Universidad Francisco de Paula Santander
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Sociedad Colombiana de Ciencias Hortícolas
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Revista Colombiana de Ciencias Hortícolas

Colombian Journal of Horticultural Science

Volume 13 / No. 1 / January-April 2019
ISSN (print) 2011-2173 ISSN (online) 2422-3719 / Doi: 10.17584/rcch

Fertilization with nitrogen on height, number of leaves and leaf area of banana / Planting method, fertilization and diazotrophs inoculation for Cantaloupe melon plants / Dormancy, container volume and biofertilizer for dwarf cashew tree seedlings / Pesticides residue in strawberry fruit / 1-methylcyclopropene and ethylene on peach fruit cv. Dorado / Resistance at *Meloidogyne incognita* with silicon dioxide / Morphoagronomic characters in tomato hybrids / flumioxazin herbicide in direct sowing onions / radish under saline stress and ascorbic acid application / Potassium silicate as a resistance elicitor in sweet corn / olives and olive oil production / essential oil of lemon balm growth with different nitrogen and light sources / cuttings defoliation and substrate type in propagation of the monkey-pepper

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SOCIEDAD COLOMBIANA DE CIENCIAS HORTÍCOLAS – SCCH

UNIVERSIDAD PEDAGÓGICA Y TECNOLÓGICA DE COLOMBIA – UPTC

UNIVERSIDAD FRANCISCO DE PAULA SANTANDER – UFPS

Revista Colombiana de Ciencias Hortícolas
Colombian Journal of Horticultural Science
Volume 13 / No. 1 / January-April 2019

ISSN (print): 2011-2173
ISSN (online): 2422-3719

INFORMATION AND CORRESPONDENCE

Sociedad Colombiana de Ciencias Hortícolas
Facultad de Ciencias Agrarias
Universidad Nacional de Colombia
P.O. Box 14490, Bogota, Colombia
Fax: (57) 1 - 316 5000 ext. 19041
soccolhort@gmail.com

Universidad Pedagógica y Tecnológica de Colombia
Facultad de Ciencias Agropecuarias
Tunja, Boyaca, Colombia
Tel.: (57) 8 - 742 2174/76
Fax: (57) 8 - 742 4321
rcch@uptc.edu.co

Universidad Francisco de Paula Santander
Facultad de Ciencias Agrarias y del Ambiente
San Jose de Cucuta, Norte de Santander, Colombia
Tel.: (57) 7 - 5776655
ing.agronomica@ufps.edu.co

Electronic version available at:
http://revistas.uptc.edu.co/revistas/index.php/ciencias_horticolas

PUBLISHED: Triannual

PUBLISHED BY
Editorial Jotamar
Tunja, Colombia - April, 2019

Publication of scientific-technical character
(Art. 4, Resolution 1508, Ministerio de Cultura of Colombia/October 3, 2000)

Internationally, the *Revista Colombiana de Ciencias Hortícolas* journal is indexed in Latindex, Google Académico, Family Farming Knowledge Platform (Plataforma de Conocimientos sobre Agricultura Familiar), Academic Search Ultimate (EBSCO), Red Iberoamericana de Innovación y Conocimiento Científico (REDIB), Ranking Rev-Sapiens, and the following databases of CAB Abstracts: AgBiotech News and Information, Agricultural Economics Database, Agroforestry Abstracts, Botanical Pesticides, Crop Physiology Abstracts, Crop Science Database, Environmental Science Database, Field Crop Abstracts, Forest Products Abstracts, Forestry Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Horticultural Science Database, Irrigation and Drainage Abstracts, Maize Abstracts, Nematological Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Genetics and Breeding Database, Plant Growth Regulator Abstracts, Plant Protection Database, Postharvest News and Information, Potato Abstracts, Review of Agricultural Entomology, Review of Aromatic and Medicinal Plants, Review of Plant Pathology, Rice Abstracts, Rural Development Abstracts, Seed Abstracts, Soil Science Database, Soils and Fertilizers Abstracts, Sugar Industry Abstracts, TROPAG & RURAL, Weed Abstracts, Wheat, Barley and Triticale Abstracts, World Agricultural Economics and Rural Sociology Abstracts.

For Colombia it is indexed in category B Publindex (Colciencias) and SciELO (Scientific Electronic Library Online). It also has a DOI (Digital Object Identifier) for all its articles.

Revista colombiana de ciencias hortícolas = Colombian journal of horticultural science / Sociedad Colombiana de Ciencias Hortícolas, Universidad Pedagógica y Tecnológica de Colombia - Vol.1, No.1 (2007) - Tunja: SCCH: Uptc, 2007 - Semestral

ISSN 2011-2173

1. Horticultura - Publicaciones Seriadas.- 2. Agricultura – Publicaciones Seriadas.- 3. Uptc - Publicaciones Seriadas

CDD 635

Publication co-financed by the Faculty of Agricultural Sciences of UPTC and the Universidad Francisco de Paula Santander

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EDITORIAL NOTE

DIEGO MIRANDA LASPRILLA
Chief editor
Revista Colombiana de Ciencias Hortícolas

The Revista Colombiana de Ciencias Hortícolas wishes to share the recognition granted by the Departamento para la Indexación de Revistas Científicas Colombianas Especializadas – Publindex (Colciencias) in Announcement 830 of 2018, placing the journal in category B. This significant achievement is indicative of the quality of the journal's articles, written by different research centers, universities, and companies that provide quality content to the journal.

Therefore, we are pleased to present to our readers the first issue of 2019, completely in English, with a digital structure that allows us to individually publish the articles and replace pagination as a citation element. However, a print version will be kept for a legal record and administrative matters, but this version will not be distributed to libraries or interested parties.

This issue contains a Fruit section that includes research on nitrogen fertilization in bananas and melons, anacardial propagation, pesticide residues in strawberry fruits and ripening applications in peach fruits.

The Vegetable section contains a varied selection of topics on inducers such as a study on resistance to *Melodoidogyne incognita* with silicon dioxide in tomatoes, the application of ascorbic acid for saline stress in radishes and the use of potassium silitate as a water stress elicitor in sweet corn. In terms of management, this sections also deals with applications of boron and calcium in tomato hybrids, improving SSF preparation with carrot and potato crop residues, and the evaluation of the herbicide flumioxazine in onions.

The Section on aromatic, medicinal and spice plants presents studies on the influence of climate on the quality of olives and olive oil produced in the tropics, as well as ammonia fertilization and types of light on the production of essential oils in *Lippia alba*.

This issue closes with a Scientific note on the asexual propagation of the monkey pepper, a promising shrub for the production of compounds.

These articles deal with issues involving edible species that are of general interest, addressing basic research and technical studies in horticultural production. Our intent is to increase discussion on the problems found in the various challenges of agricultural production.

Furthermore, we want to remind our authors to adapt the contents of their articles in accordance with the rules of publication since there are no resources or qualified personnel available to make extra adjustments. Similarly, it is mandatory that a cover image, editable graphics, research evidence, ORCID and standardized citations for authors be sent. Failure to comply with these requirements will be cause for manuscript rejection.

Doi: http://doi.org/10.17584/rcch.2019v13i1.8440

Effect of fertilization with N on height, number of leaves, and leaf area in banana (*Musa* AAA Simmonds, cv. Williams)

Efecto de fertilización nitrogenada sobre altura, número de hojas y área foliar de banano (*Musa* AAA Simmonds, cv. Williams)

JAIME TORRES-BAZURTO[1, 3*]
STANISLAV MAGNITSKIY[1]
JAIVER DANILO SÁNCHEZ[2]

Banana plantation in Uraba, Colombia.

Photo: J. Torres-Bazurto

ABSTRACT

The production of bananas (*Musa* AAA Simmonds, cv. Williams) for export in the Uraba area of Colombia represents economic and social benefits for the country, demanding improvements in agronomic practices, especially fertilization. The objective of this research was to evaluate plant height, leaf area and number of leaves in banana cv. Williams in two production cycles to adjust fertilizer recommendations according to the needs of the plants in the development phase. A sixth-generation crop was used, which was evaluated during five stages of development and two consecutive production cycles in 2011 and 2012. The multivariate approach was used for the analysis of variance of the repeated measures design, with two factors between subjects (fertilization and repetitions) and one intra-subject factor associated with the production cycle. The linear models were adjusted according to the phenological stage determined as when the vegetative structures were present, simultaneously evaluating responses in plant height, number of leaves, and leaf area. The effect of N doses on the developmental stages of the banana for production cycle and between cycles indicated that the doses of 321.8 and 483 kg ha^{-1} had the best yield. The dose of 483 kg ha^{-1} was the best one since it resulted in the highest plant growth.

Additional key words: plant nutrition; nitrogen fertilizers; growth control; Musaceae; tropical fruits.

[1] Universidad Nacional de Colombia, Facultad Ciencias Agrarias, Departamento de Agronomía, Bogota (Colombia). ORCID Torres-Bazurto, J.: 0000-0002-4731-650X; ORCID Magnitskiy, S.: 0000-0002-3715-1932
[2] Yara Colombia, North Zone Manager, Carepa (Colombia). ORCID Sánchez, J.D.: 0000-0003-1862-1748
[3] Corresponding author. jtorresb@unal.edu.co
* Article based on the PhD thesis of the first author "Absorción, distribución y acumulación de nitrógeno en banano variedad Williams en dos ciclos de producción en zona húmeda tropical".

RESUMEN

La producción de banano (*Musa* AAA Simmonds, cv. Williams) para exportación en la zona de Urabá, Colombia, representa beneficios económicos y sociales para el país, exigiendo mejorar las prácticas de manejo agronómico especialmente la fertilización. El objetivo de esta investigación fue evaluar la altura, área foliar y el número de hojas en banano cv. Williams en dos ciclos de producción para ajustar recomendaciones a valores acordes con las necesidades de la planta por fase de desarrollo. Se empleó un cultivo de sexta generación, el que se evaluó durante cinco etapas de desarrollo y dos ciclos productivos consecutivos (en los años 2011 a 2012). Se utilizó el enfoque multivariante para el análisis de varianza del diseño en medidas repetidas con dos factores entre-sujetos (fertilización y repeticiones) y uno intra-sujetos asociado al ciclo de producción. Se ajustaron los modelos lineales de acuerdo con la etapa fenológica considerada y las estructuras vegetativas presentes, evaluando simultáneamente las respuestas en altura de planta, número de hojas y área foliar. El efecto de las dosis de nitrógeno sobre las etapas de desarrollo del banano por ciclo productivo y entre ciclos permitió seleccionar las dosis de 321,8 y 483 kg ha^{-1} como las de mejor comportamiento. Sobresalió la dosis de 483 kg ha^{-1} por obtener el mayor crecimiento de la planta.

Palabras clave adicionales: nutrición vegetal; fertilizantes nitrogenados; control del crecimiento; Musaceae; frutas tropicales.

Received for publication: 24-02-2019 Accepted for publication: 29-03-2019

INTRODUCTION

The banana (*Musa* sp.) is considered one of the more important crops for diets worldwide, including the Colombian population (Muñoz, 2014). The planted area of bananas and plantains in Colombia is close to 380,000 ha (Danies, 2005), of which more than 48,000 ha corresponded to bananas for export (Quesada, 2013; AUGURA, 2014). At the global level, Colombia in 2016 was ranked 7th as a banana producer with 2,000,000 t of banana production (FAOSTAT, 2017). The export banana industry created around 40,000 direct and indirect jobs in 2004 and surpassed 60,000 jobs after 2012 (Espinal *et al*., 2005; Quesada, 2013).

Fertilization practices are an important issue in the management of banana production. Espinosa and Mite (2002), when analyzing achievements in banana fertilization practices from 1960 to 2002, indicated that, since 1992, most studies in the area were focused on adjusting the universal characteristics of banana fertilization to specific conditions of each region based on data from soil analyses. López and Espinosa (1995) proposed fertilizer ranges for N, P, K, Ca, and Mg based on nutrient contents in soil for banana producing countries in Central and South America. These authors raised the need to use soil analyses and reference values as a basis for regional adjustments of fertilizer doses (López and Espinosa, 1995).

Currently, the Uraba region faces certain challenges with productivity and fruit quality in the banana (Sánchez and Mira, 2013; Penagos, 2017); these problems originate in an inadequate balance of fertilizer use and affect investments in fertilizers, a cost that can amount to more than 40% of total production costs in bananas. Fertilizer rates in the region are traditionally lower than those suggested by Cenibanano (Colombia) for banana varieties cultivated in the area. A consequence of the management is the manifestation of nutrient disorders in the plants, such as a N deficiency, as reflected in shorter plants, smaller leaf area, generalized leaf chlorosis, and low yields. "Maturity spots" on the fruit surface generate losses in fruit quality in Uraba and are considered a physiological disorder attributed to a low availability of Ca in soils, which produces losses between 3 and 5% for a low incidence (rainy periods) and up to 18% for a high incidence (dry periods) (Sánchez and Mira, 2013). Nutrient problems in banana cultivation have made Cenibanano and various banana companies start research projects on banana nutrition in order to define optimal fertilization rates for cultivating and the area, reconciling technical, agronomic, and economic parameters.

Important morphological variables that describe the growth of banana plants include pseudostem height, number of leaves, and leaf area, which, among other factors, depend on the level of fertilization of the crop. Low levels of fertilization with N, Ca, or Zn frequently result in a reduced leaf area in bananas (Lahav and Turner, 1992), while excess (Moreira, 1999) or deficit (Freitas *et al*., 2015) N could result in a higher incidence of Sigatoka on the leaves. Adequate fertilization with N also affects plant height and leaf number in bananas (Lahav and Turner, 1992; Mustaffa and Kumar, 2012). At the same time, leaf number in *Musa* sp. serves as an important indicator of meristem changes from the vegetative to the reproductive development (Karamura and Karamura, 1995). Additionally, a minimum number of leaves, such as 12 in banana cv. Gran Nain at flowering, is required to produce fruits without problems in fruit quality or postharvest ripening (Rodríguez *et al*., 2012), while Lima *et al*. (2017) indicated that an increased number of leaves at flowering corresponded to an increased fruit length and mass in the 4th hand, as well as an increased bunch mass and extended postharvest period in cv. Nanica. Studies carried out on banana cultivars in different regions of the world have related the effects of mineral nutrition, especially with N, on these growth variables (Melo *et al*., 2010; Mustaffa and Kumar, 2012). On the other hand, in Colombia, such as in the banana producing region of Uraba, no studies have been reported on the subject, which is why it is necessary to evaluate the effect of fertilizer rates on height, number of leaves, and leaf area of the crop as part of the soil-plant relationship.

Studies in this area are important to the economic aspects of banana production in the Uraba region. The aspect that substantially affects banana production in Uraba is the excessive cost of fertilizers (Sánchez and Mira, 2013), which obliged the growers to search for alternatives that would reduce their expenses. Therefore, there is a need to improve fertilization practices and improve their efficiency, which includes adequate fertilizer doses and soil analysis as a tool to generate fertilizer recommendations. In this sense, plant growth responses to fertilizer rates could serve as an indicator of deficient or excessive applications of mineral nutrients. This research sought to evaluate the effect of fertilization with N on height, number of leaves, and leaf area in banana cv. Williams in two production cycles in Uraba, Antioquia.

MATERIALS AND METHODS

This study was carried out in the tropical humid region of Uraba-Antioquia (Colombia) in the experimental and demonstrative field of the Augura Company (Carepa, Antioquia), located at 7°46'46" N latitude and 76°40'20" W longitude at 20 m a.s.l. The soils were described as fine Fluventic Eutrudepts, clay loam over clay Fluvaquentic Eutrudept, and fine loam Vertic Endoaquept according to the USDA soil taxonomy (IGAC, 2007).

The climatic conditions at the study site included 87% average relative air humidity, maximum air temperature of 32.3°C, minimum air temperature of 23.2°C, and average air temperature of 26.7°C (the regional average was 27°C). The average solar brightness was 5 h d^{-1} for a total of 1,700 h $year^{-1}$. The average annual precipitation was 845 mm during the first production cycle (from August, 2011 to April, 2012) and 2,088 mm for the second production cycle (from February to December, 2012). Each production cycle lasted 10 months.

A banana crop (*Musa* AAA Simmonds cv. Williams) giant Cavendish subgroup, Williams clone was used. This plant has an average height of 3.5 m and is one of the two primary clones employed for export in different tropical regions of the world (Soto, 2001; Robinson and Galán, 2012). A banana plantation in the 6th productive cycle was used.

The fertilization plans recommended by Cenibanano for Uraba were used to establish the doses of fertilizers in accordance with the soil analysis (Sánchez and Mira, 2013). The nitrogen doses were (kg ha^{-1}): 1) absolute control without fertilization; 2) 0; 3) 161; 4) 321.8, and 5) 483. All treatments, except the absolute control, received the following fertilization (kg ha^{-1}): 87.1 P_2O_5, 678.8 K_2O; 50.5 CaO; 117.5 MgO; 64.2 S; 1.4 B; and 9.3 Zn. The sources of the mineral fertilizers were: Urea (46% of N), KMag (22% K_2O, 18% MgO, and 22 % S), fertiboro (10% B), solufos (30% P_2O_5, 36% Ca, 5% S, and 8% Si), ZnO (80% Zn), potassium sulphate (50% K_2O and 18% S), and KCl (60% K_2O).

Four replicates (blocks) per treatment were established, in which the treatments were randomized. Each of these replicates (blocks) was distributed based on the soil types in the experiment area. Each treatment was matched to the area units, called "botalones". Each "botalon" had an area of 1,563 m^2 and 250 banana plants, from which 15 plants were selected, with a height between 1.0 and 1.50 m. These plants formed an experiment unit and were evaluated according to the number of weeks and growth phases (Tab. 1). The agronomic management in the area was similar to that applied to the commercial banana crop in the Uraba region, except for the fertilizer doses. The fertilizer doses were split within each production cycle, for an average total of 17 applications of fertilizers per year, with approximately 3 weeks between the applications.

The phases of fruit filling and harvest during the first cycle of production coincided with the vegetative and floral differentiation phases of the second cycle. The time between the phases floral differentiation and flowering in the first cycle was extended one week because of the climatic conditions. The sampling time was selected considering the phase and time of plant development, with at least two weeks after the application of the respective fertilizer dose; in most cases, the sampling was done before the fertilizer treatments.

Table 1. Sampling times of the cv. Williams bananas according to the number of weeks and growth phases.

Phase of growth	Sampling time	Weeks of growth since sucker emergence
Vegetative	Vegetative growth	17-18
Reproductive	Flower differentiation	22-23
Productive	Flowering	39-40
Productive	Bunch filling	43-44
Productive	Harvest	50-51

Morphological variables

The following variables were measured in each growth phase:

1) Plant height (m), measured with tape from the union of the pseudostem with the corm to the last axilla of leaves;

2) Number of leaves. In each selected growth phase, the youngest fully expanded leaf was counted as the 1st leaf, then counting the number of leaves after it, considering the plant phyllotaxis;

3) Leaf area, measured on leaf number 3, using the length from the base of the leaf blade up to the apex and the width in the middle part of the leaf. The following mathematical equation from Turner (2003) was used:

$$AFT = (0.80) \times (0.662) \times L \times A \times N \qquad (1)$$

where AFT – total leaf area of the plant; L – length of the 3rd leaf (m); A – width of the 3rd leaf (m); 0.80 – proportionality factor; N – number of leaves; 0.662 – coefficient defined by the relationship between the total and the estimated leaf area.

A complete randomized block design was employed with five treatments and four replicates. The multivariate approach was used for the analysis of variance of the repeated measures design, with two factors between the subjects (fertilization and repetitions) and one intra-subject factor associated with the production cycle. The linear models were adjusted according to the phenological stage and the vegetative structures, simultaneously evaluating the responses in plant height, number of leaves, and leaf area. The data were processed using software programs SAS 9.3 (SAS Institute, Inc.), R, and Statgraphics Centurio XV (Statpoint Technologies, Inc.).

Plant height, leaf area, and number of leaves were evaluated jointly to adjust three models. One of these models described the vegetative, differentiation and flowering phenological stages, for which a repeated means design was proposed using the treatment as a factor between subjects, the cycle as an intra-subject factor and the blocks associated with the spatial variability of the soils, which facilitated adjusting the model. The other two models were defined for the phenological stages of fruit filling and harvesting; these models were performed independently; for this, the same design was used, with the same factors and blocks, to adjust the multivariate models. Additionally, since there was no interaction between the factors, contrast tests of variances between the control and other treatments were used to search for the effect of the treatments; this test was more robust than the one used in the multivariate model. A descriptive analysis was performed on the averages per treatment and phenological stage for each variable, as presented in the tables.

RESULTS AND DISCUSSION

Statistical results

The analysis of repeated measures for plant height, number of leaves and foliar area showed significant differences (P<0.0001) for the phenological stages between the vegetative stage and flowering (Tab. 2); no differences were found for the treatments or for the interaction phenological stage x treatment. In the fruit filling stage (Tab. 3), no statistical differences were found in the interaction block x treatment, but differences were found for the treatment. The contrasts between the control and the other treatments showed significant differences between the control and the treatments with N doses exceeding 321.8 and 483 kg ha^{-1}. At harvest, no significant differences were found for the factors, including the blocks, or their interactions; in addition, there were no differences observed in the control variances with other the treatments (Tab. 4). The physical soil variables (data not shown) did not affect any of the responses of the variables evaluated in the present study.

Plant height

The height of banana plants is an important parameter in crop management because it is related to plant

Table 2. Analysis of variance for plant height, number of leaves, and leaf area from the vegetative stage to flowering of the banana cv. Williams.

Source	DF	Type III SS	Mean square	F Value	Pr > F
Phenological stages (ef)	2	140602517925	70301258962	788.98	<.0001
Treatments (treat)	4	520400645.53	130100161.38	1.46	0.2302
ef*treat	8	830414365.73	103801795.72	1.16	0.3409
Error	45	4009663041.4	89103623.142		

Table 3. Analysis of variance for plant height, number of leaves, and leaf area at fruit filling of the banana cv. Williams with contrasts of variances between absolute control without fertilization (1) and the nitrogen doses (kg ha^{-1}): 0 (2); 161 (3); 321.8 (4) and 483 (5).

Source	DF	Type III SS	Mean square	F Value	Pr > F
Bloc	3	140818135	46939378	1.03	0.4145
Treat	4	1426612623	356653156	7.82	0.0024
Error	12	547483090	45623591		
Contrast	**DF**	**Contrast SS**	**Mean square**	**F Value**	**Pr > F**
1 vs. 2	1	74587772	74587772	1.63	0.2252
1 vs. 3	1	224924208	224924208	4.93	0.0464
1 vs. 4	1	1006394711	1006394711	22.06	0.0005
1 vs. 5	1	816060102	816060102	17.89	0.0012

Table 4. Analysis of variance for plant height, number of leaves, and leaf area at harvest of the banana cv. Williams with contrasts of variances between absolute control without fertilization (1) and the nitrogen doses (kg ha^{-1}): 0 (2); 161 (3); 321.8 (4) and 483 (5).

Source	DF	Type III SS	Mean square	F Value	Pr > F
bloc	3	83873165.4	27957721.8	1.77	0.2059
treat	4	12449880.2	3112470.1	0.20	0.9350
Error	12	189295132.6	15774594.4		
Contrast	**DF**	**Contrast SS**	**Mean square**	**F Value**	**Pr > F**
1 vs. 2	1	5045794.04	5045794.04	0.32	0.5821
1 vs. 3	1	7395170.06	7395170.06	0.47	0.5065
1 vs. 4	1	4011902.73	4011902.73	0.25	0.6232
1 vs. 5	1	10813499.55	10813499.55	0.69	0.4239

vigor and affects bunch harvesting (Nomura *et al*., 2017). The descriptive analysis with the averages of this variable (Tab. 5) showed an increase between the phenological stages for the two cycles as a response to plant development, with a tendency to stabilize plant height after flowering. For the first cycle, the N doses of 321.8 and 483 kg ha^{-1} resulted in the highest plant height until flowering and harvest, but, for fruit filling, this response was not so clear. For the second cycle, the effect of the N doses did not show a definite trend in most phenological stages, except at flowering and harvest, where the doses of 321 and 483 kg ha^{-1} resulted in the tallest plants. When comparing the responses of the phenological stages between cycles (Tab. 5), in the vegetative stage, the plants were taller for the first cycle than for the second; in flowering, the opposite trend occurred, when the plants were shorter in the first cycle and taller in the second one. These differences could be attributed to the changes in precipitation levels for those stages in the two cycles, when the second production cycle was characterized with very high rates of precipitation.

Mostafa (2005), while working with the cv. Williams for two cycles and N doses of 500 g/plant, reported plant heights at harvest between 2.72 and 3.08 m for the first cycle and 2.77 to 3.18 m for the second cycle, values similar to those reported (Tab. 5). Patel and Tandel (2013) found the largest height (1.62 m) with a nitrogen dose 100 g /plant, applied with drip irrigation, in banana *Musa paradisiaca* cv. Basrai. Kumar *et al*. (2003), for different cultivars of banana, achieved the highest average height (3.81 m) with doses of 480 kg ha^{-1}, while Patil and Shinde (2013) obtained the tallest plants with a nitrogen dose of 100 g/plant, supplemented with organic matter from manure and phosphorus solubilizing organisms, which resulted in the largest height (2.90 m) at harvest in banana cv. Ardhapuri.

Although Kumar *et al*. (2003) and Mostafa (2005) indicated that the higher the N dose the greater the height, this was not totally evident in the present study, where the doses of 321 and 483 kg ha^{-1} at harvest for the first cycle resulted in lower values than control (Tab. 5). This result could be attributed to the effect of nutrient recycling, such as of residual N in the soil, to the mother-sucker dependency (Bhende and Kurien, 2015), and to the climatic conditions for this period (Soto, 2001; Robinson and Galán, 2012). For the second cycle, our data were similar to that reported by other authors, when higher N doses resulted in the tallest plants (Tab. 5).

Number of leaves

The number of leaves during flowering serves as an important parameter for bunch development in bananas since these leaves are related to the plant photosynthetic rate and reflect the potential yield (Nomura *et al*., 2017). The number of leaves in the current study, according to the descriptive analysis, increased until flowering and subsequently decreased until harvest (Tab. 6), typical for this crop development (Soto, 2001). When comparing the cycles, variations between the phenological stages were

Table 5. Influence of the N dose on average plant height (m) of the banana cv. Williams.

Cycle	Dose of N (kg ha^{-1})	Growth stage				
		Vegetative	Flower differentiation	Flowering	Fruit filling	Harvest
1	Control	1.5	2.1	2.8	2.8	3.3
	0	1.6	2.0	2.5	3.1	2.9
	161	1.8	2.1	2.8	3.2	3.2
	321.8	1.8	2.1	2.9	3.1	3.1
	483	1.7	2.2	2.9	3.1	3.0
2	Control	1.3	2.2	3.1	2.6	2.7
	0	1.3	2.2	3.1	3.3	2.9
	161	1.2	2.0	3.0	3.1	2.9
	321.8	1.4	2.1	3.3	3.3	3.3
	483	1.2	1.8	3.5	3.3	3.1

observed, for example, in vegetative stage, the highest leaf number was observed in the first cycle, while at flower differentiation, the highest leaf numbers were in the second cycle (Tab. 6). These differences can be explained by the variations in the precipitation per cycle, when the second cycle had a precipitation rate exceeding 2,000 mm. In particular, cv. Williams is characterized by sensitivity in the stomata to vapor pressure deficits (Turner *et al*., 2007), which might be regarded as part of a water saving mechanism during low rainfall and, thus, contributed to a slower rate of leaf emission in the periods of low precipitation. When observing the behavior of the treatments per cycle, the application 321.8 kg ha^{-1} resulted in the highest number of leaves until flowering, while, for the second cycle, there was no predominance of any treatment for this variable.

Soto (2001) indicated that the average number of leaves at flowering for 10 banana clones, including the clone Dwarf Cavendish and the Great Dwarf, is between 10 and 15; these values (between 12 and 15), compared with the values for both cycles (Tab. 6) and for the same stage of development, could be considered typical. When our data were compared with the results of Patil and Shinde (2013), who worked with different clones, the values of the present research were low since these authors reported higher values for the stages before flowering.

Mostafa (2005), while working with Williams banana for two seasons and evaluating the effect of the N dose 784 kg ha^{-1} annual, found that the number of leaves to harvest varied between 11.6 and 13.9, different from what was found in the current research for the two cycles in the same growth stage (Tab. 6), between 4.8 and 7.5. This difference could be attributed to the practices of sanitary management, such as "leaf surgery", and the agronomic management used in the Uraba area (elimination of old leaves and those affected by Sigatoka), but there was sufficient leaf area to complete the production cycles.

Leaf area

Because leaf area is a variable that is dependent on the number of leaves, it behaved in a similar way, that is, increased in value until flowering and subsequently decreased at harvest (Tab. 7). In the stages vegetative, flower differentiation, and harvest, the leaf area was higher for the first cycle. For the other two stages (flower differentiation and fruit filling), the situation was the opposite, that is, lower values of leaf area were obtained in the first cycle, with the same explanation for these variations as seen for the number of leaves.

When observing the behavior of fertilizer treatments for this variable, a better response with the N doses 321.8 and 483 kg ha^{-1} was observed in three of the five phenological stages, including flowering, with a better response with 321.8 kg ha^{-1} between two cycles, except when flowering was the second cycle, where 483 kg ha^{-1}predominated over 321.8 kg ha^{-1}.

Table 6. Influence of N dose on the average number of leaves per plant in the banana cv. Williams.

Cycle	Dose of N (kg ha^{-1})	Growth stage				
		Vegetative	Flower differentiation	Flowering	Fruit filling	Harvest
		Number of leaves per plant				
1	Control	5.0	7.5	12.0	8.3	7.3
	0	4.8	7.8	12.3	8.8	7.5
	161	5.3	7.3	13.5	8.5	6.8
	321.8	5.3	7.3	14.0	9.8	6.8
	483	4.5	7.8	12.8	9.8	7.5
2	Control	4.8	8.5	12.3	10.8	4.8
	0	3.3	8.0	12.5	8.5	5.0
	161	3.5	8.0	11.8	10.8	4.8
	321.8	3.3	8.0	12.5	9.8	5.8
	483	4.5	7.0	12.8	10.3	5.0

Table 7. Influence of N dose on the average leaf area (m^2) in the banana cv. Williams.

Cycle	Dose N (kg ha^{-1})	Growth stage				
		Vegetative	Flower differentiation	Flowering	Fruit filling	Harvest
		Leaf area (m^2)				
1	Control	0.41	3.25	13.85	7.83	7.76
	0	0.50	2.69	12.28	9.14	6.92
	161	0.78	2.93	14.61	8.72	8.59
	321.8	0.72	3.25	15.63	11.74	7.78
	483	0.54	4.28	12.77	11.30	8.32
2	Control	0.46	4.31	13.76	8.84	5.20
	0	0.16	3.78	14.69	9.45	6.67
	161	0.24	2.47	11.58	11.30	5.13
	321.8	0.13	2.99	15.47	12.01	5.74
	483	0.73	2.33	17.30	11.75	5.56

Kuttimani *et al*. (2013), in the banana cv. Gran Nain at pre-flowering, found that different combinations of synthetic and organic fertilizers contributed to variation in the leaf area, between 12 and 18 m^2, a range which included the values of this variable in the current research at the flowering stage (Tab. 7) for the two production cycles (12.28 to 15.63 m^2 for the first cycle and 11.58 to 17.30 m^2 for the second one).

Kumar *et al*. (2003), Keshavan *et al*. (2014), Patil and Shinde (2013), when evaluating leaf area in different banana cultivars at harvest, found significant differences. The best responses in this variable were found by these authors when working with high N doses and included phosphorus solubilizing microorganisms and/or involved organic fertilization, results that show a better morphological response of the plants to high N doses. These results agree with the present research, with the treatments of 321.8 kg ha^{-1} for the two cycles and 483 kg ha^{-1} for the second cycle (Tab. 7), which presented the highest leaf area even though there were no statistical differences between the treatments, except for the one for the contrasts of variances.

In general, the research indicated a positive effect when increasing the N doses on plant height, number of leaves, and leaf area in the banana cv. Williams under the conditions of Uraba. Based on the results, a low-cost organic material is suggested for use in the banana crops, covering the difference between 321.8 and 483 kg ha^{-1} and applied in a regulated manner in order to achieve similar levels of growth. Additionally, in future research with different fertilization doses of one or several nutrients, the effect of the mother-sucker dependency on the studied variables should be investigated.

CONCLUSIONS

The effect of the nitrogen doses on banana growth per production cycle for the variables plant height, higher leaf number and leaf area was evaluated by stage of development, showing the doses of 321.8 and 483 kg ha^{-1} as the doses with the best performance. The highest rates of leaf growth were reported for various periods of crop development, such as flowering and fruit filling, except for the harvest. The dose of 483 kg ha^{-1} had the highest effect because it resulted in the greatest plant height and increased leaf number and leaf area in two production cycles.

ACKNOWLEDGMENTS

The authors express their gratitude to Augura Cenibanano (Carepa, Antioquia), Yara Colombia, and Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota for their financial support of the present study.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts the validity of the presented results at risk.

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Doi: http://doi.org/10.17584/rcch.2019v13i1.9720

Planting method, nitrogen fertilization and inoculation for diazotrophic bacteria for Cantaloupe melon plants

Método de siembra, fertilización con nitrógeno e inoculación con bacterias diazotróficas en melón Cantaloupe

EDUARDO PRADI VENDRUSCOLO[1, 3]
PAULO RICARDO OLIVEIRA[1]
LUIZ FERNANDES CARDOSO CAMPOS[1]
ALEXSANDER SELEGUINI[1]
SEBASTIÃO FERREIRA DE LIMA[2]

Melon fruit during development in protected environment.

Photo: E.P. Vendruscolo

ABSTRACT

The objective of this study was to evaluate the effects on plant development, productivity and fruit quality from combinations of planting methods, nitrogen fertilizer applications and inoculation with *Azospirillum brasilense* in Cantaloupe melons cultivated in a protected environment. A randomized blocks design was adopted with a 2×2×2 factorial scheme with five replications. The treatments consisted of combinations of planting methods (pre-established seedlings or direct field sowing), inoculation with *A. brasilense* (with or without) and nitrogen fertilization (with and without). During the vegetative phase, the height, stem diameter, number and length of plant internodes and relative chlorophyll content were evaluated. Fifty-five days after planting, the leaf nitrogen content, leaf area and dry mass were measured. At harvest, the number of days between planting and harvesting was calculated, and the fresh weight, circumference, length, bark and pulp thickness and fruit productivity were evaluated. It was verified that *A. brasilense* did not affect any of the evaluated characteristics. On the other hand, direct field seed sowing decreased the production time and provided good plant development. However, the size and productivity of the fruits were higher when pre-established seedlings were used, with or without inoculation with *A. brasilense,* fertilized with nitrogen. It was concluded that the combinations of the different sowing methods, nitrogen fertilization and inoculation with *A. brasilense* affected the development and characteristics of the Cantaloupe melon plants and fruits.

Additional key words: *Cucumis melo* L. var. *reticulatus* Naud; muskmelon; *Azospirillum brasilense*; sustainable management; soil acidification.

[1] Universidade Federal de Goiás, Escola de Agronomia, Setor de Horticultura, Goiás (Brazil). ORCID Vendruscolo, E.P.: 0000-0002-3404-8534; ORCID Oliveira, P.R.: 0000-0001-6094-743X; ORCID Campos, L.F.C.: 0000-0001-5171-5194; ORCID Seleguini, A.: 0000-0002-5762-9278

[2] Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul, Mato Grosso do Sul (Brazil). ORCID Lima, S.F.: 0000-0001-5693-912X

[3] Corresponding author. agrovendruscolo@gmail.com

RESUMEN

El objetivo de este trabajo fue evaluar los efectos sobre el crecimiento de las plantas, la productividad y la calidad de la fruta estudiando la combinación entre el sistema de siembra, fertilización con nitrógeno e inoculación con *Azospirillum brasilense* en melón cantaloupe cultivado en ambiente protegido. Se utilizó un diseño de bloques completos al azar en esquema factorial de 2×2×2, con cinco repeticiones. Los tratamientos correspondieron a: sistemas de siembra (trasplante de plántulas o siembra directa), inoculación con *Azospirillum brasilense* (con o sin) y fertilización con nitrógeno (con y sin). Durante la fase vegetativa, se evaluaron la altura, diámetro del cuello, número y longitud de entrenudos, así como el contenido relativo de clorofila total. También se obtuvo a los 55 días de la siembra el contenido de nitrógeno foliar, el área foliar y la masa seca. En la cosecha, se calculó el número de días entre la siembra y la cosecha, junto con la masa fresca, circunferencia, longitud, grosor de la corteza y pulpa, y el rendimiento de la fruta. Se verificó que la inoculación con *A. brasiliense* no alteró ninguna de las características evaluadas. Por otro lado, la siembra directa en el campo disminuyó el tiempo de producción y proporcionó un mejor desarrollo de las plantas. Sin embargo, el trasplante de plántulas y la fertilización con nitrógeno, incrementó el tamaño y la productividad de las frutas de melón Cantaloupe. Se concluyó que la combinación entre diferentes métodos de siembra, fertilización nitrogenada e inoculación con *A. brasilense* afecta el desarrollo y las características de las plantas y frutos de melón Cantaloupe.

Palabras clave adicionales: *Cucumis melo* L. var. *reticulatus* Naud.; melones; *Azospirillum brasilense*; manejo sostenible; acidificación del suelo.

Received for publication: 06-01-2019 Accepted for publication: 29-03-2019

INTRODUCTION

In twenty years, melon (*Cucumis melo* L.) production has increased by 14.5 million tons, between 1994 and 2014, and China is the top producer, with more than half of the total fruit production (FAO, 2017). In Latin America, about 1.0 million tons of melons are produced every year. Brazil provides approximately 50% of this amount, with a cultivation area of 22.000.000 (FAO, 2017).

In the majority of cases, particularly in yellow melons, which are the most produced domestically, the cultivation of melons in Brazil is carried out in the open field (Souza *et al*., 2014). However, protected environments have grown because they facilitate characteristics for management that protects against biotic and abiotic stresses (Chang *et al*., 2013) and produce fruits that have a higher physicochemical quality (Coelho *et al*., 2003; Vargas *et al*., 2008), mainly with fruits with higher value added, such as Cantaloupe melons (*Cucumis melo* L. *reticulatus* Naud.), which can result in high profitability for producers (Vendruscolo *et al*., 2017).

Cantaloupe melon plants responds positively to nitrogen fertilization with significant increases in fruit yield when high doses of nitrogen, varying from 160 to 413 kg ha^{-1}, are applied in crops cultivated under protected environment (Coelho *et al*., 2003; Fontes *et al*., 2004; Queiroga *et al*., 2011; Silva *et al*., 2014). Excessive applications of nitrogen fertilizers, however, can result in a high release of H^+ ions in the soil solution, causing acidification (Lu *et al*., 2014). Thus, alternatives that decrease the use of nitrogen fertilization and/or increase its efficiency should be the target of research, aiming at sustainability in the productive system.

The use of diazotrophic bacteria is widely discussed and has a high application potential for crops of economic interest, mainly cereals (Hungria, 2011; Araújo *et al*., 2014; Andrade *et al*., 2016; Bulegon *et al*., 2016). However, for fruit species, few results have been provided, mainly because of the absence of studies dedicated to this area. These bacteria have been observed in the production of tomato and onion seedlings. Pulido *et al*. (2003) observed positive developmental effects when inoculation of *A. brasilense* was used in seed pretreatment. In tomato production, Guevara *et al*. (2013) observed a savings of about 30% in the use of nitrogen fertilizers when the plants were submitted to inoculation with *Azospirillum brasilense*.

The objective of this study was to evaluate the effects on the vegetative and productive characteristics of combinations of planting systems, nitrogen fertilization and inoculation with *Azospirillum brasilense* in Cantaloupe melon plants grown in a protected environment.

MATERIAL AND METHODS

This study was conducted in Goiânia, Goiás state, Brazil. This municipality is located in the central region of the State, 16°40'S and 49°15'W, with an altitude of 750 m. According to Cardoso *et al*. (2014), the climate is Aw according to the classification of Köppen-Geiger, characterized by a tropical climate with a rainy season from October to April and a period with monthly precipitations below 100 mm between May and September. Mean monthly temperatures range from 20.8°C in June and July to 25.3°C in October (Cardoso *et al*., 2014). During the experiment, the climatic records of the air temperature and humidity inside the protected environment (Fig. 1), were obtained with a digital datalogger (AK172, Akso, São Leopoldo, RS, Brazil).

The protected environment used for the cultivation was an arch model, 21 m long, 7 m wide, and 2.10 ft high, with a 4 m ridge, covered with transparent plastic and the sides closed with a white anti-aphidic screen. The front and rear openings, equivalent to arches, provided greater ventilation, avoiding excessive heat inside the growing environment.

Santos *et al*. (2013) stated that the soil present in the experimental area is Latosolo Vermelho. According to the analysis established by Embrapa (Donagemma *et al*., 2011), this kind of soil has the following characteristics: Ca^{2+}=5.70 $cmol_c$ dm^{-3}, Mg^{2+}=3.00 $cmol_c$ dm^{-3}, K^+=96.00 mg dm^{-3}, P (Mehlich I)=170.00 mg dm^{-3}, organic material=23.00 g kg^{-1}, Al^{3+}=0.0 $cmol_c$ dm^{-3}, H+Al=2.40 $cmol_c$ dm^{-3} and pH ($CaCl_2$)=5.50. The granulometric analysis of the soil presented 48.00 g kg^{-1} of clay in the layer 0 - 0.20 m, following the analysis proposed by Silva (2009).

The experiment was designed in randomized blocks, in a 2×2×2 factorial scheme with five replications. The treatments consisted of combinations of planting systems (pre-established seedlings or direct field sowing), inoculation with *Azospirillum brasilense* (with or without) and the application of 120 kg ha^{-1} of nitrogen in the form of urea (with and without). Each plot had five plants, and the three central plants formed the useful plot.

For the formation of seedlings, on September 7th, 2016, seeds of Cantaloupe melon, cv. Trinity, were sown in expanded polystyrene trays containing 128 cells, filled commercial turfous substrate (Germinar, Bioflora, Prata, MG, Brazil). Twenty-three days after being sown, the seedlings were transplanted to previously prepared beds, fertilized with 4 L m^{-1} of tanned bovine manure (88.00 g kg^{-1} de M.O.; pH=7.20; 82.00 g kg^{-1} N; 3.10 g kg^{-1} P (Mehlich); 20.80 g kg^{-1} K; 3.00 g kg^{-1} Ca; 3.00 g kg^{-1} Mg; 50.00 g kg^{-1} C; 0.19 mg kg^{-1} Fe; 36.00 mg kg^{-1} Mn; 26.50 mg kg^{-1} Zn; 0.00 mg kg^{-1} Cu; 0.60 C/N ratio) and 15 g m^{-1} of Yoorin Master.

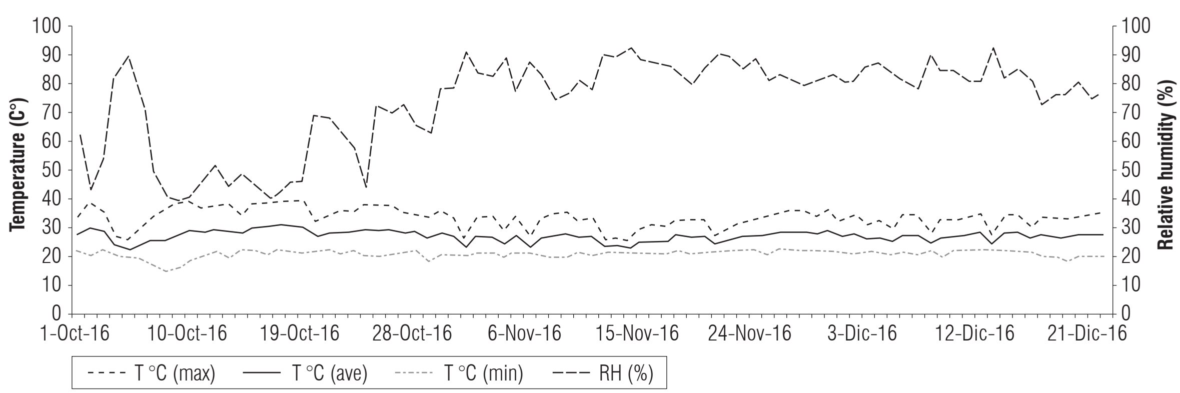

Figure 1. Summary of climatic conditions of relative air humidity and maximum, average and minimum temperature during the study. Goiânia-GO, 2016.

Meanwhile, the sowing was also carried out directly in the beds, according to the treatment. For the planting, a space of 0.45×0.8 m was used between plants and between rows (beds), respectively (Vendruscolo *et al*., 2018). The nitrogen applications were performed manually. The equivalent of a 40 kg ha^{-1} dose of urea was diluted in 500 mL of water, and the solution was applied throughout the plot, at 15, 30 and 45 d after planting. *A. brasilense* was applied in a dose of 10 mL L^{-1} of the commercial product (NITRO1000 Gramineae) as soon as the first nitrogen fertilization was carried out.

The plants were irrigated with drip tapes spaced 20 cm, vertically conducted using plastic wires as tutors. For the control of fungal diseases, whitefly and *Diaphania* spp., fungicide based on Metiram and Piraclostroblina (55% and 5% a.i.) and insecticides based on Thiamethoxam (25% a.i.) and Lambda cyhalothrin (25% a.i.) were applied at 10, 15 and 64 d after installation of the experiment.

The evaluation of the plants' biometric characteristics was carried out 15, 30 and 45 d after sowing, when the values of height, diameter at soil level, and number and length of the internodes were measured. Also, the relative content of total chlorophyll was measured with a digital chlorophyllometer (CFL1030; Falker, Porto Alegre-RS, Brazil) on a medium-plant leaf. Within 55 d, the relative contents of chlorophyll, leaf area and dry mass of the fourth leaf, taken from the plant apex, were also evaluated.

The harvest started 72 d after planting and continued for a period of 10 d. Within this period, the number of days between planting and harvesting was evaluated and the fresh fruit weight characteristics were obtained with a digital scale (W15, Welmy, Sta. Bárbara d'Oeste-SP, Brazil). The fruit circumference was measured with a metric tape, and the fruit length and bark and pulp thickness were obtained with a digital caliper (Metrotools, São Paulo, SP, Brazil). The productivity was estimated for an area of 1 ha, the soluble solids content (°Brix) was read with a manual refractometer RTA-50 (Instrutherm, São Paulo, SP, Brazil), the titratable acidity was obtained with titration with a NaOH solution (1M), and the ratio between the latter two variables provided the Ratio (SS/TA).

The data were submitted to analysis of variance, and the means were compared with the Tukey test at 5% probability, using statistical software Sisvar (Ferreira, 2014).

RESULTS AND DISCUSSION

It was verified that inoculation with *Azospirillum brasilense* did not affect the vegetative plant development in isolation. However, the planting system and the nitrogen cover fertilization influenced plant height, stem diameter, number of nodes, length of internodes and relative levels of total chlorophyll (Tab. 1).

During the entire period of evaluation of the vegetative characteristics, a higher height and number of nodes were observed in the plants grown from pre-established seedlings (Tab. 1). This result was due to uniform seedling development, maintaining constant superiority, because they were transplanted with an initial height of approximately 15 cm and with two to three nodes in the main branch.

For the stem diameter and the length of the internode, the superiority of the plants obtained with direct sowing in the beds was observed starting from the second evaluation (Tab. 1). This result may be related to the restriction of space and nutrients to which the pre-established seedlings were submitted during the initial development in the trays, resulting in a less thick stem and shortening of the initial internodes.

There was also superiority in the plants from pre-established seedlings for the relative contents of total chlorophyll in the first and second evaluation (Tab. 1). In the development of the plants obtained from direct sowing, however, this variable was equal in the third evaluation. Thus, larger leaf quantities, as well as the increase in their size, contributed to self-shading in the medium portion of the melon plants. According to Gonçalves *et al*. (2012), higher shading rates increase the relative chlorophyll content in the leaf limbus.

The nitrogen application did not interfere with the vegetative characteristics of the melon plants (Tab. 1). These results are related to favorable soil chemical characteristics, as well as to the addition of tanned bovine manure, which served as a source of nitrogen and other nutrients during the establishment and development of the melon plants.

Fifty-five days after planting, no difference was observed in the leaf nitrogen contents of the fourth leaf from the plant apex. However, throughout the evaluation of the leaf area and dry mass, it was observed that direct seeding favored leaf development (Tab. 2).

Table 1. **Plant height, stem diameter, number of nodes, internode length and total relative chlorophyll content (RCC) in melon plants cultivated in different production systems. Goiânia-GO, 2016.**

Planting system	Plant development				RCC (SPAD)
	Height (cm)	Diameter (mm)	Nº of nodes -	Internode (cm)	
15 days after planting					
Seed	10.09 b	5.26 a	4.06 b	2.49 b	40.86 b
Seedling	44.63 a	5.56 a	10.17 a	4.36 a	43.31 a
Nitrogen					
With	27.07 a	5.42 a	7.33 a	3.41 a	42.49 a
Without	27.65 a	5.40 a	6.90 b	3.44 a	41.69 a
CV%	32.49	14.78	14.97	24.13	6.65
30 days after planting					
Seed	120.67 b	8.59 a	15.42 b	7.81 a	48.02 b
Seedling	158.73 a	7.68 b	23.33 a	6.79 b	53.21 a
Nitrogen					
With	137.83 a	8.07 a	19.31 a	7.21 a	50.52 a
Without	141.56 a	8.19 a	19.44 a	7.40 a	50.71 a
CV%	14.05	10.90	10.82	10.30	6.97
45 days after planting					
Seed	215.00 b	9.56 a	27.52 b	7.82 a	49.01 a
Seedling	233.00 a	8.45 b	33.33 a	7.01 b	50.01 a
Nitrogen					
With	229.00 a	9.11 a	30.72 a	7.52 a	49.86 a
Without	219.00 b	8.91 a	30.13 a	7.31 a	49.16 a
CV%	10.71	13.45	8.97	9.13	8.36

Means followed by same letters in the columns did not differ according to the Tukey test ($P \leq 0.05$); CV: coefficient of variation.

Table 2. **Leaf area and dry mass at 55 d after planting, on melon plants cultivated in different production systems. Goiânia-GO, 2016.**

Planting system	Leaf area (cm^2)	Leaf dry mass (g)
Seed	87.41 a	0.33 a
Seedling	78.00 b	0.29 b
Nitrogen		
With	85.90 a	0.32 a
Without	79.50 b	0.30 a
CV%	17.92	21.57

Means followed by same letters in the columns did not differ according to the Tukey test ($P \leq 0.05$); CV: coefficient of variation.

The results can be attributed to the higher nutrient translocation capacity favored by the larger stem diameters obtained for these plants, including nitrogen, which also had a positive influence on leaf development. This result agrees with Brunes *et al.* (2015) who inferred that secondary stem growth represents a relevant factor to the accumulation of dry matter in the aerial part of plants once the organ obtains the function of transporting essential elements, such as water, nutrients and mineral salts.

For the transverse circumference, as well as the longitudinal circumference and productivity, an interaction between the three factors (Tab. 3) was observed. In general, it was verified that the nitrogen fertilization applied to the plants coming from pre-established seedlings, not inoculated with *A. brasilense*, resulted in a higher fruit development and, consequently, in an increased productivity (Tab. 3).

Table 3. Transversal circumference, longitudinal circumference and productivity of the Cantaloupe melon fruits according to the different production systems. Goiânia, 2016.

Transverse circumference (cm)				
Planting system	*Azospirillum brasilense*			
	With		Without	
	Nitrogen			
	With	Without	With	Without
Seed	42.21 aA1	41.55 bA1	41.63 bA1	41.19 aA1
Seedling	42.71 aA1	43.76 aA1	43.85 aA1	39.07 aB2
CV%	7.08			
Longitudinal circumference (cm)				
Planting system	*Azospirillum brasilense*			
	With		Without	
	Nitrogen			
	With	Without	With	Without
Seed	45.22 aA1	43.09 bA1	43.35 bA1	43.64 aA1
Seedling	45.03 aA1	46.33 aA1	46.84 aA1	42.15 aB2
CV%	7.94			
Produtivity (Mg ha^{-1})				
Planting system	*Azospirillum brasilense*			
	With		Without	
	Nitrogen			
	With	Without	With	Without
Seed	36.79 aA1	31.19 bA1	33.13 bA1	33.49 aA1
Seedling	38.08 aA1	40.36 aA1	41.21 aA1	30.06 bB2
CV%	24.41			

Means followed by the same lowercase letter in the columns, upper case in the rows for the factor *A. brasilense*, and the same numbers in the lines for the nitrogen factor do not differ according to the Tukey test at 5% probability; CV: coefficient of variation.

Under the experiment conditions, the productivity of the Cantaloupe melons fertilized with nitrogen reached a mean of 37.30 Mg ha^{-1}, 10.4% higher than the yield of 33.78 Mg ha^{-1} obtained in the plots without nitrogen fertilization. Similar results were observed in a study by Silva *et al*. (2014) who verified that nitrogen fertilizations up to the 160 kg ha^{-1} dose provided increases in fruit yield and quality. Results described by Fontes *et al*. (2004) and Queiroga *et al*. (2011) also showed positive responses of the development of Cantaloupe melon fruits up to doses of 373 and 413 kg ha^{-1} of N, respectively.

It was verified that the inoculation with the bacteria favored the development and the productivity of fruits in the plants obtained from direct field sowing when combined with the nitrogen cover fertilization and in the plants obtained from pre-established seedlings when nitrogen cover fertilization was not carried out (Tab. 3). The application of *A. brasilense* may not substitute nitrogen fertilization, but some studies have suggested that the inoculation of plants with these bacteria may promote better utilization of available soil nitrogen (Saubidet *et al*., 2002), improving the development of morphophysiological characteristics (Taiz *et al.*, 2017).

Despite the limited information regarding inoculation with *A. brasilense* in horticultural species, studies have demonstrated its effectiveness. Working with tomato, Guevara *et al*. (2013) observed that plant inoculation with *A. brasilense* allowed a savings of about 30% in the use of nitrogen fertilizers. Inoculation with *A. brasilense* as a seed treatment was also effective for the formation of tomato and onion seedlings, favoring development through the improvement of nutritional status (Pulido *et al*., 2003).

It was also verified that, when inoculation with *A. brasilense* or nitrogen cover fertilization was not carried out, the plants obtained from direct sowing provides greater fruit development and productivity. The larger diameter of the stem, observed in the plants obtained from direct sowing, along with the non-restriction of the space for the initial development of the roots, may have contributed to a greater exploitation of the soil, culminating in greater efficiency in the absorption and translocation of nutrients and others substances (Nibau *et al*., 2008; Brunes *et al*., 2015; Borcioni *et al*., 2016).

The treatments used in the present study did not influence the average production time, in days, between the planting and the harvest, with a mean of 76 d. However, this result shows a higher precocity of production in plants obtained from direct sowing since the formation of seedlings in the trays lasted exactly 23 d.

Thus, the development of new studies that focus on the increase of the productive capacity of plants obtained from direct sowing is recommended. The improvement of this technique will contribute to greater gains for the rural producer. Moreover, it will eliminate the costs associated with labor, structures and inputs in seedling production.

CONCLUSIONS

Different combinations of the sowing methods, nitrogen fertilization and inoculation with *A. brasilense* affected the development and characteristics of the Cantaloupe melon plants and fruits.

ACKNOWLEDGMENTS

The authors thank the Coordination of Improvement of Higher Level Personnel - CAPES for granting the scholarship to the first author.

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Quality of dwarf cashew tree seedlings as a result of methods for breaking dormancy, container volume and application of bovine biofertilizer

Calidad de plántulas de cajueiro enano como resultado de métodos de rompimiento de dormancia, volumen de matera y aplicación de biofertilizante bovino

TONI HALAN DA SILVA IRINEU[1]
MÁRIO LENO MARTINS VÉRAS[2, 5]
JOSIMAR NOGUEORA DA SILVA[1]
FRANCISCO HÉLIO ALVES DE ANDRADE[3]
RAIMUNDO ANDRADE[4]

Cashew tree seedlings, Catolé do Rocha-PB, Brazil.

Foto: T.H.S. Irineu

ABSTRACT

The dwaf cashew tree presents enormous economic relevance for the Brazilian northeast. When establishing orchards of this species, the most important phase is the formation of the seedlings. Therefore, studies aiming to improve growth aspects and seedling qualities are extremely relevant. In this sense, the present study aimed to evaluate the growth and quality of dwarf cashew tree seedlings as a result of methods for breaking dormancy, container volume and application of bovine biofertilizer. The experiment design was completely randomized, with a 2×2×5 factorial scheme and six replicates; the experimental unit consisted of five seedlings. The treatments involved two methods of breaking dormancy (scarification with sandpaper and immersion in water), two container volumes (1 and 2 kg) and five doses of bovine biofertilizer (0, 2.5, 5.0, 7.5 and 10 mL). The method for breaking dormancy with normal water for 24 hours was the most adequate for the formation of dwarf cashew tree seedlings. The doses of 7.5 and 10 mL of bovine biofertilizer provided higher growth and quality in the dwarf cashew tree seedlings. The volume of 2 kg of substrate was the most suitable volume for growing dwarf cashew trees.

[1] Federal University Rural Semiarid, Department of Plant Science, Mossoró (Brazil). ORCID Irineu, T.H.S: 0000-0003-4628-5833; ORCID Silva, J.N.: 0000-0001-9508-5526
[2] Federal University of Viçosa, Department of Plant Science, Viçosa (Brazil). ORCID Véras, M.L.M.: 0000-0001-5968-4564
[3] Federal University of Lavras, Department of Plant Physiology, Lavras (Brazil). ORCID Andrade, F.H.: 0000-0002-9699-1413
[4] State University of Paraíba, Department of Agrarian and Exact, Catolé do Rocha (Brazil). ORCID Andrade, R.: 0000-0002-0295-468X
[5] Corresponding author. mario.veras1992@gmail.com

Additional key words: *Anacardium occidentale* L.; propagation by cuttings; dormancy breaking; biofertilizers; fruits.

RESUMEN

El cajueiro presenta enorme relevancia económica para el Nordeste Brasileño. Para el establecimiento de huertos de esta espécie, la fase más importante es la formación de plántulas. Por esto, estudios para mejorar aspectos de crecimiento y calidad de plántulas son de gran importancia. En tal sentido, el objetivo del presente trabajo fue evaluar el crecimiento y calidad de plántulas de cajueiro en respuesta a métodos de rompimiento de dormancia, volumen de la matera y aplicación de biofertilizante bovino. Se utilizó el diseño experimental completamente al azar con arreglo factorial 2×2×5, seis repeticiones y la unidad experimental de cinco plántulas. Los tratamientos consistieron en dos métodos de rompimiento de dormancia (escarificación por lija e inmersión en agua), dos volúmenes de matera (1 y 2 kg) y cinco dosis de biofertilizante bovino (0; 2,5; 5,0; 7,5 y 10 mL). El método de rompimiento de dormancia con agua normal durante 24 horas fue el más adecuado para la formación de plántulas de cajueiro. Las dosis 7,5 y 10,0 mL de biofertilizante bovino proporcionaron mayor crecimiento y calidad de plántulas de cajueiro. El volumen 2 kg de sustrato fue el volumen más indicado para el cultivo de plántulas de cajueiro.

Palabras clave adicionales: *Anacardium occidentale* L.; propagación por esquejes; salida de latencia; biofertilizante; fruticultura.

Received for publication: 21-07-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is one of the most cultivated fruits in the Brazilian, northeastern semi-arid region, presenting an important crop in the generation of employment and income since it has high use potential, both for fresh consumption of pseudofruit and for processing (Araújo *et al*., 2014; Silva *et al*., 2019).

During the formation of orchards of this species, the seedling formation phase significantly contributes to the costs. Germination is a very important factor in the process of seedling formation since many species' present dormancy. Dormancy consists of the phenomenon in which the seeds of a certain species, even if they are viable and have favorable environmental conditions for germinating, stop doing so (Costa *et al*., 2010; Carvalho *et al*., 2019).

The methods most used to breaking seed dormancy include scarification (Ursulino *et al*., 2019) and immersion, which are more practical and safer methods for small farmers who desire seedlings in a short period of time. Since the cashew presents dormancy, studies on methods for breaking dormancy are extremely relevant (Mata *et al*., 2010). Therefore, it is necessary to seek more efficient methods to breaking dormancy, which is a point of paramount importance for the formation of seedlings with high quality.

Also, seedling producers face uncertainty in the choice of containers for the production of seedlings. The size of the appropriate container is an important aspect as it may influence the health of the seedlings. Studies with container volumes have shown that the volume of 2 L of substrate influences the vegetative and quality behavior of the guava and pineapple (Paiva *et al*., 2013; Diniz *et al*., 2015).

In the formation of fruit orchards, the most important step is the seedling formation phase. This phase is very demanding in terms of nutrients; therefore, fertilizer alternatives are extremely relevant because well-formed seedlings influence the quality of the orchard. Many seedlings have been using bovine biofertilizer because of the rapid absorption, making it very useful for annual and perennial crops in conventional and organic systems or in rapid treatments of nutritional deficiencies in plants.

Bovine biofertilizer has been used as a way to meet the nutritional needs of macro and micronutrient plants and organic matter; in addition, when biofertilizer is applied in liquid form, it provides greater displacement of nutrients in the soil, facilitating absorption by plant roots, thereby promoting better initial growth (Dutra *et al*., 2018; Melo Filho *et al*., 2018; Alves *et al*., 2019). According to Sá *et al*. (2013), biofertilizer, when applied via the soil, favors the activity of microorganisms and promotes better nutrient availability to the roots.

In addition to be a product rich in nutrients that are essential for plants, the bovine biofertilizer is low-priced and easily prepared by small farmers. Some studies with biofertilizers have shown positive results, being able to attenuate possible nutritional deficiencies and favor the growth of plants in the phase of seedling production (Sá *et al*., 2013; Diniz *et al*., 2015; Alves *et al*., 2019).

Because of the scarcity of studies on early dwarf cashew tree seedlings, the present study aimed to evaluate the growth and quality of cashew tree seedlings as a result of methods for breaking dormancy, container volumes and application of bovine biofertilizer.

MATERIAL AND METHODS

This experiment was conducted in 2017, for a period of 71 d in a protected environment, with 50% shading, at the State University of Paraiba - UEPB, Campus-IV, municipality of Catole do Rocha-PB, Brazil, located geographically at latitude 6°20'38" S and longitude 37°44'48" O with an altitude of 275 m, with a monthly average temperature above 18°C.

The experiment design was completely randomized, with a 2×2×5 factorial scheme and six replicates; the experiment unit consisted of five seedlings. The treatments consisted of two methods of breaking dormancy (scarification with sandpaper and immersion in water), 2 container volumes (1 and 2 dm^3) and five doses of bovine biofertilizer (0, 2.5, 5.0, 7.5 and 10 mL).

The seedlings were cultivated in containers filled with earthworm humus and soil classified as Eutrophic Flubic Neosol (Santos *et al*., 2013), which had the following characteristics: pH (H_2O)=6.85; nutrients ($cmol_c$ dm^{-3}): Ca^{2+}=2.10, Mg^{2+}=1.07, Na^+= 3.73, H=0.13, Al^{+3}=0.01, cation exchange capacity=5.94, base sum=5.8; K^+=0.01 mg dm^{-3}; organic carbon=4.55%; organic matter=7.85%; assimilable phosphorus=8.31 mg/100 g; soil texture (g kg^{-1}): sand=63.90, silt=20.65 and clay=15.45; apparently density=1.41 g cm^{-3}; field capacity humidity=11.23 g kg^{-1}; permanent wilting point humidity=6.56 g kg^{-1} and textural class=sandy loam; the soil was analyzed according to the procedures described in the methodology of Santos *et al*. (2013).

The worm humus had the following characteristics (meq/100 g): Ca=35.40, Mg=19.32, Na=1.82, K=1.41, S=57.95, H=0.00, Al=0.00, T=57.95, qualitative calcium carbonate = present and assimilable phosphorus=55.14. Others: pH H_2O (1:2.5)=7.38, eletric conductivity (EC)=2.11 dS m^{-1}.

The breaking of the dormancy in the cashew nuts was accomplished with two methods (scarification with sandpaper and immersion in water for 24 h). After treatments for dormancy, the cashew nuts were sown directly in two-pack polyethylene bags (type 1: 18×22 cm and type 2: 22×30 cm), containing 1 and 2 dm^3 of substrate, respectively.

For the production of the bovine biofertilizer, the methodology of Silva *et al*. (2012) was used. The applications of the bovine biofertilizer were done 30 d after the emergence of the plants in the container, 7 to 7 d apart, applying biofertilizer doses (0, 2.5, 5.0, 7.5 and 10 mL/plant) directly under the substrate of each container, with a total of six applications.

The bovine biofertilizer had the following chemical characteristics: pH=5.27, EC=4.81 (dS m^{-1}); N=1.30 (g kg^{-1}); P=537.0 (mg dm^{-3}); S=9.55 (mg dm^{-3}); Na=2.47 ($cmol_c$ dm^{-3}); K=1.34 ($cmol_c$ dm^{-3}); Ca=3.00 ($cmol_c$ dm^{-3}) and Mg=4.45 ($cmol_c$ dm^{-3}).

At 71 d after sowing, six plants were sampled per treatment for determinations of: height (cm) from lap bud to apical bud; stem diameter (mm); measured at a distance of 2.0 cm from the neck, using a pachymeter with an accuracy of 0.01 mm; number of leaves; root length, measured with a stepwise ruler in cm; and leaf area, where the leaves were considered to have a minimum length of 1.0 cm, measuring the leaf's main leaf length x width x adjustment factor (0.60) (Peixoto and Peixoto, 2009). The total leaf area was determined by multiplying the leaf area by the number of leaves.

Subsequently, the vegetative parts were separated and placed in paper bags and subjected to forced air circulation in an oven for 48 h at a temperature of 60°C±1.0 until constant mass was obtained and then weighed on an analytical balance with an accuracy of 0.0001 g, with the results expressed in grams. The difference between the fresh mass and dry mass provided the constant dry mass of the plants. At the end of the experiment, the Dickson Quality Index (DQI) was determined (Dickson *et al*., 1960).

The data were analyzed and interpreted with analysis of variance (Test F) and comparison of averages with the Tukey test, following Ferreira (2014), with a minimum significance of 5% probability. The analyses were performed with SISVAR statistical software v 5.0.

RESULTS AND DISCUSSION

It was observed that the interaction between the factors did not present a significant difference. The studied pot volumes statistically influenced the height, root length and leaf area of the seedlings. The methods for breaking dormancy did not influence the analyzed variables. The doses of bovine biofertilizer significantly influenced all variables.

It was observed that the height of the cashew seedlings was positively influenced by the doses of bovine biofertilizer, presenting a quadratic polynomial behavior, with higher values in height (17.36 cm) in the seedlings treated with 10 mL of bovine biofertilizer, with an increase of 14.17% in relation to the seedlings that did not receive application of bovine biofertilizer (Fig. 1A).

The results corroborate those found by Veras *et al*. (2014) who, when evaluating the growth of cashew tree seedlings subjected to doses of biofertilizer and substrate volumes, observed that the cashew had a higher yield under the application of biofertilizer at a dose of 120 mL.

The doses of biofertilizer provided a significant difference for stem diameter ($P \leq 0.01$), and the regression equation adjusted to the experiment data presented a quadratic polynomial model, in which, as the doses of biofertilizer were increased, there was an increase in diameter, with the highest value of 6.08 (mm) obtained with the dose of 10 mL (Fig. 1B).

These values are higher than those obtained by Costa *et al*. (2012), who evaluated the growth of cashews irrigated with domestic effluent and verified that the diameter of the seedlings was not influenced by the wastewater, presenting 1.0 (cm), statistically equal to that found in those irrigated with a water supply (control), values lower than those found in the present study.

The number of leaves presented a quadratic behavior, with a statistical significance ($P \leq 0.01$), obtaining a higher value (14 leaves) in the seedlings that received an application of 7.5 mL of bovine biofertilizer; as the biofertilizer dose was increased, there was a decrease in leaf number of 0.0224 with the recommended optimum dose of 7.5 (Fig. 1C).

This result is greater than that obtained by Costa *et al*. (2012) when studying cashew tree seedlings irrigated with domestic effluent, which obtained 13 leaves, found in the highest percentage of residual water.

The highest number of leaves is a result of the positive effect of the bovine biofertilizer, which may have stimulated the release of humic substances and, consequently, released nitrogen and carbon, as well as the high percentage of cation exchange capacity in the soil, thus favoring the absorption of essential nutrients (Cavalcante *et al*., 2007; Viana *et al*., 2013).

The root length showed a regression equation with respect to the effect of bovine biofertilizer doses, with an increasing linear behavior. Notably, the most marked growth was observed in the treatment with the dose of 10 mL, presenting an increase of 18.91% in relation to the control. Raising the dose showed an increase of 0.4112 in root length (Fig. 1D).

It is possible that the greater root expansion of the saplings treated with biofertilizer was due to the physical improvement provided by the humic substances present in the bovine biofertilizer (Aidyn *et al*., 2012). In passion fruit seedlings, Cavalcante *et al*. (2009) and Mesquita *et al*. (2012) observed that the seedlings showed superiority in root development when the seedlings were fertirrigated with bovine biofertilizer.

It was observed that leaf area was influenced by the doses of biofertilizer; the maximum value obtained was 28.49 cm^2 with the dose of 7.5 mL of bovine biofertilizer; as the doses of biofertilizer increased, there was a decrease (-0.0601) in the leaf area of the cashew tree seedlings (Fig. 1E). With an increase in leaf area,

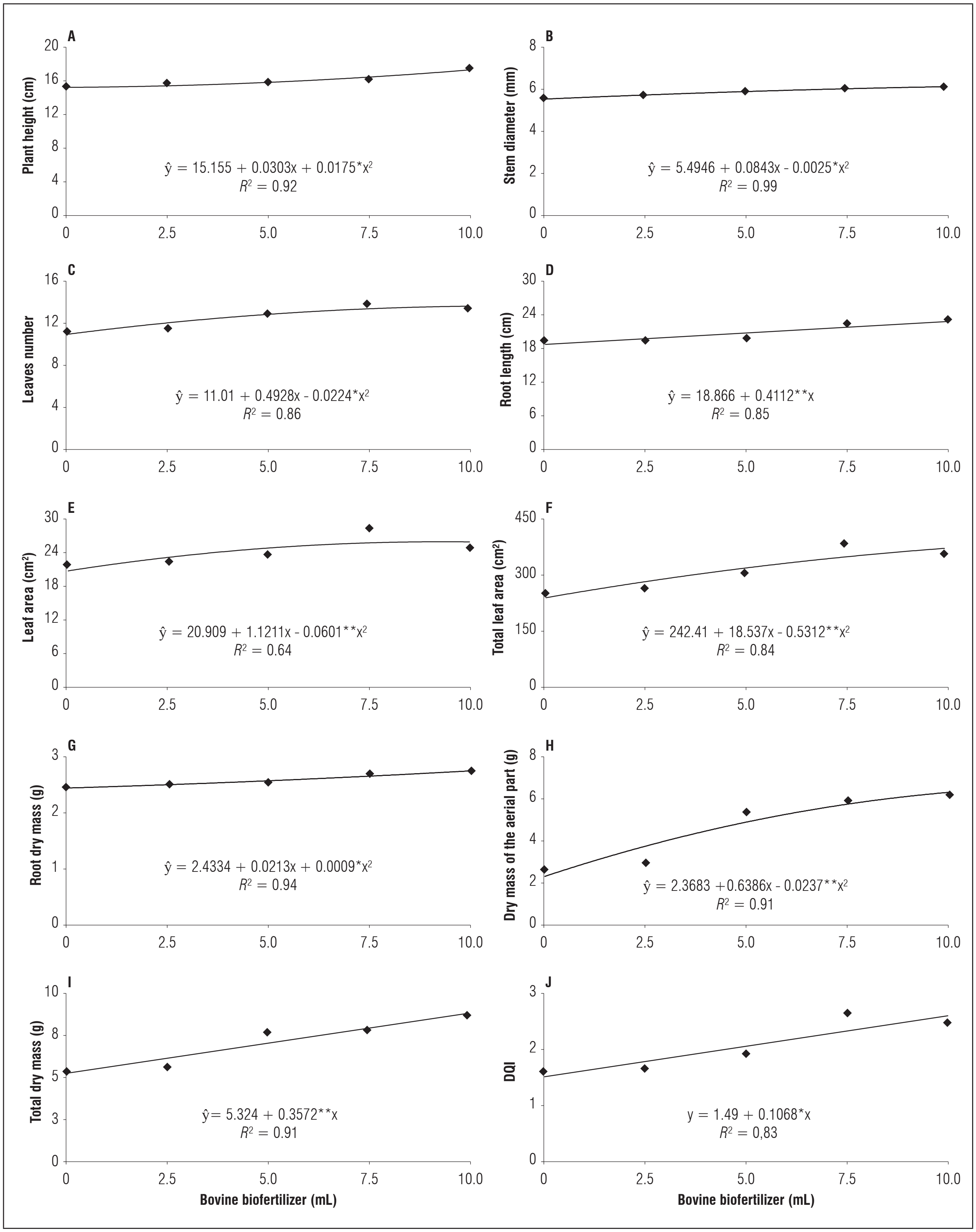

Figure 1. Height (A), stem diameter (B), number of leaves (C), root length (D), leaf area of seedling (E), total leaf area (F), root dry mass (G), dry mass of the aerial part (H), total dry mass (I) and Dickson Quality Index (J) of cashew tree seedlings submitted to doses of bovine biofertilizer.

there is greater efficiency in plants in the photosynthetic processes, as well as in the transport of solutes in the vegetal tissues. These results corroborate those obtained by Mesquita *et al*. (2012) for papaya seedlings and by Cavalcante *et al*. (2007) for guava seedlings.

The total leaf area presented a significant effect ($P \leq 0.01$), showing a quadratic polynomial behavior; the dose of 7.5 mL provided a larger leaf area (386.56 cm^2), and, beyond that dose, there was a decrease of -0.5312 in total leaf area (cm^2) (Fig. 1F).

The dry mass of the root was influenced by the doses of biofertilizer at the level of $P \leq 0.01$, presenting an increasing quadratic polynomial model. The maximum values were obtained with the dose of 10 mL, observing that, as the doses of bovine biofertilizer rose, an increase in root dry mass (Fig. 1G) was observed. The results obtained in the present study corroborate those obtained by Veras *et al*. (2014), who, when evaluating different doses of biofertilizer in the development of cashew seedlings, obtained the best results with the highest doses of biofertilizer for most of the analyzed variables.

The biofertilizer doses provided a significant difference for the dry mass of the shoot at the level of ($P \leq 0.01$), presenting a quadratic behavior. As the doses of bovine biofertilizer increased, there was an increase of 31.22 between the doses of 0 to 100 mL (Fig. 1H).

The results presented in this research were 6.22 g/plant, which corroborate the data obtained by Torres *et al*. (2014), who found similar results where the use of bovine biofertilizers promoted increases in the fresh mass of the aerial part of seedlings irrigated with water.

The regression equation adjusted to the experiment data of total dry mass presented a linearly increasing behavior, and a significant effect was observed at $P \leq 0.01$ for the biofertilizer doses. The dose of 10 mL/plant-time gave the best results in total dry mass, which was 8.75 (g/plant), presenting an increase in relation to the control of 62.33% (Fig. 1I).

The Dickson Quality Index (DQI) was statistically influenced ($P \leq 0.01$) by the Tukey test; the regression equation adjusted to the DQI data presented a quadratic behavior. The application of the 7.5 mL/plant-time dose gave satisfactory results; when the dose was increased, there was a decrease in the DQI, with a maximum value of 2.58 (Fig. 1J).

There was a significant difference between the means of the treatments of volumes for plant height, root length and leaf area of the dwarf cashew seedlings in relation to the containers used.

The increments were 6.46, 26.52 and 11.74% for plant height, root length and leaf area of the seedling, respectively, with the use of the 1 kg container in relation to the volume of 2 kg of substrate in polyethylene plastic bags. In the other analyzed variables,

Table 1. Estimated growth averages of dwarf cashew seedlings with two container volumes (V_1 and V_2) and methods for breaking dormancy (S_1 and S_2).

Variable	Container volumes		Dormancy breaking		LSD
	V_1	V_2	S_1	S_2	
Height (cm)	15.46 b	16.46 a	15.90 a	16.02 a	0.35
Stem diameter (mm)	5.74 a	5.90 a	5.73 a	5.93 a	0.07
Leaves number	12.61 a	12.65 a	12.20 a	13.07 a	0.34
Root length (cm)	18.47 b	23.37 a	20.10 a	21.74 a	0.65
Leaf area (cm^2)	22.91 b	25.60 a	23.71 a	24.80 a	0.71
Total leaf area (cm^2)	296.68 a	333.68 a	296.83 a	333.52 a	13.23
Root dry mass (g)	2.56 a	2.59 a	2.55 a	2.59 a	0.02
Dry mass of the aerial part (g)	4.64 a	4.71 a	4.65 a	4.71 a	0.03
Total dry mass (g)	6.92 a	7.30 a	6.95 a	7.27 a	0.16
Dickson Quality Index	1.94 a	2.06 a	1.97 a	2.03 a	0.07

Means followed by the same letter do not differ according to the Tukey test ($P \leq 0.05$). V_1 = 1 kg; V_2 = 2 kg; S_1 = scarification with sandpaper; S_2 = immersion in water. LSD = Least significant difference value.

it was observed that, although they did not present a significant effect for the volume, it was possible to notice that the substrate volume of 2 kg was higher than the substrate volume of 1 kg (Tab. 1).

The data found in the present study corroborate those of Veras *et al*. (2014), who studied the effect of substrate volumes on the development of cashew tree seedlings and observed that the volume of substrates did not affect the behavior of the cashew trees, as also observed by Correia *et al*. (2013) in eucalyptus seedlings when they verified that a higher volume of substrate provided the highest values for height and stem diameter at the level of the soil.

Dantas *et al*. (2013), when evaluating pineapple seedlings in different substrate volumes, observed that the volume of 2 kg was better than the 1 kg volume for the growth and dry matter characteristics of pineapple seedlings. For this reason, even if there is good availability of water, light and nutrients, dwarf cashew seedlings may have growth limited by container volume.

It was observed that the methods of breaking dormancy did not show significant effects; breaking dormancy with normal water for 24 h stood out over the scarification process with sandpaper for all analyzed variables, presenting an increase of 1.17, 1.17, and 4.60% in relation to breaking dormancy with normal water for root, shoot and total dry matter, respectively (Tab. 1). The data found in this research corroborate those of Menegazzo *et al*. (2013), who evaluated different methods of breaking dormancy and did not find satisfactory results for scarification.

Zuffo *et al*. (2014), when evaluating position and depth of sowing for emergence and initial development of *Anacardium microcarpum* Ducke seedlings, verified that the lowest depth provided the best result for dry root and shoot biomass at 1.84 and 2.93, lower than in the present research, 2.56 and 4.65, respectively.

Silva *et al*. (2015) found different results when analyzing chiseling as a method for breaking dormancy in terms of the initial growth of *açaí* seedlings and noticed that mechanical scarification provided a higher percentage of emergence and development in the seeds.

The superior results with the application of the bovine biofertilizer indicate its effectiveness as a source of nutrients and organic matter, improving soil composition and supplying nutrients that are essential for increasing crop productivity without causing damage to the environment.

In addition, the higher values can also be attributed to the nutrient supply and increase of the biological activity of the soil that the bovine biofertilizer provided; therefore, the humic substances present in the bovine biofertilizer may have improved the substrate structure and, consequently, induced an increase in cell division and permeability of cell membranes, providing greater water and nutrient uptake in the cashew tree seedlings (Khaled and Fawy, 2011).

The increase in most of the analyzed variables may have been due to the improvement in the substrate composition, increasing the aeration of the substrate and allowing greater root growth; as a consequence, there was greater transport of water and mineral salts to the seedlings. In addition, some farmers have observed the effects of bovine biofertilizer on protecting plant roots from soil pathogens, improving soil fertility and increasing crop yield (Khaled and Fawy, 2011; Celedonio *et al*., 2013).

The maximum dose of bovine biofertilizer may have resulted in a higher nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and micronutrient composition (Celedonio *et al*., 2013), which induced greater cell division and leaf expansion.

The application of bovine biofertilizer provided the highest values for growth, dry mass and quality of the cashew tree seedlings as a result of the improvement of the soil fertility with the bovine biofertilizer through the adsorption of exchangeable bases and the formation of organic complexes, besides developing negative loads (Silva *et al*., 2011). In addition, biofertilizer provides organic matter, with direct positive effects on soil, such as reduced compaction, increased water retention and improved nutrient availability (Pesakovic *et al*., 2013; Santos *et al*., 2017).

The data found for the DQI differed from those of Zuffo *et al*. (2014), who evaluated the position and depth of sowing in *Anacardium microcarpum* Ducke seedlings and found lower results, in which the highest values of the Dickson quality index (DQI) were found for the 2.0 cm sowing depth and when the positions of the nuts was thread down (A) and “back” up (E).

The higher values with the use of the 2 kg container was explained by the greater space for root growth and development of the cashew tree seedlings, improving root access to substrate moisture at depth, reducing stress resulting from the lack of water and, consequently, allowing higher growth (Stape *et al*., 2010) since the larger containers had a longer length, resulting in greater space for root growth and increasing the area of nutrient absorption.

CONCLUSIONS

The bovine biofertilizer doses of 7.5 and 10 mL provided higher growth and quality in the cashew tree seedlings.

The substrate volume of 2 kg was the most suitable volume for growing the cashew trees.

The method for breaking dormancy with normal water for 24 h was the most appropriate one for the formation of the cashew tree seedlings.

Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that that puts in risk the validity of the presented results.

ACKNOWLEDGMENT

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Pro-Rectory for Graduate Studies and Research of the Universidade Estadual da Paraíba (UEPB) for the financial support given this research.

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Doi: https://doi.org/10.17584/rcch.2019v13i1.8409

Pesticide residues in strawberry fruits cultivated under integrated pest management and conventional systems in Cundinamarca (Colombia)

Residuos de plaguicidas en frutos de fresa cultivados en sistemas de manejo integrado de plagas y convencionales en Cundinamarca (Colombia)

DAVID LÓPEZ V.[1]
MANUEL SÁNCHEZ[1]
GERHARD FISCHER[2, 4]
JOHN FABIO ACUÑA[3]
AQUILES E. DARGHAN[1]

Strawberry production based on integrated pest management in Sibate (Cundinamarca, Colombia).

Photo: G. Fischer

ABSTRACT

Because of the high susceptibility in strawberry fruits to attacks from pests and diseases, a large amount of pesticides is applied during the crop cycle and harvest period. The improper use of these substances can generate residues in agricultural products that pose a risk to human health. The objective of this study was to determine and compare pesticide residues in strawberry fruits from two different production systems distributed in the main producing areas of the Cundinamarca Department (Colombia). Eight samples of strawberry crops were collected in four producer municipalities (Guasca, Facatativa, Mosquera and Sibate) to compare different systems (conventional production *vs*. production based on Integrated Pest Management, IPM). Samples with a concentration of 394 molecules were examined using liquid and gas spectrometry. Fischer's exact test was used to determine the association between the pesticide type and residue level in the fruits, with more insecticide samples that exceeded the permitted threshold than when using fungicides. Twenty-two different molecules were detected in the analyzed samples, with 37 detection events, of which eight were reported in the IPM production systems and 29 in the conventional producers. The results revealed that nine molecules of insecticides and two of fungicides exceeded the concentrations set by Colombian regulations,

[1] Universidad Nacional de Colombia, Faculty of Agricultural Sciences, Agronomy Department, Bogota (Colombia). ORCID López V., D.: 0000-0001-5880-4376; ORCID Sánchez, M.: 0000-0003-4398-5834; ORCID Darghan, A.E.: 0000-0001-5790-1684

[2] Independent Consulter, Emeritus Researcher of Colciencias, Bogota (Colombia). ORCID Fischer, G.: 0000-0001-8101-0507

[3] Universidad Nacional de Colombia, Faculty of Engineering, Department of Civil and Agricultural Engineering, Bogota (Colombia). ORCID Acuña, J.F.: 0000-0003-1668-5836

[4] Corresponding author. gerfischer@gmail.com

but no significant differences were found between the two production systems. The calibration of equipment and applications must be improved in order to avoid over-concentration of pesticides, especially insecticides.

Additional keywords: *Fragaria×ananassa*; IPM; pesticide restriction; pesticide legislation; threshold.

RESUMEN

Debido a la alta susceptibilidad del cultivo de fresa al ataque de plagas y enfermedades, una gran cantidad de plaguicidas son aplicados durante el ciclo de cultivo. El inadecuado uso de estas sustancias puede generar residuos en los productos agrícolas, los cuales, a través de la ingesta suponen un riesgo para la salud humana. El objetivo de este estudio fue determinar y comparar la residualidad de plaguicidas en frutos de fresa provenientes de dos sistemas productivos diferentes, distribuidos en las principales zonas productoras del departamento de Cundinamarca (Colombia). Se recolectaron ocho muestras de cultivos de fresa correspondientes a cuatro municipios productores (Guasca, Facatativá, Mosquera y Sibaté), para comparar los dos sistemas diferentes (producción convencional *vs*. producción basada en el Manejo Integrado de Plagas y Enfermedades, MIPE). En las muestras se examinaron las concentraciones de 394 moléculas mediante espectrometría líquida y gaseosa. Se utilizó la prueba exacta de Fisher para determinar la asociación entre el tipo de pesticida y el nivel de residuos en frutos, encontrando que más muestras de insecticidas excedieron el umbral de residualidad permitido que las de fungicidas. Se detectaron 22 moléculas diferentes para las muestras analizadas, con 37 eventos de detección, de los cuales ocho se reportaron en cultivos del sistema MIPE y 29 correspondieron a productores convencionales. Los resultados revelaron un total de nueve moléculas de insecticidas y dos de fungicidas en concentraciones excesivas para la normatividad colombiana, pero no se encontraron diferencias significativas entre los dos sistemas de producción. Existe la necesidad de mejorar la calibración de los equipos y de las aplicaciones para evitar las sobreconcentraciones de las pesticidas, especialmente de las insecticidas.

Palabras clave adicionales: *Fragaria×ananassa*; MIP; periodo de carencia; legislación sobre plaguicidas; umbral.

Received for publication: 12-09-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The Department of Cundinamarca is the largest strawberry producer in Colombia (Agronet, 2014), particularly the municipalities of Sibate, Choconta, Guasca, Alban and Facatativa, because of its favorable soils and climatic conditions (López-Valencia *et al*., 2018). The proximity of these municipalities to the city of Bogota favors the commercialization of this fruit; this department produces and distributes more than 60% of the strawberries consumed in this country (Flores and Mora, 2010).

The strawberry is susceptible to attacks from several pests and diseases, *e.g. Phytonemus pallidus* Banks (Acari: Tarsonemidae), *Tetranychus urticae* Koch (Acari: Tetranychidae), *Frankliniella occidentalis* Perg. (Thysanoptera, Thripidae), *Lygus* sp., and *Botrytis cinerea* Pers., *Colletotrichum acutatum* (JH Simmonds), *Phytophthora* spp., *Xanthomonas fragariae* (Kennedy and King), *Sphaerotheca pannosa* (Wallr.) Lév., *Verticillium* sp., *Rhizoctonia* sp., *Pythium* sp., *and Fusarium* sp., both during the crop cycle and the post-harvest period (Maas, 1998). In order to manage this limitation, producers apply several pesticides (Wang *et al*., 2017). The intensive use of these substances in crops can contaminate the soil, air, and ground and surface water sources (Rodríguez *et al*., 2014), as well as generating collateral damage to beneficial organisms. In addition, pesticide residues in agricultural products pose a risk to human health (Nougadère *et al*., 2011). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) define pesticides as any substance or mixture of substances intended for the control of animal, plant or other species that are undesirably in production or postharvest processes (FAO, 1997).

Several studies have shown that agricultural products with pesticide residues that exceed a certain limit pose a potential risk to consumers. Adverse effects include blindness, liver diseases, increased cholesterol, neurological toxicity, alterations in the immune and reproductive system, lymphomas, prostate cancer, multiple myeloma, Parkinson's disease, infant mortality, and genetic disorders, among others (Chatterjee *et al*., 2013; Sinha *et al*., 2012; Gupta, 2006; Lozowicka, 2015). For the majority of the population, consumption is considered the principal potential route of exposure to pesticides (Nougadère *et al*., 2012; Cao *et al*., 2010; Panuwet *et al*., 2012).

As such, pesticide residues in agricultural products must be considered a public health issue that requires knowledge on and estimation of the active ingredients of these substances and the risk they pose to human health. As such, a health risk assessment should predict the likely effects of contaminants in humans during a given period (Wu *et al.,* 2014). Some studies, including Jiang *et al*. (2005), Fianko *et al*. (2011) and Ezemonye *et al*. (2015), have estimated the potential risk to human health that could be derived from the consumption of food contaminated with pesticides.

Previous studies have shown that factors such as knowledge, perception of risk and perceived control on the use of pesticides are decisive for appropriate use (Flocks *et al*., 2007; Remoundou *et al*., 2013; Damalas and Khan, 2016). Some of these studies have explored knowledge on the use of pesticides among agricultural workers, finding serious shortcomings (Damalas and Khan, 2016; Houbraken *et al*., 2016). In Colombia, improper use of pesticides prevails partly because of an outdated regulatory framework, a high rate of poverty, and low rural education (Polanco *et al*., 2014).

In today's agriculture, the use of pesticides has several benefits (Majeed, 2018), so it is presumed that it will continue to be a fundamental part of crop management strategies. However, as mentioned by the previous author, there are alternative methods for the management of pests and diseases in crops that could be more expensive than conventional practices when the environmental and social costs of the use of conventional pesticides are not taken into account. Moreover, there is a growing demand for products free of chemical residues, which encourages research and development in alternative pest and disease management (Wang *et al*., 2017).

The IPM is defined as an integrated system that keeps damage from diseases and pests below the economically acceptable level (Ehler, 2006) and is also known as the "coordinated use of complementary methods to suppress pest, weeds and diseases", reducing environmental risks by monitoring and applying physical, biological, mechanical, cultural and chemical control for sustainable management of pests (Toth *et al*., 2018). The IPM includes practices for cultural, genetic, biological, mechanical and chemical control. It promotes economic and environmental sustainability, protects human health, and delays the development of resistance in pests and diseases with knowledge-intensive systems (Epstein and Zhang, 2014). Additionally, it has guidelines that encourage farmers to produce safe and innocuous fruits (Fernandes *et al*., 2012).

Previous studies have demonstrated the efficiency and efficacy of alternative methods of pest and disease management in the reduction of pesticide residues (Fernandes *et al*., 2012; Sundaram *et al*., 2018; Houbraken *et al.*, 2016). Currently, the IPM is recognized as a mechanism that reduces risks related to the use of pesticides and ensures public health and environmental protection. IPM use is increasingly being promoted by the private sector, marketers and producers in response to consumer demand for healthy and safe food that is produced in a sustainable manner (Farfán, 2011; FAO, 2003).

For this study, a conventional production system was used that was not based on IPM concepts, maintaining practices that may be unsustainable without enough technical rigor or inadequate preharvest interval timing.

The objective of this study was to determine pesticide product residues in fresh strawberry fruits from two different production systems used on farms of the principal strawberry areas in the Cundinamarca Department.

MATERIALS AND METHODS

Production systems

Strawberry fruits from the Cundinamarca municipalities of Sibate (4°29'27"N and 74°15'34" W), Guasca (4°51'57"N and 73°52'38" W), Facatativa (4°48'53"N and 74°21'19" W) and Mosquera (4°42'28"N and 74°13'58" W) were analyzed since the Cundinamarca Department is the largest strawberry producer zone in Colombia. The conventional production systems were selected from the database of strawberry farmers in Cundinamarca found in the "Agroindustrial and Technology Corridor of Strawberry and Blackberry Project", along with practices for fertilization, pest and disease management, and harvest that were representative of each production zone. In these crops, monitoring and recording, including a semi-structured interview, were used to identify common practices among strawberry farmers for the management of pests and diseases for 8 months, which corresponded to the period between planting and harvest peaks. The inspections focused on the following aspects: (1) equipment calibration, (2) application calibration, (3) reading and monitoring the directions on pesticide labels, and (4) registration of applications in the strawberry crop.

Production systems with IPM practices implemented concepts of the Good Agricultural Practices, based on monitoring and action thresholds for phytosanitary management. In the conventional systems, the strawberry farmers did not monitor or identify thresholds of action. Crops were selected with a preventive approach, that is, the timely and adequate implementation of agricultural practices. The chosen production systems were based on a profound knowledge on cultivation and its development, pests and diseases, biological controls and environmental conditions.

For crop protection, in the conventional strawberry systems, the control of the principal pests and diseases was carried out exclusively with the application of different fungicides, insecticides and acaricides, mainly: lambda-cyhalothrin, cypermethrin, azoxystrobin, difenoconazole, tebuconazole, pyrimethanil, benzimidazole, dimethoate, chlorfenapyr and chlorpyrifos. The two compared systems were delineated depending on the phytosanitary management, but the rest of the crop management was the same (nutrition, irrigation, plantation maintenance, and harvest, etc.).

In IPM based systems, the use of other forms of control such as cultural and biological methods was privileged, leaving chemical control as the last alternative for protection, using them on rare occasions. In addition, special importance was given to crop monitoring, through which the action thresholds were implemented.

B. cinerea is one of the most limiting pathogens in strawberry crops, and *Trichoderma harzianum* Rifai and the cultural practice of removing and destroying infected material were used for management, as in IPM practices. It is important to note that, within the IPM system, an adequate fertilization plan with an appropriate amount of Ca and beneficial nutrients favored a low incidence and severity of pests and diseases in the crop.

The management of pests, such as the mite *T. urticae*, was carried out with the release of the predator *Amblyseius californicus* (syn. *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)). This intervention, in the case of the Guasca crop, drastically reduced the amount of insecticides-acaricides applied to the crop and, in the case of Sibate, completely eliminated the application of these products.

Strawberry samples

The eight strawberry samples used in the residue analysis consisted of fruits at the harvest point of different varieties, qualities and sizes, all representatives from their respective area. Each sample consisted of approximately 1 kg of fruit. The strawberry samples were not treated or handled in a special way or different from the conventional harvesting practices.

The origin of each sample was as follows: (1) two samples from the municipality of Sibate, each one from a different production system (conventional and IPM); (2) two samples from the municipality of Guasca, in this case one with IPM and the other a set of fruits from different plots, all with conventional management; (3) two samples from the municipality of Mosquera, one with IPM and the other was obtained from a local fruit market (in this case the fruits had been exposed to a shelf-life of 1 d); (4) two samples from the municipality of Facatativa, one represented the IPM and the other conventional management.

Chemical analysis

Each sample was placed in hermetically sealed bags, at temperatures between 2 and 4°C and taken to the laboratory on the same day of harvest. Once delivered to the laboratory, the samples were stored to prevent changes in their properties. The analysis was performed by the company Primoris Colombia SAS, Bogota, Colombia (ISO17025 and certificate 057-Test).

The presence and concentration of 394 pesticides (most used in agriculture, according to the codex alimentarius bases) were evaluated with gas chromatography coupled to a mass spectrophotometer (GC/MS/MS) using helium as a carrier gas and a capillary column. The instruments used included a chromatograph with an Agilent self-sampler (Santa Clara, CA) and a Waters spectrophotometer (Milford, MA), and the standards used were prepared from pure pesticides. Additionally, liquid chromatography coupled to a mass spectrophotometer (LC/MS/MS) was carried out using acetonitrile and water as the mobile phase and a C18 column. A chromatograph with self-sampler and Waters spectrophotometer were used, and the standards used were prepared from pure pesticides.

Statistical analysis

The comparison of the results was made by counting the detected events, analyzed according to Fisher's exact test (two tails), forming a two-way contingency table with the pesticide type and residues in excess or at normal values since the data were associated with the counts found in the samples with the pair of variables crossed in the table. A 90% confidence level was used to test the independence hypothesis using the SAS 9.2® software (Stokes *et al*, 2000).

The criterion of acceptance of the maximum limits of the residue in the data analysis was based on the European Community regulations (EC, 2017) since its database is extensive, and the maximum residue limits (MRLs) for strawberries are clear and specific.

RESULTS

Use of pesticides

Of the 24 strawberry farmers who applied conventional pest and disease management practices in the representative areas of Cundinamarca, as observed between July of 2016 and February of 2017, most of the farmers were men (79%).

The information on the knowledge and use of pesticides was analyzed without taking into account the location of the production system (Tab. 1). Most of the observed strawberry producers of the project performed their applications with the use of stationary pumps (96%). The preparation of the mixtures was done manually, without the use of appropriate safety equipment. In most cases, the calibration of the equipment and the calibration of the application were not performed, 87 and 100%, respectively, which makes it difficult to dose these pesticides.

For the pesticide labels, it was found that 83% of the producers did not read or follow directions, and only 8% always did it before the application. Moreover, 37% of farmers did not have a record of the products applied to the crop, and most of them (46%) recorded them sometimes.

Table 1. Conventional practices in the use of pesticides of 24 interviewed farmers.

Variable	Observation	Count
Calibration of the equipment	Always	0
	Most of the time	2
	Rarely	1
	Never	21
Calibration of the application	Always	0
	Most of the time	0
	Rarely	0
	Never	24
Reading labels	Always	2
	Most of the time	0
	Rarely	2
	Never	20
Record of application	Always	3
	Most of the time	1
	Rarely	11
	Never	9

Analysis of the samples

Of the 394 evaluated active ingredients, only 22 were present in the strawberry fruits in all evaluated locations (Tab. 2).

The municipalities with the highest number of residues of pesticides in the strawberry fruits reported were Facatativa (13), Guasca (11) and Sibate (10). Of the substances found by municipality, the pesticides in the conventional crops corresponded to Facatativa (53%), Sibate (90%), Guasca (91%) and Mosquera (100%). The most toxic substances (Category I and II) listed by municipalities were: Sibate (7 compounds), Facatativa (3 compounds), Guasca (3 compounds) and Mosquera (2 compounds). The substances most frequently found in the analyzed samples were fungicides, with a total of 22 reports in eight analyses, and only 15 reports of insecticides-acaricides were found (Tab. 2).

Of the total of 12 active ingredients belonging to insecticides-acaricides found in the strawberry fruits of all municipalities, 42% were organophosphates, 17% pyrethroids, 8% neonicotinoids, 8% carboxamide (hexythiazox), 8% propargite, 8% pyrroles and 8% tetronic and tetramic acid derivatives.

In the case of fungicides, of the 10 active ingredients found in the strawberry fruits from all municipalities, 30% were inhibitors of demethylation, 20% inhibitors of the kinase, 20% methyl benzimidazole carbamates, 10% phenylamides, 10% carboxamides and 10% aniline-pyrimidines. The most common modes of action were inhibition of membrane biosynthesis and transpiration inhibition.

Comparison between production systems

Results from the pesticide residues tested on the strawberry fruits showed differences between the conventional production systems and the systems with IPM practices, but they could not be proven

Table 2. Residues of pesticides detected in strawberry fruits from all locations (Cundinamarca-Colombia, between July, 2016 and February, 2017).

Active ingredient	Commercial name	Toxicity category	Classification	Action mode[1]
Methamidophos	Nadir® 600 SL	I	Insecticide/acaricide	1B
Chlorfenapyr	Sunfire® 24 SC	II	Insecticide	13
Trichlorfon	Profitox 80 SP	II	Insecticide	1B
Thiamethoxam	Engeo®	II	Insecticide	4A
Bifenthrin	Brigada® 100 EC	II	Insecticide/acaricide	3A
Cyhalothrin	Engeo®	II	Insecticide/acaricide	3A
Dimethoate	Roxion® 40 EC	II	Insecticide/acaricide	1B
Profenofos	Fulminator 600 EC	II	Insecticide/acaricide	1B
Chlorpyrifos	Lorsban™ 4 EC	II	Insecticide/acaricide	1B
Fluopyram	Luna® Tranquility	II	Fungicide	C2
Azoxystrobin	Amistar® TOP CS	II	Fungicide	C3
Pyrimethanil	Luna® Tranquility	II	Fungicide	D1
Spiromesifen	Oberon® SC 240	III	Insecticide/acaricide	23
Tebuconazole	Nativo® SC	III	Fungicide/bactericide	G1
Carbendazim	Carbendazim 500	III	Fungicide	B1
Epoxiconazole	Opera® SC	III	Fungicide	G1
Thiabendazole	Mertect® 500 SC	III	Fungicide	B1
Metalaxyl	Ridomil® Gold MZ 68 WP	III	Fungicide	A1
Pyraclostrobin	Opera® SC	III	Fungicide	C3
Difenoconazole	Amistar® TOP CS	III (II o III)	Fungicide	G1
Hexytiazox	Lathix 54 EC	III	Acaricide	10A
Propargite	Omite® 6EC	III	Acaricide	12C

[1]Reference code of the FRAC and IRAC. Organophosphates (1B), pyrethroids and pyrethrins (3A), neonicotinoids (4A), clofentezine, diflovidazin and hexythiazox (10A), propargite (12C), chlorfenapyr, dnoc and sulfluramid (13), tetronic and tetramic acid derivatives (23), RNA polymerase I (A1), ß-tubulin assembly in mitosis (B1), inhibition of complex II: succinate-dehydrogenase (C2), inhibition of complex III cytochrome ubiquinol oxidase (C3), methionine biosynthesis (D1), C14- demethylase in sterol biosynthesis (G1).

statistically. The lowest amount of pesticide residues was found in the crops with IPM in the installed demonstration plots of the project: Sibate 1 substance, Mosquera 0, Guasca 1, and Facatativa 6 (Tab. 3). On the other hand, the conventional systems showed the following amounts: Sibate 9 substances, Mosquera 3, Guasca 10, and Facatativa 7 (Tab. 4). Of the 37 total appearances of pesticide traces, only 8 corresponded to traces in crops with IPM (Tab. 3), that is, only 21.6%. In the fruits from the Mosquera crops, no pesticide residue was detected.

Table 3. Residues of pesticides detected in crops with IPM practices.

Municipalities	Active ingredient	Concentration (mg kg^{-1})
Sibate	Pyrimethanil	0.048
Guasca	Thiabendazole	0.027
Facatativa	Pyraclostrobin	0.050
	Carbendazim	0.026
	Epoxiconazole	0.018
	Fluopyram	0.066
	Pyrimethanil	0.300
	Trichlorfon	0.150

In the fungicide residues detected in the strawberry fruits, the active ingredient pyrimethanil was found in the IPM crops in Sibate and Facatativa. In the case of Facatativa, because of the greater number of residues of all types of pesticides, it was possible to create a two-way contingency table with the categories associated with the production system and the level of residues in the fruits.

The Fisher's exact test was used in order to determine the relationships between the qualitative variables associated with the pesticide type and residues in excess or at normal values (Tab. 5). It was found that there was a relationship of statistical dependence between the level of toxicity registered and the pesticide type applied, with a much higher amount exceeding the threshold for insecticides (9) than for fungicides (2).

Similarly, the use of different pesticides among the municipalities was evidenced since 48% of the 29 active ingredients found in the fruits were not detected in the others, and the remaining 52% were detected only in two locations at the same time. In Sibate, the conventional crops showed excessively high concentrations of cyhalothrin and carbendazim according to the Colombian Maximum Residue Levels (MRLs) (Arias *et al*., 2014).

Table 4. Residues of pesticides detected in fruits from the conventional crop system.

Farmer	Active ingredient	Concentration (mg kg^{-1})
Sibate	Cyhalothrin	0.350
	Profenofos	0.014
	Azoxystrobin	0.034
	Carbendazim	0.160
	Methamidophos	0.150
	Pyrimethanil	0.097
	Tebuconazole	0.120
	Thiamethoxam	0.220
	Trichlorfon	0.039
Guasca	Chlorpyrifos	0.040
	Propargite	0.061
	Pyraclostrobin	0.080
	Spiromesifen	0.240
	Carbendazim	0.020
	Epoxiconazole	0.048
	Hexytiazox	0.070
	Metalaxyl	0.010
	Methamidophos	1.700
	Dimethoate	0.290
Facatativa	Bifenthrin	0.030
	Pyraclostrobin	0.030
	Carbendazim	0.060
	Epoxiconazole	0.010
	Fluopyram	0.040
	Pyrimethanil	0.130
	Trichlorfon	0.670
Mosquera	Chlorfenapyr	0.086
	Azoxystrobin	0.130
	Difenoconazole	0.069

Table 5. Bivariate distribution of the sample count by type of pesticide and level of toxicity with results of Fisher's exact test.

Toxicity	Pesticide	
	Insecticide	Fungicide
In excess	9	2
Acceptable	6	22
Fisher's exact test (*P* value)		0.0008
Contingency coefficient		0.4877

DISCUSSION

Knowledge on and adequate use of pesticides by producers is of great importance since they are an essential part of strategies that seek to reduce environmental risks and damage to human health (Houbraken *et al*., 2016). Trained farmers can read and interpret the pesticide labels and perform the applications correctly; in contrast, poorly trained farmers have greater difficulties performing appropriate integrated crop management (Ibitayo, 2006; Damalas and Eleftherohorinos, 2011).

The lack of application calibration makes it possible to identify problems associated with the application of the recommended amount of pesticide according to technicians and labels. This also makes it impossible to ensure the quantity of water used or the effectiveness of the application (ICA, 2009). Bastidas *et al*. (2013) reported similar results, in which the presence of pesticide residues in passiflora crops was mainly due to the lack of implementation of Good Agricultural Practices (GAP). As indicated by Nausa (2005), strawberry farmers in Cundinamarca mostly do not receive appropriate technical assistance, so the criterion for the application of pesticides is their own experience, which is influenced by neighbors, technicians from agrochemical companies and agricultural store recommendations.

The proper use of a pesticide depends directly on the information available on its label. Therefore, a label on the container is essential and sometimes the only element available for farmers to obtain this information (PSU, 2005). Thus, the limited reading and interpretation of pesticide labels and the limited records of applications reported in this study negatively affect the environment and food safety of the harvested fruits (Pierre and Betancourt, 2007). As reported by Damalas and Khan (2016) and Guerrero (2003), the main causes of the levels of pesticide residues found in excess in fruit and vegetables include the inadequate use of doses, application frequencies and omission of the recommended time lapse between applications and the harvest.

Several studies have reported the presence of organophosphate residues in strawberry fruits. Bélanger *et al.* (1990) reported dimethoate residues in strawberry fruits up to 18 d after application. Bempah *et al.* (2011) found traces of methamidophos and dimethoate, and Guerrero (2003) recorded traces of chlorpyrifos, profenofos and dimethoate.

According to Nausa (2005), the application of commercial products such as: Lorsban®, Trapper®, Tamaron® and Monitor®, is common in strawberry crops in Cundinamarca. The active ingredients in these products are organophosphates (chlorpyrifos and methamidophos), which was the main chemical group of insecticides/acaricides with the highest number of residues found during our experiment.

Currently, in Colombia, there is no registered product for strawberries with the active ingredient cyhalothrin. However, there are reports of its use in this crop, with a residue detection period of more than 23 d (Kovacova *et al.*, 2013). The use of cyhalothrin in crops with the IPM is not recommended as it has adverse effects in non-target organisms (Kovacova *et al.*, 2013). Cyhalotrin residuality tests conducted by FAO (n.d.) confirmed that, after 3 d, the maximum concentration of this active ingredient did not exceed 0.09 mg kg^{-1}. In our results, a cyhalothrin concentration of 0.350 mg kg^{-1} demonstrated an over-application of this pesticide. Cyhalothrin is slightly toxic in terrestrial organisms and very toxic in aquatic organisms (He *et al*., 2008). Fetoui *et al*. (2009) concluded that cyhalothrin can induce oxidative stress and modification of biochemical parameters and histological aspects in the liver of rats.

Toth *et al.* (2018) determined that crops with IPM practices had lower amounts of pesticide residues than with conventional systems. However, these authors clarified that no option is completely free of residues. For this study, the IPM approach implied a set of cultural, biological and chemical strategies, as well as the selection of resistant varieties that complement each other when keeping pests and diseases at levels lower than those that cause economic damage to crops (INTA, 2013).

Under IPM practices, if the use of pesticides is required, they should be applied at the appropriate time and place, limiting applications to specific points where possible (Zhang *et al*., 2015). Based on the reports of Epstein and Zhang (2014), the IPM approach does not always reduce the use of pesticides, but can direct it to the areas with the greatest pest problems. In this way, the amount of pesticide used per unit area is reduced, along with the risk of pesticide residues in harvested foods; likewise, good implementation and the consequent efficiency in the use of pesticides can contribute to the reduction of these substances over time.

CONCLUSIONS

The results show that there are active ingredient residues of pesticides that exceed the maximum residue limits (MRLs) of Colombia in the different strawberry cultivation systems in Cundinamarca. The presence of residues was shown to be mainly related to the lack of implementation of good agricultural practices by farmers, especially for application and equipment calibration. When the calibration of equipment and applications is not performed by farmers, correct dosing of pesticides is very difficult.

The results indicate the need for frequent monitoring using pesticide residue analysis to ensure fruit safety, *e.g.* by government organizations, both for exportation and national consumption. The results highlight the urgency of developing and implementing integrated pest control packages in strawberry crops and raising awareness of the good agricultural practices in the use of pesticides.

ACKNOWLEDGMENTS

The authors express their gratitude to the institutions that are part of Agreement 2 of the *Corredor Tecnológico Agroindustrial* alliance for financing the Subproject "Research and technological development in the production systems of fruits (strawberry and blackberry) in the representative producing areas of Cundinamarca", as well as to the farmers who are beneficiaries of this subproject for providing the product and allowing the monitoring of the variables of pest and disease management in their crops.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts the validity of the presented results at risk.

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Effect of 1-methylcyclopropene and ethylene on the physiology of peach fruits (*Prunus persica* L.) cv. Dorado during storage

Efecto del 1-metilciclopropeno y el etileno en la fisiología de frutos de durazno (*Prunus persica* L.) cv. Dorado durante el almacenamiento

LUZ AMPARO MARIÑO-GONZÁLEZ[1]
CLAUDIA MARCELA BUITRAGO[1]
HELBER ENRIQUE BALAGUERA-LÓPEZ[2, 4]
EFRAÍN MARTÍNEZ-QUINTERO[3]

Measurement of color in peaches cv. Dorado.

Photo: K. Africano

ABSTRACT

The peach (*Prunus persica* L., family Rosaceae) is a drupe that is consumed fresh and used in industry; it has a climacteric behavior and, because of its high water content, is highly perishable. This study aimed to evaluate the effect of 1-methylcyclopropene (1-MCP) and ethylene on the postharvest physiology of peach fruits cv. Dorado. Harvested fruits with 100% green, 0% yellow skin color were used in a completely randomized design with four treatments: ethylene, 1-MCP, 1-MCP+ethylene and a control. After treatment, the fruits were stored at room temperature. During storage, the respiration rate, weight loss, firmness, color index of the skin, total soluble solids, total acidity and maturity ratio were evaluated. The results showed the efficiency of 1-MCP in peach cv. Dorado: 1-MCP decreased the respiration rate, color index of the skin, soluble solids and maturity ratio, while the firmness and total acidity were greater. The ethylene application showed an opposite effect, suggesting that it may regulate a large part of peach cv. Dorado ripening.

Additional key words: postharvest technology; ripeners; plant growth substances; peach postharvest; stone fruits.

[1] Universidad Pedagógica y Tecnológica de Colombia, Maestría en Fisiología Vegetal, Tunja (Colombia). ORCID Mariño-González, A.: 0000-0002-8545-505X, ORCID Buitrago, C.M.: 0000-0001-7730-0851

[2] Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias Agropecuarias, Grupo de Investigaciones Agrícolas, Tunja (Colombia); Universidad El Bosque, Departamento de Biología, Bogota (Colombia). ORCID Balaguera-López, H.E.: 0000-0003-3133-0355

[3] Universidad Pedagógica y Tecnológica de Colombia, Programa de Empresas Agropecuarias, Duitama (Colombia). ORCID Martínez-Quintero, E.: 0000-0001-8303-6284

[4] Corresponding author. helber.balaguera@uptc.edu.co

RESUMEN

El durazno (*Prunus persica* L., familia Rosaceae) es una drupa apetecida para consumo en fresco y la industrialización; presenta un comportamiento climatérico y tiene alto contenido de agua, por lo cual se considera un fruto altamente perecedero. El objetivo de este estudio fue evaluar el efecto del uso de 1-metilciclopropeno (1-MCP) y etileno sobre la fisiología poscosecha de frutos de durazno, cv. Dorado. Se utilizaron frutos cosechados con color de la epidermis 100% verde, 0% amarillo, se dispusieron en un diseño completamente al azar con cuatro tratamientos que correspondieron a etileno, 1-MCP, 1-MCP+etileno y un control sin aplicación. Luego del tratamiento, los frutos fueron almacenados a temperatura ambiente. Durante el almacenamiento se evaluó la tasa respiratoria, pérdida de peso, índice de color de la epidermis, firmeza, sólidos solubles, acidez total y relación de madurez. Los resultados mostraron la eficiencia de la aplicación de 1-MCP en durazno cv. Dorado, ya que generó significativamente menor tasa de respiración, índice de color de la epidermis, sólidos solubles y relación de madurez, mientras que la acidez total y firmeza fueron mayores. Por su parte, la aplicación de etileno mostró el efecto contrario en estos procesos, indicando que al parecer regula gran parte del proceso de maduración del fruto de durazno cv. Dorado.

Palabras clave adicionales: tecnología poscosecha; maduradores; sustancias de crecimiento vegetal; frutos de hueso.

Received for publication: 26-10-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The peach (*Prunus persica* L.) cv. Dorado is characterized by a yellow epidermis, with a light red pigmentation, gold-yellow mesocarp with red colorations around the endocarp, trichomes on the exocarp, velvety skin, and mesocarp adnate to the endocarp; additionally, it has a high sugar content and can reach up to 150 g in weight. All of these characteristics make the peach highly desirable for fresh consumption and for industrialization (Campos, 2013). Of the deciduous fruit trees, the peach is very important for Colombian agriculture, particularly in the Department of Boyaca because of its optimal edaphoclimatic characteristics, vocation and productive expertise (Puentes *et al.*, 2015). However, the peach cv. Dorado is highly perishable and has a short postharvest life (11 d in the conditions evaluated by Africano *et al.,* 2016), which limits its commercialization. This may be due to the fact that the peach is a climacteric fruit (Africano *et al.,* 2016), which means there is an accelerated increase in the ethylene production and the respiratory rate associated with ripening (Brackmann *et al.,* 2013).

Ethylene is the hormone responsible for triggering physiological, biochemical and molecular processes that guarantee fruit ripening (Binder, 2008; Balaguera-López *et al*., 2014a) by bonding to a group of protein receptors mainly located on the membrane of the endoplasmic reticulum (Chen *et al*., 2005; Serek *et al*., 2006). Ethylene allows fruits to reach optimal organoleptic characteristics for consumption; however, it is also in charge of inducing senescence, which causes agricultural products to loose nutritional and commercial value (Bapat *et al*., 2010; Balaguera-López *et al*., 2014a).

To reduce the postharvest losses of the peach cv. Dorado, it is necessary to understand the processes associated with ethylene during ripening, as a foundation for various technologies that reduce the biosynthesis and action of ethylene. Zhang *et al*. (2012) stated that the ethylene regulation of peach ripening has been thoroughly studied using biochemical, molecular and genetic techniques. However, several reviews on ethylene biosynthesis suggest that the exact role of ethylene in the ripening process is not yet fully understood (Bapat *et al*., 2010; Barry and Giovannoni, 2007) and that the knowledge gap is even greater in peach genotypes grown in the high tropics. In this regard, several studies have used the application of ethylene and 1-methylcyclopropene (1-MCP) on fruit (Zhang *et al*., 2012; Yang *et al*., 2013; Balaguera-López *et al*., 2016; Barreto *et al*., 2017).

1-MCP is the main ethylene inhibitor (Watkins, 2006), taking up the place of the ethylene receptors irreversibly and, thus, blocking the transduction chain of the signals responsible for the genetic expression related to the response to this hormone (In *et al*., 2013). The effect of 1-MCP on the physiological and biochemical processes of peach ripening has been evaluated in several studies with conflicting results: in some studies, 1-MCP blocked ethylene biosynthesis, but in others, it was ineffective (Dal Cin *et al*., 2006; Hayama *et al*., 2008; Tadiello *et al*., 2016; Wang *et al*., 2017). Liu *et al*. (2005, 2015) and Wang *et al*. (2017) found that 1-MCP delayed peach fruit ripening. However, Kluge and Jacomino (2002) and Tonetto *et al*. (2007), among others, suggested that the utility of 1-MCP is limited because it can delay peach ripening only when it is applied during the pre-climacteric state. Therefore, this study aimed to evaluate the effect of 1-MCP and ethylene, both individually and combined, on the postharvest behavior of the peach fruit cv. Dorado in order to understand ethylene-dependent ripening and the commercial potential of 1-MCP for extending the postharvest life and commercial period of this fruit.

MATERIAL AND METHODS

The peach (*Prunus persica* L.) cv. Dorado fruits came from a peach commercial crop located in Cerinza, Boyaca. The fruits were harvested with 100% green epidermis –0% yellow-red, with color values L*=62.54, a=-4.58 and b=40.81. They had a homogeneous size and good phytosanitary conditions and were taken to the postharvest laboratory of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Duitama campus, where the experiments were conducted.

Four treatments in a completely random experiment design were evaluated: 1-MCP, 1-MCP+ethylene, ethylene and a control; each treatment was replicated four times, for a total of 16 experiment units, each one composed of approximately 1,000 g of fruit. One day after harvest, the fruits were washed and disinfected with a 1% sodium hypochlorite solution, and the treatments were immediately applied. For the 1-MCP (EthylBloc®) treatment, a 0.35 mg L^{-1} 1-MCP solution was used, in which the fruits were submerged for 5 min, the adapted methodology of Choi *et al*. (2008). For the ethylene treatment, fruits were submerged in 1000 mg L^{-1} of ethephon (Ethrel® 48 SL, Bayer CropScience) for 5 min and dried at room temperature; for the 1-MCP+Ethylene treatment, the ethylene was applied 7 d after the 1-MCP application. Finally, the fruits were stored on trays of expanded polystyrene at room temperature (16 ±2°C; relative humidity of 70±12% at average). The variables were measured at 1, 4, 7, 12, 16 and 19 days of storage (DOS), until the fruits lost their commercial quality. The respiratory rate (RR; mg CO_2 kg^{-1} h^{-1}) was estimated by taking 600 g of fruit approximately and placing them in VER BC-2000 2000 cm^3 hermetic chambers (Vernier Software & Technology, Beaverton, OR) for 5 min and by determining the concentration of CO_2 with a VER CO_2-BTA sensor and the LabQuest2 interface system (Vernier Software & Technology, Beaverton, OR). The weight loss was calculated by measuring the fresh mass (the same fruits used for RR) in an Acculab VIC 612 electronic scale, 0.01 g precision (Sartorius Spain S.A., Madrid). To determine the color index (CI), the equation (1) reported by Balaguera-López *et al*. (2014a) was used; the system parameters, CIELab "L", "a" and "b", were obtained from three readings in the equatorial zone of two fruits per replication, using a Minolta CR 300 digital colorimeter (Minolta Co., Tokyo).

$$IC = (1000 \times a^*)/(L^* \times b^*) \quad (1)$$

The fruit firmness was estimated using a PCE-PTR200 digital penetrometer (PCE-Ibérica SL, Albacete, Spain) with 0.05 N approximation, and the soluble solids (SS) were measured using a Hanna HI 96803 digital refractometer (Hanna Instruments, Woonsocket, RI) with a 0-85% range and 0.1 °Brix precision. For the total acidity (TA), which was expressed as the percentage of malic acid, the volume data of NaOH, using 1 g of fruit juice, taken to 25 mL with distilled water, and adding two drops of phenolphthalein in a base-acid titration, were used. The maturity ratio (MR) was determined as the quotient between SS and TA. For the destructive measurements (firmness, SS, TA, MR), one fruit was used in each sample for experimental unity.

The obtained data were analyzed with normality (Shapiro-Wilk) and homogeneity (Levene test) tests; subsequently, an analysis of variance and a Tukey range test ($P \leq 0.05$) were conducted; the statistics software R v 3.4.2 was used.

RESULTS AND DISCUSSION

All sampling points showed statistically significant differences ($P \leq 0.05$) in the respiration rate; the treatment that resulted in the highest respiratory rate was ethylene, followed by the control, although they were not significantly different. These two treatments were significantly different from the treatments with 1-MCP. Additionally, the treatment with ethylene and the control increased the respiration rate notably after 12 d of storage, reaching values of 60.89 and

59.01 mg CO_2 kg^{-1} h^{-1}, respectively. On the contrary, the application of 1-MCP resulted in a smaller respiration rate over the storage period, showing a prolong increase from day 7 to 19, with a value of 50.13 mg CO_2 kg^{-1} h^{-1}; this result was similar to that observed in the fruits treated with 1-MCP+ethylene (Fig. 1).

The respiratory rate in climacteric fruits, including peach, depends on ethylene. Zhang *et al*. (2012) found that ethephon can accelerate energy production, glycolytic metabolism and ethylene biosynthesis, which may accelerate respiration in the tissue. In apple fruits, Yang *et al*. (2013) noted that the application of ethylene increased the respiratory rate, whereas 1-MCP decreased it, similar to that observed in this research.

The results showed that the application of 1-MCP decreased the respiration rate, perhaps because it blocks ethylene cellular receptors (Watkins, 2006). Similar studies have suggested that peach fruits reduce their respiratory rate in the presence of 1-MCP, thus, decreasing the intensity of physiological processes (Steffens *et al*., 2009; Wang *et al*., 2017). Likewise, Valero *et al*. (2004) showed that applications of 1-MCP delayed the climacteric increase in plums, whereas treatments with ethylene accelerated it. Therefore, the respiratory rate is closely related to the climacteric rate; hence, the application of 1-MCP is a viable alternative to prolong peach fruit quality. On the other hand, the increase in the respiratory rate shown by the fruits treated with 1-MCP may have been due to a rise in the amount of ethylene receptors synthetized by the tissue (Serek *et al*., 2006). Despite this, the addition of ethylene after 1-MCP did not increase the respiration, as compared to the fruits treated with 1-MCP, possibly because the low levels of ethylene in the tissues were sufficient to bind to the few existing receptors (through the effect of the 1-MCP) and induce an increase in the respiratory rate of the peach fruits.

For firmness, the peach cv. Dorado fruits showed a constant decrease in firmness during storage, which agrees with Africano *et al*. (2016) for the same cultivar. Statistically significant differences ($P \leq 0.05$) were observed starting at 7 DOS, when the fruits treated with 1-MCP had the highest firmness, and the fruits treated with ethylene had the lowest. The control fruits did not have differences from those treated with ethylene; the fruits under these two treatments lost their organoleptic quality at 16 DOS, showing a final firmness of 25.2±2.95 N for the control and 22.23±2.06 N for the ethylene treatment; the fruits treated with 1-MCP+ethylene became soft rapidly toward the end of the storage period (Fig. 2).

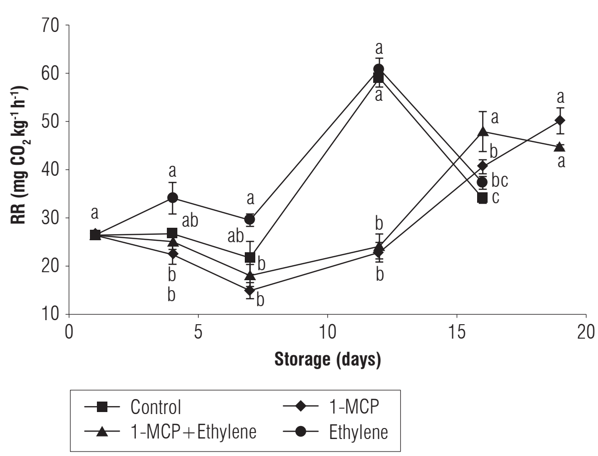

Figure 1. Effect of ethylene and 1-methylcyclopropene on respiratory rates (RR) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test (*P*≤0.05). Vertical bars on each mean indicate the standard error (*n* = 4).

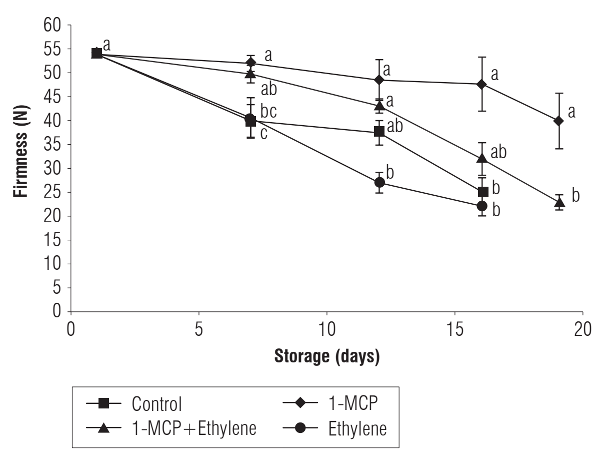

Figure 2. Effect of ethylene and 1-methylcyclopropene on the firmness of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test (*P*≤0.05). Vertical bars on each mean indicate the standard error (*n* = 4).

The loss of firmness in a fruit may be related to an increase in the activity of enzymes that degrade the cell wall in peaches, such as polygalacturonase, pectin-methylesterase, endo-β-1,4-glucanase, α-arabinosidase and β-galactosidase. The activity of these enzymes may increase in the presence of ethylene because the depolymerization of pectins and xyloglucans is regulated by ethylene (Nishiyama *et al*., 2007; Pech *et al*., 2008) and it is expected to decrease as a result of the effect of 1-MCP, which blocks ethylene activity at the cellular level; this suggests that, in peach cv. Dorado fruits, firmness may also be regulated by ethylene, even after the application of 1-MCP. Özkaya *et al.* (2016) found a reduction in the activity of pectin methyl esterase and polygalacturonase in apricot fruits. Liu *et al*. (2015), in peach cv. Yahuala, and Wang *et al.* (2017), in peach cv. CN13, found that the application of 1-MCP increased firmness in comparison with non-treated fruits. Similarly, Khan and Singh (2007) found that 1-MCP delayed the loss of firmness in *Prunus salicilina* Lindl fruits by reducing the activity of polygalacturonase, pectin esterase and endo-1,4-d-glucanase, whereas Zhang *et al*. (2012), when using proteomics on peach fruits, found that 1-MCP decreased the loss of Ca ions, suppressed the degradation of the cell wall and maintained the mechanical properties of the cellular structure.

The color index (CI) of the skin increased with storage time. There were significant differences ($P\leq0.05$) between all treatments starting at day seven of storage. The changes were smaller in the fruits treated with 1-MCP ($P\leq0.05$), showing a CI of 2.63±0.59 at the end of the storage period. The fruits treated with ethylene showed a higher index color (3.87±0.48), despite the use of ethylene 7 d after 1-MCP; the control treatment showed a CI increase during storage (Fig. 3). Lelievre *et al*. (1997) stated that color changes may be dependent or independent of ethylene, as was later suggested by Dong *et al.* (2002), who observed changes in color independent of ethylene in *Prunus armeniaca*. However, these studies indicated that color in the peach cv. Dorado is dependent on ethylene; in fact, Barreto *et al*. (2017) found that, by applying ethylene to 'Chiripá' peach fruits, the color changed more than when applying 1-MCP.

The lower CI that resulted from the application of 1-MCP in the peach fruits may have been due to the fact that this compound delays color change since it can decrease chlorophyll degradation by reducing the activity of the chlorophyllase enzyme (Watkins, 2006; Sun *et al*., 2012), which is up-regulated by ethylene. Likewise, 1-MCP also decreased color changes in the peach cv. Aurora-1 (Kluge and Jacomino, 2002) and in the peach 'Roubidoux' (Steffen *et al.*, 2009).

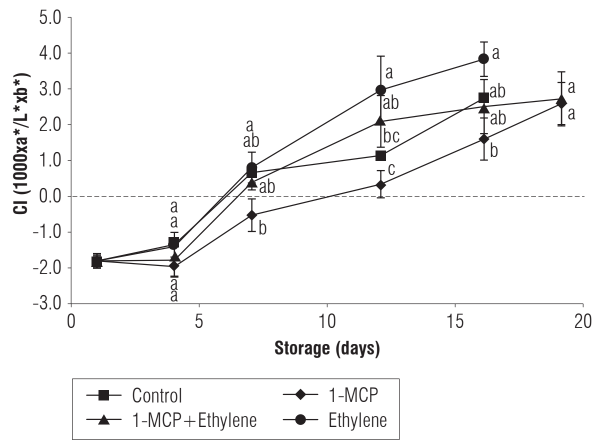

Figure 3. Effect of ethylene and 1-methylcyclopropene on color index (CI) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P\leq0.05$). Vertical bars on each mean indicate the standard error (n = 4).

The soluble solids (SS) increased gradually with storage. There were significant differences ($P\leq0.05$) between all sampling points. The fruits treated with 1-MCP accumulated less SS in every measurement, showing a value of 13.7±0.21 °Brix at the end of storage, whereas the fruits treated with ethylene, alone or in combination with 1-MCP, showed a higher concentration of SS (16.1±0.35 °Brix; Fig. 4).

The higher concentration of SS at the end of storage may be explained by the hydrolysis of starch and polysaccharides in the cell wall (Kays and Paull, 2004); in peach cv. Dorado fruits, ethylene may play a role in accelerating hydrolysis processes, while 1-MCP may delay them, which supports the hypothesis that ethylene regulates a large part of peach fruit ripening in cv. Dorado. Likewise, Zhang *et al*. (2012) found that, in peach cv. Huiyulu, 1-MCP decreased starch degradation, decreasing the glycolytic metabolism and, therefore, ripening.

Our results agree with Barreto *et al.* (2017) who, in peach cv. Chiripá, found lower SS values for fruits treated with 1-MCP than for fruit treated with ethylene; on the contrary, Tonetto *et al*. (2007) and

Steffens *et al*. (2009) did not observe any effect on SS after applying 1-MCP in the peaches 'Eldorado' and 'Roubidoux'.

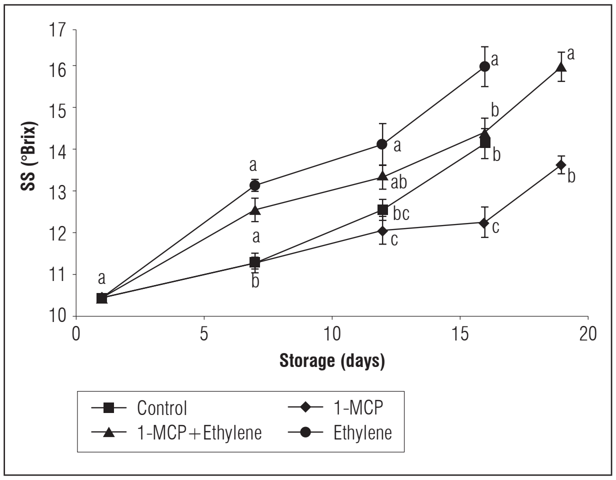

Figure 4. Effect of ethylene and 1-methylcyclopropene on soluble solids (SS) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test (P≤0.05). Vertical bars on each mean indicate the standard error (n = 4).

The total acidity (TA) showed statistically significant differences (P≤0.05) between all sampling points during storage, except at 19 DOS. For all treatments, there was a gradual decrease in acidity, which was higher for the ethylene and control treatments and lower for the 1-MCP treatment; at 19 DOS, the fruits with 1-MCP showed 0.95±0.11% TA (Fig. 5).

According to Alves *et al*. (2009), the effect of 1-MCP on the TA of peach fruits may be explained by a lower acid consumption during the tricarboxylic acid cycle through the reduction of respiration. As observed in this study, TA decreases over the storage period because organic acids are part of the respiration substrates (Kays and Paull, 2004). Similar results have been shown by Barreto *et al.* (2017) in the peach, who found higher acidity in fruits treated with 1-MCP than in fruits treated with ethylene, which accelerates the loss of TA. Liu *et al*. (2015) also observed a higher TA with 1-MCP in peaches. On the contrary, Tonetto *et al*. (2007) did not observe effects from 1-MCP on the TA of the peach 'Eldorado', similar to Steffens *et al.* (2009) for the peach cv. Roubidoux. This suggests that the effect of 1-MCP on acidity depends on the cultivar, as indicated by Ligouri *et al*. (2004).

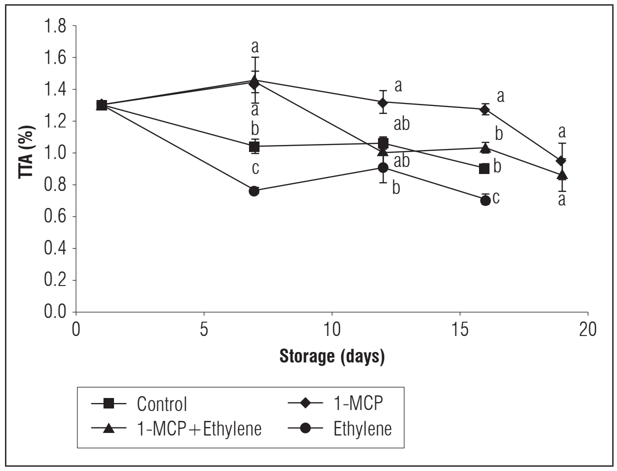

Figure 5. Effect of ethylene and 1-methylcyclopropene on total titratable acidity (TTA) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test (P≤0.05). Vertical bars on each mean indicate the standard error (n = 4).

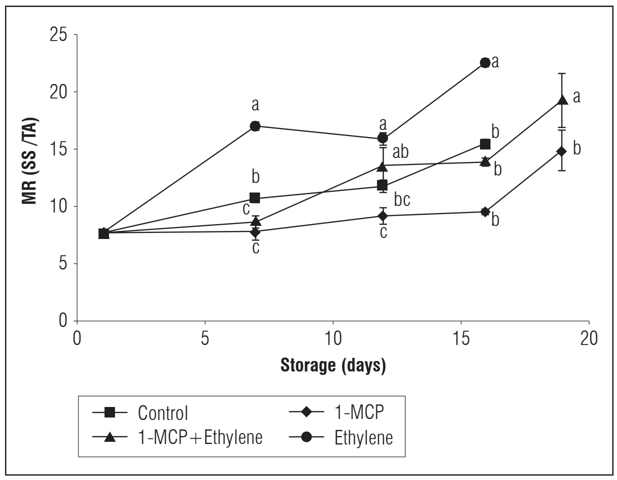

Figure 6. Effect of ethylene and 1-methylcyclopropene on maturity ratio (MR) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test (P≤0.05). Vertical bars on each mean indicate the standard error (n = 4).

The maturity ratio (MR) increased gradually throughout storage and showed significant differences (P≤0.05) between the treatments in all sampling points. The lowest values were found in the fruits treated with 1-MCP and with 1-MCP+ethylene, indicating a delay in MR (Fig. 6). The fruits treated with ethylene ripened faster, showing higher MR values over the storage period, which is a typical behavior for this variable, perhaps because of the increase in SS and decrease in TA (Ferrer *et al*., 2005; Africano *et al.,* 2015). Accordingly, the exogenous application of ethylene accelerates ripening in peach cv. Dorado fruits, whereas 1-MCP delays it; however, Tonnetto *et al*. (2007) did not find any effect from 1-MCP on the MR of the peach 'Eldorado'.

CONCLUSIONS

Our results suggest that 1-MCP decreases the respiration rate, loss of firmness, epidermis color index, total soluble solids, total titratable acidity, and maturity ratio in the peach cv. Dorado, whereas ethylene has an opposite effect. Our results demonstrate that several ripening processes in peach cv. Dorado fruits are associated with the presence of ethylene and that 1-MCP may delay ripening throughout the storage of this fruit.

ACKNOWLEDGMENTS

The authors wish to thank the "Dirección de Investigaciones, Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, Colombia" for supporting this study.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts the validity of the presented results at risk.

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Resistance induction efficiency of silicon dioxide against *Meloidogyne incognita* in tomato

Eficiencia de dióxido de silicio en la inducción de la resistencia del tomate a *Meloidogyne incognita*

EDILSON MARQUES JUNIOR[1, 2]
JORGE TADEU FIM ROSAS[1]
ALIXELHE PACHECO DAMASCENA[1]
MATHEUS ALVES SILVA[1]
GUILHERME DE RESENDE CAMARA[1]
ÂNGELO OLIVEIRA GONÇALVES[1]
WILLIAN BUCKER MORAES[1]

Tomato plants in greenhouse.

Photo: E.M. Junior

ABSTRACT

The tomato root-knot nematode is one of the main phytosanitary problems in crops. Chemical control is the phytosanitary method most used by farmers, and the study of alternative management of phytonematodes is crucial. The objective of this study was to evaluate the effect of silicon dioxide (SiO_2) on the initial development of tomato plants, as well as to determine the best dose of SiO_2 for inducing resistance to parasitism by *M. incognita*. This experiment was set up under a completely randomized design with ten treatments and five replicates in a 5×2 factorial arrangement consisting of five concentrations of SiO_2 (0, 0.15, 0.3, 0.45 and 0.6 g dm^{-3} of soil) with the presence and absence of *M. incognita*, under greenhouse conditions. The following variables were evaluated: plant height; number of leaves; fresh and dry weight of shoot; percentage of shoot dry matter; root fresh weight; number of galls; final population of nematodes; and population per gram of root. The *M. incognita* infection affected plant height, number of leaves and shoot fresh weight, while the application of SiO_2 negatively affected the formation of galls in the roots of the inoculated plants and the population per gram of root, reducing the final population of nematodes in the root system. SiO_2 also provided greater development in the tomato plants, with a significant effect on plant height. The ideal dose was 0.34 g dm^{-3} of SiO_2.

Additional key words: resistance induction; correlation; root-knot nematode; nutrition.

[1] Universidade Federal do Espírito Santo, Center for Agricultural Sciences and Engineering, Department of Agronomy, Alegre (Brazil). ORCID Junior, E.M.: 0000-0003-4216-1980; ORCID Rosas, J.T.F.: 0000-0002-3244-4816; ORCID Damascena, A.P.: 0000-0003-1374-5119; ORCID Silva, M.A.: 0000-0002-4992-9588; ORCID Camara, G.R.: 0000-0001-8181-429X; ORCID Gonçalves, A.O.: 0000-0003-3151-4875; ORCID Moraes, W.B.: 0000-0001-7478-7772

[2] Corresponding author. edilsonmarquesjr@hotmail.com

RESUMEN

Los nematodos se encuentran dentro de los principales problemas fitosanitarios del cultivo de tomate. El control químico es el método fitosanitario más utilizado por los productores y la búsqueda de medidas alternativas para su control se hacen indispensable. El objetivo de esta investigación fue evaluar el efecto del dióxido de silicio (SiO_2) en el desarrollo inicial de plantas de tomate, así como determinar las mejores dosis en la inducción de resistencia al parásito *M. incognita*. Se estableció un diseño completamente al azar, con diez tratamientos y cinco repeticiones, en arreglo factorial 5×2, consistiendo en cinco concentraciones de SiO_2 (0; 0,15; 0,3; 0,45 y 0,6 g dm^{-3} de suelo) y la presencia y ausencia de *M. incognita*, en invernadero. Se evaluaron las variables altura de la planta, número de hojas, masa fresca y seca de la parte aérea, porcentaje de materia seca de la parte aérea, masa fresca de la raíz, número de agallas, población final y por gramo de raíz de nematodos. La infección por *M. incognita* afectó las variables altura de las plantas, número de hojas y masa fresca de la parte aérea, mientras que la aplicación de SiO_2 afectó de forma negativa la formación de ramas en las raíces de las plantas inoculadas y la población por gramo de raíz, reduciendo la población final de nematodos en el sistema radicular de las plantas. La aplicación de SiO_2 también proporcionó mayor desarrollo a las plantas de tomate, presentando efecto significativo sobre la altura de las plantas, siendo 0,34 g dm^{-3} de SiO_2 la dosis ideal.

Palabras clave adicionales: inducción de resistencia; correlación; nematodo agallador; nutrición.

Received for publication: 07-03-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The cultivation of tomato (*Lycopersicon esculentum* Mill.) is notable for its high world consumption, whether raw or processed, with great socioeconomic importance (Ferreira *et al.*, 2017). Because it is a highly profitable activity with wide acceptance in the consumer market, the tomato is the most commercially important vegetable crop in Brazil, with an average production of 4 million tons of fruits harvested annually, in an area of approximately 60,000 ha (IBGE, 2016).

Of the several biotic and abiotic factors that hinder tomato production and productivity, the root-knot disease, caused by nematodes of the genus *Meloidogyne* Goeldi 1887, stands out as one of the main phytosanitary problems in this crop, limiting yield throughout the world (Rosal *et al*., 2014). Thus, this activity takes onhigh economic risk because of the high investments necessary for planting this crop (Carvalho *et al*., 2014).

Economically, the most significant nematode species in the country for tomato crops are *Meloidogyne javanica, M. incognita, M. ethiopica* and *M. arenaria* because of their pathogenicity, high losses, reduction of final product quality (Hunt and Handoo, 2009), broad geographical distribution, wide range of hosts (Pinheiro *et al*., 2014) and management complexity (Damalas and Eleftherohorinos, 2011).

Chemical control is the phytosanitary method most used by producers. Although efficient, chemical molecules are highly toxic to humans, animals and ecosystems (Damalas and Eleftherohorinos, 2011). In this context, the study of alternative measures for the control of phytonematodes has become crucial, provided that the aim is the adoption of practices that minimize pressure on the environment (Timper, 2014; Pastori *et al*., 2017; Bhatt and Sharma, 2018).

The benefits of silicon accumulation and its success in the management of various pathogens have already been demonstrated for several crops, e.g. Wang *et al*. (2017). For nematodes, success has been shown for *M. javanica* in bananas (Oliveira *et al*., 2012), rice, beans and soybeans (Mattei *et al*., 2015), for *M. incognita* in tomatoes (Melo *et al*., 2012), and for *Enneothrips flavens* Moulton 1941 in peanuts (Dalastra *et al*., 2011), among others.

It is known that the characteristics of silicon allow its use in management programs because of stiffening of plant cell walls and influence on the synthesis of toxins in the biochemical responses of plants and in the gene expression of defense proteins (Dannon and Wydra, 2004; Wang *et al.,* 2017). However, despite advances in research that promote use of this new and promising form of phytonematode management in the field, little is known about which silicon dosages are more efficient in the management of this pathogen and do not negatively affect the nutritional conditions of plants.

The objective of this study was to evaluate the effect of silicon dioxide (SiO_2) on the initial development of tomato plants, as well as to determine the best doses of SiO_2 in the induction of plant resistance to parasitism by *M. incognita*.

MATERIAL AND METHODS

This study was conducted in a greenhouse located at the Center of Agrarian Sciences and Engineering of the Federal University of Espírito Santo - CCAE/UFES, Alegre-ES (20º42' S and 41º27' W, at 269 m a.s.l.). The climate of the region, according to the Köppen classification, is type "Aw", characterized by hot and rainy summers and dry winters. The average annual temperature is 23ºC, with annual rainfall around 1,200 mm.

This experiment was set up in a completely randomized design with ten treatments and five replicates in a 5×2 factorial scheme consisting of five concentrations of SiO_2 (0, 0.15, 0.3 , 0.45 and 0.6 g dm^{-3} of soil) with the presence or absence of *M. incognita* (zero and 1,500 second stage juveniles, J2 + eggs). The SiO_2 source was the commercial product AgriSil® (98% SiO_2, pH 7.5-8), and the doses were 0, 50, 100, 150 and 200%, adapted from the recommendation of Alvarez and Ribeiro (1999).

Tomato seeds, Santa Cruz variety, were planted on a tray with 128 cells containing Bioplant® commercial substrate, and the seedlings were transplanted 21 d after sowing into pots containing 4 dm^3 of medium textured red-yellow latosol-based substrate. The fertility management was done according to the recommendation of Prezotti *et al*. (2007). Five days after seedling transplant, SiO_2 was diluted in water and applied individually via irrigation directly to the pots, with the respective doses of each treatment.

Seven days after applying the product, 1,500 second stage *M. incognita* juveniles (J2 + eggs) were inoculated in each pot. The plants were monitored constantly to avoid the presence of diseases and pests. The control of invasive plants was done manually. The soil moisture was maintained close to field capacity, at around 60%, with daily irrigation shifts.

After 30 d of nematode inoculation, the following parameters were evaluated: plant height (PH), number of leaves (NL), shoot fresh weight (SFW), shoot dry weight (SDW), percentage of dry matter (DM %), root fresh weight (RFW), number of galls (NG), final nematode population (FP), and population per gram of root (Pgr). The nematode extraction was done according to the method proposed by Hussey and Barker (1973), as modified by Bonetti and Ferraz (1981).

The data obtained for each of the evaluated variables were submitted to analysis of variance and the doses were adjusted by regression. The means of the variables related to the presence or absence of *M. incognita* were compared by t-test at 5%. The normality of the data was verified using the Kolmogorov-Smirnov test ($P\leq0.05$), and Pearson linear correlation ($P\leq0.05$) was used among the variables.

RESULTS AND DISCUSSION

The variables SFW, PH and NL were significantly affected by the presence of nematodes (Tab. 1), where in the majority of the treatments, the means differed statistically, affirming that the presence of the pathogen culminates in lower development of tomato plants, with a lower weight gain. This information is consistent with results obtained by Melo *et al*. (2012) and Moreira and Ferreira (2015), who characterize dwarfism as one of the main symptoms of tomato nematode infections (Dias *et al*., 2016). In addition to having compromised growth, the infested plants had an apparent reduction of total leaf area, because of a smaller number of leaves, which negatively affected their photosynthetic capacity. Peluzio *et al*. (1999) and Guimarães *et al*. (2009) proved that fruit filling is directly related to leaf area and number of leaves since leaves behave as a source of photoassimilates to fruits, which are sinks. According to these authors, the highest gain in leaf area occurs at the initial stage of crop development and is determinant throughout the tomato crop cycle.

For the variables SDW and RFW, the means of the plants infested by the pathogen were smaller than those not infested, but did not differ statistically,

Table 1. ANOVA of the agronomic variables comparing their means in the presence and absence of nematodes.

	SFW (kg)				SDW (kg)			
Dose	C/N	SE	S/N	SE	C/N	SE	S/N	SE
0%	0.034 a	0.0021	0.067 b	0.0048	0.011 a	0.0022	0.020 a	0.0045
50%	0.059 a	0.0083	0.062 a	0.0050	0.017 a	0.0046	0.019 a	0.0024
100%	0.053 a	0.0048	0.079 b	0.0098	0.019 a	0.0032	0.025 a	0.0069
150%	0.035 a	0.0022	0.076 b	0.0048	0.010 a	0.0026	0.015 a	0.0035
200%	0.039 a	0.0071	0.063 b	0.0053	0.074 a	0.0025	0.014 a	0.0036
	RFW (kg)				NL			
Dose	C/N	SE	S/N	SE	C/N	SE	S/N	SE
0%	0.013 a	0.0006	0.012 a	0.0029	7.6 a	0.40	8.4 a	0.40
50%	0.014 a	0.0017	0.023 b	0.0026	7.8 a	0.37	10.8 b	0.97
100%	0.020 a	0.0029	0.018 a	0.0018	8.2 a	0.58	9.6 b	0.24
150%	0.013 a	0.0014	0.013 b	0.0020	7.4 a	0.24	9.2 b	0.37
200%	0.010 a	0.0021	0.019 b	0.0028	7 a	0.55	9.2 b	0.37
	PH (m)				DM (%)			
Dose	C/N	SE	S/N	SE	C/N	SE	SN	S/E
0%	0.64 a	0.011	0.65 a	0.014	32.39 a	6.81	28.95 a	6.56
50%	0.614 a	0.025	0.73 b	0.024	30.69 a	9.55	30.29 a	2.79
100%	0.63 a	0.021	0.80 b	0.023	33.91 a	2.87	30.23 a	7.72
150%	0.57 a	0.015	0.78 b	0.028	29.89 a	7.14	19.09 a	3.90
200%	0.55 a	0.027	0.72 b	0.032	17.94 a	4.30	21.35 a	3.88

SFW: shoot fresh weight in kg; SDW: shoot dry weight in kg; PH: plant height in meters; RFW: root fresh weight; NL: number of leaves; DM (%): percentage of dry matter; C/N: plants infected by nematodes; S/N: plants not infected by nematodes; SE: standard error of the mean. Means followed by different letters differ statistically according to the t test ($P \leq 0.05$).

in the majority of the treatments. Dias *et al*. (2016) found similar results; thus, these variables do not explain with confidence the effects of this pathogen on plants at the initial stage of development. The mean of the DM (%) variable also did not differ statistically.

The analysis of variance of the effect of SiO_2 on the tomato plants showed that plant height was significantly influenced, where the use of SiO_2 resulted in higher growth. However, high doses of SiO_2 cause an inverse effect, i.e., the plants presented reduced growth, which can be explained by the indirect effect of silicon on the absorption of zinc. High doses of SiO_2 make the amount of phosphorus available in the soil high, precipitating available zinc in the form of zinc phosphate (Paim *et al*., 2006) and compromising the development of tomato plants since this nutrient is essential to the development of this crop.

Figure 1 shows the curve formed by the quadratic model that explains the growth of the tomato plants as a function of the addition of SiO_2 to the soil, where the best growth is found at the maximum point of the regression curve. Thus, the dose that provided the highest height was 116.63%, equivalent to 0.34 g dm^{-3} of SiO_2, reaching a maximum height of 0.8 m per tomato plant. Pires *et al*. (2009) found that tomato plants with greater height at the initial stage of development tend to be more productive.

The variables SFW and NL were not significantly influenced by the addition of SiO_2; however, both variables presented a positive linear correlation with plant height (Fig. 2). Porto *et al*. (2014) used these variables to infer optimal nutrition conditions for tomato plants and determined that plants that receive adequate nutrition have a certain balance between the values of the mentioned variables, affirming that plants with a greater height, weight and number of leaves are better-nourished plants. Pulz *et al*. (2008), studying the effect of silicates on potatoes, also observed that the height of plants was greater in the presence of silicon. The same authors demonstrated that higher growth of potato plants may be associated with higher availability of phosphorus in soils with a higher silicon content. The silicates exert competition for the adsorption sites of phosphorus, causing the sites to be saturated or blocked by silicate anions, increasing phosphorus adsorption (Pulz *et al*., 2008). Similarly, Prado and Fernandes (2001) also observed a higher available phosphorus content in soil treated with silicate.

For MFPA and MSPA, no significant correlation was observed between DM% and FP and NG. However, lower DM (%) indexes were obtained at the extremes (Fig. 3), that is, with the highest and lowest pathogen infection, which coincided with the lowest and highest doses of SiO_2, respectively. Therefore, both high pathogen infection and high SiO_2 doses negatively affect the development and accumulation of dry matter in these plants. Rahi *et al*. (1988) found that the dry and fresh biomass of tobacco plants infected with *M. incognita* and *M. javanica* were almost half of the biomass of uninfected plants.

The different doses of SiO_2 affected the formation of galls in the roots of the inoculated plants (Fig. 4): the higher the SiO_2 dose, the lower the number of galls. These results are similar to those found by Moreira *et al*. (2013), where the number of galls formed by *M. incognita* was lower as a result of the application of different doses of eugenol in a tomato crop. This author stated that the number of galls is directly associated with the amount of nematodes that can penetrate

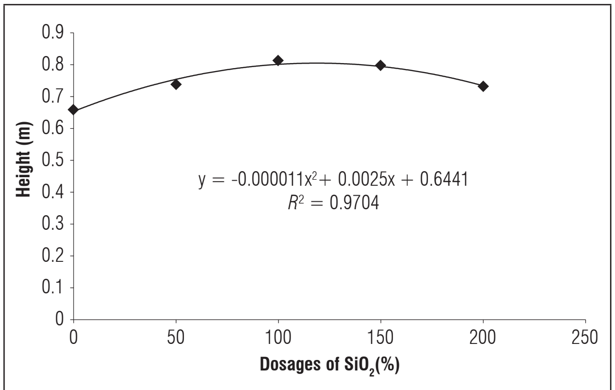

Figure 1. Effect of different dosages of SiO_2 on the height of tomato plants.

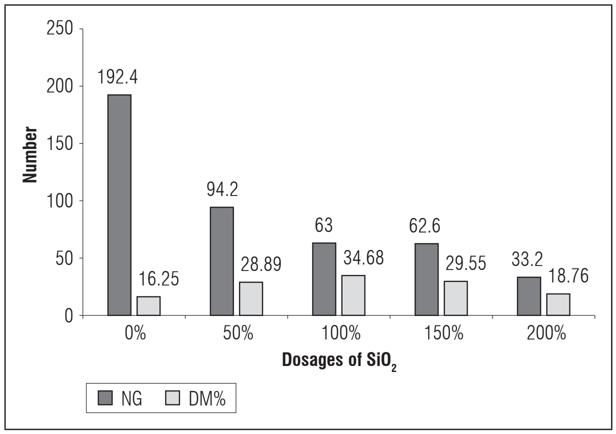

Figure 3. Relationship between the number of galls (NG) in the roots of the plants and percentage of dry matter (DM %) in the shoot at the different doses of SiO_2.

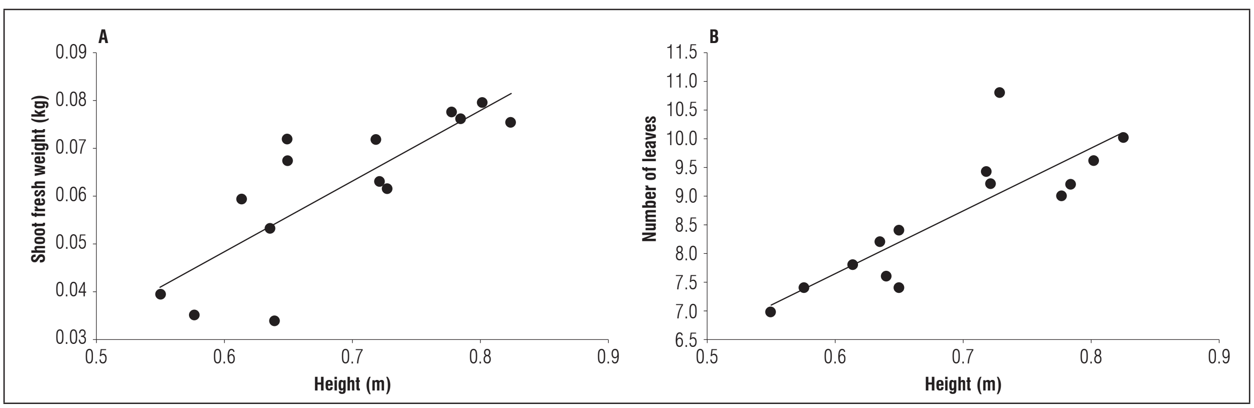

Figura 2. Correlation between plant height and the variables shoot fresh weight and number of leaves per plant. A. Pearson correlation coefficient at 5% probability (r = 0.81, $P \leq 0.0001$); B. Pearson correlation coefficient at 5% probability (r = 0.83; $P \leq 0.0001$).

and settle inside roots. Thus, a lower number of galls also means less penetration of the pathogen in the tissues of the roots, inferring that SiO_2 was able to induce resistance to the attack of the pathogen, possibly by a physical barrier formed by Si, inhibiting the penetration of the pathogen.

The final population in the roots did not show dependence on the tested SiO_2 doses, so it was not possible to propose a regression model to determine silicon concentrations capable of reducing FP. However, the number of galls had a positive correlation with the final population (Fig. 5), meaning FP depends on the number of galls, increasing as the number of galls increases. This relationship occurs because galls are modified cells where gall-forming nematodes settle and extract nutrients to complete their life cycle and multiply. Therefore, the final population of the pathogen tends to be proportional to the number of modified cells present in the roots of the infested plants (Danchin *et al*., 2016; Ralmi *et al*., 2016).

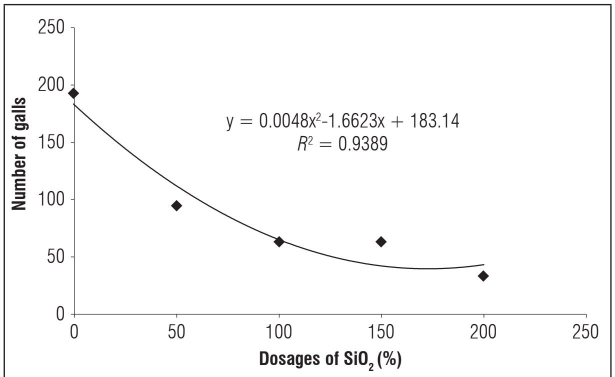

Figure 4. Effect of the different doses of SiO_2 on the number of galls (NG) in the roots of tomato plants.

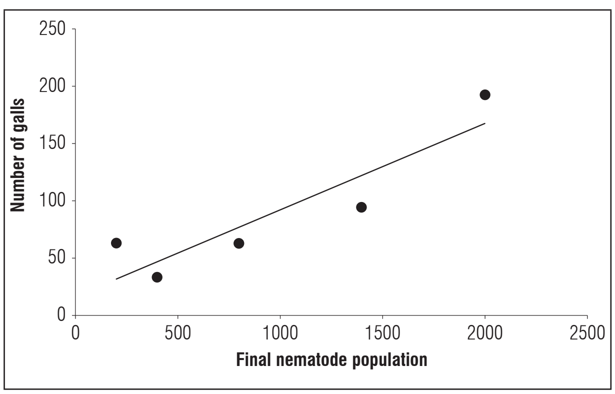

Figure 5. Correlation between the final nematode population (FP) and the number of galls (NG) in the roots of tomato plants. Pearson correlation coefficient at 5% probability (r = 0.90, P<0.035).

Another variable that was influenced by the different doses of SiO_2 was Pgr. It can be observed from Figure 6 that Pgr had lower values than the control, where silicon was not added, once again showing that silicon was able to reduce penetration and the consequent multiplication of nematodes inside the root tissues.

Several other authors have found positive results in the use of Si for the management of stresses of a biotic or abiotic origin in both tomato and other crops. Conceição *et al*. (2014) observed that the application of potassium silicate, alone or in combination with microorganisms antagonistic to the bacterial stain of melon fruits, reduced the severity of the disease. The disease index was reduced by 87%, and the the area was reduced by 65% when using Si with the antagonistic *Rhodotorula aurantiaca* Saito in the management. Cao *et al*. (2017) observed that Si can act as a mediator in tomato plants subjected to water stress, reducing the harmful effects. Sousa *et al*. (2013), studying the infection processes of *Puricularia oryzae* (Cooke) Sacc in wheat fertilized with Si, observed that the presence of Si reduced the symptoms of the disease in wheat leaves, emphasizing the possibly that, in the areas where the Si concentration was higher, the fungus penetrated less and had greater difficulty in colonizing. Mattei *et al*. (2015) observed that the hatching of *M. javanica* eggs was reduced in the presence of Si. In the tomato, a possible deposit of

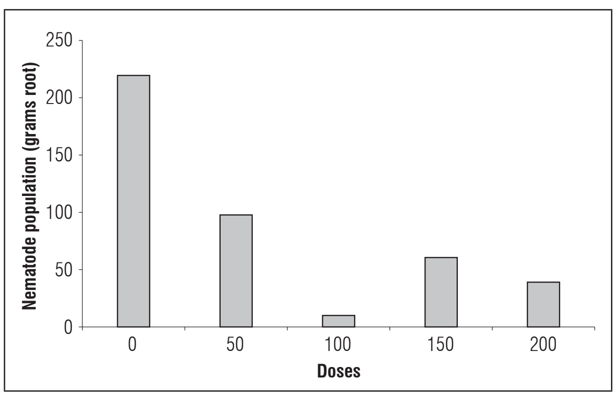

Figure 6. Relationship between the nematode population per gram of infected root and the different doses of silicon dioxide.

silicon in the root epidermis may have inhibited both the penetration of nematodes and their reproduction inside the cells.

The resistance to pathogen action conferred by the use of silicon is mainly related to the physical barrier formed by salicylic acid in the cells of the epidermis at the penetration points of the pathogen, improving the mechanical resistance of the cells. Silicon accumulates mainly in the cell wall, forming a cellulose-silicon bond that changes the natural barrier of the plant. It changes the anatomy and increases thickness as a result of a greater degree of silicification and accumulation of lignin and phenolic compounds in the lesions (Mendes *et al*., 2011), affecting the signs of recognition between the plant and the pathogen (Chérif *et al.*, 1994), the latter being a more efficient and enduring form of the resistance mechanism. Cao *et al*. (2017) affirmed that the greater accumulation of Si in the roots can maintain the integrity of the cell by improving its mechanical properties after observing a higher Si content in the roots of tomato plants that received a Si application. Besides the formation of the mechanical barrier, the presence of silicon in a plant can improve the activity of plant defense enzymes, inducing the production of antimicrobial compounds and acting in the regulation of plant protection pathways, such as the routes of jasmonic acid, salicylic acid and ethylene. Moreover, it can influence the gene expression of plant defense proteins (Bockhaven *et al*., 2013; Bhatt and Sharma, 2018). Although the protection mechanisms provided by Si were not evaluated in this study, there is sufficient evidence in the results and in the literature that confirms Si is able to reduce the penetration and reproduction of *M. incognita* in tomato plants.

CONCLUSION

The use of SiO_2 in the tomato crop provided a higher vegetative performance in the initial phase of development.

The application of SiO_2 reduced the final nematode population in the roots of the plants and was efficient in the management of *M. incognita* in this crop.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Relationship between morpho-agronomic traits in tomato hybrids

Relación entre caracteres morfoagronómicos en híbridos de tomate

MARIA INÊS DIEL[1, 3]
DÉBORA TURCHETTO ZAMBAN[2]
TIAGO OLIVOTO[1]
DIONATAN KETZER KRYSCZUN[1]
MARCOS VINÍCIUS MARQUES PINHEIRO[2]
BRUNO GIACOMINI SARI[1]
DENISE SCHMIDT[2]
ALESSANDRO DAL'COL LÚCIO[1]

Experimental tomato crop.

Photo: D.T. Zambam

ABSTRACT

The objective of this study was to identify and estimate the relationships between production component variables and fruit yields in tomatoes. This experiment was conducted in a randomized block design with a 2×3×3 factorial arrangement, with two tomato hybrids (Netuno and San Vito), three doses of boron (H_3BO_3 - 0, 2, 4 g/pit) and three calcium floral applications (no application; application every 7 days; and application every 14 days), totaling 18 treatments with four replications and 20 plants per plot. Pearson correlation coefficients were estimated between the measured variables and, after that, those with greater significance were selected for productivity with the Stepwise method and verification of multicollinearity using the number of condition and inflation factor of the variance. The correlations of the selected variables were decomposed into direct and indirect effects on fruit productivity with path analysis. There was a strong correlation between the variables, excluding the variables height, diameter and average mass of the fruits. Thus, there were cause and effect relationships between the independent variable total mass of the fruits and the principal variable total fruit yield. The variables diameter and total number of fruits did not contribute to an increased tomato production.

Additional key words: *Solanum lycopersicum*; path analysis; Pearson's correlation; multicollinearity; Stepwise; plant nutrition.

[1] Universidade Federal de Santa Maria, Crop Science Department, Santa Maria (Brazil). ORCID Diel, M.I.: 0000-0002-7905-2166; ORCID Olivoto, T.: 0000-0002-0241-9636; ORCID Krysczun, D.K.: 0000-0002-7991-8994; ORCID Giacomini Sari, B.: 0000-0003-3405-9628; ORCID Lúcio, A.D.C.: 0000-0003-0761-4200

[2] Universidade Federal de Santa Maria, Department of Agronomic and Environmental Sciences, Frederico Westphalen (Brazil). ORCID Zamban, D.T.: 0000-0003-1054-6472; ORCID Pinheiro, M.V.M.: 0000-0002-5028-7818; ORCID Schmidt, D.: 0000-0002-9963-4956

[3] Corresponding author: mariaines.diel@hotmail.com

RESUMEN

El objetivo de este trabajo fue identificar y estimar las relaciones entre variables componentes de producción y rendimiento de frutos en tomate. El experimento se realizó con diseño de bloques al azar con un arreglo factorial 2×3×3, correspondiendo a dos híbridos de tomate (Netuno y San Vito), tres dosis de boro (H_3BO_3 - 0, 2, 4 g/sitio) y calcio (si aplicación, aplicación cada 7 días y aplicación cada 14 días), para un total de 18 tratamientos, cuatro repeticiones y 20 plantas por parcela. Se estimaron los coeficientes de correlación de Pearson entre las variables medidas, posteriormente, se seleccionaron aquellos con mayor significancia para la productividad mediante el método *Stepwise* y la verificación de la multicolinealidad por el número de condiciones y el factor de inflación de la varianza. Las correlaciones de las variables seleccionadas fueron descompuestas en efectos directos e indirectos sobre la productividad de los frutos mediante el análisis de coeficientes de trayectoria. Se encontró una fuerte correlación entre las variables, excluyendo las variables altura, diámetro y masa promedio de los frutos. Por lo tanto, existen relaciones de causa y efecto entre la variable independiente la masa total de frutos y la variable principal de productividad total de frutos, y las variables diámetro y número total de frutos no contribuyeron a aumentar la productividad de tomate.

Palabras clave adicionales: *Solanum lycopersicum*; coeficientes de trayectoria; correlacion de Pearson; multicolinealidad; *Stepwise*; nutrición de plantas.

Received for publication: 26-04-2018 Accepted for publication: 29-03-2019

INTRODUCTION

Around the world, more than 5 million hectares are cultivated with tomatoes (*Solanum lycopersicum*), from which approximately 171 million tons of fruits are harvested, totaling 33.98 t ha^{-1} (FAOSTAT, 2018). These data prove the great economic and social importance of this crop. In Brazil, the cultivated area in the main producing regions totaled 37,398 ha in 2016, of which 18,674 were for table and 18,724 for industry (Kis and Carvalho, 2017). Globally, 4,782,753 ha are cultivated, with production reaching 17,7042,359 t (FAOSTAT, 2018).

Usually, when working with the tomato crop, a large number of variables are measured in order to obtain a set of data that allows the most varied types of evaluations and statistical analyses. When numerous variables are studied at the same time, correlations can be calculated between them, which are important for the selection of characteristics of interest for plant breeding (Moreira *et al*., 2013); for this, Pearson's correlation is used. However, care must be taken because, in many cases, it may not be a real measure of cause and effect, leading to misunderstandings during the interpretation of the data. In this way, path analysis is a statistical analysis capable of recognizing cause and effect relationships (Wright, 1921), unfolding the correlation coefficients in direct and indirect effects of independent variables on a dependent variable. According Rafiei and Saeidi (2005), this method is more coherent than simple linear correlations because they do not provide accurate information on each characteristic.

In this type of cause and effect relationship analysis, the presence of multicollinearity between the explanatory variables is common, and this factor may cause misunderstandings in the interpretation of the results (Cruz *et al*., 2012; Olivoto *et al*., 2017). Thus, it is extremely important to perform the multicollinearity diagnosis before carrying out the path analysis in order to obtain more accurate estimates of the direct and indirect effects on the studied dependent variable (Lúcio *et al*., 2013; Toebe and Cargnelutti Filho, 2013).

In the Stepwise method for selecting variables, the selection procedure is performed automatically with statistical packages, selecting a model with variables that explain the behavior of the dependent variable and that can be used to select variables that cause multicollinearity in the regression analysis linear (Zhang, 2016). Criteria for selecting variables include adjusted R-squared, Akaike information criterion (AIC), and Bayesian information criterion (BIC), among others (Hocking, 1976; Hosmer *et al*., 1989).

In this context, the objective of this study was to identify and estimate the relationships between the variables of production components and the total productivity of tomato fruits.

MATERIAL AND METHODS

This study was conducted at the Federal University of Santa Maria (UFSM), Campus Frederico Westphalen (27°23' S, 53°25' W and 493 m of altitude) over two years of cultivation (2012 and 2013). According to the classification of Köeppen, the region's climate is Cfa, humid subtropical, with an annual average precipitation of 1,800 mm well distributed throughout the year and subtropical from the thermal point of view (Alvares *et al*., 2013).

The soil preparation for plant cultivation was carried out with the conventional system. For the planting of the seedlings, grooves with a depth of approximately 20 cm were made, with a basic fertilization sequence, according to the soil analysis and the recommendation of the Soil Chemistry and Fertility Commission (CQFSRS/SC, 2004).

The hybrid seedlings of the Italian type group (Netuno and San Vito) were produced in polystyrene trays with 128 cells in a greenhouse. Carolina® commercial substrate was used, and, after sowing, the trays were kept in a floating system. The transplant occurred on September 4, 2012 and January 26, 2013, when they presented five definitive leaves. Spacings of 1.0 m between rows and 0.5 m between plants was used. The plants were trained vertically on a single stem with a wire when they reached 15 cm in height. Drip irrigation was used to meet the water requirements of the crop. During the cycle, the shoot leaves were removed every 2 d, and cover fertilization was carried out every 10 d, according to the soil analysis and recommendations for cultivation.

The experiment was conducted in a 2×3×3 randomized complete block design, the factors being two hybrids (Netuno and San Vito), three doses of boron (H_3BO_3 - 0, 2, 4 g/pit) and three frequencies of floral calcium applications ($CaCl_2$ at 0.6%) (no application; application every 7 d; and application every 14 d) (Plese *et al*., 1998), totaling 18 treatments with four replicates and 20 plants per plot.

At 60 d after transplanting the seedlings, when all plants had produced the seventh floral cluster, the height (ALT, in cm) of the plants was measured from the base to the apex using a tape measure. The fruits were harvested when they had yellowish spots. After harvesting, the fruits were counted and weighed daily using a digital scale, resulting in the following variables: total number of fruits per plant (NTF); total mass of fruits (MTF, g/plant); average fruit mass (MMF, g/plant); commercial fruit mass (MFC, g/plant) and non-commercial fruit mass (MFNC, g/plant); number of commercial fruits (NFC) and non-commercial fruits (NFNC); mean fruit diameter (D, cm) measured with a caliper; and total productivity (PROD, g).

In order to evaluate the relationships between the variables, Pearson's linear correlation coefficients were estimated. In order to perform path analysis without multicollinearity, the selection of the explanatory variables was done with the Stepwise method, and the following variables were selected: NTF, MTF, D, NFC and NFNC. After the selection of the variables with Stepwise, a multicollinearity diagnosis was performed between the explanatory variables through the analysis of the condition number

$$NC = \frac{\lambda \max}{\lambda \min},$$

which represents the ratio of the largest to the smallest eigenvalue of the correlation matrix, and the variance inflation factor

$VIF = \frac{1}{1-R_i^2}$, where R_j^2 the coefficient of determination is; after diagnosis, NFNC was excluded because there was high multicollinearity.

The path analysis was carried out with the Pearson correlation matrix, using the productivity variable (PROD) as the dependent variable. The path analysis coefficients were obtained with the methodology proposed by Cruz *et al*. (2012) using equation:

$$Y = P_{o1}X_1 + P_{o2}X_2 + \ldots + P_{on} + X_n + P_u \quad (1),$$

were Y is the coefficient of the dependent variable; P_o is the direct effect coefficient; X is an explanatory independent variable; P_u is the residual effect and the standardization variable.

Statistical analyzes were performed at 5% significance, with the MASS and agricolae packages available in the R program (R Core Team 2017).

RESULTS

The Pearson correlation analysis between the variables revealed several significant correlations. For the year 2012, the values showed a weak ALT correlation with the other variables. However, as expected, the NTF variable presented a strong correlation with the variables MTF, NFC, MFC (0.98, 0.93 and 0.91, respectively) and a negative correlation with the variables NFNC and MFNC (-0.65 and -0.62 respectively). The variable MTF was positively correlated with four of the ten variables: NTF, NFC, MFC and PROD (0.98, 0.94, 0.94 and 1.00, respectively) and negatively correlated with NFNC and MFNC (-0.69 and -0.66, respectively) (Tab. 1).

The variable NFC presented a significant and positive correlation with NTF, MTF, MFC, and PROD (0.93, 0.94, 0.98 and, 0.94 respectively), but was negatively correlated with NFNC and MFNC (-0.89 and -0.84. respectively). NFNC showed a positive and strong correlation with MFNC (0.94) and, with the other variables, had a negative correlation, indicating that the higher the number of non-commercial fruits, the lower the production in general. The same was observed for the variable MFNC. The MFC and PROD variables presented a strong correlation with the NTF (0.91 and 0.98), MTF (0.94 and 1.00) and NFC (0.98 and 0.94) variables, but was inversely proportional to NFNC (-0.87 and -0.69) and MFNC (-0.87 and -0.66) (Tab. 1).

For the year 2013, the relationships between the variables followed the same trend as 2012; however, they were moderately smaller, but still significant. Contrary to 2012, the variable MMF presented a higher correlation with the variables MTF, NFC, and MFC (0.56, 0.41 and 0.63, respectively) (Tab. 1).

For the two years (2012 and 2013), the Stepwise method selected the NTF, MTF, D, NFC and NFNC variables for path analysis. However, the use of these variables caused a number of conditions (NC), and the values of inflation of variance (VIF) were high; thus, the NFNC variable was discarded from the analysis, and this action corrected the multicollinearity problem. Thus, the NC values were 165 and 30 for the years of 2012 and 2013, respectively. The VIF values for the year 2012 were 27.58, 31.30, 1.20 and 9.55. For the year 2012, the multicollinearity was moderate; however, in this case, this value did not imply serious problems because they are not much above those indicated. For the year 2013, the VIF values (5.55, 7.03, 1.37 and 3.64) were low.

In the year 2012, the decomposition of linear correlations in direct and indirect effects presented a coefficient of determination of 94% and a very low residual effect, proving that the selected variables explained a large part of the observed variation. The negative and negligible effect of the direct effect of the NTF (-0.000019) and the positive and negligible effect of the direct effect of the NFC (0.000002) variables and the strong and positive correlation revealed that the correlation in the indirect effects explained the variable PROD. In the variable MTF, in which the direct effect was high and positive (1.000018), the correlation was also high and positive (1.00); the variable PROD was fully explained by the variable MTF; that is, it had a cause and effect relationship between the

Table 1. Estimates of the Pearson correlation coefficients for the variables height (ALT, cm), total number of fruits per plant (NTF), total mass of fruits (MTF, g/plant), average fruit mass (MMF, g/plant), commercial fruit mass (MFC, g/plant) and non-commercial (MFNC, g/plant), number of commercial fruits (NFC) and non-commercial (NFNC), and mean fruit diameter (D, cm) total productivity (PROD, g) of two Italian tomato cultivars produced under different doses of boron and calcium application, in 2012 (upper diagonal) and 2013 (lower diagonal).

Variables	ALT	NTF	MTF	D	NFC	NFNC	MFC	MFNC	PROD	MMF
ALT	-	0.03 ns	0.04 ns	0.18 ns	0.05 ns	-0.06 ns	0.07 ns	-0.09 ns	0.04 ns	0.08 ns
NTF	0.22 ns	-	0.98*	0.25 ns	0.93*	-0.65*	0.91*	-0.62*	0.98*	0.28 ns
MTF	0.31 ns	0.88*	-	0.26 ns	0.94*	-0.69*	0.94*	-0.66*	1.00*	0.46
D	-0.16 ns	0.02 ns	0.22 ns	-	0.36 ns	-0.41*	0.35 ns	-0.40*	0.26 ns	0.14 ns
NFC	0.09 ns	0.77*	0.84*	0.25 ns	-	-0.89*	0.98*	-0.84*	0.94*	0.40*
NFNC	0.15 ns	-0.14 ns	0.14 ns	-0.40	-0.53*	-	-0.87*	0.94*	-0.69*	-0.47*
MFC	0.18 ns	0.69*	0.89*	0.37 ns	0.95*	-0.55*	-	-0.87*	0.94*	0.48*
MFNC	0.21 ns	-0.13 ns	0.07 ns	-0.42	-0.49*	0.94*	-0.51*	-	-0.66*	-0.43*
PROD	0.31 ns	0.88*	1.00*	0.23 ns	0.84*	-0.14 ns	0.89*	-0.07 ns	-	0.46*
MMF	0.28 ns	0.10 ns	0.56*	0.48*	0.41*	-0.50*	0.63*	-0.34 ns	0.56*	-

ns - not significant; * - significant at 5% probability of error by the t-test variables. On the other hand, variable D had no cause and effect relationship with the main PROD variable (Tab. 2).

For the year 2013, the decomposition of linear correlations into direct and indirect effects showed a 99% coefficient of determination and a low residual effect, so the selected variables explained a large part of the main PROD variable. The results were similar to those of 2012, with only minor changes related to the intensity of the effect. The negligible effect of the direct effect of the NTF (0.00219) and NFC (0.00142) variables and the strong and positive correlation revealed that the indirect effects explained the variable PROD. The variable MTF demonstrated a cause and effect relationship with the PROD variable as seen in 2012, and variable D had no direct or indirect effects on the main PROD variable (Tab. 2).

DISCUSSION

The variables ALT, D, MMF did not present a significant correlation with the other variables. Similarly, Sari *et al*. (2017) evaluated the linear relationships between cherry tomato characteristics and found a weak linear effect from the variables fruit length per plant, average fruit width per plant and average fruit weight per plant on the variables number of bunches per plant, number of fruits per plant and number of fruits per bunches.

In a study carried out by Kumar *et al*. (2013), a positive relationship was observed between fruit yield per plant and number of fruits per plant. Contrary to what was found in the present study, the same authors observed that fruit weight showed a strong correlation with fruit length and fruit diameter. The relationships between characteristics may change according to the cultivar or management adopted for the crop (Fallahi *et al*., 2017). In this way, the type of management adopted in the present study, different doses of boron and calcium applications, may have interfered with the relationships between the characteristics since boron actively participates in floral induction and maintenance of flowers in plants (Perica *et al*., 2001), leading to higher fruit yields and, consequently, higher yields. In addition, boron participates in the formation of the pollen tube and fruit formation, which contribute to the non-appearance of deformed fruits, thus reducing the number and mass of non-commercial fruits. In this study, it was possible to observe that, when there is an increase in positive

Table 2. Path analysis evaluated in two cultivars of Italian tomato submitted to doses of boron and calcium applications, involving the dependent variable productivity (PROD) and the explanatory independent variables, with the split of Pearson's correlations in components of direct effect (main diagonal, underline) and indirect (in the line) for the years of 2012 and 2013.

2012					
Characteristic	**NTF**	**MTF**	**D**	**NFC**	***r***
NTF	<u>-0.000019</u>	0.981312	-0.000002	0.000002	0.98
MTF	-0.000018	<u>1.000018</u>	-0.000002	0.000002	1.00
D	-0.000005	0.261503	<u>-0.000008</u>	0.000001	0.26
NFC	-0.000017	0.938043	-0.000003	<u>0.000002</u>	0.94
Residual effect	1.17×10^{-9}				
R^2	0.94				
2013					
NTF	<u>0.00219</u>	0.87185	-0.00002	0.00109	0.88
MTF	0.00192	<u>0.99623</u>	0.00026	0.00120	1.00
D	-0.00004	0.22384	<u>0.00115</u>	0.00035	0.23
NFC	0.00169	0.84073	0.00028	<u>0.00142</u>	0.84
Residual effect	7.7×10^{-4}				
R^2	0.99				

NTF: total number of fruits per plant; MTF: total mass of fruits; D: mean fruit diameter; NFC: number of commercial fruits; *r*: Pearson's correlation coefficient.

variables, a higher fruit production results in a negative correlation with the variables NFNC and MFNC.

Calcium is an essential macronutrient for plants because it actively participates in extracellular stimuli and intracellular responses that control a large amount of endogenous processes (Edel *et al*., 2017). In addition, it has role in cell wall structure and acts on factors that control the development of plants and responses to biotic and abiotic stresses (González-Fontes *et al*., 2017). This nutrient is also important for plant growth and fruit yield (Bastías *et al*., 2010). Thus, when tomato plants are under stress or suffer a nutrient deficiency, linear relationships between variables may be different.

The high values of the coefficient of determination (R^2) and the low value of the residues indicated that there was precision in the estimates of the direct and indirect effects (Rios *et al*., 2012; Donazzolo *et al*., 2017). In the present study, the low values of NC and VIF may also have contributed to high R^2 values and low residual values in both evaluated years.

The results obtained from the path analysis were interpreted in the same way as Lúcio *et al*. (2013). That is, when the Pearson correlation coefficient was positive and indirect effects caused the direct effect negative or low, the correlation. Otherwise, if the Pearson correlation coefficient was low and the direct effect positive and high, the indirect effects were responsible for the lack of correlation. And when Pearson's correlation was negative and the direct effect positive and high, the indirect effects were eliminated from the analysis, and only the direct effect was conidered.

The use and interpretation of Pearson's correlation alone between variables can cause biased results since it does not employ the direct and indirect effects. So, the path analysis provides more reliable results that surpass the limitations of Pearson's correlation (Cruz *et al*., 2012). This was observed in the present study, that is, when the correlation between PROD and NTF was high this coefficient was used in the direct and indirect effects, it was observed that the NTF variable did not influence the PROD variable through cause and effect.

In both evaluated years, the path analysis revealed a cause and effect relationship between the MTF independent variable and the PROD dependent variable. This relationship was already expected since it is through the total mass of fruits that productivity is determined. Contrary to the present study, Sari *et al*. (2017) evaluated the linear relationships between characteristics of cherry tomatoes and concluded that production is directly related to the number of fruits produced and that the individual weight of each fruit has little influence on total production. In the present study, NTF presented low influence on the basic variable PROD. Rodrigues *et al*. (2010) observed that the variables mean weight of fruits and total number of fruits had high magnitudes of direct and indirect effects on total fruit production in salad-type tomatoes.

CONCLUSION

There were cause and effect relationships between the independent variable total mass of fruits and the main variable total fruit yield. The variables diameter and total number of fruits did not contribute substantially to an increased tomato production.

ACKNOWLEDGMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for supporting this study. We thank Paul Gallo and Duncan Hedderley for volunteering for the English grammar review.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Effectiveness of reduced doses of flumioxazin herbicide at weed control in direct sow onions

Efectividad del herbicida flumioxazin a dosis reducida para el control de malezas en cebollas de siembra directa

CHRISTIANE AUGUSTA DINIZ MELO[1, 5]
AMANDA ROCHA BARBOSA[2]
ROQUE DE CARVALHO DIAS[3]
GUSTAVO SOARES DA SILVA[4]
MARCELO RODRIGUES DOS REIS[2]

Onion prodution in the experimental area of Cooperativa Agropecuária do Alto Paranaíba (COOPADAP), Rio Paranaíba-MG, Brazil.

Photo: C.A. Diniz Melo

ABSTRACT

The recommended application of most herbicides in onion crops is after transplanting seedlings with four true leaves. In the direct sowing system, this recommendation is considered late; an alternative management is the application of reduced doses starting with a true leaf. The objective of this study was to evaluate the use of reduced doses of flumioxazin in the early phenological stages of onions on bulb yield. Two field experiments were installed, and five doses of flumioxazin (5, 10, 15, 20, and 25 g ha^{-1}) were applied in three phenological stages (1^{st}, 2^{nd}, and $1^{st}+3^{rd}$ true leaf); weed control was carried out. The results demonstrated the efficacy of reduced doses of flumioxazin on onion crop in the early stages. The dose of 20 g ha^{-1} showed use potential in the two experiments for the cvs. Perfecta and Sirius, enabling reductions of 77 to 88% of the commercial dose recommended for onions established with seedling transplanting. The application of flumioxazin in the 2^{nd} leaf reduced commercial productivity and was ineffective in the control of weeds. The application in the 1^{st} + 3^{rd} leaf, despite being an effective control, caused greater phytotoxicity and, therefore, reduced commercial productivity. The best strategy for weed management is 20 g ha^{-1} flumioxazin applied to onion plants when they reach the true first leaf stage.

Additional key words: *Allium cepa*; bulb yield; herbicide; phytotoxicity.

[1] Universidade Federal do Triângulo Mineiro, Iturama Campus, Iturama (Brazil). ORCID Melo, C.A.D.: 0000-0001-6385-2153; ORCID Reis, M.R.: 0000-0002-9940-5838
[2] Universidade Federal de Viçosa, Rio Paranaíba Campus, Rio Paranaíba (Brazil). ORCID Barbosa, A.R.: 0000-0002-4273-7399
[3] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculty of Agricultural Sciences, Botucatu (Brazil). ORCID Dias, R.C.: 0000-0001-5433-5373
[4] Universidade de São Paulo, Colégio de Agricultura Luiz de Queiroz, Piracicaba (Brazil). ORCID Silva, G.S.: 0000-0003-3267-7254
[5] Corresponding author: christiane.melo@uftm.edu.br

RESUMEN

Se recomienda en la mayoría de las veces la aplicación de herbicidas en cultivos de cebolla después del trasplante, cuando las plántulas tienen cuatro hojas verdaderas. En el sistema de siembra directa esta recomendación se considera tardía y una alternativa es la aplicación de menores dosis desde la aparición de una hoja verdadera de la cebolla. El objetivo de este estudio fue evaluar dosis reducidas de flumioxazina en estadios fenológicos tempranos de cebolla sobre el rendimiento de los bulbos. Se realizaron dos experimentos de campo y se aplicaron cinco dosis de flumioxazina (5, 10, 15, 20 y 25 g ha^{-1}) en tres estadios fenológicos (1ª, 2ª y 1ª+3ª hoja verdadera), manteniendo un control con deshierbe manual. Los resultados demostraron la eficacia de dosis reducidas de flumioxazina en el cultivo de cebolla en estadios fenológicos tempranos. La dosis de 20 g ha^{-1} mostró un potencial en los dos experimentos para cultivares Perfecta y Sirius, lo que permite reducirse entre el 77 y 88% de la dosis comercial recomendada en plántulas cebolla trasplantada. La aplicación de flumioxazina en la 2ª hoja redujo la productividad comercial y fue ineficaz en el control de malezas. La aplicación en la 1ª + 3ª hoja, a pesar de ser efectiva en el control, provocó una mayor fitotoxicidad y, por lo tanto, redujo la productividad comercial. La mejor estrategia para el manejo de malezas es 20 g ha^{-1} de flumioxazina aplicada a las plantas de cebolla cuando alcanzan la etapa de la primera hoja verdadera.

Palabras clave adicionales: *Allium cepa*; rendimiento de bulbos; herbicida; fitotoxicidad.

Received for publication: 16-04-2018 Accepted for publication: 29-03-2019

INTRODUCTION

Onions (*Allium cepa* L.) are very sensitive to interference from weeds. Onion cultivation is characterized by low soil cover because of the low plant size, little production of leaves, which are cylindrical and erect, and slow initial growth of the crops. These aspects result in low competitiveness and allow the germination and growth of weeds at practically any stage of development (Reis *et al*., 2017).

In terms of crops with a high technological level, this crop presents a high cost of production; it is common to find high levels of fertility in this production system, with systematic irrigation and intensive tillage, which promote high populations of weeds (Pereira, 2008). The management of weeds in onion crops is an important variable in the definition of productivity since this crop is negatively affected by competition with weeds. Onion productivity decreases drastically with the interference of weeds, which may reach 100% loss in the commercial production of bulbs (Jean-Simon *et al.*, 2012; Qasem, 2005). Thus, it is necessary to use control measures during the critical period of interference prevention, which is long in the onion crop and varies from 40 to 100 d (Soares *et al.*, 2003; Qasem, 2006).

Among the control methods, chemical management of weeds is widely used in this crop because of greater yield in large areas, high efficiency and lower cost. However, it requires knowledge on the use and behavior of herbicides in the soil and their selectivity to the crop, especially because the onion is highly sensitive to these products (Qasem, 2006; Uygur *et al.*, 2010; Carvalho *et al*., 2014).

In Brazil, there are few herbicide options for onion use. Linuron has been the standard for chemical weed control in onion crops since the 1980s. However, the control exerted by this herbicide has been unsatisfactory because, after 10-15 d, post-emergence herbicide applications, which may toxify the crop, and/or manual weeding are required, increasing the cost of production.

Flumioxazin (*N*-[7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2*H*-1,4-benzoxazin-6-yl]cyclohex-1-ene-1,2-dicarboxamide) may be an alternative for herbicide use in onion crops. It is an active non-ionic ingredient that exhibits low solubility in water (1.79 mg L^{-1} at 25°C) (Ferrell *et al.*, 2005) and a vapor pressure of 2.41×10^{-6} mm Hg at 22°C, indicating a low volatilization potential (Rodrigues and Almeida, 2011). It may present half-life of 17.6 d in the soil (PPDB, 2018) and undergoes degradation through hydrolysis and microorganism activity (Alister *et al*., 2008). Accordingly, flumioxazin presents little aggression to the environment; however, there are reports that, in some areas of Alliaceas in Alto Paranaíba, damage occurred through phytotoxicity, mainly with excess rainwater or irrigation and the use of high doses or doses at an inappropriate vegetative stage, making farmers afraid to use it.

Flumioxazin is registered for pre-emergence applications on weeds, 2-3 d after seedling transplanting (Agrofit, 2018). However, in the direct sowing system, applications on onion plants at the true four-leaf stage, the recommended stage for most herbicides, is considered late and ineffective against the advanced development of weeds.

Because of these problems, an alternative is to use reduced doses at the initial stages of the crop (1st, 2nd, and 1st + 3rd true leaf) since recommended doses for the onion vary from 60 to 90 g ha^{-1} ai. The objective of this study was to evaluate the efficacy of reduced doses of flumioxazin, applied in the initial phenological stages of the onion crop in terms of bulb productivity.

MATERIAL AND METHODS

Two experiments were carried out under field conditions. Experiment 1 was set in the experimental area of Cooperativa Agropecuária do Alto Paranaíba (COOPADAP), located in the municipality of Rio Paranaíba, Minas Gerais, Brazil, in a clayey Dystroferric Red Oxisol from February to July of 2015. Experiment 2 was installed in an area of commercial production on the Santo Amaro Farm, located in the municipality of Rio Paranaíba, in a dystrophic Red Oxisol with a clay texture from February to June of 2016.

In both experiments, direct sowing was used with four double lines per bed, where one million and two hundred thousand seeds per hectare were distributed. In experiment 1, cv. Perfecta was sown and, in experiment 2, cv. Sirius was used.

The planting fertilization in experiment 1 was 800 kg ha^{-1} of P_2O_5, 50 kg ha^{-1} of N and 50 kg ha^{-1} of K_2O. At 20 and 40 d after emergence, topdressing fertilization was carried out with 75 kg ha^{-1} of N and 125 kg ha^{-1} of K_2O. In experiment 2, 950 kg ha^{-1} of P_2O_5, 40 kg ha^{-1} of N and 30 kg ha^{-1} of K_2O were used. At 25 and 50 d after emergence, topdressing fertilization was carried out with 55 kg ha^{-1} of N and 130 kg ha^{-1} of K_2O. The fertilization differences between the areas were based on the chemical analyses of the soils and nutrition demand of the cultivars.

In both experiments, a randomized complete block design was used, with treatments arranged in a 5×3+1 factorial scheme, with four replicates. The first factor corresponded to the reduced doses of flumioxazin (5, 10, 15, 20 and 25 g ha^{-1}), and the second factor was the phenological stage of the onion on which the herbicide was applied (1st, 2nd, and 1st+3rd true leaf), as well as weed control. The parcels with the treatment 1st+3rd true leaf received two applications of flumioxazin in the two phenological stage of the onion at the five doses described.

The dimensions of each experimental plot were 1.5 (four double lines) × 4 m, and the two central double lines were considered as the useful area, minus 0.5 m at each end of the plot. For the application of flumioxazin (Flumyzin 500 – active ingredient concentration 500 g kg^{-1} – wettable powder formulation), a CO_2 backpack sprayer pressurized at 2 bar equipped with a bar with three flat jet tips, (fan type) 110.02, spaced at 0.5 m was used at 0.5 m above the soil with a volume of 200 L ha^{-1}.

At 15, 30 and 45 d after application (DAA) of the herbicide, counted from the application on the 1st true leaf stage, phytotoxicity evaluations were performed using a visual scale from 0 to 100%, where 0% represents the absence of symptoms and 100% is the death of the plant, and visual evaluation of the weed control (Gazziero, 1995). At 30 and 60 DAA, stand evaluations were performed by counting plants in two linear meters at three delimited points in each plot.

At 150 and 130 d after sowing, the onion was harvested in Experiments 1 and 2, respectively. One hundred plants were harvested in the useful area of each plot, and the commercial (Classes 2, 3, 4, and 5) and non-commercial (Classes 0 and 1) and total bulb yields were classified and estimated. The classification was performed according to the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP) commercial scale, based on the equatorial diameter of the bulbs in Class 0 (<1.5 cm), Class 1 (1.5 to 3.5 cm), Class 2 (3.5 to 5.0 cm), Class 3 (5.0 to 7.0 cm), Class 4 (7.0 to 9.0 cm), and Class 5 (>9.0 cm).

The data were submitted to analysis of variance by the F test ($P \leq 0.05$), and the means were plotted in figures with standard error. The experiments were analyzed separately. The software Sisvar 5.6 and Sigmaplot 10.0 were used for this.

RESULTS AND DISCUSSION

The onion toxicity was affected by the phenological stage of the crop, reduced doses of flumioxazin and cultivar. In experiment 1, at 15 DAA, the plants presented 15% injury on average (Fig. 1A), characterized by chlorotic spots on the leaves. In experiment 2, the application performed on the 2nd leaf caused increased phytotoxicity as the doses increased up to a maximum of 25% (Fig. 1D). At the time of this evaluation, the application on the 3rd leaf stage had not yet been performed because the onion plants still had two true leaves, explaining the equal results with the applications performed in the stages 1st and 1st+3rd leaf.

According to Cavaliere (2015), in order to minimize the toxicity caused by herbicides in onions, fractionation and escalation of doses have been carried out and have contributed to increased selectivity in onions, especially in the initial stages of growth of direct sowing plants. However, even with the reduction of doses, phytotoxicity may occur, as verified in the present study. In Turkey, onion phytotoxicity from herbicides is also a serious problem and, even when splitting the recommended dose of oxyfluorfen, injures have been found in plants (Uygur *et al*., 2010).

In the visual evaluations performed at 30 and 45 DAA in experiment 1, higher phytotoxicity was observed for the plants submitted to application on the 1st+3rd leaf, followed by application on the 2nd leaf and on the 1st true leaf of the crop (Fig. 1B and 1C). In experiment 2 at 30 DAA, the application on the 1st+3rd leaf caused greater phytotoxicity in the plants than at other stages, with reduction of the symptoms at 45 DAA, regardless of the treatments (Fig. 1E and 1F).

The increase in phytotoxicity in relation to the phenological stages of the crop may evidence that the tolerance of the onion to this herbicide decreases with the age of the plant and the number of applications (1st+3rd leaf). This is probably related to the increase in the number of leaves and, consequently, the leaf area for interception of the product at those initial stages of the onion planted via direct sowing. For oxyfluorfen, a herbicide of another chemical group, but with the same mechanism of action as flumioxazin, studies have shown that symptoms decreased with the age of the transplanted onion, which is due to the increase in the wax content in the leaves (Carvalho *et al*., 2014). These authors verified greater phytotoxicity when oxyfluorfen was applied at 14 d after transplanting (DAT) when compared to the application at 28 DAT.

At 60 DAA, the onion plants completely recovered from toxicity, regardless of the phenological stage (data not shown). This recovery was due to the low or absence of translocation of the herbicide in the plant, acting as a contact product. Thus, the new emitted leaves were free of toxicity symptoms. Durigan *et al*. (2005) found that, at 49 DAA of flumioxazin, onion plants recovered from severe initial toxicity when the application was made on the 5th-6th leaf of the transplanted crop.

Weed control in both experiments at 15 DAA was satisfactory in the plots where application was made on the 1st leaf (Fig. 2A and 2D). The application on the first true leaf of the onion provided weed control at an early stage of development, ensuring control effectiveness even at the smallest evaluated dose. The predominant species in experiment 1 were *Nicandra physalodes* (L.) Gaertn., *Galinsoga parviflora* Cav., *Portulaca oleracea* L., *Sonchus oleraceus* L., and *Ageratum conyzoides* L. In experiment 2, the predominant weeds were *Eleusine indica* (L.) Gaertn, *Commelina benghalensis* L., *N. physalodes* and *Gamochaeta coarctata* (Willd.) Kerguélen.

The application on the second leaf of the onion satisfactorily controlled only with the dose of 10 g ha^{-1} in experiment 1 at 15 DAA (Fig. 2A). In the other evaluations (30 and 45 DAA), this treatment was ineffective (Fig. 2B and 2C). The application of reduced doses of flumioxazin when the crop had two true leaves was not effective probably because the weeds in the area were already more developed. In experiment 2, this application stage provided acceptable control from 30 DAA, regardless of the applied dose (Fig. 2E and 2F).

At 45 DAA in experiment 1, the applications on the 1st and 1st+3rd leaves maintained control of over 90% with the 10 g ha^{-1} dose of flumioxazin (Fig. 2C). Besides controlling weeds early in the area when applied at first leaf of the onion, the additional application on the third leaf extended the residual effect of the herbicide, which also had a pre-emergence action on the weeds. In experiment 2, the application in the three stages provided satisfactory control ($>$80%) (Gazziero, 1995), particularly on the 1st and 3rd leaves, which maintained control near 100% regardless of the dose (Fig. 2F).

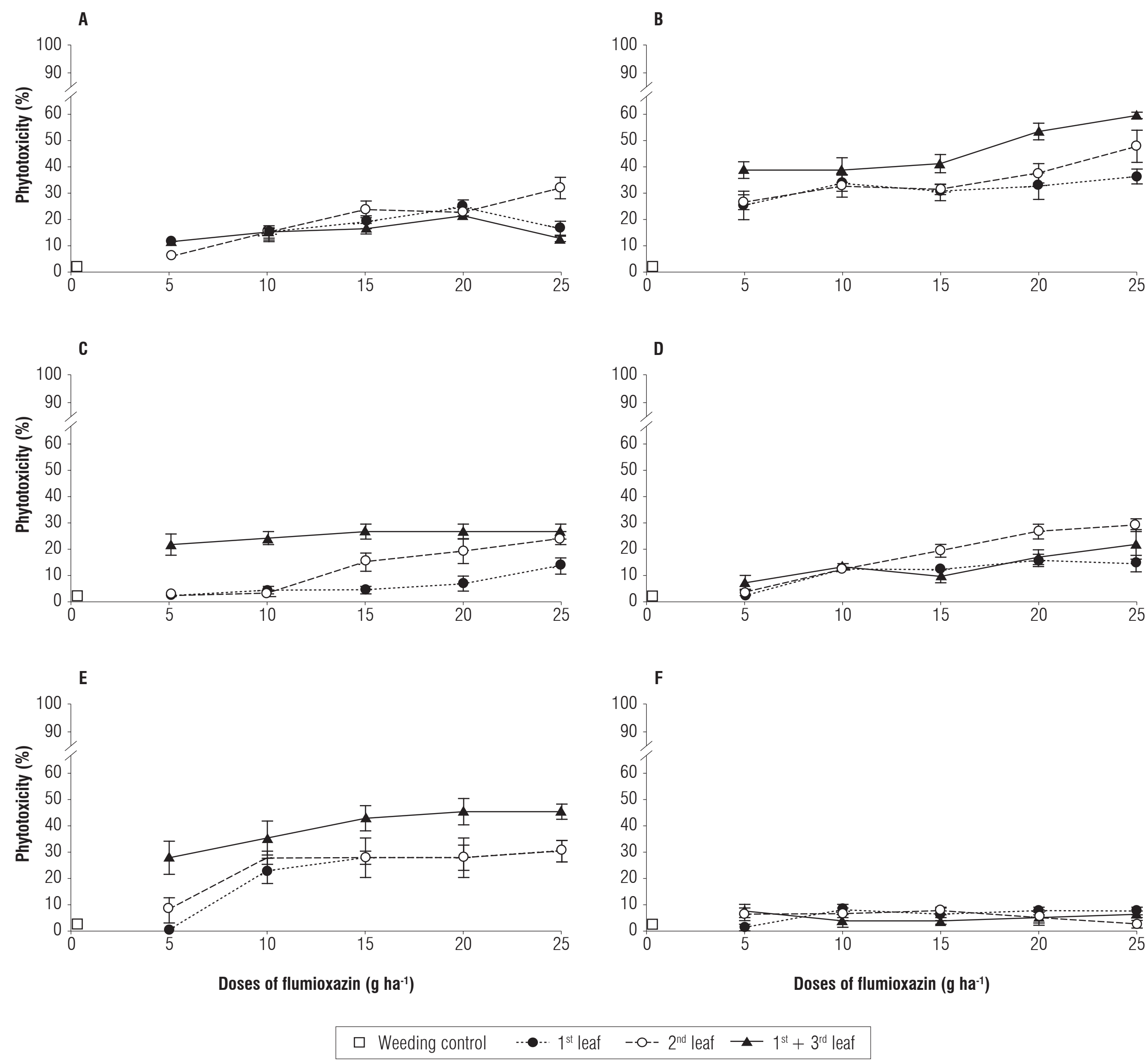

Figure 1. Phytotoxicity of onion plants evaluated at 15, 30 and 45 d after application of reduced doses of flumioxazin in experiment 1 (A, B, C) and experiment 2 (D, E, F), respectively.

In experiment 1, the stand was not altered by the application of reduced doses of flumioxazin in relation to the weeding control in the two evaluated periods (Fig. 3A and 3B). In experiment 2, the stand was reduced only with the highest dose (25 g ha^{-1}) at 30 DAA in the three application stages and, at 60 DAA, in the 1st and 1st+3rd leaf stages (Fig. 3C and 3D). The reduction in the number of onion plants was also verified by Ferreira *et al*. (2000) with an application of oxyfluorfen (0.192 kg ha^{-1}) at 19 d after sowing, reducing the initial stand by 22.6%.

Because the onion crop is very sensitive to herbicides, it is likely to suffer toxicity and be suppressed, especially in the early stages of development. Thus, depending on the dose and growth stage of the crop, phytotoxicity may be severe, irreversibly impairing the growth of the plants, leading to death and, therefore, reducing the initial population of the crop. Onion production fields established by direct sowing usually present a highly variable plant population, which was also observed in the experimental plots.

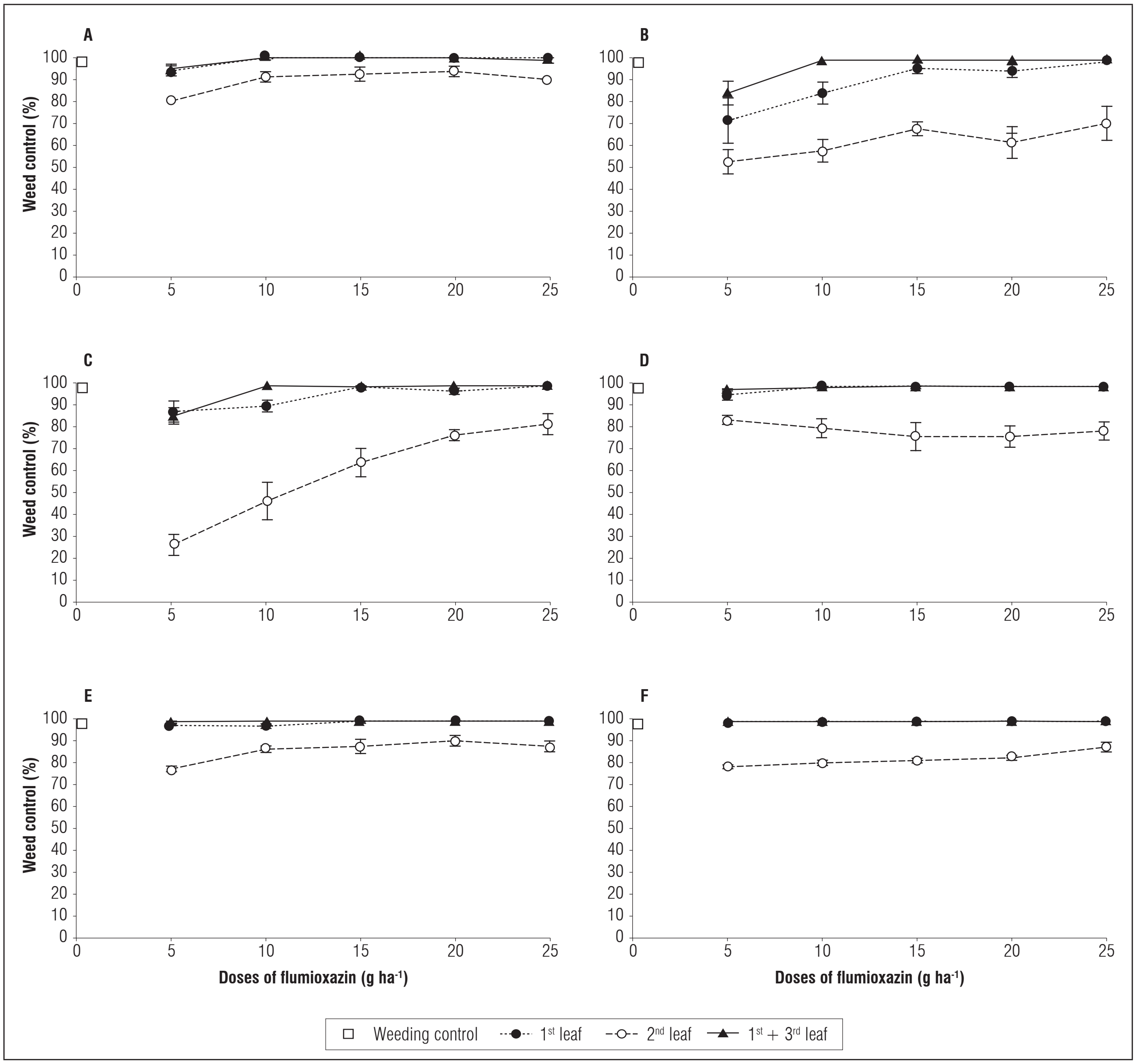

Figure 2. Weed control evaluated at 15, 30 and 45 d after the application of reduced doses of flumioxazin in experiment 1 (A, B, C) and experiment 2 (D, E, F), respectively.

The stand verified in the two experiments was different in the absence of herbicide (Fig. 3), which contributed to the difference in bulb yield observed in the two experiments (Fig. 4). The lowest number of plants per area reduced intraspecific competition, resulting in bulbs with a larger diameter and weight.

The application of flumioxazin on the 2nd and 1st+3rd leaves increased, in most doses, the bulb yield of 'Perfecta' non-commercial bulbs (Fig. 4A). The application at the first leaf was equal to or lower than the control, up to 15 g ha^{-1}, increasing the productivity of bulbs with a diameter less than 3.5 cm in the two highest doses. In experiment 2, the productivity of non-commercial bulbs was similar in the absence and presence of reduced doses of flumioxazin, except for the 20 g ha^{-1} dose applied at the 1st true leaf of the crop, which was lower than the control without an application (Fig. 4 D). Intoxication caused by herbicides may result in reduced crop growth and reduced

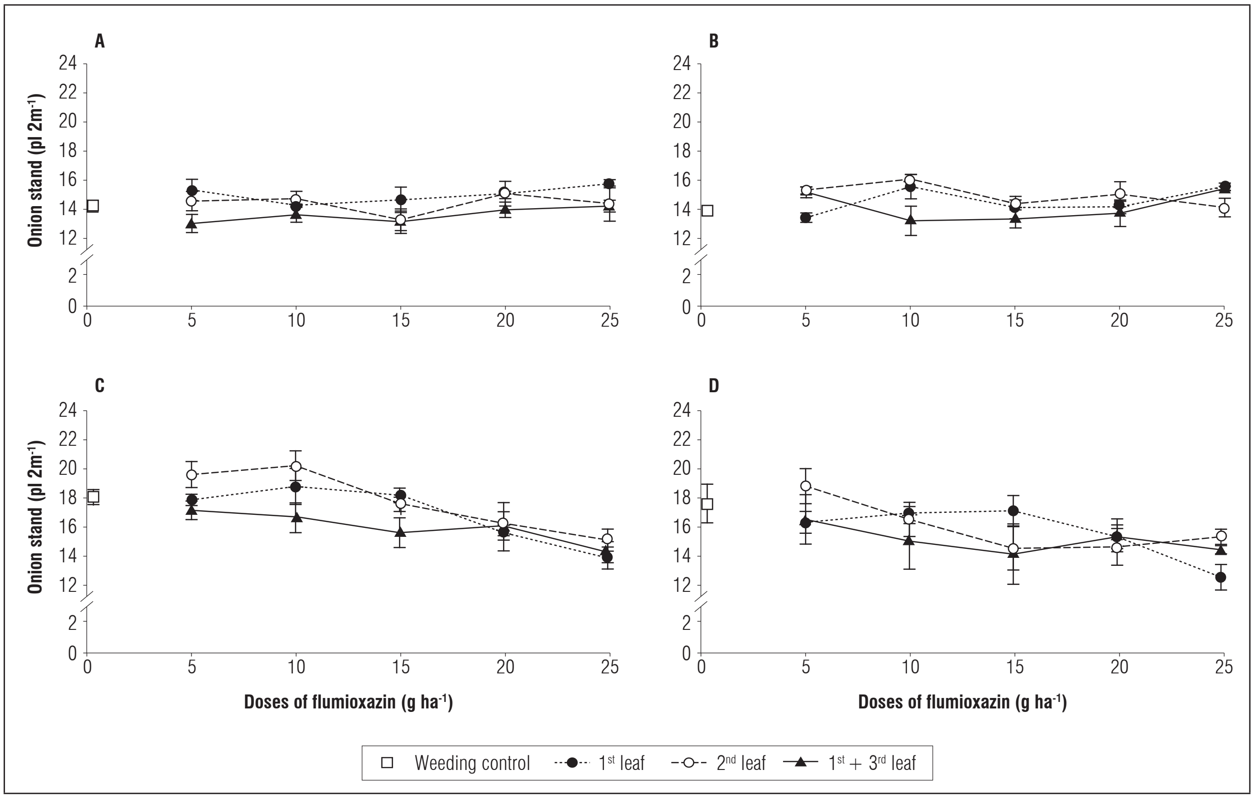

Figure 3. Onion stand evaluated at 30 and 60 d after the application of reduced doses of flumioxazin in experiment 1 (A, B) and experiment 2 (C, D), respectively.

quantity and quality of the products harvested (Robinson, 2008), even with the application of reduced doses.

The commercial and total yields in experiment 1 presented the same behavior, with emphasis on the application in the first leaf, which was superior to the other phenological stages up to the dose of 20 g ha^{-1}. The dose of 25 g ha^{-1} of flumioxazin applied on the 1st+3rd leaf reached productivity similar to the first leaf stage. However, in this experiment, the application of flumioxazin reduced the total and commercial productivity by at least 10 t ha^{-1}, as compared to the absence of herbicide (Fig. 4B and 4C). The cultivars presented different tolerances to flumioxazin, where 'Perfecta' - experiment 1 was less tolerant than 'Sirius' - Experiment 2 (Fig. 1). This fact contributed to the effect of the herbicide on the reduction of commercial and total bulb productivity and to the increase of the productivity of non-commercial bulbs, in relation to the weeding control in experiment 1.

This differential tolerance can be attributed to different foliar waxiness (Ferreira and Costa, 1982) in the two cultivars, which needs to be investigated. According to the technical information from the seed company, both cultivars have good waxiness; however, it is necessary to evaluate the thickness and composition of the cuticle and the amount of wax present in the leaves of the two cultivars to better explain the influence of these characteristics on the differential tolerance to flumioxazin and others herbicides applied post-emergence.

In experiment 2, starting with the 15 g ha^{-1} dose, a difference was found in commercial and total productivity between the stages, with superiority found in the application on the first leaf in comparison to the others. For the control without herbicides, the application on the 2nd leaf reduced the commercial and total productivity, the application on the 1st+3rd leaf was similar in all the doses and the application on the first leaf was similar up to the dose of 20 g ha^{-1} and higher at the dose of 25 g

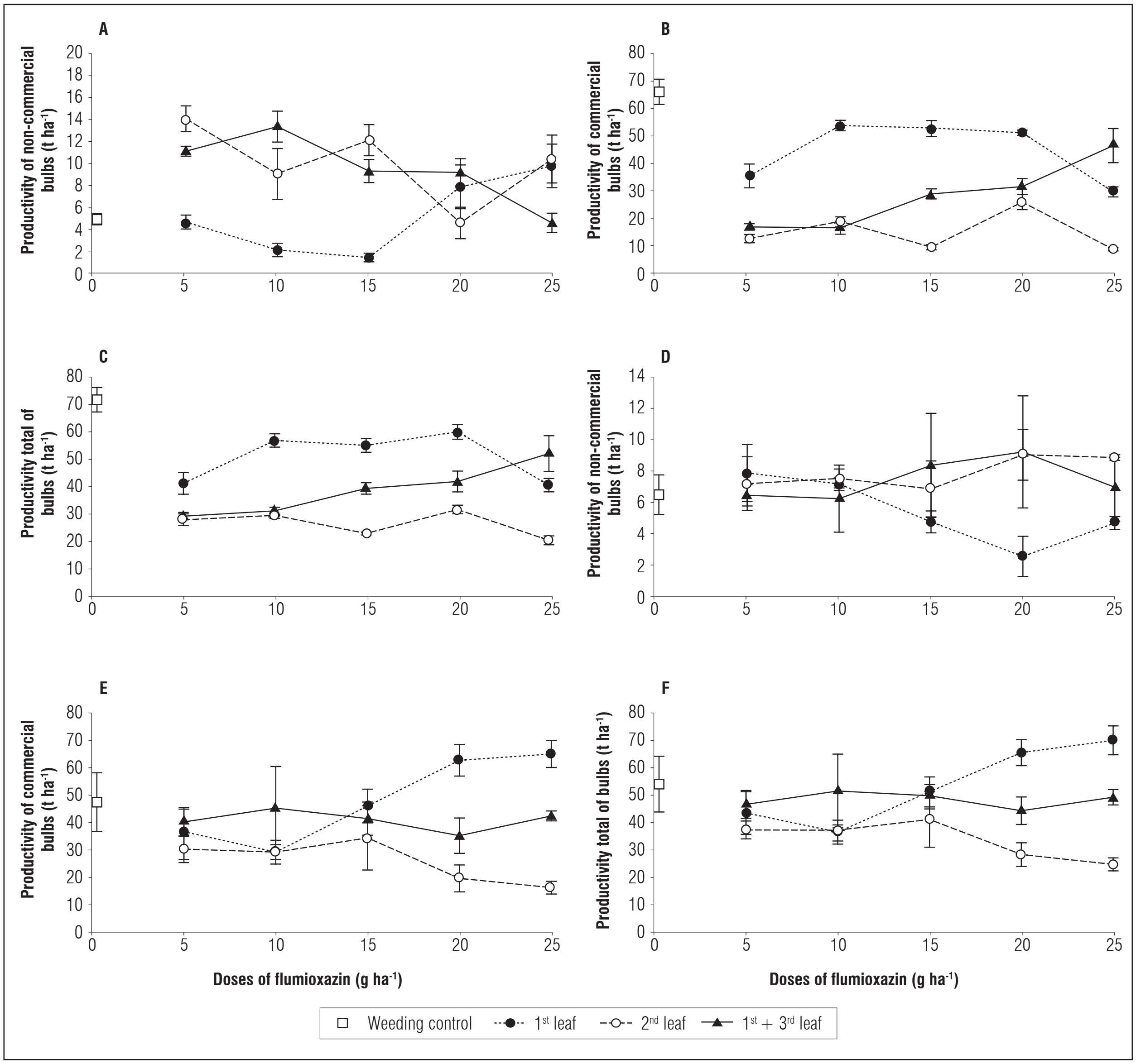

Figure 4. Productivity of non-commercial, commercial and total bulbs of onion plants submitted to reduced doses of flumioxazin at different phenological stages in experiment 1 (A, B, C) and experiment 2 (D, E, F), respectively.

ha^{-1} (Fig. 4E and 4F). In this experiment, it was evidenced that the use of reduced doses of flumioxazin may guarantee productivity equal to or even higher than the weeding control, depending on the dose applied and the phenological stage of the crop. In Iran, the use of 75% of the recommended dose of oxyfluorfen provided quality and bulb production comparable to the absence of herbicide according to a study by Abbaszadeh *et al*. (2014).

The results evidence the efficacy of reduced doses of flumioxazin in onion crops at early stages (1st, 2nd and 3rd true leaf). The dose of 20 g ha^{-1} showed use potential in the two experiments for 'Perfecta' and 'Sirius', enabling a reduction of 77% to 88% of the commercial dose recommended for onions planted with seedling transplanting. In addition, because of the lack of information in the literature on the behavior of flumioxazin in soil in tropical conditions, this reduction in the dose reduces the potential risks of environmental contamination.

CONCLUSIONS

The application of flumioxazin on the 2nd leaf reduced commercial productivity and was ineffective at controlling weeds. The application on the 1st+3rd leaf, although effective in the control, caused greater phytotoxicity and, therefore, reduced commercial productivity. The best strategy for weed management was 20 g ha^{-1} of flumioxazin applied to the onion plants when they reached the true first leaf stage.

ACKNOWLEDGEMENT

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the scholarship they granted and the Cooperativa Agropecuária do Alto Paranaíba (COOPADAP) and Santo Amaro Farm for their financial and technical support.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Compositional quality of food obtained with solid state fermentation: potato and carrot

Calidad composicional de alimentos obtenidos por fermentación en estado sólido: papa y zanahoria

ÁNGELA MIREYA RODRÍGUEZ-SALGADO[1]
LUIS MIGUEL BORRÁS-SANDOVAL[1]
CARLOS EDUARDO RODRÍGUEZ-MOLANO[1, 2]

Sample of carrots and potatoes.

Photos: A. Bohórquez-Salgado and C.E. Rodríguez-Molano

ABSTRACT

The fermentation indicators and compositional quality of food made with the solid-state fermentation of potatoes and carrots were analyzed in order to make use of foods that do not meet the requirements of consumers. Three treatments were formulated by varying the potato:carrot ratio mixed with wheat bran and corn cabbage. A microbiological analysis was performed at 0, 24 and 96 hours; the pH, crude protein (PC), ash (CZ), ethereal extract (EE), crude fiber (FC), neutral detergent fiber (FDN) and acid detergent fiber (FDA) were monitored at 0, 24, 48, 72 and 96 hours. Acidification was observed between sampling at 0 and 96 hours, with values ranging from 5.68 to 4.46, 5.73 to 4.46 and 5.69 to 4.33 for the treatments with the potato:carrot ratios 20:20, 25:25 and 30:30, respectively. Similarly, a reduction of dry matter ($P\leq0.05$) and PC increase ($P\leq0.05$) were observed from 20.1 to 26.7%, 21.1 to 27.1% and 25.4 to 27.9%, respectively for the previous ratios. No significant statistical difference was found in the variables CZ, MS, EE, FDN, FDA or FC; a significant growth of lactic acid bacteria (LAB) counts and an absence of Salmonella were evident. The proportionality in the PC increase with the potato:carrot ratios was viable with the increase of the drying material, such as wheat bran and corn cabbage.

Additional key words: feed; fermentation; waste management; crude protein; crops.

[1] Universidad Pedagógica y Tecnológica de Colombia, Grupo de investigación GIBNA, Tunja (Colombia). ORCID Rodríguez-Salgado, A.M.: 0000-0003-2187-9301; ORCID Borrás-Sandoval, L.M.: 0000-0002-3284-027X; ORCID Rodríguez-Molano, C.E.: 0000-0002-0862-3478.

[2] Corresponding author: carlos.rodriguez@uptc.edu.co

RESUMEN

Se analizaron los indicadores fermentativos y calidad composicional de un alimento por fermentación en estado sólido de papa y zanahoria; con el fin de aprovechar alimentos que no cumplen las exigencias del consumidor final. Se formularon tres tratamientos variando las inclusiones de papa:zanahoria, en mezcla con salvado de trigo y repila de maíz. Se realizó un análisis microbiológico a las 0, 24 y 96 horas; seguimiento a pH, proteína cruda (PC), cenizas (CZ), extracto etéreo (EE), fibra cruda (FC), fibra detergente neutro (FDN) y fibra detergente ácido (FDA) a las 0, 24, 48, 72 y 96 horas. Se presentó acidificación entre el muestreo 0 y 96 horas, con valores de 5,68 a 4,46; 5,73 a 4,46 y de 5,69 a 4,33 para los tratamientos con inclusiones de papa:zanahoria 20:20, 25:25 y 30:30, respectivamente. De igual forma, se observó reducción de materia seca ($P\leq0,05$) y aumento de PC ($P\leq0,05$) de 20,1 a 26,7%, 21,1 a 27,1% y de 25,4 a 27,9%, respectivamente para las anteriores inclusiones. No se encontró diferencia estadística significativa en las variables CZ, MS, E.E, FDN, FDA y FC; se evidenció un crecimiento importante de recuentos de bacterias acido lácticas (BAL) y la ausencia de *Salmonella*. La proporcionalidad en los incrementos de PC con inclusiones papa:zanahoria resulta viable con el incremento del material secante como salvado de trigo y repila de maíz.

Palabras clave adicionales: pienso; fermentación; aprovechamiento de residuos; proteína cruda; hortalizas.

Received for publication: 23-10-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The Department of Boyaca is characterized as an agricultural territory, favoring different crops, including potatoes (*S. tuberosum*) and carrots (*Daucus carota* L.). The potato has a cultivated area of 36,146 ha, average yield of 18.9 t ha^{-1}, total production of 713,592 t and large quantities of post-harvest waste; this department is ranked second for potato production in Colombia (Fedepapa, 2018). On the other hand, this department is also among the main carrot producers with a cultivated area of 1,765 ha, average yield of 38.13 t ha^{-1} (Agronet, 2015) and losses of 1% associated product deformation and alteration. The high amounts of post-harvest waste not only generate economic losses for producers but also potentially polluting agents.

Potato and carrot residues are nutritious components that can be used as animal feed with solid-state fermentation (SSF), thus avoiding possible contamination of the environment and generating a benefit to livestock production. SSF is a process that completes the carbon cycle, decomposes organic matter with fungi and bacteria that, when grown on a solid substrate, releases nutrients and energy (Peng *et al*., 2019).

SSF is characterized by microbial growth on a solid substrate (textured and porous), which has a low moisture content, in order to optimize the amount of protein and other nutrients contained in food that an animal will consume (Chen and Wang, 2017; Tosuner *et al*., 2018). In essence, it involves the use of microorganisms in order to achieve special properties in food. The growth process of the microorganisms on the substrate uses it as a source of nitrogen and nutrient salts, creating certain parameters of moisture, pH, aeration and temperature (Costa *et al*., 2018).

Fonseca-López *et al*. (2018) pointed out that carrot is a solid substrate that provides desirable sugars for the development of the fermenting microbiota in the SSF process, obtaining 16, 17 and 16.6% proteins when mixed with other materials. The objective of this research was to evaluate the nutritional and microbiological quality of food obtained with solid-state fermentation using potatoes and carrots.

MATERIALS AND METHODS

This research was carried out in the animal nutrition laboratory of the Universidad Pedagógica y Tecnológica de Colombia (Uptc), located in the city of Tunja, in the Department of Boyaca (Colombia), from April to July, 2018.

Potato and carrot crop residues from the local market of Tunja were used, which were mixed with drying materials such as: corn cabbage and wheat bran (by-products of grain and cereal plants from the municipality of Duitama), palm kernel high in fat (byproduct of the oil industry), 99.5% magnesium sulfate, 38% calcium carbonate, urea with 46% nitrogen, sugar cane (*Saccharum officinarum*) and inoculum of Lactic Acid Bacteria (LAB).

Three treatments with different potato and carrot ratios were established. The composition was as follows: (1) 20:20 potato:carrot (20% potato, 20% carrot); 21% wheat bran; 21% corn cabbage; 5% palm kernel; 2% urea; 0.5% magnesium sulfate; 0.5% calcium carbonate; 5% LAB inoculum and 5% sugar cane. (2) 25:25 potato:carrot (25% potato, 25% carrot); 16% wheat bran; 16% wheat cabbage; 5% palm kernel; 2% urea; 0.5% magnesium sulfate; 0.5% calcium carbonate; 5% LAB inoculum and 5% sugar cane. (3) 30:30 potato:carrot (30% potato, 30% carrot); 11% wheat bran; 11% wheat cabbage; 5% palm kernel; 2% urea; 0.5% magnesium sulfate; 0.5% calcium carbonate; 5% LAB inoculum and 5% sugar cane.

The inoculum followed the methodology Borras *et al*. (2017): microorganisms from a culture of heterofermentative lactic acid bacteria (LAB) of medium and rapid acidification, *Lactobacillus delbrueckiis* ssp. *bulgaricus* and *Streptococcus thermophilus* (commercial lyophilized, Liofast Y452B, SACCO®), applied to the SSF at 48 h post-preparation.

1,200 g of food were prepared per treatment, divided into three repetitions. In the SSF preparation, the main raw materials, or crop waste, (potato-carrot) were washed to remove excess organic matter and avoid an over-estimate of the ash values. The materials were then crushed to a size of approximately 4 mm, mixed manually with the rest of the ingredients until a homogeneous mixture was achieved, and finally packed in sealed polyethylene (Ziploc®) bags at 25°C to allow the fermentation process.

pH monitoring was performed at 0, 24, 48, 72 and 96 h. 5 g of sample were removed, and 45 mL of sterile distilled water were added. The preparation was stirred for 30 min on an Adams® electric stirrer, and subsequently the filtrate was obtained for measurement with an Okaton® automatic potentiometer (Oakton Instruments, Vernon Hills, IL).

The crude protein (% PC), dry matter (% MS), ash (% CZ), ethereal extract (EE), nitrogen free extract (% ELN) were also determined using the Kjeldahl method, and the neutral detergent fiber (% FDN) and acid detergent fiber (% FDA) were determined with the Van Soest *et al*. (1991) at 0, 24, 48, 72 and 96 h.

A microbiological follow-up was performed at 0, 24, 96 h for the mesophilic aerobes (CFU/mL) (AOAC 966.23.C: 2001), total coliforms (NMP) (ICMSF NMP: 2000), total and fecal coliforms (NMP) (ICMSF NMP: 2000), Clostridium Sulfite reducing spores (CFU/mL) (ISO 15213: 2003), fungi and yeasts (CFU/mL) (ISO 7954: 1987), Salmonella (AS 5013.10: 2009), lactic acid bacteria (NTC 5034: 2002).

A completely randomized design was applied. The data were submitted to the assumptions of normality with the Shapiro-Wilk statistic and homogeneity of variance levene test. The Tukey mean comparison test with a significance level of 5% was used. The statistical package used was SPSS v 23.

RESULTS AND DISCUSSION

A decrease in pH was observed for the three treatments (Tab. 1); however, there was a greater decrease between 24 and 48 h. For the measurements from 72 to 96 h, the trend achieved stability for the pH levels.

Table 1. pH changes in solid state fermentation using different potato:carrot ratios as the main materials.

Hour(s)	20:20	25:25	30:30
0	5.68±0.006 b	5.73±0.003 a	5.69±0.006 b
24	5.49±0.012 a	5.36±0.012 b	5.02±0.023 c
48	4.72±0.012 a	4.65±0.009 b	4.47±0.020 c
72	4.62±0.019 a	4.52±0.012 b	4.42±0.006 c
96	4.46±0.00 a	4.46±0.00 a	4.33±0.006 b

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P\leq0.05$); ± standard error ($n=3$).

Similar results were reported by Córdoba-Castro and Guerrero-Fajardo (2016) who pointed out that the decrease in pH in foods obtained with fermentation is due to the acids produced by biochemical reactions, especially lactic acid.

It is important to highlight that the pH values must remain in a range of 3.5 to 6.0 in order to allow an adequate growth of the microorganisms in the SSF process (Pandey *et al*., 2001; Elias *et al*., 2009). On the other hand, the decrease in pH values is related to the production of volatile fatty acids generated in the digestion of carbohydrates mediated by the microorganisms; however, lactic acid is the desired predominant acid, which is why LAB type microorganisms are added (Chundakkadu, 2005).

An increase in DM was observed in the 20:20 potato:carrot treatment (Tab. 2) because the inclusion of the substrate was lower compared to the other treatments.

Table 2. Dry matter changes in solid state fermentation using different potato:carrot ratios as the main materials.

Hour(s)	20:20	25:25	30:30
0	58.08±0.14 a	50.11±0.15 b	41.23±1.03 c
24	56.74±0.10 a	47.08±0.92 b	41.4±0.48 c
48	55.27±0.06 a	47.46±0.23 b	40.92±0.38 c
72	54.70±0.20 a	47.06±0.38 b	40.14±0.55 c
96	54.03±0.13 a	47.14±0.04 b	40.43±0.23 c

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P\leq0.05$); ± standard error ($n=3$).

The decrease in the DM% may have been related to the processes of hydrolysis of urea, fermentation of sugars (sucrose, glucose, fructose), starches, and other carbohydrates; and on a smaller scale, the decrease was also due to the deamination of peptides and amino acids. All of these processes were mediated by the microorganisms present during metabolic processes (Brea-Maure *et al*., 2015). Other studies have stated that this decrease is the result of the hydrolytic action of bacterial enzymes that produce water and volatile compounds (Carrasco *et al*., 2015; Rodríguez *et al*., 2001). Therefore, the decrease in dry matter is due to a fermentation process of carbohydrates during SSF, causing a significant increase in the concentration of volatile fatty acids (VFA) and a decrease in pH (Castillo, 2011).

The CZ values increased for the three treatments (Tab. 3), ranging from 5.5 to 7.09, evidencing the greatest increase in the treatment 30:30. Ash contains salts and oxides of different chemical elements, mainly minerals (Cárdenas *et al*., 2008).

Table 3. Ash changes (% CZ) in solid state fermentation using different potato:carrot ratios as the main materials.

Hour(s)	20:20	25:25	30:30
0	5.57±0.09 a	5.79±0.04 a	5.50±0.42 a
24	6.10±0.02 b	6.07±0.00 b	6.69±0.09 a
48	6.30±0.03 c	6.60±0.03 b	7.01±0.06 a
72	6.11±0.01 c	6.67±0.01 b	6.96±0.11 a
96	6.31±0.07 b	6.61±0.06 b	7.09±0.11 a

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P\leq0.05$); ± standard error ($n=3$).

This increase in the ash values was also reported by Pérez (1996) who, when using fermentation on *Pennisetum americanum* (L.) Leeke (pearl millet), observed that the ash value went from 1.6 to 2.1% in 72 h as a result of cellular metabolism. In another study, when SSF was carried out on the flour of the breadfruit tree, an increase in the ash values of 7.87 to 8.52% was evident (Brea *et al*., 2014). In studies carried out with different potato:carrots ratios and energy sources (molasses, glycerol), an increase in ash percentage values has been observed, obtaining results that ranged between 7.2% at 24 h (Huertas *et al*., 2016) and 5 to 7% of CZ (Cala *et al*., 2015).

Ramos (2006) pointed out that ash may be related to the energy source used in the process. However, Escudero (2012) stated that, in fermentation processes, decreases in ash levels can be observed because the microorganisms require a large amount of nutrients, among them minerals, in their biochemical processes. This is why it is important to enrich the solid matrix of the substrate with minerals and thus improve the carbon:nitrogen ratio and provide other nutritional elements required by the microorganisms.

Ash is an indicator of the mineral content of a food; however, if the values are greater than 15%, improper handling can be suspected because some kind of contamination could have occurred, such as contact with dirt (Argamentaria *et al*., 1997). According to the results obtained in the present study, no value exceeded this level, so it is evident that there was no contamination.

An increase in PC was evidenced for treatments from 0 to 96 h (Tab. 4). The food did not show significant statistical differences. However, 48 h showed an increase in the PC levels with the increasing concentrations of carrot-potatoes.

Table 4. Crude protein changes in solid state fermentation using different potato:carrot ratios as the main materials.

Hour(s)	20:20	25:25	30:30
0	20.1±2.89 a	21.1±2.99 a	25.4±1.66 a
24	25.7±0.26 a	27.4±0.25 a	27.0±1.28 a
48	26.1±0.26 c	27.8±0.68 b	30.2±0.44 a
72	26.7±0.48 a	29.1±0.20 a	25.8±2.48 a
96	26.7±0.48 a	27.1±0.22 a	27.9±3.66 a

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P \leq 0.05$); ± standard error ($n=3$).

In a previous study by Cala *et al*. (2015), there was an increase in the PC in the different sampling times, resulting in protein of 20.19 and 21.03% at 24 and 48 h, respectively, data that match the data obtained in this study (Tab. 4).

Elias *et al*. (2009) reported that an increase in protein levels was due to a synchronization between non-protein nitrogen contained in urea and carbohydrates added to the SSF, allowing the microorganisms to incorporate nitrogen and available carbon into their protoplast and forming amino acid chains and generating the synthesis of microbial proteins. When inoculating the microorganisms, a greater retention of ammonia was observed as a result of the lower pH, which increased the PC content. Additionally, when there is an increase in the PC values, if some compounds are incorporated that are sources of phosphate, nitrogen and microelements such as: calcium, iron, magnesium, manganese, copper, etc. (Ramos, 2005), the increase in PC is a result of inoculation with microorganisms, calcium carbonate and magnesium sulfate. Finally, an increase in PC values in fermentation processes was reported by Cárdenas *et al*. (2008); Rodríguez *et al*. (2001) and Fonseca-López *et al*. (2018), who obtained increases in PC from 11.5 to 16.3%, 10.1 to 13.7% and 4.34 to 19%, respectively.

The SSF was stable for the ethereal extract (EE) (Tab. 5). For the measurement time (48 h), the 20:20 and 25:25 potato:carrot treatments showed levels of 6.7% EE and 6.3% for the 30:30 potato:carrot treatment; there was no significant statistical difference ($P>0.05$). This variable corresponds to the group of nutrients called crude fat or lipids, where fat-soluble vitamins are also found.

Table 5. Ethereal extract changes in solid state fermentation using different potato:carrot ratios as the main materials.

Hour(s)	20:20	25:25	30:30
0	6.7±0.07 a	6.4±0.27 ab	5.6±0.18 b
24	6.0±0.52 a	6.1±0.07 a	5.6±0.36 a
48	6.7±0.07 a	6.7±0.50 a	6.3±0.09 a
72	6.4±0.03 a	6.4±0.27 a	6.2±0.79 a
96	6.4±0.35 a	7.2±0.35 a	6.5±0.33 a

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P \leq 0.05$); ± standard error ($n=3$).

The fiber composition (FDN and FDA) is used for the prediction of food quality since it influences the consumption of dry matter, digestibility and energy value. The FDN, FDA and FC values showed no statistical differences ($P>0.05$) between the treatments (Tab. 6).

Table 6. Composition of fibers obtained with the SSF of potatoes and carrots as the main materials.

Fibers	Hour(s)	20:20	25:25	30:30
FDN (%)	0	38.3±0.64 a	37.5±0.87 a	40.3±5.34 a
	24	41.4±0.92 a	42.1±0.70 a	44±2.39 a
	48	41.7±1.22 a	43.5±0.84 a	41.8±0.87 a
	72	40.6±0.73 a	42.7±1.09 a	42.5±1.49 a
	96	44.6±0.90 a	43.4±0.95 a	42.9±0.49 a
FDA (%)	0	13.4±0.41 a	12.5±0.06 a	12.7±0.15 a
	24	13.1±0.07 a	12.3±0.17 a	12.6±0.23 a
	48	17.5±1.17 a	14.9±0.70 a	15±0.98 a
	72	17.3±0.22 a	16.4±0.26 a	16.6±0.52 a
	96	17.1±0.87 a	16±0.90 a	18±0.19 a
FC (%)	0	11.1±0.12 a	10.3±0.17 a	10.4±0.45 a
	24	11.1±0.12 a	10.4±0.20 a	10.3±0.44 a
	48	11.2±0.09 a	11.1±0.06 a	10.9±0.26 a
	72	11.3±0.15 a	11.4±0.09 a	11.3±0.12 a
	96	11.3±0.15 a	10.4±0.09 a	11.1±0.42 a

Means followed by the same letter in the row, indicate significant difference according to the Tukey test ($P \leq 0.05$); ± standard error ($n=3$).

However, Polished *et al*. (2016) reported a decrease in HR levels in the evaluation of SSF using pear harvest residues (*Pyrus communis*) at 24, 48 and 72 h of fermentation. Ruiz et al. (2009) observed variations in FDN and FDA in silage processes, which are related to changes in soluble sugars. On the other hand, Herrera *et al*. (2014) argued that, during the fermentation of cacti, the contents of FDN and FDA were reduced with the presence of yeasts at 96 h of the fermentation process.

Cruz-Calvo and González (2000) pointed out that fiber is a set of components that have low digestibility and promote rumination and ruminal balance, that is, the main component in most ruminant production systems.

Table 7. Microbiological analysis of potato and carrot fermentation as the main SSF materials.

Microorganism	Inclusion	0 hour	24 hours	96 hours
Mesophilic aerobes (CFU/g)	20:20		$38·10^6$	$41·10^5$
	25:25	$10·10^6$	$79·10^6$	$11·10^5$
	30:30		$15·10^6$	$21·10^6$
Total coliforms (NMP)	20:20		>1 100	<3
	25:25	>1 100	1100	<3
	30:30		>1 100	<3
Fecal coliforms (NMP)	20:20		<3	<3
	25:25	3.6	<3	<3
	30:30		9.1	<3
Sulphite reducing *Clostridium* spores (CFU/g)	20:20			
	25:25	*	<10	<10
	30:30			
Fungi (CFU/g)	20:20		$10·10^2$	10
	25:25	<10	$20·10^3$	<10
	30:30		10	20
Yeasts (CFU/g)	20:20		$51·10^4$	$32·10^4$
	25:25	$32·10^4$	$80·10^4$	$30·10^4$
	30:30		$49·10^4$	$58·10^4$
Salmonella	20:20			
	25:25	*	*	*
	30:30			
Lactic acid bacteria (CFU/g)	20:20		$32·10^6$	$52·10^6$
	25:25	$10·10^6$	$79·10^6$	$78·10^6$
	30:30		$15·10^6$	$58·10^6$

*Absence 25 g

In all the treatments, a significant LAB growth count (Tab. 7) was evidenced as a sign of the effectiveness of the inoculum used, especially with the 25:25 potato:carrot ratio at 96 h of fermentation ($78·10^6$). Likewise, the absence of pathogens such as Salmonella and Clostridium and decreases in coliform counts were observed, presumably as a result of the efficiency of fermentation. It is important to note that there is no standard to compare and evaluate these results, but the figures suggest that the product is safe for animal feed.

In previous studies, LAB strains have been isolated and characterized from native microbial preparations made from agroindustrial residues, such as whey, bovine manure and ruminal contents, designed as biological inoculants for postharvest organic waste silage processes and proposed for studies on other crop residues with these inoculants (Díaz *et al*., 2014). In another study, where they evaluated the quality of a silage made with potato waste and inoculated with BAL, an increase in the amount of protein, reduction of fiber and decrease in digestibility in animals were observed (Nkosi *et al*., 2015).

CONCLUSIONS

Solid state fermentation is a process characterized by microbial growth on a solid substrate in order to optimize the amount of protein and other nutrients contained in a food when there is a favorable medium. The use of potato and carrot ratios of up to 30:30 (p/p) in the preparation of SSF foods allows for a proper decrease in the pH levels in the food, which is indicative of a good fermentation process and is related to the increase in raw protein levels in the food and stability of other nutrients.

The use of potatoes and carrots in solid-state fermentation processes is feasible as a strategy in animal feed since they can be mixed with other materials because of the fermentative capacity of efficient microorganisms, obtaining 30.2% proteins at 48 h, along with the absence of pathogens such as *Salmonella* and *Clostridium* and a decrease in coliform counts.

The Department of Boyaca is characterized as an agricultural territory, with notable potato and carrot crops occupying the largest cultivated area and total production, generating large quantities of good quality post-harvest waste that has significant nutritional components for use in animal feed. These residues are significant since they constitute a strategy of animal nutrition through fermented preparations that include efficient microorganisms, benefiting livestock production systems.

ACKNOWLEDGMENTS

The authors are thankful for the support of the Grupo de Investigacion en Bioquimica y Nutricion Animal – GIBNA of the Universidad Pedagogica y Tecnologica de Colombia, Colciencias and the Gobernación de Boyaca.

Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that they have no conflicts of interest that place the validity of the results presented herein at risk.

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Agronomic performance and gaseous exchanges of the radish under saline stress and ascorbic acid application

Rendimiento agronómico y cambios gaseosos de rábano bajo estrés salino y aplicación de ácido ascórbico

YGOR HENRIQUE LEAL[1]
LEONARDO VIEIRA DE SOUSA[1]
TOSHIK IARLEY DA SILVA[2, 5]
JOANA GOMES DE MOURA[1]
ANA GABRIELA SOUSA BASÍLIO[1]
JOSÉ SEBASTIÃO DE MELO FILHO[1]
ANDERSON CARLOS DE MELO GONÇALVES[3]
THIAGO JARDELINO DIAS[4]

Radish subjected to saline stress and ascorbic acid.
Photo: L.V. Sousa

ABSTRACT

The radish is a short-cycle vegetable that has excellent nutritional and medicinal properties. It is considered rustic, meaning it tolerates adverse conditions with the possibility of being irrigated with saline water, which creates stress. In this context, this study aimed to evaluate the effect of electrical conductivities in irrigation water and doses of ascorbic acid on the agronomic performance and gaseous exchanges of radishes. This experiment was carried out in a protected environment at the Agricultural Sciences Center of the Federal University of Paraíba, Areia, Paraíba (Brazil). The experiment design used randomized blocks with five doses of ascorbic acid (0.0, 0.29, 1.0, 1.71 and 2.0 mM) and five electrical conductivities in the irrigation water (0.5, 1.3, 3.25, 5.2 and 6.0 dS m^{-1}), with four replicates. The growth, gas exchange and production were evaluated. The doses of ascorbic acid were not significant. The increase in the electrical conductivities of the irrigation water provided a reduction in the agronomic performance and gas exchanges, except for the net photosynthesis, water use efficiency and instantaneous carboxylation efficiency, which were not significant. There was a relationship between the net photosynthesis, transpiration, internal concentration of CO_2 and water use efficiency and the stomatal conductance. The agronomic performance and gaseous exchanges of the radish culture were influenced by the salinity. The foliar application of ascorbic acid did not influence the agronomic yield and gaseous exchanges of the radishes at the tested doses.

Additional key words: *Raphanus sativus* L.; irrigation water; saline water; photosynthetic activity; antioxidant.

[1] Federal University of Paraíba, Department of Agrarian Sciences and Environmental Sciences, Areia (Brazil). ORCID Leal, Y.E.: 0000-0001-7155-3524; ORCID Vieira de Sousa, L.: 0000-0001-5846-3399; Gomes de Moura, J.: 0000-0002-4389-2956; Sousa Basílio, A.G.: 0000-0003-2873-8202; ORCID Melo Filho, J.S.: 0000-0003-3005-2795
[2] Federal University of Viçosa, Department of Plant Science, Viçosa (Brazil). ORCID Da Silva, T.I.: 0000-0003-0704-2046
[3] Federal University of Roraima, Department of Plant Science, Boa Vista (Brazil). ORCID Gonçalves, A.C.M.: 0000-0003-4151-1192
[4] Federal University of Paraíba, Department of Agriculture, Bananeiras (Brazil). Dias, T.J.: 0000-0002-7843-6184
[5] Corresponding author. iarley.toshik@gmail.com

RESUMEN

El rábano es una hortaliza de ciclo corto que presenta excelentes propiedades nutricionales y medicinales. Es considerada rústica, pues tolera condiciones adversas, con énfasis en la posibilidad de poder ser regado con agua salada, principalmente cuando se asocia a la atenuación del estrés causado. En este contexto, el presente trabajo tuvo como objetivo evaluar el efecto de conductividades eléctricas del agua de riego y de dosis de ácido ascórbico en el desempeño agronómico e intercambios gaseosos del rábano. El experimento fue conducido en un ambiente protegido, en el Centro de Ciencias Agrarias de la Universidad Federal de Paraíba, Areia, Paraíba (Brasil). El diseño experimental fue hecho en bloques aleatorizados, con cinco dosis de ácido ascórbico (0,0; 0,29; 1,0; 1,71 y 2,0 mM) y cinco conductividades eléctricas del agua de riego (0,5; 1,3; 3,25; 5,2 y 6,0 dS m^{-1}), con cuatro repeticiones. El crecimiento, los intercambios gaseosos y la producción fueron evaluados. Las dosis de ácido ascórbico no fueron significativas. El aumento de las conductividades eléctricas del agua de riego disminuyó el desempeño agronómico y los intercambios gaseosos, salvo para fotosíntesis neta, eficiencia del uso del agua y eficiencia instantánea de carboxilación, que no presentaran importancia. Hubo una relación de la fotosíntesis neta, transpiración, concentración interna de CO_2 y eficiencia del uso del agua con la conductancia estomática. El desempeño agronómico y el intercambio gaseoso del cultivo de rábano son influenciados por la salinidad. La aplicación de ácido ascórbico a través de la hoja no influye en el rendimiento agronómico y los intercambios gaseosos del rábano a las dosis probadas.

Palabras clave adicionales: *Raphanus sativus*; agua de riego; agua salada; actividad fotosintética; antioxidante.

Received for publication: 10-05-18 Accepted for publication: 29-03-2019

INTRODUCTION

The radish (*Raphanus sativus* L.) is a small vegetable that tolerates adverse conditions. Its origin lies in the Mediterranean region, and it belongs to the Brassicaceae family, which contains broccoli, cabbage, cauliflower and cabbage; however, the tuberous root is the part that is consumed, which has a reddish external color and a white flesh with a spicy flavor (Lanna *et al*., 2018).

Radish production has been stimulated because of the fact that it presents high rusticity with a short cycle crop, which means less time for harvesting, providing a fast economic return and making it a great alternative for intercropping with long cycle crops. It is appreciated for its vitamins A, B1, B2 and C, potassium, calcium, phosphorus and sulfur contents, conferring excellent medicinal properties, such as an antioxidant action, natural expectorant and coadjuvant of the digestive system (Oliveira *et al*. 2010; Cunha *et al*., 2017).

Radish crops are commonly irrigated, where the use of salt water is often the only alternative for production because quality water is increasingly scarce, especially in regions with high temperatures and low rainfall, resulting in increased salt concentration in the water (Lima *et al*., 2017). Among the abiotic stresses, saline is the most harmful to crop growth and productivity (Santos *et al*., 2012), reducing the osmotic potential of the soil solution, which can cause water deficits in plants and consequently reduce or inhibit the absorption of nutrients as a result of an ionic imbalance in the soil solution, causing phytotoxicity mainly through sodium and chloride ions (Eschemback *et al*., 2014).

In addition, saline stress can also cause plant dehydration as a result of a high salt uptake, meaning plants cannot compartmentalize and raising the concentration of these salts within plants (Parihar *et al*., 2015). In this sense, plants need greater energy expenditure to perform their vital functions, affecting growth, development and consequently productivity (Silva *et al*., 2012).

One of the mitigating methods for saline stress in plants is the application of ascorbic acid, which is an important antioxidant, protecting plants from oxidative stress, especially when caused by saline stress (Gul *et al*., 2015). It also helps the enzyme ascorbate peroxidase (APX) convert hydrogen peroxide (H_2O_2), which is a reactive oxygen species (EROS) produced in greater amounts in plants under stress that affects cellular homeostasis, playing a key role in tolerance to salinity and influencing stomatal opening, besides being easily absorbed by plants after foliar applications (Hameed *et al*., 2015).

In this context, it is necessary to establish the dose that provides the best attenuation results under saline stress in radish cultures. The aim of this study was to evaluate the effect of different electrical conductivities of irrigation water and ascorbic acid doses on agronomic performance and gaseous exchanges of the radish (*R. sativus*).

MATERIAL AND METHODS

This experiment was carried out from October to November, 2017, in a protected environment (greenhouse), in the Department of Plant Science and Environmental Sciences of the Center of Agricultural Sciences of the Federal University of Paraíba (UFPB), in the city of Areia, PB (Brazil). The soil used in the experiment was an Oxisol (Tab. 1), collected from a 0-20 cm depth layer.

This experiment was carried out in a randomized complete block design, composite central box experiment (Bortoluzzi and Alvarez, 1997), in a 5x5 factorial scheme, with four replications for five doses of ascorbic acid (AA - 0.0, 0.29, 1.0, 1.71 and 2.0 mM) and five electrical conductivities in the irrigation water (ECw - 0.5, 1.3, 3.25, 5.2 and 6.0 dS m^{-1}). A treatment that was irrigated with 0.5 dS m^{-1} water and no ascorbic acid was used to determine the irrigation depth using drainage lysimetry. The experiment plots were represented by plastic bag for the seedlings, with a capacity of 3.0 dm^3. Margaret Queen Kobayashi hybrid radish seeds were used, sowing five seeds in each plastic bag at a depth of 2 cm. On the 15 day after sowing (DAS), thinning was carried out, leaving the plants more vigorous.

The lowest salinity water (0.5 dS m^{-1}) came from the UFPB's supply system. A mixture of salts of NaCl, $CaCl_2{\cdot}2H_2O$ and $MgCl_2{\cdot}6H_2O$ and 0.5 dS m^{-1} water were added to the water with the highest salinity (1.3, 3.25, 5.2 and 6.0 dS m^{-1}), maintaining an equivalent ratio of 7:2:1 (Medeiros, 1992), measuring the ECw with a portable conductivity meter. Throughout the experiment, the plants were irrigated with the ECw that was established for each treatment, with irrigation management done using drainage lisimetry.

Foliar applications of ascorbic acid were carried out at 19 DAS with the aid of an atomizer containing the doses of ascorbic acid and the adjuvant (Tween® 80) at a concentration of 0.0002% of the syrup.

At 35 DAS, the following variables were analyzed: leaf number (LN), counting all the leaves of each plant; stem diameter (SD), using a digital caliper; leaf area (LA), with the aid of a graduated ruler to record the length and width dimensions of the leaves and then the equation $LA = C*L*f$, from Benincasa (2003), where *LA* refers to leaf area; *C* leaf length in cm; *L* leaf width in cm; and *f* correction factor for radish = 0.57; specific leaf area (SLA), according to Witkowski and Lamont (1991), where SLA = leaf area * dry leaf weight; root dry mass (RDM) and tuber dry mass (TDM), determined on a scale with a precision of 0.001 g. In order to obtain the dry root and tuber masses, the material was packed in paper bags and oven dried at a temperature of 65°C until reaching constant weight.

The gaseous exchange determinations were carried out at 34 DAS, using an infrared gas analyzer (IRGA, LI-COR - model LI-6400XT Portable Photosynthesis System) from 9:00 am to 10:00 am. The following characteristics were measured: net CO_2 assimilation (A - μmol CO_2 m^{-2} s^{-1}); stomatal conductance (gs - mol H_2O m^{-2} s^{-1}); internal CO_2 concentration (Ci - mmol CO_2 m^{-2} s^{-1}); transpiration rate (E - mmol H_2O m^{-2} s^{-1}); water use efficiency (WUE - A/E) and vapor pressure deficit (VPD - kPa).

Based on the accumulation of total dry matter and leaf area, the growth variables were determined

Table 1. Results of the chemical soil analysis used in this experiment.

pH	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^+ + Al^{3+}$	SB	CEC	V
H_2O	%	--mg dm^{-3}--		------------------------cmol$_c$ dm^{-3}------------------------							%
6.20	2.48	24.85	78.42	0.07	3.90	1.90	0.00	2.43	6.07	8.50	71.46

OM: organic matter; SB: sum of bases; CEC: cation exchange capacity; V: saturation by bases.

according to Benincasa (2003), corresponding to the relative growth rate, RGR (g g^{-1} d^{-1}), using the equation RGR = (LnW2 - LnW1) * (T2 - T1) -1, where LnW1 and LnW2 are the variation of the neperian logarithm of the dry mass between two periods, and T1 and T2 are the time variation between the periods. The leaf area ratio, LAR (cm^2 g^{-1}), was determined using the equation LAR = L * W-1, where L is the leaf area and W the total dry mass of the plant. The data obtained in the evaluations of the experiment were submitted to analysis of variance and regression using SAS University software (Cody, 2015).

RESULTS AND DISCUSSION

According to the analysis of variance, there was no interaction between the ascorbic acid (AA) and the electrical conductivities of the irrigation water (Ecw); also, there was no effect between the doses of ascorbic acid for both agronomic performance variables for the variables of gas exchange, probably because radish plants demand higher doses.

However, a difference was observed between the ECw applications in the radish plants. It was observed that, as the ECw increased (Fig. 1, 2 and 3), the values of the agronomic performance variables (leaf number, stem diameter, leaf area, specific leaf area, tuber fresh mass, root dry mass, relative growth rate and leaf area ratio) deceased.

Saline stress is the most damaging to crop growth and productivity among the abiotic stresses (Santos *et al*., 2012), causing reductions in the osmotic potential of the soil solution and consequently water deficits in plants, promoting a reduction or inhibition of the absorption of some nutrients as a result of an ionic imbalance of the soil solution and phytotoxicity, especially with sodium and chloride ions (Eschemback *et al*., 2014).

Several harmful effects caused by saline stress on plants can be related to the results obtained in the present study, where the higher salt concentrations intensified the negative reflexes in the development of the radish plants. Sousa *et al*. (2016a), evaluating

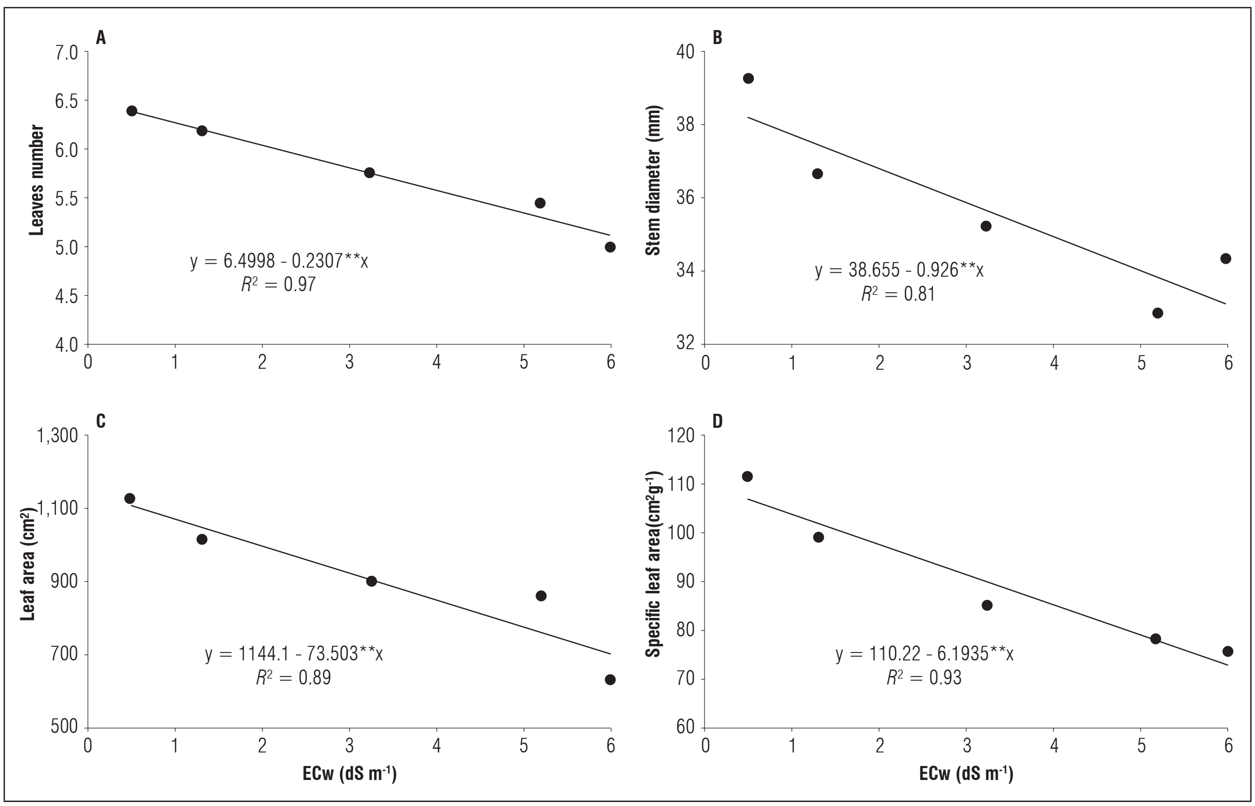

Figure 1. Leaf number (A), stem diameter (B), leaf area (C) and specific leaf area (D) of the radish plants as a function of the different electrical conductivities of the irrigation water (ECw).

the growth and productive performance of radish plants under different Ecw applications, observed results similar to those of the present study, where irrigation water with a higher electrical conductivity provided lower values for leaf area, shoot dry mass, fruit diameter, average fruit mass and productivity.

Given the variable number of leaves (Fig. 1A), it was notable that, in the lowest (0.5 dS m^{-1}) and highest (6.0 dS m^{-1}) Ecw applications, there were 6.4 and 5.1 leaves per plant, respectively, corresponding to a reduction of 20.3%. Similarly, Oliveira (2010), also working with salinity in a radish culture, obtained 6.9 leaves with the lowest Ecw application (0.5 dS m^{-1}) and 3.6 leaves with 5.0 dS m^{-1}, corresponding to a reduction of 47.6%, well above the present study even though the maximum ECw value was lower than that applied by this author. Osmotic imbalances, damage from oxygen reactive species (ROS) and ionic toxicity are the main characteristics observed in radish plants under saline stress (Sun *et al*., 2016).

As for stem diameter (Fig. 1B), a reduction of 13.4% was observed, with the respective values of 38.2 and 33.1 mm for the ECw amplitude (0.5 and 6.0 dS m^{-1}). Santos *et al*. (2016), evaluating the growth and phytomass of beet plants under different ECw applications (1.0 to 5.0 dS m^{-1}), observed that, up to an ECw of 3.0 dS m^{-1}, the stem diameter increased, with a maximum value corresponding to 33 mm, with a decrease in stem diameter values beyond this ECw value.

The leaf area of the radish plants (Fig. 1C) varied negatively between the lowest and highest ECw applications (0.5 and 6.0 dS m^{-1}), and the respective values were 1107.4 and 703.1 cm^2, corresponding to a reduction of 36.5%. Oliveira *et al*. (2012) reported that an ECw of 2.0 dS m^{-1} resulted in the highest leaf area value (497.2 cm^2), while the highest ECw (10.0 dS m^{-1}) provided the lowest leaf area value (220 cm^2), corresponding to a decrease of 55.75% for the radish culture.

When evaluating the growth and productivity of radish plants under different irrigation water salinities, 0.8, 1.5, 3.0 and 4.5 dS m^{-1}, Sousa *et al*. (2016a) obtained a leaf area of 127 and 64.1 cm^2 in the smallest and largest ECw applications, corresponding to a decrease of 49.5% in the leaf area, well above that of the present study. Srivastava *et al*. (2016), working with two varieties of radish plants under different concentrations of NaCl (0, 50, 100, 150 and 200 mM), also observed the same tendency for the variables leaf area, root and stem length and total biomass, where the higher salinity water provided lower values.

Saline stress alters the osmotic potential of the soil solution, which can cause water deficits in plants, which consequently reduce nutrient absorption, necessitating a higher energy expenditure and affecting growth, development and productivity (Silva *et al*., 2012). The results of this study evidenced a strong relationship, where the negative effect of one variable interfered, directly and negatively, with the others, explaining their similar behavior.

The highest leaf area (1107.35 cm^2 g^{-1}) was observed in the lowest ECw application (0.5 dS m^{-1}), and the lowest specific leaf area (703.08 cm^2 g^{-1}) was seen in the highest ECw application (6.0 dS m^{-1}), corresponding to a decrease of 36.5%. This negative effect was due to the inhibition of the development of the

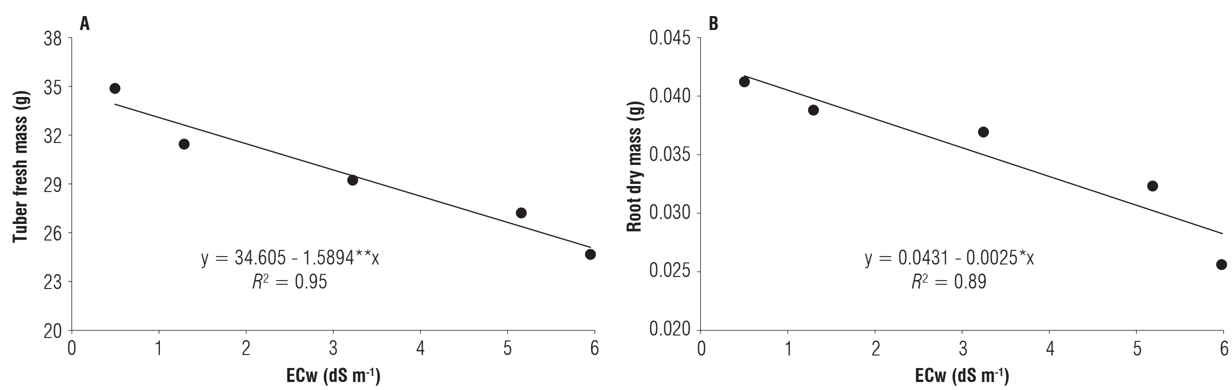

Figure 2. Fresh grass mass (A) and root dry mass (B) of the radish plants as a function of the different electrical conductivities of the irrigation water (ECw).

plants in the higher salt concentrations, reducing the leaf area and dry mass. Santos *et al*. (2017) also observed a decreasing behavior for specific leaf area in radish plants.

The tuber fresh mass and the root dry mass (Fig. 2A and B) also presented an inversely proportional behavior to the increase in the ECw applications, where the extreme results were 33.81 and 25.07 g for tuber fresh mass and s of 0.042 and 0.028 g for dry matter mas. for the lowest and highest ECw applications (0.5 and 6.0 dS m^{-1}), corresponding to reductions of 74.15 and 66.67%, respectively. The same behavior was observed by Brunes *et al*. (2013) who worked with two rice cultivars, showing a decrease in the dry mass of the roots and shoots as the ECw increased.

Sousa *et al*. (2016a), studying salinity in a radish culture, observed a fresh mass of 9.11 and 2.13 g respectively for an ECw of 0.8 and 4.5 dS m^{-1}, values well below those of the present study, but with similar behaviors and percentages of reduction (76.62%). Similarly, Oliveira *et al*. (2010), in a similar study, obtained inferior results for tuber fresh mass (19.07 and 9.12 g) using water conductivity (ECw) that varied between 0.5 and 5.0 dS m^{-1} in the radish culture.

These results were due to the high absorption of salts, which the plants could not compartmentalize, increasing the concentration of these salts in the interior and causing dehydration and consequently a reduction of the fresh mass (Parihar *et al*., 2015). According to Mekawy *et al*. (2015), another possible explanation would be the different responses of the evaluated cultivars since each genetic material has different tolerances and mechanisms under conditions of saline stress, where the one used in the present study stood out for these authors.

The leaf area ratio (Fig. 3A) was also affected by the increasing salinity, where the highest and lowest values (920.70 and 692.51 dm^2 g^{-1}) were obtained with the ECw extremes (0.5 and 6.0 dS m^{-1}), as well as the relative growth rate (0.036 and 0.024 g g^{-1} d^{-1}) (Fig. 3B).

Santos *et al*. (2014) observed the same behavior using irrigation waters with an ECw up to 4.0 dS m^{-1} in leguminous species, where waters with a higher ECw yielded lower results for the relative growth rate. The ECw increase also negatively affected the stomatal conductance (gs), the internal CO_2 concentration (Ci) and the transpiration (E) at an ECw of 3.88, 4.34 and 4.30 dS m^{-1}, respectively, with a subsequent increase in these variables at an ECw of 6.0 dS m^{-1} (Fig. 4). The reductions of these variables corresponded to 150, 20.3 and 60.6%, respectively. The net photosynthesis showed no significant effect; therefore, it was not shown in the graphs.

Such results can be justified by the presence of salts in the soil solution, reducing the water absorption capacity of the plants and causing water stress as the saline concentration increased because, in this situation, the main defense mechanism of the plants includes A, gs, Ci and E (Parihar *et al*., 2015), which is why these variables are directly related and presented similar behaviors, negatively affecting the photosynthetic apparatus of the radish and, consequently, yield.

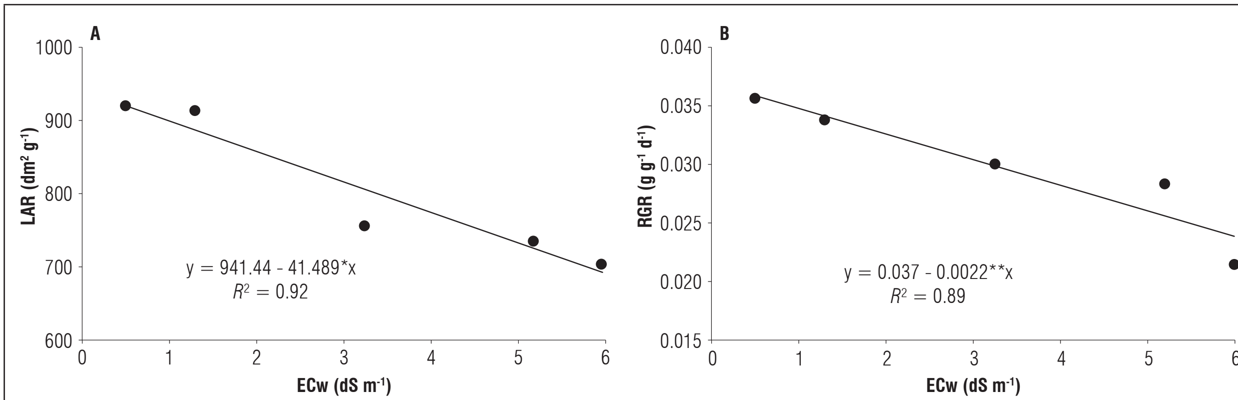

Figure 3. Leaf area ratio (A) and relative growth rate (B) of the radish plants as a function of the different electrical conductivities of the irrigation water (ECw).

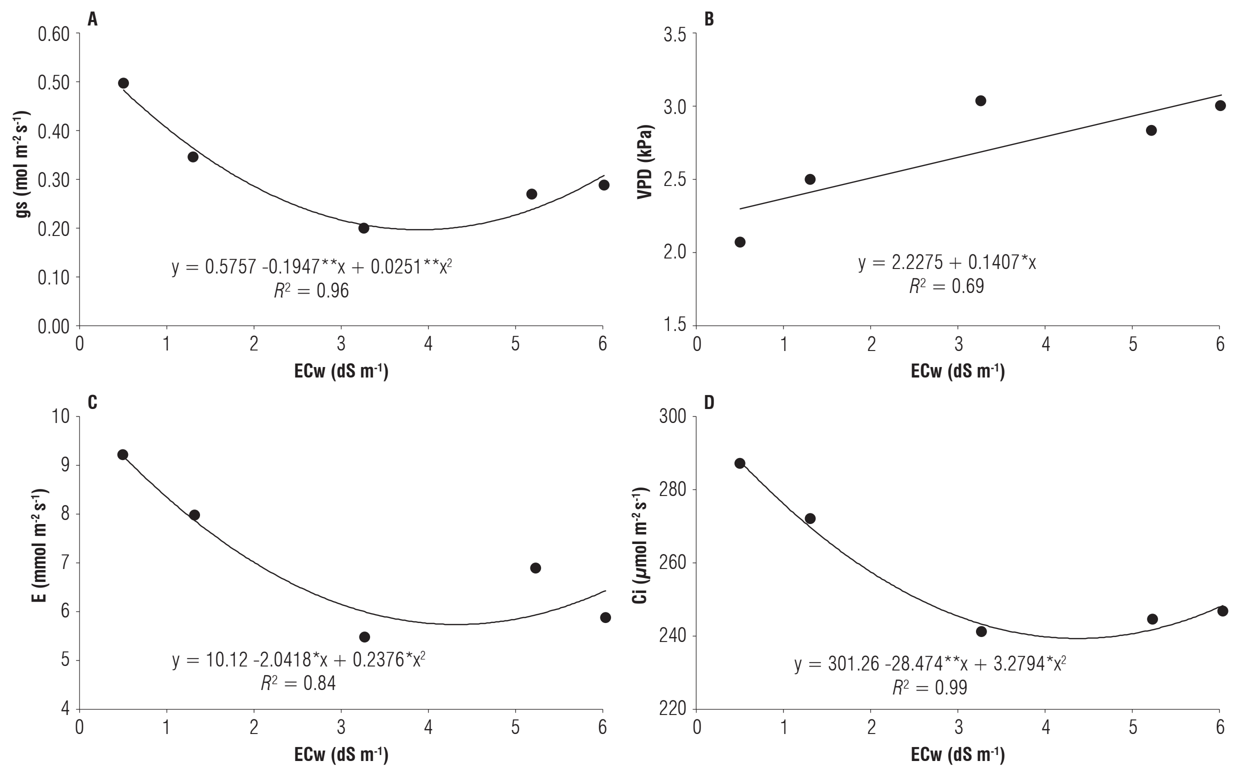

Figure 4. Stomatal conductance (gs), internal CO_2 concentration (Ci), transpiration (E) and vapor pressure deficit (VPD) of the radish plants as a function of the different electrical conductivities of the irrigation water (ECw).

Ayyub *et al*. (2016), evaluating different radish genotypes under saline stress, emphasized that stomatal conductance decreased with an increasing salinity, where a lower stomatal condutace was observed with a conductivity of 7 dS m^{-1} and that the main effect of salinity is a reduction of photosynthetic processes. Sousa *et al*. (2016b), evaluating gaseous exchanges in citrus irrigated with saline waters, observed a reduction of gs, A, E and internal efficiency of carboxylation as the ECw increased from 0.6 to 3.0 dS m^{-1}. Plants under saline stress also present a reduction of Ci because stomatal closure causes a decrease in the rate of assimilation of carbon in the leaf mesophyll (Santos and Brito, 2016). Ci is very relevant because the productivity of a plant can be understood as the product of interception of solar energy and fixed CO_2, where an adequate amount of light and higher concentrations of CO_2 provide higher photosynthetic rates. The stomatal closure impairs this activity, but, on the other hand, it reduces transpiration, reducing water loss in plants maintaining cellular turgescence and increasing photosynthetic efficiency (Taiz *et al*., 2017).

The vapor pressure deficit (VPD) was higher as the ECw increased from 0.5 to 6.0 dS m^{-1} (Fig. 4 D), ranging from 2.30 to 3.11 kPa, corresponding to an increase of 26% and evidencing that plants under salt stress are more vulnerable to a VPD, with a possible loss of water to the atmosphere.

According to Parihar *et al*. (2015), the water restriction caused by high salt concentrations in the soil solution causes plants to close their stomata to increase water use efficiency and reduce the loss of water to the atmosphere, thereby reducing gs, E, Ci and A. Barboza and Teixeira Filho (2017), working with sugarcane, observed a higher gs early in the morning, when the VPD was low and the plants were hydrated, with the reverse seen in the late afternoon when the gs decreased and VPD increased.

As the gs increased, A, E, Ci also presented higher values (Fig. 5), corresponding to increases of 77.64, 58.97 and 82.24%, respectively. However, there was a reduction in the WUE, corresponding to a decrease of 78.07%. The variations ranged from 5.52 to 9.36 for E; 237.50 to 288.79 for Ci and 2.28 to 1.78 for WUE.

According to these results, A, E, Ci and WUE had a strong relationship with the gs. The first three were directly proportional to the gs, and the latter were inversely proportional to the gs (Fig. 5). Under high gs conditions, Ci and A were favored by a greater assimilation of CO_2, and E increased as a result of a greater number of open stomata, causing the plants to lose more water and consequently reducing WUE (Taiz *et al*., 2017).

As seen in the present study, Peloso *et al*. (2017) observed a directly proportional relationship between A and gs, where the gs increase provided higher A values in a coffee crop. Similarly, Barboza and Teixeira Filho (2017) reported that E was higher as sugarcane gs increased. Ferreira *et al*. (2011) also observed a directly proportional relationship between Ci and E and the gs and an inversely proportional relationship between USA and the gs in a soybean crop.

The reduction of gs is considered one of the main factors that restrict photosynthetic activity, decreasing the CO_2 influx to the rubisco carboxylation sites within the chloroplasts and causing a decline in the photosynthetic rate. It also reduces E, providing less water loss and greater WUE (Tatagiba *et al*., 2015).

CONCLUSIONS

The electrical conductivity of the irrigation water (ECw) influenced the agronomic yield and gas exchange of the radish plants (*Raphanus sativus* L.) for gs, Ci, E and A, as a function of the gs. The foliar applications of ascorbic acid did not influence the agronomic yield or gaseous exchanges of the radish plants at the tested doses. The gs had a directly proportional relationship with A, E and Ci and an inversely proportional relationship with WUE.

Conflict of interests: this manuscript was prepared and reviewed with the full participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

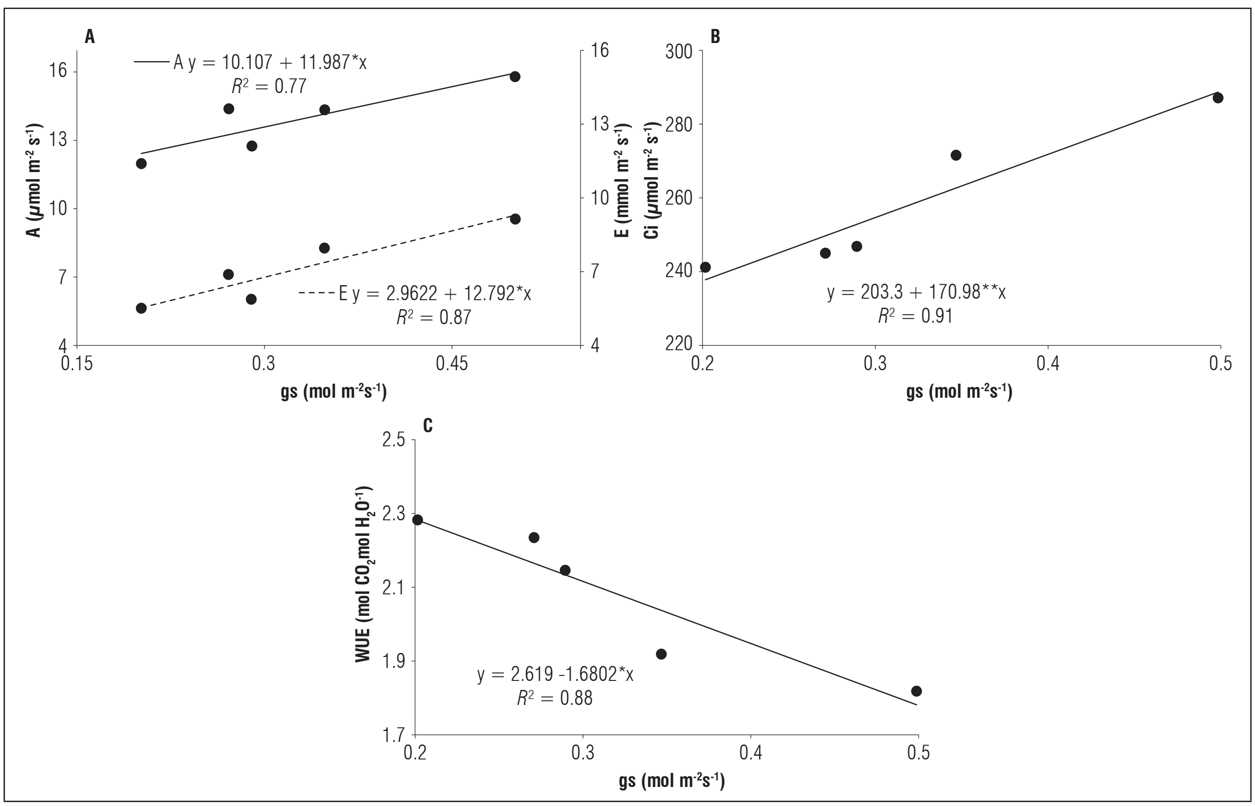

Figure 5. Net photosynthesis and transpiration (A), internal CO_2 concentration (B) and water use efficiency (C) as a function of the stomatal conductance in the radish plants subjected to different electrical conductivities of the irrigation water.

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Potassium silicate as a resistance elicitor in sweet corn yield traits under water stress

Silicato de potasio como elicitor de resistencia en las características de rendimiento de maíz dulce bajo estrés hídrico

AUSBIE L.G. ARAÚJO[1]
AMANDA M. DE ALMEIDA[2]
JOÃO DE J. GUIMARÃES[2]
FERNANDO S. DE CANTUÁRIO[2]
LEANDRO C. SALOMÃO[2]
AURÉLIO R. NETO[1]
JOSÉ M.Q. LUZ[3]
ALEXANDRE I.A. PEREIRA[2, 4]

Sweet corn plants submitted to potassium silicate and soil water tensions.

Photo: J.J. Guimarães

ABSTRACT

Plant water stress is a major problem in the Cerrado biome of Brazil. Dry periods and random climatic events cause quality and yield losses in sweet corn plants. Compounds, such as silicon (Si), are being studied to reduce the negative impacts of water stress on agricultural crops. Further tests may allow farmers to increase the use of silicon-based compounds. The objective of this study was to evaluate the production parameters of the sweet corn (*Zea mays* var. saccharata) (Poaceae) Tropical Plus® hybrid with water stress and potassium silicate doses applied with foliar spraying. A randomized block design with four soil water tensions (15, 30, 45 and 60 kPa) and four potassium silicate doses (0, 6, 12 and 24 L ha^{-1}) was used in a greenhouse. The studied factors, alone or in interaction with each other, did not affect most of the sweet corn yield parameters. The hypothesis that these results may have been partially affected by the presence of silicon are discussed. The sweet corn plant yield was affected mainly by the soil water tension of 60 kPa.

Additional key words: *Zea mays*; potassium silicate; induced resistance; resistance to water stress.

1 Instituto Federal Goiano, Campus Rio Verde Campus, Goiás (Brazil). ORCID, Araújo, A.L.G.: 0000-0001-6493-5727; ORCID Neto, A.R.: 0000-0003-0517-122
2 Instituto Federal Goiano, Campus Urutaí, Urutaí, Goiás (Brazil). ORCID, Almeida, A.M.: 0000-0003-2559-125X; ORCID Guimarães, J.J.: 0000-0001-6710-7185; ORCID Cantuário, F.S.: 0000-0002-8052-4861; ORCID Salomão, L.C.: 0000-0001-9436-1488; ORCID Pereira, A.I.A.: 0000-0001-7957-6691
3 Universidade Federal de Uberlândia, Campus Umuarama, Uberlândia (Brazil).
4 Corresponding author. aiapereira@yahoo.com.br

RESUMEN

El estrés hídrico en la planta es un problema importante en el bioma Cerrado de Brasil. Los períodos secos y los eventos climáticos aleatorios causan pérdidas de calidad y productividad en las plantas de maíz dulce. Compuestos como el silicio (Si), pueden ser estudiados para reducir los impactos negativos del estrés hídrico en los cultivos agrícolas. Pruebas adicionales pueden permitir a los agricultores aumentar el uso de compuestos a base de silicio. El objetivo de este trabajo fue evaluar los parámetros de producción de maíz dulce (*Zea mays* var. Saccharata, híbrido Tropical Plus®) con niveles de estrés hídrico y dosis de silicato de potasio aplicadas por pulverización foliar. El diseño experimental fue un diseño de bloques al azar con cuatro tensiones hídricas en el suelo (15, 30, 45 y 60 kPa) y cuatro dosis de silicato de potasio (0, 6, 12 y 24 L ha^{-1}) en invernadero. Los factores estudiados solos o en interacción no afectaron la mayoría de los parámetros de producción de maíz dulce. Se discute la hipótesis de que estos resultados pueden haber sido parcialmente afectados por la presencia de silicio. El rendimiento de la planta de maíz dulce se vio afectado principalmente por el estrés hídrico del suelo a 60 kPa.

Palabras clave adicionales: *Zea mays*; silicato de potasio; resistencia inducida; resistencia a la sequía.

Received for publication: 08-04-18 Accepted for publication: 29-03-2019

INTRODUCTION

The State of Goiás, one of the largest corn producers in Brazil (Okumura *et al*., 2013), has been experiencing droughts and poor rain distribution (Brunini *et al*., 2001). The quality and productivity of this plant, with significant economic and social importance, have been affected (Berlato *et al*., 2005). Climatic effects, such as El Niño (Li *et al*., 2011), have caused more frequent dry periods and water stress condition (Doto *et al*., 2015). Corn farmers are irrigating their crops, but at high costs (Montero *et al*., 2013). In addition, rainfall scarcity has reduced water levels in reservoirs.

Sweet corn (*Zea mays* var. saccharata) (Poaceae) cultivation is increasing in Brazil (Teixeira *et al*., 2001) in order to supply agroindustries that process raw material for commercialization. This has increased the income of small and medium sized farmers and jobs on a macro scale (Barbieri *et al*., 2005). The sweet corn cultivation system in the State of Goiás has prospects for expansion since Brazilian agriculture is one of the major producers of common corn (*Zea mays*) in the world (Okumura *et al*., 2013). Syngenta dominates the sweet corn seed market in Brazil; the Tropical Plus® hybrid has the highest commercialization.

Zea mays is tolerant to water deficits during the vegetative phase, but exhibits high sensitivity if this condition prevails during the reproductive phase (Santos *et al*., 1998). Therefore, technologies that amortize the effects of water stress in economically and socially important plants, such as sweet corn, should be assessed. Silicate fertilization has been viewed as a technology that mitigates the negative effects of stress factors on plants (Ma and Yamaji, 2006) although it is not yet widely used by Brazilian farmers. Silicon has been used as an elicitor of resistance in plants under conditions of abiotic stress, such as saline, heavy metal poisoning or water stress. The accumulation of Si in transpiration organs causes the formation of a double layer of silic, with a subtle decrease in opening of the stomata, reducing leaf transpiration, which restricts the loss of water without influencing growth (Oliveira and Castro, 2002).

The corn plant has a natural ability to respond favorably after exposure to exogenous Si sources because it accumulates this element (Takahashi *et al*., 1990). The concentration of Si in this plant is linked to aerobic respiration, increasing its accumulation in soils or hydroponic solutions that hold considerable amounts of silicon. In summation, the genes ZmLsi1 and ZmLsi6 present in corn plants are responsible for the transport of Si soil solutions to root cells and xylem cells for the balance of plants, respectively, demonstrating the adaptability of this plant to Si (Mitani *et al*., 2009).

The beneficial effects of Si on corn plants in Brazilian agricultural are still neglected in the field, while increasingly scientific results have proven the high adaptability of these plants. The fact that Brazilian soils, especially those located in the Cerrado biome, are poor in soluble Si accessible to plants (Korndörfer *et al*., 2004) may explain this fact. Farmers often avoid using silicate fertilizers for fear of increasing production costs. Nevertheless, the use of fertilizers as an exogenous source of Si in sweet corn, via soil or foliage spraying, has great potential for stimulating level of resistance to water stress (Marques *et al*., 2016). The outcomes of the present study may encourage the use of silicate fertilization in corn plants under water stress.

The aim of the present study was to evaluate responses in the production parameters of sweet corn hybrid Tropical Plus® under water stress with doses of potassium silicate sprayed on the leaves.

MATERIAL AND METHODS

This experiment was carried out in an experimental area of the "Instituto Federal Goiano-Campus Urutaí" located at Fazenda Palmital, rural region of the city of Urutaí, Goiás State (Brazil). Experimental site is located at 17°29'10 "S and 48°12'38" W, at 697 m a.s.l.

The sweet corn plants were cultivated in a greenhouse, simple arc type, with an East-West orientation, made with a metallic structure and the dimensions: 30 m length, 7 m width, and 6.2 m arc height, covered with a low-density polyethylene (LDPE) film (0.15 mm thick) and a anti-aphid mesh on the sides.

The climate of the region is classified as tropical at altitude with a dry winter and rainy summer, Cwb type according to the Köppen classification. The soil of the experimental area was classified as Dystrophic Yellow Red Latosol (Embrapa, 1999) with a sandy loam texture and physical and chemical characteristics presented in Table 1. For the physical and chemical analysis of the soil, five samples were collected inside the greenhouse. The samples were homogenized for a representative composite sample using the 0 to 20 cm and 20 to 40 cm layers of depth. The soil samples were sent to the "Laboratório Agropecuário Ltda. (SOLOGRIA)" in the city of Goiânia, State of Goiás, to obtain the technical report.

The sweet corn Tropical Plus® hybrid was used (Syngenta Seeds Ltda), provided by the company Conservas Oderich SA (Orizona, Goiás), which produces canned vegetables, including sweet corn, and who supplied the technical information needed for the sweet corn plants. The sowing was performed on April 30, 2015 with a spacing of 80×25 cm, with

Table 1. Physical and chemical properties of the soil used to grow the Tropical Plus® hybrid corn (Syngenta) in a greenhouse on the IF Goiano Urutaí campus, Goiás State, Brazil

Physical properties					
Deep	Coarse sand	Thin sand	Silte	Clay	Textural Class
(cm)	$g\ kg^{-1}$				
0-20	275	324	241	160	sandy franc
21-40	329	283	202	186	sandy franc
Chemical properties					
Deep	pH	MO	P_{resina}	H+Al	K
(cm)	$CaCl_2$	$g\ dm^{-3}$	$mg\ dm^{-3}$		$mmol_c\ dm^{-3}$
0-20	6.0	24	300	21	4.98
21-40	5.7	16	280	20	4.34
Deep	Ca	Mg	SB	CTC	V
(cm)	$cmol_c\ dm^{-3}$				%
0-20	57	22	84	104	80
21-40	55	14	73	93	78

Report issued by the Laboratório Agropecuário LTDA (SOLOGRIA), Goiânia municiplaity, Goiás State, Brazil.

three seeds per hole, 2 cm deep. The fertilization was carried out following the recommendations of Trani *et al*. (2011).

The experiment design was a randomized complete block with subdivided plots in a 4×4 factorial scheme, with four concentrations of potassium silicate (0, 6, 12 and 24 L of K_2SiO_3 ha^{-1}) and four soil water tensions (15, 30, 45 and 60 kPa), totaling 16 treatments, with four replications. The experiment consisted of four useful beds (the experiment blocks) with two planting lines per set, except for the borderlines, which were placed at the lateral ends of the greenhouse with just one planting line. Each planting line received an irrigation lateral line, a drip tube and auto-compensated driplines spaced 0.3 m apart, totaling 8 useful lateral lines and two located in the margins.

The soil adaptation was done by plowing with a mini motorized tractor, model TC 14, 14 HP (Yanmar Agritech®) with a rotary hoe. The beds were 7 m long and 1 m wide and had manually hoed, totaling 16 beds throughout the experiment.

A drip irrigation system was used, where each planting line received a 16 mm lateral irrigation line, with emitters spaced every 0.30 m. The emitters provided a flow of 1.4 L h^{-1} with a service pressure of 1 kgf cm^{-2}. The pumping system consisted of a 1 HP motor pump assembly. The fertilizer injection was carried out using the suction system of the pumping set, as pushed by a set of manual controlling valves. Soon after the pumping system was installed, a 120 Mesch screen, in addition to recording the pressure of the irrigation system. A water distribution uniformity test was executed following recommendations of Borssoi *et al*. (2012) after the irrigation system was installed. The previously calculated water distribution uniformity was 97%.

The irrigation management was done with tensiometry using soil water retention curves (Tahir *et al*., 2012) and adjusted by the model proposed by Van Genuchten (1980) with the aid of the Soil Water Retention Curve (SWRC® software) version 3.0 (Dourado-Neto *et al*., 2000). Two puncture tensiometers at depths of 20 cm and 40 cm were installed to monitor the soil water tension for each experimental plot (totaling 32 tensiometers). The readings of the soil water tension values, quantified by the tensiometers, were measured using a digital tensometer (SondaTerra® model).

In the first 30 d after planting, the same irrigation management was used for all treatments in order to assure the natural establishment of the plants. The irrigation was si milar for all plots with permanent soil moisture control at near field capacity. In the first two days after seeding, a 20 mm blade was applied, followed by daily levels close to 2 mm according to the soil water retention curve in order to maintain the soil at close to the mean matric potential (-10 kPa).

The potassium silicate was applied via foliar sprayings in the subplots with the help of a manual action costal sprayer (20 L). Drift protection was used to ensure maximum application precision and to avoid contamination of the adjacent subplots, which did not receive the doses of potassium silicate. An aggregate of four applications was done throughout the experiment.

Throughout the sweet corn plant cycle, cultural treatments were performed, such as cutting of plants and leaving a single plant per hole. The dominance of invasive plants was dealt with during the crop cycle through manual weeding. The phytosanitary control was done according to the need for prevention and control throughout the experiment.

All production parameters were quantified at 95 d after planting, in accordance with the average cycle of the Tropical Plus® hybrid, which is earlier than that of the common corn (Okumura *et al*., 2013). The following plant parameters were quantified: 1st ear (cm) height, 2nd ear (cm) height, ear length with (cm) and without straw (cm), ear diameter with (cm) and without straw (cm), ear weight with (cm) and without straw (cm), cob diameter (cm), cob fresh weight (g), number of rows per cob, unit grain depth (mm), unit grain width (mm), fresh (g) and dry (mg) weight of total grains per cob, fresh (g) and dry (mg) weight of a unit cob, fresh (g) and dry (mg) weight of total straw per plant, fresh (g) and dry (mg) stem weight, fresh (g) and dry (mg) weight of all leaves per plant, number of grains per row per ear, ear yield (t ha^{-1}), grain yield (t ha^{-1}) and industrial yield (t ha^{-1}).

All quantified data were checked for assumptions with analysis of variance. The normality was verified with the Lilliefors adherence test and, in a complementary way, visually using the symmetry of the histogram obtained by the SAEG (System of Statistical and Genetic Analysis) (Ribeiro and Melo, 2009). According to this procedure, all the quantified variables followed normal distribution and, therefore, the values of their averages, as disclosed in the figures and tables, were presented in full, without the need for transformation.

After confirmation of the implication (or not) of the factors under interaction or alone according to the ANOVA with a factorial arrangement, the means were compared using the Tukey test at the 5% probability level. Statistical analyzes (ANOVA and means tests) were done with SAEG, while the images were constructed in SigmaPlot®, v. 11 (Systat Software Inc).

RESULTS

The soil water tensions and potassium silicate doses under interaction did not affect the yield parameters 1st ear height ($F=0.20$, gl=36, $P>0.05$, CV=10, ($F=1.24$, gl= 36, $P=0.04$, CV=9.42)), 2nd ear height ($F=0.18$, ($F=1.34$, gl=36, $P=0.25$, CV=5.68)), stem-to-straw diameter ($F=1.24$, gl=($F=1.00$, gl=36, $P=0.45$, CV=20.25)), grain depth ($F=0.30$, CV=4.03) ($F=0.38$, gl=36, $P>0.05$, CV=12.41), fresh (g/ $F=0.66$, gl=36, $P>0.05$, CV=14.45) and dry ($F=0.37$, gl=36, $P>0.05$, CV=13.26), ($F=3.40$, gl=36, $P=0.41$, CV=10.12) grain weight, fresh weights ($F=0.96$, gl=36, $P>0.05$, CV=11, $F=0.67$, gl=36, $P=0.05$, CV=14.06), fresh ($F=0.67$, CV=28.07) and dry ($F=1.29$, gl=36, $P=0.27$, CV=21.08) stem weight, fresh weight ($F=0.84$, gl=36, $P>0$ ($F=1.68$, gl=36, $P=0.12$, CV=11.45)), number of grains per row ($F=0.73$, gl ($F=0.66$, $P=0.05$, CV=14.45)) and industrial yield ($F=0.05$, CV=7.79) 51, gl=36, $P>0.05$, CV=12.97), isolated or not.

The parameters spike weight ($F=3.41$, gl=36, $P=0.02$, CV=9.68), without straw ($F=4.13$, gl=36, $P=0.01$, CV ($F=6.52$, gl=36, $P=0.04$, CV=9.81), fresh cob weight ($F=(F=3.46$, gl=36, $P=0.02$, CV=5.85) and ear yield ($F=3.41$, (Fig. 1) were affected by the soil water stress (Fig. 1).

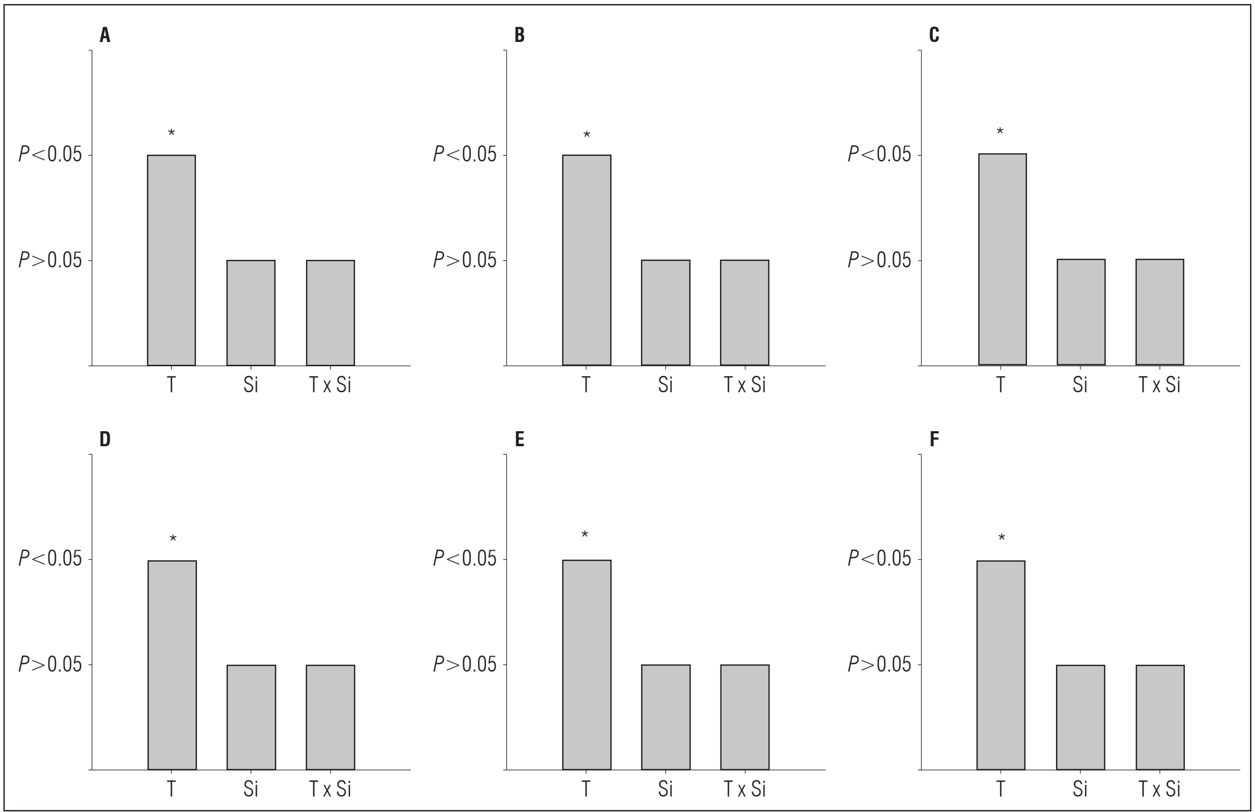

Figure 1. Summary of the ANOVA (factorial with subdivided plots) of the soil water tensions (T) (plots) and potassium silicate doses (Si) (subplots), isolated or under interaction (T x Si), for the significant production parameters (Test F) of the sweet corn (*Zea mays* var. Saccharata) hybrid Tropical Plus®. Instituto Federal Goiano, Urutaí, Goiás, Brazil. A: spike weight with straw; B: spike weight without straw; C: corn cob diameter; D: corn cob fresh weight; E: number of rows per cob; F: spike yield.

Higher values for shank weight, with and without straw, were reached at 15 and 30 kPa, as compared to the 45 and 60 kPa voltages (Fig. 2). The diameter of the cob, fresh weight of the cob and number of rows per spike were also higher with the tensions between 15 and 30 kPa (Tab. 2). The tension of 60 kPa had lower values for these parameters, including the ear yield (Tab. 2).

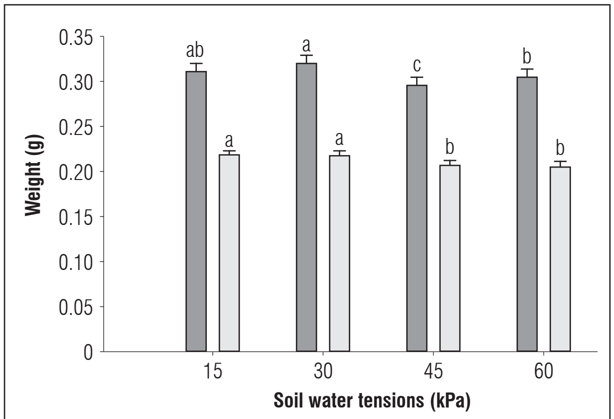

Figure 2. Weight (mean ± SEM) of sweet corn (*Zea mays* var. Saccharata) Tropical Plus® hybrid spikes with straw (dark gray bars) and without straw (light gray bars) under the effect of soil water tensions (kPa). Instituto Federal Goiano, Urutaí, Goiás, Brazil. Means with different letters, for each variable, indicate significant differences according to the Tukey test (*P*≤0.05).

DISCUSSION

The lack of effect of the soil water tensions and potassium silicate doses, isolated or under interaction, was observed on most yield parameters. The interaction between these two factors should be important for maize plants as a result of giving resistance to water stress, as previously reported (Marques *et al*., 2016). The absence of significant effects of the Si dose factor (isolated) on the productive parameters of the sweet corn did not necessarily imply that it had no influence on the results. The physiological and biochemical effects of Si under stress conditions such as water balance, reduction of oxidative stress and maintenance of adequate mineral absorption by the root system (Zhu and Gong, 2014) were entirely ignored in the present study**.** The fact that the water stress did not affect most sweet corn yield variables may have been due to the physiological and biochemical effect from the Si in the sweet corn plants. Si can mitigate water stress effects on plants of economic importance, such as *Zea mays* (Gao *et al*., 2006). This may have partially been noted in the present study with the absence of a soil water tension effect in most production parameters that normally respond to water stress.

Differences in the yield parameters were more evident when different maize genotypes were compared (Santos *et al*., 2004). The similar effect of the genetic configuration of the sweet corn Tropical Plus® hybrid in all treatments may also be responsible for the lack of effect on the evaluated yield parameters. *Zea mays* is relatively tolerant to water stress during the vegetative stage, but extremely sensitive if the stress condition persists during the reproductive stage (Song *et al*., 2010). Different soil water stresses were managed in the sweet corn plants during their entire vegetative and reproductive cycle, including in the stage between pre-flowering and grain filling. This stage is critical, where the plant can no longer satisfactorily recover from a water stress experienced in previous stages (Çakir, 2004). Therefore, the hypothesis that sweet corn adult plants have attenuated the negative effects of water stress experienced during its vegetative phase must be discarded. This would be an effective strategy for the plant to overcome stress

Table 2. Soil water tensions (SWT), cob diameter (CD), cob fresh weight (CFW), Rows number per ear (RNEAR) and spike yield (SY) (SWST) (mean ± EP1) of sweet corn (*Zea mays* var. Saccharata) Tropical Plus® hybrid (Syngenta) under different soil water stresses tensions (kPa). Federal Goiano Institute, Urutaí, Goiás, Brazil

SWT (kPa)	CD (cm)	CFW (g)	RNEAR	SY (t ha^{-1})
15	33.74 ± 0.27 a	0.216 ± 0.05 a	15.81 ± 0.19 a	27.46 ± 0.80 b
30	33.11 ± 0.28 b	0.219 ± 0.01 a	15.60 ± 0.22 ab	28.28 ± 0.82 a
45	32.56 ± 0.30 c	0.204 ± 0.09 b	15.50 ± 0.25 ab	26.88 ± 0.87 c
60	32.91 ± 0.42 d	0.184 ± 0.07 c	15.35 ± 0.20 b	26.10 ± 0.69 d

Means with different letters, per column, indicate significant differences according to the Tukey test (*P*≤0.05).

conditions experienced in the vegetative stage without reducing yield parameters in the reproductive stage (Ferreira *et al*., 2011).

The effect of the soil water tensions, with lower values for the sweet corn ear weight, with and without straw, at the higher ones, agrees with reports that indicated sensitivity to water stress (Almeida *et al*., 2016). Lower values for cob diameter and cob fresh mass under water stress conditions was reported (Parizi *et al*., 2009). These variables are strong yield indicators in corn crops because, the greater the corn mass, the greater the grain mass is (Shaheenuzzamn *et al*., 2015). This may also explain, in part, the significant differences for cob diameter and fresh weight as productivity spiked with the different soil water tensions. However, it does not fully explain why this factor did not affect the other yield parameters of the corn ear yield. The soil water tension effect may have been compensated for by the potassium silicate doses even though this factor (Si doses) did not affect most yield production parameters.

The lowest variation in the means of rows per spike, among the parameters affected by soil water tensions, may have been due to the genetic component of the evaluated Tropical Plus®hybrid, agreeing with other studies that also did not report differences (Ferreira *et al*., 2011). The decreasing number of rows per spike as the soil water stress increased was also seen in a study that evaluated the effect of different irrigation levels and calcium silicate doses on the common corn material BR 106 (Marques, 2013). The average number of rows per ear of sweet corn (independent of the soil water tensions content) was close to the 16 rows per spike that sees good acceptance on the Brazilian consumer market, reinforcing the genetic resistance of the Tropical Plus®hybrid even with water stress (Pereira *et al*., 2002).

The maximum productivity of the Tropical Plus® hybrid (~28 t ha^{-1} at 30 kPa) was higher than that indicated by the Syngenta company in Brazil (~17 t ha^{-1}) (www.syngenta.com.br). This value varies when increasing crop yields while increasing the Tropical Plus® hybrid plant population from 40,000 to 100,000 plants/ha with a maximum yield of ~9 t ha^{-1} for winter-spring cultivations and ~12 t ha^{-1} for summer crops under field conditions (Souza *et al*. 2013a; Souza *et al*. 2003b). The Vivi, Tropical Plus® and Dow SWB 551 hybrids, with 5.73, 5.35 and 2.0 t ha^{-1}, respectively, have also had similar ear yield production in the field (Teixeira *et al*., 2009). Differences in productivity results may be explained by the fact that this experiment was conducted in a greenhouse, reducing the influence of biotic and abiotic effects on the sweet corn plant production (Rosa *et al*., 2016).

The sweet corn cultivars KSC403, Merit and Obsession in Iran (Mashhad) had a similar response pattern, with decreasing growth under water stress condition (Tafrishi *et al*. 2013). The yield parameters had high sensitivity to the water stress, being a very responsive component in water stress evaluations in *Zea mays* species (Moradi *et al*. 2012).

Water stress reduces sweet corn plant productivity with losses to the corn agribusiness in Brazil. The role of silicon as an elicitor of resistance against water stress effects has been reported, and, for this reason, the non-significant effect of the Si (isolated or under interaction with the soil water tensions) should not be neglected. The spraying of potassium silicate throughout the vegetative stage on the sweet corn plants allowed for the absorption of silicon and its storage and transport to the plant yield structures (Mitani *et al*., 2009). This indicates a higher plant affinity with silicon in the vegetative stage than in the reproductive stage. Silicon may be important in post-harvest tomatoes (Marodin *et al*., 2016), another important plant in Cerrado biome agriculture.

ACKNOWLEDGMENTS

The authors thank the "Instituto Federal Goiano (IF Goiano), Campus Urutaí", the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", Conservas Oderich SA, à "Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG)" and the "Programa de Pós-Graduação em Olericultura do IF Goiano, Campus Morrinhos".

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Olives and olive oil production in the Alto Ricaurte climate region in Boyaca, Colombia

Producción de aceitunas y aceite de oliva en el clima de la región del alto Ricaurte en Boyacá Colombia

LEONARDO MAURICIO RUIZ S.[1]
DIANA CAROLINA CARVAJAL R.[2, 5]
JOSÉ FRANCISCO GARCÍA M.[3]
PEDRO JOSÉ ALMANZA-MERCHÁN[4]

Olive plants, fruits and flowers under observation.

Photos: D.C. Carvajal R.

ABSTRACT

The olive tree has expanded to several countries because of its easy adaptation to difficult edapho-climatic zones and high culinary and medicinal interest given the physicochemical composition of its fruit, including Argentina, Chile, Peru and Mexico, which have similar soil and climate conditions to the Mediterranean, where the phenological stages correspond to clearly distinct climatic seasons. However, in the Alto Ricaurte region in Boyaca, Colombia, olives do not set because of the tropical climate conditions. Because of these characteristics, the behavior of some trees, sown 4 to 30 years ago in this region, were evaluated. The temperature and precipitation were measured, and 20 branches were selected per tree, which were monitored from appearance until development; the polar and equatorial diameter of the fruits were measured until reaching maturation. A fruit and oil analysis was carried out with olives harvested in two maturation states in 2017. The temperature had a positive correlation with the phenological stages in a range of 11 to 29°C, which directly influenced the development of inflorescences and their performance. In the same year, there were two or more blooms with different intervals, where the same branch had inflorescences, flowers, freshly filled fruits and other mature fruits. As for the characteristics of the fruit and the oil, it was evident that the content of fatty acids was within the parameters required by the International Olive Council.

Additional key words: sprouting and flowering; physiological maturity; fatty acids; olive oil.

[1] Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja (Colombia). ORCID Ruiz S., L.M.: 0000-0002-0902-5605

[2] Fundación Universitaria Juan de Castellanos, Research Group AOF, Tunja (Colombia). ORCID Carvajal R., D.C.: 0000-0001-8890-3746

[3] Università degli studi di Parma, Research Group AOF, Tunja (Colombia). ORCID Garcia M., J.F.: 0000-0003-3767-6197

[4] Universidad Pedagogica y Tecnologica de Colombia (UPTC), Faculy of Agricultural Sciences, Research Group on Sustainable Agricultural Development and Production (GIPSO), Tunja (Colombia).ORCID Almanza-Merchán, P.J.: 0000-0002-9207-0617

[5] Corresponding author. dcarvajal@jdc.edu.co

RESUMEN

El olivo por su fácil adaptación a zonas edafoclimáticas difíciles, así como por el interés gastronómico, medicinal, y dada la composición bromatológica del fruto, se expandió por diferentes países como Argentina, Chile, Perú y México, que tienen condiciones de suelo y clima similares al Mediterráneo, donde sus estados fenológicos coinciden con las estaciones climáticas que son bien marcadas, a diferencia de lo que ocurre en el Alto Ricaurte Boyacá -Colombia, que por las condiciones del clima tropical, no le permiten estacionarse. Debido a estas características se evaluó el comportamiento de árboles de 4 y 30 años sembrados en esta región, donde se midió la temperatura y precipitación además de seleccionar 20 ramas por árbol, a las que se le hizo seguimiento desde el momento de la emergencia hasta su desarrollo; a los frutos se les midió el diámetro polar y ecuatorial hasta llegar a maduración, en cuento a los análisis de frutos y aceite se hizo una comparación con aceitunas cosechadas en dos estados de maduración y aceite del 2017. Arrojando como resultado que la temperatura tiene una correlación positiva sobre los estados fenológicos ya que a rangos de 11 y 29°C influyen directamente en el desarrollo de las inflorescencias y su comportamiento, además durante el año se presentan dos o más floraciones con intervalos de tiempo diverso donde se observó que en la misma rama existían inflorescencias, flores, frutos recién cuajados y otros maduros. En cuanto a las características del fruto y el aceite se evidencia que el contenido de ácidos grasos se encuentra dentro de los parámetros exigidos por el Consejo Oleícola Internacional (COI).

Palabras clave adicionales: brotación y floración; envero; madurez fisiológica; ácidos grasos; aceite.

Received for publication: 09-04-2018 Accepted for publication: 29-03-2019

INTRODUCTION

The olive tree (*Olea europaea* L.) is an evergreen tree that belongs to the species *Olea*, which has 35 species, and the Oleaceae family. It has a unique edible fruit (Kostelos and Kiritsakis, 2017). In addition, it has had gastronomic and medicinal interest since the fourth millennium before Christ. It has been used for food, cosmetics, medicines and symbols because of its physicochemical, nutraceutical, pharmaceutical and chemical characteristics (Martínez *et al*., 2014). Furthermore, its demand is based on positive effects on human health, specifically its high content of natural antioxidants and monounsaturated fatty acids (Bahloul *et al*., 2014).

Studies on the different phonological phases, reproductive blossom, morphology, physiology and flowering stage, influenced by meteorological variables (temperature, radiation, precipitation) that affect induction, differentiation and flowering, have demonstrated that these aspects directly determine the number of fruits that can be harvested (Rojo and Pérez-Badia, 2014). As a result, sprouting and flowering can be carried out between late winter and early spring with axillary buds that have a small structure and whose presence in leaves varies according to the leaf type and stage of charge (Sotomayor, 2002).

The importance of the accessory buds is based on the growth of new leaves on laden trees because the construction and rise of foliar mass increase possible potential sites for the formation of the next inflorescences (Castillo-Llanque and Rapoport, 2011; Carvajal-R. *et al*., 2018).

As a consequence, in order for the tree to grow in marginal situations, resist climate changes and adapt to modern agronomic techniques of pruning, it needs this type of buds, as seen in Peru (Tonconi, 2014). However, in Colombia, a study has not been reported because the High Ricaurte province in Boyaca is a unique zone with productive advantages (García-Molano, 2010). According to García-Molano *et al*., 2012, the Olive tree in Colombia has difficulties because the seasonal differentiation during the year is not adequate. There is not an accumulation of cold hours for floral induction, with average temperatures of 26 °C in the day and 7 °C at night; the photoperiod is almost constant during the year, which does not provide the plant a repose period because the photosynthetic activity is permanent (García-Molano and Jaramillo, 2012). Also, it has been found that floral differentiation happens through hydric stress (García-Molano and Cheverria, 2014).

On the one hand, this situation influences the flowering process throughout the year, which appears in the different phenological stages (García-Molano, 2012). The fruit quality and quantity are affected because the vegetative and reproductive growth happen at the same time. This simultaneity provokes a series of interactions between sinks (leaves, fruits, and buds) that affects the fruiting tree during its productive cycle (Castillo-Llanque and Rapoport, 2011). According to Rapoport and Moreno-Alías (2017), in olive producing regions, inflorescences are developed in the foliar axils of vegetative growth, and the knots from previous year to the flowering between July and October usually appear on the wood from last year and, in some varieties, appear over the wood from 2 years prior; while, in the Alto Ricaurte region, this situation does not occur.

On the other hand, in this region, the plants do not have the pruning management used in producing zones, where, once the harvest is finished, the plants are pruned to achieve a balance between the vegetative and reproductive cycle because of the physiological performance of the plants (Medina and Perdomo, 2015; Pérez, 2017). For instance, in Argentina, the harvest usually last over 5 months, starting in February on the table olive plantations and continuing until June and July on the olive oil plantations (Gómez del Campo *et al*., 2010). However, in Mexico, the harvest starts in August and ends in November, depending on the variety (Grijalva *et al*., 2014); while, in Chile, the harvest is between middle April and middle June, depending on the variety and the zone, and, in Peru, 97% of the production is done between April and July, and, from the end of February to April, green olives are collected, and Black Olives are picked between May and July (Cazanga *et al*., 2013).

The harvest is picked in just one period, depending on the seasons, but this does not happen in the high Andean tropics. In addition, in temperate regions, floral induction in olive trees arises in the previous summer, with temperatures between 25 and 40ºC on average, and floral initiation occurs in autumn; then, in winter, the plant gets rest, where the temperature falls below 0°C, and, in spring, the plant is activated physiologically with temperatures between 25 and 20°C (Cazanga *et al*., 2013, Torres *et al*., 2017, Rallo and Cuevas, 2017), which confirms that induction and floral differentiation in Olive producing areas are determined by thermic stress; while, in the tropics, floral induction is unknown, but floral differentiation happens through hydric stress according to García-Molano and Jaramillo (2012).

For these reasons, this article intends to distinguish the factors that theoretically define the optimal point of harvest, as well as, the effect of pruning on the physical-chemical characteristics of the fruits and physiological maturity in the Alto Ricaurte region, using the climatic conditions of olive-producing regions in the world as a reference.

MATERIALS AND METHODS

Boyaca is located at 04º 39'10" and 07º 03' 17" north latitude and 71º 57 '49" and 74º 41' 35" west longitude, in the middle of eastern Colombia. The region of Alto Ricaurte contains the villages Villa de Leyva, Sutamarchan, Tinjaca, Sachica and Raquira, which are located in the center of the department at a height of 2200 m asl on average, and the temperature is 18°C, with an overnight temperature of 5.7°C. There are also hot and dry days with an average temperature of 27ºC. Moreover, the rainfall is on average 993 mm, and there are strong winds in the months of July and August; the annual sun intensity is about 1869 h, the relative humidity is 75%, and the cloudiness is six octas according to the reports of the Institute of Meteorology and Land Adaptation IDEAM.

Firstly, the data stated in this article correspond to research carried out on olive trees planted in the region of the Alto Ricaurte 4 to 30 years ago. The measurable variables were temperature and precipitation from 2009 and 2014. This information was collected from the IDEAM Tintales weather station, using the monthly average. Secondly, in 2016 and 2017, a dataloger Exteeh-RHT20 (Extech Instruments, Waltham, MA) was installed in Huerto Olivato, which obtained the data of temperature and relative humidity. A rain measurement instrument recorded data for daily precipitation, which was measured to get the annual rate. In addition, 20 trees were selected to acquire the inflorescence length, the number of flowers and the fruit growth. 20 branches of each one were also selected, which were tracked from sprouting until development and fructification; length, number of flowers and fruits were studied.

According to the process, in step one, the fruits were measured; their polar and equatorial diameters throughout growth until maturity were analyzed. In step two, the fatty acids in the leaf were selected, 300 g from the canopy of each one of the trees, 4 and 3 years old. Likewise, the fruits and oil were compared with olives harvested in two maturity and oil stages in 2017; moreover, the leaves, fruits and oil were processed in a laboratory of the interdisciplinary research group of molecular studies from the Universidad de Antioquia, which were analyzed and interpreted.

RESULTS AND DISCUSSIONS

Flowering

The vegetative and reproductive growth of leaves, as well as, the development of fruits, are a cyclical phenomenon, both are repeated year after year. The processes that lead to fructification need two consecutive seasons, while leaf growth is completed in one year. Bud formation, floral induction and rest take place in the first season. Inflorescences, flowering, and fruit growth and development take place in the second season and conclude at maturity (Oteros *et al*., 2013; Trentacoste *et al*., 2017); however, according to the region studied, flowering usually appears in the branches that are growing, but not in those that grew the previous year; that is why the biennial cycle does not occur; additionally, there is evidence that trees can have two or more flowerings and harvests, depending on the precipitation (García-Molano *et al*., 2012).

On the other hand, in traditional olive-growing regions, winter temperature influences the flowering rate of the next season because trees are not fruitful unless they are exposed to a certain cold temperature; therefore, winter cold is the natural factor that provides the repose period to floral buds (Urbina, 2015); at the same time, dry and warm winds during the flowering period are associated with a reduction in fruit setting because the stigma is dried off, and the pollen is dehydrated (Bueno and Oviedo, 2014). Nevertheless, in the studied region, the trees were not influenced in this way because, according to the IDEAM, (2012) the temperature oscillates between 25.2 and 29.9°C throughout the day, with an average of 27.6°C, and between 6.21 and 12.3°C at night, with an average of 9.51°C (Fig. 1). Subsequently, there is not accumulation of cold hours, which determine flowering; Furthermore, the 68% relative humidity maintained pollen feasibility. This condition is an advantage because the stigma is receptive, and it helps pollination; as a consequence, harvests are matched with higher precipitation years.

Inflorescence

Temperature is the main factor, which allows the development of the Inflorescences, 72°C are required in the months of April and May to induce Inflorescence, as well as, a long exposure to cold for the optimum level of floral buds. Also, there is a dependent effect of the genotype (Rapoport, 2014; Carvajal-R. *et al*., 2018). Latitude does not have any effect on the induction of floral buds (Breton and Berville, 2013). The most fruitful buds are in

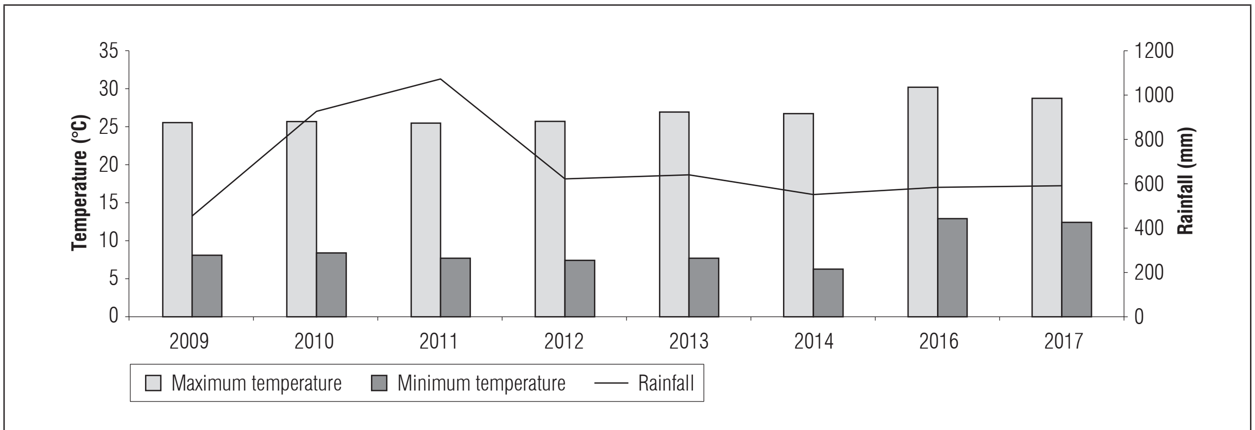

Figure 1. **Performance of the average temperature and precipitation from 2009 until 2014 and from 2016 to 2017 in Alto Ricaurte (Colombia), with a 27.6°C average temperature and an average temperature of 9.51°C during the night. The higher observed precipitations were 918.9 mm in 2010 and 1063.73 mm in 2011, and the lowest value was 450.4 mm in 2009, followed by 546.4 mm in 2014**

the central area of the branch; nevertheless, in the tropics, this is not its performance, because, there is a pattern, to bloom in new branches according to García-Molano *et al*. (2012).

Summer in the Northern and Southern Hemispheres does not have an observable effect on olives; nevertheless, it has been discovered along the study, and this effect is the floral induction phenomenon, that means the heat and the drought produce on the tree a series of chemical incentives, which are convenient for the tree. This is why, the next year will again produce plentiful flowering, as a result, the olive tree does not adapt to humid climates (Marcos, 2012).

The floral beginning includes two processes: the first one is bud predisposition to developing floral structures in the next year because of a hormonal stimulus in the induction moment, generated by the presence of fruits that have grown in the branch the previous year. This situation was not clear in the Alto Ricaurte region because the fruits grew in emerging branches. The second one is differentiation of the inflorescence and flower structures, which happens in the next year's spring in the branch that grew the previous year (Rojo and Pérez-Badia, 2014). Nonetheless, in the tropics, floral differentiation happens when precipitation starts, as was observed in the years 2001 and 2007 (García-Molano and Jaramillo, 2012). Similarly, it has been observed that olives trees have not grown because of the "El Niño" phenomenon, which happened in 2009 with longs periods of drought and rainfall of about 872.3 mm (IDEAM, 2009).

The trees stopped growth, and the apical meristems were dehydrated, which demonstrated that the olives were set, but, then in the winter of 2010 - 2011 with rainfall of 1546-1732 mm, the olive trees again began the vegetative growth, followed by flowering that would have become fruits if the winter lasted at least two months, coinciding with the study by Tapia (2012).

The floral structure called "Miñolatura" is green at first and then becomes white because of the presence of petals, with a central axis (raquis) and diverse ramifications where several flowers are located that measure between 10 and 70 mm and contain between 10 and 40 flowers. The inserted inflorescences in the proximal and distal sides of the branches have smaller dimensions (Toscano *et al*., 2015). The floral structure in the Alto Ricaurte was between 22 and 66 mm and contained between 20 and 27 flowers on average (Fig. 2). Also, the floral structure changed intensity among the varieties, tree ages, and times that tree bloomed during the year because the bloom appeared two or more times, with intervals between 6 and 8 weeks with respect to the beginning of the process (Fig. 2). This situation was observed in the years 2010 and 2011 because of rainfall in both years, as observed by (Torres *et al*., 2017), who worked with flowering, water requirements and olive oil quality as responses to new crops.

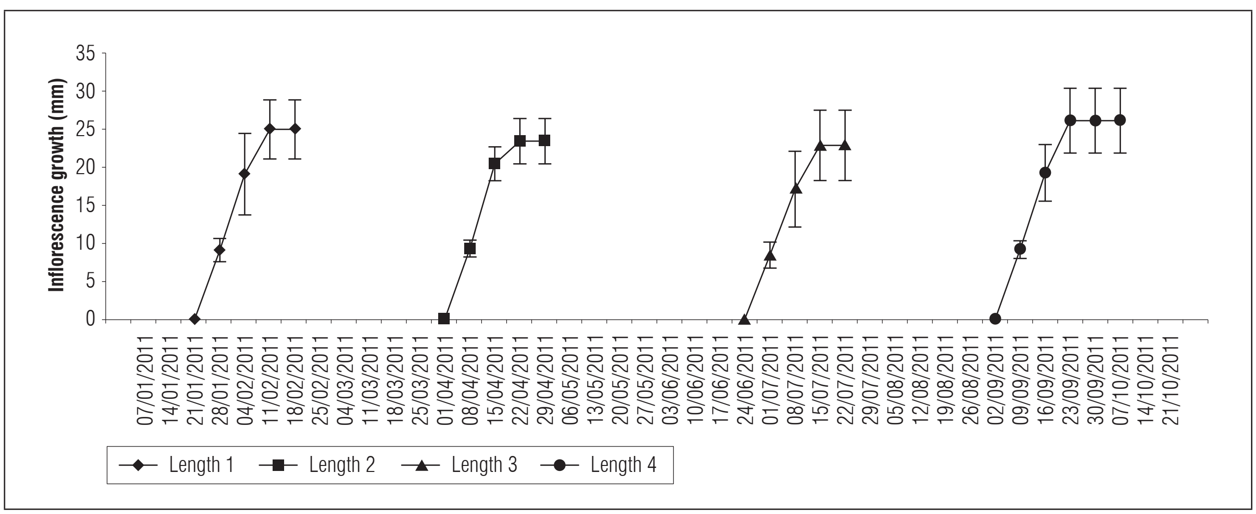

Figure 2. Growth of inflorescences olive trees in four periods (Olivanto Orchard, Alto Ricaurte, Colombia): from January to August, 2011, where trees arrive to measure between 22 and 26 mm in length and contain between 20 and 27 flowers, featuring 4 times of flowering, with intervals of 6 to 8 weeks.

Flower, pollination and fertilization

The olive tree has perfect and imperfect flowers (Rapoport and Moreno-Alías, 2017); the imperfect ones have a rudimentary or absent ovary; this is a very common phenomenon of morphological sterility, as well as, stamina or masculine gender flowers, which are fruitless (Rapoport, 2014). In the studied region, a lot of perfect and imperfect flowers were observed. For instance, for four periods marked by flowering (Fig. 3): in the first one, there were 26 flowers in inflorescence, from which 11 unfolded petals. The second one had 23 flowers with 10 that opened petals. In the third one, there were found 22 flowers, with 9 that unfolded petals, and, in the last one, there were 13 flowers, where 11 flowers were open. These data allowed us to confirm that the production of inflorescences and flowers in tropical zones has been inconstant because it is influenced by climatic, physiological and genetic factors (Beghe *et al*., 2015).

The variability and performance of flowers in the inflorescences and the opening capacity did not exceed 50% (Fig. 3), as compared to those that accomplished growth, pollination and fruit set cycle. Likewise, many of the flowers became parthenocarpic fruits and small drupes with a black color that did not grow because they were affected by the temperature, photoperiod, soil water availability, variety and low supply of pollen (because of the discrepancy of flowering). But inside, they had seeds that fell off 15 to 30 d after fruit set (Carvajal-Rodríguez and García-Molano, 2017). According to Rojo and Pérez-Badia (2014), when environmental condition are adverse or there is competition between the different organs of the plants, the premature abscission of fruits or the development parthenocarpic fruits can be induced, which may have occurred in the present study because the trees had water stress in 2009, with rainfall lower than 450.4 mm, IDEAM (2011), which provoked a prolonged vegetative recovery and then vegetative reproduction when plentiful rainfall did occur.

The olive tree needs cross-pollination, with pollen grains spread over 12 km (Seifi *et al*., 2015). Additionally, the self-incompatibility pollen-pistil is a feature of the pollination on the flowers. Hence, the flower pistil biochemically recognizes and rejects the pollen that has the same genotype (Rallo and Cuevas, 2017). Also, the olive flower has pollen a day after of another dehiscence and ends up 48 h later, consequently, the plant has viable pollen until 3 or 4 d after flowering, making sure that the pollen gets to receptive stigmas (Zhu *et al*., 2013). Nevertheless, this situation is unknown in the studied region because it has spread only by productive trees, without knowledge on compatibility between pollinating varieties or olive varieties. The duration and viability of pollen has not been identified. In the case of Huerto Olivanto, two varieties were identified; "Picual" and "Azapa", but the variety of other trees is unknown because many of them are not fruitful.

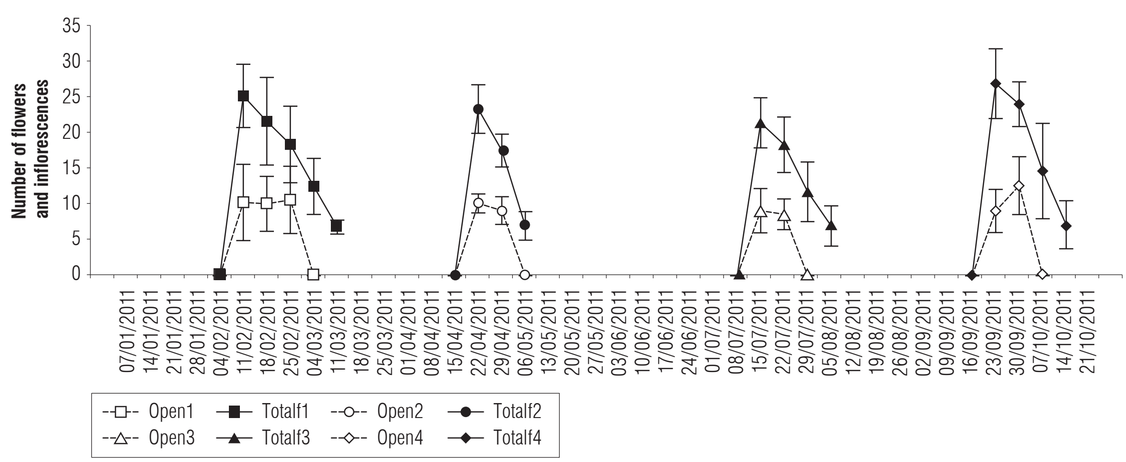

Figure 3. Number of flowers and inflorescences olive trees in four periods (Alto Ricaurte, Colombia). Bloom periods from January to August 2011, with 25, 2-23, 4-21, and 5-27, with a number of open inflorescences of 10, 6-10, 1-9, 14-12, and 6, which do not exceed 50% germinal.

Fruit set and fruit growth

The abscission process of the unfertilized ovaries and some less developed ones is triggered by fruit-setting and the growth principle (Rallo and Cuevas, 2017), and only 2% of the total flowers are set (Gómez-del-Campo and Rapoport, 2008; Rapoport and Moreno-Alías, 2017). This process shaped the drupe, which had an endocarp. Then, it started growing since the fertilization and rising size increased during the next 2 months. The mesocarp or fleshy tissue has vacuoles, where it stores the oil (Rapoport and Moreno-Alías, 2017). The outer layer or exocarp is strongly welded to the mesocarp. This tissue is composed of the monolayer epidermis with its cuticle (Beltrán *et al*., 2017) and, depending on maturity, changes color.

In the case of fruits from the Alto Ricaurte region, growth started 8 d after fruit-set, until weeks 15 and 18. Then, it began a color change. Growth was slow for four weeks approximately. After this, it increased in size for the next 2 or 3 weeks, and finally stopped at the eighth week. Subsequently, the pits began lignifying and growing, and the pulp increased in volume for nearly 30 d. Then, it started changing color, with sigmoidal growth, normal for these types of fruits (Fig. 4).

The performance of the olive trees in the tropics was special because, in the same growing period, the fruiting period may happen two or more times in the same year; consequently, when the tree flourished in two stages, it was observed that the maturity state began a week apart. That means that the harvest of these fruits can be done in the same period. Nonetheless, when 3 or more stages present in the fruiting period, its maturity is seven weeks apart from the second one. Therefore, there were different harvests in the same tree (Fig. 5), unlike regions where the olive is traditionally cultivated because flowering and harvest are concentrated in a single period.

Conversely, Navarro-Ainza and López-Carvajal (2013) confirmed that a temperature above 30°C restricts fruit-setting, and Navarro-Ainza *et al*. (2016) determined that the ideal temperature is 25°C; in this sense, the temperature in the Alto Ricaurte is on average 27.6°C, consequently, it stimulates fruit-setting, but the olive varieties in this region are not accurately known.

Summer cessation

After the pit has become hard, and the fruits have an intense green color and an expected size, they will gradually begin to accumulate reserves until maturity. At first, the fruit will accumulate reserves, such as sugar, and then it will convert them into fatty acids that will form the oil. This process is not directly formed in the fruit; it is produced by the transformation of the sugary reserves. Therefore, maturation is a process that takes place during the autumn, up to winter, in the Mediterranean region (Marcos, 2012; Seifi *et al*., 2015).

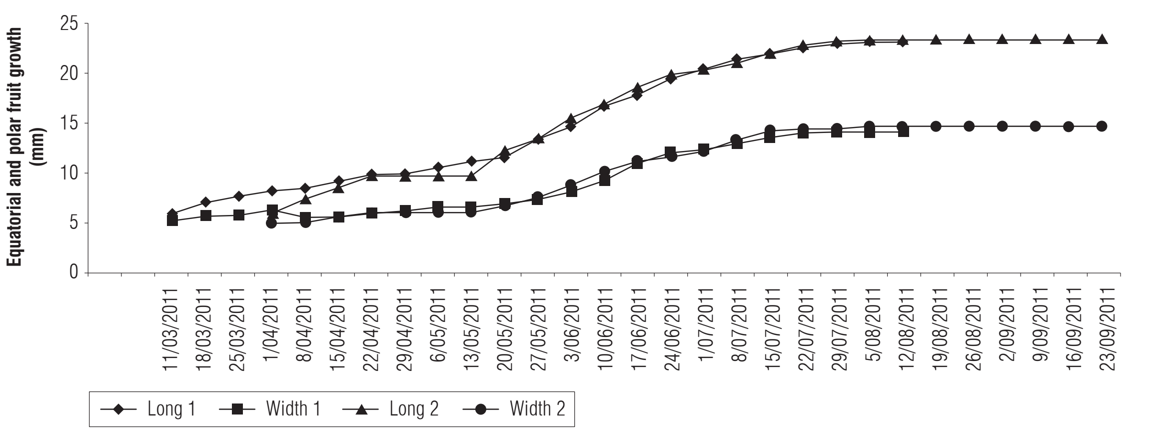

Figure 4. Measurement of equatorial and polar growth of the fruits olive tree, bloom twice between March 11th and April 1st (2011 - Alto Ricaurte, Colombia); growth shows a sigmoidal curve on May 13th, which corresponds to the 9th week of the first flowering period and the second 6 Bloom, where the increase in size of the fruits was accelerated. However, maturing for bloom 2 was extended over time.

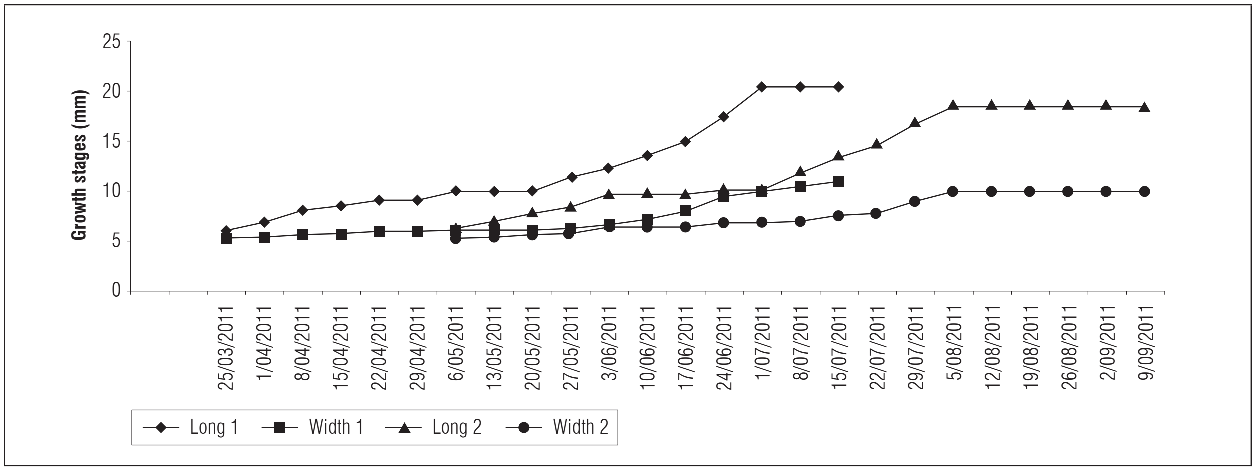

Figure 5. Different stages in the olive tree fruiting in Alto Ricaurte, Colombia. The time that elapsed between the maturing of fruits from the first flowering and the second, with 6 weeks of difference in bloom and a week difference in maturing.

Table 1. Average fatty acids contained in the olive leaves.

Age of olive trees	Methyl laureate	Methyl meristato	Methyl palmitate	Methyl stearate	Methyl oleate	Methyl linoleate	Methyl linolenate
4 years	0.17	1.15	14.56	2.59	14.53	6.22	26.8
>30 years	0.25	1.14	16.78	3.21	19.94	10.72	30.12

It has not been determined whether, in the tropics, there is summer cessation as a result of fructification spreading occurs throughout the year. But, according to physicochemical analysis in the leaves, it was found that the sugars moved by themselves to the fruits, which were sinks, forming fatty acids; this situation can happen throughout the cycle. With the climate conditions in the region, the olive can permanently do photosynthesis; it was observed that, in the leaves of the trees, more fatty acids were synthesized in the 4-year-old trees and trees older than 30 years old (Tab. 1), which may result from a mechanism of the leaves for resisting variable conditions such as temperature, intense solar radiation, and altitude, which is 2200 m.

Phase where the olive changes from green to dark violet

This is the phase in which olives change their color because of their last maturing process (Jiménez-Herrera *et al*., 2012). Hence, it is not a sudden process. The olive is green at the beginning, then, it turns yellow as a result of a strong reduction in the content of chlorophyll. Afterwards, the cells start accumulating anthocyanin, and the concentration determines the intensity of the color, from reddish to intense violet and black (Deaquiz-Oyola, 2014; Marcos, 2012). In most crops, the coloring of olives starts in the apex and keeps going until the pendulum. Then, the mesocarp gains color from outside to the violet part until the pit is formed (Beltrán *et al*., 2017). However, each kind of olive changes its color in a different way; therefore, this phase is where the olive tree variety can be recognized. For instance, some change their color earlier, as many types of 'Manzanilla' olives do, and others do so later, such as the 'Cornicabra' of Toledo. The diversity of color manifestations is so evident that some varieties can be named, such as the Royal variety (red tones) or the 'Veridical', which takes a long time to mature and many times it can be picked while still green (Marcos, 2012).

Some olives turn from green to violaceous precociously, and others keep a predominantly green color even in advanced maturity phases. This feature is affected by the charging of fruits and the watering system. Correspondingly, the color of the oil depends on the color of the olive because part of the chlorophyll remains in the oil. On the contrary, in oil made with olives that have already turned into the color expected, the yellow and orange pigments prevail. However, bitter and spicy flavors are characteristics in olive oil from premature harvests (Youssef *et al*., 2010; Jiménez-Herrera *et al*., 2012). Therefore, the period in which the olive changes the color matches the end of summer in the Mediterranean (Marcos, 2012).

This phenomenon has not been determined in the Alto Ricaurte region because the flowering and fruiting period are not uniform, which is why a tree may have inflorescences, flowers, green and mature fruits; however, in some years, there was no harvest because of the edapho-climatic conditions, as happened in 2012, 2013, 2014, 2016 and at the beginning of 2016; in this period, the trees physiologically stopped because of low precipitation (917.7 mm), a result of "La Niña" phenomenon. That is to say, in those years, the harvest was null, and the flowering fell. In general, in this region, the olives do not have diversity in color; only green (green and violet) and black have been identified in this stage; likewise, when the epicarp is totally or partially black, the fruit can be picked. It would be suitable to highlight that this behavior is proper because there is an average temperature of 27.6°C, with 13 h of light in the day.

Maturing

One of the most important parameters to indicate maturity is color variation, which is related composition (Rallo and Cuevas, 2017). In this stage, there was not only water and oil in the fruits, but also sugars, proteins, pectin, organic acids, tannins, oleuropein and inorganic components; this elements can change according to the crop, weather conditions and, maturity level (Rallo and Cuevas, 2017). Because of the phenology advance in the northwest of Argentina, the synthesis of olive takes place in the summer, when the temperatures are higher; meanwhile, in the Mediterranean, synthesis is carried out in the autumn, when the temperature is moderated; for this reason, changes in the quality of the olive oil and low levels of oleic acid are related to this phenomenon (Searles *et al*., 2011).

The qualitative components in the olive can be influenced by the environmental conditions of the production year (De La Rosa *et al*., 2015), in reference to the absolute fatty acids variations and the relation among these individual components, as well as, the relation between the oleic acid and linoleic acid ratio, and the relation among oleic acid and the palmitic and linoleic acids; by the same token, a high temperature may decrease the level of oleic acid, which is connected to an increase in palmitic acid or linoleic acids, which reduce the content of total polyphenols; while, low temperatures increase their content (Beltrán *et al*., 2017).

Fatty acid components depend on the geographical olive origin and especially the latitude, climate conditions, variety and maturity (Surra *et al*., 2015). In the Alto Ricaurte region, the temperature was about 27.6°C on average (Fig. 1) even though these temperatures were lower than summer temperatures in olive producing regions. Hence, it influenced the organoleptic composition (Tab. 2).

Table 2. Fatty acid content in fruits of the Picual variety compared with the International Olive Council.

Year	Fatty acid content in fruits of the Picual variety			
	Sample	Palmitic	Oleic	Linoleic
2012	Picual of 4 years	12625	74,285	0,615
	Picual of 30 years	13,225	71,1	0,534
2017	Fresh olives*	12,97	73,0	0,865
	Ripe olives**	13,31	72,29	0,762
	IOC***	7,5-20	55,83	3,5-21

* Picual of 10 years
** Picual of 30 years
*** Parameters International Olive Council (IOC, 2015)

Quality of the olive oil

The composition of the olive oil is determined by variety, edapho-climatic and agronomic conditions in the zone and maturity state of the fruits (Bajoub *et al*., 2015; Bakhouche *et al*., 2015). The high fatty acids of olive oil are composed of palmitic, stearic, oleic and linoleic acids (Boskou *et al*., 2006). Oleate acid is the most important component in olive oil because its quantity is higher; comparing the data of the study area with the IOC, they were within the established range and above that found by Orsavova *et al*. (2015), which was 66.4%, with the variety being unknown.

Additionally, linolenate acid is found in less quantity in olive oil; for this reason, this acid is part of the polyunsaturated fatty acids, at a proportion of 2.5 to 21% (IOC, 2015), which the European Economic Community (EEC) also established on November 6, 2003. This study showed a percentage of 3.79, which compared with the study performed by Pérez-Arquillué *et al*. (2003), whose results were 9.46%, showing that the results in this study were lower. Meanwhile, the olive oil had a lower quantity of Linolenate acid, where the content must be less or equal to 0.9, but, it is within the range according to the EEC (Tab. 3).

Table 3. Relative percentage composition of Alto Ricaurte olive oil compared to international parameters.

Oil	2012	2017	Ranges IOC
Myristrate	NR	0.017012	0.00-0.05*
Palmitate	14.21	13.3697	7.50-20.00
Stearate	1.668	1.06746	0.5-5.0
Oleate	71.18	73.756	55-83
Linoleate	4.365	3.79	2.5-21.0
Linoleate	0.945	0.77073	< ó =1.0**

*Ruiz (2015)
** European Economic Community

Myristic acid is part of the long chain of saturated fatty acids, and its appropriate range, according to Ruiz (2015), is between 0.0 and 0.05; the suitable range, according to Jamienson *et al*. (1927), must be 0.82. In both cases, the percentage was higher than the figure obtained for the Alto Ricaurte olive oil, which was 0.017%. However, Orsavova *et al*. (2015) did not report this acid either for analyses carried out in the study area in 2012.

In addition, the concentration of palmitate acid was 13.36% for the olive oil, which was between the range established by the IOC (2015); however, Sánchez *et al*. (2003) reported that the content changes depending on the variety. In the case of the Picual variety, the percentage is 11.85%, and the Morisca variety has 14.34%. Since the variety in the study area is Picual, the olive oil would have this acid within the range required by the IOC. For stearate acid, the concentration was 1.06%, within the range reported by Osada (2010), which is 0.5-5.0% according to the IOC, which, for the tropics, is within range and below that reported by (Pérez-Arquillué *et al*., 2003), which was 2.10%.

CONCLUSIONS

The production of table olives or oil in the Alto Ricaurte region is affected by the climatic conditions of the region, which result in photosynthesis throughout the year, which is why the trees do not rest and the olive color changing phase does not stop. Since the precipitation is bimodal, these plants can be fructified twice a year. However, annual rainfall below 1000 mm causes suspension of physiological activities.

ACKNOWLEDGMENTS

The authors thank the Fermented Oganic Fertilizers Research Group and the Frutalia Company in Colombia for their expertise and assistance throughout all aspects of our study in Huerto Olivanto.

Conflict of interests: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Doi: http://doi.org/10.17584/rcch.2019v13i1.7897

Content and composition of essential oil in lemon balm (*Lippia alba* (Mill) N.E.Br.) grown with ammonium and nitrate in light environments

Aceite esencial de cidrón (*Lippia alba* (Mill) N.E.Br.) cultivada con proporciones de amonio y nitrato y entornos de luz

JANDERSON DO CARMO LIMA[1, 4]
ANACLETO RANULFO DOS SANTOS[2]
ALINE DOS ANJOS SOUA[2]
MARCELO BATISTA DOS SANTOS[3]
PERICLES BARRETO ALVES[3]

Lemon balm plants in experiment.

Photo: J.C. Lima

ABSTRACT

Medicinal plants have great potential for the production of phytocompounds, which are used for various purposes, mainly in drugs and cosmetics. The aim of this study was to evaluate the essential oil from *Lippia alba* cultivated with ammonium (NH_4^+) and nitrate (NO_3^-) under light environments. The plants were subjected to five ratios of NH_4^+: NO_3^- with nutritious solutions and four light environments (red, aluminet and black mesh, and full sun). The experiment design was entirely randomized with a 5×4 interaction and five repetitions per treatment, totaling 100 experiment units. The seedlings were transplanted to 6 dm^3 plastic pots containing a mixture of washed sand + vermiculite at a ratio of 2:1. At 120 days after application of the treatments, the following parameters were evaluated: yield, content and composition of essential oil. The extraction was performed with the hydrodistillation method using drag of steam for a period of 2 hours. Nine compounds were found in the essential oil, of which neral and geranial represented 90% of the blend. Carvone was only found in the treatment with plants grown under the red mesh and with the 50:50 ratio of ammonium and nitrate.

Additional key words: essences (essential oils); artificial light; nitrogen; secondary metabolism.

[1] Universidade Estadual de Feira de Santana, Doutorando em Recursos Geneticos Vegetais, Feira de Santana (Brazil). ORCID Lima, J.C.: 0000-0002-7564-5044
[2] Universidade Federal do Recôncavo da Bahia, Centro de Ciencias Agrarias Ambientais, Cruz das Almas (Brazil). ORCID Santos, A.R.: 0000-0003-4629-3948; ORCID Soua, A.A.: 0000-0003-1522-574X
[3] Universidade Federal de Sergipe, Sao Cristovao (Brazil). ORCID Santos, M.B.: 0000-0002-3501-4475; Alves, P.B.: 0000-0002-8955-9614
[4] Corresponding author. janderson_ufrb@yahoo.com.br

RESUMEN

Las plantas medicinales tienen un gran potencial en la producción de fitocompuestos, estos son derivados para fines diversos, principalmente en la industria de medicamentos y cosméticos. El objetivo de este estudio fue evaluar el aceite esencial de *Lippia alba*, cultivada con proporciones de amonio (NH_4^+) y nitrato (NO_3^-) en entornos de luz. Las plantas fueron sometidas a cinco proporciones de NH_4^+: NO_3^-, adicionados por soluciones nutritivas, y cuatro ambientes de luz (mallas roja, aluminet y negra, y solar completo). El diseño experimental fue completamente al azar con la interacción 5×4, con 5 repeticiones por tratamiento, para un total de 100 unidades experimentales. Las plántulas fueron transplantadas a macetas de plástico 6 dm^3 de capacidad, conteniendo una mezcla de arena lavada + vermiculita en relación 2:1. Después de 120 días de la aplicación de los tratamientos, los parámetros evaluados fueron rendimiento, contenido de humedad y la composición del aceite esencial. La extracción se realizó a través de hidrodestilacion por arrastre de vapor, para un período de 2 horas. Se encontraron nueve compuestos en los aceites esenciales, el geranial y neral representaron el 90% de la mezcla, la carvona sólo se encontró en el tratamiento con plantas cultivadas bajo malla roja y con la relación de 50:50 de amonio y nitrato.

Palabras clave adicionales: esencias (aceites esenciales); luz artificial; nitrógeno; metabolismo secundario.

Received for publication: 22-03-2018 Accepted for publication: 29-03-2019

INTRODUCTION

Medicinal and aromatic plants are well known and have high acceptability by the world, including lemongrass (*Lippia alba* (Mill) N.E.Br.), also known as lemon balm, Brazilian lemongrass, field lemon balm, false melissa, field rosemary, wild rosemary, and wild cider, among others (Carmona *et al*., 2013; Ehlert *et al*., 2013). It has great economic importance domestically because of its essential oils, which have biochemicals that are of great interest for drugs since they have scientifically verified therapeutic action, besides important groups of raw material for perfumery and industries (Soares and Dias, 2013; Luz *et al*., 2014) and soothing, mild antispasmodic, analgesic, sedative, anxiolytic, and slightly expectorant properties (Matos *et al*., 2000; Nascimento *et al*., 2013). It also has some compounds with antiprotozoal, bactericidal and fungicidal activities (Tavares *et al*., 2011).

The essential oils may have different biological properties, such as a larvicidal, antioxidant, analgesic, anti-inflammatory and fungicide action (Rajkumar and Jebanesan, 2010). These oils are produced by the activation of the secondary metabolism of the plants. Factors that promote the activation of the secondary metabolism include luminosity and mineral nutrition, which are primordial for the physiological characteristics and increase vegetal production (Corrêa *et al*., 2009; Meira *et al*., 2012). Luminosity, which is essential for photosynthesis, has been studied for many years. Colored mesh, also called photoconverters, alters the spectral quality provided to plants and produces significant physiological responses in several plants (Souza *et al*., 2011).

Another indispensable factor for plant performance is mineral nutrition. Plants have macro and micronutrient requirements, including nitrogen (N), which is normally required in a greater quantity and is directly related to plant metabolism, making up constituents of important biomolecules (Bredemeier and Mundstock, 2000).

This nutrient can be absorbed in its cationic or anionic form and influences the metabolism of some plants. While studying the relationship between ammonium and nitrate, Alves *et al*. (2013) found that the dry mass yield of sunflower plants was significantly influenced by $NH4^+$ and NO_3^- ratios, concluding that, when nitrogen was supplied only in the ammoniacal form, the dry mass was severely reduced, about 38% lower for the dry mass of the aerial part, as compared to the treatment with nitrogen only in the nitric form.

When using different ratios of ammonium and nitrate in the nutrient solution for a lettuce crop, Ohse *et al*. (2017) concluded that the NH_4^+ concentration should not exceed 20% of the total nitrogen because it causes reductions in both the yield and visual quality of plants.

Therefore, the objective of this study was to evaluate the content, yield and chemical composition of essential oils from lemon grass cultivated with ammonium and nitrate under colored mesh.

MATERIAL AND METHODS

L. alba seedlings were produced from cuttings in a nursery belonging to the Center for Agrarian, Environmental and Biological Sciences of the Federal University of Recôncavo da Bahia (UFRB), Cruz das Almas-BA, Brazil. The branches used for the cuttings were produced from a matrix plant, a species that has been identified, and the exsicata is deposited in the Herbarium of the University, located in Cruz das Almas-BA, with plant trowel number HURB 8806.

The plant material was rooted in a substrate containing washed sand in polyethylene trays. After rooting, the plants with a mean height of 10 cm and root length of 12 cm were selected and transplanted to plastic vessels containing 6 dm^3 of washed sand + vermiculite at a 2:1 ratio.

The experiment was carried out in the experimental field of the Center for Agrarian, Environmental and Biological Sciences of the Universidade Federal do Recôncavo da Bahia (UFRB), in the municipality of Cruz das Almas-Bahia (12°40" S and 39°06" W, 226 m a.s.l.) from December, 2015 to April, 2016.

The experiment design was completely randomized in a 5×4 factorial scheme, with five ratios of ammonium and nitrate (100:0, 75:25, 50:50, 25:75 and 0:100) (Tab. 1) and four light environments, obtained using the colored red wavelength of 770 nm, black (shading only) and aluminized (thermal control) ChromatiNet® mesh (Polysack Plastic Industries, Negev, Israel) and full sun treatment, used as the control. Each treatment contained five replicates, one plant per pot, totaling 100 experiment units. The treatments were established based on the nitrogen (N) concentration in the solution from Hoagland and Arnon (1950). The nutrient solution was composed of macro and micronutrients, in concentrations of mg L^{-1}: N = 210, P = 31, K = 234, Ca = 200, Mg = 48 and S = 64, with pH = 5.6 (±1). The distribution of the treatments was started 8 d after transplant and acclimatization of the seedlings.

Table 1. Volume (mL) of stock solutions to form 1 L of modified nutrient solution, using ammonium and nitrate ratios (NH_4^+: NO_3^-) according to the respective treatments.

Stock solution (1 Molar)	Proportions of NH_4^+ : NO_3				
	100:0	75:25	50:50	25:75	0:100
	(mL)				
KH_2PO_4	1.0	1.0	1.0	1.0	1.0
NH_4Cl	15.0	11.25	7.5	3.75	-
KCl	5.0	1.25	5.0	3.75	-
$CaCl_2$	5.0	5.0	1.25	-	-
$MgSO_4$	2.0	2.0	2.0	2.0	2.0
KNO_3	-	3.75	-	1.25	5.0
$Ca(NO_3)_2$	-	-	3.75	5.0	5.0
Micronutrients[1]	1.0	1.0	1.0	1.0	1.0
Iron – EDTA[2]	1.0	1.0	1.0	1.0	1.0

[1]Micronutrient solution (g L^{-1}): H_3BO_3 = 2.86; $MnCl_2$ $4H_2O$ = 1.81; $ZnCl_2$ = 0.10; $CuCl_2$ = 0.04; H_2MoO_4 H_2O = 0.02. [2]Iron-EDTA solution: 26.1 g of disodium EDTA were dissolved in 286 mL of 1N NaOH + 24.9 g of $FeSO_4.7H_2O$ and aerated overnight.

Extraction of essential oil

The extraction of essential oil from the *L. alba* plants was carried out at the Phytochemical Laboratory of UFRB. At 120 d after the application of the treatments, leaves were collected to obtain the essential oil, which were individually packed in paper sacks, dried in a forced air oven at 45°C for 96 h. Afterwards, they were weighed to obtain the dry mass (g) and used for extraction of essential oil with the hydrodistillation method using drag of water vapor, equipped with a Clevenger graduated device (Santos, 2004; Oliveira *et al*., 2012).

One gram of dried phytomass at 40°C was used to determine the variation in the moisture content, and the samples were dehydrated at a temperature of 100°C to constant weight. It was necessary to join the dry material of each ammonium and nitrate ratio within each light environment to obtain the amount of phytomass sufficient for extraction of the oil, according to the methodology. Five grams of each sample were placed in a 1 L glass flask containing distilled water in sufficient volume to cover the plant material. Graduated Clevenger type appliances were used, coupled to the glass flasks, heated with thermostatic electric blankets. The extraction process was conducted for 2 h, counted from the condensation of the first drop of essential oil, and the volume extracted in the graduated column of the Clevenger was verified. Subsequently, using the Pasteur-type pipette, the oil was packed in a 2 mL glass jar, labeled and stored in a commercial freezer at -5°C until the chemical analysis was performed.

Obtaining essential oil content and yield

According to Santos (2004), the calculation of the content of essential oil (Eq. 1) was carried out with the moisture free base (BFM), which corresponded to the volume (mL) of essential oil in relation to the dry mass.

$$To: \frac{Vo}{Ms-U} \times 100 \quad (1)$$

where *To* is the oil content (%), *Vo* is the volume of oil extracted, *Ms* is the dry mass and *U* is the moisture present in dry mass.

The yield of the oil was obtained with the value of the content/100 and multiplied by the total dry mass of the leaves.

Analysis of oils

The qualitative determination was carried out in the natural products laboratory of the Federal University of Sergipe (UFS), and the essential oil analysis was performed with gas chromatography coupled to a CG-EM mass spectrometry (Shimidzu Corporation, Japan, model QP 5050A), equipped with an AOC -20i autosampler (Shimadzu) and DB-5 fused silica capillary column (30 m × 0.25 mm id, 0.25 μm film). Then, the following conditions were carried out: electron impact ionization at 70 eV; helium as the carrier gas and flow rate of 1 mL/min; an injector in the split mode (1:83) injected a volume of 0.5 μL in ethyl acetate; injected volume partition ratio of 1:83 and column pressure of 64.20 kPa; increasing temperature gradient of 4°C/min, 40 to 200°C and 20°C/min, 200 to 280°C. The injector and detector temperatures were 250 and 280°C, respectively. The mass spectra were obtained with the scan method and a scanning range of 0.50 fragments/s in the range m / z 40 - 450 Da.

The quantitative analysis of the constituents was done with a gas chromatograph equiped with a flame ionization detector using a Shimadzu GC-17 device under the following operating conditions: ZB-5MS fused silica capillary column (5% dimethylpolysiloxane) with 30 m × 0.25 mm i.d. × 0.25 μm film, using the same CG-EM conditions.

Identification of chemical constituents

The oil was analyzed simultaneously with Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Gas Chromatography with a Flame Ionization Detector (GC-FID) using a GC-2010. The qualitative analysis of the essential oils was carried out with CG/EM, while the percentage of the individual constituents was determined with CG/DIC, obtained using a detector separation system; the flow separation ratio was 4:1 (MS-FID), where the content of each component was determined based on the area of each peak related to the total area of the peaks in the chromatogram.

Each component of the oil was identified based on the retention time (considering a homologous series of C8-C18 n-alkanes), retention index (IR) applying the Dool and Kratz equation, corrected with linear regression, as well as comparing the fragmentation pattern of each component with the virtual database mass spectra (Library NIST107, NIST21 e Willey 8) and with visual comparison using the mass spectra recorded in the literature (Adams, 2007).

During the chemical analyses, the sample containing the oil of leaves grown with 100% NH_4^+ in full sun was lost because a contact reaction occurred, which made it impossible to read in the chromatograph.

RESULTS AND DISCUSSION

For the essential oil contents that were extracted (Fig. 1), it was verified that the plants grown in the nutrient solution containing the 50:50 ratio of NH_4^+:NO_3^- presented the highest average percentage value, followed by those grown with 100% of the ion ammonium under the black mesh. This was possibly due to metabolic changes during the absorption and assimilation of ammonium. For the light environments, it was observed that the plants cultivated under the black mesh presented average values higher than the others (3.12% on average), followed by red (2.86%) and full sun (2.94%).

Working in modified light environments, Chagas *et al*. (2013) observed that, in Japanes pepper mint plants, the production of essential oil was more influenced by intensity than by quality of the light. While also l studying light environments, Oliveira *et al*. (2016), found that the highest oil contents extracted from oregano plants were found in those grown under full sun. Changes in the essential oil content of *Lippia sidoides* were found by Souza *et al*. (2007) when these plants were cultivated under different percentages (75, 50 and 25%) of shading. The effects of intensity and luminous quality vary according to the species, showing the particularities of each species.

As for the essential oil yield of *L. alba* (Fig. 2), the yield was similar to that found for the content (Fig. 1), where the nutrient solution containing 50:50 NH_4^+: NO_3^- had a positive influence, obtaining the highest average value, followed by the treatment with 100% ammonium. For the light environments, the plants grown under the red mesh presented average values higher than the others (0.42 g kg^{-1}), followed by those grown under the black mesh (0.40 g kg^{-1}) and aluminet mesh (0.38 g kg^{-1}), emphasizing the affinity of this species with the light environments.

The essential oil yield varies from species to species and depends on several factors and conditions of cultivation; usually, a higher oil yield is the result of a greater accumulation of phytomass (Pinto *et al*., 2014). According to Rao (2001), most aromatic plants are sensitive to N deficiencies, and the application of N increases the production of essential oil. The metabolites produced depend on the primary metabolism of carbon, which can be divided into three distinct groups: terpenes, phenolic compounds and nitrogen compounds. These products come from the different carbons. High concentrations of N increase the demand of CH_2O for its assimilation and, consequently, energy consumption, competing with the

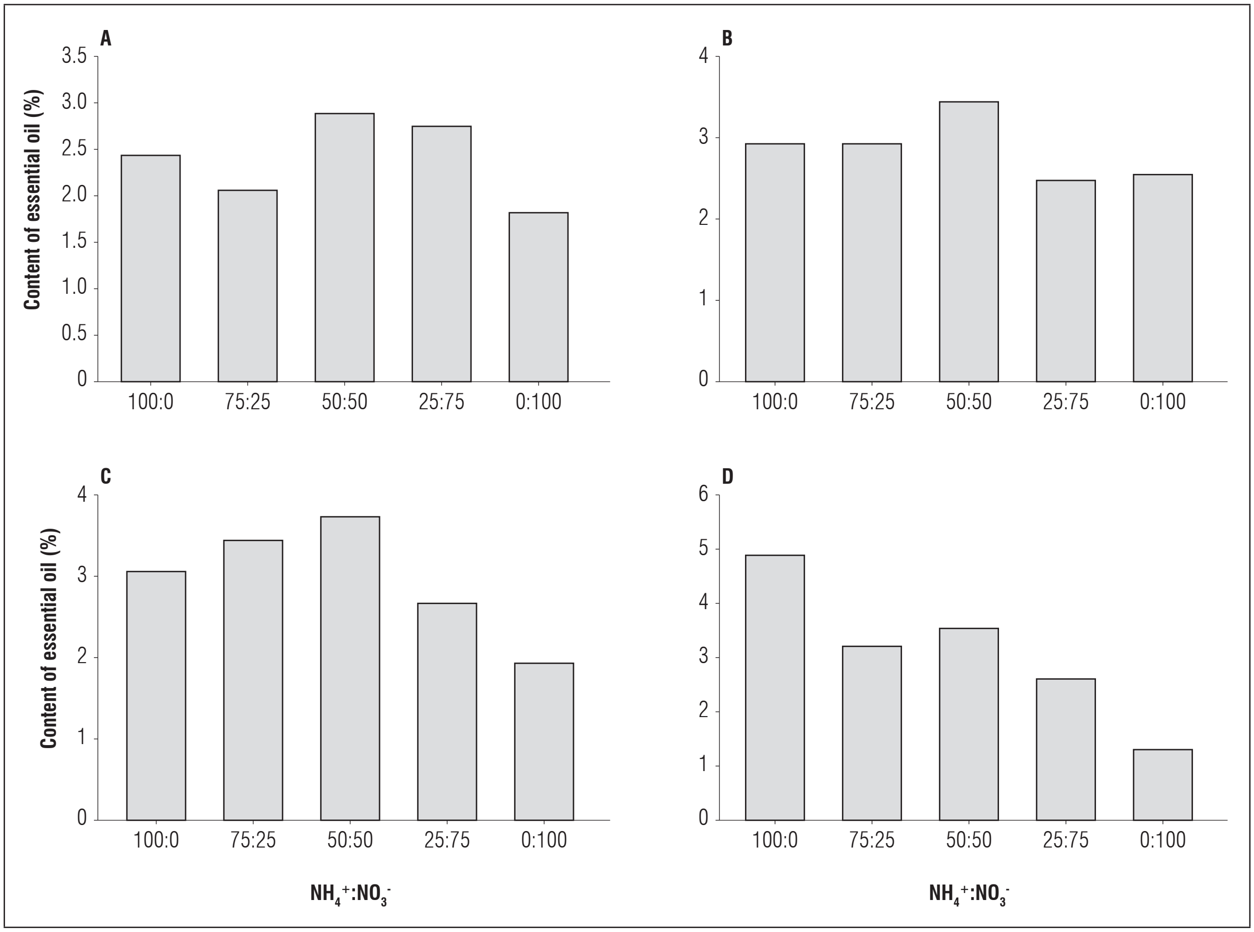

Figure 1. Content of essential oil of *Lippia alba* plants grown with NH_4^+:NO_3^- ratios and light environments: A, aluminet mesh; B, red mesh; C, full sun; D, black mesh.

primary metabolism and compromising the synthesis of these compounds (Taiz and Zeiger, 2013). It is extremely important to highlight the composition of these secondary metabolites since they are used for the production of cosmetics and pharmaceuticals, making it a very important raw material (Linde *et al*., 2016).

Among all the compounds found in the essential oil of *L. alba* leaves grown under light environments and nutrient solutions containing ammonium and nitrate, the ones that were more prominent in all the treatments were neral and geranial (Tab. 2 and 3), representing 90% of the total compounds. According to Budavari (1989), citral is a result of the mixture of geranial(citral a), and neral isomers(citral b) and has a citric odor, which is why it is used in the manufacture of certain products.

Nine different compounds were found in the *Lippia* essential oil when cultivated in light and N (Tab. 2 and 3), including: myrcene, p-cymene, linalool, neral, carvone, geranial, caryophyllene oxide, (E)-caryophyllene, terpinen-4-ol/E-isocitral, eugenol and humulene epoxide II.

The highest concentration of neral was found (Tab. 3) in the plants grown with 100% NO_3^- and under the full sun environment (48.01%), while the geranial compound had its highest content (58.2%) in the plants grown in full sun and nutrient solutions containing the 75:25 ratio of NH_4^+:NO_3^-. Probably, this happened because of the phenomenon of N nitrate absorption in relation to the ammoniacal, associated with the condition for photosynthetic activity provided by this environment.

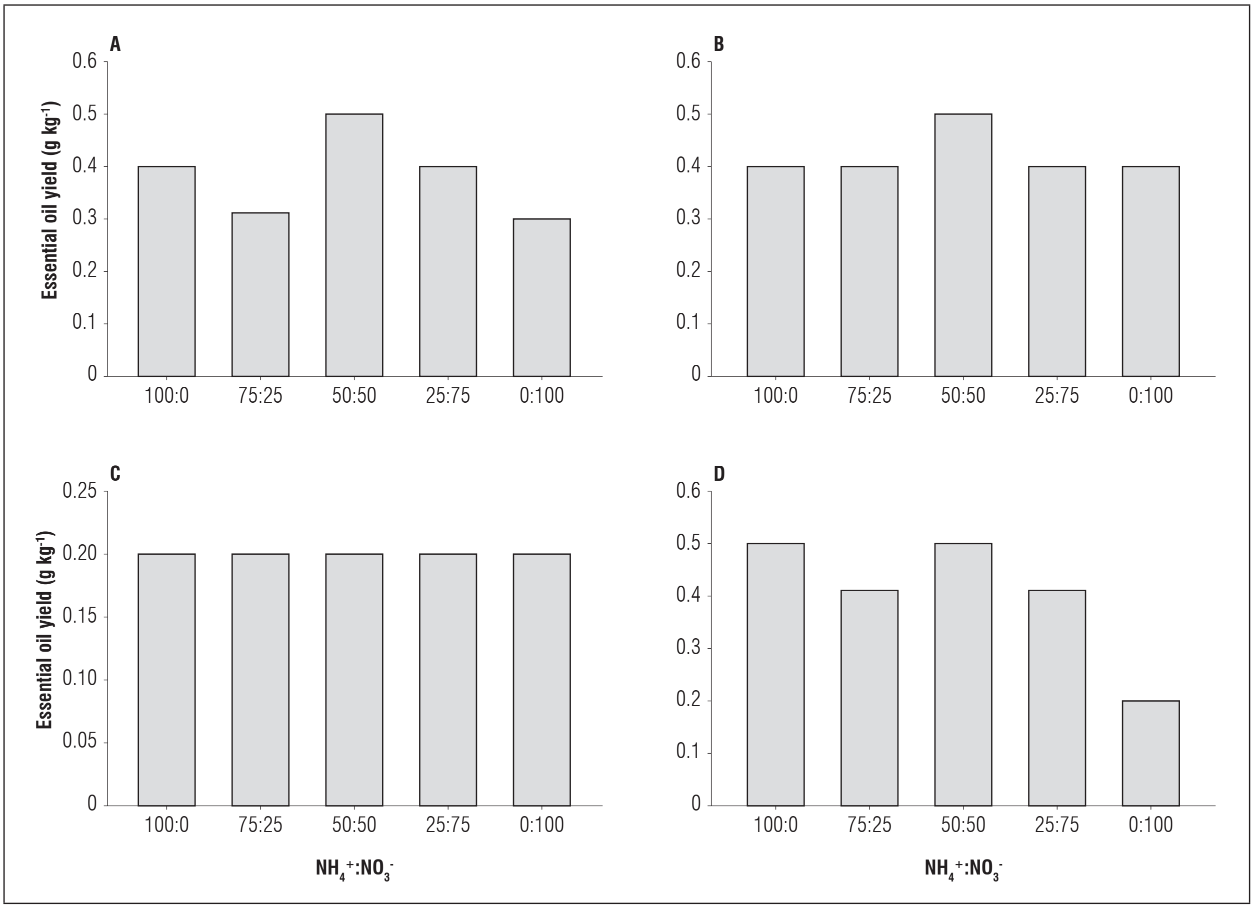

Figure 2. Essential oil yield (g kg^{-1}) of *Lippia alba* plants grown with NH_4^+:NO_3^- ratios and light enviroments: A, aluminet mesh; B, red mesh; C, full sun; D, black mesh.

Table 2. Chemical composition of the essential oil of lemon grass leaves (GC-MS: gas chromatography, GC-FID: gas chromatography coupled with flame ionization detection).

Compound	Full sun		Red mesh		Aluminet mesh		Black mesh	
	GC-MS	GC-FID	GC-MS	GC-FID	GC-MS	GC-FID	GC-MS	GC-FID
NH_4^+:NO_3^- (100:0)								
Myrcene	pp	pp	n	n	1.16	2.36	1.94	2.76
ρ-cymene	pp	pp	3.03	6.78	2.92	4.01	5.58	6.42
Linalool	pp	pp	1.44	1.54	0.98	1.36	1.29	1.47
Neral	pp	pp	35.19	33.44	35.37	35.79	35.33	32.82
Geraniale	pp	pp	54.95	53.52	53.16	53.52	50.34	52.11
Caryophyllene oxide	pp	pp	3.49	3.15	3.41	2.93	3.49	2.73
(E) –caryophyllene	pp	pp	0.96	0.76	n	n	1.19	0.96
Terpinen-4-ol/E-isocitral	pp	pp	0.98	0.76	n	n	0.84	0.70
NH_4^+:NO_3^- (75:25)								
ρ-cymene	1.17	1.97	1.42	2.49	2.41	3.50	5.87	5.96
Linalool	n	n	0.94	1.27	0.95	1.26	n	n
Neral	31.58	32.09	33.98	34.61	34.68	34.99	33.10	34.61
Geraniale	58.20	57.49	56.86	56.15	56.13	55.36	57.44	57.18
Caryophyllene oxide	6.06	5.27	4.88	4.20	4.01	3.41	3.59	2.23
(E) – caryophyllene	1.66	1.71	0.92	0.77	1.01	0.98	n	n
Humulene epoxide II	1.33	1.45	1.00	0.47	0.81	0.47	n	n
NH_4^+:NO_3^- (50:50)								
Myrcene	n	n	n	n	0.67	1.34	2.62	3.13
ρ-cymene	1.61	2.76	3.04	2.54	3.18	3.95	7.33	7.35
Linalool	n	n	1.70	1.32	1.11	1.28	1.70	1.28
Neral	32.81	33.17	43.15	32.62	34.88	34.61	35.41	34.05
Carvone	n	n	9.08	5.41	n	n	n	n
Geranial	57.03	56.37	23.93	51.37	54.85	53.72	48.58	50.40
Caryophyllene oxide	6.85	5.88	9.48	4.25	3.87	3.25	3.20	2.89
(E) – caryophyllene	1.70	1.79	2.32	0.76	0.76	1.51	n	n
Terpinen-4-ol/E-isocitral	n	n	n	n	n	n	1.16	0.87
Humulene epoxide II	n	n	2.25	0.34	0.68	0.31	n	n

pp: lost parcel; n: compound not detected.

It was also found that carvone (terpene ketone), a compound of great importance in *L. alba* leaves, was detected only in the plants grown under the red mesh and in the solution with 50:50 NH_4^+:NO_3^-; the eugenol compound only appeared in the plants grown under the aluminet mesh and in the 100% NO_3^- solution (Ehlert *et al.*, 2013).

Among the metabolites produced, terpenoids, in particular mono and sesquiterpenoids, have many functions in plants and are still poorly established for most isoprene derivatives since they involve thermoprotection effects, protection against oxidative damage, photorespiration at high temperatures and low concentrations of O_2, allelopathy and photoprotection (Taiz and Zeiger, 2013).

While studying the composition of the essential oil in native plants of *Lippia sidoides* in the city of Lavras-MG, Guimarães *et al*. (2014) found that the major constituents were carvacrol (26.44%) and 1.8-cineol (22.63%).

Table 3. Chemical composition of essential oil of lemon grass leaves (GC-MS: gas chromatography, GC-FID: gas chromatography associated with flame ionization detection).

Compound	NH_4^+:NO_3^- (25:75)							
	Full sun		Red mesh		Aluminet mesh		Black mesh	
	GC-MS	GC-FID	GC-MS	GC-FID	GC-MS	GC-FID	GC-MS	GC-FID
	%							
Myrcene	n	n	n	n	n	n	2,39	2,75
ρ-cymene	3.52	3.56	3.01	7.57	4.95	5.70	8.22	8.75
Linalool	1.74	1.08	1.24	1.64	n	n	1.58	1.67
Neral	39.76	32.89	32.51	34.32	39.22	41.95	35.75	33.56
Geranial	45.01	56.19	55.99	52.39	53.70	48.93	47.99	49.59
Caryophyllene oxide	8.01	5.25	6.13	3.39	2.13	3.39	4.08	3.65
Humulene epoxide II	1.96	1.02	1.12	0.66	n	n	n	n
NH_4^+:NO_3^- (0:100)								
Myrcene	n	n	n	n	n	n	1.84	1.78
ρ-cymene	3.88	2.87	2.08	2.39	n	n	6.46	6.71
Linalool	2.33	1.36	0.87	0.65	0.99	1.14	1.54	1.53
Neral	48.01	34.26	25.41	26.25	33.64	34.10	35.28	33.10
Geranial	35.30	56.03	42.67	42.65	56.31	55.42	50.50	52.97
Caryophyllene oxide	8.35	4.77	4.33	3.91	5.02	4.35	3.34	3.12
(E) – caryophyllene	n	n	N	n	1.27	1.27	n	n
Terpinen-4-ol/E-isocitral	n	n	N	n	n	n	1.04	0.76
Umulene epoxide II	2.13	0.69	0.80	0.61	0.94	0.87	n	n
Eugenol	n	n	N	n	1.83	2.81	n	n

N: compound not detected.

P-cymene, a compound that was detected in all treatments, is used as an antibacterial when combined with carvacrol, with synergy between them (Silva *et al*., 2010).

While studying the effect of different harvest schedules on the yield and composition of *L. alba* essential oil, Ehlert *et al*. (2013) observed that, between 8 and 10 a.m., the leaves presented the main components of the essential oil: carvone (49.48%), limonene (28.66%), sabinene (2.25%), y-terpinene (0.96%) linalool (1.30%), elemol (3.62%), and guaiol (0.56%). It is worth noting that this study was carried out in São Paulo-SP (Brazil), where the environmental conditions are different, which may have led to the biosynthesis of components that were not observed in this experiment.

Essential oils are composed of a mixture of several compounds, and the contents of each depend on the environmental factors where the plant is grown (Andrade and Casali, 1999).

These plants have the potential to adapt to different luminosity and fertility conditions because of their phenotypic plasticity (Delgado and Lopez, 2008). The composition of these essential oils can be altered by the light conditions. Batista *et al*. (2016), while working with *Lippia alba*, verified that the composition of the volatile compounds varied with light quality and chemotype, with differences that were due mainly to the amounts of eucalyptol and linalool. As for N sources, Lima *et al*. (2017) found that there were significant interactions between light environments and ammonium and nitrate ratios in the nitrogen, phosphorus and potassium contents of *Lippia alba* plants.

CONCLUSIONS

Neral and geranial were found in higher concentrations in the essential oils in the solutions with 0:100 and 75:25 NH_4^+: NO_3^-, respectively, and under full sun, representing up to 90% of the total oil constituents.

The red mesh and solution containing only N-NO_3 - favored the production of eugenol. The aluminide mesh and 50:50 ratio of NH_4^+: NO_3^- promoted the production of carvone. These compounds are of significant importance in the drug industry.

ACKNOWLEDGMENTS

The authors are grateful to Capes for funding the scholarship and developing the research.

Conflict of interests: this manuscript was prepared and revised with the participation of all the authors, who declare that they have no conflict of interests that could affect the validity of the results of the presented work.

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Doi: http://doi.org/10.17584/rcch.2019v13i1.8067

SCIENTIFIC NOTE

Effect of cuttings defoliation and different substrates on the vegetative propagation of the monkey-pepper (*Piper aduncum* L.) (Piperaceae)

Efecto de la defoliación de esquejes y diferentes substratos en la propagación vegetativa de pimienta de mono (*Piper aduncum* L.) (Piperaceae)

AUREA PORTES FERRIANI[1, 3]
DIONES KRINSKI[2]

Cuttings type and rooting performance on *Piper aduncum* L.

Photo: A.P. Ferriani

ABSTRACT

Forests constitute a valuable natural resource, especially when including non-timber forest products (NTFPs). Bioprospecting for and sustainable exploration of native species and the development of protocols for seedling production promote conservation. *Piper aduncum* L. stands out in several regions of Brazil because of several biological activities, notably action as a repellent, antimicrobial and insecticide. This study aimed to evaluate the influence of defoliation and different substrates on the cutting process. For this, an experiment was conducted in February, 2016 at the Health Plant Department, Sector of Agricultural Sciences, Federal University of Paraná (UFPR). *Piper aduncum* stem cuttings with 10 cm and 1/3 of the leaf area and defoliated cuttings. Stem cuttings were washed in running water for 5 minutes for subsequent use in three different substrates (medium sand sifted, vermiculite and commercial substrate Tropstrato HP®). The experiment design was completely randomized in a 2×3 factorial arrangement (cutting type x substrate), and it was evaluated the rooting percentage, mortality, shoot emission, number of roots and average length of the three largest roots after 60 days. The results confirmed that

[1] Universidad Federal del Paraná, Department of Agronomy, Curitiba (Brazil). ORCID Ferriani, A.P.: 0000-0002-2896-6427
[2] Universidad do Estado de Mato Grosso, Campus Eugênio Carlos Stieler, Tangara da Serra (Brazil). ORCID Krinski, D.: 0000-0002-4719-5314
[3] Corresponding author. aurea.portes@hotmail.com

the presence of leaves in *P. aduncum* stem cuttings promotes adventitious rooting. The different substrates did not exert a significant influence on the performance of the cuttings in this species.

Additional key words: propagation by cuttings; pruning; growing media; non-timber forest products (NTFPs); native spice.

RESUMEN

Los bosques constituyen un valioso recurso natural, principalmente cuando son productos forestales no maderables. La bioprospección y exploración sostenible de especies nativas, pueden desarrollar protocolos de producción de plántulas al potenciar la conservación de áreas de interés. La especie *Piper aduncum* L. se encuentra en varias regiones, presenta diversas actividades biológicas, al destacarse acciones repelente, antimicrobiana e insecticida. El objetivo de este trabajo fue evaluar la influencia de la defoliación y diferentes sustratos en el processo de propagación. Para esto, un experimento fue realizado en febrero de 2016 en el Departamento de Fitotecnia y Fitosanidad, Sector de Ciencias Agrarias, Universidad Federal de Paraná. Se utilizaron esquejes semileñosas de *Piper aduncum* con 10 cm y un 1/3 del área foliar y defoliación total. Los esquejes fueron lavados por 5 minutos en corriente de agua para posteriormente usados en tres diferentes sustratos (arena tamizada, vermiculita y sustrato comercial Tropstrato HP®). El diseño experimental fue completamente al azar con arreglo factorial 2x3 (tipo de esqueje x sustrato), y fue evaluado el porcentaje de enraizamiento, mortalidad, emisión de brotes, número de raíces y longitud media de las tres raíces más largas después de 60 días. Los resultados indicaron la necesidad de las hojas en los esquejes de *P. aduncum* para promoción del enraizamiento adventicio. Los sustratos arena, vermiculita y Topstrato no presentaron influencia significativa en el desempeño de los esquejes de la especie.

Palabras clave adicionales: propagación por estacas; poda; substratos de cultivo; produtos forestales no maderables (PFNM); pimienta nativa.

Received for publication: 06-06-2018 Accepted for publication: 29-03-2019

INTRODUCTION

Forests, in general, constitute one of the most valuable natural resources. In addition to being biodiversity centers, they are important climate regulators, rainwater drainage, and efficient soil protectors. They are also essential sources of resources for man, producing, for example, wood, cellulose, resins, tannins and essential oils, among other non-timber forest products (NTFP) (Backes, 2009).

Prospecting native species with potential for use as NTFP seeks to minimize the impact of predatory extractivism of natural areas and subsidize the exploration of new compounds of interest in a sustainable manner. The species with potential for production of compounds of interest include the genus *Piper* (Piperaceae), which occupies a prominent position. Fazolin *et al*. (2006) highlighted the variety of compounds from mixed routes (chiquimate/acetate) generating phenylpropanoic aromatic compounds (lignans and neolignans), as well as terpenes and flavonoids. For this reason, the composition of essential oils of different species of Piperaceae in Brazil has been researched under several biological and biotechnological aspects (Mesquita *et al*., 2005; Pereira *et al*., 2008; Guerrini *et al*., 2009; Oliveira *et al*., 2013; Girola *et al*., 2015; Krinski *et al*., 2018a).

Piper aduncum L., popularly known as Monkey Pepper, is native to Tropical America and widely distributed throughout the Brazilian territory. It is considered an ombrophilous species, occurring in several forest formations, preferably in soils with a high organic matter content and humidity (Lorenzi and Mattos, 2002). Morphologically, this species is described as a bush or small tree, from 2 to 7 m, showing several nodes, with membranous or chartaceous, elliptic or elliptic-oval leaves (Maia *et al*., 2000).

This species presents great potential in the recomposition of degraded areas, being a colonizer of altered areas that promotes greater natural regeneration and relative density over time (Alvarenga *et al*., 2006). Nevertheless, the main economic interest of this species is due to the production of essential oil with varied biological properties. There are reports in the literature of yields of up to 3.5% of essential oil, and the phenyl ether dillapiol is the most frequently reported major constituent (Sousa *et al*., 2008; Almeida *et al*., 2009). Among the numerous biological activities attributed to *P. aduncum* oil, repellent actions (Misni *et al*., 2009), antimicrobial agents (Guerrini *et al*., 2009) and insecticide actions stand out (Pereira *et al*., 2008; Mesquita *et al*., 2005; Krinski and Foerster, 2016; Sanini *et al*., 2017; Krinski *et al*., 2018b).

According to Elias and Santos (2016), for NTFPs to present a viable alternative that stimulates conservation and promotion of community development, some relevant aspects need to be clarified, including botanical, ecological and agronomic or silvicultural knowledge of this species. In this sense, knowledge on appropriate propagation techniques is important because it is one of the initial stages in the process of domestication of species of interest, aiming at a more rational exploitation that is not based only on extractivism.

In order to provide specific knowledge on species propagation and phytochemical research, the objective of the present study was to evaluate the influence of the presence of leaves or defoliation and the use of different substrates on the vegetative propagation of *P. aduncum* using stem cutting.

MATERIAL AND METHODS

The collection of branches with leaves from adult *Piper aduncum* plants (MBM396411 register) was carried out in February, 2016, at the Bom Jesus Biological Reserve, Antonina, Parana, Brazil. The plant material was moistened and conditioned in black polyethylene bags for transport to a greenhouse in the Agrarian Sciences sector of the Federal University of Parana, where it was kept under intermittent misting until the propagules were prepared.

Semiwoody stems generated cuttings with a 10 cm length and standard diagonal cut (bevel) at the base, straight in the upper portion, with 1/3 of the leaf area or total defoliation. After cutting, the propagules were washed in running water for 5 min. Solutions containing plant regulators were not used because the seedlings were produced in Environmental Protection Areas (EPA) where contaminant inputs are prohibited.

The planting was carried out in plastic tubes, 120 cm^3 volume, with the three substrates: medium sand sifted, vermiculite and commercial substrate Tropstrato HP®. The cuttings were kept in a greenhouse with an intermittent nebulization of 5 s every 30 min.

The experiment design was completely randomized in a 2×3 factorial arrangement (presence or absence of leaves and three substrates), with four replications and 14 cuttings per plot, comprising 56 cuttings per treatment and a total of 336 cuttings in the experiment.

After 60 d, the percentages of survival, mortality and sprouts, average number and average length of the three largest roots were evaluated. The data were submitted to analysis of variance homogeneity with the Bartlett test, variance analysis with ANOVA and means comparison with the Tukey test at 5% probability. The statistical analysis was carried out with Assistat (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

The analysis of variance showed that there were no differences among the substrates used; however, the presence of leaves was significant for all variables, except for percentage of cuttings with shoots. The interaction between the factors (presence or absence of leaves and substrates) was significant only for cutting mortality (Tab. 1).

The rooting percentages in the *P. aduncum* cuttings ranged from 2.4 to 30.8%. The highest averages were recorded in cuttings with the presence of leaves (Tab. 2) and confirmed the interespecific variation in *Piper* plant propagation, as pointed out by Dosseau (2009), Chaves *et al*. (2014), Cunha *et al*. (2015), Gomes and Krinski (2016a, 2016b), and Ferriani *et al*. (2019).

Ferriani *et al*. (2018) also verified an increase in rooting percentage responses in cuttings with leaves in

Table 1. Analysis of variance for rooting percentage (ROO), percentage of mortality (MOR), percentage of cuttings with shoot emission (SHO), number of roots (NUM) and length of the three largest roots (LEN), on cuttings of *Piper aduncum* with and without leaves depending on different substrates. Curitiba-PR (2016).

Source of variation	DF	F values				
		ROO	MOR	SHO	NUM	LEN
Presence of leaves (L)	1	26,45**	25,79**	0,17^NS^	34,17**	55,22**
Substrates (S)	2	1,40^NS^	0,50^NS^	0,17^NS^	2,84^NS^	1,47^NS^
Interaction (LxS)	2	1,40^NS^	4,79*	1,21^NS^	0,31^NS^	2,02^NS^
Residue	12	-	-	-	-	-
CV (%)		54,21	7,52	145,34	40,79	44,30

** significant at 1%; * significant at 5%; NS: not significant. DF: degrees of freedom; CV: coefficient of variation.

Table 2. Mean and standard deviation of rooting percentage, mortality, sprout emission, number of roots and mean length of the three largest roots on cuttings of *Piper aduncum* with and without leaves depending on different substrates. Curitiba-PR (2016).

	Substrates			
Cuttings	Sand	Vermiculite	Topstrato	Mean
	Rooting (%)			
With leaf	30,8±4,1 aA	18,9±4,1 aA	18,9±14,8 aA	22,9±2,9 a
Without leaf	4,8±4,1 bA	7,1±7,1 aA	2,4±4,1 bA	4,7±2,2 b
Mean	17,8±4,1 A	13,0±4,5 A	10,7±6,4 A	
	Mortality (%)			
With leaf	71,4±7,1 bA	81,0±4,1 aA	76,2±10,9 bA	76,2±8,0 b
Without leaf	95,2±4,1 aA	83,3±4,1 aA	95,2±4,1 aA	91,3±6,9 a
Mean	83,3±14,0 A	82,1±3,9 A	85,7±12,8 A	
	Sprout cuttings (%)			
With leaf	7,1±12,3 aA	2,4±4,1 aA	4,7±8,2 aA	4,7±7,9 a
Without leaf	2,4±4,1 aA	11,8±10,8 aA	4,7±4,1 aA	6,3±7,5 a
Mean	4,7±8,6 A	7,1±8,9 A	4,7±5,8 A	
	Roots number (n)			
With leaf	5,5±0,5 aA	7,1±1,5 aA	5,2±1,1 aA	5,9±1,3 a
Without leaf	2,0±2,0 bA	2,7±2,5 bA	0,3±0,6 bA	1,7±1,9 b
Mean	3,8±2,3 A	4,9±3,1 A	2,8±2,8 A	
	Roots length (cm)			
With leaf	7,7±1,4 aA	9,3±3,7 aA	11,3±2,7 aA	9,4±2,9 a
Without leaf	0,3±0,6 bA	3,2±3,2 bA	0,03±0,06 bA	1,2±2,2 b
Mean	4,0±4,1 A	6,2±4,1 A	5,7±6,4 A	

Means followed by the same uppercase letter in the rows and lowercase in the columns do not differ from each other by the Tukey test ($P \leq 0.05$).

three species of *Piper* (*P. arboreun, P. cernuum* and *P.* diospyrifolium) with indolbutyric acid (IBA) use, highlighting new study perspectives.

The mortality of *P. aduncum* cuttings was also lower in cuttings with the presence of leaves on the substrates sand and Topstrato, with no difference in the cuttings with and without leaves in the vermiculite substrate; however, a significant difference was observed for this variable in the commercial substrate (Tab. 2).

The percentage of cuttings with shoots did not present significant difference for this species because there were percentages of rooting or mortality that were significant and complementary (Tab. 2). In addition to higher rooting percentages, the presence of leaves in the *P. aduncum* cuttings promoted increases in the number and mean of the root length (Tab. 2).

The values of the rooting percentage observed for *P. aduncum* in our study, when compared with other species of the genus, were relatively low; however, the literature shows a high variation in the rooting potential of this species.

Stem cuttings of *Piper hispidum* Sw. have shown percentages higher than 85% (Cunha *et al*., 2015). Pescador *et al*. (2007) reported values higher than 60% for *Piper mikanianum* (Kunth). Steudel. Chaves *et al*. (2014) evaluated stem cuttings of three species of the genus for rhizogenic potential, where *Piper hispidum* Sw. and *Piper tuberculatum* Jacq. presented values higher than 80%, while *Piper marginatum* Jacq. did not exceeded 20% rooting.

With values similar to those observed in the present study, Mattana *et al*. (2009) reported rooting values of up to 37.5% for *Piper umbellatum* L. However, Gomes and Krinski (2016a), in the same species, obtained values between 37.5 and 60% rooting, without influence from the substrates. The longest cutting length (20 cm) had the best performance.

Even so, for *P. aduncum,* the best rooting performance in the leaf cuttings occurred because these organs are sites of synthesis of auxin and carbohydrates translocated to the base of the cuttings, accelerating the process of rooting and reducing the mortality rates (Hartmann *et al*., 2010). In addition to the synthesis of auxin and carbohydrates, it is probable that rooting and greater vigor in leaf cuttings are related to the synthesis of phenolic compounds that interact with auxin, inducing a greater vigor and percentage of root emission (Pacheco and Franco, 2008).

The significant difference for the mortality of the cuttings in the commercial substrate corroborated with the results obtained by Purcino *et al*. (2012) for two species of the genus *Ocimum* (*O. gratissimum* and *O. selloi*). Overall, the survival of the cuttings was low, differing from the values found by Dosseau (2009), who reported up to 90% survival in medium plagiotropic cuttings for *P. aduncum* in a sand substrate. Nonetheless, the author reported up to 100% mortality for basal cuttings in a vermiculite substrate.

The increase in the number of roots and mean root length verified in our study is in agreement with other studies, such as the one by Vignolo *et al*. (2014), who reported a greater number and length of roots in cuttings of three mulberry cultivars with the presence of leaves, to the detriment of cuttings without leaves.

When analyzing the percentage of cuttings with shoots, although there were no significant differences for *P. aduncum*, other species, such as those of the genus *Ocimum* studied by Purcino *et al*. (2012), have had cuttings with whole leaves or leaves reduced by half that presented percentages of shoots higher than 97 and 1.3% live cuttings, while seedlings without leaves have maintained rates of 65 and 30%. These results corroborated the relationship between leaf maintenance, as a prerequisite for sprouting, and subsequent rooting.

Together with this factor, the longest and number of roots are probably related to a higher endogenous auxin concentration because of the presence of leaves, which favors the best development of the root system (Antunes *et al*., 2000).

In addition, in *P. aduncum,* there was greater rooting in the sand substrate and higher values for the number and length of roots in the vermiculite substrate. In another *P. aduncum* study in the length of propagules and substrates, Ferriani *et al*. (2019) observed higher rooting responses (26.5%), a lower length (10 cm), without influence from the substrates and lower results with the longest cuttings (20 cm) in a vermiculite substrate.

These data are a bit different those reported by Dosseu (2009), who found higher values of survival and number and length of roots in orthotropic and plagiotropic stem cuttings of *P. aduncum* in a sand substrate, as compared to vermiculite. However, in both cases, it was observed that substrates with higher porosity presented satisfactory results.

These discrepancies in the rooting percentages showed the different genetic potentials of each species for adventitious root emission and suggested the need for specific studies for each species in order to define adequate propagation protocols (Gomes and Krinski, 2016a; 2016b; 2018a; 2018b; 2019). Further studies are indicated because of the biological and environmental importance reported in many native *Piper* species.

Thus, because of the scarcity of studies on the vegetative propagation of *P. aduncum*, complementary studies involving rejuvenation techniques, application of plant regulators and the dynamics of seasonality should be carried out on the rooting of this species.

CONCLUSIONS

The presence of leaves in *P. aduncum* stems promotes adventitious rooting. The substrates sand, vermiculite and Topstrato did not present a significant influence on the performance of *P. aduncum* cuttings under the conditions in which this experiment was carried out.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Wanderlei do Amaral for identifying and collecting this species and to the technicians of the Herbarium Curitiba Botanical Museum for the exsicate storage. The authors are also grateful to the Coordination for Improvement of Higher Education Personnel (CAPES) for its support and the scholarship granted to the second author, to researcher Erik Nunes Gomes for his assistance in collecting and making the cuttings, and to Professor Cícero Deschamps (both from UFPR / Curitiba-PR) for the availability of the substrates and the greenhouse used in the experiments.

Conflict of interests: the manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts in risk the validity of the presented results.

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Acknowledgments

When considered necessary, the authors may acknowledge the researchers or entities that contributed - conceptually, financially or practically - to the research: specialists, commercial organizations, governmental or private entities, and associations of professionals or technicians.

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- Internet citations: Author(s). Year. Title of the article. In: name(s) of the electronic publication, and/or of the website, portal or page, URL; consultation date. Example: Arafat, Y. 1996. Siembra de olivos en el desierto palestino. In: Agricultura Tropical, http://agrotropical. edunet.es; consulted: November, 2003.
- For citing proceedings: Author(s). Year. Name of the dissertation. pp. #-#. Title. Publisher, City (and country, if the city is not a capital). Example: Peet, M. 2008. Physiological disorders in tomato fruit development. p. 101. In: Book of abstracts, International Symposium on Tomato in the Tropics. Sociedad Colombiana de Ciencias Hortícolas. Villa de Leyva, Colombia.
- For theses: Author(s). Year. Title. Thesis degree. Academic institution, city (and country, if the city is not a capital). Example: Hernandez, M. 2001. Conservación del fruto de arazá (a capital). Example: Hernandez, M. 2001. Conservación del fruto de arazá (Eugenia stipitataMcVaugh) durante la poscosecha mediante de aplicación de diferentes técnicas. Doctoral thesis. Faculty of Agronomy, Universidad Nacional de Colombia, Bogota, Colombia.

International unit system (SI) or metric unit system

The *Revista Colombiana de Ciencias Hortícolas* requires the use of SI units (Système international d'unités).

The numbers of multiplication and the negative numbers of the superscripts should be used only in conjunction with SI units (for example, kg ha^{-1}). Do not place non-SI units in SI units, because the units are mathematical expressions. Reorganize the phrase respectively, for example:

- P at 20 g L^{-1}, but not 20 g P L^{-1}, nor 20 g P/L.
- The yield measured in dry mass was 10 g^{-1}, but not 10 g^{-1} of dry mass dd^{-1}.
- The active ingredient was applied at 25 g ha^{-1}, but not 25 g a.i./ha^{-1}.
- Each plant received water at 30 g^{-1} ha, but not irrigation was applied at 30 g H_2O/ha per plant.

The slant line (/) is a symbol of mathematic operation that means "division"; in science, it may be substituted by the word "per" in the meaning of "per each" and indicates rates or degrees. Use the slant lineto connect SI units with non-SI units (for example: 10°C/h or 10 L/plant). Never use the raised period and slant line in the same expression. If you mix SI and non-SI units, use the slant line first and then the word "per" in the second terminus. Never use two or more slashes (/) or the word "per" more than once in the same phrase to avoid redundancy, for example: irrigation/day per plant should be changed to: each plant was irrigated two times per day. For the totally verbal units, use one slash, such as: three flowers/ plant or 10 fruits/branch.

To cite units based on names, use lowercase, such as: "one siemens represents...". However, Celsius units should be written with the first letter capitalized.

Use the same abbreviation or symbol for the singular and plural forms of a unit (for example, 1 kg and 25 kg). Leave a blank space between the numerical value and the symbol (for example, 35 g, but not 35g), except for a percentage or °C, such as: between 14 and 20°C or growth up to 3, 6, and 9 m.

The style adopted by the *Revista Colombiana de Ciencias Hortícolas* for some units of measurements refers to the table of abbreviations and symbols of ASHS Publications Style Manual, http://www.ashs.org/downloads/ style_manual.pdf, accessed June 2011 or can be consulted in Revista Colombiana de Ciencias Hortícolas 4(1), 2010, pp. 181-184.

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