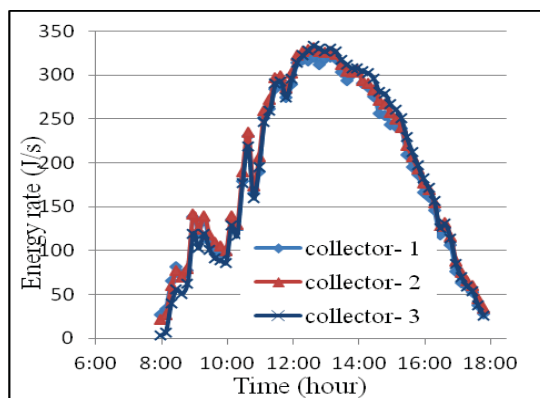


Figure 4. Energy profile of collector with similar glazing thickness.

highest average temperature of 47.2°C while double duct parallel flow and single duct front pass were 44.5 and 41.9°C respectively as shown in Figure 7.

Figure 8 shows the solar intensity versus standard time of the days the experiments were carried out. Solar intensities increased from the early hours at about 200 to 300 W/m² at 8:00 am to peak values at noon. Solar intensity curves can clearly give the weather status of the day in terms of clouds coverage. On 22/11/2011 the sky was almost clear from morning to noon while on 24/11/2011 the sky was highly covered by clouds. The highest daily solar radiation obtained during the experiments with single and double air pass was 1350 W/m² on 23 November, 2011 while the average solar intensity was 992 W/m².

Energy profiles of collectors with similar glass thicknesses

Figure 9 shows variation of solar energy for each collector model on 22/11/2011. Generally, characteristics of energy profiles in Figure 9 are similar to that of temperature profiles reported in Figure 5. From the figures it was clear that, collector energy depends on the solar intensity and varies with solar intensity fluctuations. Energy delivery starts from lowest value during the morning and start rising to maximum during the noon and then reduced towards sunset.

Low energy during the morning were caused by low angle of incidence of solar radiation on the collector surface (normally at 0 to 60°) and the fact that part of the collected energy were used in pre-heating collector and its components (Das and Chakraverty, 1991). However, with the stabilization of available energy with energy

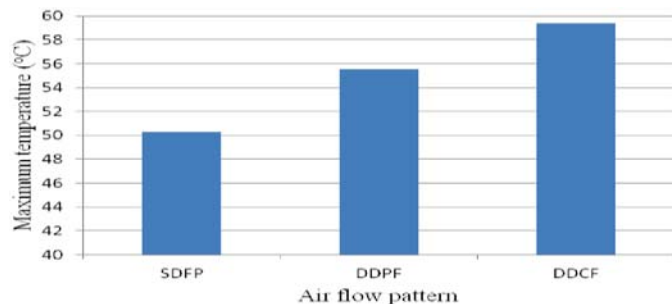


Figure 6. Maximum temperature reached in single and double air pass solar collector.

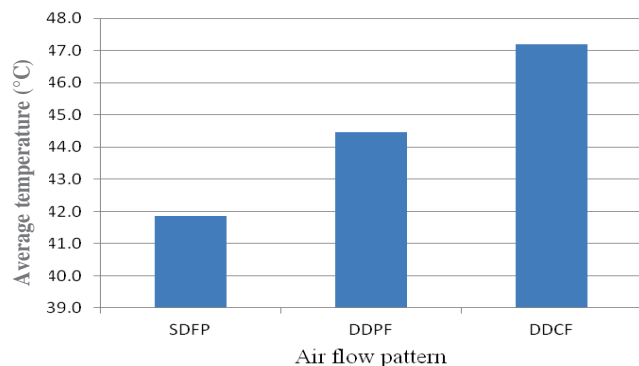


Figure 7. Average temperature for single and double duct solar collector.

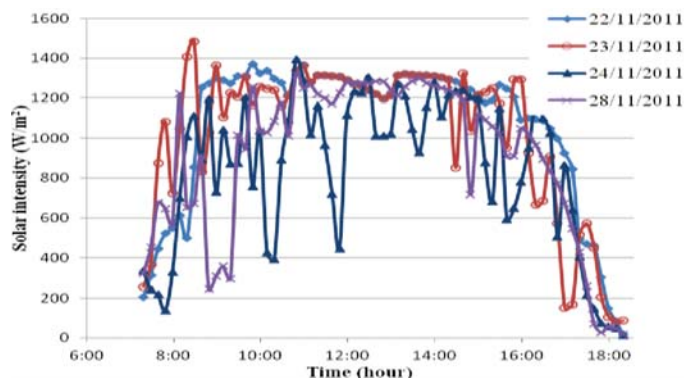


Figure 8. Comparisons of solar intensity for different days of the experiments.

stored in the absorber, from noon the energy does not fluctuate as much as solar intensity.

Analysis of performance of collector with different flow patterns

The results of the statistical analysis of variance (ANOVA) for single and double air pass in collector which

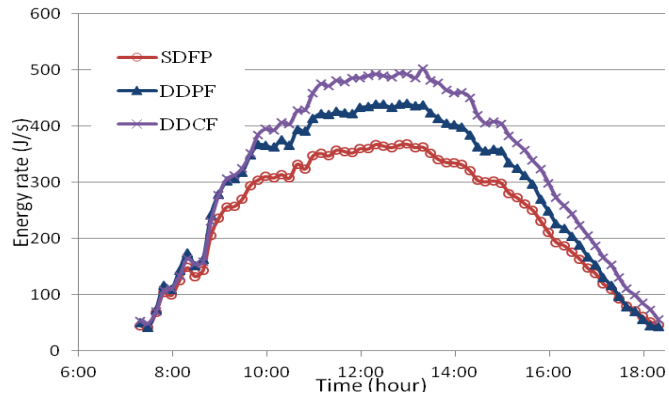


Figure 9. Energy profiles of collectors with different flows patterns 22/11/2011.

was carried out to study the significance differences between their individual means are reported in Table 2. Average performance of single duct front pass (SDFP), double duct parallel flow (DDPF) and double duct counter flow (DDCF) were 30.6, 36.1 and 38.9% respectively.

Comparisons of means for single and double pass solar collector

ascertain if there was significance difference between means of single and double pass solar collectors. From Table 3, it is evident that there is statistical significant difference between the means of single and double pass solar collectors with $p=0.002$. However, in order to identify the method to use in multiple comparison of means, Levene test of homogeneity was conducted.

From Table 4, $p>0.05$; therefore equal variance assumed (Turkey) test were used for multiple comparison of collectors means.

It is clear from Table 5 that the thermal performances of double passes solar air collectors were higher when compared to single passes. These results were in agreement with the study of Yeh et al. (2002) who outlines a considerable performance increase when employing a double-flow device instead of using a single-flow. It could be seen that, performance of single duct front pass collector (SDFP) was significant different compared to double duct parallel flow and double duct counter flow. Similarly, there were no statistical significant difference between double duct parallel flow and double duct counter flow ($p=0.235$). In double pass solar collector, air flow was from both sides of the absorber plate, and therefore it doubles heat transfer areas which in turn reduce collector thermal losses. From this study, it can be concluded that, double ducts counter flow gave the best performance and it can improve the performances of the solar collectors by 8.3% compared to single duct front pass (convictional type) as shown in Figure 10. The result was in agreement with results

Table 3. Analysis of variance of collectors with different air flow patterns.

Collectors	Sum of squares	df	Mean square	F	Significance
Between Groups	140.632	2	70.316		
Within Groups	46.145	9	5.127	13.714	0.002
Total	186.777	11			

Table 4. Results of Levene test for single and double air pass solar collector.

Levene statistic	df1	df2	Significance
3.179	2	9	0.090

Table 5. Multiple comparisons test for single and double air pass mean efficiencies.

(I) Air_flow	(J) Air_flow	Mean difference (I-J)	Standard error	Significance	95% confidence interval	
					Lower bound	Upper bound
SDFP	DDPF	-5.42500*	1.60113	0.020	-9.8954	-0.9546
	DDCF	-8.25000*	1.60113	0.002	-12.7204	-3.7796
DDPF	SDFP	5.42500*	1.60113	0.020	0.9546	9.8954
	DDCF	-2.82500	1.60113	0.235	-7.2954	1.6454
DDCF	SDFP	8.25000*	1.60113	0.002	3.7796	12.7204
	DDPF	2.82500	1.60113	0.235	-1.6454	7.2954

*: The mean difference is significant at the 0.05 level.

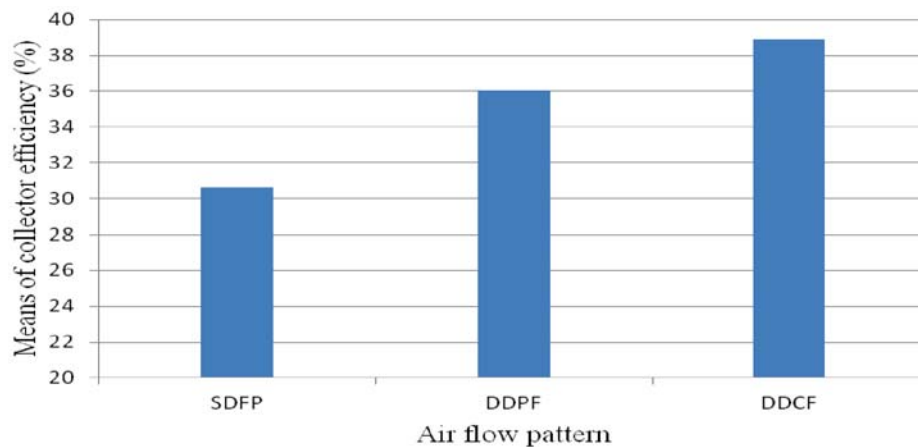


Figure 10. Means plot of performances of solar collectors with different air flow patterns.

reported by Omojaro and Abdabbagh (2010) and El-sebaili et al. (2011b) in the ranges of 7 to 19% and 7 to 9% respectively. On other hand, the result were in disagreement with the results reported by Chamoli et al. (2012), Yousef and Adam (2008) and Ramani et al. (2010) in the ranges of 10 to 15%, 10 to 12% and higher than 10% respectively. This was due to the fact that the reported studies uses aluminium plates which were

superior in thermal conductivity when compared to galvanized steel used in this study. In addition, the first author attempts to use two similar plates in double duct and compared results with single duct which contains single plates while in this study only single plate were used in double and single air passes.

Theoretically, in double duct counter flow, heat energy extracted by the flowing air in the first pass from the

glass covers is used as air preheater which in turn decreases the temperature of glass covers and heat losses to the surroundings. Therefore, the performance of double pass collectors were found to be superior compared to single pass solar air collector where air flows in one side of absorber plate. However, the application of double duct counter flow in natural convection is limited, since air needs to be forced through the two channels for efficient utilization of the system. Also more power is demanded in forced convection systems in order to overcome the effect of pressure drops due to the increased duct length. For the best performance of double duct counter flow with forced convection, it was recommended by Forson et al. (2007) and Pawar et al. (1994) that the collector length should be limited to 1.5 to 2.5 m while length to width ratio should range between 1.0 and 2.0.

Conclusion

Performances of double duct air flow pattern have been studied and compared to single duct flow pattern. The experimental results show differences in collector efficiency with change in flow pattern. From the results obtained for the different flow patterns examined, it was observed that varying the flow patterns collector efficiency appeared to significantly change. It was also observed that double duct counter flow gave efficiency of 38.2% compared to 30.6% for single duct system.

From these results it can be concluded that collector efficiency can be improved by 8.3% by altering the aerodynamics through the collector. Double pass counter flow seems to have the advantages of increased heat transfer area; reduced heat losses through glazing as well as increased turbulence hence improved heat transfer. Despite this improved efficiency, it is important to also analyse the increased collector cost and do a cost benefit analysis to arrive at an optimal situation.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

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NOMENCLATURE

SDFP: Single duct front pass (air passing over absorber plate).

DDCF: Double duct counter flow (air inter over and exit

below absorber plate).

DDPF: Double duct parallel flow (air passing in both sides of absorber plate).

A : Collector area (m^2).

F_R : Collector heat removal factor.

C_p : Specific heat capacity of air (J/Kg.K).

m : Mass flow rate (kg/s).

I : Global solar intensity reaching collector surface (W/m^2).

Q_u : Useful energy gained by air (W).

Q_{in} : Available solar energy on collector surface (W).

T_o : Temperature out of collector ($^{\circ}C$).

T_i : Air inlet temperature ($^{\circ}C$).

U_L : Heat loss coefficient ($W/m^2 K$).

α : Absorptivity

τ : Transmissivity

η : Collector efficiency

h_{fb} : Heat transfer coefficient between the fluid and base plate ($W/m^2 ^{\circ}C$).

h_{fg} : Heat transfer coefficient between the glass and fluid ($W/m^2 ^{\circ}C$).

h_{fp} : Heat transfer coefficient between absorber plate and fluid ($W/m^2 ^{\circ}C$).

$h_{r,gp}$: Radiative heat transfer coefficient between glass and absorber plate ($W/m^2 ^{\circ}C$).

$h_{r,pb}$: Radiative heat transfer coefficient between absorber and base plate ($W/m^2 ^{\circ}C$).

$T_{f1,2}$: Temperature of air in upper and lower duct respectively ($^{\circ}C$).

T_b : Base plate temperature ($^{\circ}C$).

T_g : Glass temperature ($^{\circ}C$).

T_p : Absorber plate temperature ($^{\circ}C$).

U_a : Ambient heat loss coefficient.

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Full Length Research Paper

Physical and chemical parameters, total phenols and the antioxidant activity of Pequi (*Caryocar brasiliense* Camb)

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Pequi is a Brazilian cerrado fruit with nutritive and sensory characteristics and good taste and is used in numerous typical dishes. The purpose of this study was to analyze the physical and chemical characteristics, total phenols and the antioxidant activity and the scanning electron microscope (SEM) of pequi pulp, mesocarp and peel. Physical and chemical analyses such as antioxidants and phenolic compounds compositions were performed. Physical analyses showed high variation in length, equatorial diameter, weight and volume characteristics. Green peel, dark gray mesocarp and bright yellow pulp were observed. Significant differences were observed in the physical and chemical analyses of the fruit, with the pulp showing the most relevant characteristics such as high presence of lipids and proteins. The SEM of the peel and mesocarp showed a multiform, irregular and fibrous structure while the pulp showed irregular aspect, with the presence of granules and fibers. The highest antioxidant activity ($EC_{50}=280.1$) was seen in pulp and the lowest ($EC_{50}=4.89$) in peel. However, the highest concentration (204.37 mg EAG/g) of phenols is in the peel and the lowest (26.55 mg EAG/g) in the pulp. This study concluded that the fruit pulp presents potential for body maintenance and human consumption.

Key words: Ether extract, color, pulp, reuse.

INTRODUCTION

Cerrado is a region with many fruit tree species, many of which are unexplored fruits with pleasant and exotic sensory peculiarities such as color, taste and smell. Werneck (2011) reports that pequi (*Caryocar brasiliense*

Camb.) is a fruit from the Brazilian *cerrado* with commercial and nutritional importance. Its typical exotic smell and taste are attractive characteristics. The fruit has high nutritional value, as it is a source of protein,

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Figure 1. Morphological structure of pequi (*Caryocar brasiliense* Camb.).

lipid, vitamin A, minerals and β -carotene (Alves et al., 2009). Pequi is comprised of pericarp and seed. Its pericarp is green, greenish brown epicarp, dark gray external mesocarp and yellow internal mesocarp which is the edible part of the fruit, and brown endocarp (Figure 1).

Maia et al. (2007) report that pequi is economically unexplored for oil production, as its mesocarp may be used to replace butter and its pit may be cooked and used in several typical dishes of Brazilian cerrado. The consumption of *Caryocar brasiliense* fruits is not only associated with taste and nutritional value, clinical and epidemiological studies have shown evidence indicating these fruits have immunological, therapeutic and anti-inflammatory characteristics mainly due to their antioxidant activity and presence of vitamins A and E (Saraiva et al., 2010). The antioxidant characteristics are related to phenolic compounds which present enzyme activity in the body by blocking oxidation of compounds in foods like fats, and reducing the action of reactive oxygen species (Moreira and Mancini Filho, 2004). Phenolic compounds are chemical structures that present hydroxyls and aromatic rings in simple and polymer forms which enables their antioxidant activity. There are several known sources of natural antioxidants and some are commonly found in the plant kingdom (Angelo and Jorge 2007).

Considering the above facts, the objective of this study was to evaluate the physical and chemical characteristics such as weight, diameter, proteins, lipids, total phenolics, antioxidant activity and scanning electron microscopy (SEM) of the various layers (epicarp, mesocarp and

endocarp) of pequi fruits of the region of Belo Horizonte, MG.

MATERIALS AND METHODS

General experimental procedures

The reagents used in this study were analytically pure (AP). The Folin-Ciocalteu reagent was acquired from Sigma-Aldrich, DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical from Aldrich Co. and gallic acid from Vetec. Absorption was measured using a UV-V spectrophotometer (Biospectro SP 220).

Vegetal material

Pequi fruits were manually harvested in February 2013 from native trees located in a farm in the municipality of Lagoa Santa in the metropolitan region of Belo Horizonte, MG (19° 37' 45" S and 43° 53' 23" W) and taken to the Laboratório de Frutas e Hortaliças of the Instituto Federal Goiano – Rio Verde Campus. The fruits were selected in terms of size, color, absence of mechanical injuries and spots. They were then sanitized in chlorinated water (100 mg.L⁻¹), for 15 min and dried. For the analyses of fiber and scanning electron microscope, the fruit pulp, mesocarp and peel were dried and degreased using the Soxhlet method [with petroleum ether reagent (IAL, 2008)] and ground to meal consistency at 6 mm granulometry.

Preparation of crude extracts for the antioxidant activity and total phenols

The vegetal material (1 g) for the antioxidant activity was ground to

small particles, placed in 40 mL methanol solution, 50% (V/V) in agitation for 1 h, centrifuged at 600 rpm for 15 min and the suspended particles collected. With the residue, a new extraction was conducted with 40 mL acetone solution, 70% (V/V) in agitation for 1 h, centrifuged again, and the suspended particles collected.

The two extracts were placed in a 100 mL amber volumetric flask whose meniscus was checked with water.

Determination of total phenols

1 g of the vegetal material for the analysis of total phenols was ground and solubilized in 100 mL ethanol in agitation for 1 h. Then, it was filtered and stored in an amber flask until the analysis was conducted. For the determination of total phenols present in the ethanolic extract samples, the Folin-Ciocalteu method with modifications (Sousa et al., 2007) was employed. The amount of 100 μ L was taken from the ethanolic extract and agitated with 500 μ L Folin-Ciocalteu reagent; 7.4 mL distilled water for 1 min; after which 2 mL Na_2CO_3 at 15% was added to the mix and agitated for 30 s, making up 10 mL solution. The solution was placed at rest for 2 h until reading was conducted in triplicates with absorbance at 750 nm using glass cuvettes, ethanol and all reagents as "blank", but not the sample. Total phenols (TP) were determined using the calibration curve built with gallic acid patterns (10 to 350 $\mu\text{g}/\text{mL}$) and expressed as mg of GAE (gallic acid equivalents) per g of the sample. The gallic acid calibration equation showed a correlation coefficient R^2 of 0.996.

Quantitative analysis of antioxidant activity

The antioxidant activity evaluation of pequi (peel, mesocarp and pulp) was determined through its ability to sequester DPPH free radical according to the methodology described by Sharma and Bhat (2009) with adaptations. When the DPPH solution is in contact with a substance that can donate a hydrogen atom, the reduced form of the resulting radical, presents a reduced color (Ali et al., 2009).

To determine DPPH calibration curve, a portion with different concentrations of DPPH (10, 20, 30, 40, 50 and 60 μM) was transferred to a glass cuvette and the reading conducted using a spectrophotometer at 515 nm. Methanol was used as blank for the appliance calibration. To calculate the straight-line equation, the DPPH concentrations were plotted (μM) on X axis and the respective concentrations on Y axis. The equation from the calibration curve had a correlation coefficient R^2 of 0.999.

Using the crude extract previously diluted in methanol at three concentrations (25, 50 and 100% V/V), the amount of 0.1 mL from each dilution was transferred to a test tube with 3.9 mL DPPH reagent. Readings were conducted half an hour later for sample stabilization, with the equipment previously calibrated. With the calibration curve equation and absorbance values after 30 min for every tested concentration, the concentrations expressed as EC_{50} were tested.

Centesimal composition of pequi and aspect

The physical evaluation of pequi was based on length (mm) and equatorial diameter (mm) measured with digital calipers. Volume was determined with fruit immersion in a graduated polypropylene jar with distilled water by recording the amount (mL) of fluid displaced. Fruit weight was determined using an analytical scale of three decimal places with the results expressed in grams (g).

Physicochemical composition of pequi *in natura* was determined as follows: fruit moisture according to methodology no. 925.09 of AOAC (2000) until a constant weight was obtained; ethereal extract

according to methodology no 925.38 of AOAC (2000); crude protein content according to the micro-Kjeldahl method no 920.87 of AOAC (2000); ash according to gravimetric method no. 923.03 of AOAC (2000), with calcination at 550°C and sample resting in FORNITEC muffle, model 1926, Brazil.

Color

The fruit aspect was evaluated in relation to color parameters according to the system CIELab; L^* , a^* , b^* in a colorimeter (Colorquest II, Hunter Associates Laboratory Inc., Virginia). The evaluation used 10° observation angle and D65 as standard illuminant, which corresponds to natural daylight. The results were expressed as L^* , a^* and b^* values, where L^* (luminosity or brightness) values ranging from black (0) to white (100), a^* values ranging from green (-60) to red (+60) and b^* values ranging from blue (-60) to yellow (+60).

Scanning electron microscope

For the structural analysis of peel, mesocarp and pulp meal, scanning electron microscope (SEM) was used with samples which were placed on stabs, coated with a film of gold and analyzed microscopically. The evaluation was conducted at the Multiuser Laboratory of High-Resolution Microscopy at the Physics Universidade Federal de Goiás. The analysis used Jeol scanning electron microscope, JSM – 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging.

Statistical analysis

Statistical analysis of the physical and chemical parameters and Pequi color was performed using statistical software in a completely randomized design. Means were compared using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Physical analyses of pequi fruits

Pequi fruits presented length values of 63.17 and 37.64 mm for fruit and internal mesocarp (Table 1); greater than those reported by Vera et al. (2005) (58 mm) and Oliveira et al. (2009) (55.83 mm). The equatorial diameter of pequi fruits found in this study was lower than the value (64.8 mm) reported by Vera et al. (2005). Its L^*/ED^{-1} ratio indicates pequi has a circular shape and its internal mesocarp is also circular, but with more accentuated length in relation to the fruit width.

The fruits presented mean weight of 132.04 g, close to the value (140 ± 64.91 g) reported by Cordeiro et al. (2013) in pequi fruits from Cuiabá region. However, Oliveira et al. (2009) reported lower weight (90.48 g) in a similar study. The reported difference in values confirms the high variability presented by pequi fruits. The volume of the fruit and internal ranged from 560 to 30.3, respectively. Pequi fruits presented considerable genetic variability, highlighting the differences between physical values for fruits from the same region and confirming the importance of studies on such characteristics.

Table 1. Mean and standard deviation values of length (L), equatorial diameter (ED), length/equatorial diameter (L:ED⁻¹) ratio, weight and volume of the fruit and internal mesocarp of pequi (*C. brasiliense* Camb.).

Parameter	Fruit	Internal mesocarp
Length (mm)	63.17 ± 5.36	37.64 ± 5.01
Equatorial diameter (mm)	61.42 ± 5.76	26.14 ± 5.70
L:ED ⁻¹ ratio	1.03 ± 0.08	1.44 ± 0.05
Weight (g)	132.04 ± 26.22	31.04 ± 7.92
Volume (mL)	560 ± 162.88	30.3 ± 9.00

Table 2. Mean and standard deviation values of L, a*, b* for pulp, mesocarp and peel of pequi fruits (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
L*	76.40 a ± 4.34	56.38 b ± 6.60	48.30 ^c ± 4.97
a*	25.65 a ± 5.53	4.71 b ± 2.85	-0.05 ^c ± 2.50
b*	45.66 a ± 8.29	41.12 b ± 5.90	10.74 ^c ± 3.57

Distinct small letters on the line present significant difference between each other through Tukey's test at 5% probability.

Siqueira et al. (2012) reported that the physical properties of fruits contribute to designing new equipment for post-harvest processes (drying, cleaning, classification and peeling) and storage, stressing that it may help adaptations to existing processes for better processing results.

Pequi fruit color analyses

Color is one of the attributes used in evaluating food quality; since visual analysis is one of the first felt to be used in choosing a food and also being a critical feature in choosing and accepting a product. Pequi fruit color coordinates presented significant differences for pulp, mesocarp and peel (Table 2). Coordinate L* represents how light or dark the fruit is, confirming pequi pulp is the lightest portion of the fruit, while the peel is the darkest. Pequi pulp presented 76.40 for coordinate L*, but Cordeiro et al. (2013) reported values between 48.65 and 60.08 in pequi pulp from the state of Mato Grosso, MT. Parameter a* ranges from green (-60) to red (+60); a* values for pequi pulp in this study were 25.65, lower than for pequi pulps from the state of Mato Grosso (37.27) and Goiás (85.5) (Cordeiro et al., 2013; Vera et al., 2005). Pequi pulp showed small incidence of red or green color due to the fact that the value found in this study is between the limit values of this parameter. Pequi mesocarp and peel present greenish color and low incidence of red color; thus, the values analyzed for pequi peel confirm the greenish or greenish brown color for the fruit and yellowish color for the internal mesocarp (Lima et al., 2007).

Parameter b*, ranging from blue (-60) to yellow (+60)

shows that pequi pulp, mesocarp and peel do not present blue color; pequi pulp has strong yellow color, which contributes to its commercial attractiveness *in natura*.

Physicochemical characteristics of pequi

Table 3 shows the results of pequi physicochemical characterization, presented with significant differences between the values. Pequi pulp presented 54.40 g/100 g mean values of moisture. Vera et al. (2005) reported similar mean values of moisture while studying fruits from two regions of Goiás; 48.13g/100 g for fruits from the region of Mambaí, and 54.34 g/100 g for fruits from the region of Araguapaz. In this study, pequi pulp presented lower contents of moisture and higher contents of protein, ethereal extract and ash when compared to mesocarp and peel. However, the data confirm high contents of soluble solids in the mesocarp where the moisture content is greater.

In pulp, a high protein value of (3.02 g/100 g) was observed when compared to the peel (1.06 g/100 g). Machado et al. (2013) reported 2.83 g/100 g and 2.93 g/100 g protein content in pulp and endocarp of pequi fruits. However, Sousa et al. (2011) reported 9.64 g/100 g protein content in pequi pulp.

The ethereal extract values of 56.74 g/100 g for pulp, 3.57 g/100 g for mesocarp and 0.73 g/100 g for peel were found in this study. Machado et al. (2013) reported lower values for pequi pulp and endocarp (26.30 g/100 g and 19.13 g/100 g, respectively). The food chemical composition table shows that the value of ethereal extract from pequi compares to that of fruits like babassu and avocado (Franco, 1982). However, Oliveira et al. (2006)

Table 3. Mean and standard deviation values of moisture, protein, ethereal extract, ash, soluble solids, pH, titratable acidity and pulp reducing sugars, mesocarp and peel of pequi fruits (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
Moisture (g/100 g)	54.40 c ± 0.65	83.41 a ± 0.77	73.98 ^b ± 0.28
Protein (g/100 g)	3.02 a ± 0.02	1.06 b ± 0.03	0.60 ^b ± 0.35
Ethereal Extract (g/100 g)	56.74 a ± 8.05	3.57 b ± 0.49	0.73 ^b ± 0,02
Ash (g/100 g)	0.67 a ± 0.02	0.41 a ± 0.25	0.63 ^a ± 0.01
Soluble Solids (Brix)	7.33 b ± 1.53	10.0 a ± 0.00	8.90 ^{ab} ± 0.17
pH	6.90 a ± 0.04	4.80 b ± 0.03	4.56 ^b ± 0.26
Titratable Acidity (g/100 g)	1.52 a ± 0.22	5.37 b ± 0.57	6.60 ^b ± 0.78
Reducing Sugars (g/100 g)	1.2 b ± 0.01	2.2 a ± 0.01	2.3 ^a ± 0.01

Distinct small letters on the line present significant difference between each other through Tukey's test at 5% probability.

reported that pequi oil has predominantly unsaturated fatty-acids, which makes it an oil of excellent quality. An increasing proportion of ash (0.41 g/100 g) was found in pequi mesocarp peel (0.63 g/100 g) and pulp (0.67 g/100 g); unlike the values reported by Gondim et al. (2005) who said that pequi peels present important amounts of ash when compared to pulps. This fact may be related to the greater availability of minerals in pequi pulp, which is the most tasteful portion of the fruit. Sousa et al. (2012) and Oliveira et al. (2010) found lower values than those observed in this study for pequi pulp, 0.39 and 0.6%, respectively.

The contents of soluble solids for pulp, mesocarp and peel were 7.33, 10.0 and 8.90 °Brix, respectively. Lower values (15 °Brix) were found for ripe cerrado fruits, (Silva et al., 2009). For minimally processed and stored pequi, 12 °Brix was reported after a 15-day period (Damiani et al., 2008).

Significant differences were observed when pH of pequi pulp, mesocarp and peel were compared. Pequi pulp pH (6.90) was lower than the value reported by Pinedo et al. (2010) who found 7.36 pH for pequi pulps, higher than the values of 6.58 to 6.97 reported by Vera et al. (2005). According to Chitarra and Chitarra (2005), pH values increased with reduced titratable acidity as reported in this study when comparing pequi pulp, mesocarp and peel. Souza et al. (2013) reported that unlike other tropical fruits, pequi shows low acidity.

The values of titratable acidity were 1.52, 5.37 and 6.70 g titratable acidity/100 g for pulp, mesocarp and peel, respectively. These data showed significant differences. Titratable acidity of fruits may be influenced by sample preparation, harvesting time and ripening. Pequi fruits stored in modified atmosphere (Souza et al., 2007) presented lower values than those reported in this study (0.049g titratable acidity/100g).

This study found that the amount of carbohydrate for pequi pulp, mesocarp and peel were 1.2, 2.2 and 2.3 g/100 g, respectively. Sousa et al. (2011) reported 0.40 g/100 g carbohydrate values. Santos et al. (2010)

reported 3.75 g/100 g of pulp reducing sugars in pequi fruits from the region of Barra do Bugres.

Analyses of total phenols and antioxidant activity of pequi fruits

Pequi peel showed considerable amount of total phenols, followed by pequi mesocarp and pulp with lower amounts (Table 4). Pequi peel presented 204.37 mg EAG/g for total phenols. In cerrado fruits analyzed by Roesler et al. (2007), pequi peel presented the greatest amount (209.37 ± 3.57 g GAE.kg⁻¹) of total phenols,

Pequi pulp presented a value of 26.55 mg EAG/g for phenolic compounds. However, Lima (2008) found greater values, 209 mg EAG/100 g, as well as Oliveira (2009) who found 104.12 mg EAG/100 g for *Caryocar coriaceum* species. According to Asami et al. (2003), the variation in values of phenolic compounds in the fruits is related to the use of solvents and extraction method. It is also associated with fruit species, cultivar, ripening and climate conditions. Roesler et al. (2008) reported that phenolic compounds are directly related to the antioxidant properties of the material and according to Vieira et al. (2011), a greater antioxidant activity reduces the EC₅₀ value that is, a smaller amount of extract will be required to reduce the radical by 50%. Pequi pulp is rich in lipids and pequi trees are cultivated in places with solar ray incidence, factors that help generate free radicals and consequently, antioxidant properties (Lima et al., 2007).

Considering the results, pequi fruits present potential antioxidant effect, mostly concentrated on pequi peel, followed by its mesocarp and pulp. The antioxidant activity is influenced by sample storage, plantation conditions and preparation of extract and solvents employed. Machado et al. (2013) reported that the antioxidant activity of pequi fruits is related to the presence of polyphenols, carotenoids and vitamin C in fruits.

Table 4. Mean values of total phenols and antioxidant activity for pulp, mesocarp and peel of pequi (*C. brasiliense* Camb.).

Parameter	Pulp	Mesocarp	Peel
Total phenols (mg EAG/g)	26.55 ± 5.87	139.85 ± 7.71	204.37 ± 3.71
Antioxidant activity (EC ₅₀)	280.14 ± 5.77	197.69 ± 5.11	4.89 ± 2.82

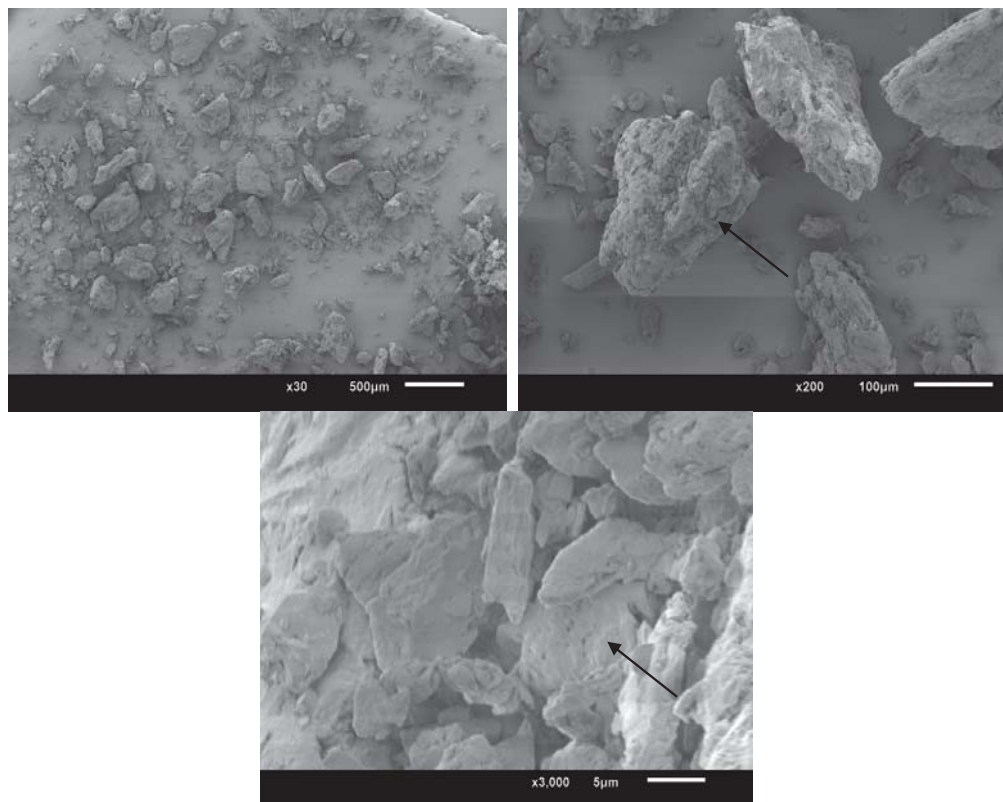


Figure 2. SEM images of pequi (*C. brasiliense* Camb.) mesocarp meal.

Scanning electron microscope (SEM)

The structural analyses of pequi mesocarp meal are illustrated in Figure 2. Degreased pequi mesocarp meal has a multiform, irregular and fibrous structure. However, the sample presented defined structures which were not observed for lyophilized pequi with sucrose treatment in which crystalline cores were created (Alves, 2007). The structural characteristics of the mesocarp suggest that its meal can have a high fiber content which may be used for human consumption. The presence of amorphous starch granules and fibrous structures was observed, as reported by Borba et al. (2005) in sweet potato meal.

The structural analyses of pequi peel are illustrated in Figure 3. Degreased pequi peel meal presented similar aspect to degreased pequi mesocarp meal analyzed in this study, therefore showing its fibrous characteristics.

Fiorda et al. (2013) reports that starch granules have characteristic circular and concave-convex structure; however, fiber structures have geometric aspect with gaps and incidence of permeable pores that contribute to water absorption. The spherical and uniform structure and geometric surfaces confirm the values of reducing sugars found in this study, with pequi peel presenting the highest values when compared to the mesocarp and pulp; results that may be interesting for human nutrition. The structural analyses of pequi pulp meal are illustrated in Figure 4. The analyses show that degreased pequi pulp meal also presented irregular aspect with granules, and surface with the presence of fibers, unlike the mesocarp meal which shows porous surface.

Essential oil in vegetal cells is retained in the cytoplasmic vacuoles. When the solvent flows during the degreasing process, these structures rupture due to mechanical or physicochemical damage and the essential

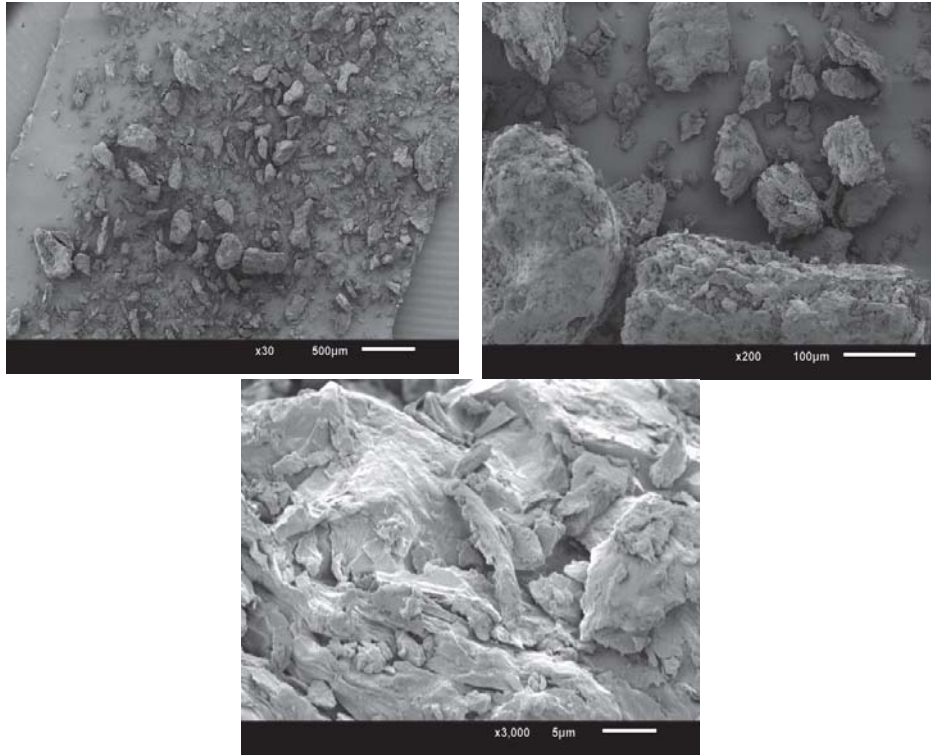


Figure 3. SEM images of pequi (*C. brasiliense* Camb.) peel meal.

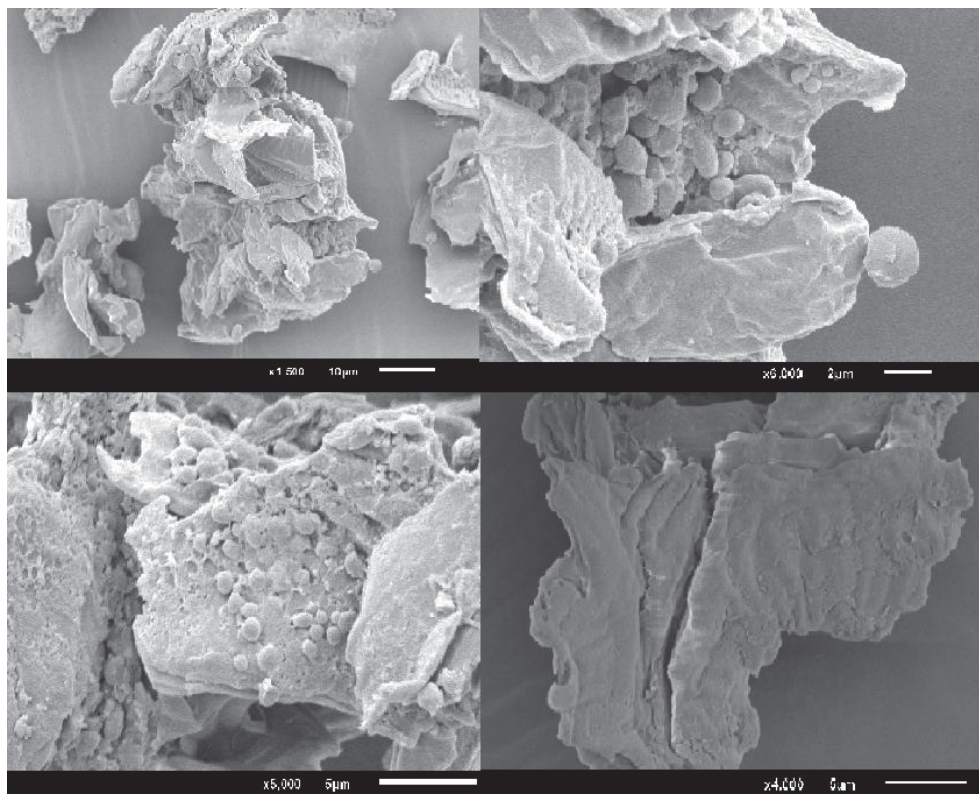


Figure 4. SEM images of pequi (*C. brasiliense* Camb.) pulp meal.

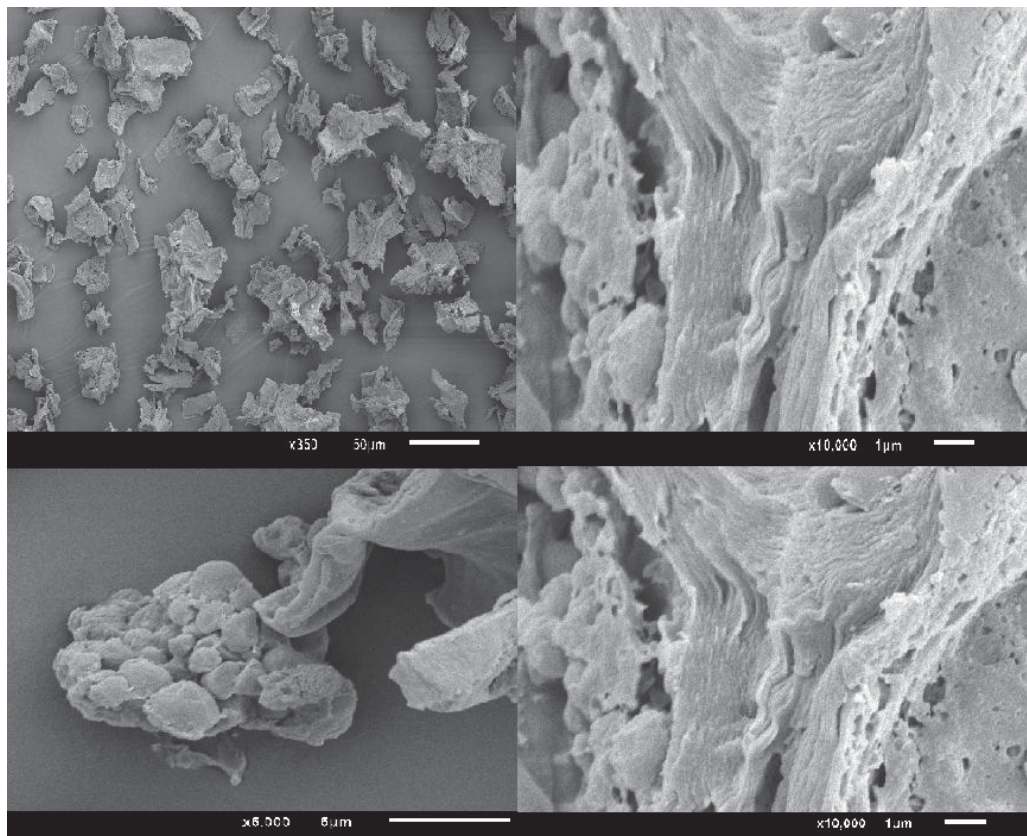


Figure 5. SEM images of pequi (*C. brasiliense* Camb.) pulp meal.

oil is released. Thus, the action of solvents used in meal degreasing may be analyzed (Figure 5) due to spaces among fibrous and starchy structures (HESS, 1975). This fact can be confirmed by analyzing the fat quantification in this study, which showed that the highest amounts of lipids are in pequi pulp and this would rupture the vacuoles of vegetal cells. Pequi pulp, with its pleasant sensory characteristics is excellent for human nutrition by adding fibers to human diet.

Conclusion

Pequi pulp, which can be used in innumerable dishes, has considerable amounts of minerals, lipids and proteins which are important nutrients for human nutrition. The high antioxidant effect of pequi pulp in relation to pequi mesocarp and skin shows the importance of this fruit to help maintain body health. However, pequi peel presented a high amount of phenols, which requires additional studies on peel utilization.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Estimation of annual yield and quality of “Valência” orange related to monthly water deficiencies

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The State of São Paulo, Brazil is the major orange producer in the world. The “Valência” orange is one of the most important cultivars for industry with a high efficiency of processed juice. Climate is the main factor of influence for citrus yield and quality and its study is fundamental for understanding the climatic requirements of the crop. The estimation of yield and quality by agrometeorological models helps to understand the effects of climate on crop cycle, besides being an option for orchards planning activities. Understanding the relationships between water deficiencies (DEF), phenological phases, yield (fruits per box (FRBOX) and quality of “Valência” orange grafted on rangpur lime (VACR) are important to improve the water use in the production areas and to provide information about water stress for plants during its cycle. The present study aimed to investigate the influence of monthly DEF on yield and quality parameters of VACR, in order to develop agrometeorological models for the main four producers regions of State of São Paulo, Brazil. Data of 13 years were used for analysis, being the period from 2001 to 2009 used for calibration and from 2010 to 2012 for validation. Multiple linear regression for model construction was used. All the developed agrometeorological models were accurate, ranging the values of mean absolute percentage error (MAPE) of 5.25 to 9.27% for mean annual yield (FRBOX) and 2.74 to 14.14% for quality (RATIO) among all regions. The angular coefficients indicate which phenological phase of VACR is more sensitive to DEF. Bauru and Limeira FRBOX was related to DEF during bud formation and vegetative dormancy. Yield at Bebedouro were related to DEF between vegetative dormancy and flowering and at Matão bud formation. Fruit quality was more sensitive to DEF during maturity at all regions.

Key words: Modeling, estimate, water deficiency, “Valência” orange.

INTRODUCTION

Brazil is the major citrus producer in the world, with the production mainly used in industrial processing for producing concentrated juice for exportation. Every five cups of orange juice consumed in the world has at least three from Brazilian fruits (Neves et al., 2010). The State

of São Paulo stands out due to three fundamental factors: economic, climatic and soil. With regard climatic and soil factors, São Paulo shows favorable conditions to citrus production due to, mild temperatures, suitable rainfall distribution, soils composition and relatively plain

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topography (Reis, 2008).

While the other factors that affect production and plant development are constant weather condition which varies from year to year and it is considered as the main factor that cause yield and quality variability (Paulino et al., 2007). Agrometeorological models are tools used to understand the influence of climatic variables on crop production, being a way to summarize the responses of the crop to the climate (Rolim et al., 2008). These models can be of great utility for planning the agricultural activities at orchards and to estimate production (processed or 'in natura' fruits). There are many examples of agrometeorological models application on estimations of yield of annual and perennial crops. Santos and Camargo (2006) developed agrometeorological models to estimate coffee yield at different regions of São Paulo, Espírito Santo, Paraná and Minas Gerais States. Bozorg et al. (2011) developed a model for wheat crop at Irã region. Araújo et al. (2014) made a modeling in order to estimate soybean yield associated to agrometeorological variables at Paraná.

Guimarães et al. (2013), elaborated models in multiple linear regressions to predict the banana "Prata" harvest time, at Guanambi, Bahia (BA) in function of yield characteristics, for example number of fruits. The main problem in multiple linear regression models is the selection of independent variables. According to Xu and Zhang (2001) there are many methods for this selection linear and non-linear multiples models, as: a) forward selection, b) backwards elimination, c) stepwise, d) leaps and bound regression, e) orthogonal descriptors, f) genetic algorithm, g) genetic population, f) choosing the operators and g) fitness of evaluation. Or according to Gujarati and Porter (2011), another option is to test all possible combinations to avoid the minimum local problems in the domain range.

Some literature was found using modelling to estimate yield and quality of "Valência" orange (Camargo et al., 1999; Volpe et al., 2002; Paulino, 2007). Citrus crops according to some studies use water deficiencies information to detect its effects at yield and quality with regards to irrigation scheduling. Panigrahi et al. (2014), have done a study about this subject and utilized this hydric information to predict yield of "Kinnow" mandarin in New Delhi, India.

Among the agrometeorological modelling papers for yield estimation, a few are exclusive dedicated to understand the water deficiencies influence at different phenological phases. For annual crops, this relation is more commonly studied, as the model tested by Monteiro and Sentelhas (2014) that used relative water deficiency at phenological phases to the calculation of actual soybean yield at different Brazil regions. For perennial crops, such analysis are not common, for most of "Valência" orange agrometeorological modelling papers search to understand effects of water stress at citrus orchards in order to determine the irrigation deficit, mainly at flowering (Pérez-Pérez et al., 2009; Rocuzzo et al.,

2014) and not to estimate yield and quality of the fruits in function of the monthly water deficiency. The "Valência" is important among sweet oranges, due to its yield and fruit size (Pio et al., 2005). At industrial view, this orange represents one of the pillars of the agroindustry, being the second cultivar most produced at the state of São Paulo. The "Valência" orange have excellent juice quality for processing, storage and transport (Coelho, 2002).

This study aim to develop agrometeorological models for estimating annual yield (mean fruits per box and fruits weight) and quality parameters (maximum of ratio, fruit sugar content, kilograms of soluble solids per hectare and mean acidity) of "Valência" orange as a function of monthly water deficiencies (DEF) for Bauru (BAU), Bebedouro (BEB), Limeira (LIM) and Matão (MAT) regions and also comprehend the relationship of annual DEF with yield and quality parameters of VACR orange.

MATERIALS AND METHODS

The regional climate data (Table 1) were obtained in daily scale from automatic meteorological station. Precipitation and air mean temperature data were organized at monthly scale from the period of 2000 to 2013 for calculation of potential evapotranspiration by the equation of Camargo (1971) (Equations 1, 2, 3, 4 and 5). Data of 13 years were divided into nine years for calibration (2001 to 2009) and three for validation (2010 to 2012).

$$ETP = 0.01 \times Q_o \times T \times ND \quad (1)$$

$$Q_o = 37.6 \times DR \times \left[\left(\frac{\pi}{180} \right) \times hn \times \sin \Phi \times \sin \delta + \cos \Phi \times \cos \delta \times \sin hn \right] \quad (2)$$

$$DR = 1 + 0.033 \times \cos \left(\frac{360 \times JD}{365} \right) \quad (3)$$

$$\delta = 23.45 \times \sin \left[\left(\frac{360}{365} \right) \times (JD - 80) \right] \quad (4)$$

$$hn = \arccos[-\tan \Phi \times \tan \delta] \quad (5)$$

where, Q_o is daily solar irradiance at the atmosphere ($\text{MJm}^{-2} \text{day}^{-1}$); DR is relatively distance from earth to sun (astronomic units); hn is hourly angle at sunrise; Φ is latitude ($^{\circ}$); δ is solar declination ($^{\circ}$); JD is Julian day; T is mean air temperature ($^{\circ}\text{C}$); ND is number of days considered.

Meteorological data were used to calculate water deficiencies (DEF) (Equation 6), from the water balance calculation by the Thornthwaite and Matter (1955) method at monthly scale with available water capacity (AWC) of 100 mm.

$$DEF = ETP - ETR \quad (6)$$

Where, DEF is water deficiency at the soil-plant system; ETP is potential evapotranspiration and ETR is actual or real evapotranspiration. Monthly quality data of ratio (Equation 7), fruit sugar content measured by optical refractometer ($^{\circ}\text{BRIX}$), kilograms of soluble solids per hectare (KGSS) (Equation 8), acidity (% citric acid) and juice percentage (Equation 9) and monthly yield data of fruits per box (FRBOX) and fruit weight (WFRUIT) of "Valência" orange (*Citrus sinensis*, L. Osbeck) grafted on rangpur lime (*Citrus*

Table 1. Regional and climatic description. Legend: Normal annual water deficiency (DEF) estimated by Thornthwaite and Matter (1955) model.

Region	Latitude	Longitude	Altitude (m)	DEF (mm)	Thorntwaite (1948) climate classification
Bauru	22° 17' 29" S	49° 33' 10" W	561	120	C ₂ sB ₄ a
Bebedouro	20° 56' 58" S	48° 28' 45" W	573	203	C ₂ dA'a
Limeira	22° 33' 53" S	47° 24' 06" W	588	132	B ₁ rB ₃ a
Matão	21° 36' 12" S	48° 21' 57" W	585	178	B ₁ rB ₄ a

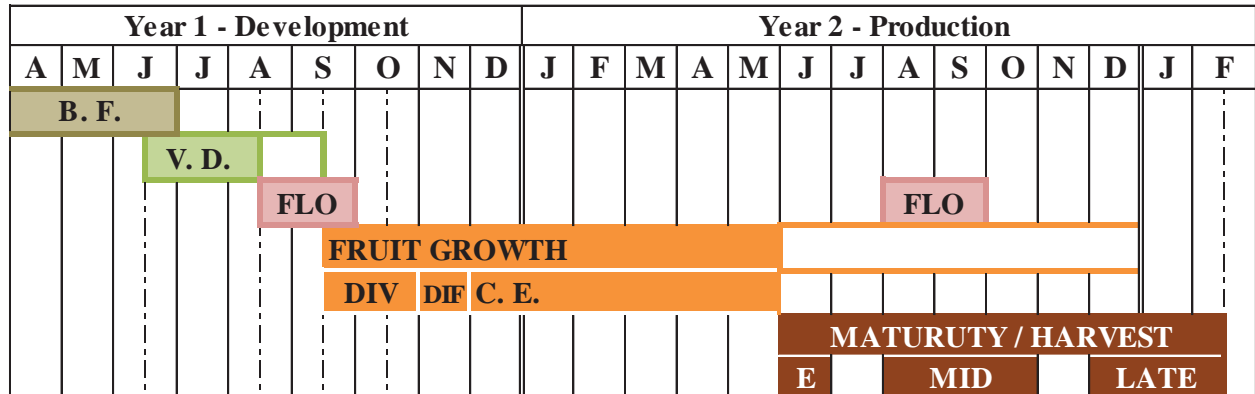


Figure 1. Mean phenology of “Valência” Orange grafted on rangpur lime for the State of São Paulo. Legend: B.F.: bud formation; V.D.: vegetative dormancy; FLO: flowering; DIV: cell division; DIF: cell differentiation; C.E.: cell expansion; E, MID e LATE: early, mid and late crops cycle lengths.

limonia, Osbeck) (VACR) were obtained from the regional producers. These data were organized as a mean of the two flowerings and only from adult orchards (more than six years). Orange plants usually have two flowerings during its cycle, induced by thermal and/or hydric stresses, but it is possible for the plant to have more flowerings with climatic stresses off season.

$$RATIO = \text{°BRIX} \times \text{ACIDITY}^{-1} \tag{7}$$

$$KGSS = JC \times \text{°BRIX} \times 40.8 \times 10^{-4} \tag{8}$$

$$\%JUICE = WJUICE \times WFRUIT^{-1} \times 100 \tag{9}$$

where, JC: juice content (L); WJUICE: juice weight (kg); ACIDITY: % of citric acid; 40.8 box weight in kg.

Multiple linear regressions (Equation 10) were applied to develop the models (Figures 6, 7, 8 and 9). The dependent variables were the yield and quality parameters and independent variables were the monthly DEF during the development and production years (Figure 1). The DEF data from April to December of the first year and from April to December of the second year were used to total the 16 possible pre-selected stresses use in the models.

$$Y = a \times X_1 + b \times X_2 + c \times X_3 + \dots + C.L. \tag{10}$$

Where, Y: yield (fruits per box), as also brix, soluble solids, ratio, acidity and fruit weight; a, b, c,.....: angular coefficients; X₁, X₂, X₃,.....: selected monthly DEF and C.L.: linear coefficient.

The main problem in multiple linear regressions is to find the combination of independent variables that brings consistency in the models. Following Gujarati and Porter (2011), the method used in

this study was to test all possible combinations of monthly DEFs with 1 to 5 independent variables in the models, totaling 6,884 tested equations for each annual yield and quality parameter. The equations were generated by a routine in Visual Basic for Applications (VBA) in MS-Excel 2010 environment.

Analyses were made to identify the multicollinearity between the independent variables (monthly DEFs). Gujarati and Porter (2011), describe that multicollinearity is not an issue if the interest is just estimation. However if exists an interest of doing a coefficients interpretation, as is the case of this study, multicollinearity causes bias on these coefficients. In this study, we chose to remove equations that have minimal correlations among independent variables greater than 0.7. The best models were classified according to statistical indices of mean absolute percentage error (MAPE) (equation 11) and precision by the adjusted determination coefficient (adjusted R²) (equations 12), selecting only regressions that are statically significant by the test-F (p-value <0.05).

$$MAPE(\%) = \frac{\sum_{i=1}^N \left(\left| \frac{Yest_i - Yobs_i}{Yobs_i} \right| \times 100 \right)}{N} \tag{11}$$

$$R^2 \text{ adjusted} = \left[1 - \frac{(1 - R^2) \times (N - 1)}{N - k - 1} \right] \tag{12}$$

where, N: number of data, Yest: estimated Y, Yobs: observed Y, k: number of independent variables.

RESULTS AND DISCUSSION

From all 6,884 generated and later tested equations for

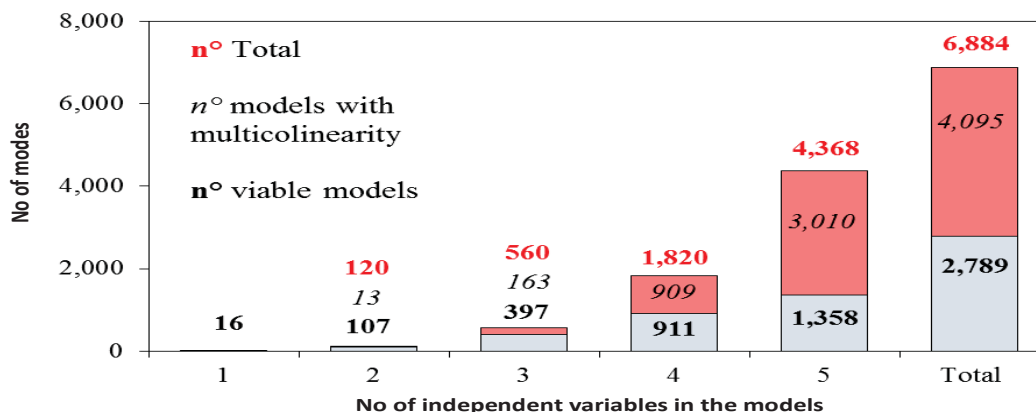


Figure 2. Number of equations generated and tested for multicollinearity for the development of agrometeorological models for estimate each parameter of quality and yield of 'Valência' orange grafted on rangpur lime in function of monthly deficiencies of development year.

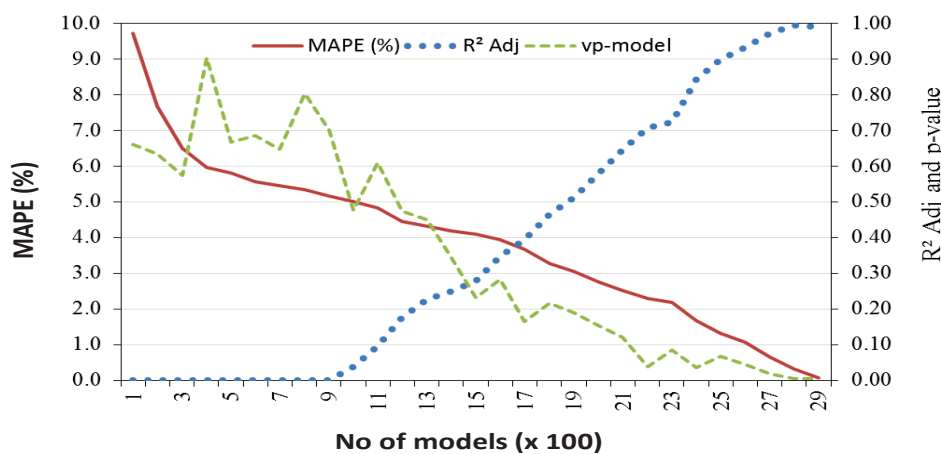


Figure 3. Example of a model classification at Bauru region according to criteria of accuracy (lowering MAPE and p-value and R^2 Adj coming close to 1.00).

each yield and quality parameters, 4,095 equations showed multicollinearity between the independent variables at the models, the remaining combinations (2,789) represent the possible models for estimation of each yield and quality parameter of each region (Figure 2). With these, the best models were selected. The method of testing all combinations of the pre-selected independent DEFs were efficient. As long as adjusted R^2 gets closer to 1.00, the p-value and the MAPE kept decreasing, coming increasingly close to zero (Figure 3). These were the criteria used to classify the best estimate model for each yield and quality parameter of VACR.

Analysis of available water variation at the studied regions proves a strong influence of DEF at yield (Figure 4) and quality (RATIO) (Figure 5) of VACR. Garcia-Tejoro et al. (2011), had a similar view for the mediterranean conditions where the available water is the most limiting factor for plant development. For São Paulo, DEF is the

most influent variable at flowering of oranges at mid-north state region and also the most active at central region, associated to low temperatures during flowering which result in reduced yield (Ribeiro et al., 2006).

Fruits per box (FRBOX) values were higher in April and reduce in December (maturity) due to the fruit size increase. In its turn, RATIO increase constantly up to the end of the year. This analysis proves that meteorological variables, especially the hydric balance elements as DEF have an interesting relation with yield and quality of VACR. An example of DEF influence at crops is demonstrated in the case of Bauru (BAU) and Bebedouro (BEB) that are regions with different characteristics where BAU have lowest temperature and lower DEF compared to BEB, however wet years (less DEF) favor yield increase at both regions. Limeira (LIM) and Matão (MAT) regions have intermediate values of DEF, being the FRBOX value higher at dry years. All the regions have

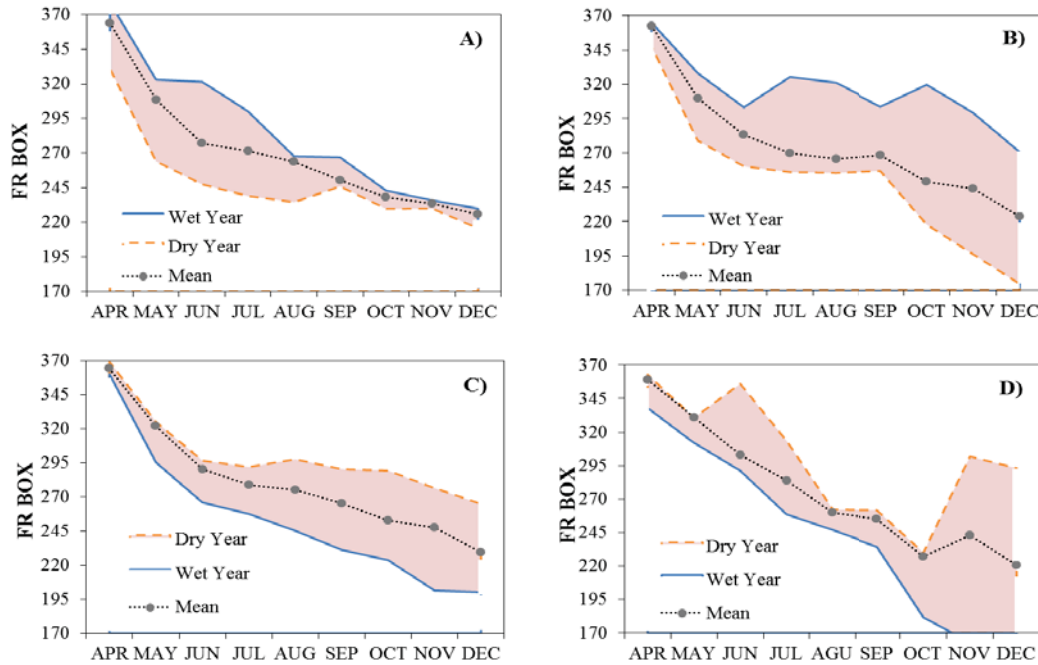


Figure 4. Yield (FRBOX, fruits per box) of “Valência” orange grafted on rangpur lime in function of the highest and the lowest water deficiency years from the period of 2000 to 2013 for four regions of State of São Paulo. A) Bauru; B) Bebedouro; C) Limeira and D) Matão.

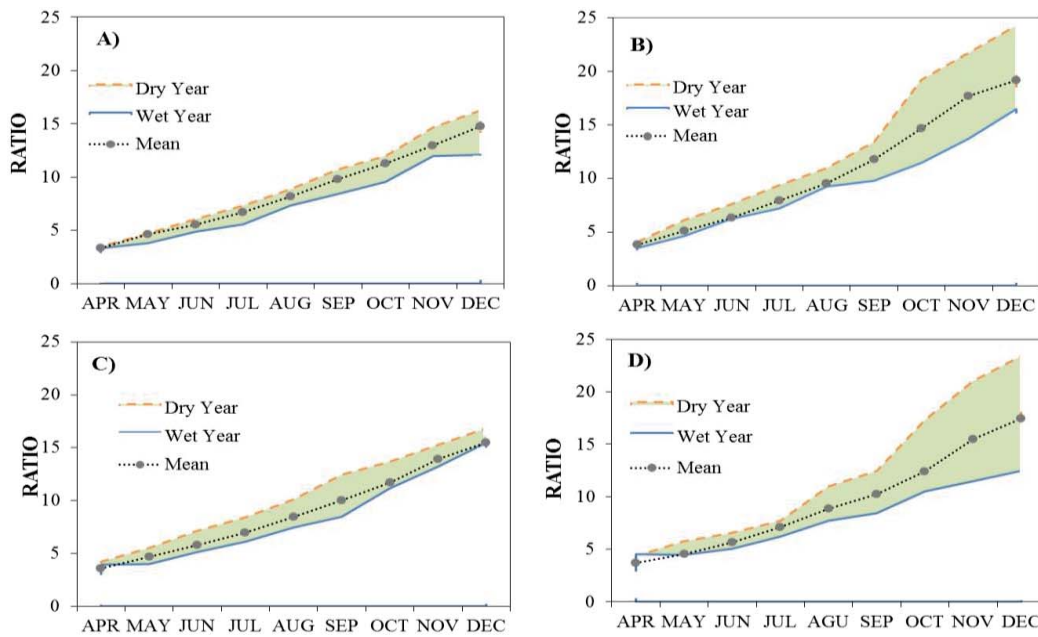


Figure 5. Quality (RATIO) of “Valência” orange grafted on rangpur lime in function of the highest and the lowest water deficiency years from the period of 2000 to 2013 for four regions of State of São Paulo. A) Bauru; B) Bebedouro; C) Limeira and D) Matão.

different relations of increase or reduction of annual DEF, thereby an analysis were performed to quantify the influence of the annual water deficit variation and the

responses this cause at average quality and yield of VACR, in annual scale (Table 2).

An evaluation of increase and reduction of 10 mm of

Table 2. Water deficiency variation and influence at annual yield and quality parameters of “Valência” orange grafted on rangpur lime from the wet and dry years of the period from 2000 to 2013. Legend: (+) increase and (-) reduce.

Bauru							
	RATIO	°BRIX	KGSS	ACIDITY	JUICE (%)	FRBOX	WFRUIT (kg)
Mean	8.56	10.28	2.44	1.47	0.58	268.71	0.16
Dry year	8.47	9.94	2.37	1.39	0.58	253.91	0.17
Wet year	7.43	9.72	2.33	1.61	0.59	278.5	0.16
(+ 10 mm DEF)	-0.0086	-0.0324	-0.0067	-0.0076	0.00	-1.4092	0.0010
(- 10 mm DEF)	0.1139	0.0565	0.0111	-0.0141	-0.0010	-0.9871	0.00
Bebedouro							
	RATIO	°BRIX	KGSS	ACIDITY	JUICE (%)	FRBOX	WFRUIT (kg)
Mean	10.67	11.19	2.56	1.36	0.56	275.18	0.16
Dry year	10.12	10.94	2.66	1.29	0.59	267.59	0.16
Wet year	9.31	10.8	2.5	1.45	0.56	272.22	0.16
(+ 10 mm DEF)	-0.0702	-0.0319	0.0128	-0.0089	0.0038	-0.9693	0.00
(- 10 mm DEF)	0.0777	0.0223	0.0034	-0.0051	0.00	0.1692	0.00
Limeira							
	RATIO	°BRIX	KGSS	ACIDITY	JUICE (%)	FRBOX	WFRUIT (kg)
Mean	8.97	10.65	2.5	1.46	0.57	280.58	0.15
Dry year	8.8	10.89	2.65	1.39	0.59	278.18	0.15
Wet year	8.33	10.5	2.48	1.57	0.58	295.83	0.14
(+ 10 mm DEF)	-0.0076	0.0107	0.0067	-0.0031	0.0009	-0.1067	0.00
(- 10 mm DEF)	0.0578	0.0135	0.0018	-0.0099	-0.0009	-1.3761	0.0009
Matão							
	RATIO	°BRIX	KGSS	ACIDITY	JUICE (%)	FRBOX	WFRUIT (kg)
Mean	9.5	11.29	2.59	1.5	0.56	281.11	0.15
Dry year	10.78	11.60	2.76	1.34	0.58	302.30	0.14
Wet year	7.31	9.82	2.21	1.57	0.55	253.12	0.18
(+ 10 mm DEF)	0.1063	0.0257	0.0141	-0.0133	0.0017	1.7597	-0.0008
(- 10 mm DEF)	0.1389	0.0932	0.0241	-0.0044	0.0006	1.7749	-0.0019

annual DEF was made and how these variations affect the annual VACR yield and quality variability. The values of the parameters of yield and quality from the dry and wet years and the mean values of the period from 2000 to 2013 were used. São Paulo having a different topography and influence from air masses has a broad climatic diversity (Rolim et al., 2007). North regions have higher air temperatures and lower occurrence of rain during winter that are favorable for orange crops production, which is the case of Bebedouro (BEB) and Matão (MAT). The recent performance of the climatic variables at Matão put this region (Bauru and Limeira) suitable for the cultivation (Ribeiro et al., 2006).

At Bebedouro, every 10 mm of annual DEF increase results in a mean reduction of (-) 0.0702 annual RATIO, while for BAU and LIM the reduction is around 0.008. However, for MAT, the increase of 10 mm of annual DEF raises to 0.1063 points of RATIO. The maximum variation of DEF at BEB during the period of study (2000 to 2013) was at 2009/2010, with an increase of DEF of 253 mm which could reduce RATIO up to 1.77 points from a year

to another. The minimized effect at BAU and LIM and the increase at MAT caused by the annual DEF variation can be due to the fact that these regions do not have water restrictions for orange crop production (Ribeiro et al., 2006). Reduced annual DEF of 10 mm, in other words, increased availability of water at the system for the plant expressed RATIO increase for all regions.

With regards to FRBOX, DEF effects were further stronger, even at regions without water restrictions for orange crop production (BAU and LIM); the reduction was significant. Each 10 mm of annual DEF increase result in a reduction of FRBOX at BAU, BEB and LIM in 1.4092, 0.9693 and 0.1067, respectively. Already at MAT, a climatic favorable region for orange crop production, each 10 mm of annual DEF increase, during the period of study, which leads to an average increase of 1.7597 FRBOX. For BEB, each increase of 10 mm of annual DEF may generate 0.97 fruits per box lose and the maximum variation (253 mm) can generate losses up to 24.36 fruits per box. The reduction of DEF, in other words, increase of available water, for BEB and MAT

Region: BAURU				Quality Parameters				Yield Parameters		
Dependent Variables				RATIO max	BRIX max	KGSS max	ACIDITY me an	FRBOX me an	WFRUIT me an	
Phenology				DEF angular coefficients						
Year 1 - Development	APR	B. F.			0.0849			1.3875		
	MAY					-0.0076				
	JUN									
	JUL	V. D.		-0.0190				2.3384		
	AUG			-0.0707	0.0173	0.0038		0.1273	-0.0004	
	SEP		FLO			0.0244			-0.0485	
	OCT					-0.0280	-0.0024	-0.0033	-0.2853	-0.0004
	NOV					0.0262	0.0056			
	DEC									
	Year 2-Production	JAN								
FEB										
MAR										
APR										
MAY										
JUN										
JUL										
AUG										
SEP			FLO		0.0638			-0.0077		
OCT										
NOV						0.0099				
DEC						0.0070				0.0002
JAN										
FEB										
Linear Coefficient				13.5827	9.7584	2.5709	1.7489	240.8113	0.1640	
p-value Regression				0.0372	0.0006	0.0471	0.0487	0.0330	0.0021	
Calibration		MAPE (%)		0.20	0.002	0.22	0.16	0.19	0.01	
		R² Adj		0.99	0.99	0.99	0.99	0.99	0.99	
Tests		MAPE (%)		6.59	4.14	7.40	7.39	5.25	2.73	
		R² Adj		0.94	0.99	0.95	0.99	0.80	0.94	

Figure 6. Agrometeorological models in function of monthly water deficiency for estimate mean and maximum annual parameters of “Valência” orange grafted on rangpur lime for Bauru region. Legend: B.F.: bud formation; V.D.: vegetative dormancy; FLO: flowering; DIV: cell division; DF: cell differentiation; C. E.: cell expansion; E: early crops; MID: midway crops; L: late crops.

showed increase at FRBOX while that for BAU and mainly for LIM had opposite effect. For these regions without restrictions for orange crop production, the reduction of annual DEF could lead to losses at yield, perhaps because of rain during the maturity phase.

An exact quantity of water applied to fill the plants needs at each phenological phase comes as an alternative to improve the water use efficiency and minimize losses at yield, by using irrigation with regulated deficiency. One of the difficulties of those techniques is in quantifying the permissible water deficiency for each phase (Carr et al., 2012). Researches about stress tolerance mechanisms and the understanding of the

interactions of those with the biotic factors are needed to help the citrus producers deal with stress problems that can result in positive or negative effects, depending on the phenological phase of the plant (Syvertsen and Garcia-Sanchez, 2014). The agrometeorological models developed in this paper relate the yield and quality parameters to the monthly DEFs of the regions (Figures 6, 7, 8 and 9), highlighting the phenological phases that are more sensitive to water stress by the use of adjusted coefficients; this can be a tool for quantify this tolerable DEF value for each phase during the plant cycle. For a better understanding of the limiting factors and estimation of yield and quality, parameters were established in

Region: BEBEDOURO				Quality Parameters				Yield Parameters		
Dependent Variables				RATIO max	BRIX max	KGSS max	ACIDITY mean	FRBOX mean	WFRUIT mean	
Phenology				DEF angular coefficients						
Year 1 - Development	APR	B. F.								
	MAY			-0.032					-0.0004	
	JUN						0.006			
	JUL	V. D.				-0.009	-0.001			
	AUG		FLO	-0.087	0.050			1.645	-0.0005	
	SEP							-0.601		
	OCT			0.069		0.005		-0.198		
	NOV									
	DEC									
Year 2-Production	JAN									
	FEB									
	MAR									
	APR			0.173			-0.003			
	MAY					0.018	0.015		-0.001	
	JUN					0.050	0.018			
	JUL					-0.022	-0.021	-0.008	-0.377	0.0004
	AUG								-0.0001	
	SEP		FLO	0.061						
	OCT					-0.017				
	NOV									
	DEC									
	JAN									
	FEB									
Linear Coefficient				17.346	11.387	3.350	1.399	251.951	0.175	
p-value Regression				0.020	0.036	0.044	0.038	0.018	0.027	
Calibration		MAPE (%)		1.09	1.38	0.62	0.49	0.81	0.41	
		R² Adj		0.97	0.86	0.94	0.95	0.91	0.96	
Tests		MAPE (%)		8.80	7.00	6.57	13.14	9.27	9.23	
		R² Adj		0.99	0.65	0.96	0.64	0.86	0.86	

Figure 7. Agrometeorological models in function of monthly water deficiency for estimate mean and maximum annual parameters of “Valência” orange grafted on rangpur lime for Bebedouro region. Legend: B.F.: bud formation; V.D.: vegetative dormancy; FLO: flowering; DIV: cell division; DF: cell differentiation; C. E.: cell expansion; E: early crops; MID: mid crops; L: late crops.

multiple linear models with monthly DEFs as independent variables during the cycle of “Valência” orange.

For BAU region (Figure 6) the estimation model of maximum RATIO (equation 13) had accuracy (MAPE) and precision (R² adj) satisfactory, with values of 6.59 and 0.94% respectively at validation and p-value of 0.0372. Considering the maximum RATIO value from the period (17.70 points), the percentage error of 6.59% represents an error by the modes of 1.16 points. At BAU, for all parameters, the most part of DEFs become concentrated at development year (1), between the phases of vegetative dormancy to the beginning of cell expansion. The yield (FRBOX) showed more sensibility to DEF at bud formation (April) and at vegetative dormancy (July), in which the angular coefficients were

higher. This result is in accordance with Camargo et al. (1999) that noticed that VACR yield is particularly sensitive to water deficiency at the beginning of flowering phase (August).

$$\begin{aligned}
 RATIO_{max\ BAU} = & -0.019 \times DEF_{JUL(1)} - 0.0707 \times DEF_{AUG(1)} - 0.0419 \times DEF_{APR(2)} \\
 & + 0.0638 \times DEF_{AUG(2)} + 0.0607 \times DEF_{SEP(2)} + 13.5827
 \end{aligned}
 \tag{13}$$

Where, (1) development year and (2) production year. Bebedouro (Figure 7) had the same performance of BAU in relation of vegetative dormancy, being the most sensitivity phase to DEF, along with the start of flowering (August). This region in company of LIM and MAT had the main inducer flowering variable, the DEF (Ribeiro et

Region: LIMEIRA		Quality Parameters				Yield Parameters		
Dependent Variables		RATIO	BRIX	KGSS	ACIDITY	FRBOX	WFRUIT	
		max	max	max	mean	mean	mean	
Phenology		DEF angular coefficients						
Year 1 - Development	APR		0.082	0.027		1.349	-0.001	
	MAY	B. F.						
	JUN		-0.160		0.009			
	JUL	V. D.	-0.011			1.057	-0.001	
	AUG					0.356	0.000	
	SEP	FLO	-0.051			0.005		
	OCT							
	NOV							
	DEC							
	Year 2-Production	JAN						
FEB								
MAR								
APR			-0.011	-0.005				
MAY			-0.192	0.082	0.029	0.017	2.187	-0.001
JUN			-0.022	-0.010		-1.560	0.001	
JUL			0.051					
AUG			0.012	0.002	-0.003			
SEP		FLO						
OCT								
NOV								
DEC								
JAN								
FEB								
Linear Coefficient		19.760	10.580	2.515	1.157	243.182	0.178	
p-value Regression		0.005	0.007	0.042	0.062	0.007	0.057	
Calibration	MAPE (%)	1.12	0.62	1.20	2.53	0.48	1.17	
	R² Adj	0.96	0.96	0.85	0.70	0.96	0.82	
Tests	MAPE (%)	2.74	4.71	4.33	8.72	7.86	9.22	
	R² Adj	0.95	0.99	0.98	0.83	0.46	0.64	

Figure 8. Agrometeorological models in function of monthly water deficiency for estimate mean and maximum annual parameters of “Valência” orange grafted on rangpur lime for Limeira region. B.F.: bud formation; V.D.: vegetative dormancy; FLO: flowering; DIV: cell division; DF: cell differentiation; C. E.: cell expansion; E: early crops; MID: mid crops; L: late crops.

al., 2006), being the performance of the flowering one of the responsible factors for losses or gains at yield. The

estimation model of mean FRBOX for BEB was significant with p-value of 0.018 and accuracy and

Region: MATÃO			Quality Parameters				Yield Parameters	
Dependent Variables			RATIO max	BRIX max	KGSS max	ACIDITY mean	FRBOX mean	W FRUIT mean
Phenology			DEF angular coefficients					
Year 1 - Development	APR	B. F.			0.008		0.833	-0.001
	MAY		0.112		-0.010	-0.006	-1.126	0.001
	JUN			0.060				
	JUL	V. D.						
	AUG				0.007		0.943	-0.001
	SEP	FLO	0.104			-0.008	-0.828	0.0005
	OCT			-0.022				
	NOV							
	DEC							
	Year 2-Production	JAN						
FEB								
MAR								
APR								
MAY					-0.011	-0.004		
JUN			0.136	-0.068				
JUL				0.036				
AUG				0.056	0.014			
SEP		FLO				0.006	0.769	-0.001
OCT			0.069			-0.004		
NOV								
DEC								
JAN								
FEB								
Linear Coefficient			7.913	11.352	2.558	1.830	259.640	0.181
p-value Regression			0.007	0.047	0.029	0.005	0.061	0.026
Calibration	MAPE (%)		4.38	1.51	1.31	1.18	2.12	1.80
	R² Adj		0.90	0.84	0.89	0.97	0.81	0.89
Tests	MAPE (%)		14.14	17.35	9.64	20.06	5.50	5.09
	R² Adj		0.62	0.97	0.99	0.99	0.99	0.98

Figure 9. Agrometeorological models in function of monthly water deficiency for estimate mean and maximum annual parameters of "Valência" orange grafted on rangpur lime for Matão region. B.F.: bud formation; V.D.: vegetative dormancy; FLO: flowering; DIV: cell division; DF: cell differentiation; C. E.: cell expansion; E: early crops; MID: mid crops; L: late crops.

precision at validation of 9.27 and 0.86, respectively. For a mean yield of 275.18 fruits per box, the percentage error of 9.27% represents an error by the model of 25.5 fruits per box approximately.

The performance of FRBOX at LIM (Figure 8) was practically the same as BAU, being the bud formation (April) and vegetative dormancy (July) phases, which are the ones that are more sensitive to DEF at the development year. Paulino et al. (2007), found that for LIM region the number of fruits per plant showed significant correlation with DEF of July to September of the development year. At production year (2) the most sensitive period for DEF at LIM is between the end of fruit growth and start of maturity (May and June). Water

restrictions during these phases can lead to losses in yield and mainly in fruit weight (Garcia-Tejero et al., 2010). The estimation model for FRBOX at LIM showed high reliability, with p-value of 0.007, and accuracy (MAPE) and precision (R² adj) at validation of 7.86 and 0.46, respectively. For a mean yield of 280.58 fruits per box, the percentage error of 7.86% represents an error by the model of 22.05 fruits per box.

At MAT (Figure 9), the bud formation phase (April) was the most sensitive to DEF, the flowering (August to September) also presented sensibility to water deficiency. Martins and Ortolani (2006), observed the same results about flowering of VACR at the region. The best estimation model of mean FRBOX was the one of this region with

greater p-value of 0.061, great accuracy and precision values of 5.5 and 0.99%, respectively. The percentage error of 5.5% represents an error by the model of 15.15 fruits per box, considering an average yield of 281.11 fruits per box.

The water stress at production year (2) at the final phases of fruit growth and maturity (from May to September) were the ones that presented more effects of DEF on quality parameters (RATIO, BRIX and KGSS). Garcia-Tejero et al. (2010), affirmed that water deficiency at maturity affects the organoleptic characteristics of fruits and mainly the quality parameters. Briefly for BAU, the RATIO is the quality parameter that is more affected by DEF on maturity. For BEB and LIM are BRIX and KGSS and for MAT is BRIX.

Generally, at humid regions that have lower annual DEF, as BAU and LIM, the parameters estimation models of yield and quality were more accurate.

Conclusion

The 'Valência' orange grafted on rangpur lime had different sensitivities to the regional climate. Yield (FRBOX) was related to water deficiencies during bud formation (April) and vegetative dormancy (July) at Bauru and Limeira regions. The FRBOX at Bebedouro was more sensitive at the end of vegetative dormancy and the start of flowering (August) at bud formation (April). For the quality parameters (RATIO, BRIX and KGSS), all regions were more sensitive to water deficiency at maturity, especially Limeira. The agrometeorological models based on monthly water deficiencies showed good performance for yield and quality estimation of VACR for all regions.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Irrigation water pricing in Awash River Basin of Ethiopia: Evaluation of its impact on scheme-level irrigation performances and willingness to pay

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Irrigation water pricing has been considered as a tool to enhance water conservation in irrigated agriculture. We have evaluated the effects of water pricing on water management practices in Awash Basin Authority (ABA). The water charge has been collected over years with the aim to generate money for the Awash Basin Authority to cover operational expenses. Both the authority and water users see the charges paid as the contribution than as water demand management tool. Widespread irrigation inefficiency and low performances are more due to low level of irrigator's knowledge to manage water, poor water conveyance and distribution systems, public ownership over large and medium-scale irrigation schemes, and inability to measure and control water. Increasing water price under such service conditions will only add burden to farmers and unlikely to be feasible. Despite these poor water delivery services, users are willing to pay relatively more than they currently pay which could increase the income of the basin authority. Improvement in irrigation water management requires strengthening of irrigation research and extension services, building the capacity of irrigators, and improving irrigation system operation and maintenance services.

Key words: Irrigation, water pricing, irrigation performance, willingness to pay.

INTRODUCTION

In developing countries like Ethiopia, agricultural sector is the dominant food supplier to the nation and sources of livelihood for more than 85% of the population. Even if the country is known for its abundant water resources potential, this dominant economic sector depends entirely

on rainfall. Moreover, 90% of the annual renewable surface water resource is shared with other neighboring countries. With low industrial development and only 16% of the population living in urban areas, agriculture can be considered as the dominate consumer of water. On the

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other hand, as a result of poor agricultural water management practices, inefficiency in water use is common. According to the Growth and Transformation Plan of the country, agricultural land equipped with small-scale irrigation will expand from 0.85 million ha during the base period (2010/2011) to 1.85 million ha at the end of the planning period (2014/2015). Irrigation development is increasingly considered as one of the strategic pillar for ensuring food security and alleviating poverty.

With increasing population and development needs in agriculture, industry, urbanization and ecosystem services as well as the trans-boundary nature of the country's water resources, the demand for water will increase. Efficient allocation and use of water becomes imperative. Nevertheless, operation and management of the existing water infrastructures such as irrigation systems is dominated by traditional practices and not well supported by scientific knowledge and tools to enhance efficiencies. These practices are characterized by poor overall performances and inefficient water uses (Awulachew and Mekonen, 2011). The major concerns emanating from such deficiencies in irrigation management are over-irrigation and related poor drainage, rise in groundwater levels, water-logging, salinity and alkalinity development in irrigated areas of the country (Wagnew, 2004; Michael and Awulachew, 2007; Zelalem, 2010; Girma and Fantaw, 2005).

Undesirable consequences of irrigation have the potential to degrade soil fertility, reduce land and water productivity and leading to significant social and economic losses to individuals, households, local communities and country in general (Hussain and Hanjira, 2004; World Bank, 2006). Owing to its flat topography and water availability, Awash River Basin (ABA) is intensively developed for irrigation since late 1950s. The country's limited large-scale irrigation projects are found in this basin. Increasing development of salinity in lower Awash Valley due to mismanagement of irrigation water represents a serious threat to sustainability of irrigation schemes (Ayenew, 2007).

Several researchers advocate water pricing as policy and economic instrument that enhance efficient use of water (Abu-Madi, 2009; Perry, 2001; Speelman et al., 2009; Dinar and Mody, 2004; Ortega et al., 2004). However, efficient allocation of water through water pricing requires getting the right pricing which in turn is sensitive to social, physical, institutional and political settings (Johansson, 2000).

Awash River Basin is the only basin in Ethiopia where irrigation water pricing is practiced. Awash Basin Authority, which was legally established in 1998 as Awash Basin River Basin Administration Agency has been responsible for integrated management of the waters of the basin. It is reestablished and named as ABA in 2000. Any significant water diversion from the river for irrigation purpose requires the approval of the authority. The ABA collects water charges on volumetric basis from all legal water users who are developing greater than 2 ha of

land. The payment is categorized into water charge (3 Birr¹ per 1000 m³ of water), operational service payment (84.10 Birr) per hectare of land served per year. The charge rate for irrigation water was set in 1994 by the then Ethiopian Water Resources Development Authority and never modified since then. Charging water use is legalized with the Ethiopian water management proclamation number 115/2005. As stated in the same proclamation, charge for water use is to be determined by the council of ministers. The country has established river basin councils and authorities for all 12 major river basins with the proclamation number 534/2007. This proclamation also stipulates the legality of charging users for water.

The impacts of water pricing on water demand and overall performance of irrigation practice in the Awash Basin has not been assessed. Hence, the aim of this paper is to evaluate the effects of water and service charges on scheme-level irrigation performances and users' willingness to pay for irrigation water.

MATERIALS AND METHODS

Description of the basin

Awash River basin is one of the major 12 river basins in the country. It is part of the Central Rift Valley in Ethiopia ranging from 8.5°N to 12°N and covering an area of about 112,696 km². The basin covers the central and northern part of the rift valley and is bounded to the west by Blue Nile Basin, to the southeast by Rift valley Lakes Basin and to the south by Wabi Shebele Basin (Figure 1). It originates from Central West part of Ethiopia, flowing 1200 Km long, and provides a number of development opportunities to the country. It is the most intensively utilized river basins in the country. Awash River originates and remains entirely in the country. The river basin has a lowest elevation of 210 m and a highest elevation of 4195 m. The total mean annual flow of the river is estimated to be 4900 million cubic meters per year. Modern irrigation in Ethiopia has started in the Awash Basin during the late 1950s with the objective of producing industrial crops (Awulachew et al., 2007). The irrigation potential of the basin is estimated to be 134,121 ha.

Data used and methods of collection

Primary data

The primary data were generated using structured questionnaires and interviews. About 29 schemes were systematically selected from legally registered irrigation water users. Generally, about 20 small-scale (command area less than 200 ha), 5 medium-scale (200 to 3000 ha) and 4 large-scale (greater than 3000 ha) irrigation schemes were included in the assessment.

The willingness to pay for irrigation, water was assessed using structured questionnaires. The interview was done using two different "bidding games". The bidding processes ascertained the respondents maximum WTP for a 1000 m³ of irrigation water which is costing only 3 Birr over years. Getting water without price was given as a starting bid and the maximum bid was set 10 Birr per 1000 m³. Then the respondents were allowed to select the final

¹ Birr is Ethiopian currency (1 Birr = 0.05139 USD)

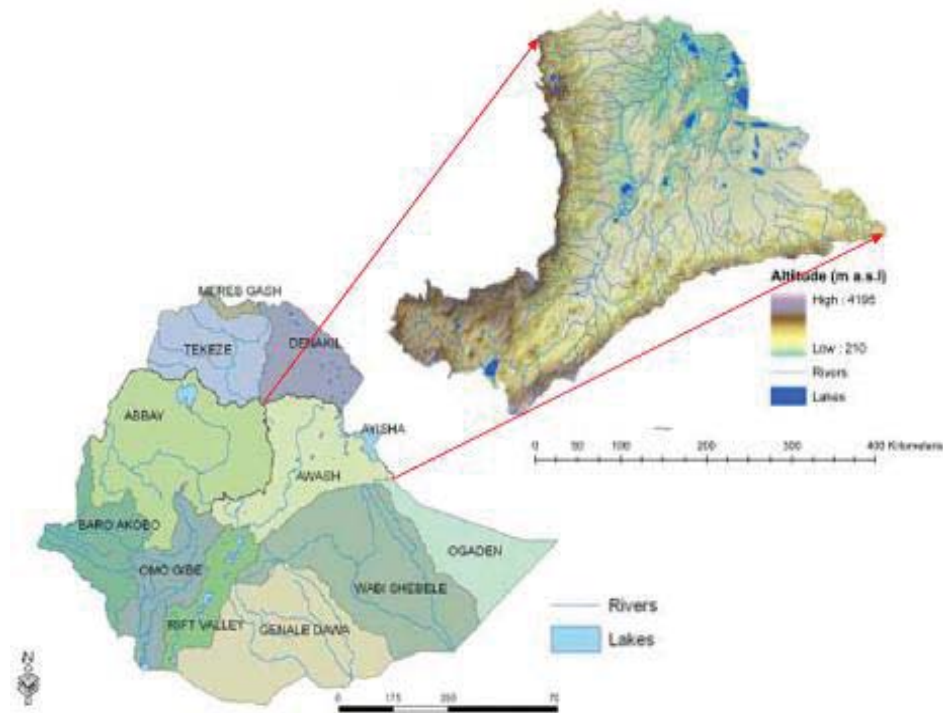


Figure 1. River Basins of Ethiopia, location and topography of Awash Basin (Awulachew et al., 2007).

price they would like to pay from 0 to 10 Birr for improved irrigation water supply.

A total of 31 irrigation water users out of 66 legally registered users were interviewed to collect information relevant to willingness to pay for irrigation water. The first round survey and related field observations and data collection were done from March to June 2011 and the second round from September to November 2012.

Secondary data

Scheme specific data such as area cultivated, amount of water diverted to each scheme each year, water fees, service charges, operation and maintenance fees collected for five consecutive production years (2005/2006-2009/2010) were collected from Awash Basin Authority. Climate data such as maximum and mean minimum temperature, relative humidity, wind speed and daily sunshine hours were obtained from nearby meteorological stations viz. Melka Worer, Metehara, Nura Era and Wonji. These data were used to estimate crop and irrigation water requirements using CROPWAT version 8.0.

Market prices of irrigated crops were obtained from the Ethiopian central statistical authority official website (<http://www.csa.gov.et>), annual reports of the Upper Awash agro-industry, sugar estates and their out growers.

Measurements of performance indicators

Irrigation performance indicators considered in this study are mainly: 1) output indicators such as land and water productivity, 2) water management indicators like: relative water supply, relative irrigation supply, and irrigation efficiency. The description of these indicators

and methods of their measurement are given in the following sections.

Land productivity

Land productivity denotes the ratio of farm output, that is, crop yield or its monetary value to cultivated (irrigated) area. Land productivity was calculated for all sampled irrigation schemes over 5 successive production years. It is expressed in terms of harvested crop yield per unit of irrigated area (tons/ha).

Water productivity (WP)

Water productivity can be expressed in terms of physical water productivity, that is, the ratio of agricultural output to the amount of water consumed. Whereas economic water productivity means the value derived per unit of water used (Vazifedoust et al., 2008; Ali and Talukder, 2008; Molden et al., 2010). WP was measured in terms of harvested crop yield per unit of water diverted or supplied (kg/m^3)

Water management indicators

Under this category, indicators relevant to measure and compare amount of water demanded and applied were used. These indicators are described as follow:

i. Relative water supply (RWS): The ratio of the total volume of water applied (irrigation plus effective rainfall) to the volume of water required to be applied during the period. Here, the later is only crop water demand.

2. Relative irrigation supply (RIS): The ratio of total volume of irrigation water delivered to the farm to the volume of irrigation water demanded (net irrigation requirement).

These two indicators have been used most often to provide a general sense of whether there is an adequate amount of water or whether the amount of irrigation water supplied is excessive (Molden, et al., 1998; Clemmens and Molden, 2007).

3. Irrigation efficiency (Ei): This is the inverse of relative irrigation water supply as given by Molden et al. (1998) and Jensen (2007).

Econometric models

Logit model was used to identify determinants of willingness to pay for irrigation water. When using the logit model, the dependent variable assumes only two values which show the occurrence and non-occurrence of events (YES or NO). In the first instance, the following factors were identified with the expectation that they affect willingness to pay of water users: educational level, household size, land size, off-farm income, access to credit, irrigation experience, slope of land, and distance to market. The main objective of the logit model is to model the relationship between these factors and the probability of household's willingness to pay for a randomly offered bid price. In this setting, the dependent variable is dichotomous and assumes 1 if the household is willing to pay the specified price level for the use of irrigation water and 0 otherwise. The binary choice model which is used to determine the farmer's average willingness to pay for irrigation water and the determinants of willingness to pay of irrigation users can be given as:

$$Y^* = X_i \beta + \varepsilon \quad (1)$$

Where Y^* is the response variable, X_i is a vector of explanatory variables, β is a vector of parameters to be estimated, and ε is the error term. However, the response variable Y^* is unobservable. The assumption is that an individual chooses to pay if the utility difference exceeds a certain threshold level, which can be set to zero. As a result, if $Y_i = 1$ (paying for water) if $Y^* > 0$ and $Y_i = 0$ (not paying for water) otherwise. Therefore, the probability that a household is willing to pay the specified bid level for irrigation water is given by:

$$P(Y_i = 1/X) = P(Y^* > 0) = P(X_i \beta + \varepsilon > 0) = F(X_i \beta) \quad (2)$$

Where, F is the cumulative distribution function. This is structural model for estimating the probability and it can be estimated either using a probit or logit model, depending on the assumption on the distribution of the error term (Green, 2003).

To estimate water users willingness to pay (WTP) for irrigation water, probit model was employed (Haneman et al., 1991). The probit model estimation of the average WTP only considers the bid values with no consideration of other factors that influence household decision on willingness to pay. Therefore, by choosing the logistic cumulative distribution function in Equation (2) for the logit model, the probability that the household is willing to pay for irrigation water is given by:

$$P(Y_i = 1/X) = P(Y^* > 0) = F(X_i \beta) = P_i = \frac{e^{Z_i}}{1 + e^{Z_i}} \quad (3)$$

Where Z_i is a linear function of n - explanatory variables (X) and can be stated as

$$Z_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \dots + \beta_k X_{ni} \quad (4)$$

If P is the probability that the household is willing to pay for the use

of irrigation water, then $1-P$, the probability of not willing to pay, is given by:

$$1 - P = \frac{1}{1 + e^{Z_i}} \quad (5)$$

However, this expression can be written as

$$\frac{P}{1 - P} = \frac{1 + e^{Z_i}}{1 + e^{-Z_i}} = e^{Z_i} \quad (6)$$

Where $P/(1-P)$ is the odds ratio or the ratio of the probability that a household is willing to pay for irrigation water supply to the probability that a household is not. Taking the natural logarithm of Equation 6, the log of the odds ratio, which is known as logit model is given by:

$$L_i = \ln\left(\frac{P}{1 - P}\right) = \ln(e^{Z_i}) = Z_i \quad (7)$$

Then the Logit Model becomes,

$$Z_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \dots + \beta_k X_{ni} + \varepsilon \quad (8)$$

In which β_0 is an intercept which indicates the log-odds in favor of paying for the use of irrigation water when the coefficients of all included explanatory variable are assumed to be zero. But, β_1 , β_2 , β_3 and \dots , β_k are slope parameters to be estimated in the model. The slope tells how the log-odds in favor of paying for the use of irrigation water change as each independent variable changes. Equation 8 was used to identify factors affecting water user's willingness to pay for irrigation water.

Assuming that the probability of irrigator's willing to pay for irrigation water supply is a linear function of bid value; the following probit model is specified to determine the average WTP of irrigation users:

$$Prob(Y=1/Bid \text{ Level}) = \beta_0 + \beta_1 Bid + \varepsilon \quad (9)$$

By dividing the intercept (β_0) by the coefficient associated with the bid value (β_1) of Equation 9, the average willingness to pay for irrigation water can be determined.

RESULTS AND DISCUSSION

On-farm irrigation performance

Land development and water use

The total amount of water diverted annually by registered water users as measured by the basin authority is given in Table 1. Mean annual water used to develop 38,157 ha of irrigated area is about 819.13 million cubic meters. This is about 16.7% of estimated annual flow of the Awash River. In return for use of this amount of water, the authority collected on average 3.9 million Birr per year which is about 197,465 US\$. This amount does not include maintenance charge which is variable based on area served and maintenance needs. For instance the maintenance charge was about 145 and 433 Birr/ha in 2006 and 2010, respectively. However, maintenance

Table 1. Number of clients, area developed, amount of water used and water charges collected every year.

Year	Number of clients	Area cultivated (ha)	Water used (million m ³)	Total cost of water service (Birr)
2006	56	37,572	844.06	3,861,730
2007	74	41,688	911.75	4,427,283
2008	76	41,427	821.53	3,961,898
2009	59	32,006	722.50	3,339,218
2010	63	38,091	795.80	3,868,557
Mean	66	38,157	819.13	3,891,737

services provided by the authority are most often limited to removal of silt from the main systems. The basin authority lacks information system for collection, management and processing of hydrological and land resources of the basin for effective planning and sustainable utilization of land and water.

Irrigation efficiency and water pricing

Figure 2 shows the irrigation efficiencies of 27 farms operated over 5 years. The efficiencies varied from 10% to more than 95%. However, 60% of the total samples considered (n = 135) operate with an irrigation efficiency of less than 35%. Whereas 75% of the total samples considered could achieve only an efficiency value of less than 45%. Higher efficiencies greater than 55% were obtained only by 14% of the total samples. The overall average irrigation efficiency is about 40%. Negative consequences of such inefficiency are widespread in middle and lower regions of the basin. Over-irrigation together with absence of proper drainage system in most of irrigation schemes in the basin has been causing rise in groundwater table, water-logging and salinity (Girma and Fantaw, 2005; Zelalem, 2010). These problems are increasingly threatening the sustainability of irrigation development in the basin (Dagnachew and Ayenew, 2006; Ayenew, 2007).

The extents of water applied to 18 cotton farms over 5 consecutive years are presented in Figure 3. Irrigation water depth applied is highly variable ranging between 228 and 4223 mm per season. Mean seasonal crop water requirement for cotton varied between 760 and 870 mm. In comparison to the water demand, about 89% of sample cotton farms and 72% of sugar cane farms are over-irrigating their farms. Surface irrigation methods such as furrow, boarder and basin irrigation are widely practiced in the basin. These water application methods together with low level of irrigator's knowledge are contributing to inefficient irrigation. The fact that most of the irrigators do not keep records of their production costs and revenue, they do not feel the income reduction effects of the low water charge.

Observed effects of over-irrigation and lack of proper drainage in the basin are wide-ranging. As a result of

groundwater rise, Beseka Lake which is highly saline lake found in the middle of the basin is increasingly expanding occupying many settlement areas and agricultural lands (Tamiru et al., 2006; Ayenew, 2007).

In principle, pricing irrigation water is expected to enhance water conservation by reducing demand (Dinar and Mody, 2004; Rogers et al., 2002; Molle and Berkoff, 2007). However, getting the right price that encourages irrigators to conserve water by remaining in irrigation business is a challenge (Johansson, 2000). Moreover, several researchers report the low price elasticity of irrigation water demand (Salman and Al-Karablieh, 2004; Doppler et al., 2002; Ruijis et al., 2008; Yang et al., 2003). Berbel and Gomez-Limon (2000) indicated that water pricing as a single instrument for controlling water use is not an appropriate means to significantly reduce agricultural water consumption. This is because consumption is not reduced until prices reach such a level that they negatively affect farm income and agricultural employment. A rational farmer may respond in different ways to falling net income resulting from higher water pricing which according to Molle et al. (2008) include: (a) saving water by improving on-farm water management practices, (b) adopting improved irrigation technology, (c) shifting cropping patterns to less water demanding crops, (d) renting out land, or discontinuing agriculture in the case of a tenant, (e) other secondary responses (illegal water use, bribery, and tampering of structures). Most of these measures are targeting more to the reduction of water demand and not enhancing production which in turn lead to decreasing income. Salman and Al-Karablieh (2004) found in the highland areas of Jordan that water prices up to US\$ 0.35/m³ reduce farmers' income without any effect on the production structure, but prices higher than US\$ 0.35 reduce the cultivated area and drive most agricultural production alternatives into unprofitable situations. Speelman et al. (2009) also reported that further increases in water prices beyond a certain level have not only limited additional effect on the efficient use of water because the higher prices do not only decreases water use but also reduce the profit of the farmers. At higher water pricing rate some farmers which are not profitable anymore may quit from farming activities which leads to water saving at sectoral level (Speelman et al., 2009) but

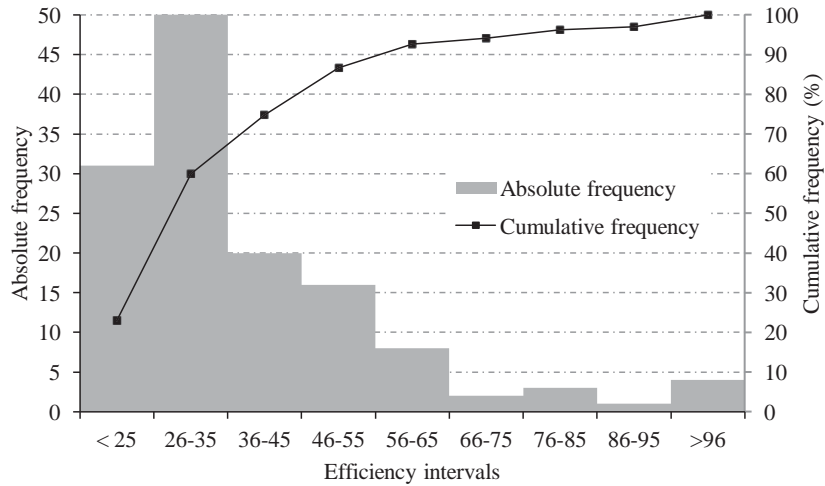


Figure 2. Absolute and cumulative frequency of irrigation efficiency in Awash Basin (total number of farms 27 * 5 years data, n = 135, average efficiency = 40%).

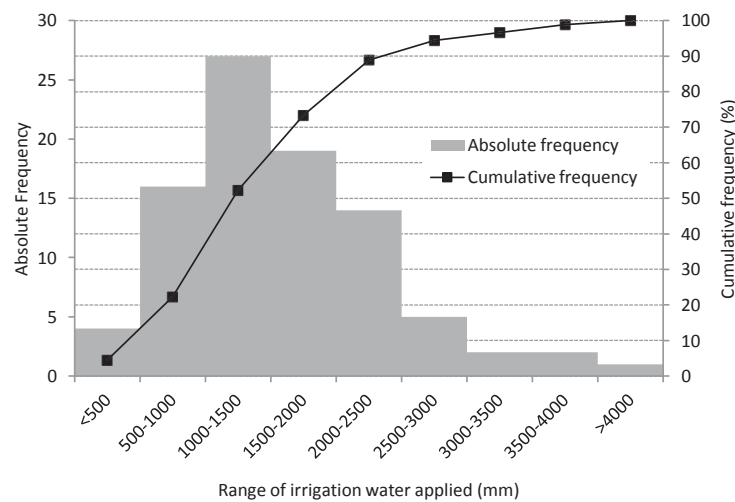


Figure 3. Irrigation water applied (mm) to 18 cotton farms over 5 consecutive years (2005-2010).

leads to reduced agricultural production which in turn affects negatively the livelihoods of rural community.

The pricing level practiced in Awash River Basin (0.00015 US\$/m³) is low compared to 0.33 US\$/m³ Palestine (Abu-Amadi, 2009), 0.024 US\$/m³ in Jordan (Doppler et al., 2002), 0.02 US\$/m³ in Morocco (Dinar and Mody, 2004). Comparison of farms operating under water pricing and non-pricing system in the basin indicated that there is no significant difference in their overall performance including irrigation efficiency (Kibrom, 2012). This leads to the conclusion that the current pricing mechanism as a financial incentive is not adequate to encourage irrigators to conserve water and also invest in water saving irrigation technologies.

On the other hand, it is difficult to relate inefficiencies observed in the basin with low level of pricing. Because, excess application of water than the crop demand is attributed to the “more water input – more yields” perception of local irrigators, lack of knowledge about soil – water – plant relationships and uncertainty of getting water for the next irrigation and associated risk aversion tendency of farmers. Moreover, poor water delivery, distribution and application systems are contributing to water losses and hence inefficiencies. Most of all, the pricing scheme and mechanism of collecting charges are focusing more on fund raising for the authority than encouraging efficient water management and covering service costs. Proclamation number 129/1998 legalizes

Table 2. Crop productivity (tons/ha).

Year	Cotton (18 farms)				Sugarcane (6 farms)				Onion (3 farms)			
	Mean	Min	Max	St.D	Mean	Min	Max	St.D	Mean	Min	Max	St.D
2006	2.45	1.31	3.50	0.64	176.6	145.0	200.0	23.3	16.8	12.5	20.0	3.88
2007	2.66	1.60	3.60	0.50	172.8	135.0	200.0	25.0	18.4	13.2	22.0	4.61
2008	2.56	1.50	3.20	0.57	173.8	116.7	205.0	36.8	16.5	14.0	18.0	2.18
2009	2.44	1.31	3.30	0.60	182.9	116.6	212.5	38.5	19.3	13.5	23.0	5.11
2010	2.34	1.20	3.50	0.63	182.9	116.6	212.5	38.5	17.9	13.8	20.0	3.52
Mean	2.49	1.38	3.42	0.59	177.8	126.0	206.0	32.88	17.8	13.4	20.6	3.90

the basin authority to collect water charges to cover its budget requirements. In some instances, group of farmers are using common off-take structures. The amount of water measured at these control points is divided by the number of served farms to determine the water charge. Under such condition where sometimes involved farms are equally charged for water, there is no incentive to conserve water. Although the principle of volumetric water pricing system is adapted, inability to accurately measure the quantity of water provided to or received by each irrigator remains a crucial problem.

Output performances

Productivity (tons/ha)

Productivity performance of irrigated crops can be measured using indicators such as yield or monetary value of the total produce per unit of area. Most often the land productivity is measured in terms of yield per units of land used. The values of land productivity in terms of yield per hectare for cotton, sugarcane and onion over consecutive five years (2006-2010) are given in Table 2. The values varied not only from year to year but also between farms within the year.

The five years consecutive observation of cotton productivity in eighteen farms in the Awash Basin showed results that varied from 1.2 to 3.6 tons/ha. Under optimum irrigation practices at Melka Werer research center in the same Basin, the productivity of cotton was reported to be 3.5 tons/ha (Tilahun, 2010) which is close to the maximum production obtained from eighteen farms over five years. The mean productivity across the years ranges from 2.34 to 2.66 tons/ha. Pereira et al. (2009) reported an average productivity of about 3.7 tons/ha for cotton in central Asia under full irrigation.

The productivity of sugarcane as measured in six farms over five years ranged from a minimum of 116.6 tons/ha to a maximum of 212.5 tons/ha. The standard deviation (St.D) varied from 23.3 to 38.5 tons/ha. This productivity gap represents huge potential that could be tapped through improvement of management practices. FAO (2012) considers 120 tons/ha of fresh cane yield as a

good yield worldwide. The productivity of onion ranged from 12.5 to 23.0 tons/ha and the average is about 18 tons/ha. Compared to the production under research condition at Meka Werer in the same Basin which is about 35 tons/ha (Tilahun, 2010), the production obtained from three farms over five years is low. Kumar et al. (2007) reported that an average productivity of onion in Punjab areas of India under full irrigation was about 32 tons/ha which is by far higher than this result.

Water productivity (WP)

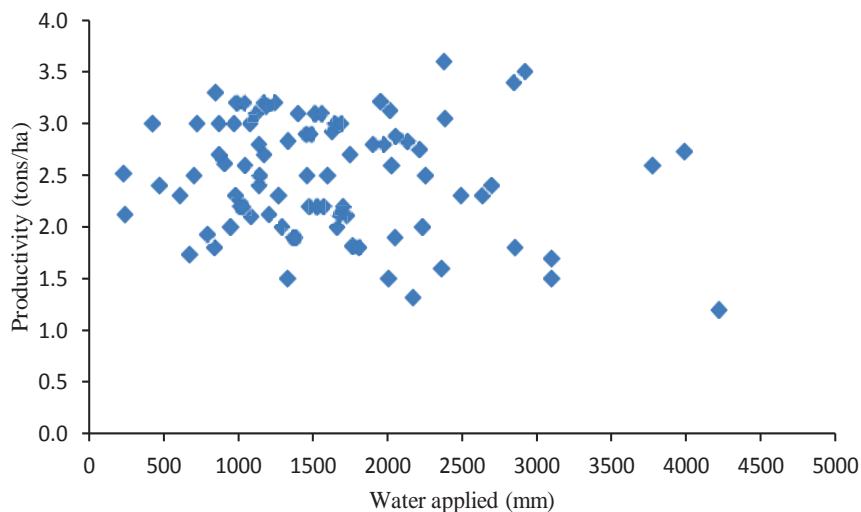
The basic idea behind relating the amount of crop yield produced to the amount of water used is that the water consumed by transpiration is in exchange for the assimilation of carbon dioxide (through plant stomata) leading to the production of biomass (that is, the total volume of vegetative matter produced) of which usually only a part is harvested as yield (Perry et al., 2009). However, it is difficult most often to make separation between water used for transpiration and evaporation. As a result total amount of water applied to the field during the production period was considered to measure WP (Ali and Talukder, 2008).

The mean water productivity of crops considered is presented in Table 3. The values of WP ranged from 0.03 to 0.52 kg/m³ for cotton, 4.4 to 13.5 kg/m³ for sugarcane and 1.0 to 1.8 kg/m³ for onion. Evidently, inefficiencies discussed in the previous sections have negative influences on WP. In areas like Awash Basin, where water scarcity is not yet an issue, WP has got less attention. Irrigators are less interested in high WP value unless the resulting crop yield is increased.

Zwart and Bastiaansse (2004), after reviewing more than 16 published sources from different countries, have found that the water productivity of cotton varies between 0.10 and 0.37 kg/m³. The results found in Awash River Basin shows maximum water productivity value of about 0.52 kg/m³ which is greater than literature value. Regassa et al. (2007) found that the water productivity of cotton and sugarcane in India is 1.70 and 5.95 kg/m³ under conventional irrigation, respectively. The authors provided evidences of enhanced water productivity by

Table 3. Average water productivity of different crops (kg/m^3).

	2006	2007	2008	2009	2010	Mean	St. D	Min/Max
Cotton	0.15	0.17	0.15	0.15	0.17	0.16	0.09	0.03/0.52
Sugarcane	6.9	8.1	7.0	8.2	9.2	7.9	2.7	4.4/13.5
Onion	1.3	1.5	1.4	1.6	1.6	1.5	0.23	1.0/1.8

**Figure 4.** Productivity of cotton versus depth of water applied (18 farmers over 5 seasons).

adoption of water saving irrigation technologies. Compared to the water productivity range of 3 to $10 \text{ kg}/\text{m}^3$ given by Molden et al. (2010) for onion, the values found here are low.

Under current condition wherein water scarcity is not a problem, concerns about WP is related to mitigation of undesirable consequences of over-irrigation such as water-logging, salinity and yield reduction. The WP in the basin could be enhanced by improvement of the irrigation management practices which are currently inefficient as described in the previous sections.

Figure 4 shows the relationship between amounts of water applied and cotton yield per unit of land cultivated. The common trend of crop-water-production function depicts that the crop yield will increase with increasing water application up to a certain optimum level which results a maximum crop yield beyond which additional water application results in decreasing productivity. However, the results found here show decreasing trend indicating excess application portion of crop-water-production function.

The results show that there are significant productivity differences between irrigators who applied more or less same amounts of water. This might be attributed to differences in management practices including timing of water application and other inputs such as fertilizers. This

variation in water use efficiency suggests that there is significant scope for inefficient farms to improve their practices to use water and other inputs more productively.

Irrigation water supply performances

Water supply performances were evaluated using three performance indicators, that is, relative irrigation supply, relative water supply and water delivery ratio. The results are presented in Tables 4 and 5.

The results show that the values of all indicators considered are greater than unity implying that water supplied to the farms were in excess of the requirements. The average relative irrigation supply (RIS) was greater than 3 under cotton and sugarcane farms. This indicator compares specially the amount of irrigation water diverted to the fields with the amount of irrigation water actually required.

The relative water supply (RWS) on the other hand relates the total volume of water applied (irrigation plus effective rainfall) to the volume of water required by the crops. The values of RWS range from 2.18 to 2.38 for cotton, from 1.69 to 2.50 for sugarcane and from 1.65 to 2.39 for onion. Results of RIS and RWS under this

Table 4. Average seasonal relative irrigation supply (RIS).

Crop	2006	2007	2008	2009	2010	Mean
Cotton (18 farms)	3.27	2.83	3.68	3.07	2.89	3.14
Sugarcane (6 farms)	3.86	3.29	4.03	3.33	2.10	3.32
Onion (3 farms)	2.95	2.57	3.35	3.99	1.65	2.90

Table 5. Average seasonal relative water supply (RWS).

Crop	2006	2007	2008	2009	2010	Mean
Cotton (18 farms)	2.38	2.18	2.56	2.29	2.32	2.35
Sugarcane (6 farms)	2.50	1.97	2.38	2.00	1.69	2.11
Onion (3 farms)	2.25	2.39	2.35	2.44	1.65	2.21

section validate the finds of oversupply of water discussed under irrigation efficiency.

Performance of charge collection

Mean annual collected charges by ABA in relation to area developed and annual farms revenues are presented in Table 6. The water charge collected from farms ranges from 1.5 to 3.4% of the total farm revenue which is comparatively very low.

The largest proportion of payment is due to service charges and O&M. These cost categories are not related to amount of water used. Rather they are dependent on area cultivated and hence cannot encourage irrigators to use water efficiently. As argued by Speelman et al. (2009), the effect of irrigation charges on agricultural water use efficiency might be insignificant if irrigation water costs represent too small a proportion of the total production costs. However, as the total costs of production inputs are not considered here, it is difficult to judge the value of water in relation to total revenue.

Results of econometric models

Determinants of WTP

The results of the logit model, to identify factors affecting water user's willingness to pay for irrigation water are presented in Table 7. As it can be seen from the coefficient column, some factors such as educational level, household size, land size, land slope and distance to market are negatively related to WTP. Whereas factors like bid level, household income, off-farm income, access to credit, experience of irrigation are positively related to WTP.

Relatively educated respondents had the fear of increased price if they identify themselves willing to pay.

Speelman et al. (2008) found that education has got insignificant impact on efficiency of water which they attributed to low average education level in their samples. Family size is also negatively related to willingness to pay off a household.

A household with access to credit and off-farm activities is willing to pay for irrigation water than those households with no access. Irrigators with longer irrigation experiences are also more willing to pay than those relatively short periods of experience.

The land size is also negatively related to WTP. This is because, as the size of the land increases so does the total annual water charges and this decreases the willingness to pay off a household for irrigation water supply.

However, all the regressors have a significant impact on the willingness to pay of farmers for irrigation water supply, as the LR statistic is 16.39, whose p value is about 0.00889, which is very small.

However, a more meaningful interpretation of logit model is in terms of odds ratio, which are obtained by taking the antilog of the various slope coefficients. Thus, if the antilog of the off-farm income coefficient of 0.2159 is considered, then the result will be 1.2410. This suggests that farmers who are participating in off-farm activities are more than 1 times likely to pay for irrigation water than those who have no access to off-farm activities.

Average WTP for irrigation water

The average willingness to pay of irrigation users using the probit model is determined as: Average WTP = β_0/β_1 where β_0 is a constant term and β_1 is the coefficient of the bid level. The results of the model are presented in Table 8.

The result from the probit model shows that the average willingness to pay off a farm household for

Table 6. Annual water charges in relation to total revenue from cotton (mean over 5 years).

Farm number	Land cultivated (ha)	Total revenue (*1000Birr)	Charges (Birr)			% charge to total revenue
			Water	Service	O&M	
1	10.0	220	409	782	2,344	1.6
2	39.2	634	2,126	3,065	8,872	2.2
3	15.8	221	869	1,235	3,172	2.4
4	8.4	151	385	657	2,120	2.1
5	137.0	1,644	7,576	10,711	29,082	2.9
6	15.0	177	1,203	1,173	3,718	3.4
7	38.2	682	1,776	2,986	8,028	1.9
8	1035.4	19,508	39,035	80,944	214,386	1.7
9	2116.7	33,955	92,596	165,482	444,797	2.1
10	30.0	646	1,000	2,345	6,368	1.5
11	23.0	252	1,761	1,798	4,882	3.4
12	24.4	373	1,454	1,908	5,210	2.3
13	2375.3	43,137	128,052	185,702	546,946	2.0
14	21.4	272	1,121	1,673	5,140	2.9
15	11.0	217	523	860	2,335	1.7
16	54.8	861	1,932	4,285	12,452	2.2
17	64.4	1,202	1,793	5,035	15,796	1.9
18	327.0	6,896	9,130	25,565	74,725	1.6

irrigation service is 88 Birr per hectare of land served per year. This is about 11% more than the current level of pricing, that is, 78.11 Birr. The average willingness to pay of irrigation users for irrigation water is found to be 3.50 Birr per 1000 m³ instead of the current charge which is 3 Birr.

Conclusions

The current irrigation water pricing system in Awash Basin has got no influence on the performance of irrigation practices. The water charge adapted by the basin authority is fixed in 1994 by the then Ethiopian Water Resources Development Authority. Although, irrigation development is proceeding quite intensively and the need for proper management of the river basin becomes imperative, the price level has never changed since then. It is apparently low compared to the pricing levels practiced in many countries (Easter and Liu, 2005). According to Ethiopian Water Resources Management Regulations (115/2005), Council of Ministers is responsible to determine water charges. Nevertheless, charge for irrigation water has not been legally determined.

Moreover, the current collection of water and service charges in the basin has been serving more, the purposes of fund raising to enable the basin authority exist than providing improved water services. Given insignificant contribution of the collected water and service charges to water management, water users are

willing to pay 17 and 11% more water and service charges respectively compared to the current prices. Even with the additional money, the users are willing to pay for the current poor level of services they are getting which is low; this extra amount could be used to improve the water delivery services.

It is quite clear that increasing the water charge to the level of WTP found in this study would increase the income of the basin authority but reduces that of the farmer's income without much impact on water management practices. So far, water scarcity is not felt as a problem in the basin. As a result, the basin authority and irrigators have not been worried about water allocation and efficiency in water use. However, the fact that Awash River basin is the most intensively developed basin in the country, the importance of its sustainable use and management should not be overlooked. Over-irrigation coupled with lack of proper drainage system in the basin is adversely affecting agricultural production and the environment (Zelalem, 2010; Ayenew, 2007; Dagnachew and Ayenew, 2006; Tamiru et al., 2006; Wondimagedgne and Abere, 2012).

Large-scale irrigation schemes which are the dominant users of the basin's water are operated by public agencies and funded from public resources. Hence, the change in operational procedures followed by these agencies can effectively be made through government policies than a simple rise in water charge. These schemes divert several hundred million cubic meters of water every year. Hence, there is huge water saving potential in these schemes which could be tapped

Table 7. The regression result of the determinants of the WTP from Logit Model.

Variable of the model	Coefficient	Standard error	Z	P > (Z)
Bid level	0.0377	0.0719	0.5300	0.5990
Educational level	-0.4201	0.2592	-1.6200	0.1050
Household size	-0.2601	0.2738	-0.9500	0.3420
Household income	0.0001	0.0000	1.6500	0.1000
Land size	-0.0210	0.0261	-0.8100	0.4200
Off-farm income	0.2159	1.3279	0.1600	0.8710
Access to credit	0.3991	1.5528	0.2600	0.7970
Irrigation experience	0.0727	0.0901	0.8100	0.4190
SLOPE	-0.1291	1.5750	-0.0800	0.9350
Distance to market	-0.0468	0.0622	-0.7500	0.4510
CONSTANT	-1.9261	70751	-0.2700	0.7850

Number of observations = 31, Likely hood ratio (LR) $\chi^2(10) = 16.39$ Prob > $\chi^2 = 0.00889$, Log likelihood = -10.47955, Pseudo $R^2 = 0.4389$.

Table 8. The Regression Results of the Probit Model.

WTP	Coefficient	Standard Error	Z	P> (Z)
Bid level	0.070237	0.0359	1.9500	0.0510
CONSTANT	6.178598	3.2566	-1.900	0.058

Number of observations = 31, Likely hood ratio (LR) $\chi^2(10) = 12.24$, Prob > $\chi^2 = 0.0005$, Log likelihood = -12.5572, Pseudo $R^2 = 0.3276$.

through improvement of irrigation management. Pricing water will have only limited influence on public agency operated schemes as the money is paid from the public pocket.

Given the existing low level of farmer's knowledge about irrigation, widespread poverty and food insecurity, lack of capacity to afford improved technologies, government ownership over large-scale irrigation schemes, it is unlikely that increased pricing will translate into water management improvement. Introduction of increased pricing policies without improving water provision and other input services would add burden to small-scale farmers and will not be feasible.

The pricing system in the basin has been serving two purposes. First, generating revenue for the basin authority to exist and operate. Second, covering maintenance costs of primary canals which most often are limited to removal of silt. So, neither irrigators nor the authority considers water pricing as a measure of water demand management. The basin authority needs to build its capacity to be able to monitor the water resources of the basin, ensure efficient allocation and management of the basin's water. Most of all, implementation of volumetric water pricing system requires capacity to measure the amount of water used by individual farms and improvement of water delivery and distribution systems to minimize losses. This requires high investment and operational capacities.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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Full Length Research Paper

Monitoring of outcrops waters quality in the watershed UBÁ creek

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This study aimed to monitor the effects of anthropic action on outcrops waters quality located in the Ubá city - Brazil. For this study, ten outcrops waters had their physical, chemical and microbiological characteristics monitored throughout the year 2013, for five times. The results showed that there was a great environmental degradation and only 30% of outcrops waters monitored presented adequate fresh consumption of the standard portability point of view, making it necessary interventions to ensure its quality.

Key words: Potability, pollution, water analysis.

INTRODUCTION

Groundwater corresponds to approximately 90% of the freshwater in the world and about 1.5 billion people depend on these supplies (UNEP, 2008). However, the available quantities are not always sufficient to supply the urban area and, in this case, the population suffers from water scarcity that can be aggravated by failure to control pollution that affects the aquifers (Foster et al., 2010).

In Brazil, about 39% of cities are supplied by groundwater, and several of them supply all their water requirements using this type of supply which in addition meets directly to the population and are used in industry, agriculture, leisure and among others (ANA, 2010; BRASIL, 2009).

The increased demand for water in the cities associated with the impacts of intense urbanization, leads to a worrying situation for the future sustainability of urban public water supply, especially in some Brazilian metropolitan regions (SRH, 2006). The reduction of

quantity and the degradation of water affect the society as a whole (BRASIL, 2007).

Groundwater is responsible for the supply of more than 50% of water demand for all human needs, for food and regulation of rivers, streams and many lakes and ponds, enabling them to continue flowing in the dry season. Groundwater generally have high standard of physical-chemical and bacteriological quality and are captured in outcrops waters or wells that can be built close to the areas of consumption (BRASIL, 2009).

The quality of surface water and groundwater for human consumption must satisfy standards of quality and portability, ensuring that their physical, chemical and biological characteristics are within recommended standards by the World Health Organization (WHO). Owing to the fact that water is vital to the activities of the human body, hygiene and food preparation. In Brazil, these standards are defined in Decree 2914 of 2011 of

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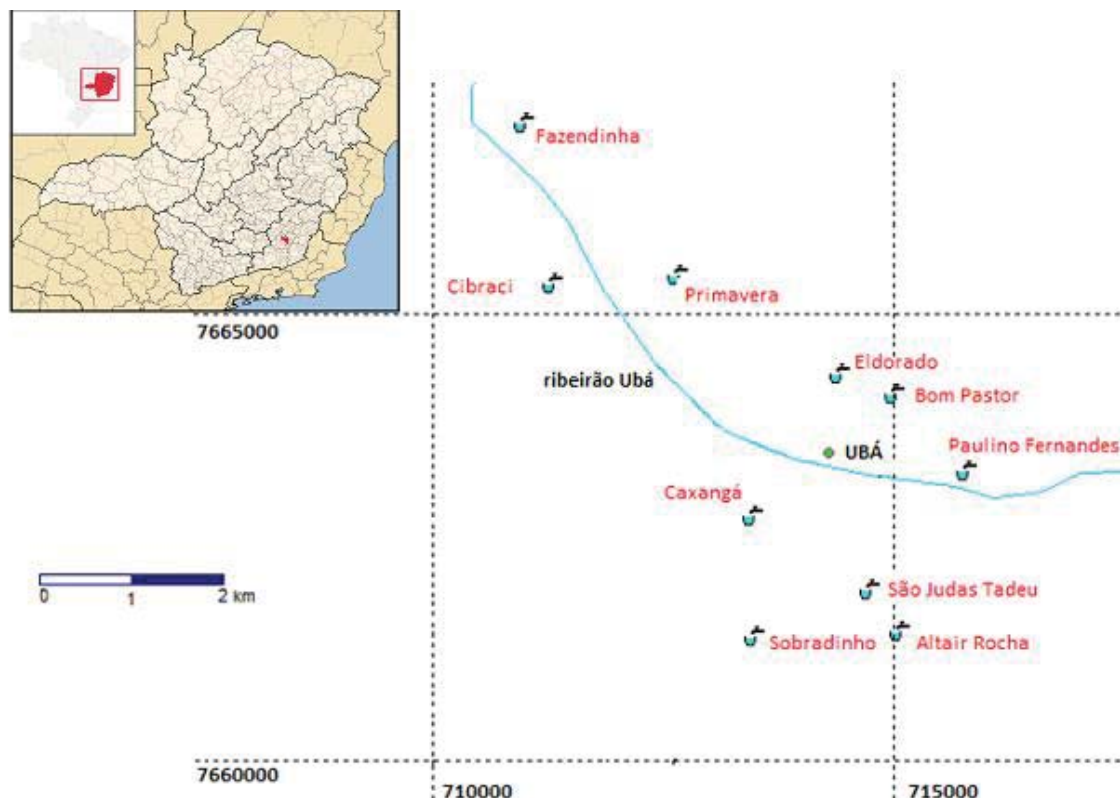


Figure 1. Geographical Location of outcrops waters monitored.

the Ministry of Health of Brazil.

The pollution caused by human activities, the increase in world population, over consumption and the high degree of wastage are factors that put at risk the availability of fresh water. Owing to its strategic importance for present and future generations, our groundwater reserves require special care in order to ensure its preservation and use of rational and sustainable manner.

Considering the great importance of groundwater for the maintenance and expansion of urban development and the possibility of contamination by waterborne diseases, this study aimed to monitor outcrops of waters quality in the Ubá city –Brazil.

METHODOLOGY

The study area comprised of an urban route of watershed of the Ubá or Miragaia creek, pertaining watershed of the Paraíba do Sul river, which has 33 km long and drains an area of 254 km², which represents 62.3% of the area Ubá city - Brazil (UBA, 2011).

The predominant soils in the region are loamy, presenting 55% of wavy relief and 40% hilly with elevations ranging from 300 m (southern county region) and 900 m (north-east region of the city) and an average rainfall in the region is 1,272 mm yr⁻¹ (UBA, 2011). To evaluate the effects of anthropic action on the groundwater quality, ten outcrops waters had the physical, chemical and microbiological characteristics monitored throughout the year

2013, for five times.

Figure 1 presented the geographic location of monitoring points, corresponding to outcrops waters located in districts Fazendinha, Cibraci, Primavera, Eldorado, Bom Pastor, Paulino Fernandes, Caxangá, São Judas Tadeu and Sobradinho e Altair Rocha. The determinations of temperature and pH values were carried in situ, while for the other characteristics, water samples were taken in pre-sterilized flask, packed in a plastic box with ice, being conducted, immediately, to the Laboratory of Water Analysis of the Minas Gerais University – Uba Unit. In these samples, the following analyzes were carried: turbidity, electrical conductivity (EC), chloride, hardness, nitrate, total coliform (TC) and fecal (CF), according to the methods described in APHA (1998).

RESULTS AND DISCUSSION

The mean values and respective standard deviations of the physical and chemical characteristics of the ten outcrops waters monitored over year 2013 are presented in Table 1. The temperature is one of the standards, or organoleptic characteristics, waters quality, associate to the sensitivity in living organisms, which make it an attractive water or not for consumption. When changing the temperature of aquatic system which is so significant to the point of changing their quality, it is described as thermal pollution (PERCEBON, 2005).

The temperatures recorded (20.6 to 24°C) did not reflect any human activity occurring in the waters, verifying only

Table 1. Mean values and respective standard deviations of the physical and chemical characteristics of the outcrops waters monitored.

Outcrop water	pH	T	Turbidity	EC	Choride	Hardness	N-NO ₃ ⁻
		°C	UNT	µS cm ⁻¹ mg L ⁻¹ mg L ⁻¹	
Bom Pastor	7.66 ± 0.17	22.4 ± 2.06	0.04 ± 0.02	107.6 ± 6.50	19.3 ± 0.73	53.0 ± 3.74	0.748 ± 0.080
Eldorado	7.42 ± 0.11	22.4 ± 2.06	8.30 ± 1.40	101.2 ± 8.04	28.8 ± 1.94	33.6 ± 2.94	2.661 ± 0.240
Primavera	7.19 ± 0.15	22.4 ± 2.06	0.04 ± 0.02	63.6 ± 7.05	28.8 ± 1.94	10.2 ± 1.33	3.930 ± 0.070
Cibraci	7.00 ± 0.06	22.4 ± 2.06	0.04 ± 0.02	21.5 ± 5.82	28.8 ± 1.94	2.6 ± 1.20	0.264 ± 0.010
Sobradinho	7.30 ± 0.08	24.0 ± 2.76	0.04 ± 0.02	63.9 ± 4.26	28.8 ± 1.94	24.0 ± 2.19	0.469 ± 0.030
Altair Rocha	7.38 ± 0.10	24.0 ± 2.76	0.04 ± 0.02	53.4 ± 5.38	28.8 ± 1.94	16.6 ± 1.20	1.365 ± 0.150
São Judas Tadeu	7.30 ± 0.09	22.4 ± 2.06	0.04 ± 0.02	42.5 ± 4.44	19.5 ± 2.57	14.0 ± 1.67	1.478 ± 0.130
Fazendinha	6.19 ± 0.15	21.6 ± 1.74	14.72 ± 1.23	105.6 ± 6.07	21.7 ± 2.22	22.4 ± 1.50	4.552 ± 0.440
Caxangá	6.42 ± 0.08	21.6 ± 1.74	0.04 ± 0.02	176.4 ± 6.68	35.4 ± 2.92	44.6 ± 2.80	5.265 ± 0.360
Paulino Fernandes	6.62 ± 0.10	20.6 ± 1.74	11.9 ± 1.59	49.9 ± 6.15	21.9 ± 1.89	21.8 ± 1.83	0.345 ± 0.030

T - Temperature, EC – Eletrical Conductivity.

an elevation of their levels due to the increase of air temperature during the sample taking, once the city presented a humid tropical climate, with average annual temperature of 21°C (UBA, 2011). The recorded pH range (6.19 - 7.66) is regular, which is in accordance with the quality standard of surface water in the CONAMA Resolution 357/2005 and, potability, according to Decree 2914/2011 of the Ministry of Health Brazil, not being evidenced a spatial pattern of occurrence. These results are in accordance with studies of surface water quality of the Ubá creek conducted by Carvalho et al. (2004) and, separately, do not indicate any effects of human activity on the quality of water evaluated.

The outcrops waters located in districts Eldorado, Fazendinha and Paulino Fernandes showed turbidity values above the standard of acceptance for human consumption without previous treatment, as Ordinance no 2914/2011 of the Ministry of Health of Brazil, which establishes the limit of 5 NTU, complementing the microbiological requirements.

Although, the turbidity may be of natural origin, not bringing direct health hazards, is aesthetically displeasing in potable water, and suspended solids can provide shelter for pathogenic microorganisms (Von Sperling, 2005). In the case of outcrops waters in evaluation, the observed values may be related both to natural causes, such as the misuse of the soil, anthropogenic and the contamination by sewage.

According Mouchrek Filho and Nascimento (2005), the electrical conductivity is an indirect measure of anthropogenic effects because it depends on the temperature and ionic concentrations, indicating the quantity of existing salts in water. Thus, the outcrops waters located in Bom Pastor, Eldorado, Fazendinha and Caxangá presented values for the electrical conductivity parameter above 100 mS cm⁻¹, indicating impacted environments according to Mouchrek Filho and Nascimento (2005).

Regarding the organoleptic properties potability

analyzed, both the values of chloride as the hardness of all outcrops waters were monitored within the limits established by the Ministry of Health of Brazil, according to Decree no 2914/2011. Similar results were obtained by Carvalho et al. (2004), when analyzing the surface waters of Ubá creek along urban stretch, including nearby launch of industrial effluents. Also, Silva and Araújo (2003), analyzed water samples from groundwater in Feira de Santana (BA), obtained 100% of the samples with parameters of chlorides and hardness within the recommended standard, indicating that these parameters require high concentration to change the quality of water.

The occurrence of nitrogen compounds in their different oxidation states is an indicative of contamination of the aquifer and possible inadequate sanitary conditions. The nitrate in excess, causes two adverse health effects, which are the induction of methemoglobinemia, especially in children and, the potential formation of nitrosamines and nitrosamides, both in carcinogenic (Scorsafava et al., 2010; Nascimento, 2005; Barbosa, 2005). Therefore, the maximum permitted value established by Ordinance 2914/2011 of the Ministry of Health of Brazil is 10 mg L⁻¹ N-NO₃⁻ in potable water.

Therefore, analyzing Table 1, it is verified that the consumption in nature of outcrops waters would not cause this disease. However, with regard to contamination by anthropogenic activities, outcrop water located in Eldorado, Primavera, Fazendinha and Caxangá, that is, 30% of water supplies, N-NO₃⁻ concentrations showed higher 3 mg L⁻¹, which was configure. According to Alaburda and Nishihara (1998), these water supplies are contaminated.

Scorsafava et al. (2010), studied the quality of water from wells and outcrop water for human consumption in the state of São Paulo, found that nitrate concentration was higher than those allowed by legislation in 15% of the wells and 30% of outcrop water. Similar results were obtained by Freitas et al. (2001) who studied water samples from wells on Fluminense Park in Rio de

Table 2. Average values and respective standard deviations of the microbiological characteristics of the outcrops waters monitored.

Outcrop	TC	FC
	MPN/100 ml	
Bom Pastor	7.5 ± 1.3	Aus.
Eldorado	201.2 ± 13.9	24.1 ± 1.6
Primavera	Aus.	Aus.
Cibraci	Aus.	Aus.
Sobradinho	Aus.	Aus.
Altair Rocha	104.0 ± 9.5	20.0 ± 0.9
São Judas Tadeu	18.9 ± 1.8	4.1 ± 0.5
Fazendinha	10.167.0 ± 270.0	1.512.5 ± 76.0
Caxangá	162.4 ± 12.0	Aus.
Paulino Fernandes	6.878.0 ± 145.0	998.5 ± 53.0

TC - Total coliforms, FC - coliforms, MPN - most probable number.

Janeiro, found that 30% of the samples exceeded the maximum amounts permitted by legislation. The mean values and respective standard deviations of the microbiological characteristics of the ten outcrops waters monitored over year 2013 are presented in Table 2.

Determination of the concentration of total coliforms assumes significance as an indicator parameter of the possibility of pathogenic microorganisms responsible for the transmission of waterborne diseases. The presence of fecal coliforms indicates the possibility of other enteric pathogens and fecal contamination, serving as an indicator of sanitary quality of water (Moura et al., 2009; Silva, 2000). According to Decree 2914/2011 of the Ministry of Health of Brazil, in terms of microbiological, the potability of water can be determined by the absence of counting total and fecal coliforms in 100 ml of sample.

Observing Table 2, it is verified that the outcrops waters located in Bom Pastor, Eldorado, Altair Rocha, Sao Judas Tadeu, Fazendinha, Caxangá and Paulino Fernandes, that is, 70% of water supplies, did not present good conditions for natural consumption, necessary disinfection process is needed in order to make them suitable for consumption, due to the high degree of contamination. Similar results were obtained by Lima and Freitas (2007), when studying the water quality of wells and outcrops waters in the urban perimeter of Uberaba city, consumed by a segment of the population. These authors found that 40% of the wells and 75% of outcrops waters had inadequate water for human consumption, being in disagreement with current standards required by Ordinance 2915/2011. Regarding Ubá creek, Carvalho et al. (2004) found that surface waters close to urban perimeter were contaminated by total and fecal coliforms.

Considering the monitored parameters, it was observed that only 30% of the outcrop monitored had been presented suitable for consumption "in nature" as required by standards of potability, indicating significant environmental degradation and high risk of contamination

by the water-transmitted diseases. The quality of water supplies monitored are directly associated with the characteristics of their location, such as reduced health infrastructure, soil erosion, proximity to places of effluent discharge and inadequate state of preservation of outcrop water. Although the city has the privilege of having high water supplies, both groundwater as surface water, these supplies are suffering from pollution by domestic and industrial effluents, making it necessary to ensure quality and quantity of water for present and future generations.

Conclusion

According to the results, it can be concluded that only 30% of outcrops monitored were presented as suitable for consumption "in nature" according to the point of view of potability, indicating that the sources groundwater in the region has suffered from significant environmental degradation, and this requires interventions to ensure its quality.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Growth, yield and quality responses to plant spacing in potato (*Solanum tuberosum*) varieties

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A field experiment was conducted from November to March during the 2012 / 2013 planting season at Africa University farm, Mutare, Zimbabwe to evaluate the effects of plant spacing and different potato varieties on growth, yield and quality of potato (*Solanum tuberosum* L.). The experiment was designed as a randomized complete block design with 4 × 3 factorial arrangements of treatments. The first factor was plant spacing (in-row spacing); 20, 25, 30 and 35 cm. The second factor was varieties: BP1, KY20 and Mnandi. Very close spacing produced a high number of small sized tubers leading to reduced marketable yield. The highest stem count was observed at high plant densities, the lowest at low plant densities. At low density plantings, leaf number per plant was high and at high density plantings the leaf number was reduced across all varieties. BP1 and KY20 exhibited the highest specific gravity and Mnandi had the least. Results of the study suggest that a wider spacing of 90 by 35 cm can be advantageous for all the three varieties since all the varieties compensated for the additional spacing to produce the highest marketable yield yet with less seed thus reducing production cost. Also from the study, an in-row spacing of 25 cm can be used by seed producers since the highest number of medium sized tubers was obtained and this size is normally used as seed.

Key words: Plant spacing, potato yield, potato quality, potato varieties.

INTRODUCTION

Many factors influence potato yield and quality and among these are cultivar, plant population, soil type, weather conditions, water management, fertilization, seed piece size, pests and diseases (Khalafalla, 2001). Planting density strongly affects yield and more tubers and yield per square meter are expected at higher planting densities (Karafyllidis et al., 1996). Bussan et al. (2007) and Creamer et al. (1999), argued that optimizing

plant density was one of the most important practices in potato production management, as it affects seed cost, plant development, yield and the quality of the crop.

According to Love and Thompson-Johns (1999), plant spacing studies are among the earliest researches that were carried out in potato production. Even though a lot of research has been done on this topic, Masarirambi et al. (2012) stated that more information is still required on

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Table 1. Plant height in cm recorded at 30, 60 and 90 days after emergence.

Variety	Plant height (cm)								
	30 DAE			60 DAE			90 DAE		
	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi
Spacing									
20	27.58	27.94	27.63	46.90 ^a	62.93 ^c	48.37 ^a	53.17 ^a	66.67 ^b	55.35 ^{ab}
25	25.55	29.26	27.48	47.80 ^a	65.82 ^e	48.47 ^a	55.62 ^{ab}	69.15 ^{bc}	56.15 ^b
30	25.38	25.64	28.10	65.90 ^e	65.77 ^e	55.09 ^b	69.60 ^c	70.33 ^c	70.80 ^c
35	26.90	26.93	27.77	66.67 ^e	66.90 ^e	66.65 ^e	76.82 ^d	69.50 ^c	69.87 ^c
Means	25.30^b	26.95^c	22.74^a						
Lsd Vrt		1.35			1.34			1.38	
Lsd SP		1.56			1.55			1.60	
Lsd Sp*Vrt		2.702			2.685			2.774	
P val SP		NS			*			*	
P val Vrt		*			*			*	
P val SP*Vrt		NS			*			*	
CV%		12.10			5.60			5.30	

*, Significance at $P < 0.05$; NS, non-significance at $P > 0.05$. The means not sharing a common letter in a column differ significantly at 0.05. Vrt, varieties; SP, spacing.

interrelationships of plant populations and tuber sizes in relation to growth and subsequent yield. Plant population studies in potato are thought to be never out dated because newly developed cultivars have unique tuber characteristics and evolving industries constantly come up with new tuber size requirements. Barry et al. (1990) and Güllüoğlu and Arıoğlu (2009) noted that the optimal planting density differed depending on environmental conditions and cultivars.

Although it is generally accepted that total yield increases with increasing plant density while the percentage of large tubers decreases, Creamer et al. (1999) argued that varieties differed in their ability to compensate for wider gaps as the plant population is reduced. The current study sought to find out how different plant spacing affects the growth, yield and quality of three potato varieties.

MATERIALS AND METHODS

The study was carried out at Africa University Farm (AU) in Mutare, Zimbabwe (18°53'70, 3°S: 32°36'27.9"E) at 1104 m above sea level. The planting material was obtained from the Seed Potato Association in Zimbabwe. Three early maturing varieties were used, namely BP1, Mnandi and KY20, and were planted at 20, 25, 30 and 35 cm in the row. The row to row spacing was 90 cm. All crop management and fertilizer applications were done as per standard practice. The experiment was a 3 × 4 factorial laid out as a RCBD of 4 blocks. The gross plot size was 3.6 m × 3.6 m and the harvest plot was 2 m × 2 m. Total yield, marketable tuber yield, plant height, stem count, tuber density, leaf counts, tuber size category, dry matter content and specific gravity of tubers were measured subjected to the Analysis of Variance (ANOVA) using Genstat Discovery 3 edition. Whenever the treatment was significant; Fisher's least significance difference (LSD) test was used for mean separation at $p = 0.05$.

RESULTS

Effect of spacing and variety on plant height at 30, 60 and 90 days after emergence (DAE)

At 30 DAE there was neither density effect nor variety by density interaction. However, varieties showed significant differences ($p < 0.05$). Based on stem height, the varieties could be arranged as Mnandi < BP1 < KY20 (Table 1). These results show that variety KY20 was generally taller than the other varieties. The significant planting density differences ($p < 0.05$) with regard to plant height observed at 60 and 90 DAP were dependent on variety. With regard to BP1, at 60 DAE, the in row spacing of 20 and 25 cm gave the same plant height while the spacing at 30 and 35 cm were the same.

Mnandi behaved similarly at low in row spacing but plant height increased progressively with increase in spacing above 25 cm while for KY20 the plant height was low ant 20 cm and the same for all spacings above 20 cm. The response by KY20 was maintained at 90 DAE. The plant height of BP1 increased progressively with increase in intra-row spacing while that of Mnandi increased progressively up to 30 cm and thereafter remained unchanged (Table 1).

Effect of plant spacing and variety on leaf count per plant at 30, 60 and 90 days after emergence

From 30 to 90 DAE the response of leaf counts to planting density was dependent on variety (Table 2). Generally, at 30 DAE all varieties showed similar growth habit in terms of leafiness at 30 and 35 cm intra-row

Table 2. Leaf counts per plant at 30, 60 and 90 days after emergence.

Variety	Leaf counts								
	30 DAE			60 DAE			90 DAE		
	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi
Spacing									
20	113.17 ^b	112.83 ^b	92.42 ^a	224.83 ^a	248.68 ^d	225.00 ^a	318.08 ^b	319.00 ^b	308.17 ^a
25	128.17 ^c	138.17 ^d	117.67 ^b	234.50 ^b	262.42 ^e	243.33 ^c	338.83 ^d	339.80 ^d	326.85 ^c
30	165.75 ^f	162.42 ^f	147.08 ^e	287.08 ^f	283.58 ^f	261.08 ^e	356.33 ^f	357.75 ^f	346.92 ^e
35	164.33 ^f	165.17 ^f	146.17 ^e	287.58 ^f	287.17 ^f	263.25 ^e	377.83 ^h	377.50 ^h	361.00 ^g
Lsd Vrt		2.64			2.35			1.36	
Lsd SP		3.00			2.71			1.57	
Lsd SP* Vrt		5.289			4.703			2.724	
P val SP		*			*			*	
P val Vrt		*			*			*	
P val SP*Vrt *		*			*			*	
CV%		4.80			2.20			1.00	

*, Significance at $P < 0.05$; NS, non-significance at $P > 0.05$. The means not sharing a common letter in a column differ significantly at 0.05. Vrt, varieties; SP, spacing.

spacing. Leafiness was lower at the lowest plant spacing for all varieties although Mnandi had the lowest leafiness at 20 and 25 cm. Mnandi also showed the lowest leafiness at 30 and 35 cm. At 90 DAE all varieties showed a progressive increase in leafiness with increase in intra-row spacing length. Once more Mnandi had the least leafiness (Table 2).

Effect of spacing and variety on tuber size distribution

Number of small sized tubers

Generally the proportion of small sized tubers increased with increase in plant density. At a spacing of 20 cm there were more small tubers for Mnandi than BP1 and KY20. At 25 cm BP1 had the most small tubers (Table 3). At the standard spacing (30 cm) the number of small tubers was the same for all varieties but the same as those for the higher density (20 cm in-row spacing) for KY20 and Mnandi. As the in-row spacing increased to 35 cm KY20 showed the least while there was no change for BP1 and Mnandi (Table 3).

Number of medium sized tubers

As shown in Table 3, there was a significant ($p < 0.05$) interaction between variety and plant spacing on the number of medium sized tubers. BP1 was not affected by plant spacing with regard to medium tubers. Mnandi was affected by spacing but the optimal was at 25 cm while for KY20 the optimal was at 25 and 30 cm in row spacing.

Number of large sized tubers

The response of varieties with regards to large tubers varied according to plant spacing (Table 3). Reducing the plant population resulted in an increase in the proportion of the large sized tubers and this applied to all the three varieties. For BP1 and Mnandi increasing plant spacing to 35 cm did not increase the size of large tubers while for KY20 it did.

Number of oversized tubers

As shown in Table 3, plant spacing had a significant ($p < 0.05$) effect on the number of oversized tubers while no varietal differences were apparent. Overall, the proportion of oversized tubers was high at the 35 cm only for BP1 and KY20.

Effect of spacing and variety on stem count

The number of stems per plant was influenced significantly ($p < 0.05$) by both variety and plant spacing. For BP1 and Mnandi the stem count was least at 20 cm and unchanged from 25 to 35 cm. For KY20 it increased with increase in spacing but remained unchanged after 30 cm.

Spacing and variety effect on tuber density at harvesting

The tuber density decreased with increase in spacing for

Table 3. Tuber size categories (arcsine transformed data).

Variety	Tuber size category											
	Small			Medium			Large			Oversize		
	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi
Spacing												
20	0.754 ^d	0.839 ^{de}	0.918 ^e	0.522 ^a	0.460 ^a	0.465 ^a	0.291 ^{ab}	0.267 ^a	0.282 ^{ab}	0.151 ^c	0.151 ^a	0.151 ^a
25	0.834 ^{de}	0.334 ^{bc}	0.361 ^{bc}	0.464 ^a	0.868 ^b	0.885 ^b	0.265 ^a	0.378 ^b	0.331 ^{ab}	0.151 ^a	0.151 ^a	0.151 ^a
30	0.308 ^{bc}	0.334 ^{bc}	0.399 ^c	0.487 ^a	0.803 ^b	0.448 ^a	0.717 ^{cd}	0.389 ^b	0.752 ^d	0.174 ^a	0.151 ^a	0.151 ^a
35	0.292 ^b	0.171 ^a	0.329 ^{bc}	0.417 ^a	0.434 ^a	0.531 ^a	0.635 ^c	0.769 ^d	0.709 ^{cd}	0.206 ^b	0.307 ^c	0.151 ^a
Lsd Vrt		0.0471		0.0519			0.0556		0.0135			
Lsd SP		0.0471		0.0599			0.0482		0.0156			
Lsd SP*Vrt		0.0815		0.1037			0.0964		0.0271			
P val SP		*		*			*		*			
P val Vrt		*		*			*		*			
P val SP*Vrt		*		*			*		*			
CV%		20.6		22.8			24.7		19.7			

*, Significance at $P < 0.05$; NS, non-significance at $P > 0.05$. The means not sharing a common letter in a column differ significantly at 0.05. Vrt, varieties; SP, spacing. † The means not sharing a common letter in a column differ significantly at 0.05. Small-25 mm to 37.5 mm in diameter; Medium- 37.5 mm to 50 mm in diameter; Large-50.00 mm to 56.25 mm in diameters and oversized-56.25 mm to 62.25 mm in diameter.

all varieties but more so for Mnandi than for the other two varieties (Table 4).

Effect of spacing and variety on total yield

Generally, the total yield rose with a decrease in plant population, reaching a peak at the standard spacing of 30 cm for BP1 and KY20 but at 35 cm for Mnandi (Table 4).

Effect of spacing and variety on marketable yield

Essentially marketable yield (medium + large + oversized tubers) increased with decrease in plant population showing an optimum at the standard population density (90 × 30 cm) (Table 4). The lowest density reduced yield for Mnandi but not for the other two varieties

Specific gravity

There was a significant interaction ($p < 0.05$) between the variety and plant spacing on specific gravity. Generally there was a rise in specific gravity up until it reached a peak at the standard spacing of 30 cm and a further increase in in-row spacing led to a fall in specific gravity. BP1 showed a steady increase in specific gravity when in-row spacing was increased from 20 to 25 cm and when the in-row spacing was increased to 30 cm, the specific gravity was statistically the same as that obtained when an in-row spacing of 25 cm was used. Increasing the in-row spacing from 30 to 35 cm led to a sharp fall in the specific gravity. KY20 responded almost in the same way

with BP1 on this parameter. When in-row spacing was increased from 20 cm to 25 there was a sharp increase in the specific gravity and it remained constant when a spacing of 30 cm was used. Increasing the in-row spacing to 35 cm resulted in a sharp fall in the specific gravity. Cultivar Mnandi did not show any increase in specific gravity when in-row spacing was increased from 20 to 25 cm. It showed a steady increase when the in-row spacing was increased to 30 cm but increasing the in-row further did not result in any changes as the specific gravity.

In general it can be seen that higher densities (20 and 25 cm) and lower densities (35 cm) resulted in a reduction in specific gravity.

As for the effect of variety on specific gravity the results showed a significant differences ($P < 0.05$) with BP1 and KY20 exhibiting the highest values and Mnandi having the least Table 5.

Dry matter

Dry matter was affected differently for the different varieties. For BP1 and KY20 the optimum was at 25 and 30 cm while for Mnandi it was at 30 and 35 cm (both unchanged).

DISCUSSION

Effect of spacing and variety on plant height

Generally, the three varieties responded differently to plant height when exposed to different plant spacing.

Table 4. Number of stem counts, tubers per plant, total yield (t/ha) and the marketable yield (t/ha).

Variety	Variety											
	Stem count			Tuber density			Total Yield (t/ha)			Marketable yield		
Spacing	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi	BP1	KY20	Mnandi
20	2.91 ^a	2.75 ^a	2.66 ^a	18.25 ^d	16.67 ^c	16.42 ^d	16.35 ^b	16.54 ^b	15.00 ^a	14.47 ^a	14.74 ^{ab}	14.83 ^{ab}
25	6.50 ^d	3.75 ^b	6.66 ^d	15.67 ^{bc}	15.50 ^b	13.80 ^c	15.48 ^{ab}	16.18 ^b	14.48 ^a	15.21 ^{abc}	14.01 ^a	14.35 ^a
30	6.38 ^d	5.00 ^c	6.41 ^d	12.67 ^a	12.67 ^a	10.17 ^b	21.77 ^f	21.89 ^f	17.67 ^c	16.98 ^d	17.29 ^d	16.44 ^c
35	6.41 ^d	5.08 ^c	6.41 ^d	12.67 ^a	12.67 ^a	9.50 ^a	20.33 ^e	19.44 ^e	18.24 ^d	16.47 ^{cd}	17.51 ^d	15.98 ^b
Lsd Vrt		0.37			0.61			1.599			0.72	
Lsd SP		0.40			0.71			1.847			0.83	
Lsd SP*Vrt		0.6955			1.070			1.082			1.448	
P val SP		*			*			*			*	
P val Vrt		*			*			*			*	
P val SP*Vrt		*			*			*			*	
CV%		16.90			11.10			13.10			35.5	

*, Significance at P<0.05; NS, non-significance at P>0.05. The means not sharing a common letter in a column differ significantly at 0.05. Vrt, varieties; SP, spacing. .

Table 5. Specific gravity and dry matter percentage.

Variety	Specific gravity (g/m ²)			Dry matter (%)		
	BP1	KY20	Mnandi	BP1	KY20	Mnandi
Spacing						
20	1.07350 ^a	1.07050 ^a	1.06550 ^a	18.83900 ^{bc}	18.20600 ^b	17.15100 ^a
25	1.07650 ^b	1.08120 ^c	1.06925 ^b	19.47200 ^{cd}	20.47400 ^e	17.94200 ^{ab}
30	1.08000 ^b	1.07900 ^b	1.07825 ^d	20.21000 ^{de}	19.99000 ^{de}	19.84100 ^{de}
35	1.07200 ^a	1.07000 ^a	1.07725 ^c	18.52200 ^b	18.1000 ^b	19.63000 ^{ce}
Lsd Vrt		0.00252		0.53220		
Lsd SP		0.00218		0.46090		
Lsd SP*Vrt		2.702		0.9218		
P val SP		*		*		
P val		*		*		
P val SP*Vrt		*		*		
CV%		12.6		3.4		

*, Significance at P<0.05; NS, non-significance at P>0.05. The means not sharing a common letter in a column differ significantly at 0.05. Vrt, varieties; SP, spacing. .

KY20 was taller than the other two varieties which were the same. These differences are likely due to varietal differences that could be associated with their canopy structure or other growth habit like internode length, a parameter we did not measure in this study. Simongo et al. (2011) attributed differences in stem height to the differences that the cultivars had in canopy structure. The canopy structure has an effect on photosynthesis as it increases the rate at which incoming solar radiation is intercepted. This occurs when the canopy has features that increase photosynthesis like erect leaves. Overall, a canopy that favours a higher photosynthetic rate will have a higher growth rate and stem growth.

Effect of plant spacing and variety on leaf count per plant

The phenomenon that at low densities leafiness was high disagreed with Masarirambi et al. (2012) perhaps because we worked with different plant densities. We found a high number of leaves at low plant densities attributable, perhaps, to decreasing inter-plant competition for water, light and nutrients at highest plant density. The observed leafiness would be expected to have an impact on dry matter accumulation, with leafy varieties showing an advantage (Table 2) over the less leafy ones.

The leafiness at low densities might also be explained by the fact that all the varieties were responding to the availability of growth requirements at low plant densities thus favoring branching. This agrees with Vander Zaag et al. (1990) who also found a high number of leaves at low plant densities.

Effect of spacing and variety on tuber size distribution

Number of small sized tubers: In general, it can be seen that the higher densities resulted in smaller tubers across all varieties. This is in agreement with Getachew et al. (2013) who concluded that tuber bulking of individuals at close spacing were reduced and resulting in small tubers. Khalafalla (2001), Mutetwa, (2010) and Love and Thompson-Johns (1999) also found closer spacing to result in smaller tuber sizes. In a similar work, Rieman et al. (1953) reported that Russet Burbank (a variety) had a tendency of producing many but small tubers regardless of plant density. Therefore, different varieties have different capacities of producing different tuber sizes based on number of tubers that a particular variety can set.

Number of medium sized tubers

The different varieties responded differently to the different plant spacing. These differences might be due to the differences of the genetics of the three varieties. The differences might be influenced by the number of tubers that the variety inherently sets. In a similar experiment Rieman et al. (1953) reported that Russet Burbank had a tendency of producing many tubers but their size was small. So in this case the varieties that produce more of the medium sized tubers would have produced tubers in moderation such that food is almost equally distributed to all the plants in the same manner.

Number of large sized tubers

Reducing the plant population resulted in an increase in large sized tubers and this applied to all the three varieties. This may be because of few sinks available per unit area that resulted in less competition between the individuals at low plant densities. More resources were channeled to each individual tuber at low density plantings resulting in a high number of large sized tubers. In other studies (Güllüoğlu and Arıoğlu 2009; Love and Thompson-Johns 1999) larger numbers of large sized tubers occurred when a wider spacing was used because of availability of growth requirements for the growth of the tubers.

Number of oversized tubers

The varieties produced a small proportion of oversized tubers. However at 90 × 35 cm BP1 and KY20 had higher tubers in this category a difference that should be varietal. In general, oversized tubers are not common for some varieties. Perhaps an increase in plant spacing to 90 × 45 cm would have produced marked differences.

Effect of spacing and variety on stem count

The number of stems per plant was influenced significantly ($p < 0.05$) by both variety and plant spacing. For BP1 and Mnandi the stem count was least at 20 cm and unchanged from 25 to 35 cm. For KY20 it increased with increase in spacing but remained unchanged after 30 cm.

Generally the stem count was least at 20 cm and unchanged from 25 to 35. This could have been a result of high competition at the lowest spacing. This disagrees with the findings of Masarirambi et al. (2012) who found out that the number of stems increased with an increase in plant density. However, if one compares their data within our range (90 × 20 cm to 90 × 35 cm) one can observe that they did not observe any differences. Nielson et al. (1989) found a relationship between eye numbers and stem density and postulated that this was varietal. When they tested two varieties Russet Burbank and Nooksack cultivars they found out that Russet Burbank averaged twice as many eyes per seed tuber compared to Nooksack tubers of equal size. Thus more stems would be produced on russet Burbank compared to Nooksack.

Spacing and variety effect on tuber density

The phenomenon of having high number of tubers at high densities as found in our study can be explained by the fact that at low density plantings fewer sinks are produced per unit area and these increased as the planting density increased. This is in agreement with Patel et al. (2002) and Karafyllidis et al. (1996) who found that tuber numbers were more at higher plant densities than lower plant densities. However, this is in contrast with Masarirambi et al. (2012) and Güllüoğlu and Arıoğlu (2009) who concluded that the availability of space had an effect on number of tubers formed. They pointed out that the greater the space the higher the number of tubers because space availability has an imposing effect on number of tubers formed. At all plant spacings, the number of tubers differed among the different varieties with Mnandi having the least and KY20 and BP1 having the highest. According to Thompson and Taylor (1974) argues that the number and size of tubers is genetically controlled and the number may be 3 to 60 tubers per

plant. When they carried out their studies on different varieties at different densities they found out that Pentland Marble variety had more tubers/m² compared to Maris Peer variety which had the least number at high densities. Wurr et al. (1993) also concluded that stem number was varietal.

Effect of spacing and variety on total yield in tonnes/hectare

Generally, the total yield rose with a decrease in plant population, reaching a peak at the standard spacing of 30 cm. The plant spacing of 35 cm, although it had a much greater advantage of accumulating more assimilates it could not compensate for yield at wider spacing resulting in it having a lower yield compared to that of an in-row spacing of 30 cm. This can be attributed to the extended amount of foliage that was produced resulting in the total yield obtained to be low as most of the assimilates supported the haulm growth at the expense of the tuber growth. This agrees with Getachew et al. (2013) who also observed a fall in total yield when the in-row was further increased to 40 cm.

At all the in-row spacings that were used, it can be said that total yields were higher for BP1 and KY20, with Mnandi having the least. This can be attributed to the genetic make-up of the different varieties as shown by the number of leaves that were produced by each of the varieties. When the leaf counts were taken at 90 DAP (Table 2), BP1 and KY20 had the highest leaf mean values and their yield at the end is almost similar, again suggesting the leaf number influenced yield.

Effect of spacing and variety on marketable yield

This phenomenon of having marketable yield rising with a decrease in plant population might be because of less inter-plant competition at low plant densities. Plants were able to efficiently use the available growth requirements and that had a direct effect on yield. Masarirambi et al. (2012) also noted that when there was an intensive competition such as the one experienced at high densities there was an earlier set in of inter plant competition for growth resources such as light, water and nutrients resulting in a decrease in relative growth rate. This contradicts Khalafalla (2001) that at relatively high plant densities the marketable yield was high.

KY20 had the highest marketable yield followed by BP1 and Mnandi respectively at 30 and 35 cm and for 20 and 25 cm the marketable yield was just the same. These differences could have been attributed to the difference in the genetic make-up of the varieties.

Specific gravity

Specific gravity was influenced both by variety and

planting density. However, this important parameter is influenced by a many factors including variety, planting density, nutrition, planting time, seed quality, irrigation and many others. A fall in specific gravity at high densities across all varieties could be accounted for by the intense competition among the plants. Therefore, the amount of nutrients that was partitioned to each individual plant was less as compared to sparsely planted plants. In similar studies Getachew et al. (2013), Vander Zaag et al. (1990) and Burton (1948), agreed with our findings, concluding that there was a rise in specific gravity up until it reached a peak and then fell. They also found out that specific gravity increased with an increase in the plant spacing. Getachew et al. (2013) and Fonseka et al. (1996) showed a fall in the specific gravity at very sparse densities after reaching its peak, in this case at 35 cm. This was attributed to the minimum competition among the plants. Competition led to a continual growth of the vegetative parts thus leading to less assimilates being channelled to the tubers. Veeranna et al. (1997) reported that growth parameters and specific gravity were improved by wider plant spacing and this agrees with the findings of the study.

As for the effect of variety on specific gravity the results showed varying specific gravity across all the plant spacing used as shown in Table 5. This can be explained by the fact that different varieties have different specific gravities. Myhre (1959) in his studies on factors affecting specific gravity he concluded that different varieties have different specific gravity.

Dry matter percentage

The lower dry matter at the highest plant densities may have been caused by intra-competition among the plants. In similar studies Getachew et al. (2013), Vander Zaag et al. (1990) and Burton (1948), also found out that percentage dry matter increased with an increase in the plant spacing. Getachew et al. (2013) and Fonseka et al. (1996) pointed out that the fall in the percentage dry matter at very sparse densities after reaching its peak, in this case at 35 cm can be attributed to the minimum competition among the plants which then led to a continual growth of the vegetative parts thus leading to less assimilates being channeled to the tubers. Veeranna et al. (1997) (cited in Mutetwa, 2010) reported that growth parameters and dry matter accumulation were improved by wider plant spacing and this agrees with the findings of the study.

This explains why there was higher dry matter percentage as those varieties that had more leaf counts have the capacity to carry out more photosynthesis and were able to produce more assimilates which were then channeled to the sinks below ground. Wurr (1974) in similar studies found out, as we did, that tuber dry-matter percentage was varietal.

Conclusion

In this study we showed many important findings: In general, plant height increased progressively with increase in intra-row spacing depending on variety while leafiness was generally lower at the lowest plant spacing for all varieties although all varieties showed a progressive increase in leafiness with increase in intra-row spacing length. Generally the proportion of small sized tubers increased with increase in plant density but varieties differed in this response while there was no consistent trend with regard to medium tubers, with some varieties showing more medium tubers as plant density decreased. Reducing the plant population resulted in an increase in the proportion of the large sized tubers and this applied to all the three varieties and the proportion of oversized tubers was high at the 35 cm only for some varieties and not others. The number of stems per plant was influenced significantly by both variety and plant spacing while the tuber density decreased with increase in spacing for all varieties but more so for Mnandi than for the other two varieties. The total yield and marketable yield rose with a decrease in plant population, reaching a peak at the standard spacing of 30 cm. As for specific gravity there was a significant interaction between the variety and plant spacing. Generally there was a rise in specific gravity up until it reached a peak at the standard spacing of 30 cm and a further increase in in-row spacing led to a fall in specific gravity. Lastly dry matter was affected differently for the different varieties.

Conflict of Interest

The authors have not declared any conflict of interest.

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