

Article

Potassium doses on the ecophysiological characteristics of 'Syrah' grapevine grown at São Francisco River Valley, Brazil

Agnaldo Rodrigues de Melo Chaves^{1*}, Davi José Silva¹, Saulo Tarso Aidar¹, Luciana Martins Santos², Karinne Albuquerque Campos de Prado², Bruno Ricardo Silva Costa¹

> ¹Brazilian Agricultural Research Corporation, EMBRAPA Semiárido, Petrolina, PE, Brazil ²University of Pernambuco, Petrolina, PE, Brazil *Corresponding author, e-mail: agnaldo.chaves@embrapa.br

Abstract

The nutrients availability can lead to changes on grapevines physiological behavior, which results in a great importance of studies regarding the application of potassium doses. The aim of this research was to evaluate the ecophysiological behavior of grapevine cultivar 'Syrah' growing under different potassium doses by fertigation, according to the determination of gas exchange, chlorophyll a, fluorescence and pigments index. Five years old plants, grafted on 'Paulsen 1103' rootstock, in a trellis system and planted at theBebedouro Experimental Field, located in Petrolina, PE, Brazil, were evaluated. The experiment was composed by five potassium doses (0, 20, 40, 80 and 160 kg ha⁻¹), applied by fertigation. The potassium sources were potassium sulfate, potassium nitrate and potassium chloride. The evaluations of the ecophysiologyical parameters were performed at four different times (07am, 10am, 1pm and 3pm) throughout the day on the flowering stage and the first and second fruit growth stages, determining gas exchange and chlorophyll a fluorescence and the pigments index was evaluated at the same grapevines leaves one time. Considering each evaluation period, it was observed that, according to the magnitude of the results for gas exchange, chlorophyll a fluorescence and pigments index, is not possible to indicate the best potassium dose. The climatic conditions during each period of evaluation influenced most the eco-physiological variables than the applied potassium doses, while the changes on pigments index were due to leaves development during the plant cycle.

Keywords: photosynthesis, transpiration, chlorophyll a fluorescence

Doses de potássio no comportamento ecofisiológico de videira 'Syrah' cultivada no Submédio do Vale do São Francisco

Resumo

A disponibilidade de nutrientes proporciona alteração no comportamento fisiológico das plantas, tornando de grande importância o estudo da aplicação de diferentes doses de potássio em videira de vinho. O objetivo desta pesquisa foi avaliar o comportamento ecofisiológico da videira 'Syrah' cultivada em diferentes doses de potássio aplicadas via fertirrigação, analisando as trocas gasosas, a fluorescência da clorofila a e o índice de pigmentos. Foram utilizadas plantas com cinco anos de idade, enxertadas sobre 'Paulsen 1103', cultivadas em espaldeira e implantadas no Campo Experimental de Bebedouro, Petrolina-PE. O experimento foi constituído de cinco doses de potássio (0, 20, 40, 80 e 160 kg ha⁻¹) aplicados via fertirrigação utilizando como fontes o sulfato de potássio, o nitrato de potássio e o cloreto de potássio. As avaliações foram realizadas em quatro horários (07:00, 10:00, 13:00 e 15:00 h) ao longo dia na fase de florescimento e nas 1ª e 2ª fases de crescimento do fruto, consistindo em determinar os parâmetros de trocas gasosas, da fluorescência da clorofila, enquanto o índice de pigmentos foi avaliado nas mesmas datas e nas mesmas folhas de videira uma única vez. Considerando-se cada data de avaliação de forma independente, se observou que a magnitude dos resultados dos parâmetros de trocas gasosas e da fluorescência da clorofila a obtidos neste trabalho não sofreram influência das doses de potássio e que as condições climáticas reinantes em cada período de avaliação influenciaram mais as respostas ecofisiológicas do que as doses de potássio aplicadas, enquanto a alteração no índice de pigmentos foi em função do desenvolvimento das folhas ao longo do ciclo.

Palavras-chave: fotossíntese, transpiração, fluorescência da clorofila a

Introduction

Potassium plays an important role in plant nutrition, influencing many physiological processes such as carbohydrates translocation, protein synthesis, enzymes activation, stomata opening and closure, turgor pressure regulation and control of CO_2 input and H_2O exit in stomata and the subsequent concentration at plant mesophyll cells during the transpiration process (Schreiner et al., 2013).

During the photosynthetic process, an inadequate potassium supply associated to a high global radiation incidence on leaves may cause reduction in the consumption of ATP and NADPH produced on the photochemical phase of photosynthesis, since it may happen an insufficient supply of CO₂ to be fixed by Ribulose 1,5-bisphosphate carboxylase and oxygenase (Rubisco) in biochemical photosynthesis phase due to the stomata closure (Praxedes et al, 2006; Schreiner et al., 2013). This ATP and NADPH accumulation will increase the synthesis of reactive oxygen species, which, in high concentrations, may damage proteins, pigments and photosystems, reducing the role of chlorophyll in the photochemical phase of photosynthesis and the substrates availability for the photosynthesis biochemical phase(Pinheiro et al, 2004; Chaves et al., 2008).

In the São Francisco River Valley region is recorded a high incidence of global radiation that can promote the synthesis of reactive oxygen species, which in high concentrations may cause damage in electron transport chain components, reducing the capacity of radiation absorption by the plant pigments and its use on chlorophyll a fluorescence of the plants (Pompelli et al, 2010).

During the wine elaboration process, the potassium content present in the wine must affects both quality and preparation costs, since the physic-chemical, biological and organoleptic characteristics have an important role in wine pH (Daut & Fogaça, 2008). The pH is greatly influenced by the cultivation techniques such as potassium fertilization, plant material, maturation stage and the technology applied for wine elaboration (Chaurel, 2006), conditions that can promote changes in vinification. When wine must have a high initial pH, this needs to be adjusted during the wine process with tartaric acid addition, which is a very common procedure in global wine industry, when the grapevines are grown in soils that have problems with high potassium levels (Daut & Fogaça, 2008). High potassium concentrations in must may promote excessive losses of tartaric acid, which is precipitated as potassium bitartrate, causing difficult and expensive costs in pH adjustment (Mpelasoka et al., 2003). To minimize these costs in tartaric acid correction it is necessary to obtain fruits with less potassium, since high potassium concentrations in wine and must will require the addition of high levels of free SO₂ or SO₂ during the vinification process, providing inadequate aromas that harm wine quality (Reyner, 2007).

During berries maturation stage of Pinot Noir, Cabernet Sauvignon and Merlot grapevines, Fogaça et al. (2007) described that the increased levels of potassium absorbed by fruits increased the must pH values. This condition, related to the fact that some soils of the São Francisco River Valley present high natural potassium content, provided by the source material and pedogenesis processes (Cunha et al., 2010), may reduce the expansion capacity for grapevines cultivation areas in this region, due to the effect of potassium in wine quality.

Thus, the major goal of this study was to investigate the ecophysiological mechanisms in Syrah grapevines growing under different potassium doses applied by fertigation, through the evaluation of the gas exchange, chlorophyll a fluorescence and pigment index.

Materials and methods

The study was carried out with five years old grapevines cv 'Syrah' growing in field and grafted on 'Paulsen 1103' rootstock, spaced in 3.0×1.0 m between rows and plants, respectively, in trellis system and installed at Bebedouro Experimental Field, located in Petrolina, PE, Brazil (latitude 9°8'8,9'' S, longitude 40°18'33.6'', altitude 373 m). The experiment was constituted of five K₂O doses (0, 20, 40, 80 e 160 kg ha⁻¹) and designed in randomized blocks with four replications. The experimental unit in each

block consisted of 16 plants and the pruning was realized in May 30, 2014. The irrigation system adopted was the drip irrigation with 2 emitters per plant, spaced 0.5 m each one, with flow rate of 2.0 L h⁻¹ and potassium fertilization was applied by fertigation using an electric injection pump. Weekly applications of the potassium doses were accomplished during a period of ten weeks, with 40% of the K_2O doses applied at the first stage of the crop cycle (4 weeks before flowering) and 60% applied at the second stage of the crop cycle (6 weeks after flowering). Potassium sulphate, potassium nitrate and potassium chloride were use as potassium sources as described in Tables 1 and 2.

	Potassium Sulphate	Potassium Nitrate			
K ₂ O doses (kg ha ⁻¹)	Amount (g)	Amount (g)			
0	0	0			
20	192	0			
40	384	0			
80	768	0			
160	768	835			

Table 2. Potassium Sulphate, Potassium Nitrate and Potassium Chloride proportions used in applications from July06th to August 15th, 2014.

Weekly aplication	Potassium Sulphate	Potassium Nitrate	Potassium Chloride		
K ₂ O doses (kg ha-1)	Amount (g)	Amount (g)	Amount (g)		
0	0	0	0		
20	213	0	0		
40	427	0	0		
80	853	0	0		
160	853	557	213		

Before starting the fertilizer applications, soil samples were collected from all experimental units in the depths of 0-20 and 20-40 cm (Table 3). From this sample, soil pH, electrical conductivity, organic carbon, exchange cations, potential acidity and available P_2O_5 were analyzed, as

described by Embrapa (1997). From soil solution extractors installed in the experimental area, it was evaluated the nitrate and potassium ions concentration. The ions concentration was determined by the procedure described in Silva (2009).

 Table 3. Soil analysis of 0-20 cm and 20-40 cm depths in the experimental area.

K ₂ O	CE	рН	МО	P ₂ 0 ₅	K ₂ 0	Na	Са	Mg	H+AI	Sb	CTC	V
kg ha-1	dS m ⁻¹		g kg-1	mg dm⁻³	cmol _c dm ⁻³					%		
	0-20 cm											
0	0.35	6.1	6.1	26.3	0.12	0.1	2.2	0.9	1.4	3.4	4.7	70
20	0.44	6.2	9.7	43.4	0.14	0.0	2.4	0.9	1.7	3.5	5.2	67
40	0.43	6.3	10.7	27.1	0.13	0.0	2.3	1.1	1.3	3.6	4.9	74
80	0.51	6.6	8.4	41.1	0.14	0.0	2.6	1.2	1.1	4.1	5.1	80
160	0.41	6.5	13.0	28.0	0.15	0.0	2.1	1.0	1.3	3.2	4.5	75
20-40 cm												
0	0.19	6.0	7.3	49.0	0.07	0.0	2.0	0.9	1.3	3.1	4.4	69
20	0.20	5.9	5.3	45.0	0.08	0.0	1.8	0.8	1.4	2.8	4.2	68
40	0.26	6.1	6.1	35.8	0.08	0.0	2.0	0.8	1.4	2.9	4.3	68
80	0.35	6.6	11.6	59.0	0.12	0.0	2.6	1.1	1.1	3.8	5.0	77
160	0.28	6.2	7.7	54.4	0.12	0.0	1.9	0.8	1.4	2.9	4.3	68

The ecophysiological parameters were measured at four different times during the day (07am, 10am, 1pm and 3pm) in July 07, August 07 and September 03, 2014. These days

of measurements were chosen based on the flowering stage and first and second fruit growth stages, as described by Soares & Costa (2009). The net CO₂ assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), leaf-toair vapor pressure deficit (δe), internal to ambient CO_2 concentration ratio (C_i/C_a), leaf temperature, intrinsic water use efficiency (A/g_{c}) and instant water use efficiency (A/E) were measured under artificial photosynthetic photon flux (PPF) of 1,300 μ mol m⁻² s⁻¹ and CO₂ ambient concentration (390 ppm), with a portable open-flow gas exchange (Li-6400XT, Li-Cor, Nebraska, EUA). The initial fluorescence light adapted (F'_{0}) , maximum fluorescence (F'_m) , constant fluorescence (F's), adapted variable and maximum fluorescence ratio (F'_{V}/F'_{m}) , quantum yield of PSII electron transport (Φ FSII), photochemistry dissipated (q_{p}) , non-photochemistry dissipated (q_{N}) and electron transport rate (ETR) was measured with the same equipment used for the gas exchange measurements. The evaluations were made in two central plants in each experimental unit, using healthy leaves located in the opposite side of the bunches.

The pigments index (chlorophyll a, b and total) was determined using the portable chlorophyll meter device ClorofiLOG (Falker Automação Agrícola Ltda., Brazil) at the same days of the gas exchange and chlorophyll a fluorescence evaluations and the measurements were also made in healthy leaves. The results were expressed in mean and mean standard error, which is applied in ecophysiologics studies.

Results and discussion

Climate data were obtained from an automatic agro-meteorological station located 60m from the experimental area. During all evaluated periods, air temperature increased at 3pm (30° C), while air relative humidity above 60% was observed at 07am, decreasing throughout the day and reaching the smallest values at 3pm (Figure 1A, 1B and 1C), while global radiation intensity showed higher values between 10am and 1pm (Figure 1C). Air temperature, air relative humidity and incident global radiation are essential to explain the balance of gas exchange in plants, since higher values of air temperature and lower values of relative air humidity provide higher values of δe that promotes plant stomata closure to prevent excessive water loss, a condition that provides decreasing in CO₂ availability to A.



Figure 1. Time course of: A - air temperature; B - relative air humidity; C - global radiation in flowering stage, 1st and 2nd fruit growth stages in 2014 obtained from automatic agrometeorological station at Bebedouro Experimental Field, Petrolina, PE, Brazil.

Com. Sci., Bom Jesus, v.7, n.3, p.362-371, Ago./Out. 2016

Higher values of air temperature and global radiation intensity and smaller values for relative air humidity were observed at September 03rd, which may cause alterations in physiological processes, gas exchange and chlorophyll a fluorescence, due to adjustments on plant metabolism to avoid water loss and to prevent physiologic disorders, such as excessive dehydration, which is harmful to plant development. Teixeira et al. (2012) described that high average air temperature values are unfavorable to grapevines in which pruning is performed in May, while physiological limitations can occur when the pruning is carried out in October.

Considering each evaluation period independently, it was observed that the plants had similar magnitude of gas exchange for all different potassium doses (Figure 2). High A and g_s values were observed at 07am, for all evaluations.

Lower values of A and g_s were observed in September 03rd, which may be associated to higher δe values during the day, which provides stomata closure to prevent excessive water loss, a common condition of sensitive plants in response to the increased δe , as observed in perennial plants by Pine et al. (2005).

Values of A, g_s , E and δ e (Figures 2A, 2B, 2C and 2D) were mostly influenced by climatic conditions than the potassium doses, indicating the importance of environmental conditions in plant physiological processes. Chaves et al. (2008, 2012) reported the major influence of climatic conditions on the gas exchange parameters evaluation in coffee, assuming that δe increases as the relative air humidity reduces, with reduction of stomata opening in order to minimize the excessive water loss, but it will reduce the CO₂ entry in stomata for its use during A (Schreiner et al., 2013).



Figure 2. Time course: A - net CO_2 assimilation rate (A); B - stomatal conductance (gs); C - transpiration rate (E); D - leaf-to-air vapor pressure deficit (δ e) in flowering phase, 1st and 2nd fruit growth phases in 2014 in wine trees 'Syrah' cultivated in five K₂O doses (kg ha⁻¹). Each point represents the mean ± SD of eight observations.

Com. Sci., Bom Jesus, v.7, n.3, p.362-371, Ago./Out. 2016

No changes were observed in A/g_s , A/E, C_i/C_a and leaf temperature at the three evaluation periods for all potassium doses (Figures 3A-3D). Lower values for A/E and higher values for leaf temperature may be associated to air temperature and global radiation intensity,

which have a lot of influence on the physiological processes. Schreiner et al. (2013) evaluating four K_2O doses (control, 10, 20 and 50 % of control) in Pinot Noir grapevines did not observed differences in A and $g_{s'}$ both assessed in the flowering and in fruit growing stages.



Figure 3. Time course: A - internal to ambient CO₂ concentration ratio (C₁/C_o); B - leaf temperature; C – efficiency of intrinsic water use (A/g₂); D - instant efficiency of water use(A/E) in flowering phase, 1st and 2nd fruit growth stages in 2014 in 'Syrah' grapevines cultivated under five K₂O doses (kg ha⁻¹). Each point represents the mean ± SD of eight observations.

The values of F'_0 and F'_v/F'_m over the three evaluation periods were not different according to potassium doses, but lower values were observed in September 03 (Figures 4A and 4D), which may be due to the higher global radiation value observed on that day (79.819 watts m⁻²) compared to the data recorded on July 07 (313.056 watts m⁻²) and August 07 (270.252 watts m⁻²). Similar results of F'_v/F'_m were obtained by Schreiner et al. (2013) in a study applying different K₂O doses (control, 10, 20 and 50% of the control) in Pinot Noir vines, where no difference

between treatments was observed.

The Φ FSII values were similar between treatments, but higher values were observed in July 07 (Figure 5A). High Φ FSII values throughout the day may be related to greater overall incident radiation as described by Chaves et al. (2008), which is the natural condition of the plant responding to the global radiation increase during the day. Smaller values of q_p and *ETR* on August 07 (Figure 5B and 5D) may be associated to higher overall radiation rate in that period, since these two parameters are related to the electrons flow at electron transport chain of photosynthesis, when ATP and NADPH is produced for CO_2 fixation in biochemical photosynthesis phase (Pinheiro et al., 2004; Chaves et al., 2008).

The q_p values associated to q_N values (Figures 5B and 5C) indicate that there was no damage to photosystems of the electron transport chain.



Figure 4. Time course: A - initial fluorescence adapted to light (F'_0) ; B - maximum fluorescence (F'_m) ; C - constant fluorescence (F's); D - variable and maximum fluorescence ratio in an open system (F'_v/F'_m) during flowering stage, 1st and 2nd fruit growth stages in 2014 for 'Syrah' grapevines grown under five K₂O doses (kg ha⁻¹). Each point represents the mean ± SD of eight observations.

Reductions in Φ FSII are often associated with increasing in non-photochemical dissipation of the absorbed energy, resulting in *ETR* decreases in plants exposed to radiation and that have problems in stomata opening for the ATP and NADPH consumption (Pinheiro et al, 2004). However, it can be noticed that damage in photosystems of the evaluated plants did not happen, considering the similar values of q_N . Consequently, the magnitude of ATP and NADPH production must not have been very different between plants growing under different potassium doses, as noticed by Schreiner et al. (2013) in Pinot Noir vine at the mentioned conditions. The values of pigments index, *a*, *b* and total chlorophyll (Figures 6A-6B) presented similar behavior within the evaluated periods. Comparison between periods showed that higher values were observed in July 07 and September 03, indicating that different potassium levels did not affected the pigment content. The changes during the evaluation periods were related to plants physiological development conditions. These results indicated that pigment degradation due to high global radiation did not occur, which could be related to the potassium role in chlorophyll synthesis, indicating that this nutrient did not limited the pigments synthesis.



Figure 5. Time course: A - quantum yield of PSII electron transport (Φ FSII); B photochemical dissipation (q_p); C - non-photochemical dissipation (q_n); D - electron transport rate (*ETR*) in flowering stage, 1st and 2nd fruit growth stages in 2014 in'Syrah' grapevines cultivated under five K₂O doses (kg ha⁻¹). Each point represents the mean ± SD of eight observations.



Figure 6. Pigments index: A - chlorophyll a; B - chlorophyll b; C - total chlorophyll a in flowering stage (July 07 = white columns) and for the 1st (August 07 = black columns) and 2^{nd} (September 03= dashed columns) fruit growth stages in 2014 for 'Syrah' grapevines grown under five K₂O doses (kg ha⁻¹). Each point represents the mean ± SD of eight observations.

Com. Sci., Bom Jesus, v.7, n.3, p.362-371, Ago./Out. 2016

The absence of the effect of potassium doses in the physiological processes observed in this study corroborates the results obtained by Silva et al. (2014), when no effect was observed for productive capacity in plants grown at the same experimental area and with the same potassium doses. These results may have been obtained due to the soil chemical characteristics (Table 3), which records previous cultivations, with the application of numerous fertilizers that build the soil fertility. This information becomes important because it reveals that in areas with a pre-established crop, it is possible to apply lower amounts of fertilizer, which does not cause reduction of plants physiological and productive capacity, in addition to providing financial and environmental profits.

Conclusions

The results described in this study showed that the potassium doses did not affected the grapevines physiological processes and the possible differences over the assessment periods were due to physiological conditions of plant development and to the prevailing climate conditions for each evaluation period.

References

Albuquerque, A.H.P., Viana, T.V.A., Marinho, A.B., Sousa, G.G., Azevedo, B.M. 2013. Irrigação e fertirrigação potássica na cultura da videira em condições semiáridas. Pesquisa Agropecuária Tropical 43: 315-321.

Chaurel, J.Y. 2006. Effect of potassium fertilization on soil, grapevine and wine in granitic soil. Progress Agricole et Viticole 123: 455-462.

Chaves, A.R.M., Ten-Caten, A., Pinheiro, H.A., Ribeiro, A., DaMatta, F.M. 2008. Seasonal changes in photoprotective mechanism of leaves from shaded and unshaded field-grown coffee (*Coffea arabica* L.) trees. Trees 22: 351-361.

Chaves, A.R.M., Martins, S.C.V., Batista, K.D., Celin, E.F., DaMatta, F.M. 2012. Varying leaf-to-fruit ratios affect branch growth and dieback, with little to no effect on photosynthesis, carbohydrate or mineral pools, in different canopy positions of field-grown coffee trees. Environmental and Experimental Botany 77: 207-218.

Cunha, T.J.F., Petrere, V.G., Silva, D.J., Mendes, A.M.S., Melo, R.F., Oliveira Neto, M.B., Silva, M.S.L., Alvarez, I.A. 2010. Principais solos do semiarido tropical brasileiro: caracterização, potencialidades, limitações, fertilidade e manejo. In: Sa, I.B., Silva, P.C.G. Semiárido Brasileiro: pesquisa, desenvolvimento e inovação. Embrapa Semiárido, Petrolina, Brasil. 402p.

Daudt, C.E., Fogaça, A.E. 2008. Efeito do ácido tartárico nos valores de potássio, acidez titulável e pH durante a vinificação de uvas Cabernet Sauvignon. Ciência Rural 38: 2345-2350.

EMBRAPA. Manual de métodos de análises de solo. 1997. 2ª Edição, Embrapa Solos, Rio de Janeiro, Brasil, 212p.

Fogaça, A.O., Daut, C.E., Dorneles, D. 2007. Potássio em uvas II. Analise peciolar e sua correlação com o teor de potássio em uvas viníferas. Ciência e Tecnologia de Alimentos 27: 597-601.

Mpelasoka, B.S, Schachtman, D.P., Treeby, M.T., Thomas, M.R. 2003. Review of potassium nutrition in grapevines with special emphasis on berry accumulation. 2003. Australian Journal of Grape and Wine Research 9: 154-168.

Pinheiro, H.A., DaMatta, F.M., Chaves, A.R.M., Fontes, E.P.B., Loureiro, M.E. 2004 Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. Plant Science 167: 1307-1314.

Pompelli, M.F., Martins, S.C.V., Antunes, W.C., Chaves, A.R.M., DaMatta, F.M. 2010. Photosynthesis and photoprotection in coffee leaves is affected by nitrogen and light availabilities in winter conditions. Journal of Plant Physiology 167: 1052-1060.

Praxedes, S.C., DaMatta, F.M., Loureiro, M.E., Ferrão, M.A.G., Cordeiro, A.T. 2006. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (Coffea canephora Pierre var. kouillou) leaves. Environmental and Experimental Botany 56: 263-273.

Reyner, A. 2007. Manuel de viticulture, 10a Edition, Editora Tecnology & Documents, Paris, França, 532 p.

Schreiner, R.P., Lee, J., Skinkis, P.A. 2013. N, P, and K Supply to Pinot noir Grapevines: Impact on vine nutrient status, growth, physiology, and yield. American Journal of Enology and Viticulture 64: 26-38.

Silva, F.C. 2009. Manual de análises químicas de solos, plantas e fertilizantes, 2a Edição, Embrapa Informação Tecnológica; Rio de Janeiro, Brasil, 627 p.

Silva, D.J., Silva, A.O., Bassoi, L.H., Costa, B.R.S.,

Teixeira, R.P., Souza, D.R.M. 2014. Adubação orgânica e fertirrigação potássica em videira 'Syrah' no Semiárido. Irriga 1: 168-178.

Soares, J.M., Costa, F.F. Irrigação. 2009. In: Soares, J.M., Leão, P.C.S. (Eds) A Vitivinicultura no Semiárido Brasileiro. Embrapa Semiárido, Petrolina, Brasil, p. 351-427.

Teixeira, A.H.C., Tonietto, J., Pereira, G.E., Angelotti, F. 2012. Delimitação da aptidão agroclimática para videira sob irrigação no nordeste brasileiro. Revista Brasileira de Engenharia Agrícola e Ambiental 16, 399–407.