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Full Length Research Paper

Evaluation of highland maize at Bule hora District of Southern Oromia, Southern Ethiopia

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In order to evaluate the performance of highland maize, an experiment was conducted at Bule hora district of southern Ethiopia by introducing five highland maize from Ambo Plant Protection Research Center and Bako Agriculture Research Center, during the main cropping season of 2010 and 2011. The experiment was carried out in a randomized complete block design with three replications. The data were recorded on plant height, ear height, number of ears/plot, grain yield and 1000 grain weight. All the varieties showed significant differences with each other for all the parameters studied. Variety Jibat had the highest grain yield of 9.677t/ha. The highest plant height of 205.1 cm was recorded from BH660. The maximum number of ear per plant was recorded from AMH851(Jibat). The maximum value for 1000 grain weight was shown by AMH851 (405 g). Therefore, maize variety AMH851 (Jibat) was found to be the most promising, which has the potential to increase the average yield of maize in Bule hora district and is therefore recommended for the replacement of the low yield land race of the area.

Key words: Highland maize, Bule hora, Jibat, AMH51.

INTRODUCTION

In Ethiopia, maize (*Zea mays* L.) exceeds all other cereal crops in terms of annual production and productivity. Maize is increasingly an important component of diets across the country. It is considered as a major source of protein ranking only behind meat, fish and legumes in terms of yearly protein production (Dasbak et al., 2008). The grain is similarly rich in vitamins and fats and makes the crop match suitably, as an energy source, with root and tuber crops per unit measure (Kling, 1991; Dasbak et al., 2008). Though, maize is the most dominant cereal crop in Borana mid highland of southern Ethiopia, its production is hampered by biotic and abiotic stresses among these problem, lack of improved seed is the commonest problem in the study area. In Borana zone of southern Oromia, maize is one of the major cereal crops widely cultivated during the main cropping season (Ganna) and production of this crop in this area is far below the average national yield. This is due to the lack of improved maize varieties that are widely adapted to the area and the presence of maize stalk borer during the epidemic year. The cause of low productivity has been ascribed partly to the use of traditional low-yielding open-pollinated varieties (MOFA-SRID, 2006). At present, there is a growing demand for use of hybrid maize, hybrids have uniform production, higher yielding and are stable in performance as well as in other plant characteristics (unpublished source material).

In Bule hora distric of Borana mid highland, the

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Table 1. Maize varieties used for the trials.

S/N	Variety	Year release	Altitude (masl)	Rain fall (mm)	Breeder/maintainer	Source
1	AMH 851 (Jibat)	2009	1800-2600	1000-1200	Ambo	EIAR
2	AMH 850 (Wenchi)	2008	1800-2600	1000-1200	Ambo	EIAR
3	AMH800 (Argane)		1800-2500	1000-1200	Ambo	EIAR
4	BH670	2002	1700-2400	1000-1500	Bako	EIAR
5	BH660	1993	1600-2400	1000-1500	Bako	EIAR

EIAR = Ethiopia Institute of Agricultural research.

problem of the area is well documented and still no efforts have been made so far to improve production and productivity of maize. In this area, farmers look for high yield variety, adaptable and resistant to disease like maize leaf rust (*Puccinia sorghi*), southern maize blight and maize stalk borer. In the study areas, farmers continuously grow maize which are local and late maturing which takes 8-9 months as well as have low yield and exposed to disease for a long period of time. This paper tried to evaluate five improved highland maize for its adaptability and better agronomic performance at the study areas and areas with similar agro-ecology in the Borana mid highland.

Objective

To identify high land maize variety that is adaptable, with higher yielder and better agronomic performance under Bule hora district of southern Oromia.

MATERIALS AND METHODS

Five improved release highland maize varieties were introduced from Ambo Plant Protection Research Center for its adaptability and yield trial at Bule hora district for two consecutive cropping season of 2011 and 2012. The RCBD with three replication was used and a total plot area of 4.2 m x 3 m. And an inter and intra-spacing of 70 x 30cm were used, respectively. All the recommended field management and fertilizer were used as per the recommendation for the maize (Table 1).

Data analysis

All the agronomic data were recorded and subjected to analysis. Analysis of variance was performed using the GLM procedure of SAS Statistical Software Version 9.1 (SAS, 2007). Effects were considered significant in all statistical calculations if the P-values were ≤ 0.05. Means were separated using Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance (ANOVA) for different plant traits recorded is given in Table 2. According to ANOVA, the varieties differ significantly for their plant height (cm), ear height (cm), ear per plant, TSW (g) and grain yield (t/ ha) (Table 2).

Plant height (cm)

All the varieties showed significant difference for plant height (Table 2). Among the tested cultivars, BH660 had the highest plant height (205.1 cm) followed by BH670 (188.8 cm), while short statured plants of 145 cm were recorded from AMH850 (wanchi). Hussain et al. (2011) reported differential pattern of maize varieties for plant height.

Ear height (cm)

The difference in ear height (cm) in this study was found to be significant owing to the genetic variation among the evaluated maize varieties. Still, the BH660 had the maximum ear height (113.1 cm), while the shortest ear height was recorded for AMH850 (66.6 cm). These results got sufficient substantiation with the findings of Hussain et al. (2011) and Nazir et al. (2010)

Number of ears per plant

Significant differences were recorded among evaluated highland for their ear number per plant (Table 2). Variety AMH851 (Jibat) gives the maximum number of ears per plant (1.95.0), while the minimum number of ear per plant was recorded from Bh660 (1.40). Other researchers also reported genetic variations among different maize hybrids (Ihsan et al., 2005; Haq et al., 2005).

Thousand seed weight (TSW)

Grain weight is an important yield and yield component of maize and varies from maize variety to maize. The result of TSW significantly differs among the varieties (Table 2). The maximum value for 1000-grain weight was obtained from AMH851 (405.00 g), while the minimum value was obtained from cultivar BH660 (295 g). The observed

Table 2. Mean performance of yield and yield component of highland maize at Bule hora district of Sothern Oromia, Southern Ethiopia, during 2011.

S/N	Trt	2010						2011						
		DF	DM	Ph (cm)	EL (cm)	TSW	Yld (t/ha)	DF	DM	Ph (cm)	EL (cm)	E P	TSW	Yld (kg/ha)
1	AMH 851	107^{a}	150.8^{b}	140^{ab}	59.5^{ac}	375^{a}	9.021^{a}	107^{a}	150.8^{a}	162.8^{cd}	74.8^{b}	1.95^{ab}	405^{a}	9.677^{a}
2	AMH850	107.3^{a}	146.3^{a}	159^{ab}	68.8^{abd}	305^{bf}	7.71^{b}	107.25^{a}	146.3^{a}	145.4^{a}	66.6^{b}	1.7^{ab}	365^{bf}	8.211^{ae}
3	AMH800	108.5^{a}	149.8^{b}	166.5^{a}	80^{a}	291.67^{cgj}	6.31^{cf}	108.5^{a}	149.5^{a}	181.8^{bc}	96.8^{a}	1.6^{ab}	325^{cfi}	6.534^{bef}
4	BH670	108^{a}	150.5^{b}	143.3^{ab}	63^{acd}	285^{dhkm}	5.83^{dgi}	108^{a}	150.5^{a}	188.8^{ab}	101.3^{a}	1.85^{a}	315^{dgik}	6.079^{cefg}
5	BH660	108.5^{a}	148^{b}	164.3^{a}	77.3^{ab}	240^{eiln}	4.54^{ehj}	108.5^{a}	148^{a}	205.1^{a}	113.1^{a}	1.4^{b}	295^{ehjl}	5.334^{defg}
Mean		107.5	148.6	148.7	65.9	2993.3	6.68	107.7	149.1	176.3	90.5	1.67	341	7.167
Lsd (5%)		3.8	4.4	37.9	10.50	4.5	3.12	4.2	5.8	19.1	18.3	0.43	15	3.09
CV (%)		2.4	2.2	17.1	34.3	13.6	12	2.5	2.5	7	18.33	17.2		29.2

Letters with similar letters were non-significant while letters different letters were significant. Lsd= Least significant difference, DF= date to flowering, DM= date to maturity, Ph= plant height (cm), EL= ear length (cm), TSW = thousand seed weight (gm), Yld = yield (t/ha), EP= ear per plant.

Table 3. Means of cob length, cob diameters, kernel row per plant and kernel per row of five highland maize evaluated at Bule hora district during 2011 cropping season.

Variety	COL (cm)	COD (cm)	KRPC	KPR
AMH851 (Jibat)	13.9	4.5^{a}	15.1^{a}	31.9^{a}
AMH850 (wonchi)	13.4	4.4^{a}	13.9^{bf}	29.8^{ae}
AMH800 (Arganne)	13.3	4.0^{a}	13.3^{cfi}	28.6^{beg}
BH670	12.4	4.0^{a}	13.0^{dgij}	26.9^{cegh}
BH660	12.2	3.8^{c}	12.9^{ehij}	26.2^{dfgh}
LSD (5%)	1.2	0.4	0.8	3.3
Mean	13.04	4.14	13.64	28.68
CV (%)	6.6	7.1	6.26	11.35

COL = Cob length (cm), COD = cob diameters (cm), KRPC = kernel row per cobs, KPR = kernel per row.

differences may be attributed to the genetic makeup of the varieties. In support of this finding, different researchers have reported significant amount of variability in different maize populations studied.

Grain yield (t/ha)

Significant differences were obtained for grain yield among different varieties used in this trial. Maize variety AMH51 (Jibat) showed higher grain yield (9.677 t/ha), while variety BH660 produced lower grain yield (5.334 t/ha). Similar result corroborated with result reported by Akbar et al. (2009) who evaluated and identified high yielding maize varieties among different genotypes tested.

Among the improved high land maize varieties evaluated for their adaptability at bule hora, the longest cob length was obtained from Jibat (AMh51) and not significantly different from others and similar result was obtained for cob diameters. The highly significant result was obtained for kernel row per cobs and kernel per row and the highest result was obtained from Jibat (AMH51) maize varieties (Table 3).

Akposoe top cross hybrid TZEI-W-POP DT STRC3 x TZEI-5 was statistically similar to

Aburohemaa in cob length.

Generally, mean of cob length was 13.6 cm and ranged from 12.6 (Aburohemaa) to 13.4 cm (Omankwa) for the checks and 12.4 (Fu 2090 DWDP x TZEI-22) to 16.5 cm (Fu 2080 DWFP x TZEI-19) for the top cross hybrids (Table 3).

Cob diameter

For cob diameter, all the seven highest yielding top cross hybrids were statistically the same and were not different from the three checks. Overall, the mean cob diameter was 4.2 cm, and ranged from 4.2 (Omankwa) to 4.4 cm (Aburohemaa) for the checks and 3.3 (Fu 2090 DWDP x TZEI-48) to 4.6 cm (TZEI-W-POPDTSTRC4 x TZEI-3) for the top cross hybrids (Table 3).

Kernel row cob-1

Among the top cross hybrids, TZEI-W-POP DT STRC3 x TZEI-1 had the maximum number of rows per cob. TZEI-W-POP DT STRC3 x TZEI-1, Fu 2090 DWDP x TZEI-30 and Fu 2080 DWDP x TZEI-1 were statistically the same. TZEI-W-POP DT STRC3 x TZEI-5, Fu 2080 DWFP x TZEI-4, Fu 2090 DWDP x TZEI-46 and TZEI-W-POP DT STRC3 x TZEI-39 were also statistically the same. On the contrary, TZEI-W-POP DT STRC3 x TZEI-1, Fu 2090 DWDP x TZEI-30 and Fu 2080 DWDP x TZEI-1 were statistically different from TZEI-W-POP DT STRC3 x TZEI-5, Fu 2080 DWFP x TZEI-4, Fu 2090 DWDP x TZEI-46 and TZEI-W-POP DT STRC3 x TZEI-

Conclusion

From this experiment, it is concluded that the cultivar AMH851 (Jibat) remained superior in its yield and yield component during the experimental years. Therefore, this variety is recommended for Bule hora area and for similar agro-ecology of the zone. The results of this study revealed that there is a considerable amount of difference among the variety which could be used for further improvement in maize breeding.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sunflower seed treatment with growth inhibitor: Crop development aspects and yield

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Sunflower is one of the most important oleaginous crops designated for edible oil production in the world. However, Brazilian growers still do not have enough knowledge, and technical expertise, for expanding the sunflower crop yield through the adoption of new technologies, like the utilization of plant growth regulators (paclobutrazol). The aim of this work was to evaluate the effects of different doses (0, 50, 100, 150 and 200 mL ha^{-1}) of the growth regulator paclobutrazol on agronomic characteristics of sunflower crop in Uberlandia, Minas Gerais State, Brazil. Treating the seed with paclobutrazol decreased the size of the disk flowers and the plant height, while the number of days to complete the development cycle increased. The yield was not affected by the different doses. The use of paclobutrazol in seed treatment is an interesting tool to manage the development of the sunflower crop, as it affects desirable parameters which does not reduce seed yield.

Key words: *Helianthus annuus* L., paclobutrazol, biorregulator.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the main oleaginous crops designated for edible oil production in the world. In the 2014/2015 agricultural year, a yield of 1,376 kg ha^{-1} was reached in Brazil, resulting from 109.4 thousand hectares cultivated with this crop (CONAB, 2015).

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Considering the higher resistance to heat, drought and cold than most of the species grown in Brazil (Lima et al., 2014), sunflower areas expanded with about 25% in 2014/2015 as compared to the agricultural year of 2013/2014 (CONAB, 2015). This crop has received more attention due to its good results as an option to succession planting and crop rotation, animal feed, honey production and functional human nutrition (Faria et al., 2015; Ungaro et al., 2009). Furthermore, since 2010, the Brazilian government demands that all diesel commercialized in Brazil should be admixed with at least 5% of biodiesel (Matsumoto et al., 2015).

Even with the increasing demand of this product, Brazilian production is still low, for example only 1.5 Russian production, and is about half of the Israeli average yield (FAO, 2015). Growers in Brazil still do not have enough experience with sunflower; neither have capacitated technical assistance to expand the yield of this crop. One example of applicable technology is the utilization of plant hormones and growth regulators, which is insufficiently explored in this crop (Spitzer et al., 2011)

One of the currently used plant growth regulators in agriculture is paclobutrazol. This compound is a triazole derivative known for its inhibitory activity against sterol and gibberellin synthesis (Rademacher, 2000; Khan et al., 2009). It affects some aspects of the plant such as height and flowering, due to alterations in phytohormone levels (Kim et al., 2012). These modifications are interesting for sunflower production due to the high susceptibility of this crop to lodging, which is correlated to its considerable height and weight of its disk flower (Sposaro et al., 2008).

However, its results depend on the applied concentrations, number, the moment of applications, and target (foliar or soil) (Almeida And Pereira, 1996; Koutoubras et al., 2014). Furthermore, there are just a few studies on the use of paclobutrazol in seed treatment (Melo et al., 2014, 2015). Due to these facts, the utilization of this hormone in the sunflower crop still requires more studies.

The aim of this work is to evaluate the effects of different doses of seed treatment with paclobutrazol in agronomic characteristics related to the sunflower crop production in Uberlandia, Minas Gerais State, Brazil.

MATERIALS AND METHODS

This work was conducted in 2015 at an experimental area in Heliagro Agricultura e Pecuária (24°20'32'' S; 53°51'36'' W), located in Uberlandia, Brazil. The region has an average altitude of 830 m and weather classified as Aw according to Köppen classification and it is considered a tropical wet climate but with a pronounced dry season (Ribeiro et al., 2013).

It was established as a randomised block design, with five replications for each treatment. The treatments were based on different doses of paclobutrazol: 0 (Control), 50, 100, 150 and 200 mL ha^{-1}. Each plot consisted of four rows of six meters with a space of 0.7 m from the other, comprising 80 plants each one; the distance between plots was 1 m.

The paclobutrazol solution was obtained from a commercial product registered as Cultar 250 SC. The different doses were applied inside a plastic bag with the seeds, followed by intense agitation for good coverage of the seeds. The chosen variety of sunflower was the simple hybrid Helio 251, recommended for all the Central Brazilian territory (Porto et al., 2008)

The sowing was in March manually and jab "matraca" planter was used. After emergence and initial development of seedlings, some of them were removed to avoid intraspecific competition and to establish a population of 45,000 plants ha^{-1}.

During sowing, 350 kg ha^{-1} of the fertilizer 8-20-20, related to the concentrations of nitrogen, phosphorus and potassium, respectively, was applied in the furrows. Twenty days after the crop emergence, there was a side dressing fertilization with 150 kg ha^{-1} of ammonium nitrate. Also, there were applications of 10 kg ha^{-1} of sodium octaborate, diluted in water and applied to the soil while sowing and ten days after the crop emergence. The fertilization was based on soil fertility analysis to meet the nutritional requirements of the crop described by Ribeiro et al. (1998).

For the evaluations, only the two central rows of each parcel were considered. The height of the plants was measured from the soil to the upper leaf at flowering (considering 50% of the plants with 50% open flowers). The average size of the disk flowers was assessed at harvesting by measuring the length between the extremities of the structure. After the manual harvest, the achenes were taken to the laboratory to be separated from the seeds, which were weighted to determine the production of each parcel and from those results, the estimated yield (kg ha^{-1}) was calculated.

The results were submitted for normality test of residuals (Shapiro-Wilk test) and homogeneity of variance (Bartlett test) using the SPSS software. The variance analysis was assessed using F-test with 5% significance variance analysis. The choice of the regression model was based on the significance of the regression coefficients and on the highest value of the determination coefficient; in both cases, the SISVAR (Ferreira, 2011) software was used.

RESULTS AND DISCUSSION

The sunflower plants presented distinct results in response to the different doses of paclobutrazol in the following characteristics: size of the disk flowers, height of the plants and duration of the productive cycle (Table 1). However, the weight of the grains after the harvest did not present any differences due to the treatments. The biggest disc flowers were observed with 50 mL ha^{-1} dose of paclobutrazol, followed by the dose of 100 mL ha^{-1}, while the higher doses gave similar or even lower results as compared to the control (Figure 1).

This occurred because gibberellins have an inhibitory effect on the flowering process (Pereira et al., 2014). As paclobutrazol inhibits the synthesis of this hormone in plants and interfer with the oxidation of ent-kaurene to

Table 1. Variance analysis table for development and yield parameters of sunflower under different doses of paclobutrazol in seed treatment.

Sources of variance	DF	DD	SY	HP	DC
Treatments	4	4.06*	0.06 NS	0.03*	26.06**
Block	4	1.76NS	0.08NS	0.01 NS	1.66 NS
Residue	16	0.98	0.06	0.01	3.63
VC		5.16	28.48	5.22	2.47

VC: Variation coefficient; DF: degree of freedom; DD: diameter of the disk flower; SY: seed yield; HP: height of plants; DC: days to complete the development cycle. Values followed by NS were not significant at 5% of significance; *Significant at 5%; **significant at 1%.

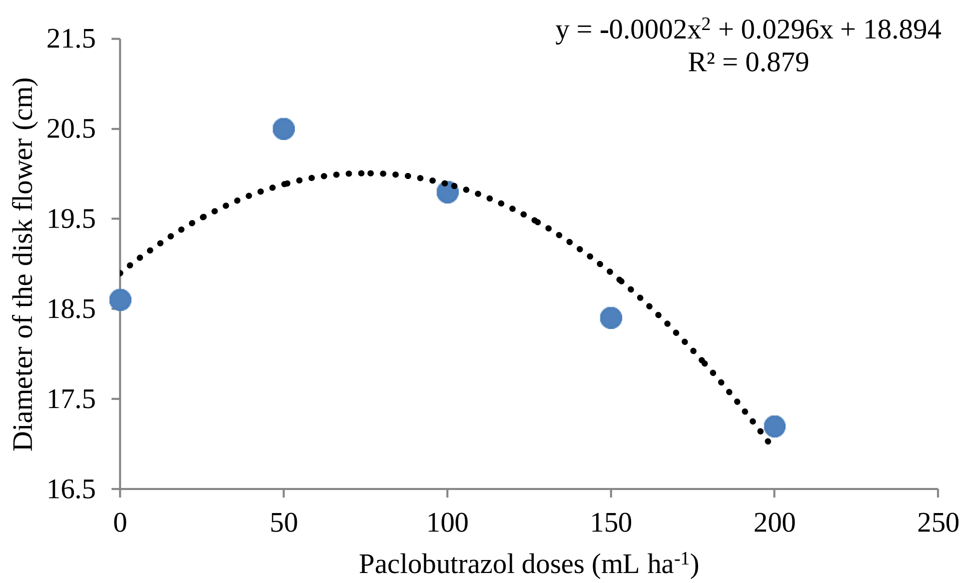

Figure 1. Size of the disk flowers under an increasing dose of paclobutrazol in sunflower seed treatment.

kaurenoic acid in the pathway of gibberellins (Almeida and Pereira, 1996; Zheng et al., 2012), lower doses of this growth inhibitor might be beneficial to the flowering development of several crops. However, higher doses have a negative effect on this parameter, as also observed by Wanderley et al. (2007), who noticed that high doses of paclobutrazol resulted in smaller disc flowers in sunflower under the hydroponic system.

The height of the plants at flowering showed a concentration dependence following paclobutrazol treatment (Figure 2). The control treatment had the highest average plant height (1.89 cm), while the treatments with paclobutrazol presented lower values.

Wanderley et al. (2014) noticed that in ornamental sunflower produced in a hydroponic system, the reduction of the height reached even 90%, while Koutoubras et al. (2014) reported a decrease of 11.1%. So it is important to highlight that different genotypes of sunflower might present variable responses to paclobutrazol. Lower plant growth resulting from the utilization of paclobutrazol was also reported in several other crops (Hua et al., 2014; Peng et al., 2014; Koutoubras and Damala, 2015). Gibberellin promotes stem growth, especially as a result of its effect on cell elongation (Zhang et al., 2011; Yang et al., 2012). By inhibiting gibberellins, paclobutrazol also reduces the plants' height. This is a very desirable result for the sunflower crop as tall plants and heavy discs make plants

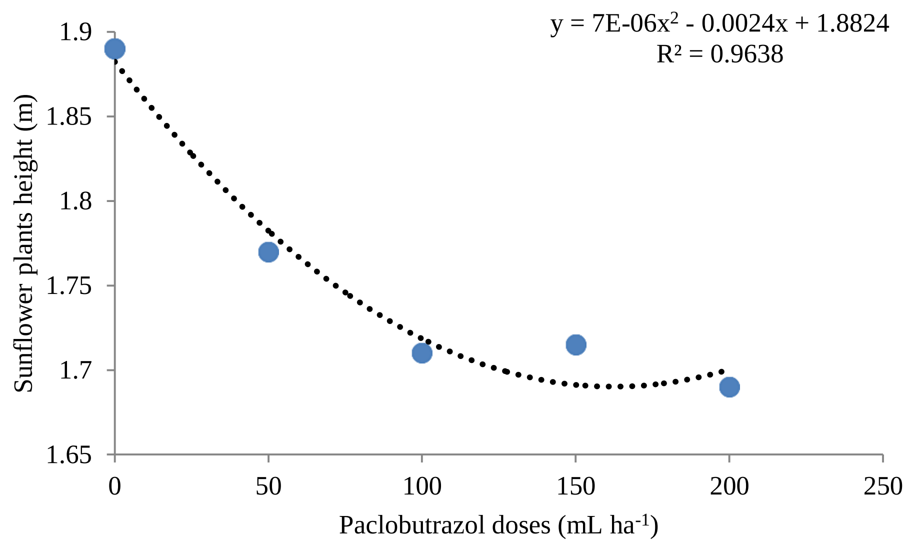

Figure 2. Average height of sunflower plants at flowering under increasing doses of paclobutrazol in seed treatment.

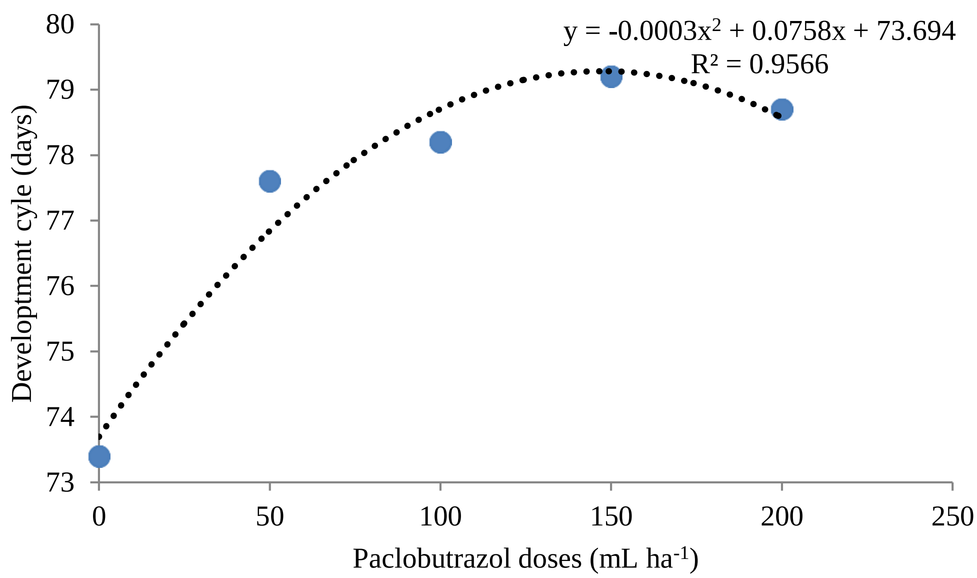

Figure 3. Number of days from sowing to physiological maturity of sunflower plants under increasing doses of paclobutrazol in seed treatment.

very susceptible to lodging (Koutoubras et al., 2014; Silva et al., 2015). This way, shorter sunflower plants might be a strategy to reduce losses in the mechanized harvest.

Seeds treated with paclobutrazol need longer time to complete development, in some cases almost an additional week (Figure 3). Thus, the treatment without paclobutrazol was faster in reaching the physiological maturity. This might be related to a delay in the emergence of the shoots caused by the growth inhibitor. Melo et al. (2014) noticed that tomato seeds treated with paclobutrazol had a reduced germination rate and seedling emergence; this is related to the antagonizing effect of gibberellin, which is an essential hormone to germination.

Another reason for the longer cycle is the delayed development of leaf primordia into expanded leaves. This result was reported by Almeida and Pereira (1996), while assessing foliar applications of paclobutrazol in sunflower crop. With a smaller quantity of expanded leaves, photosynthesis is less effective, indicating a longer time for the plant to complete its vegetative cycle.

A longer productive cycle can be a problem for Brazilian conditions. In this country, sunflower is usually produced in the same fields after another crop such as soybean in the same year. With a higher amount of days in the field, the plants are more exposed to aggressive diseases like white mold (Markell et al., 2015) and weather conditions like wind damage. The results show an undesirable effect of the growth inhibitor in this regard, implying that the use of paclobutrazol in seed treatment should be followed by rigorous phytosanitary strategies.

The seed yield did not show any statistical differences between the treatments (Table 1). The average yield was relatively low (1044.92 kg ha^{-1}) when compared with the Brazilian average yield (FAO, 2015), but this is the consequence of an unexpected drought in the area during the experiment. The yield responses found in this study were similar to those found by Koutoubras et al. (2014), who reported that a single foliar application of paclobutrazol resulted in shorter plants, but without the detriment of any yield parameters, like seed weight or number of achenes per disk flower.

However, paclobutrazol also increases yield in other crops (Tekalign and Hammes, 2005; Hua et al., 2014; Gatan and Gonzalez, 2015). This indicates that paclobutrazol might be a valuable tool for sunflower production as a growth regulator, as it reduces plant height and does not negatively affect yield. However, as it causes a longer development cycle, further researches involving environmental factors (as diseases occurrence) are needed for a clearer conclusion on the potential of this tool.

Conclusions

The use of paclobutrazol in seed treatment might be an interesting tool to manage the development of the sunflower crop under propitious conditions for plant lodging, as it reduces height without affecting seed yield. More studies are necessary to evaluate if the longer time to complete the development cycle can be harmful to sunflower in the presence of abiotic or biotic stresses.

Conflict of Interests

The authors have not declared any conflict of interests.

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Nitrate and potassium leaching and the response of the common bean to different irrigation blades

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The aim of this study was to determine the effects of increasing irrigation levels on the movement of nitrate and potassium in clay soil and on crop yields for the common bean irrigated by central pivot under Brazilian Cerrado conditions. The experiment was conducted in Urutaí, Goiás, Brazil, in Dystrophic Oxisol clayey soil in 2014. Four irrigation regimes were tested, which were equivalent to 50, 100, 150 and 200% of crop evapotranspiration (ETc), estimated using the Class A tank method. The plots were arranged in a randomized complete block design with four replications; the soil solution was evaluated seven times during the cycle. Potassium chloride with 58% K_2O was used as the potassium source, and calcium nitrate with 15.5% total nitrogen (N) as the nitrogen source. To collect the soil solutions, extractors were installed at depths of 0.20, 0.40, 0.60, 0.80 and 1.00 m. The concentrations of nitrate (NO_3^-) and potassium (K^+) were determined without filtering or digestion. At harvesting, the production components and yield were evaluated. The NO_3^- and K^+ levels within the soil profile varied between treatments, showing nutrient leaching below the root system for the higher irrigation treatments. Therefore, the application of appropriate irrigation techniques should reduce the leaching of NO_3^- and K^+ and lead to higher yields for the common bean.

Key words: Bean, aspersion, water management, loss of NO_3^- and K^+.

INTRODUCTION

The Brazilian Cerrado has been intensively explored since the early 1970s and is currently one of the most important areas of the country in terms of production of grains and meat. The soils are originally acidic and present low availability of essential nutrients for plants (Vendrame et al., 2010). Thus, they require the use of fertilizers to enable

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the production of crops.

One of the problems that occur, due to the intensive use of fertilizers in farming systems is the leaching phenomenon involving complex interaction between soil hydrology, water and nutrient uptake by plants and management practices (Van et al. 2006). Soluble ions from correctives, fertilizers and the decomposition of organic matter are potentially available to plants. However, during periods of high rainfall, intensity or excessive irrigation, there can be significant water drainage, facilitating the downward movement of these ions. For this reason, leaching is a constant concern that requires regular monitoring to prevent the loss of nutrients and environmental pollution (Santos et al., 2002).

Beans are historically one of the main food consumed in Brazil and in the world (Barbosa and Gonzaga, 2012), with important nutritional role to humans as a source of vitamins, fiber, iron, isoflavones, phosphorus, magnesium, calcium, zinc and mainly protein for the population of low income (Broughton et al., 2003).

The common bean (*Phaseolus vulgaris* L.) is a plant that requires high levels of nutrients due to its short lifecycle (70-110 days) and reduced, shallow root system, in which approximately 76 to 90% of its roots are found in the first 0.30 m of soil (Stone, 2002). Among the nutritional deficiencies found in bean crops, nitrogen (N) deficiency is the most common. Approximately, 50% of the total N absorbed is exported to the grain, making it necessary to determine the correct dose and time of application to provide adequate nutrition to the plant until the beginning of flowering to increase the number of pods per plant (Carvalho et al., 2001). The application of excessive N doses, in addition to increasing economic costs, can have serious environmental risks, whereas insufficient doses can limit productivity (Santos et al., 2002).

Nitrate (NO_3^-) is the mineral form of N in the soil that results from the application of nitrogen fertilizer or organic matter mineralization. When NO_3^- in the soil solution is not absorbed by plants or immobilized by soil microbiota, it can be easily leached, as it has a negative charge and is not adsorbed well by soil particles, which exhibit predominantly negative charges (Primavesi et al., 2006).

Special attention should also be paid to the use of potassium (K) fertilizer, as K is the second most important mineral nutrient required by vegetable species after N (Marschner, 1995). In particular, the bean has two periods of high K demand. The first occurs during flower bud differentiation, when crops absorb on average of 1.7 kg K ha^{-1} day^{-1}, and the second period occurs at the end of flowering and the onset pod formation, when crops absorb 2.2 to 3.3 kg K ha^{-1} day^{-1} (Rodrigues et al., 2013).

K present in the soil solution moves vertically, mainly through drainage water, and can be transported through leaching to depths inaccessible to the root system (Oliveira and Boas, 2008). This movement of potassium in the soil profile primarily depends on soil texture type (Neves et al., 2009), cation exchange capacity (CEC) and water regime, as well as fertilizer dosage and solubility (Rosolem et al., 2006).

Even though modern technologies have led to higher bean crop yields, irrigation efficiency remains a problem because irrigation management is still lacking and the producer fearing the crop to suffer from water stress, usually uses excessive application of water. Out of all water used in the world, 70% is for irrigation (Evett and Tolk, 2009). According to a study conducted by Company Energy of Minas Gerais (CEMIG), if irrigation were to be implemented rationally, approximately 20% water and 30% of the consumed energy could be saved (Lima et al., 1999).

As energy costs represent approximately 7% of the total cost of bean production (FAEG, 2015), optimal irrigation management practices would yield savings of only 2.1% of the total production cost, which producers generally consider negligible, leading them to prioritize other expenses such as manuring, which represents approximately 28% of the total production cost. However, correct irrigation practices have other benefits besides saving electricity, such as reducing nutrient loss by leaching due to the high mobility of N and K in the soil.

Therefore, the aim of this study was to determine the effects of increasing irrigation levels on the movement of nitrate and potassium in clay soil and its influence on the productivity of bean crops irrigated by center pivot in Cerrado conditions.

MATERIALS AND METHODS

Site description

The experiment was conducted between July and November of 2014 in an area irrigated by central pivot at the Federal Institute Goiano (IF Goiano), Campus Urutaí, Goiás, Brazil, in dystrophic Oxisol clayey (Embrapa, 2006), 17° 28' 41" South latitude and 48° 11' 35" West longitude, at an altitude of 823 m. The climate, according to Köppen, was classified as Cwa (tropical altitude), with dry winters and hot and rainy summers.

Before installing the experiment, the authors calculated the water distribution uniformity at the pivot (irrigated area = 19.63 ha, relief inclined = 5% of slope, blade applied to 100% = 2.32 mm and CUC = 82%), performed water infiltration tests in the soil (TIB = 159 mm h^{-1}), constructed a water retention curve for the soil for the 0 to 0.30 m layer (θ FC at 10 kPa = 0.2335 m^3 m^{-3}, θ criticism at 35 kPa = 0.1697 m^3 m^{-3} and θ PWP at 1500 kPa = 0.1149 m^3 m^{-3}) and analyzed the chemical and physical properties of the soil (Table 1). The percentage of macropores and micropores in the soil were approximately 26.80 and 23.35%, respectively.

Experiment conduction

After the desiccation of spontaneous vegetation was performed

Table 1. Chemical and physical soil characteristics.

Layer (m)	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	K	N	O.M.	CEC effective	CEC pH 7,0	Clay	Silt	Sand	Ds
			($cmol_c$ dm^{-3})			(mg dm^{-3})		(g kg^{-1})	($cmol_c$ dm^{-3})			(%)		(g cm^{-3})
0.0-0.2	5.8	4.6	1.7	0.0	4.3	63	600	29	6.46	10.76	45.1	12.1	42.8	1.34
0.2-0.4	5.7	3.6	1.4	0.1	3.8	20	600	21	5.15	8.85	45.2	12.7	42.1	1.26
0.4-0.6	5.7	2.1	1.0	0.2	3.1	13	400	17	3.33	6.23	46.3	12.5	41.2	1.13
0.6-0.8	5.7	1.4	0.8	0.1	2.4	10	400	12	2.33	4.63	45.1	13.1	41.8	1.10
0.8-1.0	5.4	0.9	0.7	0.1	2.2	7	400	11	1.72	3.82	43.0	14.0	43.0	1.11

Ds: soil density.

mechanically on 10 July 2014, dry beans (cultivar Pearl) were sown at a spacing of 0.50 m between rows and 15 seeds per m. At the time of sowing, 450 kg ha^{-1} formulated fertilizer was applied with the following composition: 4% nitrogen, 30% phosphorus, 16% potassium, 2% calcium, 0.6% manganese, 0.54% boron and 0.27% zinc.

During cultivation, the culture was irrigated using 34 water blades with the Class A tank method, and the tank was located approximately 500 m from the experimental area. To estimate the crop evapotranspiration (ETc), the coefficient of the tank (Kp) was fixed at 0.7 (Cunha et al., 2013) and an adapted version of the bean crop coefficient (Kc) proposed by Silva and Stone (1999) was used: 00-14 days after emergence (DAE) Kc = 0.49; 15-24 DAE Kc = 0.69; 25-34 DAE Kc = 0.77; 35-44 DAE Kc = 0.90; 45-54 DAE Kc = 1.06; 55-64 DAE Kc = 0.89; 65-74 DAE Kc = 0.74; 75-84 DAE Kc = 0.48; and 85-95 DAE Kc = 0.27.

The first irrigation was performed one day after sowing (DAS), to raise the surface layer of 0.15 m to field capacity (FC) for all treatments. Another four irrigations were performed every two days to replace water lost to evaporation and ensure seed germination, which occurred at eight DAS. The sixth and last common irrigation for all treatments was performed with the aim of restoring ETc and raised the FC of the soil profile from 0 to 0.30 m. Afterward, irrigations were performed every three days, and the bean was subjected to four different water regimes (treatments) characterized by water slides of 50, 100, 150 and 200% ETc. The plots were arranged in a randomized complete block design with four replications located 200 m from the center of the pivot with 15 m between them. Each plot cultivation consisted of six bean lines 5-m in length.

As top dressing, 522 kg ha^{-1} calcium nitrate fertilizer with 15.5% total Nitrogen (14.4% N-Nitric and 1.1% N-Ammonium) was applied, which was made available in two applications at the beginning of the V3 and V4 stages of the bean crop. During bean development, all relevant cultural and phytosanitary treatments recommended by Barbosa and Gonzaga (2012) were performed.

To collect the soil solution, solution extractors were installed in the bean planting row at the center of the plots, one per depth at 0.20, 0.40, 0.60, 0.80 and 1.00 m, which were used for seven extractions. With the aid of a hand pump, the extractors were used to apply a vacuum pressure of approximately 70 kPa 24 h after an irrigation, and the solutions were collected six hours after the application of vacuum. The first six extractions were performed at 12, 33, 48, 63, 78 and 92 DAS, with the crops in the field, and the seventh and final extraction was performed 124 DAS, fifteen days after the bean harvest. In the four days preceding the last extraction, daily irrigation levels equivalent to 23 mm were applied to moisten the soil and ensure solution extraction at all depths of treatment. The extracted soil solutions were assayed directly without filtering or digestion of nitrate (NO_3^-) or potassium (K^+). The NO_3^- concentration was determined using a HORIBA LAQUAtwin nutrient meter, model B-343 and the K^+ concentration using a HORIBA LAQUAtwin nutrient meter, model B-731 (TRACOM EQUIPAMENTOS LTDA, São Paulo - SP, Brazil).

The bean harvest was performed at 109 DAS. At harvest, the plants located two-m from one of the central lines of each plot were counted to determine the final plant population, and then ten consecutive plants were collected from the useful area of each plot to evaluate the following production components: number of pods per plant, number of grains per pod and mass of 100 grains. At the time of determination of grain moisture, which was performed using a Gehaka Agri G800 moisture meter, the mass per hectoliter for each sample was also determined. The grain yield was obtained using the manual pull-off method on two four-m lines in the floor area of each parcel, disregarding 0.5 m from each end. Plants were air-dried and subjected to manual threshing, and the grains obtained were weighed; the data were processed in kg ha^{-1} with humidity adjusted to 13%.

After the harvest of bean crop, a final set of soil samples were collected for chemical analysis to compare soil metrics before and after the experiment.

Data analysis

The final population results for plant number, yield components and bean yield were subjected to analysis of variance and the variables that showed significant differences at 5% probability of error were analyzed via regression for the quantitative factor (irrigation blades) to determine the model that best predicted the relationships between variables.

RESULTS AND DISCUSSION

The treatments received the following water slides (mm) based on percentage of ETc: treatment 50% (rain = 112, irrigation = 215, total = 327), treatment 100% (rain = 112, irrigation = 377, total = 489), treatment 150% (rain = 112, irrigation = 539, total = 651) and treatment 200% (rain = 112, irrigation = 701, total = 813).

The dynamic water balance during the bean development

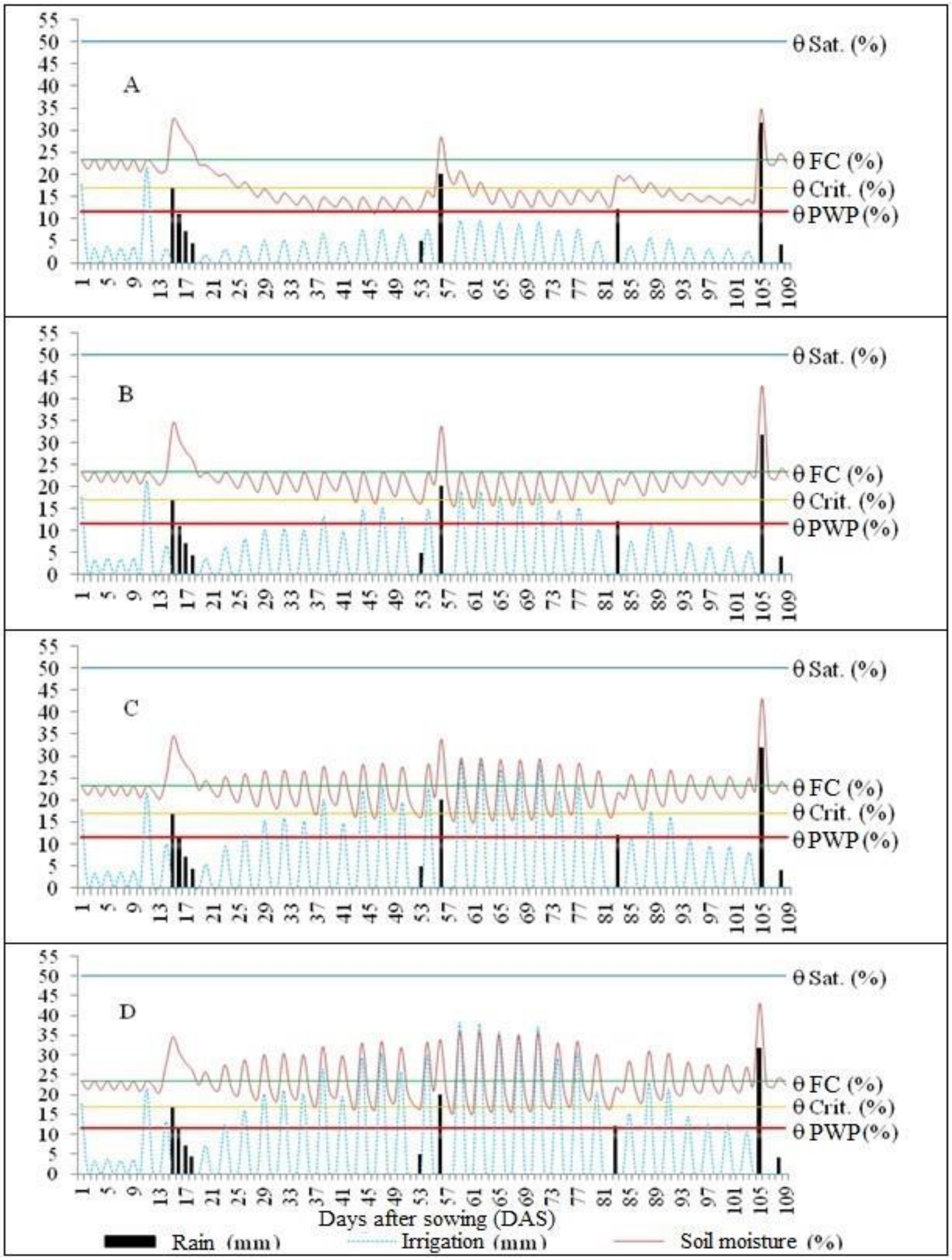

Figure 1. Dynamic water balance in the soil. Treatment with 50% (A), 100% (B), 150% (C) and 200% (D) ETc for the bean crop. For 1 to 10 DAS, the root system was considered to reach a depth of 0.15 m, and afterward to a depth of 0.30 m.

cycle with a water replacement regime of only 50% ETc was first analyzed (Figure 1A). Note that with the exception of the initial period of crop establishment (from the 1 to 13 DAS), as well as some periods following

Table 2. Nitrate and potassium concentrations (mg dm^{-3}) in the soil solution at different depths for the irrigation treatments with 50% (A), 100% (B) 150% (C) and 200% (D) ETc.

Depth (m)	Days after sowing (DAS)													
	12		33		48		63		78		92		124	
	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+
(A)														
0.20	64	10	385	27	-	-	-	-	-	-	-	-	94	6
0.40	-	-	83	3	17	1	44	1	-	-	-	-	139	4
0.60	-	-	50	4	60	4	84	6	-	-	-	-	39	3
0.80	-	-	24	1	25	1	27	1	-	-	-	-	21	1
1.00	-	-	14	2	13	2	12	2	-	-	-	-	10	2
B														
0.20	109	13	445	34	730	39	434	16	76	7	-	-	22	3
0.40	-	-	35	2	42	1	74	2	22	2	-	-	27	1
0.60	-	-	14	3	13	1	11	1	9	1	10	2	21	1
0.80	-	-	15	3	13	3	9	2	7	2	7	1	8	2
1.00	-	-	14	4	11	3	9	3	9	2	8	2	7	2
C														
0.20	84	4	510	8	888	20	85	6	11	2	17	1	24	3
0.40	-	-	103	1	225	1	165	1	44	1	21	1	22	1
0.60	-	-	43	2	88	1	37	1	96	1	62	1	20	1
0.80	-	-	23	4	28	4	22	3	13	2	15	2	24	2
1.00	-	-	17	4	17	4	15	3	13	2	12	2	13	2
(D)														
0.20	57	7	398	15	443	5	53	2	9	2	13	2	15	3
0.40	-	-	174	5	410	6	99	3	9	1	12	2	24	2
0.60	-	-	46	2	93	1	56	1	65	1	40	1	25	1
0.80	-	-	146	4	64	3	61	2	54	2	52	1	39	2
1.00	-	-	11	3	11	2	12	2	54	2	47	2	42	2

rainfall (15, 16, 17, 18, 53, 56, 83, 105 and 108 DAS), moisture levels in the soil remained between the critical moisture level and the permanent wilting point (PWP), leading to water deficits during nearly every culture cycle.

For the 50% ETc water treatment, it was not possible to extract soil solutions at all given times and depths due to low humidity in the soil. When solutions were extracted, the highest concentrations of NO_3^- and K^+ during the crop cycle (from the 1st to the 6th extractions) were at a depth of 0.20 m (Table 2A). High nutrient levels, primarily for NO_3^-, were found at depths of 0.40, 0.60 and 0.80 m at 33, 48 and 63 DAS, most likely due to the influence of rainfall at these times, leading to the leaching of nutrients to these layers. The last extraction at 124 DAS, which occurred fifteen days after the bean harvest, showed high concentrations of NO_3^-, in the two soil layers in particular, which were not absorbed by the bean crop, possibly due to low soil moisture when replacing only 50% of the ETc.

Figure 1B shows the dynamic water balance during the bean development cycle with a water replacement regime of 100% ETc. With the exception of certain periods under the influence of rain, which increased soil moisture above the FC, most of the time soil moisture levels remained between the FC and the critical moisture, indicating correct irrigation management throughout the cycle.

Table 2B shows that during the growing cycle with 100% ETc replacement, higher concentrations of NO_3^- and K^+ were present in the 0.20 m layer, the region in which most of the been root system is located. Intermediate values of NO_3^- were found at a depth of 0.40 m, which may have been due to the influence of rains during the experiment. At layers of 0.60 m and deeper,

Table 3. Chemical characterization of the clay soil after the bean harvest for treatments with different irrigation blades.

Layer (m)	Treatment	K	N	Treatment	K	N
		(mg dm^{-3})			(mg dm^{-3})	
0.00-0.20	50% [(1)]	160	800	150%	112	800
0.20-0.40	50%	72	500	150%	44	800
0.40-0.60	50%	116	500	150%	32	500
0,60-0.80	50%	36	400	150%	24	400
0.80-1.00	50%	28	400	150%	20	400
0.00-0.20	100%	100	800	200%	96	800
0.20-0.40	100%	36	1000	200%	44	800
0.40-0.60	100%	32	500	200%	28	600
0.60-0.80	100%	32	400	200%	20	500
0.80-1.00	100%	28	400	200%	24	600

[(1)] 50, 100, 150 and 200% ETc, representing the percentage of water applied by irrigation, based on ETc.

only low concentrations of NO_3^- and K^+ were found. Failure to extract solutions at depths of 0.20 and 0.40 m at 92 DAS was likely to be due to a decrease in irrigation water, as the crop was already in the maturation stage. Extractions performed after crop harvesting in this treatment did not show high concentrations of NO_3^- and K^+ in the soil solution, indicating that most of these nutrients were absorbed by the culture or were retained in soil colloids.

The dynamic water balance during treatment with a water replacement regime of 150% ETc (Figure 1C) revealed that soil moisture levels fluctuated between the critical moisture level and points above the FC, which is indicative of excess irrigation and leading to water loss through percolation to layers below the root system.

Table 2C shows that for the irrigation regime based on 150% ETc, high K^+ concentrations were found in soil solutions collected 0.20 m from the ground surface, whereas the concentrations of NO_3^- varied at the different depths analyzed. Up to 48 DAS, the highest NO_3^- levels were found in the first 0.20 m of soil, although high levels were present up to 0.80 m below the surface. After 63 DAS, the highest NO_3^- concentrations were measured 0.40 m from the surface, and at 78 and 92 DAS, the highest concentrations of this of the nutrient were found in the 0.60 m layer. Extractions performed after harvesting for this treatment did not show high concentrations of any analyzed nutrient, suggesting they were retained in soil colloids, diluted in the soil solution or leached to lower soil layers.

Figure 1D illustrates the dynamic water balance during a water replacement regime of 200% ETc. In this treatment, soil moisture levels varied between the critical moisture level and points well above the FC, again indicating excessive irrigation. In this treatment, water seepage losses to layers below the root system were even more evident.

Table 2D shows that even with the use of irrigation levels based on 200% ETc, the highest K^+ concentrations in soil solutions are found at 0.20 and 0.40 m below the ground surface, whereas NO_3^- concentrations fluctuated greatly. Up to 33 DAS, the highest NO_3^- concentrations were within the first 0.20 m of soil, although high concentrations were found up to 0.80 m. At 48 DAS, the highest NO_3^- concentrations were found in the 0.20 and 0.40 m layers, again with high values up to 0.80 m. After 63 DAS, the highest concentration of NO_3^- were present in the 0.40 m layer, with high values up to 0.80 m. By 78 and 92 DAS, NO_3^- concentrations were already low in the 0.20 and 0.40 m layers and high in the 0.60, 0.80 and 1.00 m layers. The NO_3^- concentration measurements after harvesting showed low values for NO_3^- in the 0.20 m layer, which increased up to 1.00 m, confirming the leaching of NO_3^- to deeper soil layers in this treatment.

By comparing the chemical characteristics of the soil after bean harvest (Table 3) with those from before planting (Table 1), it can be observed that there was a substantial increase in K, which was retained in the soil at all depths in all the four treatments, with the highest values in the layer at 0.00 to 0.20 m. This is likely why we observed low K concentrations in the soil solutions. The K values found in the soil in the 50% ETc treatment were much higher than that in the other treatments, primarily in the upper soil layers. Therefore, it appears that low soil moisture levels in this treatment must have affected the rate of potassium uptake by the crop, leading to a greater amount of this nutrient being adsorbed on soil colloids.

With respect to N, high N-NO_3^- concentrations were

Table 4. Summary of the analysis of variance with sources of variation, degrees of freedom, mean squares, coefficient of variation (CV), calculated F and P-values for the number of plants per hectare (NP), number of pods per plant (NPP), mass of 100 grains (M100G), plant length (PL), number of grains per pod (NGP), mass per hectoliter (MH) and grain productivity (PROD).

Sources of variation	Degrees of freedom	Mean squares						
		NP	NPP	M100G (g)	PL (cm)	NGP	MH (kg)	PROD (kg ha^{-1})
Blocks	3	2946223958	16.67	2.09	44.36	0.08	1.73	20868
Blades	3	622265625	14.03	1.10	1117.79	1.21	14.04	889950
Residue	9	900130208	5.15	2.75	53.28	0.10	2.75	42578
CV (%)		13.34	19.58	6.59	7.21	6.93	2.16	9.82
Calculated F *		0.69	2.72	0.40	20.98	11.61	5.10	20.90
P-value *		0.57	0.09	0.76	0.00	0.00	0.02	0.00

*The values of calculated F and P-value refer to the blades.

observed in soil solutions for all treatments. The main difference between the treatments was that for the 50 and 100% ETc regimes, the highest concentrations of NO_3^- were found in the 0.20 m layer throughout the crop cycle, whereas for the 150 and 200% ETc treatments, the NO_3^- levels remained highest in the 0.20 m layer only until 48 DAS. After this period, the highest concentrations of NO_3^- were found in deeper soil layers. Treatment with 150% and 200% of ETc caused NO_3^- levels to be highest in the 0.60 and 0.80 m layers, respectively, demonstrating the leaching of NO_3^- to soil layers below the bean root system. Dynia (2000) found that NO3 has high mobility in tropical soils with ion accumulation between 2.20 and 4.60 m depth in loamy soil, and between 3.40 and 6.00 m depth in sandy soil, therefore, much below the exploration root zone of most crops.

In this work, only the concentrations of NO_3^- and K^+ in the soil solution were measured, so it was not possible to quantify the losses of these nutrients to soil layers below the bean root system. However, it can be concluded that nutrient loss was most relevant to NO_3^- for treatments with irrigation blades of 150 and 200% ETc, as high concentrations of this nutrient were found in the deeper soil layers, inaccessible to the culture root system. According to Sharpley and Halvorson (1994), high concentrations of N and K in surface water and groundwater have caused not only environmental problems but also affected human health. According to Goulding et al. (2008), better management of essential nutrients for the plants is needed to promote sustainable agriculture. The number of plants per hectare, number of pods per plant and mass of 100 grains did not show significant differences ($p \geq 0.05$) between treatments, whereas plant length, number of seeds per pod, weight per hectoliter and grain yield did show significant differences ($p < 0.05$) (Table 4). The number of plants per hectare was not significantly affected likely because differentiation between water slides occurred after crop establishment.

For plant length, the regression curve (Figure 2A) that best fit the data was the second-order polynomial. Based on this model, optimal plant length would be achieved with an approximately 175% ETc irrigation regime. According to Costa et al. (2008), the reduction in plant height is due to the fact that water stress reduces the turgor of cells and, consequently, their growth. A small leaf area means less perspiration, conserving a limited water supply in the soil for a longer period, thus, reduction of leaf area can be considered as the first line of defense against dryness (Taiz and Zeiger, 2009).

For the number of grains per pod, the regression curve (Figure 2B) that best fit the data was the second-order polynomial. Based on this model, the highest number of seeds per pod would be achieved with water blades of approximately 179% ETc. According to Stone and Moreira (2000), water restriction in the reproductive phase promotes reduction in the number of grains per pod, due to egg abortion.

For mass per hectoliter, the regression curve (Figure 2C) that best fit the data was the second-order inverse polynomial. Based on this model, the highest mass per hectoliter would be obtained with water blades of approximately 131% ETc.

For grain yield, the regression curve (Figure 2D) that best fit the data was the second-order inverse polynomial. Based on this model, the highest productivity would be obtained using water blades of approximately 100% ETc. It can be observed in the curve that there is a marked increase in productivity between 50 and 100% ETc, whereas the increases in productivity for 150 and 200% ETc are more modest.

According to Silveira and Stone (2004), the common bean is sensitive to deficiency and excess in the soil. Thus, to obtain high yields, one should avoid the deficit or

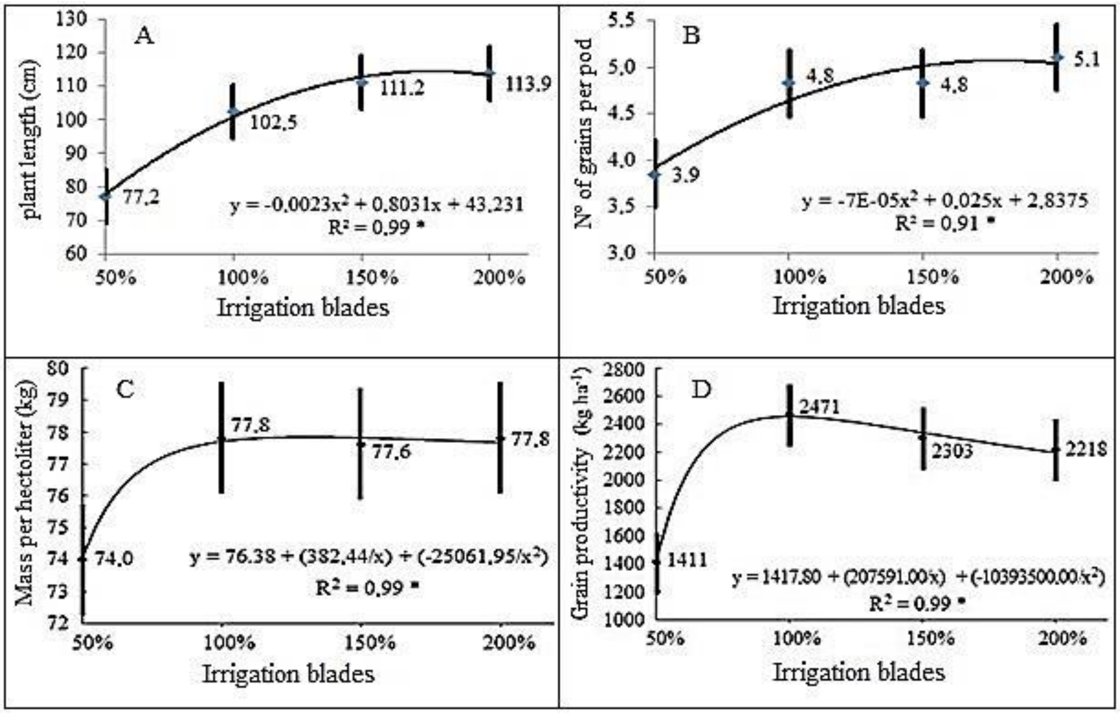

Figure 2. Adjustment of averages: plant length (A), number of grains per pod (B), mass per hectoliter (C) and grain productivity (D) depending on irrigation blade. The 50, 100, 150 and 200% represent the percentage of irrigation water applied based on ETc. Vertical lines indicate standard deviation. *Significant at 5% probability ($p < 0.05$).

excess water in the soil at any stage of the crop cycle (Posse et al., 2010).

When comparing the average yields for the 50 (1411 kg ha^{-1}), 150 (2303 kg ha^{-1}) and 200% (2218 kg ha^{-1}) ETc treatments with that of the 100% ETc treatment (2471 kg ha^{-1}), the results were as follows: treatment with 50% ETc produced a 57% yield, treatment with 150% ETc produced a 93% yield and treatment with 200% ETc produced a 90% yield.

For the conditions in this study, it was found that water deficits were more harmful to bean yield than excess water. According to Nobrega et al. (2004), the duration, intensity, frequency and time of stress can interfere with most morphological and physiological processes in plants, adversely affecting yield components. Low water availability in the soil is a crucial limiting factor in bean production, particular during the three critical stages of germination, flowering and grain filling (Soratto et al., 2003).

Conclusions

Water blades above 100% ETc in clay soil cultivated with the common bean cause nitrate and potassium leaching and reduce the efficiency of these fertilizers. Water deficits lead to lower losses of nitrate and potassium through leaching. However, water deficits also lead to significant yield reductions. Proper irrigation management in the common bean (100% ETc), combined with appropriate timing and doses of nitrogen and potassium, provides the highest yield of grains.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Performance of a water ram built with disposable bottles

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The hydraulic ram is a device that makes use of the energy of a "water fall" to raise part of the water flow to a higher elevation by merely using the energy of the water hammer. Even though a small water volume is compressed, the device may be a solution for water supply on family farms, for home use, animal drinking troughs and watering of vegetables. Current study evaluated the performance of a hydraulic ram constructed with disposable bottles replacing the conventional air chamber model made of cast iron. Experiment was conducted at the College of Agronomic Sciences in Botucatu SP Brazil. Equipment´s performance was assessed by employing different volumes of air chambers (0.25; 0.6; 1.0 and 2.0 L) at the following pumping pressures: 70, 105, 140, 175, 210 and 245 kPa. Results showed that 0.6 and 1.0 L bottles showed higher pressure flow. Within the conditions given for polyethylene tubes, the effectiveness of the device occurred only at a maximum 1:7 ratio (slope/elevation).

Key words: hydraulic ram pump, polyethylene terephthalate, outflow.

INTRODUCTION

The hydraulic ram is a simple and inexpensive device that was introduced for water supply to the rural and deprived areas of the third world countries. The use of these pumps, it is hoped that it can solve the problems of lack of adequate water for drinking, agriculture and animal husbandry, etc. and therefore be useful in preventing rural to urban migration (Fatahi-Alkouhi et al., 2015).

A hydraulic ram functions by a water hammer caused by closing a valve that interrupts the water flow from a supply to a higher hydraulic head (Abate and Botrel, 2002), with the lifting of water through no outside power source. According to Horne and Newman (2005), the hydraulic ram does not need any external power source;

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maintenance and function are simple, without any need of specialized manpower; costs and deployment are low; and it may be run 24 h a day. The hydraulic ram is used in low-head hydropower and highly suitable on small farms. Hydraulic rams may be manufactured at non-industrial very low costs with PVC tubes and polyethylene (PET) bottles as alternatives for the normally cast iron air chamber (CERPCH, 2002).

PET-built hydraulic ram is a relatively very recent device. Although there is scanty information on the device, it is highly relevant in situations where no other type of water pumping is available. Analyses evaluate its performance according to the characteristics of its construction and supply flow, water pressure, loss and yield. Needless to say, the latter factor is the most important item for users. The function of the hydraulic ram during the whole day, continually repeated in 20 to 100 beat cycles per min, depends on the supply flow which pressurises the water intermittently (Jennings, 1996). Thus, there is a continuous water supply without any sort of interruption. The apparatus is self-starting and no oiling is required (RHM, 2014). Supply flow rates or available flow for the hydraulic ram depend on the characteristics of its construction and on the material used. Cararo et al. (2007) employed alternative PET material and reported flows between 21.28 and 46.81 L min^{-1}. The authors emphasized that variation was due to the device´s construction type. Hydraulic rams manufactured in Brazil function with a flow between 5 and 150 L min^{-1} (Azevedo Netto and Alvarez, 1988).

Compressed water volume is related to the ratio between low and high-head water and on the material used in the supply. Abate and Botrel (2002) used PVC and galvanized steel supply tubes and had the highest yield with a hydraulic ram featuring PVC tubes up to 4.2 m level difference. They underscored that galvanized steel tubes are the most efficient.

Mohammed (2007) noted at the development of a hydraulic ram pump that it would conveniently alleviate the problem of water supply to the mass populace; ideally, verifying different combinations of the supply and delivery heads and flows, stroke length and weight of the impulse valve, volume of the air chamber and size of the snifter valve, etc.

When compared to steel ones, PVC tubes have two different characteristics that affect yield: the first favors yield and is characterized by low internal roughness, with low flow loss; the second, unfavorable to yield, is characterized by the tubes´ elasticity which absorbed the water hammer impact (Abate and Botrel, 2002).

CERPCH (2002) reported that in PET bottle hydraulic rams, the elevation height must be 2 to 8 times the height of the fall of the source water, proportionate to the diameter of input and output tube. The device must be placed at a sufficient level difference so that it may operate, in other words at a minimum of 1 m and a maximum of 10 m (the support pressure of the PET bottle should be taken into account). As a rule, level difference is produced artificially by a small dam and using the river´s level difference.

The iron-manufactured air chamber has a greater rigidity with regard to the impact of the water hammer when compared to that in PET bottle air chamber. In fact, the former has low attenuation to the hammer impacts and thus a better yield than the latter. PET bottles have only a relative resistance since they normally support a 200 kPa pressure from its normal contents (Cararo et al., 2007).

Current assay evaluates the hydraulic characteristics of a hydraulic ram made from PET bottles with different volumes and submitted to several pressures while employing polyethylene tubes for supply.

MATERIALS AND METHODS

Assay was conducted in the Laboratory of Irrigation Equipments of the Department of Rural Engineering of the Faculdade de Ciências Agronômica (UNESP), Botucatu SP Brazil. Tested treatments comprised of four volumes (disposable bottles): 0.25, 0.6, 1.0 and 2.0 L and five pressure levels: 105, 140, 175, 210 and 245 kPa. Bottle lids had a 22 mm diameter.

Supply reservoir comprised a 200 L metal tank with water level kept at a height of 3.5 m relative to the hydraulic ram. Water flowed from the reservoir through a polyethylene tube with internal diameter 22.5 mm, so that the equipment could be tested. Part of the total water volume was deployed to the hydraulic ram and was pressured through a tube with internal diameter 10.68 mm; the remaining water or excess water overflowed through the safety valve. Gauges for pressure control were installed and pressure was monitored by digital manometer with 0 - 7 kgf cm^{-2} range.

Figure 1 shows the hydraulic ram, developed with a PET bottle, its components are: 1 - inlet; 2- pressure flow; 3- PET bottle; 4 - vertical retention valve; 5- bottom valve. Water volume in the pressure tube and safety valve were collected with 10 L pails during 1 min.

Samples were weighed and rates transformed into discharges. Supply discharge of the hydraulic ram with PET bottle was obtained from pressure and excess discharges. The sum of the two discharges was equal to total supply discharge. Discharges were necessary for the functioning and the elevation of a section by the water hammer.

A chronometer determined the number of impacts by the safety valve in 1 min. The spring was calibrated so that the number of impacts would be between 50 and 60. Each bottle type had three replications at same pressures 70, 105, 140, 175, 210 and 240 kPa, while measuring discharge in liters per minute for pressure and excess water. Yields were calculated by Equation 1.

$$\eta\% = \frac{q * H}{(Q+q) * h} * 100 \quad (1)$$

Where:
η% = yield in %;
q = pressure discharge in $m^3 s^{-1}$;
Q = discharge in safety valve in $m^3 s^{-1}$;
h = height of supply water in m;
H = pressure height in m.

Analysis of variance was performed to verify data dependence using F-test at 5 % probability. R^2 rate was the parameter to verify adjustments of regression curves to reported data.

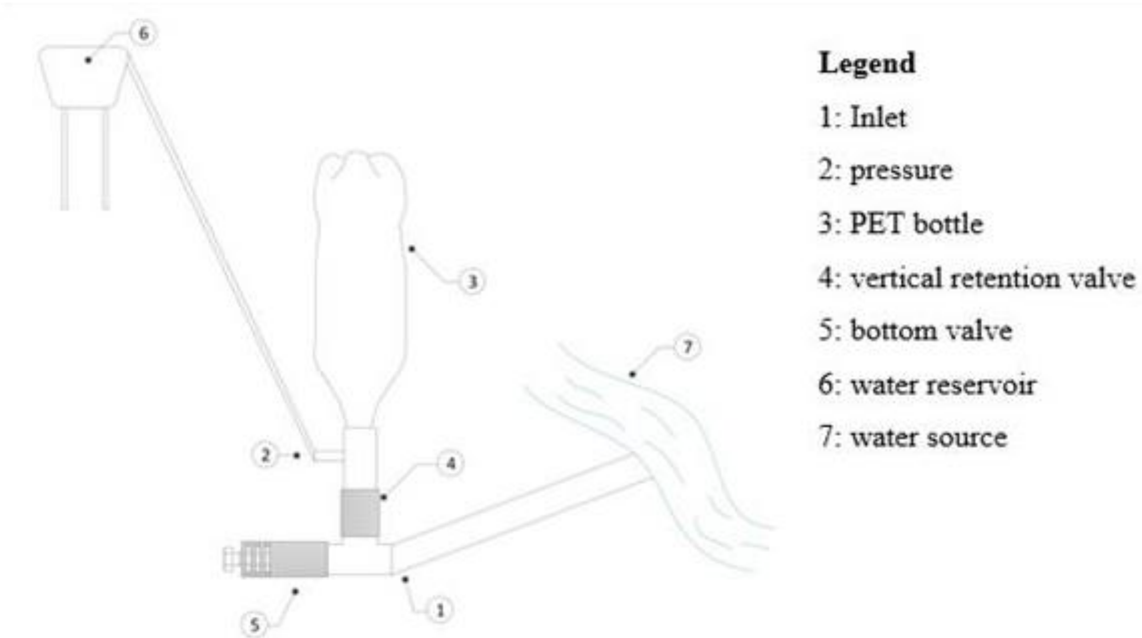

Figure 1. Hydraulic ram made of a PET bottle.

RESULTS AND DISCUSSION

Collected data were analyzed and compared taking into consideration the performance of the hydraulic ram of the PET bottle for volumes 0.25; 0.6; 1.0 and 2.0 L and pressures 70, 105, 140, 175, 210 and 245 kPa (Table 1). The hydraulic ram pumped between 0.24 and 5.1 L min^{-1}. On the other hand, Cararo et al. (2007) reported rates between 0.58 and 11.76 L min^{-1}, with a 4.36 m level difference relative to the level of the hydraulic ram. Abate and Botrel (2002) studied galvanized steel and PVC supply tubes in a commercial hydraulic ram and reported rates between 2.39 and 6.68 L min^{-1}. Variations were caused by the material (galvanized steel and PVC) and water supply heights employed (2.1; 3.8 and 4.7 m).

The four different volume-sized bottles evaluated decreased pumped pressure discharge as pressure increased. When pressures were analyzed separately for the different volumes under analysis, no statistical difference was registered among the flows, with the exception of the volumes 0.6 and 1.0 L at pressure 240 kPa. Variable volumes of excess water did not have any significant difference in the treatments under analysis. Table 2 shows the water volume which has not been used in the pressure pump, or rather, excess discharge. Variations of water loss from excess water during the assay reached 67% (pressure at 70 kPa, with 1 L bottle) and 97.47% (pressure at 245 kPa and 0.25 L bottle) respectively. Rates demonstrate high water loss by the equipment, characteristic of its deployment. Results corroborated those by Cararo et al. (2007) with loss rates between 64.72 and 98.16%.

During laboratory assays, the handling of the equipment did not always permit the precise adjustment of pre-established pressures by gauges and manometers since the equipment had pressure variations during work caused by the water hammer. Table 3 shows mean pressures (for the desired pressures) for each volume.

Pre-established pressures prior to the assay were 70, 105, 140, 175, 210 and 245 kPa. Averages of the four replications during the assay were respectively 69.10, 104.62, 143.61, 173.86, 209.73 and 243.66 kPa.

Bottles with volumes 0.25 and 2 L, at pressure 245 kPa, had lower yield rates than those by CERPCH (2002) and by Abate and Botrel (2002), who described yield between 30 and 60% and between 42.93 and 78.32% respectively.

According to Zárate Rojas (2002), yield the hydraulic ram mainly depends on the relationship between fall height from the reservoir to the hydraulic ram, on the height of the equipment elevation to the upper reservoir and to the preciseness of the manufactured apparatus. Tests with the PET bottles used as air chambers at different volumes and pressures provided the relationship between rates of pumped water caused by level difference and the relationship between level difference

Table 1. Mean rates for pumped and excess water flow in treatments with PET bottles of different volumes under different pressures.

Volume (L)	Pressure (kPa)					
	70	105	140	175	210	245
Highest pumped water flow (L min^{-1})						
0.25	5.10[a]	3.05[a]	2.30[a]	1.58[a]	0.95[a]	0.29b
0.6	4.90[a]	2.27[a]	2.43[a]	1.88[a]	1.25[a]	0.51[a]
1.0	4.65[a]	2.88[a]	2.27[a]	1.75[a]	1.02[a]	0.58[a]
2.0	5.01[a]	3.00[a]	2.32[a]	1.66[a]	0.97[a]	0.24[b]
Excess pumped water (L min^{-1})						
0.25	12.21[a]	11.88[a]	12.88[a]	12.36[a]	12.15[a]	11.44[a]
0.6	10.89[a]	13.06[a]	14.78[a]	15.02[a]	12.92[a]	11.25[a]
1.0	9.44[a]	10.36[a]	12.49[a]	13.34[a]	12.28[a]	12.37[a]
2.0	10.24[a]	11.34[a]	12.30[a]	12.49[a]	11.36[a]	8.46[a]
CV = 5. 29%						

No significant difference for same lettered values according to Tukey´s test at 5% probability.

Table 2. Percentage of total volume of water used in pressure and excess pump, yield and discharge in supply (pressures).

Volume (L)	Pressure (kPa)					
	70	105	140	175	210	245
Volume of water under pressure (%)						
0.25	29.48	20.43	15.17	11.31	7.26	2.52
0.6	31.03	20.00	14.09	11.14	8.84	4.36
1.0	32.99	21.76	15.38	11.62	7.68	4.46
2.0	32.85	20.93	15.88	11.75	7.85	2.77
Volume of excess water (%)						
0.25	70.51	79.57	84.82	88.68	92.73	97.47
0.6	68.96	79.99	85.90	88.85	91.15	95.63
1.0	67.00	78.24	84.61	88.37	92.31	95.53
2.0	67.14	79.07	84.11	88.24	92.14	97.22
Yield (%)						
0.25	58.97	61.28	60.70	56.56	43.59	17.34
0.6	62.06	44.36	56.40	55.70	53.05	29.92
1.0	66.00	65.29	61.53	58.11	46.13	30.63
2.0	65.71	62.79	63.55	58.76	47.14	19.04
Total discharge in supply (L min^{-1})						
0.25	17.31	14.93	15.18	13.93	13.09	11.73
0.6	15.80	16.33	17.21	16.90	14.17	11.76
1.0	14.09	13.24	14.77	15.09	13.30	12.94
2.0	15.24	14.34	14.62	14.15	12.32	8.70

and pumped water. Table 4 shows rates of pumped water discharge obtained in the assay.

PET bottles with 0.6 and 1.0 L provided the highest rates of pumped flow, with the best performance among the others being a 1:3 ratio. Thus, it may be inferred that air chambers with 0.6 and 1.0 L at 245 kPa had the best yield when submitted to the highest level difference/ pumping pressure ratio, evaluated in the experiment.

Table 3. Mean rates of pressures and yields for different volumes of PET bottles.

Volume (L)	Pressure (kPa)					
0.25	70.13	103.52	144.78	178.05	211.58	245.57
0.6	68.67	104.33	143.00	173.00	208.00	244.00
1.0	68.80	105.32	143.33	172.20	209.67	243.55
2.0	68.80	105.32	143.33	172.20	209.67	243.55
Means	**69.10**	**104.62**	**143.61**	**173.86**	**209.73**	**243.66**
	Yield (%)					
0.25	58.97	61.28	60.70	56.56	43.59	17.34
0.6	62.06	44.36	56.40	55.70	53.05	29.92
1.0	66.00	65.29	61.53	58.11	46.13	30.63
2.0	65.71	62.79	63.55	58.76	47.14	19.04

Table 4. Rates of pumped water discharge according to the different pumped water heights verified in current assay.

Height of pumped water (m)	Ratio level difference/Pumped water	Discharge (L min^{-1})			
		0.25	0.6	1.0	2.0
7.0	1:2	5.10	4.90	4.65	5.01
10.5	1:3	3.05	3.27	2.88	3.00
14.0	1:4	2.30	2.43	2.27	2.32
17.5	1:5	1.58	1.88	1.75	1.66
21.0	1:6	0.95	1.25	1.02	0.97
24.0	1:7	0.29	0.51	0.58	0.24

Yield rates in Table 3 were at 17.34% for 245 kPa pressure with 0.25 L PET bottle, since maximum yield of 66% occurred with 70 kPa pressure with 2 L PET bottle. Results confirmed data by Cararo et al. (2007) with 11.04 and 59.28% rates. The 0.6 L bottle had the best pumping discharge for level difference/pumping pressure of 1:7 ratio, with a possible delivery of 700 L day^{-1}.

Linear regression was the best that was most adequate for the pumping pressure flow within the different pressures and conditions studied. Figure 2 shows the linear regression for pressures 70, 105, 140, 175, 210 e 245 kPa and evidenced the device´s hydraulic behavior. Although, current hydraulic ram pumped flows between 0.06 and 5.37 L min^{-1}, following proper construction conditions and height of pressure flow, maximum discharge could be higher if the supply tubes were made of more rigid material. High elasticity of the polyethylene absorbed the impact of the water hammer and reduced its efficiency.

According to Suarda and Wirawan (2008) the back head pressure caused by the water hammer in the drive pipe decreases from 103.87 m without air vessel to 37.85 m with air vessel. Moreover, pressure shock in delivery pipe increases from 0.29 m without air vessel to 2.9 m with air vessel.

Therefore, they showed that fixing an air vessel could increase sharply the efficiency of the hydraulic ram from 0.72 to about 19.45%. The 0.6 and 1.0 L polyethylene bottles had the best results, mainly for the highest-level difference/pressure flow ratios. Hammer impact failed to occur at pressures over 245 kPa on the water flow. The air in the bottle was released and the system stopped functioning. The developed hydraulic ram pumped up to 739.15 liters of water a day for a 1:6 ratio level difference/pressure flow ratio, with the 0.6 L PET bottle.

Conflict of interest

The authors have not declared any conflict of interest.

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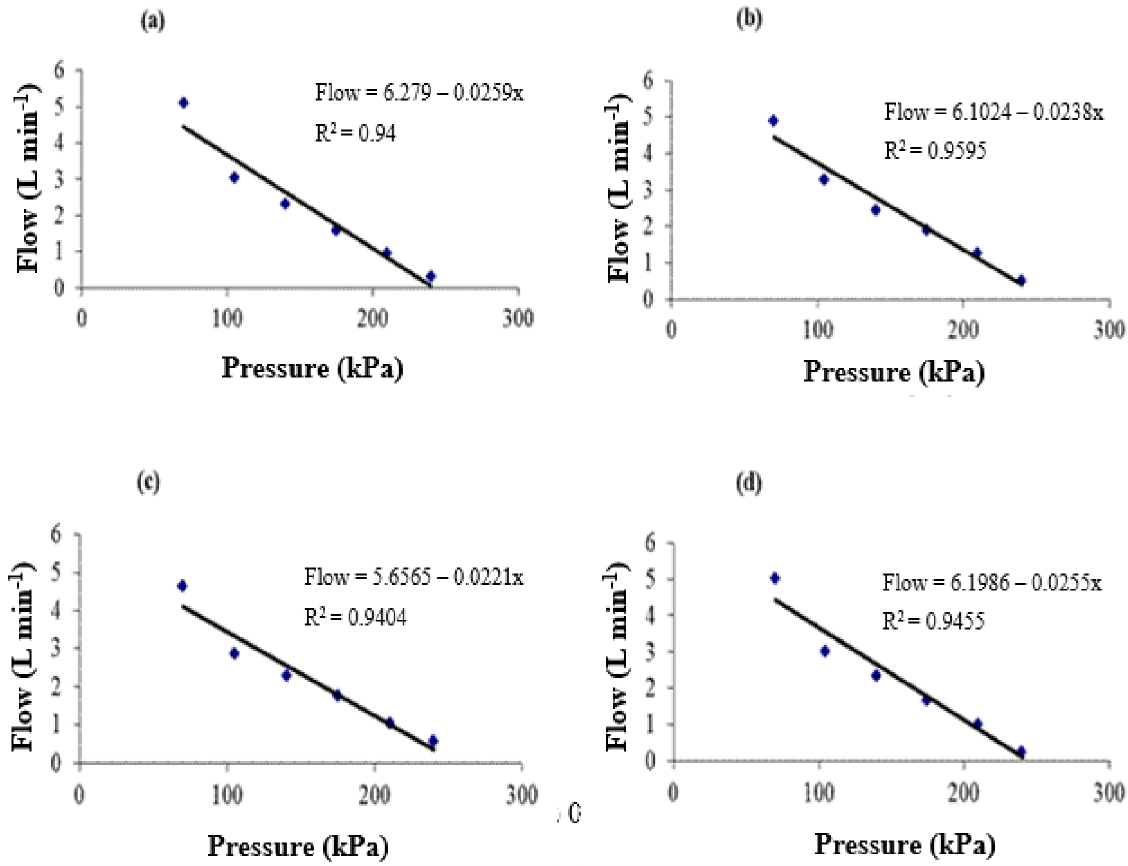

Figure 2. Linear regression for flow: (a) 0.25 L; (b) 0.6 L; (c) 1.0 L e (d) 2.0 L.

scientific project.

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Full Length Research Paper

Estimation of the flower buttons per inflorescences of grapevine (*Vitis vinifera* L.) by image auto-assessment processing

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The aim of this research is to develop a reliable tool by a special method, combining image processing based on watershed algorithm, and a predictive model to estimate automatically the flowers number per inflorescence. Eighty images of *Vitis vinifera* L. inflorescence (the Cardinal cultivar) were processed. Watershed algorithm was used for the image processing and this was followed by statistical analysis that provides robust predictive estimation of the flower button number. The results show a robust estimation, compared to manual flowers counting, with strong correlation. The developed algorithm shows that the watershed algorithm was able to provide an automatic assessment of the flower button number in the inflorescence. The method used is more robust and provides a more significant level compared with recent studies. In the applied research in viticulture, it is crucial to improve knowledge of yield forecasting and to study the fruit set rates estimation. The technique is used to determine, with a higher significance level, the fruitiness rate of grapevine at the early stage of flowering.

Key words: Flower button, fruit set rate, grapevine, image processing, watershed.

INTRODUCTION

Determining fruit set rates requires a flower button counting at stage H (separate flower buttons) of Baggiolini code (1952) in the development cycle of the vine, and at another stage (J). The first attempt of counting procedure is done smoothly and cautiously by manual manipulation of a lot of inflorescences of vines. This monotonous procedure has some technical and spatiotemporal constraints. The manual flower counting is very difficult,

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and deals with many problems having influence on the requested research results. The main problem is the large number of flower buttons (several hundreds or thousands) that can be grouped into clusters (cluster compactness). This may cause a loss in flower buttons during manipulation. Other disadvantages are the possible severe natural conditions of experimentation and time constraints.

The research of Bessis (1960) suggests a method for determination of the flower number. It is based on a linear regression between the cluster length and its richness in flower. He affirmed a positive relationship. This is actually the common method for flower assessment.

More recently, counting objects by using computer simulation has been of great research interest involving important methodological problems of image processing. Researchers have developed various techniques. Formerly, Girard et al. (2009) have developed a method of counting flexible oblong objects that may overlap. This method uses a combination of image processing morphological and statistical filtering.

Moreover, Vallotton and Thomas (2008) have introduced a system based on algorithms of image processing for counting the number of hairs and its length. Whereas, Sossa et al. (2003) have proposed a technique for counting objects in an image without separating the conglomerates of objects, this technique is based on the skeletonization. Similarly, in applied biological processes, Guérin et al. (2004) studied the feasibility of counting of wheat grain by colored images based on texture analysis.

In viticulture, Poni et al. (2006) found a relatively strong correlation between actual and manual count flower numbered on prints. In order to operate the automatic assessment, Cubero et al. (2014) developed a fast and accurate method for detecting and removing the pedicel in images of berries. This method is based on a novel signature of the contour. Diago et al. (2014) developed a simple and robust machine vision methodology to be applied on image taken under field conditions in order to estimate automatically the number of flowers per inflorescence. The later work is taken as reference for our comparative statistical analysis by comparing the robustness and the significance level of the methods.

About the applications on the grapevine, Dunn and Martin (2004) and Tardaguila et al. (2010) developed a program, which recognizes automatically the grapes from a digital image of the canopy of Cabernet Sauvignon grapevine. This method is used to predict vineyard yield. In order to count berries in grapes, Font et al. (2014) presented an automatic method for counting red grapes from high-resolution images of vineyards taken under artificial lighting. The proposed method is based on detecting the specular reflection peaks from the spherical surface of the grapes. Ivorra et al. (2015) propose a three dimensional computer vision approach to assessing grape yield components based on new 3D descriptors. More advanced methodologies applied in viticulture is found in the studies of Nuske et al. (2011), Liu et al. (2013), Nuske et al. (2014), Aquino et al. (2015), Diago et al. (2015), Font et al. (2015) and Schöler et al. (2015).

The main objective of this work is to develop a new method automatic assessment system using image processing and based on watershed in order to detect and separate contiguous flower buttons. It will be pursued by a comparative statistical analysis to obtain a predictive estimation of the flower assessment in the vineyard, which the utility lies in determining fruit set rate.

MATERIALS AND METHODS

In this section, the image acquisition and the process analysis carried on these images and flower counting are described. In order to get a better counting system, a large number of images, which are considered as learning data are worked on. The study was carried out with 80 grapevine inflorescences from different Cardinal (*Vitis vinifera* L.) cultivar.

The images were taken in the field conditions using a Bridge Digital Camera (Sony HX100v) at stage H (separate flower buttons) of Baggiolini (1952) code against a dark background to assure a high contrast. The camera was set to automatic mode and the distance between the inflorescence and the camera lens was not fixed because of the inflorescence size. In the same time, a cautious and precise manual counting was carried in the field for each photographed inflorescence.

Images were processed using Matlab (v7.14) in particular the Image Processing Toolbox. The proposed algorithm is divided in two parts. In a first step, a pre-treatment is necessary to prepare the image. Then, the watershed is applied to the processed images to obtain the final segmentation of flower buttons.

The pre-treatment is based on image processing. It is a very interesting scientific topic that provides more applications (Serra, 1982; Soille, 2002). The objective is to prepare the image by cleaning (noise elimination) or by reducing the information quantity to be processed in order to keep only the most significant information. Consequently, this step is based on the mathematical morphology. The basic idea is to compare objects to be analyzed with another object of a known shape called "structuring element".

Therefore, to prepare our image (Figure 1a), the following steps were realized: (*i*) The images are converted to grayscale (Figure 1b). (*ii*) The images background is removed using the operation "Top Hat", which represents a residue for amplifying the contrast (Figure 1c). (*iii*) The resulting image is then converted into a binary image by using thresholding (Figure 1d). The function *graythresh* automatically computes an appropriate threshold to use to convert the grayscale image to binary (*iv*) The flower buttons are separated by morphological operations (Erode the binary image with a disk structuring element using "*imerode*" function, then remove small objects by the function *bwareaopen* and *imopen*) (Figure 1e).

The watershed introduced for image analysis is one of the most powerful methods to accomplish the delineation steps in image segmentation chains. The watershed technique allows partitioning the image pixels into a set of connected regions, separated by a closed contour. It is an adapted tool for segmentation (Cousty,

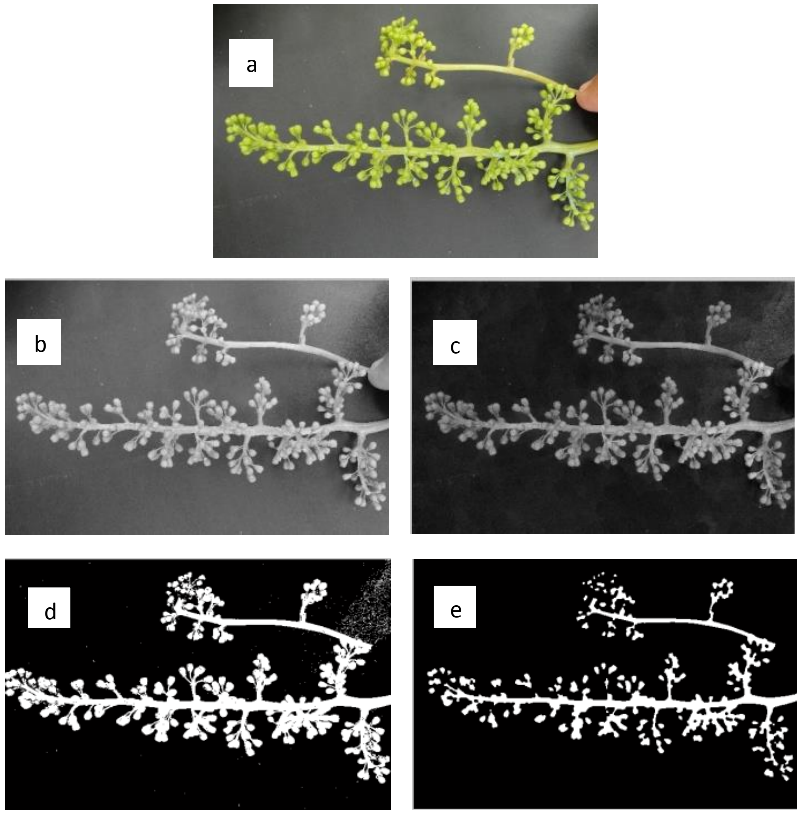

Figure 1. (a) The original image; (b) The image converted to grayscale; (c) Separating the background from the flowers clusters; (d) The image after binarisation; (e) Separation of flower buttons by morphological operations.

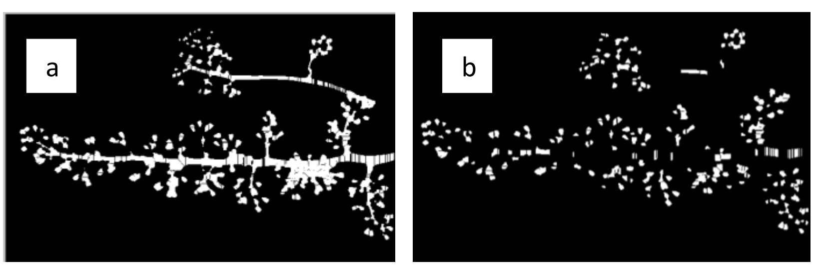

Figure 2. (a) Image after watershed function application; (b) Estimation of flower buttons number after elimination of stalk objects.

2007; Najman and Cousty, 2014; Dias and Nonato, 2015). It is the principal tool of morphological segmentation. Its major advantages, according to Meyer (2012), are the following: (*i*) It produces closed contours: To each minimum or to each marker corresponds one region. (*ii*) Flooding a topographic surface fills some minima and the watershed of the flooded surface has less catchment basin. The catchment basins of successive floodings form a hierarchical segmentation. (*iii*) It is possible to flood a surface so as to impose minima at some predetermined places: This leads to marker based segmentation.

This technique was used to separate contiguous flower buttons (Figure 2a) where it is possible to label the obtained objects. For the proposed automatic counting method, it should take into account the following issues: (1) The flower buttons are grouped in clusters (superimposed flower buttons), which are not being handled by the simple counting. (2) The flower button size differs from one image to another, depending on the distance between the camera and the inflorescence. (3) There are inflorescences containing flower

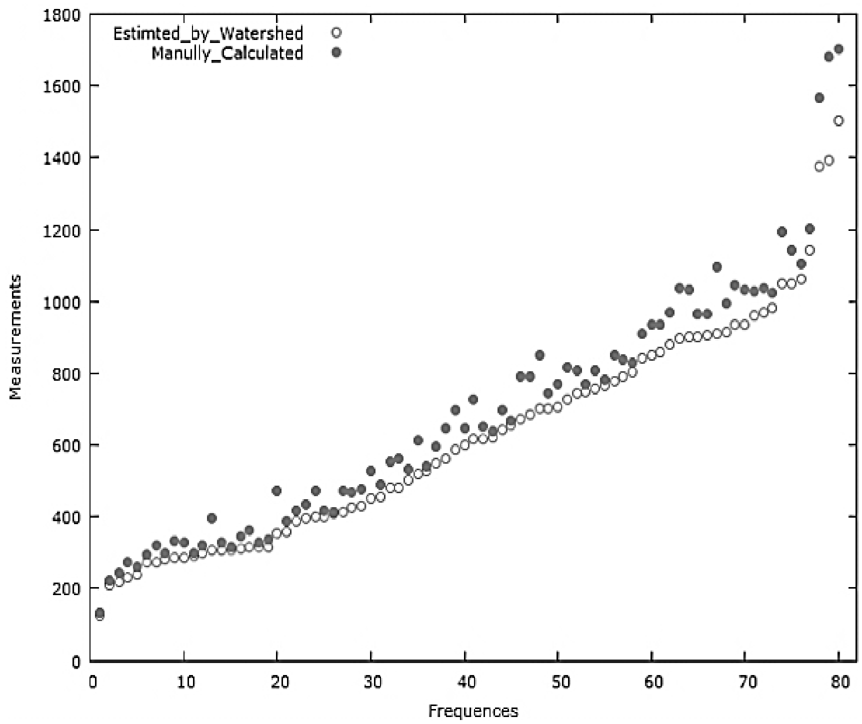

Figure 3. Graphic of the manually counted and those estimated by watershed.

buttons with different sizes in the same image. (4) The image contrast has an influence on the separation of the inflorescence from the background due to light during the image acquisition. (5) The bad separation of raffles that appears before taking images results a superimposed flower button. (6) The emergence of additional objects. These issues require changes in the developed algorithm (threshold adjustment and structural element size) from one image to another in order to eliminate the additional objects, and to separate the inflorescence from background for better detection of flower buttons.

The main objective was the determination of the correlation between manual counting (MC) procedure and automatic counting estimated by Watershed (EW) of flower buttons in our proposed method. Evaluation of the performance of the developed method was carried by the correlation coefficient (r) and the coefficient of determination (R^2). Statistical analysis was conducted using SAS (v9.1).

RESULTS AND DISCUSSION

To determine the number of flower buttons per inflorescence on the image, the objects were divided into three categories: Flower buttons, objects removed because of the smaller size, the unidentified objects (stalks).

By eliminating the stalk and the small objects, a better estimation of the number of flower buttons was obtain (Figure 2b). The number found by the program (Figure 2b) is 283 flowers on the total of 301 manually counted. This reflects an approximation of manually calculated number.

After the algorithm implementation on the total images, results named “Estimated by Watershed” (EW) were gotten. They are graphically presented with the manually counted measurements (MC) in Figure 3.

The uniform lightness from an image to another is corrected by the elimination of background (distinguishing inflorescence from background) that makes the method independent of lightness. This involves changes in the level from a grayscale image to another. The difference in brightness between different images of the database shows that the method is robust for this factor.

A sensitivity to the shape and size of the morphological filter shows that a change in size of the structuring element "disk" is required for each picture due to volume of flower buttons.

The noise has the effect to perturb the labelling of objects (flower buttons) by the existence of more objects. However, the threshold easily removes these very small objects.

Both measures showed a strong correlation (with R^2: 0.99), which leads us to draw that regression line as shown in Figure 4.

Poni et al. (2006) found, by a regression between actual (real) flower number and the number of flowers

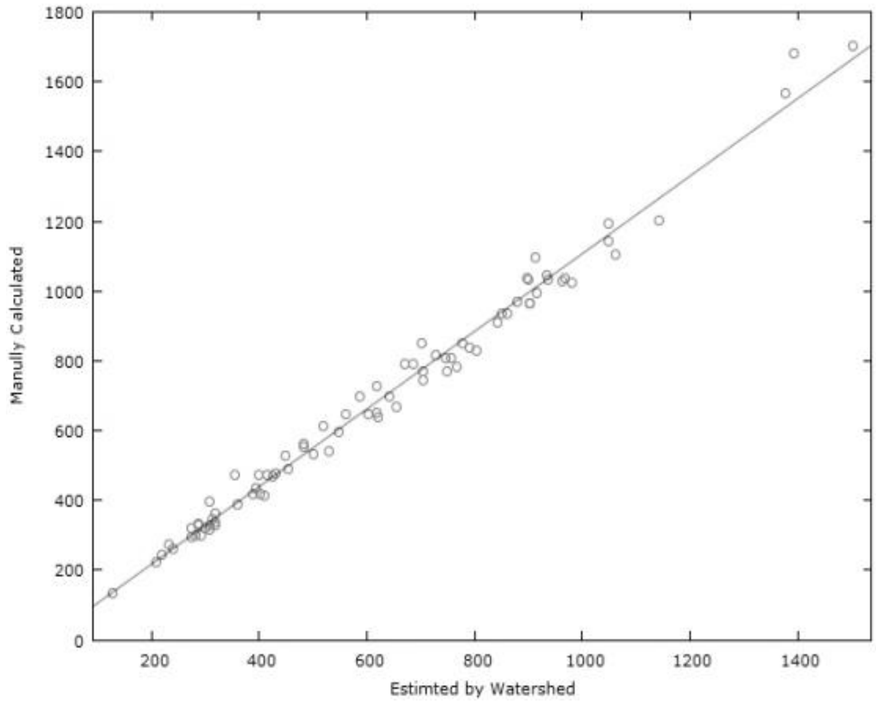

Figure 4. Relationship between the number of flower buttons manually counted and those estimated automatically by watershed (R^2= 0.988 at p<0.00001].

counted on photo prints, that the coefficient of determination was 0.88 and 0.87 for the two worked varieties. Compared with our results based on automatic counting, it seems to be more significant and presents a higher correlation (R^2=0.98).

Diago et al. (2014), by using the same software but with another function in toolbox labeled *bwconncomp*, have similar findings. Comparing our results with Diago et al. (2014, 2015) results, regardless of the used technique, it seems that our results have a more significant level. They found the coefficient of determination between 0.76 and 0.89 among the four studied cultivars.

The flower buttons assessment is done for a single upper surface (a perpendicular view of the image, where flower buttons on the other side of the inflorescence were invisible in images and consequently, undetectable by the algorithm). This makes the counting of the total flower buttons per inflorescence impossible. For this reason, we explain the underestimation of the occurred flower number.

To solve this problem, the implementation of a regression method giving low counting error (with no influence on the results) is required. Through the linear regression method, we could obtain predicted values (Figure 5). By comparing the predicted values with those counted manually, the subject of our estimation, we have found that there is a strong correlation with quite a strong significance with a coefficient of determination of 0.99 (with p<0.00001).

To evaluate the error in the resulting model, the analysis of the linear regression estimator has shown that the values of residuals are reduced. In fact, they have low values by which its standard error of 34.4.

Returning to the precedent example, the number found by the prediction is 312 flowers, which is closer to the manual count (301 flowers) than that obtained from the watershed program (283 flowers). In order to get closer to the manually counted values, our research has shown that we should add 5% of the estimated value to the original one.

Most studies of flower number per cluster were used to estimate fruit set. Therefore, account of the flower button number per inflorescence is essential for accurate assessment of fruit set. In this research paper, we have developed a method of grapevine flower buttons assessment per inflorescence by image processing. This method is based on image processing techniques (The mathematical morphology and the watershed), with a comparative statistical analysis. The comparative analysis has shown that the applied technique presents a more significant level and high robustness compared with some previous studies.

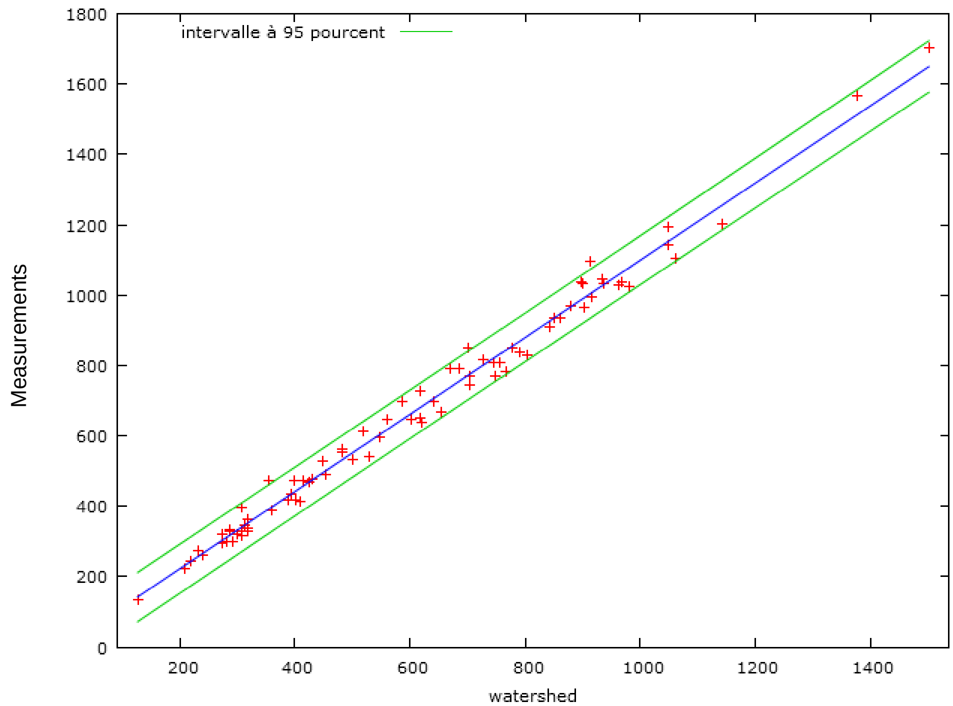

Figure 5. Graphical presentation of predictions with confidence interval of 95%.

Our study is in progress to overcome the difficulties of automatic counting and to implement a reliable tool to estimate the flower buttons per inflorescence automatically, which will be very useful and may be of great help for researchers seeking for the determination of estimated fruit set of grapevine. In the future research, efforts will focus on the correlation estimation between the automatic grape counting results and the ripeness degree of the clusters of grapes and on applying the proposed counting method to other grape varieties and to other families of fruit to estimate yield production.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Induction of genetic variability and plant development in palisade grass evaluated in M2 mutants

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The objective of this study was to evaluate the induction of genetic variability and plant development of palisade grass (*Urochloa brizantha* L.) M2 mutants in acclimatization to subtropical climate condition. The mutagenic agent methyl methanesulfonate (MMS), at a dosage of 0.5%, was used to induce mutation in 4,000 seeds of the cultivar Marandu. Thirty five plants survived after the induction of MMS mutagen agent. These plants were isolated transplanted in experimental area to advance the generation. Seeds produced by plants were sown in pots allocated in Biosystems Organized Development incubator and subjected to cold stress at 0°C in seedling stage. Surviving seedlings per family were transplanted to field conditions in Southern Brazil. The plant development was evaluated in the M2 generation during the agricultural years of 2012-2013 and 2013-2014. Genetic variability analyzes were carried out with 21 traits, based on the average Euclidean distance and the relative contribution proposed by Singh (1981). Chemical mutation induction with MMS generates genetic variability in palisade grass, enhancing the selection of superior genotypes in subtropical climate conditions. The chemical induction of mutations with the MMS mutagen provided genetic variability in the population of *U. brizantha* with formation of 18 divergent groups during the research.

Key words: *Urochloa brizantha* L., plant breeding, genetic recombination, forage, multivariate analyses.

INTRODUCTION

The species of genus *Urochloa* are grown in tropical and subtropical regions of the southern hemisphere. The Brazilian cultivated land area has increased due to the species hardiness and adaptation capacity to different climatic conditions. Those characteristics highlight the country as a big exporter of this species seeds, having commercial relations with approximately 40 countries (CARDOSO et al., 2014).

The genetic breeding of this species faces difficulties to increase the genetic variability at the germplasm banks

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due to the influence of apomictic reproduction mode and the presence of different polyploidy levels. A research was carried out with *Urochloa brizantha* accesses in the germplasm bank of the Brazilian Agricultural Research Corporation - EMBRAPA with a total of 222 accesses. The results showed that all accesses are polyploid, which two are diploid, 157 are tetraploid, 41 are pentaploid, and 22 are hexaploid. These factors hamper sexual reproduction due to the chromosome parity and the ploidy level (Resende et al., 2008).

Among the challenges of genetic breeding, the formation of *Urochloa* segregating populations is difficult to reach because of the difficulties in increasing variability in this species (Valle et al., 2009). Currently, the steps of introduction, evaluation and selection are necessary to launch new cultivars of *Urochloa* (Araújo et al., 2008). Research related to the existent genetic diversity on the germplasm banks of *Urochloa* have been conducted by Ambiel et al. (2010) and Garcia et al. (2013). These researchers found that the genetic variability index is considered low. Moreover, Fuzinatto et al. (2012) studied the possibility of crosses between different species of *Urochloa* and indicated that chromosomal irregularity and apomixis are factors that contribute increasing difficulties in exploring the genetic variability and fixing traits of interest.

Studies regarding the induction of mutations present potential for exploitation to expand the genetic variability in *Urochloa*, since the chemical mutation induction is characterized as an alternative to generate genetic variability for species such as *Urochloa*. This species exhibit difficulty in generating new natural recombination because of its narrow genetic basis (Muller, 1927). In Brazil, research with the mutation induction in the Poaceae family species were performed by Coimbra et al. (2004) and Souza et al. (2005), which indicated success in increasing genetic variability and changes in desired traits, standing out as an alternative to generate genetic variability. A mutation is inclined to be a specific change in a gene or act on chromosomes (Allard, 1971), which highlight successive random changes in nucleotides. In this way, it can provide combinations that generate genetic variability amplification (Taiz and Zeiger, 2013).

The use of appropriate methodologies to evaluate *Urochloa* accesses is important to characterize new individuals. The unweighted pair group method with arithmetic mean (UPGMA) is utilized to assess the variability in forage grasses. Totti et al. (2001) utilized the same method in *Paspalum* accesses, describing it as appropriate due to the consistency in distinguishing accessions. Furthermore, studies with ecotypes of *U. brizantha* also observed the efficiency of UPGMA clustering method in the study of genetic diversity (Torres et al., 2015).

In addition to the genetic diversity, it is important to study the canopy growth and development. Thereby, regrowth and vegetative growth are important for assessing the carrying capacity of animals in the pastures (Lenzi, 2012; Souza et al., 2013). However, seed production should also be considered in forage crops breeding since there is a direct positive relation between the inflorescences number and increased seed yield (Bean, 1972). The objective of this study was to evaluate the induction of genetic variability and plant development of palisade grass (*U. brizantha* L.) M2 mutants in acclimatization to subtropical climate condition.

MATERIALS AND METHODS

The experiment was carried out in the Plant Breeding and Plant Production Laboratory of Federal University of Santa Maria located at coordinates of 27°S23'26" and 53°25'43"W, altitude of 461.3 m in the state of Rio Grande do Sul, southern Brazil. Soil structural properties were based on soil analysis carried out at the experiment area. The soil is characterized by 60% of clay, pH 6.3 and 2.3% of organic matter demonstrating good fertility levels for phosphorus, potassium, and micronutrients. A base fertilization with 20 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5, and 80 kg ha^{-1} of K_2O (NPK only in rate of 05-20-20) was performed prior to the experiment installation.

Chemical mutation induction on 4,000 viable seeds of *U. brizantha* cv. Marandu was held with the mutagen methyl methanesulfonate (MMS) at a dose of 0.5% according to the methodology adopted by Coimbra et al. (2004) in order to assess the genetic variability. After exposure to the mutagen, the seeds were sown in pots with soil and commercial substrate. The preselection was performed at 21 days after sowing (DAS), when the seedlings were exposed to the controlled temperature of 0°C, which is the frost formation temperature, in B.O.D. incubator. The surviving seedlings per family were transplanted to field conditions on March 15, 2011, forming the basis population that originated the progenies studied in this research, corresponding to the M2 generation.

Transplanted seedlings were exposed to low temperatures condition reaching up to 0°C during winter time, until seed production in the following year. Seeds were collected from 35 individuals that gave rise to M1 generation. The seeds that gave rise to M2 generation were allocated with the check varieties *U. brizantha* MG5, *U. brizantha* BRS Piatã, and *U. brizantha* Marandu sowed in rows on April 3rd, 2012. The plant development was evaluated in the M2 generation during two agricultural years. Experimental units consisted of two meters in length and 0.45 m of distance between genotypes. Potassium nitrate at 0.2% was used at the sowing row to break dormancy. The experimental design used for the accesses allocation was the augmented blocks of Federer, where check varieties were allocated with three repetitions.

Twenty one traits were considered in the analysis to study the genetic variability among different genotypes. The studied traits are final height in the 1st year (FH1), final number of inflorescence in the 1st year (FNI1), final number of tillers (FNT), average value of chlorophyll (AVC), final height in the 2nd year (FH2), final number of inflorescences in the 2nd year (FNI2), regrowth notes index after frost (RNF), number of seeds rows per ear (NRE), length of the last open leaf (LOL), width of the last open leaf (WOL), flower stem length (FSL), length of the inflorescence shaft (LI), racemes number per inflorescence (RNI), length of the basal raceme (LBR), number of spikelets per basal racemes (NSBR), stigma color (SC), anther color (AC), length of the rachis hairiness (LRH), density of limbo hairiness (DLH), density of sheath hairiness (DSH), and density of

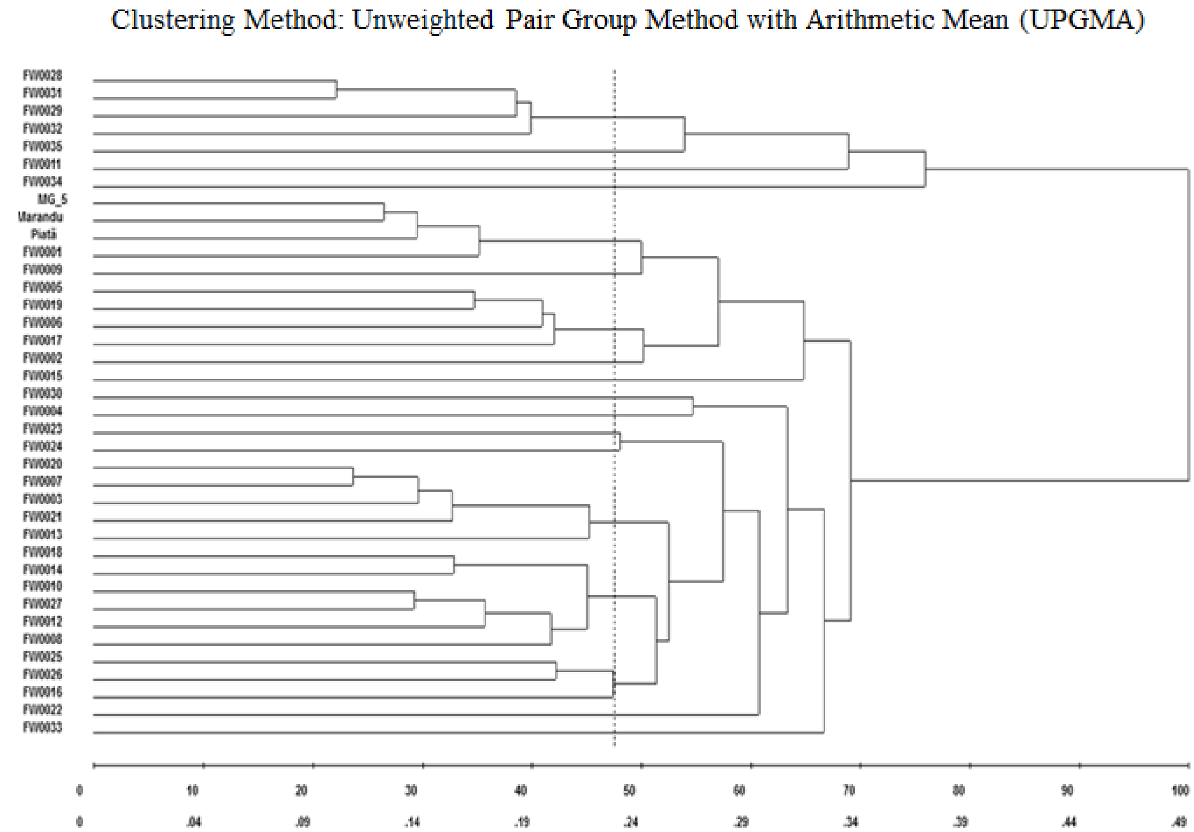

Figure 1. Dendrogram formed by the UPGMA clustering method based on the average eucledian distance, representing the dissimilarity between thirty five M2 mutants accesses of *U. brizantha* and check varieties *U. brizantha* MG-5, *U. brizantha* Piatã, *U. brizantha* Marandu formed from 21 morphological traits carried out in subtropical climate in two agricultural years in Frederico Westphalen, southern Brazil.

the stem hairiness (DTSH).

Evaluations were carried out weekly after the beginning of genotypes emergence to assess the proposed traits FNT, AVC, and RNF. The other assessments to measure the variability were carried out when the genotypes reached full flowering and the final tillers establishment at 140 DAS. The UPGMA method was used for the dissimilarity evaluation and to study the relative contributions of the studied traits.

Assessments of growth and morphological traits were performed weekly to perform the genotypes acclimatization analysis, as follows: Weekly growth rate in the first year: the sowing of the M2 generation was held on April 3rd, 2012, measuring up the weekly development of the studied accesses on observing height in cm, measured from the beginning of the emergency to full flowering of the genotypes; weekly growth rate in the second year: After standardization cut, the weekly development of studied accesses was measured in height (cm). The observations were started before the first frost formation, on July 17th, 2013, corresponding to the start of regrowth until full flowering; appearance of inflorescence in the second year: weekly observation was proceeded to evaluate the appearance of inflorescences, assessing the present inflorescences in the corresponding evaluation units of the week. These evaluations started on September 18th, 2013 being carried out until the full flowering of all genotypes.

Growth variables and inflorescence appearance were submitted to polynomial regression to assess the behavior of accesses in subtropical conditions. Both accesses with better performance and the check varieties were evaluated. Statistical analyzes were performed utilizing Microsoft Office Excel® application and Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

According to Figure 1, there was the formation of 18 distinct groups with average dissimilarity of 23.51% and 17 groups demonstrated dissimilarity in relation to the check varieties. The three check varieties and the genotype FW 0001 were grouped together with unusual characteristics that provide them high similarity. After the measurement of the canopy morphological traits based on 21 studied traits (Table 1), it appears that 97% of the progeny have dissimilarity in relation to check varieties. There was the formation of a group of six genotypes, a group of five genotypes, three groups with four genotypes, one group of three genotypes and 12 groups with one genotype, showing that the UPGMA method was also effective for this research, as also reported in other studies with forage grasses (Totti et al., 2001; Torres et al., 2015).

Detected genetic dissimilarity demonstrates that mutation induction generated high genetic variability,

Table 1. Relative contribution of 21 traits according to the methodology proposed by Singh, referring to population formed by mutants accesses (M2 generation) of *U. brizantha* in acclimatization to subtropical conditions and check varieties *U. brizantha* MG-5, *U. brizantha* BRS Piatã, *U. brizantha* Marandu in two agricultural years in Frederico Westphalen, southern Brazil.

Variable	S.j	Relative contribution (%)
Final height in the 1st year	991994.2	5.9599
Final number of inflorescence in the 1st year	664143.1	3.9901
Final number of tillers	12156.93	0.073
Average value of chlorophyll	84783.39	0.5094
Final height in the 2nd year	573419.7	3.4451
Final number of inflorescences in the 2nd year	5261030	31.6081
Regrowth notes index after frost	1749.806	0.0105
Number of seeds rows per ear	3845.444	0.0231
Length of the last open leaf	41354.95	0.2485
Width of the last open leaf	63.18086	0.0004
Flower stem length	4999732	30.0382
Length of the inflorescence shaft	3393389	20.3874
Racemes number per inflorescence	4490.728	0.027
Length of the basal raceme	51446.97	0.3091
Number of spikelets per basal racemes	556985.1	3.3463
Stigma color	1114.222	0.0067
Anther color	1064	0.0064
Length of the rachis hairiness	845.5463	0.0051
Density of limbo hairiness	293.2346	0.0018
Density of sheath hairiness	315.1235	0.0019
Density of the stem hairiness	349.5309	0.0021

S.j. Relative contribution as criterion Singh (1981).

which was also found in other studies with Poaceae family species, as these proceeded in Brazil with oat (Coimbra et al., 2004; Souza et al., 2005). This increase in variability provides high importance to *Urochloa*, mainly due to reproductive difficulties that are provided by the presence of apomixis, present in the genotypes of *U. brizantha* studied in the Brazilian germplasm bank (Resende et al., 2008; Valle et al., 2009) and chromosomal irregularities (Fuzinatto et al., 2012).

The presence of dissimilarity is associated with the efficiency of chemical induction of mutations with the mutagen MMS associated with preselection applied on the progenies. Cold temperatures simulation was applied during preselection and it is characterized as a strategy to eliminate at an early time individuals with low tolerance to cold. At the same time, it allows to select progenies that have cold tolerance, which can provide benefits to the breeding program, selecting promising genotypes for this trait of interest. According to a study performed in Ona, state of Florida, forage selection for cold tolerance was promising as they found herdability of 0.39 to 0.50 for the trait cold tolerance in two crop cycles. These positive results enabled new selections in later generations (Jank et al., 2002).

The use of chemicals for mutation induction provides random nucleotide changes over the genome, which can generate individuals with agronomic interest (Allard, 1971). Among the mechanisms developed by plants throughout evolution to increase cold tolerance, it stands out the greater accumulation of amino acids and sugars (Alcázar et al., 2011), action of anti-freeze proteins (Wang et al., 2006), and greater fluidity of the plasma membrane exposed to stress by cold (Taiz and Zeiger, 2013).

According to the analysis of the 21 variables (Table 1), there was different inputs depending on the studied trait. Among all studied traits, FNI2 (31.60%), FSL (30.03%), LI (20.38%), FH1 (5.95%), FNI1 (3.99%), FH2 (3.44%), and NSBR (3.34%) were highlighted with greater contributions. The remaining 14 variables contribute individualy with values below 1%. The study of dissimilarity among genotypes is important to proceed with the screening of individuals with potential to move forward in a breeding program. In order to register a new cultivar, it must have distinctness from other cultivars by a minimum number of descriptors having homogeneity and stability (Brazil, 2011).

Regarding the analysis of genotypes acclimatization, the greater development (Figure 2) regarding the linear growth of check varieties and genotypes more acclimated to the study conditions was explained by the polynomial regression curve of second degree order, with emphasis

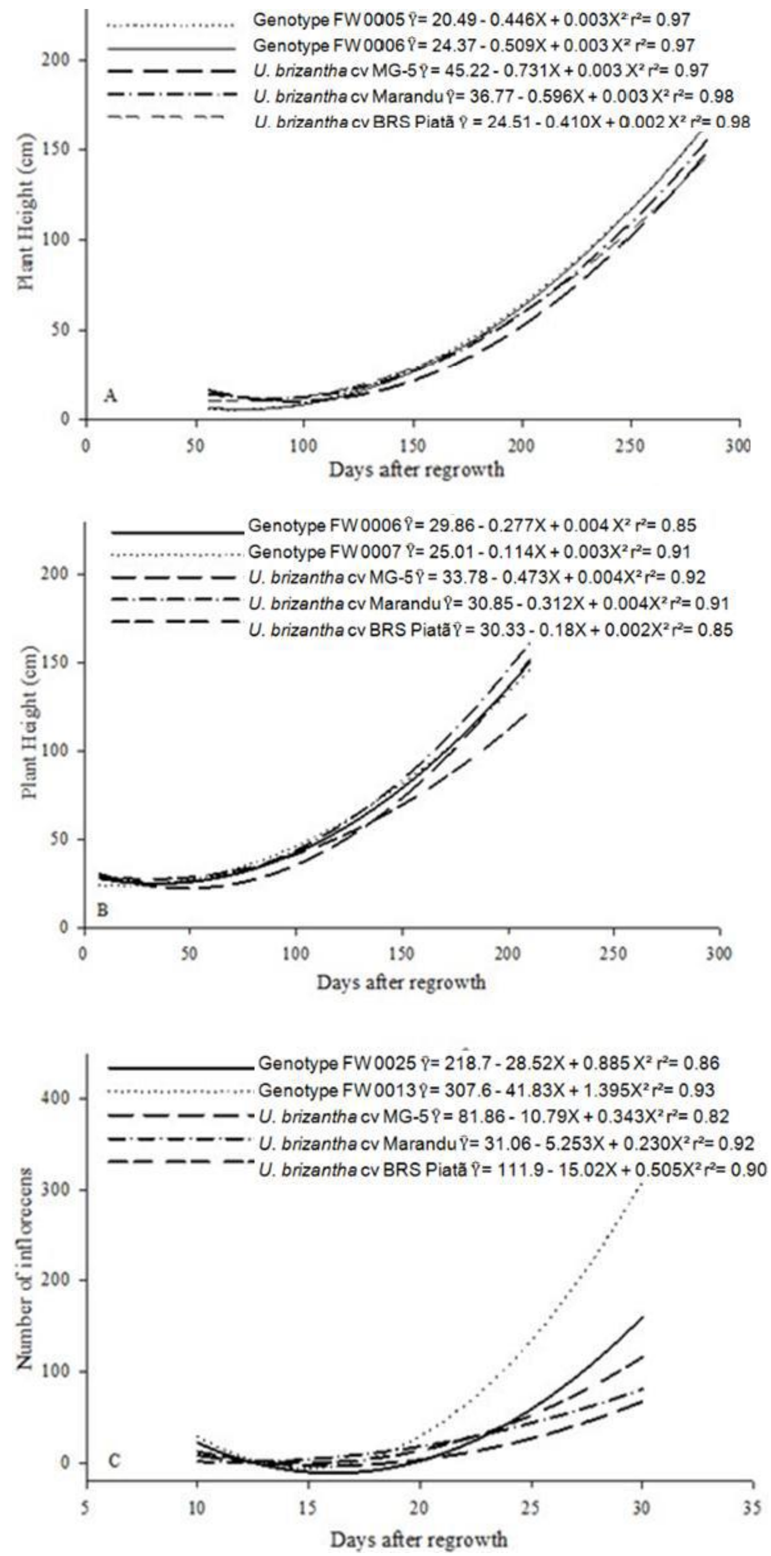

Figure 2. Linear growth of *U. brizantha* genotypes expressed in centimeters with measurements from sowing to full flowering (A), regrowth to full flowering (B), and emergence of inflorescences expressed in units per evaluation plot (C) referring to a population formed by mutants accesses in M2 generation and check varieties *U. brizantha* MG-5, *U. brizantha* BRS Piatã, *U. brizantha* Marandu in subtropical climate in two agricultural years in Frederico Westphalen, southern Brazil.

on access FW 0005 and FW 0006. The proper initial growth before the flowering is important as the initial flowering moment is characterized as the optimal resting point on forage species. At this time, there is a high assimilates translocation from the aerial part to the roots, keeping the reserves available for the forage regrowth. Thereby, grazing performed before or after the optimum point of rest may degrade the pasture (Lenzi, 2012). The experimental precision during the study was considered appropriate due to the crop development from sowing to flowering presented r^2 above 0.97, regrowth to flowering showed r^2 above 0.85, and appearance of inflorescences time exhibited r^2 above 0.82.

The beginning of flowering corresponded to 208 DAS, as the estimated value for growth rate of *U. brizantha* MG-5 was 22.96 cm, *U. brizantha* BRS Piata was 25.75 cm, and for *U. brizantha* Marandu was 42.59 cm. These values are low compared to the better mutant genotypes that expressed 48.29 cm for the FW 0006 genotype and 57.51 cm for the FW 0005 genotype. The estimated values expressed influence of increased cold tolerance in the initial period. Therefore, the main characteristics required for cold tolerance in subtropical regions are the use of more vigorous seedlings, tillering capacity, presence of axillary tillers, and fast regrowth after periods of low temperatures (Souza et al., 2013).

The growth in the second year of evaluation corresponds to regrowth (Figure 2). It is explained by polynomial regression curve of second degree order, where the beginning of flowering was used to estimate the regrowth height with estimated height values of 34.14 cm for *U. brizantha* MG-5, 37.23 cm for *U. brizantha* BRS Piata, 50.36 cm for *U. brizantha* Marandu, 53.54 cm for FW0006, and 53.92 cm for FW0007, that were the better accesses. The mutant genotypes presented greater values than *U. brizantha* Marandu, that was the best check variety for this trait. It is necessary reliable phenotypic expressions that can form the basis for evaluation under field conditions in order to have proper selection and discard of genotypes (Cruz, 2005). This statement can be attributed to the FW0006 genotype, which was expressed among the best individuals related to its growth in two years. This access has potential for further applications in a breeding program.

Regarding the emergence of inflorescences in the second year of evaluation (Figure 2), it showed development explained by the polynomial regression curve of second degree order, with trend line estimated in the full flowering of 66 inflorescences for *U. brizantha* MG-5, 115 inflorescences for *U. brizantha* BRS Piata, 80 inflorescences for *U. brizantha* Marandu, 308 inflorescences for access FW0013, and 159 inflorescences for access FW0025. The planning and selection decision is a vital moment for a breeding program. However, the topic of associating high matter production and seed production of the genotypes should present a balance because genotypes with low seed production may have compromise the economic viability of its commercial release. Moreover, evaluating and selecting the trait number of inflorescences in *Setaria sphacelata* was considered feasible due to the 17% increase in the number of inflorescences in relation to the original population (Jank et al., 2007).

In accordance with analysis of vegetative growth for the studied traits, genotypes with greater growth and regrowth were not in the group with greater seed production. Moreover, the increase in inflorescences mass is a main factor to increase seed production (Bean, 1972). Thus, the selection of individuals that meet market demands for quality forage production combined with proper levels of seed production should be performed during the process of advancing generations.

Conclusion

The chemical induction of mutations with the mutagen methyl methanesulfonate provided genetic variability in the population of *U. brizantha* with formation of 18 divergent groups during the research. The access FW0006 presented proper plant development, standing between the two best accesses during the two years of study. It demonstrates potential for further studies in the breeding program with aim of selecting genotypes with cold tolerance in subtropical regions.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physical and hydraulic properties of a Latosol influenced by land use and management changes

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Changes to soil use can modify the soil's physical and hydraulic properties, affecting its potential for productivity. This study aimed to characterize the physical and hydraulic properties of a clayey Dystroferric Red Latosol under the following land uses: conventional tillage (CT), direct drilling systems (DD), and native forest (NF). The study was conducted in Londrina (PR), Brazil, (23°23′ S, 50°11′ W and altitude of 585 m). Soil samples were collected at depths of 0 to 0.10, 0.10 to 0.20, 0.20 to 0.30, and 0.30 to 0.40 m. The following properties were evaluated: size distribution of solid particles, particle density, soil bulk density, total porosity, macroporosity, microporosity, water infiltration, and soil water retention curve. Conventional tillage and DD of this land modified soil physical and hydraulic properties from that under NF. The NF soil had greater organic matter content in its surface layer, a greater number of macropores, lower density, and less water retention capacity than soils from the CT and DD systems. At a precipitation rate of 70 mm h^{-1}, only the CT system exhibited surface run-off. This was due to rupturing of the porous system and a lower infiltration rate. In contrast, plant residues in the DD system protected the soil structure against damage caused by direct impact due to raindrops, allowing for total infiltration of simulated rainfall events. The NF soil is important in extracting and replenishing groundwater stores. However, it does not retain more water than the other systems in the surface layers.

Key words: Soil water retention, soil water infiltration, direct drilling system, Dystroferric Red Latosols, conventional tillage.

INTRODUCTION

The state of Paraná in Brazil underwent a period of great change in land use starting at the beginning of the twentieth century. This period saw the replacement of native forests by agricultural lands (Gubert Filho, 1998). The removal of plant cover and adopting unsuitable soil management practices with intensive cultivation

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considerable accelerated the erosion and compaction process, resulting in the degradation of agricultural soils because of a rapid decline in organic matter content (Castro Filho and Logan, 1991). The change in ground cover also triggered changes to water balance, causing reduced water infiltration rates and increasing surface runoff, in addition to increasing evaporation and reducing transpiration.

Rates of water infiltration into soil, along with the hydraulic conductivity of saturated soils, are two of the most important physical properties used to understand accelerated erosion. Water infiltration into the soil profile is considered a property that controls leaching, runoff and crop water availability (Franzluebbers, 2002). Portable rainfall simulators are useful to measure infiltration rates in the field (Roth et al., 1985; Santos et al., 2016). Thus, determining the infiltration rate plays a crucial role, given the direct relationship between erosion and ability of water to infiltrate and move into deeper layers of the soil profile (Roth et al., 1985).

The direct drilling (DD) system has often been recommended as a soil management technique for tropical and subtropical climates. This is due to the accumulation of surface residues and tilling only the sowing line, which supports higher carbon content in surface soil (Calegari et al., 2006). Furthermore, permanent plant cover at the soil surface provided by this agricultural system favors the physical conditions necessary for seed germination and initial crop growth (Vanlauwe et al., 2014). The use of green manure has the potential to improve soil chemistry, as well as its biological and physical properties (Zaccheo et al., 2016). In addition to plant cover, seeders equipped with drilling shafts in direct drilling systems provide a physical environment beneficial for establishing crops by increasing total porosity, macroporosity, and by reducing soil bulk density and mechanical resistance to penetration in the sowing line (Nunes et al., 2014). In direct drilling systems, straw mulches applied to the soil surface improves soil water status, reduces hydraulic gradients and soil temperature during the growing season by providing an insulation layer, especially in the period of crop establishment (Siczek et al., 2015). In contrast, where conventional tilling is part of soil preparation, there is a reduction in organic matter content and soil macroporosity compared with forest and DD soils (Dalmago et al., 2009).

Soil bulk density at the surface layer may increase in the years following DD implementation, which is due to soil compaction caused by the machinery used to conduct growing operations (Secco et al., 2005). As organic matter levels and biological activities increase, however, soil structure improves while soil bulk density gradually decreases (Assis and Lanças, 2005). The water available for crop cultivation largely determines the level of climate risk. Soils with high organic matter content provide better conditions for profile rooting and exploitation, resulting in greater yields in non-irrigated conditions. The volume of water stored in soil tends to be greater at higher (more positive) potentials when planted without tilling in forest soils and becomes lesser as the potential declines (Dalmago et al., 2009). The aim of this work was to characterize the physical and hydraulic properties of a Dystroferric Red Latosol after conventional tillage and direct drilling, and compare it to soil in native forest.

MATERIALS AND METHODS

This study was conducted at the Experimental Station of the Agronomic Institute of Paraná (IAPAR) in Londrina (PR), Brazil, (23°23′ S and 50°11′ W), where the altitude was 585 m. The region has a Cfa climate type, which according to Köppen classification, is described as humid and subtropical with hot summers. The average annual temperature is 21.1°C. The temperature during the hottest (January) and coldest (July) months are 23.9 and 16.9°C, respectively. The average annual precipitation is 1,610 mm. December, January, and February are the wettest months, while June, July, and August receive the least rainfall (Caviglione et al., 2000).

The soil at the study area is described as a clayey Dystroferric Red Latosol according to the Brazilian soil classification system (Santos et al., 2013). It is similar to a Ferralsol (Food and Agriculture Organization of the United Nations - FAO, 2006), and a very fine, ferruginous, isothermic rhodic happludox according to USDA Soil taxonomy (Soil Survey Staff, 1998). Additional details regarding the mineralogical and chemical characteristics were reported by Castro Filho and Logan (1991). In all three soil management types, particle size composition exhibited clay levels greater than 75 dag kg^{-1} throughout the soil profile (Table 1). Conventional tillage (CT) uses one operation with disc plough at a depth between 20 and 25 cm and two disking with the light disc harrow for leveling the ground and preparing the seedbed. This area was maintained for 10 years by cultivating winter cover crops black oats (*Avena strigosa* Schieb), oil seed radish (*Raphanus sativus* L. var. Oleiferus Metzg), or white lupins (*Lupinus albus* L.). In the summer, it was seeded with black mucuna (*Mucuna pruriens*) and *Crotalaria spectabilis*. During the flowering stage, cover crops were treated with herbicides while the soils were broken up and then smoothed using a harrow. The area was kept uncovered and weeded by hand to collect physical and hydraulic data. To determine soil-physical hydric properties, the area was cleaned with hand hoeing weeding.

The direct drilling system (DD) area was, for 8 years, maintained with soybean with an inter-row spacing of 0.5 m during the summer, and black oats (IAPAR Ibiporã 61 variety) with an inter-row spacing of 0.17 m during the winter. The seeder for direct drilling was equipped with narrow tires, which opened a narrow slot into the mulch-covered soil. This equipment was pulled using a medium tractor according to National Association of Motor Vehicle Manufacturers (ANFAVEA, 2011) classification, New Holland®, model TL 75, 4 × 2 TDA with 78 horse power). This tractor was equipped with 12.4 to 24 front tires (0.31 m) with a diagonal structure and 18.4 to 30 rear tires (width: 0.47 m). The native forest (NF) comprised two hectares in a secondary mixed hardwood with a highly diverse flora that was maintained as a legal reserve. Soil samples were collected using a Dutch auger. This was to determine the total organic carbon content in each of the three systems (DD, CT, and NF). Samples were taken from four different depths (0 to 0.10 m, 0.10 to 0.20 m, 0.20 to 0.30 m, and 0.30 to 0.40 m), with three replicates for each. The samples were carbon oxidized by wet

Table 1. Particle size distribution of Dystroferric Red Latosol at four depths under a conventional tillage system (CT), a direct drilling system (DD), and in a native forest (NF) in Londrina (PR), Brazil.

Land uses / Soil managements	Depth (m)											
	0 – 0.10			0.10 – 0.20			0.20 – 0.30			0.30 – 0.40		
	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand	Clay	Silt	Sand
	--- dag kg^{-1} ---			--- dag kg^{-1} ----			--- dag kg^{-1} -			--- dag kg^{-1} -		
CT	78	17	5	76	17	7	77	16	7	79	14	7
DD	79	15	6	82	11	7	81	12	7	81	12	7
NF	76	14	10	76	14	10	77	13	10	79	13	8

combustion with potassium dichromate (Walkley and Black, 1947). Data analysis was conducted according to the methods reported by Pavan et al. (1992). To conduct physical and hydraulic analyses, undisturbed soil samples were collected in between the rows after the summer harvest and/or following the management of undergrowth plants and before sowing winter species. For this purpose, trenches were dug in March 2011 and soil samples were collected horizontally and then were inserted in the soil, using Köpecky cylinders of 0.05 m height and internal diameter with sharp edges and an internal volume of 98.17 cm^3.

Soil particle density was determined using the volumetric flask method. Soil bulk density, total porosity, macroporosity, and microporosity were determined using methods described by EMBRAPA (1997). Soil samples were weighed and placed on a suction table measuring 0.32 m × 0.54 m × 0.10 m, which was protected by a screen and parchment paper for 48 h. The height of the water column was set to obtain a matrix potential of -6 kPa. The samples were then returned to the table and adjusted to obtain a matrix potential of -10 kPa and until the weight was constant. The samples were then weighed and placed on ceramic plates inside a Richard's pressure chamber set at potentials of -33, -100, -300, -500, and -1,500 kPa. This was for drainage and to obtain soil moisture retention curves. Soil water retention curves were obtained by adjusting the soil's volumetric water content (θ, in cm^3 cm^{-3}) and the soil matrix potential (ψm, - kPa). Soil water retention curve modeling was carried out using the computer program, Soil Water Retention Curve (SWRC), version 3.0 beta (Dourado Neto et al., 2001). A constraint (m = 1 – 1/n) (Mualem, 1976) was applied to the model we used, which was first proposed by Mualem-Genuchten (van Genuchten, 1980) (Equation 1):

$$\theta = \theta_s + \frac{\theta_s - \theta_r}{[1+(\alpha.\Psi_m)^n]^m} \quad (1)$$

where θ is the volumetric soil water content (cm^3 cm^{-3}), θ_{sat} is saturated soil water content (cm^3 cm^{-3}), θ_{res} is the residual soil water content (cm^3 cm^{-3}) at a matrix potential equal to 1,500 kPa (van Genuchten 1980; Pires et al., 2008); α, n, and m are adjustment parameters for the equation. The water available at a depth range of 0.0 to 0.04 m was calculated as the difference between the water retained in the soil samples at a matrix potential of -10 kPa and -1,500 kPa (Silva et al., 2006). Soil bulk density, total porosity, macroporosity, microporosity, and soil organic carbon content were all analyzed statistically. All experiments were conducted according to a randomized block design, with split-plots and four replicates. Land uses and soil management (of DD, CT and NF were considered the main factor, while sampling depths (0 to 0.10 m, 0.10 to 0.20 m, 0.20 to 0.30 m, and 0.30 to 0.40 m) were used as the split-plot. The results were analyzed using an analysis of variance (ANOVA), with an F-test at 95% probability. Means were compared using Tukey's test. All statistical analyses were performed using Sisvar software, version 5.1 (Ferreira, 2011).

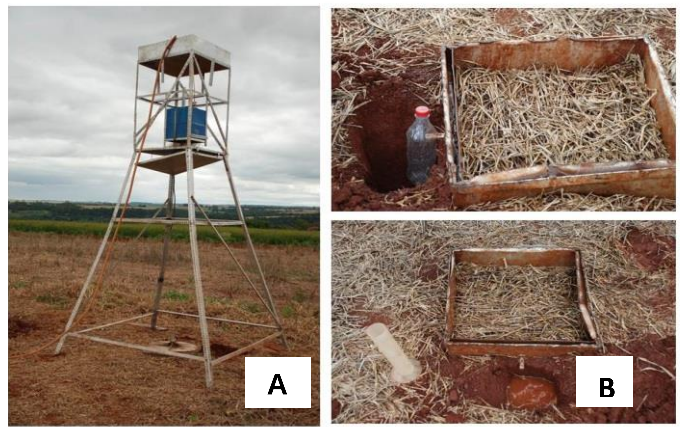

Figure 1. Portable rainfall simulator (A) and galvanized steel sheet collector (B).

Water infiltration and water loss in the three land use systems was evaluated in March 2011 using a portable rainfall simulator developed by Roth et al. (1985), which was installed at a height of 3 m. The simulator was calibrated for rainfall of 70 mm h^{-1} for 60 min. The infiltration index was calculated as the difference between rainfall intensity and surface runoff. The terrain slope at the three sites was approximately 2%.

A 0.12 m^2 sheet of galvanized steel was dug into the soil at a depth of 0.25 m to perform the procedure. The metal sheet was comprised of a gutter and a spout to collect surface runoff into a test tube at 2 min intervals. Three measurements were taken at different sampling points at each of the land use types (Figure 1).

RESULTS AND DISCUSSION

Soil samples from the CT and DD sites exhibited higher bulk densities and microporosity (Figure 2a and 2d), as well as lower total porosity and macroporosity, than samples from NF (Figure 2b and 2c). This was at the four depths tested. At a soil depth of 0 to 0.1 m, our results were similar to those reported by Pagliai et al. (2004) for a soil submitted to ripper equipment or minimum tillage compared to conventional tillage. They observed that soil macroporosity (pores with effective diameter greater than 50 μm) at a depth of 0 to 0.1 m in conventionally tilled soil was lower than soils under minimum tillage or ripper

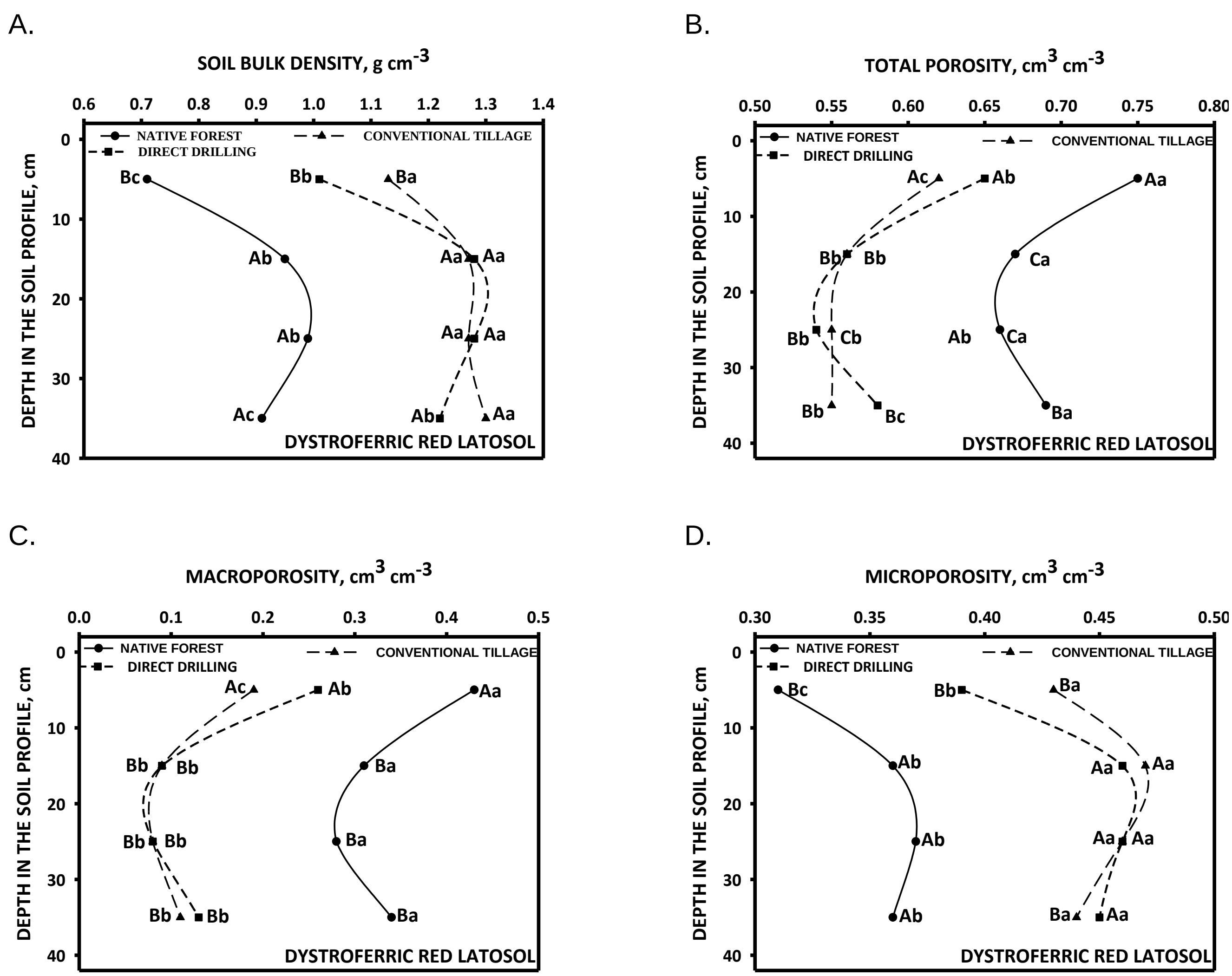

Figure 2. Physical properties of the clayey Dystroferric Red Latosol under different uses and management types at four depths: (0.0-0.10 m; 0.10-0.20 m; 0.20-0.30 m; 0.30-0.40 m). A: soil bulk density; B: total porosity; C: macroporosity and D: microporosity. Means followed by the same lower case letters (within the same soil depth compared among land uses) and upper case letters (within land uses compared among different soil depths) were not significantly different according to Tukey's test ($p > 0.05$).

sub soiling. Minimum disturbance of soil at this depth could improve its physical properties, which can be beneficial for root growth and the mechanical actions of the seeder.

As suggested earlier by Derpsch et al. (1991) for this tropical clayey Latosol, soil bulk densities greater than 1.25 g cm^{-3} could restrict root growth, aeration and water permeability. Other criteria, which could be adopted for classifying soil physical conditions, are based on pore size distribution (Pagliai et al., 2004). These authors have also shown that a more developed surface crust in conventionally tilled soils may cause a decrease in soil porosity. In addition, the soils in this system are prone to soil compaction and subsurface plough pan.

Based on the afore-mentioned criteria, soil layers ranging from 0.1 to 0.2 m and 0.2 to 0.3 m under CT and DD are compacted and are therefore considered not favorable to root growth and water distribution in the soil profile. Despite changes to soil physical properties being possibly related to soil structure damage, the higher microporosity in those soils could be the result of an increase to available water for plants (Sidiras and Vieira, 1984; Derpsch et al., 1991; Pagliai et al., 2004).

It is likely that the physical and hydraulic properties of Dystroferric Red Latosol following CT are more subject to changes imposed by land management compared to the DD system. This is consistent with observations reported by Sidiras and Vieira (1984). These authors reported that CT soil was more susceptible to variations in soil bulk density caused by tractor wheels during sowing. These results are related to lower structure stabilization and to the soil load-bearing capacity of soils subjected to conventional tillage (Dias Junior and Pierce, 1996). At depths of 0 to 0.10 m and 0.30 to 0.40 m, soil bulk density, total porosity, and macroporosity were not considered as constraints to plant growth and water infiltration into the soil. This was despite soil bulk density values in the surface layer increasing by 34% under DD conditions, when compared to NF soil, and by 42% under CT conditions (Figure 2). In clayey Dystroferric Red Latosol with reduced tillage and CT, Argenton et al. (2005) reported there were increased soil bulk densities between 71 and 86% for depths ranging from 0.05 to 0.10 m, and between 10 and 16% for depths ranging from 0.30 to 0.40 m. Machine use and unsuitable soil moisture conditions lead to permanent damage to the soil structure (Dias Junior and Pierce, 1996). This is the result of the pressure applied to the ground surface (Argenton et al., 2005) by tires or active parts of the equipment that exceed the soil's ability to withstand the weight (Silva et al., 2003). Such heavy weights are transmitted to various depths through stress distribution (Araujo-Junior et al., 2011). Soil bulk density increase of 27 and 37% were recorded after applying an equivalent amount of pressure (900 kPa) on samples of oxidic-gibbistic red yellow latosol and clayey kaolinitic yellow latosol (Silva et al., 2006).

In two clayey Oxisols (81 and 83 dag kg^{-1} clay) of the West region of the State of Paraná, Brazil, Assouline et al. (1997) reported that soil compaction differed when subjected to compressions ranging from 50 kPa to 1,000 kPa. Those researchers also showed that beyond the similarity in particle size distribution and soil bulk density of both soil types, there were several differences in their physicochemical properties, particle thickness and crystallinity, all of which affect soil stability. In addition to changes in soil bulk density, Silva et al. (2006) reported there was a reduction in the average diameter of stable aggregates in water. This was at a total porosity volume of 17 and 23%, reflecting reductions in macroporosity by 53 and 67%, and increases in microporosity by 35 and 23%, respectively, for yellow red latosol and yellow latosol in samples subjected to 900 kPa. In the present study, the reduction in total porosity at depths ranging from 0.0 to 0.10 m, when compared to NF soil (0.75 cm^3 cm^{-3}), was 14% for soil under the DD system (0.65 cm^3 cm^{-3}) and 18% (0.62 cm^3 cm^{-3}) under the CT system (Figure 2). At a depth range of 0.0 to 0.10 m, the sowing of winter undergrowth plants and commercial plants in the summer in CT and DD systems may have positively changed their physical properties based on the seeder's active parts (circular blade, sowing drill, fertilizer dispensers, seed sowing drill, and tamping wheel). In addition, there was an accumulation of plant residues and land preparation through plowing and using a harrow.

Sowing both summer and winter crops may contribute to favorable structural conditions at the surface of DD soils (Nunes et al., 2014). Another aspect worth mentioning is that the pressure exerted by these parts of the seeder may change the pressure applied to the sowing layer, as noted by (Reis et al., 2006), for compacting components. Furthermore, Reis et al. (2006) noted that the sowing shaft reduced the soil bulk density from 1.14 kg dm^{-3} to 1.00 kg dm^{-3} at a maximum depth of 8.0 cm. The low soil bulk densities observed in the NF (Figure 2a) may be due to the thick layer of undergrowth that has been deposited over the years, making the NF surface soil levels highly organic and porous (Assis and Lanças, 2005; Centurion et al., 2007). Greater total porosity values (macro and microporosity) at a depth range of 0.0 to 0.15 m in CT soils compared with DD in Dystroferric Red Latosol were observed by Silva and Rosolem (2001). The effect of the undergrowth on the physical properties of the soil is cumulative and requires years of management before a significant difference become apparent (Laurani et al., 2004). In the present study, the DD system was used for eight consecutive years and already showed changes in the soil's surface levels. In the CT system, with management incorporating winter cover crops, such as black oats cultivar Iapar 61 used as green manure, improvements and differences in the soil's properties were not very pronounced in comparison to those in the DD system. Forest soil contained higher total organic carbon content than that found in agricultural soils at depths of 0.0 to 0.10 m and 0.10 to 0.20 m (Figure 3). This may be attributed to greater organic residue provided by the tree canopy, in addition to the interception of incident radiation, which minimizes oxidation of the soil's organic matter by directly radiating the soil surface. Conventional tillage promoted a mean decrease of 43% in soil organic carbon content at soil surfaces of 0 to 0.10 m (Figure 3). Due to the short duration under DD (8 years), no differences were observed in the organic carbon content in the soil compared to CT.

At a depth range of 0.20 to 0.30 m, the total organic carbon content observed in the soil under the CT system was similar to that observed in the NF and greater than that of DD soil. This may have been the result of soil layer inversion caused by soil preparation in the CT system, which distributes carbon to greater depths, increasing organometallic bonds and reducing microbial decomposition. The CT system introduced more carbon than the DD system in the layers evaluated as a result of crop rotation that incorporates undergrowth plants. Similar results, with respect to the total organic carbon content of the soil, were reported by Argenton et al. (2005), who confirmed that the association of cover crops with corn, stabilized the carbon content at certain depths regardless of the management system (DD or CT). It was expected that over the years, the DD system will gradually increase the carbon content in the soil profile because of the cumulative effect of adding plant residues to the surface layers, thereby improving the soil's physical properties (Calegari et al., 2006). The results of the adjustment parameters using the Mualem-Genuchten

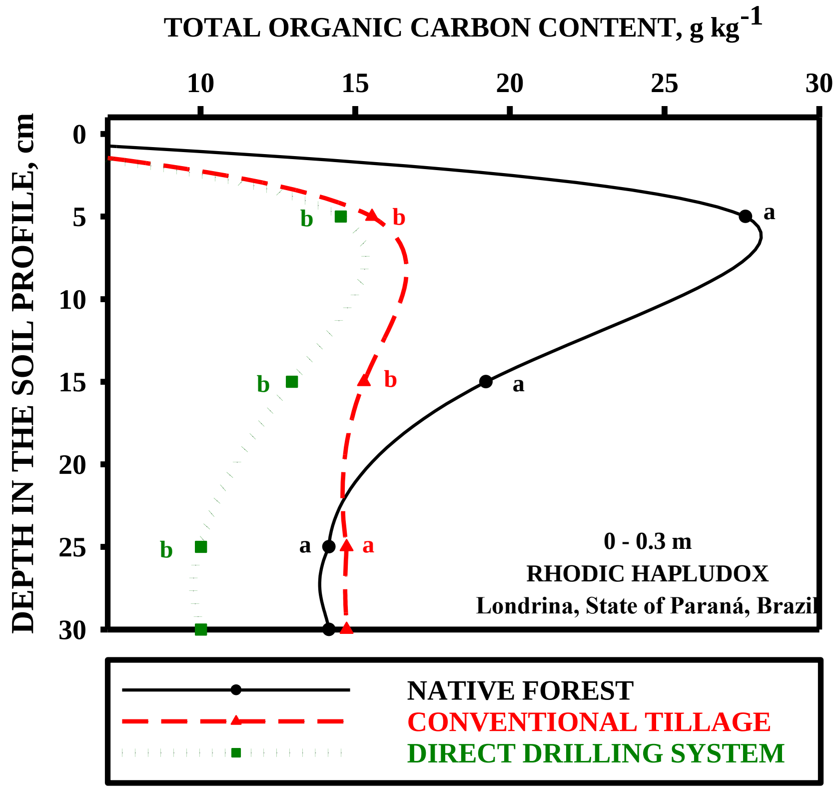

Figure 3. Total organic carbon content of a clayey Dystroferric Red Latosol under the following land uses and management types: conventional tillage system (CT) and direct drilling system (DD). These were compared to native forest (NF) soil. Samples were taken at three depths. Means followed by the same lower letter within the same depth were not significantly different according to Tukey's test ($p > 0.05$).

model for the water retention curves for the LVdf in native forest and different soil management systems are presented in Table 2. The model was adjusted to the data, with a coefficient of determination (R^2) between 0.87 and 0.99, all of which were significant at 1% ($P < 0.01$) probability according to the F-test.

Soil water retention curves from the three environments at four depths are displayed in Figure 4. At a depth range of 0.0 to 0.10 m, it was observed that CT soil provided greater water retention values among the potentials, ranging from -0.32 kPa to -1,500 kPa. Soil porosity was modified by CT soil preparation (reducing total porosity and macroporosity, while increasing microporosity) (Figure 2). With regards to NF soil (□v–10 kPa = 0.2973 $cm^3\ cm^{-3}$), the Dystroferric Red Latosol's water retention rate, at a matrix potential equal to -10 kPa (field capacity) under the CT system (□v–10 kPa = 0.4026 $cm^3\ cm^{-3}$) increased by 30%. The DD system (□v–10 kPa = 0.3761 $cm^3\ cm^{-3}$), however, increased by 20% in relation to water retention at -10 kPa when compared with NF soil. On the other hand, the CT system increased the water retention rate by 6% relative to the DD system (Figure 4). This result was also observed by Sidiras and Vieira (1984), who noted that a modification of the porous space in the traffic line of an 85 HP tractor showed a positive effect on the ability of the soil to retain water. A similar effect was observed when growing wheat, soybeans, and turnips, relative to the soil between the wheel tracks, because pore volumes were reduced if their diameters were greater than 10 µm.

In the 0.10 to 0.20 m layer, NF soil retained less water at all matrix potentials, when compared to soil from CT and DD systems (Figure 3). Due to the increased macropore volume and low soil bulk density (Figure 2), these physical and hydraulic properties do not contribute to water storage. In the 0.30 to 0.40 m layer, there was greater water retention at less negative potentials than in the other environments. Nevertheless, the total volume of water retained in the NF was less at all depth ranges, which bolsters the forest's role in maintaining soil quality, providing water absorption, preventing run-off, replenishing the groundwater table, and recycling water via transpiration. Furthermore, the possibility that forest soil at the higher depths could hold more water should not be discarded. However, these measurements were not evaluated in the present study. The volume of

Table 2. Parameters of the model Mualem-Genuchten after adjusting water retention curves by the clayey Dystroferric Red Latosol under different land uses and at different depths.

Parameters	0.0 - 0.10	0.10 - 0.20	0.20 - 0.30	0.30 - 0.40
	Native Forest Soil, NF depth (m)			
θ_{sat} (cm^3 cm^{-3})	0.67	0.63	0.59	0.65
θ_{res} (cm^3 cm^{-3})	0.19	0.26	0.28	0.25
α (kPa^{-1})	9.89	3.32	1.73	3.07
N	1.33	1.41	1.47	1.43
M	0.25	0.29	0.32	0.30
R^2	0.98	0.99	0.98	0.99
F Value	1,126.45	3,858.83	1,032.65	2,795.00
Significance (%)	< 0.001	< 0.001	< 0.001	< 0.001
	Conventional Tillage Soil, CT depth (m)			
θ_{sat} (cm^3 cm^{-3})	0.56	0.55	0.53	0.54
θ_{res} (cm^3 cm^{-3})	0.28	0.36	0.35	0.35
α (kPa^{-1})	1.19	0.41	0.45	0.48
N	1.31	1.46	1.40	1.62
M	0.24	0.31	0.29	0.38
R^2	0.92	0.87	0.88	0.94
F Value	214.03	156.03	169.42	355.80
Significance (%)	< 0.001	< 0.001	< 0.001	< 0.001
	Direct Drilling Soil, DD depth (m)			
θ_{sat} (cm^3 cm^{-3})	0.62	0.52	0.52	0.54
θ_{res} (cm^3 cm^{-3})	0.26	0.33	0.34	0.34
α (kPa^{-1})	3.52	0.30	0.38	0.63
N	1.32	1.36	1.34	1.39
M	0.24	0.26	0.25	0.28
R^2	0.97	0.97	0.97	0.97
F Value	686.85	559.36	642.81	840.61
Significance (%)	< 0.001	< 0.001	< 0.001	< 0.001

θ_{sat} = volumetric soil water content at saturation (cm^3 cm^{-3}), θ_{res} = volumetric residual soil water content (cm^3 cm^{-3}) at a matrix potential of 1,500 kPa (van Genuchten, 1980, Pires et al., 2008); α, n, and m are adjustment parameters for the equation with its limitations. The R^2 corresponds to the coefficient of determination. The F value was calculated for the equation.

available water varied between 9 and 12% at all depths, which was similar to values (9 and 10%) reported by Faria and Caramori (1986) for the same soil under CT conditions. The volume of water stored in the soil profile at a maximum depth of 0.40 m was greater under DD conditions (42 mm) and lower in the forest (38 mm), whereas soil under CT provided 39.71 mm of storage.

Soil-water infiltration

Surface runoff in the NF and DD environments did not occur following 60 min of precipitation at intensity of 70 mm h^{-1}. In contrast, runoff from the CT environment was observed. This was attributed to the distribution of solid particles in the soil, which were quantified by increased soil bulk density values and changes to the porous system (a reduction in the total porous volume and macroporosity) (Figure 2). Following the three rain simulation events, the CT environment experienced a loss of water totaling 24.1 mm, 11.5 mm, and 24.0 mm, respectively. The ground cover in the CT environment was between 2 and 3%, whereas that of the DD environment was approximately 80 to 90%. In an area composed of the same Dystroferric Red Latosol under the DD system, and without plant coverage, water infiltration was constant for up to 10 min after simulating rain at an intensity of 68 mm h^{-1} (Roth et al., 1985). It was noted that after 60 min of rain, water infiltration decreased to 5 mm h^{-1}. Nevertheless, it is clear that plant cover in the DD system is efficient at providing better conditions for water infiltration into soils, thus, preventing

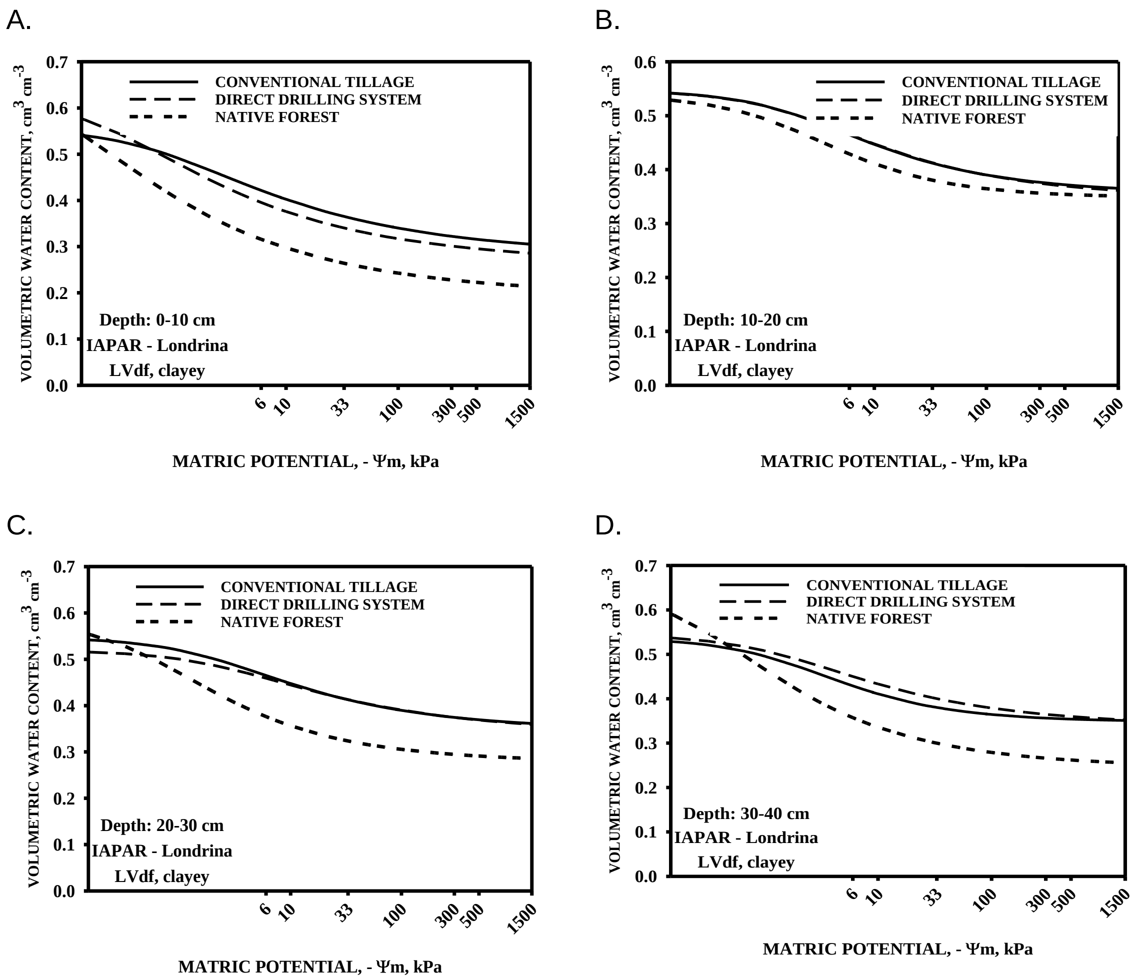

Figure 4. Water retention curves for the Dystroferric Red Latosol in a conventional tillage system (CT), a direct drilling system (DD), and a native forest (NF) in Londrina (PR), Brazil, at depths of 0.0–0.10 m (A), 0.10–0.20 m (B), 0.20–0.30 m (C), and 0.30–0.40 m (D).

erosion and contributing to increased water storage and the replenishment of groundwater. The presence of organic matter in the surface NF soil profile reduced soil bulk density and increased macroporosity, thus, reducing its ability to retain water at a maximum depth of 0.40 m (Franzlubbers, 2002; Araujo-Junior et al., 2011). On the one hand, those researchers suggested that the particle density of organic matter was considerably lower than that of mineral soil. On the other hand, in both NF soil and DD soil, total water infiltration occurred at an intensity of 70 mm h^{-1}, whereas that of the CT demonstrated surface runoff.

Insufficient surface undergrowth of the CT system's soil leads to the formation of surface level crusting. This is a surface layer of variable thickness created by the disaggregation of soil by water droplets, resulting in reduced water infiltration (Sidiras and Vieira, 1984, Araujo-Junior et al., 2011, 2015). In a test that simulated rain at an intensity of 85 mm h-1 on the same Dystroferric Red Latosol used in this study, Araujo-Junior et al. (2015) showed that water infiltration can be reduced to 7 mm h^{-1} in areas with insufficient plant cover. This result reinforces the fundamental role that plant cover plays in maintaining the physical and hydraulic properties of surface level tropical soils, as well as in the replenishment of springs and in precipitation recycling, also enabling the total infiltration of precipitated water and the exit of water through transpiration.

Conclusion

1. Soil use in CT and DD systems lead to changes in the physical and hydraulic attributes of the soil, thus, increasing soil bulk density and microporosity, reducing total porosity volume and macroporosity beneficial for water movement by the clayey Dystroferric Red Latosol in relation to native forest soil.

2. The CT system maintained total organic carbon content at 0.20 to 0.30 m depth similar to that observed in NF and greater than that of DD.
3. Permanent soil cover in the DD system, proportioned by plant residues, protected the soil structure against damage caused by direct impact of raindrops, maintained soil bulk density lower than critical limits, total porosity, macroporosity and enhanced water infiltration compared to conventional tillage soil.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Economic evaluation of milk production in family farm, with cattle feed with soybean meal

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Accounting can play an important role as a management tool, through information to enable planning, control and decision making, turning farms into companies with the capacity to monitor the evolution of the sector, especially with regard to the objectives and functions of financial management, cost control, crop diversification and comparison of results. Soybean meal, half-whole can be an excellent alternative to livestock dairy and beef cattle and pig farming. The study aims to evaluate the production of milk in function of soybean meal feed from cold press extruder. The study was conducted in São Miguel do Iguaçu, in a rural property. We evaluated eight animals, four of which received commercial feed and four soybean meal from extruder press. The cost of production and the simple payback was calculated and discounted. The payback was found as of year one.

Key words: Agriculture family, economic viability, dairy, soybean meal.

INTRODUCTION

Family farming has been strongly developed in the Brazilian context, due to current economy and in order, these producers can be sustainable and improve environmental conditions, agroecological alternatives are searched, such as: use of digesters, solar and photovoltaic energy, the development of biofuels and materials are re-used (Carvalho and Marin, 2016). With this, family farms can be defined as one in which the family, at the same time owns the means of production and takes the job in the establishment productive. The historical analysis of the Brazilian agricultural economy shows that dairy farming was the most penalized by public policies. The effects caused by price-fixing, are still alive in the memory of the producer. The agroenergy deals with the manufacture and use of the various types of biofuels, which are derived from activities in rural areas, such as agriculture, livestock and forestry (Wanderley, 1999).

Thus, the owner of the property is also the administrator; it is important to know the available resources on their property and adopt appropriate technologies that enable the producer to reduce costs,

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Figure 1. Image of the cold extruder press which is extracting the oil and as a by-product produces the soybean meal. Source: www.bindgalvão.com.br.

ensure its sustainability and the permanence in the activity (Matos, 2002). According to Vilckas (2004), the development and implementation of planning in the rural sector is a major challenge, given that the enterprises in this sector are subject to many variables, such as the dependence on natural resources, the seasonality of the market, the perfectibility of products, the life cycle of plants and animals and the maturing time of the products. The country manager has the need for knowledge of natural resources, capital market knowledge validity of the products and their applications, to know biology and meteorology. The success of rural enterprise is not only high productivity through modern techniques, but also the control of production costs.

With regard to a sustainable alternative for family farming, there is the use of materials such as pie oilseeds which is a byproduct of oil manufacture and could be discarded and thereby can be used for other functions such as shows, Costa et al (2005), which used the sunflower pie to feed pigs in the growing-finishing phases, they found that the meal provided the same performance levels that the feed used. Already, Santos et al (2005) used the filter pie for the production of seedlings of vegetables and Furlan (2001) replaced the sunflower by soybean meal in feed for broiler chickens.

And for the feeding of ruminants, poultry and swine a study to replace the commercial food by soybean meal is being studied. This soybean meal has a good quality with high digestibility (Britzman, 2001), when in suitable moisture states according to Islabão (1986). The soybean composition is 38% crude protein, 82% total digestible nutrient and 20% oil, and due to these characteristics, its use is very viable for feed milk production and it can also be a rich source of energy in diets fattening bullocks (Harris Junior, 1990; Feijó, 1996).

The soybean meal is a protein source that currently has been widely used for animal feed; soybean meal represents about 79% of the soybean. During the oil extraction this process occurs, heating of soybean meal, which helps to increase their nutritional qualities (Thiago, Silva, 2003). In order to assist the development of sustainable practices and provide energy re-use in family farming, this work aims to evaluate the production of milk in feed function with soybean meal from extruder press cold.

MATERIALS AND METHODS

The experiment was conducted in the municipality of São Miguel do Iguaçu located at latitude 25° 20 '53 "and longitude 54° 14' 16" with an average altitude of 312 m and a population of 25,769 (IBGE, 2010), in a family farm which has the size of 11.1 ha or 4,586 acres. In this property, there is a creation of 30 dairy cows, which is the main activity besides cereal crops such as corn, and soybean where corn is used to feed cattle and soybean as part of crop rotation. To obtain the soybean meal it was used an extruder press of Bindigalvão mark (Figure 1), which extracts the extra virgin oil and therefore provides as residue the toasted meal ready for use in animal feed. The cost for the use of the machine in operation is R$ 1,50 per hour worked.

For the experiment it was used eight cows of the property. Four received the bran obtained from the extruder and four continued to receive the feed normally used in the property for a period of fifteen days. The composition of the commercial feed is shown in Table 1, while the soybean meal was produced from 60 kg from which 8 liters of oil were extracted and 52 kg of soybean meal. To facilitate understanding, the extruder feed will be called soybean meal produced by the extruder press and commercial feed. The economic feasibility analysis of the study was performed from the values of the project's cash flow, using economic indicators such as net present value, which takes into account the effect of time on the monetary values, using the average rate of attractiveness of0.4583% per month, or 5.64% per year, which is the long term

Table 1. Description of commercial feed produced by the Cooperative LAR.

Commercial feed (produced by LAR Cooperative).
Corn with up to 6% of flamed, up to 1% impurity and humidity up to 12%.
Soybean meal 46% (Hypro)
Wheat with PH 80 and up to 1% of impurity
Tortuga Mineral Premix
Calcium phosphate
salt
Limestone

Table 2. Description of values for the deployment of an extruder press, expressed in Reais (R$).

Description	Amounts (R$)
Machine Value	R$ 79.500,00
10x10m Shed value	R$ 70.000,00

interest rate - TJLP set by the Central Bank of Brazil (FEDERAL REVENUE BRAZIL, 2011) and payback, which shows the time to raise the capital invested. For the project, it was used the average of the balances of cash flows of nine years, discounting the last year as it has the return of the residual value of goods and machines.

RESULTS AND DISCUSSION

Table 2 shows the values for the deployment of the extruder press, whereby the press and a 10x10 m shed for the accommodation of the machine is necessary, in this case the property value is also considered. For biodiesel production physical labor will be required, that is, a person per day. For the cost of feed production in the press 5.5 kw/bag is spent with power X R$ 0.18 = R$ 0.99. Machine energy cost in operation approximately of R$ 1.50 per hour worked. In Figure 2, it can be seen, the final result of milk production with soybean meal and the commercial feed. The production was between 309 to 371 liters with the average of 338.5 L in fifteen days, with two daily milk collections and when compared to the other meal it is observed that feeding with the soybean meal increased milk production. The animals fed with the diet type 1 produced 1354 L while the diet type 2 animals produced 959 L in fifteen days. This result disagrees with Garcia et al. (2006) that found that, introducing the sunflower meal in ruminant feed was not influential in consumption and weight gain.

The average production of industrial feed was 239.75 liters, varying in 358 to 177 liters of production. Restle et al. (2004) tested the exchange of sorghum grain by soybean hull, where there was an increase in weight gain and reduced feed conversion that can be explained by the improvement in the rumen, thereby making better use of the fiber. About the waste of biofuels industry Almeida (2005) noted that in dairy cows using babassu bran oil residue, it increased milk production and improved economic feasibility, when 20% of soybean meal was added. To Paulino et al. (2006b) supplementation with soybean provided mass gains of 0.140 Kg/day compared to supplementation with mineral mix, promoting bigger production per area, less time occupation of pastures and working capital, according to the authors, whole grains can also be used without impairing the productive performance and may even minimize processing costs.

Since Torres et al. (2003) tested the addition of sugarcane bagasse to dairy cattle feed, bagasse increases dry matter intake, the accuracy level should be used in accordance with the performance and economics, but weight gain is decreased. The animals fed with diet type 2 had production of less than 29.17% that the animals fed with diet type 1, as shown in Figure 2. Halachmi et al. (2004) replaced corn silage by soybean hulls and it was observed, an increase in milk production in 38.5 Kg/day with soybean hulls and 36.3 Kg/day and corn silage, it was also observed an increase in total production of fat for the diet. Thus, Paulino et al. (2002) found no difference in weight gain in animals when compared two soy supplements (one with whole grains and the other with soybean meal) in the finishing phase, but found an increase in carcass yield which was 52.96%. Silva Neto and Basso (2005) stated that the consolidation of dairy farming for family production is fundamental to present a regular source of income and especially by the extent in terms of market. Thus, the competition at low levels means that milk production is still an option for a large number of producers (Wilkinson, 1997).

In simple payback for commercial feed the return is then obtained in the first year in 1 year and 2 months, the investment can be considered satisfactory as per the return. The project evaluation involves a set of techniques that seek to establish viability parameters, usually these parameters are described by the payback (Bruni, Famá and Smith, 1998). As for the extruder feed payback is over 9 months and 24 days. As shown in Figure 2, the initial investment is R$ 149,500.00 and can yield profits of up to R$ 700,000.00 for the use of soybean meal, giving a difference of R$ 295,000.00 gain when compared to the commercial feed gains. Non-exact methods (Casarotto and Kopittke, 2010) or criteria in current terms (Buarque, 1989) do not consider the effects of time on the money value. The main methods in this category are: simple payback, simple profitability and cost-benefit ratio in current terms (not updated) as shown in Figure 3. With the discounted payback indicator, there is the payback times using commercial feed throughout the year 2 and the extruder feed over 1 year, as can be seen in Figure 4. To Britzman et al. (2001), the discounted payback period is the number of years

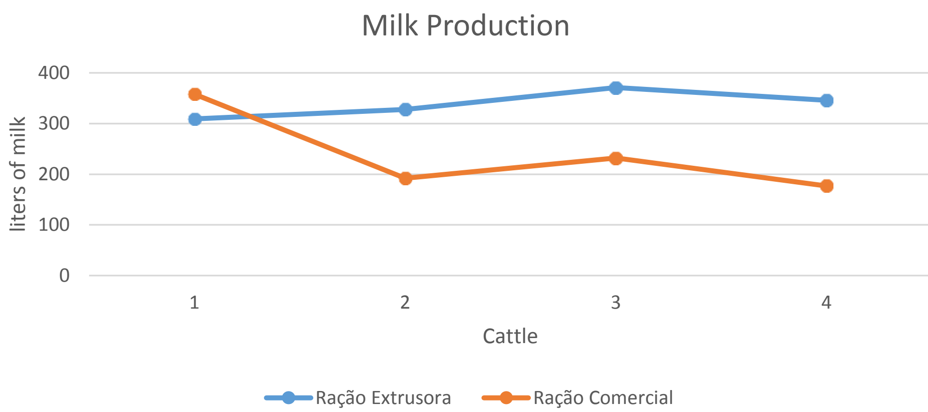

Figure 2. Total milk production using conventional soybean meal and soybean meal obtained from cold press extruder.

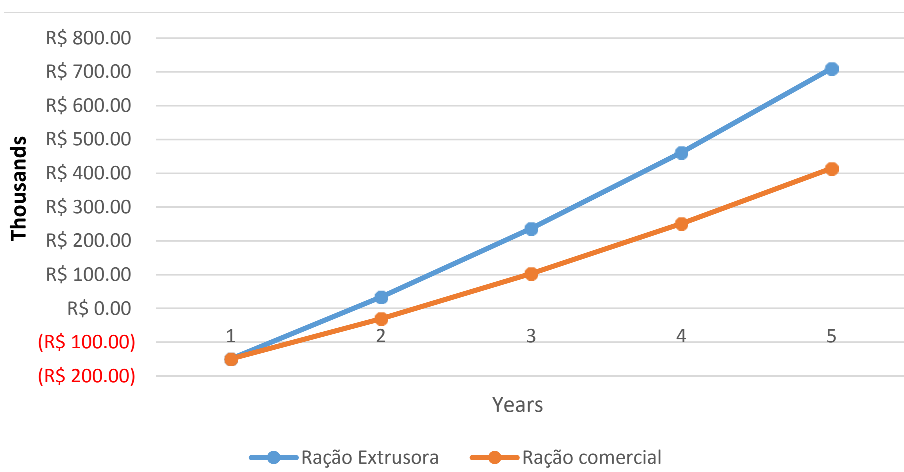

Figure 3. Graph showing the relation of the simple payback of the two types of feed. Amounts in reais.

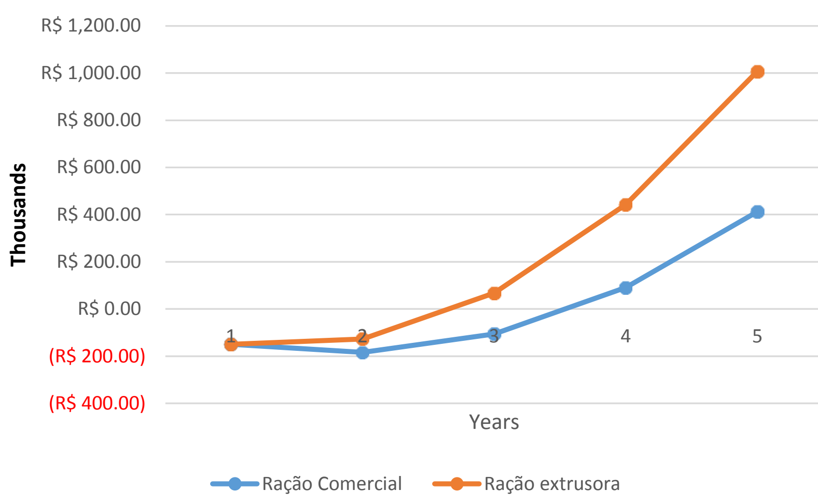

Figure 4. Payback discounted the commercial and extruder feed. Values represented in Reais (R$).

required to recover the investment of the net flow of discounted cash.

In the case of payback discounted, there are the current investment values targeting the data on the net present value, and then, there are the recoveries of investments using soybean meal produced by the extruder press, where the return will be in the first year since the great increased productivity. According to Assaf Neto et al. (2005) it is in the payback recovery period of investment and identification that the capital made is recovered by means of cash flows. Brigham and Houston (1999) state that the lower the cost of the project the best for the investor. According to Braga (1989) the lower the period of investment recovery the lower the risk.

Conclusions

1. The return through the simple payback comes through 9 months and 24 days for the extruder feed.
2. The initial investment of the producer for the implementation of oil extraction is high, but the discounted payback period is from year one.
3. Animals fed the soybean meal diet produced 1354 liters while animals fed with commercial feed produced 959 liters in fifteen days of the experiment.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional status of soil and plant and nutrient discrimination factor of some irrigated olive orchards at the North West of Egypt

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A study was conducted during 2014 to 2015 seasons on olive orchards; cultivars (Koroneki, Dolce and Manzanillo) grown on sandy soil under drip irrigation and irrigated with different levels of water salinity from 0.95 to 4.3dS/m^{-1}, in various locations in Cairo-Alexandria road, to evaluate the nutritional status through soil testing and leaf analysis. The soils had very high pH and low organic matter, potassium, iron and manganese, different levels of EC, $CaCO_3$, P, Na concentrations ranged between low and high, and different levels of Ca, Mg, Zn and Cu ranged between low and medium. Significant negative correlation was found between pH and Zn in soil. In olive leaves, the levels of N, P, Na, Fe and Cu concentrations ranged between low and high, K and Ca concentrations were between low and sufficient; Mg, Mn and Zn concentrations were sufficient. A highly significant positive correlation between leaf Mg concentrations and yield was found. Olive trees showed that minerals of needle ranked from greatest to least as K > Ca > Mg indicating that olive trees have more ability (preferability) to take up K in higher quantities than Ca and Mg. Olive varieties differ also in ability (preferability) to take up micronutrients.

Key words: Olive orchards, soil testing, plant analysis, nutrient status.

INTRODUCTION

Egypt has a great potential for olive production. Olive sector occupies high importance and is very promising. There is a need to increase the quantity and value of olive crop to improve the agricultural income. Olive trees do much better through controlled balanced nutrient supply. Most of olive plantations in Egypt are grown in sandy soils which fertility is low. Best nutrients management measures are required to grow olive trees successfully on such soils.

Olive trees are concentrated in three areas in Egypt:

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North Sinai, the Alexandria Cairo road and Siwa. The cultivated varieties are pruned every year in order to stimulate fruit production. Plants need enough nutrients to be able to grow into strong and healthy trees. Fertilizers are crucial in olive orchards as this will help in the production of healthy trees.

Soil and plant analysis can be used to evaluate the nutritional status of the olive trees and nutrient availability in the soil to supply the trees with nutrients requirement (Benitez et al., 2002; Chatzistathis and Therios, 2009; Chatzistathis et al., 2010; Sarrwy et al., 2010 ; Toplu et al., 2010). Researchers studied the nutritional status of olive trees and the efficiency of fertilizers management system, such as El-Gazzar et al. (1974), Tekin et al. (1994), Kamal (2000), Xiloyannis et al. (2000), El-Hassanin et al. (2001), Soyergin et al. (2002), Shaaban and El-Fouly (2005), Fayed (2010), Rodrigues et al. (2012) and El-Fouly et al. (2014). Also, some researchers studied the affect of olive cultivar on leaf nutrient, such as Jordao et al. (1999) and Saidana et al. (2009).

Most of olive orchards in this area are fertilized based on expérience of other areas in Egypt or abroad. The aim of this study is to use soil testing and leaf analysis on some representative farms with different fertilization mesures to examine the nutritional status and yield of the orchards.

This information can lead to examine the necessity of working out fertilization recommendation based on the analysis, expected yield (removal) and prevailing practices in the orchard.

MATERIALS AND METHODS

This study was carried out at two successfully seasons (2014 to 2015) in farms located at km 48 to 65 of Cairo-Alexandria road in the north west of Egypt, as representatives for this olive trees growing area. Area is located at semi-arid climate with an annual precipitation < 50 mm. Five farms were chosen representing the area and varieties planted, Moderate pruning has been done and drip irrigation is used.

Soil and water sampling

Representative soil samples were collected from each of the studied orchards at depth of 30 to 60 cm from the zone of the root tips of the trees under the end of canopy in November of each year. Collected soil samples were air dried, ground to pass through a 2 mm screen places in plastic bottles and kept for physical and chemical analysis. Water samples were collected in 1 L bottles from each farm and kept for chemical analysis. Total of 30 soil and water samples were taken.

Leaf sampling

Leaf samples were collected around the tree from the fully mature leaves of spring at 4 to 7 month–old young shoots. Samples were washed with tap water, 0.001 N HCL and distilled water, respectively, dried at 70°C for 24 h and ground in a stainless steel mill, then passed through a 40 mesh nylon sieve and stored in plastic bottles. Total of 100 leaf samples were taken.

Chemical analysis

Particle size distribution was carried out using hydrometer method, pH and EC were measured in the 1:2.5 water extract, and total calcium carbonate was determined using calcimeter method (Page,1982), and soil organic matter by potassium dichromate (Walkley and Black, 1934). Phosphorus was extracted using sodium bicarbonate (Olsen et al., 1954). Potassium, calcium, magnesium and sodium were extracted using ammonium acetate (Jackson, 1973). Iron, manganese, zinc and copper were extracted using DPTA (Lindsay and Norvell, 1978).

Plant material was digested using an acid mixture consisting of nitric, perchloric and sulfuric acids in the ratio of 8:1:1 (v/v), respectively (Chapman and Pratt, 1978). Nitrogen was determined in the dry plant material using the boric acid modification described by Ma and Zuazage (1942) and distillation was done using a Buechi 320- N2-distillation unit. Phosphorus was photometrically determined using the molybdate vanadate method according to Jackson (1973). Potassium, Ca and Na were determined using flame photometer (Genway). Magnesium, Fe, Mn, Zn and Cu were determined using the Atomic absorption spectrophotometer (Perkin Elemer 1100 B). The soil data were evaluated using the criteria published by Ankerman and Large (1974) and Silvertooth, (2001), whereas the leaf analysis data were evaluated according to the criteria in Plant Analysis Handbook (Jones et al., 1991).

Data were subjected to statistical analysis using Costate Statistical package, in order to calculate means, standard deviations (SD) and the possible correlations (r^2) (Anonymous, 1989).

Discrimination factor (DF)

Comparative element ratios for plant samples and for soils were calculated as index of specific metal uptake and thus relative metal requirements by the plant. The equation DF= (concentration of total nutrient a/ concentration of total nutrient b in leaves) / (concentration of extractable metal a in soil/metal b concentration in soil). DF value>1 means plant preference for metal a and DF value<1 means plant preference for metal b (Aulio and Ojala, 1983; Baligar et al., 1979).

RESULTS AND DISCUSSION

Field practices

Table 1 shows the most important agricultural operations applied in the olive groves. The data of these shows variations in cultivars cultivated, age, the number of trees/ha., chemical fertilizer and organic and in productivity.

Irrigation water showed salinity ranged from 608 to 2758 mgL^{-1}. It is known that olive trees withstand salinity up to 5000 ppm, as well as the acidity ranged between neutral and slightly above neutral Table 2.

Soil properties and its nutrients status

Values of soil characteristics and available nutrient

Table 1. Information on general conditions and field management practices.

Farm	cultivar	Tree age(year)	Distance (M)	Organicfertilizer (kg/ tree)	No.trees/ha	Fertilization (g/tree/year)			Micro-nutrients	Yieldkg/tree
						N	P	K		
El-Salam	Koroneki, Dolce	8	3.5x6	50	476	450	150	400	Applied as fertigation every week	35-45
El-FIFA	Koroneki, Manzanillo	10	6x6	40	276	1034	776	690	Three foliar sprays/season	35
LiLi	Manzanillo	15	4x4	15	600	359	111	343	Three foliar sprays/season	40
IFA	Dolce	10-12	6X7	10	238	165	320	384	Boron foliar spray/season	40-50
Heli	Koroneki	20	6x5	-	333	428	114	140	One foliar spray/season	20-30

Table 2. Chemical analyses of irrigation water.

Parameter	El-Salam	El-FIFA	LiLi	IFA	Heli
	Value				
EC dS/m (ppm)	3.56 (2278)	1.49 (954)	1.38 (883)	0.95 (608)	4.31 (2758)
pH	7.61	7.31	7.49	7.55	7.80

concentrations were evaluated according to Ankerman and Large (1974) and Silvertooth (2001) (Table 3).

Soil properties

Data in Table 4 shows that soil texture is sandy, it's known that coarse-textured soils are suffering from nutrient deficiency. The values of the EC, are ranged from 0.15 to 3.53 dS/m which is classified between very low and high saline soil while, $CaCO_3$ values are ranged from 2.33 to 9.17. According to Anter et al. (1973) the soil is considered as calcareous soil when the $CaCO_3$ are 8% or over. The soils were found to differ in their $CaCO_3$ content. Soils are low in organic matter (O.M) content. Soil organic matter is used as an indicator of soil fertility. As soil organic matter had a positive relationship with total content of N in soil, thus it is expected to be low in N.

Nutrient concentrations in the soil

Values in Table 3 were used to evaluate the concentrations of different nutrients in the soil. Data in Table 5 show that soil content of P ranged between very low and middle. On the other hand, the range of K was between very low and low levels, in spite of the fact that all farms use potassium fertilizers. The soil content of Ca and Mg ranged between low and medium and Na ranged between low and very high. Regarding micronutrients, soil test showed that Fe and Mn ranged between very low to low whereas, Zn and Cu are very low to medium. From Tables 4 and 5 it is clear that all farm soils are sandy and have low organic matter which reflected on different decline of macro and micro nutrient contents in soils.

Table 6 shows the sufficient values of macro and micronutrients for olive leaves (dry weight basis). Data in Table 7 shows that levels of both N and P, ranged between low and high, and K, Ca and Mg levels were between low and medium. Micronutrients ranged between low and high for Fe, while tests showed average level for each of the Mn and Zn. The level of Cu ranged between low and high. In this regard, some researchers pointed out that indeed fertilizer is useful when the leaf nutrient drops below the sufficient level (Hartmann, 1958; Fernadez-Escobar, 1999).

Table 7 shows that nutrient concentrations of

Table 3. Tentative rating values of soil fertility status.

Soil test	Very low	Low	Medium	High	Very high
CaCO3%	< 0.5	0.5-2	2.1-8	8.1-30	31-45
Organic matter (O.M),%	< 1.0	1-2	2.1-3	3.1-5	>5.0
Electric conductivity (E.C.) dS/m	< 0.1	0.1-0.2	0.3-0.4	0.5-0.7	> 0.7
pH	< 5.8	5.9-6.6	6.7-7.2	7.3-8.5	> 8.5
Macronutrients (mg/100 g)					
Phosphorus (P)	< 0.5	0.5-1.1	1.2-2.7	2.8-4	> 4
Potassium (K)	< 11.7	11.8-20	21-30	31-47	> 47
Calcium (Ca)	10-40	41-90	91-140	141-500	> 500
Magnesium (Mg)	< 11	11-29	30-180	> 180	-
Sodium (Na)	< 20	20-25	26-30	> 30	-
Micronutrients (ppm)					
Iron (Fe)	< 5	5-10	11-16	17-25	> 25
Manganese (Mn)	< 5	5-8	9-12	13-30	> 30
Zinc (Zn)	< 0.5	0.5-1.5	1.6-3	3.1-6	> 6
Copper (Cu)	< 0.3	0.3-0.8	0.9-1.2	1.3-2.5	> 2.5

Table 4. Physico-chemical characteristics of the investigated soil.

Character	El-Salam		El-FIFA		LiLi	IFA	Heli
	Koroneiki	Dolce	Koroneiki	Manzanillo	Manzanillo	Dolce	Koroneiki
	Mean+S.D						
Sand%	85±1	89.5±1	86±2	78±6	90.5±0.8	90.8±2	88.1±1.15
Silt%	4±1	3.3±1	4±2	9±2	3.3±1.0	2.0±2	2.7±1.15
Clay%	11±1	7.2±0	10±1	13±3	6.2±1.7	7.2±0	9.2±0.00
Texture	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy
pH(1:2.5)	8.40±0.06H	8.05±0.1H	8.28±0.06H	8.39±0.44H	8.45±0.20H	8.76±0.1H	7.72±0.10H
E.C dS/m^{-1}	0.15±0.03L	3.53±0.74H	1.36±0.17H	1.29±0.89VH	0.08±0.04VL	0.34± 0.03M	0.35±0.03M
$CaCO_3$ %	2.33±0.12M	2.40±0.4M	9.17±0.80H	8.80±3.67H	7.2±3.2M	1.47±0.23L	1.80±0.20L
O.M %	1.37±0.31L	1.12±0.09L	0.17±0.02VL	0.83±0.34VL	0.24±0.15VL	0.15±0.02VL	1.70±0.12L

Mean value ± standard deviation (SD); VL= very low; L= low; M= medium; H= high.

leaves are better than that of the soils, probably due to fertilization programs used in these farms. However, still most of the nutrients without the appropriate level in some cultivars in some farms, for example:

Low potassium in Koroneiki and Dolce cultivars in El-Salam farm as well as in Dolce in IFA farm and in Koroneiki in Heli farm, also low calcium in Koroneiki in El-FIFA farm and in Dolce in IFA farm. Although, Mg was in an adequate level, but it's at the lowest level of range as in El-FIFA farm and in the LiLi farm.

For micronutrients, Dolce cultivar is suffering from low iron in El-Salam and IFA farms as well as low zinc and Manzanillo cultivar suffering of copper in LiLi farm. Table 7 shows also that each cultivar within each farm has a different nutrient concentrations, in spite of every farm received equal rates of fertilization and type. These results agrees with the studies of Jordao et al. (1999) and Saidana et al. (2009). It could be concluded from these results that all farms suffer from nutrient imbalances, which differ according to cultivar.

High significant positive correlation was found between the content of silt and Ca which may be high to cation exchange capacity (CEC) of silt and a positive significant correlation was found between the content of silt and clay which their particles are overlapping with each. Also a positive high significant correlation was found between the EC and Na where NaCl has direct effect on the values of electric soil conductivity. A positive significant correlation was found between the EC and Mn, and between the content of Na and Mn. However, a positive high significant correlation was found between the content of Zn and Cu.

Table 5. Available macro- and micro-nutrients concentrations of the investigated soils.

Element	El-Salam		El-FIFA		IFA	LiLi	Heli
	Koroneiki	Dolce	Koroneiki	Manzanillo	Dolce	Manzanillo	Koroneiki
				mg/100g			
P	1.60±0.24 M	2.30±0.33 M	0.49±0.05 VL	2.52±0.27M	0.91±0.13 L	0.53±0.05 L	0.83±0.05 L
K	3.6±0.17VL	11.1±0.73 VL	19.3±0.58 L	13.7±5.24L	12.1±0.60 L	12.8±7.4 L	13±1 L
Ca	94±0.5 M	71.2±32 L	84.5±5 L	337.2±64.4H	195±13 H	64±24 L	60±3.5 L
Mg	30±1 M	31±1 M	13.8±1 L	33±3.5M	23±2 L	33.5±17 M	33.2±2.1 M
Na	13±1Vl	190±10 VH	145±1 VH	29.55±15.37M	16±1 VL	9±3 VL	20±1 L
				mg/1000g			
Fe	10.6±0.32 L	3.6±0.4 VL	9.7±0.2 L	2.55±0.75VL	2.3±0.3 VL	1.7±0.75 VL	10.4±0.17 L
Mn	2.47±0.15Vl	5.3±1.2 L	1.9±0.1 VL	1.27±0.15VL	0.5±0.1 VL	1.5±0.86 VL	1.5±0.10 VL
Zn	0.82±0.04 L	0.80±0.03 L	1.5±0.1 L	1.27±0.25L	0.11±0.01 VL	0.39±0.15 VL	2.17±0.25 M
Cu	0.18±0.02 Vl	0.17±0.0.02 Vl	1.03±0.06 M	0.40±0.26L	0.12±0.0.01 VL	0.23±0.19 VL	1.2±0.10 M

Mean value ± standard deviation (SD); VL= very low; L= low; M= medium; H= high; VH= very high.

Table 6. Sufficient values of macro and micronutrients for olive leaves (dry weight basis).

Nutrient	Values	Nutrient	Values
	%	ppm	
N	1.5-2.0	Fe	90-124
P	0.10-0.30	Mn	>20
K	0.80-1.30	Zn	>10
Ca	1.0-1.43	Cu	4-9
Mg	0.10-0.30	B	19-150
Na	< 0.20	-	-

Source: Hartmann et al. (1966), Bouat, (1968) and Recalde and Chaves (1975).

Table 7. Nutrient concentrations of leaf samples.

Cultivar element	El-Salam		El-FIFA		IFA	LiLi	Heli
	Koroneiki	Dolce	Koroneiki	Manzanillo	Dolce	Manzanillo	Koroneiki
				%			
N	2.9±0.18 H	1.63±0.14 S	1.66±0.24 S	1.56±0.16 S	1.31±0.11 L	1.31±0.23 L	2.10±0.06 S
P	0.36±0.11 H	0.11±0.01S	0.26±0.02 S	0.09±0.01L	0.12±0.01S	0.08±0.02 L	0.24±0.01 S
K	0.58±0.02 L	0.53±0.10 L	1.01±0.44 S	1.22±0.38 S	0.50±0.06 L	1.35±0.13 S	0.59±0.01 L
Ca	1.61±0.31S	1.32±0.12 S	0.49±0.03 L	2.00±0.28 S	0.39±0.02 L	1.54±0.15 S	1.92±0.13 S
Mg	0.35±0.03 S	0.44±0.02 S	0.30±0.02 S	0.14±0.03 S	0.24±0.02 S	0.18±0.02 S	0.21±0.03 S
Na	0.63±0.04 H	0.18±0.04 S	0.24±0.05 H	0.03±0.01 L	0.10±0.06 L	0.05±0.01L	0.83±0.10 H
				ppm			
Fe	144±11 H	78±6 L	108±18 S	144±32 H	40±8 L	175±15 H	146±11 H
Mn	37±1 S	28±5 S	45±2 S	34±4 S	26±2 S	26.8±4.2 S	48.3±0.76 S
Zn	39±1 S	16±3 S	25±1 S	28±1 S	20±2 S	45.5±6.6 S	36±3.12 S
Cu	17±1 H	11±3 H	9.5±0.5 S	23±7 H	4.3±2.6 S	3.4±1L	13.8±2.25 H

Mean value ± standard deviation (SD), VL= very low, L= low, S= sufficient, H= high.

Table 8. coefficient between soil characteristics as well as nutrient concentrations of the soil.

Nutrient	Silt%	Clay %	pH	E.C dS/m	$CaCO_3$ %	O.M %	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Sand%	-.944**	-.95**	0.001	-.042	-0.525	-0.20	-0.58	-0.03	-0.724	-0.09	0.108	-0.112	0.153	-0.385	-0.119
Silt%	-	0.794*	0.078	0.147	0.637	0.052	0.678	0.095	0.887**	0.085	-0.057	-0.175	-0.154	0.188	-0.059
Clay%	-	-	-0.077	-0.062	0.365	0.325	0.431	-0.04	0.496	0.088	-0.146	0.372	-0.136	0.531	0.275
pH	-	-	-	-0.314	0.173	-0.74	-0.14	-0.09	0.249	-0.07	-0.329	-0.537	-0.328	-0.758*	-0.629
E.C dS/m	-	-	-	-	0.028	0.101	0.569	0.196	-0.060	-0.01	0.893**	-0.204	0.854*	0.071	-0.099
$CaCO_3$ %	-	-	-	-	-	-0.51	0.071	0.581	0.674	-0.65	0.153	-0.127	-0.397	0.040	0.147
O.M %	-	-	-	-	-	-	0.423	-0.53	-0.157	0.547	-0.063	0.525	0.356	0.560	0.232
P	-	-	-	-	-	-	-	-0.36	0.540	0.448	0.227	-0.265	0.493	-0.018	-0.421
K	-	-	-	-	-	-	-	-	0.091	-0.52	0.367	-0.125	-0.233	0.329	0.575
Ca	-	-	-	-	-	-	-	-	-	0.085	-0.279	-0.458	-0.406	-0.112	-0.253
Mg	-	-	-	-	-	-	-	-	-	-	-0.407	-0.273	0.157	0.015	-0.283
Na	-	-	-	-	-	-	-	-	-	-	-	0.053	0.768*	0.129	0.114
Fe	-	-	-	-	-	-	-	-	-	-	-	-	0.051	0.668	0.648
Mn	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-0.205
Zn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.897**

r* 0.05 = 0.754 (significant at 5% level) r** 0.01 = 0. 874 (significant at 1% level).

Under drip irrigation, increasing EC values with no efficient system for washing salts from soil, where salts are concentrated at the edges of humidity area, some of the elements to be maintained on the particles of clay and silt and thus may affect them available for absorption by trees which affects the growth of leaves and thus on productivity.

On the other hand, highly significant negative correlation was found between sand and both of silt and clay, when it increases the percentage of sand particles thus less percentage of clay and silt particles. On the contrary, significant negative correlation was found between pH and Zn where Zn tends to be less available when soil pH is above 7.5. (Table 8).

Correlation between leaf nutrient concentrations and yield of the olive trees

Data of Table 9 showed that there is a significant positive correlation between leaf contents of N and both of P and Na and between P and Na, while fertilization with P promoted growth and increase N uptake, and this may also led to increase in Na uptake. Also, highly significant positive correlation was recorded between Mg, and yield increase the percentage of sand in the soils of these farms, meaning that these soils have exchange capacity low and thus led to lower content of magnesium available for absorption.

Micronutrient ratios (Discrimination factor) for olive

Discrimination factor of micronutrient pairs for olive leaf were calculated for each of the five locations and listed in Table 10. It could be noticed that Df values for K/Ca, K/Mg and Ca/Mg were > 1. Accordingly, olive leaves taken from trees grown on sandy soils showed Df values in a pattern of K > Ca > Mg indicating that olive trees have more ability (preferability) to take up K in higher quantities than Ca and Mg under sandy soil conditions, except in Dolce variety in IFA Farm where Mg was more preferred than Ca which may be due to low Mg in soil and lower Mg ratio to Ca in soil.

On the other hand, olive varieties differ in ability (preferability) to take up micronutrients where Koroneiki have more ability (preferability) to take up Mn in higher quantities than the other micronutrients and Manzanillo have more ability (preferability) to take up Fe in higher quantities than the other micronutrients while Dolce have more ability (preferability) to take up Zn in higher quantities than the other micronutrients under the conditions of each farm.

Conclusion

The results show that soils of orchards are poor sandy soils with non-adequate levels of P, K and micronutrients. The different fertilization regimes used resulted in imbalances in nutrients contents in trees. In order to optimize fertilizer use, soil testing and leaf analysis should be used as a base for calculating fertilizer needs, and each farm should be treated individually. There is also preference in the different cultivars in the uptake of different nutrients. This also should be taken into consideration when working out fertilizers recommendations.

Table 9. Correlation coefficient between leaf nutrient concentrations and yield of the olive trees.

Nutrient	P%	K%	Ca%	Mg%	Na%	Fe ppm	Mn ppm	Zn ppm	Cu ppm	Yield/ha
N%	0.878*	-0.421	0.368	0.359	0.797*	0.314	0.488	0.350	0.509	0.282
P%	-	-0.395	-0.014	0.354	0.778*	0.166	0.678	0.261	0.271	0.225
K%	-	-	0.230	-0.617	-0.527	0.620	-0.077	0.443	0.035	-0.325
Ca%	-	-	-	-0.291	0.340	0.745	0.177	0.525	0.645	-0.180
Mg%	-	-	-	-	0.183	-0.437	-0.070	-0.450	-0.089	0.908**
Na%	-	-	-	-	-	0.265	0.722	0.341	0.276	0.027
Fe ppm	-	-	-	-	-	-	0.324	0.876**	0.330	-0.174
Mn ppm	-	-	-	-	-	-	-	0.194	0.389	-0.257
Zn ppm	-	-	-	-	-	-	-	-	0.009	-0.167
Cu ppm	-	-	-	-	-	-	-	-	-	-0.220

r* 0.05 = 0.754 (significant at 5% level) r** 0.01 = 0. 874 (significant at 1% level).

Table 10. Discrimination factor for micronutrients in olive leaves.

Farm cultivar	El-Salam		El-FIFA		IFA	LiLi	Heli
	Koroneiki	Dolce	Koroneiki	Manzanillo	Dolce	Manzanillo	Koroneiki
K/Ca	9.47	2.56	9.04	14.88	2.06	4.40	1.43
K/Mg	13.81	3.37	2.41	21.00	3.95	19.63	7.17
Ca/Mg	1.47	1.30	0.27	1.40	0.19	4.48	5.05
Fe/Mn	0.91	4.10	0.47	2.12	0.33	5.78	0.44
Fe/Zn	0.29	1.08	0.67	2.56	0.10	0.88	0.85
Fe/Cu	0.14	0.33	1.21	0.98	0.49	6.96	1.22
Mn/Zn	0.32	0.26	1.42	1.21	0.29	0.15	1.94
Mn/Cu	0.16	0.08	2.58	0.47	1.45	1.21	2.80
Zn/Cu	0.50	0.31	1.80	0.38	5.05	7.87	1.44

Conflict of Interests

The authors have not declared any conflict of interests.

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Yield and vigor of corn seeds under the influence of flooding periods

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The aim of this work was to characterize the physiological performance and some of the yield attributes of maize seeds in response to periods of temporary flooding. The study was conducted in an experimental design of randomized blocks, with four treatments composed by four replications, being evaluated the germination, the first count of germination, the germination speed index, the thousand seed weight, the number of seeds per ear, the number of rows per ear, the electrical conductivity in seeds, the length of shoot and primary root and the dry matter of shoot and primary root. Thousand seed weight, number of seeds per ear, number of rows per ear and electrical conductivity in seeds were reduced when plants were exposed to a 72 h flooding period. The flooding period of 72 h adversely affects the growth, the physical characteristics and the vigor of maize seeds.

Key words: *Zea mays* L., number of seeds, thousand seed weight, electrical conductivity, initial growth.

INTRODUCTION

Corn belongs to the family Poaceae. It is cultivated in tropical, subtropical and temperate climates. The area cultivated with this species in Brazil in the first 2014/15 the second harvest was 9,587.6 ha. The yield in the main regions with corn cultivation in southern Brazil, under favorable soil and weather conditions, is 12-13 t ha^{-1} (Conab, 2015). This species responds to the interaction of climatic elements. Solar radiation, precipitation and temperature are factors that exert the greatest influence on the culture because they affect the physiology of plants (Brachtvogel et al., 2009). In general, soil flooding reduces the diffusion of ethylene and increases its endogenous concentration in plant tissues (Yin et al., 2009), leading to a reduction in leaf area and leaf area

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ratio (Gazolla Neto et al., 2012). It also increases the concentration of starch in leaves (Irfan et al., 2010) and reduces photosynthetic rates due to retro-inhibition (Araya et al., 2006), and forming short chain aliphatic organic acids in the soil (Schmidt et al., 2010). The metabolic pathway used is anaerobic fermentation, has a lower energy production (Bailey Serres and Voesenek, 2008).

Seed quality relates to environmental conditions under which the plant and the seed develop, comprising characteristics that determine the value for sowing (Marcos Filho, 2005). For its determination, the germination test is used. This test is conducted under suitable environment conditions in order to reproduce conditions closer to those found in the field, and therefore, tests such as electrical conductivity and plant growth, concerning mass and length, are developed (Peske et al., 2012). The change in the metabolism and photosynthetic rates alters plant growth and development. It may reduce seed quality and cause a reduction in yield attributes. There may be different responses according to species and genotypes (Pryor et al., 2006). By studying the effect of soil flooding on soybean plants, Ludwig (2010) observed a reduction in the physiological performance of seeds of plants exposed to flooding. Thomas and Costa (2010) reported that flooding during the vegetative stage has a negative effect on plant growth and consequently on the potential yield of seeds.

For *Brachiaria* spp. plants, Caetano and Dias-Filho (2008) noted a decrease in relative growth rate in three accessions from six accessions studied. Except for one accession, the authors noted that flooding reduced net photosynthesis, stomatal conductance and transpiration of accessions. Dias-Filho (2005), studying *Brachiaria brizantha* cv. Marandu, *B. decumbens* and *B. humidicola* on flooded soils, observed a high negative correlation between starch accumulation in leaves and photosynthetic capacity of these species. In this context, the aim of this study was to characterize the physiological performance and some attributes of corn seed yield in response to temporary flooding periods.

MATERIALS AND METHODS

The experiment was conducted at a didactic and experimental field located at the *Campus* Capão do Leão of the Federal University of Pelotas. Its geographical location is 31º52' S and 52º21' W. The values for physiological performance were determined at the Laboratório Didático de Análise de Sementes do Departamento de Fitotecnia, Programa de Pós-Graduação em Ciência e Tecnologia de Sementes (Didactic Laboratory of Seed Analysis of the Department of Plant Science, Graduate Program in Science and Seed Technology). To obtain the seeds used in determining response to flooding periods, a cultivation of a corn genotype (*Zea mayz* L.) was performed from propagation material with 95% germination. This material was collected from the municipality of Ametista do Sul, Rio Grande do Sul state, at geographical coordinates 27º21' S and 53º10' W.

The plants were grown in a Hydromorphic Planosol with a sandy loam texture (Embrapa, 1999). The treatments consisted of periods of flooding in the vegetative stage between V8 and V9, after 75 days of sowing, as follows: zero (soil kept at field capacity); 24; 48 and 72 h of flooding. The spacing between lines and plants was 45 and 36 centimeters, respectively, resulting in a population density of 55,000 plants ha^{-1} (Borghi and Crusciol, 2007). The soil correction was performed in accordance with a previous analysis and the recommendation by the (Cqfs, 2004). The irrigation was made when there were drought periods. To establish the soil flooding, "marachas" (rows) were opened in the vicinity of each plot, characterizing a beam system. Soil flooding periods were imposed from overflow, with a water depth of 20 mm above the soil, within beams. After each period, the opening of "marachas" (rows) of beams was performed, and the withdrawal of the water blade and soil drainage was performed.

After the end of the development cycle, at 150 days after sowing, seeds were harvested with moisture of 35%, and the ears were dried in an oven at 30°C until they reached a moisture of 14%. Subsequently, seeds were threshed by hand. To evaluate the effects of flooding periods on physiological performance and yield of corn seeds, the following tests were performed:

a) Electric conductivity test: conducted with four replications, with 25 seeds, using a predetermined paste. Seeds were placed in polyethylene containers for soaking in 75 ml of deionized water and then placed in a BOD germinator at 25°C. After 6, 12 and 24 h of soaking, the readings were performed on a DM-32 conductivity meter. The results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.
b) Mass of thousand seeds: determined from eight subsamples with 100 seeds. The results were expressed in grams (g) according to the Rules for Seed Analysis (Brasil, 2009).
c) Number of rows of seed per ear: determined by direct counting using four subsamples with ten ears per treatment. The results were expressed as average number of rows of seed per ear.
d) Number of seeds per ear: direct counting of the number of seeds per ear. For this, ten subsamples with 10 ears per treatment were used. The results were expressed as average number of seeds per ear.
e) Germination: The test was conducted with four subsamples with 50 seeds per treatment, and the seeds were disposed between three sheets of germination paper, moistened with water at a ratio of 2.5 times the mass of the dry paper. Paper rolls were formed and then transferred to a BOD growth chamber at 25°C. The evaluation was performed seven days after sowing and the results were expressed as percentage of normal seedlings (Brasil, 2009).
f) First counting of germination: performed jointly to the germination test, four days after sowing, according to Rules for Seed Analysis. The results were expressed as percentage of normal seedlings (Brasil, 2009).
g) Germination speed index: obtained from daily counts of germinated seeds with a minimal root protrusion of 3 to 4 mm. Counts were performed to obtain the constant number of germinated seeds and the germination speed index was established according to the recommendation by (Nakagawa, 1994).
h) Shoot and primary root length of seedlings: four subsamples with 10 seedlings were selected at the end of the germination test. The shoot length was obtained by measuring the distance between the insertion of the basal portion of the primary root, and the primary root length was measured by the distance between its apical and basal parts. The results are expressed in millimeters per seedling (mm $seedling^{-1}$).
i) Shoot and primary root dry mass: obtained from four subsamples with 10 seedlings at the end of the germination test. For this, the seedlings were packaged in brown paper envelopes and dried in a forced ventilation oven at 70°C until constant weight. The results are expressed in milligrams per seedling (mg $seedling^{-1}$).

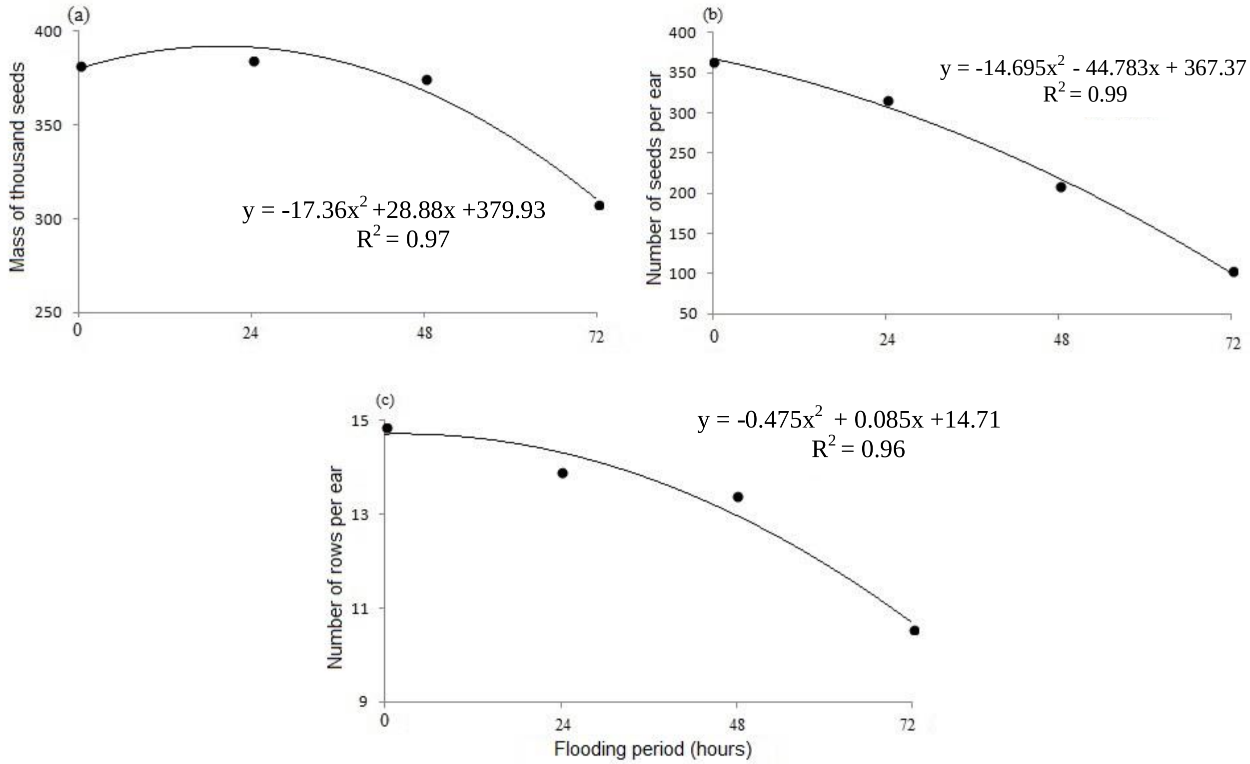

Figure 1. Mass thousand seeds (a), number of seeds per ear (b) and number of rows per ear (c) in corn (*Z. mays* L.) grown under condition of periods of temporary flooding of 0; 24; 48 and 72 h.

The experimental design was randomized blocks with four treatments. Data were submitted to analysis of variance. When significant at 5% probability, the results were expressed by orthogonal polynomials.

RESULTS AND DISCUSSION

There was no significant difference at 5% probability for germination, first counting, germination speed index, root length and root dry matter when evaluating the physiological performance of corn seeds subjected to temporary flooding periods (data not shown). However, the response to imposed stress depends on the inherent characteristics of each species. Gehling et al. (2015) observed that rye seeds produced under the influence of flooding, reduced germination by reducing the number of normal seedlings and decrease the vigor due to a lower number of germinated seeds per day. There was a reduction in the mass of thousand seeds upon increasing the flooding period (Figure 1a). The mass of thousand seeds reached the maximum response point at 20 h, leading to a reduction of 74% in yield for seeds produced in 72 h of flooding compared to those produced at field capacity. This physical attribute of seeds is related to the amount of assimilates allocated during their development, and seeds that develop in a more favorable environment, tend to reach a higher mass of thousand seeds (Peske et al., 2012).

It should be noted that seed weight depends on the size and duration of the functioning of the photosynthetic apparatus and photosynthate translocation efficiency of the seed filling period and soil and climatic conditions during the development of the mother plant and seeds (Vieira Junior and Dourado Neto, 2008). In this sense, soil flooding alters the hormonal balance of the plant so that, in water overflow situations, plants reduce the diffusion of ethylene out of the cells into the root system by increasing the endogenous concentration, resulting in senescence and leaf fall (Yin et al., 2009).

During flooding, carbon fixation by the photosynthetic process is adversely affected due to a lower oxygen diffusion and reduction of gas exchange between the root system and pore spaces where they develop (Magalhães and Souza, 2011), resulting in a decreased production of ATP and an inappropriate plant development (Horchani et al., 2008).

The number of seeds per ear fitted the quadratic model with a high coefficient of determination ($R^2 \geq 0.99$). It reduced by increasing the flooding period, reaching the maximum response point at 37% (Figure 1b). These results can be explained because flooding occurred between the V8 and V9 development stages, and the number of seeds per ear was defined between the V9 and V12 stages (Magalhães and Souza, 2011). In the

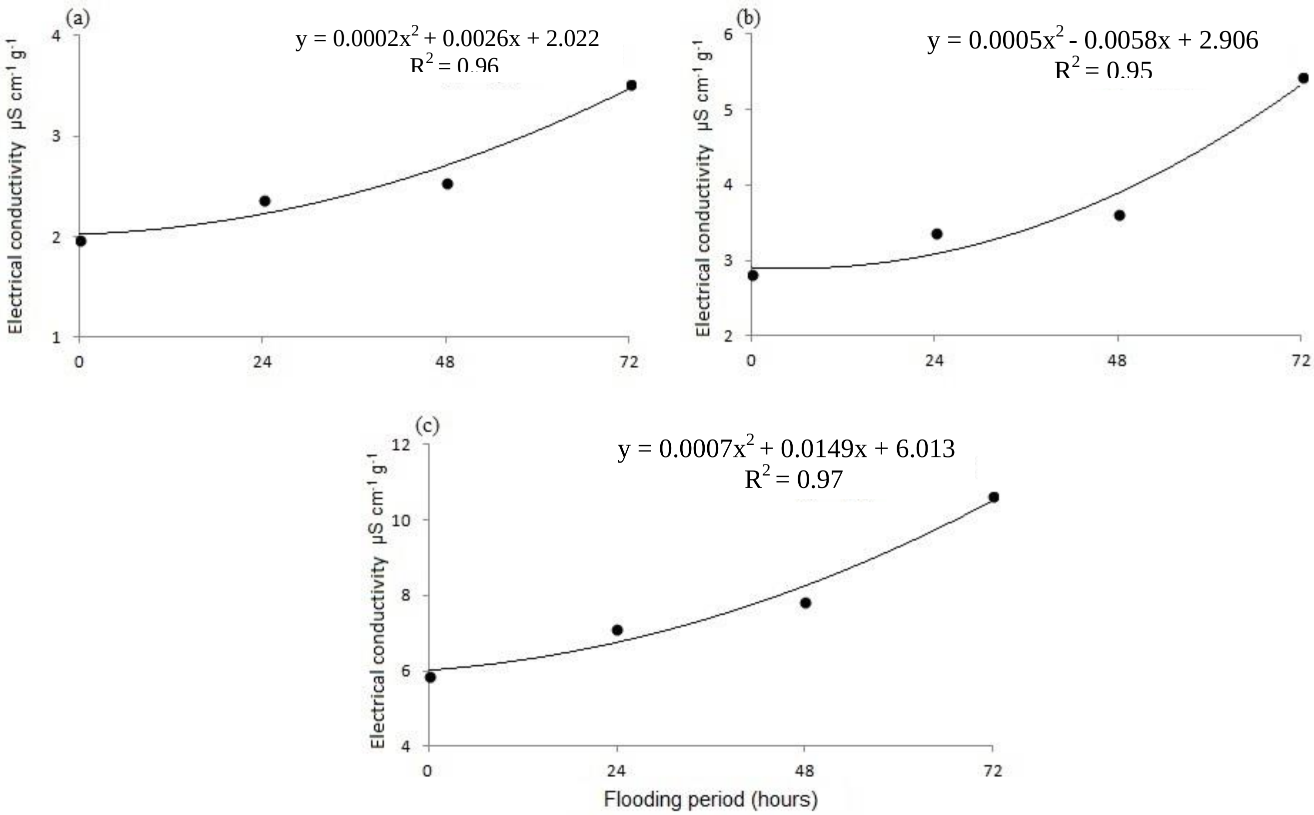

Figure 2. Electrical Conductivity after 3; 6 and 24 h (a; b; c) corn seed imbibition (*Z. mays* L.) produced by plants under different periods of flooding, and 0 (DC); 24; 48 and 72 h temporary flooding.

V12 development stage, the occurrence of environmental stress adversely affects the number of ovules per row, and consequently the number of seeds. However, the level of damage caused to the ear depends on the duration and intensity of the stress. In a short and high intensity period, damage may occur at any place of the ear (Magalhães and Souza, 2011). The number of rows per ear fitted the quadratic model with a high coefficient of determination ($R^2 \geq 0.96$), reaching the maximum response point at 2.15% (Figure 1c). Regarding ears produced by plants kept at field capacity, there was a reduction of 0.95, 1.45 and 4.30% in the number of rows of plants subjected to flooding periods of 24, 48 and 72 h, respectively. According to Magalhães et al. (2002), the number of rows per ear is defined at the V8 stage, which corresponds to the plant vegetative stage with eight leaves. Therefore, any stress occurring at this development stage of corn plants may adversely affect the formation of the number of rows per ear.

The electrical conductivity (Figure 2) evaluated in seeds subjected to different flooding periods after 3, 6 and 24 h of soaking fitted the quadratic model with a high coefficient of determination ($R^2 \geq 0.99$). There was an increase in the release of electrolytes with the increasing stress imposed and soaking time. After 3 h of soaking, there was a superiority of 0.41, 0.58 and 1.55% (Figure 2a) in the release of electrolytes from seeds produced by plants subjected to temporary flooding of 24, 48 and 72 h, respectively, compared to seeds produced at field capacity. After 6 h of soaking, the seeds produced with a temporary flooding of 24, 48 and 72 h showed a superiority in exudate bleaching of 0.56, 0.79 and 2.61% (Figure 2b), respectively, compared to seeds at field capacity. At 24 h of soaking, seeds produced in 24, 48 and 72 h were superior than those produced at field capacity at 1.27, 1.97 and 4.76% (Figure 2c), respectively. In this sense, at all evaluated times of soaking, seeds produced by plants subjected to flooding showed less ability to reorganize the cell membrane system. The efficiency in keeping the integrity of cell membranes is essential for the resumption of an efficient respiration process via oxidation, which provides energy, through the compounds, for the development of seedlings, maintaining a high relation with the maximum expression of seed vigor (Marcos Filho, 2005).

The lesser reorganization capacity of the cell membrane system may be related to the change in the pattern of development of the species, which, according to Hua Wu et al. (2009), shows a classic seed development pattern, presented by orthodox seeds,

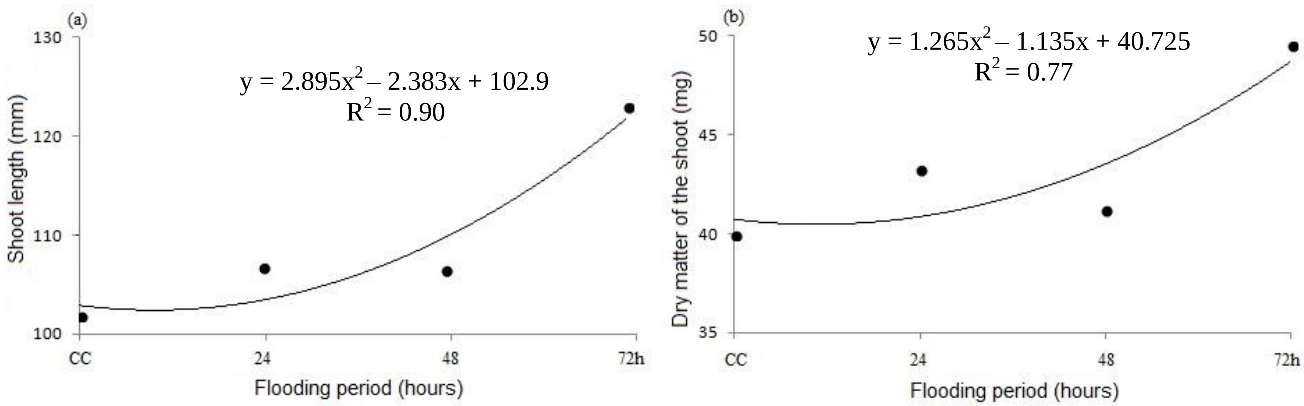

Figure 3. Length (a) and dry matter of the shoot (b) of maize seedlings (*Z. mays* L.) subjected to flooding. Where: 0; 24; 48 and 72 h temporary flooding.

comprising the synthesis and allocation of carbohydrates, lipids and proteins. In the final formation phase, there is the deposition of LEA proteins and raffinose oligosaccharides, which act to stabilize cell membranes providing tolerance to a dehydration process (Ferreira and Borghetti, 2008). Stress may change the preference of the metabolic drain for certain compounds, reducing its deposition and seed quality (Gehling et al., 2015). Shoot length and dry matter fitted the quadratic model ($R^2 \geq$ 0.91 and 0.77). There was a significant increase in these two growth attributes in seedlings from seeds produced under the influence of 72 h of temporary flooding (Figure 3). For shoot length and dry matter, they were, compared to seeds produced by plants kept at field capacity, an increase of 21 and 10% in seedlings originated from seeds produced under 72 h of flooding. The increase in shoot length contributes to the increase in dry matter (Burgos et al., 2004). In seedlings from seeds produced under the influence of flooding, there was evidence of higher carbon allocation efficiency, even when produced seeds achieved a greater release of electrolytes according to electric conductivity tests (Figure 2). The allocation of dry matter relates with efficiency of hydrolysis and translocation of assimilates. It is thus, an indication of seed quality (Carvalho et al., 2011). For the species *B. brizantha*, which is from the same family as corn, some cultivars produce a greater shoot dry mass when subjected to flooding (Kroth et al., 2015). Flooding negatively affects corn plants concerning yield, mass of thousand seeds, number of seeds per ear, number of rows per ear and seed vigor. However, when evaluating the quality of seeds and the physiological performance of seedlings, there was an increase in shoot length and dry matter.

Conclusion

Temporary flooding in the studied development stages led to the results obtained. Temporary flooding affects physiological performance and yield attributes of corn seeds according to stress period; With the increase in the flooding period in up to 72 h, there is an increase in electrolyte leaching in corn seeds; Seeds produced in 72 h of flooding showed a reduced mass of thousand seeds, number of seeds per ear and number of rows per ear.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Forage yield components of various alfalfa (*Medicago sativa* L.) cultivars grown on salt-affected soil under rainfed and irrigated conditions in a Mediterranean environment

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In Algeria, the area allocated to crop fodders in particular, the alfalfa (*Medicago sativa* L.) is very limited as compared to other crops. Some obstacles hinder the development of crops especially in irrigated perimeters. A field experiment was conducted to highlight the adaptation of sixteen (16) alfalfa varieties from different origins (Algeria, Australia, France, Italy, Morocco, Tunisia and USA) to drought, soil salinity and to evaluate the dry matter yields in the experimental station INRAA of Hmadena in the Lower-Cheliff plain in two cropping seasons, 2005/2006 and 2006/2007. The tests were conducted in a saline soil under two water regimes, rainfalls and irrigation with the maximum evapotranspiration. The evaluation focuses on dry matter yield, plant height and stand density. The results obtained in two campaigns show the encouraging production of dry matter of some varieties such as "Ameristrand 801S" and the weak production of the local variety "Tamantit", a reduction in the number of plants per square meter from one year to another, and an average height of the plants which differs from a variety to another. The number of harvests per season was normally between 4 and 6. It is concluded that some varieties are suitable for dry matter production in salt affected soil under both water regimes in the area of Hmadna in the Lower-Cheliff plain (Algeria).

Key words: Algeria, alfalfa, cultivar, dry matter yield, plant height, stand density.

INTRODUCTION

Alfalfa, *Medicago sativa* L. is one of the most widespread forage plants in the world. Grown in equatorial regions to the limits of the Arctic Circle, however, its greatest development is found in warm temperate areas. Alfalfa

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Table 1. Monthly average temperature (T) and monthly total rainfall (R) of the growing seasons, 2005/2006 and 2006/2007.

Month	2005		2006		2007	
	T_m(°C)	Rainfall (mm)	T_m(°C)	Rainfall (mm)	T_m(°C)	Rainfall (mm)
September	24.61	14.9	25.58	0.7	26.32	34.00
October	21.44	20.9	22.93	1.8	20.42	47.00
November	14.12	75.9	17.19	0.3	15.56	32.2
December	11.57	36.8	13.01	70.4	10.90	5.4
January	7.67	4.10	09.20	29.6	10.71	46.3
February	13.86	50.80	09.75	71.6	13.42	24.7
March	14.08	14.50	13.96	7	12.74	56.5
April	16.03	3.7	19.46	17.3	15.24	108
May	23.25	0.7	24.77	37.6	21.08	19.2
June	27.37	0.2	26.16	00	25.28	0
July	30.67	0.1	31.57	0.4	29.76	0.4
August	27.69	00	28.12	0	29.06	0

Table 2. Some characteristics of the studied soil (0-35 cm depth).

Characteristics	Values	Characteristics	Values
Clay (%)	45.50	Ca meq/100 g	28.34
Sand (%)	12.12	Mg meq/100 g	6.17
Silt (%)	42.34	Na meq/100 g	2.51
pH	7.72	K meq/100 g	1.46
CE dS/m	6.89	P_2O_5 ass	89.88
Bulk density	1.78	Mo %	3.81

usually has the highest feeding value of all common hay crops. Addition of alfalfa to lamb diets enhances production and profits (Rong et al., 2014).

Perennial alfalfa is one of the most adapted species to drought (Lemaire 2006). Its life cycle gives it the ability to contribute to the sustainability of rain fed cropping systems (Volaire and Norton, 2006). The alfalfa plant can cover its needs from the water loss through evaporation and the loss of soil, it is also vulnerable to wind and hydric erosion. The global area occupied by alfalfa is about 12.5 million hectares producing 324.5 million tons in 2013 (FAOSTAT, 2015). African production is about 5.32 million tons over an area of 185 000 ha. In 2013, Algeria produced only 29,000 tons on an area of 3,000 ha (FAOSTAT, 2015). In arid Mediterranean areas, alfalfa is often irrigated and often challenged with a saline stress. Search for varieties best adapted to this situation is a priority (Ibriz et al., 2004). In Algeria, the establishment of performance tests from introduced species and varieties could be a way of research in order to choose the best perennial alfalfa cultivars. The main objective of this study is to evaluate some forage yield components of 16 alfalfa, *M sativa* cultivars from different origins under the climatic conditions of the region of Hmadena in north-western of Algeria.

MATERIALS AND METHODS

Site description and climatic conditions

The tests were performed during two successive campaigns: 2005/2006 and 2006/2007, on sixteen (16) alfalfa varieties from different origins, at the experimental station of Hmadna, Algeria (35° 54 'N and 0° 47 'E with an altitude of 48 m) belonging to the National Institute of Agronomic Research of Algeria. The study area is characterized by a semi-arid to arid climate trend where irrigation is essential for crops. Monthly average temperature (T) and monthly total rainfall (R) of the growing seasons 2005/2006 and 2006/2007 are shown in Table 1. The soil of the experimental area is of a loamy-clay texture with a higher electrical conductivity of the irrigated environment relative to 6.0 vs. 5.20 dS m^{-1} which was consistent with the expected increase of soil salinity caused by irrigation with moderately saline water featuring 3.40 dS m^{-1} electrical conductivity (Annicchiarico et al., 2010). Some physical and chemical characteristics of the studied soil of the experimental area are presented in Table 2.

Plant materials, experimental design and cultivation practices

The experiment was conducted at Hmadna on fields cultivated under two water regimes: a rainfed system without any irrigation supply during the two experimental seasons and an irrigated system were the quantity of water supplied is equal to the ETM. Seed bed preparation included ploughing, disk harrowing and cultivation. Weed control was performed manually. Sowing was

Table 3. Names and origins of the tested cultivars.

Name of cultivars	Origin	Name of cultivars	Origin
Ecotipo siciliano	Italy	Gabes 2355	Tunisia
Prosementi	Italy	Magali	France
ABT 805	USA	Melissa	France
Ameristrand 801S	USA	Coussouls	France
Mamuntanas	USA	Africaine	Morocco
Tamantit	Algeria	Rich2	Morocco
Sardi 10	Australia	Erfoud1	Morocco
Siriver	Australia	Demnat	Morocco

performed by hand during the second week of October 2004 with 25 kg ha^{-1} seed rate for all varieties which is considered appropriate to combine forage and seed production (Lloveras et al., 2008). As these are selected cultivars, seed purity was 100% as well as germination percentage. Sixteen perennial alfalfa cultivars were placed on two plots with 4 replications with row spacing of 0.20 m. Each plot is consists of 16 micro plots of 2.50 m length and 2 m wide with a spacing of 0.20 m between microplot and 0.4 m between blocks. Table 3 shows the name of each variety and origin country. Harvest started as soon as 75% of seedlings in the elementary plot reached 0.35 m height for the winter season and 50% of flowering in most of plots for the spring and summer seasons.

The cuts are made at 0.05 m from the ground level and the yield is calculated in dry matter (DM). Each collected sample was weighed and passed to the drying oven for 80°C during a period of 48 h. Four cuts were performed for test in rainfed and 6 cuts for irrigated test.

The density of the initial and final stand were evaluated by counting the number of plants per m^2 for each of the 16 varieties in each replicate for both tests (rainfed and irrigated). Plant height was recorded on 18 plants taken randomly by repetition and for each variety in both trials.

Statistical analysis

The analysis of variance test (ANOVA) was applied on the data with mean separation of 5%. Levels of significance averages of various repetitions were calculated and analyzed by the statistical software (STATBOX 6.0.4.) and the used device is the unifactorial total randomization by the test of Newman and Keuls ($P_{0.05}$ and $P_{0.01}$).

RESULTS AND DISCUSSION

Stand density

Statistical analyses of each test and for each season show that there are no significant differences between all the varieties studied concerning the numbers of plant/m^2. Whereas, high significant differences were obtained among stand densities when compared the results of 2005/2006 season with those of 2006/2007 for tests conducted in rainfed. Significant differences existed also between the results of tests conducted in irrigated for the year 2005/2006 and 2006/2007. The result of statistical analysis of densities between the rainfed tests and irrigated one for the season 2006/2007 reveals insignificance.

The highest density was marked for the variety, Ecotipo Siciliano with 72 plants /m^2 recorded in 2005/2006 in the test leads in irrigated. This variety kept its values in first rank for the test carried out in rainfed with 50 plants per m^2 for the same year. On one hand, these results join those of Chocarro and Lloveras (2014) who found a stand density reaching 70 plants per m^2 with spacing of 20 cm between rows which is the same spacing used in this experiment.

On the other hand, the lowest densities were recorded in “Tamentit”, African and Demnat203 cultivars with 30, 32.5 and 32.5 plants per m^2 respectively for the test carried out in rainfed and 55, 40 and 50 plants per m^2 respectively in the irrigated trial for the same season (2005/2006), with a regression of density for the same varieties in 2006/2007. The stand densities at the end of each campaign are given in Table 4.

Plant height

As one of the most important forage yield components, the average plant heights of alfalfa seedling were calculated during all seasons of 2005/2006 and 2006/2007 in both water regimes (Table 5). The results show that the highest plants are between 28.56 and 28.06 cm in Ameristand 801S and Melissa varieties, respectively, during 2005/2006 under rainfed. The height of the local variety seems low with an average of 20.37 cm. For the tests in irrigated plots in the same season (2005/2006), the heights of the plants were higher than in rain with 37.58 cm for Ameristand 801S and 36.63 cm for Demnat.

In addition, for the second season (2006/2007), the Ameristand 801S variety had the highest average with 36 cm for the test in rainfed and 39.91 cm for the test in irrigated. The average heights for the local variety Tamentit were 32.37 cm in rainfed and 33.37 in irrigated.

Statistical analysis of data showed highly significant differences among all cultivars examined for two campaigns from both tests (rainfed and irrigated). These results are proximate to those found by Mikic et al. (2005) where they obtained significant differences in the heights of plants among all studied cultivars and Katic et al. (2006) obtained plants heights which varied between 36.4 and 68.9 cm in alfalfa grown between 2003 and 2004 in Serbia.

The averages of plant height obtained in these experiments seem lower than those found by Mikic et al. (2005) and Katic et al. (2006). This could be explained by the soil type of the study, which is known by its salinity and by the climate which is semi-arid and also by the use of different plant material. Mikic et al. (2005) and Katic et al. (2006) obtained plant heights varied between 36.4 and

Table 4. Stand density per m^2 of the 16 alfalfa varieties.

Season	2005/2006		2006/2007	
	Rainfed	Irrigated	Rainfed	Irrigated
Cultivars	Plants/m²	Plants/m²	Plants/m²	Plants/m²
Ecotipo siciliano	50	72.5	22.5	37.5
Prosementi	35	52.5	15	25
ABT 805	35	55	17.5	40
Ameristand 801S	32.5	67.5	12.5	42.5
Mamuntanas	47.5	60	22.5	37.5
Tamantit	30	52.5	10	27.5
Sardi 10	32.5	52.5	12.5	35
Siriver	35	42.5	17.5	32.5
Africaine	32.5	40	15	22.5
Gabes-2355	45	50	17.5	27.5
Magali 1	37.5	57.5	22.5	27.5
Melissa	37.5	60	20	37.5
Cousouls	37.5	55	17.5	32.5
Rich 2	37.5	42.5	15	32.5
Erfoud 1	35	50	17.5	37.5
Demnat 203	32.5	50	10	37.5
SEM	13.05	16.45	8.22	10.18
Prob.	0.70	0.37	0.49	0.23
Sig.	N.S	N.S	N.S	N.S
C.V (%)	35.24	30.62	49.68	30.59

NS: Non-significant; Sig: significance level; SEM: standard error mean; CV: coefficient of variance.

68.9 cm in alfalfa grown between 2003 and 2004 in Serbia.

Comparison of average heights of alfalfa plants obtained in rainfed tests in the 2005/2006 season with the averages of 2006/2007 shows non-significant differences between the two seasons. Meanwhile, highly significant differences were obtained when comparing the average heights of plants in irrigated test for the season of 2005/2006 to 2006/2007.

Forage yield

In this study, the forage DM yield (DM) on both experiments in irrigated and rainfed during 2005/2006 and 2006/2007 period were presented. The number of the cuts per season was 6 in irrigated plots and 4 cuts in rainfed. Bellague et al. (2008) obtained 4 alfalfa cuts for the same varieties in rainfed and 6 cups for irrigation in 2004. Fanlo et al. (2006) obtained six alfalfa cuts in a test carried out in irrigated plots and 3 cuts in rainfed tests conducted in Girona (Spain) which is characterized by similar climatic conditions of the region.

The results of field trials studied under different water regimes show a dry matter yield varying from 6.13 to 13.44 t ha^{-1} for all varieties in irrigated conditions. Therefore, in rainfed conditions, the yield varied between 2.27 and 4.69 t ha^{-1}. For the 1st season in the rainfed plots, the most productive varieties in order of importance are: Ameristrand 801S, Mamuntanas, Melissa and Riche2 and less productive are: Tamentit, Prosementi, Coussouls and Magali and for irrigated plots, the varieties, Ameristrand 801S, Mamuntanas, Melissa and Riche2 were the most productive and Tamentit, Prosementi, Coussouls and Magali were less productive varieties (Table 6). For the 2nd season (2006/2007), the variety Mamuntanas, Erfoud, Riche2 and Siriver had the best yield in DM. Unlikely, varieties such as Tamentit, Prosementi, Demnat and Coussouls still had the lowest yield. Tables 7 and 8 show the forage yield results by cuts and by period (in dry and irrigated) for both seasons. Khelifi et al. (2008) achieved an average DM yield balance of 2.59 to 7.11 t ha^{-1} for the same varieties with rainfed trial conducted in the area of Algiers which is characterized by soil and climate of optimum conditions than the study area. For the irrigated trial, yields obtained range from 2.59 to 11.64 t ha^{-1}.

Benabderrahim et al. (2008) showed that the cultivar "Gabes" originating from the Tunisian oases presents small quantities of dry matter. The best fresh matter

Table 5. The plant heights of 16 alfalfa varieties for 2005/2006 and 2006/2007 under rainfed and irrigated conditions.

Cultivars	2005/2006		2006/2007	
	Rainfed	Irrigated	Rainfed	Irrigated
	Height (cm)		Height (cm)	
Ecotipo siciliano	21.68^{de}	34.41^{ab}	36^{a}	37^{abc}
Prosementi	18.56^{e}	29.58^{b}	31.62^{a}	34.04^{bcd}
ABT 805	23.5^{cd}	32.38^{ab}	33.37^{a}	34.87^{bcd}
Ameristand 801S	28.56^{a}	37.58^{a}	36^{a}	39.91^{a}
Mamuntanas	27^{abc}	35.17^{ab}	31.75^{a}	37.87^{abc}
Tamantit	20.37^{de}	30.21^{b}	32.37^{a}	33.37^{cd}
Sardi 10	24.31^{bcd}	33.88^{ab}	35.31^{a}	36.58^{abc}
Siriver	24.06^{bcd}	32.38^{b}	32.12^{a}	36.04^{abcd}
Africaine	21.43^{de}	30.63^{b}	32.5^{a}	34.33^{bcd}
Gabes-2355	24.31^{bcd}	33.17^{ab}	32.37^{a}	35.54^{abc}
Magali 1	18.75^{e}	32.17^{ab}	32.31^{a}	31.83^{d}
Melissa	28.06^{ab}	33.54^{ab}	34.75^{a}	37.87^{abc}
Cousouls	19.87^{de}	32.88^{ab}	31.56^{a}	33.46^{cd}
Rich 2	23.06^{cd}	30.92^{ab}	35.68^{a}	33.87^{cd}
Erfoud 1	24.56^{bcd}	35.96^{ab}	33.81^{a}	38.83^{ab}
Demnat 203	23.81^{bcd}	36.63^{ab}	35.93^{a}	38.75^{ab}
SEM	2.018	2.91	1.778	2.015
Prob.	0	0.007	0.00021	0
Sig.	**	**	**	**
C.V (%)	8.68%	8.77%	5.29%	5.61%

NS= no significant; *:significant at P < 0.05; **:Highly significant at P < 0.01. a, b, c, d: homogeneous group (numbers with the same letter are not significant different at P < 0.05). Sig: Significance level. SEM: standard error mean, CV: coefficient of variance.

yield is recorded in the cultivar Sardi (Australian origin). The cultivar, African (Moroccan origin) was most productive dry matter. Fanlo et al. (2006) showed that the average yield in rainfed conditions is between 1.3 and 6.3 t ha^{-1} and that of irrigated test is higher with an average of 8 to 22.5 t ha^{-1}. These results on the influence of the varieties on the dry matter yield correspond to those of Stanisavljević et al. (2012) who recorded an average DM yield between 2.59 and 6.54 t ha^{-1} depending on cultivars. Average DM yields among 18.8 and 7.48 t ha^{-1} have been reported during an experimentation in Spain by Delgado et al. (2013) and Chocarro and Lloveras (2014). The statistical analyses of data show that there are highly significant differences between varieties in relation to the yields. The average of DM yield ranges from 8.6 to 20.4 t ha^{-1} depending on the results obtained by Katic et al. (2006) who confirm that these changes are significantly dependent on the cultivar. Van Heerden (2012) reported that the average of DM yield of all varieties in rainfed trial increased in the second year as compared to the first season from 3.36 to 4.75 t ha^{-1}. Otherwise, for the irrigated tests, the average yield in the first season (9.53 t ha^{-1}) is higher than that of the second campaign (8.57 t ha^{-1}). This increase in production of rainfed test is mainly due to the good distribution of rainfall during the spring season (Van Heerden, 2012).

Karagic et al. (2005) demonstrated that the climatic conditions of the cultivation region have a great effect on the yield of alfalfa which varied from 5.38 to 8.85 t ha^{-1}. The variations in this study area were mainly subject to the influence of climatic conditions.

Conclusion

The main objective of this study was to assess forage yield components of 16 alfalfa, *M. sativa* L. varieties from different origins under soil and climatic conditions of the Hmadna region in the North West of Algeria during two campaigns in 2005/2006 and 2006/2007.

Based on the results obtained in the experiment, it is suggested to cultivate varieties Ameristrand 801S, ecotipo Siciliano Mamuntanas and Erfoud1 under an irrigation system at maximal evapotranspiration, other cultivars as Mamuntanas, Melissa, and Erfoud1 Siriver can be conducted under rainfed.

In this region characterized by a semi-arid to arid tendency and clay loamy soils with moderate salinity to

Table 6. Dry matter forage yield t ha^{-1} of 16 alfalfa varieties for the 2005/2006 and 2006/2007 seasons in rainfed and irrigated conditions.

Cultivars	2005/2006		2006/2007	
	Rainfed	Irrigated	Rainfed	Irrigated
	DM yield		DM yield	
Ecotipo siciliano	3.51^{cd}	11.38^{b}	5.14^{bcd}	9.75^{b}
Prosementi	2.32^{e}	7.96^{f}	3.92^{fg}	7.41^{g}
ABT 805	3.44^{cd}	9.86^{d}	4.53^{ef}	8.97^{cde}
Ameristand 801 S	4.29^{ab}	13.44^{a}	4.64^{de}	10.94^{a}
Mamuntanas	4.68^{a}	11.09^{b}	6.42^{a}	9.94^{b}
Tamantit	2.27^{e}	7.57^{fg}	3.61^{g}	7.79^{fg}
Sardi 10	3.55^{cd}	10.53^{cd}	4.80^{cde}	9.09^{cd}
Siriver	3.92^{bc}	8.95^{e}	5.13^{bcd}	8.97^{cde}
Africaine	3.15^{d}	6.13^{h}	4.19^{efg}	6.81^{h}
Gabes-2355	3.69^{cd}	8.52^{e}	4.48^{ef}	8.49^{de}
Magali 1	2.55^{e}	8.53^{e}	4.60^{de}	6.99^{h}
Melissa	3.99^{bc}	9.52^{d}	5.58^{b}	8.37^{ef}
Cousouls	2.37^{e}	11.26^{b}	4.18^{efg}	7.86^{fg}
Rich 2	3.35^{cd}	7.34^{g}	5.25^{bc}	7.14^{h}
Erfoud 1	3.49^{cd}	11.35^{b}	5.70^{b}	9.17^{c}
Demnat 203	3.14^{d}	9.03^{e}	3.93^{fg}	8.36^{ef}
SEM	2.916	2.859	2.968	3.337
Prob.	0	0	0	0
Sig.	**	**	**	**
CV	8.68%	3.00%	6.23%	3.92%

NS= no significant; *: significant at P < 0.05; **: Highly Significant at P < 0.01. a, b, c, d: homogeneous group (numbers with the same letter are not significant different at P < 0.05). Sig: Significance level. SEM: standard error mean, CV: Coefficient of variance.

Table 7. Forage yield of alfalfa per cuts in both water regimes for 2005/2006.

Cultivars (2005/2006)	Forage yield in irrigated test ($t.ha^{-1}$)						Forage yield in rainfed test ($t.ha^{-1}$)			
	1st cut	2nd cut	3rd cut	4th cut	5th cut	6th cut	1st cut	2nd cut	3rd cut	4th cut
Ecotipo siciliano	1.03	2.78	2.35	2.07	1.91	1.24	1.43	0.94	0.72	0.42
Prosementi	0.42	1.48	1.87	1.70	1.55	0.94	0.97	0.56	0.52	0.26
ABT 805	1.58	2.55	1.91	1.73	1.34	0.71	2.15	0.52	0.51	0.26
Ameristand 801 S	2.16	2.64	2.54	2.37	2.36	1.37	2.31	0.90	0.81	0.27
Mamuntanas	1.76	2.73	2.03	1.97	1.68	0.93	2.43	1.01	0.78	0.45
Tamantit	0.87	0.99	1.49	1.35	1.80	1.07	0.95	0.51	0.57	0.25
Sardi 10	1.58	2.15	2.05	1.88	1.75	1.12	1.74	0.93	0.64	0.25
Siriver	1.34	2.08	2.00	1.66	1.23	0.64	1.87	1.0	0.64	0.41
Africaine	0.55	1.15	1.22	1.37	1.23	0.62	1.12	0.72	0.95	0.37
Gabes-2355	1.53	1.63	1.49	1.33	1.54	1.01	1.84	0.59	0.90	0.36
Magali 1	0.77	2.09	1.45	1.77	1.53	0.92	0.78	0.59	0.81	0.38
Melissa	1.60	2.11	1.86	1.65	1.46	0.85	1.67	0.96	0.97	0.41
Cousouls	1.14	2.57	2.36	2.00	2.02	1.17	1.14	0.54	0.45	0.25
Rich 2	0.93	1.75	1.35	1.54	1.17	0.61	1.66	0.68	0.60	0.42
Erfoud 1	1.40	2.35	2.34	1.92	2.07	1.27	1.50	0.77	0.79	0.44
Demnat 203	1.15	1.40	2.35	1.37	1.81	1.34	1.46	0.64	0.74	0.30

Table 8. Forage yield of alfalfa per cut in both water regimes for 2006/2007.

Cultivars (2006/2007)	Forage yield in irrigated test (t.ha^{-1})						Forage yield in rainfed test (t.ha^{-1})			
	1st cut	2nd cut	3rd cut	4th cut	5th cut	6th cut	1st cut	2nd cut	3rd cut	4th cut
Ecotipo siciliano	0.51	1.51	4.20	1.92	1.28	0.33	1.72	2.28	0.69	0.50
Prosementi	0.58	0.85	3.10	1.61	1.05	0.23	1.08	2.02	0.53	0.31
ABT 805	0.88	1.36	4.01	1.71	0.76	0.26	1.52	2.19	0.51	0.31
Ameristand 801 S	1.35	1.69	3.70	2.22	1.64	0.35	1.67	1.90	0.81	0.26
Mamuntanas	0.96	1.74	4.16	1.84	0.96	0.29	2.71	2.49	0.79	0.43
Tamantit	0.78	0.86	2.60	1.87	1.42	0.27	1.02	1.55	0.55	0.51
Sardi 10	0.88	1.27	3.39	1.97	1.25	0.34	1.60	2.02	0.64	0.55
Siriver	0.83	1.21	3.88	1.83	1.02	0.21	1.87	2.35	0.61	0.31
Africaine	0.51	0.68	2.76	1.45	1.09	0.31	1.40	1.57	0.63	0.60
Gabes-2355	0.95	0.95	2.96	1.64	1.46	0.53	1.37	1.61	0.89	0.61
Magali 1	0.38	0.78	3.23	1.44	0.92	0.25	1.60	2.03	0.78	0.19
Melissa	0.74	1.25	3.19	1.67	1.22	0.31	1.81	2.24	0.91	0.64
Cousouls	0.37	1.18	3.71	1.49	0.98	0.15	1.46	2.12	0.44	0.17
Rich 2	0.59	1.06	3.03	1.38	0.92	0.16	1.76	2.38	0.69	0.44
Erfoud 1	1.00	1.33	2.76	2.30	1.40	0.39	1.80	2.44	0.78	0.58
Demnat 203	1.10	0.96	2.77	1.63	1.42	0.49	1.31	1.47	0.75	0.41

cultivate both cultivars Mamuntanas and Erfoud1 as long as these two cultivars gave acceptable yields in MS during the two campaigns under both water regimes, is proposed, especially the cultivar Erfoud1 of Moroccan origin from region a similar to this study area which gave satisfactory forage yields.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agroclimatic risk of development of *Diaphorina citri* in the citrus region of Nuevo Leon, Mexico

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Diaphorina citri **is the insect vector of the disease, Huanglongbing (HLB) which is the most devastating citrus disease worldwide. It is necessary to identify potential risks; this can be done based on favorable thermo-pluviometrical conditions for the vector. The present study aims to identify potential risks in the Nuevo Leon citrus region in areas, which have not yet been devastated by the citrus disease. The presence of HLB and the population growth of the** ***D. citri*** **will be influenced by four factors involved in the epidemiological system: the disease-prone host - citrus fruits; the pathogen agent; the biology of the vector; and the climate conditions that foster the vector establishment and reproduction. With information from 65 weather stations, some indices were estimated: potential generations of the vector per year, days with favorable temperature and suitable rainy days for the vector, area planted with citrus fruits, and finally, the potential risk index. This shows that the Nuevo León citrus region is a HLB free region. The method can be applied to citrus producing areas which have not been devastated by the citrus disease worldwide.**

Key words: Citrus greening disease, climate conditions, Huanglongbing, potential risks.

INTRODUCTION

Of the worldwide production of citrus fruits, the bulk is achieved in eighteen countries that together produce 115.5 million tons (FAOSTAT, 2014). Production has been affected by pests and diseases, including the deadly bacterial disease to the vector Huanglongbing (HLB), also known as citrus greening disease, which is considered to be the greatest threat to citrus cultivation worldwide. The agents are Gram-negative bacteria of the genus *Candidatus* Liberibacter, which includes three species: *C.* Liberibacter africanus, *C.* Liberibacter americanus and *C*. Liberibacter asiaticus; the last named is the most widely distributed species in Asia and America because of its tolerance of high temperatures (Bové, 2006; Narouei et al., 2016). HLB is restricted to the phloem of the host citrus plant, and in citrus crop, it is transmitted by vegetative means such as grafts. In nature, it is transmitted by the psyllid insects *Triozaerytreae* and *Diaphorina citri*, the latter having been first detected in China more than 100 years ago (da Graça et al., 2008).

Until 2003, HLB was restricted to two continents, Africa and Asia. It was reported on the American continent for

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the first time in 2004, in Araracuara, São Paulo, Brazil, where the presence of *C.* Liberibacter asiaticus was detected (Colleta-Filho et al., 2004); in 2005 its presence was confirmed in Florida, USA, followed by Cuba in 2007 and Louisiana, USA in 2008.

The disease affects various links in the chain of citrus production, thereby reducing employment opportunities in harvesting, manufacture of machinery and boxes, and transport and final consumer price of the fresh fruit. In addition, it destabilizes industries engaged in processing the product: producers of juices, concentrates, oil extracts and dried peel, and the transport and final consumer price of these.

The ability of the Asian citrus psyllid (ACP), *Diaphorina citri*, to transmit HLB constitutes a serious threat. This small insect feeds on the leaves and stems of citrus trees and related species such as jasmine and curry leaves. It can be spread by anthropogenic factors such as the commercial and tourist activities using aircraft, ships, trucks and cars, and also by anemochorous dispersion, like the wind.

Citrus production in Mexico ranks fourth worldwide, after Brazil, China and the USA. There are over 334,658.68 ha across twenty-three States in Mexico, which together account for an annual production in excess of 4 million tons of fruit, with an approximate value of US 461 million dollars in 2012.

HLB was first recorded in 2009 in the states of Yucatan and Quintana Roo, owing to the proximity to, and trade with, Cuba, the USA (Florida and Louisiana) and the Dominican Republic. Investigation has been scarce and isolated: Lopez et al. (2009) noted the lack of a master research plan and recommended the activation of an emergency plan. The memorandum NOM-EM-047-FITO-2009 from the national body for health and food safety in agriculture established phytosanitary actions leading to the implementation of a monitoring program that includes sampling, diagnosis, inspection and monitoring for the timely detection of the introduction and spread of HLB in Mexico, as well as the application of phytosanitary measures required for controlling it, including the delimitation of areas under phytosanitary control, disposal of infected materials, vector control, sampling inspection and restriction and/or control of the movement of plant material from the host species (Diario Oficial de la Federación, 2009).

The disease was expected to spread gradually towards citrus-producing areas on the slopes bordering the Gulf of Mexico; however, in April 2010, the bacterium made a turn; it was detected in Mexican lemon trees in the municipality of Tecoman, Colima, a state bordering the Pacific. To date, the disease has been detected in 13 of Mexico's 23 citrus production states (SENASICA-DGSV, 2014).

There is yet no cure for the affected trees. Based on current regulations and experiences in other citrus regions of the world, the strategy for managing HLB includes disposal of infected material and, keeping orchards productive as long as possible, learning to live with the disease, and applying strict vector-control methods.

The presence of HLB in Africa, Asia and America led to studies on its economic and social impact in affected countries; in all cases, the results point to huge losses associated with the disposal of infected trees and the rise in production costs due to management practices to prevent the disease (Taylor et al., 2016).

Diaz et al. (2014) focused on the environmental conditions that foster the presence, abundance and population growth rate of *D. citri*. Other lines of research have developed indices to delimit agroclimatic risk areas (Moschini et al., 2010).

Areas not affected by the disease include the Nuevo Leon citrus region, an area recognized as the birthplace in Mexico for the cultivation of the orange (*Citricus sinensis*): the first juice-processing plants in Latin America were established there. Orange production in 2014 was 204,750 tons; Nuevo León ranked fifth at national level.

However, some areas have not yet been affected by HLB, even some that are contiguous with affected areas. It is necessary to identify potential risk, and this can be based on thermo-pluviometrical conditions favorable to the vector. The present study aims to identify potential risk for areas in the Nuevo Leon citrus region, a region that is not yet affected by the disease.

MATERIALS AND METHODS

HLB is fostered when temperature, vector insects and the developmental stage of citrus trees combine and interact. This agrosystem, generically known as an epidemiologic triangle (Bové, 2006; Batool et al., 2007; da Graça et al., 2008) has served in the present study as a basis for considering additional environmental conditions: rainfall, relative humidity, photoperiod and insect dispersion by large air masses. Consideration of all these parameters together has allowed the configuration of the Pathosystem Tetrahedron (Figure 1).

In this case, the disease-prone host is the orange tree, the pathogen agent is the *Candidatus* Liberibacter asiaticus (CLas), the vector is *D. citri*, and the environmental conditions are primarily climatic ones (temperature, precipitation, relative humidity, photoperiod and the dynamics of large air masses).

In order to determine each of the factors involved in this tetrahedron, the literature on the environmental conditions (climate) that favor each life stage of *D. citri* was reviewed (Yang et al., 2006; Bové, 2006; Batool et al., 2007; Diaz et al., 2014; Moschini et al., 2010). The environmental conditions in the study region were ascertained from analysis of the climate records of 65 stations included in the Computerized Climate System database (CLICOM) of the National Meteorological Service (SMN, 2014). The data used included daily maximum and minimum temperatures and daily precipitation. The reliability of these data was tested by path and homogeneity tests. The average climatic values were also obtained from SMN. Statistics of the planted and harvested areas of the various citrus fruits were obtained from the national agricultural information system.

The concept of potential generation of the vector, refers to the number of generations that can be produced, according to the

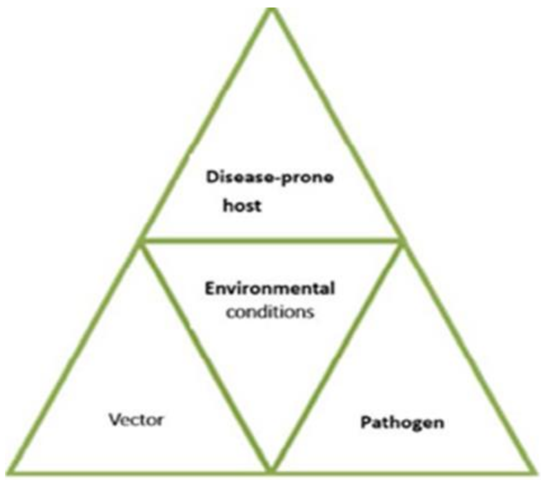

Figure 1. Pathosystem tetrahedron.

biology of the vector and the environmental conditions. One generation of *D. citri* requires 211 degree-days to complete its development, with a threshold temperature of 13.5°C (Diaz et al., 2014; Moschini et al., 2010). The mean daily temperature of each station was used here to calculate the degree-days (GD), at each station.

$$GD_i = GD_{i-1} + (Tmed_i - 13.5)$$

Where, $Tmed_i$ is the average of the maximum and minimum temperature for each day at that station, and 13.5°C is the baseline.

$$Tmed_i = (T\max_i + T\min_i)/2$$

When the cumulative value is 211°-days, one generation of the psyllid would have been completed. Once the potential number of generations has been calculated, the Potential Generations Index (PGI) is calculated for that station:

$$PGI_i = PG_i / Max(PG)$$

where: PGI_i : potential generations index for the *i*-th station; *PGi*: is the number of potential generations calculated for that station; *Max (PG)* is the maximum number of potential generations observed for all stations.

The number of days with favorable temperature conditions refers to the number of days with temperatures favoring a more rapid development of *D. citri* eggs, nymphs and adults. The number of days that meet the condition of minimum temperature, 15°C and maximum temperature, 32°C were identified, this being the temperature range reported to foster the development of the vector (Moschini et al., 2010). Once the number of days for each station has been determined, the index of days with favorable temperature conditions is calculated as:

$$IDFC_i = DFC_i / Max(DFC)$$

Where, $IDFC_i$ is the index of days with favorable temperatures for the *i*-th station, DFC_i is the number of days with favorable temperatures for the *i*-th station, *Max (DFC)* is the maximum number of favorable days observed for all the stations.

With the precipitation data obtained for each station and considering the rainfall requirement of more than 50 mm for the vector insect according to Moschini et al. (2010), the number of days that meet this requirement, that is, the number of days with favorable precipitation is calculated as follows:

$$IDFP_i = DFP_i / Max(DFP)$$

Where, $IDFP_i$ is the index of number of days with favorable rainfall for the *i*-th station, DFP_i is the number of days with favorable conditions for the *i*-th station, *Max (DFP)* is the maximum value of favorable days observed for all the stations.

The maximum orange-planted area known at the municipal level defined as Index of Planted Area, was calculated according to the following formula:

$$IPA_i = PA / Max(PA)$$

Where, IPA_i is the index of the area planted with citrus recorded for the *i*-th station, PA_i is the area planted with citrus recorded for the *i*-th station, *Max (PA)* is the maximum area planted with citrus observed for all stations.

Based on the average indices previously calculated, the potential risk index to identify areas at risk of a potential impact of *D. citri*, the HLB vector, was estimated as:

$$RI_i = PGI_i + IDFC_i + IDFP_i + IPA_i / 4$$

where: PGI_i Potential generations index for the *i*-th station, $IDFC_i$: index of days with favorable conditions for the *i*-th station, $IDFP_i$: index of days with favorable precipitation for the *i*-th station, IPA_i: index of area planted with citrus recorded for the *i*-th station.

The calculated indices were grouped in a database associated with weather stations, which are georeferenced. These data were exported to a Geographic Information System (GIS). ArcGis software 10 allowed the database to be linked to a digital map; this software contains the Geostatistical Analyst Tool, which performs spatial interpolation processes. The study area lies in the central west portion of the State of Nuevo Leon (24° 30' − 25° 30' N; 99° − 100° 20' W). It includes the municipalities of Allende, Cadereyta Jimenez, General Teran, Hualahuises, Montemorelos, Linares and Rayones. The total citrus area amounts to 25 589 ha (Figure 2). Because of the geographical location, altitude above sea level (between 300 and 1,300 m) and dominant meteorological phenomena across the citrus-growing region, the prevailing climate is semi-warm subhumid, (A)Cw, with temperatures between 18 and 22°C and summer rainfall, and small areas with a regime intermediate between summer and winter, (A)Cx'w.

In general, rainfall was not abundant. In low-altitude land, annual precipitation ranges from 400 - 600 mm. Most of the citrus region receives 600 - 800 mm, and only in the small areas does annual rainfall fluctuate between 800 and 1 200 mm.

RESULTS

In the study area, temperatures ranged from -2 to 40°C. Minimum temperatures varied between -2 and -8°C from October to February but rose thereafter, exceeding 10°C from May to August. Maximum temperatures exceeded 35°C and rose towards midsummer, reaching a peak of 40°C in May. Both minimum and maximum temperatures are limiting factors for the vector, since it is intolerant to

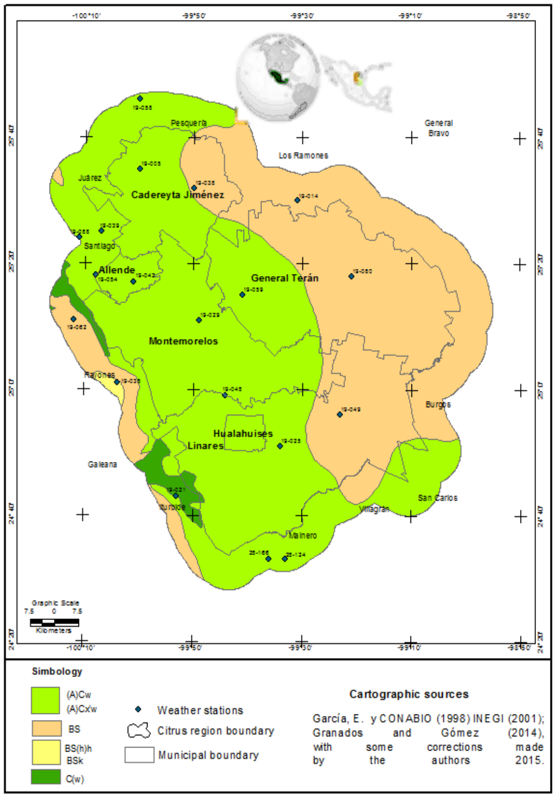

Figure 2. Climate across the Nuevo Leon citrus region. Inset: Location in Nuevo Leon State.

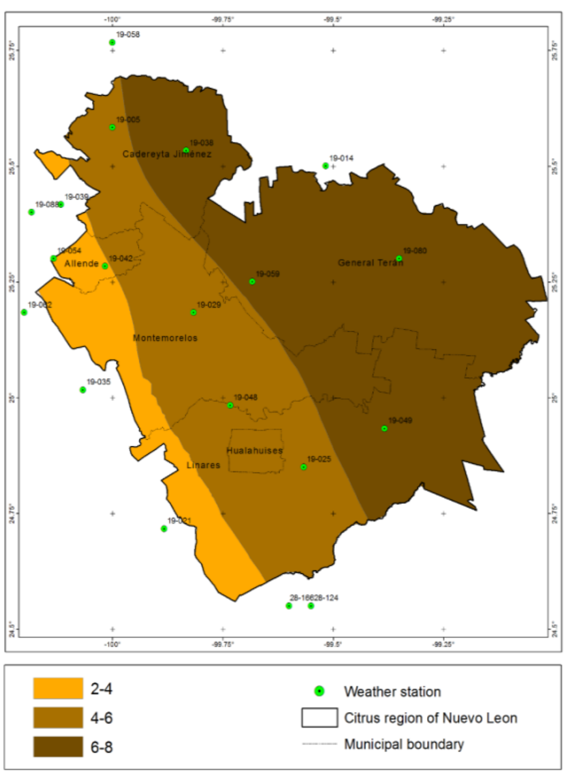

Figure 3. Potential generations of *D. citri* per season.

extreme conditions. The relative humidity is variable, being above 60% from May to September when monthly precipitation exceeds 50 mm. September is usually the month with the highest precipitation; the average value recorded for that month was 105 mm.

The derived number of potential generations indicated that between 2 and 8 generations of *D. citri* may be developed per year in the citrus region (Figure 3); 2−4 on the outskirts of the Sierra Madre Oriental, a temperate area with temperatures between 12 and 18°C (western portion of the Allende, Montemorelos and Linares municipalities); 4−6 in important areas of Cadereyta Jimenez, Montemorelos and Linares; and 6−8 towards the Gulf Coastal Plain, where warm conditions prevail (22−24°C), corresponding to the eastern portion of Cadereyta Jimenez and Linares, and the entire General Teran.

The mean number of favorable days for the development of the vector in the citrus region ranges from 21 to 90 days per year: 61−90 days in General Teran and the eastern portion of Cadereyta Jimenez and Linares municipalities; 41−60 days in Hualahises and the central portions of Cadereyta Jimenez, Linares and Montemorelos; and 21−40 in Allende and the western portions of Montemorelos and Linares, areas in the vicinity of the Sierra Madre Oriental where the altitudinal gradient leads to a drop in temperature (Figure 4).

The highest number of days with favorable precipitation for the development of the vector occurs in the central portion of the citrus region. This central area receives 400 to 600 mm of rainfall per year, exceeding the 50 mm on only 117 days per year. The region's western portion received more than 50 mm of rain during up to 78 days per year and the respective index ranged between 0.1 and 0.4. In General, Tera, and northeastern Cadereyta Jimenez, Montemorelos and Linares, the rainfall exceeded 50 mm on 17 - 39 days per year, and here the index is also 0.1 – 0.4 (Figure 5).

Orange plantations comprised between 1,000 and 40,000 ha in the citrus municipalities (Figure 6). The municipality with the largest area with oranges was General Teran, with 39 231 ha, followed by Linares, Cadereyta Jimenez, Montemorelos, Allende and Hualahuises, respectively. Then, some municipalities may experience more economic consequences than others.

The risk index (Figure 7) indicated the risk level associated with the development of *D. citri* and the potential incidence of HLB in the citrus region. The municipalities of General Teran, Cadereyta, Montemorelos and Linares have a high potential risk, with an index that ranges between 0.61 and 0.90. There is a lower risk to the west region, with an index of 0.21- 0.60.

DISCUSSION

From the detection of the disease in citrus plants in

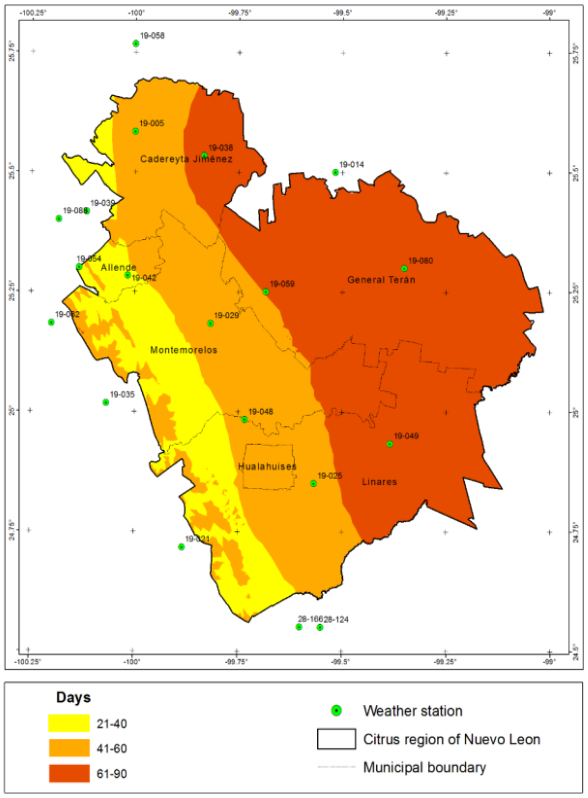

Figure 4. Number of days with temperatures favorable to *D. citri.*

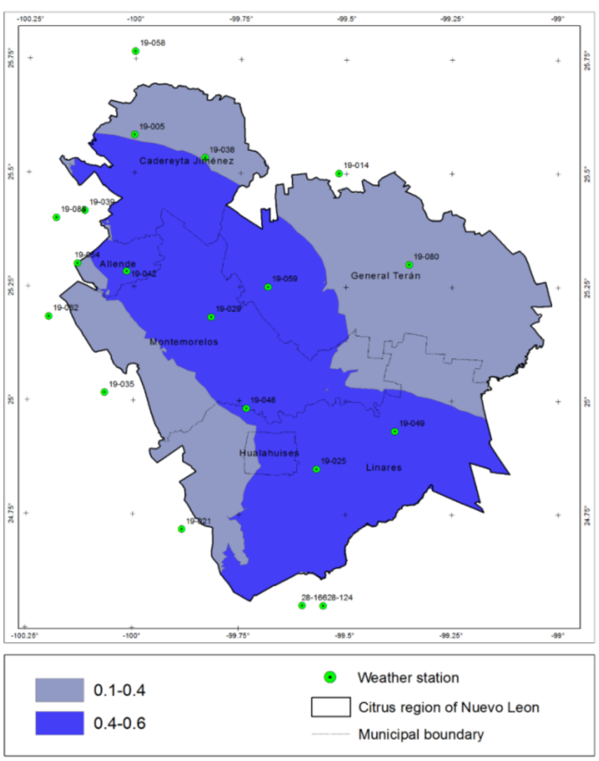

Figure 5. Index of days with rainfall favorable to *D. citri.*

America in 2004, research works on the biology of *Diaphorina citri,* the disease vector, have increased (Halbert and Manjunath, 2004; Narouei et al., 2016). Grafton-Cardwell et al. (2013) stated that climate exerts a number of direct effects on the life cycle and reproduction of *D. citri*.

Although, in Mexico, the presence of HLB was detected a little later, research on this topic was also conducted (Lopez et al., 2009), but no cure was found for HLB to date. It has been suggested that the affected areas should adopt an integrated and appropriate strategy, including the elimination of diseased trees, use of non-contaminated plants, pesticide application and biological control of the psyllid vector. Investigations are currently ongoing, and Tabachnick (2015) has stressed that the search for further solutions to reduce the devastating impact on citrus fruit production is urgent.

This search should also include research focused on disease-free citrus production areas, to diagnose the potential risk, such as studies that include the geo-technologies: spatial modelling, simulation of risk areas and map algebra (Magarey et al., 2007; Ladányi, 2010; Gutierrez et al., 2011; Rosa et al., 2011; LANGIF, 2015; NAPPFAST, 2015; Narouei et al., 2016; Taylor et al., 2016).

Investigations at a country scale were carried out by Torres-Pacheco et al. (2013), and delimited potential regions and degrees of risk based on the environmental affinities of *Diaphorina citri*, as well as the range and phenology of host plants. In southeastern Mexico, a region characterized by high temperatures (> 25°C) and precipitation (>1100 mm), the potential existence of up to 27 generations were quantified.

On a regional basis, in northern Mexico, the potential distribution of *D. citri* in Nuevo Leon was analyzed, based on the synchrony between phytophage and host, by calculating and determining the spatial distribution of specific temperature and precipitation indicators (days degree and days with high probability of rainfall, respectively) throughout the year. The most vulnerable areas were located in the warm (22 - 24°C air temperature) and semi-dry (400-600 mm precipitation) Gulf of Mexico highland, which includes the eastern area of Cadereyta Jimenez and Linares and the entire General Teran municipalities; in this area, the phytophage may put the Nuevo Leon citrus plantations and the industry at high risk within just 6-8 generations.

Prevention-oriented research contributes to scientific information that will allow the development of actions to reduce vulnerability to this citrus disease (LANGIF). The information derived from this work may be regarded as preventive and represents an instrument that will support the planning of work schemes and the proposal of new courses of action aimed at reducing the effect of this disease. Besides, key stakeholders should be involved, including specialists in natural areas, socio-economic disciplines and leading producers who promote new

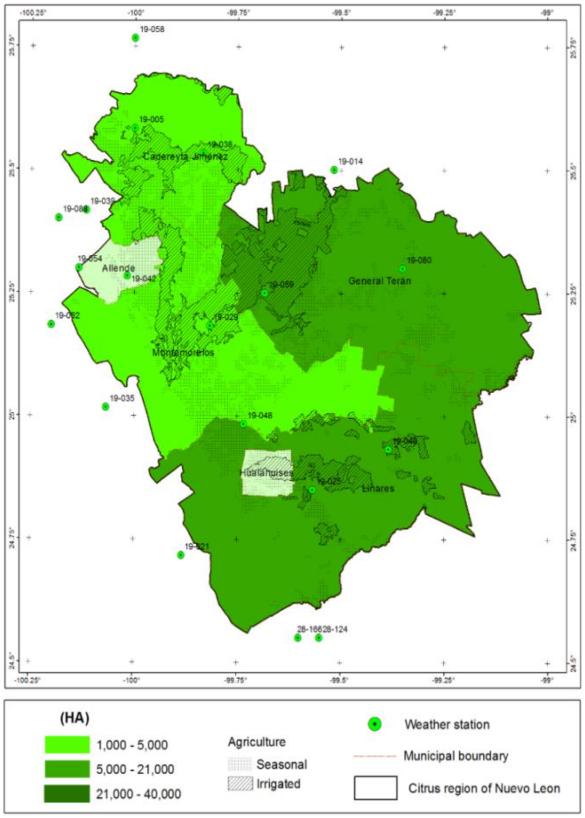

Figure 6. Index of the agricultural area.

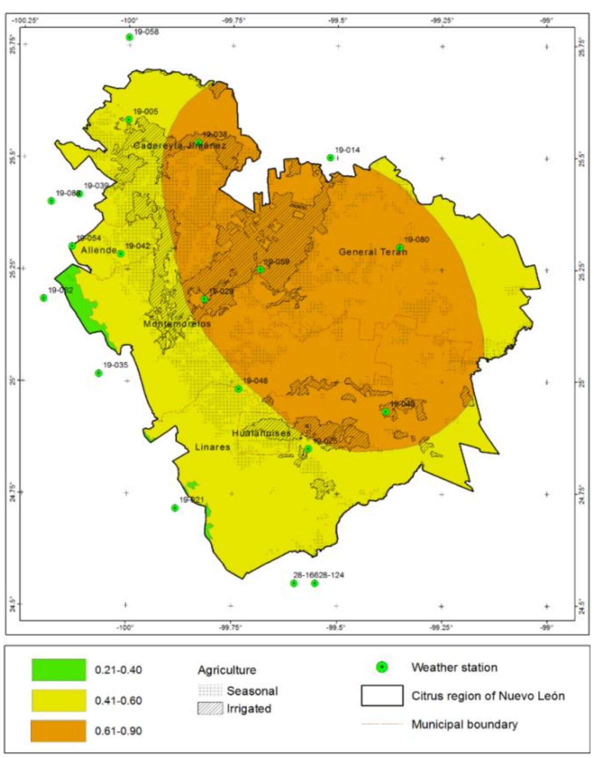

Figure 7. Risk index for the development of *D. citri* in the Nuevo Leon citrus region.

agricultural approaches for these plantations.

Conclusions

The spatial representation of phytosanitary risk, integrated from the analysis of the main thermo-pluviometric variables quantified here, allowed the evaluation of potential danger of vector, *D. citri* establishment in this region. Although, non-vector psyllids were present, conditions in the study area were seemingly suboptimal for the development of the vector; accordingly, the Nuevo Leon citrus region is free of the danger of HLB. The most important climatic element was the distribution and variation of temperature, which sometimes exceeded 40 or fell below 3°C. Lower temperatures would hinder the establishment and development of the psyllid population by slowing down its metabolism and, hence, disrupting embryonic development. The maps show this to be particularly applicable in the western part of the Nuevo León citrus region, where the development of the vector is limited by temperature extremes associated with altitude and by the smaller areas planted with the host trees.

The infected production areas are remote from this region, but the dispersal of psyllids carrying HLB cannot be ruled out, since this may be encouraged by atypical atmospheric phenomena such as variations in wind speed and direction, and incident light. Further analysis of these phenomena should produce more comprehensive indices and thereby improve the spatial representation of risk. The anthropogenic components must be strictly controlled, as established by the phytosanitary standard, NOM-EM-047-2009, in order to prevent the transport of infected specimens into the study region.

Conflict of interests

The authors have not declared any conflict of interest.

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