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Full Length Research Paper

Relative forage preference by camel (*Camelus dromedarius*) as influenced by season, sex and age in the Sahel zone of north western Nigeria

Alkali, H. A.[1*], Muhammad B. F.[2], Njidda, A. A.[1], Abubakar, M.[3] and Ghude, M. I.[4]

[1]Department of Animal Science, Federal University, Kashere, P.M.B. 0182, Gombe State, Nigeria.
[2]Federal College of Education (Technical), Bichi, Kano State, Nigeria.
[3]Animal Production Program, Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria.
[4]Veterinary Section, Nassarawa Local Government Council, Kano State, Nigeria.

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A study on effect of age, sex and seasonal variation in forage preference of camel was conducted in the Sudano-Sahelian zone of north western Nigeria. A total number of 12 camels were used in the study. Data were collected at an interval of 5 min for each category of animal for 3 consecutive days in dry and rainy seasons using scan sampling method. Based on the observation, the number of plants preferred in this study by young camel calves was less than that preferred by matured camel. Matured animals consume mostly diversified, thorny and taller plant species which might not be easily accessed by the camel calves. *Leptadania hastata* was found to be the most preferred forage during the rainy season, while *Ziziphus mauritiana* was the preferred forage during dry season with mean feeding time of 87.33 and 46.66 min/day, respectively; while the least preferred forage during the rainy and dry seasons were *Acacia sieberiana* and *Bauhinia rufescens* with mean feeding time of 0.11 and 15.00 min/day, respectively. Browse species found in the study area are extremely important as feed for camels.

Key words: Camel, preference, forage, grazing.

INTRODUCTION

The camels in tropical Africa are raised on natural pastures which decline both in quality and quantity from the rainy season to the dry season (Smith et al., 1991). The marked fluctuations in seasonal weights and irregular growth of animals are as a result of changes in their nutritional status. Subsistence farmers could not afford to feed supplementations in order to illuminate dry season growth checks; they solely rely on crop residues and some browses to supplement the grazing during the dry season (Mohammed and Hoffman, 2006). Most of these forages are potential feed resources which degrade readily in the rumen (Njidda, 2012). The common

*Corresponding author. E-mail: alkalindukku@gmail.com. Tel: +2347034906020.

challenges camel herders face in keeping their animals is the declining feed resources both in quality and quantity as a result of drought and in this context, fodder trees and shrubs emerge as key resources allowing the herds to endure up to the end of the dry season. The Sudano-Sahelian zone of north western Nigeria is identified to have a diverse forage species that can support livestock production (Muhammed, 2013). Large population of camel herds from neighboring countries are seen migrating into this part of the country in search of feed (Umaru and Bello, 2013; Kalla et al., 2008). However, there is inadequate information on the usefulness of these forages as feed resources for camel. The selective livestock grazing has diverse and far reaching implications for herdsmen, resource managers and researchers. Knowledge of specific species grazed provides insight into various facets of diet quality, stocking rate, livestock distribution and effect of defoliation on the subsequent well-being of forage plants. Most studies on camel feed preference (Kassily, 2002; Ouedraogo-Kone et al., 2006; Mengli, 2006) failed to address the effect of sex, age and seasonal variations in the camel feed preference. This paper was therefore designed with main objective of investigating the forage utilization and preference in the zone to enable us improve and fully exploit the potentials of the available forages and effectively understand the camel ecology and effective rangeland management and to further enable us develop a reticulated camel production system in northern Nigeria.

MATERIALS AND METHODS

Study area

The forage preference study was conducted in Ilela Local Government Area of Sokoto State, North Western Nigeria. The State covers a land area of 25,973 square kilometers with a population of 4,244,399 million. It lies to the north west of Nigeria on 13°04N5°14E and shares a common boundary with Niger Republic to the North, Katsina State to the East, Kwara State to the South and Benin Republic to the West. It has an average temperature of 28.3°C and is one of the hottest cities in the country; however, the maximum day time temperature is generally under 40°C most of the year. The highest recorded temperature is 47°C which is also the highest recorded temperature in the country. The mean annual rainfall ranges between 500 and 1300 mm.

Forage preference sampling

Scan sampling method was used to determine the forages preferred in both rainy and dry seasons as described by Guevara et al. (1996), Zhao et al. (2006) and Chimsa et al. (2013). In this technique, animals were closely followed (3 to 5 m) and monitored during feeding to ensure accurate identification of the plant consumed at an interval of 5 min. The time spent by the camel on each forage is thus recorded in minutes/day. A total number of twelve (12) camels were used in the study. The observations were made on four categories of camel (adult, young, male and female) for three consecutive days, both in the morning and in the afternoon. Morning observations were recorded between 9.00 am and 12:00 pm and afternoon observations were made between 2.00 pm to 5:00 pm.

Experimental design and statistical analysis

The study was laid down in a completely randomized block design and all data generated were analyzed using the GLM procedure of GenStat 10.

RESULTS

Seasonal variation in forage preference by the camels

Seasonal variation in camel forage preference is presented in Table 1. There was significant ($P<0.05$) difference in the forages preferred in both dry and rainy season. During the wet season, camels showed high preference for *Leptadania hastata* with a mean feeding time of 87.33 min/day followed by *Boscia angustifolia* 62.50 min/day. The least preferred forages during the wet season were *Cacia arerah, Faidherbia albida* and *Maerua crassifolia* with a mean feeding time of 0.99 min/day each. In the dry season, camels showed high preference for *Z. mauritania* and *P. pentandrus* 46.66 min/day each. The only forage that was not affected by the season was *Bauhinia rufescens* with an average feeding time of 46.66 min/day in wet season and 22.91 min/day in dry seasons, respectively.

Effect of age on forage preference by the camels

Age had significantly ($P<0.05$) affected preference of the forages (Table 2). Young calves had high preference for *I. oblongifolia* with a mean feeding time of 65.21 min/day followed by *P. pentandrus* 54.17 min/day. The least preferred forages by camel calves were *S. birrea, F. albida* with an average feeding time of 3.75 min/day and 5.08 min/day. Adult camels showed high preference for *Z. mauritania* and *S. birrea* with a mean feeding time of 54.17 and 45.83 min/day. The least foraged species by the adult animal includes *P. tomentosa* 3.75 min/day and *M. crassifolia* 13.75 min/day.

Effect of sex on forage preference by the camels

There was significant ($P<0.05$) difference in diet selection between male and female camels (Table 3). Male animals showed high preference for *P. pentandrus*, 50.83 min/day followed by *B. angustifolia*, 45.00 min/day, while the female camels preferred *I. oblongifolia* 49.58 min/day. Consumption of *C. arerah, Guiera senegalensis and P. tomentosa* were not significant ($P>0.05$) between the two sexes.

Table 1. Effect of season on camel forage preference.

Browse species	Hausa name	Feeding time (minutes/day)		
		Dry season	Wet season	t-values
Indigofera oblongifolia	Birgu	40.00	53.75	-1.41
Ziziphus mauritania	Magarya	46.66	27.08	-5.04
Maerua crassifolia	Jirga	11.16	0.99	-5.61
Bauhinia rufescens	Kargo	22.60	46.66	-7.05
Boscia angustifolia	Anza	22.91	62.50	-8.11
Phyllanthus pentandrus	Geza	46.66	52.08	-0.92
Faidherbia albida	Gawo	15.42	0.99	9.17
Acacia sieberiana	Farar kaya	26.67	0.11	-12.34
Cacia arerah	Marga	9.58	0.99	-5.35
Balanite aegyptiaca	Aduwa	24.58	0.92	-8.83
Leptadania hastata	Yadiya	20.50	87.33	-8.03
Guiera senegalensis	Sabara	25.78	56.67	-4.67
Acacia nilotica	Bagaruwa	45.95	48.33	6.12
Selerocarpa birrea	Danya	30.32	47.92	7.44
Pelgularia tomentosa	Patakka	15.15	17.50	4.37

Table 2. Effect of camel age on forage preference.

Browse species	Hausa name	Feeding time (minutes/day)		
		Young	Adult	t-values
Indigofera oblongifolia	Birgu	65.21	28.75	-.056
Ziziphus mauritania	Magarya	33.75	54.17	- 0.84
Maerua crassifolia	Jirga	5.42	13.75	0.16
Bauhinia rufescens	Kargo	34.58	27.08	-0.92
Boscia angustifolia	Anza	48.33	37.08	-0.45
Phyllanthus pentandrus	Geza	54.17	40.58	0.48
Faidherbi aalbida	Gawo	5.08	8.33	0.43
Acacia sieberiana	Farar kaya	15.42	18.41	-0.57
Cacia arerah	Malga	5.42	15.42	0.15
Balanite aegyptiaca	Aduwa	8.75	15.75	-0.49
Leptadania hastata	Yadiya	48.58	37.50	-0.49
Guiera senegalensis	Sabara	39.17	35.42	0.55
Acacia nilotica	Bagaruwa	17.58	26.58	0.70
Selerocarpa birrea	Danya	3.75	45.83	0.59
Pelgularia tomentosa	Patakka	13.75	3.75	0.21

DISCUSSION

Feed choice according to Guevara et al. (1996) by animal is a highly sophisticated process, developed through evolution, to maximize the efficiency of energy use in food harvesting. Both animal and forage attributes affect diet selection. Animal attributes include species, class of animal, productive function, prior conditioning and experience. Forage factors affecting diet selection according to Dereje and Uden (2005) include chemical composition and physical characteristics of the feed. In this study, it was observed that these had significant impact on the dietary selection of forages consumed. Camels showed high preference for grass/legumes such as *L. hastata* and *B. angustifolia* and shift their dietary preference to mainly thorny plants, tree branches and twigs during the dry season. This confirms the reports that the dromedary camel shows a feeding preference of annual and ephemeral plants and only when these dry off do shrubs and trees forages begins to dominate its diet (Mukasa-Mugerwa, 1981; Schwartz et al., 1992; Kassily, 2002; Chimsa et al., 2013). Wei (1979)

Table 3. Effect of sex on forage preference.

Browse species	Hausa name	Feeding time (minutes/day)		t-values
		Male	Female	
Indigofera oblongifolia	Birgu	44.17	49.58	-.054
Ziziphus Mauritania	Magarya	34.58	39.17	-0.82
Maerua crassifolia	Jirga	10.00	9.17	0.16
Bauhinia rufescens	Kargo	34.50	27.08	-0.94
Boscia angustifolia	Anza	45.00	40.12	0.47
Phyllanthus pentandrus	Geza	50.83	47.92	0.48
Faidherbia albida	Gawo	7.08	8.33	0.34
Acacia sieberiana	Farar kaya	15.42	11.25	0.55
Cacia arerah	Malga	5.42	4.12	0.15
Balanite aegyptiaca	Aduwa	13.83	10.75	-0.49
Leptadania hastata	Yadiya	42.75	45.58	-0.49
Guiera senegalensis	Sabara	39.17	37.50	0.53
Acacia nilotica	Bagaruwa	24.58	23.45	0.70
Selerocarpa birrea	Danya	21.25	26.67	0.61
Pulgularia tomentosa	Patakka	6.25	11.25	0.21

reported that camels can survive in a natural and semi wild conditions of roaming and grazing in the range throughout the year feeding on coarse, thorny plants and those forages with unfavorable flavors. This study further confirms the report of Schwartz et al. (1992) that deep rooted trees and large evergreen bushes are usually the only reliable sources of forages for camels during drought and dry season.

The number of plants preferred in this study by young camel calves was less than that preferred by matured camel. The calves exhibited preference for mostly annual plants especially during the wet season as they become more available. This variation in the preference between young and adult camels might not be unconnected with their ability and experience to browse the diversified plants by the adult which are inaccessible by the calves. Dereje and Uden (2005) also reported that the percentage time spent by camel browsing preferred plants species by matured and young calves were 80 and 87% during wet and dry season. This study showed that body size and/or age had influenced dietary preference of camel significantly. Camel calves showed high preference for shrubs/legumes which constitute their chief dietary component while adult and matured camels feed mostly on thorny and taller plants. Some authors (Ouedraogo-Kone, 2006; Chimsa et al., 2013) reported that the adult camels spent more time walking, resting and rubbing against trees and sexual activities as compared to the young animals.

In the present study, it was also found that *I. oblongifolia* was the most frequently preferred plant species by the camel calves, while *Z. mauritania* is the most preferred forage by the matured camels. This great variation might be due to high water content and succulent edible leaves with high CP and low fiber content than any other species. Rutagwenga (1985), Kassily (2002) and Towhidi (2007) reported that these species have high CP and low fiber. However, in contrast to this study, Chimsa et al. (2013) reported that *Opuntia ficus indicus* was the most preferred forage by the camel calves. This variation indicated that the order of preference for plant species changes and depends upon location, rangeland composition and availability of feed resources.

The dietary preference of forages by male and female camels also differed in this study. Male animals showed high preference for *P. pentandrus,* while the female showed preference for *I. oblongifolia.* A number of differences between the sexes in both species' composition of a diet and its quality have been reported in ungulate species. Main et al. (1996) reported that in ungulates, sexes segregate because sexual differences and body size lead to different energy requirements and hence food selection. This variation between the male and female is also attributed to their body size. Many studies (Illius and Gordon, 1992; Van Soest, 1994) reported that there is a variation in terms of their metabolic rate which is invariably related to body weight, decreasing with increasing body weight, while rumen volume and gut capacity remain a constant fraction of body weight. Similarly, Van Soest (1994) also reported that larger ruminants posses larger rumen and have slower passage rate of food than smaller ones and this may assume that within a species, males are more efficient in utilizing energy than the females. The females therefore, need to compensate for this digestive inferiority by either increasing foraging efficiency or by selecting higher quality forage (high nitrogen levels) than that

which is consumed by males. In addition, energy expenditure and transfer of nutrients through lactation increase selectivity in reproducing females for food sources rich in nitrogen, sodium or calcium (Clutton-Brock et al., 1986b; Iason et al., 1986). Sexually dimorphic males and females may, therefore, select different plant species or habitats with differing plant and nutrient availability and hence segregate in space (Main et al., 1996). Males and females often use different habitat types, but also overlap in habitat use (Villaret and Bon, 1995). In contrast to this study, Bleich et al. (1997) found no difference between the sexes in either habitat choice or ingested plant quality.

Conclusion

In general, animal factors (animal species, sex, age and feed demand), behavioral factors (grazing, social and previous experience) and availability of plant species present (chemical and physical characteristics, and abundance) were reported as major factors for plant selectivity by foraging camel on natural range. Further studies on the relationship between forage quality and intake in camel should be investigated.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Bleich VC, Bowyer RT, Wehausen JD (1997). Sexual Segregation in Mountain Sheep: Resources or Predation? Wildlife Monogr. 134:1-50.

Chimsa YY, Mummed M, Kurtu Y (2013). Forage Preference Of Camel Calves (Camelus Dromedarius) In Eastern Ethiopia. J. Anim. Plant Sci. 23(5):1236-1241.

Clutton TH, Albon SD, Guinness FE (1986). Great expectations: dominance, breeding success and offspring sex ratios in red deer. Anim. Behav. 34:460-471.

Dereje M, Uden P (2005). The Browsing Dromedary Camel I. Behaviour, Plant Preference and Quality of Forage Selected. Anim. Feed Sci. Technol.121:297-308.

Guevara JC, Stasi CR, Estevez OR (1996). Seasonal Specific Selectivity by Cattle on Rangeland in Monte Desert of Mendoza, Argentina. J. Arid Environ. 34:125-132.

Illius AW, Gordon IJ (1992). Modelling the nutritional ecology of ungulate herbivores: evolution of body size and competitive interactions. *Oecologia* 89:428-434.

Iason GR, Duck CD, Clutton-Brock TH (1986). Grazing and reproductive success of red deer: the effect of local enrichment by gull colonies. J. Anim. Ecol. 55:507-515.

Kalla DJU, Zahraddeen D, Yerima J (2008). Reproductive Performance of One Humped Camel at the Komodugu-Yobe River Basin, Nigeria. WBC/ICAR Satelite Meeting on Camelid Reproduction pp. 77-81.

Kassily FN (2002). Forage Quality and Camel Feeding Patterns in Central Boringo, Kenya. Livest. Production Sci. 78:175-182.

Main M, Weckerly F, Vernon C (1996). Sexual segregation in ungulates: new directions for research. J. Mammal. 77:449-461.

Mengli Z, Walters DW, Jin Y (2006). Bactrian Camel Foraging Behaviour in Haloxylon ammeodendron Desert of inner Mongolia. Appl. Anim. Behav. 99:330-343.

Mohammed I, Hoffmann I (2006). Management of Drought Camels (*Camelus dromedarius*) in Crop-livestock Production System in North West – Nigeria. Livest. Res. Rural Dev. 17:12.

Muhammed A (2013). Place of Biodiversity in Ecosystems' Efficiency In Nigeria. British J. Earth Sci. Res. 1(1):10-17.

Mukasa-Mugerwa E (1981). The Camel (Camelus dromedaries): A Bibliographical Review. ILCA Monograph 5. International Livestock Centre for Africa, Addis Ababa, Ethiopia http://www.ilri.org/InfoServ/Webpub/Fulldocs/Monono5/Toc.htm

Njidda AA, Olatunji EA, Raji AY (2012). Semi arid browse forages: Their antinutritive substances and *in sacco* neutral detergent fibre and organic matter degradability. J. Agric. Vet. Sci. 1(6):31-36.

Ouedraogo-Kone S, Kabore-Zoungrana CY, Ledin I (2006). Important Characteristics of Some Browse Species in an Agrosilvopastoral System in West Africa. Agroforestry Syst. 74:213-221.

Rutagwenda T (1985). The control of important camel diseases in the integrated Project of Arid Lands Study area. Camel disease and Productivity in the arid lands of Northern Kenya. Integrated Project in the Arid Lands (IPAL) technical Report No. E-7, Germany pp. 9-70.

Schwartz HJ (1992). Common range forage species preferred by Camels and their nutritive value. In The One-Humped Camel in Eastern Africa: A pictorial guide to diseases, health care and management. Verlag Josef Margraf, Germany.

Smith OB, Idowu OA, Odunlami O (1991). Comparetive Rumen Degradability of Forages, Browse, Crop Residues and Agricultural by-products. Livest. Res. Rural Dev. 3(2):1011-1017.

Towhidi A. (2007). Nutritive value of some herbage for dromedary Camel in Iran. Pak. J. Biol. Sci. 10(1):167-170.

Umaru MA, Bello A (2013). Reproduction in the One Humped Camel (*Camelus dromedarius*) in Semi Arid Nigeria. S. J. 2(1):1-7.

Van Soest PJ (1994). Nutritional Ecology of Ruminants**.** Cornell University Press ltd 476 p.

Villaret JC, Bon R (1995). Social and spatial segregation in alpine ibex (*Capra ibex*) in Bargy, French Alps. Ethol. 101:291-300.

Wei D (1979). Chinese camels and their productivities. In Proc. Workshop on Camels and their Productivities 18-20 December, Khartoum, Sudan.

Zhao M, Walter WD, Guodong H, Jin Y (2006). Bactrian camel Foraging Behaviour in Haloxy Ammodendron Desert of Inner Mongolia. Appl. Anim. Behav. Sci. 99:330-343.

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Full Length Research Paper

Effects of associated co-inoculation of *Bradyrhizobium japonicum* with *Azospirillum brasilense* on soybean yield and growth

Glaucia Cristina Ferri[1]*, Alessandro Lucca Braccini[2], Fernanda Brunetta Godinho Anghinoni[1] and Lucas Caiubi Pereira[1]

[1]Graduate Program in Agronomy, State University of Maringá, Brazil.
[2]Department of Agronomy, State University of Maringá, Brazil.

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The continuous development of soybean cultivars aiming at higher yields can result in a demand of N supply higher than the standard inoculation with *Bradyrhizobium* species. In this context, *Azospirillum* species, a nitrogen-fixing microorganism found in the rhizosphere of various plant species, may be studied as a way of providing soybean crop with the part of the N it needs. Employing a completely randomized design with four replicates, this study aimed to evaluate the agronomic performance of two different methods of standard inoculation of *Bradyrhizobium* spp. associated with the co-inoculation with *Azospirillum* spp. The tested treatments consisted of the absence of N fertilizer and inoculant, full N recommendation, two single inoculations with *Bradyrhizobium* spp. (in-furrow and seed-applied) combined with an in-furrow application of *Azospirillum* spp. At R_2 growth stage, the nodule number, nodule dry weight, shoot dry biomass and shoot N content were the variables evaluated, whereas at R_8 the grain yield, thousand seed weight and grain N content were assessed. The results demonstrated that the co-inoculation of *Bradyrhizobium* spp. with *Azospirillum brasilense* is beneficial to soybean yield, mainly using the in-furrow method of application for *Bradyrhizobium* spp., associated to the in-furrow co-inoculation with *Azospirillum* spp.

Key words: *Glycine max*, inoculation, nitrogen content, yield.

INTRODUCTION

Soybean [*Glycine Max* (L.) Merr.] production is among the agricultural activities that has presented the most outstanding economic increase worldwide (Dall'Agnol et al., 2010), reaching a prominent place in the global economy. The crop plays a significant role as feedstock in oil edible production or in providing the raw material needed to obtain grain for animal feeding. According to the United States Department of Agriculture, the global grain production of soybean in 2015/2016 crop season was of 312,672 million of tons, which represent an increase of about 41% comparative to the 2005/2006 crop season (USDA, 2016).

*Corresponding author. E-mail: ferriglaucia@hotmail.com.

Nitrogen (N) is an indispensable plant nutrient related to amino acid synthesis, protein production, chlorophyll and enzymes associated with vegetable growth and development (Taiz and Zeiger, 2004; Hawkesford et al., 2012). In soybean, constituting around 1 to 5% of the plant dry matter, N is the second major nutrient demanded by the crop. It is estimated that for 1 ton of grains produced, 80 kg of N are demanded by the crop (Hungria et al., 2007). As an essential element, the N balance affects root and shoot formation and the photosynthesis process, impacting thus on the partitioning of photo assimilates at the entire plant level (Taiz and Zeiger, 2004).

In Brazil, the world's second-largest soybean producer (USDA, 2016), the success of this crop has also been attributed to the benefits obtained from the seed inoculation with *Bradyrhizobium* species, bacteria able to enzymatically reduced the atmospheric N into ammonia, which is then assimilated by plant tissues as nitrogenous compounds. The symbiosis between the bacterium and plants is an association that has been pointed out to be capable in providing crop with the N level that it needs (Hungria et al., 2006; Hungria et al., 2015).

A sprayed application of *Bradyrhizobium* spp. in the planting furrow of soybean cultivation is a consolidated agricultural practice in Brazil that can simultaneously be performed in sowing operation or soil fertilization (Embrapa, 2011). However, the continuous improvement of cultivars aiming at higher yields can result in a demand of N supply higher than the standard inoculation with *Bradyrhizobium* spp. (Hungria et al., 2015).

In this context, *Azospirillum* species, a N-fixing microorganism found in the rhizosphere of various plant species, have been reported as a way of providing crop with the part of the N it needs through biological N fixation (Pedraza et al., 2009; Hungria et al., 2010). Beneficial responses to *Azospirillum* spp. inoculation in maize or wheat have been reported in field conditions (Domingues Neto et al., 2013). For soybean, however, considering the diverse environmental conditions in which the crop is cultivated in Brazil, it needs further studies on the effects of the inoculation of this bacterium (Hungria et al., 2013)

On this point, this study aimed to evaluate the agronomic performance of two inoculation methods (seed and in-furrow) of *Bradyrhizobium* spp. in soybean crop combined with an in-furrow co-inoculation with *Azospirillum* spp., both compared to the standard inoculation technique (single inoculation with of *Bradyrhizobium* spp.) used in the crop management.

MATERIALS AND METHODS

The experiment was carried out during the soybean crop year of 2015/2016 at the Iguatemi Research Station (FEI) of the State University of Maringá (UEM), in Maringá, in northwestern Paraná State, located at latitude 23°25' south and longitude 51°57' west of Greenwich and with an average altitude of 540 m. The region's climate and soil are, respectively, classified as Cfa just as Köppen classification (Caviglione et al., 2000) and Typical Red Dystrophic Argisol according to the Brazilian Classification System (Embrapa, 2013).

Experiment installation and conduction

Under tillage conservation system, sowing took place on October 21st 2015 in a completely randomized design with four replicates. The plots comprised of eight rows of 5 × 0.45 m apart; however, the harvesting area of each experimental unit consisted of only 10.8 m², since the lateral rows and the end boundaries of the central portion were not considered as a way of minimizing the border effect described in Petersen (1994).

BMX Potência RR, an indeterminate growth habit cultivar classified as semi-early for the maturation cycle (relative maturity groups 6.7), was employed in the experiment. Previously sowing and inoculation, seeds were treated with an insecticide/fungicide product based on pyraclostrobin + thiophanate-methyl + fipronil (Standak Top®, 200 ml 100 kg^{-1} of seeds). Sowing was then performed by uniformly distributing 12 viable seeds per linear meter. Apart from the N supply and the inoculation with *Bradyrhizobium japonicum* (standard inoculation), soil correction and fertilization, weed control as well as pest and disease management were all conducted as recommended by the Brazilian Agricultural Research Corporation (Embrapa, 2011).

Inoculants and inoculation methods

The strains AbV5 and AbV6 at the concentration of 2×10^8 CFU ml^{-1} (Masterfix Gramíneas®) were used as source of *Azospirillum brasilense*, whereas a blend of the strains Semia5019 and Semia5079 at 5×10^9 CFU ml^{-1} (Masterfix®) were the source of *B. japonicum*. Both inoculants were liquid commercial and the strains used were found to be the most effective in biological N fixation (Hungria et al., 2010).

The methods of inoculation consisted either of mixing the products with the seeds or of applying them in the planting furrow prior sowing (Table 1). In the first method, after inoculation, seeds were left to dry in the shade for a period no longer than 2 h, while in the second one, the inoculants was applied via a CO_2 propelled backpack sprayer (with 2 bar constant pressure and a flow of 0.35 L min^{-1}) equipped with a pipe containing one XR 110 02 nozzle (type TeeJet Series), which provided a spray volume of 100 L ha^{-1} working at a height of 50 cm from the target and at a speed of 1 m s^{-1}, reaching a 50 cm wide strip of application.

A detailed scheme of the treatments describing the doses and the inoculation methods used is shown in Table 1. Two controls treatments were performed: the first consisted purely of a non-inoculated treatment (T1), whereas the second was a non-inoculation control that received N fertilizer (T2). Using urea (46% of N) as source, N fertilizer was applied at 200 kg ha^{-1}, split into two applications of 50% each, one at sowing and the other at the R_2 (55 days after sowing) growth stage (Hungria et al., 2015). The other treatments consisted of either inoculation of *B. japonicum* (standard inoculation) or of co-inoculation of *B. japonicum* with *A. brasilense*. While *B. japonicum* followed an in-furrow (T5 and T6) or seed application (T3 and T4), *A. brasilense* was directly sprayed in the sowing furrow (T4 and T6) just as performed in Hungria et al. (2015).

Agronomic characteristics and yield evaluation

At the R_2 growth stage (55 days after sowing), ten plants were randomly collected from the harvesting area of each plot in order to

Table 1. Scheme of the treatments describing the N fertilizer level, the control treatments, the doses and the inoculation methods of *B. japonicum* associated, or not, with the co-inoculation with *A. brasilense* (Maringá, Paraná, Brazil, 2015/2016).

Treatments	N dose (kg ha^{-1})	*Bradyrhizobium japonicum*		*Azospirillum brasilense*	
		Doses* (ml)	Inoculation method	Doses* (ml)	Inoculation method
T1	0	0	-	0	-
T2	200	0	-	0	-
T3	0	200	Seeds	0	-
T4	0	200	Seeds	200	Furrow**
T5	0	600	Furrow**	0	-
T6	0	600	Furrow**	200	Furrow**

*ml 100 kg^{-1}seed or ml ha^{-1} according to the method. ** Total volume of 100 L ha^{-1}.

evaluate the nodule number (NN), the nodule dry weight (NDW), as well as the shoot dry biomass (SDB) and shoot N content (SNC). On the other hand, the grain yield (GY), the thousand seed weight (TSW) and the grain N content (GNC) were assessed at R_8 (135 days after sowing), which is the complete physiological maturity of the cultivar (Veloso et al., 2006; Hungria et al., 2006).

Nodule number (NN) and nodule dry weight (NDW)

The ten samples were collected in each plot using a shovel to excavate a block of soil of 0.40 × 0.40 m by 0.20 m deep. In the laboratory, nodules were removed from roots and then counted and eventually dried in a forced-air oven at 65 ± 2°C for 72 h (Brasil, 2009).

Shoot dry biomass (SDB) and shoot N content (SNC)

Once collected, the plants were placed in paper bags, identified and dried in a forced-air oven at 65 ± 2°C for 72 h (Brasil, 2009) and eventually weighted. The average SDB data were expressed in g plant^{-1}. Dried shoots were then ground (18 mesh) and subjected to sulfuric digestion to determine total SNC as documented in AOAC (2000) with the modifications proposed by Vitti et al. (2001), SDB data were expressed in grams per plant (g pl^{-1}), while SNC as percent of N content in the dry matter.

Grain yield

From five to eight days after the complete physiological maturity (R_8 stage), harvest was manually performed collecting all the plants of the evaluated area of each plot. Pods were mechanically threshed and then impurities were removed in order to obtain a very clean lot. After proper moisture content adjustment of 13% (Brasil, 2009), the cleaned grains were weighed and the average data were converted into kg ha^{-1}.

Thousand seed weigh (TSW)

After the moisture adjustment described earlier, it was determined by weighing 8 subsamples of 100 seeds for each field plot, with an analytical scale accurate to 1 mg. For all plots, the coefficient of variation was less than four, and the results were multiplied by 10 (Brasil, 2009).

Grain N content (GNC)

From the cleaned seeds portion, it was determined through the method of sulfuric acid digestion using a micro Kjeldahl distillation apparatus, as described in AOAC (2000) with the modification pointed out in Vitti et al. (2001). GNC data were expressed as percent of dry matter.

Statistical analysis

All analyses were performed using the statistical software Sisvar (Ferreira, 2011). The data were submitted to the Shapiro-Wilk test ($p<0.10$) and Levene test ($p<0.10$) to verify the normal distribution and homoscedasticity, respectively. Each variable was subjected to analysis of variance at 10% probability and when it was significant, the means were compared by Fishers' protected t-test LSD (Least Significant Difference) ($p\leq0.10$) according to Banzatto and Kronka (2008).

RESULTS AND DISCUSSION

The results obtained in the crop season of 2015/2016 for the soybean nodulation and growth, the total N accumulated in shoots and grains as well as, the results of the yield compounds are shown in Table 2. All the variables showed statistically significant at 10%, indicating thus that the two tested inoculation methods of *B. japonicum* associated to an in-furrow co-inoculation with *A. brasilense* significantly affected the crop.

Nodule number (NN) and nodule dry weight (NDW)

Regardless of the application method, all treatments in which the standard inoculation (*B. japonicum*), associated or not to the co-inoculation (*B. japonicum* + *A. brasilense*), showed higher values for NN and NDW than the non-inoculated treatments (T1 and T2) at the R_2 physiological stage (55 days after sowing). On this point (Table 2), the in-furrow inoculation with *B. japonicum* associated to the in-furrow application of *A. brasilense* provided significantly superior results among the tested

Table 2. Nodule number (NN), nodule dry weight (NDW), shoot dry biomass (SDB) and shoot N content (SNC) at R_2stage, as well, the grain yield (GY), the thousand seed weight (TSW) and grain N content (GNC) at$R_8$of the soybean cultivar BMX Potência RR in response to standard inoculation (*B. japonicum*) and to the co-inoculation (*B. japonicum +A. brasilense)* in Maringá, Brazil, crop season 2015/2016.

Treatments	NN (No. pl^{-1})	NDW (g pl^{-1})	SDB (g pl^{-1})	SNC (%)	TSW (g)	GNC (%)	GY (kg ha^{-1})
T1	49.50^{e}	0.49^{e}	9.58^{e}	6.62^{f}	120.48^{f}	7.85^{d}	1843.62^{e}
T2	54.75^{e}	0.59^{d}	12.97^{d}	7.21^{e}	131.06^{e}	8.51^{c}	2196.44^{d}
T3	61.92^{d}	0.66^{c}	15.01^{c}	7.56^{d}	137.88^{d}	8.75^{c}	2333.16^{d}
T4	74.50^{c}	0.69^{c}	15.85^{c}	7.92^{c}	140.72^{c}	9.13^{b}	2.579.44^{c}
T5	95.83^{b}	0.76^{b}	16.82^{b}	8.36^{b}	143.95 ^{b}	9.39^{b}	2788.45^{b}
T6	103.00^{a}	0.95^{a}	26.63^{a}	9.37^{a}	150.31^{a}	9.97^{a}	3354.58^{a}
Means	73.25	0.69	16.14	7.84	137.4	8.93	2515.95
C.V. (%)	4.88	7.32	15.72	2.76	2.78	2.78	4.13

Within columns, means followed by same letter do not significantly differ from each other ($p < 0.10$ LSD).

treatments.

As expected, the lowest NN and NDW values were observed in treatments T1 and T2. Fact that can be explained by the absence of the standard inoculation, indicating thus that the supply of N for soybean crop is needless when the strains Semia 5019 and Semia 5079 of *B. japonicum* are used prior sowing (Mendes et al., 2003; Zilli et al., 2006).

Regarding the NDW, T3 (single seed inoculation) and T4 (co-inoculation) did not differ significantly from each other, which means that the in-furrow co-inoculation with *A. brasilense* had no influence on this nodulation response. Further, in the presence of *A. brasilense* (T4), higher NN value was observed. A contrasting finding was pointed out in Hungria et al. (2015), in which the co-inoculation did not increase the NN comparative to standard inoculation; however, no incompatibility between the tested inoculants was found when the same method of application was employed.

Hungria et al. (2007) affirmed that at R_2 growth stage, a NN ranging from 15 to 30 nodules by plant and a NDW ranging from 0.10 to 0.20 g pl^{-1} are the adequate nodulation parameters in which the bacteria are able to supply the full N demand of the crop. However, as pointed out in Hungria et al. (2013), the higher results found in the present study may be due to the enrichment of soil bradyrhizobial population as the field area used has been continuously cultivated with inoculated soybean.

Shoot dry biomass (SDB) and shoot N content (SNC)

The SDB was significantly increased by the in-furrow inoculation of *B. japonicum*, relative to the non-inoculated controls (T1 and T2) or to the seed-inoculated treatments (T3 and T4). The same performance was found for SNC.

Further, once co-inoculated with *A. brasilense*, the treatments T4 and T6 provided equal or superior SNC and SDB values than those in which the single inoculation was performed, regardless of the method of application (Table 2). In this context, Hungria et al. (2015) summarized that it is unclear whether for soybean the benefits of co-inoculation of *B. japonicum* with *A. brasilense* are merely due to an intensification of N_2 fixation rates, or if other any secondary factor is involved. On the other hand, for maize, Domingues Neto et al. (2013) found an increase in the dry biomass of both the shoots and roots as a result of foliar application of *Azospirillum* spp. Bashan and Dubrovsky (1996) proposed that by affecting root roles, *Azospirillum* spp. participates in the partitioning of the carbon and minerals at the entire plant level. Later, Bashan et al. (2004) pointed that the positive response of dry matter accumulation to *A. brasilense* inoculation could be attributed to the phytohormones excreted by this bacterium.

Thousand seed weight (TSW) and grain N content (GNC)

As yield or quality components, the TSW and GNC were positively influenced by the inoculation of *B. japonicum* associated, or not to an application of *A. brasilense.* Moreover, treatments submitted to the in-furrow co-inoculation (T4 and T6) performed better than those in which only the standard inoculation was performed (T3 and T5).

In the bibliography, contrasting results have been recorded about the effects of the inoculation with *A. brasilense* on TSW. In this regard, while in Biari et al. (2008), Costa et al. (2015) and Morais et al. (2016) found an increase in seed weigh of maize after inoculation; on the other hand, no significant interaction between inoculation and seed weigh was reported by Novakowiski et al. (2011). Also, Dobbelaere et al. (1999) reported that inoculations with *A. brasilense* did not impact GNC in

maize.

Apart from the non-inoculated treatment T1, to which no N fertilizer was supplied, all treatments inoculated with *B. japonicum* (co-inoculated, or not, with *A. brasilense*) showed GNC values within the range considered as suitable for this crop (Martinez et al., 1999), but slightly lower than those reported by Hungria et al. (2015). Therefore, *B. japonicum* was able to provide the full N demand of the crop.

Grain yield (GY)

The in-furrow co-inoculation with *A. brasilense* and *B. japonicum* employed in T6 provided significant yield increase compared to the other treatments, mainly to those non-inoculated (T1 and T2). Interestingly, overall GY revealed the same trend observed earlier in NN, NDW, SDB, SNC, TSW and GNC, in which the highest values were observed in T6 followed in a decreasing order by treatments T5 and T4, with significant differences among them.

Such as observed in this work, Pedraza et al. (2009) and Hungria et al. (2010) found that in non-leguminous, *A. brasilense* promoted increase in the plant growth and yield compounds. Regardless of the co-inoculation, among the two tested standard inoculation of *B. japonicum,* the in-furrow application (T5 and T6) showed superior GY performance than the seed-inoculated treatments (T3 and T4). However, the co-inoculation with *A. brasilense* performed in T4, provided superior NN, SNC, TSW, GNC and GY results than the single seed-inoculated treatment (T3).

Brandão Júnior and Hungria (2000) documented that for *Rhizobium* spp. establishment in the substrate, the minimum concentration of viable bacteria in the inoculant must be hundred times higher than that naturally found in the soil. Based on this finding, it is plausible to suggest that the inoculant doses of *A. brasilense* combined with *B. japonicum* application used in T4 and T6 reached the minimum bacteria concentration able to form a robust soil colony.

As discussed in Araújo et al. (2015), under limited N availability, plants cannot produce sufficient root exudates, which act as sign to influence the ability of *A. brasilense* to colonize soil by horizontal movement or to survive in the rhizosphere (Mark et al., 2005). On this point, based on the understanding of Hallmann et al. (1997) and Saubidet et al. (2002), it is plausible to suggest that *B. japonicum* provided crop with an adequate N supply to sustain its growth and yield and, therefore, the plants were able to provide *A. brasilense* with the needed rate of carbon compounds for its soil establishment.

In the absence of oxygen, *A. brasilense* was found to present higher rate of N fixation (Dobbelaere et al., 1999). Nevertheless, the increase in the growth and yield in co-inoculated treatments of soybean with this bacterium may not only be credited to N biological fixation, since the excretion of hormones from the association plant-bacterium can increase the number and the mean diameter of lateral and adventitious roots (Dobbelaere et al., 2003), which could have intensified the plant nutrient absorption and thus the productivity (Bashan and De-Bashan, 2010).

Conclusion

The results demonstrated that co-inoculation with *B. japonicum* and *A. brasilense* is beneficial to soybean crop. The co-inoculation promoted grain yield increment ranging from 10 to 20% relative to standard inoculation (single application of *B. japonicum*), with the highest value observed using the in-furrow method of application of *B. japonicum* associated to an also in-furrow co-inoculation with *A. brasilense*.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

AOAC (2000). Association of Official Analytical Chemists. Official methods of analysis of A.O.A.C Internacional. 17 th ed. Gaitherburg, 1-2.

Araújo EO, Mercante FM, Vitorino ACT (2015). Effect of nitrogen fertilization associated with inoculation of *Azospirillum brasilense* and *Herbaspirillum seropedicae* on corn. Afr. J. Agric. Res. 10(3):137-145.

Banzatto DA, Kronka SN (2008). Experimentação Agrícola. 4 th ed Jaboticabal: FUNEP 237p.

Bashan Y, Dubrovsky JG (1996). *Azospirillum* spp. participation in dry matter partitioning in grasses at the whole plant level. Biol. Fertil. Soils 23:435-440.

Bashan Y, De-Bashan LE (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth - a critical assessment 2010. Adv. Agron. 108:77-136.

Bashan Y, Holguin G, De-Bashan LE (2004). *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. 50:521-577.

Biari A, Gholami A, Rahmani HA (2008). Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant prowth promoting rhizobacteria in arid region of Iran. J. Biol. Sci. 8(6):1015-1020.

Brandão Junior O, Hungria M (2000). Efeito de doses de inoculante turfoso na fixação biológica do nitrogênio pela cultura da soja. Rev. Bras. Ciênc. Solo 24(3):527-535.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS. 399p.

Caviglione JH, Kiihll LRB, Caramori PH, Oliveira D (2000). Cartas climáticas do Paraná. Londrina: IAPAR, CD.

Costa RRGF, Quirino GSF, Naves DCF, Santos CB, Rocha AFS (2015). Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize. Pesq. Agropec. Trop. 45(3):304-311.

Dall'Agnol A, Lazarotto JJ, Hirakuri MH (2010) Desenvolvimento, mercado e rentabilidade da soja brasileira. Circular Técnica 74. Embrapa Soja. Londrina.

Dobbelaere S, Croonenborghs A, Trys A, VandeBroek A, Vanderleyden J (1999). Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. Plant Soil 212:155-164.

Dobbelaere S, Vanderleyden J, Okon Y (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci. 22:107-149.

Domingues Neto FJ, Yoshimi FK, Garcia RDG, Miyamoto YR, Domingues MCS (2013). Desenvolvimento e produtividade do milho verde safrinha em resposta à aplicação foliar com *Azospirillum brasilense.* Enciclopédia Biosfera 9(17):1030-1040.

Embrapa: Empresa Brasileira de Pesquisa Agropecuária (2011). Tecnologias de produção de soja – região central do Brasil 2012 e 2013. Londrina: Embrapa Soja, 264 p. (Sistemas de Produção, 15).

Embrapa: Empresa Brasileira de Pesquisa Agropecuária (2013). Sistema brasileiro de classificação de solos. Embrapa Solos. Rio de Janeiro, Brasil. 306 p.

Ferreira DF (2011). Sisvar: a computer statisticalanalysis system. Ciênc. Agrotec. 35(6):1039-1042.

Hallmann J, Quadt–Hallmann A, Mahaffee WF, Kloepper JW (1997). Bacterial endophytes in agricultural crops. Can. J. Microb. 43:895-914.

Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Skrumasager I (2012). Functions of macronutrients. In: Marschner P (ed.). Marschner's mineral nutrition of higher plants. 3rd ed. London: Elsevier. pp. 135-178.

Hungria M, Campo RJ, Mendes IC (2007). A importância do processo de fixação biológica de nitrogênio para a cultura da soja: componente essencial para a competitividade do produto brasileiro. Londrina, Embrapa Soja, 80 p (Documentos, 283).

Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331:413-425.

Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, Mendes IC, Arihara J (2006). Nitrogen Nutrition of Soybean in Brazil: Contributions of Biological N_2 Fixation and of N Fertilizer to Grain Yield. Can. J. Plant Sci. 86:927-939.

Hungria M, Nogueira MA, Araujo R (2015). Soybean seed coinoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. Am. J. Plant Sci. 6:811-817.

Hungria M, Nogueira MA, Araujo RS (2013). Tecnologia de coinoculação da soja com *Bradyrhizobium* e *Azospirillum*: incrementos no rendimento com sustentabilidade e baixo custo. In: Reunião de Pesquisa de Soja da Região Central do Brasil 33, Londrina.

Mark GL, Dow JM, Kiely PD, Higgins H, Haynes J, Baysse C, O'Gara F (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. Proc. Nat. Acad. Sci. United States Am. 102(48):17454-17459.

Martinez HEP, Carvalho JG, Souza RB (1999). Diagnose foliar. In: Ribeiro AC, Guimarães PTG, Alvarez VVH (eds) Recomendação para uso de corretivos e fertilizantes em Minas Gerais: 5ª Aproximação. Viçosa, Comissão de Fertilidade do Solo do Estado de Minas Gerais. pp. 143-168.

Mendes IC, Hungria M, Vargas MAT (2003). Soybean response to starter nitrogen and *Bradyrhizobium* inoculation on a Cerradooxisol under no-tillage and conventional tillage system. Rev. Bras. Ciênc. Solo 27:81-87.

Morais TP, Brito CH, Brandão AM, Rezende WS (2016). Inoculation of maize with *Azospirillum brasilense* in the seed furrow. Rev. Ciênc. Agron. 47(2):290-298.

Novakowiski JH, Sandini IE, Falbo MK, de Moraes A, Novakowiski JH, Cheng NC (2011). Efeito residual da adubação nitrogenada e inoculação de *Azospirillum brasilense* na cultura do milho. Semin. Ciênc. Agrar. 32:1687-1698.

Pedraza RO, Bellone CH, de Bellone S, Sorte PMB, Teixeira KRD (2009). *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. Eur. J. Soil Biol. 45:36-43.

Petersen R (1994). Agricultural field experiments: Design and analysis. Marcel Dekker Inc., New York, USA.426 p.

Saubidet MI, Fatta N, Barneix AJ (2002). The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. Plant Soil 245:215-222.

Taiz L, Zeiger E (2004). Fisiologia Vegetal.Trad. Santarém, E R et al., 3. ed. Porto Alegre: Artmed. 719p.

USDA (2016). United State Department of Agriculture – Foreign Agricultural Service. Available on: http://apps.fas.usda.gov/psdonline. Acessed on: 02 September 2016.

Veloso CAC, Viégas I de JM, Oliveira RF de, Botelho SM (2006). Amostragem de solo e planta para análise química. Belém, PA: Embrapa Amazônia Oriental. 39p.

Vitti GC, Camargo MAF, Lara C (2001). Síntese de análise químicas em tecido vegetal. Piracicaba: Departamento de Solos e Nutrição de Plantas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo.

Zilli JE, Marson LC, Campo RJ, Gianluppi V, Hungria M (2006). Avaliação da fixação biológica de nitrogênio na soja em áreas de primeiro cultivo no cerrado de Roraima. Embrapa Roraima. 9p. (Comunicado Técnico, 20).

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Full Length Research Paper

Estimated damage caused by ramularia leaf spots on cotton

Thiago Alexandre Santana Gilio[1*], Dejânia Vieira de Araújo[2], Kelly Lana Araújo[3], Thomas Edson Fregonese[2], Rodrigo Chimenez Franzon[1] and Jaqueline Aguila Pizzato[2]

[1]Universidade Estadual de Maringá (UEM), Maringá, Paraná, Brazil.
[2]Universidade do Estado de Mato Grosso (UNEMAT), Tangará da Serra, Mato Grosso State, Brazil.
[3]Universidade do Estado de Mato Grosso (UNEMAT), Cáceres, Mato Grosso State, Brazil.

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The objectives of this study are to establish damage functions for the *Ramularia areola* pathosystem and characterize the damage in cultivars susceptible and resistant to it. The experiment was conducted in the city of Tangará da Serra in the 2012/2013 harvest season. The experimental design was randomized blocks with four replications in a factorial arrangement of four cultivars × four gradients. The plots were divided into two subplots (twin plots). The division I received fungicide applications at intervals of 15, 30 and 45 days and no applications to establish the gradients of the disease, and the division II received weekly applications of fungicide in order to be kept without the disease or at minimum levels. FiberMax 993, FMT 701, FMT 707 RX and IAC 25 RMD cultivars were used with different reactions to ramularia spots. Disease severity was weekly determined and used to calculate the area below the disease progress curve (ABDPC). In addition to area below the severity progress curve (ABSPC), the variables related to the following production components were obtained: seed cotton productivity, damage, percentage of damage to productivity, average number of bolls per plant, weight of one hundred seeds, percentage of fiber, and components related to fiber technological characteristics. Analyses of variance were conducted and data were adjusted by the linear integral model. The ABSPC variable showed a high relation with damage and percentage of damage to productivity. Estimates reached levels of up to 975.42 kg ha^{-1}, or 36.83%, of damage to productivity.

Key words: Damage, functions, *Ramularia areola*, *Gossypium hirsutum*, fiber technological characteristics (HVI).

INTRODUCTION

Brazil is the world's fifth largest cotton producer (FAO, 2016). In Brazil, cotton lint production in the 2014/2015 harvest was 1,562,800.00 tons in a cultivated area of 976.2 hectares. 64% of this area is located in the Midwest

*Corresponding author. E-mail: thiago_gilio@hotmail.com.

region, followed by the Northeast region with 32% (CONAB, 2016).

As in many cultures, there are diseases responsible for negative factors, causing damage to crops and losses in agricultural activity. Among the most important cotton diseases, is the ramularia leaf spot, considered by many researchers as the most important disease of cotton in Brazil (Cia and Salgado, 2005).

Ramularia leaf spots were diagnosed for the first time in 1890 in the state of Alabama, United States (Atkinson, 1890). Subsequently, the disease was found in several places that cultivated cotton. All cultivated species of the genus *Gossypium* were hosts to the disease (Bell, 1981). Ramularia leaf spot, also known as ramulariosis, false mildew, areolate mildew, grey mildew or cotton ramularia are caused by the phytopathogenic fungus *Ramularia areola* GF Atk. [syn. = *Ramularia gossypii* (Speg.) Cif., *Cercosporella gossypii* Speg.] in its asexual or anamorphic form and in its sexual or telemorphic form by *Mrycosphaerella areola* Ehrlich and FA Wolf (Atkinson, 1890; Suassuna and Coutinho, 2007).

This disease may occur in all plant development stages, causing lesions to both sides of leaves, resulting in a reduction in the photosynthetic area. It accounts for significant damage to productivity and fiber quality of the culture. Favorable climatic conditions for the development of the pathogen are temperatures between 12 and 32°C, the optimal range being 25 and 30°C, and a relative humidity above 80% (Rathaiah, 1977). According to Mohan et al. (2006), the disease worsens in a relative humidity over 90 or 91% and in temperatures from 23 to 27°C.

In recent years, with the increase of the cotton production area, cultivars susceptible to this disease are been used with a greater precocity. Therefore, this increased the potential for damage, being then considered as one of the major cotton diseases (Utiamada et al., 2003). According to Andrade Junior and Galbiere (2014), in the Brazilian center-western region, yield losses caused by Ramularia leaf spot are about 20 to 30% without control (Andrade Junior and Galbiere, 2014), therefore, the potential of damage to production caused by ramularia leaf spots can reach up to 68% in susceptible cultivars (Shivankare and Wangikar, 1992).

Variations in susceptibility levels of cultivars occur probably due to emergence of new races of the pathogen and the use of cultivars that do not have resistance (Polizel et al., 2008). Girotto et al. (2013) found genetic and phenotypic variability in isolates of the fungus, *Ramularia areola* thus studies to establish the pathogen races are needed.

Traditional evaluation models and quantification of damage are obtained by disease variation in different plots and by relative levels of severity of the disease in relation to production. Thus, damage can be caused by a simple linear regression, where the variable disease is the independent variable, and the reduced productivity, whether in kilograms per hectare or percentage, is the dependent variable (Zadoks, 1985; Amorim and Bergamin Filho, 2011). The damage equation provides biological variables to calculate the economic damage threshold. Thus, the equation becomes necessary for decision-making regarding improvement programs or integrated disease management programs (Amorim and Bergamin Filho, 2011), since there are no cotton cultivars with a satisfactory resistance to ramularia leaf spot. Thus, the use of fungicides is necessary to ensure production (Cia and Salgado, 2005).

This study aimed to establish the damage functions for ramularia leaf spot on cotton plants and to characterize the damage to cultivars resistant and susceptible to ramularia.

MATERIALS AND METHODS

The experiment was conducted in the city of Tangará da Serra, Mato Grosso State. The experimental area is located 320 m above sea level, at 14°37'10" S and 59°29'09" W. The soil of this area was classified as a dystrophic clayey red latosol (Embrapa, 1999).

The field sowing was performed on December 18, 2012, with a density of eight plants per meter. The fertilization was performed according to Souza and Lobato (2004), where 25 kg ha^{-1} of N, 100 kg ha^{-1} of P_2O_5 and 40 kg ha^{-1} of K_2O were used at planting. The topdressing was applied 30 days after sowing with 50 kg ha^{-1} of N and 40 kg ha^{-1} of P_2O_5, and at 50 days with 50 kg ha^{-1} of N.

The inoculation of the plants was made at 45 days after plant emergence in the early reproductive stage (B1). The inoculum was obtained from the washing of cotton leaves with symptoms of Ramularia. The suspension was adjusted to a concentration of 4×10^5 conidia mL^{-1} with a Neubauer chamber. The adhesive spreader Tween 20 (0.01%) was added to the final suspension, and inoculations were performed with a portable back sprayer until runoff.

The experimental design was randomized blocks in a 4 × 4 factorial (four cultivars and four gradients of disease), with four replications, totaling 16 treatments. The cultivars FiberMax 993, FMT 701, FMT 707 and IAC 25 RMD were used, each one with differential reactions to ramularia leaf spot (Table 1). In Additional to that to obtain different levels of disease in each cultivar, sprayings were performed with the mixture of fungicides, trifloxystrobin (60 $g.ha^{-1}$) + prothioconazole (70 $g.ha^{-1}$) + mineral oil (500 $mL.ha^{-1}$), at intervals between applications of 15 days for the first gradient, 30 days for the second gradient, 45 days for the thirty gradient and no application for gradient fourth.

The experimental plot consisted of eight lines of 5 m in length with a spacing of 0.9 m. Each plot was divided into two equal subplots (twin portions), that is, each division consisted of four rows, being the division I with the disease at different gradients and division II without the disease (or with minimal disease). To keep the division I without disease, applications of fungicide were performed at intervals of seven days. The useful area for evaluations was composed of two main lines in each division of the plot, in which severity and yield components evaluations were performed, and samples were collected for fiber technological analysis. On the border of each plot was sown an infector line with the susceptible cultivar FMT 701 to homogenize the inoculum pressure in the experiment. The control of insects-pests and weeds was performed when necessary by raising the level of damage.

At 20 days after inoculation, symptoms of ramularia leaf spots were identified in the experiment and the evaluations of disease severity began. The evaluations were performed at intervals of seven days until 152 days after planting (DAP). The disease severity

Table 1. Cultivars, reaction to ramularia leaf spots and respective breeders and holding institutions.

Cultivars	Breeder/holding	Reaction to ramularia spots	Cycle
Fibermax 993	Bayer Seeds	Susceptible	Late
FMT 701	Fundação Mato Grosso	Mod. Susceptible	Late
FMT 707 RX	Fundação Mato Grosso	Resistant	Medium
IAC 25 RMD	Inst. Agronômico de Campinas	Resistant	Medium

[1]Reactions regarding the level of resistance were obtained from breeders/holding agencies. [2](Mod) Moderately.

was evaluated by percentage of diseased leaf area with the diagrammatic scale proposed by Aquino (2008) with nine severity levels: 0.05, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 67.20%.

For the evaluation of the disease, six plants of the useful area of each division in each plot were previously marked. These plants were also used in determining the percentage of fiber, one hundred seed weight and analysis of fiber characteristics.

Severity data of all evaluations were used to calculate the area below the severity progress curve (ABSPC) according to Shaner and Finney (1977):

$$\mathrm{ABSPC} = \sum_{i=1}^{n-1} [(N_i + N_{i+1})/2](t_{i+1} - t_i)$$

Where, n is the number of evaluations, x is the severity of the disease at time t_i and (t_i+1-t_i) is the interval between two consecutive evaluations.

The real and potential productivity was obtained from the manual harvest of all plants in four central meters of the useful area of each division in each plot. The weighing was measured with a digital electronic scale with accuracy of 0.01 g, and data were converted to kilograms per hectare. The real productivity was estimated from the harvest and the weighing of divisions of the plots that were subjected to different disease gradients. The estimated productivity was obtained by harvest and weighing of the healthy divisions of the plots.

To obtain the loss in productivity in kilograms per hectare (kg ha^{-1}) and in percentage, the following equations were used (James et al., 1968; Nkalubo et al., 2007):

$$\text{Damage in Kg. ha}^{-1} = \mathrm{PP} - \mathrm{RP}$$

and

$$\text{Damage in \%} = \frac{\mathrm{PP} - \mathrm{RP}}{\mathrm{PP}} \text{ x } 100$$

where PP is potential productivity and RP is real productivity.

During harvesting, the total number of bolls of the six marked plants in each division was counted to obtain the average of bolls per plant. 20 bolls were sampled to obtain the percentage of fiber and the one hundred seed weight. In addition, analyses with a high volume instrument (HVI) device were performed to obtain fiber characteristics, length, uniformity index, resistance index, stretch, micronaire, yellowing degree, maturity, short fiber content and CSP reliability index.

The damage in these production components and fiber characteristics were obtained by subtracting the means of each component in the division under the effect of different disease gradients minus the means of the components obtained from the healthy division (Amorim and Bergamin Filho, 2011).

Weather temperature (°C) and relative humidity (%) data during the experiment were obtained from the National Institute of Meteorology (INMET) at the weather station installed in the city of Tangará da Serra, located approximately 100 m from the experiment site. With hourly temperature and relative humidity records, the number of optimal hours for germination of *R. areola* conidia per month was calculated. Temperatures between 25 and 30°C and a relative air humidity greater than or equal to 80% were considered as ideal climatic conditions (Rathaiah, 1977).

Analyses of variance were conducted and data were adjusted by the linear integral model Y = a+bx, where y is damage and x is ABSPC (Schneider et al., 1976). In all analyzes, the software Genes was used (Cruz, 2006).

RESULTS AND DISCUSSION

The temperature was favorable for the germination of conidia and the development of *R. areola* throughout the experiment (Figure 1a). The number of favorable air humidity hours also occurred throughout the cycle, with peaks between 25 and 30, 49 and 55, 67 and 73, 91 and 97, and 151 and 153 days after planting (DAP), reducing after 154 DAP until the end of the cycle (Figure 1b).

Ramularia spots, like most diseases, are influenced by climate, which is crucial to the pathogen development of this disease. Rathaiah (1977) and later Curvêlo et al. (2010), studying the penetration of *R. areola*, observed that the highest germination of conidia occurred in the temperature range of 25-30°C and an air humidity above 80%, and that the greater penetration of the fungus occurred in the nighttime wetting cycle and daytime drying cycle. The authors also found that in the process of germination and penetration, the germ tube of this fungus tolerates up to 16 h of drying (humidity above 20%) for a few cycles and then returns to the process of germination and penetration.

The analysis of variance was performed for all variables evaluated. However, there was a significant interaction ($p \leq 0.01$) between cultivars and disease gradients only regarding the ABSPC of the disease, damage in kilograms per hectare and percentage of damage.

In cultivars under different ramularia spot gradients, damage to productivity ranged from 104.00 to 975.42 kg ha^{-1}, equivalent to 4.33 and 36.72%, respectively (Table 2). The cultivars Fibermax 993 and FMT 701 had the highest percentages of damage, reaching 36.83 and 36.72%, the highest gradients of the disease,

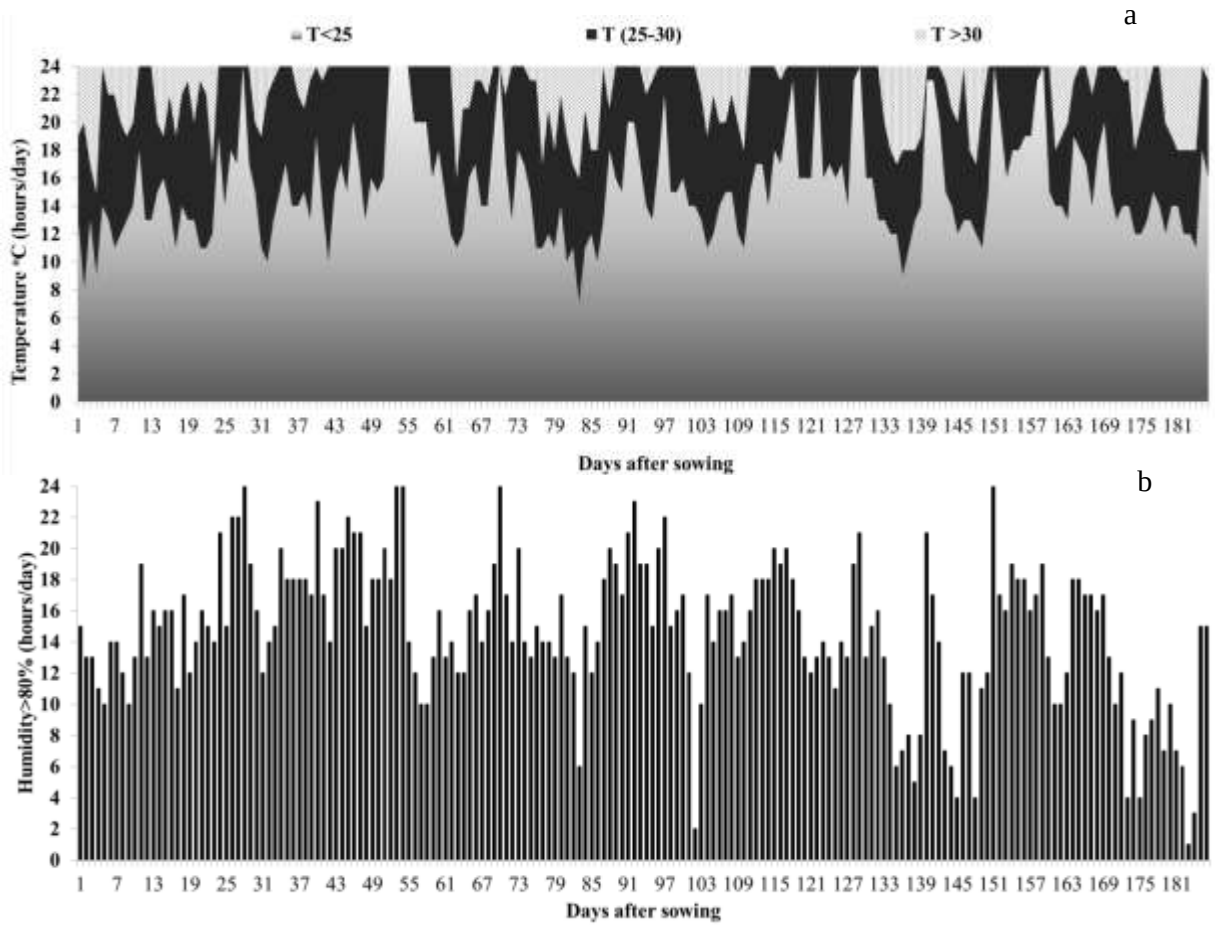

Figure 1. Climatic temperature and relative humidity data during the experiment. A- Number of hours of daily temperature <25°C, 25-30°C and >30°C; B- number of hours with a relative humidity higher than 80%. Tangará da Serra, 2012/2013 harvest.

respectively. The cultivar FMT 707 had the lowest damage percentages, varying between 4.33 and 19.86% among disease gradients, followed by IAC 25, wherein damage percentages ranged from 10.24 to 25.08% (Table 2). Ascari et al. (2016) has observed yield demages of 30.82% comparing control and uncontrol plots in first sowing.

Cultivars had different ABSPC indexes and percentages of damage to productivity. The graphical representation of the linear equation of damage in function of ABSPC shows a significant increase of the damage with the increase in ABSPC, wherein for each ABSPC unit, there was an increase of 0.0375% in damage to productivity, with a coefficient of determination of 96.89% (Figure 2a). Brito et al. (2007), studying the effect of cercosporiosis on commercial corn hybrids, also found a linear increase in damage to productivity due to the increase of the ABSPC index.

When cultivars and their gradients were considered in a same Equation (Figure 2b), the equation components were similar to those obtained by the cultivars: for each ABSPC unit, there was an increase of 0.041% in damage to productivity, but the coefficient of determination was 82.11%. Even so, it can be considered as a high relation of ABSPC with productivity, given the fact that coefficients above 80% are considered acceptable.

Comparing damage percentage equations in function of ABSPC, the relations were significant for both susceptible cultivars ($p \leq 0.01$) and resistant cultivars ($p \leq 0.05$). For the cultivars Fibermax 993 and FMT 701 (Figure 3a and b), the damage function equation showed that for each ABSPC unit, there was a 0.0745 and 0.0412% damage to productivity, respectively, with coefficients of determination of 98.33 and 97.70%. However, considering the cultivars, FMT 707 and IAC 25 RMD (Figure 3c and d), one ABSPC unit increased damage to productivity by 0.238 and 0.143%, respectively. The coefficients of determination ranged from 98.4 to 97.79%, with the exception of the cultivar IAC 25, with a coefficient of determination of 84.22%, thus demonstrating a high relation between damage to productivity and ABSPC (Figure 3).

Table 2. Area below the severity progress curve (ABSPC), real and potential productivity, and damage to productivity caused by Ramularia spots under different disease gradients and cotton cultivars in Tangará da Serra, MT, 2012/2013 harvest.

Cultivar	Disease gradient	ABSPC	Real productivity (Kg ha^{-1})	Potential productivity (Kg ha^{-1})	Damage	
					Kg ha^{-1}	(%)
FM 993	1	309.22	1,660.00	1,982.50	322.50	16.55
FM 993	2	447.21	1,937.50	2,570.00	632.50	24.61
FM 993	3	538.89	1,497.50	2,251.25	753.75	34.14
FM 993	4	589.52	1,548.75	2,438.75	890.00	36.83
FMT 701	1	182.24	1,833.75	2,233.75	400.00	17.88
FMT 701	2	397.41	1,931.25	2,542.50	611.25	24.08
FMT 701	3	527.79	1,728.75	2,508.75	780.00	31.27
FMT 701	4	642.87	1,680.00	2,655.42	975.42	36.72
FMT 707	1	20.90	2,280.00	2,384.00	104.00	4.33
FMT 707	2	32.97	1,617.50	1,856.25	238.75	13.49
FMT 707	3	60.76	1,835.00	2,165.00	330.00	15.07
FMT 707	4	76.63	1,868.75	2,331.25	462.50	19.86
IAC 25	1	175.96	1,962.50	2,173.75	211.25	10.24
IAC 25	2	185.16	1,938.75	2,228.75	290.00	12.82
IAC 25	3	227.68	1,707.50	2,068.75	361.25	17.81
IAC 25	4	272.58	1,527.50	2,036.25	508.75	25.08

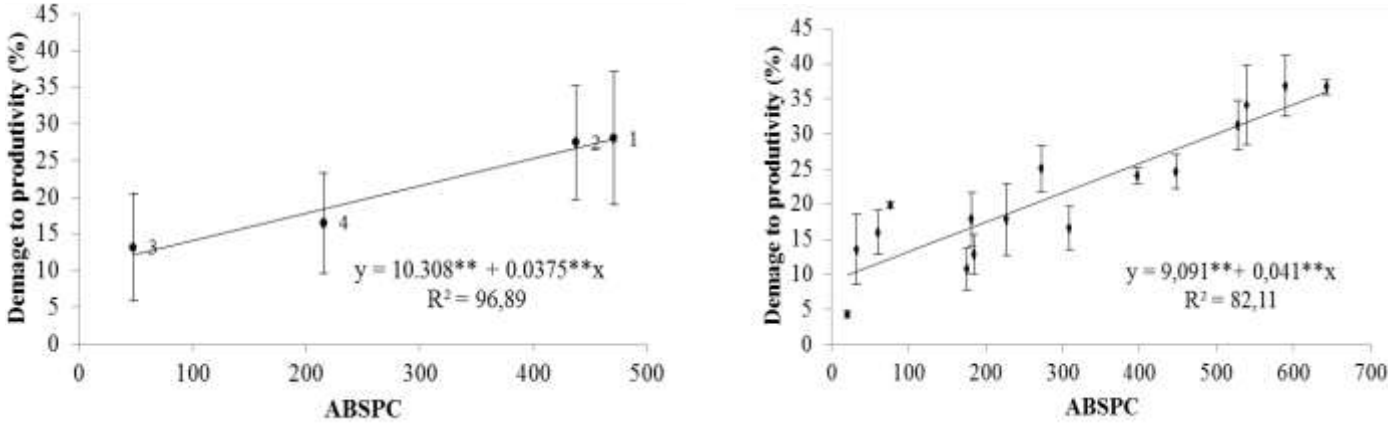

Figure 2. Relation between damage to productivity and ABSPC for cotton cutivars Fibermax 993 (1), FMT 701 (2), FMT 707 (3) and IAT 25 (4) (a), and for all cultivar and gradient treatments (1b).

For several other pathosystems, relations were found between ABSPC and damage to productivity by the integral linear model, as reported by Hartman et al. (1991) for Asian soybean rust, Godoy and Canteri (2004) for mildew and Cercospora in soybeans, Brito et al. (2007) for corn Cercospora, Leite et al. (2006) for sunflower Alternaria, Hikishima et al. (2010) for Asian soybean rust, and Fiallos (2011) for common bean blight. ABSPC is a variable recommended by some authors because it represents the epidemic of the disease as a whole in all plant cycles (Bergamin Filho and Amorim, 1996).

In plants with an indefinite cycle, it is common to find a relation between disease and production because leaves are not fixed (Amorim and Bergamin Filho, 2011). However, the high relation in the pathosystem under study may be because the use of regulators ultimately determines the cotton cycle.

Although, the total damage is greater in susceptible cultivars, damages by ABSPC units were higher in resistant cultivars (Figure 3c and d), exceeded by up to six times the susceptible cultivars (Figure 3a and d). This may be related to a greater tolerance by susceptible cultivars used herein considering the severity of the disease.

The relationship between ABSPC and some productivity

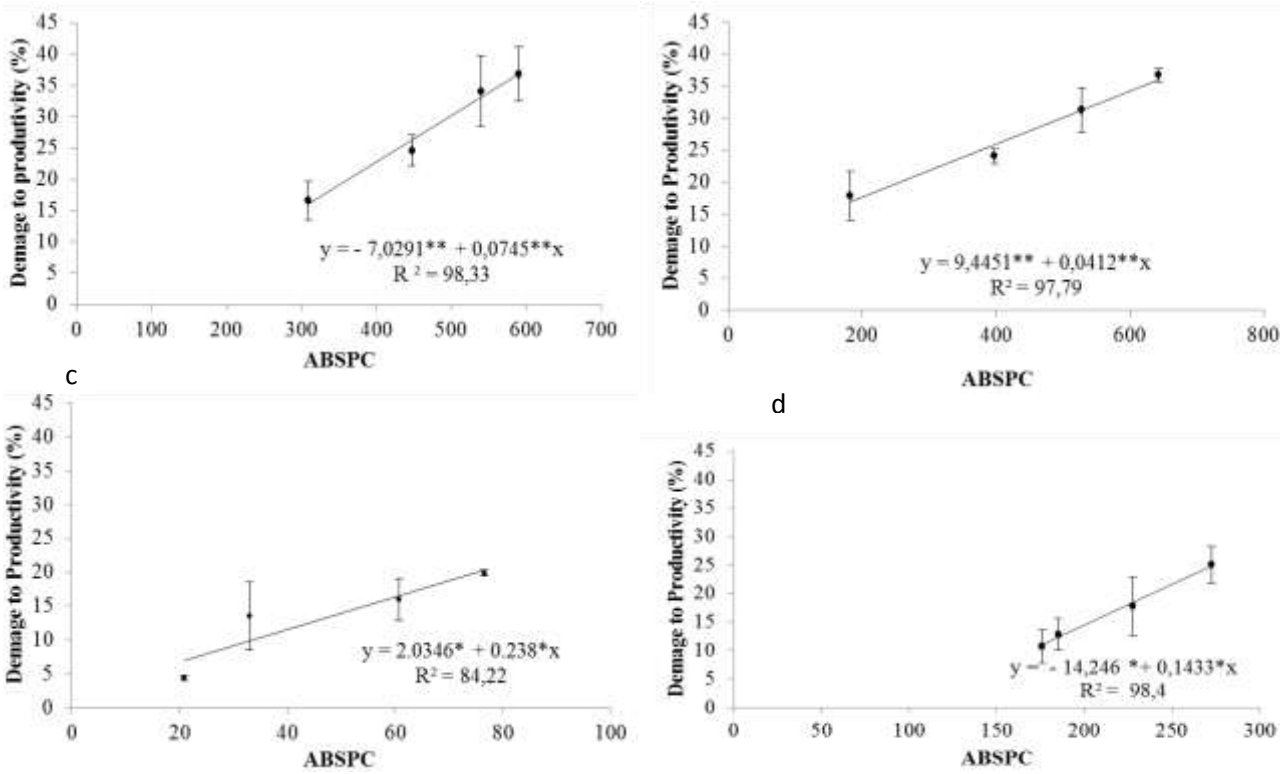

Figure 3. Relation between damage to productivity and ABSPC for cotton cultivars Fibermax 993 (a), FMT 701 (b), FMT 707 (c) and IAC 25 RMD (d). Tangará da Serra, MT, 2012/2013 harvest.

Table 3. Angular coefficients of the regression line between characteristics related to productivity and fiber characteristics (HVI) in function of area below the severity progress curve of Ramularia spots on cotton (y = a+bx, where y is damage and x is ABSPC).

Characteristics related to productivity	Intercept (a)	Slope (b)	Coefficient of determination (R^2)
No. of bolls/plant	1.6418	0.0007	0.53^{ns}
Damage to productivity ($Kgha^{-1}$)	156.6140	1.1446	85.85**
Weight of 100 seeds	0.4068	-0.0023	16.02^{ns}
Fiber percentage	5.7084	-0.0058	0.78^{ns}
Fiber characteristics (HVI)	Intercept (a)	Slope (b)	R^2
Length	0.3173	-0.0005	3.03^{ns}
Uniformity Index	0.5262	-0.0007	3.86^{ns}
Resistance index	0.6591	-0.0019	53.17*
Stretching	-0.0111	0.0001	2.09^{ns}
Micronaire	0.05326	0.00002	0.02^{ns}
Degree of yellowing	-0.1504	0.0004	2.46^{ns}
Maturity	302.2838	2,975.1829	1.97^{ns}
Short fiber index	-0.4657	0.00091	5.92^{ns}
Reliability index	115.0120	-0.2861	16.23^{ns}

* and **Significant at 1 and 5%, respectively. ^{ns}Not significant.

components, estimated by the same linear equation, are shown in Table 3. There was no significant relation of ABSPC on production components: number of bolls per plant, weight of one hundred seeds and percentage of

fiber.

The production component damage to productivity (kg ha^{-1}) suffered a significant interference of ABSPC, with a coefficient of determination of 85.85%. There was damage of 1 $kgha^{-1}$ by ABSPC unit of the ramularia spot (Table 3). Polizel et al. (2008), evaluating 16 cotton genotypes in Uberlândia, MG, in experimental field conditions, found no significant correlation between severity of ramularia spots and production.

As for fiber technological properties, there was a significant relation only for fiber resistance, in which for each ABSPC unit, there was a reduction of 0.0019 gf tex^{-1}. However, the coefficient of determination was 53.17%, which little explains what occurred (Table 3). Cia et al. (2008), studying 16 cotton genotypes in various regions of Brazil regarding resistance to multiple diseases, including ramularia spots, did not identify a relation between disease and quality of fiber.

Conclusions

The relation between ABSPC, damage in kilograms per hectare and damage percentage was significant, and the model y = a+bx may be used to estimate the damage to these components in function of ABSPC. As for fiber characteristics, the ABSPC of ramularia spots negatively influenced only the resistance index.

Percentages of damage to productivity caused by ramularia spots reached levels of up to 36.83% in susceptible cultivars. Even in the cultivar with the highest resistance to damage to productivity, percentages reached levels up to 19.86%.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Andrade Junior ER, Galbieri R (2014). Eficiência de fungicidas no controle de mancha de ramulária em algodoeiro, na safra 2013/14 no Mato Grosso. Circular técnica n°12/IMA, Cuiabá 8 p.

Amorim L, Bergamim Filho A (2011). Fenologia, patometria e quantificação de danos. In: Amorim L, Rezende JAM, Bergamim Filho A. (Edt.). Manual de Fitopatologia. Princípios e Conceitos. São Paulo. Agron. Ceres 1:517-540.

Aquino LA, Berger PG, Rodrigues FÁ, Zambolim L, Hernandez FR, Miranda LM (2008). Elaboration and validation of a diagrammatic scale to quantify areolate mildew on cotton. Summa Phytopathol. 34(4):361-363.

Ascari JP, de Araújo DV, Dias LDE, Bagatini GJ, Mendes IRN (2016). Severity of ramularia leaf spot and seed cotton yield in different sowing times. Rev. Caatin. 29(3):603-610,

Atkinson GF (1890). A new *Ramularia* on cotton. Bot. Gaz. 15:166-168.

Bell AA (1981). Areolate mildew. In: Watkins GM. (ed.). Compendium of Cotton Diseases. APS Symp. Ser., St. Paul, Minnesota, United states 87 p.

Bergamin Filho A, Amorim L (1996). Doenças de plantas tropicais: epidemiologia e controle econômico. São Paulo SP. Editora Ceres 299 p.

Brito AH, Von Pinho RG, Pozza EA, Pereira JLAR and Faria Filho EM (2007). Efeito da cercosporiose no rendimento de híbridos comerciais de milho. Fitopat. Bras. 32:472-479.

Cia E, Salgado CL (2005). Doenças do algodoeiro (*Gossypium* spp.). In: Kimati, H. et al. (Eds.). Manual de fitopatologia: doenças das plantas cultivadas, 4ed. São Paulo: Agron. Ceres 2:41-52.

Cia E, Fuzatto MG, Kondo JI, Luders RR, Galbieri R, Carvalho LH, Ito MF, Ruano O, de Almeida WP, Adriano Borges De Oliveira3, da Cunha HF, Chiavegato EJ, Aguiar PH, Rossetto R, Martins ALM, Pettinelli Júnior A, t Bolonhezi D, Foltran DE, Kasai FS, Bortoletto N (2008). Reação de cultivares e linhagens de algodoeiro às principais doenças que ocorrem em regiões produtoras do Brasil. Rev. Ceres 55(8):518:524.

CONAB – Companhia Nacional de Abastecimento (2016). Acompanhamento de safra brasileira: grãos, décimo levantamento, julho 2016. Disponível em: <http://www.conab.gov.br> Acesso em: 29 de agosto de 2016.

Cruz CD (2006). Programa Genes: Biometria. Editora UFV. Viçosa (MG) 382p.

Curvêlo CRS, Rodrigues PGB, Rezende DC (2010). Microscopia eletrônica de varredura do processo infeccioso de *Ramularia areola* em folhas de algodoeiro. Trop. Plant Pathol. 35(2):108-113.

Embrapa (1999). Centro Nacional de Pesquisa de Solos. Sistema brasileiro de classificação de solos. Brasília, DF: Embrapa Produção da Informação; Rio de Janeiro: Embrapa Solos 412 p.

FAO - Food and Agriculture Organization (2016). Disponível em: <http://www.fao.org>. Acesso em: 29 de agosto de 2016.

Fiallos FRG (2011). Modelo de ponto crítico para estimar danos causados pela mela na cultura do feijoeiro. Cienc. Tecnol. 4(1):1-4.

Girotto L, Marangoni MS, Matos JN, Galbieri R, Almeida WP, Mehta YR (2013). Identification of phenotypic and genotypic variability among the isolates of Ramularia areola of Brazilian Cotton. Am. J. Plant Sci. Temuco 4(9):1893-1898.

Godoy CV, Canteri MG (2004). Efeito da severidade de oídio e crestamento foliar de cercospora na produtividade da cultura da soja. Fitopatol. Bras. 29:526-531.

Hartman GL, Wang TC, Tschanz AT (1991). Soybean rust development and quantitative relationship between rust severity and soybean yield. Plant Dis. 75:596-600.

Hikishima M, Canteri MG, Godoy CV, Koga LJ, Silva AJ (2010). Quantificação de danos e relações entre severidade, medidas de refletância e produtividade no patossistema ferrugem asiática da soja. Trop. Plant Pathol. 35:96-103.

James WC, Jenkins JEE, Jemmentt JL (1968). The relationship between leaf blotch caused by *Rhynchosporium secalis* and losses in grain yield of spring barley. Ann. Appl. Biol. 62:273-288.

Leite RMVBC, Amorim L, Bergamin Filho A (2006). Relationships of disease and leaf area variables with yield in the *Alternaria helianthi*–sunflower pathosystem. Plant Pathol. 55:73-81.

Mohan P, Mukewar PM, Singh VV, Singh P, Khadi BM, Amudha J, Deshpande VG (2006). Identification of sources of Resistance to grey mildew disease (*Ramularia areola*) in diploid Cotton (*Gossypium arboreum*). Technical Bulletin 34, CICR. Disponível em: http://www.cicr.org.in/. Acesso em: 29, agosto, 2016.

Nkalubo S, Melis R, Ópio F (2007). Yield loss associated with anthracnose disease on Ugandan market-class dry bean cultivars. Afr. Crop Sci. Conf. Proc. 8:869-874.

Polizel AC, Juliatti FC, Penna JCV, Hamawaki OT (2008). Reação de genótipos de algodoeiro quanto à severidade de manchas foliares. Biosci. J. 24(3):8-12.

Rathaiah Y (1977). Spore germination and mode of cotton infection by *Ramularia areola*. Phytopathology 67:351-357.

Schneider RW, Willians RJ, Sinclair JB (1976). Cercospora leaf spot of cowpea: models for estimating yield loss. Phytopathology 66:384-388.

Souza DMG, Lobato E (2004). Adubação com nitrogênio. In: Sousa DMG, Lobato E, eds. Cerrado: correção do solo e adubação. 2.ed. Planaltina, Embrapa Cerrados pp. 129-144.

Shaner G, Finney RE (1977). The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathol. 67:1051-1056.

Shivankar SK, Wangikar PD (1992). Estimation of crop losses due to grey mildew disease of cotton caused by Ramularia areola. Indian Phytopathol. 45(1):74-76.

Suassuna ND, Coutinho WM (2007). Manejo das Principais Doenças do Algodoeiro no Cerrado Brasileiro. In: Freire, E. C. Algodão - No Cerrado do Brasil. Brasília: Associação Brasileira dos Produtores de Algodão 918 p.

Utiamada CM, Lopes JC, Sato LN, Roim LFB, Kajihara L, Occhiena EM (2003). Controle químico da ramulária (*Ramularia areola*) e ferrugem (*Phakospora gossypii*) na cultura do algodoeiro In: IV Congresso Brasileiro do Algodão, Goiânia GO.

Zadoks JC (1985). On the conceptual basis of crop loss assessment: the threshold theory. Ann. Rev. Phytopathol. 23:455-473.

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Full Length Research Paper

Effect of organo-mineral fertilizer and poultry litter waste on sugarcane yield and some plant and soil chemical properties

Lucélia Alves Ramos*, Regina Maria Quintão Lana, Gaspar Henrique Korndörfer and Adriane de Andrade Silva

Universidade Federal de Uberlândia – Avenida Amazonas s/n – bloco 4C – sala 111 – Bairro Umuarama – CEP: 38400-302 - Uberlândia-MG – Brazil.

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Application of organic wastes in sugarcane (*Saccharum officinarum)* is an alternative possibly replacing or supplementing mineral fertilizers. This study aimed to evaluate the effects of poultry litter, organo-mineral fertilizer and mineral fertilizer on soil chemical properties, yield and plant content of macronutrients in sugarcane. The experiment was conducted at the Jalles Machado Mill in Goianésia-GO, from July 2009 to April 2010, as randomized complete blocks design (RCBD). The mineral fertilizer was applied at the following doses: 66; 120 and 82 kg ha^{-1} of N, P_2O_5 and K_2O, respectively; poultry litter and organ-mineral fertilizer were applied at doses of 3.0, 6.0 and 9.0 t ha^{-1}, in addition to the control (no fertilization). The following variables were analyzed: organic matter, macronutrients in soil and leaves and sugarcane yield. The productivity of sugarcane was higher with poultry litter and organo-mineral fertilizer in relation to mineral fertilization, and organo-mineral fertilization was superior to poultry litter. There was an increase in soil phosphorus levels in soil by fertilizing with poultry litter and organo-mineral fertilizer. Levels of soil K, Ca, Mg contents, acidity and soil organic matter, and levels of macronutrients in sugarcane did not change by the application of poultry litter and organo-mineral fertilizer comparing with mineral fertilizer. The use of organo-mineral fertilizer, as well as pure poultry litter is recommended.

Key words: Wastes, biofertilizers, compost.

INTRODUCTION

The crop yield of sugarcane is governed by several factors intrinsic to the cultivar, climate, soil and management practices of the culture. However, all these factors interact with each other and it is up to professionals working with this culture to integrate the effects of these factors to achieve greater economic productivity (Gama 2007).

Fertilizers and pesticides have been frequent targets of criticism because of their elevated costs involved in production of sugarcane, or in other cultures. The high prices caused by the fact that raw materials for the production of fertilizers and pesticides are imported.

*Corresponding author. E-mail: luceliaar@hotmail.com Tel: (34) 99168-3428.

Table 1. Soil chemical characterization in two depths Jalles Machado Mill, 2009.

Profile (cm)	pH	P	K^+	Al^{3+}	Ca^{2+}	Mg^{2+}	SB	T	V	m	M.O.
		mg dm^{-3}	---------- $cmol_c$ dm^{-3} ----------						---------- % ----------		g kg^{-1}
00-25	4.9	12.5	0.04	0.02	1.9	0.7	2.64	5.5	47.7	0.7	28.5
25-50	5.2	2.9	0.05	0.00	2.8	0.7	3.55	6.2	57.4	0.0	29.4

pH – $CaCl_2$; P e K - Extrator Mehlich (HCl 0.05 mol L^- + H_2SO_4 0.025 mol L^-); Ca, Mg e Al - Extrator KCl 1 mol L^{-1};; T - CTC potencial (a pH 7.0);V - saturação por bases; m - saturação por alumínio (Embrapa, 2009). MO – método colorimétrico.

About 60% of nitrogen, 50% of phosphorus and 90% of potassium used in manufacturing of fertilizers in Brazil are imported from other countries (Vieira 2009).

Proper nutrition of sugarcane is demonstrably recognized as the main cause of increments of crop yield. While mineral fertilization is widely used to supply nutrients and represent a large part of production costs, seeking alternatives benefitting sugarcane and ethanol producers by reducing their costs represents a major contribution of research institutions.

With the growth of poultry production in our country supply of animal wastes such as poultry litter has been increased. Thus, their application of organic wastes is being considered as a promising and cost-effective method to confront this challenge. These wastes have been demonstrated to provide plant nutrients and organic matter to the soil required for crop production (Roy et al., 2015). Besides that their use in agriculture as an alternative source of nutrients is important both to properly dispose of these products in order to avoid environmental pollution and to reduce high costs of synthetic fertilizers (Portugal et al., 2009).

Poultry litter is a mixture of substrate, feces, feathers and feed scraps used in bedding of broiler sheds (Chen et al., 2013). Chicken litter is usually recycled as an organic fertilizer or soil amendment for direct application to agricultural land (Enticknap et al., 2006). This residue can be used to improve physical and chemical properties of soil and the productivity of some cultures (Silva et al., 2009).

Besides animal waste, various materials are obtained from the production of sugar and ethanol as boiler ash and filter-cake. Knowledge of their composition and possible uses in crops allows their application in the form of organo-mineral fertilizers, which can provide a greater environmental control, considerable savings in fertilizer costs (Santiago and Rosseto, 2009) and significant results in productivity of sugarcane.

An alternative for the use of poultry litter and by-products of sugar and ethanol production is composting of these residues together with mineral fertilizers to improve their chemical and physical properties (Chen and Jiang, 2014). Thus, the use of waste, both of animal and industrial origin, as nutrient sources in sugarcane production is of paramount importance, because, in addition to contributing to environmental sustainability by no longer having a status of polluters, they become an alternative in soil fertilization, decreasing the use of mineral fertilizers.

Therefore, our objective was to evaluate productivity, content of macronutrients in sugarcane leaves and chemical characteristics of soil as a result of application of poultry litter and organo-mineral fertilizer in relation to mineral fertilizer in soil.

MATERIALS AND METHODS

The experiment was conducted at the Jalles Machado Mill, in Goianésia- GO, from July 2009 to April 2010, using the IAC91-1099 sugarcane variety. The soil is classified as dystrophic red-yellow latosol, clay texture (589 g kg^{-1} of clay) with the following chemical properties (Table 1).

The experiment was conducted as a randomized complete blocks design with 2 x 3 +1 + 1 strata (poultry litter and organo-mineral fertilizer x three doses + an additional treatment (mineral fertilizer) + a control treatment), eight treatments and four replications, totaling 32 plots. experimental plots had 5 rows of sugarcane spaced 1.5 m apart, with 10 m in length, with a total area of 75 m^2. The treatments were: poultry litter and organo-mineral fertilizer, whose chemical properties are shown in Table 2, at doses of 3.0, 6.0 and 9.0 t ha^{-1}; mineral fertilization (additional treatment) at doses of 66 kg ha^{-1} of N (ammonium nitrate), 120 kg ha^{-1} of P_2O_5 (monoammonium phosphate) and 82 kg ha^{-1} of K_2O (potassium chloride); as well as a control without fertilization.

The organo-mineral fertilizer had been prepared for a period of 41 days and received the following proportions of materials: 55% of filter cake, 17.8% of boiler ash, 17.8% of poultry litter and 7.1% of agricultural gypsum. At the time of application on the field 1.1% of ammonium nitrate and 1.2% of potassium chloride were added to this fertilizer.

Planting was carried out in July 2009, after five years of cane cultivation, from 2003 to 2008 and application of 1 t ha-1 of lime and 10 t ha-1 of filter cake, made in 2005. After sugarcane harvesting, soil received application of lime, gypsum and itafós phosphate, and then soybean was planted. After soybean harvesting, in 2009, cane was planted.

Leaf samples were collected 150 days after treatment application using a methodology described by Korndörfer and Ramos (2008) to analyze macronutrients (N, P, K, Ca Mg and S). Sugarcane was harvested 210 days after treatment application, weighed to obtain stalk yield and soil samples were collected between rows 0-20 and 20-40 cm depth. Analyses of plant macronutrients, soil chemical properties (pH, Ca, Mg, K and P) and organic matter of soil were carried out in the Soil Analysis Laboratory (LABAS) of the Institute of Agricultural Sciences, Federal University of Uberlândia, following the methodology described by Embrapa (2009).

The data were analyzed and means were compared by Dunnet test (Assis 2011). The means were compared using Tukey test and

Table 2. Chemical properties of poultry litter and organo-mineral fertilizers.

Determinations	Poultry litter		Organo-mineral fertilizer	
	Dry base (110ºC)	Natural humidity	Dry base (110ºC)	Natural humidity
pH in $CaCl_2$ (0.01 mol L^{-1})	----	8.2	----	8.20
Total organic matter (%)	61.80	48.22	59.90	32.20
Organic Carbon (%)	23.69	18.49	23.10	12.46
Relation C/N (C total/N total)	12/1	12/1	18/1	18/1
Relation C/N (C org./N total.)	8/1	8/1	13/1	13/1
Total humidity (%)	----	21.98	----	46.08
Total nitrogen (%)	2.81	2.19	1.81	0.98
Total phosphorus (%)	3.72	2.90	3.11	1.68
Total potassium (%)	3.64	2.84	3.98	2.15
Total calcium (%)	4.38	3.42	5.50	2.97
Total magnesium (%)	0.73	0.57	0.65	0.35
Total sulfur (%)	0.63	0.49	3.41	1.84
Total boron (mg kg^{-1})	55	43	25	13
Copper (mg kg^{-1})	394	307	270	146
Iron (mg kg^{-1})	11092	8654	6521	3516
Total manganese (mg kg^{-1})	783	611	826	445
Zinc (mg kg^{-1})	611	477	460	248
Total sodium (mg kg^{-1})	5146	4015	5075	2737

CTC: Rodella. A.A.; Alcarde, J.C.; N –[N Total] = sulfuric digestion; P, K, Ca, Mg, S, Cu, Fe, Mn, Zn = Nitro perchloric digestion; B = Colorimetric Azomethine -H.

a statistical program Assistat at 5% significance level (Assis 2011).

RESULTS AND DISCUSSION

Stalk yield

Stalk yield, was significantly higher ($p < 0.05$) when organo-mineral fertilizer was used than poultry litter (Table 3). The highest stalk yield (13.0 t ha^{-1}) was obtained with the application of 9 t ha^{-1} of organo-mineral fertilizer, and mineral fertilizers (control) (23.0 t ha^{-1}).

Although no significant difference was observed ($p > 0.05$), the treatments with 3 and 6 t ha^{-1} of poultry litter influenced positively sugarcane productivity, being superior to the control treatment and mineral fertilization, producing 13.0 and 10.9 t ha^{-1} of stalks more, respectively (Table 3).

Higher yield can be expected with the application of poultry litter and organo-mineral fertilizer in the similar areas in subsequent years, since the residual effect and increased mineralization of these products can contribute to the improvement of such parameters. Assis (2007), evaluating the influence of fertilization with poultry litter on productivity, bromatological attributes and absorption of nutrients in *B. decumbens*, noted that the influence of fertilization on yield occurred only in the second harvest and was more significant with larger doses of poultry litter.

Rao and Veeranna (1998) found that the use of combined doses of cattle manure with mineral fertilizers produced higher yields of sugarcane. It should be expected that the substitution of mineral fertilizer by fertilizing with organic waste, could be an alternative, as already pointed out by Matsuoka et al. (2002) mentioning that the production of sugarcane using organic waste is feasible with similar agricultural yields to those obtained with mineral fertilizers and Anjos et al. (2007) who concluded that it is possible to replace minerals by organic fertilizers without losses of stalk yields and artisan brown sugar.

Thus, the use of pure poultry litter or together with waste from the production of sugar and ethanol and mineral fertilizers, or as an organo-mineral fertilizer, is an alternative which can be used to replace mineral fertilizers.

Chemical properties of soil

There were no differences significant ($p > 0.05$) between soil pH (Table 4). There was a slight reduction in pH where mineral fertilizers were applied, which can be explained by fertilization with ammonium nitrate (Table 4).

Almeida Júnior (2010), comparing the effects of organic and mineral fertilization on soil and plant characteristics of sugarcane culture, found that mineral fertilization significantly reduced soil pH. According to the author, this

Table 3. Stalk yield of sugarcane with three poultry litter doses (CF) and organo-mineral fertilizer (FO).

Treatment (t ha $^{-1}$)	Stalk yield, t ha^{-1}		
	CF	FO	Mean
Mineral fertilization [1]	138.8		
Control [2]	128.2		
3	141.2	147.8	144.5 ^{A}
6	139.1	149.9	144.5 ^{A}
9	132.9	152.0 *	142.4 ^{A}
Mean	137.7 ^{b}	149.9 ^{a}	
CV % = 7.94; DMS CF and FO = 9.52 ; DMS Dunnet = 22.56			

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control: no fertilizers.

Table 4. pH ($CaCl_2$) of soil as influenced by poultry litter doses (CF) and organomineral fertilizer (FO).

Treatment (t ha $^{-1}$)	CF	F O	Mean
Mineral fertilization [1]	4.9		
Control [2]	5.2		
3	5.3^{ns}	5.1^{ns}	5.2^{A}
6	5.2^{ns}	5.0^{ns}	5.1^{A}
9	5.0^{ns}	5.1^{ns}	5.0^{A}
Mean	5.2^{a}	5.5^{a}	
CV% = 5.94; DMS CF and FO = 0.25; DMS Dunnet = 0.60.			

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control: no fertilizers.

Table 5. Phosphorus (mg dm^{-3}) and potassium (cmolc dm^{-3}) levels in soil (depending on the application of three doses of chicken litter (CF) and organo-mineral fertilizer (FO).

Treatment (t ha $^{-1}$)	Phosphorus		Mean	Potassium		Mean
	C F	C O		C F	C O	
Mineral fertilization [1]		19.3			0.04	
Control [2]		12.5			0.04	
3	31.0*	18.8^{ns}	24.9^{AB}	0.05^{ns}	0.04^{ns}	0.05^{A}
6	17.1^{ns}	19.0^{ns}	18.0^{B}	0.05^{ns}	0.05^{ns}	0.05^{A}
9	34.1*	29.9*	32.0^{A}	0.05^{ns}	0.05^{ns}	0.05^{A}
Mean	27.4^{a}	22.5^{a}				
CV % = 32.1; DMS CF and FO = 6.2 ; DMS Dunnet = 14.7				CV % = 17.5; DMS CF and FO = 0.006; DMS Dunnet = 0.01		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at0.05 significance*; [1] recommended fertilization; [2] Control: no fertilizers-

was probably due to the use of ammonium sulfate in mineral fertilizer, which can acidify soil by the formation of two protons (H^+) for each nitrified NH_4^+ ion.

There was an increase significant ($p < 0.05$) in the availability of P in soil caused by the use of poultry litter and organo-mineral fertilizer (Table 5). As for K, there was no significant difference ($p > 0.05$) between treatments in the supply of this nutrient to soil (Table 5). According to CFSEMG (1999), the K contents in soil are considered low.

The increased availability of P in soil can be explained by the increase of the amount of directly available P due to the presence of this nutrient in the waste and its indirect action, which has the ability to improve soil properties (Oliveira, 2000).

Novais et al. (2007) reported that organic material

Table 6. Levels of calcium and magnesium in soil (cmolc dm^{-3}) depending on the application of three doses of poultry litter (CF) and organo-mineral fertilizer (F O).

Treatment (t ha^{-1})	Calcium		Mean	Magnesium		Mean
	C F	F O		C F	F O	
Mineral fertilization [1]	1.3			0.4		
Control [2]	1.7			0.6		
3	1.8^{ns}	1.5^{ns}	1.6^{A}	0.6^{ns}	0.5^{ns}	0.5^{A}
6	1.4^{ns}	1.4^{ns}	1.4^{A}	0.4^{ns}	0.5^{ns}	0.4^{A}
9	1.5^{ns}	1.5^{ns}	1.5^{A}	0.4^{ns}	0.6^{ns}	0.5^{A}
Average	1.5^{a}	1.4^{a}		0.5^{a}	0.5^{a}	
CV % = 33.0; DMS CF and FO = 0.4; DMS Dunnet = 1.0				CV % = 42.0; DMS CF and FO = 0.2 ; DMS Dunnet = 0,4		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control : no fertilizers.

Table 7. Contents of aluminum (cmolc dm^{-3}) and organic matter (kg dag^{-1}) in soil due to the application of three doses of chicken litter (CF) and organo-mineral fertilizer (FO).

Treatment	Aluminum		Mean	Organic matter		Mean
	CF	FO		CF	FO	
Mineral fertilization [1]	0.04			2.0		
Control [2]	0.05			2.1		
3 t ha^{-1}	0.05^{ns}	0.08^{ns}	0.06^{A}	2.3^{ns}	2.1^{ns}	2.2^{A}
6 t ha^{-1}	0.05^{ns}	0.09^{ns}	0.07^{A}	2.0^{ns}	2.1^{ns}	2.1^{A}
9 t ha^{-1}	0.04^{ns}	0.01^{ns}	0.03^{A}	2.2^{ns}	2.2^{ns}	2.2^{A}
Average	0.05^{a}	0.06^{a}		2.1^{a}	2.2^{a}	
CV % = 118.0; DMS CF and FO = 0.05; DMS Dunnet = 0.1				CV % = 9.9; DMS CF and FO = 0.2; DMS Dunnet = 0.4		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control : no fertilizers

added to soil through the application of waste such as poultry litter, brings about, in general, decreased adsorption and increased availability of phosphorus for plants. Santos et al. (2009) reported that the availability of phosphorus in soil caused by the use of organic residues possibly occured due to the action of organic and humic acids and alcohols, which provide substances such as phenols and contribute to increased availability of this nutrient

There was no significant difference ($p > 0.05$) between the treatments with mineral fertilizer, poultry litter doses and organo-mineral fertilizer regarding Ca and Mg levels in soil (Table 6). Nevertheless, the average levels of available Ca, where poultry litter and organo-mineral fertilizer were applied, were higher than mineral fertilizer, possibly due to the lack of Ca source. The average supply of Ca to soil was 1.5 cmolc dm^{-3} with poultry litter and 1.4 cmolc dm^{-3} with organo-mineral fertilizer. According to CFSEMG (1999), the levels of Ca in all treatments, are considered medium, i.e. are between the range of 1.21 and 2.4 cmolc dm^{-3}.

There was no significant difference ($p > 0.05$) between control treatment and where mineral fertilizer, poultry litter and organo-mineral fertilizer were used regarding Al content and soil organic matter (Table 7).

The average Al content is considered low to very low, according to CFSEMG (1999), which was due to lime and gypsum application applied before planting. Low aluminum contents are correlated with the mean pH values in $CaCl_2$ which was 5.2, when poultry litter was used and 5.5 with the organo-mineral fertilizer (Table 4). The high coefficient of variation (CV%) for levels of exchangeable Al in soil are due to low levels of DMS of poultry litter and organo-mineral fertilizer, that is, anything below or above the DMS is detected by the statistical analysis, which increases CV%.

Although the levels of soil organic matter were not altered by the application of poultry litter and organo-mineral fertilizer (Table 7), according to CFSEMG (1999), the values of soil organic matter are within limits considered medium, which are 2.01 – 4.0 dag kg^{-1}.

Portugal et al. (2009), studying the effects of different poultry litter doses for two consecutive years in *Brachiaria brizantha* cv. Marandú also found no significant changes in levels of soil organic matter. Similar result was found by Silva (2005) who did not obtain changes in soil organic

Table 8. Foliar nitrogen and sulfur in sugarcane as a result of application of three poultry litter doses (CF) and organic fertilizer (FO).

Treatment (t ha $^{-1}$)	Nitrogen, g kg^{-1}			Sulfur, g kg^{-1}		
	CF	FO	Mean	CF	FO	Mean
Mineral fertilization [1]	16.2			1.3		
Control [2]	16.5			1.4		
3	16.3^{ns}	16.2^{ns}	16.2^{AB}	1.4^{ns}	1.5^{ns}	1.4^{A}
6	16.8^{ns}	16.6^{ns}	16.7^{A}	1.4^{ns}	1.4^{ns}	1.4^{A}
9	15.7^{ns}	16.2^{ns}	16.0^{B}	1.4^{ns}	1.4^{ns}	1.4^{A}
Mean	16.3^{a}	16.3^{a}	16.2^{AB}	1.4^{a}	1.4^{a}	
CV% = 3.4; DMS CF and FO = 0.5; DMS Dunnet = 1.1				CV% = 9.3; DMS CF and FO = 0.1; DMS Dunnet = 0.3		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control: no fertilizers.

matter at 0-20 cm and 20-40 cm depth.

Another factor to consider is that this study was developed in a region where conditions favor decomposition of organic matter. Silva (2005), evaluating the influence of mineral fertilization and poultry litter on soil chemical properties, mentioned that a continuous application of organic waste might increase the level of this attribute in soil, although high temperature, moisture and microbial activities contribute to the decomposition of organic matter.

No significant increases of pH, nutrient content and soil organic matter were achieved where poultry litter and organic fertilizer were used in relation to mineral fertilization. Santos et al. (2009), evaluating the effects of organic and mineral fertilization on soil chemical properties, nutritional status and yield of sugarcane, showed that pH, organic matter, K, Mg and Al in soil were not affected by doses of organic compound. This effect can be attributed to the fact that organic and organo-mineral fertilizers have nutrients associated with organic compounds, which give them a gradual solubility, that is, the total content is not fully soluble in water gradually releasing the nutrients with time, with lower availability at first (Luz and Korndörfer, 2011).

According to Silva (2009), a long-term monitoring of an area allows an assessment of the effect of organic fertilizers, which may improve chemical, physical and biological conditions of soil, ensuring a sustainability of the area. A controlled application of organic fertilizer during the cultivation cycle can deliver nutrients in a way that meets their removal.

According to the record provided by the mill, the areas are quite homogeneous with medium fertility, which may explain the lack of response to most chemical characteristics delivered with poultry litter, organo-mineral compound and mineral fertilizer.

Regarding the lack of response of chemical properties and organic matter in soil, one must consider the form of soil sampling in the areas. The poultry litter, organo-mineral and mineral fertilizers were applied accompanied by cultivation of the crop, but on the other hand soil sampling was carried out between rows. This form of sampling between the rows may distort, or not show real effects of fertilizers on chemical characteristics and levels of soil organic matter.

Leaves macronutrients contents

There was no significant difference ($p > 0.05$) among the applications of poultry litter, mineral and organo-mineral fertilizer in relation to levels of foliar N and S (Table 8). According to Raij et al. (1996), an adequate leaf N range in sugarcane is 18-25 g kg^{-1}. Thus, the values of nutrients are below levels which are considered adequate.

The lack of response of N content in leaves to the application of litter may be related to the fact that part of it is still in organic form, which determines a lower efficiency of nutrient uptake by sugarcane (Lourenço et al., 2010). Canabarro et al. (2003) reported that organic N present in organic waste such as chicken litter, has a low mineralization rate and the decomposing microorganisms of the carbonaceous compounds of these materials immobilize part of N in the waste, decreasing the absorption of this nutrient by plants.

Regarding foliar S, there was no significant difference ($p > 0.05$) between the mineral fertilizer, poultry litter and organic fertilizer (Table 8). The average content of S in leaves with poultry litter and organo-mineral fertilizer application, was 1.4 g kg^{-1} (Table 8), and according to Raij et al. (1996), S values are below the 1.5 to 3.0 g kg^{-1} range, which is considered suitable in sugarcane.

Sulphur has high power of lixiviation and due to undeveloped root system early in the crop cycle there is low uptake of soluble S from soil with much of the nutrient amenable to lixiviation. Another point to consider is that in soil SO_4^{-2} is predominantly adsorbed by mineral and organic particles (Alvarez et al., 2007).

There was no significant difference ($p > 0.05$) between the use of poultry litter, organo-mineral and mineral fertilizer regarding foliar P concentrations (Table 9). However, according to Raij et al. (1996), the average

Table 9. Foliar phosphorus and potassium in sugarcane as a result of application of three poultry litter doses (CF) and organic fertilizer (FO).

Treatment (t ha $^{-1}$)	Phosphorus, g kg^{-1}			Potassium, g kg^{-1}		
	CF	FO	Mean	CF	FO	Mean
Mineral fertilization [1]	1.5			26.4		
Control [2]	1.5			26.1		
3	1.6	1.5	1.6	27.1^{A}	27.9	27.5^{A}
6	1.4	1.5	1.4	26.1^{A}	28.5	27.3^{A}
9	1.6	1.5	1.6	28.5^{A}	29.1	28.8^{A}
Mean	1.5^{a}	1.5^{a}		27.3^{a}	28.5^{a}	
CV % = 7.1; DMS CF and FO = 0.1; DMS Dunnet = 0.2				CV % = 5.8; DMS CF and FO = 1.3; DMS Dunnet = 3.2		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control : no fertilizers.

Table 10. Foliar calcium and magnesium in sugarcane as a result of application of poultry litter doses (CF) and organic fertilizer (FO).

Treatment (t ha $^{-1}$)	Calcium (g kg^{-1})			Magnesium (g kg^{-1})		
	CF	FO	Average	CF	FO	Average
Mineral fertilization [1]	3.0			1.9		
Control [2]	3.2			2.0		
3	3.0	3.1	3.0^{A}	1.8	1.8	1.8^{A}
6	2.9	2.8	2.9^{A}	1.7*	1.7*	1.7^{A}
9	2.7	2.7	2.7^{A}	1.9	1.7*	1.8^{A}
Average	2.9^{a}	2.8^{a}		1.9^{a}	1.7^{a}	
CV % = 8.6; DMS CF and FO = 0.2; DMS Dunnet = 0.5				CV % = 7.8; DMS CF and FO = 0.1; DMS Dunnet = 0.3		

Means followed by different lowercase letters in lines and uppercase in columns differ by Tukey test at 0.05 significance*; [1] recommended fertilization; [2] Control: no fertilizers.

values of this nutrient in the leaves are within the 1.5 to 3.0 g kg^{-1} range, which is considered appropriate for this culture.

The application of poultry litter and organo-mineral fertilizer did not differ significantly ($p > 0.05$) from the mineral fertilizers for plant K content (Table 9). Although not changed, plant K content is within the 10-16 g kg^{-1} range considered adequate in the cultivation of sugarcane (Raij et al. 1996). Teixeira (2013), evaluating bioavailability of P and K from mineral and organic fertilizers, mentioned that with the permanence of the root system in soil a higher P uptake is expected by ratoon, especially with the application of organo-mineral fertilizer, which has slower solubility and a higher residual effect, and could provide more P to the crop.

There was no significant difference ($p > 0.05$) between the application of poultry litter, mineral and organo-mineral fertilizer regarding leaves Ca and Mg content (Table 10). The average levels of Ca, where poultry litter, mineral and organo-mineral fertilizer were applied, were within the 2.0 to 8.0 g kg^{-1} range, which is considered adequate. Similar to Ca, Mg levels were also considered within the proper range, which is 1.0 to 3.0 g kg^{-1} (Raij et al., 1996).

At these experimental conditions, the mineral fertilizer, poultry litter and organo-mineral fertilizer did not efficiently supply certain macronutrients, both to soil and to plant, remaining below the appropriate levels proposed by Raij et al. (1996) for sugarcane.

Silva (2009), working with the application of organic waste in corn (*Zea mays*), also noted little variation in nutrient levels in soil and plants. In addition, according to the author, one can attribute this behavior of waste to the fact that an experimental area is naturally homogeneous with medium fertility. Thus, there is the necessity to follow the process in a long term enabling the observation of the performance of organic waste and organo-mineral compounds, which may improve chemical, physical and biological conditions of soil, ensuring the sustainability of the area. It is assumed that the application of these materials in a controlled manner during more crop cycles will provide nutrients in a way that meets the removals.

Conclusions

1) The stalk yield of sugarcane was higher with the poultry litter and organo-mineral fertilizer application

compared to mineral fertilizers, and organo-mineral fertilizers were superior than poultry litter;
2) There was an increase of soil phosphorus content by fertilizing with poultry litter and organo-mineral fertilizer;
3) K, Ca, Mg contents acidity and soil organic matter were not altered by the application of poultry litter, organo-mineral fertilizer and mineral fertilizers;
4) Plant contents of macronutrients of sugarcane were not altered by the application of poultry litter, mineral fertilizer and organo-mineral fertilizers.
5) The use of organo-mineral fertilizer, as well as pure poultry litter is recommended.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Almeida Júnior AB (2010). Adubação orgânica em cana-de-açúcar: efeitos no solo e na planta. Recife**:** Universidade Federal Rural de Pernambuco. 58 p.

Alvarez VH, Roscoe R, Kurihara CH, Pereira NF (2007). Enxofre. In: Novais RF, Alvarez VH, Barros NF, Fontes RLF, Cantarutti RB, Neves JCL (eds.). Fertilidade do solo. 1 ed. Viçosa: Soc. Bras. Ciênc. do Solo. cap. 6:595-644.

Anjos IA, Andrade LAB, Garcia JC, Figueiredo PAM, Carvalho GJ (2007). Efeitos da adubação orgânica e da época de colheita na qualidade de matéria-prima e nos rendimentos agrícola e de açúcar mascavo artesanal de duas cultivares de cana-de-açúcar (cana-planta). Ciênc. Agrotecnol. 31(1):59-63.

Assis DF (2007). Produtividade composição bromatológica de *Brachiaria decumbens* após segundo ano de aplicação de dejetos de aves e suínos. Uberlândia: Universidade Federal de Uberlândia 101 p.

Assis F (2011). Assistat - Assistência Estatística. Disponível em: http://www.assistat.com/indexp.html. Acesso em: 23 fev. 2011.

Canabarro DVN, Giacomini SJ, Aita C, Da Ros CO, Guidini E, Marques MG (2003). Acúmulo de nitrogênio e produtividade de milho com o uso de dejetos de suínos manejados na forma líquida e em cama sobreposta. In congresso brasileiro de ciência do solo (Vol. 29).

Chen Z, Diao J, Dharmasena M, Ionita C, Jiang X, Rieck J (2013). Thermal inactivation of desiccation-adapted Salmonella spp. in aged chicken litter. Appl. Environ. Microbiol. 79(22):7013-7020.

Chen Z, Jiang X (2014). Microbiological safety of chicken litter or chicken litter-based organic fertilizers: rev. Agric., 4(1):1-29.

Comissão de fertilidade do solo do estado de minas gerais (1999). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação, 180 p.

Enticknap JJ, Nonogaki H, Place AR, Hill RT (2006). Microbial diversity associated with odor modification for production of fertilizers from chicken litter. Appl. Environ. Microbiol. 72:4105-4114.

Gama AMJ (2007). Sistema de rotação e adubação fosfatada na cultura da cana-de-açúcar no cerrado. Uberlândia: Universidade Federal de Uberlândia 86 p.

Korndörfer GH, Ramos LA (2008). Diagnose foliar em cana-de-açúcar. In: Prado RM, Rozane E, Vale DE, Correia RAM, Souza HA (eds.). Nutrição de plantas: Diagnose foliar em grandes culturas. Jaboticabal: CAPES/FUNDUNESP, cap. 12:211-219.

Lourenço KS, Corrêa JC, Ernan PR, Lopes LS, Nicoloso RS (2010). Absorção de NPK pelo feijoeiro adubado com camas de aves e fertilizantes minerais. In: congresso brasileiro de ciência do solo 33. Anais... Uberlândia/MG: SBCS (CD Rom).

Luz PHC, Korndörfer GH (2011). Reciclagem de subprodutos na agricultura. In: Vasconcelos H P (Ed.). Contribuições para a produção de alimentos: Ideias para uma agricultura eficaz. São Paulo: Nova Bandeira Produções Editoriais 1:123-143.

Matsuoka S, Margarido LAC, Lavorenti NA, Elias Júnior, Pinell DM (2002). Comportamento de variedades de cana-de-açúcar em um sistema orgânico de produção. In: congresso nacional da stab, 8. Anais... Recife: STAB. p. 301-308.

Novais RF, Smyth TJ, Nunes FN (2007). Fósforo. In: Novais RF, Alvarez VH, Barros NF, Fontes RLF, Cantarutti RB, Neves JCL (eds.) Fertilidade do solo. 1 ed. Viçosa: Soc. Bras. Ciênc. do Solo, cap. 8:472-537.

Oliveira FC (2000). Disposição de lodo de esgoto e composto de lixo urbano num Latossolo Vermelho-Amarelo cultivado com cana-de-açúcar. Piracicaba: Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo 247 p.

Portugal AF, Ribeiro DO, Carballal MR, Vilela LAF, Araújo EJ, Gontijo MFD (2009). Efeitos da utilização de diferentes doses de cama de frango por dois anos consecutivos na condição química do solo e obtenção de matéria seca em Brachiaria brizantha cv. Marandú. In: Anais.... Florianópolis: EMBRAPA suínos e aves p. 137-142.

Raij BV, Cantarella H, Raij BV, Cantarella H, Quaggio J, Furlani A (1996). Outras culturas industriais. In: Raij B van et al. (coord.) Recomendações de adubação e calagem para o Estado de São Paulo. 2.ed. Campinas: Instituto Agronômico & Fundação IAC, pp. 233-236.

Rao S, Veeranna VS (1998). Substitution of inorganics nitrogen through local organic manure in sugarcan. J. Maharashtra Agric. Universities, Pune 23:104-106.

Roy M, Das R, Kundu A, Karmakar S, Das S, Sen PK, Debsarcar A, Mukherjee J (2015). Organic Cultivation of Tomato in India with Recycled Slaughterhouse Wastes: Evaluation of Fertilizer and Fruit Safety. Agric. 5:826-856.

Santiago AD, Rossetto R (2009). Adubação: resíduos alternativos. Disponível em: http://www.agencia.cnptia.embrapa.br/gestor/canadeacucar/arvore/CONTAG01_39_711200516717.html. Acesso em: 24 ago. 2009.

Santos ET, Heinrichs R, Figueiredo PAM, Paschoaloto JR, Fruchi VM, Lisboa LAM (2009). Atributos químicos do solo e estado nutricional da cana-de-açúcar submetida à adubação orgânica e mineral. Disponível em: http://www.dracena.unesp.br/#!/eventos/sicud_2009/anais_agronomia.php. Acesso em: 13 out. 2011.

Embrapa (2009). Serviço Nacional de Levantamento e Conservação de Solos. Manual de análises químicas de solo, plantas e fertilizantes. Embrapa informação tecnológica, 2 ed. 627 p.

Silva AA (2005). Potencialidade de recuperação de pastagens de *Brachiaria decumbens* fertilizada como camas de aviário e fontes minerais. Uberlândia: Universidade Federal de Uberlândia, 152 p.

Silva AA (2009). Viabilidade técnica e econômica da implantação da biodigestão anaeróbia e aplicação de biofertilizante nos atributos de solo e plantas. Jaboticabal: Faculdade de Ciências Agrárias e veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho” 168 p.

Teixeira WG (2013). Biodisponibilidade de fósforo e potássio provenientes de fertilizantes mineral e organomineral. Uberlândia: Universidade Federal de Uberlândia 99 p.

Vieira MX (2009). Eficiência agronômica da adubação de soqueira de cana-de-açúcar com cloreto de amônio. Piracicaba: Universidade de São Paulo - Escola Sup. Agric. Luiz de Queiroz 134 p.

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Physical and chemical characteristics and drying kinetics of turmeric (*Curcuma longa L.*)

Maria Siqueira De Lima[4], Marco Antônio Pereira Da Silva[1]*, Geovana Rocha Plácido[1], Caroline Cagnin[3], Núbia Ferreira Vieira[4], Ruthele Moraes Do Carmo[1], Rânio Cesar Francisco Da Silva[1], Carlos Frederico De Souza Castro[1], Márcio Caliari[2] and Richard Marins Silva[3]

[1]Faculty of the Graduate Program in Animal Science, Instituto Federal Goiano - Rio Verde Campus, CP 66, 75901-970, Rio Verde, Goiás, Brazil.
[2]Graduate Program in Food Science and Technology, Universidade Federal de Goiás, CP 131, 74690-900, Goiânia - GO, Brazil.
[3]Graduate Degree in Food Engineering – Instituto Federal Goiano – Rio Verde Campus, CP 66, 75901-970, Rio Verde, Goiás, Brazil.
[4]Graduate Program in Food Technology – Instituto Federal Goiano - Rio Verde Campus, CP 66, 75901-970, Rio Verde, Goiás, Brazil.

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This study aimed to characterize the physical and chemical composition, antioxidant activity, essential oil yield, drying kinetics and fit to mathematical models, color parameters, particle size and scanning electron microscopy of fresh turmeric rhizomes (*Curcuma longa* L.). The physical and chemical composition of turmeric showed technological interest, with standards consistent with those reported in the literature. The essential oil has potential for further studies and applicability in food products, as well as use as preservative with antioxidant action. The Midilli model was the one that best fit the drying kinetics of turmeric. The results also allowed analyzing soluble, insoluble and total fibers and SEM, and it was found that there is perspective of using this raw material for the development of new products.

Key words: Turmeric, antioxidant activity, drying kinetics.

INTRODUCTION

The contribution of synthetic additives to the advancement of the sensory characteristics of foods and improvement of their life-of-shelf is unquestionable, which significantly contributes to the progress of food industries. However, global trends seek solutions to the partial or total substitution of synthetic by natural substances in order to

*Corresponding author. E-mail: marcotonyrv@yahoo.com.br. Tel: +55 64 981221172.

improve the quality of foods (Filho et al., 2000).

Within a wide range of natural dyes, turmeric or saffron (*Curcuma longa* L.) is an orange-yellowish rhizome that provides the extract with its main pigment been curcumin. Turmeric has three structurally analogous curcuminoid dyes belonging to the class of diferuloylmethane ($C_{21}H_{20}O_6$) (Hamerski et al., 2013). Isolation and application of these compounds show properties that contribute to results that have proven that this pigment has therapeutic value, anti-inflammatory, anti-cancer, antibacterial and antifungal activity against foodborne pathogens and as preservative in food formulations (Naidu et al., 2009; Wang et al., 2009; Akran et al., 2010).

Currently, turmeric has increased participation in food products, mainly as dye in pasta, mustards, sauces (curry), cheeses, eggs and snacks like potato chips, and is also used in margarine and meat with antioxidant purposes (Volp et al., 2009).

Among various studies in literature related to the inherent properties of turmeric essential oils, the *in vitro* antimicrobial (Péret-Almeida et al., 2008) and antioxidant activities stand out (Jayaprakasha et al., 2006), which contribute to the interest in the development of new turmeric-based products.

In this context, due to the limited information concerning the preparation of turmeric-based products, this work aimed to evaluate rhizomes regarding physical characteristics and proximate composition, antioxidant activity, essential oil yield, drying kinetics and fit to mathematical models, color parameters, particle size and scanning electron microscopy.

MATERIALS AND METHODS

Fresh *C. longa* L. rhizomes collected in municipality of Rio Verde, GO, under geographical coordinates: 17°37'38, 26"S, and 50°45'18, 94"W, altitude 704 m above sea level, were used using hoe and manual pull-off into the ground at random. Rhizomes were then transported to the Laboratório de Frutas e Hortaliças, Instituto Federal Goiano – Campus Rio Verde, GO. Fresh rhizomes were selected, sorted cleaned, and stored at room temperature. Part of fresh rhizomes was packed in plastic bags, sealed and stored under refrigeration (4°C) until moment of specific analysis.

Methods

Sample preparation

For sample preparation, turmeric rhizomes underwent no pre-drying treatment. After cleaning with hypochlorite solution at 150 ppm for 15 min, the film involving rhizomes was removed using stainless knife. A fraction of fresh rhizomes was ground in a food processor to analyze the antioxidant activity and extraction of essential oils. The other fraction underwent manual slicing to obtain slices with 2.3 ± 0.1 mm in thickness, which were submitted to drying processes in an oven with forced air, with velocity for 5 m/s, circulation at temperature of 65°C to complete dryness to obtain the drying curve. After drying, the samples were ground, vacuum packed in bags and stored polyethylene temperature at 20° C until the time of subsequent analyzes.

Physical characterization of fresh turmeric rhizomes

The physical characterization of fresh rhizomes was performed as follows: fruit weight was determined by weighing in analytical scales with accuracy of three decimal places, and the results expressed in grams (g), length (mm) and equatorial diameter (mm) measured using digital calipers. The length/equatorial diameter ratio was determined by dividing the length by the diameter. Volume was determined by immersing the fruit in graduated polypropylene jar with distilled water, recording the volume (ml) of liquid displaced.

Proximate composition of turmeric flour

The physical and chemical composition of turmeric flour was determined as follows: moisture according to methodology No. 925.09 of AOAC (2000) up to constant weight; ether extract according to methodology No. 925.38 of AOAC (2000); crude protein content as the micro-Kjeldahl method No. 920.87 of AOAC (2000); ash, according to gravimetric method of AOAC (2000) No. 923.03, with calcination at 550°C and sample remaining in FORNITEC furnace model 1926, Brazil. Total, soluble and insoluble fiber were determined using the enzymatic-gravimetric method proposed by AOAC (2000), which consisted of screening the turmeric flour (50 mesh sieve - 0.297 mm) to submit to enzymatic digestion with amylase, protease and amyloglucosidase and subsequent to precipitation of soluble fiber in the presence of 78% ethanol. Analyses were performed in triplicate.

Quantitative analysis of the antioxidant activity

Assessment of the antioxidant activity of turmeric (ground fresh rhizome) was determined by the ability of scavenging the DPPH free radical, according to the methodology described by Pérez-Jiménez and Saura-Calixto (2006). The results were expressed as EC_{50} that relates to the reduction of 50% of the initial concentration of DPPH, this value was obtained by linear regression.

Extraction of essential oils and yield

For the extraction of turmeric essential oils by hydrodistillation, the Clevenger apparatus adapted to a round bottom flask was used. Fresh rhizomes were ground and weighed (300 g) and dissolved in 1000 ml of distilled water (AOAC, 2000). After extraction, the essential oil was stored under refrigeration in amber bottle. Essential oil yield was calculated based on the green and dry matter in moisture-free basis (MFB), using the formula proposed by Santos et al. (2004):

Equation 1: $\mathrm{To} = \frac{\mathrm{Vo}}{\mathrm{m} - \frac{\mathrm{mxU}}{100}}$

Where, To = essential oil yield (%); Vo = oil volume extracted (ml); m = sample weight (g); U = sample humidity (%).

Drying kinetics

For the drying kinetics, 1000 g of sliced sample with mean thickness of 2 mm were weighed and dried in oven with forced circulation in nylon screens trays, where the mass loss was monitored during the dehydration process until samples reached constant mass, with weighing performed at regular intervals of 20

Table 1. Mathematical models adjusted to the fresh turmeric drying kinetics.

Model designation	Equation
Lewis	RX=exp(-kt)
Page	RX=exp(-kt^b)
Henderson and Pabis	RX=a*exp(-kt)
Wang and Singh	RX=1+a*t+b*t^2
Logarithm	RX=a*exp(-k*t)+c
Midilli	RX=a*exp(-kt^b)+c*t
Diffusion Approach	RX=a*exp(-k*t)+(1-a)*exp(-k*b*t)

where: RX- water ratio (dimensionless); a, b, c, k, n- model constants; t- time (min).

min. Upon reaching the constant weight, the oven temperature was set to 105°C with the remaining samples for 24 h. After this period, the equilibrium water content was obtained.

From the weight loss data of samples during drying and the equilibrium water content, water ratios were calculated (Equation 1) and the water ratio curves as a function of the drying time were drawn. The temperature and the relative humidity of the environment outside the drying chamber were monitored by thermo hygrometer. The relative humidity inside the drying chamber was obtained through the basic principles of psychometrics, using the GRAPSI software.

Equation 2: $MR = \frac{U - U_e}{U_i - U_e}$

where: MR- moisture ratio, dimensionless; U- Water content in the product at time t, decimal dry basis (kg water kg^{-1} of dry matter); Ue- Equilibrium water content in the product, decimal dry basis (kg water kg^{-1} of dry matter); and Ui- Initial water content of the product, decimal dry basis (kg kg^{-1} of dry water).

The fresh turmeric experimental drying data were fit to mathematical models often used to represent the drying of agricultural products, as shown in Table 1, also using the statistical computer Statistics 2.0 software for adjustment of different mathematical models.

Color parameters

Instrumental color parameters (L*, a* and b*) of turmeric samples, fresh turmeric flour and compressed seasoning were analyzed by Hunter Lab Colorimeter, Model Color Quest II (Hunterlab, 1998).

Particle size

The particle size profile of turmeric flour was evaluated in vibrating equipment (Produtest) composed of six screens and background, with openings ranging from 1.41 to 0.053 mm (Dias and Leonel, 2006). To determine the uniformity index (UI), R% values obtained from coarse, medium and fine sieves were separately summed up and the result was expressed as percentage. Mean geometric diameter (MGD) was calculated with the equation of Handerson and Perry (1955), adapted to express the result in mm: MGD (mm) = 104.14 × 2^{MF}

Statistical analysis

Statistical analysis of data was performed using the ASSISTAT Software, through linear regression, variance statistical averages (Silva & Azevedo, 2009).

RESULTS

The physical characteristics of turmeric rhizomes are shown in Table 2. The mean weight, length, equatorial diameter, L/ED ratio and turmeric volume results were 16.41 g; 67.79 mm; 15.80 mm; 4.28 and 17.16 ml, respectively.

The average weight of turmeric rhizomes of this study were higher than those selected for planting reported by Chaves et al. (2011) using turmeric rhizomes with weights ranging from 5 to 15 g. According to diameter and length data (Table 2), variation coefficient values (VC%) were 0.13 and 0.27%, respectively, indicating homogeneity with low dispersion.

The geometrical characteristics of rhizomes showed the relationship of elongated length and diameter, indicating that the greater the L/ED ratio, the greater the length, where the variation coefficient value (VC%) of turmeric rhizomes was 0.23%, showing homogeneity with low dispersion. Ferreira et al. (2012) observed correlations between length and diameter, indicating the possible indirect selection of the amount of mass. The mean values and standard deviations of turmeric physicochemical parameters are shown in Table 3.

By studying turmeric according to culture and planting location, farming practices, fertilizer use and maturity of rhizomes, Krishnamurthy et al. (1975), Souza and Gloria (1998) and Cecilio Filho et al. (2000) analyzed samples collected from different regions of the state of Minas Gerais and showed the following proximate composition of turmeric rhizomes: protein from 7.01 to 8.51%; fiber from 5.50 to 7.22%, ash from 6.44 to 7.81% and starch from 35.30 to 39.9%, respectively.

Dried turmeric rhizomes showed average composition of 13.1% of water; 6.3% of proteins; 5.1% of fats; 69.4% of carbohydrates; 3.5% of ash and 2.6% of fibers. The content of turmeric curcuminoids may vary between 2 and 9%, depending on geographic conditions (Esatbeyoglu et al., 2012). In general, starch is the component present in greater proportion, as observed in this study by scanning electron microscopy.

The moisture content of turmeric flour in the present study was 7.83%, which was consistent with results found in the literature. The average value is within limits for vegetable flour established by legislation, which is 15% (ANVISA, 2005), ensuring product quality, since according to Barboza et al. (2006), low moisture content contributes to lower water activity, which is the amount of water available for microbial growth and most bacteria do not grow at water activities less than 0.91.

The antioxidant properties of *Cúrcuma longa* L. are of great interest in the food industry, in which curcumin is the major dye. This was already achieved in the dried samples. Value of CE_{50} = 338.9 g/L was determined by

Table 2. Descriptive results of turmeric weight, length, equatorial diameter, length/equatorial diameter (L/ED) and volume.

Variable (n=200)	Mean	SD	Minimum value	Maximum value	VC (%)
Weight (rhizome weight - g)	16.41	7.56	2.30	39.00	0.46
Length (rhizome - mm)	67.79	18.07	12.43	110.08	0.27
Equatorial diameter (rhizome - mm)	15.80	2.12	10.74	22.29	0.13
L / ED ratio	4.28	0.96	0.88	6.58	0.23
Volume (volume rhizome - mL)	17.16	7.97	5.00	40.00	0.46

n = Number of samples, SD = standard deviation VC = variation coefficient.

Table 3. Mean values and standard deviation of protein, ether extract, moisture, ash and fiber contents of turmeric flour (*Curcuma longa* L.).

Parameters	Mean and standard deviation
Protein (g/100 g weight edible protein)	8.28 ±0.46
Ether extract (g/100 g edible lipid weight)	4.07 ±0.24
Moisture (g/100 g weight of dry matter)	7.83 ±0.15
Ash (g/100 g weight of dry matter)	7.77 ±0.30
Soluble dietary fiber (g/100 g weight edible fibers)	1.01 ±0.50
Insoluble dietary fiber (g/100 g weight edible fibers)	8.66 ±0.58
Total dietary fiber (g/100 g weight edible fibers)	10.60 ±1.32

the percentage of DPPH scavenging for the antioxidant activity of the fruit.

Parize et al. (2006) reported in turmeric samples CE_{50} values of 242.1 and 501.4 µl/ ml, and turmeric pattern of almost 97% of curcumin showed CE_{50} value equal to 19.15 g/L, indicating that the higher the curcumin concentration, the lower the CE_{50} value and the higher the antioxidant activity.

Research methodologies for the study of curcuminoids have proven their oxidizing activity through the DPPH method with samples with concentration of up to 88, 80 and 68% and CE_{50} values of 56, 62 and 73 ppm, respectively (Naidu et al., 2009).

The average essential oil yield obtained in this study was 2.33%, with extract of light color, characteristic odor, giving the flavor of fresh rhizome. This yield was determined by the mass/mass ratio that showed value among those found in literature, but lower than 2.5 to 7.0% (Krishnamurthy et al., 1976; Viasan et al., 1989; Leung and Foster, 1996), 4.5 to 5.8% by Govindarajan (1980) and 4.4% reported by Péret-Almeida et al. (2008).

Table 5 shows the coefficients of the Midilli model adjusted for the drying of turmeric. The results of the particle size analysis are shown in Table 6, which shows the retention percentages of turmeric flour in sieves. Turmeric flour showed relative proportion among particles (UI) of 0.98% for coarse particles; 38.45% for medium particles and 60.57% for fine particles. The geometric mean diameter (GMD) was 0.046 mm.

The results on the calorimetry of sectioned rhizomes, turmeric flour and seasoning (*Curcuma longa L.*) are shown in Table 7.

DISCUSSION

No comparative data in relation to insoluble, soluble and total fiber content of turmeric were found in literature, but the study results showed satisfactory levels, indicating that turmeric is a source of dietary fiber.

According to Spiller (2001), dietary fiber plays an important role in reducing the risk of many diseases, especially the water soluble fraction, which has received much attention due to its various physiological functions.

The antioxidant properties were evaluated by various lipid peroxidation tests, as well as methods of DPPH radicals and chelating metals by Singh et al. (2010), which showed in their results that alpha-turmerone, an important component in fresh rhizome, is only smaller in dry rhizomes. In addition, the beta-turmerone content of dry rhizome is lower than the amount found in fresh rhizomes.

Among the methods used of the extraction of essential oils, extraction by hydro distillation was the method chosen for this work because the process uses water as a solvent, which is able to extract essential oils and not leaving toxic residues after extraction, even with the results by Naghetini (2006), who reported that extraction with non-polar solvent is simpler and faster and shows higher yield, 4.4 mL/100g, as compared to hydro

Table 4. Determination coefficient values (R^2), root of the mean square error (RMSE) and mean relative error (P) for mathematical models used in the drying process of turmeric (*Curcuma Longa* L.).

Model designation	65°C		
	R^2 (%)	RMSE	P (%)
Lewis	97.89	0.0817	0.0112
Page	99.93	0.1085	0.0149
Henderson and Pabis	98.36	0.2779	0.0382
Wang and Singh	99.32	1.4383	0.0182
Logarithm	98.84	8.55×10^{-11}	1.17×10^{-11}
Midilli	99.97	0.0016	0.0002
Diffusion Approach	97.90	0.0817	0.0112

Table 5. Coefficients of the Midilli model adjusted for the drying of turmeric.

Temperature (°C)	Midilli Model	R^2
65	$RX=1.004298*\exp(-0.978102*t^{1.671694})+0.005705*t$	99.97

Table 6. Particle size of turmeric flour (*Curcuma longa* L.).

Tyler	Holes (mm)	(PRi). g	(%R)	Ki	Ki ×%R
24	0.707	0.98	0.98	6	5.88
32	0.5	5.22	5.22	5	26.1
60	0.25	20.7	20.7	4	82.8
100	0.15	12.53	12.53	3	37.59
150	0.105	1.42	1.42	2	2.84
270	0.053	59.15	59.15	1	59.15
Bottom	0	0	0	0	0
TOTAL		**100**	**100**		**214.36**

Pri (g) = Weight retained in the sieve; % R = percentage retained on each sieve; Ki = conventional constant factors from 0 to 6 Ki x; R = total product obtained; Fineness Module (FM) = total product obtained x total product retained; UI = Uniformity index; mean geometric diameter (MGD) = 104.14 x 2 ^ FM = 0.046 mm.

distillation, whose yield was 2.8 ml/100 g.

According to Gounder and Lingamallu (2012), the higher essential oil yield was obtained from cured rhizome when compared with other rhizomes under study; however, 28 chemical compounds were identified in the oil from fresh rhizomes, some of which were not present in the oil from dried rhizomes due to losses during the processing steps (Cousins et al., 2007), demonstrating good alternatives to increase the antioxidant activity of foods during manufacturing.

Vilela and Artur (2008) conducted a survey of turmeric drying curves for different temperatures, wind speed and rhizome cuts and suggested that for faster drying and hence lower energy expenditure, the diameter of the material should be smaller. To be considered a good fit, the model must achieve linear regression coefficient values (R^2) higher and near as possible to the unity (1.0) and, concurrently, P (%) and RMSE values should be lower and near zero.

For the temperature used in drying turmeric, it was found that the models used satisfactorily fitted the experimental data, with Midilli model showing higher R^2 values (Table 4) and lower P (%) and RMSE values, adjusting better to the data observed, as compared to other adjustments.

The drying constant (k) for the Midilli model, which shows the effect of external drying conditions, was significant. Sousa et al. (2011) analyzed twelve models, and the Midilli model presented the best fit to describe the drying curves of turnip. According to Goneli et al. (2014), who evaluated the drying kinetics of black sage leaves and observed that several researchers working with other species of medicinal plants also concluded that the Midilli model was the best fit to experimental drying

Table 7. Mean values and standard deviation of the color parameters of sectioned rhizomes, turmeric flour and seasoning (*C. longa* L.).

Samples	Parameters		
	L*	a*	b*
1 (Rhizomes)	50.49 ±0.52[b]	25.57 ±2.38[a]	33.83 ±1.69[b]
2 (Flour)	65.11 ±0.17[a]	21.60 ±0.25[b]	40.40 ±1.00[a]

Different small letters in the column differ significantly at 1% probability.

data.

The analysis of color components L*, a* and b* in the samples demonstrated a significant variation in results, with L* ranging from 50.49 to 65.11.

For chromaticity coordinate a*, the color component ranges from (-60) green to (60) red. Turmeric samples showed values ranging from 21.60 to 25.57, which became more positive, indicating a tendency for red.

Chromaticity coordinate b* ranged from (-60) blue to (60) yellow, showing variation from 33.83 to 40.40 and a tendency to yellow on all samples. Turmeric flour was classified as orange-yellowish, with higher b* value, that is, high-intensity of yellow chroma, which is associated with the original color of turmeric. Tonnensen and Karlsen (1985) studied the effect of temperature on curcumin and found that is up to 100°C; there was no significant loss of curcumin, but at 125°C, there was degradation of 15.25% of pigment in relation to the initial content. Some researchers have demonstrated the physicochemical properties of yellow pigment obtained from turmeric in liquid and crystallized form maintained color at 80% for 6 months (Joshi et al., 2009).

Uniformity in particle size distribution is more important than particle size itself, because it facilitates good distribution of water in the mass. Thus, one should give preference to flour having particles of uniform size, especially those that pass through sieve number 30 (0.600 mm) and are retained in sieve number 60 (0.25 mm) (Guerreiro, 2006).

Regarding the raw material used in the food industry, particle size below 200 µis recommended to provide uniform color, and particle size equal to that of special wheat flour is desirable in the production of pasta, as this particle size presents no problems of rhizome particles to "stain" the mass during preparation (Marinozzi, 2002). Therefore, the turmeric flour under study showed greater amount retained in sieve 270 (0.053 mm), but it is within standards set by legislation (Brazil, 1996).

The oil is then held in vacuoles located in the cytoplasm. When these are disrupted by mechanical or physicochemical damage, oils retained is released, which for having a low boiling point, exhale a strong aroma (Hess, 1975).

Turmeric has not been used by the starch industry in Brazil because starch extraction is considered a secondary use, and rhizomes are only used to obtain dye and, in small proportions, for direct use in cooking (He et al., 1998). Given the number of research conducted on this topic, there is a great perspective of using residue from the extraction of essential oils and dyes as raw material for the production of starch.

Conclusion

With respect to turmeric characterization (*Curcuma longa* L.), it was possible to find specific properties, and the significant antioxidant activity confirms the potential use of the curcumin dye with preservative function in functional foods. It was found that the Midilli model presents the best fit to experimental drying data.

Insoluble, soluble and total dietary fiber showed satisfactory levels, indicating that turmeric as a source of dietary fiber should be studied in future studies, which should evaluate the profile of these compounds of great importance for the food industry.

Conflict of interests

The authors have not declared any conflict of interest.

REFERENCES

Akran M, Shahab-Uddin AA, Usmanghani K, Hannan A, Mohiuddin E, Asif M (2010). Curcuma longa and curcumin: a review article. Roman. J. Biol. Plant Biol. 55(2):65-70.

ANVISA - Agência nacional de vigilância sanitária (2005). Resolução RDC n° 263, de 22 de setembro de 2005. Technical regulation for cereal products, starches, flour and bran. Available in: http://www.suvisa.rn.gov.br/contentproducao/aplicacao/sesap_suvisa /arquivos/gerados/resol_263_set_2005.pdf>. Accessed on: 30 October 2013.

AOAC - Association of official analytical chemists (2000). Official methods of analysis of the Official Analytical Chemists International. 17ed.

Barboza LMV, Waszczynskyj N, Freitas RJS (2006). Microbiological evaluation of yerba-maté (Ilex paraguariensis St. Hil.). Rev. Inst. Adolfo Lutz 65(2):123-126.

Cecilio Filho AB, Souza RJ, Braz LT, Tavares M (2000). Curcuma: medicinal, spice and of other potential use plant. Ciênc. Rural 30(1):171-175.

Chaves FCM, Berni RF, Pinheiro JB, Vaz APA (2011). Influence of seed weight in the production of turmeric rhizomes. In: Congresso brasileiro de olericultura, Anais P 51.

Cousins M, Adelberg J, Chen F, Rieck J (2007). Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (Curcuma longa L.) grown in vitro. Ind. Crops Prod. 25(2):129-135.

Dias LT, Leonel M (2006). Phisico-chemical characteristics of cassava flours from different regions of Brazil. Ciênc. Agrotecnol. 30(4):692-700.

Esatbeyoglu T, Huebbe P, Ernst IM, Chin D, Wagner AE, Rimbach G (2012). Curcumin-from molecule to biological function. Angewandte Chemie International Edition 51(22):5308-5332.

Goneli ALD, Nasu AK, Gancedo R, Araújo WD, Sarath KLL (2014). Drying kinetics of Cordia verbenacea DC. leaves. Rev. Bras. Plantas Med. 16(2):434-443.

Gounder DK, Lingamallu J (2012). Comparison of chemical composition and antioxidant potential of volatile oil from fresh, dried and cured

turmeric (Curcuma longa) rhizomes. Ind. Crops Prod. 38:124-131.
Guerreiro L (2006). Dossiê técnico: massas alimentícias. REDETEC.
Hamerski L, Rezende MJC, Silva BV (2013). Using Nature's Colors for Meet Consumer Wishes: Natural substances such as dyes Industry Food. Rev. Virtual Quím. 5(3):394-420.
Handerson SM, Perry RL (1955). Agricultural process engineering. New York: John Wiley and Sons.
He XG, Lin LZ, Lian LZ, Lindenmaier M (1998). Liquid chromatography-electrospray mass spectrometric analysis of curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.). J. Chromatogr. 818:127-132.
Hess D (1975). Plant physiology. New York: Springer-Verlag, 99-116.
Hunterlab (1998). User's manual with universal software versions 3.5. Reston.
Jayaprakasha GK, Jaganmohan-Rao L, Sakariah KK (2006). Antioxidant activities of curcumin demethoxycurcumin and bisdemethoxycurcumin. Food Chem. Bark. 98(4):720-724.
Joshi P, Jain S, Sharma V (2009). Turmeric (Curcuma longa) a natural source of edible yellow colour. Int. J. Food Sci. Technol. 44(12):2402-2406.
Krishnamurthy N, Mathew AG, Nambudiri ES, Shivashankar YS, Lewis YC, Natarajan CP (1976). Oil and oleoresin of turmeric. Trop. Sci. 18:37-45.
Krishnamurthy N, Padmadabai R, Natarajan CP, Kuppuswamy S (1975). Colours Content of Turmeric Varieties and Studies on its' Processing. J. Food Sci. Technol. 12:37-45.
Leung AY, Foster S (1996). Encyclopedia of common natural ingredients used in food, drugs and cosmetics. 2. ed. New York: John Wiley & Sons P 870.
Marinozzi G (2002) Study of the productive chain of saffron (Curcuma longa L.) and the local production system of Mara Rosa/GO region. In: UFG. Estabelecimento de tecnologia para o fortalecimento do agronegócio do açafrão (*Curcuma longa* L.) em Mara Rosa-GO. Goiânia: UFG/CNPq. pp. 83-90.
Naghetini CC (2006). Physicochemical characterization and antifungal activity of essential oils of turmeric. Dissertação (Master Scientiae em Ciência de Alimentos) – Programa de Pós Graduação em Ciência de Alimentos, Universidade Federal de Minas Gerais.
Naidu MM, Shyamala BN, Manjunatha JR, Sulochanamma G, Srinivas P (2009). Simple HPLC method for resolution of curcuminoids with antioxidant potential. J. Food Sci. 74(4):312-318.
Parize AL, Cola G, Brighente IMC, Souza TCR (2006). Antioxidant activity of turmeric dye encapsulated in microparticles and chitosan films. 34a Reunião Anual da Sociedade Brasileira de Química-Departamento de Química, Universidade Federal de Santa Catarina.
Péret-Almeida L, Naghetini CC, Nunan EA, Junqueira RG, Glória MBA (2008). In vitro antimicrobial activity of the ground rhizome, curcuminoid pigments and essential oil of *Curcuma longa* L. Ciênc. Agrotecnol. 32(3):875-881.
Pérez-Jiménez J, Saura-Calixto F (2006). Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Res. Int. 39(7):791-800.
Santos AS, Alves SM, Figueiredo FJC, Rocha Neto OG (2004). System description and essential oils extraction methods and determination of moisture biomass in the laboratory. Embrapa Amazônia Oriental,6, Comun. Técn. P 99.
Silva FASE, Azevedo CAV (2009). Principal components analysis in the software assistat-statistical attendance. in: World congress on computers in agriculture, 7, reno-nv-usa: American society of agricultural and biological engineers.
Singh G, Kapoor IPS, Singh P, Heluani CS, Lampasona MP, Catalão CAN (2010). Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (Curcuma longa Linn.). Food Chem. Toxicol. 48(4):1026-1031.
Sousa KA, Resende O, Chaves TH, Costa LM (2011). Drying kinetics of wild radish (*Raphanus sativus* L.). Rev. Ciênc. Agron. 42(4):883-892.
Souza CRA, Glória MBA (1998). Chemical analysis of turmeric from Minas Gerais, Brazil and comparison of methods for flavor free oleoresin. Brazil. Arch. Biol. Techonol. 41(2):218-224.
Spiller GA (2001). Dietary fiber in prevention and treatment of disease. In G.A. Stryer, L., Tymoczko, J.L e Berg, J.M., Bioquímica, 3ª edição, Editora Guanabara Koogan, São Paulo, 2001.
Viasan AC, Menon AN, Rad MJ, Narayanan CS, Mathew AG (1989). Chemical analysis of some cultivars of Curcuma longa Linn. J. Food Sci. Technol. 26:293-295.
Vilela CAA, Artur PO (2008). Drying of *Curcuma longa* L. in different shapes. Ciênc. Tecnol. Aliment. 28(2):387-394.
Volp ACP, Renhe IRT, Stringuetann PC (2009). Bioactive natural pigments. Aliment. Nutr. 20(1):157-166.
Wang Y, Lu Z, Wu HLF (2009). Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens. Int. J. Food Microbiol. 136(1):71-74.

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Soybean as bioindicador of residual effect of 2,4-D herbicide in an oxisol from the Brazilian cerrado

Maria Aparecida Peres-Oliveira, Edna Maria Bonfim-Silva*, Vinicius Melo da Silva, Tonny José Araújo da Silva and Helon Hébano de Freitas Sousa

Federal University of Mato Grosso, Rondonópolis, Brazil.

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Invasive plants, besides competing for space, also compete for water, light, and nutrients against the crop, what may cause drastic production cuts. Chemical control through herbicides is the most widely used method to control these plants. This study aimed to evaluate the persistence of the 2,4-D (dichlorophenoxyacetic acid) herbicide in soybean (*Glycine max* L.) crops in an Oxisol from the Brazilian Cerrado. The experiment was conducted in a greenhouse using a randomized block design, 5x3 factorial, five application periods (0, 3, 5, 7 and 10 days before sowing), and three herbicide doses (0, 750 and 1500 g e. a. ha^{-1}), in four repetitions. Herbicides were sprayed with the assistance of knapsack sprayer at constant pressure (kept through CO_2 compressed). Residual effect was assessed by emergence speed index (ESI), visual plant phytotoxicity, plant height, and shoots and root dry biomass. Persistence of 2,4-D herbicide was short in soybeans planted in an Oxisol. Periods from 0 to 3 days before sowing were the most harmful to soybeans, that is, the closer the spraying was from sowing, higher was its damage to the crop. The 1500 g e. a. ha^{-1} dose had a higher residual effect.

Key words: Persistence, bioassay, *Glycine max*, dichlorophenoxyacetic acid.

INTRODUCTION

In relation to the increase in grain production, Brazil is estimated to cover an area of 58.5 million hectares, what makes agriculture one of the most important activities in the country, especially in the state of Mato Grosso, the largest national soybean (*Glycine max* L.) producer. In 2015, Brazil's Central-West region accounted for 42% of soybean production, and is also the country's main agricultural region (CONAB, 2015).

Weed occurrence in agricultural areas may reduce crop yields, resulting in large losses (Fontes, 2003). Weeds are those that directly or indirectly harm human activity (Silva, 2007a), as they compete against the crop for space, water, light and nutrients, may act as pest and disease hosts, exercise allelopathic effects, and reduce biodiversity, among other problems (Vasconcelos et al., 2012).

In order to mitigate weed impacts, it is necessary to adopt control measures, whether mechanical, physical,

*Corresponding author. E-mail: embonfim@hotmail.com.

Table 1. Chemical and grain size characterization of an Oxisol in the 0.0 to 0.20 m deep layer.

pH	P	K	Ca	Mg	H	Al	SB	CTC	V	O.M.	Sand	Silte	Clay
$CaCl_2$	mg dm^{-3}				$Cmol_c$ dm^{-3}				%	g dm^{-3}		g kg^{-1}	
4.8	1.4	23	0.4	0.2	5.4	0.8	0.8	6.8	9.7	27.1	423	133	444

chemical or biological. Of these, chemical control was and still be the most widely used measure (Pitelli, 1987). On the other hand, the use of chemicals may become a risk, requiring care. According to the National Health Surveillance Agency (ANVISA, 2013), the Brazilian pesticide market expanded 190% in the last decade, more than twice than the global market (93%).

Despite the beneficial effects that pesticides have in that kind of control, excessive or incorrect use may lead to soil, water, and crop contamination, affecting non-target organisms (Mohiddin et al., 2015; Xiao et al., 2012). When the molecules of a given herbicide are applied in the environment, the final destination is usually the soil. Incorrect chemical use in these soils is a serious threat to the health of humans, animals, and aquatic systems worldwide (Karam et al., 2009; Belo et al., 2007). Concern on this topic has increased, which justifies soil studies aimed at avoiding phytotoxicity of succeeding crops.

Considering the soil as the probable destination of most chemicals, both in pre- or post-emergence application, herbicides are subject to physical and chemical processes that lead their destination in the environment, which may follow different pathways (Law, 2001; Oliveira and Brighenti, 2011). Among processes that may occur with herbicides in the soil, persistence and leaching are the most common.

Herbicide persistence is the ability of a compound to show its residual effect, in order to prevent weed development in a certain area. On the other hand, herbicides with long bioactivity may cause subsequent damage to sensitive crops, that is, successor plants in a crop rotation system or crop consortium (Dan et al., 2012a,b). There are many processes that influence herbicide persistence: dissipation, evaporation, leaching, surface runoff, molecule absorption by the plant, biological, physical and chemical degradation (Silva et al., 2007b), and the own initial dose (Blanco and Oliveira, 1987).

The 2,4-D (2,4-dichlorophenylacetic acid) herbicide is a growth regulator that has a similar effect to the auxin hormone (Ashton and Crafts, 1973). Belonging to the phenoxy compound family, it consists of salts or esters with high molecular weight and low volatility, derived from phenoxyacetic acid (Saad, 1978). It is used as herbicide for selecting narrow-leaved plants, causing higher phytotoxicity in broad-leaved species, and is widely used in weed desiccation before soybean sowing, as this herbicide has short to intermediate soil persistence.

An alternative to evaluate herbicide residual effect in the soil is the use of plant species that are highly sensitive to the herbicide of interest (bioindicators). The technique aforementioned is known as bioassay (Inoue et al., 2002; Nunes and Vidal, 2009). Bioassay advantages over other techniques are its simplicity, low cost, and the possibility of detecting the biologically active amount of the herbicide. In addition, it is directly applicable to field conditions (Lima et al., 1999).

The aim of this study was to evaluate 2,4-D (dichlorophenoxyacetic acid) herbicide persistence in an Oxisol from the Brazilian Cerrado through bioassay, using the soybean crop as bioindicator.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at 16º 28 'south latitude, 50° 34' west longitude and 284 m altitude. The experiment used a randomized block design, 5x3 factorial, five application periods (0, 3, 5, 7 and 10 days before sowing), and three 2,4-D herbicide doses (0, 750 and 1500 g e.a. ha^{-1}), in four repetitions.

Each experimental unit consisted of pots with 5 dm^3 capacity containing eight soybean plants of TMG 132 cv. (seeded at 5 cm depth). The soil used in this study was an Oxisol collected in the Rondonópolis region, MT, in depths from 0 to 0.20 m. After collection, the soil was sifted a 4-mm mesh, and was subsequently characterized by chemical and grain size analysis (according to EMBRAPA's methodology (1997), as shown in Table 1). The soil was kept at 80% of field capacity moisture content, according to the methodology proposed by Bonfim-Silva et al. (2011) throughout the study.

Herbicide were sprayed with the assistance of knapsack sprayer at constant pressure (kept through CO_2 compressed) equipped with spray nozzle XR 11002 and with consumption corresponding to 200 L ha^{-1}. Herbicide persistence in the soil was evaluated by emergence speed index (ESI), visual soybean phytotoxicity (with a score ranging from 1 to 5, where 1 corresponds to no injury and 5 to plant death) (SBCPD, 1995), plant height (cm), and shoot - BDM (g) and root dry matter - BDM (g) at 26 days after sowing, at the end of the study. Statistical analysis was conducted in accordance with the polynomial regression model.

RESULTS AND DISCUSSION

In the Emergence Speed Index - ESI parameter, only the 1500 g e.a. ha^{-1} dose (the highest dose in the experiment) was significant, linearly reducing emergence speed as herbicide and sowing application approached (Figure 1a).

Regarding periods in each dose applied, only the periods of 0, 3 and 5 days before sowing were significant. The period of 0 days before sowing caused higher

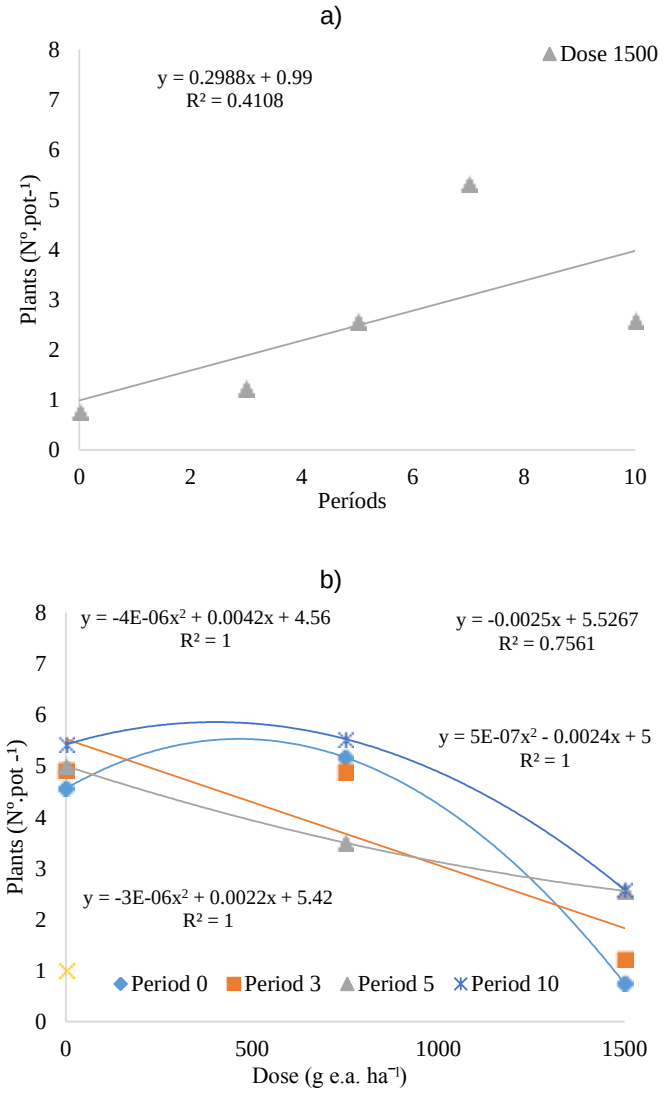

Figure 1. Emergence speed index of soybean seeds in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

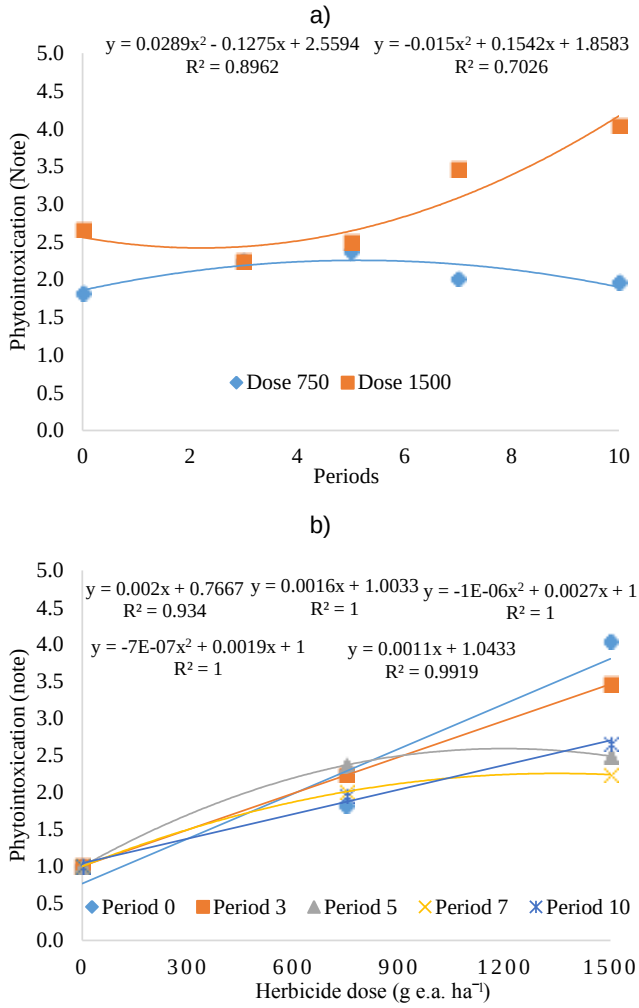

Figure 2. Visual phytointoxication of soybean plants in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

emergence speed reductions. The intermediate dose (750 g e.a. ha^{-1}) provided the highest emergence speed, even at 0 days before sowing (Figure 1b). Schäfer et al. (1999) found that 2,4-D (2,4-Dichlorophenoxyacetic acid) exerted hormonal action when applied in small doses. According to Mortensen et al. (2012), 2,4-D acts as a herbicide that controls dicotyledonous weed species. However, 2,4-D also has hormonal action, acting as a synthetic auxin which can be used as plant growth regulator.

For the other periods (3 and 5 days before sowing), increasing doses caused linear emergence speed reduction, which was mitigated as spraying distanced sowing. A lower emergence speed index was observed when the highest 2,4-D dose was applied at sowing (0 days before sowing), result also found by Silva et al. (2011) and Peres-Oliveira et al. (2016).

According to Procópio et al. (2008), herbicides that have residual activity in the soil are important inputs to ensure commercial crop yields, especially for crops with long weed interference periods. However, after that period is over, which often coincides with crop canopy closing, herbicide presence in the soil may become undesirable, and may result in carryover (Belo et al., 2007). The 2,4-D herbicide has short to intermediate persistence in soils. In normal doses, 2,4-D residual activity does not exceed four weeks in clay soils and hot weather (Silva et al., 2007a). In this parameter, it was observed that the herbicide did not exercise residual action in periods distant from soybean sowing.

Soybean phytotoxicity (Figure 2) was observed in all

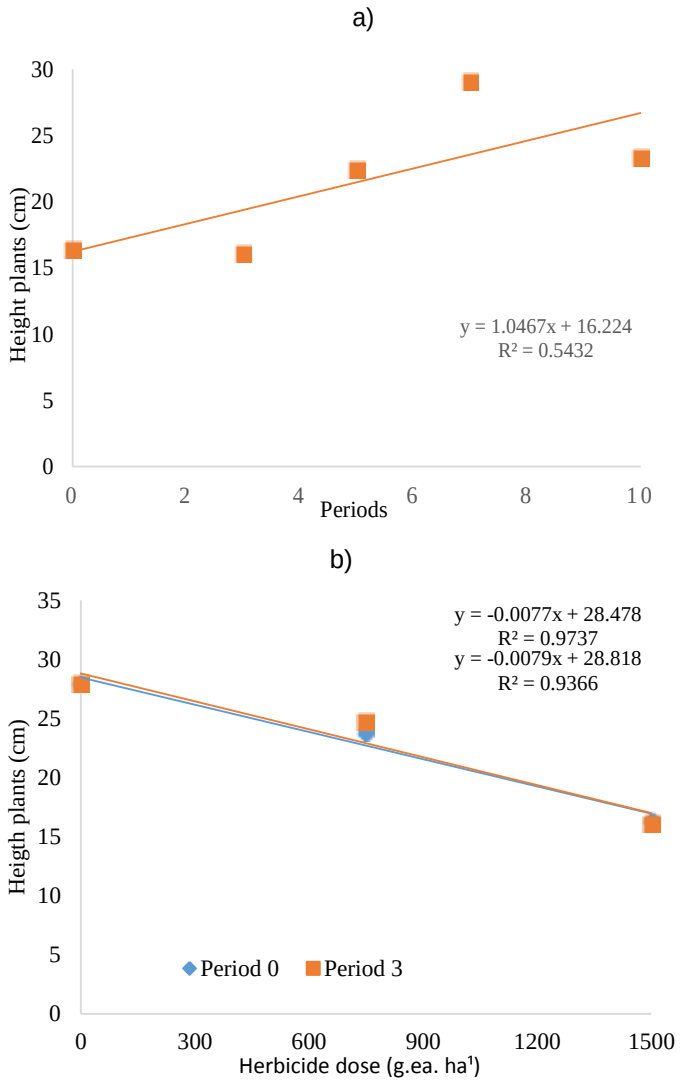

Figure 3. Soybean plant height in Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

application periods for both doses. The first symptoms, which occurred 10 days after sowing, were mild injuries, insufficient to cause yield reductions. Phytotoxicity visual symptoms observed were leaf shriveling, petiole epinasty and rib bleaching. One of the most common techniques to identify and quantify herbicides in the soil or water is to use bioassays through indicator plants with easy cultivation, fast development, and high sensitivity to the herbicide evaluated (Nyffeler et al., 1982; Souza et al., 1999). According to Thill (2003), intoxication symptoms produced in the leaves of various dicotyledonous plants by auxinic herbicides are easily characterized. Therefore, they are widely used to detect residues of these herbicides in the soil.

Studies with cotton (Constantin et al., 2007) and soybean (Silva et al., 2011) crops showed symptoms such as leaf shriveling and petiole epinasty. In both studies, 2,4-D herbicide intoxication caused bleaching of leaf blade ribs, symptom also found in this study. In general, the main 2,4-D effects in the plant are leaf shriveling, leaf bending to the underside, stem bending to the soil and rigidity (with cracks sometimes) or swelling in almost its entire length. In addition, barks are parted, branches and roots appear within these cracks, developing shoots stop growing, poorly-constituted organs appear, and plants lose their green color, yellowing or even dying (Saad, 1978).

Doses of 750 and 1500 g e.a.ha^{-1} were significant. The 1500 g. e.a.ha^{-1} dose provided higher crop damage and higher phytotoxicity scores. As spraying and sowing came closer, phytointoxication scores increased, causing from light injuries to plant death (Figure 2a).

Similar results were observed by Silva et al. (2011), when they used the 1005 g e.a. ha^{-1} dose in soybean. Farinelli et al. (2005) used the same dose in a millet crop and did not find phytotoxicity effects. The 750 g. e.a.ha^{-1} dose caused slight injuries, which were insufficient to affect plant growth and/or yield.

All periods (0, 3, 5, 7 and 10 days before sowing) were significant (Figure 2b), where periods of 0 and 3 days before sowing had the highest phytotoxicity scores. Symptoms such as severe injuries and reduced growth were observed, which may drastically reduce yield. The farther the spraying was conducted from sowing, lower was the plant damage.

When some herbicides reach the soil, their redistribution and degradation processes begin, which may be extremely short, as for some simple and non-persistent molecules, or may last for months or years, for highly persistent compounds (Filizola et al., 2002).

Auxinic or auxin mimic herbicides were the first selective organic herbicides for weed control. They are still extensively used in rice, corn, wheat, sugarcane, and pastures due to their characteristics (Thill, 2003). They are latifolicide products, and 2,4-D has short to intermediate soil persistence, which, according to Silva et al. (2007a), may cause intoxication in sensitive species, such as soybean, bean, cotton, and other dicotyledonous, when these are grown in areas where the herbicide was applied.

In the plant height variable (Figure 3), only the 1500 g e.a.ha^{-1} dose was significant, causing plant height reduction. Plant height reduction was increased as spraying approached sowing date (Figure 3a). Pacheco et al. (2007) observed a reduction from 7 to 27% in millet (*Pennisetum americanum* L.) plant height using the 1005 g e. a. ha^{-1} dose.

Regarding the outcome of doses over periods, only the periods of 0 and 3 days before sowing were significant. As the period between spraying and sowing decreased along dose application, plant height was gradually reduced. Silva

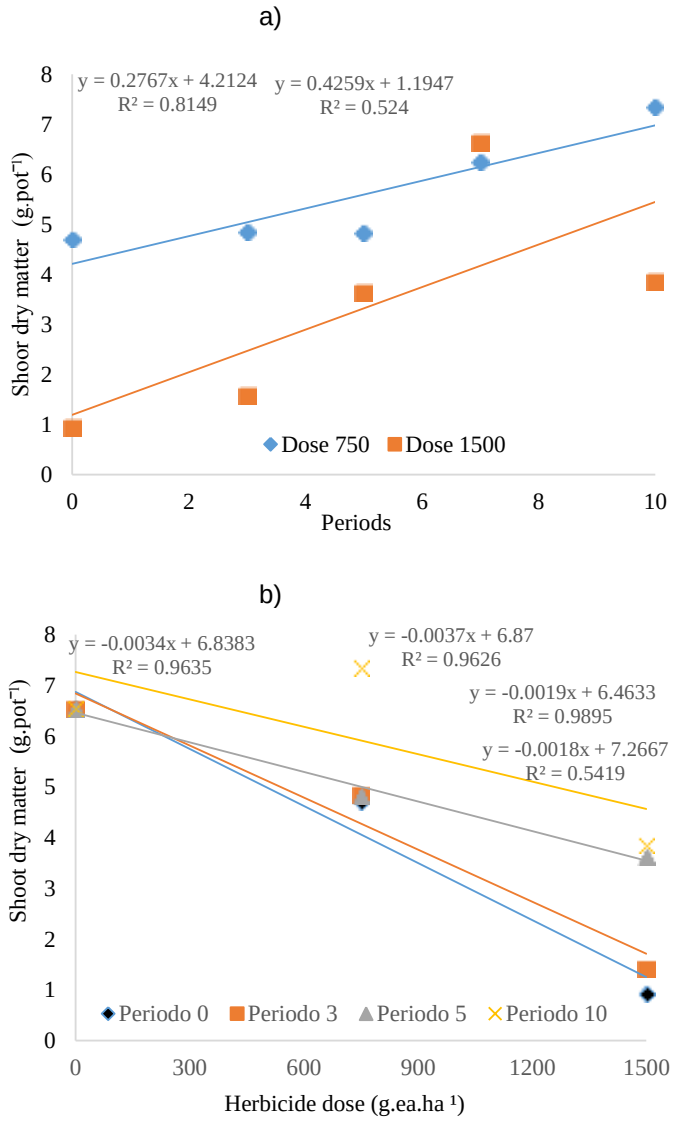

Figure 4. Soybean shoots dry matter in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant at 0.1, 1 and 5% respectively.

et al. (2011) observed lower height at 0 days before sowing, and herbicide dose increase caused significant soybean height reduction.

Santos et al. (2013) observed plant height decrease in plants that are sensitive to auxinic herbicides, and this variable was inversely proportional to 2,4-D dose increase in a short period between herbicide application and sowing. The 2,4-D herbicide has short soil persistence, allowing for sowing susceptible crops two weeks after application (Rodrigues and Almeida, 2011).

For shoot dry matter (Figure 4), only 750 and 1500 g e.a.ha^{-1} doses were significant. Dry matter had better increases in more distant periods between spraying and sowing, that is, both doses linearly reduced shoot dry matter as spraying approached sowing (Figure 4a). The 1500 g e.a.ha^{-1} dose was the most severe and reduced shoot dry matter.

Regarding the assessment of days according to each dose applied (Figure 4b), except for period 7, all other periods were significant (0, 3, 5 and 10 days before sowing). The period of 0 days before sowing drastically reduced shoot plant development, leading to lower dry matter content, i.e., the short time period damaged dry matter production. Root dry matter reduction was due to the fact that herbicides such as 2,4-D induce intense cell proliferation in tissues, causing leaf and stem epinasty, besides phloem interruption, preventing photoassimilate movement from leaves to the root system (Silva et al., 2007b).

Decrease was evidenced as the dose increased, a tendency that continued in all other periods (0, 3, 5 and 10 days before sowing) and causing shoot dry matter reduction (Figure 4b). Increasing the dose may significantly reduce dry matter biomass during application periods of 3 and 14 days before sowing (Silva et al., 2011). Peres-Oliveira et al. (2016) studied a mixture of 2,4-D and glyphosate, and found the lowest shoot dry matter index at 0 days before sowing, in contrast with the evaluation at 14 days before sowing, which had the highest shoot dry matter increase. The farther the spraying was from sowing, higher was crop development.

In the root dry matter variable (Figure 5), only the 1500 g e.a.ha^{-1} dose was significant, where it reduced dry matter as spraying approached sowing (Figure 5a). As spraying distanced from sowing, root yield increased.

Regarding the assessment of days according to each dose applied (Figure 5b), only the periods of 0 and 3 days before sowing were significant. Root dry matter was influenced by the distance between sowing and herbicide spraying in both applied doses. The period of 0 days before sowing drastically reduced root development, resulting in a lower dry matter content. As the dose increased, reduction became more evident. This behavior could also be observed in the period of 3 days before sowing.

Similar results were found by Silva et al. (2011), where dry matter was reduced in all treatments, with the lowest values observed at 0 days before sowing. In this study there was no statistical difference for 2250 and 3000 doses in relation to application periods.

Conclusion

Persistence of 2,4-D herbicide was short in an Oxisol for soybean; Periods of 0 and 3 days before sowing were the most harmful to soybeans, that is, the closer the spraying was from sowing, higher was the crop damage. The 1500 g e. a. ha^{-1} dose had a higher residual effect in the soil for soybean.

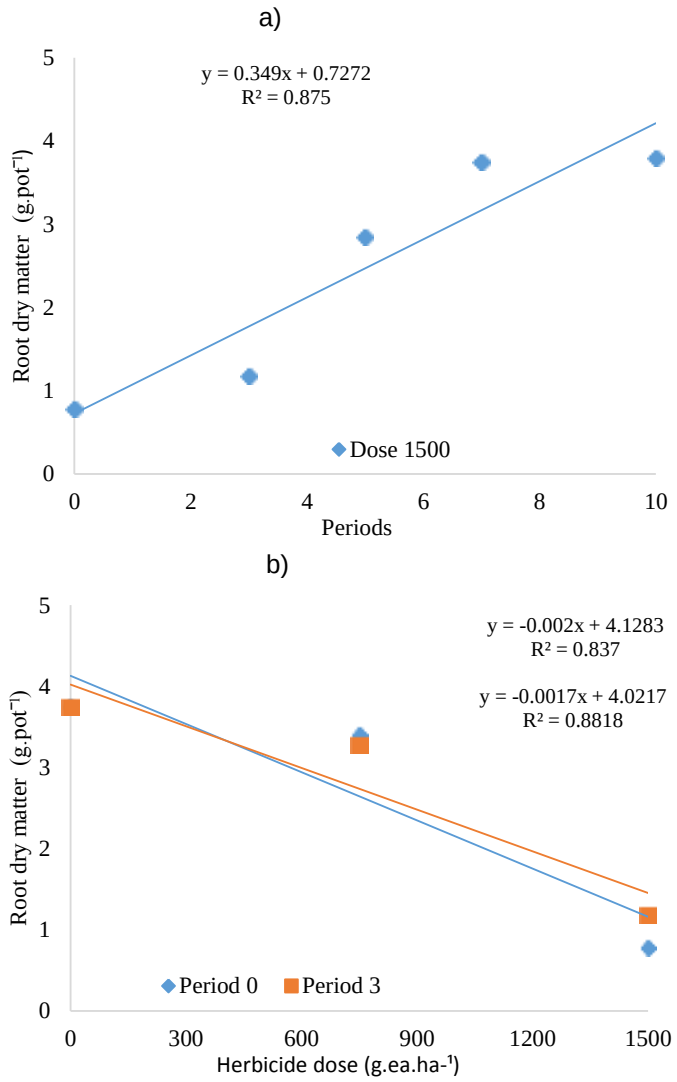

Figure 5. Soybean root dry matter in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant at 0.1, 1 and 5% respectively.

Conflicts of Interests

The authors have not declared any conflict of interests.

REFERENCES

ANVISA - Agência Nacional de Vigilância Sanitária (2013). Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA). Relatório de Atividades de 2011 e 2012. Brasília: Agência Nacional de Vigilância Sanitária.

Ashton FM, Crafts AS (1973). Mode of action of herbicides. New York: John Wiley.

Belo AF, Santos EA, Santos JB, Ferreira LR, Silva AA, Cecon PR, Silva LL (2007). Fitorremediação de solo adubado com composto orgânico e contaminado com trifloxysulfuron-sodium. Planta Daninha 25(2):251-258.

Blanco HG, Oliveira DA (1987). Persistência de herbicidas em Latossolo Vermelho Amarelo em cultura de cana-de-açúcar. Pesqui. Agropecu. Bras. 22(7):681-687.

Bonfim-Silva EM, Silva TJA, Cabral EA, Kroth BE, Rezende D (2011). Desenvolvimento inicial de gramíneas submetidas ao estresse hídrico. Rev. Caatin. 24(2):180-186.

CONAB - Companhia Nacional de Abastecimento (2015). Acompanhamento da safra brasileira grãos. ISSN 2318-6852. 1(4) - Safra 2014/15 Quarto Levantamento Bras. pp. 1-95.

Constantin J, Oliveira Júnior RS, Fagliari JR, Pagliari PH, Arantes JGZ, Cavalieri SD, Framesqui VP, Gonçalves DA (2007). Efeito de sub-doses de 2,4-D na produtividade do algodão e suscetibilidade da cultura em função de seu estádio de desenvolvimento. Eng. Agríc. 27:24-29.

Dan HA, Dan LGM, Barroso ALL, Oliveira Neto AM, Guerra Naiara (2012a). Resíduos de herbicidas utilizados na cultura da soja sobre o milho cultivado em sucessão. Rev. Caatin. 25(1):86-91.

Dan HA, Dan LGM, Barroso ALL, Procópio SO, Oliveira Júnior RS, Braz GBP, Alonso DG (2012b). Atividade residual de herbicidas usados na soja sobre o girassol cultivado em sucessão. Cienc. Rural 42(11):1929-1935.

EMBRAPA. Centro Nacional de Pesquisa de Solos (1997). Manual de métodos de análise de solos. 2 ed. rev. e atual. Rio de Janeiro: EMBRAPA.

Farinelli R, Penariol FG, Lemos LB (2005). Eficiência do herbicida 2,4-D no controle de *Raphanus raphanistrum* L. em pós-emergência na cultura do milheto. Res. Bras. Milho Sorgo 4:104-111,.

Filizola HF, Ferracini VL, Sans LMA, Gomes MAF, Ferreira CJA (2002). Monitoramento e avaliação do risco de contaminação por pesticidas em água superficial e subterrânea na região de Guairá. Pesqui. Agropecu. Bras. 37(5):659-667.

Fontes JRA (2003). Manejo Integrado de Plantas Daninhas. Documentos 113. Planaltina.

Karam D, Silva JAA, Foloni LL (2009). Potencial de contaminação ambiental de herbicidas utilizados na cultura do milho. Rev. Bras. Milho Sorgo 8(3):247-262.

Inoue MH, Marchiori Jr O, Oliveira Jr RS, Constantin J, Tormena CA (2002). Calagem e o potencial de lixiviação de imazaquin em colunas de solo. Planta Daninha 20(1):125:132.

Law SE (2001). Agricultural electrostatic spray application: a review of significant research and development during de 20th century. J. Electrostat. 51-52:25-42.

Lima RO, Oliveira MF, Silva AA, Magalhães JV (1999). Comportamento do herbicida flumioxazin em solo com diferentes doses de calcário. Rev. Ceres 46(268):607-613.

Mohiddin GJ, Shrinivasulu M, Maddela NR, Manjunatha B, Rangaswamy V, Kaiser ARK, Asqui JCM, Rueda OD (2015). Influence of the insecticides acetamiprid and carbofuran on arylamidase and myrosinase activities in the tropical black and red clay soils. Environ. Monit. Asses. 187:388.

Mortensen DA, Egan JF, Maxwell BD, Ryan MR, Smith RG (2012). Navigating a critical juncture for sustainable weed management. BioScience 62(1):75-84.

Nunes AL, Vidal RA (2009). Seleção de plantas quantificadoras de herbicidas residuais. R. Ecotoxicol. Meio Ambiente 19(1):19-28.

Nyffeler A, Gerber HR, Hurle K, Pestemer W, Schmidt RR (1982). Collaborative studies of dose-response curves obtained with different bioassay methods for soil-applied herbicides 22(4):213-222.

Oliveira MF, Brighenti AM (2011). Comportamento dos herbicidas no ambiente. In: Oliveira Jr. R S, Constantin J, Inoue M H. Biologia e manejo de plantas daninhas. Curitiba: Omnipax pp. 264-304.

Pacheco LP, Petter FA, Câmara ACF, Lima DBC, Procópio SO, Barroso ALL, Cargnelutti Filho A, Silva IS (2007). Tolerância do milheto (*Pennisetum americanum*) ao 2,4-D. Planta Daninha 25(1):173-179.

Peres-Oliveira MA, Bonfim-Silva EM, Silva VM, Vieira ECS (2016). Persistence of 2, 4-D and glyphosate in a Cerrado soil, Brazil. Afr. J. Agric. Res. 11(11):912-919.

Pitelli R A (1987). Competição e controle das plantas daninhas em áreas agrícolas. Série Técnica IPEF 4(12):1-24.

Procópio SO, Carmo ML, Pires FR, Cargnelutti Filho A, Braz GBP, Silva WFP, Barroso ALL, Silva GP, Carmo EL, Braz AJBP (2008). Fitorremediação de solo contaminado com picloram por capim-pé-

de-galinha-gigante (*Eleusine coracana*). Rev. Bras. Ciênc. Solo 32(6):2517-2524.
Rodrigues BN, Almeida F S (2011). Guia de herbicidas. 6ª Edição. Londrina-PR.
Saad O (1978). A vez dos herbicidas. 2.ed. São Paulo: Nobel.
Santos DP, Broga RR, Guimarães FAR, Passos ABRJ, Silva DV, Santos JB, Nery MC (2013). Determinação de espécies bioindicadoras de resíduos de herbicidas auxínicos Rev. Ceres 60(3):354-362.
Schäfer G, Koller OC, Sartori IA (1999). Retenção de frutos de laranjeiras de umbigo 'monte parnaso' em função da aplicação de 2,4-d, ácido giberélico e da anelagem de ramos. Cienc. Rural 29(4):639-644.
Silva FML, Cavalieri SD, São José AR, Ulloa S M, Velini E D (2011). Atividade residual de 2,4-D sobre a emergência de soja em solos com texturas distintas. Rev. Bras. Herb. 10(1):29-36.
Silva AA, Ferreira FA, Silva LRF (2007a). Herbicidas: classificação e mecanismo de ação. In: Silva A A, Silva J F. Tópicos em manejo de plantas daninhas. Viçosa, MG: UFV. pp.83-148.
Silva AA, Vivian R, Oliveira Junior RS (2007b). Herbicidas: Comportamento no Solo. In: Silva A A, Silva J F. Tópicos em manejo de plantas daninhas. Viçosa, MG: UFV. pp. 189-248.
SBCPD - Sociedade Brasileira da Ciência das Plantas Daninhas (1995). Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. Londrina: SBCPD.
Souza AP, Prates HT, Ferreira FA, Reis E Jordão CP (1999). Lixiviação do glyphosate e do imazapyr em solos com diferentes texturas e composição química. II. Método analítico. Planta Daninha 17(2):245-262.
Thill D (2003). Growth regulator herbicides. In: Weller S C, Thill D, Bridges D C, van Scoyoc G E, Graveel J G, Turco Júnior R F, Goldsbrough P, Ruhl, GE, Holt H A, Reicher ZJ, Whitford F. WHITFORD, F.. (Eds.) Herbicide action course. West Lafayette: Purdue University pp. 267-275
Vasconcelos MCC, Silva AFA, Lima RS (2012). Interferências de plantas daninhas sobre plantas cultivadas. Agropecu. Cient. Semi-Árido 8(1):01-06.
Xiao W, Wang H, Zhu Z, Zhang J, He Z, Yang X (2012). Bioremediation of Cd and carbendazim cocontaminated soil by Cd-hyperaccumulator Sedum alfredii associated with carbendazim-degrading bacterial strains. Environ. Sci. Pollut. Res. Int. 20(1):380-389.

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Full Length Research Paper

Biological control of the bacterial wilt *Ralstonia solanacearum* by bioprotector with fungi chitosan from *Cunninghamella elegans* on tomatoes

Wagner Silva Oliveira*, Iwanne Lima Coelho, Jéssica Rafaella Sousa Oliveira, Maria Camila Barros Silva Leite, Thatiana Montenegro Stamford Arnaud, Newton Pereira Stamford and Emmanuella Vila Nova Silva

Department of Agronomy, Federal Rural University of Pernambuco, Av. Dom Manoel de Medeiros, s/n, 52171-900, Recife, Pernambuco, Brazil.

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Fertilization is one of the most important means to improve plant production and nutrient uptake. Tomatoes plants are very exigent on fertilizers and sensitive to diseases. For satisfactory yield and fruit quality soil fertility and diseases need to be controlled. The aim of this paper was to evaluate the effectiveness of a bioprotector that contains fungi chitosan as an alternative biofertilizer, which releases nutrients and induces resistance against tomatoes wilt by *Ralstonia solanacearum* bacteria. The treatments were: 1- soluble NPK fertilizers (NPKF) in recommended rate (RR), 2- bioprotector (NPKP) with fungi chitosan in half of recommended rate (50% RR), 3– NPKP 100% RR, and 4– NPKP 150% RR. Treatments without bacterial inoculation (PO) and with bacterial inoculation (P1) were added to evaluate the plant characteristics (plant height, shoot diameter, number of leaves in ramification, fresh and dry matter of shoots). The symptom classes used to observe the induction of resistance were: (--) plants with no disease symptoms; (-) plant with slight symptoms; (+) plants with drastic symptoms and (++) plants died. Plants receiving the soluble fertilizers (NPKF) showed drastic disease symptoms one week after *R. solanacearum* inoculation, and all the plants died two weeks after inoculation. Plants with NPKP that contains *Cunninghamella elegans* in rates 50, 100 and 150% RR induced resistance for bacterial disease and promote better plant characteristics. The results showed that the bioprotector displays normal characteristics. The protector may be used as alternative for conventional fertilizers, especially inducing resistance for bacterial control.

Key words: *Lycopersicon lycopersicum*, *Ralstonia solanacearum*, biopolymers, organic bioprotector, phytopatogenic bacteria, resistance induction.

INTRODUCTION

Soluble fertilizers are of great importance for plant growth and yield but their use by low-income farmers is prohibitive due to the high price. Furthermore, the soluble nutrients may lixiviate to the deeper soil layers and can promote environmental problems (van Straaten, 2007). In a modern and sustainable agriculture, the application of soluble fertilizers and soil amendments are applied for increment of food production, meet economic criteria to

increase soil fertility and to minimize environmental damage (Stamford et al., 2008).

In general, Brazilian soils contain low available P and K content and these nutrients need to be supplied by the farmer. So, to increase the use of renewable natural sources of phosphate, it is necessary to study the effectiveness of different fertilizers (Araújo et al., 2008). An alternative for effective and economic fertilization is the use of biofertilizers made from phosphate and potash rocks with elemental sulfur inoculated with *Acidithiobacillus.* This combination achieves greater nutrient availability since the bacteria produce sulfuric acid, and thus increase both phosphorus and potassium availabilities, with results comparable to traditional fertilizers in several experiments with different economic crops (Stamford et al., 2006; van Straaten, 2007; Stamford et al., 2008).

Nitrogen is one of the most important nutrients due to its role in proteins and nucleic acids, and PK rock biofertilizers have no available N content to promote normal plant growth, although, in mixture with earthworm compound, inoculated with free living diazotrophic bacteria, has been shown to be effective as a N source (Lima et al., 2010).

Chitosan from crustaceous has been frequently used in assays to increase resistance against plant pathogens (Berger et al., 2013), while at the same time, it has greater chelating properties as compared to other natural biopolymers, and can release nutrients to the environment (Boonlertnirun et al., 2008; Goy et al., 2009). On the other hand, chitosan from fungi biomass, as compared to that from crustaceous sources, is independent of seasonal factors, and allows simultaneous extraction of chitin and chitosan (Franco et al., 2004). Up to now, there are no reports on its use as a bioprotector (Franco et al., 2004).

This paper aims to evaluate the effectiveness of the bioprotector (NPKP) with addition of fungi chitosan from *Cunninghamella elegans*, on characteristics of tomatoes grown in a Brazilian tableland Argisol. The NPKP bioprotector compared with the mineral soluble fertilizer (NPKF) showed possibility for use as alternative for replacement of conventional NPK soluble fertilizer and especially for use as fungicide.

MATERIALS AND METHODS

Production of the bioprotector

The PK rock biofertilizers were produced at the Federal Agricultural University of Pernambuco (UFRPE) in accordance with El Tarabily et al. (2006) and Stamford et al. (2007). Analysis of the P and K biofertilizer by the Embrapa (2009) methodology showed: (P-biofertilizer)- pH = 3.8, available P (A) = 60 (g kg^{-1}) and (K biofertilizer) - pH = 3.3, available K = 10 (g kg^{-1}).

The production of the biofertilizer (NPKB) was processed by mixing PK rock biofertilizers with organic biofertilizer (earthworm compost) enriched in N by inoculation with the selected free-living bacteria *Beijerinckia indica* (NFB 10001), in accordance with Lima et al. (2010). The analysis of the earthworm compound presented: pH 7.95; organic carbon (100.7 g kg^{-1}); total N (8.6 g kg^{-1}); total S (2.98 g kg^{-1}); total P (1.12 g kg^{-1}). The rock biofertilizer (PKB) and the organic biofertilizer (OB) were mixed in proportion 1:4 (PKB: OB), inoculated with free-living bacteria (NFB 10001) and maintained in incubation for 30 days.

The bioprotector (NPKP) represents the biofertilizer (NPKB) by addition of *C. elegans* (UCP 542), fungi that contains chitosan in their cellular wall (Franco et al., 2004). The fungus *C. elegans* was purified in Petri dishes in potato dextrose agar (PDA) grown for 10 days at 28°C. The monosporic culture of the *C. elegans* was obtained growing the Mucorales fungus in Potato Dextrose (BD), in 2000 mL Erlenmeyers flasks (containing 1000 mL) kept under shaking (180 rotations per minute) for 96 h at 28°C. The culture diluted in distilled water (20 L^{-1}) was applied by manual irrigation. For production of the bioprotector (NPKP), the NPKB from PK rocks was mixed with earthworm compound and incubated for 30 days. The chemical analyses of the bioprotector (NPKP) at the final period of incubation showed: pH = 6.4, total N = 20 g kg^{-1}; available P = 21 g kg^{-1} and available K = 19 g kg^{-1}.

Site, soil and experimental conditions

A greenhouse experiment was realized (November to December 2015) using samples of a “Yellow Argisol medium texture” (Embrapa, 2013) with low available P and K, and predominantly cultivated with horticultural crops, tropical fruits and cowpea legume. The chemical analyzes of soil, collected at 0-20 cm deep, showed: pH (H_2O) = 6.2; organic matter (g kg^{-1}) = 12.31; P (Mehlich 1) = 2 mg dm^{-3}; exchangeable cations ($cmol_c$ dm^{-3}) K = 0.22; Ca = 1.05; Mg = 0.6; Al = 0.4. The physical analyzes showed: particle density (g cm^{-3}) = 2.61; bulk density (g dm^{-3}) = 1.40; sand (g kg^{-1}) = 700; lime (g kg^{-1}) = 100 and clay (g kg^{-1}) = 200.

One month before transplanting to the pots at the greenhouse experiment, the seedlings of tomato (UC 82) purchase from the Isla Pak Industry, were grown in polypropylene trays (450 cells) with the commercial substrate “Vivatto Slim”. The seedlings were manually planted in November 02, 2015, and after 13 days of growth, they were transplanted to pots with soil (4 kg). Irrigation were processed daily, based in the pot weight, applying distilled water to maintain the moisture near field holding capacity. The NPK fertilizers treatments were applied at the planting date, before seedling transplantation. The current cultural practices were realized in accord with the usual recommendations for commercial tomatoes cultivated in the Brazilian rainforest region.

The greenhouse experiment was conducted in randomized block design, with four replicates. The fertilization treatments were: (1)-treatment with conventional NPK fertilizer (NPKF) in recommended rate (RR), (2)- bioprotector (NPKP) with fungi chitosan in half of recommended rate (50% RR), (3)- NPKP 100% RR, and (4)- NPKP 150% RR. All fertilization rates followed the current recommendation for irrigated tomatoes in Pernambuco (IPA, 2008). Treatments without bacterial inoculation (PO) were used to

*Corresponding author. E-mail: wagneragronomo@gmail.com. Tel: 55-81-33206198. Fax: 55-81-33206220.

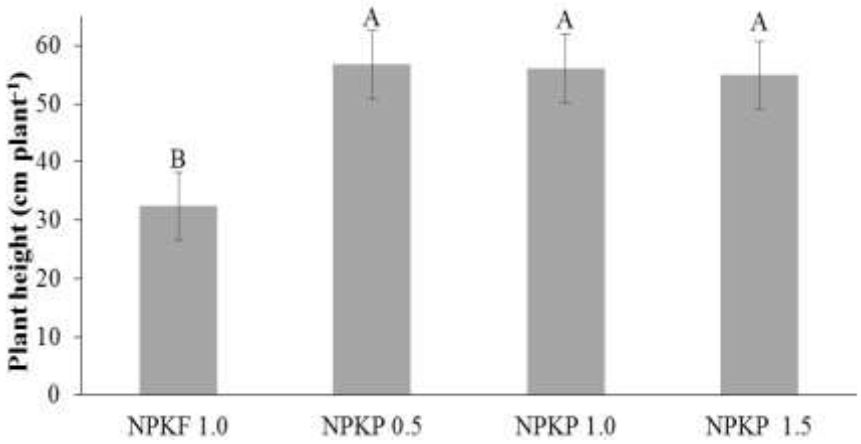

Figure 1. Plant height (45 days after transplantation) affected by the fertilization treatments NPKF 1.0 (100% recommended rate- RR), NPKP 0.5 (50% RR), NPKP 1.0 (100% RR), NPKP 1.5 (150% RR). Means with the same letter are not different by the Tukey test ($p < 0.05$).

evaluate the plant characteristics and the plants with bacterial inoculation (P1) were used to evaluate tomato resistance to *Ralstonia solanacearum*. The analysis of soil fertility evaluates the influence of the fertilization treatments and the resistance for the plant disease tomatoes wilt promoted by *Ralstonia solanacearum* bacteria in plants after inoculation of the pathogen was observed.

The pathogenic bacteria *R. solanacearum* was isolated from an area with tomatoes plants characterized with bacterial wilt symptoms, confirmed and identified by biochemistry analyzes. Isolation was processed in NYDA (dextrose 10 g, meal extract 3 g, yeast extract 5 g. peptone 3 g and agar-agar 18 g), by the continuous risks method, and Petri dishes were incubated for 48 h at 28°C. Healthy plants were inoculated by making a semicircular cut in the root system with a sterilized scalpel, and was added, per pot 20 mL of bacterial suspension (UFC $5x10^8$ mL^{-1}) in accordance with Garcia et al. (2013).

Determinations and statistical analyzes

After 45 days of growth, when flowering was beginning, the plants with PO treatment (not inoculated with pathogenic bacteria) were harvested to determine the plant characteristics (height, diameter, number of ramification and fresh and dry shoot weight). Soil samples were collected immediately after the plant harvest, for analysis of fertility (soil pH, available P and K and exchangeable Ca^{+2}, Mg^{+2} and Al^{+3}).

One week after the pathogen inoculation, the disease symptoms classified into: (--) plants with no disease symptoms; (-) plants with slight disease symptoms (+) plants with drastic disease symptoms and (++) plants died were observed.

The statistical calculations for plant characteristics and soil analysis used SAS 9.2 (SAS Institute 2011) through analysis of variance and means comparison using Tukey's test at probability $p \leq 0.05$.

RESULTS AND DISCUSSION

Tomatoes plants characteristics

Plants receiving bioprotector (NPKP) were significantly ($P<0.05$) higher, with larger diameter and had more leaves in ramifications than plants receiving the soluble conventional fertilizer. There were no significant differences between the bioprotector treatment applied in the three rates (50, 100 and 150% RR).

The plant characteristics (height, shoot diameter and average leaves in ramifications) are present in Figures 1, 2 and 3. The results showed positive and significant increase with application of the different bioprotector treatments, compared with the soluble fertilizer treatments. In a general, the best results were found with NPKP applied in the higher rates, and the soluble fertilizer (NPKF) showed the lowest results.

In reference to fresh and dry shoot weight (Figures 4 and 5), the best results were obtained when the NPKP was applied in higher rates (100 and 150% RR), when compared with NPKP in the lower rate (50% RR) and NPKF in recommended rate, which achieved the lowest results. The obtained results showed the effectiveness of the NPKP that promote good nutritional response in tomato plants in the greenhouse experiment.

In two consecutive harvest of lettuce, applying the biofertilizer (NPKB), Lima et al. (2007) reported the positive and significant effect when compared with soluble fertilizer (NPKF). Similar results on melon grown in soil of the Brazilian semiarid region were described by Oliveira et al. (2014), Costa et al. (2011) and Moura et al. (2007).

In reference to the bioprotector effects, in general, the best results were shown, when compared with the soluble fertilizer treatment, which can be due to the metabolic action of the oxidative bacteria *Acidithiobacillus* that acidify the soil and the acidity release nutrients contained in the rocks used to produce the bioprotector. Stamford et al. (2006, 2008) in greenhouse experiments reported positive and significant effects of the PK rock

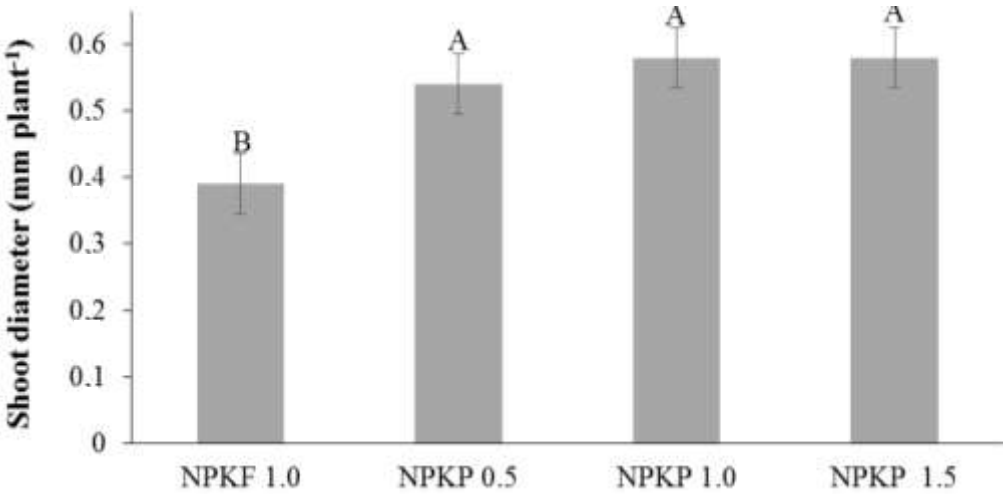

Figure 2. Shoot diameter with 45 days after transplantation as affected by the fertilization treatments NPKF 1.0 (100% recommended rate- RR), NPKP 0.5 (50% RR), NPKP 1.0 (100% RR), NPKP 1.5 (150% RR). Means with the same letter are not different by the Tukey test ($p < 0.05$).

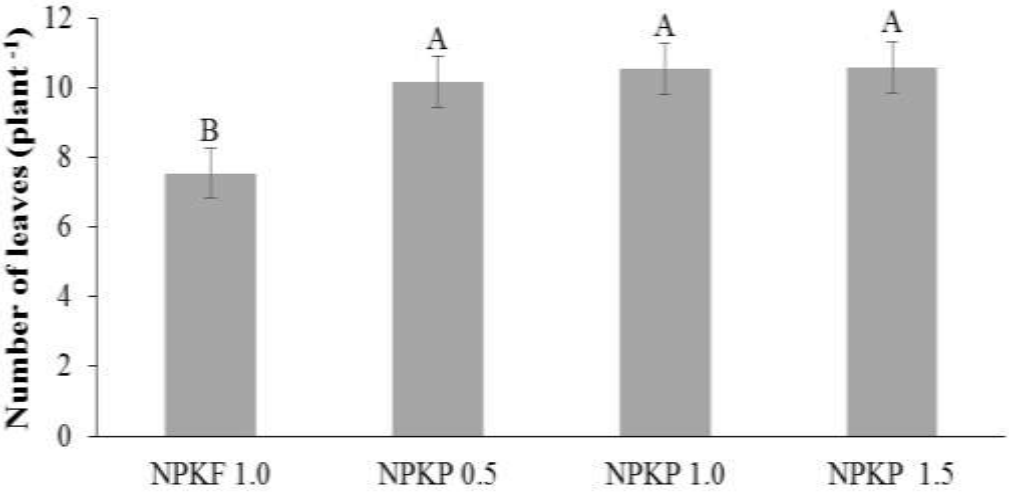

Figure 3. Number of leaves in ramifications with 45 days after transplantation as affected by the fertilization treatments NPKF 1.0 (100% recommended rate - RR), NPKP 0.5 (50% RR), NPKP 1.0 (100% RR), NPKP 1.5 (150% RR). Means with the same letter are not different by the Tukey test ($p < 0.05$).

biofertilizers inoculated with *Acidithiobacillus* in some characteristics of sugarcane and observed best effectiveness when comparing the PK soluble fertilizer. When applying the rock biofertilizer in higher amount, the authors observed reduction in plant characteristics, probably promoted by the soil acidification due to the low pH of the rock biofertilizers (pH 3.0 to 3.5).

The best results for the plant characteristics display the same behavior obtained when the different rates of the bioprotector treatments were applied. The obtained results are in accordance with Echart and Cavalli-Molina (2001) and Degenhardt et al. (1998) that observed damage in the plant growth and inhibition on roots development as affected by the acidification that reduce soil pH, especially in the presence of exchangeable aluminum, which may promote nutritional deficiency and reduction in shoot and root growth.

Soil analyses

The soil analyzes determined in soil collected after tomatoes harvest at 45 days of growth are present in Table 1. The treatment with NPKF applied in recommended

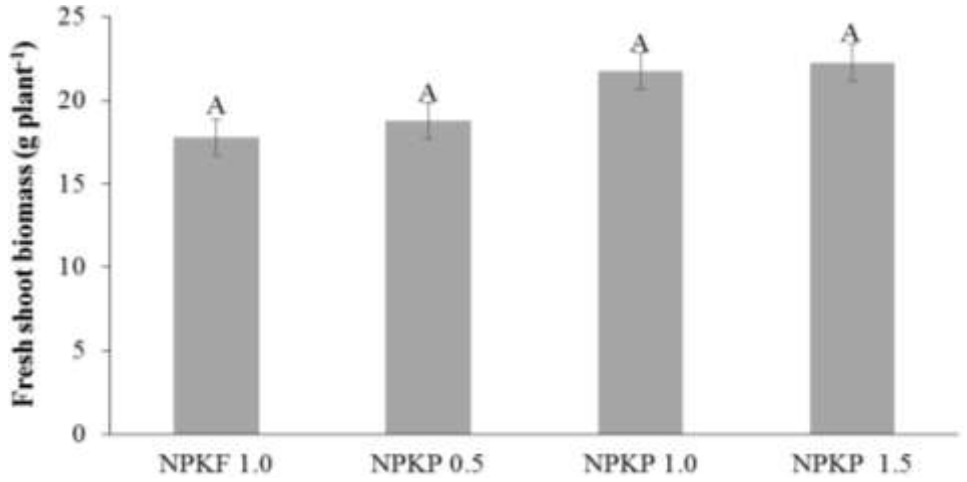

Figure 4. Fresh shoot biomass of tomato plants with 45 days after transplantation as affected by the fertilization treatments NPKF 1.0 (100% recommended rate - RR), NPKP 0.5 (50% RR), NPKP 1.0 (100% RR), NPKP 1.5 (150% RR). Means with the same letter are not different by the Tukey test ($p < 0.05$).

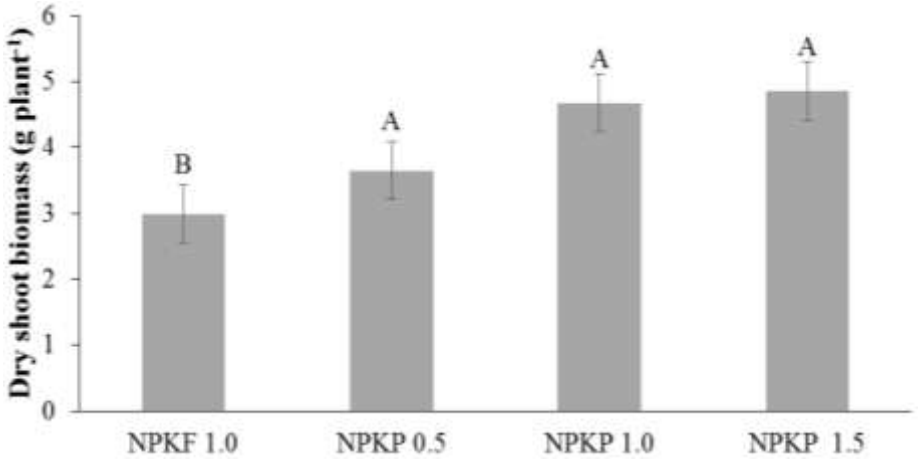

Figure 5. Dry shoot biomass of tomato plants with 45 days after transplantation as affected by the fertilization treatments NPKF 1.0 (100% recommended rate - RR), NPKP 0.5 (50% RR), NPKP 1.0 (100% RR), NPKP 1.5 (150% RR). Means with the same letter are not different by the Tukey test ($p < 0.05$).

Table 1. Soil analyzes after harvesting tomatoes plants submitted to fertilization treatments with NPKF 100% recommended rate (RR), NPKP 50% RR, 100% RR, and 150% RR, inoculated with *R. solanacearum*.

Fertilization treatments	pH	Exchangeable			Available P
		Al	Ca	Mg	
	H_2O	---------- $cmol_c\ dm^{-3}$ ----------			$mg\ dm^{-3}$
FNPK 100% RR	4.6b	1.00a	1.45a	1.0b	30b
PNPK 50% RR	5.3a	0.02b	1.08a	3.2a	40a
PNPK 100% RR	5.4a	0.02b	1.11a	4.9a	46a
PNPK 150% RR	5.5a	0.01b	1.18a	5.1a	50a

Data with the same letter have no statistical difference by the Tukey's test ($p \leq 0.05$)

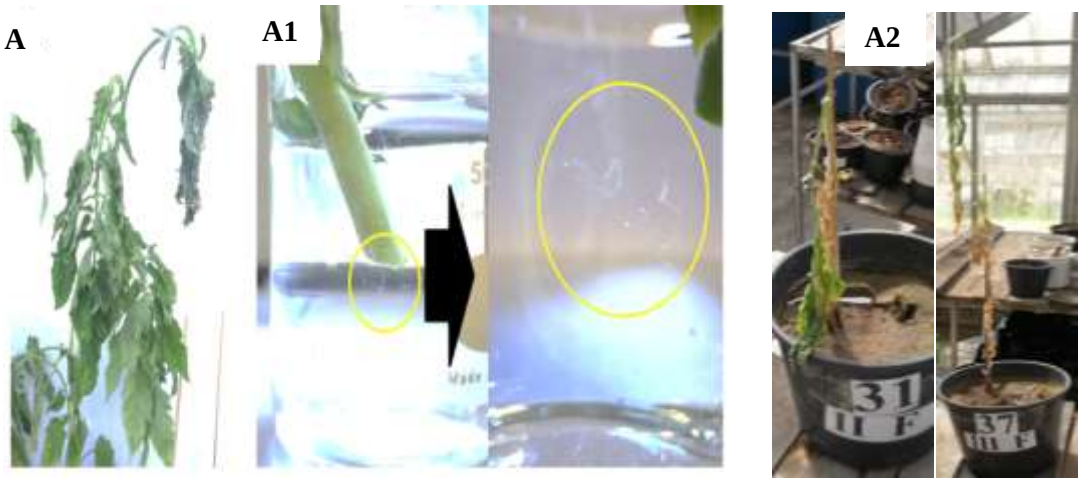

Figure 6. A- Tomato plants with wilt symptoms of the disease promoted by the *R. solanacearum* bacteria with treatment NPKF 100% (recommended rate) at one week after bacteria inoculation. A1- plant exudate with evident reaction of the wilt disease by the test in the symptomatic tissue showing the bacteria grown in the base of stem. A2- Tomato plants died at two weeks after wilt bacteria inoculation.

Table 2. Disease symptoms showed by tomatoes plants one and two weeks after inoculation with *Ralstonia solanacearum* as affected by the different fertilization treatments (Bioprotector at 50%, 100% and 150% Recommended Rate - RR and soluble Fertilizers 100% RR).

Response in growth	One week (mg plant^{-1})	Two weeks (mg plant^{-1})
NPKP 50	(--)	(-)
NPKP 100	(--)	(--)
NPKP 150	(--)	(--)
NPKF* 100	(+)	(++)

*Plants with no symptoms of disease (--); Plants with slight symptoms of disease (-); Plants with drastic symptoms of disease (+); plants that died (++).

rate revealed lower pH, higher Al, and also did not differ among the different rates of NPKP applied. Although, the experimental design confirmed these results because in the production of bioprotector the low pH is neutrized by the addition of organic matter as earthworm compound that present very high pH (pH 7.9).

The results of nutrients in soil especially Mg and P are in accordance with the literature of biofertilizer produced with phosphate and potash rocks. The addition of sulfur inoculated with *Acidithiobacillus* may be taken to confirm the production of sufficient sulfuric acid by the bacteria to increase P and Mg solubility as previously proposed by Stamford et al. (2008, 2009). A secondary possibility for causal mechanism is a direct effect of the chitosan as proposed by Kowalski et al. (2006) and Goy et al. (2009). The values of P in the soil are higher than in the NPKF treatment, because chitosan increase the levels of N and P in the substrates as proposed by Kowalski et al. (2006) and Goy et al. (2009).

Resistance to wilt disease (*R. solanacearum*)

The tomatoes wilt symptoms were observed in Figure 6. One week after inoculation, all plants that received the treatments with soluble mineral fertilizers (NPKF) showed drastic symptoms of the wilt disease (Figure 6 1A) and the effects were confirmed by the plant exudate test in the symptomatic tissue (Figure 6 A1). Statistical analysis was not necessary because the wilt disease occurred in plants with application of soluble fertilizer (NPKF) and all the tomatoes plants died two weeks after the pathogenic inoculation (Figure 6 A2). The plants that received the fertilization treatments with NPKP with the different rates do not present wilt disease and maintained satisfactory growth (Table 2).

Hayward (1994) reported that several factors influence the success of infection promoted by *R. solanacearum* bacteria, and the most important is cultural practice, especially fertilization. Berger et al. (2013) observed the

effects of fungi chitosan in the activity of some enzymes and on growth of cowpea plants, in a table land soil from the Brazilian Northeast, rainforest region with low content in P and K nutrients, applying fungi chitosan and biofertilizer (NPKP), although, in the study, symptoms of disease (*Fusarium oxysporum*) were not observed in cowpea plants supplied with soluble fertilizer (NPKF).

Plants not supplied by sufficient nutrients such as N, P and K, influence growth and may contribute to increase in the susceptibility of diseases as reported by Ghormade et al. (2010) and Guazzelli et al. (2007). However, the plants of the experiment were normally supplied in nutrients at the 54 days of growth and these arguments, and the plants with NPKF only showed symptoms of disease after the pathogen inoculation.

Chitosan release from the biomass of *C. elegans* fungi, during the process of PNPK production probably acts in the protection against *R. solanacearum* inducing plant resistance, and therefore may act in the mineralization process, releasing nutrients for plants absorption as reported by Boonlertnirun et al. (2008).

The reduction of bacterial wilt disease by the action of compounds that induce resistance such as acibenzolar-S-methyl (Araujo et al., 2008), were tested for *Pseudomonas* (Peixoto, 1997); cultural processes such as biofumigation and solarization (Baptista et al., 2006) are not proven to control bacterial wilt, and these practices are expensive and produce problems in the soil and in the environment; although, there is no study on tomato wilt control in the literature by application of biofertilizers and bioprotector.

Conclusions

The application of bioprotector with fungi chitosan controls tomatoes bacterial wilt with no addition of fungicides, while its fertilizer effects allowed normal plant growth than conventional soluble fertilizer. The bioprotector may be used in replacement of NPK fertilizers.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Araujo FF, Tiritan CS, Pereira HM, Caetano Júnior O (2008). Development of mays and soil fertility under application of organic matter and phosphorite. Braz. J. Agric. Eng. Environ. 12:507-511.

Baptista MJ, Lopes CA, Souza RB, Furumoto O (2006). Effect of solarization and biofumigation in autumn, in the incidence of bacterial wilt and potatoes yield. Braz. J. Hortic. 24:99-102.

Berger LRR, Stamford NP, Santos CERS, Freitas ADS, Franco LO, Stamford TCM (2013). Plant and soil characteristics affected by biofertilizers from rocks and organic matter inoculated with diazotrophic bacteria and fungi that produce chitosan. J. Soil Sci. Plant Nutr. 13:592-603.

Boonlertnirun S, Boonraung C, Suvanasara R (2008). Application of Chitosan in Rice Production. J. Metals Mat. Min. 18:47-52.

Costa CLL, Batista JE, Costa Junior CO, Santos AP, Silva ML (2011). Application of phosphate fertilizer in melon grown in calcareous soils. Rev. Verde. 6:7-11.

Degenhardt J, Larsen PB, Howell SK, Kochian LV (1998). Aluminum resistance in the *Arabidopsis* mutant a1r-104 is caused by an aluminum-induced increase in rhizosphere pH. Plant Physiol. 117:19-27.

Echart CL, Cavalli-Molina S (2001). Aluminum toxicity: effects, mechanisms for tolerance and genetic control. Ciência Rural 31:531-541.

El Tarabily KA, Soaud AA, Saleh ME, Matsumoto S (2006). Isolation and characterization of sulfur bacteria, including strains of *Rhizobium* from calcareous soils and their effects on nutrient uptake and growth of maize (*Zea mays* L.). Austr. J. Agric. Res. 57:101-111.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (2009). Manual de Análises Químicas de Solo, Plantas e Fertilizantes. Brasília 627 p.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária (2013). Sistema brasileiro de classificação de solos. Brasília 306 p.

Franco LO, Maia RCC, Porto ALF, Messias AS, Fukushima K, Takaki GMC (2004). Heavy metal biosorption by chitin and chitosan isolated from *Cunninghamella elegans* (IFM 46109). Braz. J. Microbiol. 35:243-247.

Garcia AL, Lima WG, Souza EB, Michereff SJ, Mariano RLR (2013). Characterization of *Ralstonia solanacearum* causing bacerial wilt bell pepper in the state of Pernambuco, Brazil. J. Plant Physiol. 95:237-245.

Ghormade V, Deshpande MV, Paknikar KM (2010). Perspectives for nanobiotechnology enabled protection and nutrition of plants. Biotechnol. Adv. 29:792-803.

Goy RC, Britto D, Assis OBG (2009). A Review of the antimicrobial activity of chitosan. Polym. Sci. Technol. 9:241-247.

Guazzelli MJ, Meirelles L, Barreto R, Gonçalves A, Motter C, Rupp LC (2007). Aplicação da teoria da trofobiose no controle de pragas e doenças: uma experiência na serra gaúcha. Agricultural 4:16-19.

Hayward AC (1994). Systematics and phylogeny of *Pseudomonas solanacearum* and related bacteria. In: Bacterial Wilt: The *Pseudomonas solanacearum* disease and its causative agent. Wallingford, CAB International pp.123-135.

Instituto Agronômico de Pernambuco – IPA. (2008). Recomendações de adubação para o estado de Pernambuco. 212p.

Kowalski B, Terry FJ, Herrera L, Peñalver DS (2006). Application of soluble chitosan in vitro and in the greenhouse to increase yield and seed quality of potato minitubers. Potato Res. 49:167-176.

Lima FS, Stamford NP, Sousa CS, Lira Junior MA, Malheiros SMM, Van Straaten P (2010). Earthworm compound and rock biofertilizer enriched in nitrogen by inoculation with free living diazotrophic bacteria. World J. Microbiol. Biotechnol. 26:1769-1777.

Lima RCM, Stamford NP, Santos CERS, Dias SHL (2007). Lettuce yield and chemical attributes of a Latosoil as affected by PK rock biofertilizer application. Braz. J. Hortic. 25:224-229.

Moura PM, Stamford NP, Duenhas LH, Santos CERS, Nunes GHS **(**2007). Effectiveness of rock biofertilizer with *Acidithiobacillus* on melon grown in the San Francisco Valley. Braz. J. Agric. Sci. 2:1-7.

SAS Institute (2011). The SAS 9.2 software. Statistical analysis System for Windows**.** Procedure guide for personal computer. Cary (CD-ROM).

Oliveira WS, Stamford NP, Silva EVN, Silva CERS, Freitas AD, Arnaud TMS, Sarmento BF (2014). Biofertilizer produced by interactive microbial processes affects melon yield and nutrients availability in a

Brazilian semiarid soil. Austr. J. Crop Sci. 8:1124-1131.
Peixoto AR (1997). Controle biológico da murcha bacteriana do tomateiro, por *Pseudomonas* spp. fluorescentes. Ciênc. Rural 27:153-160.
Stamford NP, Moura PM, Lira Junior MA, Santos CERS, Duenhas LH, Gava CAT (2009). Chemical attributes of an Argisol of the Vale do São Francisco after melon growth with phosphate and potash rocks biofertilizers. Braz. J. Hortic. 27:447-452.
Stamford NP, Lima RA, Lira Junior MA, Santos CERS (2008). Effectiveness of phosphate and potash rocks with *Acidithiobacillus* on sugar cane yield and their effects in soil chemical attributes. World J. Microbiol. Biotechnol. 24:2061-2066.
Stamford NP, Santos PR, Santos CERS, Freitas ADS, Dias SHL, Lira Junior MA (2007). Agronomic effectiveness of biofertilizers with phosphate rock, sulphur and *Acidithiobacillus* in a Brazilian tableland acidic soil grown with yam bean. Biores. Technol. 98:1311–1318.
Stamford NP, Lima RA, Lira Junior MA, Santos CERS, Dias SHL (2006). Rock biofertilizers with *Acidithiobacillus* on sugarcane yield and nutrient uptake in a Brazilian soil. Geomicrobiol. J. 23:261-265.
Van Straaten P (2007). Agrogeology - the use of rocks for crops. Enviroquest, Cambridge, Ontario, Canada 440 p.

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Nutritional diagnosis of sugarcane varieties in a Yellow Oxisol during three agricultural seasons

Vinicius Santos Gomes da Silva[1*], Mauro Wagner de Oliveira[2], Daniele Costa de Oliveira[3], Terezinha Bezerra Albino Oliveira[2], Manoel Gomes Pereira[4] and Carlos Henrique de Castro Nogueira[2]

[1]Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros, s/n, 52171-900, Recife, Pernambuco, Brazil.
[2]Centro de Ciências Agrárias, Universidade Federal de Alagoas, BR 104, Km 85, s/n, 57100-000. Rio Largo, Alagoas, Brazil.
[3]Departamento de Agronomia, Escola Superior de Agricultura Luiz de Queiróz, Universidade de São Paulo, Av. Pádua Dias, 11, 13418-900, Piracicaba, São Paulo, Brazil.
[4]Departamento de Pesquisa, Usina Triunfo, Vila Triunfo, s/n, 57680-000, Boca da Mata, Alagoas, Brazil.

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Leaf analysis is one of the main methods used for the evaluation of nutritional requirements in sugarcane culture, contributing to a better management of fertilizers. This study aimed to evaluate the nutritional status of sugarcane varieties by quantifying the leaf content of macro and micronutrients of the +3 leaf. The varieties RB92579, RB867515, VAT90-212 and SP813250 were evaluated in a Yellow Oxisol at Fazenda Jequiá, Anadia, Alagoas (AL) state, during plant-cane, first and second regrowth cycles. The design was randomized blocks consisting of four varieties and five replications. Varietal differences were observed in leaf contents of macro and micronutrients. However, no variety had a higher leaf content regarding any element.

Key words: Macronutrients, micronutrients, nutritional status, production system, *Saccharum* spp.

INTRODUCTION

Sugarcane has a socio-economic importance for Brazil generating employment, income and exchange values (Zuanazzi and Mayorga, 2010; Marin and Nassif, 2013). Its average productivity is 76.9 t ha^{-1}, which corresponds to less than 25% of the biological potential of the crop. The low productivity is caused, among other factors, by inadequate fertilization in relation to culture requirements (Conab, 2016).

*Corresponding author. E-mail: vinicius.agro2008.1@gmail.com Telephone: 55-81-33206237. Fax: 55-81-33206200.

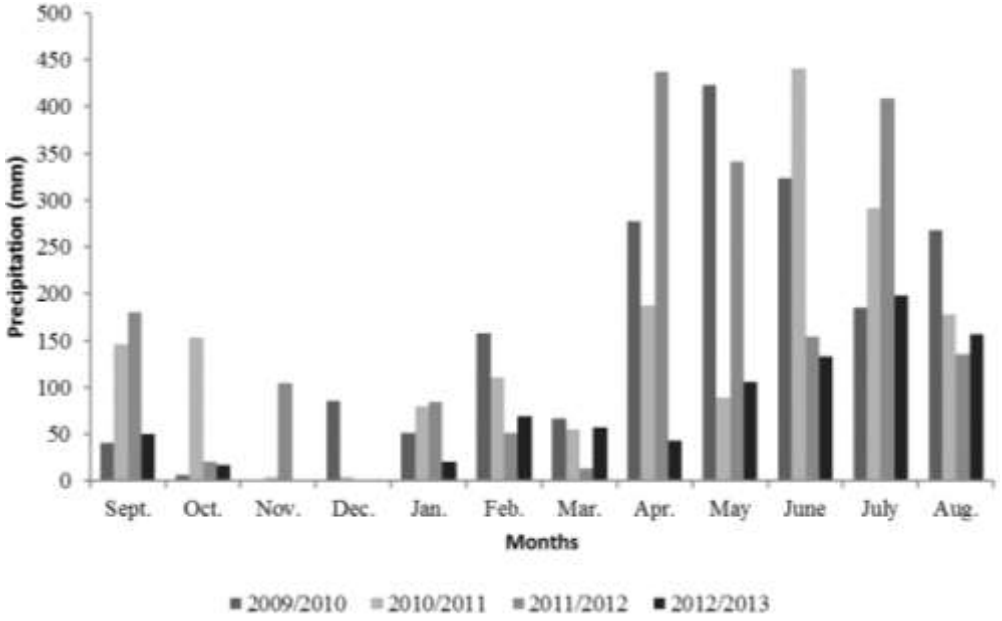

Figure 1. Monthly precipitation during the period studied.

Due to its high biomass production, sugar cane extracts and accumulates high amounts of soil nutrients (Calheiros et al., 2012; Mishra et al., 2014; Meena et al., 2015). Oliveira et al. (2010) evaluating the extraction and export of macronutrients by 11 sugarcane varieties in Ultisol, obtained averages of 0.91, 0.13, 1.71, 1.18 and 0.44 kg t^{-1} culm N, P, K, Ca and Mg, respectively. Thus, the accumulation of nutrients by sugarcane shows the need for an adequate fertilization for the crop to achieve high yields. Furthermore, fertilization represents a significant percentage of the production costs of sugarcane crops. Thus, it must follow strict optimization criteria to obtain a higher productivity and a lower cost (Otto et al., 2010).

The optimization can be achieved by a proper application of nutrients regarding the quantity, timing and form of application. The diagnosis of plant nutritional status is a tool for planning, evaluating and calibrating the fertilization recommendations of a crop. Its use is essential to sugarcane production as the nutritional status of sugarcane influences photosynthetic rates and the metabolism of sucrose, directly affecting the productivity, longevity and profitability of crops (Malavolta et al., 1997; Raij, 2011).

The diagnosis can be performed by evaluating the results of chemical foliar analysis. It allows identifying and correcting deficiencies and nutritional imbalances in plants, and it monitors and evaluates the efficiency of a particular crop fertilization and soil fertility program (Deus et al., 2012). In this sense, the proper use of the results obtained from leaf analyses contribute to a rational use of inputs, promoting the nutritional balance of plants, providing greater crop yield and increasing safety in the use of fertilizers (Souza et al., 2011; Santos et al., 2013).

The literature reports appropriate ranges for nutrients levels using a well-nourished +3 leaf of sugarcane. These values were between 16 and 21, 1.5 and 3.5, 6 and 16, 2 and 10, 1.0 and 3.6 and 1.3 to 3.0 g kg^{-1} for N, P, K, Ca, Mg and S, respectively. Regarding micronutrients, the leaf content ranges considered adequate for Cu, Fe, Mn and Zn are between 6 and 50, 8 and 17, 40 and 500, 25 and 250, and 10 and 50 mg kg^{-1}, respectively (Orlando Filho, 1983; Malavolta et al., 1997; Raij, 2011). These results were obtained under different soil and climatic conditions in Alagoas with varieties that are practically no longer cultivated in that state. This may hinder the use of leaf analysis as a tool for identifying and correcting deficiencies and nutritional imbalances.

In face of such considerations, this study was conducted to evaluate by leaf analysis the nutritional status of four varieties of sugarcane, representing more than 50% of the sugarcane crop area in Alagoas, during plant-cane, first and second regrowth cycles.

MATERIALS AND METHODS

The research was conducted in the municipality of Anadia, state of Alagoas (09°41'04" S and 36°18'15" W). The experimental area belongs to the Triunfo Plant located in the municipality of Boca da Mata, AL. The climate of the experiment area is rainy tropical with dry summers, according to the Köppen classification. The average annual rainfall is 1500 mm (Figure 1) and the average annual

Table 1. Results of chemical analyses of soil samples at the layers 0-20 cm and 20-40 cm.

Layers	pH	P	K	Ca	Mg	Al	H+Al	SB	T
cm	-	mg dm^{-3}				cmolc dm^{-3}			
00-20	5.9	103.0	40	1.8	0.8	0.0	3.80	2.70	6.5
20-40	5.0	21.6	20	0.6	0.3	0.6	4.62	0.95	5.57

Prof	T	V	m	MO	Zn	Fe	Mn	Cu	B
cm	cmolc dm^{-3}	%		Dag kg^{-1}	mg dm-^{3}				
00-20	2.70	42	0	1.8	2.5	75.6	9.7	1.1	0.4
20-40	1.55	17	39	0.8	0.4	53.4	0.3	0.2	0.3

pH in H_2O (Ratio 1:2.5). P K, Fe, Zn, Mn, and Cu: Mehlich extractor. Ca, Mg and Al: KCl extractor. H+Al: Calcium acetate extractor. B: Hot water extractor, S: Monocalcium phosphate in acetic acid extractor.

temperature is 29ºC. The relief varies from flat to gently rolling.

The soil of the experimental area was classified as a dystrophic Yellow Oxisol (Embrapa, 2013) with a medium texture. Before installing the experiment, soil chemical analyses were performed. The chemical characterization was carried out on samples collected at layers 0.0-0.2 m and 0.2-0.4 m (Table 1). The correction of soil acidity was performed using dolomitic limestone at 150 kg ha^{-1} calculated by increasing base saturation to 60%, according to Oliveira et al. (2007). After the application of limestone, the soil was plowed and meshed. Then, grooves were opened. The planting density was 15-18 buds per furrow meter.

The experimental design was randomized blocks with five replications consisting of four varieties of sugarcane: SP813250, RB867515, RB92579 and VAT90212. They were grown in plots with six grooves and 10-m length, spaced 1 m, totaling 60 m^2 of total area. The four central lines, six meters long, totaling 24 m^2, were considered as the useful area of each plot. The varieties were chosen because the sum of their cultivated areas was greater than 50% of the area planted with sugarcane in the state of Alagoas during the 2010/2011 season, one year before implementing the experiment.

The soil fertilization was based on the recommendation by the Triunfo Plant according to the results of soil analysis (Table 1), 60, 100 and 150 kg ha^{-1} of N, P_2O_5 and K_2O were applied. The sources were ammonium sulfate, simple superphosphate and potassium chloride, respectively. They were applied at the bottom of the grooves. The plant-cane cycle was harvested after 14 months. After the harvest, the research continued on the first and second regrowth cycles, each cycle during 12 months. In these cycles, all treatments received 500 kg ha^{-1} of the formula 20-05-25.

The leaf analysis was performed at the maximum growth stage of the plants, eight months after planting the plant-cane cycle and six months after cutting the plant-cane and the first regrowth. For the evaluation of nutritional status, the +3 leaf was sampled. 20 leaves were randomly collected within the useful area of each plot. The sampled leaves were washed in deionized water. Then, the median third of the leaf blades, discarding the midrib, was separated for chemical analysis. Then, the samples were dried at 65°C in a forced-air circulation oven until constant mass and ground in a Wiley mill. The leaf blade was analyzed for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), following the methods described by Malavolta et al. (1997). The nitrogen was extracted by sulfuric acid digestion following the Kjeldahl method. The boron was extracted by dry digestion and determined by the muffle method. The other nutrients were extracted by nitric-perchloric digestion. The P was colorimetrically determined by the development of the blue color by reducing the phospho-molybdenum complex. K, Ca, Mg, Mn, Zn, Cu and Fe were determined by atomic absorption spectrophotometry and the S was obtained by turbidimetry of barium sulfate.

Statistical analyses were performed using the software Sisvar (Ferreira, 2011). The variables were subjected to analysis of variance by F test. For variables with a significant F, the means were compared by Scott Knott test at 5% probability.

RESULTS AND DISCUSSION

Tables 2, 3 and 4 show the mean squares of the analysis of variance and the mean test for nitrogen, phosphorus, potassium, calcium, magnesium and sulfur in the middle third of the +3 leaf of sugarcane varieties VAT90212, RB92579, RB867515 and SP813250 during the plant-cane, first and second regrowth cycles.

For leaf nitrogen contents, there was a significant difference by F test during the plant-cane and the first regrowth cycles (Table 2). The cane plant variety RB92579 showed a higher leaf content (15.16 g kg^{-1}). The other varieties did not show differences. Comparing the leaf N contents of the four varieties during the plant-cane cycle with those reported by Orlando Filho (1983), Malavolta et al. (1997) and Raij (2011), the values were below those considered as appropriate concentrations. During the first regrowth, the highest leaf contents (19.16 and 19.35 g kg^{-1}) were observed for the varieties RB92579 and SP813250, respectively. In this cycle, four varieties were at a suitable concentration according to the authors above.

During the second regrowth and similar to the plant-cane, the overall mean for leaf nitrogen content was below the appropriate as mentioned by Orlando Filho (1983), Malavolta et al. (1997) and Raij (2011). Oliveira et al. (2007) stated that the nutrient content in the limbo of the +3 leaf cannot be related to the quantity of

Table 2. Average macronutrient contents (N and P) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Nitrogen (g kg^{-1})				Phosphorus (g kg^{-1})			
	PC	FR	SR	Average	PC	FR	SR	Average
VAT90212	13.2^{b}	17.5^{b}	14.8	15.1	1.6^{b}	2.3^{b}	1.6^{b}	1.8
SP813250	13.7^{b}	19.3^{a}	14.2	15.7	1.8^{b}	2.5^{b}	1.5^{b}	1.9
RB867515	13.9^{b}	16.6^{b}	14.3	14.9	1.4^{a}	2.0^{a}	1.4^{a}	1.6
RB92579	15.1^{a}	19.1^{b}	15.4	16.5	1.4^{a}	1.9^{a}	1.4^{a}	1.6
Average	14.0	18.1	14.7	15.6	1.6	2.2	1.5	1.7
Source of variation	**DF**	**Mean squares**						
Varieties	3	3.36**	9.07**	1.56^{ns}	-	0.21**	0.39**	0.03**	-
Blocks	4	2.91	0.81	5.48	-	0.02	0.02	0.008	-
Residue	12	0.51	1.73	1.43	-	0.02	0.03	0.005	-
CV (%)		5.35	7.25	8.13	-	10.14	8.58	5.06	-

* and** - significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

Table 3. Average macronutrient contents (K and Ca) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Potassium (g kg^{-1})				Calcium (g kg^{-1})			
	PC	FR	SR	Average	PC	FR	SR	Average
VAT90212	9.7^{b}	8.8^{a}	8.0^{b}	8.8	3.0^{a}	4.6^{a}	3.3^{b}	3.6
SP813250	8.4^{a}	8.8^{a}	8.0^{b}	8.4	3.9^{b}	4.8^{a}	3.2^{a}	3.9
RB867515	8.7^{a}	8.9^{a}	7.0^{a}	8.2	3.4^{a}	4.5^{a}	3.5^{b}	3.8
RB92579	8.4^{a}	10.0^{a}	7.9^{b}	8.7	4.0^{b}	4.8^{a}	2.8^{a}	3.8
Average	8.8	9.1	7.7	8.5	3.6	4.7	3.2	3.8
Source of variation	**DF**	**Mean squares**						
Varieties	3	1.97*	1.58^{ns}	1.09*	-	1.00*	0.14^{ns}	0.42**	-
Blocks	4	2.61	1.63	1.17	-	0.32	0.29	0.25	-
Residue	12	0.58	0.92	0.40	-	0.14	0.23	0.04	-
C.V(%)	-	8.84	10.49	8.27	-	10.62	10.29	6.89	-

* and** - significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

accumulated nutrients in the shoot biomass. When the plant grows and consequently accumulates more dry matter, there is a dilution of such elements in the biomass, including leaves. This effect has been called "dilution effect."

In sugarcane reform areas, the application of liming and the soil preparation (plowing and harrowing) increase soil microbial activity and there is a greater mineralization rate of the soil organic matter (Ferreira et al., 2015), especially residues and rhizomes of the previous crop. Thus, the nitrate content in the soil solution increases. This increase, combined with a high phosphorus availability in the soil resultant from fertilization, results in an increased efficiency of nitrate uptake by plants (Oliveira et al., 2007; Isaac et al., 2011). Magalhães (1996) showed that the absorption and translocation of nitrogen for corn plants are greatly influenced by the phosphorus endogenous availability: plants with a higher phosphorous endogenous availability have a lower Km (Michaelis-Mentem constant) and a higher inflow rate (Malavolta et al., 1997; Oliveira et al., 2007).

The leaf P concentration was influenced by the varieties during the three crop cycles (Table 2). VAT90212 and SP813250, both during plant-cane and first and second

Table 4. Average macronutrient contents (Mg and S) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Magnesium (g kg^{-1})				Sulfur (g kg^{-1})			
	PC	FR	SR	Average	PC	FR	SR	Average
VAT90212	3.5^{b}	3.6^{b}	2.5^{c}	3.2	1.2^{a}	1.9^{b}	1.7^{a}	1.6
SP813250	3.4^{b}	3.3^{b}	1.9^{b}	2.8	1.5^{b}	1.7^{a}	1.5^{a}	1.5
RB867515	3.6^{b}	3.8^{b}	2.6^{c}	3.3	1.5^{b}	1.9^{b}	2.1^{b}	1.8
RB92579	2.4^{a}	2.1^{a}	1.4^{a}	1.9	1.2^{a}	1.6^{a}	1.4^{a}	1.4
Average	3.2	3.2	2.1	3.7	1.38	1.80	1.70	1.6

Source of variation	DF	Mean squares						
Varieties	3	1.62**	2.69**	1.52**		0.13*	0.08^{ns}	0.47**	1.62**
Blocks	4	0.08	0.15	0.15		0.01	0.008	0.054	0.08
Residue	12	0.11	0.17	0.04		0.04	0.02	0.029	0.11
C.V(%)		10.30	12.83	9.81		15.36	9.03	10.09	10.30

* and **Significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

regrowth, showed a leaf P greater than RB867515 and RB92579. Averaging each cycle of leaf P of VAT90212 and SP813250 and comparing them with an average of leaf P of RB867515 and RB92579, it is observed that, during the plant-cane and the first regrowth, the leaf P of VAT90212 and SP813250 was about 20%, but in the second regrowth it decreased to 10%. The contents of phosphorus in leaf, during the plant-cane cycle, are of the similar as observed by Vasconcelos et al.(2014).

Considering the high availability of P in the soil, which in the layer 0-20 cm had 103 mg dm^{-3} of phosphorus extracted with Mehlich 1, the plants, particularly during the plant-cane cycle, should also have a high leaf P content. However, both in plant-cane and the second regrowth, leaf contents were at the limit established by Raij (2011) but lower than values advocated by Orlando Filho (1983) and Malavolta et al. (1997).

In a study conducted at the Triunfo Plant with RB867515 during the first regrowth cycle in a soil with a high P content (average values above 30 mg dm^{-3} of phosphorus extracted by Mehlich), Oliveira et al. (2011) found leaf P values lower than 1.6 g kg^{-1}, characterizing, according to Malavolta et al. (1997) and Orlando Filho (1983), an inadequate supply of this element, however, the productivity of RB867515 was 166 tonnes of culms per hectare. Considering this and other observations of high-productive sugarcane fields and leaf P levels lower than 2.0 g kg^{-1}, Oliveira et al. (2011b) pondered whether the reference values set by Orlando Filho (1983) and Malavolta et al. (1997) would be suitable for the evaluation of the nutritional state of Alagoas sugarcane fields.

Significant differences were found by F test for leaf potassium contents during plant-cane and second regrowth cycles (Table 3). During the plant-cane cycle, the leaf potassium content for VAT 90212 was approximately 15% higher than other varieties. However, during the second regrowth, the K content in the +3 leaf of RB92579 was 13% lower than the others. In the three crop cycles, leaf contents were below the sufficiency range mentioned by Malavolta et al. (1997) and Raij (2011), but at appropriate concentrations when the values reported by Orlando Filho (1983) are taken as a reference. For this author, the minimum adequate content would be 6.0 g of K per kg of dry matter. Potassium contents in the +3 leaf, during the plant-cane cycle, are of the similar as mentioned by Oliveira et al. (2016).

There was a variety effect in leaf calcium contents during plant-cane and second regrowth cycles (Table 3). The leaf contents during the three cycles were below the proper range reported by Malavolta et al. (1997). For the first sugarcane regrowth, the leaf contents were in a range considered adequate by Orlando Filho (1983) and Raij (2011): 2.0-8.0 g of calcium per kg of dry matter in the +3 leaf. Since the soil received dolomitic lime in a quantity sufficient to increase the base saturation to 60% (Oliveira et al., 2007) and because of soil analyses conducted in each plot after the implementation of the experiment (data not shown), it was found that the saturation increased to 60% or more. The inadequate supply of calcium should not have occurred.

Calcium levels in the +3 leaf, during the plant-cane cycle, are of the same order of magnitude as mentioned by Faroni et al. (2009), Oliveira et al. (2011b) and Omollo et al., (2016). During the first regrowth, an average content of 4.72 g kg^{-1} was observed. It was lower than that obtained by Rozane et al. (2008), who obtained a content of 5.6 g kg^{-1} for the first harvest of SP791011. In

Table 5. Average micronutrient contents (Zn and Fe) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Zinc (g kg^{-1})				Iron (g kg^{-1})			
	PC	FR	SR	Average	PC	FR	SR	Average
VAT90212	15.2^{b}	14.0^{a}	15.0^{a}	14.7	45.4^{a}	56.6^{a}	47.2^{a}	49.7
SP813250	12.4^{a}	13.2^{a}	12.8^{a}	12.8	99.4^{a}	58.6^{a}	49. 0^{a}	69.0
RB867515	15.8^{b}	13.8^{a}	14.2^{a}	14.6	48.0^{a}	55.8^{a}	49.0^{a}	50.9
RB92579	13.8^{a}	12.6^{a}	12.6^{a}	13.0	46.2^{a}	62.2^{a}	55.4^{a}	54.6
Average	14.3	13.4	13.6	13.7	59.7	58.3	50.1	56.0

Source of variation	DF	Mean squares						
Varieties	3	11.53*	2.00^{ns}	6.58^{ns}	3499.51^{ns}	40.73^{ns}	64.85^{ns}	11.53*
Blocks	4	9.67	2.32	2.70	5202.25	131.42	67.07	9.67
Residue	12	2.57	0.62	8.66	3327.68	65.19	57.97	2.57
C.V(%)		11.22	5.90	21.57	96.55	13.93	15.18	11.22

* and **Significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

the second regrowth, leaf calcium contents were similar to those obtained by Prado and Pancelli (2008).

The magnesium contents in the +3 leaf differed among varieties during the three cycles (Table 4). The values are within the range considered adequate by Orlando Filho (1983). The average levels during plant-cane and first regrowth cycles, compared to values mentioned by Raij et al. (1996) and Malavolta et al. (1997), are above the indicated concentration range.

The RB92579 variety had the lowest concentrations during plant-cane, first and second regrowth cycles. During plant-cane, the results obtained were higher than those observed by Píperas et al. (2009). In the first regrowth, leaf contents were 28% higher than those observed by Mendes (2006). In the second regrowth, the average leaf Mg content was similar to that obtained by Prado and Pancelli (2008).

There were significant differences in plant-cane, first and second regrowth cycles regarding leaf sulfur contents (Table 4). The average levels in the three cycles were below the levels indicated by Malavola et al. (1997), but within the range recommended by Orlando Filho (1983). The leaf S content during plant-cane was similar to that observed by Faroni et al. (2009), but, during regrowth, the levels increased. A "dilution effect" may have occurred on the plant-cane.

The average levels of macronutrients in the middle third of the +3 leaf of the varieties VAT90212, RB92579, RB867515 and SP83250 during plant-cane, first and second regrowth cycles showed the following descending order of concentration: nitrogen, potassium, calcium, magnesium, phosphorus and sulfur, coinciding with that mentioned by Prado and Pancelli (2008) and Oliveira et al. (2011a). However, for the plant-cane cycle, there are reports of a potassium leaf content higher than nitrogen (Mendes, 2006).

Tables 5, 6 and 7 show the mean squares of the analysis of variance and the mean test for zinc, iron, manganese and boron in the middle third of the +3 leaf of sugarcane varieties VAT90212, RB92579, RB867515 and SP813250 during the plant-cane, first and second regrowth cycles.

During the plant-cane cycle, there was a significant difference only for leaf zinc levels (Table 5). During the first regrowth, there was a variety effect for copper, and during the second regrowth there were significant differences for manganese and boron. Based on the compilation by Oliveira et al. (2007), the lowest values of leaf micronutrients considered adequate are 6.0, 9.0, 40.0, 25.0 and 10.0 mg kg^{-1} for boron, copper, iron, manganese and zinc, respectively. Thus, the sugarcane varieties of this study, during the plant-cane cycle, would have an adequate supply of boron, iron and zinc (Tables 5 and 6). However, only the variety VAT90212 was not deficient in Mn (Table 7). In the first and the second regrowth cycles, there was a general micronutrient deficiency according to the reference values established by Malavolta et al. (1997).

Copper deficiency has been widespread in many sugarcane fields: from the northeast of Minas Gerais state to Rio Grande do Norte (Oliveira et al., 2011b). In the vast majority of these soils, the copper content extracted by Mehlich is lower than 0.80 mg dm^{-3}, a critical value according to Marinho and Albuquerque (1981) based on studies conducted in Alagoas.

The availability of micronutrients in the soil, estimated by chemical extractors, varied widely in terms of methods and extractors used for the chemical analysis of the soil.

Table 6. Average micronutrient contents (Cu and B) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Cooper (g kg^{-1})				Boron (g kg^{-1})			
	PC	FR	SR	Average	PC	FR	SR	Average
VAT90212	2.2^{a}	6.6^{b}	3.8^{a}	4.1	7.1^{a}	9.8^{a}	4.4^{b}	7.1
SP813250	2.2^{a}	7.4^{b}	4.4^{a}	4.6	7.1^{a}	9.4^{a}	3.4^{a}	6.6
RB867515	2.2^{a}	6.6^{b}	3.8^{a}	4.2	6.3^{a}	8.1^{a}	3.4^{a}	5.9
RB92579	1.8^{a}	5.8^{a}	4.0^{a}	3.8	7.3^{a}	9.8^{a}	3.4^{a}	6.8
Average	2.1	6.6	4.0	5.9	6.9	9.2	3.7	6.6

Source of variation	DF	Mean squares							
Varieties	3	0.20^{ns}	2.13*	0.400^{ns}	-	1.01^{ns}	3.19^{ns}	1.07*	0.20^{ns}
Blocks	4	0.70	0.57	0.250	-	1.18	64.34	1.42	0.70
Residue	12	0.36	0.34	0.316	-	0.45	3.90	0.18	0.36
C.V(%)	-	28.83	8.86	14.07	-	9.63	21.28	11.72	28.83

* and **Significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

Table 7. Average micronutrient contents (Mn) in the +3 leaf of sugarcane during the maximum growth phase of plant-cane (PC), first regrowth (FR) and second regrowth (SR) cycles.

Varieties	Manganese			
	PC	FR	SR	Average
VAT90212	28.0^{a}	38.6^{a}	28.0^{b}	31.53
SP813250	22.8^{a}	39.0^{a}	22.2^{a}	28.0
RB867515	22.6^{a}	31.8^{a}	29.6^{b}	28.0
RB92579	20.0^{a}	33.0^{a}	19.0^{a}	24
Media	23.3	35.6	24.7	27.8

Source of variation	DF	Mean squares			
Varieties	3	56.18^{ns}	69.60^{ns}	122.73**	-
Blocks	4	166.70	420.20	170.42	-
Residue	12	18.93	69.43	17.85	-
CV (%)	-	18.63	23.41	17.11	-

* and **Significant at 5 and 1% probability, respectively, by F test; ^{ns} = not significant at 1% probability. Means followed by the same letter do not differ statistically by Scott-Knott test at 5% probability.

analysis should be associated to the history of the area, especially to the record of their deficiency in previous crops (Oliveira et al., 2007, 2011b; Raij, 2011).

Conclusions

The varieties RB92579, RB867515, VAT90212 and SP813250 show differences in leaf contents of macro and micronutrients, but no variety has a higher content regarding any element.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Calheiros AS, Oliveira MW, Ferreira VM, Barbosa GVS, Santiago AD, Aristides EVS (2012). Produção de biomassa, de açúcar e de proteína em função de variedades de cana e de adubação fosfatada. Semin: Cien. Agrar. 32:809-818.

Conab (Companhia Nacional de Abastecimento) (2016). Acompanhamento da safra brasileira: cana-de-açúcar. Primeiro Levantamento. 66p.

Deus JAL, Barreto JHB, Soares I, Souza NCS, Sales JAF, Oliveira Filho, JS (2012). Mathematical chance in determination of nutritional status of peanut. Biosci. J. 28:115-123.

Embrapa (Empresa Brasileira de Pesquisa Agropecuária) (2013). Sistema Brasileiro de Classificação de Solos. Centro Nacional de Pesquisa de Solos: Rio de Janeiro. 353p.

Faroni CE, Trivelin PCO, Franco HCJ, Vitti AC, Otto R, Cantarella H (2009). Estado nutricional da cultura de cana-de-açúcar (cana-planta) em experimentos com 15N. Rev. Bras. Ci. Solo. 6:1919-1927.

Ferreira, DA, Franco, HC, Otto, R., Vitti, AC, Fortes, C., Faroni, CE, Garside, AL, Trivelin, PC (2015). Contribution of N from green harvest residues for sugarcane nutrition in Brazil. GCB Bioenergy. 8:859-866.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciênc. Agrotecnol. 35:1039-1042.

Isaac ME, Harmand JM, Drevon JJ (2011). Growth and nitrogen acquisition strategies of Acacia senegal seedlings under exponential phosphorus additions. J. Plant Physiol. 168:776-781.

Magalhães JV (1996). Absorção e translocação de nitrogênio por plantas de milho *(Zea mays, L.)* submetidas a períodos crescentes de omissão de fósforo na solução nutritiva. Viçosa, MG. 76p. Dissertação de mestrado em Solos e Nutrição de Plantas – Universidade Federal de Viçosa.

Malavolta E, Vitti GC, Oliveira SA (1997). Avaliação do estado nutricional das plantas: princípios e aplicações. 2.ed. Piracicaba: ABPPF 319 p.

Marin F, Nassif DS (2013). Mudanças climáticas e a cana-de-açúcar no Brasil: Fisiologia, conjuntura e cenário futuro. Rev. Bras. Eng. Agríc. Ambient. 12:232-239.

Marinho ML, Albuquerque GAC (1981). Efeito do cobre e do zinco na produção de cana-de-açúcar em solos de tabuleiros de Alagoas. Bras. Açuc. 98:41-50.

Meena BS, Ram B, Narolia, RS, Singh, P. (2015). Yield, quality, nutrient uptake and economics of spring sugarcane (*Saccharum officinarum*) as influenced by balanced fertilization in clay loam soils of Rajasthan. Indian J. Agron. 60:457-463.

Mendes LC (2006). Eficiência nutricional de cultivares de cana-de-açúcar. Viçosa, MG. 46 p. Dissertação de mestrado em Solos e Nutrição de Plantas – Universidade Federal de Viçosa.

Mishra AK, Shukla SK, Yadav DV, Awasthi SK (2014). Iron, manganese and sulphur uptake and nutrients availability in sugarcane based system in subtropical India. Sugar Tech. 16:300-310.

Oliveira ECA, Freire FJ, Oliveira RID, Freire MBGDS, Simões Neto, DE; Silva, SAMD (2010). Extração e exportação de nutrientes por variedades de cana-de-açúcar cultivadas sob irrigação plena. Rev. Bras. Cienc. Solo 4:343-1352.

Oliveira ECA, Freire JF, Oliveira RI, Oliveira AC, Freire MBGS (2011a). Acúmulo e alocação de nutrientes em cana-de-açúcar. Rev. Ciênc. Agron. 42:579-588.

Oliveira MW, Freire FM, Macêdo GAR, Ferreira JJ (2007). Nutrição mineral e adubação da cana-de-açúcar. Inf. Agropec. 28:30-43.

Oliveira MW, Magrini JL, Lyra, FEV, Valduga GR., Pereira MG, Tenorio CJM, Aristides EVS (2011b). Produção da RB867515 influenciada pela aplicação de substâncias húmicas, aminoácidos e extrato de algas marinhas. Stab. 30:30-33.

Oliveira RI, Medeiros MRFA, Freire CS, Freire FJ, Neto DES, Oliveira ECA (2016). Nutrient partitioning and nutritional requirement in sugarcane. Aus. J. Crop Sci. 10-69-75.

Omollo JO, Semu E, Msaky J, Owuor P (2016). Effects of Cropping Systems and Agricultural Lime on Soil Properties and Nutrient Content of Sugarcane on Acidified Soils of Kisumu County, Kenya. Am. J. Agric. Forest. 44:97-111.

Orlando Filho J (1983). Nutrição e adubação da cana-de-açúcar no Brasil. Instituto do Açúcar e do Álcool. Programa Nacional de Melhoramento da Cana-de-Açúcar. Planalsucar. 368 p.

Otto R, Vitti GC, Luz PHC (2010). Manejo da adubação potássica na cultura da cana-de-açúcar. Rev. Bras. Ci. Solo. 34:1137-1145.

Píperas GV, Creste JE, Eche FR (2009). Uso do DRIS na avaliação do estado nutricional da cana-de-açúcar. Ceres 56:556-563.

Prado RDM, Pancelli MA (2008). Resposta de soqueiras de cana-de-açúcar à aplicação de nitrogênio em sistema de colheita sem queima. Bragantia 67:951-959.

Raij BV (2011). Fertilidade do solo e manejo dos nutrientes. Piracicaba: International Plant Nutrition Institute.

Rozane DE, Prado RM, Romualdo LM, Pancelli AM, Franco C (2008). Amostragem de folha em cana-de-açúcar submetida à adubação nitrogenada. Rev. Agric. 83:8-18.

Santos EF, Donha RMA, Magno CM (2013). Faixas normais de nutrientes em cana-de-açúcar pelos métodos ChM, DRIS E CND e nível crítico pela distribuição normal reduzida. Rev. Bras. Cienc. Solo. 37:1651-1658.

Souza HA, Hernandes A, Romualdo LM, Rozane DE, Natale W, Barbosa JC (2011). Folha diagnóstica para avaliação do estado nutricional do feijoeiro. Rev. Bras. Eng. Agríc. Ambient. 15:1243-1250.

Vasconcelos RL, Prado RM, Campos CNS, Caione G, Almeida HJ, Moda LR, Mello FT, Dela Marta CC (2014). Sources of phosphorus with sugar cane filter cake on the nutritional status and productivity of sugar cane (*Saccharum officinarum L*) cultivated in red-yellow latosoil. Aus. J. Crop Sci. 8:1467-1474.

Zuanazzi JAS, Mayorga P (2010). Phytoproducts and economic development. Quím. Nova 33:1421-1428.

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Full Length Research Paper

Growth of *Piper nigrum* L. and nutrients cycling by intercropping with leguminous species

Evelyn Trevisan[1], Fábio Luiz Partelli[1], Marcos Góes de Oliveira[1], Fábio Ribeiro Pires[1] and Heder Braun[2]*

[1]Department Plant and Biology Science, University Center of North Espírito Santo, Federal University of Espírito Santo, São Mateus, Espírito Santo State, Brazil.
[2]Post-Graduate Program of Agroecology, Maranhão State University, Campus São Luís, São Luís, Maranhão State, Brazil.

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Nitrogen (N) fixation is performed efficiently by leguminous plants, which can then be used as 'green manure' to reduce the need for synthetic fertilizer (especially N) and thus sustainably provide satisfactory crop yields with low production costs. The aim of this study was to determine the effect of intercropping with leguminous species used as cover crops on branch growth and nutrient cycling of pepper plants. The experiment was carried out in an indoor system with a randomized block design with four replicates. The treatments consisted of seven leguminous plant species which were planted as cover crops for black pepper plants: *Canavalia ensiformis*, *Mucuna deeringiana*, *Mucuna pruriens*, *Cajanus cajan*, *Crotalaria juncea*, *Crotalaria spectabilis* and the uncovered control. Samples of leaves and branches were harvested from flowering leguminous plants to quantify the production of dry matter. In addition, macro- and micro-nutrients content was measured in the legumes. The growth of the main branch of the pepper plants was measured at 30 and 60 days after the leguminous cover crops were cut. The leguminous plants, *M. pruriens*, *C. ensiformis*, *C. cajan* and *C. juncea* provided higher accumulation of nutrients and dry matter production in the pepper plants. At 60 days after cutting, intercropping with *C. ensiformis* supported more growth of pepper plants as determined by main branch measurements.

Key words: Black pepper, cover crops, soil fertility management, soil conservation.

INTRODUCTION

The black pepper (*Piper nigrum* L.), belonging to the family, Piperaceae, is a perennial plant of Indian origin which is cultivated in many tropical regions. Due to its peculiar taste, this plant is commonly used for food flavoring, preparation and food processing, characterizing it as an important spice which is sold worldwide (Hussain et al., 2011; Nair, 2011).

Among black pepper-producing countries, Brazil stands out as the third largest producer of this crop, with more than 40 thousand tons produced annually. In the Brazilian

*Corresponding author. E-mail: hederbraun@gmail.com.

states of Para and Espírito Santo, 79 and 13% of agricultural production is represented by black pepper cultivation, respectively. Espírito Santo Farmers have better access to technologies such as irrigation systems in approximately 80% of the area planted with this crop, which has led to higher yields as compared to Para (Partelli, 2009). In addition to these technologies, these farmers use legumes as a source of nutrients for the cultivated plants to ensure greater agricultural sustainability.

The impact of conventional agriculture on the environment is easily perceived, compromising the sustainability of agricultural activity. Sustainable agriculture seeks to combine aspects of socio-cultural, economic and environmental research in order to implement conservative agricultural practices, such as the use of 'green manure' as opposed to industrial soil additives (nitrogen-N fertilizer), to promote the sustainability of ecosystems. A common form of 'green manure' is provided by the cultivation of legumes alongside food crops, which promotes nutrient cycling, provides biological fixation of atmospheric N (Partelli et al., 2011; Moda et al., 2014), favors the density and diversity of edaphic microorganisms (Ferreira et al., 2010), improves soil structure (Cunha et al., 2010) and can be an efficient strategy to maintain productivity in the low-fertility soils of the humid tropical (Moura et al., 2010; Aguiar et al., 2010) as compared to fertilization with industrially produced mineral N only.

The effectiveness of green manure provided by legumes is superior to any other group of plants, because of the specific symbiotic associations that legumes form with N fixing bacteria, which result in a high N concentration in the plant (Partelli et al., 2011; Souza et al., 2015). Besides providing N, leguminous plants may enhance the quality of soil organic matter, and consequently the ability to exchange cations (Partelli et al., 2011).

While it is well established that soil fertility can be improved by substituting mineral N for naturally occurring N_2 fixers, resulting in satisfactory yield with relatively low production costs, there are limited studies available in the literature describing the use of leguminous intercrops as green manure to promote the growth of pepper plants. Given this, the present work aimed to establish the usefulness of leguminous intercrops in pepper plants by quantifying the growth of the branches of black pepper plants and nutrient cycling provided by legumes.

MATERIALS AND METHODS

The experiment was conducted in smallholder farmer's field which is located in the municipality of Jaguare, Espírito Santo-Brazil (18° 54' 20" S, 40° 04' 34" W and altitude of 80 m) in Oxisol dystrophic soil (Santos et al., 2013) of sandy-clay texture. The climate is classified as Aw according to the Köppen climate classification and is tropical with a dry winter and a rainy summer (Alvares et al., 2013).

The experiment was conducted in black pepper plants planted in a randomized block design with four replicates, consisting of seven treatments. Each treatment block was planted alongside a different species of legume to be used for green manure: *Canavalia ensiformis* (jack bean), *Mucuna deeringiana* (mucuna dwarf), *Mucuna pruriens* (velvet bean), *Cajanus cajan* (pigeon pea), *Crotalaria juncea*, *Crotalaria spectabilis* and control (without green manure).

The black pepper plants were planted with 3.0 m between rows and 1.8 m between plants, under a sprinkler irrigation system. Each experimental unit consisted of two rows of seven plants, totaling 75.6 m². Within each experiment unit, the leguminous plants were planted in pits by hand, in January 2012. Two rows of leguminous plants were planted, with 0.4 m between plants and 0.5 m between rows, one on each side of the rows of pepper plants. On average, four legume seeds per hole were planted.

In order to measure the dry biomass production of the various leguminous plants, about 300 g of fresh biomass consisting of branches and leaves were collected from each experimental unit (one or two plants, depending on the species) at 70 days after sowing, when the plants were flowering. These were obtained from the four central meters of each row in each experimental unit, comprising an area of 24 m². Subsequently, these plants were dried in an oven with forced air ventilation at 65°C, until they reached a constant mass. After drying, the plant material was weighed on an analytical balance. Immediately after weighing, the plant material was ground in a Wiley mill and a subsample of ground plant material was digested in concentrated sulfuric acid. These samples were analyzed for macronutrient content according to the methodology described by Silva (2009). The nutrient content of the aerial parts of these leguminous plants was obtained by multiplying the weight of dry matter by the nutrient content measured in that dry matter.

In order to assess the effectiveness of each leguminous plant species as green manure, the vegetative growth of the apical branches of the pepper plants was measured at 30 and 60 days after cutting the leguminous plants (DAC). The distribution of the data was assessed using Lilliefors test, and transformed using the function $y = \text{Log}(x+10)$. Subsequently, a one way analysis of variance (ANOVA) was performed on the transformed data. Duncan test ($P \leq 0.05$) was used to compare the treatment means of all available variables. All statistical analyses were performed with the statistical software ASSISTAT 7.7 beta (Silva and Azevedo, 2006).

RESULTS AND DISCUSSION

In this study, the effect of using green manure generated by leguminous intercropping for six species of legumes was determined for black pepper plants by measuring the nutrient content of the legumes as well as the growth of the pepper plants. The legumes, *Mucuna* and *C. ensiformis* generated higher average dry biomass, which did not differ significantly from the average of legumes *C. cajan* and *C. juncea* (Table 1). This is similar to the findings of Partelli et al. (2011), who studied the cycling of nutrients in coffee plants. According to Partelli et al. (2011), this increase in biomass production by these legume species as compared to other leguminous plants studied, can be attributed to their generally increased seasonal growth.

It is known that dry matter yield in these species is lower in soil without supplementation by mineral fertilizer and without liming, due to a greater need for soil fertility

Table 1. Dry matter production of leguminous plant grown alongside with pepper plants.

Treatments	Dry matter (kg ha^{-1})
Mucuna pruriens	2949.67^{a}
Canavalia ensiformis	2301.96^{a}
Cajanus cajan	2231.67^{ab}
Crotalaria juncea	1387.64^{ab}
Crotalaria spectabilis	567.73^{bc}
Mucuna deeringiana	522.90^{c}
CV[1] (%)	8.35

Means followed by the same letters in the same column do not differ by Duncan test (p>0.05). [1]Coefficient of variation.

for these species (Nyatsanga and Pierre, 1973; Howard and Rees, 1996). For this study, the low dry biomass production demonstrated by these legumes may indicate that the soil fertility under study conditions may have been insufficient. This shows that the choice of cover crops must be adapted to the local climate and soil conditions in cases where high biomass production capacity is required. Higher amount of macro/micro-nutrients was measured in *M. pruriens* and *C. ensiformis* followed by *C. cajan* and *C. juncea* (Table 2), probably because these species present the greatest bio-matter production index (Table 1) and ability to adapt to the study climate.

Similar results were reported by Silva et al. (2014) when studying the effect of straw, nutrient levels and soil cover for plant coverage seeded in summer for directly sowed beans. These authors observed that the greatest accumulation of N, P, K, Ca, and S was obtained in *M. pruriens*, and attributed the results to higher biomass of the shoots produced by this crop. The accumulation of nutrients depends on the species used, the phenological stage, the dry matter production of shoots and the cultivation period (Partelli et al., 2011).

Studies revealed that the capacity to accumulate nutrients in the tissues of legumes contributes to the ability of these species to recycle nutrients from the soil and atmosphere, and later (after cutting and decomposition) make them available to plants in 'green manure' (Oliveira et al., 2002). These desirable characteristics are augmented by the beneficial role of cover plants to the soil, where they reduce the flow of water loss at the surface and reduce soil erosion and the loss of nutrients, increase the amount of available organic matter, and provide improved nutrient bio-availability for plants (Partelli et al., 2011; Souza et al., 2015).

The ability of the cover crops used in this study to absorb nutrients and immobilize them followed a similar trend regarding the production of dry biomass. The most efficient legume for green manure would ideally be efficient both at accumulating and absorbing nutrients and producing the most biomass. Another aspect to consider is the environmental contribution of intercropping afforded by soil protection, which reduces losses by processes such as leaching, due to better soil structure, and also reduces the entrainment of particles by erosion and others that promote loss in quality soils (Chaves et al., 1997).

Intercropping with the leguminous plants *C. ensiformis*, *C. juncea* and *C. cajan* provided the highest average growth of pepper branches at 30 days after cutting (Table 3). At 60 days after cutting, the pepper plants grown alongside the species *C. ensiformis* had the highest average growth of apical branches. These results indicate that the growth of pepper plants is improved with complementary fertilization arising from the decomposition of plant material from intercropped leguminous plants. This improvement may be attributed to the greater dry matter production (Table 1) and accumulation of macronutrients measured for *C. ensiformis* (Table 2) because of their proven ability to fix nitrogen in the soil and their contribution to integral agricultural production systems, which is responsible for growth and development of pepper plant. According to Tabaldi et al. (2012), leguminous plants, *C. ensiformis* are adapted to conditions of low fertility and high temperatures, have the fast growing ability, are capable of producing large amounts of biomass in a short period of time, and are capable of uptake nutrients from deeper layers, ensuring a large residual effect for the culture.

At 30 and 60 days after cutting, the species, *M. deeringiana and M. pruriens* did not differ from the control treatment. These results indicate that these two species may provide inadequate green manure for pepper plants; however, these species could still be used as a cover crop to maintain soil moisture. The choice of legume species for cover crop is related to the quantity and quality of material produced by the legume, with the amounts of nutrients released from the waste during the decomposition process required to meet the needs of subsequent cultures (Mendonça and Stott, 2003). The other species may be referred to as high quality, or important for nutrient cycling, in this regard. Still, these results indicate the phytotechnic benefit of implementing a consortium of plants grown alongside legume species.

The species, *M. pruriens* produced the highest average dry biomass and the highest accumulation of macro and micronutrients measured. On the other hand, this species apparently contributed the least to the growth of nearby pepper plants. This may be because this species is a natural climber which somehow entangled the branches of the pepper plants, resulting in reduction in vegetative growth. For this reason, *M. pruriens* is not indicated for intercropping with pepper plants, unless it is plowed under before it begins to climb.

Conclusions

Leguminous *M. pruriens*, *C. ensiformis*, *C. cajan* and *C.*

Table 2. Accumulation of macronutrients and micronutrients in shoots of leguminous grown alongside with pepper plants.

Treatments	Macronutrients (kg ha^{-1})					
	N	P	K	Ca	Mg	S
Canavalia ensiformis	92.65^{a}	5.66^{ab}	43.16^{ab}	48.87^{a}	6.90^{a}	5.59^{a}
Mucuna pruriens	85.27^{a}	6.87^{a}	64.54^{a}	38.67^{ab}	6.46^{a}	5.81^{a}
Cajanus cajan	61.70^{ab}	4.77^{ab}	33.47^{b}c	23.41^{b}c	4.32^{ab}	3.72^{ab}
Crotalaria juncea	38.46^{ab}	2.31^{ab}	19.94^{b}c	14.21c	3.30^{ab}	1.91^{b}
Mucuna deeringiana	21.33^{b}	1.39^{ab}	9.15c	7.80c	1.34^{b}	1.11^{b}
Crotalaria spectabilis	18.95^{b}	0.82^{b}	9.93c	8.37c	1.24^{b}	1.12^{b}
CV[1] (%)	12.11	8.35	11.71	11.28	6.17	5.56

	Micronutrients (g ha^{-1})				
	Fe	Zn	Cu	Mn	B
Canavalia ensiformis	250.9^{b}	85.2^{b}	11.5^{ab}	200.3^{a}	59.9^{ab}
Mucuna pruriens	436.6^{a}	150.4^{a}	23.6^{a}	209.4^{a}	88.5^{a}
Cajanus cajan	183.0^{b}c	75.9^{b}	11.2^{ab}	93.7^{b}c	64.7^{ab}
Crotalaria juncea	99.9^{b}c	73.5^{b}	5.6^{b}	144.3^{ab}	43.7^{b}
Mucuna deeringiana	55.4c	37.1^{b}	5.2^{b}	79.5^{b}c	20.4^{b}
Crotalaria spectabilis	43.7c	26.7^{b}	2.8^{b}	31.8c	16.5^{b}
CV (%)	0.44	0.18	0.6	0.27	0.14

Means followed by the same letters in the same column do not differ by Duncan test (p>0.05). [1]Coefficient of variation.

Table 3. Vegetative growth (cm) of the main branches of pepper plans, 30 and 60 days after cutting the leguminous plants.

Treatments	Branches growth (cm)	
	30 Days	60 Days
Control	9.2^{b}	12.9^{e}
Canavalia ensiformis	16.8^{a}	22.4^{a}
Crotalaria juncea	14.9^{a}	18.9^{b}
Cajanus cajan	14.4^{a}	18.0^{bc}
Crotalaria spectabilis	11.4^{b}	15.8^{cd}
Mucuna deeringiana	11.6^{b}	14.1^{de}
Mucuna pruriens	10.9^{b}	13.0^{e}
CV[1] (%)	18.93	13.43

Means followed by the same letters in the same column do not differ by Duncan test (p>0.05). [1]Coefficient of variation.

juncea had the highest measured accumulation of nutrients and dry biomass production among the legume species considered in this study. Of these, *C. ensiformis* apparently contributes most to the growth of black pepper plants at 60 days after cutting.

Conflict of interest

The authors did not declare any conflict of interest.

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REFERENCES

Aguiar ACF, Bicudo SJ, Sobrinho JRSC, Martins ALS, Coelho KP, Moura EG (2010). Nutrient recycling and physical indicators of an alley cropping system in a sandy loam soil in the pre-Amazon region of Brazil. Nutr. Cycl. Agroecosyst. 86(2):189-198.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013). Koppen's climate classification map for Brazil. Meteorol. Z. 22(6):711-728.

Chaves JCD, Pavan MA, Calegari A (1997). Adição de matéria seca da parte aérea e nutrientes através da utilização de plantas para cobertura em culturas perenes e seus efeitos sobre a reação do solo. Braz. Arch. Biol. Techn. 40(1):47-55.

Cunha EQ, Stone LF, Moreira JAA, Ferreira EPB, Didonet AD (2010). Atributos físicos do solo sob diferentes preparos e coberturas influenciados pela distribuição de poros. Rev. Bras. Eng. Agríc. Ambient. 14(11):1160-1169.

Ferreira EPB, Santos HP, Costa JR, De-Polli H, Rumjanek NG (2010). Microbial soil quality indicators under different crop rotations and tillage management. Rev. Ciênc. Agron. 41(2):177-183.

Howard JB, Rees DC (1996). Structural basis of biological nitrogen fixation. Chemistry 96(7):2965-2982.
Hussain A, Naz S, Nazir H, Shinwari ZK (2011). Tissue culture of black pepper (*Piper nigrum* L.) in Pakistan. Pakistan J. Bot. 43(2):1069-1078.
Mendonça ES, Stott DE (2003). Characteristics and decomposition rates of pruning residues from a shaded coffee system in Southeastern Brazil. Agrofor. Syst. 57(2):117-125.
Moda LR, Santos CLR, Flores RA, Borges BMM, Andrioli I, Prado RM (2014). Resposta do milho cultivado em sistema de plantio direto à aplicação de doses de nitrogênio e cultivo de plantas de cobertura em pré-safra. Biosci. J. 30(1):178-187.
Moura EG, Serpa SS, Santos JGD, Sobrinho JRSC, Aguiar ACF (2010). Nutrient use efficiency in alley cropping systems in the Amazonian periphery. Plant Soil. 335:363-371.
Nyatsanga T, Pierre WH (1973). Effect on nitrogen fixation by legumes on soil acidity. Agron. J. 65:936-940.
Oliveira ALM, Urquiaga S, Dobereiner J, Baldani JI (2002). The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. Plant Soil. 242:205-215.
Partelli FL (2009). Nutrition of black pepper (*Piper nigrun* L.) - a Brazilian experience. J. Spices Aromatic Crops 18(2):73-83.
Partelli FL, Vieira HD, Ferreira EPB, Viana AP, Espindola JAA, Urquiaga S, Boddey RM (2011). Biologic dinitrogen fixation and nutrient cycling in cover crops and their effect on organic Conilon coffee. Semina: Cienc. Agrar. 32(3):995-1006.
Prabhakaran NKP (2011). Agronomy and economy of black pepper and cardamom. The “King” and “Queen” of Spices. London, Elsevier Science Publishing, 366 p.
Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha TJF, Oliveira JB (2013). Sistema Brasileira de classificação do solo. 3rd ed. Brasília: Embrapa Solos, 353 p.
Silva FAS, Azevedo CAV (2006). A new version of the Assistat - Statistical Assistance Software. In: World Congress on Computers in Agriculture, 4, Orlando. Proceedings... Reno, Am. Soc. Agric. Biol. Eng., pp. 393-396.
Silva FC (2009). Manual de análise química de solos, plantas e fertilizantes. 2ª.ed. Brasília, DF: Embrapa.
Silva MP, Arf O, Sá ME, Abrantes FL, Bertin CLF, Souza LCD, Arruda N (2014). Palhada, teores de nutrientes e cobertura do solo por plantas de cobertura semeadas no verão para semeadura direta de feijão. Rev. Agrarian. 7(24):233-243.
Souza JL, Guimarães GP, Favarato LF (2015). Desenvolvimento de hortaliças e atributos do solo com adubação verde e compostos orgânicos sob níveis de N. Hortic. Bras. 33(1):19-26.
Tabaldi LA, Vieira MC , Zárate NAH, Silva LR, Gonçalves WLF, Pilecco M, Formagio ASN, RP Gassi, Padovan MP (2012). Cover crops and their effects on the biomass yield of *Serjania marginata* plants. Cienc. Rural 42(4):614-620.

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Growth comparison of 22 genotypes of conilon coffee after regular pruning cycle

José Maria Dalcomo[1], Henrique Duarte Vieira[2]*, Adésio Ferreira[3] and Fábio Luiz Partelli[4]

[1]Instituto Federal do Espírito Santo, Campus Alegre, ES, Brazil.
[2]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Campos dos Goytacazes, RJ, Brazil.
[3]Departamento de Produção Vegetal, Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alegre, ES, Brazil.
[4]Departamento de Ciências Agrárias e Biológicas, Universidade Federal do Espírito Santo,Centro Universitário Norte do Espírito Santo, São Mateus, ES, Brazil.

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A regular pruning cycle (RPC) reduces costs when implementing crop treatments and facilitates semi-mechanized harvesting. However, there is information on plant development under this system. Thus, this study aimed to assess the new branch growth of 22 genotypes of *Coffea canephora* (conilon) after a regular pruning cycle. The assay was conducted at Cachoeiro de Itapemirim, Southern Espírito Santo State, Brazil. Measurements of 12 morphoagronomic traits were performed, and their relationships with temperature and rainfall were studied. The branch vegetative growth rates varied seasonally throughout the assessment period, with higher growth rates and higher temperatures, even not extreme, during rainy periods and lower growth rates and milder temperatures during the dry season. The logistic model with best fit to describe pattern of cumulative growth by genotypes following RPC was sigmoid for all the studied traits.

Key words: *Coffea canephora*, growth curve, seasonality, temperature.

INTRODUCTION

Coffee stands out as a worldwide product of great importance and is cultivated in more than 80 countries, with approximately 143.4 million of 60-kg bags production in 2015. Approximately, 35% of total production was *Coffea canephora* (conilon or robusta) (ICO, 2016). Brazil is the largest coffee producer and exporter, having harvested 43.24 million bags in 2015 on 1.99 thousand hectares with the planted species *C. arabica* (Arabica) and *C. canephora*. Conilon coffee accounts for approximately 26% of coffee produced in country and has been used for many years in mixtures with Arabica coffee to enhance flavour of drink and to prepare soluble coffee (CONAB, 2016).

The conilon coffee tree is characterized by

*Corresponding author. E-mail: vieira.henrique101@gmail.com.

reproduction through allogamy (cross- fertilization). Therefore, use of asexual propagation becomes necessary to obtain productive varieties with a defined maturation cycle (Bragança et al., 2001). Furthermore, productivity of plants propagated by cutting is higher than that of plants propagated by grafting (Partelli et al., 2014). Conilon coffee varieties are usually polyclonal, formed by sets of genotypes (Barbosa et al., 2014; Leroy et al., 2014; Dalcolmo et al., 2015), usually grouped according to their maturation cycle, which varies from early to late (Fonseca et al., 2004).

A coffee plantation should have a canopy architecture that promotes optimal gas exchanges to be effective (DaMatta et al., 2007). Coffee plant growth may be affected by environmental stresses common in agricultural systems (Batista-Santos et al., 2011; Partelli et al., 2014; Ramalho et al., 2014; Rodrigues et al., 2016). There are numerous biotic and abiotic factors responsible for growth changes, which may act directly or indirectly and usually act jointly. Therefore, it is difficult to identify primary factor influencing a given growth pattern (Ivoglo et al., 2008). Furthermore, various genotypes and several parts of same plant may have different responses, growing at different rates and in different seasons (Pereira et al., 2007).

The vegetative growth of *C. arabica* coffee tree branches is noticeably seasonal in latitudes where Brazilian coffee production is concentrated, with maximum growth coinciding with rainy seasons with hot and long days, and reduced growth coinciding with dry seasons with cold days and short photoperiods (Barros et al., 1997; Silva et al., 2004; Amaral et al., 2006; Ferreira et al., 2013). Growth seasonality would be associated with fluctuations in minimum air temperature, lower than 14°C (Amaral et al., 2006).

Seasonality is a variable phenomenon affecting species and variety of *C. canephora.* Growth-rate reductions begin when field temperatures are lower than 17°C (Partelli et al., 2010) for most genotypes (Partelli et al., 2013). This pattern is explained by conilon coffee evolution, which occurred under ecological conditions of African lowlands (Davis et al., 2011). Low temperature affects several components of conilon coffee photosynthetic mechanism, reducing stomatal conductance, net photosynthesis, photochemical efficiency of photosystem II, thylakoid electron transport and enzymatic activity, lipid classes and fatty acids, even changing composition and structure of complexes of photosynthetic pigments (Partelli et al., 2009; 2011; Batista-Santos et al., 2011; Scotti-Campos et al., 2014).

The growth rates of conilon coffee plants decline with age (Bragança et al., 2015). Therefore, pruning and thinning are routine practices among conilon farmers, who use these practices as instruments of rejuvenation and maintenance of crop productivity (Pereira et al., 2007). A regular pruning cycle (RPC), wherein crop shoots are completely renewed every four or five years, is currently an option. Pruning is also aimed to promote and reduce costs of implementing crop treatments and semi-mechanized harvesting.

Understanding vegetative growth is a key tool both for assessing physiological status of plants and in crop-management practices. That's why this study aimed to examine growth, assessing 12 morphoagronomic traits in 22 genotypes of *C. canephora* and relating these traits to climate conditions temperature and rainfall conditions.

MATERIALS AND METHODS

The study was conducted in Cachoeiro de Itapemirim municipality, Espírito Santo state, Brazil. The experiment was planted in June 2005, in a randomized block design, with 55 treatments (genotypes of *C. canephora* var. Conilon, belonging to breeding programme of Capixaba Institute of Research, Technical Assistance and Rural Extension (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, INCAPER – Espírito Santo) and four replicates. Each plot consisted of one row with five plants, spaced 3.0 x 1.2 m, and second, and fourth plants were considered the useful plot. The methods used were same as those recommended by the INCAPER for cash crops, with increased supplemental irrigation.

A total of 51 clonal genotypes resulting from phenotypic selection of mother plants from farms in Castelo region, Espírito Santo state - ES (termed Castelo Assessment [Avaliação Castelo – AC]), three clonal genotypes of cultivar INCAPER 8142 (conilon Vitória) and one clonal genotype of open-pollinated cultivar EMCAPER 8151 (Robusta Tropical), all belonging to breeding programme of INCAPER, ES, were studied. All plants were submitted to a regular pruning cycle (RPC), maintaining two of five orthotropic stems of plants and removing plagiotropic branches that had produced grains in more than 50% of their rosettes, after completing fourth harvest (September 2010).

A total of 22 genotypes were selected from the original experiment to study growth: the 18 most promising clones from the group Avaliação Castelo (AC02, AC03, AC12, AC13, AC22, AC24, AC26, AC27, AC28, AC29, AC30, AC35, AC36, AC37, AC39, AC40, AC43 and AC46), in addition to three genotypes of cultivar conilon Vitória (12V - early, 02V - medium and 13V - late) and cultivar robusta tropical (RT), based on assessments conducted in years 2006, 2007, 2008, 2009 and 2010 (four harvests), regarding productivity criteria, production stability, maturation uniformity, grain size, vigour and rust tolerance. The following traits were assessed in branches grown after RPC during 2010/2011 crop year:

1) Number of new orthotropic branches per plant (OBN), assessed by direct count of new branches with length equal to or higher than 10 cm. Five new branches were kept in each plant to originate new crown, detaching others;
2) Dry matter of removed orthotropic branches (ODM), assessed by weighing following drying in an oven at 65°C for 72 h;
3) Length of new orthotropic branches (OBL), assessed measuring distance between insertions of five new branches with old and their apical meristems (cm);
4) Diameter of new orthotropic branches (OBD), using a standard measurement in central region of second internode of each of five branches (mm);
5) Number of orthotropic nodes (ONN), assessed by direct count in each of five branches;
6) Number of new plagiotropic branches (PBN), assessed by direct count in each of five new orthotropic branches;
7) Length of new plagiotropic branches (PBL), measured in two branches selected per plant, one on each side of crop row and

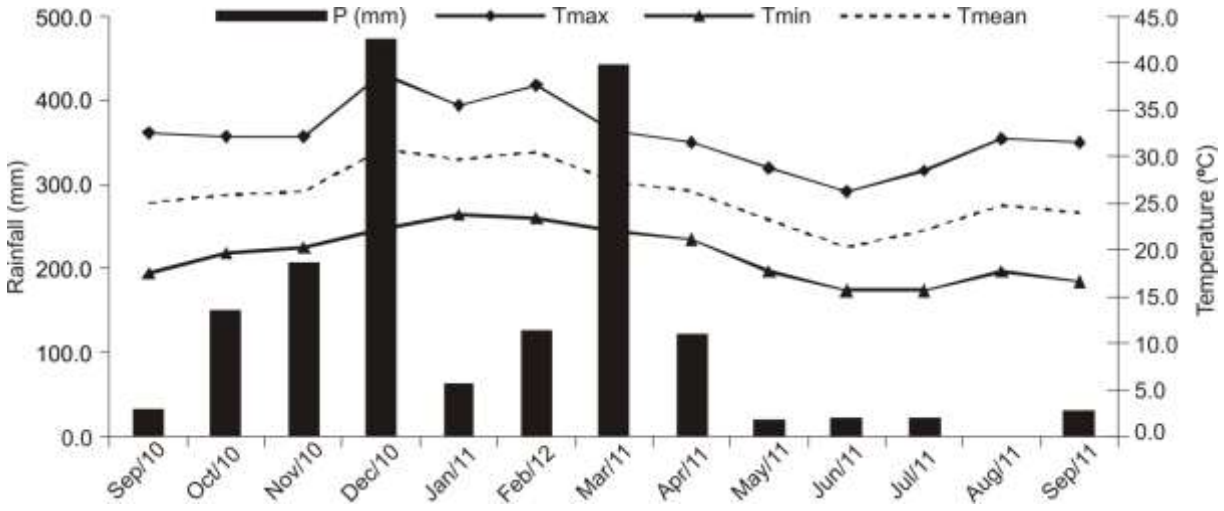

Figure 1. Maximum (Tmax), mean (Tmean) and minimum (Tmin) air temperatures and rainfall (mm) recorded in September 2010 to September 2011 period in the experimental area, Cachoeiro de Itapemirim, Espírito Santo state, Brazil.

calculated measuring to the distance between insertion of those branches in orthotropic branch and their apical meristem (cm);
8) Number of plagiotropic nodes (PNN), assessed by direct count in selected branches;
9) Number of leaves emerged in plagiotropic branches (PLN), directly assessed in the selected branches mentioned in item 7;
10) Maximum crown diameter (MCD), measured in transverse direction to crop row, with ends of longest branches as limits (cm);
11) Orthotropic internode length (OIL), calculated by ratio between monthly growth of each orthotropic branch and number of orthotropic nodes that emerged in a month (ONN; cm);
12) Plagiotropic internode length (PIL), calculated by ratio between monthly growth of plagiotropic branches and number of nodes that emerged in a month (PNN) in both of selected branches (cm).

The monthly growth rates were assessed for all traits evaluated, calculated by the difference between values recorded in current and previous months, divided by number of days between assessments. The cumulative growth was also assessed for traits OBN, ODM, OBL, OBD, ONN, PBN, PBL, PNN, PLN and MCD, calculated by difference between values recorded in the current month and at beginning of the experiment.

Supplemental irrigation was performed through sprinkling, applying one 25-mm rainfall simulation per irrigation, two in year 2010 (09/20 and 10/13) and five in year 2011 (02/08; 06/03; 08/29; 08/30 and 09/06). Rainfall was assessed using a pluviometer installed on experimental area, and temperatures were assessed through readings on a digital thermometer of brand E 7427 (CALARM; Figure 1).

The analysis of variance (P≥0.01) was performed using free software programming language R (R Foundation for Statistical Computing, 2012) and monthly cumulative growth averages for 10 traits during year. The scheme of plots subdivided in time with genotypes in plot and assessment seasons in subplots was used for this analysis. Regression analyses were subsequently performed per trait for worst- and best-performing genotypes and overall cumulative average (OCA) of 22 genotypes.

The overall average of 22 genotypes (AGT_X) per trait and season assessed was calculated based on daily growth rates, and values were used to design a graph showing variation in growth rate of each trait regarding assessment season.

RESULTS AND DISCUSSION

The emergence of new branches in plants submitted to an RPC occurred rapidly, which enabled first assessment 30 days after pruning (10/29/2010). September is a season when conilon coffee plant naturally resumes its growth in southern region of Espírito Santo State, according to Amaral et al. (2007) and Libardi et al. (1998), as well as corroborating data from northern regions of States of Rio de Janeiro (Partelli et al., 2010) and Espírito Santo (Partelli et al., 2013). Growth resumption was also found in that period in the present study, given favourable climate conditions (Figure 1). This pattern was further strengthened by breaking apical dominance and changing source-sink relationship through pruning, by increase in minimum air temperature in late September, by supplying supplemental irrigation on 09/20 and by return of rainy season on 09/28, among other contributing factors.

Set of 22 genotypes of conilon coffee for traits OBN, ODM, OBL, OBD, ONN, PBN, PBL, PNN, PLN and MCD are shown in Figure 2. The graphs representing the cumulative monthly growths are the period from 09/20/2010 to 09/20/2011. The logistic models describing a sigmoid pattern were best fitted to represent growth form after RPC for all the traits

The pattern obtained was notably similar to that reported by Bragança et al. (2015), who measured conilon coffee plant growth until sixth year of age. The authors explained that woody plants, such as coffee, should show an initial dry weight accumulation of organs and/or tissues typical of a linear model of growth.

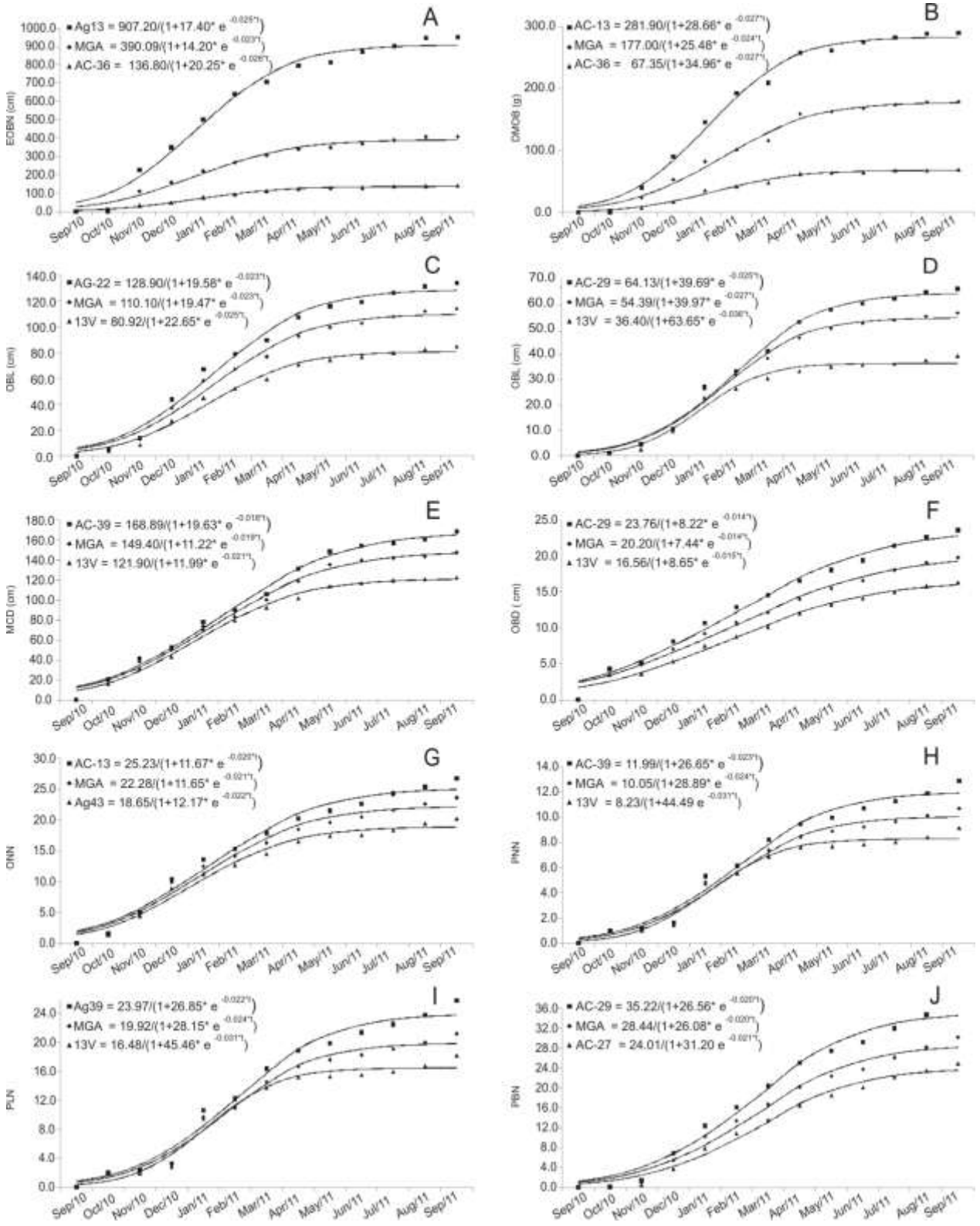

Figure 2. Measured values and regression curves fitted to describe the cumulative growth pattern of the worst- and best-performing genotypes and of the overall cumulative average (OCA) of the 22 genotypes for the following traits: A) emerged orthotropic branch number (OBN); B) dry matter of orthotropic branches (ODM; g); C) orthotropic branch length (OBL; cm); D) plagiotropic branch length (PBL; cm); E) maximum crown diameter (MCD; cm); F) orthotropic branch diameter (OBD; mm); G) orthotropic node number (ONN); H) plagiotropic node number (PNN); I) plagiotropic leaf number (PLN); J) plagiotropic branch number (PBN).

However, internal control mechanisms modify that relationship throughout their growth, and most appropriate way to describe growth becomes a sigmoid curve.

The genotype AC-36 (136.58) grew lowest number of orthotropic branches (OBN) at end of thr period assessed. AC-13 (905.10) was genetic material that grew better (OBN). The average OBN was 389.58 new branches (Figure 2A). Those orthotropic branches weighed on average, 176.19 g after being removed and dried (ODM), ranging from 67.24 (AC-37) to 281.45 g (AC-28; Figure 2B). The five orthotropic branches remaining in plant, intended for new crown formation, had a height of 109.58 cm (OBL). The highest genotype measured 128.26 cm (AC-22), and shortest was 80.71 cm (13V; Figure 2C). The selected plagiotropic branches had an average length of 54.26 cm (PBL), ranging from 36.39 (13V) to 63.84 (AC-29; Figure 2D). The tallest genotype (AC-39) had a maximum crown diameter (MCD) of 165.94 cm, smallest diameter was 121.28 cm (13V), and average crown diameter of all the genotypes was 147.46 cm (Figure 2E). The orthotropic branches of genotype AC-29 (22.78 mm) had largest diameter (OBD), and 13V (16.00 mm) had smallest; average diameter was 19.27 mm (Figure 2F). An average of 22.13 nodes emerged per orthotropic branch (ONN), with one genotype producing 24.98 nodes (AC-13) and another producing only 18.88 nodes (AC-43; Figure 2G).

The average plagiotropic branch had 10.00 nodes (PNN); one genotype had branches with 11.89 nodes (AC-29), and another had only 8.27 nodes (13V; Figure 2H). A total of 20.54 leaves emerged in each plagiotropic branch (PLN) on average, ranging from 18.14 (AC-35) to 24.93 (AC-39; Figure 2I). An average of 28.21 plagiotropic branches (PBN) emerged in each orthotropic branch, ranging from 23.59 (AC-27) to 34.58 (AC-9; Figure2J).

The following daily rates were assessed when comparing overall cumulative average growth of 22 genotypes (OCA) with time elapsed between first and last assessments (361 days): 1.13 branches (OBN), 0.49 g (ODM), 3.16 mm (OBL), 0.055 mm (OBD), 0.065 nodes (ONN), 0.08 branches (PBN), 1.56 mm (PBL), 0.029 nodes (PNN) and 0.059 leaves (PLN). However, the growth rates of coffee plant shoots (considering the growth of orthotropic and plagiotropic branches, formation of nodes and leaf expansion, among others) are known to vary seasonally, according to climate conditions, particularly rainfall and temperature regimes, although photoperiod also has an effect (Libardi et al., 1998; Amaral et al., 2007; Ronchi and DaMatta et al., 2007; Partelli et al., 2010; 2013), which shows need to analyze coffee plant growth in different seasons.

The daily growth rates showed extremely similar seasonal performances in 12 traits, when comparing monthly growth rates in period of 09/20/2010 to 09/20/2011 (Figure 3) and rainfall and temperature curves for same period (Figure 1). Furthermore, the variation in growth rates was somewhat similar to rainfall and temperature curves in that period. The growth rates were high during rainy periods and periods with higher temperatures, even not extremely so. Conversely, growth rates were lower during the dry season and periods with milder temperatures. That similarity between curves was also reported by several authors (Libardi et al., 1998; Amaral et al., 2007; Ronchi and DaMatta, 2007; Partelli et al., 2010; 2013).

The minimum air temperature began to increase in mid-September 2010, remaining above 17°C until mid-May 2011 (Figure 1). Most of active growth occurred in that period, showing two peaks in growth curves: a first, often larger peak, distributed between months of November to January, and a second peak, less intense, recorded in months of March and April (Figure 3), which was also recorded by Amaral et al. (2007), with small discrepancies in dates of occurrence.

The transient decreases in growth intensity noted between both peaks in February may be explained as a consequence of limitations imposed by maximum air temperatures (Amaral et al., 2007; Libardi et al., 1998; Silva et al., 2004), which remained above 35°C in December, January and February, with a possible association with excessive rainfall (Amaral et al., 2007), which amounted to 328.5 and 328.0 mm in November and December, respectively (Figure 1).

Dardengo et al. (2009) report that decreases in growth rates in this period may also be associated with prolonged drought (43 days of drought occurred between 01/15 and 02/27, a period during which only one 25-mm irrigation could be performed). It is also noteworthy that conilon coffee plants show metabolic losses due to drought and also respond differently to soil water deficit (DaMatta et al., 2003; Pinheiro et al., 2004; Praxedes et al., 2006). The decreased growth rates in that period may also be attributed to fast grain filling because fruits are strongest drains, and therefore, coffee plants grow less in that phase, according to com Amaral et al. (2007). The highest number of orthotropic branches (OBN) emerged in November, with a rate of 3.25 branches day^{-1} (Figure 3A). The emergence of many orthotropic branches is a desirable trait of genotypes, given the need to cut and renew crown. However, such material is undesirable given demand for manual labour required for its removal and energy expenditure to produce dry matter that will be discarded.

The genotypes showed highest growth rates of orthotropic branches (OBL) and maximum production of orthotropic nodes (ONN) in December, with rates of 8.1 mm day^{-1} (Figure 3C) and 0.17 nodes day^{-1} (Figure 3G), respectively. The rate of 8.1 mm day^{-1} was similar to rates found in some genotypes by Partelli et al. (2013) in northern Espírito Santo State in high-yielding crops, albeit well above rates reported by Partelli et al.

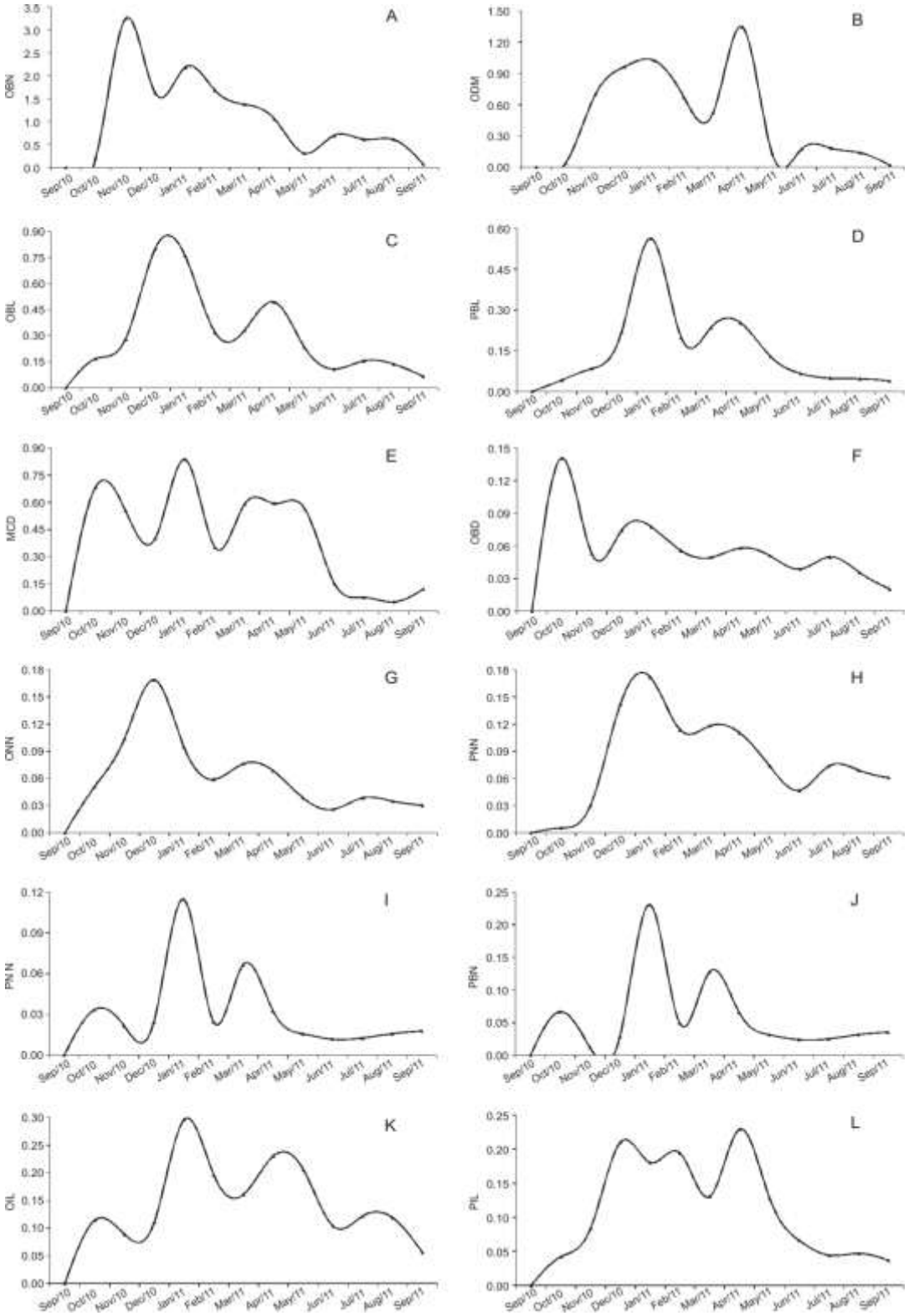

Figure 3. Variation of growth rates among the assessed seasons regarding the following traits: A) emerged orthotropic branch number (OBN; branches day^{-1}); B) dry matter of orthotropic branches (ODM; g day^{-1}); C) orthotropic branch length (OBL; cm day^{-1}); D) plagiotropic branch length (PBL; cm day^{-1}); E) maximum crown diameter (MCD; cm day^{-1}); F) orthotropic branch diameter (OBD; mm day^{-1}); G) orthotropic node number (ONN; nodes day^{-1}); H) plagiotropic node number (PNN; nodes day^{-1}); I) plagiotropic leaf number (PLN; leaves day^{-1}); J) plagiotropic branch number (PBN; branches day^{-1}); K) orthotropic internode length (OIL; cm day^{-1}); L) plagiotropic internode length (PIL; cm day^{-1}).

(2010) in northern region of Rio de Janeiro State in crops with low productive potential. The high growth rate may be explained by age of new branches, which were stimulated by changing source-sink relationship through pruning and good crop management.

The growth peaks of traits of plagiotropic branch length (PBL), maximum crown diameter (MCD), orthotropic branch diameter (OBD), plagiotropic branch number (PBN), plagiotropic node number (PNN), plagiotropic leaf number (PLN) and orthotropic internode length (OIL) occurred in January, with the following rates: 5.6 mm day^{-1} (Figure 3D), 8.4 mm day^{-1} (Figure 3E), 0.08 mm day^{-1} (Figure 3F), 0.17 branches day^{-1} (Figure 3J), 0.11 nodes day^{-1} (Figure 3H), 0.23 leaves day^{-1} (Figure 3I) and 2.9 mm day^{-1} (Figure 3K), respectively.

The highest values of traits dry matter of orthotropic branches (ODM) and plagiotropic internode length (PIL) were recorded in April, with rates of 1.35 g dry matter day^{-1} (Figure 3B) and 2.3 mm day^{-1} (Figure 3L), respectively. The peaks of those two traits occurred late, most likely as a form of compensatory growth (Ronchi and DaMatta, 2007) following return of rainy season in March and compensating for delay that occurred during drought between 01/15 and 02/27 (Figure 1). This pattern may also be associated with end of grain-filling period and the fact that no limitation occurred due to temperature, with maximum and minimum values in that month being 31.5 and 21.1°C, respectively.

The minimum air temperatures were below 17°C from mid-May to September 2011, a period during which lowest growth rates were also recorded. The rates of ONN, PBN, PNN and PLN traits decreased to their lowest values in June, when minimum air temperature was 15.7°C, by approximately 0.3 mm day^{-1}, 0.05 branches day^{-1}, 0.01 nodes day^{-1} and 0.02 leaves day^{-1}, respectively. The lowest rate of MCD was recorded in August, at a minimum temperature of 17.0°C, which only grew at 0.5 mm day^{-1}. The other traits showed lower values of growth rates in September, under a minimum temperature of 16.6°C, with a growth of 0.06 branches day^{-1} (OBN), 0.02 g D.M. day^{-1} (ODM), 0.6 mm day^{-1} (OBL), 0.4 mm day^{-1} (PBL), 0.2 mm day^{-1} (OBD), 0.6 mm day^{-1} (OIL) and 0.4 mm day^{-1} (PIL). Those results are consistent with the current understanding that field temperatures below 17°C are associated with decreased growth rates of conilon coffee plants (Partelli et al., 2010, 2013).

Conclusions

The logistic model describing a sigmoid pattern was best fit for all the studied traits, representing cumulative growth form after a regular pruning cycle. The growth rates varied seasonally throughout the year, with higher rates during rainy periods and higher temperatures, even not extremely so, and lower rates during dry season and milder temperatures. The growth rate of 8.1 mm day^{-1} may be explained by the age of new branches, which were stimulated by changing source-sink relationship through pruning.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Amaral JAT, Rena AB and Amaral JFT (2006) Crescimento vegetativo sazonal do cafeeiro e sua relação com fotoperíodo, frutificação, resistência estomática e fotossíntese. Pesqui. Agropec. Bras. 41:377-384.

Amaral JAT, Lopes JC, Amaral JFT, Saraiva SH and Jesus Jr WC (2007). Crescimento vegetativo e produtividade de cafeeiros conilon propagados por estacas em tubetes. Ciênc. Agrotecnol. 31:1624-1629.

Barbosa DHGS, Rodrigues WP, Vieira HD, Partelli FL, Viana AP (2014). Adaptability and stability of conilon coffee in areas of high altitud. Gene. Mole. Res. 13:7879-7888.

Barros RS, Mota JWS, DaMatta FM and Maestri M (1997). Decline of vegetative growth in *Coffea arabica* L. in relation to leaf temperature, water potential and stomatal conductance. Field Crops Res. 54:65-72.

Batista-Santos P, Lidon FC, Fortunato A, Leitão AE, Lopes E, Partelli F, Ramalho JC (2011). The impact of cold on photosynthesis in genotypes of *Coffea* spp. - Photosystem sensitivity, photoprotective mechanisms and gene expression. J. Plant Physiol. 168:792-806.

Bragança SM, Carvalho CHS, Fonseca AFA, Ferrão RG (2001). Variedades clonais de café Conilon para o Estado do Espírito Santo. Pesquisa Agropecuária, Brasileira, 36:765-770.

Bragança SM, Martinez HEP, Leite HG, Santos LP, Lani JA, Sediyama CS, Alvarez VH (2015). Acumulação de matéria seca pelo cafeeiro conilon**.** Rev. Ceres. 57:048-052.

CONAB (2015). Companhia Nacional de Abastecimento. Café: Safra 2016: Primeira Estimativa. Disponível em: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_01_20_17_01_56_boletim_cafe_-_janeiro_2016.pdf. Acesso: 05 de abril de 2016.

Dalcolmo JM, Vieira HD, Ferreira A, Lima WL, Ferrão RG, Fonseca AFA, Partelli FL (2015). Evaluation of genetic divergence among clones of conilon coffee after scheduled cycle pruning. Gene. Mole. Res. 14:15417-15426.

DaMatta FM, Chaves ARM, Pinheiro HA, Ducatti C, Loureiro ME (2003). Drought tolerance of two field-grown clones of *Coffea canephora*. Plant Sci. 164:111-117.

DaMatta FM, Ronchi CP, Maestri M, Barros RS (2007). Ecophysiology of coffee growth and production. Braz. J. Plant Physiol. 19:485-510.

Dardengo MCJD, Reis EF, Passos RR (2009). Influência da disponibilidade hídrica no crescimento inicial do cafeeiro conilon. Biosci. J. 25:1-14.

Davis AP, Tosh J, Ruch N, Fay MF (2011). Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data implications for the size, morphology, distribution and evolutionary history of *Coffea*, Bot. J. Linnean Soc. 167: 357-377.

Ferreira EPB, Partelli FL, Didonet AD, Marra GER, Braun H (2013). Crescimento vegetativo de *Coffea arabica* L. influenciado por irrigação e fatores climáticos no Cerrado Goiano. Semina: Ciênc. Agrár. 34:3235-3244.

Fonseca AFA, Ferrão MAG, Ferrão RG, Verdin-Filho AC, Volpi PS, Zucateli F (2004). 'Conilon Vitória - Incaper 8142': improved *Coffea canephora* var. kouillou clone cultivar for the state of Espírito Santo. Crop Breed. Appl. Biotechnol. 4:503-505.

ICO (2016). International Coffee Organization. Trade Statistics. Statistics. Disponível em: http://www.ico.org/prices/po-production.pdf.

Ivoglo MG, Fazuoli LC, Oliveira ACB, Gallo PB, Mistro JC, Silvarolla MB, Toma-Braghini MASAKO (2008). Divergência genética entre progênies de cafés robustas. Campinas, Bragantia 67:823-831.

Leroy T, De Bellis F, Legnate H, Musoli P, Kalonji A, Solórzano RGL, Cubry P (2014). Developing core collections to optimize the management and the exploitation of diversity of the coffee *Coffea canephora.* Genetica 142:185-199.

Libardi VCM, Amaral JAT and Amaral JFT (1998). Crescimento vegetativo sazonal do cafeeiro (*Coffea canephora* Pierre var. Conilon) no sul do Estado do Espírito Santo. Rev. Bras. Agrometeorol. 6:23-28.

Partelli FL, Araújo AV, Vieira HD, Dias JRM, Menezes LFT, Ramalho JC (2014). Microclimate and development of 'Conilon' coffee intercropped with rubber trees. Pesqui. Agropec. Bras. 49:872-881.

Partelli FL, Batista-Santos P, Scotti-Campos P, Pais IP, Quartin VL, Vieira HD, Ramalho JC (2011). Characterization of the main lipid components of chloroplast membranes and cold induced changes in *Coffea* spp. Environ. Exp. Bot. 74(1):194-204.

Partelli FL, Marré WB, Falqueto AR, Vieira HD, Cavatti PC (2013). Seasonal vegetative growth in genotypes of *Coffea canephora*, as Related to Climatic Factors. J. Agric. Sci. 5:108-116.

Partelli FL, Vieira HD, Silva MG, Ramalho JC (2010). Seasonal vegetative growth of different age branches of Conilon coffee tree. Semina: Ciências Agrárias, Londrina 31:619-626.

Partelli FL, Vieira HD, Viana AP, Batista‑Santos P, Rodrigues AP, Leitão AE, Ramalho JC (2009). Low temperature impact on photosynthetic parameters of coffee genotypes. Pesqui. Agropec. Bras. 44:1404-1415.

Pereira SP, Guimarães RJ, Bartholo GF, Guimarães PTG, Alves JD (2007). Crescimento Vegetativo e produção de cafeeiros (*Coffea arabica* L.) recepados em duas épocas, conduzidos em espaçamentos crescentes. Ciênc. Agrotecnol. 31:643-649.

Pinheiro AH, DaMatta FM, Chaves ARM, Fontes EPB, Loureiro ME (2004). Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. Plant Sci. 167:1307-1314.

Praxedes SC, DaMatta FM, Loureiro ME, Ferrão MAG, Cordeiro AT (2006). Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. *kouillou*) leaves. Environ. Exp. Bot. 56:263-273.

R Foundation For Statistical Computing R (2012). A language and environment for statistical computing. Disponível em: <http://softlibre.unizar.es/manuales/aplicaciones/ r/fullrefman.pdf>. Acesso em: 05 setembro de 2012.

Ramalho JC, DaMatta FM, Rodrigues AP, Scotti-Campos P, Pais I, Batista-Santos P, Leitão AE (2014). Cold impact and acclimation response of *Coffea* spp. plants. Theoretical and Experimental Plant Physiol. 26:5-18.

Rodrigues WP, Martins MQ, Fortunato AS, Rodrigues AP, Semedo JN, Simões-Costa MC, Máguas C (2016). Long-term elevated air [CO2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. Global Change Biol. 22:415-431.

Ronchi CP, DaMatta FM (2007). Aspectos fisiológicos do café Conilon In: Café Conilon. (Ferrão RG, Fonseca AFA, Bragança SM, Ferrão MAG, De Muner LH, eds.). Vitória: Incaper.

Scotti-Campos P, Pais IP, Partelli FL, Batista-Santos P, Ramalho JC (2014). Phospholipids profile in chloroplasts of *Coffea* spp. genotypes differing in cold acclimation ability. J. Plant Physiol. 171:243-248.

Silva EA, DaMatta FM, Ducatti C, Regazzi AJ, Barros RS (2004). Seasonal changes in vegetative growth and photosynthesis of *Arabica coffee* trees. Field Crops Res. 89:349-357.

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