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Full Length Research Paper

Influences of inter and intra row spacing on yield, yield component and morphological characteristics of onion (*Allium cepa* L.) at Western Amhara region

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Appropriate spacing enables the farmers to keep appropriate plant population in the field. Hence, it can avoid over or less population in a given plot of land which has negative effect on yield and quality of onion. Spacing of 40 × 20 × 10 cm between furrow, row and plants, respectively has been used for onion production in Ethiopia. But producers complain 10 cm intra row spacing produces large bulb size which is not preferred by consumer for home consumption. To optimize onion productivity, full package of information is required. To fill this gap field experiment was conducted to study the effect of inter and intra row spacing on the bulb yield and yield components of onion at Ribb in Fogera, Woramit in Bahir Dar and Koga in Mecha districts in 2014. The interaction of the lowest inter and intra row spacing mature earlier by 15 days compared to highest inter and intra row spacing. Forty nine percent of medium sized bulbs were produced with the interaction of 15 and 6 cm inter and intra row spacing while only 32% of medium sized bulbs were produced by the interaction of 25 and 8 cm inter and intra row spacing. Small sized bulb distribution decreased as intra row spacing increased while large bulb sized distribution increased when intra row spacing increased. The highest bulb weight (102 g) was produced by the interaction of highest inter and intra row spacing while the lowest bulb weight (45 g) was produced by the lowest inter and intra row spacing. Interaction of inter and intra row spacing of 15 and 4 cm respectively, scored the 1st highest marketable bulb yield (39 ton ha^{-1}) and interaction of 15 and 6 cm inter and intra row spacing scored the 2nd highest marketable bulb yield (36 ton ha^{-1}). The result revealed that the earlier recommendation (20 and 10 cm inter and intra row spacing) produced more number of medium and large sized bulbs with a yield penalty of 8.0 and 10 ton per ha^{-1} compared to interaction of inter row spacing of 15 cm with intra row spacing of 6 and 4 cm, respectively. Therefore, the interaction of inter and intra row spacing of 15 with 6 cm is recommended as a first option for producers who wishes to produce maximum bulb yield with maximum medium sized bulbs. It is also confirmed that earlier recommendation is better as a second option for producers who wishes to produce for export market which requires medium and large sized bulbs of onion.

Key words: Onion, inter, intra, spacing, bulb, yield.

INTRODUCTION

Among the common irrigated vegetables, onion (*Allium cepa* L.) ranks number one both in area coverage and local consumption in Ethiopia. In 2012, the total production of onion in Ethiopia was about 3,281,574 tons from 30,478 ha of land with average yield of 10.76 ton per ha^{-1} (FAO, 2012). Particularly, it is the popular vegetable grown under irrigation in most of the traditional and the recent modern irrigation schemes in Ethiopia. However, the largest production of onion is not supported with improved production practices like spacing to improve its productivity and bulb quality. To avoid nutrient competition sufficient spacing between plants and rows is vital to get maximum yield in given land. Appropriate spacing enables the farmer to keep appropriate plant population avoid over and less population in a given plot of land which has negative effect on yield and quality (EARO, 2004). Seedlings are widely transplanted with the spacing of 40 × 20 × 10 cm between furrow, row and plants, respectively in Ethiopia. Recently research results confirmed that spacing of 10 cm between plants produced large bulb size. On the other hand individual consumers do not prefer these large sized bulbs for home consumption (EHDA, 2011). For example, Geremew et al. (2010) recommended intra row spacing of 4 cm for 'Nasik' Red and 'Adama' Red varieties, and 6 cm for 'Bombay' Red variety, in central rift valley areas of Ethiopia. Whereas, according to Tadesse (2008), onion farmers in Fogera area do not keep the recommended spacing. They do not use any measurement to keep the spacing. Because of this there is no specific distance between both rows and plants. According to Yemane et al. (2013) finding the largest bulb size was produced by the larger intra-row spacing (10 cm), showed highest rotting percentage compared to intra-row spacing (5 and 7.5 cm). Bosekeng (2012) also observed that large plants grown at wider spacing are associated with split bulbs and sensitive to a cold stimulus causing bolting. To optimize onion productivity, full package of information is required (Lemma and Shimeles, 2003). Plant population needs to be optimized. The optimum use of spacing or plant population has dual advantages. It avoids strong competition between plants for growth factors such as water, nutrient and light. In addition optimum plant population enables efficient use of available cropland without wastage (Geremew et al., 2010).

The average productivity of onion in Amhara region was 13.76 t ha^{-1} which was very low as compared to other onion producing countries (CSA, 2012). This is attributed to shortage of improved technologies, limited awareness on the production practice that affect to exploit the full potential benefits of the crop. There is also lack of location specific researches on onion in particular and for other crops in general. In addition, the quality of the produce is also inferior due to pre-harvest and post-harvest biotic and abiotic factors. Therefore, in view of those gaps on onion production and productivity, the study was conducted to evaluate the influence of inter and intra row spacing on bulb yield and physical bulb quality of onion under western Amhara conditions.

MATERIALS AND METHODS

Description of the study areas

Woramit

Woramit is located in North-western part of Bahir Dar town on the shore of Lake Tana in Ethiopia. It is located at 11°38′ N and 37°10′ E which is about 563 km North of the capital city, Addis Ababa. The area has an altitude of 1800 m above sea level. It has warm and humid climate with distinct dry and wet seasons. The mean daily maximum temperature is 29.5°C in April. The mean daily minimum temperature is 6.2°C in January. The area receives a mean annual rainfall of 800 to 1250 mm. The area is characterized as mild altitude agro-ecology. The soil at the Woramit experimental site is Nitosol. The soil is moderately acidic (pH 6.4) with soil texture of sand (13%), silt (33%) and clay (54%). It has very low organic matter content (3.9 %). Available phosphorus content is low (6.3 mg/kg). It has medium total nitrogen contents (0.16%).

Koga

Koga is located in the North West part of Ethiopia in Mecha woreda. It is located between 11° 10' and 11° 25' North latitude and 37° 2' and 37° 17' East longitude in Blue Nile basin, which is about 540 km North of the capital city, Addis Ababa. The mean annual rainfall recorded at Merawi station is 1480 mm, of which 90% falls in the months May to October. The monthly mean temperature is 25.8°C. The elevation is 1960 m above sea level, and the slope ranges from nearly flat to 5%. The area is characterized as mild altitude agro-ecology. The soil at the Koga experimental site is Nitosol. The soil is strongly acidic (pH 5.1-5.3) with high exchangeable acidity (1.54-5.23) and high exchangeable Al^{3+} (0.92-2.88 cmol kg^{-1}) content. It has very low organic matter content (2.34-4.44%). Available phosphorus content is low (3.54-8.69 ppm). It has medium total nitrogen contents (0.18-0.24%).

Ribb

Ribb is located in North West part of Ethiopia in Fogera woreda. It is located at 11°44' to 12° 03' North and 37°25' to 37°58' East which is about 605 km North of the capital city, Addis Ababa. Its altitude is 1774 m above sea level. It receives 1400 mm mean annual rain fall. The mean daily maximum temperature is 30°C. The mean daily minimum temperature is 11.5°C. The area is characterized as mild altitude agro-ecology. The soil at the Ribb experimental site is fluvisol (an alluvial deposit). The soil has high available phosphorus (36.71 ppm) and very low to low total nitrogen contents (0.003). The CEC is high (33.00 cmol kg^{-1}). The soil is strongly acidic with high exchangeable acidity and high exchangeable Al^{3+} content. It has very low organic matter content. Available phosphorus content is low.

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Experimental materials, treatments and experimental design

Onion variety used for the study was 'Bombay red'. The experiment consists of three inter row spacing (15, 20 and 25 cm) and four intra row spacing (4, 6, 8, 10 cm) which was arranged in 3 × 4 factorial combination in Randomized Complete Block Design with three replications.

Experimental procedure

When seedlings reached at appropriate stage for transplanting, it was transplanted on 6 m^2 gross plot size (3 × 2 m). Four, three and two central double rows were maintained for the inter row spacing of 15, 20 and 25 cm, respectively. 25, 30 and 40 cm furrow width were also maintained for the inter row spacing of 15, 20 and 25 cm, respectively for irrigation water application (Figure 1). Based on the recommendation of each testing sites, 100 and 285 kg DAP and Urea ha^{-1} for Ribb, 300 and 100 kg DAP and Urea per ha^{-1} for Koga and 200 and 100 DAP and Urea ha^{-1} for Woramit was applied. The whole rate of DAP was applied at the time of transplanting while Urea was applied in two splits, half at the time of transplanting and half at 45 days after transplanting. 0.75 litter per hectare base of Selecron® 720 EC, was applied every two weeks interval for the control of onion trips. Other agronomic management practices were applied according to the national recommendation.

Data collection

Morpho-phenological traits

Days to maturity (DM): The actual number of days from seedling transplanting to the field to a day at which more than 90% of the plants in a plot showing yellowing of leaves was recorded to determine the days to physiological maturity.

Plant height (PH) (cm): The mean height of ten randomly selected plants was measured and divided by number of plant taken. It was measured using ruler from the soil surface to the tip of the leaves at bulb development stage.

Leaf number per plant (LN): The mean number of leaves produced by ten randomly selected plants. It was recorded at bulb development stage and expressed as number of leaves per plant.

Yield components

Bulb diameter (BD) (cm): The mean bulb diameter of ten randomly selected plants. It was measured by using caliper at the widest point in the middle portion of the matured bulb and expressed in cm.

Average bulb weight (ABW) (g): Average fresh weight of ten randomly selected marketable bulbs was measured by using digital balance from central rows of each plot and expressed in gram.

Bulb size distribution (%): Two holes with different size (65 cm and 45 cm diameter) were prepared on thick carton paper sheet. Bulbs which were not passed through 65 cm hole were considered as big bulbs, bulbs which were passed through 65cm hole but not passed through 45 cm hole were considered as medium bulbs and bulbs which were passed through 45 cm hole were considered as small bulbs. Numbers of different sized bulbs were converted in percent by the following formula.

$$\% = \frac{number\ of\ different\ sized\ bulbs}{Total\ bulbs} x\ 100$$

Bulb yield

Marketable bulb yield (MBY) (t ha^{-1}): Total weight of clean, disease and damage free bulbs were measured per net plot and converted to tha^{-1}.

Unmarketable bulb yield (UMBY) (t ha^{-1}): Total weight of under sized bulb,
decay, physiological disorder such as thick necked, split and bolters were measured per net plot and converted to t ha^{-1}.

Total bulb yield (TBY) (t ha^{-1}): Total weight of marketable and unmarketable bulbs were measured per net plot and converted to t ha^{-1}.

Statistical analysis

Analysis of variance (ANOVA) was computed using SAS (9.00 version) software. Duncan multiple range tests at 5% probability level was carried out for mean separation. Excel micro soft program was used to draw graphs.

RESULTS AND DISCUSSION

Morpho-phenological traits

The analysis of variance result revealed that all morpho-phenological attributes including plant height, number of leaves per plant and days to 90% plant maturity was significantly (P<0.05) influenced by location. The significant location effect (P<0.05) demonstrates that there were location difference in terms of weather conditions, soil physical and chemical properties which determines the general growth and yield of onion as it is described in site descriptions. Although the performance of onion growth and yield was differing from location to location, the overall effect of the treatments have similar trend from location to location. All these traits were also significantly (P<0.05) affected by both the main effect inter and intra row spacing. With the same manner inter row, intra row spacing and location interaction significantly (P<0.05) influenced all morpho-phenological attributes tested. All possible two way interactions (inter x intra, inter × location and intra × location) significantly (P<0.05) influenced plant height and 90% plant maturity except leaf number per plant (Table 1).

Plants grown with the combination of the highest inter row spacing of 25 cm and the highest intra row spacing of 10 cm recorded the highest value (51.42 cm) for plant height. On the other hand plants grown with the combination of the lowest inter row spacing of 15 cm and lowest intra row spacing of 4 cm recorded the lowest plant height value (45.35 cm). It is justified by Khan et al. (2002) that due to high

Table 1. Mean squares from analysis of variance (ANOVA) for growth physical quality and yield and yield related traits of onion.

Source of variation	df	Mean squares										
		DM	PH	BD	LN	Bulb size distribution			BW	MBY	UMBY	TBY
						Small (%)	Medium (%)	Large (%)				
Inter row	2	516**	94**	1.55**	9.82*	4590**	188**	5187**	9215**	2784359051**	69365486**	3723845995**
Intra	3	278**	36**	1.31**	24.89**	2457**	314**	3268**	6570**	196651242**	64576751^{ns}	484727238 **
Rep	2	8^{ns}	0.48^{ns}	0.12^{ns}	1.09^{ns}	7.48^{ns}	4.88^{ns}	18^{ns}	128^{ns}	638791^{ns}	1190486^{ns}	1817297^{ns}
Loc	2	1027**	2237**	134.4**	165.40**	8272**	935**	9311**	8194**	2726418981**	68387708**	2828449051**
Inter*Intra	6	9*	14*	0.29*	0.65^{ns}	109**	116**	146**	556**	52342307*	4493881*	45070918*
Inter*loc	4	75**	85**	0.19^{ns}	3.90^{ns}	406**	323**	143**	61^{ns}	122323287**	13440486^{ns}	193860579**
Intra*loc	6	10*	9^{ns}	0.25*	0.59^{ns}	161**	726**	238**	175^{ns}	34059691^{ns}	4658696*	28413696^{ns}
Inter*intra*loc	12	175**	18**	0.09^{ns}	1.99^{ns}	74**	177**	159**	191^{ns}	37546590*	2512631^{ns}	36463627*
Error	70	3	5	0.11	2.87	21.74	29.28	24.99	114	19426618	1734295	18964542

*, ** and ns- significant (P<0.05), highly significant (P<0.01) and non significant, respectively. DM, Days to maturity, PH, plant height, BD, bulb diameter; LN, leaf number; BW, bulb weight; MBY, marketable bulb yield; UMBY, unmarketable bulb yield; TBY, total bulb yield.

competition among the closest plant spacing, onion produced least response for plant height. Plants grown with the combination of highest inter row spacing of 25 cm and highest intra row spacing of 10 cm took longer time (125 days) for maturity. On the other hand plants grown with the combination of the lowest inter row spacing of 15 cm and the lowest intra row spacing of 4 cm took shortest time 110 days for maturity. The combination of the lowest inter row spacing (15 cm) with lowest intra row spacing (4 cm) mature earlier by 15 days as compared to highest inter row spacing (25 cm) with highest intra row spacing (10 cm). The delay in maturity due to wider spacing could be possibly due to the fact that plants in wider intra-row spacing did not compete for resources (nutrients, sun light, water and space) so that they prolonged their vegetative stage. The current study identified the advantage of using closer spacing for early bulb yield to fetch maximum price from the early market.

Yield components

Bulb size distribution

The analysis of variance result revealed that bulb size distribution viz. small (<45 mm in diameter), medium (45-65 mm in diameter) and big (>65 mm in diameter) was significantly influenced by the main effect inter, intra and location. All these traits were also significantly affected by inter row, intra row spacing and location interaction. All possible two way interactions (inter × intra, inter × location and intra × location) influenced onion bulb size distribution (Table 1).

More than 50% of small sized bulbs was produced with the interaction effect of the lowest inter row spacing of 15 cm and lowest intra row spacing of 4 cm, while 16% of small bulbs were produced with the interaction effect of highest inter row spacing of 25 cm and highest intra row spacing of 10 cm. Forty nine percent of medium sized bulbs were produced with the interaction effect of 15 and 6 cm inter and intra row spacing, respectively while only 32% of medium sized bulbs were produced by the interaction effect of 25 and 8 cm inter and intra row spacing. Forty eight percent of large sized bulbs were produced by the interaction effect of highest inter row spacing of 25 cm and highest intra row spacing 10 cm while only 8% of large sized bulbs produced by the interaction effect of the lowest inter row spacing of 15 cm and lowest intra row spacing of 4 cm. The current result indicated that small sized bulb distribution decreased as intra spacing increased while large bulb sized distribution increased when intra row spacing increased (Figure 2). Consumer's preferences regarding to bulb size of onion, medium sized (45-65 mm) bulbs are most preferable for most household conditions. Large sized bulbs (>65 mm) are mostly preferred for hotel, restaurant and export market in Ethiopian conditions.

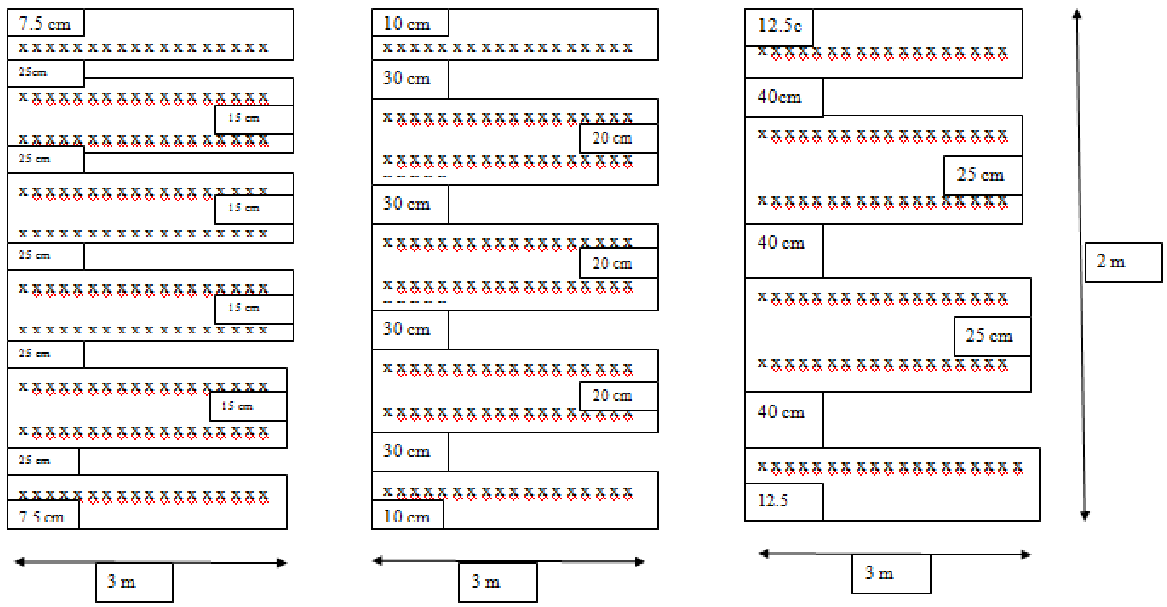

Figure 1. Spatial arrangements of rows, ridges and furrows on the plot for each inter row spacing treatment (15, 20 and 25 cm).

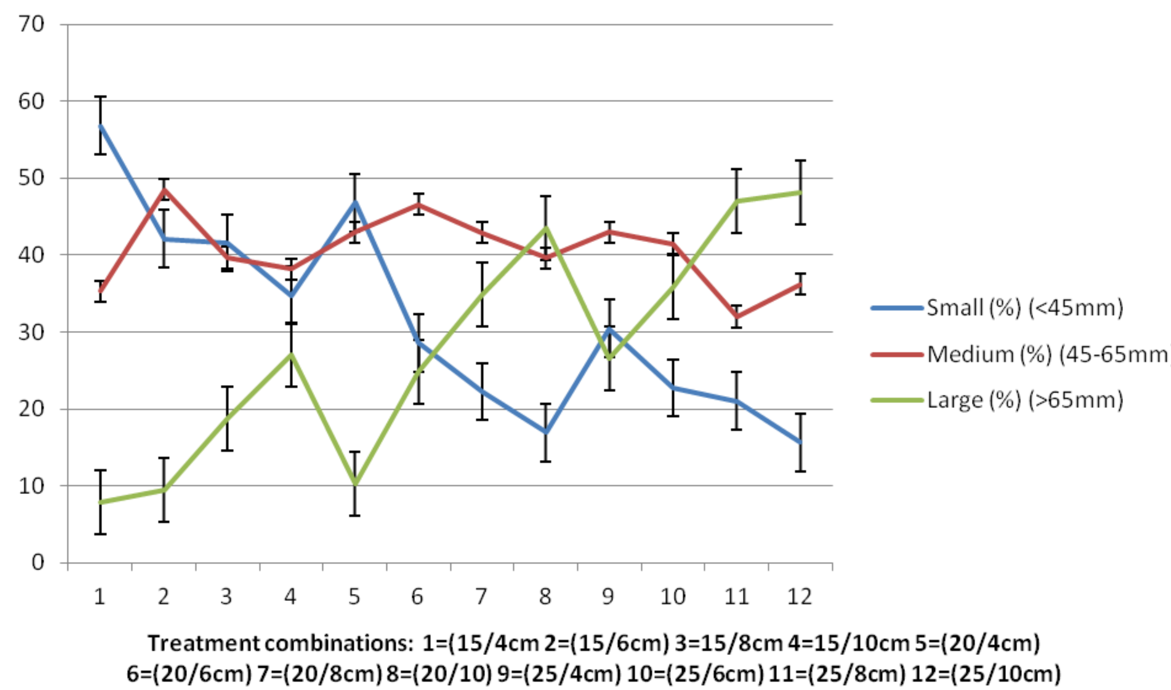

Figure 2. Bulb size distribution as affected by inter and intra row spacing of onion.

Average bulb weight (g)

The analysis of variance result revealed that bulb weight was significantly influenced by the main effect inter, intra and location. This traits was also significantly affected by the interaction of inter row and intra row spacing. All possible way interactions (inter × location, intra × location and inter × intra × location) was not statistically affected onion bulb weight except two way interaction inter and intra row spacing (Table 1). The highest bulb weight (102

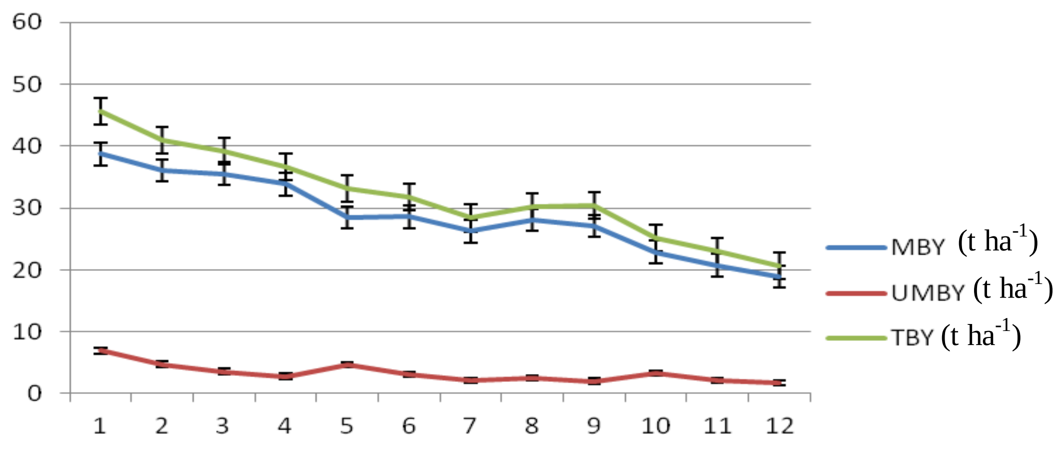

Figure 3. Onion bulb yield trend as affected by inter and intra row spacing of onion.

g) was produced by the interaction effect of highest inter row spacing of 25 cm with highest intra row spacing of 10 cm while the lowest bulb weight (45 g) was produced by the lowest inter row spacing of 15 cm with lowest intra row spacing of 4 cm. The increment in bulb weight due to increase in intra-row spacing might be due to the growth of taller plants with higher number of leaves causing higher synthesis and transportation of photosynthetic product from source to sinks and plants widely spaced experienced little or no competition for limited growth resources compared to closely spaced plants as described by Khan et al. (2002) and Biswas et al. (2003).

Average bulb diameter (mm)

The analysis of variance result revealed that bulb diameter was significantly influenced by the main effect inter, intra and location. This traits was also significantly affected both two way interaction of inter row by intra row spacing and intra by location. Two way interaction of inter by location and three way interaction of (inter × intra × location) was not statistically affected bulb diameter (Table 1). Significantly the first highest bulb diameter (7.12 mm) was produced by the interaction effect of 25 cm inter and 8 cm intra row spacing and the second highest bulb diameter (7.08 cm) was produced by the interaction of 20 cm inter row and 10 cm intra row spacing without statistically non-significant bulb diameter. On the other hand significantly lowest bulb diameter (6.20 cm) was produced when bulbs have been produced by the interaction effects of lowest inter row spacing of 15 cm and lowest intra row spacing of 4 cm. The present result was in line with that of Khan et al. (2002) who reported that wider intra-row spacing (12 cm) gave larger bulb diameter (5.13 cm) of onion. Whereas the closest(9 cm) spaced plants gave the lowest bulb diameter (3.80 cm). The increase in bulb diameter at wider spacing could be attributed to availability of more nutrients and moisture due to less competition effects in different physiological and metabolic processes through increase in dry matter production..

Onion bulb yield (t ha^{-1})

Marketable and total bulb yield per hectare

The analysis of variance result revealed that both marketable and total bulb yield was significantly influenced by the main effects inter, intra and location. Both traits were also significantly affected two way interaction of inter × intra and inter × location except intra × location. With the same manner inter row, intra row spacing and location interaction influenced both marketable and total bulb yields (Table 1). The interaction effect of the lowest inter row spacing of 15 cm with lowest intra row spacing of 4 cm scored the first highest marketable and total bulb yield of 39 and 46 t ha $^{-1}$, respectively. The interaction effect of the lowest inter row spacing of 15 cm with intra row spacing of 6 cm scored the second highest marketable and total bulb yield of 36 and 41 t ha $^{-1}$, respectively with statistically non-significant yield difference between the two treatment combinations. On the other hand interaction effect of highest inter row spacing of 25 cm with highest intra row spacing of 10 cm scored the lowest marketable and total bulb yield of 19 and 21 t ha^{-1}, respectively (Table 2). Generally, bulb yield increases significantly as population density increases (Figure 3). The current study indicated

Table 2. Interaction effect of inter and intra row spacing on bulb size distribution and yield of onion combined over locations.

Treat	DM	PH (cm)	BD (cm)	Bulb size distribution			BW (g)	MBY (t ha^{-1})	UMBY (t ha^{-1})	TBY (t ha^{-1})
				Small (%) (<45 mm)	Medium (%) (45-65 mm)	Large (%) (>65 mm)				
15-4	110.66^{f}	45.35^{c}	6.20^{e}	56.78^{a}	35.29^{cd}	7.91^{g}	45.54^{d}	38.71^{a}	6.95^{a}	45.66^{a}
15-6	112.11^{f}	46.91^{bc}	6.35^{de}	42.09^{bc}	48.47^{a}	9.43^{g}	49.11^{cd}	36.1^{a}	4.75^{b}	40.92^{ab}
15-8	116.00^{e}	47.35^{bc}	6.58^{bcd}	41.60^{bc}	39.67^{bcd}	18.71^{f}	51.85^{cd}	35.55^{a}	3.57^{bc}	39.13^{b}
15-10	117.5^{de}	45.17^{c}	6.41^{cde}	34.79^{cd}	38.16^{bcd}	27.04^{de}	69.30^{b}	33.86^{ab}	2.8^{cd}	36.71^{bc}
20-4	116.11^{e}	47.5^{bc}	6.60^{bcd}	46.78^{b}	42.98^{abc}	10.23^{g}	47.55^{d}	28.45^{c}	4.68^{b}	33.13^{cd}
20-6	117.8^{de}	48.27^{abc}	6.64^{bcd}	28.59^{de}	46.59^{ab}	24.8^{ef}	73.64^{b}	28.60^{bc}	3.11^{bcd}	31.71^{cd}
20-8	119.33^{cd}	49.98^{ab}	6.81^{ab}	22.25^{ef}	42.92^{abc}	34.82^{cd}	72.03^{b}	26.22^{cd}	2.13^{cd}	28.362^{de}
20-10	123.88^{ab}	48.62^{abc}	6.75^{bc}	16.91^{f}	39.63^{bcd}	43.45^{ab}	99.73^{a}	28.08^{cd}	2.55^{cd}	30.13^{de}
25-4	116.88^{de}	46.17^{bc}	6.22^{e}	30.47^{d}	42.95^{abc}	26.57^{ef}	64.88^{bc}	27.12^{cd}	2.04^{cd}	30.48^{d}
25-6	121.66^{bc}	49.80^{ab}	6.67^{bcd}	22.72^{ef}	41.49^{abcd}	35.78^{bc}	79.17^{b}	22.88^{de}	3.35^{bcd}	25.22^{ef}
25-8	122.11^{bc}	49.75^{ab}	7.12^{a}	21.00^{f}	32.00^{d}	47.00^{a}	96.84^{a}	20.75^{e}	2.15^{cd}	22.91^{f}
25-10	125.11^{a}	51.42^{a}	7.08^{a}	15.65^{f}	36.18^{cd}	48.15^{a}	101.9^{a}	18.86^{e}	1.80^{d}	20.67^{f}
R^2	0.95	0.93	0.97	0.96	0.85	0.96	0.88	0.90	0.83	0.92
Sig.	**	**	*	**	**	**	**	*	*	*
CV%	1.54	5.03	5.05	14.85	13.31	17.88	15.09	12.30	31.91	10.89

Means in the column with the same letter are none statistically significant at (5%). DM, Days to maturity, PH, plant height, BD, bulb diameter; LN, leaf number; BW, bulb weight; MBY, marketable bulb yield; UMBY, unmarketable bulb yield; TBY, total bulb yield.

that beyond 15 and 6 cm inter and intra row spacing, the number of small sized bulbs per hectare increased with statistically non-significant bulb yield difference. Bleasdale (1966) and Frappell (1973) reported similar results by stating the total yield of ripe bulbs increased with increasing number of plants per square meter until an optimum was reached and thereafter the yield declined. Kantona et al. (2003) concluded that, planting densities significantly affected the onion bulb yield.

Unmarketable yield

The analysis of variance result revealed that unmarketable bulb yield was significantly influenced by the main effects inter, intra and location. This trait was also significantly affected by the two way interaction of inter × intra and intra × location except inter × location. Three way interaction of inter × intra × location was none statistically influenced unmarketable bulb yield (Table 1). The highest unmarketable bulb yield of 7 ton per ha^{-1} was obtained when onion was produced with lowest inter and intra row spacing 15 and 4 cm, respectively. Whereas, the lowest unmarketable bulb yield of 2 ton per ha^{-1} was obtained when onion was produced with the highest inter and intra row spacing of 25 and 10 cm, respectively. This is for the reason that close spacing resulted in high undersize bulb as the consequence of higher competition between plants. This finding was in agreement with similar study of Seck and Baldeh (2009) who concluded that plant density has an impact on unmarketable bulb size.

CONCLUSIONS AND RECOMMENDATIONS

Bulbs produced by the interaction effect of 15 cm inter with 4 and 6 cm intra row spacing of mature earlier by 15 and 13 days compared with wider inter and intra row spacing of 25 and 10 cm, respectively. Producing two weeks early bulb yield by using closer spacing may play a significant role for producers to fetch maximum price from the early market. Maximum percent of medium sized bulbs were produced by the interaction effect of15 and 6 cm inter and intra row spacing. The maximum individual bulb weight (102 g) was produced interaction effect of wider inter and intra row spacing of 25 and 10 cm, respectively. Whereas minimum individual bulb weight (45 g) was produced by combination of 15 and 4 cm inter and intra row spacing, respectively. The combination of inter and intra row spacing of 15 and 4 cm respectively, scored the 1st highest marketable bulb yield and the combination 15 and 6 cm inter and intra row spacing scored the 2nd highest marketable bulb yield with non-significant yield difference with interaction of 15 and 4 cm inter and intra row spacing, respectively. Inter intra row spacing beyond 15 and 6 cm did not bring significant bulb yield difference. It implies that inter intra row spacing of 15 and 6 cm are optimum to obtain the highest marketable bulb yield of onion with maximum number of medium sized bulbs. The result revealed that the earlier recommendation (20 and10 cm inter and intra row spacing) produced more number of medium and large sized bulbs with a yield penalty of 8.0 and 10 ton per ha^{-1} compared to interaction of inter row spacing of 15 cm with intra row spacing of 6 and 4 cm, respectively. Therefore, the interaction of inter and intra row spacing of 15 with 6 cm is recommended as a first option for producers wishes to produce maximum bulb yield with maximum medium sized bulbs. It was also confirmed that earlier recommendation (20 and 10 cm inter and intra row spacing) is better as a second option for producers who wishes to produce for export market which requires medium and large sized bulbs.

Conflict of Interests

The authors have not declared any conflict of interests.

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Corn yield as a function of amounts of nitrogen applied in bands

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The aim of the current paper is to study the effect of different amounts of nitrogen applied in bands of corn hybrids on variables related to corn plant growth and its yield components. The experiment was carried out under no-tillage system at Guarapuava, PR, Brazil, throughout the period of October 1st 2009 to March 20th 2010. The treatments resulted in the combination of two simple hybrids of corn (P30R50 and AG8025) and six doses of nitrogen applied in bands (0; 75; 150; 225; 300 and 375 kg N ha^{-1} in urea form). The treatments were 12 arranged in a 2x6 factorial design of randomized blocks in four replications. The corn hybrid AG8025 had a small number of leaves, smaller rows of grains per stalk and insertion height of primary stalk compared to the hybrid P30R50. However, with a greater plant height, higher overall dry phytomass and productivity point out the influence of genetic variability on the crop. Nitrogen doses influenced significantly biological variables, such as plant height at the phenological stages V_9 and R_1, insertion height of the primary stalk, branch diameter, number of photosynthetically active leaves at R_1, stalk diameter, one thousand grains weight and productivity. 295 kg de N ha^{-1} dose provided estimated yield of 13,032.93 kg ha^{-1}. Factors, such as hybrids and N doses, did not affect harvest index, whose average value corresponded to 0.52. Yield was positively correlated to most of the variables in the study, outstanding yield components, such as dry phytomass of grains per stalk and overall phytomass of the aerial part per plant. It is concluded that knowing the effect of N on corn plant physiology makes the characterization of yield possible and helps in the selection of corn plant as a parameter for N management in bands.

Key words: *Zea mays* L., yield component, nitrogen, harvest index.

INTRODUCTION

Corn (*Zea mays* L.) belongs to the Poaceae family, and has C_4 mechanism for carbon fixation. Such a feature,

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associated with its leaf area, makes it utilizes global solar radiation for the conversion of mineral carbon into organic one. Thus, among the cereals of economic importance, corn has the greatest potential to produce phytomass which makes it to give a high productivity at a given site (Sangoi et al., 2007).

Nevertheless, the productivity potential of the crop is highly dependent on essential nutrients. Among them, nitrogen (N) is considered as the nutrient required by plants in great amounts. It leads to high yield (Fontoura and Bayer, 2009; Holland and Schepers, 2010); its appropriate dose and right time application make the crop to yield well. Corn yield might be affected in high or low intensities in the phenological stage (Subedi and Ma, 2005).

Knowledge of the effect of N on corn crop physiology under different environments and management systems, whether by means of variables related to the crop growing season, yield components, as well as the accumulation of dry phytomass (Shanahan et al., 2008), contributes to knowing the right moment to apply N in bands, rationalize nitrogen fertilization in bands so as to reduce the environmental impact of the N applied in the soil and water, mainly as a nitrate form (Alcântara and Camargo, 2010).

The aim of the current study is to scrutinize the effect of N doses applied in bands in corn hybrids on biological variables related to growth and development of corn plants, yield components and overall dry phytomass of the aerial part. It also aims to examine whether there is correlation of such variables with yield to establish a feasible N management for corn plants at Guarapuava, PR, Brazil.

MATERIALS AND METHODS

The field experiment was conducted under no- tillage system at the region of Entre Rios, in the municipality of Guarapuava, PR, Brazil [latitude 25º32'S, longitude 51º28'W and altitude of 1,126 m] between October 1^{st} and March 20^{th} of 2010. The climate of the site, according to Köppen's classification, is humid subtropical bereft of dry season and with frequent severe frosts (Peel et al., 2007). Mean annual rainfall at the region is 1.942 mm. Monthly mean air temperature is 16.8°C with maximum and minimum values corresponding to 23.1°C and 12.4°C, respectively (Simepar, 2011).

Throughout the crop growing season the overall amount of rainfall was above 35 year historical average, along with variations of 5.1 mm for the month of November and of 114.7 mm for the month of January. There was crop rotation over the last years at the experimental area for winter/summer year season as follows: wheat/soybean (2006), oat/maize (2007), barley/soybean (2008), and in 2009 oat preceded maize crop. The type of soil prevailing in the experiment area is latosol with a depth of roughly 2 m, good physical conditions, and with a high potential for agricultural use. The chemical characteristics of the soil are seen in Table 1.

Treatments that resulted from the combination of two simple hybrids were classified as precocious cycle for maize - Pioneer 30R50 and Agroceres 8025 (P30r50 and AG8025) - and 6 doses of N applied in cover (0, 75, 150, 225, 300 and 375 kg of N ha^{-1} in the form of urea). The treatments comprise a 2x6 factorial design of randomized blocks with four replications. Plots consisted of 8 lines of 5 m long and space of 0.75 m, occupying a total area of 30 m^2. Sowing was performed manually on October 1^{st} of 2009 shortly after the incorporation of 8-30-20 fertilizer formula plus 0.4% of Zn to reach a plant population of 69.722 plants ha^{-1}. Cultural practices were adopted in the crop according to its occurrence and recommendation for the crop in the field.

N doses were applied once manually in the total area on November $9^{th,}$ 2009 with the plants at V_5 stage. Under the V_3 stage, out of the two central lines, 4 plants per $plot^{-1}$ were selected and identified for determination of the following variables: plant height, branch diameter, insertion height of the primary stalk, number of photosynthetically active leaves, number of grain rows per stalk, number of grains per row, number of total expanded leaves, length of stalk, diameter of stalk, one thousand grain weight, dry phytomass of grains per stalk, overall dry phytomass of the aerial part per plant, and harvest index.

The determination of initial plant population was preformed at V1 stage and the final one at harvest. This was done by taking into accout all the plants along 5 meter from the four central lines in order to obtain a mean number of plants per linear meter, which was extrapolated to plants per hectare.

Final productivity was assessed by means of the manual harvest of the ears from a useful area of 13.5 m^2, mechanical threshing, determination of dry phytomass of grains per stalk and extrapolation of the values for kg ha^{-1}, correcting it to a water content of 13%.

Experimental data obtained from each variable were subjected to analysis of variance through the SAS statistical program (SAS, 2008). Whenever the interaction between the hybrids and N doses was significant, and when effect of N doses was observed, a study of regression was carried out by means of illustrations provided by graphs made by the Excel program. The degree of correlation and agreement between the variables measured herein was expressed by the coefficient of Pearson correlation.

RESULTS AND DISCUSSION

The AG8025 corn hybrid (56.52; 159.04 and 243.47 cm) demonstrated the highest plant heights (PH) at all phenological stages assessed (V_5, V_9 and R_1) in relation to the P30R50 hybrid (46.51; 141.12 and 238.25 cm). Fluctuations on the PH among corn hybrids at the R_1 stage were also observed by Subedi and Ma (2005), showing values between 187 and 232 cm, with lower values for the P30R50 and AG8025 hybrids at R_1 stage. Probably such discrepancies observed between the hybrids might reflect the variation on its genetic origins regardless of the N doses applied in bands.

The PH at V_9 stage increased with the N application up to the dose of 285 kg ha^{-1}, determined as the dose of a maximum technical efficiency, which corresponded to an estimated height of 159.52 cm (Figure 1a). The PH at R_1 stage increased up to the dose of 285 kg ha^{-1}, corresponding to 247.36 cm (Figure 1b) and revealing a high N requirement between the stages V_9 and R_1. In the

Table 1. Chemical characteristics of the soil at the experimental area classified as Brume Latosol. Ponta Grossa, PR, Brazil. 2012.

Attributes	Unities	Depth (cm)		
		0-10	10-20	20-40
pH in $CaCl_2$		5.4	4.7	4.7
H + Al	$cmol_c\ dm^{-3}$	5.35	8.36	9.01
Al changeable	$cmol_c\ dm^{-3}$	0.0	0.3	0.4
Ca changeable	$cmol_c\ dm^{-3}$	6.9	4.0	3.1
Mg changeable	$cmol_c\ dm^{-3}$	2.4	1.5	1.3
K changeable	$cmol_c\ dm^{-3}$	0.57	0.38	0.25
P	$mg\ dm^{-3}$	22.9	6.0	2.4
C-organic	$g\ dm^{-3}$	32	21	19
CTC at pH 7	$cmol_c\ dm^{-3}$	15.22	14.24	13.66
CTC effective	$cmol_c\ dm^{-3}$	9.87	6.18	5.05
Sat. per bases (V)	%	64.8	41.3	34.0
Sat. per Al (m)	%	0.0	4.9	7.9
Sat. per Ca	%	45.3	28.1	22.7
Sat. per Mg	%	15.8	10.5	9.5
Sat. per K	%	3.7	2.7	1.8
Ratio Ca/Mg		2.9	2.7	2.4
Ratio Ca + Mg/K		16.3	14.5	17.6

H + Al: Buffer solution SMP; Al, Ca and Mg changeable: KCl 1 mol L^{-1}; P and K: Mehlich-1 and C-organic: Walkley-Black. Source: Laboratory of Soil Fertility of the State University of Ponta Grossa.

work idealized by Silva et al. (2005), the corn PHR1 responded up to the dose of 171 kg of N ha^{-1}, reaching 223 cm. However, Gomes et al. (2007) observed a linear increase of PH for corn as a function of N doses, reaching 222 cm with an application of 150 kg de N ha^{-1} in bands.

The PH of the P30F33 hybrid assessed by Tomazela et al. (2006) under the dose of 100 kg of N ha^{-1} corresponded to 260 cm at R_1 stage. This makes it viable to characterize the genotypic differences among corn hybrids for PH, since in the current study, P30R50 and AG8025 hybrids at R_1 stage with an estimated dose of 285 kg de N ha^{-1}, that is, 185 kg of N ha^{-1} less, showed a lower value of PH (247.36 cm). The correlation among PHV_5, PHV_9, PHR_1 and yield was significant (p<0.01) and increasing (r=0.52; 0.77 e 0.80**, respectively). The PHR_1 showed also a high correlation with branch diameter at R_1 stage (BDR_1), number of photosynthetically active leaves ($NPALR_1$), number of grains per row (NGR), dry phytomass of grains per stalk (DPGS), overall dry phytomass of the aerial part per plant (ODPAP), corresponding to 0.77, 0.72; 0.69; 0.67 and 0.66**, respectively. Such associations indicate that an increase in PH was accompanied by the number of photosynthetically active leaves, and as a result by the overall dry phytomass of aerial part, culminating in a high net photosynthesis and yield.

The corn hybrid P30R50 revealed a higher insertion height of the primary stalk (IHPS) compared to the AG8025 hybrid (125.17 against 117.31 cm), differing by 6.7%. With regard to the variable identification of the leaf of the primary stalk (ILPS), the primary stalk of the P30R50 hybrid has been located on average a leaf above (at leaf 13) in relation to the position of the stalk of the AG8025 hybrid. Such variations might be ascribed to the genetic variability among hybrids. In the same way, Subedi and Ma (2005), by analyzing three corn hybrids, also found the influence of genetic variability for the variables IHPS (88.0; 97.0 and 76.0 cm) and ILPS (11, 12 and 10). The correlation between IHPS and yield was of 0.43**, although no correlation was found between ILPS and corn yield. The estimated dose in 285 kg of N ha^{-1} in bands was the one that promoted the highest IHPS, corresponding to 127.22 cm (Figure 1c). Silva et al. (2005) obtained responses up to the dose of 158 kg of N ha^{-1}, allowing for the estimation of the IHPS in 122.69 cm.

The branch diameter (BD) was influenced by the corn hybrids and N doses applied in bands at V_9 (BDV_9) stage, as well as only by the N doses at the R_1 stage (BDR_1). The BDV_9 of the P30R50 hybrid (26.34 cm) was 2.8% higher than the BDV_9 of the AG8025 hybrid (25.63 cm), a

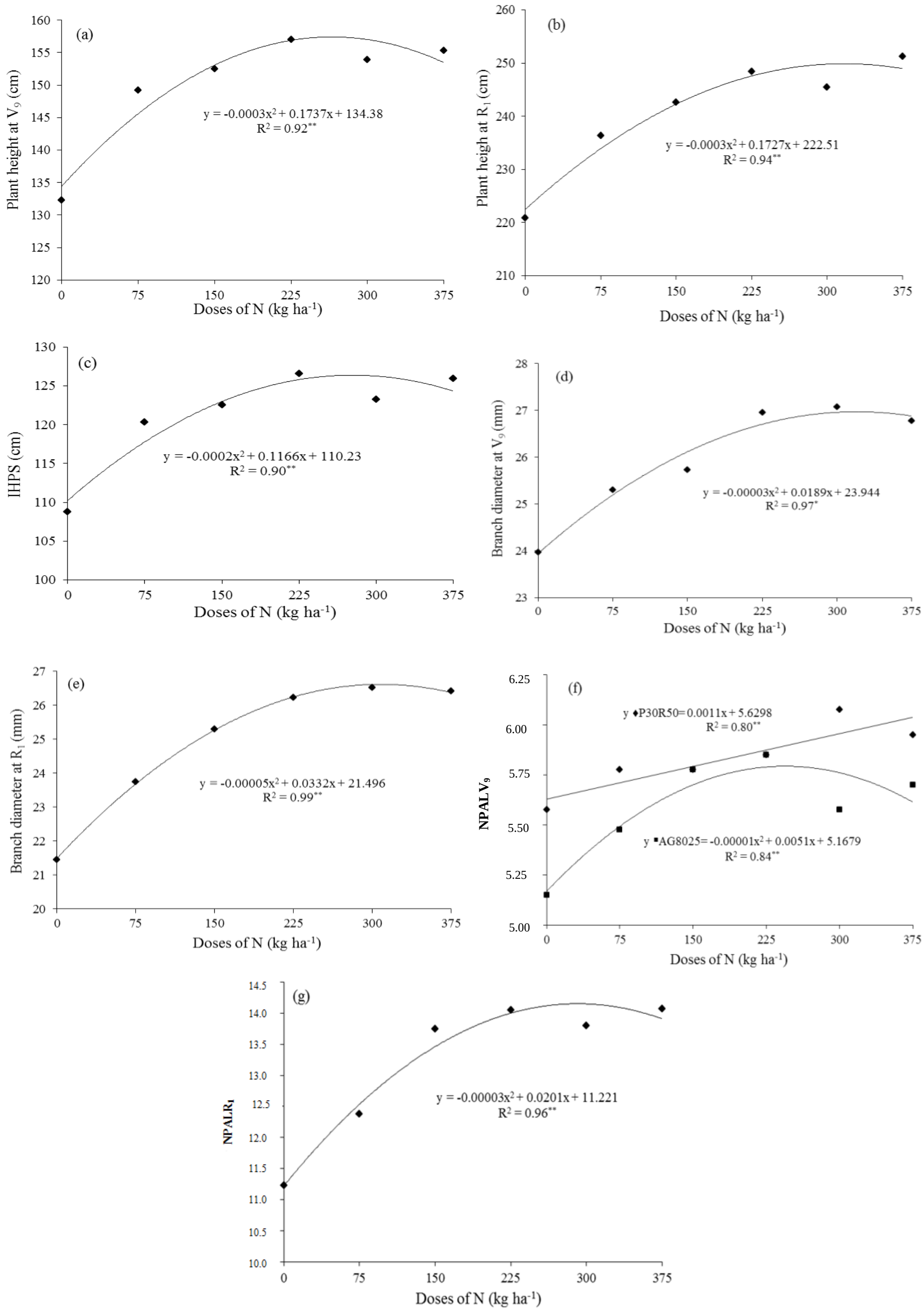

Figure 1. Height of corn plant at the phenological stage V_9 (a), height of corn plant at the phenological stage R_1 (b), insertion height of the primary stalk (IHPS) (c), diameter of corn plant branches at the phenological stage V_9 (d), diameter of corn plant branches at the phenological stage R_1 (e), number of photosynthically active leaves per plant at the phenological stage V_9 ($NPALV_9$) (f), number of photosynthically active leaves per plant at the phenological stage R_1 ($NPALR_1$) (g) as a function of corn hybrids and N doses applied in bands. * $p<0.05$ and ** $p<0.01$. Ponta Grossa, PR, Brazil. 2012.

difference not observed at the stage R_1; its average BD was 24.97 mm. Dourado Neto et al. (2003) found an average value of BDR_1 of 30.8 mm for the hybrids AG1051, AG7575 and DKB911, whereas Tomazela et al. (2006) obtained a BD of 22.5 mm for the P30F33 hybrid at the same stage, with values higher and lower than the average.

Regression analysis studies resulted in estimated 26.92 and 27.01 mm. Maximum values of BDV_9 and BDR_1 corresponded, respectively, to the doses of 305 and 330 kg of N ha^{-1} applied in bands (Figures 1d and e). The difference between BD estimated as the peak at V_9 and R_1 stages was minimum (0.09 mm), but the consumption at the R_1 stage was 25 kg of N ha^{-1} higher at the V_9 stage, a fact that demonstrates a BD stabilization for the corn hybrids from V_9. The correlation between BDV_9, BDR_1 and yield was 0.50** and 0.71**, respectively. The BDR_1 also showed a high correlation with $NPALR_1$, NGR, DPGS and ODPAP (r= 0.88; 0.72; 0.67 and 0.66**, respectively).

P30R50 hybrid was superior in the number of photosynthetically active leaves per plant (NPAL) at V_5 (3.36), V_9 (5.83) and R_1 (13.48) stages as opposed to AG8025 for the same stages, with increases of 6.33% (3.16); 4.29 % (5.59) and 4.01% (12.96), respectively. P30R50 also demonstrated a large number of total expanded leaves (NTEL) in relation to AG8025 (18.50), with a 4.05% difference for both hybrids. The outcomes indicate that P30R50 had a higher NPAL per plant than that obtained for AG8025.

Subedi and Ma (2005) also found significant differences for NTEL with fluctuations of 16 to 20 leaves per plant. Sangoi et al. (2007) obtained for the simple hybrid DKB909 after three years of evaluation a NTEL of 20.7; 19.2 and 19.1 - values roughly similar to that of the simple hybrids P30R50 and AG8025 (19.25 and 18.50, respectively). By comparing NTEL with NPALR1 we evidenced that there was a significant reduction in NPAL along all its extension. Faced with such a fact we might infer that there was N remobilization (Seebauer et al., 2004), as well as carbonic skeletons (Taiz and Zeiger, 2013) to the stalks and/or roots of the corn plants. The number of senescent leaves was of 5.77 leaves for the P30R50 hybrid and 5.54 for the AG8025 hybrid.

The NTEL was not influenced by the N doses in bands. This is likely owing to N amounts applied at the sowing date, as well as the available N in the soil which met the initial crop needs, since the differentiation of the leaves takes place up to the V_6 stage (Ritchie et al., 2003). And also due to the fact that up-dated corn hybrids absorb less than 15% of the total N up to the V_7 stage (Shanahan et al., 2008). Such an up take of N corresponds to the phenological stage where there is an accumulation of about 5% of the overall dry phytomass.

For the variable $NPALV_9$ there was an increasing linear response of the crop to the N doses applied in bands for the P30R50 hybrid and a quadratic response for the AG8025 hybrid (Figure 1f). N doses significantly affected $NPALR_1$; however both corn hybrids performed in the same fashion and revealed a similar behavior (Figure 1g). Sangoi et al. (2007), applying bands of 100 kg of N ha^{-1} at V_5, obtained after 3 years of assessment for the simple hybrid DKB909 an $NPALR_1$ of 13.6; 13.7 and 10.9, corresponding to yields of 11,750; 11,760 and 9,500 kg ha^{-1}, respectively. In the current work the maximum estimate of $NPALR_1$ was 1.86 times the mean value obtained throughout a three year evaluation by Sangoi et al. (2007). However, N requirement of the crop was much higher (230 kg of N ha^{-1}). The correlation between yield and NPAL was positive at V_9 and R_1 stages (r= 0.33** and 0.70**, respectively). There were also positive correlations for $NPALR_1$ and NGR, DS, DPGS, ODPAP and HI with r Pearson coefficients of 0.68**, 0.58**; 0.59**; 0.53** and 0.41**, respectively. $NFTER_1$ correlated only with NFG (r= 0.29**).

For both initial (IPP) and final plant populations (FPP) there was neither effect of the studied factors nor interaction among them on the variables assessed herein. Mean IPP was of 73,958.44 and FPP was of 69.722,25 plants ha^{-1}, corresponding to a 5.73% reduction in yield. For the FPP evaluation only plants with stalks were taken into account. Nevertheless the difference of 4,236.19 plants ha^{-1} between the IPP and FPP might be attributed mainly to a delay of emergence of the seedlings, which certainly did compromise growth of the aerial part and root system of the crop.

The intraspecific competition effect existing among the corn plants in the field might have been intensified within plants with delaying emergence. A reduction in the number of stalks per area or an increase in the occurrence of plants bereft of stalks is an indication of competition that takes place during the vegetative stage (Merotto et al., 1999). Sterility in corn, according to Sangoi (2000), might occur even in hybrids of precocious cycle that result in low plant height, low NPAL, more erected leaves, small corn tassels along with a small variation amplitude (expressed in days) between the development of male and female inflorescences. IPP correlated positively only with FPP (r= 0.72**) and FPP with yield (r= 0.35**).

Sangoi et al. (2009), making use of a population of 75,000 plants ha^{-1} for the simple hybrid P30F53, obtained a dry phytomass of grains per stalk (DPGS) of 181.18 g and a yield of 12,634 kg ha^{-1}. Comparing to a FPP of 69,722.25 plants ha^{-1} found in the current study, this corresponded to a DPGS of 202.47 g and a yield of 11,684 kg $ha^{-1.}$ This indicates that an increase in FPP might cause a reduction in DPGS, but an increase in yield.

Corn hybrids impinged significant effects on variables such as number of grain rows per stalk (NGRS), number of grains per row (NGR), length of stalk (LS), one thousand grain weight (1000GW) and stalk diameter (SD). However, the factor N doses had a significant effect on the variables NGR, LS, 1000GW and SD along with interactions only for the NGR and SD variables.

The NGRS was higher for the P30R50 hybrid (15.35) in comparison to the AG8025 (14.69), with a difference of 4.5%. However, for the variables 1000GW and SD there was a superiority of the AG8025 hybrid in relation to the P30R50 (358.08 g and 50.87 mm), a 33.86 g and 0.58 mm difference corresponding to 9.46 and 1.14%, respectively. Though the NGRS has been lower for the AG8025 hybrid, the SD and HI (Figure 2b) were high, resulting in a higher 1000GW and an increase in crop yields. There was no positive association between NGRS and yield, since NGRS was not influenced by the N doses applied in bands.

In order to estimate the number of grains per stalk (535.76), it is necessary to multiply NGR (35.67) by NGRS (15.02), which corresponded to a 1000GW of 375.01 g. Similar results were also obtained by Tomazela et al. (2006) for the P30F33 hybrid, whose mean number of grains per stalk (NGS) was 522.75, corresponding to a 1000GW of 346.95 g. Nevertheless, Sangoi et al. (2007) found throughout a three year assessment for the DKB909 hybrid 447, 397 and 400 grains per stalk, corresponding therefore to a mean 1000GW of 333; 324 and 300 g, respectively.

Fernandes et al. (2005) obtained for the simple hybrids AG9010 and DKB333B, triple hybrid CO32, double hybrid XB8010, cultivars BR106 and Sol da Manhã a NGR of 482.89; 506.41; 534.35; 523.31; 459.39 and 466.88, corresponding to a 1000GW of 275.10; 277.70; 272.70; 285.90; 233.90 and 269.10 g, respectively. The 1000GW were all lower than those obtained for the simple hybrids P30R50 and AG8025, whereas the triple hybrid CO32 and double hybrid XB8010 did show similar calculated values for NGS (535.76). The discrepancies at stake were ascribed possibly to genetic basis of the hybrids and also to environmental factors.

A regression study revealed a quadratic response of the crop to the N doses applied in bands either for the P30R50 or AG8025 hybrids (Figure 2a) as a function of the maximum doses estimated in 185 and 335 kg of N ha^{-1}, respectively, corresponding to 36.04 and 38.38 grains per stalk (Figure 2a). There was great variation amplitude of crop response to N doses between hybrids for NGR. Silva et al. (2005) did obtain for such variable a lower dose (154 kg of N ha^{-1} in bands), demonstrating therefore a quite similar value of NGR (38.55).

Discrepancies noted between the maximum doses for technical efficiency estimated for NGR, compared to the outcomes obtained by Silva et al. (2005), evidenced that there are different responses of the crop to N demand for the hybrids that lead to distinct yield estimates. Silva et al. (2005) obtained by means of the estimated dose 166 kg of N ha^{-1} a yield of 6,709.37 kg ha^{-1} against a 13,032.93 kg ha^{-1} yield found in the current work with the estimated dose of 295 kg of N ha^{-1} (Figure 2g). Data on the productivity potential are rather dependent on N availability, other productivity components, as well as the accumulation of dry phytomass at each environment. Owing to the favorable meteorological conditions of Guarapuava, PR, along with cultural practices adopted in the corn crop which were conducive to increase productivity potential, we drew the conclusion that the responses of the crop to the highest N doses applied in bands were justifiable for NGR of both P30R50 and AG8025 hybrids - an observation that might be confirmed by the high correlation between NGR and yield (r= 0.70**). The NGR also showed a high correlation with DPGS (r= 0.84**) and ODPAP (r= 0.76**).

Maximum stalk length (SL) for the P30R50 corn hybrid was estimated to be 17.64 cm with a dose of 190 kg of N ha^{-1}, whereas the AG8025 showed a linear response to N doses applied in bands (Figure 2 b). The correlation between SL and yield was high (r= 0.70**). The SL was also associated with a high Pearson correlation coefficient for DPGS (r= 0.88**), ODPAP (r= 0.85** and 1000GW (r= 0.71**). Mean SL obtained herein, irrespective of the hybrid and N doses applied was 17.99 cm, being higher than that obtained by Subedi and Ma (2005) for three corn hybrids (15.9; 16.0 and 16.2 cm), pointing out a likely influence of genetic differences.

The response of the crop for the diameter of stalk (DS) as a function of N doses in bands was increasing linearly (Figure 2c). The correlation analysis revealed a strict relationship between DS and yield (r= 0.71**), DPGS (r= 0.82**) and ODPAP (r= 0.79**). Nevertheless, the mean value of DS obtained in the current work was 51.2 mm, evidencing those thresholds reported by Subedi and Ma (2005) when subjected to statistical analyses for three hybrids (42.0; 43.0 and 45.0 mm).

One thousand grain weight (1000GW) variable responded significantly to N doses applied in bands regardless of the hybrid in consideration (Figure 2d). The fit was quadratic, being 265 kg of N ha^{-1}, an estimated dose in bands that lead to the maximum 1000GW, which was 389.10 g. The correlation between 1000GW and yield was positive (r= 0.66**), as well as between the first and DPGS (r= 0.82**) and ODPAP (r= 0.81**).

The responsiveness reached herein differs from that obtained by Silva et al. (2005), since such authors observed a linear effect for 1000GW in relation to N doses applied in bands. However, it was estimated for the dose of 180 kg of N $ha^{-1,}$ a value of 1000GW equivalent to 317.86 g. This was below the one reported by Sangoi et al. (2007), where they applied 100 kg of N ha^{-1} at the phenological stage V_5, whose values were

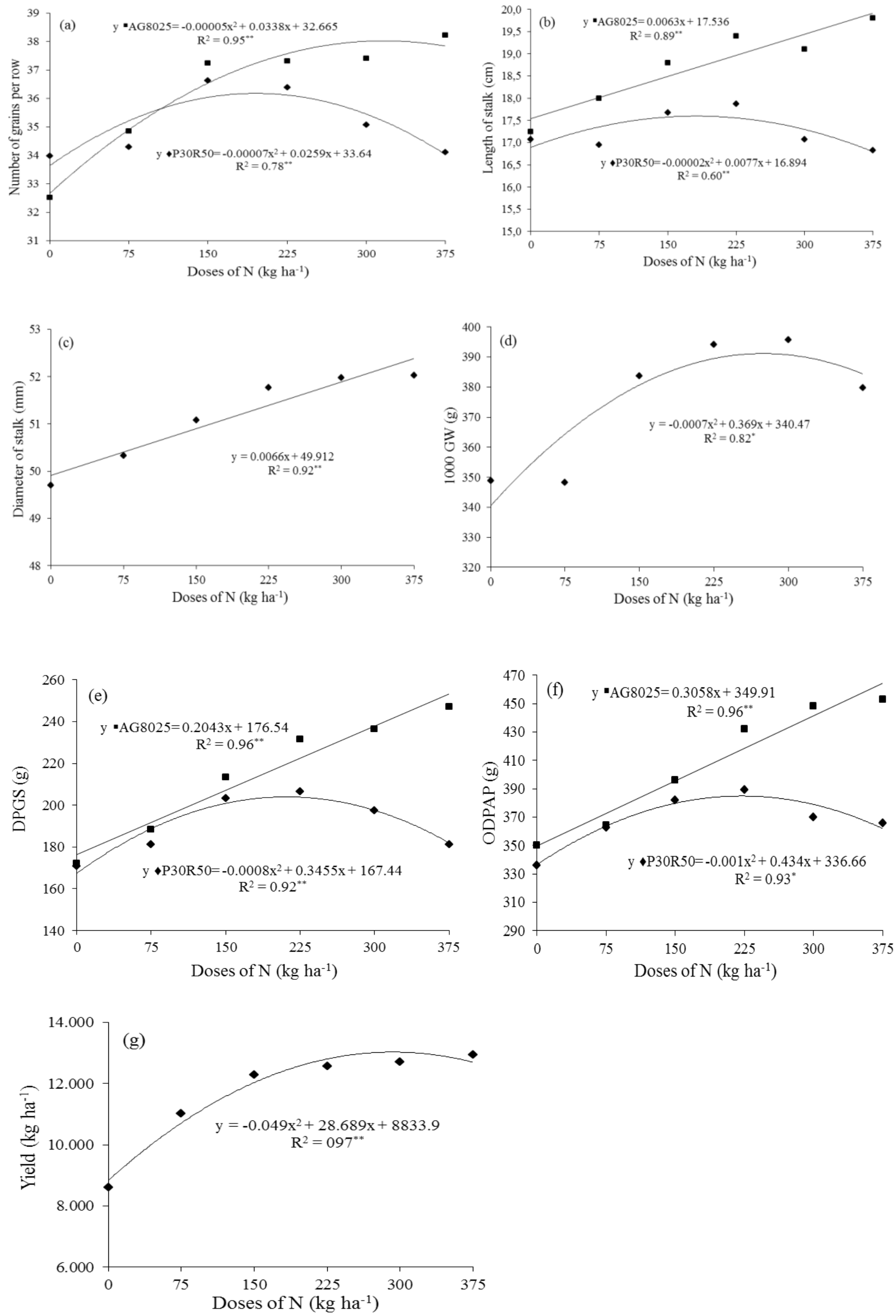

Figure 2. Number of grains per row (NGR) (a), length of stalk (LS) (b), diameter of stalk (DS) (c), 1000 grain weight (1000GW) (d), dry phytomass of grains per stalk (DPGS) (e), overall dry phytomass of the aerial part per plant (ODPAP) (f) and yield (g) as a function of corn hybrids and N doses applied in bands. * $p<0.05$ and ** $p<0.01$. Ponta Grossa, PR, Brazil. 2012.

339; 332 and 305 g for the simple hybrid DKB909 during a three-year assessment. For Gomes et al. (2007), N doses did not influence 1000GW, whose mean value was 320.40 g. 1000GW correlated positively with NGR, LS and DS (0.59; 0.71 and 0.71**, respectively).

The variable dry phytomass of the straw of the aerial part per plant (DPSAPP) was influenced only by the corn hybrids. Variables, such as dry phytomass of grains per stalk (DPGS) and overall dry phytomass of the aerial part per plant (ODPAP), were significantly affected by both factors and interaction between corn hybrids and N doses applied in bands. The ODPAP correlated positively with NGR, LS, DS, 1000GW, ODPAP, DPGS and yield (0.76; 0.85; 0.79; 0.81; 0.86; 0.93 and 0.73**, respectively). Pearson correlation coefficients between DPGS and NGR, LS, DS, 1000GW, ODPAP and yield were 0.84; 0.88; 0.82; 0.82; 0.61 and 0.77**, respectively. Taking into account the regression analysis between ODPAP and NGR, LS, DS, 1000GW and yield r coefficients were 0.47; 0.60; 0.56; 0.60 and 0.50**, respectively.

The ODPAP was the highest (8.33% in average) for the AG8025 hybrid (192.39 g) compared to P30R50 (177.59 g). Coherency in AG8025 hybrid showed the highest plant heights (PH) at all the phenological stages evaluated, even having presented 0.75 leaves less at R_1 for P30R50 hybrid. The highest ODPAP caused the AG8025 hybrid to have a photosynthetically efficiency above P30R50 along with a high 1000GW and DPGS, making it become more productive. This corroborates the findings of Cui et al. (2009), who stated that more productive genotypes are those that produce the largest amounts of dry phytomass at a given site. Thus the highest yield obtained by the AG8025 hybrid might be ascribed to the largest accumulation of ODPAP, DPGS and ODPAP for the P30R50 hybrid.

There was an increasing linear relationship of the DPGS for the AG8025 hybrid (96%) and a 92% fit of the data to the second degree equation for the P30R50 hybrid. The maximum value estimated by means of the fitted equation for the P30R50 hybrid was of 204.74 g with the dose of 215 kg of N ha^{-1} applied in bands (Figure 2e).

Sangoi et al. (2007) obtained DPGS of 157, 153 and 115 g during a three-year evaluation for the simple corn hybrid DKB909 with an application of 100 kg of N ha^{-1} in bands at stage V_5. Whereas Gomes et al. (2007) found a DPGS of 183 g with a dose of 150 kg of N ha^{-1} in bands, whose values were below that obtained herein (202.47 g) irrespective of the hybrid and N dose applied in bands.

The fact that DPGS has responded in a quadratic and linear way to the N doses for P30R50 and AG8025 hybrids resulted in increments of ODPAP, once the variable ODPAP did not respond to N doses applied in bands. The unfolding of N doses within each corn hybrid for ODPAP revealed for the P30R50 hybrid a fit to the quadratic equation. Its estimated dose of 215 kg of N ha^{-1} led to a yield of 383.75 g of ODPAP, whilst AG8025 hybrid showed a linear response (Figure 2f).

The mean value obtained for the variable HI was 0.52, being similar to that of two corn hybrids (Pioneer 3905 and Pioneer 30F06 Bt) and superior to Maizex LF850 RR hybrid studied by Subedi and Ma (2005), whose values were of 0.54; 0.51 and 0.46, respectively. The HI obtained herein for the P30R50 and AG8025 hybrids are in accordance with those obtained by Echarte and Andrade (2003), who reported that corn simple hybrids had shown a more stable HI irrespective of the amount of ODPAP or yield of grains per plant. HI correlated positively with NGR, LS, DS, 1000GW, DPGS and yield (0.56; 0.45; 0.44; 0.41; 0.61 and 0.45**, respectively).

The AG8025 hybrid, besides showing a higher yield as opposed to P30R50 hybrid, also showed a high ODPAP and ODPAP, reinforcing such a relevance and necessity of accumulation of dry phytomass to express productivity potential of the crop at a given site (Cui et al., 2009). Moreover, such a mean HI obtained herein (0.52) falls into the most frequent ranges for a high productive potential of corn hybrids (0.50 to 0.55), being 0.60 the threshold reported for most of the crops in the literature (Sharma-Natu and Ghildiyal, 2005). HI showed a significant correlation with yield (0.45**).

The AG8025 hybrid yield (12,438.81 kg ha^{-1}) was superior in relation to the P30R50 hybrid (10,929.21 kg ha^{-1}), resulting in a difference of 13.81%. Argenta et al. (2003) obtained a difference of 2,900 kg ha^{-1} between the P32R21 and Premium hybrids, whose yields were 12,400 and 9,500 kg ha^{-1}, respectively. Discrepancies in yield for corn hybrids were also found by Ferreira et al. (2009), with a maximum yield of 10,553 kg ha^{-1}.

Variables such as NGR, LS, DPGS and ODPAP were simultaneously affected by the factors, corn hybrids and N doses applied in bands; however, interaction between such factors was not observed for yield. These results prove that yield of corn hybrids comes from the expression of each one of such components, as well as from the accumulation of dry phytomass as influenced by N availability.

A fitted second degree equation (Figure 2g) allowed the identification of the dose 295 kg of N ha^{-1} in bands, with which the maximum yield is to be achieved (13,032.93 kg ha^{-1}). Hurtado et al. (2009) obtained an estimated maximum yield of 9,210 kg ha^{-1}, corresponding to the dose of 242 kg of N ha^{-1} applied in bands. Nevertheless, Holland and Schepers (2010) over the course of three years of evaluation reached the conclusion that the dose of 200 kg of N ha^{-1} caused the crop to attain yields of 11,530, 12,110 and 13,660 kg ha^{-1}.

Sangoi et al. (2009) reported that the application of two doses of 100 kg of N ha^{-1} in bands at phenological stages of V_4 and V_{10} of the Ritchie scale (Ritchie et al., 2003) resulted in yields of 12,634 kg ha^{-1} for the simple P30F53 hybrid. Such an outcome is quite similar to yields obtained in the current work for the AG8025 hybrid under the dose of 295 kg of N ha^{-1} in bands with only one application at V_5. This is important because the P30F53 simple hybrid is largely grown in production fields of the region of Campos Gerais, Paraná, Brazil.

The assessment of amounts of N on corn plants for most of the variables evaluated in the current study revealed a significant effect. However, for variables such as NTEL and NGRS no effect of N applied in bands was observed in production fields of corn. Nevertheless a connection between N responsiveness, as well as differentiation timing and definition of plant variables, indicates the possibility of managing N at different stages under sustainable approaches in agricultural systems.

The scrutiny of corn plants by means of variables related to crop growth and development, yield components, as well as accumulation of dry phytomass in compliance with N availability allows for the characterization of corn yield – a fact that might be confirmed by the positive correlation between corn yields with most of the studied variables herein. There is high correlation between yield and NGR (0.70**); LS (0.70**); DS (0.71**) and 1000GW (0.66**), as well as dry phytomass of grains per stalk (r = 0.77**) and overall dry phytomass of the aerial part per plant (0.73**). Moreover, from the characterization of corn yield the importance of the plant as a parameter to manage N in bands might be taken into account in order to maximize productivity and minimize the costs of production in a given corn farming system.

Conclusions

The N application at the phenological stage V_5 allowed for the identification of differentiated responses of the studied variables, including lack of response for some of them. This permitted characterization of the effect of N on corn hybrids and also the identification of phenological stages for N management, where V_7-V_8 was considered to be the limits for N application in bands under the environmental conditions in this study.

Knowledge of the influence of N doses either on the variables related to crop growth and development, yield components or accumulated overall dry phytomass and its dependence relationship with yield at each production field might lead to the formation of yield, and to manage nitrogen fertilization in bands under sustainable approaches in agricultural systems.

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Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Transmission and effects of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton seeds

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This paper aimed to evaluate the transmission of *Fusarium oxysporum* f. sp. v*asinfectum* (*FOV*) and the effects of this pathogen on the initial development of cotton plants following inoculation of seeds. Two cultivars (susceptible and resistant) and two strains (most and least aggressive) of the pathogen were used in this study. The inoculation method was based on the contact between seeds and fungal colonies on substrates containing mannitol. Percentage of *FOV* in seeds and the percentage of seed germination were evaluated by blother test and germination test, after inoculation. Emergence of seedlings and speed index, initial and final stands, size and dry weight of the plants were verified in trays containing soil substrate. Disease severity, pathogen transmission and plant infection, from seed to plant, were determined in separate trial on plants. Occurrence of the pathogen was higher when inoculum potential was increased for all variables analyzed. The number of normal seedlings, determined by seed germination test, decreased when the incidence of the pathogen in the seed was increased. The same occurred to other variables, in which there was difference between cultivars where IAC 20-233 presented the best performance. No significant differences were found between strains for emergence speed index, initial and final stands variables. Transmission and infection rates were increased according to the inoculum potentials increasing and the maximum pathogen transmission rate, from seed to plant was around 50%.

Key words: Transmissibility, pathogenicity, fusarium wilt disease, cotton.

INTRODUCTION

Fusarium wilt, caused by *Fusarium oxysporum f. sp. vasinfectum* (Atk.) W.C. Snyd. & H. N. Hans, is one of the most important diseases of cotton (*Gossypium hirsutum* L.) causing severe yield losses worldwide. The disease can reduce cotton productivity to intolerable levels in countries located specially in Africa, Asia, USA, South America and Oceania (Davis et al., 1996, 2006; Wang et al., 2006). The pathogen is considered to be soil-borne with a parasitic phase in the plant and a saprophytic phase in the soil or in plant debris after harvest (Wang et al., 2006). In addition to soil, *FOV* can be disseminated by infected seeds and plant materials and by contaminated tools (Hillocks and Kibani, 2002). The occurrence of this pathogen on seed lots of cotton is variable, ranging from 0.6 to 47% (Kulkarni, 1934; Veigas, 1935; Perry, 1962; Bennett et al., 2008). However, the mechanisms of seed infection of *FOV* and its transmission from seed to seedling and seedling to plant still not fully understood and disserve our attention.

The use of resistant cultivars has been the most effective strategy to control diseases like *FOV* wilt in several crops. However, the success of breeding programs to control wilt disease in cotton depends on the understanding of the population structure of *FOV* as well as how the pathogen is transmitted (Wang et al., 2006). In addition, other difficulties faced in this pathos system are the presence of other organisms that have been found associated to wilted cotton plants during their early stage of seedling development. Symptoms of *FOV* wilt in cotton can be identified at both early and late stages of plant development depending upon the aggressiveness of the isolate (Smith et al., 1981; Kim et al., 2005; Davis et al., 2006).

Information on specific resistance of cotton cultivars to *FOV* wilt that are transmitted through seed is presently absent. This information, in addition to others of epidemiological nature, is of fundamental importance as that pathogen has been proposed to be part of the list of non-quarantine regulated pests in Brazil (Machado, 1994). Therefore, the establishment of health standards of *FOV* in seed lots as a part of the seed certification programs in Brazil could give a good contribution to provide healthier planting material. Thus, the objectives of this study were to evaluate the transmission rate of two strains (most and less aggressive) of *FOV* from cotton seeds to emerged plants and to study the effect of this infection on the development of two cultivars of cotton (susceptible and resistant) in the initial stage of plant under controlled conditions.

MATERIALS AND METHODS

Seed

Seeds of cotton, cultivars IAC 20-233 and FM 966, considered resistant and susceptible to Fusarium wilt, respectively, were used in the present study. Both cultivars were provided by Agronomic Institute of Campinas (IAC), Sao Paulo, Brazil. All seed batches used in this research were submitted initially to germination and health tests, following descriptions in the Brazilian Rules for Seed Analysis (Brazil, 2009). The germination test was carried out on towel paper, in which 25 seeds were distributed evenly on each roll pad, with four pads per treatment. For the health test blotter method was used following description in BRASIL (2009), except that 2,4-D (Sodium dichlorophenoxyacetate) at 10 ppm concentration was incorporated to blotter substrate (Neergaard, 1979; Machado and Langerak, 1993). These preliminary evaluations were conducted at the Seed Pathology Laboratory of the Plant Pathology Department and at the Seed Analysis Laboratory of the Agronomy Department, both in the Federal University of Lavras, Minas Gerais, Brazil.

Seed inoculation

The two strains of *FOV*, used were selected according to their virulence levels presented in previously pathogenicity test carried with 49 isolates.

Seeds were inoculated using the method of osmoconditioning as described by Machado et al. (2004). According to this methodology, colonies of the selected strains were initially produced on SNA medium under incubation at 25 ± 2°C, with photoperiod of 12 h for seven days. Using a Neubauer chamber, conidial suspensions were prepared with 20 ml sterile water to obtain a final concentration of 10^6 spores/ml. One millimeter of each suspension for each strain was distributed in Petri dishes on PDA medium amended with 46.3 g of mannitol (osmotic restrictor) per liter, to provide an osmotic potential of -1.0 Mega-Pascal (MPa). The amount of mannitol was indicated and adjusted according to the software MPPS (Michel and Radcliffe, 1995). The amended medium with mannitol was used to achieve different inoculum levels on infected seeds for preventing the germination seed (Machado et al., 2004). After three days of Petri dishes incubation, the strains were grown on the agar amended medium, completely. Then, seeds of the selected cultivars, were previously immersed in sodium hypochlorite 2% solution for 1 min, washed in sterile water three times and were dried over night at room temperature. After that, disinfested seeds were distributed evenly on the developing colonies of the *Fusarium* strains, organized in a single layer on the top of the agar amended medium. All Petri dishes were then incubated at temperature of 25 ± 2°C, were kept for 48, 72 or 108 h. Agar amended medium without inoculum was used with seeds of both cultivars as controls. Following each incubation period, seeds were removed from the dishes and placed on filter paper pads to dry during two days at room conditions.

Seed health analysis and germination test after inoculation of seeds

Inoculated seeds were surface disinfected as described previously. Seeds were tested by the PCNB Agar method as described by Sousa et al. (2008), with addition of mannitol at concentration indicated by the software MPPS (Michel and Radcliffe, 1995). To achieve -1.0 Mega-Pascal (MPa), 46.3 g of mannitol were used for each liter of medium. After seven days of incubation, at temperature of 22 ± 2°C and a photoperiod of 12 h, the seeds were examined in a stereomicroscope (Coleman®, 40x) for the presence of *FOV*. Identification of pathogen was based on morphological features of the conidia (shape, size and arrangement), conidiogenous cell formation (mono- or polyphialides) as well as growth pattern, pigmentation and conlony shape (Leslie and Summerrell, 2006). The occurrence of *FOV* was registered in a

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total of 200 seeds and the incidence was determined as percentage of each *FOV* strain in the sample of seeds.

For determination of germination test, 200 seeds of each cultivar inoculated with *FOV* were used and, unlike the seed health test, seeds were not previously disinfected. At the germination test was determinated the percentage of normal seedlings. Both health and germination tests was performed according to the Brazilian Rules for Seed Analysis (BRASIL, 2009) and were conducted in completely randomized design with eight and four replicates, respectively.

Transmission of *F. oxysporum* f. sp. *vasinfectum* from inoculated seeds to emerged plants

To study of the transmission of *FOV* from seed to seedlings, seeds were sowed in polyethylene pots of 5 kg capacity and trial conducted for 45 days. Ten seeds were sown for each treatment on a substrate composed by a mixture of autoclaved soil and sand in the proportion of 1:1. The experiment was in randomized design with for replicates an conducted in growth room at 25 ± 3°C with a photoperiod of 12 h. The transmission of the pathogen was calculated based on the disease severity (DS), infection rate (IR) and transmission rate (TR). The evaluation of DS was performed by observing symptoms on the leaves and stems and basing on a grading scale varying from 0 to 3, in which, 0 = healthy plants, 1 = wilted plants 2= dead plants and 3 = plants not emerged. The data were analyzed by applying the formula described by McKinney (1923). IR and TR were determined using the methodology adapted from Teixeira and Machado (2003). To determine the IR, stem fragments of emerged plants were sampled and cut out about three centimeters above the substrate line and then sectioned longitudinally and decontaminated respectively with sodium hypochlorite (2%) for one minute. Disinfected fragments were placed on Petri dishes with PCNB medium and then incubated at 22± 2°C, with photoperiod of 12 h for seven days (Sousa et al., 2008). After this period, the plates were examined for the occurrence of mycelial growth of *FOV* on the fragments and on the culture medium, using stereomicroscope (Coleman®, 40x). The TR was determined based on the mathematic relation between infection percentage (IR) and the incidence percentage (I) of that pathogen as recorded on the tested fragments of cotton plants and on inoculated seeds by the health testing.

Effects of *F. oxysporum* f. sp. *vasinfectum* on the growth of cotton seedlings

The assays were carried out in polyethylene boxes with dimensions of 48 × 29 × 10 cm containing substrate composed of soil and sand (1:1) previously treated with methyl bromide. Two hundred seeds were sown per treatment, 50 seeds per box. The experiment was conducted in a growth room with temperature of 25±3°C and a photoperiod of 12 h for 25 days after sowing (DAS). The variables observed were: Emergence Speed Index (ESI), Initial and Final Stand (IS and FS), Seedling Height (SH), and Dry Weight (DW).

The emergence of seedlings was determined by daily counts of seedlings until the stabilization of the number of seedlings for three consecutive days. The ESI was calculated according to Maguire (1962) by equation:

$$ESI = \sum_{i=1}^{n} Ni/Di$$

Where ESI = Emergence Speed Index; Ni = Number of emerged seedlings the 1st count, 2nd count, ... nth count, respectively; Di = Number of days after sowing the 1st count, 2nd count, ... nth count, respectively.

The values for the IS and FS were recorded at 8 and 25 days after sowing, respectively, and the number of plants recorded at these two periods was converted in percentage of emerged seedlings. Data on seedling height (cm) were obtained by measuring, with a ruler, ten emerged plants taken at random, per replicate, by cutting them at the ground level. Then all sectioned plants were submitted to drying in forced air oven (Marconi® model MA035) at temperature of 50°C for 72 h. Plant material was weighed using a semi-analytical scale (Gehaka® model AG 200) and the results were reported in grams (g).

Statistical analysis

The analysis of variance, from all the variables, were carried out in a factorial 4 × 2 × 2, where the factors were four exposure times of seeds to pathogen (0, 48, 72 and 108 h); two cultivars (IAC 20-233 and FM 966); and two strains (CML 1098 and CML 1135) using a computer statistical analysis system (SISVAR) (Ferreira, 2011). The data were processed using the □x or □x + 0.5 transformations when necessary and the means between treatments were compared by mean comparison test (Tukey test, P ≤ 0.05) or by regression according to the nature of the data.

RESULTS AND DISCUSSION

Initially germination and health tests, of both cultivars of cotton, IAC 20-233 (resistant) and FM 966 (susceptible), presented 85 and 72% of germination and 1.5 and 0% of incidence of *FOV*, respectively.

It was observed that percentage of germination of seeds in both cultivars was proportionally lower whereas the inoculum potentials in the seeds increased, represented by exposure times of the seeds to the pathogen (Figure 1). Comparing the effects of the isolates on the cultivars germination, it was observed that the cultivar IAC 20-233 presented better performance than the cultivar FM 966 in the presence of the two isolates whereas no difference was noticed between the isolates CML 1098 and CML 1135 for the cultivar IAC 20-233 (Table 1). This result can be directly traced not only to the cultivar resistance but also to the initial physiological quality of the cultivars used.

Analyzing the initial profile of the seeds, cultivar IAC 20-233 showed better physiological condition than the cultivar FM 966 (Figure 2). This result was observed due to the higher percentage of seed germination of this cultivar in relation to cultivar FM 966. Despite being considered 'resistant' to Fusarium, the presence of *FOV* in seeds of cultivar IAC 20-233 provided germination reduction of 21 and 22% for the strains CML 1098 and CML 1135, respectively. The occurrence of *FOV* on seeds was significantly different among the times of seed exposition to the pathogen or, in other words, according to the increasing inoculum potential. The increase in the pathogen incidence on inoculated seeds through all inoculum potentials proved the efficiency of this methodology which allowed the presence of the pathogen

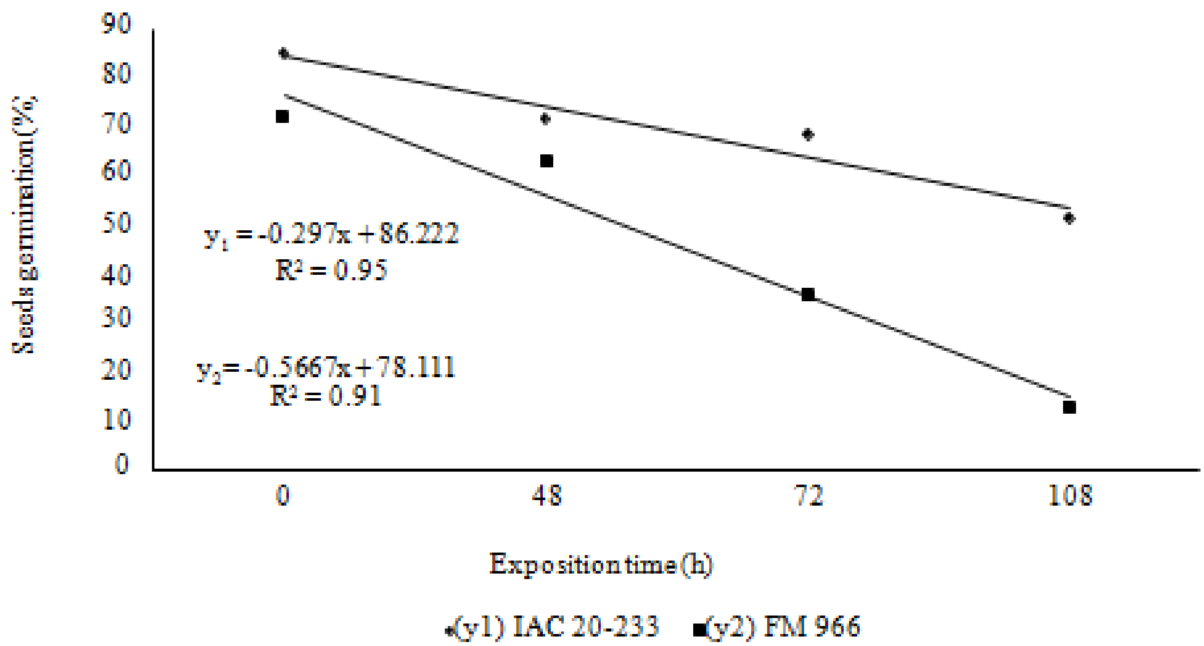

Figure 1. Cotton seeds germination from the cultivars IAC 20-233 and FM 966 inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*, related to the time of exposition to the pathogen.

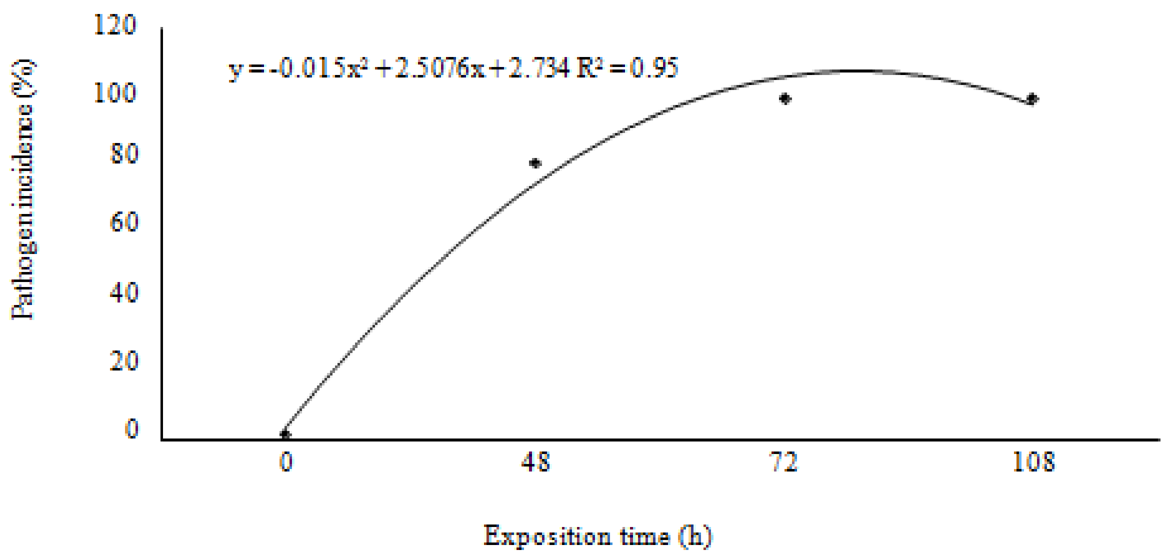

Figure 2. Incidence of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton seeds according to the exposition time.

in high levels in seeds. Accordingly, similar results and observations from other papers support this as the most appropriate technique (Teixeira and Machado, 2003; Teixeira et al., 2005; Araújo et al., 2006; Sousa et al., 2008).

The effect of inoculum potential on the seeds, observed in the health test, was reflected in the DI measured in cotton plants 45 days after sowing. There was an increase of the DI while the inoculum potential obtained by the time of inoculation increased from 0 to 108 h of seed exposition to the pathogen for both cultivars studied. The highest DI was obtained for the cultivar FM 966 (89.2%) whereas for the cultivar IAC 20-233, considered susceptible was 47.5% (Figure 3).

According to DI results of the work, Sousa et al. (2008) found an increase in DI while the exposure time of seeds

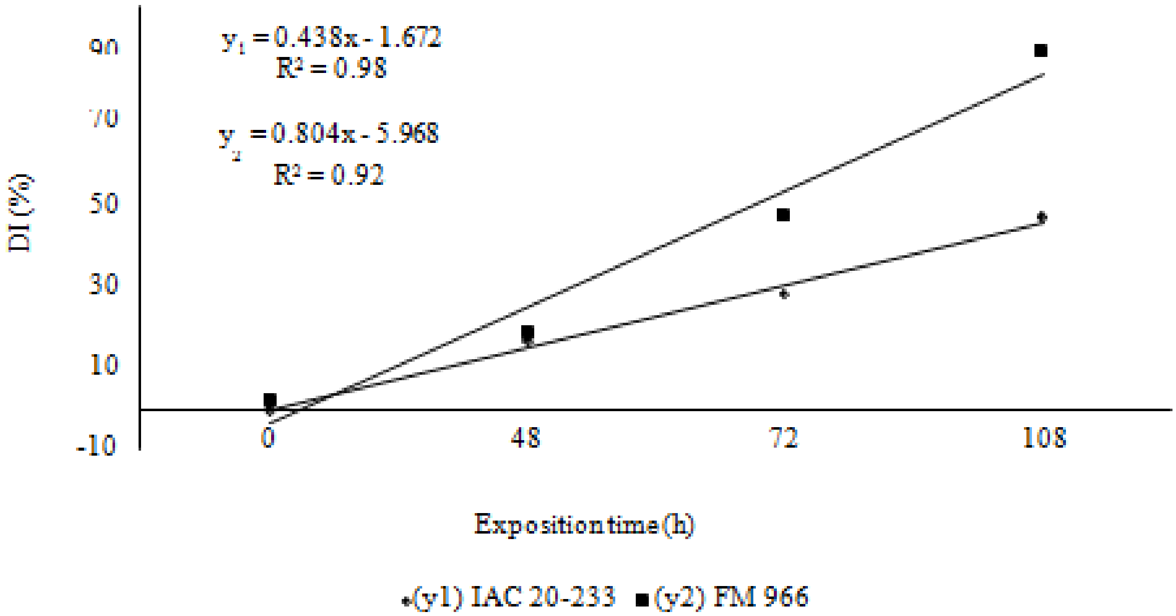

Figure 3. Disease index (DI) of cotton plants (45 days after sowing) related to the IAC 20-233 and FM 966 cultivars, from seeds inoculated with *Fusarium oxysporum* f. sp. *vasinfectum* in different exposition times.

to *FOV* was from 24 to 120 h for all three seed inoculation methods used. In the other work, Teixeira et al. (1997) also observed that the exposure time (0, 15 and 30 h) influenced the DI of cotton seedlings, grown from seeds inoculated with *Colletotrichum gossypii* South. Additionally, the authors reported the increase of the DI in both desinfected and non desinfected seeds after inoculation. On the other hand, Araújo et al. (2006), evaluating the pathosystem *C. gossypii* South. var. *cephalosporioides* A.S. Costa in cotton plants, found that the inoculum potential did not increase significantly the incidence and severity of seedling disease in cotton in all exposure time evaluated (36, 72 and 108 h) even though these rates have been high starting in the shortest period of the seeds exposure to the pathogen.

The influence of strains in severity was greater for the cultivar FM 966 than for the cultivar IAC 20-233 (Table 1). In this context, the resistance factor may be clearly noticed in the infection process and transmission of the pathogen from seed to plant.

In this study, one of the objectives was to investigate the infection rates and the transmission of the pathogen aiming to clarify this gap on the infection process. The results observed show the significant difference between the times of exposure to the pathogen reinforcing that the inoculum potential does make difference in the infection and transmission process. As the assessment of the rates was made in plants that remained alive until 45 days after sowing. The results about the rates of infection and transmission observed in this study may be related to the low percentage of emergence observed, especially in the time of 108 h. This may have occurred due to the death of seeds, caused by high infection by the pathogen at 108 h.

Thus the rates of infection and transmission, measured in stem fragments of cotton plants were higher in proportion to the increase of the inoculum potential, reaching a maximum of 51% in both cases. The point of maximum infection and transmission was observed at 78 h of contact between the seeds and the pathogen. From that point on only a small reduction in the values was observed for both the rates (Figure 4).

In this context, it was clear that the infection of the plant tissues may be directly related to the inoculum potentials reached by the seeds. From these results, it is assumed that the transmissibility of *FOV* from cotton seeds is effective and can reflect higher or lower rates of infection and transmission, depending on the level of seed infection presented.

In a similar study conducted with corn kernels inoculated with *Acremonium strictum* Gams, Teixeira and Machado (2003) observed, however, that the infection rate, as measured in the aerial parts of the plants 28 days after sowing, was higher according to the increase of the inoculum potential of the seeds (0 to 120 h). Nevertheless, no difference was reported for the transmission rate at 24, 72 and 120 h, differing only in time 0 h.

Reports of natural infection of cotton seeds from symptomatic plants have been described in Tanzania with levels around 47% (Perry, 1962). However, values below 10% of infection have been more common, particularly when seeds are originated from non-

Table 1. Seed germination, disease index (DI) in plants with 45 days, emergence speed index (ESI) and final stand for 25 days related to both strains of *Fusarium oxysporum* f. sp. *vasinfectum* (CML 1098 and CML 1135) and both cotton cultivars (IAC 20-233 and FM 966).

Strains	Seed germination (%)*		DI (%)*		ESI*		Final stand (%)*	
	IAC 20-233	FM 966	IAC 20-233	FM 966	IAC 20-233	FM 966	IAC 20-233	FM 966
CML 1098	64.0[Aa]	33.0[Bb]	28.9[Ab]	52.2[Aa]	35.01[Aa]	25.08[Ab]	35.0[Aa]	26.0[Ab]
CML 1135	63.0[Aa]	41.0[Ab]	33.3[Ab]	52.5[Aa]	31.41B[a]	25.67[Ab]	33.0[Aa]	24.0[Ab]
CV (%)	16.08		20.36		8.85		15.34	

*Means within a column and line followed by the same letter were not different (Tukey P > 0.05); CV: Coefficient of variation.

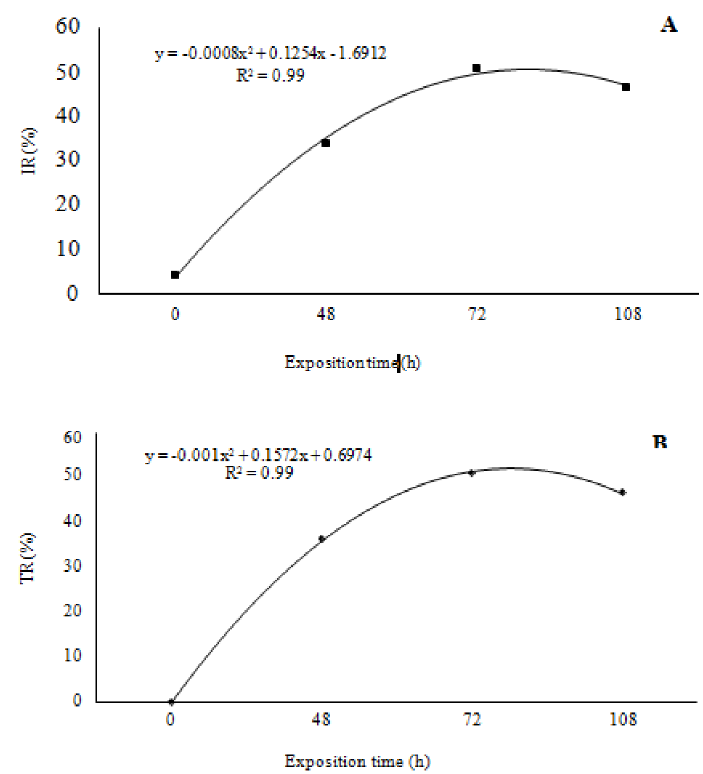

Figure 4. Infection Rate (IR) (A) and Transmission Rate (TR) (B) of *Fusarium oxysporum* f. sp. *vasinfectum* evaluated in cotton plants (45 days after sowing), depending on the exposition times to the pathogen.

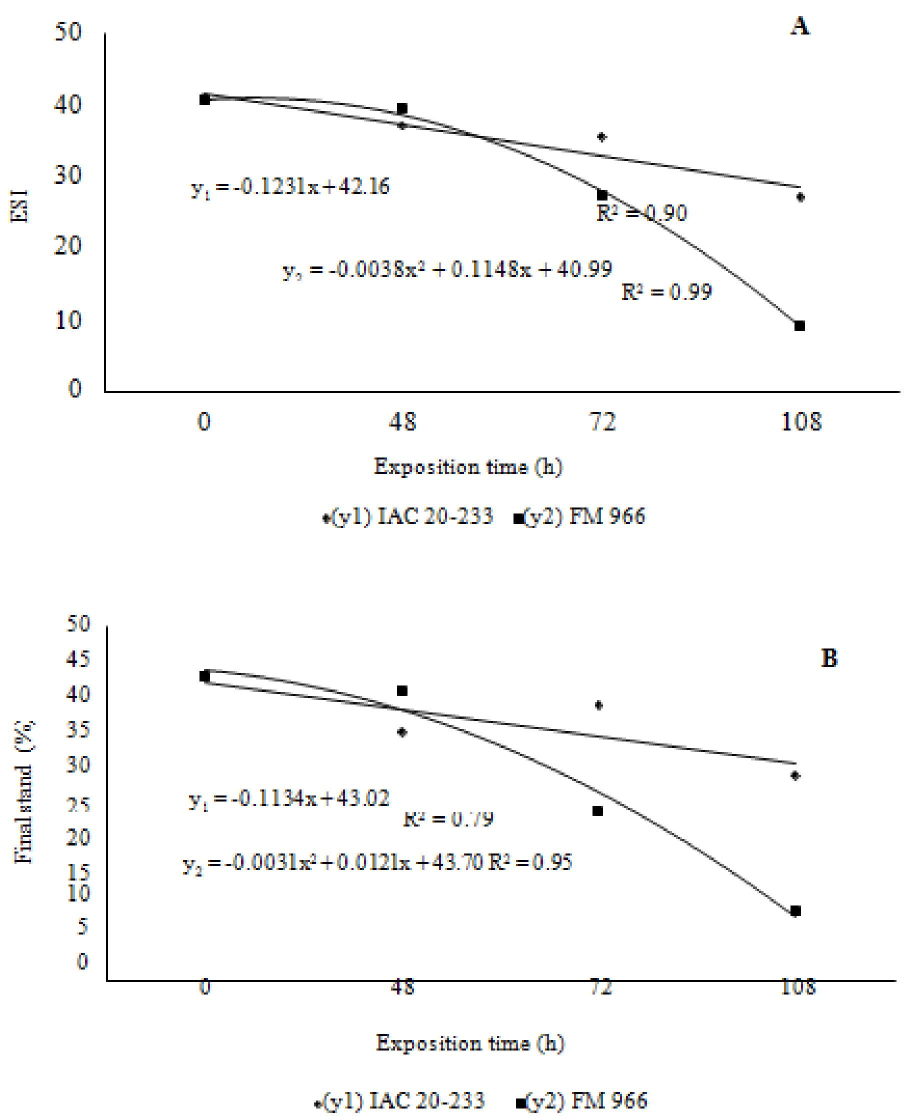

Figure 5. Emergence speed index (ESI) (A) and final stand (B) of cotton cultivars, IAC 20-233 and FM 966 related to the exposition times of seeds to the pathogen.

symptomatic cultivars that have moderate resistance (Hillocks, 1992). According to Bennett et al. (2008), low seed infection rates may influence the capacity of the pathogen to survive to acid-delinting and subsequently infect seedlings. The same authors have observed that *FOV* race 4 is able to infect the cottonseed in California field conditions confirming the pathogen seed transmission ability.

Other noticeable results were observed such as the reduction in both seed vigor, represented by the emergence speed index (ESI), as well as in the final stand (FS), according to the inoculum potential increasing for both the cultivars evaluated. As a whole, as the inoculum potential in the seeds increased the germination and vigor of it was significantly reduced and influencing the initial and final stands as well. The reduction was greater in cultivar FM 966, considered susceptible to *Fusarium* wilt and this result could be related to the health tests and germination standard (Figure 5).

Results about initial and final stands obtained here are consistent with those obtained by Sousa et al. (2008), who observed decrease in ESI and on final stand of cotton seedlings inoculated with *FOV*. In another work, studying the influence of various pathogens in cotton, among which *FOV* was tested, the variables in question were also negatively affected by the difference in osmotic potential and exposure time of seeds to the pathogens (Machado et al., 2004).

Comparing the isolates used, even though their effects on the final stand were not observed for both cultivars in the IVE for cultivar IAC 20-233, not only a difference was noticed but it was also verified that the

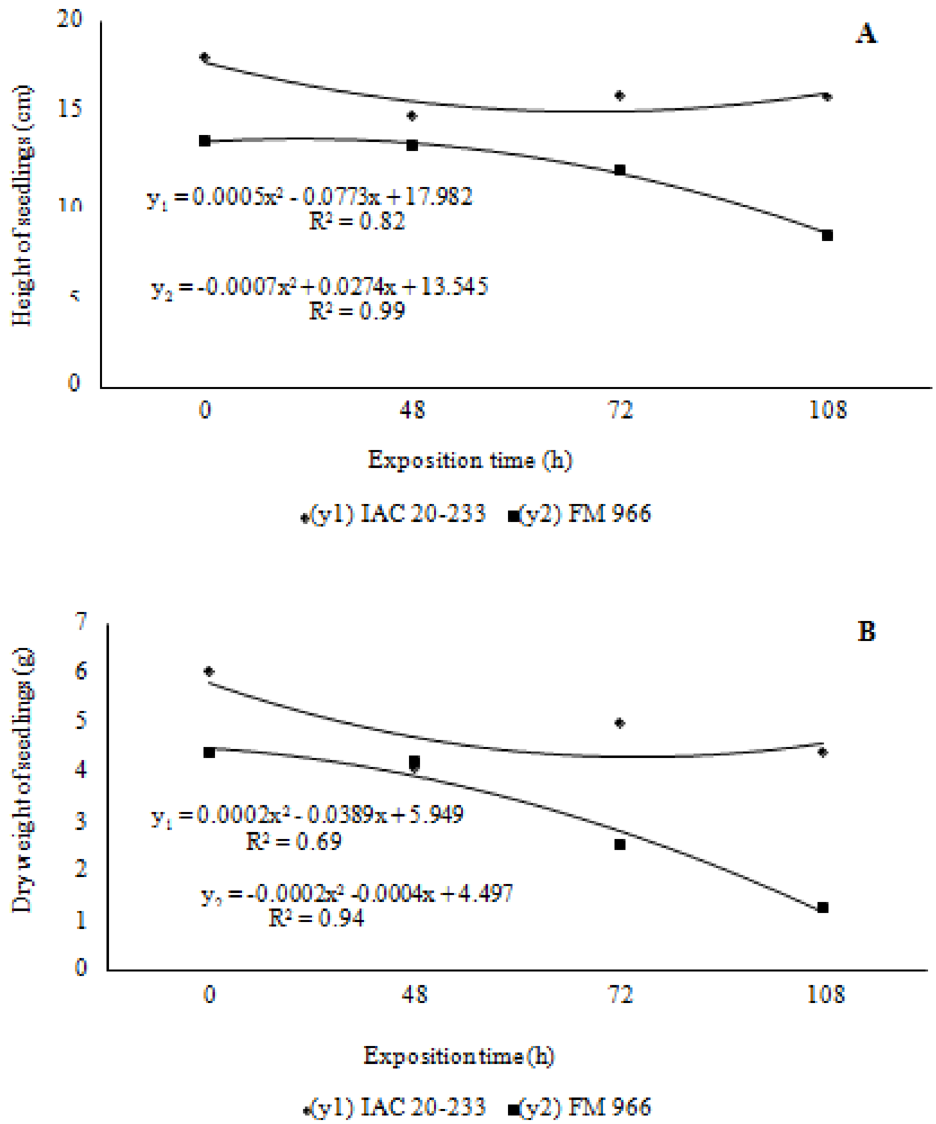

Figure 6. Height (A) and shoot dry weight (B) of seedlings of the cotton cultivars, depending on the time of exposition to the pathogen.

CML 1135 strain significantly reduced the emergence rate compared to the CML 1098 strain (Table 1).

In the same way, the inoculum potential significantly reduced the percentage of cotton seedlings, while this was increased from 36 to 108 h of seeds infected by *C. gossypii* var. *cephalosporioides* (Araújo et al., 2006). In contrast, different results were observed by Teixeira and Machado (2003), in which the effect of the priming (osmotic restriction) and exposure time increased the ESI for corn seedlings from seeds inoculated with *A. strictum*, and the highest values were observed at the points of 72 and 120 h. This stimulation at the speed of emergence was also observed by Carvalho (1999), in which the solute mannitol added to the culture medium of -1.0 Mega-Pascal (MPa) for up to 120 h, caused a priming effect, stimulating the emergence of bean seedling.

The effect of exposure time or inoculum potential on the seedling height and dry weight was gradually similar for both cultivars. However, the reduction noticed for the cultivar IAC 20-233 was lower than that observed in cultivar FM 966 (Figure 6).

Despite the amount of inoculum present in seeds, seedlings, cultivar IAC 20-233 presented more resistance, resulting in higher development and production of dry weight, even when time increased from 0 to 108 h of exposure. Likewise, the height and dry weight of maize seedlings inoculated with *A. strictum* were lower with increasing time of exposition to the pathogen (0, 24, 72 and 120 h), showing the effect of inoculation on growth of seedlings (Teixeira and Machado, 2003).

The differences between both cultivars in this work showed clearly the genetics of host resistance taking place against the pathogen during the vegetative period. This fact may be considered with great relevance to better understanding all the events related to pathogen transmission from seeds, as well as to improve measures of diseases control on seed production fields.

Conclusion

In general, the findings of this study made it possible to see the differences between both cultivars in respect of genetics of host resistance and the inoculum potencial,where the occurrence of the pathogen were higher when inoculum potential was increased for all variables analyzed. The number of normal seedlings decreased when the incidence of the pathogen in the seed was increased. The same occurred to other variables, in which there was difference between cultivars where IAC 20-233 presented the best performance. Also, no significant differences were found between strains for emergence speed index, initial and final stands variables and the transmission and infection rates were increased according to the inoculum potentials increasing and the maximum pathogen transmission rate, from seed to plant was around 50%.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Irrigated sorghum and cowpea after wet-season rice as a pathway out of subsistence agriculture in the Senegal River Valley in Mauritania

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Livestock is more important to the Mauritanian economy (13% of National Gross Domestic Product) compared with just 4% from crop production. It is surprising, therefore, that irrigation has so far contributed little to animal productivity given the limited carrying capacity and irregular inter-annual production of the extensive rangeland, and the need to import fodder concentrates to sustain livestock during the *hot-dry season* (April to June). Our hypothesis is that growing irrigated sorghum and cowpea planted late in the *mild-dry season* (December-January after rice harvest (November-December) would improve profitability and sustainability of irrigation schemes. The objective is to replace fodder concentrates, currently purchased abroad, by fodder grown in irrigation schemes. To test our hypothesis we performed an extensive survey of 12 villages located within a significant gradient of rainfall from West to South-East along the northern bank of the Senegal River and used a simulation model constructed to study interactions between traditional and irrigated grain and/or fodder crop. The results of this study reveal how the introduction of irrigated sorghum and cowpea sown late in the *mild-dry season* would, even at relatively moderate yields (2.8-4.1 and 1.4-2.1 t grain ha^{-1}, for sorghum and cowpea, respectively), provide additional grain required by smallholder farmers and reduce costs of livestock production (30%). In addition, the introduction of these crops increases by 31 to 54% the household net agricultural production of households having only small ruminants and by 14 to 23% of households having both small ruminants and cattle. Extending the irrigated cropping season shares the depreciation costs over more crops and improves the sustainability of the irrigation schemes. The regions that would most benefit from these additional irrigated crops are Trarza and Gorgol where the demand and hence prices of sorghum and cowpea grain and fodder are higher than in the other regions.

Key words: Mauritania, livestock, irrigated rice, irrigated sorghum, irrigated cowpea, household net agricultural production.

INTRODUCTION

In Mauritania, rangeland pasture production and cropping of high land (*Dieri*) and/or depressions (*Bas–fonds)* where local runoff accumulates provide the best opportunities for primary production during the *wet season* (July to September). In addition, during the *mild-dry season* (October to March, a dry period with relatively moderate temperatures), flood-recession agriculture is practiced along the bank of the Senegal River both on land close to the river that floods every year (*Falo*) and also in depressions within more extensive plain areas that flood variably from year to year (*Walo*). Pearl millet is the predominant crop in *Dieri*, sorghum in *Bas-fonds* and *Walo* and maize in the *Falo*. These cereals are usually grown associated with cowpea (Comas et al., 2012).

Once the *wet season* has finished, inland transhumant livestock gradually migrate south towards the river where they increasingly compete with sedentary livestock of the Senegal River villagers for fodder and watering until the following *wet season*. While sedentary herds can complement rangeland pasture with crop residues that become available in October (*Dieri* and *Bas-Fonds*), December (irrigated rice) and February (*Walo* and *Falo*) a significative feed deficit occurs during the *hot-dry season* (April-May to June-July) when farmers must buy fodder concentrates.

In response to the prolonged drought of 1968 to 1973, about 40,000 ha of *Walo* land were transformed in the 1980s into irrigated rice schemes protected from flooding by levee banks, although nowadays less than 50% of that area remains in production (Mateos et al., 2010). In general, farmers produce one rice crop per year leaving the land bare the remaining months despite continuing availability of water. Considering the importance of livestock in the Mauritanian economy (13% of National Gross Domestic Product compared to 4% from crop production), its profitability, and the limited carrying capacity and irregular inter-annual production of the rangeland together with the need to import fodder concentrates, it is surprising that irrigation contributes little to livestock production. At present, it is limited to direct grazing of paddy straw after harvest and more recently sale of paddy straw and bran to newly establish peri-urban dairy farming in the capital Nouakchott and the regional centers, Rosso and Boghé (Corniaux et al., 2001). To support the dairy development, importation of groundnut meal has increased markedly to average around 9,600 t annually during the 2000s (Direction Générale des Douane, 2012).

Our hypothesis is that growing irrigated sorghum and cowpea crops for animal fodder during the *mild-dry season* after rice harvest in November-December, would improve profitability and sustainability of irrigation schemes. For this, it must be demonstrated that the required investment is affordable, the production costs of grain and fodder are below market price and that there is no clash for labour with the traditional flood recession *Walo* crops that are planted from October onwards. Both sorghum and cowpeas are well known in the region and less demanding water than rice. To test our hypothesis we have used data from an extensive survey of 12 villages located within a significant gradient of rainfall from West to South-East along the northern bank of the Senegal River (Comas et al., 2012), and a simulation model (Connor et al., 2008) constructed to study interactions between traditional crops and rice in the region that included an optional second irrigated grain and/or fodder crop planted late during the *mild-dry season.* Proof of concept is an essential prelude to, and an encouragement for, validation by experimentation.

MATERIALS AND METHODS

Surveyed villages

All households of 12 villages with small and medium-sized community-managed irrigation schemes (< 100 ha) spaced along the northern bank of the Senegal River were surveyed, ten in 2008 and two in 2009 (1,584 households in total). This area comprises, from West to South-East, the south-west of Trarza Region and southern parts of Brakna, Gorgol and Guidimaka (Figure 1). Survey information was recorded during personal interviews conducted by trained local technicians. Details of the survey were presented in Comas et al. (2012). In addition to information about farming systems and household characteristics, the following specific information on the management of livestock was collected; (1) numbers of cattle, donkeys, sheep and goats, (2) milk and meat production, (3) quantities and costs of inputs (wages of shepherds and costs of animal health products, and of fodder concentrates for the *hot-dry season* before the first rains) and (4) household consumption of milk and meat. In addition, a weekly survey of (5) retail prices of meat (beef, mutton and goat), milk (cow, sheep and goat), concentrated feed and fodder (sorghum stover, cowpea haulms and rice straw) was made from October 2008 to October 2009 in the riverside towns of Rosso, Boghé, and Kaédi to provide local data to supplement official statistics.

Stocking was expressed as tropical livestock units (TLU, live weight of 250 kg) using conversion ratios of 0.7, 0.5 and 0.1 to estimate TLU for cattle, donkeys and small ruminants, respectively. Daily dry matter intake needs of livestock were estimated at 2.75% of live weight, amounting annually to 2500 kg dry matter per TLU.

Simulation of irrigated sorghum and cowpea

Simulations of sorghum and cowpea crop development, growth and yield, and demand for irrigation water were developed from Connor

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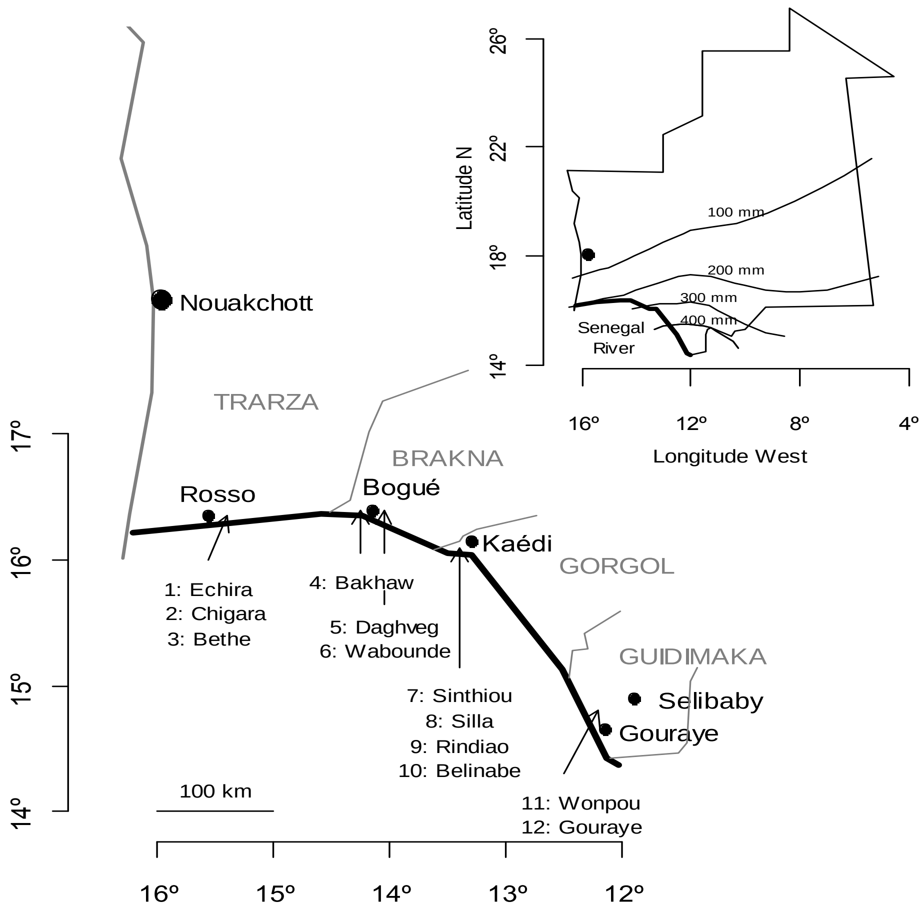

Figure 1. Rainfall zones in Mauritania and location of 12 surveyed villages along the northern bank of the Senegal River. Map based on data from AGRHYMET.

et al. (2008). Variables used were daily rainfall, maximum and minimum temperatures, and sun hours. In addition, daily maximum and minimum relative humidity and wind speed were used to calculate reference crop evapotranspiration (ETo) using the Penman-Monteith equation. Meteorological data were available from three weather stations in Trarza (Rosso, 16.30º N, 15. 48º W), Gorgol (Kaedi, 16.09º N, 13. 30º E) and Guidimaka (Selibaby, 15.09º N, 12. 11º W) for the period 1961 to 2010.

These models integrate the effect of weather conditions, soil type, and management (crop, sowing time, yield and labor, costs of production and market prices) on the productivity of crops. Soils are defined according to rooting depth, water-holding capacity, drainage rate, and adequate fertility. Simulations of growth and yield were made through to maturity for crops sown from December (day 360) to early February (day 35). This option was chosen to avoid the subsequent *hot-dry season* of high water demand and uncertain crop productivity.

Current household net agricultural production

Current costs of production for traditional crops and irrigated rice were obtained from Comas et al. (2012). Livestock operating costs combine health products, shepherding, and supplementary fodder during the *hot-dry season*. The latter costed at market value. Distribution of production costs between milk and meat was made proportional to their mean market values. Household productivity of milk and meat was calculated from the number of cattle, sheep and goats per household, average yields of milk and meat, number of productive animals in the herd and average market price. This was done individually for the four regions in the survey using the following additional parameters: non-adult mortality rate (%), adult mortality rate (%), number of years required to reach reproductive maturity, reproduction rate (%), number of animals born at each birth, number of productive years of females. According to local experts (Kane, 1995) and Kane personal communication these parameters were set as follows: for cows, 15 and 10%, 4 years, 58%, 1 animal, and 11 years, respectively, and for small ruminants: 27.5 and 20%, 1 year, 90%, 1.2 animals and 8 years. Yield of carcass meat is taken as 52% of live weight according to the data in Mauritania. Current household net agricultural production was calculated as the sum of net production of traditional crops, rice and livestock.

Household net agricultural production in the new scenario

Household net agricultural production in the new scenario was calculated by adding to the net production of traditional crops, rice and livestock calculated previously the net production of crops produced after rice harvest and the related improvement to livestock production. Direct cost of production of irrigated *late mild-season* crops incurred includes labor, tractor for tilling the soil before planting, consumption of diesel fuel, fertilizer, seed, and pesticides, plus an interest charge of 15% p.a. for three months (sowing to harvest) according to yield obtained by simulation. Fodder production from crop residues was calculated as 2.5 times grain mass. Depreciation costs of construction of the irrigation perimeter and for purchase and maintenance of pumping equipment were established as in Comas et al. (2012) and distributed between rice and irrigated *mild-season* crops according to the consumption of diesel. Supplementary fodder during the *hot-dry season* was costed according to production while fodder concentrate, if needed, was costed at market price.

Simulation and statistical analysis software

Models for crop simulation analysis were constructed in Systems Thinking for Educational and Research (STELLA); details can be found in Connor et al. (2008). Computing and statistical analyses were made using statistical program of R Development Core Team. Since the distribution of most indices showed a positive skewed distribution, data were expressed in logarithmic form before statistical analysis. To test for differences between villages, analysis of variance and subsequent mean separation were performed using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Climate in the study region and survey overview

Monthly average values of rainfall, ETo and temperature for Trarza, Gorgol and Guidimaka from 1961 to 2010 are presented in Figure 2. Rainfall is concentrated from June to October and shows a strong West to South-East increase (231-516 mm year^{-1}). ETo follows the same seasonal trend at each site; rising to highest values in May and then decreasing during the rains. ETo is greater in Trarza and Gorgol than in Guidimaka due to strong winds blowing from the Sahara to the western regions during the *hot-dry season*. Temperatures remain relatively low during the *mild-dry season* and are similar in all regions (average daily means 20 to 25°C) but increase markedly during the *hot-dry season* reaching monthly averages of 31ºC at Rosso, and of almost 35°C at Kaedi and Selibaby before falling significantly with the onset of rains. With high temperature and ETo, the *hot-dry season* is a particularly harsh period for crops, animals and humans.

On average almost a quarter (22%) of surveyed households have no livestock, nearly half (45%) have only small ruminants, very few have only cattle (4%), but nearly a third (29%) have both small ruminants and cattle. The analysis reveals that there is no clear trend in household type with increasing rainfall from West to South-East (Figure 3). However, it reveals that there are significant differences in the distribution of TLU per resident between households that only have small ruminants and those having both small ruminants and cattle, with median values of 0.09 and 0.37, respectively. While there are no significant differences along the gradient of rainfall for those having only small ruminants, there are for those having both small ruminants and cattle.

The analyses presented in this study to evaluate the impact of livestock on household economy and capacity to meet food requirements, focus on households having small ruminants and both small ruminants and cattle with current access to irrigation. These have the capacity to add a second crop after wet-season rice; they comprise 50 and 63% of households having only small ruminants and small ruminants and cattle, respectively. For these households, labor is readily available (median number of adults active in agriculture is 5).

Survey data (Table 1) show that average productivity of animals varies markedly between regions, showing a strong West to South-East increase, positively correlated with rainfall. The difference is seen especially in milk production (270 to 480 kg per cow and 45 to 120 kg per sheep or goat) in response to the availability of green fodder. This distinction is not significant in meat production, indicating than meat production is less affected by the lack, or quality, of fodder than milk production. Human consumption of fresh milk and meat are essentially similar in all villages. Average annual consumption is 144 and 30 kg per person of fresh milk and meat, respectively, consistent with FAO data on consumption of these foods in Mauritania. Survey data reveal that fodder concentrate is required annually from 120 to 60 days from West to South-East. The survey established a clear trend in the average supply of fodder concentrates, amounting to 430 to 130 kg TLU^{-1} year^{-1} from West to South-East. Average daily quantities supplied to livestock are 2.9 kg DM TLU^{-1} day^{-1}, well below the recommended intake (6.8 kg DM TLU^{-1} day^{-1}). Average fodder concentrate supplied to livestock is 60% groundnut meal and 40% paddy bran.

In households that have only small ruminants, the median value of the ratio of household milk and meat production to consumption is 0.15 and only 1% of these households produce more milk and meat than they consume. In households having both small ruminants and cattle, by contrast, the median value is 0.51 with 25% of households producing more than they consume. Along the valley this ratio is fairly stable for both types of households. However, especially in the South-East, there are some households with livestock production well in excess of consumption. This pattern is similar to the distribution of TLU per resident, that is, households that produce least are in the center of the valley.

Market prices

Market prices of both sorghum and cowpea remain fairly high and stable because households consume most of their production. Survey data show annual market prices for grain in Trarza, Brakna and Gorgol in the ranges 148 to 165 MRO kg^{-1} for sorghum and 160 to 180 MRO kg^{-1} for cowpea and somewhat lower in Guidimaka (MRO: Ouguiya, the Mauritanian local currency, 1 € = 385 MRO)

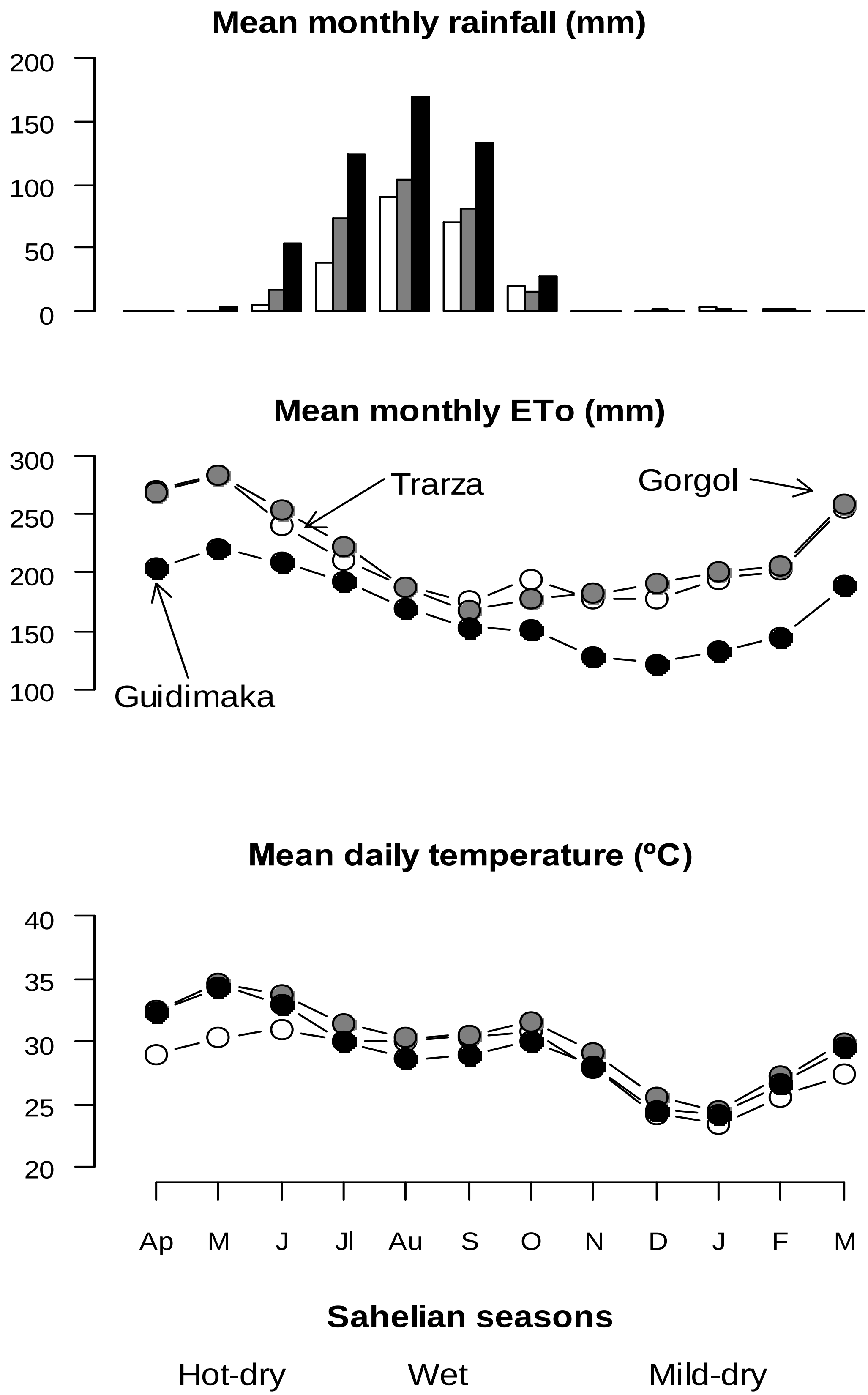

Figure 2. Annual sequences of mean monthly rainfall, mean monthly reference crop evapotranspiration (ETo), and mean daily temperature for three sites along the Senegal River Valley.

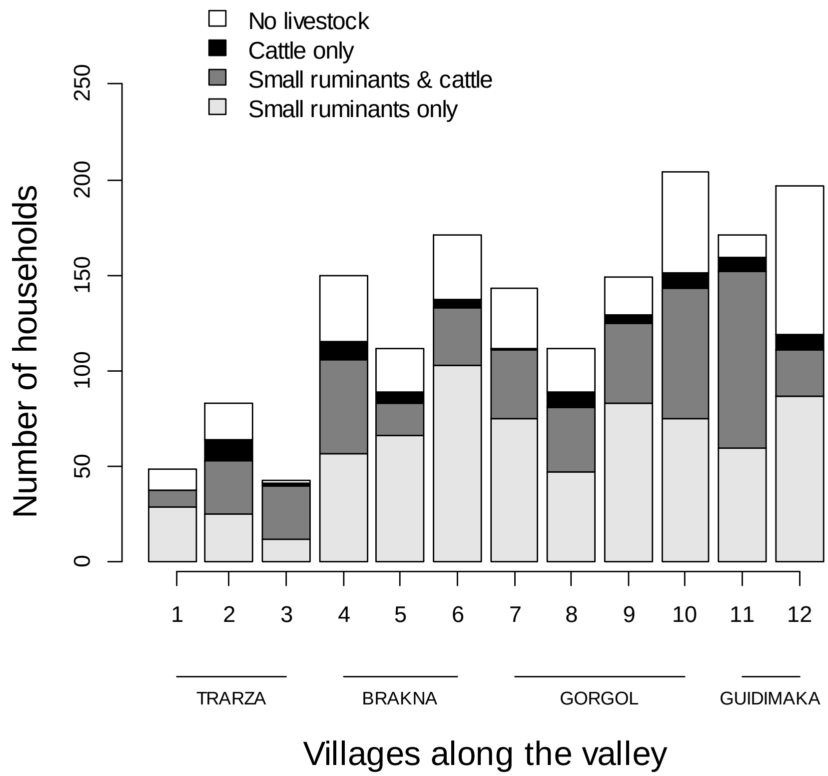

Figure 3. Distribution of household types classified into four groups according to their livestock holdings in 12 villages distributed West to South-East along the northern bank of the Senegal River Valley.

Table 1. Average annual yields of milk and meat and supply of fodder concentrates by small ruminants (sheeps and goats) and cattle in amounts per head and duration of supply in four regions of the Senegal River Valley, Mauritania.

Small ruminants		**Region (from West to South-East)**		
		Trarza	**Brakna and Gorgol**	**Guidimaka**
	days	90	120	120
Fresh milk yield	kg head^{-1}year^{-1}	45	79	120
Meat yield	kg head^{-1}year^{-1}	18	19	20
Concentrated feed supply	days	120	90	60
	kg head^{-1}year^{-1}	30	18	9
Cattle				
	days	90	120	120
Fresh milk yield	kg head^{-1}year^{-1}	270	420	480
Meat yield	kg head^{-1}year^{-1}	105	113	120
	days	120	90	60
Concentrated feed supply	kg head^{-1}year^{-1}	300	180	90

(Table 2). Market price of sorghum stover ranges from 32 to 12 MRO kg^{-1}, from West to South-East, and that of cowpea haulms from 60 to 20 MRO kg^{-1}. Average milk market price increases from Trarza to Gorgol for both small ruminants (200 to 350 MRO kg^{-1}) or cow (227 to 349 MRO kg^{-1}), but it is much lower in Guidimaka (200 and 120 MRO kg^{-1}, respectively). Average market prices of meat follow a decreasing trend of 1,160 to 900 MRO kg^{-1} for small ruminants and 950 to 700 MRO kg^{-1} for calf meat from West to South-East. During the *hot-dry*

Table 2. Average market prices and costs of production at 50 to 75% simulated yield, and labour in four regions in the northern bank of the Senegal River Valley.

Market prices (MRO kg^{-1})		**Region (from West to South-East)**		
		Trarza	**Brakna-Gorgol**	**Guidimaka**
Sorghum	Grain	164	148-165	130
	Stover	32	25	12
Cowpea	Grain	180	160-170	140
	Haulms	60	50	20
Rice	Grain	74	90-89	95
	Straw	9	12	5
Rice bran		20	20	10
Groundnut meal		120	125	130
Milk	Small ruminants	200	300-350	120
	Cattle	227	266-349	200
Meat	Small ruminants	1160	925-900	900
	Cattle	950	792-775	700
Current production costs (MRO kg^{-1})				
Rice	Grain	69	62	76
	Straw	8	8	4
Milk	Small ruminants	77	37	15
	Cattle	138	100	23
Meat	Small ruminants	449	105	111
	Cattle	563	254	80
Production costs in the new scenario (MRO kg^{-1})				
	Grain	58	51	63
	Straw	7	7	3
Irrigated sorghum	Grain	54-36	55-37	56-37
	Stover	10-7	9-6	5-3
Irrigated cowpea	Grain	60-40	62-41	71-47
	Haulms	20-13	19-13	10-7
Milk	Small ruminanats	64-37	28-26	13
	Cattle	78-60	50-41	18-15
Meat	Small ruminanats	370-320	77-73	100-96
	Cattle	328-251	128-105	63-53
Labor (MRO/day)		2000	1500	1000

385 MRO = 1€ (tttp://www.Mauritania.mr/fr/index.php).

season, average market price of paddy bran averages 18 MRO kg^{-1}. Market price of groundnut meal increases slightly from West to South-East, from 120 to 130 MRO kg^{-1} (Table 2).

Current household net agricultural production

Differences in current production costs of milk and meat between regions are much greater than those in market prices mainly due to differences in availability of pasture and crop residues, the latter mostly from the larger areas of traditional crops rather than from rice. Higher rainfall in the South-East (Guidimaka) makes pasture and crop residues from traditional crops more available than in the West (Trarza) making costs of production of livestock in the South-East lower in the West where more feed concentrate is used. From West to South-East, average cost of milk production ranges from 138 to 15 MRO kg^{-1} and of meat, from 563 to 80 MRO kg^{-1}. In Guidimaka, the region of highest rainfall, production costs are well below of those of other regions (Table 2).

For households having only small ruminants, median current household net agricultural production is 370,000 MRO per household, equivalent in value to about 230 working days (average daily cost is 1,500 MRO (Figure 4). The contributions of the various products are: livestock 51% (57% milk and 43% meat), traditional crops

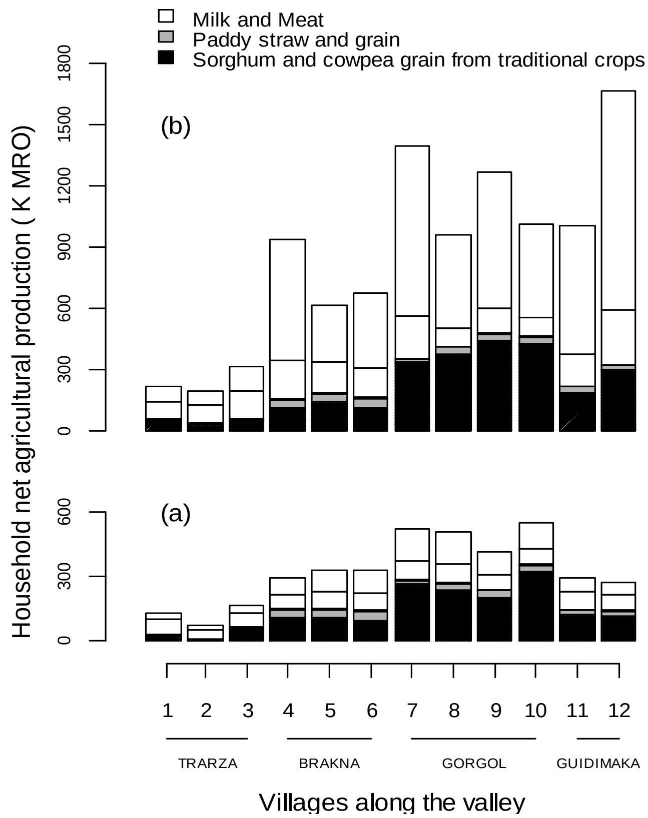

Figure 4. Current median net agricultural production in households with access to irrigation (a) having small ruminants (S) and (b) both small ruminants and cattle (SC) in 12 villages along the northern bank of the Senegal River Valley arranged from West to South-East.

42% (100% grain), and rice 7% (86% grain and 14% straw). By contrast, median current net agricultural production of households having both small ruminants and cattle it is 996,000 MRO per household, equivalent to 665 working days. Livestock contribute most, 78% (79% milk, 21% meat), followed by traditional crops 19% (grain) and rice 3% (87% grain, 13% straw). These data reveal the difference of the economies of these two types households in livestock production, particularly for milk. In the most arid region, Trarza, these differences are much smaller. These data also show that rice production returns much less than traditional crops and livestock.

Inclusion of irrigated late mild-dry season crops in the current scenario

At current average grain yields (3.5 t ha^{-1}), production of one rice crop per year in small and medium-sized collective perimeters (< 100 ha) can barely cover the cost of production and cannot repay loans or maintain pumping equipment and the irrigation schemes themselves (Comas et al., 2012). Analyses by Borgia et al. (2013) reveal how conditions of irrigation schemes fall easily into a spiral of degradation unless external financial support is provided. The record of abandonment of these irrigation schemes demonstrates that such funds are not commonly available (Mateos et al., 2010; García-Bolaños et al., 2011; Borgia et al., 2012). However, experimental data show that rice yields can be significantly increased with a good management of nutrients and weeds (van Asten et al., 2003; Haefele et al., 2001, 2004; Poussin et al., 2003, 2006) and by transplanting proper seedlings (García-Ponce et al., 2013). The proposal presented here to counter this spiral of degradation is the inclusion of irrigated sorghum and cowpea crops grown late in the mild-dry season (from December to March). The objective is to replace fodder concentrates, currently purchased mostly abroad to sustain livestock during the ensuing hot-dry season, with fodder grown in the irrigation schemes. The idea of cultivating irrigated sorghum and cowpea in the mild-dry season is not new. In fact, field trials were conducted by IRAT (Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières) and ISRA (Institut Sénegalais de Recherches Agricoles) in the late 1960s (Chantereau

1983), and subsequently by CNRADA (Centre National de Recherche Agronomique et de Développement Agricole) (Sy Aly, personal communication). What is new, is growing irrigated sorghum and cowpea planted late in the mild-dry season (December or January), after rice harvest in November-December. The impact of this delay of planting on productivity was unknown because, apart from some recent attempts to promote forage crop production for peri-urban milk producers (Toutain et al., 2009), there has been no experimental work during this season in Mauritania. For this option to be feasible, it must be demonstrated that the required investment is affordable and the production costs of grain and fodder are below market price. The decision to develop a second irrigated crop to replace fodder concentrate should be a collective decision taken by the cooperative as a whole.

Since flood recession cropping on Walo remains an option during the mild-dry season there exists potential for clashes for workload with irrigated crops sown late in the mild-dry season. In practice, however, there are opportunities to attend to both activities because of differences in timing of cropping operations and the small areas involved. In traditional Walo land, cropping starts in October-November and harvest is well underway or completed in February. By contrast, irrigated mild-dry season crops are sown after rice harvest at the end of December and harvested in March. In addition, given the small areas involved (median of 1.3 ha per household) this operations can be performed by one active person (Comas et al., 2012) while survey data indicate availability of 5 adults per household. Furthermore, unlike the traditional lands that are often located at 2 to 5 km from villages, irrigated plots are close by, facilitating the most time-consuming operations like bird control and harvesting.

Simulation of yield and development of irrigated late mild-season crops

Crop simulation results show that as planting is delayed (from late December to late January) sorghum yield increases more in Trarza (5.2 to 5.9 t ha^{-1}), which is cooler than Gorgol and Guidimaka (5.2 to 5.5 t ha^{-1}). In contrast, the productivity of cowpea, that is less adapted to high temperatures, increases in Trarza (2.6 to 3.0 t ha^{-1}), but decreases in Gorgol (2.5 to 2.2 t ha^{-1}) and Guidimaka (2.7 to 2.5 t ha^{-1}). On the other hand, simulated irrigation water requirement of sorghum and cowpea sown late in the mild-dry season (day 360) are 85 and 39%, respectively, of rice in the wet season, ranging from 960 to 700 mm (day 200) from West to South-East (Figure 5). Since rainfall during this period is negligible, the pattern of irrigation water requirement reflects increasing ETo associated with increasing radiation, wind speed and temperature (Figure 2). In all regions irrigation requirement increases as sowing date is delayed. The smaller irrigation water requirement at Guidimaka compared with the other two regions derives from lower ETo (Figure 4). Sorghum requires more water than shorter-season cowpea and produces more biomass. For the first planting date (day 360), average irrigation requirements are 800, 760 and 490 mm in Trarza, Gorgol and Guidimaka, respectively. Both water requirements and simulated yield of cowpea are about half those of sorghum. This is relevant because irrigation water is about 22% of the total costs of irrigated crop production. These results also show that yields in the mild-dry season were higher than in the wet season, arguably a result of greater solar irradiance under cloudless skies (Connor et al., 2008).

Simulation analysis shows that the introduction of irrigated crops sown late in the mild-dry season (equal areas of sorghum and cowpea) increases annual mean grain production by 62 kg per resident at 50% of yield obtained by simulation, and to 92 kg per resident at 75%. These are relatively high values with respect to current average annual production of these grains from traditional crops (104 kg per resident) and the annual consumption of sorghum and cowpea, which together with wheat and maize amount to around 120 kg per resident (Comas et al., 2012). On the other hand, simulation results show that annual fodder supplied by irrigated crops planted late in the mild-dry season (2/3 sorghum stover and 1/3 cowpea haulms) is 408 kg DM per TLU at 50% of simulated yield and 612 kg DM per TLU at 75% which represents 64% and almost 100% of fodder requirement during the subsequent hot-dry season at 50 and 75% yield obtained by simulation, respectively.

Household net agricultural production in the new scenario

Using conservative grain yields, ranging between 2.8 to 4.1 and 1.4 to 2.1 t ha^{-1}, for sorghum and cowpea, respectively, the analysis of production costs of irrigated sorghum and cowpea establishes a positive margin, relative to annual average market price, for both grain and fodder of 40 to 24%. In addition, replacing fodder concentrates by fodder from irrigated mild-dry season crops reduces costs of livestock fodder during the hot-dry season, with an average saving relative to fodder concentrate of 20 to 30% for small ruminants and of 40 to 50% for cattle. Furthermore for the same expenditure these irrigated mild-dry season crops also produce highly valued grain for human consumption at a cost competitive with the market price. Finally, irrigated mild-dry season crops contribute to the amortization charges of the irrigation scheme, currently borne entirely by wet season rice production. On average this offers an average cost saving of milk and meat production from small ruminants of 20% relative to fodder concentrates.

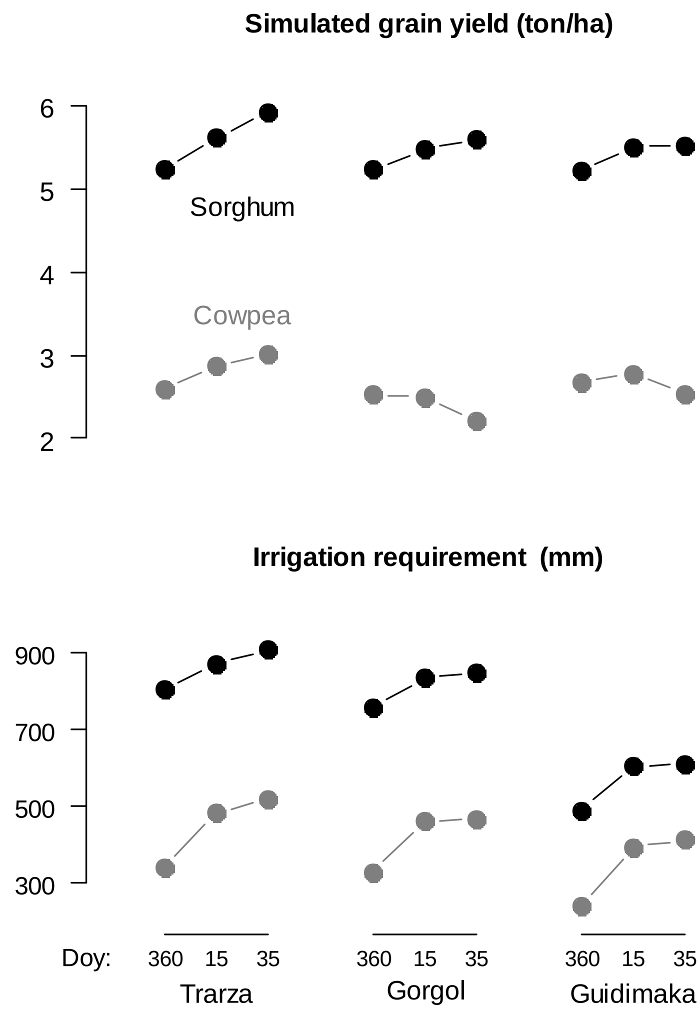

Figure 5. Simulated yields (t ha^{-1}) and irrigation requirement (mm) of sorghum and cowpea sown at doy 360 (25 Dec), 15 (14 Jan) and 35 (3 Feb) at three sites in the Senegal River Valley. Doy 300 marks the average start of the *mild-dry season* which lasts until around day 90 of the following year.

The biggest savings occur with cattle with mean cost reduction of 40% in Trarza, Brakna and Gorgol, although less in Guidimaka (20%) where less concentrate is required (Table 1). Inclusion of irrigated mild-dry season crops also provides opportunity for families with few livestock to earn cash. This applies especially to households having only small ruminants because they have fewer livestock (median 0.08 TLU per resident) compared those with both small ruminants and cattle (median 0.45 TLU per resident).

Estimated median household net production of sorghum and cowpea grain in the new scenario range from 77,000 to 135,000 MRO per household at yields ranging between 2.8 to 4.1 and 1.4 to 2.1 t grain ha^{-1}, for sorghum and cowpea, respectively. The households that would most benefit from irrigated second crops are those of Trarza and Gorgol (Table 2) where the prices of sorghum and cowpea grain are higher than in the other regions (Table 1). On the other hand, estimates of median annual household saving in production of livestock with fodder from irrigated mild-dry season crops range from 4,000 MRO per household with only small ruminants to 37,000 to 52,000 MRO for those with both small ruminants and cattle. The biggest savings are obtained in Trarza, where median annual savings are 7,000 to 9,000 and 44,000 to 74,000 MRO per household having only small ruminants and those having both small ruminants and cattle, respectively (Table 3). These greater savings can be explained by the small areas of traditional crops and pastures there relative to other regions. In addition, households that do not consume all the fodder produced by the second irrigated crop have surplus for sale. This possibility is especially open to households having only small ruminants, for which

Table 3. Increase in median household net production (sales and savings) derived from irrigated late *mild-dry season* crops (sorghum and cowpea) at 50 to 75% simulated yield for households having either only small ruminants (S_l) or small ruminants and cattle (SC_l).

Net production	Household type	Region				
		Trarza	Brakna	Gorgol	Guidimaka	Overall
		Increase in median annual net production (× 10^3 MRO per household)				
Sale of grain	S_l & SC_l	80-140	67-120	77-135	51-98	77-135
Sale of surplus fodder	S_l	32-63	20-45	21-48	32-63	24-52
	SC_l	0-1	0	0	0	0
Savings in the production of livestock	S_l	7-9	3-4	4	1	4
	SC_l	44-74	37-53	47-63	15-22	37-52
Savings in rice production	S_l & SC_l	17	18	18	18	18
Annual overall increase	S_l	134-224	112-194	123-209	104-181	114-198
	SC_l	147-250	130-214	147-237	87-162	141-232

median annual net production obtained from the sale of surplus fodder range from 24,000 to 52,000 MRO. The median value for households having both small ruminants and cattle is zero. Households in Trarza and Guidimaka benefit most because stocking rate and fodder demand are greater than in other regions.

In summary, the introduction of irrigated sorghum and cowpea sown late in the mild-dry season increases household median annual net agricultural production of households having only small ruminants by 114,000 to 198,000 MRO, while for households having both small ruminants and cattle these corresponding values are 141,000 to 232,000 MRO. The largest contribution to that increase is in the production of grain sorghum and cowpea (54 to 63%) and in the production of fodder specially for to households having only small ruminants (3 to 27%), followed by reduced costs of rice production because of sharing scheme costs (9 to 16%) and by smaller cost of feeding the animals during the ensuing hot-dry season (3 to 27%). The result of differences in cropping patterns, stocking rates and population between regions confers most benefit from irrigated late mild-dry season crops in Trarza and Gorgol where the prices of sorghum and cowpea grain and fodder are higher than in the other regions.

Conclusion

The results of this study reveal how the introduction of irrigated sorghum and cowpea crops sown late in the mild-dry season would, even at relatively moderate yields (2.8 to 4.1 and 1.4 to 2.1 t grain ha^{-1}, for sorghum and cowpea, respectively), provide additional grain required by smallholder farmers and reduce costs of livestock production (30%). Initially, the advantages would be greatest for farmers who have sufficient animals to utilize all the extra fodder, but for those with fewer animals there would be opportunity for sale of surplus to a ready market. Substantial areas of irrigated rice are available for this development and for extending the benefits to numerous villages and households. In addition to the direct requirements of village households, there is also a growing market for animal fodder to support intensive milk production for increasing urban populations. Given this additional and growing demand, advantage from intensification of currently under-utilized irrigated land and extension to previously abandoned areas would flow more widely and could promote more development of irrigation and agriculture generally. The regions that would most benefit from the introduction of irrigated second crops are Trarza and Gorgol. These promising results encourage further research to validate the proposed concept in the field. Particularly, attainable yield and harvest index of sorghum and cowpea grown late in the mild-dry season should be evaluated experimentally and on-farm. Also, there is uncertainty about how sorghum and cowpea grain prices would evolve if these crops were grown late during the mild-dry season extensively. Market research should accompany the introduction of irrigated late mild-dry season crops.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Concepts and key issues of ethnoveterinary medicine in Africa: A review of its application in Zimbabwe

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This review is aimed at highlighting the issue of ethno-veterinary medicine (EVM) and the progress and limitations in its application in Zimbabwe.It is a fact that animal diseases are a major constraint to livestock production and development, particularly in communities living in marginal areas affected with endemic pathogens, vectors and diseases. These communities do not have access to modern veterinary information and services or are less economically endowed albeit coping with enormous animal health problems. Their animal health care management systems are based on people's own local and inherent indigenous knowledge of EVM. It is crucial to learn, evaluate, and without being biased and ethnocentric, promote and integrate the beneficial facets of traditional animal health care practices into current primary livestock health care delivery services. This review article highlights some developments in understanding and recourse to EVM.It also discusses a variety of issues related to the use and understanding of EVM in many different ways by different stakeholders in Zimbabwe. The review further outlines the possible way forward.

Key words: Ethnoveterinary medicine, animal health care systems, key issues, way forward, Zimbabwe.

INTRODUCTION

Parasites present a periodic problem particularly to grazing livestock as they are always exposed to and, therefore, constantly reinfected (Masikati, 2010). The levels of infestation for untreated animals raised in natural settings tend to fluctuate with seasonal metabolisms. It has been observed in goats and sheep that the highest levels of parasites correspond to periods of change: change in location (e.g. buildings to pasture); change in diet or use of food (e.g. lactation to maintenance diet) (Duval, 1994). The use of synthetic drugs against internal and external parasites only provides short term solutions, thus it has to be done consistently in order to maintain low parasitic concentrations in livestock. However, this is mainly possible when supported by donor aid in Zimbabwe. As long as the funds remain available, synthetic drugs will be the main methods of controlling parasites in livestock production systems (Masimba et al., 2011).The reliance on donor funds is, however, not sustainable.

In the absence of funds, farmers face the challenge of scarcity, erratic supply and/or prohibitive costs of synthetic drugs or veterinary services and they usually revert back to more appropriate and sustainable traditional systems of animal health care (Mathias and McCorkle, 2004). According to the Word Health Organisation (WHO), at least 80% of people in

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developing countries depend largely on indigenous practices for the control and treatment of various diseases affecting both human beings and animals (Jabbar et al., 2005). In Zimbabwe for example the Department of Veterinary Services used to supply acaricides to all communal dips in the rural areas, but since the economic depletion many dip tanks have not been operational as the Department failed to supply these acaricides (Mwale et al., 2005). Initially some farmers resorted to using commercial acaricides but the prices of these acaricides became deterrent that most farmers were unable to dip or spray their cattle. Eventually, these resource-limited farmers resorted to using locally available medicinal plants to control livestock diseases, ectoparasites and other related vectors (Mutibvu et al., 2012). Mwale et al. (2005) acknowledged that in Zimbabwe EVM is gaining recognition at the expense of conventional drugs especially because of its greater accessibility, lower costs and apparent effectiveness. The objective of this paper is to highlight the main issues in the reappraisal and to point out possible ways forward for use of EVM in Zimbabwe. It is important to first prompt a reappraisal of the potential and limitations of both ethno and modern veterinary medicine.

Limitations of orthodox livestock health care practices

The major problems associated with the use of synthetic chemicals include; environmental pollution, development of resistant strains (Dipeolu and Ndungu, 1991), and delay in the development of immunity in young animals (Dipeolu, 1991). Most products are sold with English instructions for use and safe handling rather than the local language which can be understood by local users who are in most cases illiterate hence the frequency of incorrect application is high. In Kenya adulteration of commercial livestock drugs such as synthetic anthelmintics has been established to be a common practice leading to resistance of some disease causing organisms in livestock (Githiori, 2004).Resistant strains of ticks were reported to have developed against benzene hexachloride, dichlorodiphenyltrichloroethane (DDT) and toxaphane in a period ranging between 18 months to five years (Kayaa, 2000). Some internal parasites have developed resistance against deworming drugs asbenzimidazole, levamisole and even Ivermectin because of too frequent use (Duval, 1994). Studies carried out by Kayaa (2000) have also revealed that synthetic dewormers slow the decomposition of manure. The annual cost for importing acaricides has been estimated at US$9.3 million in Zimbabwe (Perry et al., 1990), US$16 million in Kenya (Tatchell et al., 1986), US$10 million in Zambia (Pegram et al., 1988), US$26 million in Tanzania (Kagaruki, 1997) and Uganda (Okello-Onen and Nsumbuga-Mutaka, 1997). The overall loss due to ticks has thus been estimated at US$720 million per year (Dipeolu and Ndungu, 1991).

Cultural livestock health care practices

Ethno-veterinary medicine (EVM) provides alternatives for controlling both internal and external parasites in livestock production systems that are environmentally friendly, relatively cheap and not prone to development of resistant parasitic strains. Ethno-veterinary medicine involves the use of indigenous beliefs, knowledge, skills, methods and practices pertaining to the health care of animals (Mathius-Mundy and McCorkle, 1989).Perhaps of more importance is the fact that herbal remedies are known to be broad spectrum and therefore may be a future answer to resistance development of pathogens to conventional drugs (Mwale et al., 2005). Ethnoveterinary medicine knowledge varies from region to region as well as between and within communities. Documentation of this traditional knowledge is limited in many developing countries including Zimbabwe. Storage of this knowledge is solely depended on the collective memory of just a few entrusted persons within communities for it is just not common 'knowledge' for everybody. It has been transmitted across generations by an oral tradition which according to Matekaire and Bwakura (2004) is in danger of extinction. A survey conducted by Masimba et al. (2011) also reiterated that the knowledge of ethnoveterinary practices was mostly passed down generations orally. Their study revealed the sources of EVM knowledge as grandparents and parents (61.5%), friends (20.5%), neighbours (12.5%), and others (6.5%). The situation is worsened by rapid socio-economic, technological and environmental changes (Sarasan et al., 2011). Documentation of ethno-veterinary practices is critically urgent so that the knowledge can be preserved, plants conserved and sustainably managed for the control of livestock diseases.

Limitations of Ethno-veterinary medicine

As wide spread as it is, the practice of EVM has lagged behind that of its counterpart (modern veterinary medicine) mainly because the practice was secretly done and its information hidden in the gray literature (Mathias, 2004). Ethno-veterinary practice has its own limitations as well. Over-harvesting threatens the diversity of habitats which includes semi-arid woodland and savanna habitats. Diversity is also threatened by high deforestation rates and overexploitation (Wanzala et al., 2005). Efforts are needed to propagate and cultivate those species most at risk. A better scientific understanding of where these plants grow and how they work will optimise their collection and sustainable use. Also all plants are not necessarily harmless, so a better understanding of their chemistry can help to evaluate the associated risks. For example, nicotine, a well known compound in tobacco plants is actually insecticidal and the plant is consequently used for this purpose by some farmers. However, nicotine is also well known for its toxicity to man and so the use of tobacco plants for this purpose needs to be carried out with care (Mathias, 2004). As indicated earlier EVM knowledge is not universally recognized as a valid method of disease control as it differs from region to region as well as within communities. It has been developed through trial and error and deliberate experimentation, therefore the practice is less systematic and less formalized (Matekaire and Bwakura, 2004).In Zimbabwe as in other African countries many veterinarians and decision makers have not examined its potential and/or have been trained to ignore or ridicule it. There is still need for the validation, documentation and acknowledgement of EVM in Zimbabwe among other tropical countries.

Merits of ethno-veterinary medicine

However, despite these limitations, EVM has been scientifically proven to control a wide spectrum of common livestock diseases such as diarrhea, wounds, coccidiosis and reproductive disorders (Mlambo et al., 2011; Mwale et al., 2005; Matekaire and Bwakura, 2004). In recent years a growing number of researchers from varied fields have studied, valued, confirmed, validated and documented the potential effectiveness of the traditional animal health management systems in native and local communities (Wanzala et al., 2005).Research has also provided an understanding of the plant chemistry and modes of action for plant species already used by many farmers. Research results have revealed several pesticidal plants that can be used reliably and safely to treat livestock. Legal registration of these botanical products is usually not required for their promotion. This approach offers sustainable strategies directed towards developing sound and appropriate animal health care systems suitable and relevant to rural communities in improving livestock performance and production and, hence, livelihood(Stevenson et al., 2010).

Integrating ethno-veterinary medicine in modern livestock health care systems

Like any other knowledge systems, EVM is very dynamic in its management and practice (Wazala et al., 2005). As a result of this dynamism, many ethnopractitioners find themselves in a situation where they complement EVM with modern veterinary medicine, especially in cases where EVM is limited and or may be deemed dysfunctional (Martin et al., 2001; Mathias, 2004). Enhancement of this approach is most likely to spur research and development of EVM and undoubtedly enable it to make immense strides in the development of the livestock industry. This however, requires good knowledge and understanding of EVM limitations and successes, needs and circumstances of local practitioners, mutual understanding and co-operation between conventional veterinarians and ethnopractitioners and respect to Intellectual Property Rights (IPR).

According to a survey conducted in Gutu District, Zimbabwe by Masimba et al. (2011) none of the households relied on conventional medicines alone in treating poultry diseases. Ninety five percent of the households used traditional medicines only whilst the rest employed a combination of traditional and conventional remedies.

Common herbs used for ethno-veterinary disease control

Mari et al. (2005) carried out a diagnostic survey of the use of herbal plants in poultry health management in a small-scale farming area in Masvingo, Zimbabwe. They revealed the therapeutic value of some of the indigenous practices and emphasized on the need to document indigenous knowledge systems before they are lost. Their surveys showed that the majority of farmers were familiar with pesticidal plants, but very few actually used them. This highlights the need to foster the actual use of these plants, to maximise the benefits they can bring to resource-limited farmers. They listed herbs that are commonly used by smallholder farmers to treat livestock in Zimbabwe. The listed herbs included: *Boswellia serata* (frankincense), *Adansonia digitata* (baobab lemonade), *Adenium multiflorum*(impala lily), *Aloe spicata*, *Aloe vera* (burn plant), *Cussonia arborea* (Chibwabwa/Chipombola), *Cycnium adonense* (the ink plant), *Cyperus articulatus* (jointed flatsedge), *Allium sativum* (garlic), *A. spicata*, *Lycopersicon esculentum*, *Myrothamnu sflabellifoilius*, *Lannea stullmannii*, *Ficus burkei*, *Sarcostemma viminale*, *Capsicum annum*, *Parinaria curatellifolia*, *Albizia gummisera*, *Albizia adianthifolia*, and soot. Of these *A. vera* was reported to be arguably the most important as it is found in many geographical regions and is effective against a wide range of diseases and ailments (Mwale et al., 2005). This is attributed to its several pharmacological properties which include antibacterial, antifungal, antivenin as well as strong immunological properties (Mwale et al., 2005). Mwale et al. (2005) also reported that *A.vera* contain acetylated mannans or acemannan sugars which are responsible for boosting immunity in animals, hence *Alloe* species has wide range of uses. Externally *A. vera* has been used to treat wounds (mixed with used engine oil in rural areas), skin irritations including burns, bruises and abrasions as well as general inflammatory skin disorders (Mwale et al., 2005). The authors went on to indicate that *A*. *vera* has anti-allergy and anti-inflammatory properties because of the presence of glycoproteins and anthraquinones which block the regeneration of thromboxanes and inhibit bradykinin. Table 1 shows common animal conditions and their perceived remedies as recorded by Matekaire and Bwakura (2004):

Small-scale farmers' attitude towards veterinary services

Matekaire and Bwakura (2004) reported results from a survey they carried out in Mashonaland East, West and Central, Zimbabwe. The survey revealed that approximately 95% of communal area farmers never avail themselves of the veterinary services offered by the Government except for cattle dipping, which is legally mandatory. The farmers cited prohibitive costs of drugs and services provided, while others perceived the veterinary health service as an organization that destroys livestock in the event of disease outbreaks, and thus would not seek assistance from them. According to the study carried out by Matekaire and Bwakura (2004) traditional animal healers in Zimbabwe have less to offer in the treatment and control of epidemic and endemic infectious diseases like foot and mouth disease, rinderpest, anthrax, and acute life-threatening bacterial diseases. However, they can cope with a reasonable spectrum of common symptoms and conditions such as septicaemia, diarrhoea, wounds, colds, worms, and reproductive disorders.In addition, they have been successful with numerous bacterial diseases including coccidiosis, mycobacteriosis, plague and a wide spectrum of coliform diseases.

Most of the non-conventional treatment of ailments involved the use of plant parts except for the use of soot (Mwale et al., 2005). Matekaire and Bwakura (2004) also reported that every household made use of *A.vera*. Over 95% of the households acknowledged the use of *Sarcostemma viminale* intreating ailments in poultry.Plant part used was similar across all the four villages, implying this knowledge is common and generally shared among households within a common setting (Matekaire and Bwakura, 2004).

Ethnoveterinary control of external parasites

Ticks are external parasites on cattle that cause tick worry and disturbs animals from reaching their potential productivity (Norval et al., 1994). They also cause tick borne diseases (TBD) which reduce cattle productivity and, if left untreated, may result in their death. Conventional control for TBD vectors in Zimbabwe involves the use of conventional acaricides as cattle dips or sprays. Regular acaricides treatment of cattle is expensive and so, for economic reasons, the Government of Zimbabwe is no longer enforcing a policy of strict tick control (Norval et al., 1994). It is likely that reduced tick control will result in the spread of *Amblyomma* ticks to previously uninfested areas (Norval et al., 1994). Farmers ought to make use of medicinal plants that have been proven to be effective against ticks.

Evaluation of the effectiveness of botanical extracts

Preliminary surveys carried out by Madzimure et al. (2011) reported *Lippia javanica* (Verbencaceae), *Strychnos spinosa* and *Solanum panduriforme* (incanum) as plants with acaricidal properties in Zimbabwe. Subsequent studies by Madzimure et al. (2011) revealed that *L*. *javanica*is effective in controlling cattle ticks that cause morbidity and spread fatal blood diseases in livestock. Tick counts on cattle treated with plant extracts at an application rate of 10% were as low as on cattle treated with the standard Amitrazbased commercial product (Tickbuster). While some tick parasitism remained on these cows (approximately 10% of that found on untreated cows), peripheral blood samples showed no haemoparasites in the treated cattle, implying that animals did not suffer from clinical tick-borne diseases after treatment with *L.javanica* extracts. Further studies were carried to confirm efficacy and determine toxicity of these plant species. In this experiment the efficacy and toxicity of the plants were evaluated against a positive control of an Amitraz based commercial acaricide (Tickbuster). Water leaf extracts (5, 10 and 20% w/v) and fruit extracts in the same concentrations at v/v were sprayed on Mashona animals for six weeks and daily tick counts collected. The efficacy trials carried out at Henderson Research Station showed that all the plants had acaricidal properties ($P < 0.05$). There was no significant difference between Tickbuster and 10% *L. javanica* treatment. In the other plant species the 5% treatment was more effective than the higher concentrations. Acaricidal activity was attributed to the various chemical constituents of the plants.

In the toxicity experiments water extracts of the plant materials were orally administered to sexually mature Balb/c mice. Overall mortality data was high in the *S. spinosa* treatments (83.4%) and relatively lower in *L. javanica* (37.5 %) (Nyahangare et al., 2012). Acute toxicity was attributed to some secoiridoids in *S. spinosa* and methylxanthines in *L. javanica*. The results show that while the plant extracts are pesticidal they have potential negative health effects to users if they are used incorrectly (Stevenson et al., 2010). More chemistry work still needs to be done though for optimal exploitation of the acaricidal plants.

Table 1. Traditional remedies for the treatment of commonly encountered disease conditions in farm animals in Zimbabwe.

Animal condition	Remedy [Common name, (Genus, species)]	Botanical consistency[a](%)	Consistencyof veterinary usage[b] (%)	Method of application
Septic wounds	Muvengahonye (*Canthium*spp.)	61	89	Fresh leaves are ground and applied to the wound
	Muvheva (*Kigeliaafricana*)	42	53	The inner core of dried fruit is applied as a powder on the wound
	Murenja(*Cassius quandrangularis*)	34	68	Fruit is crushed and the fluid applied to wound
	Gavakava (*Aloe spp.*)	33	72	Dry leaves are crushed and the powder applied
Eye problems	Nhundurwa(*Solanumindicum*)	74	83	Fruit is crushed and the fluid is applied to the eye
	Snail's shell	51	43	Shell is ground to powder and applied to the eye
Bloat	Munhanzva(*Pauzzoziamixta*)	39	65	Leaves crushed and water added; animal made to swallow mixture
	Chin'aic(Phlegmostomium)	55	51	Mix with table salt, add water; animal made to swallow mixture
Coccidiosis	Gavakava(*Aloe* spp.)	88	92	Grind fresh leaves and add to drinking water
Worms	Muzhozho(*Venoniaamygdalina)*	49	73	Add water to ground fresh leaves; animal made to swallow mixture
	Banana(*Musa paradisiacal*)	57	44	Add water to crushed fresh roots; animal made to swallow mixture
	Gavakava(*Aloe* spp.)	78	65	Add water to crushed fresh leaves; animal made to swallow mixture
Newcastle disease	Gusha(*Sesanumangustifolius*)	33	87	Crush fresh fruit and add to drinking water in poultry
Retained afterbirth	Munhanzva(*Pauzzoziamixta*)	63	71	Fresh leaves are crushed and the slippery paste inserted into the vagina.
Fertility	Gomarara (*Loranthus spp.)*	55	36	Feeding fresh leaves to rabbits improves kidding rate
Snake bite	Munyoka(*Amaranthusgneizaus*)	27	74	Add water to crushed fresh roots; animal made to swallow mixture
	Banana(*Musa paradisiacal*)	36	54	Add water to crushed dried roots; animal made to swallow mixture

[a]Frequency of association of a particular plant species with a particular or perceived medicinal value.[b]Frequency of a particular plant species being associated with or used to treat a particular disease.[c]Chin'ai refers to soot-/carbon-tainted grass that accumulates in the thatched roofs of kitchens as a result of burning firewood, cow dung, or coal from cooking.

Tephrosia vogelii

Literature suggests that *Tephrosia vogelii* (TV) can be used as an acaricide. Researchers at Makoholi Research Station, in collaboration with ICRAF, tested and verified the effectiveness of *T. vogelii* as a biopesticide on cattle in 2009. It was concluded from preliminary results that TV is a very promising biopesticide that, with ascertainment and further testing, could be disseminated to a wider audience.

CONCLUSION

Indeed, it is true that ignoring ethno-veterinary medicine in today's development would mean losing a very important and special component in life history of mankind that definitely would have made a difference for better. However, although EVM practices are evidently gaining popularity in various Zimbabwean communities, a lot of claims have not been validated by research. Research is also necessary as some plants used in EVM may have deleterious health implications on humans and animals; hence, advice on their safety should accompany promotion of their use. Documentation of EVM is critical but it offers resourcesto poor farmers an effective, low cost, sustainable and environmentally friendly pest management strategy.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Optimization of Blaney-Morin-Nigeria (BMN) model for estimating evapotranspiration in Enugu, Nigeria

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The Blaney-Morin-Nigeria (BMN) model was developed for the estimation of reference evapotranspiration (ET_o) in Nigeria using commonly available meteorological parameters. In the development of the model, there were some perceived shortcomings which are believed to affect the reliability of *ET_o* estimation, particularly in some specific locations in Nigeria, due to varying environmental factors. This study re-examined the model by using the non linear regression model based on *Levenberg-Marquardt Algorithm* (Excel Solver) to minimize the error space and generate new model constants specific for Enugu. A 25-year (1989 to 2014) monthly record of climatic variables (Solar radiation, Temperature, Relative Humidity and Wind speed) for Enugu were collected from the Nigeria Meteorological Agency (NIMET), Abuja in Nigeria and used. The newly generated model constants, *H* and *m*, were 392.2 and 1.19, respectively. Reference evapotranspiration for Enugu [$ET_{o(nBMN)}$] was thereafter estimated using the optimized BMN model and compared with that calculated using the FAO56-PM model [$ET_{o(FAO56-PM)}$]. The optimized model showed a more accurate estimation of ET for Enugu as indicated by the higher correlation coefficient of 0.82 compared to 0.76 for the original BMN, lower mean absolute error (MAE) of 0.133 compared to 0.42 and root mean square (RMSE) of 0.3641 compared to 0.44. The χ^2 of 0.135 obtained using the optimized model was also low showing that the new model gives a better estimation of ET for Enugu, Nigeria.

Key words: Evapotranspiration, Blaney-Morin-Nigeria model, FAO56-PM model, Levenberg-Marquardt Algorithm, Enugu, Nigeria.

INTRODUCTION

Water availability is one of the important factors considered in Agriculture. Water is provided to crop through natural precipitation and subsurface moisture. In many areas of the world where rainfall is insufficient to meet the crop water demand, irrigation system is often used to offset the water deficit. Water is lost from crops through evaporation and transpiration. Evaporation is the process of liquid water conversion to water vapour and removal from the evaporating surface. Transpiration includes the vaporization of liquid water contained in

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plant tissues and the water removal to the atmosphere. Evapotranspiration (ET), which is the combination of evaporation and transpiration, is also known as the consumptive use or actual evapotranspiration (Allen et al., 1998).

The rate of ET is determined using different approaches. As indicated by Ilesanmi et al. (2012), these approaches may be broadly classified into direct and indirect measurement. The direct measurement include the use of lysimeters (Ayoade, 1988, 1988a; Meissner et al., 2010) and atmometers (Diop et al., 2015), energy balance/budget approach (Adeboye et al., 2009; Farahani, et al., 2007), the soil water budget method (Phene et al., 1990; Evett and Parkin, 2005; Farahani et al., 2007), the use of pan evaporimeters (Ayoade, 1988, 1988a; Howell et al., 1991; Young et al., 1996; Yang et al., 2000; Gavilan et al., 2007), satellite remote sensing (Bastiaanssen et al., 2005; Tang et al., 2009) and the Eddy-Covariance method (Shuttleworth, 1993, 2007). Direct measurement of evapotranspration is usually not feasible in many situations because it is expensive and relatively time consuming (Igbadum et al., 2006). The indirect measurement involves the use of empirical models developed to estimate ET using meteorological data. These models range from simple expressions which relate ET to temperature and/or radiation to models having extensive data requirement. The models may be classified into three: Temperature-based models (Thornthwaite, Blaney-Cridle, Blaney-Morin and McCloud models); radiation-based models (Turc, Hargreaves, Hargreaves-Samani, Priestly-Taylor and the Makkink Formula) and a combination approach based on original Penman model which consists of radiation and aerodynamic part (Jacobs and Satti, 2001; Alexandris et al., 2008, Ilesanmi et al., 2012). Penman's model has been modified over the years because it produces good results when applied over different climatic regions (Allen et al., 1998). One of its modification is the FAO56 Penman-Monteith model (FAO56-PM) which has been adjudged to be the best estimator of Reference Evapotranspiration (ET_o) and has been recommended as the model to be used for the estimation of ET_o from meteorological data (Allen et al., 1998; Alexandris et al., 2008) if all meteorological data is available.

In Nigeria, as in most developing countries, the meteorological data needed for the calculation of ET_o using models such as the FAO56-PM are most of the time not readily available (Adeboye et al., 2009). For Nigeria, therefore, a temperature based model - the Blaney-Morin-Nigeria (BMN) - developed by Duru (1984) has been adjudged to be the best for the Nigerian condition by the Nigerian Institute of Agricultural Engineers (NIAE). In the development of this model, there are some perceived shortcomings which are believed to affect its reliability for ET estimation in Nigeria. These shortcomings have been exhaustively discussed by Idike and Aneke (2002), Idike (2005) and Ilesanmi et al. (2012) among other researchers. This study was therefore carried out to generate a modified version of the BMN model that is specific for Enugu, Nigeria.

MATERIALS AND METHODS

Study area

Enugu, which is the capital city of Enugu State, in Nigeria, was used for the study (Figure 1). It is lies within Latitude: 6°26′33″ N, Longitude: 7°30′07″ E with an elevation of 227 m above sea level. Located in the tropical rain forest zone of Nigeria, Enugu's vegetation is derived savannah. The area has a humid climate with a mean daily temperature of 26.7°C, a mean relative humidity of 72% and an average annual rainfall of 2000 mm. The humidity is highest between March and November when it attains a mean value of over 80%. As in the rest of West Africa, there are two major seasons: the rainy season and dry season. The rainy season lasts from April to November while the dry season lasts from January to April. In between, is the Harmattan season which is characterized by very low relative humidity and a very dry and dusty wind. Like the rest of Nigeria, Enugu is hot all year round (Wikipedia, 2016).

Data source

Monthly weather data for Enugu covering a period of 25 years (1989 - 2014) was collected from the Nigeria Meteorological Agency (NIMET), Abuja. The data includes solar radiation, maximum and minimum temperature, relative humidity and wind speed.

Model optimization

The Blaney-Morin-Nigeria (BMN) model developed for the estimation of reference evapotranspiration in Nigeria by Duru (1984) formed the basis for the optimization exercise. The model is given by:

$$ET = \frac{rf(0.45T_a+8)(H-R^m)}{100} \quad (1)$$

Where, rf is the ratio of monthly radiation to annual radiation, T_a the mean monthly temperature (℃), R the mean monthly relative humidity, *H* and *m* are model constants given by Duru (1984) as 520 and 1.31 respectively. These model constants were used to initialize the BMN model as well as the weather data from NIMET. Thereafter, the non linear regression model based on *Levenberg-Marquardt Algorithm* [Excel Solver] (Levenberg, 1944; Marquardt, 1963) was used to minimize the error space and generate new model constants specific for Enugu. Reference evapotranspiration for Enugu [$ET_{o(nBMN)}$] was then estimated using the newly optimized BMN model. The determined ET was then compared with that estimated using the FAO56-PM model [$ET_{o(FAO56-PM)}$] based on grass.

Model accuracy

Four criterion were used to test the accuracy of the new model constants for estimating ET. They are:

1. The mean absolute error (MAE): This gives the mean difference

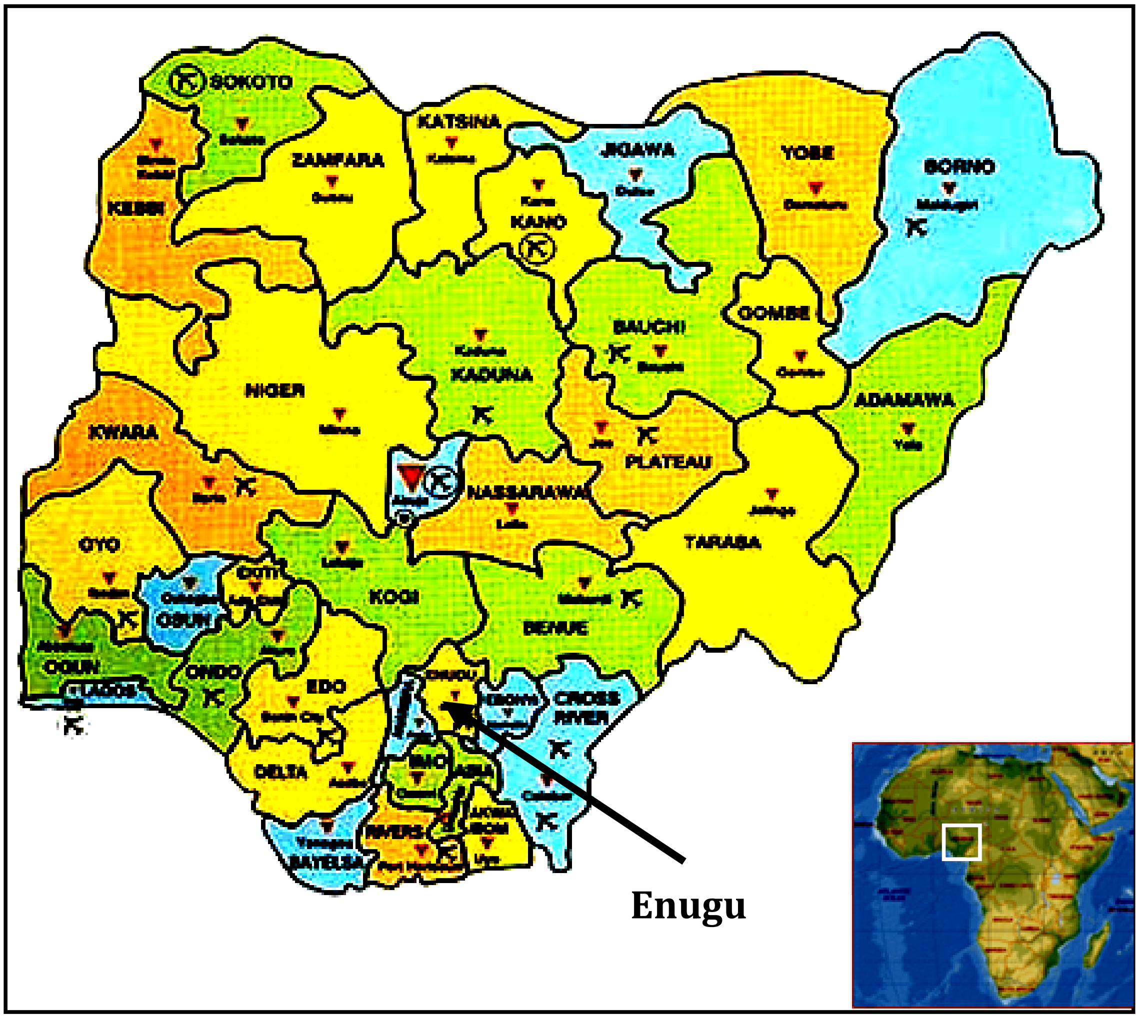

Figure 1. Map of Nigeria showing Enugu, the study location.

between the observed and predicted values.

$$MAE = N^{-1}\sum_{i=1}^{N}|Pi - Oi| \quad (2)$$

Where; Pi are thepredicted values, Oi theobserved values and N the number of observations. It is more accurate for small or limited data.

2. Chi square: This is a validation model similar to MAE but for larger data.

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N - Z} \quad (3)$$

where; χ^2 is Chi square, N thenumber of observations, Z thenumber of constants, $MR_{exp,i}$ the i^{th} experimental data and $MR_{pre,i}$ the i^{th} predicted data

3. The Root Mean Square Error (RMSE): Similar in operation with the Mean Absolute Error (MAE) but preferred for use with large data.

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{1/2} \quad (4)$$

Where; RSME is the root mean square error, N the number of observation, $MR_{exp,i}$the i^{th} experimental data and $MR_{pre,i}$ the i^{th} predicted data

4. The Coefficient of Correlation (r): Investigates the level of similarities between two sets of values.

$$r = \frac{\sum PO - \frac{\sum P \sum O}{N}}{\sqrt{\left(\sum P^2 - \frac{(\sum P)^2}{N}\right)\left(\sum O^2 - \frac{(\sum O)^2}{N}\right)}} \quad (5)$$

Where; r is the Correlation coefficient, $\sum P$ the sum of predicted values, $\sum O$ the sum of observed values and N the number of observations.

In Equations 2 to 5, observed or experimental data refer to ET_o values calculated using the FAO56-PM model while predicted refers to values determined using the optimized BMN model. According to Duru (1984), the closer to zero the values of MAE, RMSE, and χ^2, the better performed the respective model, whereas the closer to unity (1.0) the value of *r*, the better the model. Also, according to Duru (1984), "from a design and safety standpoint, a model that over predicts should be preferred to one that under predicts and the better model is one that over predicts to a lesser degree" (Ilesanmi

Table 1. The monthly mean ratio of monthly to annual solar radiation (rf), mean monthly temperature (T_a), and the mean monthly relative humidity(R), as well as the ET computed using FAO56-PM [$ET_{o(FAO56-PM)}$] and that computed using the newly optimized BMN [$ET_{o(FAO56-PM)}$].

Month	rf	T_a(°C)	R (%)	$ET_{o(FAO56-PM)}$(mm/d)	$ET_{o(nBMN)}$(mm/d)
JAN	0.08	26.18	58.19	3.77	4.04
FEB	0.08	28.55	60.22	4.41	4.47
MAR	0.09	29.15	65.86	4.67	4.52
APR	0.09	28.42	73.34	4.42	4.13
MAY	0.09	27.08	77.50	4.05	3.72
JUN	0.08	26.01	79.84	3.64	3.45
JUL	0.08	24.90	82.40	3.10	3.27
AUG	0.09	24.69	82.76	2.92	3.31
SEP	0.09	25.17	81.46	3.27	3.41
OCT	0.08	26.10	78.71	3.53	3.47
NOV	0.08	26.90	71.48	3.70	3.62
DEC	0.08	26.46	67.62	3.46	3.60
MEAN	**0.08**	**26.63**	**73.28**	**3.74**	**3.75**

Table 2. Comparison of Duru (1984) and the new BNM model constants.

BMN models	H	m
Duru (1984) (Nigeria)	520	1.33
New BMN (Enugu)	392.2	1.19

et al., 2012).

RESULTS AND DISCUSSION

Weather data

The monthly mean ratio of monthly to annual solar radiation (rf), the temperature (T_a) and relative humidity(R) over the study period are shown in . The ratio, rf, ranged from 0.08 to 0.09 with a mean of 0.08. The mean monthly temperature ranged from 24.9 to 29.15°C while the relative humidity ranged from 58.19 to 82.76%.

Estimated model constants

A comparison of the old (Duru, 1984) and the newly developed model constants are shown in Table 2. From the table, it is seen that while the model constants estimated by Duru for *H* and *m* were 520 and 1.33 respectively, the newly estimated values for the constants using the Levenberg-Marquardt Algorithm were 392.2 and 1.19, respectively. These values are lower than those of Duru (1984). The lower values agree with the result obtained by Ilesanmi et al. (2012) who used similar method to recalibrate the BMN model for estimating ET_o for some other Nigerian locations.

The optimized BMN model for Enugu is, therefore, of the form:

$$ET_o = \frac{rf(0.45T+8)(392- R^{1.19})}{100} \quad (6)$$

Comparison between FAO56-PM and New BMN model's

Figure 2 and Columns 5 and 6 of Table 1 show a comparison of the values of the reference evapotranspiration for Enugu calculated using FAO56-PM [$ET_{o(FAO56-PM)}$] and the new BMN [$ET_{o(nBMN)}$] models. The values calculated using the optimized BMN model ranged from 3.31 in August to 4.52 in March with an annual average of 3.75. The ones calculated using FAO56-PM ranged from 2.92 in August to 4.67 in March. The $ET_{o(nBMN)}$ values were lower than those of $ET_{o(FAO56-PM)}$ between March and June and between October and November. The reverse is the case in December and January and between the months of July and September. Also high values of ET_o were obtained during the hottest part of the year (which also corresponds to period of high insolation), that is, between February and April, while low values were recorded during the cold rainy season which occurs around May to September. This is to be expected since temperature and solar radiation are important factors in evapotranspiration (Allen et al., 1998).

The plots of the predicted and actual ET for some typical years (2006 and 2014) are shown in Figure 3. The same pattern, as observed for the mean values over

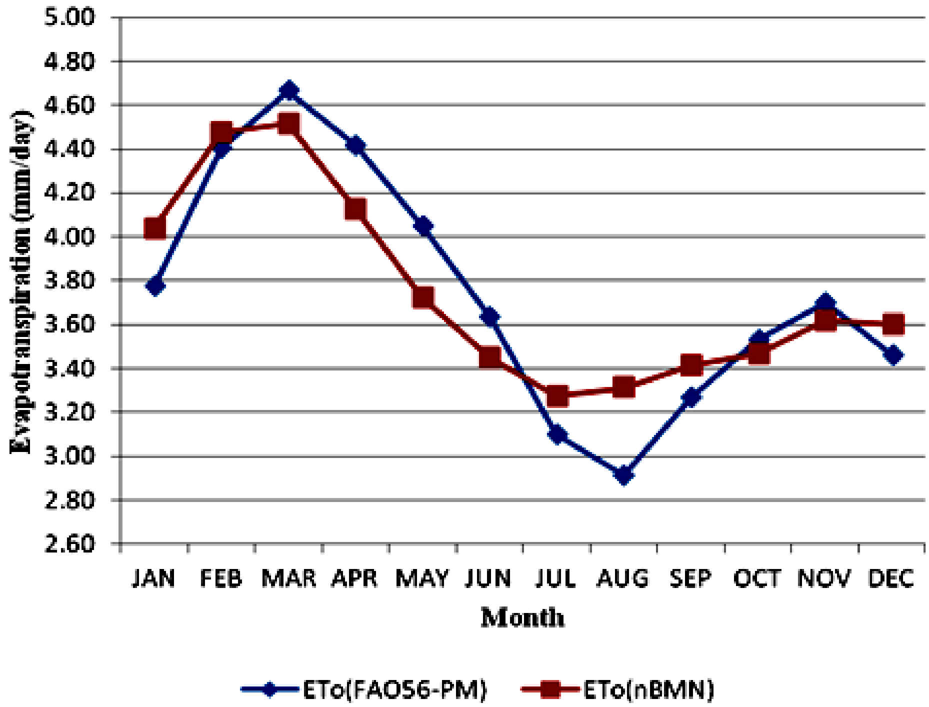

Figure 2. Comparison of mean values of reference evapotranspiration for Enugu calculated over the study period using FAO56-PM model [$ET_{o(FAO56-PM)}$] and the optimized BMN [$ET_{o(nBMN)}$] model.

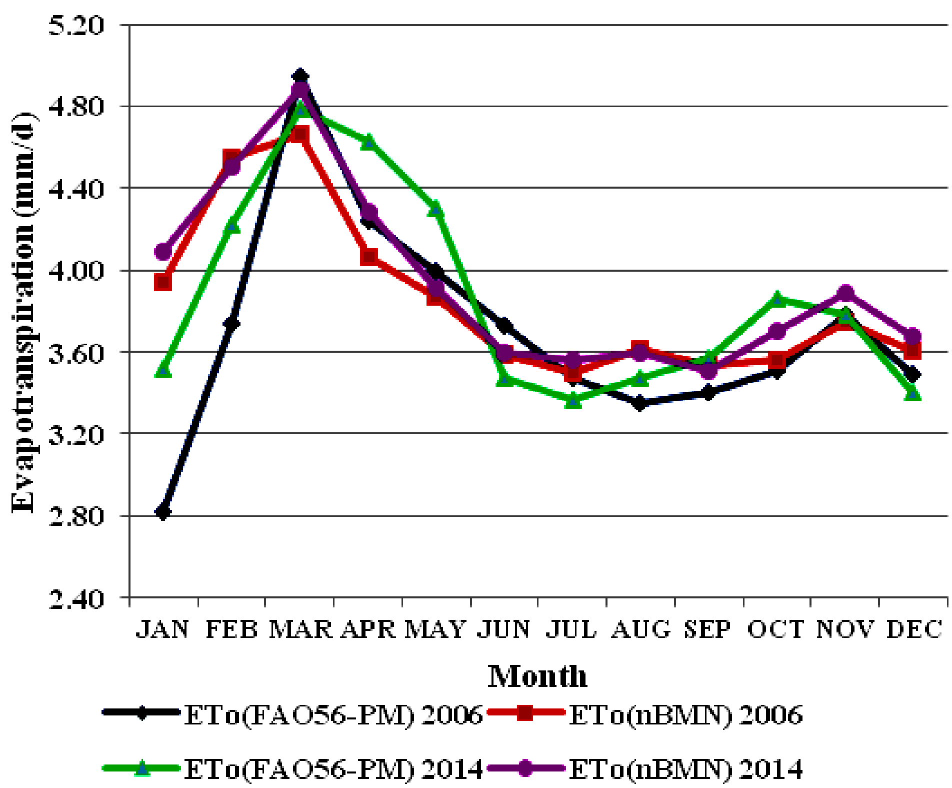

Figure 3. Comparison of mean values of reference evapotranspiration for Enugu for some typical years (2006 and 2014) using FAO56-PM model [$ET_{o(FAO56-PM)}$] and the optimized BMN [$ET_{o(nBMN)}$]model.

Table 3. Observed and predicted values of the constants and the models validation values of the new bmn for enugu.

BMN models	H	m	MAE	RMSE	χ^2	*r*
BMN (Duru, 1984)	520	1.33	0.42	0.44	-	0.76
New BMN (Enugu)	392.2	1.19	0.133	0.3641	0.135	**0.82**

the study period, is also exhibited for the shown typical years.

The mean absolute error (MAE), root mean square (RMSE), Chi square (χ^2) and correlation coefficient (*r*) for the optimized BMN model are shown in Table 3. Also shown in the table are similar values as obtained by Duru (1984) using the original BMN model. The MAE and RMSE of 0.133 and 0.3641, respectively, are lower than those obtained with Duru's model which was 0.42 and 0.44, respectively. The correlation coefficient of 0.82 was higher than that of Duru's model which was 0.76. As stated earlier, the closer to zero the values of MAE, RMSE, and χ^2, the better the model, whereas the closer to unity (1.0) the value of *r*, the better. The estimated MAE and RMSE are lower than that of Duru showing that the modified model gives a better estimate of ET for Enugu, Nigeria. Although Duru (1984) did not compute the χ^2 value for his model, the value for the developed model was 0.135 which is relatively low indicating a good prediction. Also the correlation coefficient of 0.82 shows a better prediction when compared with Duru's model with an *r* value of 0.76.

The fact that optimized BMN gave a better estimation of ET_o is to be expected. Among the perceived shortcomings in the development of Duru's model was the fact that the relevant meteorological data used in developing the model was obtained from only one location (Zaria, Northern Nigeria); yet the model was developed for use throughout Nigeria and possibly the West African sub-region (Idike, 2005). Manual iteration was also used during its development and this is prone to errors (Ilesanmi et al., 2012) and hence likely to lead to lesser reliability.

Compared to the correlation coefficient (*r*) values computed by Duru (1984) for other Nigerian locations, the correlation coefficient of 0.82 obtained for Enugu using the optimized BMN model was the highest. For example the highest correlation coefficient recorded by Duru was 0.80 for Lagos while the lowest was 0.62 for Gombe in Northern Nigeria. Also the MAE of 0.133 and RMSE of 0.3641 obtained with the optimized BMN model were lower that similar values determined for other Nigerian location by Duru. The lowest MAE and RMSE recorded by Duru in his model were 0.40 and 0.40, respectively (Duru, 1984). The values were obtained for Akure and Lagos, respectively.

Conclusion

This study outlined the effect of the corrections done to the old BMN model for the estimation of evapotranspiration. From the results obtained during the data analysis, the newly developed location specific BMN model proved to be a more accurate estimator of ET_o when applied to Enugu location. This is because this work utilized a standard statistical procedure in its calibration rather than the manual iteration method previously employed by Duru (1984) in the development of the original model. The overall result indicates that the Blaney-Morin-Nigeria model is location specific and that the newly generated models fared better and has improved performances than the BMN for the location considered for this study. For further study, other models such as Priestly-Taylor Model, Hargreaves-Samani Model could also be used to further re-examine the BMN model for this and other study areas. Also actual field data could be used to test the accuracy of the new model instead of the data generated using FAO56-PM model.

Conflict of Interests

There is absolutely no conflict of interests known to the author with regards to this manuscript.

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Full Length Research Paper

Forage intake and performance of cattle in silvo-pastoral systems and monoculture of Marandu in Pre-Amazon region

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This work aimed to evaluate dry matter intake (DMI) and performance of F1 Nellore x Guzera with live weight (LW) of 180±15 kg (5 months) on pastures of *Brachiaria brizantha* cv. Marandu in silvo-pastoral systems composed of babassu palm (*Attalea speciosa*) and monoculture systems in Pre-Amazonic region of Maranhão state, Brazil. Animals were evaluated in four systems: 0, 80, 131 and 160 adult palms ha^{-1}, characterizing monoculture (MC), low density (LD), average density (AD) and high density palm trees (HD), respectively, during the rainy (RS) and dry season (DS). Comparing seasons, only DMI in MC and AD were affected. DMI was between 2.6% LW for the RS and 2.8% LW for the DS, These values are very close as suggested by NRC, which is 2.7% LW for animals in this category. Higher average daily gain per ha was observed in animals kept in pastures with LD (0.750 g.day^{-1} and 84.37 kg.ha^{-1}, respectively). It was observed that animal performance was influenced by density of palm trees and the DMI by season, probably by the sward structure. Animal performance and production forage biomass were higher in 0 and 80 palm systems. This greater forage biomass allow higher stocking rate and, consequently higher animal production per ha.

Key words: Silvo-pastoral systems, pasture, season, Guzerá, Nelore, matter intake, daily gain.

INTRODUCTION

Animals raised on pasture where the forage is the only food source should consume sufficient amounts of

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nutrients for an acceptable production, because deficiency or low intake of any essential nutrient can affect animal performance. Dry matter intake (DMI) is influenced by palatability, nutritional value and forage availability, and the DMI is the main limiting factor of the animal productivity. In addition, the pastoral environment presents strong influence on these effects. Based on these features, there is a search for alternative systems to minimize negative effects of the environment and forage nutritional aspects, thereby increasing animal productivity.

A feasible alternative to solve problems caused by traditional livestock, where the grass is based on monoculture of forage species, is the silvo-pastoral systems (SPS). These systems are the intentional combination of trees and grazing animals in the same area at the same time and managed in an integrated manner, with the goal of increasing productivity per unit area. On these systems, interactions occur in all directions and at different magnitudes.

In silvo-pastoral systems, the presence of trees may retain and/or improve the quality of the soil by favoring the control erosion, the nutrient cycling and the addition of organic matter; use the solar radiation, more efficiently than in pastures in monocultive and capture nutrients and soil moisture at different depths, decreasing the dependence on external inputs of nutrients or establishing a more positive relationship between the benefit/cost.

According to characteristics of each ecosystem, you can adapt the native forests to SPS, thus preserving common trees of certain regions. In the State of Maranhão, Northeast region of Brazil, for example, the consortium of the mature babassu palm (*Attalea speciosa* Mart.) with pasture is called “traditional SPS”. This palm tree stands out in dry forest of the Amazon forest, mainly in Maranhão, where the state concentrate about 53% of the all babassu forest of the Brazilian territory. Despite the great importance that the babassu palm has in the state, little is known about its ecological properties, its effects on vegetation and soil, its efficient management and the ideal density of palm trees in the pasture. All these factors influence the animal performance, especially in the Pre-Amazonic Maranhão, in which livestock predominates within the state.

Few researches were done on animal under silvo-pastoral system. However, it is recognized that the information on the forage intake by animals in grazing system is important for most efficient recommendations on nutritional plan, regarding animal response and productivity per area (Aroeira et al., 2005; Paciullo et al., 2008).

This work was conducted with the objective of assessing the weight gain and feed intake of cattle grazing *Brachiaria brizantha* CV. Marandu in silvo-pastoral systems and monoculture, during rainy and dry seasons.

MATERIALS AND METHODS

The experiment was conducted at Água-Viva farm, in the municipality of Matinha-Baixada Maranhense region, with geographical position 45°00´40.9´´ W longitude and 03°06´55.5´´ S latitude. Forage species used was *B. brizantha* CV. Marandu and the arboreal species babassu palm *Attalea speciosa* Martius which was already established on the property.

Experimental design, animals and housing

Animals (Nelore x Guzerá with average of 180 ± 15 kg) were evaluated in four systems which are Marandu grass monoculture and three densities of babassu palms trees with more Marandu grass (SPS), corresponding to 0, 80, 131 and 160 adult palms.ha^{-1}, respectively. These systems characterize monoculture (MC), low density (LD), average density (AD) and high density palm trees (HD). Animals were placed on experimental units in completely randomized design (CRD) with subdivided plots arrangement, getting on the plots the densities of palms and in the subplots, the rainy and dry seasons. The data were grouped in two periods: the rainy season (April to June/July 2013) and dry season (June/July 2013 to October 2013).

Data regarding the average monthly rainfall calculated from a data series of 30 years and rainfall and temperature during the experimental period are presented in Figure 1. The annual precipitation varied from 2,000 mm annually, with the greatest concentration during the experimental period between the months of April to June. The maximum and the minimum temperatures were about 32 and 23ºC, respectively.

The total area used in the experiment was eight hectares, subdivided into four paddocks of two hectares, managed under continuous stocking, with five Nelore x Guzerá per experimental unit. Over time, regulators animals were placed and removed of each paddock to avoid possible sub or super grazing. The remainder of animals was kept in a reserve paddock (6 ha) and used in the experimental units as regulators animals, whenever there was need of stocking rate adjustment to keep the pastures in the predetermined height of 35 cm. All animals received water and mineral mixture *ad libitum*, and health management as recommended by Embrapa beef cattle.

Before the establishment of the experiment, samplings were made for characterization of soil fertility in the 0-20 cm layer. As can be seen in Table 1, all paddocks presented soil with characteristics of average fertility and independent of the treatment, the corrections of soil acidity were performed on the basis of the data of the analysis performed by lifting method of base saturation to 70%. The preparation of the area and corrective practices were carried out between October and November 2011, and the replanting of the grass in open areas within the paddocks, where the soil was exposed (no grass), was conducted between the months of January and February of 2012.

The fertilization in paddocks of monoculture, 80, 131 and 160 palms/ha was as follows: nitrogen (N) in the form of urea in 150, 150, 150 and 150 kg.ha^{-1}, respectively; phosphorus (P_2O_5) in the

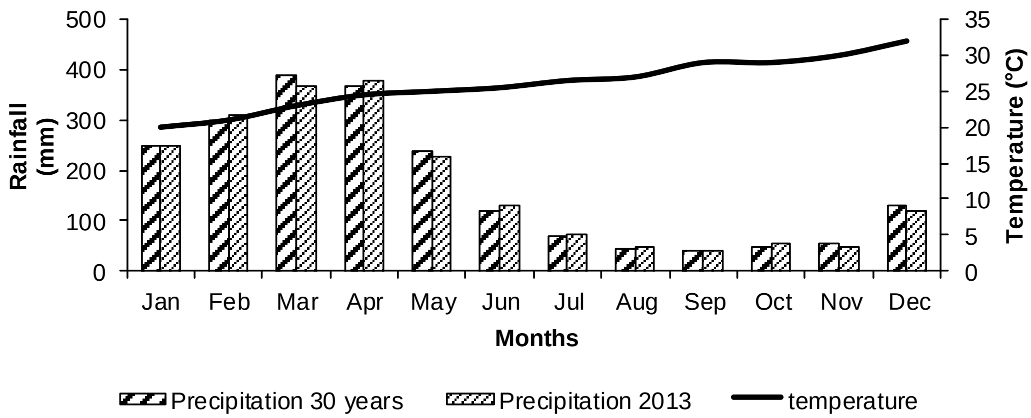

Figure 1. Average monthly rainfall calculated from a data series of 30 years and observed monthly rainfall and temperature during the experimental period in the year of 2013. Source: INMET (adapted).

Table 1. Chemical soil attributes in silvo-pastoral systems with different densities of babassu palms and monoculture before the experiment.

System[1]	pH	M.O	P	K	Ca	Mg	H+Al	Al	B	CTC
	$CaCl_2$	--------g dm$^{3-(1)}$----------			-------------- mmol$_c$ dm$^{3-(1)}$ -----------------					
MC	4.8	23	21	32	17	6	42	2	19	68
LD	5.2	23	9	40	21	9	27	1	20	61
MD	5.0	22	23	26	22	10	42	2	20	77
HD	4.8	23	11	37	20	7	44	1	28	75

System[1]	V	S	Cu	Fe	Zn	Mn	B
	----- % -----		------------------------------- mg dm$^{3-(1)}$ ------------------------------				
MC	38	5	0.4	104	4.4	47.3	0.19
LD	56	7	0.4	149	4.2	33.6	0.20
MD	45	6	0.4	61	4.6	54	0.20
HD	41	10	0.4	105	4.1	44	0.28

[1]Babassu palm density: MC = Grass monoculture (no trees); LD = Low density (80 palms.ha^{-1}); AD = Average density (131 palms.ha^{-1}); HD = High density (160 palms.ha^{-1}).

form of simple superphosphate in 150, 150, 150 and 100 kg.ha^{-1}; potash (K_2O) in 60, 60, 60 and 60 kg.ha^{-1}; and dolomite (limestone) in 1100, 270, 1550 and 1260 kg.ha^{-1}.

Canopy height was measured weekly, with ruler graduated in centimeters, in 30 random points by paddock. The height of each point corresponded to the average height of the canopy around the ruler.

Sampling and measurements

Forage biomass assessments were held every 28 days. To determine the productivity of forage (PF) representative samples of the paddocks were taken using squares of 0.25 m^2. The squares were placed at random in representative points of grazing at the time of sampling and the plants contained within each square were cut low to the ground every 28 days. After weighting, samples were separated into morphological components leaves, stems and dead material. These components were also dried in an oven and the proportion of each component was expressed as a percentage of the total weight. In possession of the proportions of each component in dry matter, it was determined the relationship leaf: stem ratio (LSR).

For the estimation of dry matter intake (DMI) only 20 of 40 animals were used, being five (repetitions) per system. Chromium oxide (Cr_2O_3) was administered orally, with the help of a rubber pipe, straight into the esophagus in doses of 10 g, once a day. The procedure was held for eight days, and in the first five days, the balance of intake and excretion of the indicator was obtained, and from the sixth day, samples of fresh feces were collected (around 300 g) directly from the rectum of animals, so that there was no contamination with dried feces or soil. The first day sampling was at 4:00 pm, the second at noon and the last was at 8:00 am, three days of collection was done. Then, the samples were frozen at -18°C. Then, the samples were homogenized, packed in aluminum dishes and pre-dried in oven of forced ventilation of 65°C up to present the constant weight. Then, they were processed in mill type "Wiley" with 1 mm sieve and grouped in proportion to have the composite samples of each animal.

The chromium concentration was estimated using an atomic absorption spectrophotometer, as methodology proposed by Williams et al. (1962), described by Silva and Queiroz (2009). Fecal total production was determined by the following equation proposed by Berchielli et al. (2005): Total feces production (g DM/d) = Amount of Cr_2O_3 (g)/ Concentration of Cr_2O_3 in fecal DM (g/gDM). The DMI was estimated by the following equation: DMI = Fecal production/(1 – IVDMD). For the calculation of the rate of recovery of fecal indicator, the following equation was used: Rate of recovery = (Fecal production by indicator x 100)/Fecal production by total collection. The calculation of recovery rate consisted of using data produced with total administered chrome and total excreted chrome, estimated by calculated fecal production (FPc).

The average weight of the animals in a period was used as reference to relate intake as proportion of live weight. Evaluation of the palatability was realized on collection of selected forage by animals in the form of simulated grazing during the months of June and September 2013. Dry matter (DM) and crude protein (CP) contents were calculated by Kjeldahl digestion, according to recommendations of the AOAC (1990) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) following the procedures of Van Soest (1994). The *in vitro* digestibility of dry matter (IVDMD) was according to Tilley and Terry (1963).

Every 30 days, all animals were weighed to follow their performance. Performance was calculated by average daily gain ($kg^{0,75}$) of animals. Also, the number of days on which the animals remained regulators in pasture was counted, to enable the estimation of the rate of manning of pastures, where along the experiment and in accordance with the availability of forage these animals walked in and out of pastoralist environments. Animal weight gain (WG) per hectare ($kg.ha^{-1}$) was obtained by multiplying the average daily gain by the number of animals (evaluators and regulators) maintained in the paddocks by month.

Statistical analyses

The data were grouped into two periods: rainy season (April to June/July 2013) and dry season (June/July 2013 to October 2013). Initially, the data were submitted to normality test (Crame-Von Misses) and scapular (Levene) to show the assumptions and were submitted to variance analysis by F-test. In the case of significant difference, comparison of averages was by SNK at 5% probability. The statistical analyses were conducted with the PROC GLM from SAS 9.0 (2002), with the option of repeated measurements in time, characterized by periods of assessment in each season of the year.

RESULTS AND DISCUSSION

There was no influence of period, density of palm trees and monoculture on chemical composition of the Marandu grass (P>0.05) during the voluntary intake trials (Table 2). This result is surprising, regarding the variation of the nutritional value of grass in SPS exposed to shading as compared to monoculture (Paciullo et al., 2007; Soares et al., 2004). According to Moreira et al. (2009), shaded plants present more levels of crude protein as the shading results in a larger cell size of the plants, increasing its cellular content and, consequently, crude protein.

Competition for nutrients is something common among tree species and grasses in SPS, in which the root system of the species used is well developed and possibly ended up competing with the Marandu grass for water and nutrients. Its root system is composed of two types of roots, a thick one responsible for storage of carbohydrates and a broad set of thin roots responsible for "pumping" of mobile nutrients leached to the deeper layers of the soil. By growing up together, the tree component and grass present competition for nutrients and water depending on the species, which probably occurred in the SPS. Therefore, their nutritional values are not different from the monoculture, being evident in dry season, as observed with a greater amount of senescent grass around the babassu plants. This competitive effect is not a possible allelopathic effect, it is for nutrients and water and as confirmed by Da Silva and Firmo (2008) who studied the allelopathic effects of Marandu grass on germination of babassu and did not find positive responses.

Moura (2004) noted that in areas with large densities of babassu, there was a negative linear effect of increasing density on protein content, indicating a possible competition for water and nutrients. Such effect did not occur in this work because the layout of palm trees at random may have interfered with this competition. In the dry season, the animals ingested larger quantities of forage on Mono and AD palms than in rainy season (P<0.05) (Table 3).

In all the systems, the forage production during cycles was above the average required according to the number of animals in each pastoral environment. The most critical system in relation to forage availability was HD during the dry season (Figure 2). However, the stocking rate in this environment was adjusted according to the availability of forage in that time. According to Gomide et al. (2001), intake is reduced when the supply of forage is less than 4-6% of live weight (LW). In this work, in any moment, this critical range of availability was reached, because there was a strict control of the quantity of animals in each silvo-pastoral system.

The forage intake was not affected by Marandu grass, since the nutritional value did not differ among treatments

Table 2. Chemical composition (% of dry matter) of the Marandu grass, collected in simulated grazing on silvo-pastoral systems with different densities of the babassu palms and monoculture in rainy and dry periods.

Season	System[1]				CV^2 (%)	MSE^3	P-value
	MC	LD	AD	HD			
Crude protein (%DM[7])							
Dry	6.42	6.71	6.42	7.29	20.03	1.34	0.0549
Rainy	7.71	7.58	7.88	9.19		1.98	0.0612
Neutral detergent fiber (%DM)							
Dry	72.60	70.79	68.30	70.53	14.02	12.23	0.0745
Rainy	69.83	59.91	69.74	68.35		11.76	0.0713
Acid detergent fiber (%DM)							
Dry	56.07	53.71	51.64	54.17	5.05	18.32	0.0675
Rainy	52.98	52.94	54.26	53.03		16.98	0.0634
Lignin (%DM)							
Dry	8.87	7.98	10.04	7.91	20.07	2.32	0.0512
Rainy	7.82	8.23	9.09	9.29		2.45	0.0543
Cellulose (%DM)							
Dry	47.21	47.21	47.21	47.21	21.3	19.98	0.0682
Rainy	45.16	45.16	45.16	45.16		20.17	0.0743
Hemicellulose (%DM)							
Dry	16.53	16.53	16.53	16.53	23.5	9.87	0.0534
Rainy	16.86	16.86	16.86	16.86		8.85	0.0577

[1]Babassu palm density: MC = Grass monoculture (no trees); LD = Low density (80 palms.ha^{-1}); AD = Average density (131 palms.ha^{-1}); HD = High density (160 palms.ha^{-1}).[2] CV = Coefficient of variation; [3] MSE= Means standard errors.

Table 3. Average values of dry matter intake (kg DM) crossbred cattle (Nellore x Guzerá), in silvo-pastoral systems in rainy and dry seasons.

System[1]	Season		CV^3 (%)	MSE^4	P-value
	Rainy DMI^2 (kg)	Dry DMI (kg)			
MC	6.82^{Ba}	7.41^{Aa}	37.69	2.32	0.0234
LD	6.43^{Aa}	6.72^{Aa}		3.31	0.0681
AD	6.29^{Ba}	6.55^{Aa}		3.29	0.0133
HD	6.52^{Aa}	6.45^{Aa}		3.56	0.0627

Means followed by the same uppercase letter (lines) and minuscule (columns) do not differ by SNK at 0.05. [1]Babassu palm density: MC = Grass monoculture (no trees); LD = Low density (80 palms.ha^{-1}); AD = Average density (131 palms.ha^{-1}); HD = High density (160 palms.ha^{-1}).[2]DMI = Dry matter intake; [3]CV = Coefficient of variation; [4]MSE= Means standard errors.

and by forage availability. This variation observed for the dry matter intake of animals kept in monoculture and AD palm trees is more related to the structure of the pasture during the two periods. On monoculture, there was a greater forage production in DS than in RS, with an increase of 3.22%, which increase grazing period on

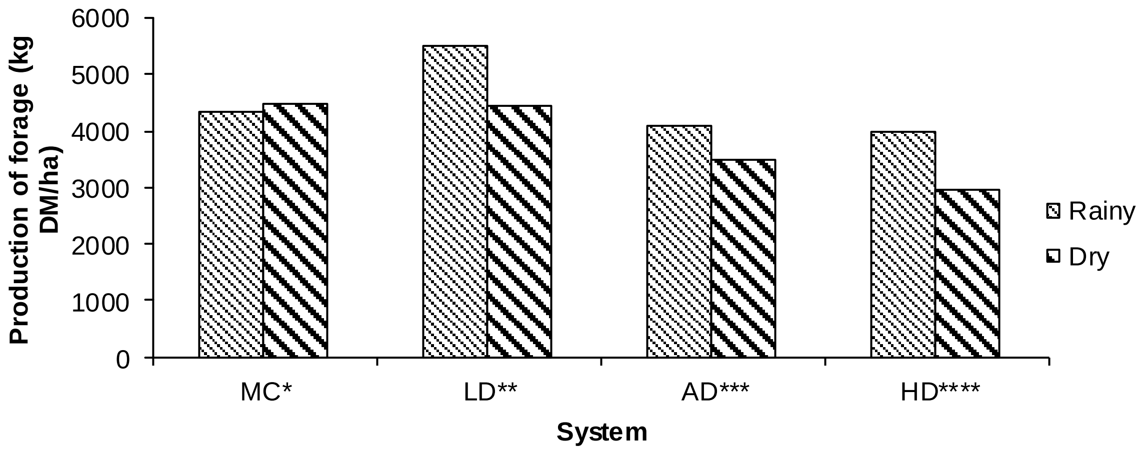

Figure 2. Production of forage in silvo-pastoral systems and monoculture. *monoculture; **80 palm trees.ha^{-1}; ***131 palm trees.ha^{-1}; ****160 palm trees.ha^{-1}.

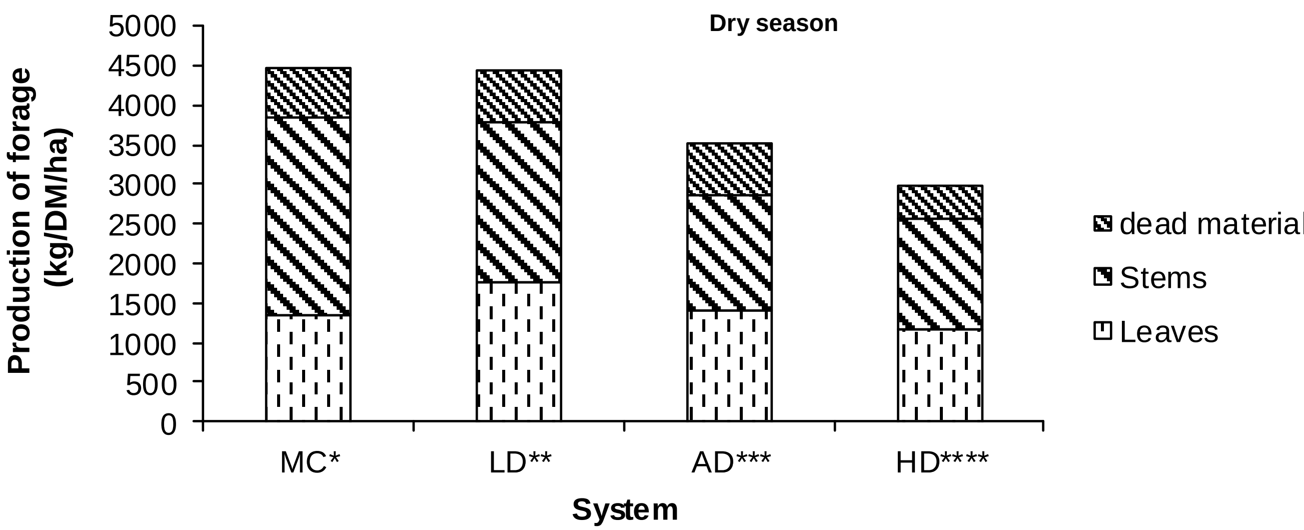

Figure 3. Production of forage in relation to amounts of leaves, stems and dead material in silvo-pastoral systems and monoculture during the dry season. *Monoculture; **80 palm trees.ha^{-1}; ***131 palm trees.ha^{-1}; ****160 palm trees.ha^{-1}.

fields and, consequently, the DMI. However, there was a greater participation of stem fraction, which was about 1,120 kg.ha^{-1} greater than the fraction of leaves and there was in addition, a significant 14.28% of dead material (Figure 3).

Stems as compared to leaves fraction increased during the dry season in all the systems, but in the AD palms, this relationship was less pronounced, being only 5.4% higher. Already in the other systems, there were 45.16; 13.75 and 17.14% higher proportion of stems for monoculture, LD and HD palms, respectively, justifying the greater DMI in the AD palms during the dry season.

Regarding the availability of stems, it is worth mentioning the greater participation during the rainy season only in monoculture, with 160 kg.ha^{-1} higher than the leaves fraction, which probably could have interfered with the DMI, but as compared to the SPS, there was no difference. In the AD and HD palms, there was a greater

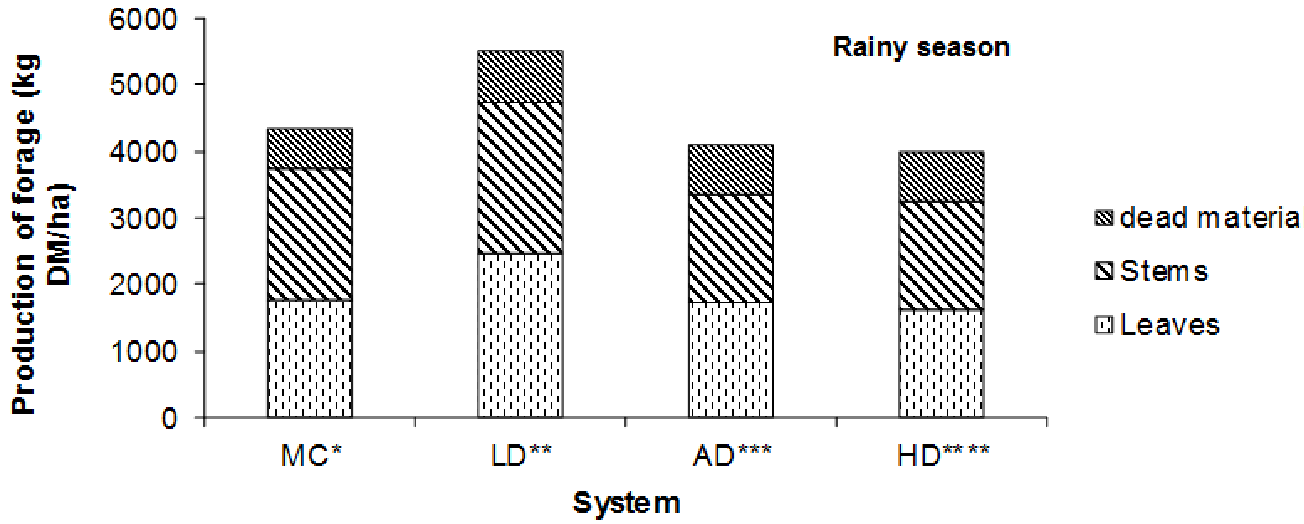

Figure 4. Production of forage in relation to amounts of leaves, stems and dead material in silvo-pastoral systems and monoculture during the rainy season. *Monoculture; **80 palms.ha^{-1}; **80 palm trees.ha^{-1}; ***131 palm trees.ha^{-1}; ****160 palm trees.ha^{-1}.

Table 4. Recovery rate of chromium oxide for crossbred cattle (Nellore x Guzerá) in silvo-pastoral systems in the rainy and dry season.

System[1]	RR[2] (%)		Mean	CV[5] (%)	[6]MSE	P- value
	RS[3]	DS[4]				
MC	92.35	93.23	92.29		21.76	0.0745
LD	91.32	92.41	91.86		17.87	0.0713
AD	90.78	91.45	91.11	27.8	20.98	0.0645
HD	91.00	92.21	91.60		19.87	0.0675
Mean	91.36	92.32				

Means followed by the same uppercase letter (lines) and minuscule (columns) do not differ by SNK at 0.05. [1]Babassu palm density: MC = Grass monoculture (no trees); LD = Low density (80 palms.ha^{-1}); AD = Average density (131 palms.ha^{-1}); HD = High density (160 palms.ha^{-1}), [2]RR = Recovery rate; [3]RS = Rainy season; [4]DS = Dry season; [5]CV = Coefficient of variation; [6]MSE= Means standard errors.

concentration of dead material over the period, with 770 and 630 kg.ha^{-1}, respectively (Figure 4).

The values of the recovery rate of indicator, during the experimental period for the four evaluated systems are in Table 4. There were no differences for both season and densities (P>0.05). In this work, it is observed that there was on average, a higher concentration of Cr_2O_3 during the dry season due to the amount of fiber in forage being higher on average in that period, where the NDF was 70.55 and 66.97% for dry and rainy season, respectively. In addition, the lignin and FDA contents were high in all the systems, which contributed to this difference between the periods, as the fibrous carbohydrates and lignin retain greater quantity of the indicator in the feces.

Detmann et al. (2001), by applying this methodology, estimated that DMI was 3.11% LW for beef cattle (F1 Limousin x Nellore) in *Brachiaria decumbens* pasture. Therefore, as much as this technique has its negative points, the values found here are acceptable according to the literature. Whereas, in grazing conditions, real intake is not known, and the values estimated by chromic oxide can only be compared to those obtained from tables of requirements in similar studies and methods. The data concerning the average daily gain (ADG) and gain per area (WG, kg.ha^{-1}) in each system are in Table 5. In general, it was observed that animals kept in LD palms had higher ADG, gaining on average, 0.75 kg$^{0.75}$.day^{-1}, followed by monoculture (0.65 kg$^{0.75}$.day^{-1}), AD palms

Table 5. Weight gain of crossbred cattle (Nellore x Guzerá) in silvi-pastoral systems composed of Marandu grass and different densities of babassu palm trees and monoculture.

System[1]	ADG[2] ($g.kg^{0,75}$)	WG[3] ($kg.ha^{-1}$)	CV[4] (%)
MC	0.65 [b]	73.12 [b]	
LD	0.75 [a]	84.37 [a]	25.5
AD	0.63 [b]	70.87 [b]	
HD	0.58 [b]	65.25 [b]	
P-value	0.0234	0.0324	
[5]MSE	0.202	14.23	

Means followed by the same letter do not differ by SNK at 0.05. [1]Babassu palm density: MC = Grass monoculture (no trees); LD = Low density (80 $palms.ha^{-1}$); AD = Average density (131 $palms.ha^{-1}$); HD = High density (160 $palms.ha^{-1}$), [2]Average daily gain; [3]Weight gain; [4]Coefficient of variation; [5]MSE= Means standard errors.

(0.63 $kg^{0.75}.day^{-1}$) and HD palms (0.58 $kg^{0.75}.day^{-1}$), which did not differ among them (P>0.05).

The ADG is within the expected value for animals in this category, non-suckling calves and without supplementation, according to Malafaia et al. (2004). Data on animal performance in silvipastoral systems are scarce in the literature, notably referring to the tree component densities mainly in SPS in which tree are native components, as in the case of babassu palm tree.

In work carried out in pastures of *B. brizantha* CV. Marandu, established in silvipastoral system with eucalyptus, body weight gain of Nellore steers of ages similar to those used in this work ranged from 392 and 892 $g.steer^{-1}$ day (Bernardino et al., 2011). These authors considered moderate weight gains for animals grazing Marandu grass, when faced with results obtained in monoculture and highlighted the potential use of silvo-pastoral systems in the production of beef cattle.

The largest ADG observed in animals maintained in LD palms is due to the intake of high amount of leaves allowed by the structure of the canopy. As showed in Figure 3, the forage production on LD palms was superior to other systems in both periods, with an average of 4,945 $kg.ha^{-1}$. Moreover, the leaf fraction, which is the favorite part of ruminants, was the highest in this system.

Average daily gainof over 500 g for animals raised exclusively on pastures, as observed in this work, showed reasonable performance of crossbred steers during the growing phase. The gain per area shows the same tendency as the ADG, with the higher value in the LD palms system presenting the best values, with gain of 84.37 $kg.ha^{-1}$, followed by monoculture, AD and HD palms, which did not differ among them (P>0.05). The system with 80 palm trees showed the highest stocking rate over time, and then animal production by area was bigger. Bernardino et al. (2007) evaluated the performance of steers on SPS of eucalyptus and *B. brizantha* and observed gain of 57 $kg.ha^{-1}$, lower than the obtained value in the present study.

Based on forage production (Figures 3 and 4) and the data of the relation of forage mass over the months, it can be considered that in all the pastoral systems, there was a moderate grazing intensity, because the use of grazing has allowed the maintenance of plants considered palatable. However, it did not allow the increase in its production, except in the monoculture in which there was an increase of 3.22%. As discussed earlier, it was an increase considered undesirable because there was only an increase of stems.

The stocking rate throughout the experimental period varied depending on the monthly availability of forage, in which in the beginning of the experiment there was the need for a large stocking rate, because the effect of fertilization in single dose caused the a significant production. The average stocking rate throughout the experimental period were 2.11, 2.35, 2.26 and 2.15 $AU.ha^{-1}$ for monoculture, LD, AD and HD palms, respectively (Figure 5).

In monoculture and HD palm systems stocking rate declined significantly throughout the experiment. On monoculture, for example, even with an increase in the production of forage, the availability of leaves was critical during the dry season. On HD palms the forage availability was the most affected, in which from one period to another there was a decrease of 25.06%. This was probably influenced by shading, since 160 palm trees/ha at random provides a higher shade, interfering with photosynthetic capacity of the grass. As a way to get light, there was a greater increase of stems, about 17.41% greater than the leaves component.

Babassu palm trees of the experiment were adults, with overall average of 7.5 m shaft height (base of the cup). The architecture (a form of inverted pyramid) causes the degree of shading to be much larger than other tree species used in SPS as the eucalyptus, for example, in which much higher density is used. This great shading provided by babassu palms can compromise the diffused light penetration in the pasture. However, in the system with LD palms, forage production remained steadily, and then the stocking rate in this pastoral environment was greater.

Conclusions

Palm trees density and the DMI affect the animal performance. The 80 palm trees system has better animal performance and forage production over time, which makes the stocking rate and livestock production

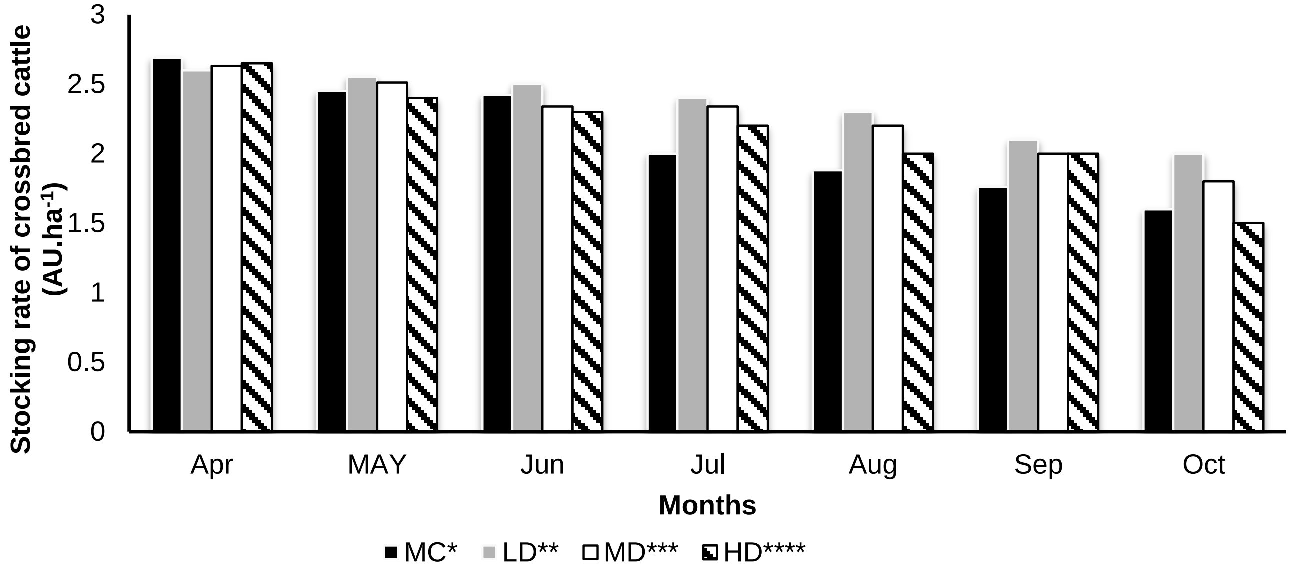

Figure 5. Stocking rate of crossbred cattle (Nellore x Guzerá) in AU.ha^{-1}, in silvo-pastoral and monoculture systems throughout the experimental period in the year, 2013. *Monoculture; **80 palm trees.ha^{-1}; ***131 palm trees.ha^{-1}; ****160 palm trees.ha^{-1}.

per area higher.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Broccoli growth and nutritional status as influenced by doses of nitrogen and boron

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Proper growth and development of broccoli may be affected by the application of N and B. Accordingly, the objective herein was to verify the effects of N and B application on growth and the nutritional status in the vegetative phase of broccoli. The experimental design was a completely random one in a factorial (4 × 2 +1) scheme, with four doses of B (0.25, 0.50, 1.00, and 2.00 mg dm^{-3}) and two doses of N (200 and 600 mg dm^{-3}), and the check treatment (no B and no N), with four repetitions. The green color index, the accumulation of N and B in the plant aerial part, leaf area, and the aerial part dry matter content were evaluated. The interaction between nitrogen and boron was not important for the green color index, N and B accumulation, leaf area and dry matter production of broccoli in the vegetative phase. However, the isolated effects of nitrogen fertilization and boron doses were beneficial for broccoli development.

Key words: Fertilization, *Brassica oleraceae* var. italic, vegetative phase.

INTRODUCTION

Broccoli (*Brassica oleracea* var. italica) is one of the vegetable species most widely grown and consumed in the world (Pizetta et al., 2005), highlighted mainly for its nutritional value as a source of various compounds, such as vitamins, minerals, antioxidants (Umar et al., 2013a), as well as its anticancer properties (Yoldas et al., 2008).

Among the cultural practices capable of increasing the productivity and the quality of broccolis, the equilibrated application of nutrients, especially N and B (Kojoi et al., 2009), is one of the most important. Nitrogen, the second most demanded nutrient by the crop, plays important role on the plant vegetative growth. Previous research has

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indicated that nutrients have important effects on broccolis productivity and quality (Belec et al., 2001; Moniruzzaman et al., 2007; Ambrosini et al., 2015).

Boron, needed by the plants in low quantities, is the most important micronutrient also due to its interaction with N (Campagnol et al., 2009). This interaction between N and B may result from non competitive inhibition (Malavolta et al., 1997). Thus, high doses of N may reduce the absorption of B, leading to physiological disturbances. The B deficiency may be induced by the plant rapid growth promoted by N (Filgueira, 2003).

Research works with boron in broccoli is restricted to the reproductive phase, since its larger demand and importance since pollen grain germination up to the pollen tube growth reflecting on fecundation and growth of the flowers (Prado, 2008). During the vegetative phase, B demand is relatively low in comparison with the reproductive one. But little is known about the effects of boron specially when in association with N in excess.

The hypothesis that the plants submitted to the N in excess could reduce the amount of accumulated B without hampering dry matter production since the low demand of that micronutrient during the vegetative phase, and thus, the interaction between N and B is lowly probable as long as B is not deficient.

Having those facts in mind, the objective of this research work was to verify the effects of the application of N and B on the growth and nutritional status of broccoli plants during the vegetative phase of growth.

MATERIALS AND METHODS

The study was carried out under greenhouse conditions at the Jaboticabal, Brazil campus of the Paulista State University (UNESP) from August to October of 2013. Samples of a red dystrophic latosol (Embrapa, 2013), taken from the 0 to 20 cm top layer, were used to fill the vases. The chemical analysis (based on methodology described in Raij et al. (2001)) of the soil showed its composition to be of: pH in $CaCl_2$ = 5.5, organic matter (MO) = 8.0 g dm^{-3}, P resin = 6.0 mg dm^{-3}, K = 1.4 $mmol_c$ dm^{-3}, Ca = 16 $mmol_c$ dm^{-3}, Mg = 9.0 $mmol_c$ dm^{-3}, B = 0.2 mg dm^{-3}, Cu = 0.2 mg dm^{-3}, Fe = 4.0 mg dm^{-3}, Mn = 4.2 mg dm^{-3}, Zn = 0.3 mg dm^{-3}, H + Al = 15 $mmol_c$ dm^{-3}, sum of bases (SB) = 26.4 $mmol_c$ dm^{-3}, cation exchange capacity (CEC) = 41.4 $mmol_c$ dm^{-3} and bases saturation (V) = 63.5%. Boron content of the soil was considered low, according to Raij et al. (1997) (0 to 0.20 mg dm^{-3}).

Four replications of each treatment were distributed in the greenhouse according to a completely random design in a factorial scheme of 4 × 2 + 1, with four doses of B (0.25, 0.50, 1.00, and 2.00 mg dm^{-3}) supplied by borax and two doses of N (200 and 600 mg dm^{-1}) supplied by urea. The check treatment included no B and no N. Each experimental unit was formed by a 3 dm^3 vase with one plant.

The doses of 1.0 mg dm^{-3} of B, supplied by borax, and 200 mg dm^{-3} of N, supplied by urea, indicated as the adequate ones for broccolis (Malavolta, 1981) were used to determine the treatments of this experiment. To all treatments, 270 mg dm^{-3} of K (supplied by potassium chloride) and 300 mg dm^{-3} of P (supplied by simple superphosphate) were added, according to Malavolta (1981).

Three weeks old broccoli plantlets (produced in 200 cell polystyrene trays) of the cultivar 'ramoso-santana' were used. Three plantlets were planted per vase. A week after transplantation, the plantlets were thinned so as to leave only one plant per vase. The plants, during the experimental period, were watered twice a day so as to keep soil moisture at 70% of its maximum retention capacity. The action of insect pests was controlled by the application of the indicated insecticides.

Two months after transplantation, during the vegetative phase, the green color index of the plants in each vase was evaluated, read in a recently developed leaf at its median part at the side of the central nervure with the help of an equipment of the OPTI-Sciences™, model CCM – 200 and the leaf area with the help of an area meter model LI - 3100™. At that moment, the aerial part of the plant was taken which was submitted to washing in deionized water followed by drying in a forced ventilation oven at 65°C till a constant weight was reached. After that, the plant dry matter content was determined and the material was ground in a Willey mill and the contents of N and B determined according to methods described in Bataglia et al. (1983). Then by combining the weight of these nutrients and the plant dry matter weight, the accumulation of these nutrients was calculated.

The data were submitted to the analysis of variance by the F test at 5% of probability. When the F value was statistically significant, the N means were compared by the Tukey test at 5% of probability and the B means analyzed by a polynomial regression analysis. The software ASSISTAT, version 7.6 beta was used.

RESULTS AND DISCUSSION

N doses affected only the green color index and the accumulation of N, independently of the application of boron (Table 1). A similar result was reported by Ambrosini et al. (2015) in broccoli cultivation.

The highest dose of N promoted the largest increment in the green color index, independently of boron dose (Table 1). A similar response was reported by Dufault (1988) in broccolis plants growing in green house and by Arjona and Greig (1984) in broccolis cultivar Green Comet, with a detected increase in chlorophyll content. Ambrosini et al. (2015) also reported that for the broccoli cultivar BRO 68, nitrogen application stimulated the synthesis of chlorophyll in the leaves, resulting in improved nutritional quality.

This result may be justified by the role played by N in the constitution of the chlorophyll molecule (Malavolta et al., 1997), since there was a larger accumulation of N in the broccolis plants resulting from the increment in N doses (Table 1). Another hypothesis is that the presence of N in the leaves favored the assimilation of CO_2 in the photosynthesis process, promoting an increase in the net photosynthesis rate and consequently an increase in the chlorophyll content of the leaves (Li et al., 2013).

There was an effect of B doses on the accumulation of N and B in the plant (Table 1). The boron doses

Table 1. Green color index (GCI), accumulation of nitrogen and boron, leaf area and dry matter of broccoli in the vegetative phase in nitrogen function and boron rates, Jaboticabal, SP, Brazil, 2013.

Treatment	GCI	N Accumulation	B Accumulation	Leaf area	Dry matter
Doses of nitrogen (N)	-	g per plant	mg per plant	cm^2	g per plant
200 mg dm^{-3}	35.75^B	62.36^B	0.11	470.57	3.59
600 mg dm^{-3}	46.50^A	89.37^A	0.12	452.99	3.39
(N)	8.20**	8.22**	0.02^{NS}	0.71^{NS}	0.29^{NS}
Doses of boron (B)	3.01^{NS}	3.54*	2.83*	0.99^{NS}	1.01^{NS}
N × B	1.12^{NS}	0.54^{NS}	2.84^{NS}	2.42^{NS}	2.25^{NS}
Factor × Control	14.12**	19.43**	20.74**	19.31**	24.20**
CV (%)	19.9	19.2	21.9	24.5	22.2

Means followed by the same capital letter in the column, does not differ from each other by the Tukey test at 5% probability. ^{NS}not significant and **, *significant at 1 and 5% probability by F test.

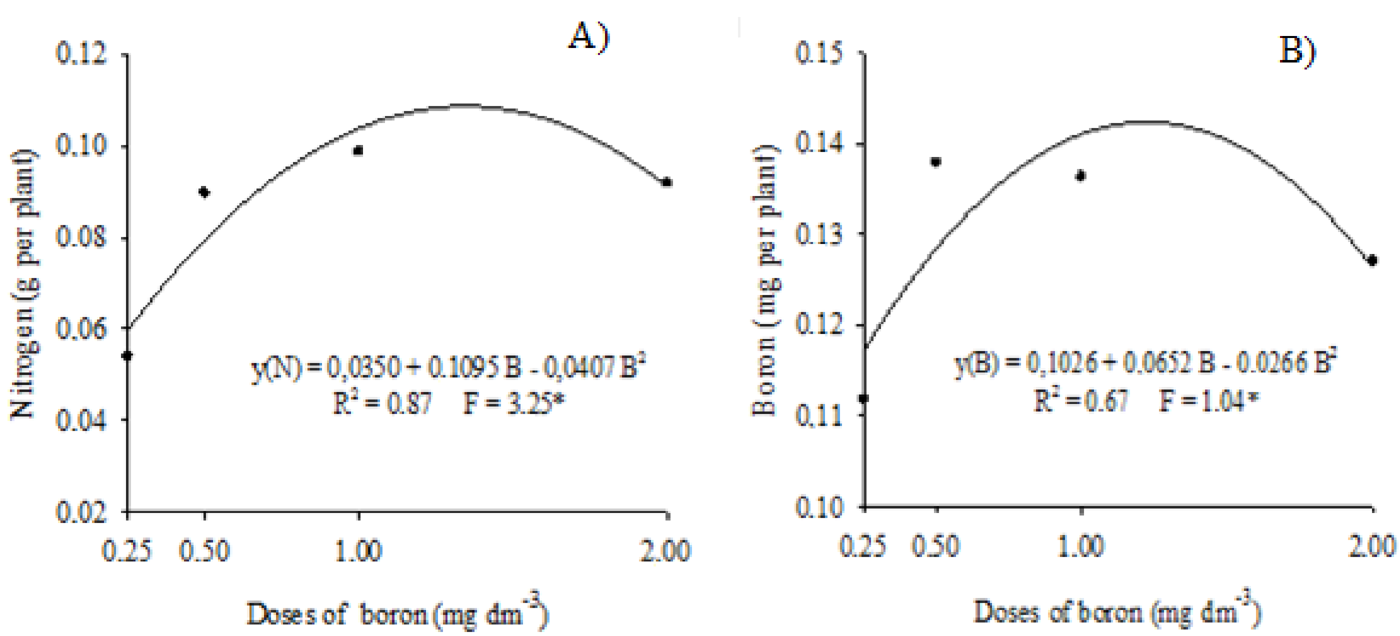

Figure 1. Accumulation of nitrogen (A) and boron (B) expressed in g per plant for broccoli in the vegetative phase in function of boron doses.*Significant at 1% probability by the F-test.

promoted a quadratic increase in the accumulation of foliar N, with a maximum value of 0.11 g per plant when the N dose reached 1.34 mg dm^{-3} (Figure 1A) and also in the accumulation of B, with a maximum value of 0.143 per plant when the dose was of 1.22 mg dm^{-3} (Figure 1B). The absence of interaction between B and N doses during the vegetative phase (Table 1), may be explained by the relation that exists between these two nutrients only in the reproductive phase due to the demand of B being higher during that phase as determined by the germination of the pollen grain and growth of the pollen tube and also to the fact that N accelerates plant growth thus inducing B deficiency (Shelp et al., 1995). Since during the vegetative phase flowering does not take place, the amount of B needed by the plant are low so that a deficiency of that nutrient is less likely as well as an interaction with nitrogen.

In relation to the interaction factorial × check treatment, an effect of the interaction for the studied variables was observed (Table 1). This result may be explained by the importance of the roles played by N and B in the plant life when both nutrients are at deficient levels and plants growing without those nutrients (Malavolta et al., 1997).

Conclusion

The interaction between nitrogen and boron was not important for the green color index, N and B accumulation, leaf area and dry matter production of broccoli in the vegetative phase. However, the isolated effects of nitrogen fertilization and boron doses were beneficial for broccoli development.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Alternative control of *Corynespora cassiicola* in papaya seedlings and fruits by *Cinnamomum zeylanicum* essential oil

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The study aimed to evaluate the efficiency of essential oil concentrations on *Cinnamomum zeylanicum* leaves as a possible fungicide against mycelial growth, and the sporulation of *Corynespora cassiicola* in papaya seedling and fruit. The study performed three experiments, namely comparison of the anti-*C. cassiicola* activity of: 1 µL mL^{-1} *C. zeylanicum* essential oil, commercial fungicide, and control-treatment; comparison of the anti-*C. cassiicola* activity of five essential oil concentrations (0.0, 0.5, 1.0, 2.0, and 4.0 µL mL^{-1}) and a commercial fungicide (150 g ha^{-1} i.a.); and the effect of applying essential oil concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 µL mL^{-1}) before and after the application of mycelium disk fungus. It was found that the essential oil from *C. zeylanicum* inhibits mycelial growth and sporulation of the *C. cassiicola* fungus. The essential oil was able to maintain lower percentage of leaves with lesions in papaya seedling up to 14 days after the inoculation. The essential oil derived from *C. zeylanicum*, applied as a preventive treatment, is efficient in controlling the size of lesions in papaya fruits, however, the effect is not observed if the essential oil is applied after infection.

Key words: *Carica papaya*, target-spot, natural pesticides, inhibitory effect.

INTRODUCTION

The excessive use of fungicidaes to increase the fruit papaya yield gave rise to many negative effects including the appearing of diseases in all crops, including papaya crops. The presence of diseases can cause severe economic losses that reduce papaya fruit yield and marketability. The main papaya diseases are viruses such as Mosaic (Papaya ringspot virus - PRSV) and papaya sticky disease (Papaya meleira virus - PMeV),

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nematoses (*Meloidogyne incognita*), fungal diseases such as Alternaria spot (*Alternaria alternate* [Fr.] Keissl), anthracnose (*Colletotrichum gloesporioides* Penz) and the disease known as target-spot (*Corynespora cassiicola* [Berk & M. a;. Curtis] CTWei), all disease widely spread in most producing areas of world, including Brazil (Porter et al., 2014). Furthermore, in Maranhão State the presence of ideal climate and temperature conditions are response to provide the disease target-spot (*Corynespora cassiicola*) (Silva, 2011).

Target-spot is characterized by the appearance of injuries, which can affect the leaves, stem and fruits. Hundreds of injuries can occur in a single leaf leading to their premature loss as a result of foliar abscission. These injuries are pale yellow color, and vary from 1 to 2 mm in diameter, but in some cases can reach 10 mm in diameter, with necrotic centres. In some cases, the injury presents in the leaf might be superficially and easily cracked. The symptom of this disease on fruit is characterized by small and rounded injuries, approximately 1 mm in diameter. The developments of the disease on fruit occur rapidly, and can affect the fruit pulp, rendering it commercially unviable. Among the strategies to control the target-spot disease is the use of fungicides (Ventura et al., 2004; Liberato and Mctaggart, 2006).

Fungicides mainly used to control the disease are from dithiocarbamates, phthalonitrile, copper oxicloride, chlorothalonil or tebuconazole groups, which are also used for controlling other fungal diseases of papaya plants (Oliveira and Filho, 2006). It is known that the disorder use of fungicides can cause serious damage to the environment and human health. Hence, alternative sources of fungicide for disease control, especially those that could replace chemicals, cost effective and use in low or no residual power are necessary (Ootani et al., 2013).

The potential of essential oils to stop or inhibit mycelial growth has been studied as an alternative to the exclusive use of fungicides. Costa et al. (2011) reported that the eugenol is one of the main compounds found in clove essential oil (*Syzygium aromaticum* [L.] Merr. & L. M. Perry) *in vitro*. These authors showed that the utilization this essential oil was effective on the mycelial growth of *Rhizoctonia solani* Kun*, Fusarium solani* (Mart.) Sacc. and *Fusarium oxysporum Schlecht.* Studies performed by Carlos et al. (2010) reported that the essential oil of medicinal plant *Achillea millefolium* L., in the concentration of 200 µL, inhibited 63% of mycelial growth and 98 to 100% of the sporulation and the spore germination. Similarly, Carnelossi et al. (2009) using both *in vitro* and *in vivo* techniques showed a positive effect in the control of anthracnose in papaya fruit using essential oil of lemongrass leaves (*Cymbopogon citratus)*, eucalyptus (*Eucalyptus citriodora* L.), mint (*Mentha arvensis*L) and tarragon (*Artemisia dracunculus* L.).

Recent studies on the use of plant essential oil have been used as a new strategy for the control of plant disease. Studies have been performed to improve the antimicrobial activity and stability of *C. zeylanicum* (commonly known as cinnamon) essential oil with or without chitosan nanoparticles against *Phytophthora drechsleri*, the causal agent of cucumber (*Cucumis sativus* L.) fruit rot (Mohammadi et al., 2015). In addition, studies also reported the efficacy of chitosan in combination with *Zataria multiflora* or *C. zeylanicum* essential oil to inhibit *P. drechsleri in vitro* and on artificially infected cucumbers (Mohammadi et al., 2016). Those authors reported that *C. zeylanicum* combined with chitosan coatings can be an effective method for extending cucumber shelf-life and the combined treatments (chitosan with *C. zeylanicum* and chitosan with *Zataria multiflora*) were able to reduce fungal decay in the range of 77 to 85%, compared with the control at day 9.

For the development of natural products as an alternative to the use of synthetic fungicides, *in vitro* experiments are needed to verified the essential oil effectiveness on the pathogens. After the identification of essential oil efficiency on pathogen control new tests *in vivo* are needed to establish the optimum essential oil concentrations for specific inhibition of single or a group of pathogens (Combrinck et al., 2011).

The study hypothesized that essential oil have potential as environmentally safe alternative for the control of plant disease in relationship with the synthetic fungicides. Despite these natural products potentially great importance, few studies have focused specifically on the effects of *C. zeylanicum* on *C. cassiicola* in the humid tropic of Brazil. The aim of this work was to:

i) Evaluate the efficiency of the essential oil obtained from *C. zeylanicum* as a potential fungicide on mycelial growth and the sporulation of *C. cassiicola in vitro.*
ii) Evaluate the effect of various essential oil concentrations on the control of target-spot in papaya seedling *in vivo.*
iii) Evaluate the efficiency of this oil in the preventative and curative control of *C. cassiicola* in papaya fruit.

MATERIALS AND METHODS

Experimental site and obtainment of isolate

The experiments were conducted in Phytopathology Laboratory and greenhouse under constant relative humidity, temperature and irrigation conditions, at the State University of Maranhão, São Luís, Maranhão State. The predominant climate type in São Luis is mesothermal tropical and humid with pluviometrics precipitations of approximately 1900 mm, high relative humidity (±82%) and mean temperatures of 26°C. *C. cassiicola* (registry: MGSS-092) isolate was obtained from the fungus collection named "Prof. Gilson

Soares da Silva"/ UEMA. The isolate has been conserved in potato dextrose agar (PDA), which has been preserved by continuous sub-culturing.

Leaves samples and extraction of essential oil

In this study, the leaves were used only. Leaves samples used for extraction of the essential oil were obtained from *C. zeylanicum*, collected in the experimental area of Medicinal Plants of the Federal University of Maranhão, São Luís, Maranhão State, Brazil. Subsequently, the leaves were placed in paper bags and dried in an oven at 70°C until constant weight was achieved. After oven drying to a constant weight, extraction of the essential oil was performed at the laboratory of the Medicinal Plants at the Federal University of Maranhão. Distillation process was used for the extraction of essential oils.

Each distillation process used a quantity of 100 g of dry leaves of *C. zeylanicum* coarsely chopped. Distillation was performed using a modified Clevenger's glass apparatus. The distillation started after a heating time of 40 min. Subsequently, the extraction process was carried out for 3 h after the first drop of distillate until complete exhaustion of the leaves. Condensation was carried out continuously with water chilled to 10°C. The same extraction process was performed three times. The essential oil extracted was recovered, dried with anhydrous sodium sulphate and stored in a refrigerator at 4°C in tightly closed amber vials, and it was used for analysis and various functional biological tests. This process was performed in normal conditions (1.0 bar, 25°C). Chromatography analysis indicated that eugenol is the main volatile compound of extracted oil from *C. zeylanicum*. This component is present in essential oils or extracts of many other plants, and it is used as an alternative to control diseases.

Experimental design

Experiment 1

The experiment was carried out in a randomized experimental design, with 10 replicates, in a split-plot arrangement, with three treatments in the main plots and the nine periods of daily measurements in the subplots. Each Petri dish consisted of one replicate. Each Petri dish contained *C. cassiicola* fungal colonies. The experiment consisted of three treatments as follows: the essential oil from *C. zeylanicum* applied at 1 µL mL^{-1}; commercial fungicide (Tebuconazole) applied with the rates of 150 g ha^{-1} i.a., as per schedule recommendation from the manufacturer; and control treatment, comprising the fungi cultivated in PDA incubation without application of essential oil and fungicide. The subplots consisted of nine periods of daily measurements after inoculation.

The PDA was autoclaving at 121°C for 15 min. The treatments were added to melting PDA medium at 45°C. Subsequently, 20 ml of this mixture was transferred to petri dishes with 9 cm of diameter. Five-millimeter diameter discs were obtained from the fungal colonies and transferred to the center of Petri dishes with PDA medium. Petri dishes were closed with plastic film and stored in a BOD (Biological Oxygen Demand – SPLabor Model SP-225) type chambers at 25°C with a photoperiod of 12 h. The Petri dishes were closed with plastic film to avoid possible transpiration of the compounds and contamination by other microorganisms.

Evaluation of the effect of the essential oil on mycelial growth was performed by daily evaluations of colony diameter in two perpendicular axes for a period of 9 days after 48 h of initial fungus transplantation. This initial time was necessary for the control treatment in order to fill the whole Petri dish. Mycelial growth speed rate (MGSR) was measured daily as described by Oliveira (1991):

$$\mathrm{MGSR} = \frac{\sum(\mathrm{D}-\mathrm{Da})}{\mathrm{N}}$$

Where, D = current average, Da = average diameter of the previous day, and N= number of days after inoculation. At 10 days after incubation to allow for mycelial growth of *C. cassiicola*, the percentage inhibition of mycelial growth (PIC) was evaluated as described by Bastos (1997):

$$\mathrm{PIC} = \frac{\text{growth in the control - growth in the treatment}}{\text{growth in the control}} \, x\, 100$$

Evaluation of sporulation was performed after 10 days of incubation. 10 ml distilled water was pipetted onto the Petri dishes and a Drigalski handle was used to produce conidia. After rapid manual shaking and scraping with a sterilized soft bristle brush, Petri dish contents were filtered in sterilized gauze and quantified in a Neubauer chamber.

Data obtained in the first experiment were statistically examined by analysis of variance (ANOVA) and regression. Means of qualitative factors were compared by the Tukey test at significance level up to 5% probability. The quantitative factor and models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level value of determination coefficient (R^2), and according to the biological phenomenon**.** The R^2 was obtained by relationship between regression sum and treatment sum of square.

Experiment 2

Six seeds of Sunrise Solo variety were sown in pots with 5 L capacity. The substrates used in the seeds germination comprised of soil and autoclaved manure in the proportion of 3:1 v/v. 10 days after sowing, plants were thinned to three plants per pot. Sixty days after germination, papaya seedlings were sprayed with spores suspensions of the *C. cassiicola*. After incubation, plants were kept in a humid chamber in greenhouse conditions for 48 h. Then, papaya seedlings were maintained in constant relative humidity, temperature and irrigation conditions for 28 days of evaluation period.

For inoculum prepararation, *C. cassiicola* isolates were transferred to Petri dishes containing PDA and were maintained in B.O.D. type chambers, at temperature of 25°C, with a photoperiod of 12 h, for 7 days. Then, 20 ml of distilled water was added to the Petri dishes and conidia were collected from the flooding dishes by gently scraping the colony surface with a bent glass rod. Subsequently, conidia counts were performed with the aid of a Neubauer chamber. Conidia suspensions were adjusted to approximately 1×10^5 conidia mL^{-1}.

The experiment was carried out in a randomized experimental design, with 6 replicates, in a split-plot arrangement, with five essential oil concentrations and commercial fungicide with active ingredient Tebuconazole (Folicur®) in the main plots and the four periods of measurements, in 7-day intervals in the subplots. Each pots contain one replicate. The five essential oil concentrations were: 0.0, 0.5, 1.0, 2.0, and 4.0 µL mL^{-1}. The commercial fungicide was applied at a rate of 150 g ha^{-1} i.a., as per schedule recommendation from the manufacturer. The subplots consisted in

7-day intervals at 7, 14, 21 and 28 days after inoculation. First disease symptoms were observed at three days after inoculation. The treatments were applied on papaya seedlings after appearing the first disease symptoms. Dimethyl sulfoxide (DMSO) (1% in distilled water) was used as a solvent to solubilize the essential oil. This solution was then applied to each sample using a 1.0 L hand sprayer.

The evaluated parameters included total number of leaves, number of plants with disease and the severity of the target-spot disease measured as the percentage of leaves with lesions and the total leaf area with lesions. The total leaf area with lesions calculation was performed at 28 days after inoculation. Then, for each pot, four leaves were collected from middle to upper level of the seedlings and were scanned in gray scale with resolution of 75 dpi (pixel/inch). The standardization of leaf area and the total leaf area with lesions were performed through the ImagemJ program, with distance adjusted in pixel for 29.52 pixel. The percentage of growth inhibition (P.I.C.) of lesions was also calculated according to the following equation:

$$\mathrm{PIC} = \frac{\text{growth of lesions in control - growth of lesions in treatment}}{\text{growth of lesions in control}} x100$$

Data obtained during second experiment were statistically examined by ANOVA and regression. The mean of each essential oil concentration was compared with that of commercial fungicide by the Dunnett's test at significance level up to 5% probability. Regression models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level, value of determination coefficient (R^2) and according to the biological phenomenon**.** The R² was obtained by relationship between regression sum and treatment sum of square.

Experiment 3

Healthy papaya fruit of the Sunrise Solo cultivar were acquired at intermediate maturation stage. The fruit were washed in running water, and immersed in a solution of 2% (v/v) sodium hypochlorite for 1 min. Then, the fruit were rinsed with sterile distilled water for three times and were kept in room temperature until there were completely dried. This experiment was conducted in a completely randomized design with five replicates. Five essential oil concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 µL mL^{-1}) of *C. zeylanicum* were used. Each replicate comprised of one fruit. The essential oil concentration of 0.0 µL mL^{-1} comprised of the fruits containing the mycelium disk and sterile distilled water.

For the preventative treatment, essential oil concentrations were applied 24 h before pathogen inoculation. DMSO (1% in distilled water) was used to solubilize the essential oil at various concentrations for the treatments. The application of solution containing essential oil was performed with a small handheld sprayer of 1.0 L capacity. For assessment of curative treatment with the essential oil, orifices of approximately 2 mm in depth were performed in the middle part of the fruit and later 5 mm diameter mycelium discs were inserted. The fruits were placed in transparent plastic bags and kept in plastic containers in a humid chamber for 24 h. The fruits were sprayed with essentials oils and kept in the humid chamber for 72 h. Thereafter, the fruits were kept inside the plastic containers during six days for the disease symptoms development.

The evaluation of number of infested fruits and severity of the disease (diameter of lesions) were performed six days after the inoculation. The P.I.C. of lesions was also calculated. The results obtained from the third experiment were examined by regression analysis. Regression models were chosen based on the significance of regression coefficients using the *t*-test at 5% probability level, value of determination coefficient (R^2), and according to the biological phenomenon. The R² was obtained by relationship between regression sum and treatment sum of square. The SAEG (version 9.1, 2007) software was used for all statistical analysis.

RESULTS AND DISCUSSION

Mycelial growth and conidium production of *C. cassiicola*

There was a significant effect of treatments on mycelial growth of *C. cassiicola* fungus through out of the experiments (Table 1). The treatment using essential oil derived from *C. zeylanicum* was found as efficient as the commercial fungicide treatment in inhibiting fungal mycelial growth. Target-spots were observed on untreated papaya leaves. However, there was no mycelial growth of *C. cassiicola* fungus in essential oil and fungicide treated papaya leaves. Thus, these results showed the efficiency of *C. zeylanicum* essential oil for the control of spot-target. Increased periods of daily measurements after inoculation resulted in a significant increase in the mycelial growth of the *C. cassiicola* fungus according to a linear model (Figure 1). The control treatment provides the mycelia growth normally. The MGSR for the control treatment was of 0.18 cm day^{-1}.

Essential oil obtained from bark and leaves of *C. zeylanicum* was reported by Ranasinghe et al. (2002) which provide inhibitory effect on mycelial growth of the fungi *Colletotrichum musae* (Berk & Curt.) Arx (causing the disease known as anthracnose), *Lasiodiplodia theobromae* and *Fusarium proliferatum* (Matsuhima) Nirenberg (responsible for rot in rhizomes of banana) *in vitro*. Combrinck et al. (2011) also performed 100% efficiency of *C. zeylanicum* essential oil on mycelial growth inhibition of the pathogens *L. theobromae*, *C. gloeosporioides*, *Alternaria citrii*, *Botrytis cinerea* and *Penicillium digitatum*. Carnelossi et al. (2009) observed total or partial inhibition of *C. gloeosporioides* sporulation with the use of essential oils of lemon grass, eucalyptus, mint and tarragon. Besides, Lorenzetti et al. (2011) verified the effect of essential oil of various species on the control of the fungus *Botrytis cinérea* in strawberry gray with concentrations of 125 and 1000 ppm. The *C. zeylanicum* oil was found to efficiently affect both mycelial growth and germination of fungi spores.

The inhibitory effects were due to the presence of eugenol, a majority compound present in the essential oil of *C. zeylanicum* (cinnamon) was reported by Carnelossi et al. (2009) and Lorenzetti et al. (2011). This substance is responsible for causing: alterations in the cytoplasmic membrane; interruption of the motive power of protons, of

Table 1. Mycelial growth (cm) *in vitro* affected by essential oil from *C. zeylanicum* (1 µL mL^{-1}).

Treatment	Days after the inoculation									PIC[1]	n^0 spores (x10^5) [2]
	1	2	3	4	5	6	7	8	9		
Control	1.6^b	2.6^b	3.2^b	3.5^b	4.0^b	4.4^b	4.7^b	5.8^b	6.1^b	-	2.64 ^{b}*
Essential oil	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a	100	0.0 ^{a}
Fungicide	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a	0^a	100	0.0 ^{a}

*Means followed by the same lower case letter in the column do not differ by Tukey test at 5% probability.[1]Percentage of mycelial growth inhibition (PIC). [2]Sporulation of *C. cassiicola* evaluated at 10 days after the inoculation.

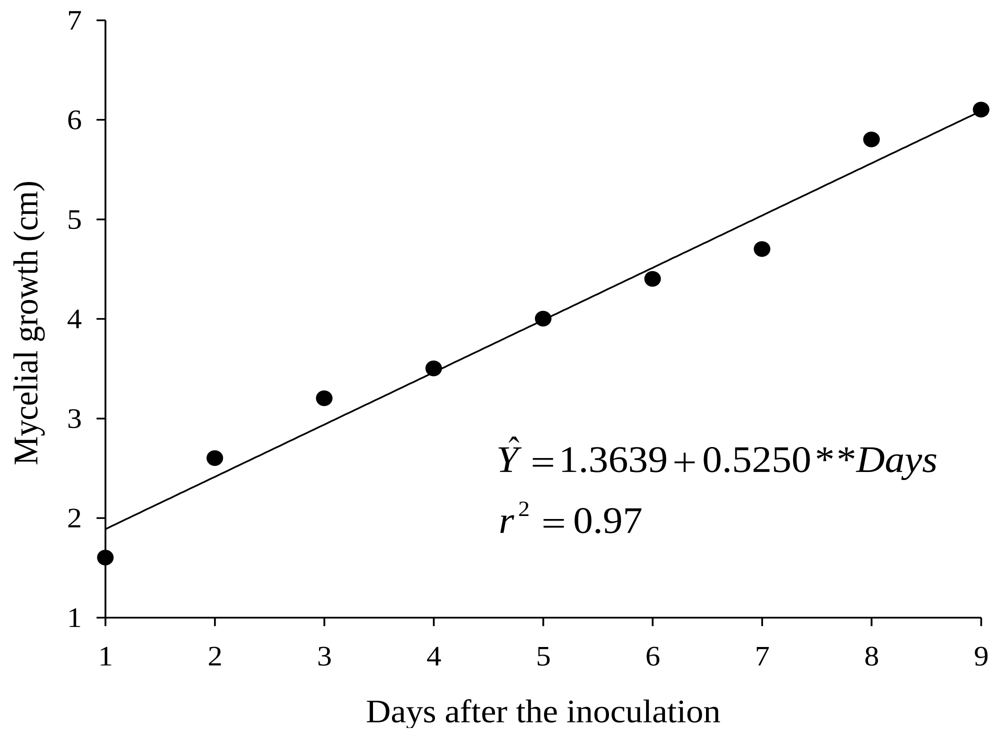

Figure 1. Mycelial growth (cm) as function of days after inoculation for control treatment. ** Significant at 1% of probability by the *t*-test (** Significant at 1% of probability by the *t*-test).

the electrons flux, of the active transport; and coagulation of the cellular content of filamentous fungi (Davidson, 1997; Abbaszadeh et al. 2014). Costa et al. (2011) reported that the essential oil obtained from cloves (*Syzygium aromaticum*) presents a huge concentration of eugenol and promotes morphological alteration in the vacuoles; disorganization of cell contents; and decrease of the cell wall distinctness beyond lower turgescence in cells of mycelia treated with this oil.

These mechanisms could explain the inhibitory action observed in the experiment, which showed the inhibition of *C. cassiicola* fungus by *C. zeylanicum in vitro* attributed, to confirm the essential oil of cinnamon as promising agent on the control of *C. cassiicola* fungus.

Control of target-spot in papaya seedling

Under greenhouse conditions, all seedlings showed symptoms of the disease three days after inoculation with fungus. The quick establishment of pathogen is probably due to the high susceptibility of the papaya plant to *C. cassiicola*, as reported by Oliveira (2007), first disease symptom occurs at 96 h after inoculation with fungus. There was no statistically significant difference between each essential oil concentration compared with fungicide treatment on measures of total leaf number for all time points after inoculation, and at 21 and 28 days after inoculation for percentage of leaves with lesions (Table 2). At 7 and 14 days after inoculation, there was a

Table 2. Total leaf number and percentage of leaves with lesions caused by *Corynespora cassiicola* on papaya seedling treated with various essential oil concentrations of *C. zeylanicum.*

Treatments	Days after inoculation			
	7	14	21	28
	Total leaf number			
Fungicide	8.0	7.6	7.7	7.0
0.0	8.8^{NS}	8.5^{NS}	7.1^{NS}	5.9^{NS}
0.5	6.7^{NS}	6.1^{NS}	6.8^{NS}	7.4^{NS}
1.0	9.1^{NS}	7.6^{NS}	8.2^{NS}	8.0^{NS}
2.0	6.2^{NS}	5.9^{NS}	5.7^{NS}	5.3^{NS}
4.0	7.4^{NS}	5.9^{NS}	5.9^{NS}	5.2^{NS}
	Percentage of leaves with lesions			
	7.8	14.7	28.6	28.1
0.0	27.5*	36.0*	51.8^{NS}	44.4^{NS}
0.5	25.9*	38.7*	43.7^{NS}	35.0^{NS}
1.0	21.8*	29.2*	31.3^{NS}	30.9^{NS}
2.0	13.2^{NS}	24.5^{NS}	25.2^{NS}	24.9^{NS}
4.0	8.2^{NS}	11.0^{NS}	24.8^{NS}	25.4^{NS}

*and ^{NS}: Means differ and do not differ from the fungicide treatment, respectively, at 5% probability by Dunnett's test.

statistically significant difference between 0.0; 0.5 and 1.0 µL mL^{-1} essential oil concentrations when compared with fungicide treatment on the measure of percentage of leaves with lesions (Table 2). However, there was no statistically significant difference of 2.0 and 4.0 µL mL^{-1} essential oil concentration when compared with fungicide treatment on percentage of leaves with lesions.

By visual analyses, at higher essential oil concentration (4.0 µL mL^{-1}), the presence of higher quantities of discolored spots on leaves were detected. This spots found on leaves of papaya seedlings are non-characteristic of the target-spot disease. These results could have potentially been caused by the phytotoxicity effect on leaves of papaya seedlings, which invalidated the concentration as promoter of the control of *C. cassiicola* in papaya seedlings. Furthermore, the discoloration spots appearing could have been the cause for the fungal not to grow on the leaves of papaya seedlings.

As described by Liberato and Mctaggart (2006), the leaf of the papaya plant is highly susceptible to target-spot, and when there is a considerable number of a lesion on the leaf, the disease causes premature leaf shedding. Martins et al. (2012) also reported that the highly aggressive fungus reduces the photosynthetic capacity of papaya leaves, and has a direct effect on foliar abscission.

The essential oil concentrations and the commercial fungicide were not able to lessen premature leaf shedding caused by the target-spot disease over the days assessed (Figure 2A). As per the *in vitro* experiment, where there was a reduction of the mycelial growth and sporulation, it was expected that in presence of *C. zeylanicum* essential oil, the *C. cassiicola* infection is suppress, thus this increased the longevity of the papaya leaves. These results were different to those reported by Médice et al. (2007) who verified that the presence of essentials oil of citronella (*C. nardus* [L.] Rendle), eucalyptus, neem (*Azadirachta indica* A. Juss.) and thyme (*Thymus vulgaris* L.) were capable of keeping the soybean plant leaves green and photosynthetically active.

The study also observed that the essential oil and commercial fungicide were able to keep the low percentage of infected leaves until the 14th day of evaluation (Figure 2B). There was a trend showing a reduction in the percentage of infected leaves between the 21st and the 28th days of evaluation (Figure 2B). The reduction in the percentage of infected leaves over the same time interval coincides with the major reduction in the number of leaves presented in this treatment (Figure 2A). This fact is related to the attack of *C. cassiicola* and it was reported by Ventura et al. (2004) and Liberato and Mctaggart (2006).

Similar effects were described by Médice et al. (2007), where a decrease was observed in the percentage of leaves with pustule of the *Phakopsora pachyrhizi* Syd. & P. Syd. after the application of essential oil from eucalyptus up to seven days after inoculation. However, the essential oil of thyme was able to reduce the incidence of disease until the 36th day of evaluation, which may be attributed to the higher or lower capacity of these oils in low active concentrations to maintain their action over time at ambient conditions. These results reinforce the idea of the necessity of new applications of *C. zeylanicum* essential oil (except at the concentration of 4 µL mL^{-1}) in controlling target-spot in papaya seedling, and in doing so, avoid continuous new fungal infections on papaya leaves. Essential oil concentrations presented no effects on leaf number for all four evaluation time points. Increased days after inoculation resulted in a significant increase in the percentage of leaves with lesions according to a linear model for all evaluation time points (Table 3).

Essential oil concentrations had no effect on leaf area and total lesioned area (Table 4). The averages were 64.8 cm^2 and 2.0 cm^2 for leaf area and total lesioned area, respectively. There was no statistically significant difference between each essential oil concentration compared with fungicide treatment on leaf area, and total lesioned area in papaya seedlings (Table 4). However, it was noted that the action of different concentrations of *C. zeylanicum* essential oil did effect the percentage of growth inhibition of lesions for the treatments of 1.0 µL mL^{-1} and 2.0 µL mL^{-1}, representing 66 and 54% of

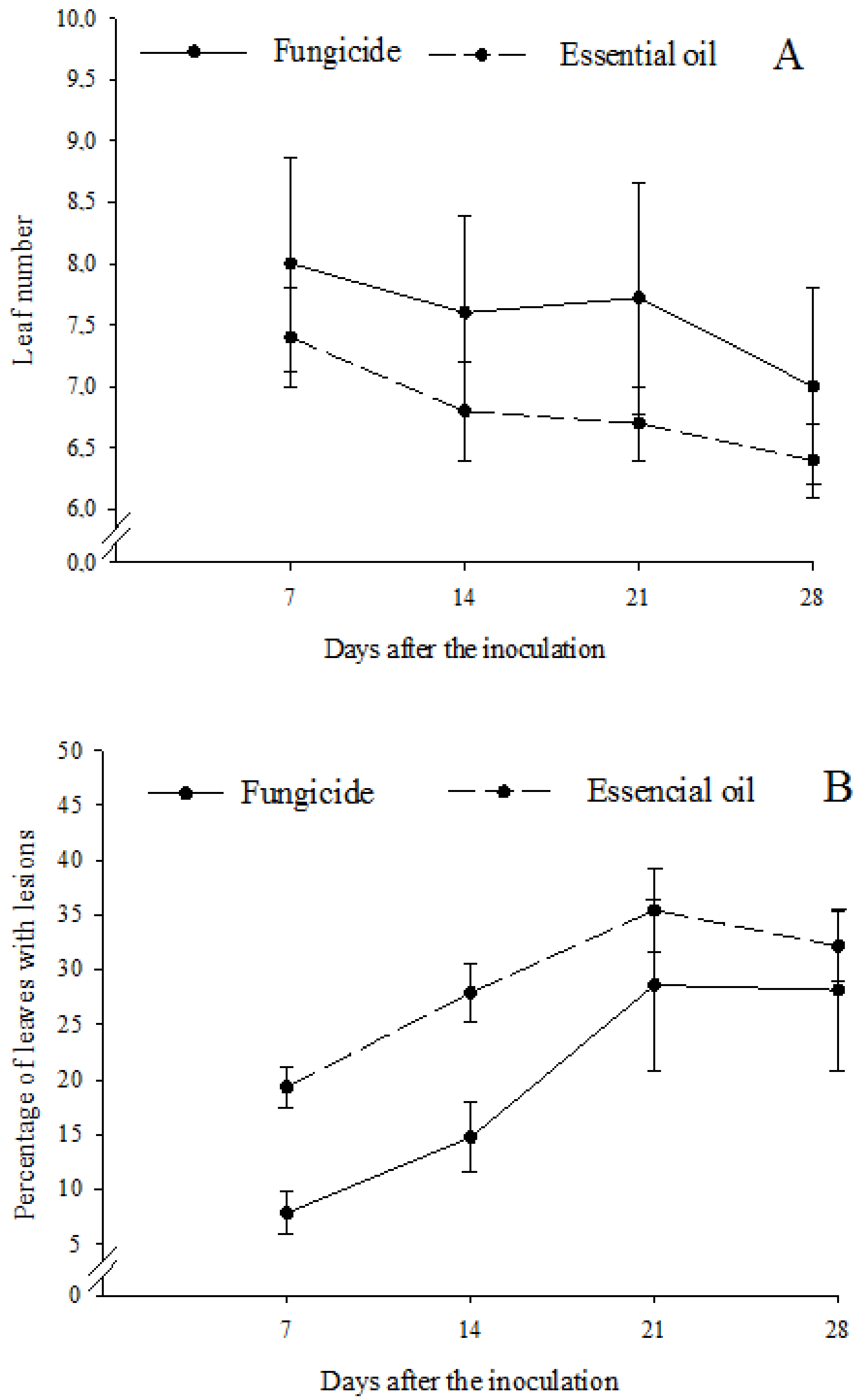

Figure 2. Leaf number (A) and percentage of leaves with lesions (B) affected x days after inoculation. B*ars denote standard error* of the mean.

inhibition, respectively, in relation to the control (Table 4).

Similar results obtained in another pathosystem were reported by Lorenzetti et al. (2012), efficiency of essential oils on reduction of severity of diseases. They found significant results with the essential oils of citronella and eucalyptus on the control of lemon grass leaf rust,

Table 3. Adjusted model of leaf number and percentage of leaves with lesions caused by *C. cassiicola* on papaya trees treated with different concentrations of *C. zeylanicum* essential oil.

Days	Adjusted model	r^2/R^2
	Leaf number	
7	$\hat{Y}$ = 7.4; SE = 0.4	-
14	$\hat{Y}$= 6.8; SE = 0.4	-
21	$\hat{Y}$ = 6.7; SE = 0.3	-
28	$\hat{Y}$ = 6.4; SE = 0.3	-
	Percentage of leaves with lesions	
7	$\hat{Y}$=26.9625 - 5.0959**X	0.93
14	$\hat{Y}$=38.0125 - 6.7550**X	0.95
21	$\hat{Y}$=44.7275 - 6.2450°X	0.68
28	$\hat{Y}$=38.2850 - 4.11°X	0.65

** and °: Significant at 1 and 10% of probability by the *t*-test, respectively. SE = standard error of the mean.

Table 4. Effect of *C. zeylanicum* essential oil on total area and total lesioned area (severity) caused by the *C. Cassiicola* fungus in papaya seedling at 28 days after inoculation with the pathogen.

Treatments	Leaf area (cm^2)	Total lesioned area (cm²)[1]	Percentage of control
Fungicide	**42.0**	**0.42 (2,3)**	**20.7**
0.0	86.0^{NS}	0.35^{NS} (2.9)	0.0
0.5	53.5^{NS}	0.43^{NS} (2.4)	18.2
1.0	69.8^{NS}	0.23^{NS} (1.0)	66.5
2.0	54.6^{NS}	0.26^{NS} (1.3)	54.3
4.0	60.4^{NS}	0.43^{NS} (2.4)	15.6

^{NS}: Means differ and do not differ from the fungicide treatment, at 5% probability by Dunnett's test, respectively. [1]Values were transformed to Log(x+1). Values in parentheses indicate values originals.

obtaining a smaller percentage of infected leaves after 30 days of application of the essential oil when compared to the control.

The range that provides high percentage of disease control was 1.0 to 2.0 µL mL^{-1} (Table 4). These results are important in emphasizing that new research is necessary in tropical humid Brazil. This new research needs to define an optimum essential oil concentration that provides greater control of the disease in papaya crops for current conditions.

Preventative and curative control of lesions in fruits of papaya

Increased essential oil concentrations resulted in a significant decrease in the preventative ability of treatments, when considering lesion size, according to a linear plateau response (Figure 3). An essential oil concentration of 0.52 µL mL^{-1} led to the smallest estimated size lesion. After this concentration, the lesion size was constant. Essential oil concentrations presented no effects to lesion size in curative treatments. The lesion size was 11.48 cm (Table 5). These results show that after the infection of papaya fruits, the percentage of inhibition of disease is too low. Thus, it is necessary to focus efforts on the preventative treatment of fungal infection in papaya fruits.

Perhaps the presence of essential oil before inoculation with fungus (preventative treatment) was capable of providing an environment of protection in the fruit, thereby creating a barrier to the establishment and development of lesions. In the curative treatments, the time between the inoculation and the treatment should have been sufficient to fully establish the fungus in the fruit. Another consideration is the fast process of papaya

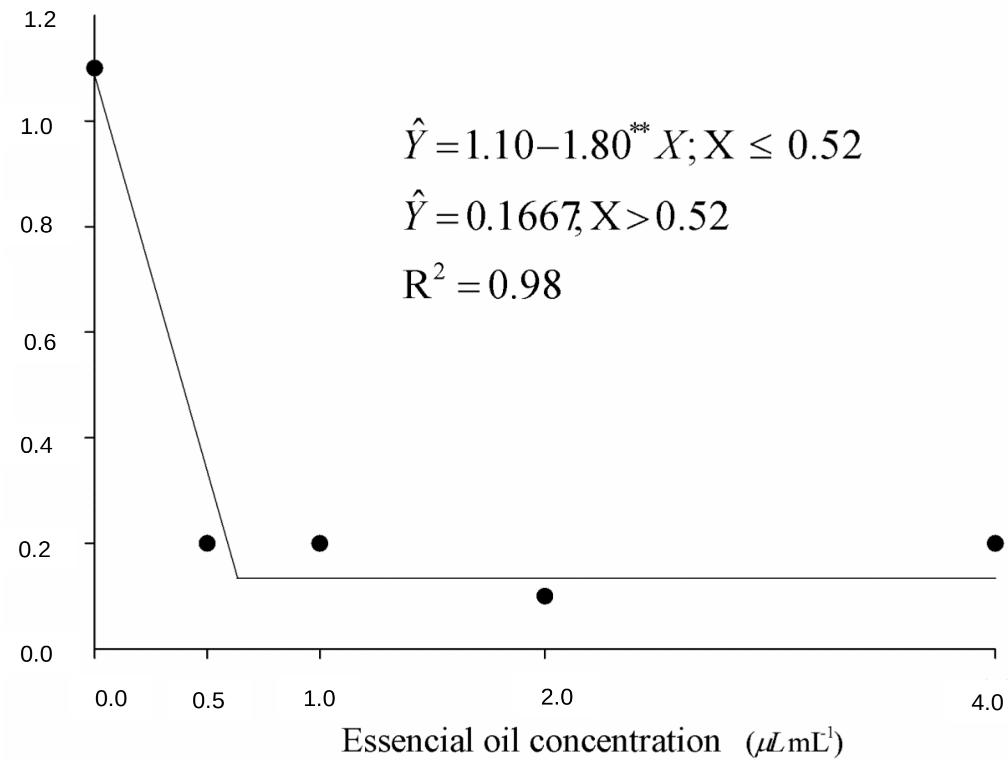

Figure 3. Lesion size (cm) in response to preventative treatment with essential oil at various concentrations **(**** Significant at 1% of probability by the *t*-test. *Values (Y) were transformed* to √x).

Table 5. Lesion size (cm) in curative treatments affected by essential oil concentration in papaya fruits at 7 days after the inoculation of the pathogen.

Treatments	Lesion size (cm)	Percentage of control
0.0	10.8	0
0.5	12.5	-15
1.0	10.9	0
2.0	9.0	17
4.0	11.7	-0.08
-	$\hat{Y} = \bar{Y} = 11.48$	-

fruits ripening, mainly in the presence of pathogen, which stimulates the production of ethylene, the hormone responsible for ripening. This could explain why curative treatments with essential oil did not result in pathogen control.

Similar results obtained by Carnelossi et al. (2009), used of the essential oil of *C. citratus* and *Eucalyptus citriodora* (at 1% concentration) in the control of *C. gloeosporioides,* the causal agent of papaya anthracnose in postharvest. The fruits were preventatively treated and subsequently inoculated after 24 h, presented higher control of disease, attesting to the potential use of essential oils as an alternative to the application of commercial fungicides in the prevention of disease. The study results showed that the application of essential oil from *C. zeylanicum* could be considered as an effective method to inhibit fungal growth, to reduce the use of synthetic fungicides in papaya seedling, and could be used as a preventative control for *C. cassiicola* in papaya fruits. In the future, more laboratory and field studies are suggested for further practical validation of *C. zeylanicum* essential oil on *C. cassiicola* control in papaya seedlings and postharvest fruits.

Conclusions

Essential oil from *C. zeylanicum* has inhibitory effect of on the mycelial growth and sporulation of the *C. cassiicola* fungus. The essential oil keeps the low percentage of leaves with lesions in papaya seedling up to 14 days after the inoculation. The essential oil from *C. zeylanicum*, applied as a preventative treatment, is effective in controlling lesion size. Essential oil has no effect in controlling lesion size after infections of *C. cassiicola* have already been established in papaya fruits. The *C. cassiicola* essential oil concentration used to obtain the lower infection of *C. cassiicola* is 0.52 µL mL^{-1} which is applied before the infection in fruits.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Chemical characteristics of fruits of two species of *Physalis* under nitrogen fertilization

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Although small fruits, with some exceptions, are not yet as popular in Brazil, some farmers have invested in this market and the genus *Physalis* can be an important alternative source of income due to its high added value and possibility of cultivation in small areas. To date, there are no specific fertilizer recommendations for even the most cultivated species (*Physalis peruviana*) and therefore the goal of this study was to evaluate the effects of nitrogen fertilization on quality of fruits of *Physalis peruviana* and *Physalis pubescens*. Plants were grown in 5 L pots containing soil and maintained in a controlled environment, in greenhouse. Treatments consisted of five N levels (kg ha^{-1}): 0, 200, 250, 300 and 350, with seven replicates per treatment. Variables evaluated were total soluble sugars and reducing sugars, phenolics, pH, titratable acidity and fruit diameter. Nitrogen increased soluble sugars (350 kg ha^{-1}) and reducing sugars (300 kg ha^{-1}), mainly in *P. pubescens*. There was a slight increase of juice pH, but concentrations of total phenols and citric acid decreased with increasing levels of N. Fruit diameter of the two species of *Physalis* was increased by N fertilization, and the best N level was 300 kg ha^{-1}.

Key words: *Physalis peruviana*, *Physalis pubescens*, reducing sugars, titratable acidity, total phenolics.

INTRODUCTION

Physalis is a plant of the family Solanaceae and according to El Sheikha et al. (2010) there are about 453 species, 100 of which are well known, and Mexico is considered the center of diversity of the genus (Whitson, 2012). Several species have shown important biological activities that turn them potential sources of pharmacological products (Tomassini et al., 2000; Wu et al., 2005; Arun and Asha, 2007).

The fruit of *Physalis* is sweet and slightly acid, with high levels of vitamins A, C, phosphorus and iron, as well as flavonoids and phytosterols (Rufato et al., 2008). The production and quality of the fruit depends on the balance of various natural and anthropomorphic environmental factors; among them, the soil fertility is the easiest to control and its proper management can result not only in increments of crop yield, but higher quality of the fruit (Valencia et al., 2003), through biochemical and physiological processes occurring in plants (Ferreira et

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Table 1. Soil analysis.

Organic matter	P mg dm^{-3}	K $cmol_c$ dm^{-3}	Ca $cmol_c$ dm^{-3}	Mg $cmol_c$ dm^{-3}	pH $CaCl_2$	V (%)	pH SMP	SB $cmol_c$ dm^{-3}	H + Al $cmol_c$ dm^{-3}
60.31	3.46	0.40	4.86	2.10	4.6	44.20	5.30	7.36	9.29

al., 2006). Among them the photosynthetic activity and the rate of photosynthate translocation directly interferes with fruit quality, which can be understood as a set of different individual internal (color, odor, pH, soluble solids, etc.) and external characteristics (size, shape, color, etc.).

Nitrogen (N) has great effect on growth and absorption rates of other elements by the plant, and is one of the most important nutrients for plant nutrition. Nitrogen fertilization affects vegetative and reproductive characteristics of plants, enhances and increases the protein and nucleic acid synthesis, and promotes vegetative growth and the formation of floral and fruit buds. In Brazil, many crops are fertilized empirically, thus the experience of farmers prevails: But this may imply economic losses by a lack or excess of nutrients, which may result in changes in the characteristics of the fruit with decreased organoleptic quality.

Therefore, the objective of this study was to evaluate the effects of different levels of nitrogen on the chemical and physical characteristics of *Physalis peruviana* L. and *Physalis pubescens* L.

MATERIALS AND METHODS

Seedlings of *P. peruviana* L. and *P. pubescens* L. were obtained by growing them in a greenhouse, from seeds of plants at Universidade Tecnológica Federal do Paraná, Brazil. The experiment was conducted from February to October, for two years (2011 and 2012), under 28 ± 2°C in the greenhouse. Seeds were placed on Styrofoam trays containing substrate (fertile humus), and three seeds were sown per cell, with thinning after 20 days, leaving one seedling per cell. When these were approximately 15 cm high (70 days old) they were transplanted to pots (5 L) containing soil as substrate, which was analyzed to perform fertilization (Table 1) with pH corrected to 5.8.

The experimental design was completely randomized with a factorial (2 × 5) arrangement of treatments: Species (*P. peruviana* L. and *P. pubescens* L.); and five N (as urea) levels (kg ha^{-1}): 0, 200, 250, 300, 350; with 7 replications per treatment. The results were used for an ANOVA and treatment means were analyzed by orthogonal contrasts ($p \leq 0.05$), using ASSISTAT software. Other two macronutrients (P and K) were maintained on fixed doses: 600 kg ha^{-1} P_2O_5 and 500 kg ha^{-1} KCl.

Due to the uneven ripening of the fruit, samples were taken weekly and analyses performed at Laboratory of Biochemistry and Plant Physiology of the UTFPR. Fruit were harvested when the calyx was yellow-greenish.

Fruit were weighed (Marte AY220 digital scalel) and then macerated by hand and filtrated. Variables evaluated were: pH (Tecnopon mPA-210 digital pH meter), titratable acidity (ascorbic acid); concentrations of total soluble sugars (TSS), using the methodology proposed by Dubois et al. (1956) and reducing sugars (RS), through the dinitrosalicylic acid (Miller, 1959) both by spectrophotometry (UV-1800 Shimadzu spectrophotometer UV-1800) with glucose as standard. Total phenolic compounds were determined by spectrophotometry using the methodology described by Makkar et al. (1993) with tyrosine as standard.

RESULTS AND DISCUSSION

The different levels of nitrogen resulted in variations in the levels of metabolic compounds evaluated in both species of *Physalis*. With respect to the concentrations of soluble sugars, the fruits of both species showed increase with higher levels of nitrogen up to 300 kg ha^{-1} (Figure 1). In all levels of nitrogen tested, *P. pubescens* showed initial value of TSS greater than that of *P. peruviana*, responding better to the increments of the nutrient applied. Ferreira et al. (2006) suggest that increasing amounts of sugars until a given level of nitrogen are due to the biosynthesis of these compounds during photosynthesis, which are subsequently translocated to the fruit in the process of development and growth thereof. Bénard et al. (2009) reported higher concentrations of total sugars but at low nitrogen levels.

The reductions in TSS concentrations at levels above 300 kg ha^{-1} nitrogen may be the result of greater vegetative growth, which was observed in the experiment, in height and dry weight of shoots, besides the greater number of fruits, particularly in *P. pubescens* (data not shown).

Nitrogen stimulates the activation of meristematic tissues of the plant, leading to increased consumption of storage sugars (Nabinger, 1996; Alexandrino et al., 2004), thus in *Physalis* nitrogen fertilization above 300 kg ha^{-1} stimulates greater vegetative growth resulting in consumption of storage sugars to sustain this energy expenditure, hence reducing the levels to be translocated to the fruit.

This intense growth and subsequent translocation of sucrose for fruit and its inversion during maturation resulted in increases in the concentration of reducing sugars dependent on nitrogen levels in *P. pubescens*, with little variation in the levels of these compounds in *P. peruviana* (Figure 2). These responses confirm the ability of the first to respond differently to increasing levels of fertilization, possibly for being a more rustic species, not subjected to constant selection processes, even the most simple.

In tomato, Ferreira et al. (2006) found no change in total soluble solids, mainly represented by glucose and fructose, with increased level of N, which is also observed in *P. peruviana*, differently from *P. pubescens*

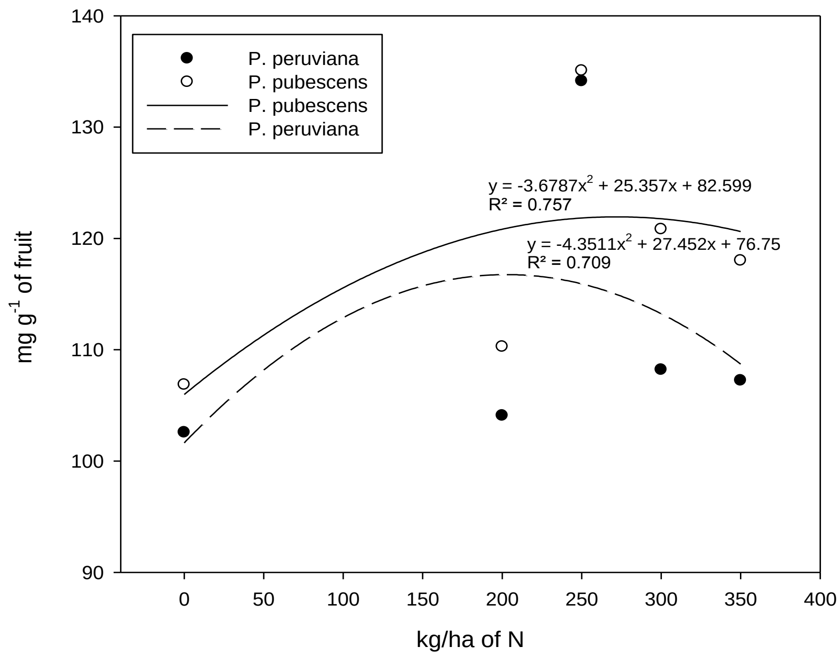

Figure 1. Concentrations of total soluble sugars (TSS) in fruits of *P. peruviana* and *P. pubescens* under different nitrogen levels.

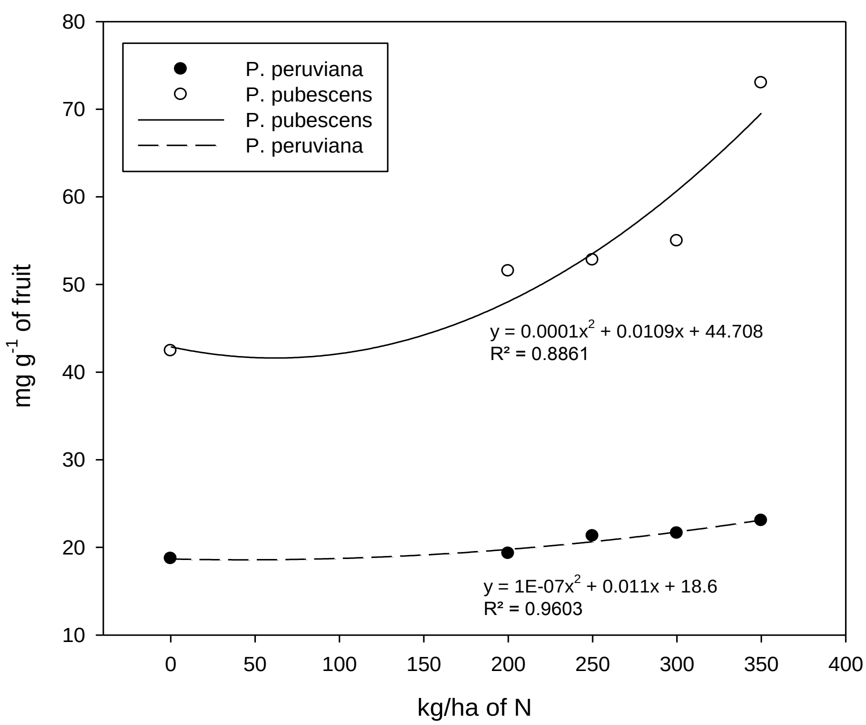

Figure 2. Concentrations of reducing sugars (RS) in fruits of *P. peruviana* and *P. pubescens* under different nitrogen levels.

and tomato in a study developed Valencia et al. (2003). In another experiment with tomato, reducing sugars were present at higher concentrations in reduced levels of N (Bénard et al., 2009).

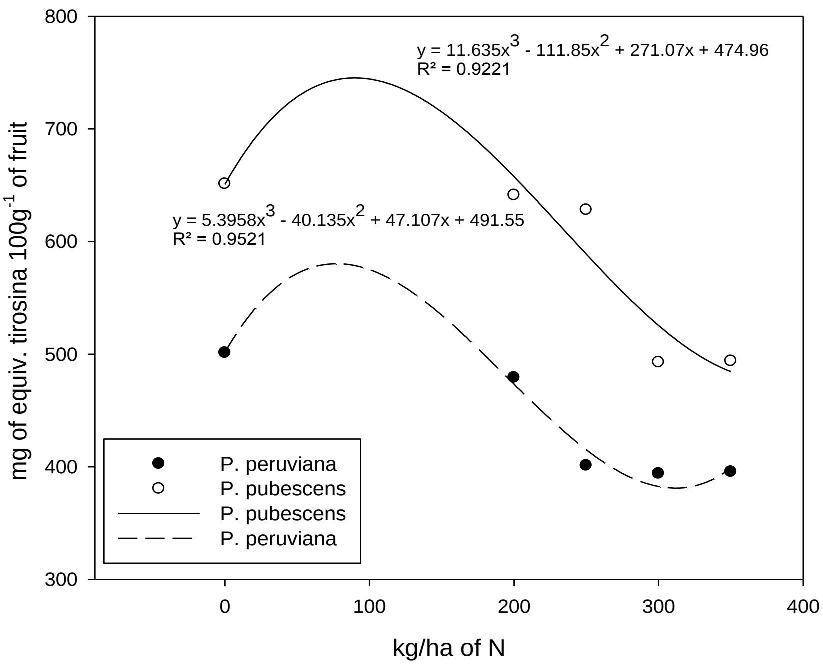

Figure 3. Total phenolic content in fruits of *P. peruviana* and *P. pubescens* under different nitrogen levels.

The sugar content in fruit of *Physalis*, especially in *P. pubescens*, imparts a sweeter flavor to this species, which was confirmed by taste tests conducted with the community (data not shown).

In the analyses of total soluble phenolic compounds, the results were the opposite of primary compounds (sugars). From the dose of 200 kg ha^{-1} nitrogen in both *Physalis* species it was verified a cubic trend with increasing nitrogen levels. Between the two species, *P. pubescens* showed the highest values of phenols (Figure 3), similarly to that observed in tomato with higher concentrations of some phenolic compounds at lower nitrogen levels (Bénard et al., 2009).

The synthesis of phenolic compounds increases under stress, especially when the use of carbon for growth and/or reproduction decreases due to lack of nutrients (Kandil et al., 2004). When N limits growth of plants, there is an accumulation of carbohydrates in their tissues, according to the carbon/nutrient balance hypothesis (Bryant et al., 1983), thus this accumulation directs carbon to the synthesis of secondary metabolites (Hamilton et al., 2001), which seems to occur in *Physalis*. Coelho et al. (2012) also observed a decrease in the concentration of total phenolics with increasing level of N in leaves of potato plants, following a quadratic model.

The results are consistent with the inverse relationship between the synthesis of primary metabolites during the growth/development of plant and production of secondary metabolic compounds, including phenols. Thus, the phenol synthesis depends on the balance of sucrose and fruit are the preferred sinks, and therefore nutrients and carbohydrates are directed to their development as well as to other parts of the plant, rather than to synthesis and accumulation of phenolic compounds.

In relation to citric acid in *Physalis* fruit, it was observed that the concentrations showed a quadratic response with increases up to 200 kg ha^{-1} (Figure 4). *P. peruviana* showed the highest concentrations of citric acid, giving a more acidic flavor to fruit, when compared to *P. pubescens*, also confirmed by taste tests (data not shown).

Bénard et al. (2009) also obtained a reduced acidity with increasing nitrogen, resulting in less acidic characteristics to tomato fruit. Conversely, in muskmelon, there was an increase in the percentage of citric acid with higher N levels (Coelho et al., 2003). According to Blanco and Folegatti (2008) the citric acid content in tomato fruit increases with higher cation: Anion ratio in order to maintain electro neutrality in fruit tissues. As N is absorbed by plants preferentially in the form anion (NO_3^-), may have occurred an imbalance in this ratio, with increased N uptake in relation to other nutrients, such as K^+ (Marshner, 1995).

These changes were also proved by direct measurement of pH (Figure 5), with elevation in values with increase of N for both species, but smallest variations in pH values were observed for *P. pubescens*. The increase in N levels may have increased fruit turgidity indirectly causing a drop in acidity values, due to the dilution of acids (Paula et al., 1991), which may have

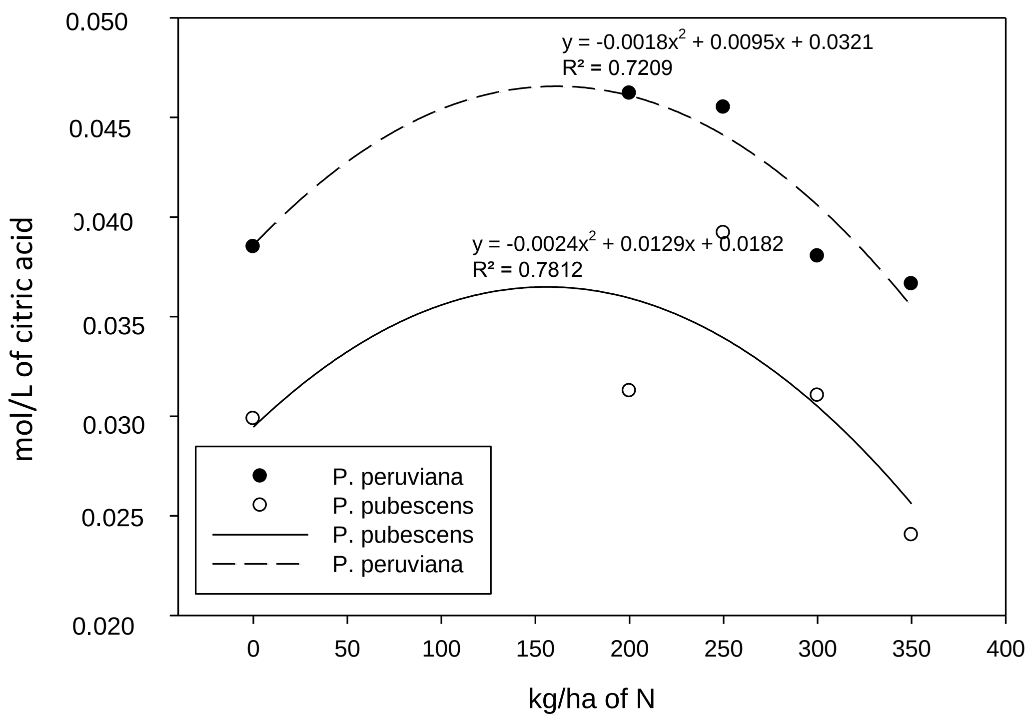

Figure 4. Titratable acidity in fruits of *P. peruviana* and *P. pubescens* under different nitrogen levels.

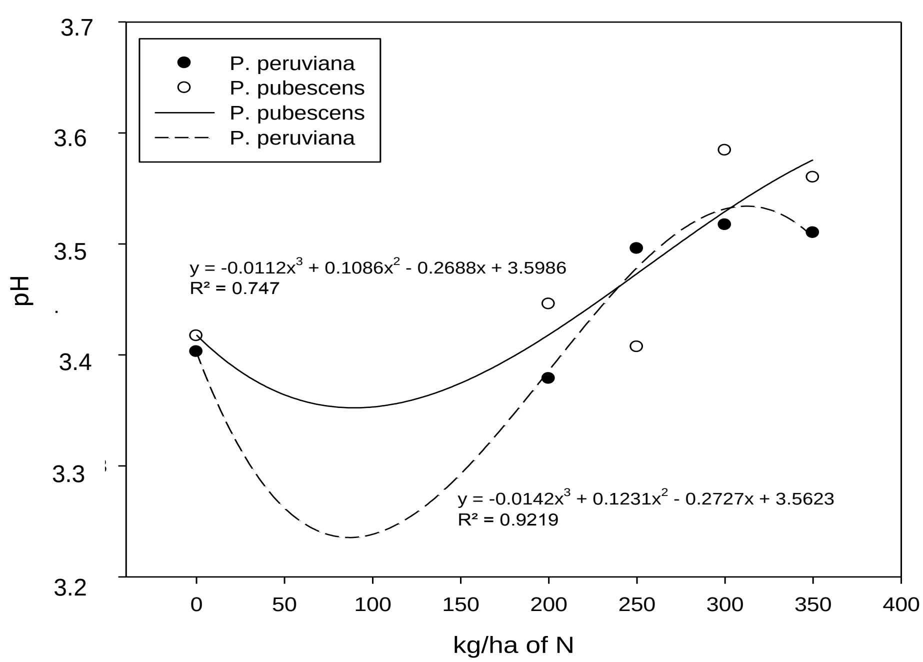

Figure 5. Values of pH in fruits of *P. peruviana* and *P. pubescens* under different levels of nitrogen fertilization.

occurred in *Physalis*.

Similar results were verified by Silva et al. (2008), which registered that the increase in pH with increasing N levels is justified by the lower concentration of acids accumulated in guava fruit.

Fruit quality comprises chemical characteristics, size

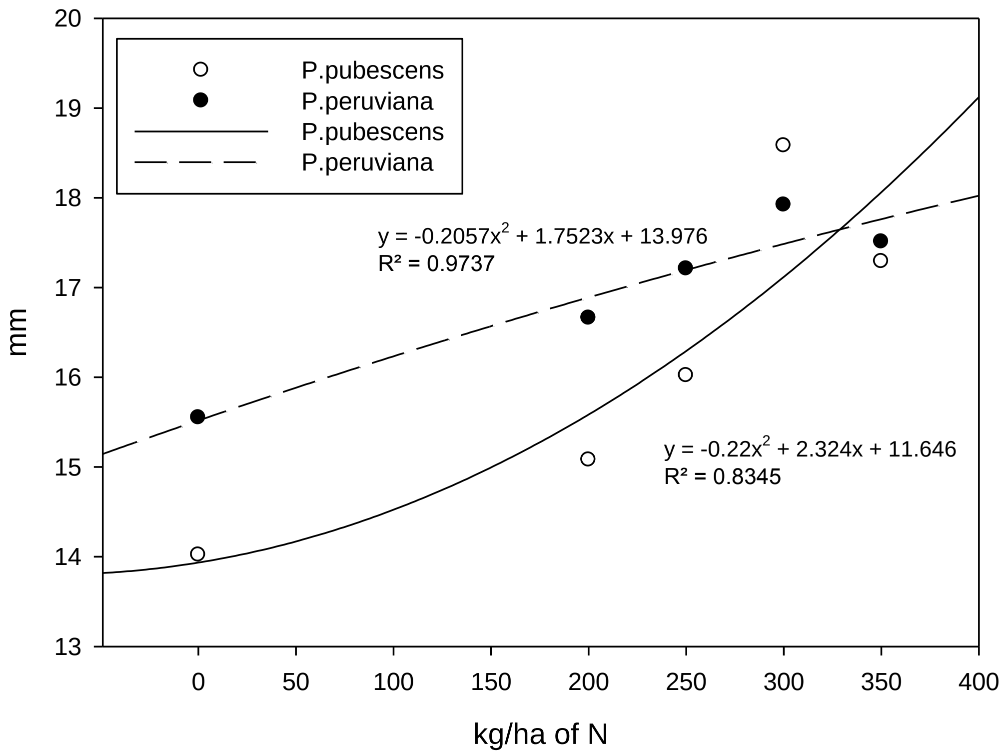

Figure 6. Diameter of fruits of *P. peruviana* and *P. pubescens* under different levels of nitrogen fertilization.

and number of fruit. In this way, we measured the diameters of the fruits of *Physalis* and as a result, the nitrogen positively affected this parameter up to the level of 300 kg ha^{-1}, as shown in Figure 6. Fruit size was increased with fertilization, which was expected until reaching the threshold of nutritional need of the plant, from which the excess promoted no increased yield, which indicates that above the optimal value occur waste and losses, if the main goal is fruit production.

Increases in fruit diameter with increase in nitrogen fertilization were also reported by Coelho et al. (2003) and Queiroga et al. (2007), working with melon, by Pedrosa et al. (2012) with pumpkin, and by Brito Neto et al. (2011) with sunrise solo papaya, however with a decrease in size from a given level of nitrogen. According to these authors, the deficiency or excess of N affects the size and quality of the fruit, since high levels tend to increase the number rather than the size of the fruit, which seems to occur slightly in *P. peruviana* at a level of 350 kg ha^{-1}.

Conclusions

In short, the results evidenced the positive effect of nitrogen in greenhouse cultivation for two species of *Physalis*. In general, 300 kg ha^{-1} can be considered the best level under the conditions of the experiment, which led to the set of positive characteristics that determine fruit quality, especially for *P. pubescens*.

Conflict of Interests

The authors have not declared any conflict of interests.

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Physiological quality of hybrid maize seeds through respiratory and enzymatic activities

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With the increased demand for high quality maize seeds, the market absorbs innovations that add speed and reliability in the tests that enable differentiation of potential performance of seed lots. This study aims to evaluate the combination of the physiological tests with the expression of enzymes of respiratory route for differences in vigor levels in lots of hybrid seed corn. The percentage of germination, germination first count, emergence on tray, index of emergency speed, accelerated aging and respiration rate were evaluated by Pettenkoffer and Titulation method. Furthermore, the expression of alpha amylase, alcohol dehydrogenase, malate dehydrogenase and pyruvate decarboxylase was analyzed. With the results, it is determined that the vigor tests analyzed, together with the tests of enzymatic expressions of respiratory route were efficient to detect different levels of force in hybrid seed corn. Lot 1 had higher physiological quality while lot 2 had the lowest vigor compared to the other lots. With the vigor tests and expression of enzymes, it was possible to differentiate the quality of the seeds and therefore it can be recommended as reliable tests for defining the quality of seed lots.

Key words: Respiration, enzyme expression, vigor, viability.

INTRODUCTION

The search for increase in productivity has raised the technological level in the maize crop fields. Consequently, there is increase in the demand for quality seeds and investment in the seeds sector; maize seeds utilized represented 91% in the last crop season (Abrasem, 2015).

One of the first requirements for the success of maize hybrid seed performance is their physiological quality (Marcos, 2005). The attributes which determine the seeds quality are related to genetic, physical, physiological and sanitary factors. These attributes can be characterized by germination, vigor and longevity tests which show their viability (Moterle et al., 2011; Nerling et al., 2013).

To meet the demand for maize, seeds analyses have become an indispensable tool for obtaining quality control data, mainly from the final maturation stage (Krzyzanowki et al., 1999).Many tests contribute to increased technology for seeds production, quick analysis of quality processing and consequently increased productivity and low costs. With this, seeds companies aim to upgrade tests, like germination and vigor tests, in order to have results with behavior equal to the seeds sown in the field (Santos et al., 2004).

The tests used to evaluate the physiological quality correspond to the somatory of two fundamental parameters: viability and vigor. Viability is valid if the germination test brings out maximum potential seeds and produces normal seedlings on favorable conditions (Carvalho and Nakagawa, 2000). Many a time, the seedlings that emerge from field can be considerably lower compared to those obtained from the germination tests in the laboratory (Bhering et al., 2003). This shows the necessity of obtaining complementary information. To complement this information, vigor tests are used, which evaluate the potential of seeds germination and the fast development of normal seedlings under a wide variety of environmental conditions (Aosa, 1983). Thus, apart from physiological tests, other techniques have been used to verify the degree of seeds deterioration, like respiratory and enzymatic analyses (Devi et al., 2007; Lamarca, 2009).

Respiratory activity can be evaluated by gas exchanges and consists of manometric O_2 consumed or measured CO_2 liberated. It is sensitive and requires few amounts of materials (Crispim et al., 1994). Furthermore, it presents a considerable quality seeds. It is an important complementary test compared to the traditional one for determining vigor of seeds lots (Mendes et al., 2009; Dode et al., 2012). Another way to verify the processes related to seeds quality is the evaluation of enzymatic activity of respiratory route. The alterations in the normal pattern of these enzymes lead to a respiratory disorders, causing problems in the seeds quality and vigor (Devi et al., 2007). However, for this work, important enzymes were selected which are related to the seeds respiratory activity

Thus, the objective of this work is to correlate the respiratory activity and enzymatic expression from respiratory route to determine the seeds' physiological quality; specifically to differentiate the levels of vigor of hybrids maize seed lots.

MATERIALS AND METHODS

The research was conducted in the Central Laboratory of Seeds of the Agricultural Department at Universidade Federal de Lavras (UFLA), in Lavras, MG. Five lots of hybrid maize seeds with different levels of vigor were used.

The seeds water content was determined by the oven method at 105°C for 24 h (BRASIL, 2009), using two replications of 50 seeds of each treatment. After this period, the seeds were taken to desiccators until the samples cooled down. Later, the seeds dry weight was obtained. The results were expressed in percentage.

The germination test was conducted with four replications of 50 seeds; they were sown in moistened germitest paper in the proportion of 2.5 ml of water per g of paper. Seeds were kept in germinator, regulated with temperature of 25°C and the evaluations of normal seedlings were done in two counts: the first on the fourth day and the last one on the seventh day after sowing. The results were expressed in medium percentage of normal seedlings of the four replications (BRASIL, 2009).

For the calculation of emergence speed index, daily evaluations were done from the beginning of seedlings emergence, counting of the number of emerged seedlings until the stabilization of the stand. At the final test, with the daily data of the number of emerged seedlings, the emergence speed index was calculated, according to the formula proposed by Maguire (1962):

$$E.S.I. = (E_1/N_1) + (E_2/N_2) + \ldots + (E_n/N_n),$$

where: E.S.I = emergence speed index; E1, E2 and En = number of normal seedlings computed in the first count, second count and in the last count; N1, N2 and Nn = number of sowing days at 1ª, 2ª and at last count.

In the germination test, there were normal seedlings on the fourth day after sowing. For the accelerated aging test, plastic transparent boxes (11.5 x 11.5 x 3.5 cm) like mini-cameras were used, where the seeds were distributed to form a uniform layer. 40 ml of distilled water was added, creating an environment of 100% of air relative humidity. The boxes were closed and kept in aging chamber (B.O.D.), regulated at 41°C for 96 h. The respiratory activity was evaluated according to the physic-chemical methods given by Pettenkoffere and Titulation, which evaluates the quantity of CO_2 liberated and O_2 consumed respectively from seeds respiration (Crispim et al., 1994). The results obtained were correlated with the other tests to determine the seeds' physiological quality. For the Pettenkofer method, the respiratory activity was determined using four flasks: the first two contained sodium hydroxide (NaOH), the third was conditioned with seeds analyzed and the fourth had barium hydroxide ($Ba(OH)_2$).The flasks were closed with silicone, and connected by a tube; the air flux was controlled through a tap. After two hours of seeds exposure, two aliquots of supernatant were taken for titulation. In each aliquot

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Table 1. Vigor tests: medium values of first count (FC), germination (G), emergence in trays (E), emergence speed index (GSI), accelerated aging test (AA) and respiratory taxes activity by the Pettenkofer (RESP Pet) method, and by the Titulation test (RESP Tit) method for five lots of hybrids maize seeds.

Lots	FC	G(%)	E(%)	GSI	AA	RespPet	RespTit
01	91^a	99^a	98^a	3^a	99^a	460.50^a	3.16^a
02	77^c	96^a	82^b	5^c	82^c	209.75d	6.62^c
03	88^b	99^a	98^a	4^b	93^b	365.25^b	5.39^b
04	87^b	98^a	98^a	4^b	91^b	313.50^c	5.76^b
05	92^a	98^a	99^a	4^b	94^b	351.25^b	5.58^b
CV (%)	2.58	1.44	2.77	1.04	3.36	6.35	5.90

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability.

were added two drops of phenolphthalein reagent color and later submitted for titulation with hydrochloric acid (HCl). The volume of HCl used until the "turn point" was proportional to the quantity of $BaCO_3$ presented in the solution, which is also proportional to the quantity of CO_2 from the seeds respiratory activity. From the stoichiometric calculations, it was possible to obtain the quantity of liberated CO_2 during the process of seeds respiration. The result was expressed in quantity of liberated carbon dioxide per g of seeds per hour.

For the titulation method transparent plastic boxes were used (gearbox type) for supporting the seeds. In the deep of each gerbox were placed 40 ml of KOH solution at 0.1 N (Figures 3 and 4) and after were closed to avoid gas exchanges with the environment. Each of the four replications of 50 seeds was placed on blotter paper moistened with 2x the seeds' weight. The gearboxes were kept in cold chamber (type B.O.D.) for a period of 24 h at constant temperature of 25°C.

After this period, drops of phenolphthalein reagent color were added in a sample of 25 ml of KOH solution, per replication; and were submitted for titulation with HCl 0.1 N. In the "turning point" the volume of HCl spent in each tested replication was registered. This volume of HCl which is directly related with the quantity of CO_2 fixed for the KOH solution is from respiration.

For the Pettenkofer and titulation methods, the results were expressed in mg of CO_2 and mg of O_2 per g of dry seeds respectively, according to the following formulas:

Formula already simplified for Pettenkofer:

$$(W-L) \times C / MS$$

Where, W: white reading test; L: reading of HCl volume spent to neutralize the KOH submitted for respiration; C: correction factor (3.52); MS: seeds dry matter.

Formula already simplified for titulation:

$$(Lb-Ls) \times 1.10^5/h*g$$

Where, Lb: white reading test (mL); Ls: sample reading (mL); H: length of stay on the device (hours); G: mass of used seeds (g).

For the enzymatic evaluations, two samples of 25 seeds of each treatment were collected. These seeds were sown in germitest paper and after 72 h, they were macerated with PVP and liquid nitrogen in ice. Later they were stored at -86°C, until the extraction moment. For the enzymes extraction, the extraction buffer (Tris HCl 0.2 M pH 8 + 0.1% of β-mercaptoethanol) was added in the proportion of 250 ɥL for 100 mg of seeds powder. The material was homogenized in vortex and kept in refrigerator during 12 h followed by the centrifugation at 14000 rpm for 30 min at 4°C. Then, 60 µL of supernatant in polyacrilamide gel was applied. The electrophoretic run was realized in a discontinuous polyacrylamide gel system at 7.5 (separating gel) and 4.5% (concentrating gel) using Tris-glycine pH 8.9 as standard buffer in the gel electrode system. The running was performed at 150 V for 5 h.

At the end of running, the gels were revealed for the enzymes alpha amylase (α-AMI- EC 3.2.1.1.), malate dehydrogenase (MDH- EC 1.1.1.37.), alcohol dehydrogenase (ADH - EC 1.1.1.1) and pyruvate decarboxylase according to the protocols established by Alfenas, (2006). Complete randomized experimental design was used for the five materials, with four replications. The data were statistically interpreted using variance analyses for all the tests with the aid of SISVAR® statistical program (Ferreira, 2011). For comparing the averages, the Scott-Knott test at 5% of probability was used. The simple linear correlation coefficient (r) of Pearson was determined between the values obtained in the tests used for the evaluation of the physiological quality of seeds. The evaluation of the gels was realized on transilluminator, being considered the variation of intensity of bands.

RESULTS AND DISCUSSION

The medium water content of the seeds in the test was 13.1, with maximum variation of 1%. Minimum variation of water content is important between the materials to avoid increased deterioration process and formation of products which cause immediate damages, like free radicals, found in the final result (Marcos, 2005).

Vigor tests use specific situations of stress to preview the relative behavior of lots in field. This is because a variable number of tests must be used for the results to be coherent and consistent (Carvalho and Nakagawa, 1988; Woodstock, 1973). Thus, various tests were applied to maize seed of 5 lots. For the tests used to evaluate the characteristics of the seeds' vigor were observed significant differences between the lots, with exception of the results observed in the germination test (Table 1).

Table 2. Coefficient of simple correlation (r)between germination (G), emergence in trays (E), emergence speed index (ESI), accelerated aging (AA)and respiratory taxes activity by the Pettenkofer (RESP Pet) method, and by the Titulation test (RESP Tit) method for five lots of hybrids maize seeds.

Parameter	G	E (%)	GSI	AA	RESP Pet	RESP Tit
FC	0.8565^{ns}	0.9510^{*}	-0.0631^{ns}	0.9375^{*}	0.8626^{**}	-0.6611^{ns}
G (%)		0.8972^{*}	-0.2217^{ns}	0.9187^{*}	0.9136^{*}	-0.7442^{ns}
E (%)			0.1205^{ns}	0.8777^{*}	0.7930^{ns}	-0.5555^{ns}
GSI				-0.3619^{ns}	-0.9092^{*}	0.9370^{*}
AA					0.9831^{**}	-0.8743^{**}
RESP Pet						-0.9347^{*}

The results of vigor tests in all the seeds were consistent. This means that the lots were classified in a pattern in most part of the tests. Similar results were found by Pereira (2012), on evaluating the physiological quality of pepper and chili seeds through vigor tests and respiratory activity. This observation was different from the one related by Caliari and Silva (2001) and Castro (2011) who studied 45 and 16 lots of maize seeds, respectively, and obtained different results based on the vigor test used. The first germination count, realized to facilitate the conduction of germination test, can be considered a vigor test, because the germination speed is one of the first characteristics to be affected by the deterioration process of seeds (Vieira et al., 1994). This test is based on the principle that the lots with highest percentage of normal seedlings on the fourth day of germination are the most vigorous. It is a quick and an important test, since the uniformity and emergence speed of seedlings bare the most important compounds inside the actual concept of seeds vigor (Willyder, 2010).

In this work, it was possible to observe that the first count of germination (FC) differentiated the lots into three levels, separating lots 01 and 05 as having better quality and lot 02 as having lower quality; however, there was no difference for the germination (G%) between the lots (Table 1). The emergence test on trays (E%) separated the lots only in two levels which were statistically different: lot 02 was the less vigorous and the others were considered statistically equal. For the emergence speed index (ESI), lot 01 presented less number of days for medium emergence of seedlings. However, it is possible to infer that this lot presented better quality. Lot 02 necessitated more days for seeds germination, being the less vigorous lot as compared to the others.

The accelerated aging test (AA) is based on the increase of seeds deterioration by the exposure of these seeds to adverse conditions of high temperature and humidity (40 to 45°C and 100% UR). These are the most related environmental factors related to seeds deterioration (Delouche and Baskin, 1973). For this test, three levels of differentiation were determined in similar way to the others tests: lot 01 presented better quality and lot 02 presented lower quality.

Regarding the respiration test done for the seeds, determined by the Pettenkofer method, it was possible to separate statistically the five lots of maize seeds into three levels of vigor. According to Mendes et al. (2009) this test is an alternative or an important complement to the traditional tests for the determinations of seeds lots vigor, because it is a practical, simple and cheap test. So, in seeds with high vigor were observed the higher values of respiration by the liberation of CO_2 characterizing the integrity of mitochondria. According to Bewley and Black (1994), the integrity of mitochondria in the viable embryos increases from the beginning of imbibition process, which becomes more efficient in ATP production. Thus, vigorous seeds breathe more compared to those seeds with less vigor, in the same period of time.

Castro (2011) observed this differentiation in maize seeds; Pereira (2012) in pepper and chili seeds; Dranski et al. (2013), in canola seeds and Venske et al. (2014) in cotton seeds. By the titulation method, it was also possible to separate statically the five lots of maize seeds in three levels of vigor. Thus, in seeds with high vigor was observed higher consumed O_2 and consequently increase in the CO_2 production. The high consumed O_2 was observed for lot 1 and consequently higher vigor of seeds, contributing also to the integrity of mitochondria (Bewley and Black, 1994). For lot 02, there was lower consumption of O_2 as compared to the other lots (Table 1).

In relation to the simple linear correlation analysis (Table 2) between the results observed in the tests used for the evaluation of physiological quality of maize seeds, there was significant correlation between the results of respiratory activity measured by the Pettenkofer method and those observed in the first count of germination test, with (r) of 0.8626, germination with (r) de 0.9136, emergence speed index with (r) of -0.9092 and aging with (r) of 0.9831.

By the titulation method, there was significant

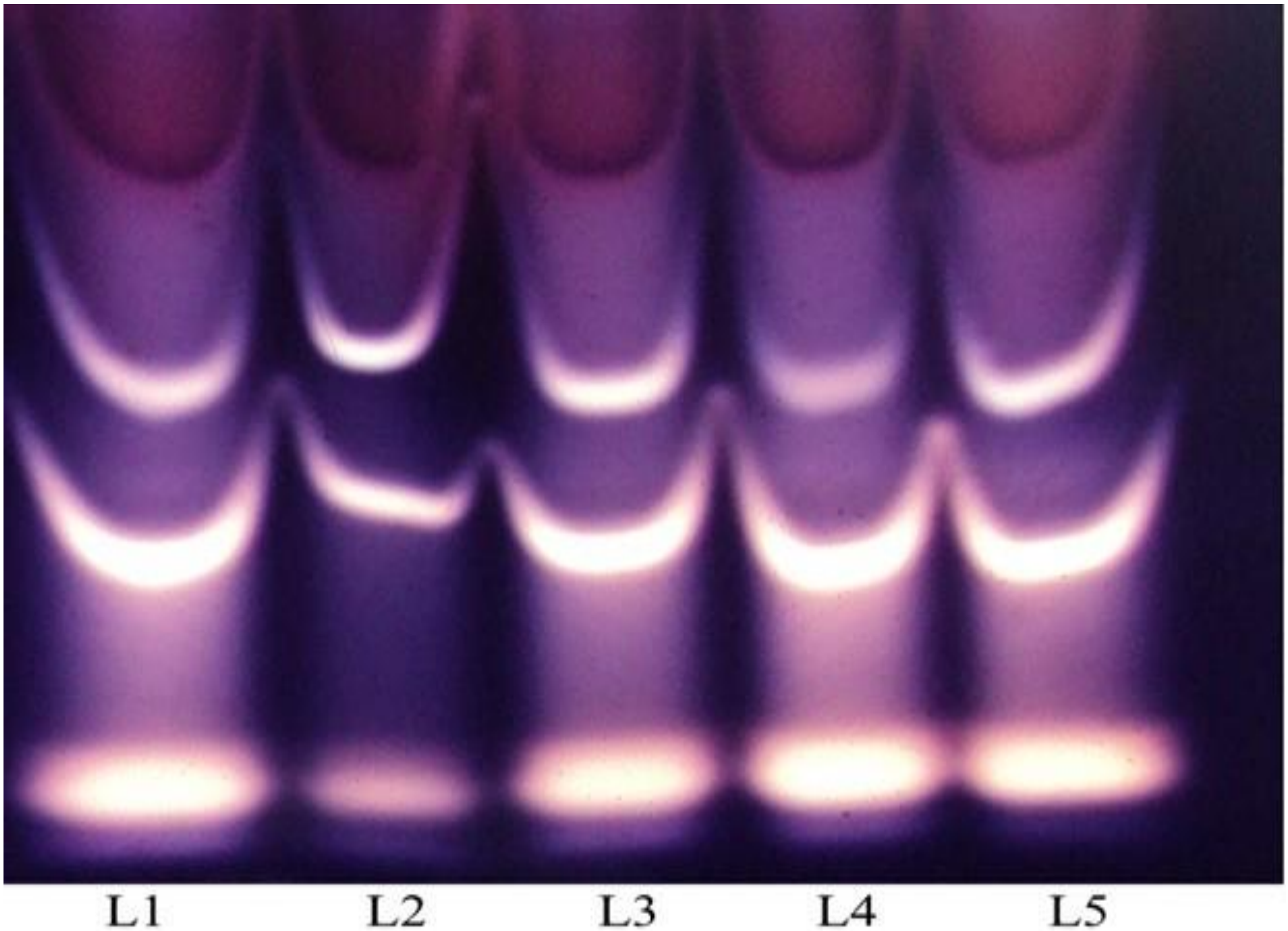

Figure 1. Expression of alpha amylase enzyme (α-AMI) in seeds of five commercial hybrids of maize with different levels of vigor.

correlation between the results of respiratory activity and those results observed in the tests of emergence of seedling speed index with (r) of 0.9370 and for the aging with (r) of -0.8743 and the Pettenkofer method with (r) of –0.9347. Crispim et al. (1994) observed significant correlations between the results observed in the titulation methods and other tests used for the evaluation of physiological quality of soybean seeds. Pereira (2012) also observed correlation between the results obtained in the physiological tests and Pettenkofer and titulation tests for pepper and chili seeds, reinforcing the importance of evaluating the seeds respiratory activity for complementing the germination and vigor tests. In relation to the enzymatic analysis, the alpha amylase expression (Figure 1) was higher in lot 01 and their lower expression was in lot 02, indicating a correlation of the expression of this enzyme with the vigor tests (Table 1). Alpha amylase enzyme is a hydrolytic enzyme that acts on starch; it assists in the supply of energy present mainly during the beginning of seeds germination, contributing to higher vigor of seeds. Consequently, it provides substrates for plant use and thus, ensures higher quality of seeds emergence as it occurs in lot 01 (Nedel et al., 1999).

In maize, the alpha amylase enzyme, when promotes the starch hydrolyses, makes available the carbohydrates required for the embryo development, contributing to the germinative process (Franco et al., 2002). However, Oliveira (2013) points out that beyond the amylases genes, various others genes can be involved in the character control of seeds' physiological quality, for example, genes related directly with respiration.

In relation to the malate dehydrogenase expression presented in Figure 2, it was observed higher expression of this enzyme for lots 1 and 5, and lower expression in lot 02, indicating again, a correlation of this enzyme

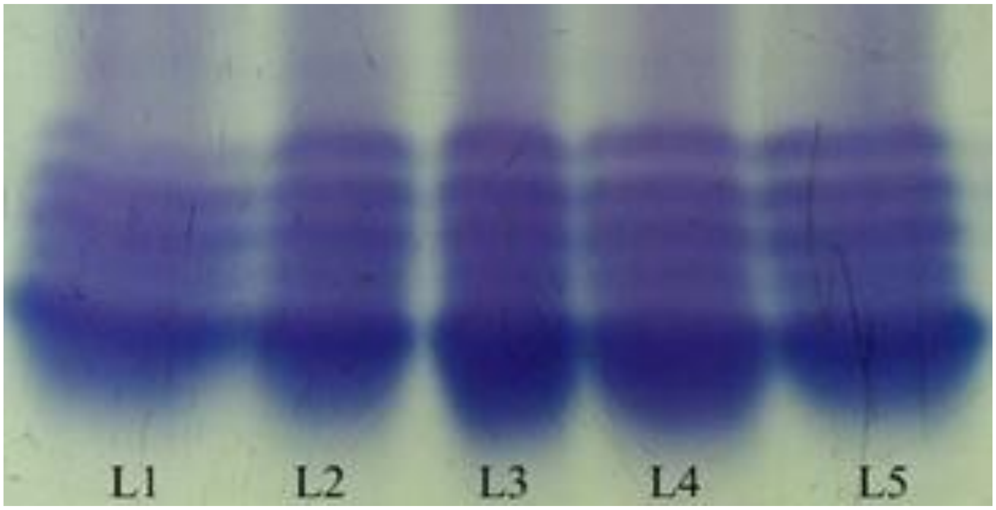

Figure 2. Expression of enzyme malatodesidrogenase (MDH) in seeds of five commercial hybrids of maize with different levels of vigor.

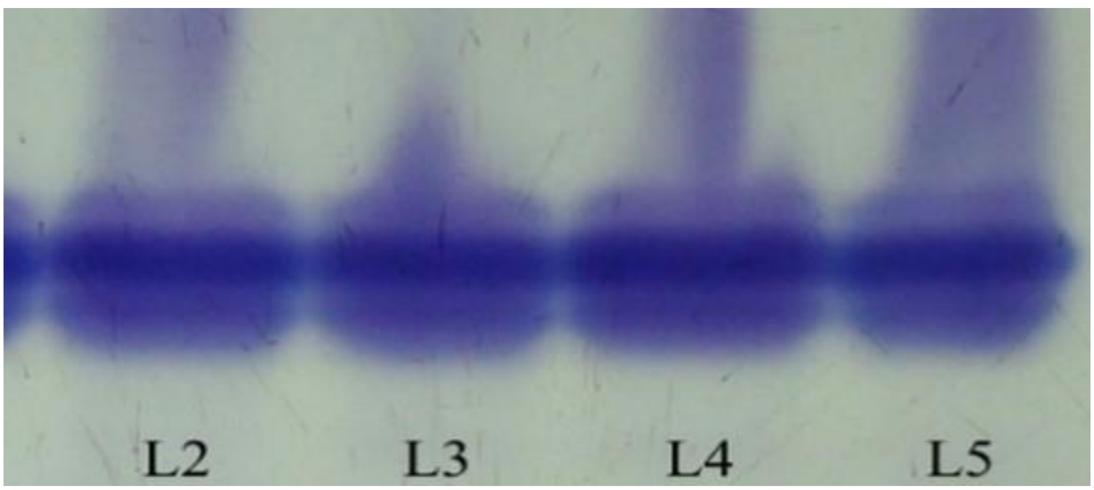

Figure 3. Expression of enzyme alcohol dehydrogenase (ADH) in seeds of five commercial hybrids of maize with different levels of vigor.

expression with the vigor tests realized. The malate dehydrogenase enzyme has an important function in the production of NADH for the Krebs Cicle, participating in the respiratory process. Thus, it is present in different cellular compartments and so, expressing higher intensity of bands in seeds lots with higher respiratory taxes (Shatters et al., 1994).

The correlation of these results can be associated to the activity of this enzyme. MDH enzyme acts in the generation of energy to important metabolic process, like seeds germination; it participates in the movement of malate through the mitochondrial membrane generating energy and fixing CO_2 in plants (Taiz and Zeiger, 2009). It was not possible to infer differences in the expression of alcohol dehydrogenase enzyme activity (Figure 3). Thus, in this work, alcohol dehydrogenase was not effective for determining the quality of lots. According to the results obtained in Table 1, the lots presented significant differences in the characteristics of vigor evaluated, which cannot be observed in bands of the enzyme alcohol dehydrogenase. On the other hand, Castro (2011) observed that the electrophoretic profile of ADH

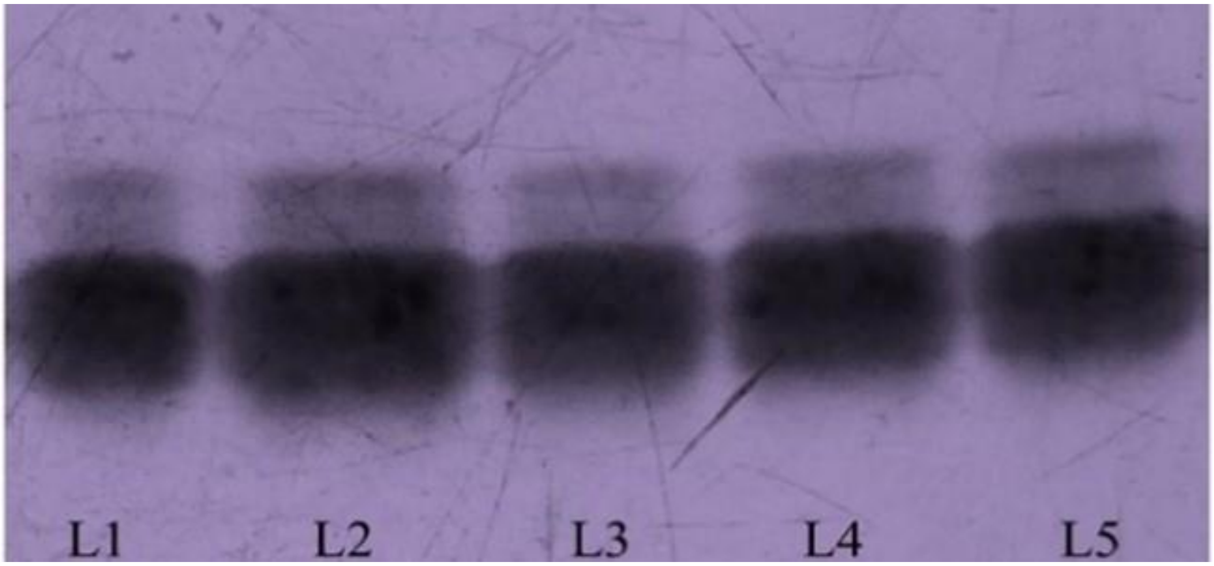

Figure 4. Expression of enzyme pyruvate decarboxylase (PDC) in seeds of five commercial hybrids of maize with different levels of vigor.

enzyme for maize lots submitted for respiratory activity evaluation presented reduction in the intensity for the lot with lower physiological quality.

These results can be justified once the ADH enzyme is involved in the process of converting acetaldehyde to ethanol and the accumulation of acetaldehyde has been related to the deterioration of seeds. Zhang et al. (1994) report that, when the activity of alcohol dehydrogenase enzyme decreased, seeds becomes more susceptible to the deleterious effects of acetaldehyde, which can be an important factor that accelerates the deterioration of seeds during storage. Analyzing the expression of pyruvate decarboxylase enzyme (Figure 4), it was observed higher expression of this enzyme in lot 02, which according to the vigor tests presented in Table 1, corresponded to the lot with lower quality. Thus, in seeds with low vigor, the respiratory activity can be compromised affecting aerobic route and consequently the anaerobic route. Consequently, toxic products such as acetaldehyde and ethanol are accumulated. In this process, the pyruvate decarboxylase enzyme converts pyruvate from glycolysis to acetaldehyde which is reduced to ethanol in anaerobic route (Ferreira and Borghetti, 2007).

With this, the analyses of expression profiles of enzymes involved in the respiratory process are an important tool that complements the physiological tests for the separation of seeds lots in respect to their physiological quality. Alterations in the activities of these enzymes influence the metabolic processes of synthesis that are linked to respiratory processes and consequently influence the seed vigor and seedling development which depend on adequate availability of accumulated reserves (Marini et al., 2013).

Conclusions

The physiological tests and the enzymatic analyses applied to five evaluated lots were efficient to detect different levels of quality in hybrid maize seeds. Through the physiological and enzymatic tests, it was possible to verify that lot 1 presented higher vigor, while lot 2 presented lower vigor compared to the other lots. There are correlations between the vigor of maize seeds and the expression of enzymes from respiration route, except the alcohol dehydrogenase enzyme, because it was not possible to infer differences in its expression.

Conflict of Interests

The authors have not declared any conflict of interests.

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