AGRONOMIA COLOMBIANA

Doi: 10.15446/agron.colomb

VOLUME XXXVIII, No. 3 SEPTEMBER-DECEMBER 2020 ISSN 0120-9965

Tarifa Postal Reducida No. 2015-404 4-72 La Red Postal de Colombia, vence 31 de Dic. 2020

Centro Editorial
Facultad de Ciencias Agrarias
Sede Bogotá

UNIVERSIDAD
NACIONAL
DE COLOMBIA

AGRONOMIA COLOMBIANA

VOLUME XXXVIII | No. 3 | SEPTEMBER-DECEMBER 2020 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

PUBLICATION OF A SCIENTIFIC-TECHNICAL NATURE BY THE FACULTY OF AGRICULTURAL SCIENCES OF THE UNIVERSIDAD NACIONAL DE COLOMBIA, BOGOTA

Publication registered at the Ministerio de Gobierno Resolution No. 00862 of March 24, 1983

Information, correspondence, subscription and exchange:

Revista Agronomía Colombiana
Faculty of Agricultural Sciences,
Universidad Nacional de Colombia

P.O. Box 14490, Bogota-Colombia

Phone: (571) 316 5355 / 316 5000 ext. 10265

Fax: 316 5176

E-mail: agrocol_fabog@unal.edu.co

Electronic version available at:

http://www.scielo.org.co

http://www.revistas.unal.edu.co/index.php/agrocol

http://agronomia.unal.edu.co

ISSN: 0120-9965 (Print)

ISSN: 2357-3732 (Online)

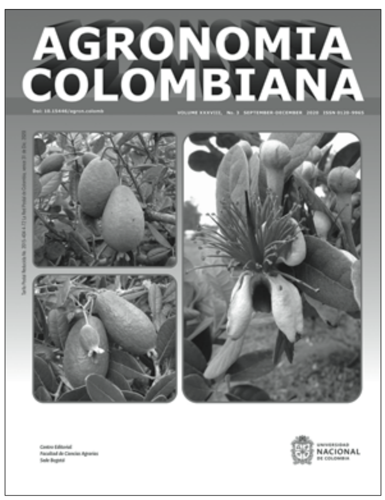
Published: Triannual
Number of copies: 20

Editorial assistance
César Albarracín
Juan Carlos Anduckia Ávila
Ana María Díaz
Luz Grass Bernal
Stanislav Magnitskiy

Translator and English proofreader
Thomas Defler
César Cabra

Electronic Design and Development
Isabel Sandoval

Printer
DGP Editores



Our cover:

Influence of some environmental factors on the feijoa (Acca sellowiana [Berg] Burret): A review. Article on pages: 388-397.

Agronomía Colombiana is a technical-scientific publication classified by Colciencias in category C of the Índice Nacional de Publicaciones Seriadas y Científicas y Tecnológicas (Publindex) (Colombia). The journal is indexed in the Scientific Electronic Library Online (SciELO) and Scopus. Internationally, the journal is referenced in Redalyc, Latindex, AGRIS (FAO), ResearchGate, Family Farming Knowledge Platform (***Plataforma de Conocimientos sobre Agricultura Familiar***), and integrated in CABI Full Text and the following databases of CAB-ABSTRACTS: Agricultural Engineering Abstracts, Agroforestry Abstracts, Crop Physiology Abstracts, Field Crop Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Irrigation and Drainage Abstracts, Maize Abstracts, Nematological Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Growth Regulator Abstracts, Postharvest News and Information, Potato Abstracts, Review of Agricultural Entomology, Review of Aromatic and Medicinal Plants, Review of Plant Pathology, Rice Abstracts, Seed Abstracts, Soils and Fertilizers, Sugar Industry Abstracts, Weed Abstracts y World Agricultural Economics and Rural Sociology Abstracts.

The authors of the articles submitted to the journal Agronomía Colombiana must be aware of and avoid scientific misconduct related to: scientific fraud in all or part of the data of the study and data falsification and manipulation; dishonesty due to fictitious authorship or gifting or exchange of co-authorship, duplicate publications, partial or complete, in different journals and self-plagiarism by reusing portions of previous writings; citation omission, citation copying without consultation and excessive self-citation, among others.

AGRONOMIA COLOMBIANA

VOLUME XXXVIII | No. 3 | SEPTEMBER-DECEMBER 2020 | ISSN (print): 0120-9965 / ISSN (online): 2357-3732

TABLE OF CONTENTS

SCIENTIFIC NOTE

APPENDIX / ANEXOS

After three years as Editor-in-Chief of *Agronomía Colombiana*, the time has come for me to step down and contribute to other areas of the Faculty of Agricultural Sciences where my work is needed. It has been my privilege but also a great responsibility to serve as editor of a scientific journal, even more now, when performance appraisals are focused on quantitative outputs in all spheres of life. Additionally, taking the reins of the journal, after the direction of Professor Gerhard Fischer, was a double challenge since *Agronomía Colombiana* adopted the format of a modern journal and set a new benchmark for agricultural research in the country thanks to his vision. The challenges were overcome, and the journal continued its journey. It is time now for me to pass the baton to Professor Stanislav Magnitskiy who, I am sure, will take the journal in new, exciting directions. As I leave, I feel grateful for the opportunity to learn and understand the breadth and depth of a publication like *Agronomía Colombiana* in a context such as that of tropical agriculture. My best wishes to the new editorial team, I say goodbye in hopes that my contribution to the journal may help to greater recognition of its role in the dissemination of scientific knowledge related to agriculture.

In this farewell editorial, I would also like to pay tribute to Professor Alejandro Chaparro. I had the chance of editing some of his papers during my term at Agronomía Colombiana. Professor Chaparro was a prolific author and his contributions to our Journal include more than 10 published manuscripts. For this reason, this issue of *Agronomía Colombiana*, my last one as Editor-in-Chief, is dedicated to his memory. Alejandro was not only an author but also a friend, and sometimes a challenger in some areas where our technical and scientific views overlapped. His critical

Después de tres años como Editor en Jefe de la *Revista Agronomía Colombiana*, ha llegado el momento de hacer un relevo y aportar en otros frentes de la Facultad de Ciencias Agrarias en los cuales se requiere mi trabajo. Ocupar el cargo de editor de una revista científica es un privilegio y a la vez una gran responsabilidad, más ahora que la evaluación del desempeño se da de manera tan cuantitativa en todos los órdenes. Adicionalmente, asumir las riendas de la revista después de la dirección del Profesor Gerhard Fischer era de partida un reto doble, pues de alguna manera *Agronomía Colombiana* adoptó el formato de una revista moderna y se convirtió en referente de la investigación agraria del país, gracias a la visión personal del profesor. Los retos se asumieron y la revista continuó su camino. Ahora damos paso para que el profesor Stanislav Magnitskiy recoja el testigo y conduzca la revista a nuevos y más emocionantes escenarios. Con agradecimiento, me llevo la sensación de haber aprendido el oficio, comprendiendo la dimensión de una publicación como *Agronomía Colombiana* en un contexto como el de la agricultura tropical. Los mejores deseos al nuevo equipo editorial, me despido con la esperanza de que mi aporte a la revista pueda contribuir a un mayor reconocimiento de su labor en la divulgación del conocimiento científico alrededor de la agricultura.

En esta nota de despedida, también quisiera hacer un breve homenaje al profesor Alejandro Chaparro, a quien edité varios artículos en mi paso por Agronomía Colombiana. El profesor Chaparro fue un prolífico autor y sus contribuciones a nuestra revista incluyen más de 10 manuscritos publicados. Por esta razón, este número de *Agronomía Colombiana*, el último que edito, está dedicado a su memoria. Alejandro no sólo fue autor, vision encouraged me to work harder to support my ideas. Thank you, Alejandro, for all the lessons learned, may you rest in peace.

también fue amigo y varias veces contradictor en algunos ámbitos donde nuestras visiones técnicas y científicas se cruzaron. Su visión crítica me llevó a esforzarme más en la sustentación de mis ideas. Gracias Alejandro por todas las enseñanzas, paz en tu tumba.

MAURICIO PARRA QUIJANO
Editor en jefe
Revista Agronomía Colombiana

Gene action and heritability in bi-parental crosses of sunflower

Acción genética y heredabilidad en cruces biparentales de girasol

Mohamed Ali Abdelsatar[1]* and Tamer Hassan Ali Hassan[1]

ABSTRACT

A field experiment was conducted in the contrasting locations of Kafr-El-Hamam/Sharkia and Al-Arish/North Sinai agricultural research stations of the Agricultural Research Center in Egypt. We estimated the additive and dominance genetic variances as well as heritability in the broad and narrow senses for sunflower yield and its attributes in the cross L350 × L355 using North Carolina Design-III. The magnitude of the additive variances for all studied traits at both locations in proportion to the phenotypic variances was larger than the dominance variances. The average degree of dominance was greater than the unit, as also confirmed by the high narrow-sense heritability for most of the studied traits. The genetic improvement may be achieved through the selection of genotypes with larger head diameter and heavier seed weight which also showed moderate to high values of narrow-sense heritability. Thirty-two sunflower backcrosses were grouped into eight distinct clusters/groups by canonical analysis.

Key words: North Carolina Design-III, average degree of dominance, selection criteria, cluster.

RESUMEN

Se realizó un experimento de campo en lugares contrastantes de las estaciones de investigación agrícola Kafr-El-Hamam/Sharkia y Al-Arish/Norte del Sinaí en el Centro de Investigación Agrícola en Egipto. Se estimaron la variación genética aditiva y dominante, así como la heredabilidad en sentido amplio y restringido para el rendimiento y atributos de girasol en el cruce L350 × L355 usando North Carolina Design-III. La magnitud de las variaciones aditivas para todos los rasgos estudiados en ambos lugares en proporción a las variaciones fenotípicas fue mayor que las dominantes, y el grado promedio de dominancia fue mayor que la unidad, como también lo confirma la alta heredabilidad de sentido estrecho para la mayoría de los rasgos estudiados. La mejora genética se puede lograr mediante la selección de genotipos con mayor diámetro de capítulo y mayor peso de semilla que también mostraron valores, moderados a altos, con rasgos de heredabilidad de sentido estrecho. Treinta y dos retro cruzamientos de girasol se agruparon en ocho clústeres/grupos distintos mediante análisis canónico.

Palabras clave: diseño de Carolina del Norte-III, grado promedio de dominancia, criterios de selección, agrupamiento.

Introduction

The genetic improvement of sunflower yield and its quality mainly relies on considerable genetic variability in the population. Various breeding procedures such as North Carolina design III (NCD III) can be used for creating new genetic variability, which is obtained by selecting plants in the F_2 generation with crossing to both inbred parents. NCD III also provides more information on the nature of gene action governing either additive or dominance inheritance of seed yield and its component traits in populations. However, it assumes the absence of epistasis as mentioned by Acquaah (2012). Hence, simple mating designs improve seed yield and its components.

The additive gene action was more important than the non-additive one in contributing to the variability of the head diameter (Gvozdenović *et al.* 2005), 1000-seed weight and seed yield (Ortis *et al.* 2005), plant height, seed number/head, 1000-seed weight, and seed yield (Karasu *et al.*, 2010). Abd EL-Satar (2017) and Abdelsatar *et al.* (2020) also confirmed a predominant role of the additive gene action in the control of the days to 50% flowering, days to physiological maturity, plant height, head diameter, number of green leaves/plant, and seed oil content. However, the seed weight/plant took an opposite trend. Thus, selection for improving these traits would be effective in early generations. Nevertheless, the importance and predominance of non-additive or dominance effects was detected for plant height (Gvozdenović *et al.,* 2005; Shankar *et al.,* 2007), days to 50% flowering (Shankar *et al.,* 2007), and seed yield (Parameshwarappa *et al.,* 2008; Karasu *et al.,* 2010; Chandra *et al*., 2011). Similarly, Azad *et al.* (2016) concluded the predominance of the non-additive gene action

Received for publication: 12 February, 2020. Accepted for publication: 21 July, 2020.

Doi: 10.15446/agron.colomb.v38n3.84472

[1] Oil Crops Research Department, Field Crops Research Institute, Agricultural Research Center, Giza (Egypt).
[*] Corresponding author: mohamedtemraz1@yahoo.com

in inheritance of all the studied traits except for days to maturity. Additionally, many of the yield-attributing traits in sunflower are under the influence of non-additive gene action as reported by Shinde *et al.* (2016), Abd EL-Satar *et al.* (2017), Lakshman *et al.* (2019) and Ahmed *et al.* (2019). Consequently, according to the previous authors, the improvement of these traits can be performed by using hybrid varieties. Seed weight/plant is considered a complex trait; hence, attention must be paid to correlation and path analyses at both phenotypic and genotypic levels for improving seed weight/plant. Vidhyavathi *et al.* (2005) concluded that plant height and head diameter were considered the best selection criteria for improving seed weight/plant. Similarly, 100-seed weight and head diameter had the highest direct and indirect influence on seed weight/plant as Abd EL-Satar *et al.* (2017) mentioned.

Cluster analysis by D^2 statistics is a powerful tool in measuring the divergence of tested genotypes on yield and its attributes. Thirumala *et al.* (2005) grouped 94 sunflower genotypes into 10 clusters based on D^2 values. Moreover, Ram *et al.* (2018) classified the thirty-two genotypes of sunflower into six clusters using the Tocher method.

Given the previous points, this research aimed to estimate additive and dominance genetic variance, and heritability in broad and narrow senses for sunflower yield and its attributes. Additionally, the selection criteria for improving seed weight/plant were determined using correlation and path analyses at both phenotypic and genotypic levels, and the bi-parental crosses were classified using cluster analysis.

Materials and methods

Plant material

The F_1 cross (i.e., L_{350} x L_{355}) was selected based on its yield performance in F_1 evaluation, with the highest heterotic effects over mid- and better parents for further breeding (Abd EL-Satar *et al.,* 2015); this population was used for generating bi-parental progenies.

F_1 seeds of the tested cross (L_{350} x L_{355}) were sown on March 5, 2018 at the Al-Arish agricultural research station of the Agricultural Research Center, in Egypt, and F_1 plants were selfed at the flowering stage to produce the F_2 seed generation. F_2 seeds were sown on June 5, 2018 along with their two parents at the Kafr-El-Hamam agricultural research station of the Agricultural Research Center, in Egypt. Sixteen plants were randomly selected from the F_2 population and grouped in four sets. Each one of these plants was crossed as the male parent with each of the two parents as a female parent to produce 16 crosses of F_2 x P_1 and 16 of F_2 x P_2 as in the North Carolina Design III.

Field evaluation trials

The 32 bi-parental crosses, and the two parents, were hand-planted on adjacent plots without separators on June 5, 2019 by using a randomized complete block design with three replicates in the four sets at the two locations of Kafr-El-Hamam and Al-Arish agricultural research stations of the Agricultural Research Center in Egypt.

Soil samples of each location were analyzed to determine their compositions and chemical properties following Jackson (1973), as shown in Table 1. Wheat was planted before in the winter season of 2018/2019 in both locations.

Each replicate was grown in a plot consisting of 3 rows. Each row was 4 m long and 60 cm apart, with plants spaced at 30 cm within rows. The cultural practices were followed as recommended for sunflower production in the region.

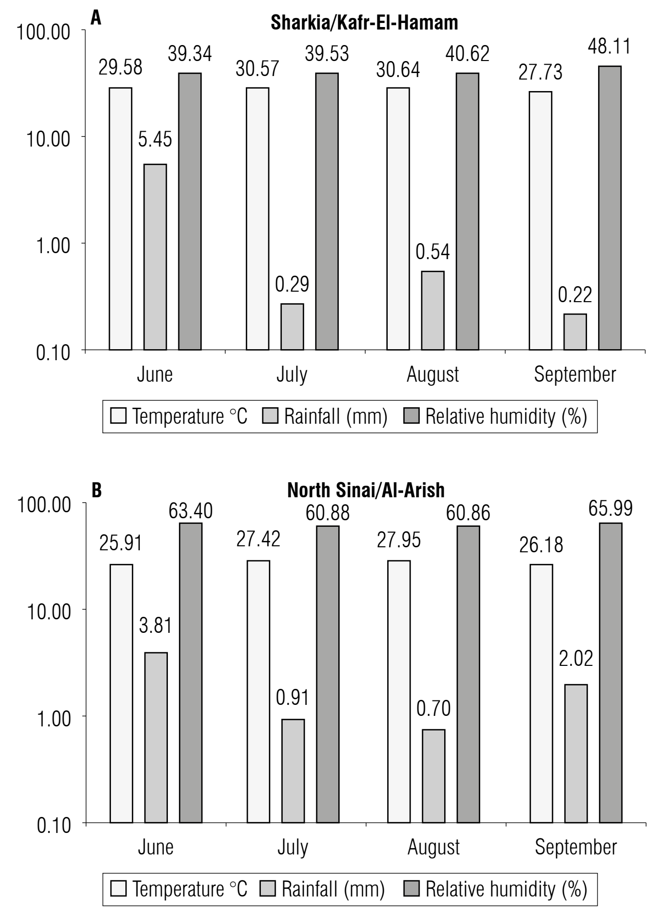


FIGURE 1. Monthly temperature, rainfall, and relative humidity during the period of sunflower growth in the 2019 season at both locations.

TABLE 1. Latitude and longitude of the two experimental field stations and soil composition and chemical properties of the upper 30 cm of the experimental soil.

Property	Sharkia/ Kafr-El-Hamam	North Sinai/ Al-Arish
Latitude	30°61'N	31°13'N
Longitude	31°50'E	33°80'E
Soil composition		
Sand (%)	17.42	72.35
Silt (%)	35.59	25.22
Clay (%)	46.99	2.43
Soil texture	Clay loam	Sandy loam
Chemical analysis		
Concentration of N (mg kg^{-1})	152	14.42
Concentration of P (mg kg^{-1})	9.52	4.75
Concentration of K (mg kg^{-1})	456	31.42
Electrical conductivity (ds/m)	0.62	4.76
pH	7.62	7.95

Data collection

At harvest, ten competitive plants were randomly selected from the first and third rows in each plot to measure plant height (cm), head diameter (cm), 100-seed weight (g), and seed weight/plant (g). The seed weight/plant was adjusted to a 15.5% seed moisture, whereas the days to physiological maturity were determined on a plot basis. Seed yield/m^2 was recorded from the plants located in the second row as the middle one, which was 4 m long for a total of 2.4 m^2 in each experimental plot. These values were then converted to yield ha^{-1}. Seed oil content was determined after drying seeds at 70°C for 48 h, by the Soxhlet extraction technique, using diethyl ether (AOAC, 1990). Gas-liquid chromatography (Aglent 6890 GC, USA) was used for the determination and identification of fatty acids methyl esters such as oleic acid and linoleic acid following Zygadlo *et al.* (1994) methodology in the Central Laboratory of the Food Technology Research Institute, ARC, Egypt.

Statistical analysis

The analysis of North Carolina design III was performed as outlined by Comstock and Robinson (1948) to estimate different genetic components. The phenotypic and genotypic correlation coefficient (Weber and Moorthy, 1952) and phenotypic and genotypic path analysis (Dewey and Lu, 1959) were determined. Additionally, the grouping and arrangement of genotypes by canonical analysis (Rao, 1952) based on D-square statistics (D^2) developed by Mahalanobis (1936) were carried out.

Results and discussion

Analysis of variance (ANOVA)

Mean squares of North Carolina design III due to females/sets and males/set (Tabs. 2-3) were highly significant for all the studied traits at both locations and their combined analysis. These results indicate that there is great diversity among families, and hence, it would be effective for improving these traits. Females x males/sets interaction mean squares at both locations and their combined analysis were highly significant for all the studied traits as shown in Tables 2 and 3, suggesting that some divergent dominance could play a role across the specific crosses and across the sets. The presence of highly significant effects of the locations on all the studied traits (Tab. 3) showed that environmental factors influenced the phenotypic expression for all the studied traits. Table 3 shows that the mean squares due to the interaction between location and females, males, and females by males were highly significant for all the studied traits. However, the interaction between the location and males for plant height, head diameter, seed weight/plant, seed yield ha^{-1} and oleic acid did not reach a significant level. The significance of these traits due to the interaction the location with previous populations indicates that the location has sufficient environmental variability to lead to fluctuations in the ranking of these population components. Similar results were reported by Abd EL-Satar *et al.* (2015), Abd EL-Satar (2017) and Abdelsatar *et al.* (2020).

Furthermore, female variances (Tab. 2) contributed to the largest relative proportion of variances compared to males and female x male for all the studied traits at the two locations. These results indicate that these differences among females led to obtain great genetic diversity among their crosses with males, so they can be considered as desirable/preferred in future sunflower breeding programs.

Mean performance

The mean performance of all the studied traits for the male x female interaction of backcrosses are shown in Supplementary material 1-3. The results revealed significant differences among the bi-parental crosses for all the studied traits at both locations.

In general, set 2 of backcrosses with P1 obtained the shortest period to physiological maturity (85.00 d and 77.58 d), and the shortest (dwarf) parents (158.65 cm and 152.37 cm) at Kafr-El-Hamam and Al-Arish, respectively. However, the first order of sets was the Set 3 of backcrosses with P2 for head diameter (25.62 cm and 18.29 cm), seed weight/plant (35.46 g and 28.68 g), seed yield ha^{-1} (2458.88 kg $ha^{-1}$and

TABLE 2. Mean squares of North Carolina design III for all the studied traits in the bi-parental sunflower crosses at Kafr-El-Hamam (K) and Al-Arish (A) in the 2019 season.

SOV	Df	K	A	K	A	K	A
		Days to maturity		Plant height		Head diameter	
Sets	3	433.13**	204.84**	565.53**	325.12**	124.97**	50.97**
Replications	8	5.16	2.38	15.81	11.85	1.11	0.85
Female in sets	4	282.23**	140.51**	765.26**	385.42**	52.09**	20.06**
Male in sets	12	153.94**	68.34**	280.75**	141.15**	41.4**	16.57**
F x M in sets	12	49.81**	22.37**	120.15**	78.71**	31.4**	10.77**
Error	56	3	1.2	7.54	5.85	0.97	0.63
SOV	**Df**	**100-seed weight**		**Seed weight/plant**		**Seed yield ha^{-1}**	
Sets	3	1.09**	2.53**	39.05**	55.03**	253115.07**	233842.96**
Replications	8	0.02	0.04	1.93	0.86	1672.04	3267.29
Female in sets	4	3.91**	1.29**	61.58**	86.5**	266613.26**	197240.36**
Male in sets	12	1.94**	2.15**	114.2**	84.62**	159450.80**	120517.96**
F x M in sets	12	1.59**	0.49**	55**	52.2**	105253.29**	85103.32**
Error	56	0.04	0.03	0.94	2.08	2982.03	2862.77
SOV	**Df**	**Seed oil content**		**Oleic acid**		**Linoleic acid**	
Sets	3	44.42**	12.18**	7.3**	19.7**	252.36**	154.71**
Replications	8	0.13	0.33	0.15	0.14	1.2	0.82
Female in sets	4	22.46**	11.81**	4.6**	12.09**	90.66**	70.1**
Male in sets	12	28.56**	10.41**	3.38**	8.82**	86.27**	42.02**
F x M in sets	12	13.12**	7.51**	2.02**	6.11**	54.76**	36.2**
Error	56	0.36	0.39	0.1	0.31	0.97	1.11

SOV: Source of variance; Df: Degree of freedom.

*, ** Significant at 0.05 and 0.01 probability level, respectively.

TABLE 3. Mean squares of North Carolina design III for all studied traits in the bi-parental sunflower crosses across two locations in the 2019 season.

SOV	Df	DM	PH	HD	100 SW	SWP	SYH	OC	OA	LA
Replications	16	0.09	0.64	0.44	0.02	0.44	614.6	0.08	0.05	0.1
Location(L)	1	4417.92**	4329.06**	1753.59**	269.21**	2362.51**	10867013.7**	573.01**	105.01**	435.73**
Set (S)	3	616.8**	868.88**	167.76**	2.81**	90.59**	482914.4**	51.24**	25.4**	399.51**
L x S	3	527.65**	583.42**	190.12**	23.34**	220.4**	1027324.0**	61.9**	15.5**	138.08**
Crosses (C)	31	161.13**	368.71**	45.09**	2.3**	132.64**	232122.8**	24.89**	9.31**	101.88**
Female (F)	4	410.3**	1104.44**	67.91**	3.36**	140.18**	446944.7**	32.25**	15.68**	158.56**
Male (M)	12	165.63**	265.09**	17.47**	2.55**	86.2**	97518.1**	13.47**	3.44**	67.98**
F x M	12	113.87**	319.26**	76.38**	2.27**	209.72**	353150.8**	40.07**	15.38**	142.36**
L x C	31	9.45**	22.17**	3.77**	0.83**	5.27**	10182.0**	3.12**	0.87**	4.47**
L x F	4	28.32**	62.57**	10.37**	2.45**	10.51**	19941.6**	6.05**	2.21**	7.88**
L x M	12	8.36*	7.64	0.9	0.27**	3.58	4681.8	1.8**	0.2	3.29*
L x M x F	12	6.61**	28.78**	5.38**	1.07**	6.53**	14974.6**	4.26**	1.31**	5.62**
Error	112	2.62	8.58	0.88	0.04	1.65	3187.4	0.4	0.22	1.18

SOV: Source of variance; Df: Degree of freedom

DM: Days to maturity, PH: Plant height, HD: Head diameter, 100 SW: 100-seed weight, SWP: Seed weight/plant, SYH: Seed yield ha^{-1}, OC: Seed oil content, OA: Oleic acid and LA: Linoleic acid.

*, ** Significant at 0.05 and 0.01 probability level, respectively.

1944.79 kg ha^{-1}), seed oil content (41.76% and 44.08%), oleic acid content (29.42% and 31.62%) and linoleic acid content (52.57% and 54.58%) at Kafr-El-Hamam and Al-Arish, respectively. Moreover, the heaviest weight of 100 seeds was detected in the Set 2 of backcrosses with P1 (7.52 g) at Kafr-El-Hamam and in the Set 3 of backcrosses with P2 (5.25 g) at Al-Arish.

Overall, backcrosses with the second parent were slightly inferior (desirable) for earliness in days to maturity (0.91% and 0.74%) than overall backcrosses with P1, which were slightly inferior (desirable) for plant height (0.14% and 0.01%) at Kafr-El-Hamam and Al-Arish, respectively. However, the overall backcrosses with the second parent were slightly superior (desirable) for head diameter (0.88% and 0.31%), seed weight/plant (4.48% and 0.94%), seed yield ha^{-1} (0.83% and 2.09%), seed oil content (0.19% and 0.98%), oleic acid content (0.44% and 1.30%) and linoleic acid content (3.45% and 3.75%) than overall backcrosses with the first parent at Kafr-El-Hamam and Al-Arish, respectively.

Generally, the backcrosses had the highest mean values of all the studied traits at Kafr-El-Hamam, except for quality traits such as seed oil content and oleic and linoleic acids which exhibited the highest proportion at Al-Arish. Based on their mean performance, the backcrosses M4 x P2/Set 3 and M1 x P1/Set 4 performed well for most studied traits and consequently, seed weight/plant at both locations. This is maybe due to the aggregation of favorable genes from different traits in these superior backcrosses. The genetic variability among bi-parental sunflower crosses is consistent with those reported by Shinde *et al.* (2016), Abd EL-Satar *et al.* (2017) and Lakshman *et al.* (2019).

Gene mode of action

The estimation of additive (σ^2A) and dominance variances (σ^2D) along with heritability (h^2_{NS}) and average degree of dominance (ā) at both contrasting locations and the combined analysis are shown in Table 4. A significant variance of all the studied traits was a good indicator for computing both additive and dominance components. Hence, either hybridization breeding or selection in segregating generations is recommended for bi-parental crosses improvement. The magnitude of the additive variances for all the studied traits at both locations in proportion to the phenotypic variances was larger than dominant ones and the average degree of dominance was greater than the unit. Hence, the selection of these traits in early segregating generations to accumulate additive genes would be successful for producing superior inbred lines to be used in hybrid breeding programs (Tab. 4). Similar results were found by Karasu *et al.* (2010), Abd EL-Satar (2017) and Abdelsatar *et al.* (2020). The relative proportion variance of additive to phenotypic variance was larger than the dominance variance for days to maturity, plant height, and 100-seed

TABLE 4. Estimation of genetic parameters, additive and dominance variance, and degree of dominance in an F_2 population of sunflower backcrosses at the contrasting locations of Kafr-El-Hamam (K) and Al-Arish (A) in the 2019 season.

Parameters	K	A	K	A	K	A
	Days to maturity		Plant height		Head diameter	
δ^2_A	100.62	44.76	182.14	90.20	26.96	10.63
δ^2_D	31.21	14.12	75.07	48.58	20.29	6.76
$h^2_{(b)}$	97.77	98.01	97.15	95.96	97.99	96.51
$h^2_{(n)}$	74.63	74.51	68.80	62.37	55.91	58.98
(ā)	0.79	0.79	0.91	1.04	1.23	1.13
Parameters	100-seed weight		Seed weight/plant		Seed yield ha^{-1}	
δ^2_A	1.26	1.41	75.51	55.02	104312.5	78436.79
δ^2_D	1.03	0.31	36.04	33.41	68180.84	54827.04
$h^2_{(b)}$	98.12	98.20	99.16	97.70	98.30	97.90
$h^2_{(n)}$	54.05	80.61	67.12	60.79	59.45	57.62
(ā)	1.28	0.66	0.98	1.10	1.14	1.18
Parameters	Seed oil content		Oleic acid		Linoleic acid	
δ^2_A	18.80	6.68	2.18	5.67	56.86	27.27
δ^2_D	8.50	4.75	1.28	3.86	35.86	23.39
$h^2_{(b)}$	98.69	96.66	97.16	96.81	98.96	97.85
$h^2_{(n)}$	67.95	56.50	61.32	57.57	60.69	52.67
(ā)	0.95	1.19	1.08	1.17	1.12	1.31

δ^2_A: Additive variance; δ^2_D: Dominance variance; $h^2_{(b)}$: Broad-sense heritability; $h^2_{(n)}$: Narrow-sense heritability and (ā): Average degree of dominance.

weight as shown in the combined analysis (Tab. 5). On the other hand, the ratio of dominance to phenotypic variance was more significant than the additive variance, and the average degree of dominance was greater than the unit. These results were obtained for the head diameter, seed weight/plant, seed yield ha^{-1} and seed oil content as well as for oleic and linoleic acids as shown in the combined analysis (Tab. 5). This effect could be related to genetic interactions (Comstock and Robinson, 1948) and confirm the greatest role of dominance variance in the inheritance of these traits. However, selection in the later generations would be more effective than early selection for these traits. These findings were agreement with Lakshman *et al.* (2019) and Ahmed *et al.* (2019).

Estimates of broad sense heritability at both locations and their combined analysis were more than 95% for all the studied traits, indicating higher importance of the genetic effects of these traits. Moreover, moderate to high estimates of narrow-sense heritability were detected in all the studied traits at both locations, indicating that the effect of additive genetic variance was pronounced compared to dominance genetic variance (Tab. 4). These findings were agreement with Abd EL-Satar (2017) and Abdelsatar *et al.* (2020). Selection for all traits should be highly effective in this population. Regarding the combined analysis, days to maturity, plant height, and 100-seed weight are the highest heritable traits (Tab. 5). Therefore, selection for these traits should be highly effective in improving this population at early segregating generations. On the other hand, the estimates of narrow-sense heritability were low to moderate for head diameter, seed weight/plant, seed yield ha^{-1} and seed oil content as well as for oleic and linoleic acids. The selection of these traits in early segregating generations is not efficient but it would be effective in later generations. These findings were agreement with Abd EL-Satar (2017) and Abdelsatar *et al.* (2020) who reported a predominant role of additive gene action in the control of days to 50% flowering, days to physiological maturity, plant height, head diameter, number of green leaves/plant, and seed oil content. However, seed weight/plant showed an opposite trend.

Association of traits and path analysis as powerful selection criteria

Phenotypic and genotypic correlation

Phenotypic and genotypic correlations were estimated between seed weight/plant and its attributes for 32 sunflower genotypes based on the average of two locations (Tab. 6). Seed weight/plant was positively and significantly or highly significantly associated with days to maturity, plant height, head diameter, and 100-seed weight at phenotypic and genotypic levels. These results indicate that the largest diameter of head with moderate value of narrow-sense heritability and the heaviest weight of 100-seed with a high value of narrow-sense heritability across locations can be considered of the main components to improve seed weight/plant; hence, selection will be more effective for these traits. The same results were obtained by Abd EL-Satar *et al.* (2017) who found that head diameter and 100-seed weight had the highest direct and indirect influence on seed weight/plant.

TABLE 5. Estimation of genetic parameters, additive and dominance variance, and degree of dominance in an F_2 population of sunflower backcrosses across the locations of Kafr-El-Hamam and Al-Arish in the 2019 season.

Parameters	DM	PH	HD	100 SW	SWP	SYH	OC	OA	LA
δ^2_A	52.42	85.82	5.52	0.76	27.54	30945.45	3.89	1.08	21.56
δ^2_D	20.45	51.47	12.59	0.45	35.32	58825.37	7.08	2.52	24.24
$h^2_{(b)}$	99.40	98.97	99.20	99.46	99.56	99.41	99.40	98.99	99.57
$h^2_{(n)}$	71.51	61.87	30.25	62.54	43.62	34.27	35.26	29.73	46.88
(ā)	0.88	1.10	2.14	1.09	1.60	1.95	1.91	2.16	1.50

DM: Days to maturity, PH: Plant height, HD: Head diameter, 100 SW: 100-seed weight, SWP: Seed weight/plant[1], SYH: Seed yield ha^{-1}, OC: Seed oil content, OA: Oleic acid and LA: Linoleic acid. δ^2_A: Additive variance, δ^2_D: Dominance variance; $h^2_{(b)}$: Broad-sense heritability; $h^2_{(n)}$: Narrow-sense heritability and (ā): Average degree of dominance.

TABLE 6. Pooled phenotypic (above diagonal) and genotypic (below diagonal) correlations of 32 sunflower bi-parental crosses across two locations.

Traits	Days to maturity	Plant height	Head diameter	100-seed weight	Seed weight/plant
Days to maturity	1.000	0.874**	0.678**	0.466**	0.556**
Plant height	0.899**	1.000	0.575**	0.339	0.427*
Head diameter	0.695**	0.583**	1.000	0.368*	0.769**
100-seed weight	0.471**	0.342	0.365*	1.000	0.528**
Seed weight/plant	0.564**	0.430*	0.786**	0.529**	1.000

*, ** Significant at 0.05 and 0.01 probability level, respectively.

Phenotypic and genotypic path analysis

Path analysis is an important tool to partition the correlation coefficients into direct and indirect effects of attribute traits on seed weight/plant.

Genotypic correlations recorded higher values than phenotypic correlations regarding the direct and indirect effects of seed weight attributes on seed weight, suggesting the negligible role of the environment on the genotypic expression (Tab. 7 and Fig. 2). Maximum positive direct effects at both, phenotypic and genotypic levels, were observed for head diameter (*P*=0.695, *G*=0.732) with a moderate value of narrow-sense heritability, followed by 100-seed weight (*P*=0.288, *G*=0.291) with high value of narrow-sense heritability. Therefore, a preferred improvement may be achieved by selecting genotypes with a larger head diameter and heavier seed weight. On the other hand, a negative direct effect on seed weight/plant at phenotypic and genotypic levels was exerted by plant height (*P*=-0.112, *G*= -0.114).

Furthermore, the highest indirect effects at phenotypic and genotypic levels on seed weight/plant were detected in days to maturity (*P*=0.471, *G*=0.509) through head diameter followed by head diameter *via* plant height (*P*=0.400, *G*=0.426) and 100-seed weight (*P*=0.256, *G*=0.267). These results may be considered as identical to the previous results of correlation at both phenotypic and genotypic levels (Tab. 7 and Fig. 2). The above-mentioned results of direct and joint effects indicate that the direct selection of head diameter and 100-seed weight will be more effective in improving seed weight/plant. Days to maturity, head diameter, and 100-seed weight exhibited negative indirect effects at phenotypic and genotypic levels on seed weight/plant through plant height. These results agreed with those of Abd EL-Satar *et al.* (2017). The residual effect was recorded with 0.579 and 0.556 at phenotypic and genotypic levels, respectively. These results indicate that the independent traits that are included at the phenotypic and genotypic path analysis explained 42.15% and 44.42% of the total variation, respectively, in seed weight/plant. The highest residual effects of phenotypic and genotypic path analyses indicate that the presence of other traits that are

TABLE 7. Pooled phenotypic (*P*) and genotypic (*G*) path analysis of 32 sunflower backcrosses for seed weight/plant across two locations.

Traits		Days to maturity	Plant height	Head diameter	100-seed weight	Correlation with seed weight/plant
Days to maturity	P	0.048	-0.098	0.471	0.135	0.556**
	G	0.020	-0.102	0.509	0.137	0.564**
Plant height	P	0.042	-0.112	0.399	0.098	0.427*
	G	0.018	-0.114	0.426	0.100	0.430*
Head diameter	P	0.033	-0.065	0.695	0.106	0.769**
	G	0.014	-0.066	0.732	0.106	0.786**
100-seed weight	P	0.023	-0.038	0.256	0.288	0.529**
	G	0.010	-0.039	0.267	0.291	0.529**
Residual	P			0.578		
	G			0.556		

*, ** Significant at 0.05 and 0.01 probability level, respectively. Highlighted values indicate a direct effect and normal values indicate an indirect effect.

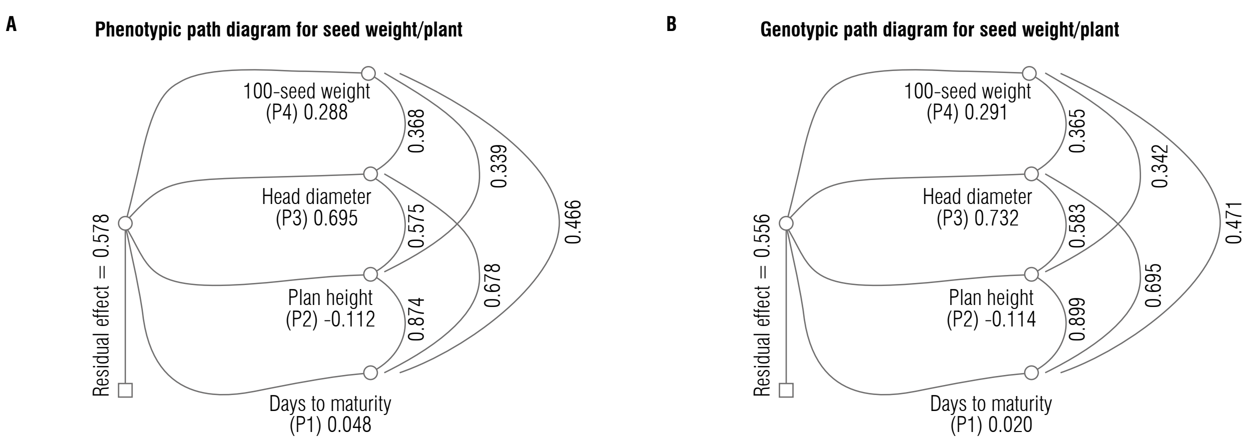


FIGURE 2. Pooled A) phenotypic and B) genotypic path diagram for seed weight/plant across the two locations.

not included in the present study is associated with the highest effect on seed weight/plant.

Genetic divergence

Thirty-two sunflower backcrosses were grouped into eight distinct clusters/groups by canonical analysis, as shown in Supplementary material 4 and Figure 3. Data indicated that the evaluated sunflower backcrosses were widely divergent, so they were scattered into eight clusters. The first cluster was the largest with 14 backcrosses, followed by the fourth cluster with six and the second with four backcrosses. The third and fifth clusters had two backcrosses and the seventh and eighth clusters had one backcross each.

Supplementary material 5 shows a comparison of cluster means on the average of both locations. The shortest period to physiological maturity (78.67 d) along with the lowest values of plant height (151.79 cm), head diameter (14.90 cm), seed yield ha^{-1} (1899.31 kg ha^{-1}), seed oil content (33.07%), oleic acid (26.42%) and linoleic acid (34.44%) were spotted in the third cluster, whereas the longest period to physiological maturity (101.0 d) and the highest values of plant height (181.84 cm), head diameter (27.73 cm), 100-seed weight (8.40 g), seed weight/plant (42.21 g), seed yield ha^{-1} (2810.79 kgha^{-1}), seed oil content (42.67%), oleic acid (30.56%) and linoleic acid (54.95%) were found in the seventh cluster.

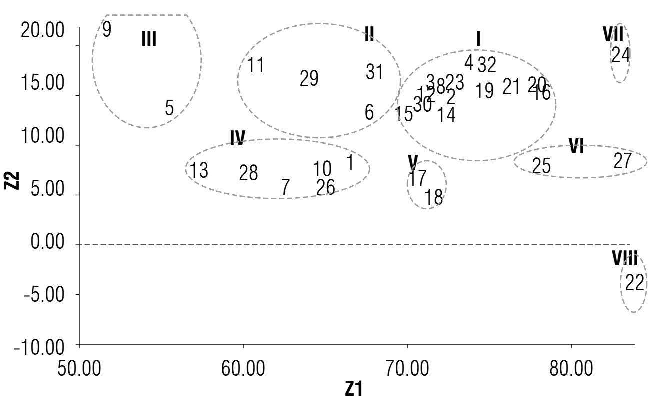


FIGURE 3. Scatter diagram of 32 sunflower backcrosses across two locations based on their canonical vectors superimposed with clustering. Code of figures as in Supplementary material 4.

Conclusion

Based on the results mentioned above, it can be concluded that selection of all the studied traits at both locations in early segregating generations to accumulate additive genes would produce superior inbred lines to be used in hybrid breeding programs. The genetic improvement may be achieved through the selection of genotypes with a larger head diameter and heavier seed weight. Moreover, the genetic variations were observed among backcrosses can be used in the future programs for sunflower improvement.

Literature cited

Abd EL-Satar, M.A. 2017. Genetic analysis of half diallel matting with different methods and their comparisons for yield and its associated traits in sunflower under saline soil stress conditions. Helia 40(66), 85-114. Doi: 10.1515/helia-2017-0001

Abd EL-Satar, M.A., A.A.E.H. Ahmed, and T.H.A. Hassan. 2017. Response of seed yield and fatty acid compositions for some sunflower genotypes to plant spacing and nitrogen fertilization. Inf. Process. Agric. 4(3), 241-252. Doi: 10.1016/j.inpa.2017.05.003

Abd EL-Satar, M.A., R.M. Fahmy, and T.H.A. Hassan. 2015. Genetic control of sunflower seed yield and its components under different edaphic and climate conditions. The 9th Plant Breeding International Conference, September 2015. Egypt. J. Plant Breed. 19(5), 103-123.

Abdelsatar, M.A., E.M.M. Elnenny, and T.H.A. Hassan. 2020. Inheritance of seed yield and yield-related traits in sunflower. J. Crop Improv. 34(3), 378-396. Doi: 10.1080/15427528.2020.1723767

Acquaah, G. 2012. Principles of plant genetics and breeding. 2nd ed. Wiley-Blackwell, Oxford, UK.

Ahmed, M.A., M.A. Abdelsatar, M.A. Attia, and A.A. Abeer. 2019. GGE biplot analysis of line by tester for seed yield and its attributes in sunflower. RUDN J. Agron. Anim. Ind. 14(4), 374-389. Doi: 10.22363/2312-797X-2019-14-4-374-389

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of official analytical chemists, Virginia, USA.

Azad, K., G. Shabbir, M.A. Khan, T. Mahmood, Z.H. Shah, F. Alghabari, and I. Daur. 2016. Combining ability analysis and gene action studies of different quantitative traits in sunflower by line x tester. Crop Res. 51(2).

Chandra, B.S., S. Kumar, A.R.G. Ranganadha, and M.Y. Dudhe. 2011. Combining ability studies for development of new hybrids over environments in sunflower (*Helianthus annuus* L.). J. Agric. Sci. 3(2), 230-237. Doi: 10.5539/jas.v3n2p230

Comstock, R.E. and H.F. Robinson. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating average degree of dominance. Biometrics 4(4), 254-266.

Dewey, D.R. and R.H. Lu. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agron. J. 51(9), 515-518. Doi: 10.2134/agronj1959.00021962005100090002x

Gvozdenović, S., J. Joksimović, and D. Škorić. 2005. Gene effect and combining abilities for plant height and head diameter in Sunflower. Genetica 37(1), 57-64. Doi: 10.2298/GENSR0501057G

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall of India Private Limited. New Delhi.

Karasu, A., M. Oz., M. Sincik, A.T. Goksoy, and Z.M. Turan. 2010. Combining ability and heterosis for yield and yield components in sunflower. Not. Bot. Hort. Agrobot. Cluj-Napoca 38(3), 260-264.

Lakshman, S.S., N.R. Chakrabarty, and P.C. Kole. 2019. Study on the combining ability and gene action in sunflower through line x tester matting design. Electron. J. Plant Breed. 10(2), 816-826. Doi:10.5958/0975-928X.2019.00109.1

Mahalanobis, P.C. 1936. On the generalized distance in statistics. Proc. Natl. Inst. Sci. India. 2, 49-55.

Ortis, L., G. Nestares, E. Frutos, and N. Machado. 2005. Combining ability analysis for agronomic traits in sunflower (*Helianthus annuus* L.). Helia 28(43), 125-134.

Parameshwarappa, K.G., J. Ram, and B.S. Lingaraju. 2008. Heterosis and combining ability for seed yield, oil content and other agronomic traits involving mutant restorer lines in sunflower (*Helianthus annuus* L.). J. Oilseeds Res. 25(1), 8-12.

Ram, J.J., U.K. Singh, S.K. Singh, and B. Krishna. 2018. Study of genetic diversity in Sunflower (*Helianthus annuus* L.). Int. J. Curr. Microbiol. App. Sci. 7(5), 2266-2272. Doi: 10.20546/ijcmas.2018.705.263

Rao, C.R. 1952. Advanced statistical methods in biometrical research. John Wiley and Sons, New York, USA

Shankar, V.G., M. Ganesh, A.R.G. Ranganatha, A. Suman, and V. Shridhar. 2007. Combining ability studies in diverse CMS sources in sunflower (*Helianthus annuus* L.). Ind. J. Agric. Res. 41(3), 171-176.

Shinde, S.R., R.B. Sapkale, and R.M. Pawar. 2016. Combining ability analysis for yield and its components in sunflower (*Helianthus annuus* L.). Int. J. Agric. Sci., 12(1), 51-55.

Thirumala, R.V., A.R.G. Ranganatha, M. Ganesh, K. Srinivasulu, and P.V. Rao. 2005. Assessment of genetic divergence in gene pool inbreds and elite lines of sunflower (*Helianthus annuus* L.) J. Oilseeds Res. 22(1), 168-171.

Vidhyavathi, R., P. Mahalakshmi, N. Manivannan, and V. Muralidharan. 2005. Correlation and path analysis in sunflower (*Helianthus annuus* L.). Agric. Sci. Digest 25(1), 6-10.

Weber, C.R. and B.R. Moorthy. 1952. Heritable and nonheritable relationship and variability of oil content and agronomic traits in the F_2 generations of soybean crosses. Agron. J. 44(4), 202-209. Doi: 10.2134/agronj1952.00021962004400040010x

Zygadlo, J.A., R.E. Morere, R.E. Abburra, and C.A. Guzman. 1994. Fatty acids composition in seed oils of some Onagraceae. J. Am. Oil Chem. Soc. 71(8), 915-916.

SUPPLEMENTARY MATERIAL 1. Mean performance of days to maturity, plant height and head diameter for bi-parental sunflower crosses at Kafr-El-Hamam (K) and Al-Arish (A) in the 2019 season.

Bi-parental crosses	Days to maturity (d)				Plant height (cm)				Head diameter (cm)			
	K		A		K		A		K		A	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
M1/Set 1	87.33	82.00	79.00	76.33	163.40	155.30	156.47	151.33	18.70	15.30	14.10	11.80
M2/Set 1	93.33	87.33	82.67	79.00	165.63	163.80	158.27	156.60	22.92	19.73	16.07	14.10
M3/Set 1	92.00	83.00	81.67	76.33	165.20	155.30	156.97	151.33	22.58	16.60	15.73	12.90
M4/Set 1	97.33	92.33	87.33	82.33	179.91	165.58	163.53	158.27	26.42	22.91	18.67	16.20
M1/Set 2	80.67	83.67	75.67	77.33	153.20	183.24	147.33	167.97	14.50	17.83	11.21	13.17
M2/Set 2	85.33	93.33	78.00	83.33	161.30	168.20	154.23	158.20	17.83	23.40	13.83	16.53
M3/Set 2	83.67	89.33	76.33	80.00	155.30	164.50	151.33	156.60	17.03	20.77	13.10	14.87
M4/Set 2	90.33	97.33	80.33	86.67	164.80	181.17	156.60	163.30	22.00	25.80	15.07	18.30
M1/Set 3	88.67	96.00	79.67	85.33	164.50	174.00	156.60	161.73	24.33	23.97	16.90	17.47
M2/Set 3	96.00	101.67	85.33	90.67	174.13	184.01	161.23	170.77	24.67	27.28	17.63	19.43
M3/Set 3	94.33	92.33	83.67	83.00	168.20	165.87	159.73	157.67	23.60	23.51	16.20	16.10
M4/Set 3	97.00	109.67	86.67	92.33	178.94	186.87	164.90	176.80	25.10	27.73	18.30	20.17
M1/Set 4	100.00	85.33	89.33	78.00	183.24	161.27	167.97	152.63	27.07	18.03	19.23	13.17
M2/Set 4	86.00	91.00	78.00	81.33	159.53	165.00	153.93	157.67	17.40	21.68	13.10	15.43
M3/Set 4	100.00	89.00	89.33	80.00	183.24	164.53	167.97	156.97	27.07	20.27	19.23	14.77
M4/Set 4	109.67	95.00	93.33	84.67	188.43	174.00	181.67	161.13	14.50	23.97	11.80	16.53
LSD 5%	2.83		1.79		4.49		3.96		1.61		1.30	

SUPPLEMENTARY MATERIAL 2. Mean performance of 100-seed weight, seed weight/plant and seed yield ha^{-1} for bi-parental sunflower crosses at Kafr-El-Hamam (K) and Al-Arish (A) in the 2019 season.

Bi-parental crosses	100-Seed weight				Seed weight/plant				Seed yield ha^{-1}			
	K		A		K		A		K		A	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
M1/Set 1	6.37	6.61	4.24	3.41	28.23	27.48	23.90	18.97	2067.38	1884.31	1603.10	1335.00
M2/Set 1	7.28	6.38	4.84	4.24	34.94	34.02	28.13	23.90	2131.04	2068.97	1775.71	1603.10
M3/Set 1	7.28	5.21	4.80	3.41	35.52	27.97	28.77	18.97	2131.04	1942.72	1732.46	1509.68
M4/Set 1	7.98	7.28	5.27	4.81	38.52	35.95	31.63	28.83	2561.11	2113.65	1992.62	1746.59
M1/Set 2	8.56	6.28	3.41	4.20	25.77	28.37	18.63	20.40	1914.32	2033.02	1335.00	1508.65
M2/Set 2	6.35	7.36	4.20	4.95	25.80	33.12	22.03	29.47	2033.81	2188.02	1521.51	1783.02
M3/Set 2	8.17	6.57	5.37	4.51	27.97	34.52	19.40	26.00	1981.91	2084.36	1501.43	1656.90
M4/Set 2	7.01	7.62	4.58	5.29	35.63	38.49	27.00	31.43	2113.81	2425.11	1709.60	1901.75
M1/Set 3	6.65	7.55	4.51	5.15	27.73	37.48	25.10	30.03	2083.49	2255.48	1656.90	1830.16
M2/Set 3	6.50	6.05	4.42	4.78	28.56	25.62	23.80	20.47	2076.35	2610.40	1639.60	2056.11
M3/Set 3	7.32	7.33	4.84	4.83	37.01	36.51	28.87	28.83	2176.20	2158.81	1799.92	1766.51
M4/Set 3	7.63	8.40	6.26	6.25	38.45	42.21	30.73	35.40	2397.38	2810.79	1874.52	2126.35
M1/Set 4	5.57	7.53	3.97	3.92	39.37	30.43	32.27	21.20	2583.10	2041.83	1992.62	1525.56
M2/Set 4	6.05	6.90	3.95	4.73	24.81	34.95	21.47	27.83	2007.82	2093.44	1535.56	1725.00
M3/Set 4	6.19	7.45	5.30	4.57	39.37	34.52	32.27	25.50	2583.10	2092.57	1992.62	1693.41
M4/Set 4	7.37	8.13	5.22	4.97	27.10	37.28	18.63	29.33	1919.68	2247.94	1341.51	1814.52
LSD 5%	0.34		0.29		1.59		2.36		89.32		87.51	

LSD 5%: Least significant difference at α=0.05.

SUPPLEMENTARY MATERIAL 3. Mean performance of seed oil content, oleic acid content and linoleic acid content for bi-parental sunflower crosses at Kafr-El-Hamam (K) and Al-Arish (A) in the 2019 season.

Bi-parental crosses	Seed oil content				Oleic acid content				Linoleic acid content			
	K		A		K		A		K		A	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
M1/Set 1	37.84	33.43	42.51	39.17	27.72	26.55	29.35	27.18	44.13	34.42	47.29	42.93
M2/Set 1	40.70	38.05	43.15	42.51	28.45	27.82	30.30	29.35	47.67	44.61	50.86	47.63
M3/Set 1	39.82	36.21	42.78	41.31	28.39	26.55	30.01	27.18	47.11	38.80	48.96	42.93
M4/Set 1	41.82	40.16	44.21	42.97	29.34	28.45	31.14	30.23	43.89	47.44	46.89	49.35
M1/Set 2	32.71	33.35	36.58	41.95	26.29	27.35	25.15	28.11	34.45	37.42	41.11	45.71
M2/Set 2	37.40	40.76	42.22	43.19	27.38	28.22	28.73	29.98	43.41	47.00	46.56	49.78
M3/Set 2	37.12	39.07	41.61	42.51	27.15	28.12	28.07	29.46	42.07	45.93	44.80	48.35
M4/Set 2	40.60	41.64	43.52	44.41	28.18	29.34	29.70	31.35	46.41	53.06	48.49	55.05
M1/Set 3	39.07	41.52	42.51	44.11	28.12	28.95	29.45	31.03	45.70	52.18	48.08	53.70
M2/Set 3	38.49	42.67	42.51	44.41	27.95	29.71	29.41	32.06	45.15	54.95	47.75	56.54
M3/Set 3	41.34	40.16	43.43	43.11	28.49	28.45	30.69	30.51	49.73	48.18	51.78	50.30
M4/Set 3	41.85	42.67	44.41	44.67	29.71	30.56	31.55	32.89	53.06	54.95	54.96	57.76
M1/Set 4	42.47	37.35	44.41	42.26	29.71	27.45	31.93	28.72	42.66	43.63	45.71	46.44
M2/Set 4	37.35	39.94	42.37	42.73	27.59	28.35	28.70	29.82	42.91	46.59	46.40	49.26
M3/Set 4	42.47	39.20	44.41	42.54	29.71	28.01	31.93	29.62	54.26	45.73	55.54	47.86
M4/Set 4	35.34	41.43	38.28	43.80	26.29	28.59	25.92	30.75	38.32	51.82	41.92	53.36
LSD 5%	0.98		1.03		0.52		0.92		1.61		1.73	

LSD 5%: Least significant difference at α=0.05.

SUPPLEMENTARY MATERIAL 4. Grouping of 32 sunflower backcrosses in clusters based on the canonical analysis for all the studied traits across two locations.

Cluster	No. of backcrosses	Backcrosses
I	14	2 (M_2 x P1/Set_1), 3 (M_3 x P1/Set_1), 4 (M_4 x P1/Set_1), 8 (M_4 x P2/Set_1),12 (M_4 x P1/Set_2),14 (M_2 x P2/Set_2), 15 (M_3 x P2/Set_2),16 (M_4 x P2/Set_2), 19 (M_3 x P1/Set_3), 20 (M_4 x P1/Set_3), 21 (M_1 x P2/Set_3), 23 (M_3 x P2/Set_3),30 (M_2 x P2/Set_4), 32 (M_4 x P2/Set_4).
II	4	6 (M_2 x P2/Set_1), 11 (M_3 x P1/Set_2), 29 (M_1 x P2/Set_4), 31 (M_3 x P2/Set_4).
III	2	5 (M_1 x P2/Set_1), 9 (M_1 x P1/Set_2).
IV	6	1 (M_1 x P1/Set_1), 7 (M_3 x P2/Set_1), 10 (M_2 x P1/Set_2), 13 (M_1 x P2/Set_2), 26 (M_2 x P1/Set_4), 28 (M_4 x P1/Set_4).
V	2	17 (M_1 x P1/Set_3), 18 (M_2 x P1/Set_3).
VI	2	25 (M_1 x P1/Set_4), 27 (M_3 x P1/Set_4)
VII	1	24 (M_4 x P2/Set_3).
VIII	1	22 (M_2 x P2/Set_3).

SUPPLEMENTARY MATERIAL 5. Mean value of nine quantitative traits of the eight clusters for 32 bi-parental crosses across two locations.

Cluster	DM	PH	HD	100 SW	SWP	SYH	OC	OA	LA
I	88.57	164.84	23.47	7.38	36.31	2219.81	40.77	28.64	48.58
II	82.33	157.80	18.77	7.38	31.74	2046.31	37.93	27.61	44.01
III	78.67	151.79	14.90	7.59	26.62	1899.31	33.07	26.42	34.44
IV	84.75	164.73	17.14	6.27	27.05	2000.74	36.25	27.15	40.883
V	87.42	164.12	24.50	6.58	28.15	2079.93	38.78	28.04	45.43
VI	94.67	175.60	27.07	5.88	39.37	2583.10	42.47	29.71	48.46
VII	101.00	181.84	27.73	8.40	42.21	2810.79	42.67	30.56	54.95
VIII	96.17	177.39	27.28	6.05	25.62	2610.40	42.67	29.71	54.95

DM: Days to maturity, PH: Plant height, HD: Head diameter, 100 SW: 100-seed weight, SWP: Seed weight/plant, SYH: Seed yield ha^{-1}, OC: Seed oil content, OA: Oleic acid and LA: Linoleic acid.

Sowing date and maize response to the splitting of nitrogen side-dressing fertilization

Fecha de siembra y respuesta del maíz al fraccionamiento de la fertilización nitrogenada en la superficie

Hugo François Kuneski[1]*, Luis Sangoi[1], Antonio Eduardo Coelho[1], Lucieli Santini Leolato[1], Marcos Cardoso Martins Júnior[1], Vander de Liz Oliveira[1], and Rafael Leandro Scherer[1]

ABSTRACT

The splitting of nitrogen side-dressing fertilization with the application of a nitrogen dose at maize tasseling may increase grain yield, mainly when the crop is sown at the most favorable date to achieve high productivity. This research was carried out to evaluate the effect of sowing date on the response of maize to the splitting of nitrogen side-dressing fertilization at different growth stages. Two sowing dates were tested: preferential (10/15/2015 and 09/20/2016) and late (12/5/2015 and 12/5/2016). Six nitrogen side-dressing management treatments were evaluated at each sowing date: control without N; complete nitrogen rate applied at phenological stages V5, V10 and VT; ½ N rate at V5 + ½ N rate at V10; and ⅓ N rate at V5 + ⅓ N rate at V10 + ⅓ N rate at VT. The N rate was equivalent to 300 kg ha^{-1}. Grain yield ranged from 8.9 to 15.3 t ha^{-1} in Lages and from 7.4 to 16.4 t ha^{-1} in Atalanta. There were no significant differences in grain yield and agronomic efficiency of nitrogen use (AE) among the treatments with the entire N side-dressing fertilization at V5 and V10 and those when the nutrient was split into two or three times, regardless of sowing date. AE was higher when the crop was sown at the preferential sowing date than in the late sowing date, regardless of N side-dressing application. The splitting of nitrogen fertilization up to the crop tasseling did not increase maize grain yield or AE.

Key words: *Zea mays* L., growth stage, sowing period, fractionation.

RESUMEN

El fraccionamiento de la fertilización nitrogenada en la superficie, con la aplicación de una parte de nitrógeno en el panojamiento, puede aumentar el rendimiento de los granos de maíz, principalmente cuando el cultivo se siembra en la fecha más favorable para obtener una alta productividad. El objetivo de esta investigación fue evaluar los efectos de la fecha de siembra sobre la respuesta del maíz al fraccionamiento de la fertilización nitrogenada en la superficie y a los estados fenológicos. Se evaluaron dos fechas de siembra: preferencial (15/10/2015 y 20/09/2016) y tardía (5/12/2015 y 5/12/2016). En cada fecha de siembra fueron analizados seis tratamientos de aplicación de nitrógeno en la superficie: testigo sin N; aplicación de dosis completa de N en V5, V10 y VT; ½ aplicación de N en V5, + ½ aplicación de N en V10; y ⅓ de la aplicación de N en V5 + ⅓ de la aplicación de N en V10 + ⅓ de la aplicación de N en VT. La dosis de nitrógeno utilizada fue de 300 kg ha^{-1}. El rendimiento de granos varió entre 8.9 a 15.3 t ha^{-1} en Lages y 7.4 a 16.4 t ha^{-1} en Atalanta. No hubo diferencias significativas en el rendimiento de granos ni en la eficiencia agronómica del uso de nitrógeno (EUN) entre los tratamientos con aplicación de la dosis completa de N en los estadíos V5 y V10 y aquellos en los que el nutriente se dividió dos o tres veces, independiente de la fecha de siembra. La EUN fue mayor en la siembra realizada al en la fecha preferencial que en la fecha tardía, independientemente del manejo de la aplicación nitrogenada en la superficie. El fraccionamiento de la fertilización nitrogenada en la superficie hasta VT no incrementó el rendimiento de granos ni la EUN del maíz.

Palabras claves: *Zea mays* L., estadío de crecimiento, periodo de siembra, fraccionamiento.

Introduction

Nitrogen is the most unstable element in the soil and also the nutrient that has the largest impact on maize grain yield (Cantarella, 2007). The splitting of nitrogen side-dressing fertilization at different growth stages is an alternative for enhancing the efficiency of nitrogen fertilizers and to mitigate N losses. This is possible due to the higher N uptake promoted by the syncronization between nitrogen fertilization and maize demand for the nutrient (Silva *et al.*, 2005).

Received for publication: 11 March, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.85637

[1] Centro de Ciencias Agroveterinarias, Universidade do Estado de Santa Catarina, Santa Catarina (Brazil).
* Corresponding author: hugokuneski@outlook.com

Several factors are involved in maize response to the splitting of nitrogen side-dressing fertilization: soil type, weather conditions, cropping system, sowing time, hybrid type, N source, growth stage, and the method of nitrogen application (Sangoi *et al*., 2016). The splitting of nitrogen side-dressing fertilization is usually recommended when high N rates are used on sandy soils and rainy conditions (Fontoura & Bayer, 2009).

Only 5% to 10% of the total nitrogen is taken up by maize until the growth stage V6, according to the scale proposed by Ritchie *et al.* (1993), when the growing point is transformed into a floral primordium (Sangoi *et al*., 2010). Nearly 75% of the total N absorbed by the plant during its cycle is concentrated between V6 and tasseling (Sangoi *et al*., 2016). For this reason, the commission of soil chemistry and fertility recommends that N side-dressing fertilization should be performed between the growth stages V4 and V8 in one or two applications, at the beginning of the period of greatest foliar expansion and large increments in plant height (Comissão de Química e Fertilidade do Solo, 2016).

An experiment carried out by DeBruin and Butzen (2014) in the United States of America showed that modern maize hybrids can take up 50% of total nitrogen after flowering. Therefore, late nitrogen fertilizations carried out at tasseling may delay foliar senescence and increase the crop phostosynthetic activity during grain filling. These physiological changes may enhance kernel weight and increase grain yield. The results obtained by Kosgey *et al.* (2013) and Ning *et al.* (2014) reinforce this tendency, showing that modern hybrids have the capacity to take up and assimilate great amounts of N after flowering. Therefore, splitting nitrogen side-dressing fertilization up to tasseling may be important to maximize grain yield.

The sowing date is a determinant factor for optimizing maize agronomic performance. The choice of sowing date is specific for each production region. It is affected by environmental conditions, especially temperature, precipitation, and the availability of solar radiation. When there is no water limitation, the most favorable sowing date for maize in southern Brazil is the beginning of spring (September-October) (Serpa *et al*., 2012). Sowings performed between the middle of September and the middle of October favor the coincidence between flowering and the period of the year with long days, high solar radiation availability, and temperatures ranging from 20 to 30°C. This is the time when maize has maximum leaf area (Sangoi *et al*., 2010). Despite that, late sowings performed at the end of spring (November-December) and at the beginning of summer (December-January) are common in southern Brazil, when maize is grown after other crops with higher economic importance, such as tobacco, onion, and garlic (Forsthofer *et al*., 2004).

The positive effect of splitting N side-dressing fertilization up to maize tasseling may be affected by the sowing date. At late sowings, the soil and air temperatures are high during the early stages of the crop, and this stimulates the plant vegetative growth and development. However, the grain filling period will occur in late summer (February-March) and early autumn (March-April), when the temperature and solar radiation are low and less favorable to N uptake from the soil and transportation of it to the kernels. Thus, the efficiency of side-dressing part of N rate at tasseling as a strategy to increase grain yield may be compromised at late sowing dates.

This study was based on the hypothesis that the effect of splitting nitrogen side-dressing fertilization as a management strategy to improve grain yield and nitrogen efficiency use is higher when maize is sown at a favorable time, at the beginning of spring. The objective of the experiment was to assess the effect of the sowing date on maize response to the splitting of nitrogen side-dressing fertilization at different growth stages.

Materials and methods

Two field experiments were set in Lages (2015/2016) and Atalanta (2016/2017) in the State of Santa Catarina, southern Brazil. The experimental site of Lages is located at 27°50’35” S, 50°02’45” W and 849 m a.s.l. and the experimental site of Atalanta is located at 27°26’03” S, 49°42’06” W at 586 m a.s.l. The soils were classified as Distrofic red Nitossol (Lages) and Distrofic Haplic Cambisol (Atalanta) (Santos *et al*., 2018). The results of soil analysis performed at the plowable layer (0 to 20 cm) in August of each growing season are shown in Table 1. Maize was sown after black oat (*Avena strigosa*) desiccation at both experimental sites. Winter coverage was killed with an application of glyphosate (1200 g a.i.) 45 d before each sowing date.

A randomized block design was used with treatments arranged in split plots and 4 repetitions. Two sowing times were tested in the main plots: preferential (beginning of spring) and late (end of spring). The preferential sowing dates were 10/15/2015 (Lages) and 09/20/2016 (Atalanta). The late sowing dates were 12/5/2015 (Lages) and 12/5/2016 (Atalanta). Six nitrogen side-dressing management treatments were assessed in the split plots: control (without N);

TABLE 1. Soil properties in the 0 to 20 cm layer of the experimental areas during the 2015/16 (Lages) and 2016/17 (Atalanta) growing seasons.

Locality	Clay	pH		OM	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}
-	g kg^{-1}	H_2O	SMP	g kg^{-1}	---mg dm^{-3} ---		------ cmolc dm^{-3} ------		
Lages	530	5.1	5.2	46.0	13.0	195.0	7.01	2.45	0.30
Atalanta	452	5.7	6.2	22.5	80.1	22.5	7.55	2.07	0.00

SMP - Shoemaker-McLean-Pratt; OM - organic matter; P and K were extracted with Mehlich-1; Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with KCl 1 mol L^{-1}.

complete N rate applied at V5 (vegetative grown stage - fifth leaf collar); complete N rate applied at V10; ½ N rate applied at V5 and ½ N rate applied at V10; ⅓ N rate applied at V5, ⅓ N rate applied at V10; and ⅓ N rate applied at VT (tasseling); and complete N rate applied at VT, according to a scale proposed by Ritchie *et al.* (1993) (Tab. 2). Each split plot comprised four rows, with 0.7 m row spacing, 0.19 m plant spacing and 6.0 m length. All data were collected in the two central rows of each split plot, except for 0.5 m at the end of each line, in an area of 7.0 m^2.

The experimental areas were fertilized with a mixture of phosphorus, potassium, and nitrogen on each sowing date, based on the results of soil analyses carried out yearly and in accordance with the recommendations of the commission

TABLE 2. Characterization of the fertilization on each sowing date and N side-dressing managements. Lages (2015/2016) and Atalanta (2016/2017).

Treatments	Fertilization on each sowing date (kg ha^{-1})			N side-dressing managements (N kg ha^{-1})		
	K_2O	P_2O_5	N	V5	V10	VT
Control	200	300	30	-	-	-
V5	200	300	30	300	-	-
V10	200	300	30	-	300	-
VT	200	300	30	-	-	300
V5+V10	200	300	30	150	150	-
V5+V10+VT	200	300	30	100	100	100

Growth stages according to the scale proposed by Ritchie *et al.* (1993): V5 (vegetative growth stage - fifth leaf collar); V10 (vegetative growth stage - tenth leaf collar); VT (tasseling).

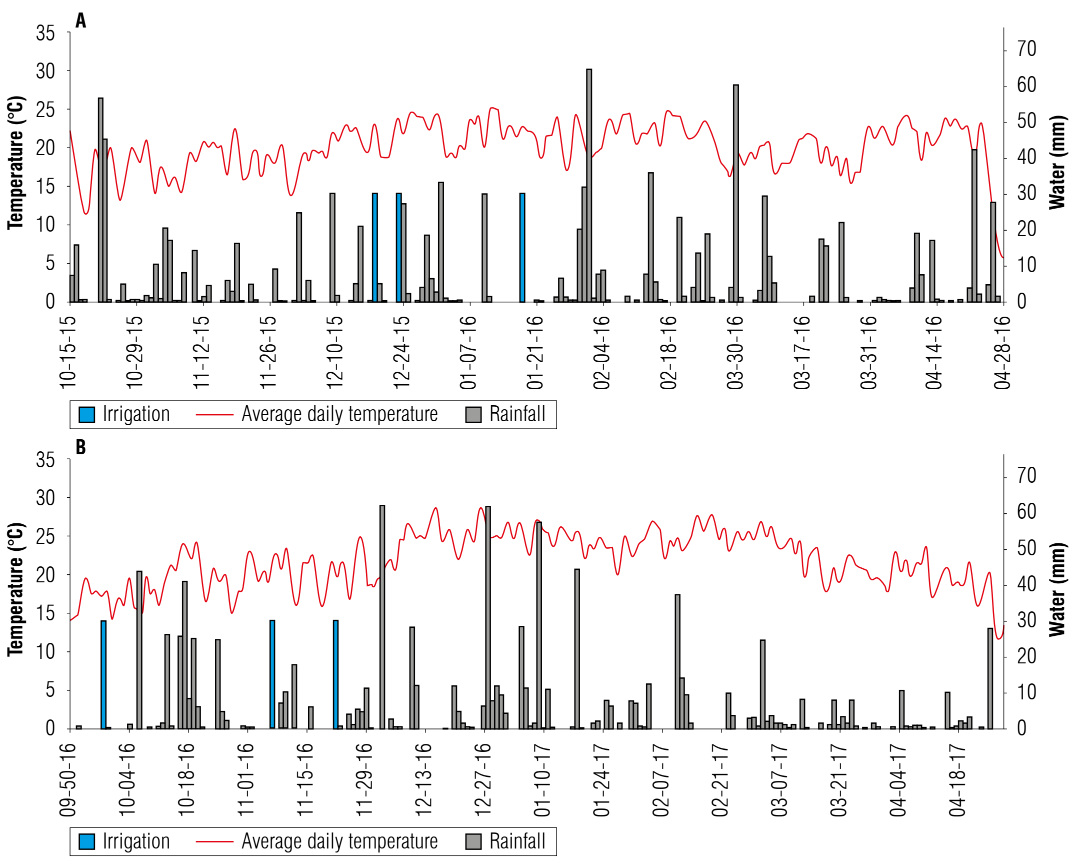


FIGURE 1. Average daily temperature, daily rainfall and irrigation of maize cycles from seedling to harvest during A) 2015/2016 (Lages-SC) and B) 2016/2017 (Atalanta-SC) growing seasons.

of soil chemistry and fertility (Comissão de Química e Fertilidade do Solo, 2016) to achieve a grain yield of 21 t ha^{-1}. The sources used to supply N, P, and K were urea (45% N), triple superphosphate (46% P_2O_5), and potassium chloride (60% K_2O), respectively. The rates applied at sowing were equivalent to 30 kg ha^{-1} N, 300 kg ha^{-1} P_2O_5, and 200 kg ha^{-1} K_2O (Tab. 2). Fertilizers were distributed on the soil surface close to the plant rows. Nitrogen was side-dressing at a rate of 300 kg N ha^{-1}, according to the growth stages defined for each treatment (Tab. 2). The experiment was planted by hand in a no-tillage system, with manual seeders and marked strings for a plant population of 75000 plants ha^{-1}. The hybrid tested was P30F53YH. The experiments were irrigated to maintain soil moisture close to the field capacity (Fig. 1).

Weed control was carried out with two herbicide applications. The first one was performed at preemergence immediately after sowing and with a combination of atrazine (1259 g a.i. ha^{-1}) and metolachlor (1250 g a.i. ha^{-1}). The second application was performed after maize emergence, when plants were at V4 with tembotriona (100 g a.i. ha^{-1}). Insects and diseases were controlled whenever necessary so that they did not to interfere with the crop development.

Green leaves were measured when maize reached the R1 growth stage (silking), to determine the leaf area and leaf area index (LAI). These determinations were repeated periodically every 14 d, up to 70 d after silking, when the kernels were physiologically ripened. Leaf area per plant (LA) was calculated using the following expression according to the method described by Borrás *et al.* (2003):

$$LA = L \times W \times 0.75 \quad (1)$$

where L is the length of the leaf, W is the width of the leaf, and 0.75 is the correlation coefficient.

The ears were manually harvested on 04/02/2016 and 05/02/2016 in the first year, and 03/02/2017 and 04/28/2017 in the second year. The ears were mechanically threshed. The kernels were oven dried at 60°C until they had constant weight using a drying oven with circulation/air exchange (MA035/1152, Marconi, Piracicaba, SP, Brazil). Afterwards, grain yield and its components were determined and expressed at the standard moisture of 13%.

Agronomic efficiency of nitrogen use (AE) expressed in kg kg^{-1} was determined according to Fageria and Baligar (2005), using the following formula:

$$AE = (GYf - GYu)/Na \quad (2)$$

where AE is the agronomic efficiency, GYf is the fertilized grain yield, GYu is the unfertilized grain yield, and Na is the application rate of side-dressing N.

The data were examined by analysis of variance using the F test with the software ASSISTAT 7.7 (E Silva & de Azevedo, 2016). The F values were considered significant at the error probability level of 5%. When the treatment differences were significant, the means were compared by the Tukey's test. The LAI variations during grain filling were assessed by polynomial regression analysis, testing the linear and quadratic models. Average comparisons were carried out at the significance level of 5%.

Results and discussion

The leaf area index (LAI) was affected by the interaction between sowing date, nitrogen side-dressing fertilization treatment, and days after silking (Tab. 3). There was a reduction of LAI from silking to kernel physiological maturity in all six nitrogen side-dressing management treatments due to the gradual leaf senescence occurred during grain filling (Fig. 2). The LAI values at each sampling time and their rate of decrease during the sampling period of treatments with the entire N fertilization at V5 and V10 or split into two and three times were similar at both experimental sites, regardless of the sowing date. Such behavior shows that dividing side-dressing into two or three growth stages neither delayed leaf senescence nor increased the crop LAI at the end of grain filling. The control without N showed a higher decrease of LAI values whereas the treatment with all nitrogen side-dressing fertilization at VT showed the lowest decrease of this variable along the 70-day sampling period. These differences are related to the important role of nitrogen on chlorophyll formation and its effect on keeping leaves photosynthetically active for a longer period (Sangoi *et al.*, 2016).

Grain yield ranged from 8.9 to 15.3 t ha^{-1} in Lages and from 7.4 to 16.4 t ha^{-1} in Atalanta (Tab. 4). It was affected by the interaction between sowing date and nitrogen side-dressing fertilization in Lages (Tab. 3). When maize was sown at the preferential time, the control and the treatment with complete N fertilization at VT showed lower yields than the other treatments. At the late sowing date, there were no differences between grain yield of the treatments with nitrogen side-dressing fertilization and the control. This behavior shows that grain yield response to nitrogen fertilization was higher when maize was sown at the beginning of spring (September/October). The highlands of the State of Santa Catarina, where the experimental site of Lages is located, have a long and cold winter.

TABLE 3. F-values and significances according to the analysis of variance for grain yield, weight of 1000 grains, grains per ear and agronomic efficiency of N use as a function of the main effect of sowing date and nitrogen side-dressing management and agronomic efficiency of N use as a function of the main effect of sowing date, nitrogen side-dressing management and days after silking.

SV	SD	NSDM	SDxNSDM	DAS	SDxDAS	NSDMxDAS	SDxNSDMxDAS
DF	1	5	5	5	5	25	25
2015/2016 (Lages-SC)							
GY	11.2273^{ns}	15.5383**	6.5588**	-	-	-	-
GW	4.6708^{ns}	10.9596**	0.7836^{ns}	-	-	-	-
GE	27.9211*	5.7070**	2.3709^{ns}	-	-	-	-
AE	25.2284*	3.4486*	2.5536^{ns}	-	-	-	-
LAI	31.4945*	4.4587**	1.4498^{ns}	492.0726**	6.0787**	5.6660**	3.2579**
2016/2017 (Atalanta-SC)							
GY	8.1533*	51.8202**	1.4450^{ns}	-	-	-	-
GW	12.9454*	45.7972**	2.1787^{ns}	-	-	-	-
GE	3.8681^{ns}	34.7024**	0.4869^{ns}	-	-	-	-
AE	6.5999^{ns}	7.8824**	0.8398^{ns}	-	-	-	-
LAI	0.5239^{ns}	35.4390**	1.2505^{ns}	610.9370**	10.4832**	9.9511**	2.4922**

F-test. ** significant at 1% probability level ($P<0.01$); * significant at 5% probability level (0.01d”0.05); ^{ns} - not significant ($P>0.05$). SV - source of variation; SD - sowing date; NSDM - nitrogen side-dressing management; DAS - days after silking; DF - degree of freedom. GY - grain yield; GW - weight of 1000 grains; GE - grains per ear; AE - agronomic efficiency of N use. LAI - leaf area index.

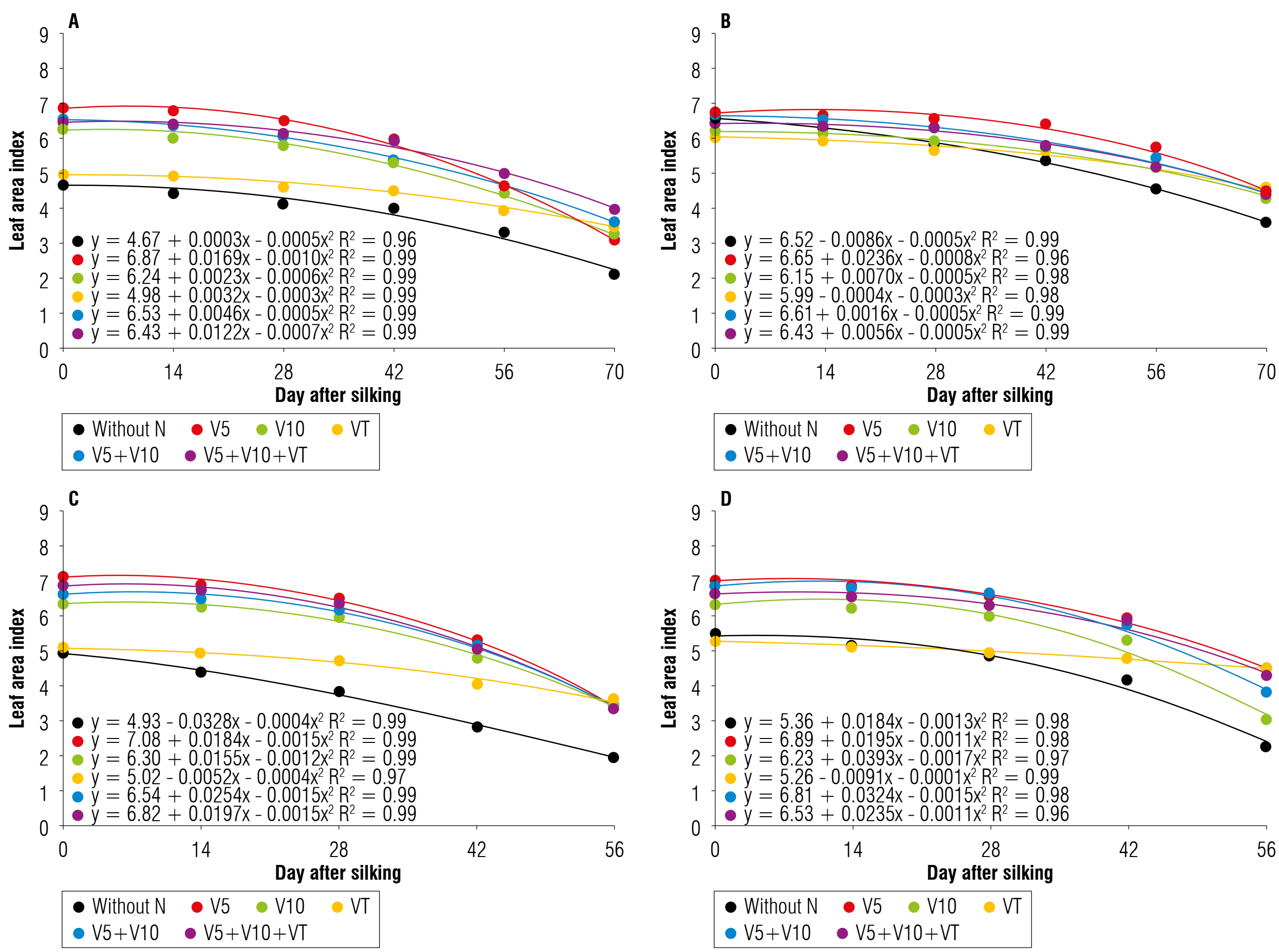


FIGURE 2. Leaf area index (LAI) during maize grain filling as affected by sowing date and nitrogen side-dressing fertilization in Lages A) 10/15/2015; B) 12/05/2015), and Atalanta C) 09/20/2016; D) 12/05/2016).

When maize is grown in early spring, the soil temperature is still low. The low temperature values delay soil organic matter mineralization and decrease the speed of *Avena strigosa* straw decomposition (Fontoura & Bayer, 2009). Both factors reduce N availability to the crop, enhancing its response to N sidre-dress fertilization.

In the 2015/2016 experiment, grain yield was greater at the preferential sowing date than at the late sowing date when nitrogen side-dressing fertilization was applied at V5 and V10 at one or more growth stages (Tab. 3). An opposite trend was observed in the control without N fertilization which obtained higher productivity when maize was sown in December rather than in October. At both sowing dates, there were no significant differences among treatments with the entire nitrogen rate applied in V5 or V10 and treatments where N side-dressing was split into two (V5+V10) or three (V5+V10+VT) times.

Grain yield was affected by the main effects of the sowing date and nitrogen side-dressing management system in Atalanta (Tab. 3). The treatments with N fertilization in V5, V10, V5+V10 and V5+V10+VT did not show significant differences and were more productive than the treatments without N and with complete fertilization in VT, on the average of two sowing dates (Tab. 4). Grain yield was higher when maize was sown in September than in December, on the average of six nitrogen side-dressing fertilization treatments.

The main hypothesis of this work was based on the results of experiments conducted in the northern hemisphere by DeBruin and Butzen (2014), Kosgey *et al.* (2013) and Ning *et al.* (2014). According to this hypothesis, splitting N side-dressing fertilization in order to supply part of the N rate at crop tasseling is an effective stategy to potentialize grain yield when maize is sown at the preferential time at the beginning of spring. The data gathered in Lages and Atalanta did not confirm this hypothesis. Trials carried out by Cardoso *et al.* (2011), Cruz *et al.* (2008) and Panison *et al.* (2019) in different production regions of Brazil also did not detect increments of maize grain yield with the fractioning on N side-dressing fertilization.

The divison of N side-dressing rates at different growth stages with the application of some amount of N fertilizer at VT has a large potential to increase maize grain yield when farmers grow hyper-early hybrids and achieve high kernel productivities (DeBruin & Butzen, 2014). Although the N rate (300 kg ha^{-1}) was defined as aiming to reach yield levels of 21 t ha^{-1}, the maximum grain productivity recorded in the experiment was 16.4 t ha^{-1}. Furthermore, the hybrid P30F53YH does not have a hyper-early cycle and shows great production stability at different enviroments. It is possible that these two characteristics have mitigated the postive effect of splitting N side-dressing fertilization on grain yield in this research. A third factor that may have contributed to the grain yield behaviour is the soil type where the experiments were carried out. At both experimental sites, the soils contain more than 400 g kg^{-1} of clay (Tab. 1). The chance of having a positive kernel yield response to the splitting of N side-dressing is greater in sandy soils, where N losses by leaching are more pronounced (Cantarella, 2007).

Grain yield was higher at the preferential sowing date than at the late sowing date when nitrogen was side-dressing to the crop. This behaviour is related to the more favorable climatic conditions for reaching high yields, verified when maize is sown at the beginning of spring in southern Brazil (Sangoi *et al.*, 2010). Late sowings decrease maize potential yield due to the low temperatures and less solar radiation available during grain filling (Mundstock & Silva, 2005).

In Lages, the control without N showed higher kernel productivity when the sowing was performed in December rather than in October. Such response emphasizes the effect of the sowing date on nitrogen availability derived from soil organic matter content and *Avena strigosa* straw decomposition. This effect is accentuated in the highlands of the State of Santa Catarina, which show low soil temperatures at the end of winter (August-September). The higher soil temperatures registered at the end of spring stimulate organic matter mineralization and *Avena strigosa* straw decay, enhancing N availability for maize development at late sowing dates (Fontoura & Bayer, 2009).

There was a significant increment on maize productivity with the entire N fertilization at VT compared to the control (Tab. 3). Numerically, the yield increase was equivalent to 3.4 and 4.7 t ha^{-1} at the preferential sowing time, for the growing seasons of 2015/2016 and 2016/2017, respectively (Tab. 4). These data corroborate the statements of DeBruin and Butzen (2014) and Ning *et al.* (2014), showing that modern hybrids have great capacity to uptake nitrogen during grain filling.

At both growing seasons, control plants produced less kernel weight than the other treatments with N fertilization (Tab. 4). Nitrogen supply is directly related to the crop leaf area index. Maize LAI decreased more drastically during grain filling when N

TABLE 4. Grain yield, weight of 1000 grains, grains per ear and agronomic efficiency (AE) of N use of maize in two sowing dates and six nitrogen side-dressing management treatments.

Sowing date	Growth stage of N side-dressing fertilization						Mean	C.V. (%)
	Control	V5	V10	VT	V5+V10	V5+V10+VT		
Grain yield (t ha^{-1})								
2015/2016 (Lages-SC)								
10/15	8.9 bC	15.3 aA	14.8 aAB	12.2 aB	15.2 aA	15.0 aA	13.6	
12/05	11.5 aA	13.0 bA	12.0 bA	12.9 aA	13.9 bA	12.8 bA	12.7	6.3
Mean	10.2	14.2	13.4	12.5	14.6	13.9		
C.V. (%)	7.6							
2016/2017 (Atalanta-SC)								
09/20	8.3	15.2	15.2	13.0	15.7	16.4	14.0 a	
12/05	7.4	13.5	12.6	10.4	12.7	13.0	11.6 b	12.3
Mean	7.9 C	14.3 A	13.9 A	11.7 B	14.2 A	14.7 A		
C.V. (%)	8.1							
Weight of 1000 grains (g)								
2015/2016 (Lages-SC)								
10/15	348	397	404	404	400	419	395 NS	
12/05	356	389	390	391	393	396	386	3.4
Mean	352 B	393 A	397 A	397 A	396 A	407 A		
C.V. (%)	3.7							
2016/2017 (Atalanta-SC)								
09/20	277	336	340	336	344	345	329 a	
12/05	276	333	317	337	324	331	320 b	2.9
Mean	277 B	334 A	328 A	337 A	334 A	338 A		
C.V. (%)	3.1							
Grains per ear (n°)								
2015/2016 (Lages-SC)								
10/15	382	512	504	446	521	468	472 b	
12/05	475	517	480	480	504	474	488 a	1.9
Mean	428 B	515 A	492 A	463 AB	513 A	471 AB		
C.V. (%)	7.1							
2016/2017 (Atalanta-SC)								
09/20	352	523	517	444	616	537	481 NS	
12/05	327	471	474	380	465	467	431	19.6
Mean	340 C	497 A	496 A	412 B	419 A	502 A		
C.V. (%)	7.0							
AE (kg kg^{-1})								
2015/2016 (Lages-SC)								
10/15	-	21.6	19.7	11.2	21.2	20.5	18.9 a	
12/05	-	5.1	1.9	4.6	8.1	4.5	4.9 b	64.3
Mean	-	13.4 AB	10.8 AB	7.9 B	14.7 A	12.5 AB		
C.V. (%)	29.0							
2016/2017 (Atalanta-SC)								
09/20	-	22.8	23.0	9.8	24.5	26.8	21.4 a	
12/05	-	20.2	17.4	10.1	17.7	18.8	16.9 b	29.1
Mean	-	21.5 A	20.2 A	10.0 B	21.1 A	22.8 A		
C.V. (%)	27.41							

V5 - five expanded leaves; V10 - ten expanded leaves; VT - tasseling, according to the growth stage scale proposed by Ritchie *et al.* (1993); preferential sowing date - early spring (10/15/2015 and 09/20/2016); Late sowing date - end of spring (12/05/2015 and 12/05/2015); means followed by the same lowercase letter in the column and uppercase letter in the row do not differ significantly by the Tukey test at the significance level of 5% ($P<0.05$); NS - not signifficant ($P\geq0.05$). C.V. - coefficient of variation.

side-dressing was not performed (Fig. 2) which contributed to a decrease of the 1000 grain mass in the control. Such a tendency was also reported by Cruz *et al.* (2008) and Cardoso *et al.* (2011). The splitting of N fertilization, combined with the application of ⅓ of the N rate at tasseling was expected to delay leaf senescence, increase grain filling duration, and enhance kernel mass, especially at the preferential sowing date. The hyphotesis was not confirmed because there were no significant differences for LAI and 1000 grain mass among treatments with N side-dressing carried out at growth stages one (V5 or V10), two (V5+V10) or three (V5+V10+VT) .

In 2015/2016, the control obtained ears with a lower number of kernels than the treatment with N fertilization at V5 and V10 (Tab. 4). The ears produced fewer grains when maize was sown in October rather than in December, on the average of N side-dressing fertilization treatments. In the growing season of 2016/2017, the control and the treatment with complete N side-dressing fertilization in VT obtained ears with a lower kernel number than the other treatments that contributed to the lower yields of these two treatments. This behavior confirms the observations of Bortolini *et al.* (2000) and Forsthofer *et al.* (2006), showing that the kernel number per ear is the yield component that affects maize productivity the most.

The agronomic efficiency of nitrogen use (AE) was affected by the main effects of sowing date and nitrogen side-dressing fertilization (Tab. 3). This variable was higher at the preferential sowing date rather than at the late sowing date at both experimental sites, regardless of the nitrogen side-dressing fertilization treatment (Tab. 4). At both experimental sites, the lowest AE values were recorded in the treatment with complete N fertilization in VT. The supply of the complete N rate at maize tasseling can recover partially the damages caused by nitrogen defficiency during the crop vegetative stages. This was confirmed by the weight of 1000 grains behaviour, which did not show statistical differences among the treatments with N fertilization at V5, V10 or VT (Tab. 4). However, the application of the complete N rate at VT could not totally compensate the negative effects of the lack of nitrogen between V5 and VT when the number of ears per plant and the number of potential kernels per ear were defined (Sangoi *et al.*, 2010). Therefore, nitrogen defficiency up to maize flowering reduced grain yield and AE when the whole side-dressing fertilization was performed at VT.

At both growing seasons, the AE values were lower than 25 kg of side-dressing N per kg of kernel (Tab. 4). Mota *et al.* (2015) and Sangoi *et al.* (2015) obtained AE values between 30 and 60 under similar experimental conditions, depending on the N rate and source. The lower yield than that planned for the nitrogen rate used (21 t ha^{-1}) in the trials contributed to reduce AE. The low nitrogen efficiency to enhance grain production probably contributed to the differences in the absence of productivity among treatments with complete N fertilization at V5 or V10 and treatments where the side-dressing operation was split into two or three times.

Conclusions

The splitting of N side-dressing fertilization into two (V5+V10) or three (V5+V10+VT) growth stages does not increase maize grain yield or AE, compared to the complete N application at V5 or V10, regardless of the sowing date.

The AE is higher when maize is sown at the beginning of spring, regardless of the nitrogen side-dressing fertilization system.

The complete nitrogen side-dressing fertilization at VT can help to recover partial damages to grain yield caused by no nitrogen aplication during maize vegetative development.

Author's contributions

HFK formulated the research goals and aims, applied the statistical techiques to analyze the study data, and wrote the original draft of the manuscript; LS formulated the research goals and aims and reviewed the manuscript; AEC and LSL reviewed the manuscript and performed the experiments; MCMJ, VLO, and RLS developed the methodology, performed the experiments and collected the data, and all authors revised and agreed to the published version of the manuscript.

Literature cited

Borrás, L., Maddoni, G. A., & Otegui, M. E. (2003). Leaf senescence in maize hybrids: plant population, row spacing and kernel set effects. *Field Crops Research, 82*(1), 13–26. https://doi.org/10.1016/S0378-4290(03)00002-9

Bortolini, C. G., Silva, P. R. F., & Argenta, G. (2000). Sistemas consorciados de aveia preta e ervilhaca comum como cobertura de solo e seus efeitos na cultura do milho em sucessão. *Revista Brasileira de Ciência do Solo, 24*(4), 897–903. https://doi.org/10.1590/S0100-06832000000400021

Cantarella, H. (2007). Nitrogênio. In R. F. Novais, V. H. Alvarez, N. F. Barros, R. L. F. Fontes, R. B. Cantarutti , & J. C. L. Neves (Eds.), *Fertilidade do solo* (pp. 375–470). Sociedade Brasileira de Ciência do Solo.

Cardoso, S. D. M., Soratto, R. P., Da Silva, Â. H., & De Mendonça, C. G. (2011). Fontes e parcelamento do nitrogênio em cobertura, na cultura do milho sob plantio direto. *Revista Brasileira de Ciências Agrarias*, 6(1), 23–28. https://doi.org/10.5039/agraria.v6i1a739

Comissão de química e fertilidade do solo. (2016). *Manual de calagem e adubação para os estados do Rio Grande do Sul e Santa Catarina*. Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul.

Cruz, S. C. S., Pereira, F. R. D. S., Santos, J. R., De Albuquerque, A. W., & Da Silva, E. T. (2008). Parcelamento da adubação nitrogenada na cultura do milho irrigado em sistema plantio direto. *Revista Brasileira de Engenharia Agrícola e Ambiental, 12*(4), 370–375. https://doi.org/10.1590/S1415-43662008000400006

DeBruin, J., & Butzen, S. (2014). Nitrogen uptake in corn. *Crop Insights, 24*(4), 1–8.

E Silva, F. D. A. S., & De Azevedo, C. A. V. (2016). The Assistat Software version 7.7 and its use in the analysis of experimental data. *African Journal of Agricultural Research, 11*(39), 3733–3740. https://doi.org/10.5897/AJAR2016.11522

Fageria, N. K., & Baligar, V. C. (2005). Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy, 88*, 97–185. https://doi.org/10.1016/S0065-2113(05)88004-6

Fontoura, S. M. V., & Bayer, C. (2009). Adubação nitrogenada para alto rendimento de milho em plantio direto na região centro-sul do Paraná. *Revista Brasileira de Ciência do Solo, 33*(6), 1721–1732. https://doi.org/10.1590/S0100-06832009000600021

Forsthofer, E. L., Silva, P. R. F. D., Argenta, G., Strieder, M. L., Suhre, E., & Rambo, L. (2004). Desenvolvimento fenológico e agronômico de três híbridos de milho em três épocas de semeadura. *Ciência Rural, 34*(5), 1341–1348. https://doi.org/10.1590/S0103-84782004000500004

Forsthofer, E. L., Silva, P. R. F. D., Strieder, M. L., Minetto, T., Rambo, L., Argenta, G., Sangoi, L., Suhre, E., & Silva, A. A. D. (2006). Desempenho agronômico e econômico do milho em diferentes níveis de manejo e épocas de semeadura. *Pesquisa Agropecuária Brasileira*, *41*(3), 399–407. https://doi.org/10.1590/S0100-204X2006000300005

Kosgey, J. R., Moot, D. J., Fletcher, A. L., & McKenzie, B. A. (2013). Dry matter accumulation and post-silking N economy of 'stay-green' maize (*Zea mays* L.) hybrids. *European Journal of Agronomy, 51*, 43–52. https://doi.org/10.1016/j.eja.2013.07.001

Mota, M. R., Sangoi, L., Schenatto, D. E., Giordani, W., Boniatti, C. M., & Dall'Igna, L. (2015). Fontes estabilizadas de nitrogênio como alternativa para aumentar o rendimento de grãos e a eficiência de uso do nitrogênio pelo milho. *Revista Brasileira de Ciência do Solo, 39*(2), 512–522. https://doi.org/10.1590/01000683rbcs20140308

Mundstock, C. M., & Silva, P. R. F. D. (2005). *Manejo da cultura do milho para altos rendimentos de grãos*. Evangraf.

Ning, P., Li, S., Li, X., & Li, C. (2014). New maize hybrids had larger and deeper post-silking root than old ones. *Field Crops Research, 166*, 66–71. https://doi.org/10.1016/j.fcr.2014.06.009

Panison, F., Sangoi, L., Durli, M. M., Leolato, L. S., Coelho, A. E., Kuneski, H. F., & Liz, V. O. D. (2019). Timing and splitting of nitrogen side-dress fertilization of early corn hybrids for high grain yield. *Revista Brasileira de Ciência do Solo, 43*, Article e0170338. https://doi.org/10.1590/18069657rbcs20170338

Ritchie, S. W., Hanway, J. J., & Benson, G. O. (1993). *How a corn plant develops*. Special report no. 48. Iowa State University of Science and Technology and Technology Cooperative Extension Service.

Sangoi, L., Silva, P. R. F. D., Argenta, G., & Rambo, L. (2010). *Ecofisiologia da cultura do milho para altos rendimentos*. Graphel.

Sangoi, L., da Silva, L. M. M., Mota, M. R., Panison, F., Schmitt, A., de Souza, N. M., Giordani, W., & Schenatto, D. E. (2015). Desempenho agronômico do milho em razão do tratamento de sementes com *Azospirillum* sp. e da aplicação de doses de nitrogênio mineral. *Revista Brasileira de Ciência do Solo, 39*(4), 1141–1150. https://doi.org/10.1590/01000683rbcs20140736

Sangoi, L., Silva, P. R. F. D., & Pagliarini, N. H. F. (2016). *Estratégias de manejo da adubação nitrogenada em milho na região sul do Brasil*. Graphel.

Santos, H. G. D., Jacomine, P. K. T., Anjos, L. H. C. D., Oliveira, V. A. D., Lumbreras, J. F., Coelho, M. R., Almeida, J. A. D., Araujo Filho, J. C. D., Oliveira, J. B. D., & Cunha, T. J. F. (2018). *Sistema Brasileiro de Classificação de Solos*. Embrapa.

Serpa, M. D. S., da Silva, P. R. F., Sangoi, L., Vieira, V. M., & Marchesi, D. R. (2012). Densidade de plantas em híbridos de milho semeados no final do inverno em ambientes irrigados e de sequeiro. *Pesquisa Agropecuária Brasileira, 47*(4), 541–549. https://doi.org/10.1590/S0100-204X2012000400010

Silva, E. C. D., Ferreira, S. M., Silva, G. P., de Assis, R. L., & Guimarães, G. L. (2005). Épocas e formas de aplicação de nitrogênio no milho sob plantio direto em solo de cerrado. *Revista Brasileira de Ciência do Solo, 29*(5), 725–733. https://doi.org/10.1590/S0100-06832005000500008

Antioxidant compounds in diploid potato: Effect of the foliar application of magnesium and manganese

Compuestos antioxidantes en papa diploide: Efecto de la aplicación foliar de magnesio y manganeso

María Margarita López-Rodríguez[1]* and Carlos Eduardo Ñústez-López[1]

ABSTRACT

The potato is a good source of compounds with high antioxidant activity such as phenolic acids, anthocyanins, and carotenoids. These contents can be affected by agronomic practices such as fertilization. This research evaluated the effect of foliar fertilization with magnesium and manganese on the content of phenolic compounds, anthocyanins, and carotenoids, and the antioxidant activity of cooked tubers of four potato cultivars of the Phureja Group. We determined the antioxidant activity and the content of total phenol, total anthocyanins, phenolic acids and carotenoids. The cultivar Violeta showed the highest content of total phenols and the highest antioxidant activity. A significant effect of magnesium fertilization was observed that increased the content of total phenols in the cultivar Criolla Colombia and reduced it in cultivars Violeta and Milagros. These results suggest a high influence of the genotype on the response of the evaluated variables. Therefore, the cultivar Violetta is recommended for the *in vivo* study of antioxidant activity, as it showed the highest values when compared to the other cultivars.

Key words: Phureja Group, phenolic acids, anthocyanin, carotenoids.

RESUMEN

La papa es una buena fuente de compuestos con alta actividad antioxidante como los ácidos fenólicos, las antocianinas y los carotenoides, cuyos contenidos se pueden ver afectados por prácticas agronómicas como la fertilización. El presente estudio evaluó el efecto de la fertilización foliar con magnesio y manganeso sobre el contenido de compuestos fenólicos, antocianinas y carotenoides, y la actividad antioxidante en tubérculos cocidos de cuatro cultivares de papa del Grupo Phureja. Se determinó la actividad antioxidante y el contenido de fenoles totales, antocianinas totales, ácidos fenólicos y carotenoides. El cultivar Violeta presentó los mayores contenidos de fenoles totales y la mayor actividad antioxidante. Se observó un efecto significativo de la fertilización con magnesio, que aumentó el contenido de fenoles totales en el cultivar Criolla Colombia y lo redujo en los cultivares Violeta y Milagros. Estos resultados sugieren alta influencia del genotipo sobre la respuesta de las variables evaluadas. Como consecuencia, el cultivar Violeta es recomendado para el estudio de actividad antioxidante *in vivo*, teniendo en cuenta que mostró los mayores valores, comparado con los demás cultivares.

Palabras clave: Grupo Phureja, ácidos fenólicos, antocianina, carotenoides.

Introduction

The potato (*Solanum tuberosum* L.) is considered the fourth most important crop in terms of planted area and the third most consumed food by humans. This tuber plays a key role in food security and constitutes a high portion of the human diet in developing countries (Ezekiel *et al.*, 2013; Devaux *et al.*, 2014; FAO, 2018). Potato tubers are consumed worldwide and are cooked in different ways. However, eating unpeeled boiled potatoes is predominant in many regions (Camire *et al.*, 2009).

Potatoes contain a wide range of phytochemicals such as phenolic acids, anthocyanins and carotenoids, and diploid potatoes known as the Phureja Group are considered an important source of these phytochemicals (Pillai *et al.*, 2013; Narvaez-Cuenca *et al.*, 2018). Hydroxycinnamic acids are the predominant acids in potatoes, and within these, chlorogenic acid is the most abundant (Mattila and Hellström, 2007; Piñeros *et al.,* 2017). Anthocyanins are water-soluble phenolic compounds that belong to the flavonoid group and are responsible for the red, blue and purple color in fruits and vegetables (Fang, 2015; Tierno *et al.,* 2015). They are also present in the skin and flesh of potato tubers (Brown, 2008). Carotenoids are lipophilic pigments responsible for the yellow, orange, and red colors in vegetables (Rodríguez-Amaya, 2018). These phytochemical compounds are metabolites with antioxidant activity

Received for publication: 10 May, 2019. Accepted for publication: 9 November, 2020.

Doi: 10.15446/agron.colomb.v38n3.79629

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).

[*] Corresponding author: mmlopezr@unal.edu.co

that protects cells from oxidative stress damage, play an important role in the tuber organoleptic properties and provide multiple benefits for the human body (Craft *et al.*, 2012; Ezekiel *et al.*, 2013; Bellumori *et al.*, 2017).

The concentration of these compounds with antioxidant activity in potato tubers is affected by factors such as the genotype, environment, agronomic crop management, and postharvest processes (André *et al.*, 2009; Ezekiel *et al.*, 2013; Lachman *et al.*, 2016). Among the agronomic factors, fertilization is important because a balanced management of nutrients ensures that plants can reach their genetic potential reflected in the quality and yield parameters (Gómez, 2005).

There is scarce information about the effect of plant nutrition on the content of phenolics and carotenoids or their antioxidant activity in potato tubers. In Colombia, research on macro and micronutrients has been conducted solely to analyze their effect on yield and quality of commercial potato cultivars (Gómez *et al.*, 2006; Palacios *et al.*, 2008). Magnesium (Mg) and manganese (Mn) are two of the essential plant nutrients involved in multiple biological functions (Farzadfar *et al.*, 2017). Although these elements have been studied in different topics such as tuber yield and quality (Pérez *et al.*, 2008; Villa *et al.*, 2011), there are no reports in connection with their antioxidant activity.

The purpose of this research was to evaluate the effect of foliar applications of magnesium and manganese on the antioxidant activity, total phenol, total anthocyanins, phenolic acids, and carotenoid contents of four diploid potato cultivars, Criolla Colombia, Paola, Milagros and Violeta.

Materials and methods

Plant material

Tubers of diploid potato cultivars with different colors of skin and flesh were used for this study. The cultivars Criolla Colombia and Paola had yellow skin and flesh; the cultivar Milagros had red skin and yellow flesh, and the cultivar Violeta had purple skin and purple and white flesh (Fig. 1).

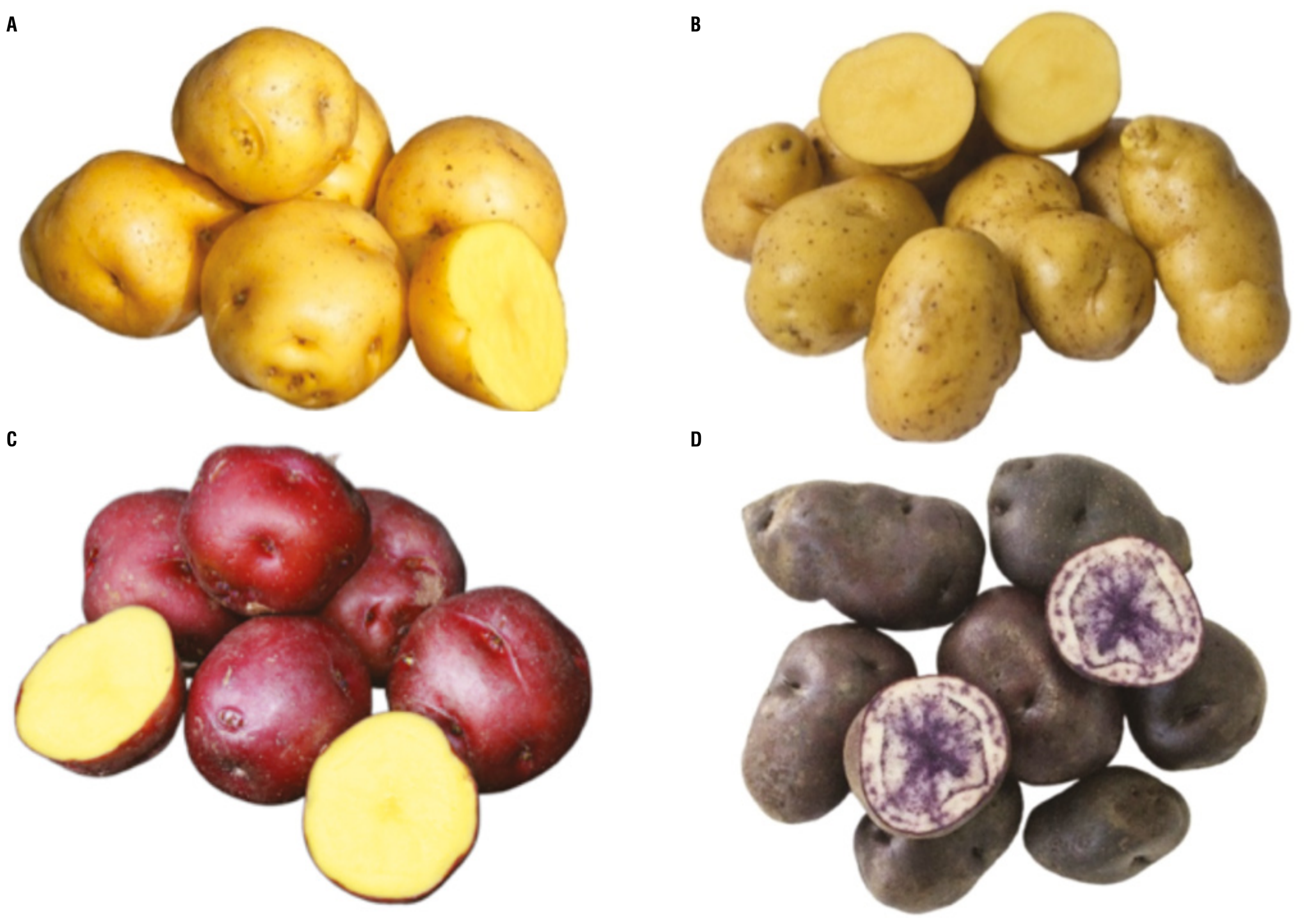


FIGURE 1. Tubers of cultivars A) Criolla Colombia, B) Paola, C) Milagros and D) Violeta.

Chemicals

Ethanol, methanol, Folin Ciocalteu reagent, 2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), acetate trihydrate, iron(III) chloride hexahydrate, glacial acetic acid, chlorhidric acid, sodium carbonate and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Panreac. Gallic acid was obtained from Alfa. The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and standards of zeaxanthin, antheraxanthin and lutein were obtained from Sigma Aldrich. Acetonitrile, butyl hydroxytoluene (BHT), hexane, and triethylamine were obtained from J.T. Baker. Standards of chlorogenic acid and caffeic acid were obtained from Chromadex. Standards of neochlorogenic acid and cryptochlorogenic acid were obtained from PhytoLab. Water was obtained by a Millipore system (EMD Millipore Corp, Billerica, MA, USA).

Foliar fertilization with magnesium and manganese

The field experiment was conducted at the experimental farm Centro Agropecuario Marengo of the Universidad Nacional de Colombia, located in Mosquera, Cundinamarca, Colombia (4°41'04.44" N, 74°12'57.46" W, 2543 m a.s.l.) with an annual average precipitation of 750 mm and annual average temperature of 14°C, from October 2016 to January 2017. The characteristics of the soil used in the experiment are shown in Table 1. The experimental design consisted of a randomized block with three replicates. Four treatments were evaluated: 1) control (without Mg or Mn applications), 2) 450 g ha^{-1} of Mg, 3) 300 g ha^{-1} of Mn, and 4) combination of elements, 450 g ha^{-1} of Mg and 300 g ha^{-1} of Mn. The experimental unit was 66 plants per cultivar. Applications were split into three foliar sprays, at 60, 75 and 90 d after planting. The source of Mg and Mn used were chelates (EDTA). Basal fertilizer [10-20-20 (N-P_2O_5-K_2O)] was applied at a rate of 25 g per seed tuber at sowing. Potato tubers were harvested at 120 d after planting and a sample of each replicate was collected for cooking.

Cooking process

For each treatment, four tubers of approximately similar size (40-50 g) were selected. Whole and unpeeled tubers were washed with tap water and placed in a stainless-steel pot containing boiling water. Cooking time tests were performed on each cultivar by piercing the tubers with a knife. The following cooking times were used: 20 min for cultivars Criolla Colombia and Paola and 19 min for cultivars Milagros and Violeta. After cooking, the samples were cooled at room temperature, placed in sealed plastic bags and frozen at -80°C. The samples were then freeze-dried and pulverized with a blender. Each sample was stored in an amber glass flask at room temperature until use.

Extraction of phenolic compounds

Phenolic compounds were extracted using the methods reported by Piñeros *et al.* (2017) and Burgos *et al.* (2013). Fifty mg of lyophilized sample was extracted with 1 ml of methanol/water/acetic (50:50:0.1, v/v/v) using sonication at 4°C for 10 min. The mixture was centrifuged at 5000 rpm at 4°C for 15 min and the supernatant was collected. Extraction was performed five times. The five supernatants were mixed, flushed with nitrogen gas, and stored at -20°C until further analyses.

Determination of total phenols

Total phenol content was determined using the Folin Ciocalteu method (Waterhouse, 2002). An aliquot 20 µl of the extract solution was taken into a cuvette, and then 1.58 ml of deionized water was added. After 8 min at room temperature, 300 µl of a sodium carbonate solution (20%, w/v) was added, and the mixture was left to stand for 90 min in the dark at room temperature. Absorbance was measured at 765 nm with a spectrophotometer (SmartSpec Plus, BIORAD, Richmond, CA, USA). The total phenol concentration was calculated using a gallic acid calibration curve (r^2 = 0.9984) ranging from 10 to 100 µg ml^{-1}. Data were expressed as mg of gallic acid equivalents per 100 g of potato dry weight (DW).

Determination of antioxidant activity

Antioxidant activity was determined by ABTS and Ferric ion Reducing Antioxidant Power (FRAP) assays. For ABTS assays, the procedure was carried out according to the method of Re *et al.* (1999) with some modifications. The radical ABTS was activated by mixing 2.5 mM of a potassium persulfate solution and 7 mM of an ABTS solution in equal quantities and allowing them to react for 16 h in the dark. The solution was then diluted with 99% ethanol to obtain an absorbance of 0.70 at 734 nm using a spectrophotometer.

TABLE 1. Chemical and physical characteristics of the soil used in the experiment.

pH	CO	Ca	K	Mg	Na	Al	ECEC	P	Cu	Fe	Mn	Zn	B	Texture
	%	meq 100^{-1}						mg kg^{-1}						
5.6	3.6	17	1	5.9	0.8	0	25.5	86	1.2	249	3.4	50	0.4	Clay Loam

ECEC - Effective cation exchange capacity.

Briefly, 10 µl of each phenolic extract sample was added to 1 ml of ABTS solution into a cuvette. Absorbance was measured at 734 nm. The antioxidant activity was calculated using a trolox acid calibration curve (r^2 = 0.9975) ranging from 50 to 800 µM. Data were expressed as µM of trolox equivalents per 100 g of potato DW.

The FRAP assay was carried out according to Benzie and Strain (1996) with some modifications. The FRAP solution was obtained by mixing 50 ml of 300 mM acetate buffer, pH 3.6; 5 ml of 10 mM TPTZ solution, 5 ml of $FeCl_3 \cdot 6H_2O$ and 2.4 ml of deionized water. Briefly, 30 µl of each phenolic extract sample were added to 90 µl of deionized water and allowed to react with 900 µl of FRAP solution for 5 min in a cuvette. Absorbance was measured at 593 nm with a spectrophotometer. The antioxidant activity was calculated using a trolox acid calibration curve (r^2 = 0.9975) ranging from 50 to 800 µM. Data were expressed as µM of trolox equivalents per 100 g of potato DW.

Determination of total anthocyanins

Total anthocyanin content was determined according to Burgos *et al.* (2014). For anthocyanin extraction of cultivars Criolla Colombia, Paola and Milagros 40 mg of lyophilized sample and 20 mg for cultivar Violeta were used. Lyophilized samples were extracted with 1 ml of a methanol/1.5 M chloridric acid (80:20, v/v) solution using sonication at 4°C for 5 min and placed in a water bath at 80°C for 5 min. The mixture was centrifuged at 5000 rpm at 4°C for 5 min and the supernatant was collected. Extraction was performed four times for cultivars Criolla Colombia, Paola and Milagros, and six times for cultivar Violeta. The supernatants were mixed and stored at -20°C until analysis.

Then, 1.5 ml of extract was taken into a cuvette. Absorbance was measured at 700, 545 and 515 nm with a spectrophotometer. Total anthocyanin concentration was calculated using as reference the extinction coefficient (3.02×10^4) and the molecular weight (718.5 g L^{-1}) of malvidin-3-p-coumarylglycoside for the sample of cultivar Violeta and the extinction coefficient (2.73×10^4) and the molecular weight (486.5 g L^{-1}) of pelargonidin-3-glucoside for samples of cultivars Criolla Colombia, Paola and Milagros. Data were expressed as mg of anthocyanin per 100 g of potato DW.

Determination of phenolic acids

The phenolic extracts were filtered through disposable nylon syringe filter units (0.2 µm, Thermo Scientific, Waltham, MA, USA).

The phenolic extract was analyzed according to Narváez *et al.* (2013). Separation was performed using a Hypersil gold RP-C18 column (150 mm × 2.1 mm, 1.9 µm, Thermo Scientific). The mobile phase was composed of water/acetonitrile/acetic acid (99:1:0.1, v/v/v) (solvent A) and acetonitrile/acetic acid (100:0.1, v/v) (solvent B). The elution program consisted of isocratic conditions at 0% B for 5 min; linear gradient from 0 to 60% B for 18 min, and from 60 to 100% B for 1 min; isocratic conditions at 100% B for 3 min; linear gradient from 100 to 0% B for 1 min, and conditioning in isocratic starting conditions at 0% B for 7 min. The volume of injection was 5 µl. The flow rate was 400 µl/min. Detection was performed at 325 nm.

Identification was based on the comparison of retention time and UV spectra of standards. Quantification of chlorogenic acid, cryptochlorogenic acid, and neochlorogenic acid were based on the calibration curve with chlorogenic acid ranging from 0.1 to 10 mg L^{-1} (r^2 = 0.9956). Quantification of caffeic acid was performed by using a calibration curve with caffeic acid ranging from 0.05 to 10 mg L^{-1} (r^2 = 0.9994). The results were expressed as mg 100 g^{-1} of potato DW.

Determination of carotenoids

Carotenoids were identified and quantified by reversed phase (RP) - ultrahigh performance liquid chromatography (UHPLC) with diode array detection (DAD) (Dionex Ultimate 3000, Thermo Scientific, Waltham, MA, USA).

Extraction was carried out according to Maurer *et al.* (2014). Under dim light conditions, 250 mg of lyophilized sample were weighed into an amber plastic centrifuge tube and 2 ml of acetone/methanol (2:1, v/v), containing 0.5% (w/v) butylhydroxytoluene (BHT), were added, followed by 1 ml of hexane. The mixture was sonicated at 4°C for 20 min and 1.5 ml of cold 1 mol L^{-1} aqueous sodium chloride was added to the extract. The mixture was centrifuged at 5000 rpm at 4°C for 10 min. The supernatant was collected and stored at 4°C. Extraction was performed three times. The three supernatants were mixed and concentrated to 1 ml at 20°C under a nitrogen gas atmosphere. The concentrated extract was filtered through a disposable nylon syringe filter unit (0.2 µm, Thermo Scientific, Waltham, MA, USA) and analyzed by RP-UHPLC-DAD. Separation was performed using an AQUITY UPLC RP18 column (150 mm × 2.1 mm, 1.7 µm, Waters, Milford, MA, USA) with a temperature of 20°C. The mobile phase was composed of ethyl acetate/triethylamine (100:0.25, v/v) (solvent A), acetonitrile/triethylamine (100:0.25, v/v) (solvent B), and acetonitrile/water/triethylamine (50:50:0.25, v/v/v) (solvent C). The elution program consisted of isocratic conditions at 95% B for 1 min; linear gradient from 95 to 65% B for 12 min; isocratic conditions at 65% B for 2.5 min; linear gradient from 65 to 95% B for 2 min; and conditioning in isocratic starting conditions at 95% B for 9.5 min. The volume of injection was 5 µl. The flow rate was 250 µl/min. Detection was performed at 450 nm.

Zeaxanthin, antheraxanthin, and lutein were identified in the extracts by comparing their retention times and UV-Vis spectrum against external standards. Quantification of each carotenoid was based on calibration curves of standards ranging from 1 to 100 mg L^{-1} ($r^2 \geq 0.9993$). Results were expressed as mg 100 g^{-1} of potato DW.

Statistical analysis

All analyses were performed using the statistical software R (2019). Data were subjected to analysis of variance (ANOVA), and means were compared using the Tukey's test at $P \leq 0.05$.

Results

Total phenolic content

The foliar fertilization with Mg (450 g ha^{-1}) had a significant effect on the cultivar Criolla Colombia, contrasting with cultivars Milagros and Violeta that showed a reduction when compared to the control. There was no effect on the cultivar Paola. The foliar fertilization with Mn (300 g ha^{-1}) had a significant effect only on the cultivar Milagros reducing the total phenolic content when compared to the control. The two elements in combination did not show a significant effect on any of the cultivars. The cultivar Violeta showed the highest mean value for the total phenolic content and it was significantly higher than the cultivars Criolla Colombia and Paola (Tab. 2).

Antioxidant activity by the ABTS method

The foliar fertilization had a significant effect on the cultivars Milagros and Violeta while the other cultivars did not show a response. These significant effects were observed with Mn (300 g ha^{-1}) and Mg (450 g ha^{-1}), respectively, that led to a reduction in the antioxidant activity when compared to the control treatment. The cultivar Violeta had the highest average value and was significantly superior to the other cultivars (Tab. 2).

Antioxidant activity by the FRAP method

The foliar fertilization had a significant effect on the cultivar Criolla Colombia. The fertilization with Mg (450 g ha^{-1}) increased the antioxidant activity compared to the control. The cultivar Violeta showed the highest mean value compared to the cultivars Criolla Colombia, Paola and Milagros (Tab. 2).

Total anthocyanin content

The foliar fertilization had a significant effect on the cultivar Milagros with Mg (450 g ha^{-1}) and Mg and Mn (450 and 300 g ha^{-1}) compared to the control, showing a reduction in the anthocyanin content. There were no significant effects of the foliar fertilizer application on the rest of cultivars. The cultivar Violeta showed the highest mean value for the total anthocyanin content among all cultivars (Tab. 2).

Phenolic acid content

Figure 2 shows a chromatogram with the results for the quantification of phenolic acids with the UHPLC. There was a retention time of 9.64 min for the neochlorogenic acid, 11.31 min for the chlorogenic acid, 11.56 min for the cryptochlorogenic acid, and 11.87 min for the caffeic acid. To generate the chromatogram, data were collected from samples of the cultivar Violeta as a control (no treatment with Mg and Mn). A range from 9 to 13 minutes was considered as this range provides the highest peaks for the acids under study.

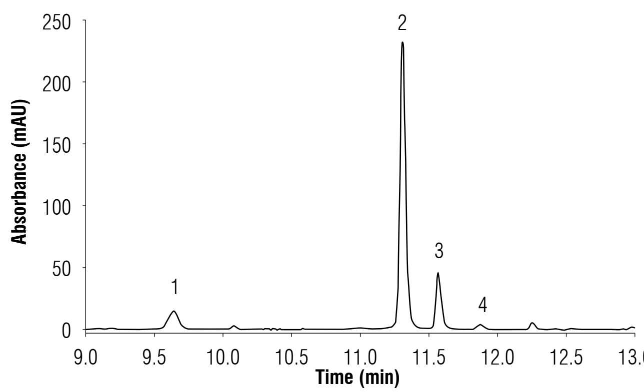


FIGURE 2. Chromatogram of phenolic acids quantified in boiled potato tubers from cultivar Violeta recorded at 325 nm. 1: Neochlorogenic acid; 2: Chlorogenic acid; 3: Cryptochlorogenic acid, and 4: Caffeic acid.

The foliar fertilization had a significant effect on the cultivar Criolla Colombia with the treatment Mg and Mn (450 and 300 g ha^{-1}) showing an increase in the content of chlorogenic acid and neochlorogenic acid compared to the control. Similarly, the treatment Mg (450 g ha^{-1}) significantly increased the neochlorogenic and caffeic acid contents compared to the control. In cultivar Milagros, the Mg (450 g ha^{-1}) significantly decreased the cryptochlorogenic acid and caffeic acid content compared to the control. The same effect was observed with the Mn (300 g ha^{-1}) on the caffeic acid content. No significant effect from the fertilization treatment was observed on the cultivar Paola (Tab. 2), and only a significant reduction in the chlorogenic acid was observed in the cultivar Violeta when it was treated with 450 g ha^{-1} of Mg.

From all quantified acids, chlorogenic acid was predominant. The cultivar Violeta showed the highest mean values for chlorogenic, neochlorogenic and cryptochlorogenic acids. These values were significantly higher when compared to the other cultivars. In the case of the caffeic acid, the cultivar Paola showed the highest mean value, statistically higher among all cultivars (Tab. 2).

Carotenoid content

Figure 3 shows a chromatogram with the results for the quantification of carotenoids with the UHPLC. There was a retention time of 3.67 min for antheraxanthin, 4.52 min for lutein, and 4.81 min for zeaxanthin. To generate the chromatogram, data were collected from the samples of the cultivar Criolla Colombia as a control (no treatment with Mg and Mn). A range from 3 to 5 min was considered as this range provided the highest peaks for the carotenoids under study.

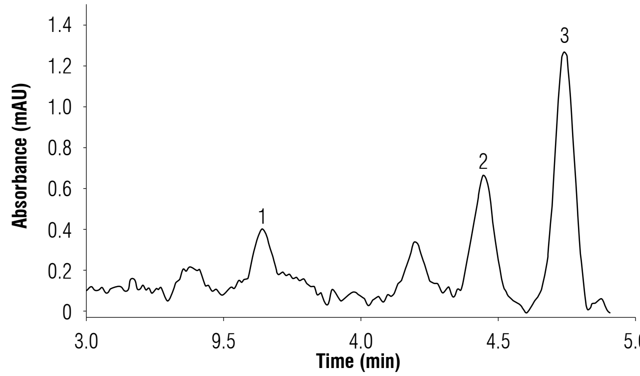


FIGURE 3. Chromatogram of the carotenoids quantified in boiled potato tubers from cultivar Criolla Colombia recorded at 425 nm. 1: antheraxanthin, 2: lutein and 3: zeaxanthin.

Lutein content did not show a significant effect from the fertilization treatments compared to the control in the four cultivars evaluated. Cultivar Milagros showed the highest mean value for lutein, but it was not significantly different from the cultivar Criolla Colombia, which showed the lowest mean value (Tab. 2).

There was no significant effect of the treatments on the content of antheraxanthin in the cultivars Criolla Colombia,

TABLE 2. Antioxidant activity, phenolic compounds, and carotenoids of cooked potato tubers of four diploid cultivars with Mg and Mn foliar fertilization.

Cultivar	Treatment	Total phenols mg GAE/100g DW		ABTS μM Trolox/100g DW		FRAP μM Trolox/100g DW		Anthocyanins mg/100g DW		Chlorogenic acid mg/100g DW	
C. Colombia	0	177.65 ± 40.97	b	988.89 ± 535.69	-	1269.70 ± 206.14	b	0.54 ± 0.41	-	33.31 ± 9.25	b
	Mg	259.58 ± 14.61	a	1350.00 ± 190.61	-	1672.72 ± 82.07	a	1.04 ± 0.45	-	59.73 ± 7.24	ab
	MgMn	220.61 ± 26.55	ab	849.00 ± 246.53	-	1480.30 ± 193.67	ab	1.10 ± 0.47	-	69.39 ± 20.89	a
	Mn	213.80 ± 24.76	ab	888.89 ± 152.99	-	1413.64 ± 72.95	b	0.93 ± 0.18	-	40.74 ± 5.31	ab
	Colombia*	217.91 ± 39.75	c	1019.44 ± 359.54	b	1459.09 ± 204.78	c	0.90 ± 0.43	b	50.79 ± 18.35	b
Paola	0	255.59 ± 40.42	-	1261.11 ± 437.37	-	1792.21 ± 195.19	-	0.24 ± 0.23	-	110.55 ± 25.00	-
	Mg	261.46 ± 31.78	-	1255.56 ± 307.44	-	1982.68 ± 240.79	-	0.37 ± 0.45	-	95.30 ± 17.82	-
	MgMn	270.14 ± 29.73	-	1516.67 ± 34.96	-	1859.31 ± 282.59	-	0.29 ± 0.24	-	97.64 ± 12.69	-
	Mn	295.26 ± 73.84	-	1555.56 ± 442.05	-	2158.01 ± 377.98	-	0.46 ± 0.33	-	124.01 ± 50.24	-
	Paola*	270.61 ± 46.81	b	1397.22 ± 353.85	b	1948.05 299.18	b	0.34 ± 0.31	b	106.87 ± 28.34	ab
Milagros	0	352.30 ± 27.00	a	1966.67 ± 188.56	a	2492.42 ± 389.60	-	21.47 ± 1.53	a	126.62 ± 21.66	-
	Mg	313.33 ± 11.46	b	1644.44 ± 183.38	ab	2169.19 ± 273.18	-	16.14 ± 2.42	b	95.16 ± 23.50	-
	MgMn	350.19 ± 9.07	a	1994.44 ± 223.52	a	2366.16 ± 137.09	-	16.17 ± 0.83	b	103.91 ± 14.44	-
	Mn	310.05 ± 24.80	b	1572.22 ± 297.71	b	2159.09 ± 219.57	-	22.42 ± 2.59	a	102.27 ± 11.02	-
	Milagros*	331.47 ± 27.36	ab	1794.44 ± 286.52	b	2296.72 ± 290.21	b	19.05 ± 3.50	b	106.99 ± 19.94	a
Violeta	0	522.25 ± 23.17	a	3672.22 ± 214.39	a	4848.48 ± 637.93	-	86.86 ± 6.59	-	230.00 ± 12.55	a
	Mg	454.41 ± 58.74	b	2750.00 ± 583.76	b	4015.15 ± 547.28	-	76.43 ± 9.30	-	187.62 ± 33,23	b
	MgMn	503.94 ± 70.52	ab	3544.44 ± 475.47	a	4643.94 ± 678.88	-	92.52 ± 25.35	-	225.99 ± 25.54	ab
	Mn	485.87 ± 40.68	ab	3216.67 ± 273.05	ab	4568.18 ± 876.22	-	79.23 ± 14.17	-	213.76 ± 14.93	ab
	Violeta*	491.62 ± 54.42	a	3295.83 ± 530.91	a	4518.94 ± 721.06	a	83.76 ± 15.92	a	214.34 ± 26.22	a

Treatments: 0 (without fertilization), Mg (450 g ha^{-1}), MgMn (450 and 300 g ha^{-1}), and Mn (300 g ha^{-1}). ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid), FRAP (Ferric ion Reducing Antioxidant Power). Different letters after the mean values indicate significant differences by the Tukey test ($P < 0.05$) ± Sd. *Cultivar mean. ND: Not detected.

TABLE 2. Continuation. Antioxidant activity, phenolic compounds and carotenoids of cooked potato tubers of four diploid cultivars with Mg and Mn foliar fertilization.

Cultivar	Treatment	Neochlorogenic acid mg/100g DW	Cryptochlorogenic acid mg/100g DW	Caffeic acid mg/ 100g DW	Lutein mg/100g DW	Antheraxanthin mg/ 100g DW	Zeaxanthin mg/ 100g DW
C. Colombia	0	3.64 ± 0.42 b	5.50 ± 1.20 -	3.12 ± 0.76 b	32.37 ± 11.20 -	17.45± 2.19 -	100.16± 21.06 b
	Mg	6.14 ± 0.19 a	10.55 ± 1.25 -	6.11 ± 0.49 a	49.16 ± 28.64 -	45.07± 30.48 -	171.38± 36.63 a
	MgMn	5.55 ± 1.01 a	10.61 ± 3.96 -	5.50 ± 1.16 ab	43.61 ± 32.10 -	30.84± 23.13 -	66.44± 34.60 b
	Mn	4.62 ± 0.17 ab	6.81 ± 0.62 -	4.39 ± 0.85 ab	32.56 ± 6.80 -	20.70± 8.29 -	90.23± 10.94 b
	Colombia*	4.99 ± 1.10 b	8.37 ± 3.01 B	4.78 ± 1.40 b	39.43 ± 20.61 b	28.51± 20.15 -	107.05± 47.25 a
Paola	0	6.97 ± 1.34 -	13.62 ± 3.04 -	11.28 ± 2.72 -	92.66 ± 18.17 -	38.08± 20.02 -	13.28± 2.71 -
	Mg	6.76 ± 0.59 -	12.56 ± 1.82 -	10.30 ± 1.46 -	72.79 ± 16.26 -	27.77± 13.62 -	7.95± 3.28 -
	MgMn	6.43 ± 0.13 -	12.35 ± 0.53 -	10.40 ± 1.52 -	67.43 ± 13.74 -	15.74± 10.10 -	7.22± 2.57 -
	Mn	6.66 ± 0.53 -	14.52 ± 3.63 -	11.75 ± 0.90 -	75.26 ± 30.76 -	14.27± 9.58 -	7.21± 6.91 -
	Paola*	6.71 ± 0.70 b	13.26 ± 2.36 ab	10.93 ± 1.64 a	77.03 ± 20.30 ab	23.96± 15.63 -	8.91± 4.49 b
Milagros	0	5.45 ± 0.33 -	14.57 ± 1.68 A	3.88 ± 0.65 a	127.22 ± 8.19 -	13.27± 3.91 -	32.45± 6.91 -
	Mg	5.14 ± 0.49 -	11.61 ± 1.38 B	2.85 ± 0.17 b	124.03 ± 33.82 -	22.20± 8.34 -	29.58± 9.63 -
	MgMn	5.26 ± 0.32 -	12.49 ± 1.08 ab	3.11 ± 0.23 ab	131.73± 14.56 -	22.28± 15.93 -	26.03± 5.17 -
	Mn	5.04 ± 0.08 -	12.09 ± 0.52 ab	3.03 ± 0.26 b	106.13± 0.91 -	12.87± 1.77 -	25.34± 3.92 -
	Milagros*	5.22 ± 0.33 b	12.69 ± 1.58 ab	3.22 ± 0.52 b	122.28± 19.02 a	17.66± 9.23 -	28.35± 6.49 b
Violeta	0	28.91 ± 1.94 -	46.61 ± 1.68 -	4.58 ± 0.65 -	47.88± 4.97 -	ND	ND
	Mg	26.90 ± 3.55 -	37.53 ± 1.38 -	3.99 ± 0.17 -	45.33± 11.38 -	ND	ND
	MgMn	30.91 ± 6.59 -	49.76 ± 1.08 -	4.60 ± 0.23 -	51.17± 15.11 -	ND	ND
	Mn	24.87 ± 0.50 -	39.01 ± 0.52 -	4.24 ± 0.26 -	57.52± 6.48 -	ND	ND
	Violeta*	27.90 ± 4.06 a	43.23 ± 7.44 A	4.35 ± 0.41 b	67.14± 63.78 ab	ND	ND

Treatments: 0 (without fertilization), Mg (450 g ha^{-1}), MgMn (450 and 300 g ha^{-1}), and Mn (300 g ha^{-1}). Different letters after the mean values indicate significant differences by the Tukey test ($P<0.05$) ± Sd. *Cultivar mean. ND: Not detected.

Milagros and Paola. These cultivars showed similar mean values without significant differences. This carotenoid was not detected in the cultivar Violeta (Tab. 2).

There was a significant effect of fertilization with Mg (450 g ha^{-1}) on the content of zeaxanthin in the cultivar Criolla Colombia compared to the control, showing an increase in the carotenoid. In cultivars Paola and Milagros, the treatments did not have a significant effect, while in cultivar Violeta it was not detected (Tab. 2).

Discussion

There is very little information about the effect of foliar fertilization with magnesium and manganese in potato. However, documented research findings on fertilization with magnesium in potato plants are in line with the responses observed in this study for the cultivars Milagros and Violeta. Klein *et al.* (1982) found that the fertilization of potato crops with this element significantly reduced the content of phenols and suggests a possible effect of the phenolase enzymes. Despite not finding significant differences, Hamouz *et al.* (2006) observe that the content of phenolic compounds decreased with magnesium fertilization. Phenolase enzymes such as polyphenol oxidase are associated with the degradation of anthocyanins and oxidation of phenolic compounds in plants (Ruenroengklin *et al.*, 2009). In the potato, these enzymes are present in the tubers (Tian *et al.*, 2016).

The effect of manganese has been reported in other species with a possible action in the activity of phenolase enzymes. Farzadfar *et al.* (2017) evaluated the activity of the enzyme polyphenol oxidase in *Tanacetum parthenium* plants fertilized with this element. The results showed a reduction in the activity of the enzyme related to a greater content of phenolic compounds; however, in this study, Mn did not show an effect on these compounds in the four cultivars evaluated.

From the treatment responses observed, this research emphasizes the important influence of the genotype, as reported by many authors who attributed a wide variation in genotypic differences of antioxidant activity, phenolic compounds, and carotenoids in potato (Andre *et al*., 2007; Reddivari *et al*., 2007; Lachman *et al*., 2012; Tierno *et al*., 2016; Oertel *et al*., 2017; Cuéllar-Cepeda *et al*., 2019).

The responses observed in this study showed a greater influence of the genotype on the treatments applied, as has been reported by numerous authors who attribute the differences in antioxidant activity and the content of phenolic and carotenoid compounds in potatoes to a wide genotypic variation (Andre *et al*., 2007; Reddivari *et al*., 2007; Lachman *et al*., 2012; Tierno *et al*., 2016; Oertel *et al*., 2017; Cuéllar-Cepeda *et al*., 2019).

The observed higher content of total phenols and antioxidant activity in the cultivars Milagros and Violeta compared to cultivars Criolla Colombia and Paola, correspond with the studies of Lachman *et al.* (2012), Navarre *et al.* (2011) and Rytel *et al.* (2014). These studies record values between two and three times higher in red and purple potato tubers compared to yellow tubers. The high antioxidant activity observed in the cultivar Violet coincides with the reports of Reyes *et al.* (2005); Lachman *et al.* (2008); Valiñas *et al.* (2017) and Cerón-Lasso *et al.* (2018), who show that varieties of potato with purple flesh contain higher amounts of antioxidant compounds, where anthocyanins are the most representative.

There was a high variation in the concentration of phenolic acids among the cultivars evaluated. However, chlorogenic acid exhibited a greater content in all cultivars. These results coincide with the studies by Narváez-Cuenca *et al.* (2013) in whole potato tubers of commercial cultivars of Colombia, Piñeros *et al.* (2017) in cooked potato tubers of different varieties and cultivars of Phureja Group of Colombia, Rytel *et al.* (2014) in processed yellow and colored potato tubers of Czech Republic, and Gutiérrez-Quequezana *et al.* (2020) in whole purple potato tubers of Switzerland. The total contents of chlorogenic, cryptochlorogenic, and neochlorogenic acids were lower in the cultivars Criolla Colombia, Paola and Milagros than in the cultivar Violeta that attained values between two and five times higher. These results are consistent with those reported by Ezekiel *et al.* (2013) and Bellumori *et al.* (2017), who found a much higher concentration of phenolic acids in purple potato tubers.

The carotenoid content showed variation among cultivars. In the cultivars of yellow flesh ('Criolla Colombia', 'Paola' and 'Milagros') lutein, antheraxanthin, and zeaxanthin were detected and quantified. In the cultivar Violeta, only lutein was detected. These responses are coincident with Tatarowska *et al.* (2019) and Kotíková *et al.* (2016), where the content and type of carotenoids detected were different between cultivars. The higher contents of carotenoids were associated with yellow cultivars compared with the purple ones, and the presence of only lutein in a cultivar similar to Violet (skin and flesh of purple color) was reported.

Conclusions

The responses of the evaluated variables to the treatments applied were different for each cultivar, showing an important effect of the genotype.

Foliar fertilization with Mg had an effect on total phenol content and antioxidant activity. These values increased in cultivar Criolla Colombia whereas they decreased in cultivar Violeta.

Foliar fertilization with Mn reduced the antioxidant activity by the ABTS method and the content of total phenols and caffeic acid in the cultivar Milagros. No significant effects were observed in the other cultivars.

The content of carotenoids showed high variability among the cultivars. In the cultivar Violeta (purple skin and flesh), contents of antheraxanthin and zeaxanthin were not detected, while lutein was detected in all cultivars with similar contents.

Foliar application of Mg and Mn cannot be recommended as a general agronomic practice if the purpose is to increase the content of phenolic compounds, carotenoids and antioxidant activity in cultivars of the Phureja Group, as there are genotype-specific responses.

This study allows a suggestion for the use of the cultivar Violeta in further studies to determine *in vivo* antioxidant activity. This is a reference cultivar for diploid potato breeding regarding characteristics associated with antioxidant activity.

Acknowledgments

This study was supported by the "Convenio Especial de Cooperación Derivado 2. Corredor Tecnológico Agroindustrial-CTA", and the financial support of Sistema General de Regalías, la Secretaría de Ciencia y Tecnología del Departamento de Cundinamarca, la Secretaria Distrital de Desarrollo Económico de Bogotá D.C., Universidad Nacional de Colombia, and AGROSAVIA.

Literature cited

Andre, C.M., M. Ghislain, P. Bertin, M. Oufir, M.D.R. Herrera, L. Hoffmann, J. Hausman, Y. Larondelle, and D. Evers. 2007. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. J. Agric. Food Chem. 55(2), 366-378. Doi: 10.1021/jf062740i

André, C.M., M. Oufir, L. Hoffmann, J.F. Hausman, H. Rogez, Y. Larondelle, and D. Evers. 2009. Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andean potatoes (*Solanum tuberosum* L.). J. Food Compost. Anal. 22(6), 517-524. Doi: 10.1016/j.jfca.2008.11.010

Bellumori, M., M. Innocenti, M. Michelozzi, L. Cerretani, and N. Mulinacci. 2017. Coloured-fleshed potatoes after boiling: promising sources of known antioxidant compounds. J. Food Compost. Anal. 59, 1-7. Doi: 10.1016/j.jfca.2017.02.004

Benzie, F. and J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. Anal. Biochem. 239(1), 70-76. Doi: 10.1006/abio.1996.0292

Brown, C.R., R.W. Durst, R. Wrolstad, and W. de Jong. 2008. Variability of phytonutrient content of potato in relation to growing location and cooking method. Potato Res. 51, 259-270. Doi: 10.1007/s11540-008-9115-0

Burgos, G., W. Amoros, L. Muñoa, P. Sosa, E. Cayhualla, C. Sánchez, C. Díaz, and M. Bonierbale. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. J. Food Compost. Anal. 30(1), 6-12. Doi: 10.1016/j.jfca.2012.12.001

Burgos, G., L. Muñoa, P. Sosa, E. Cayhualla, R. Carpio, and T. Felde. 2014. Procedures for chemical analysis of potato and sweetpotato samples at CIP's Quality and Nutrition Laboratory. CIP Communication and public awareness department (CPAD), Lima. Doi: 10.4160/9789290604440

Camire, M.E., S. Kubow, and D.J. Donnelly. 2009. Potatoes and human health. Crit. Rev. Food Sci. Nutr. 49(10), 823-840. Doi: 10.1080/10408390903041996

Cerón-Lasso, M., A.F. Alzate-Arbeláez, B.A. Rojano, and C.E. Ñústez-López. 2018. Composición fisicoquímica y propiedades antioxidantes de genotipos nativos de papa criolla (*Solanum tuberosum* Grupo Phureja). Inf. Tecnol. 29(3), 205-216. Doi: 10.4067/S0718-07642018000300205

Craft, B.D., A.L. Kerrihard, R. Amarowicz, and R.B. Pegg. 2012. Phenol-based antioxidants and the *in vitro* methods used for their assessment. Compr. Rev. Food Sci. 11(2), 148-173. Doi: 10.1111/j.1541-4337.2011.00173.x

Cuéllar-Cepeda, F., M. Parra-Galindo, J. Urquijo, L. Restrepo-Sánchez, T. Mosquera-Vásquez, and C. Narváez-Cuenca. 2019. Influence of genotype, agro-climatic conditions, cooking method, and their interactions on individual carotenoids and hydroxycinnamic acids contents in tubers of diploid potatoes. Food Chem. 288, 127-138. Doi: 10.1016/j.foodchem.2019.03.015

Devaux, A., P. Kromann, and O. Ortiz. 2014. Potatoes for sustainable global food security. Am. J. Potato Res. 57(3-4),185-199. Doi: 10.1007/s11540-014-9265-1

Ezekiel, R., N. Singh, S. Sharma, and A. Kaur. 2013. Beneficial phytochemicals in potato - a review. Food Res. Int. 50(2), 487-496. Doi: 10.1016/j.foodres.2011.04.025

FAO. 2018. Commodity balances - crops primary equivalent. URL: http://www.fao.org/faostat/en/#data/BC (accessed 10 April 2018).

Fang, J. 2015. Classification of fruits based on anthocyanin types and relevance to their health effects. Nutrition 31(11-12), 1301-1306. Doi: 10.1016/j.nut.2015.04.015

Farzadfar, S., F. Zarinkamar, and M. Hojati. 2017. Magnesium and manganese affect photosynthesis, essential oil composition and phenolic compounds of *Tanacetum parthenium*. Plant Physiol. Biochem. 112, 207-217. Doi: 10.1016/j.plaphy.2017.01.002

Gómez, M. 2005. Guía técnica para el manejo nutricional de los cultivos: diagnóstico, interpretación y recomendación de planes de fertilización. Microfertisa, Bogota.

Gómez, M., M. López, and Y. Cifuentes. 2006. El manganeso como factor positivo en la producción de papa (*Solanum tuberosum* L.) y arveja (*Pisum sativum* L.) en suelos del altiplano Cundiboyacense. Agron. Colomb. 24(2), 340-347.

Gutiérrez-Quequezana, L., A.L. Vuorinen, H. Kallio, and B. Yang. 2020. Impact of cultivar, growth temperature and developmental stage on phenolic compounds and ascorbic acid in purple and yellow potato tubers. Food Chem. 326, 126966. Doi: 10.1016/j.foodchem.2020.126966

Hamouz, K., J. Lachman, P. Dvořák, M. Jůzl, and V. Pivec. 2006. The effect of site conditions, variety and fertilization on the content of polyphenols in potato tubers. Plant Soil Environ. 52(9), 407-412. Doi: 10.17221/3459-PSE

Klein, L.B., S. Chandra, and N.I. Mondy. 1982. Effect of magnesium fertilization on the quality of potatoes: total nitrogen, nonprotein nitrogen, protein, amino acids, minerals, and firmness. J. Agric. Food Chem. 30(4), 754-757. Doi: 10.1021/jf00112a032

Kotíková, Z., M. Šulc, J. Lachman, V. Pivec, M. Orsák, and K. Hamouz. 2016. Carotenoid profile and retention in yellow-, purple- and red-fleshed potatoes after thermal processing. Food Chem. 197(A), 992-1001. Doi: 10.1016/j.foodchem.2015.11.072

Lachman, J., K. Hamouz, M. Orsák, V. Pivec, and P. Dvořák. 2008. The influence of flesh colour and growing locality on polyphenolic content and antioxidant activity in potatoes. Sci. Hortic. 117(2), 109-114. Doi: 10.1016/j.scienta.2008.03.030

Lachman, J., K. Hamouz, M. Orsák, V. Pivec, K. Hejtmánková, K. Pazderů, P. Dvořák, and J. Čepl. 2012. Impact of selected factors - cultivar, storage, cooking and baking on the content of anthocyanins in coloured-flesh potatoes. Food Chem. 113(4), 1107-1116. Doi: 10.1016/j.foodchem.2011.07.077

Lachman, J., K. Hamouz, M. Orsák, and Z. Kotíková. 2016. Carotenoids in potatoes - a short overview. Plant Soil Environ. 62(10), 474-481. Doi: 10.17221/459/2016-PSE

Mattila, P. and J. Hellström. 2007. Phenolic acids in potatoes, vegetables, and some of their products. J. Food Compost. Anal. 20(3-4), 152-160. Doi: 10.1016/j.jfca.2006.05.007

Maurer, M., J. Mein, S. Chaudhuri, and H. Constant. 2014. An improved UHPLC-UV method for separation and quantification of carotenoids in vegetable crops. Food Chem. 165, 475-482. Doi: 10.1016/j.foodchem.2014.05.038

Narváez-Cuenca, C.E., J.P. Vincken, C. Zheng, and H. Gruppen. 2013. Diversity of (dihydro) hydroxycinnamic acid conjugates in Colombian potato tubers. Food Chem. 139 (1-4), 1087-1097. Doi: 10.1016/j.foodchem.2013.02.018

Narváez-Cuenca, C.E., C. Peña, L.P. Restrepo-Sánchez, A. Kushalappa, and T. Mosquera. 2018. Macronutrient contents of potato genotype collections in the *Solanum tuberosum* Group Phureja. J. Food Compost. Anal. 66, 179-184. Doi: 10.1016/j.jfca.2017.12.019

Navarre, D.A., S.S. Pillai, R. Shakya, and M.J. Holden. 2011. HPLC profiling of phenolics in diverse potato genotypes. Food Chem. 127(1), 34-41. Doi: 10.1016/j.foodchem.2010.12.080

Oertel, A., A. Matros, A. Hartmann, P. Arapitsas, K.J. Dehmer, S. Martens, and H.P. Mock. 2017. Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols. Planta 246, 281-297. Doi: 10.1007/s00425-017-2718-4

Palacios, C.A., S. Jaramillo, L.H. González, and J.M. Cotes. 2008. Efecto de la fertilización sobre la calidad de la papa para procesamiento en dos suelos antioqueños con propiedades ándicas. Agron. Colomb. 26(3), 487-496.

Pérez, L., L. Rodríguez, and M. Gómez. 2008. Efecto del fraccionamiento de la fertilización con N, P, K y Mg y la aplicación de los micronutrientes B, Mn y Zn en el rendimiento y calidad de papa criolla (*Solanum phureja*) variedad Criolla Colombia. Agron. Colomb. 26(3), 477-486.

Pillai, S.S., D.A. Navarre, and J. Bamberg. 2013. Analysis of polyphenols, anthocyanins and carotenoids in tubers from *Solanum tuberosum* Group Phureja, Stenotomum and Andigena. Am. J. Potato Res. 90(5), 440-450. Doi: 10.1007/s12230-013-9318-z

Piñeros-Niño, C., C.E. Narváez-Cuenca, A.C. Kushalappa, and T. Mosquera. 2017. Hydroxycinnamic acids in cooked potato tubers from *Solanum tuberosum* group Phureja. Food Sci. Nutr. 5(3), 380-389. Doi: 10.1002/fsn3.403

R, Core Team. 2019. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna. URL: http://www.R-project.org/. (accessed 5 May 2018).

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9-10), 1231-1237. Doi: 10.1016/S0891-5849(98)00315-3

Reddivari, L., A.L. Hale, and J.C. Miller. 2007. Determination of phenolic content, composition and their contribution to antioxidant activity in specialty potato selections. Am. J. Potato Res. 84, 275-282. Doi: 10.1007/BF02986239

Reyes, L., J. Miller, and L. Cisneros-Zevallos. 2005. Antioxidant capacity, anthocyanins and total phenolics in purple- and red-fleshed potato (*Solanum tuberosum* L.) genotypes. Am. J. Potato Res. 82, 271-277. Doi: 10.1007/BF02871956

Rodríguez-Amaya, D.B. 2018. Update on natural food pigments - a mini-review on carotenoids, anthocyanins, and betalains. Food Res. Int. 124, 200-205. Doi: 10.1016/j.foodres.2018.05.028

Ruenroengklin, N., J. Sun, J. Shi, S.J. Xue, and Y. Jiang. 2009. Role of endogenous and exogenous phenolics in litchi anthocyanin degradation caused by polyphenol oxidase. Food Chem. 115(4), 1253-1256. Doi: 10.1016/j.foodchem.2009.01.040

Rytel, E., A. Tajner-Czopek, A. Kita, M. Aniołowska, A.Z. Kucharska, A. Sokół-Łętowska, and K. Hamouz. 2014. Content of polyphenols in coloured and yellow fleshed potatoes during dices processing. Food Chem. 161, 224-229. Doi: 10.1016/j.foodchem.2014.04.002

Tatarowska, B., D. Milczarek, E. Wszelaczyńska, J. Pobereżny, N. Keutgen, A.J. Keutgen, and B. Flis. 2019. Carotenoids variability of potato tubers in relation to genotype, growing location and year. Am. J. Potato Res. 96(5), 493-504. Doi: 10.1007/s12230-019-09732-9

Tian, J., J. Chen, F. Lv, S. Chen, J. Chen, D. Liu, and X. Ye. 2016. Domestic cooking methods affect the phytochemical composition and antioxidant activity of purple-fleshed potatoes. Food Chem. 197(B), 1264-1270. Doi: 10.1016/j.foodchem.2015.11.049

Tierno, R., D. Hornero-Méndez, L. Gallardo-Guerrero, R. López-Pardo, and J.I. Ruiz de Galarreta. 2015. Effect of boiling on the total phenolic, anthocyanin and carotenoid concentrations of potato tubers from selected cultivars and introgressed breeding lines from native potato species. J. Food Compost. Anal. 41, 58-65. Doi: 10.1016/j.jfca.2015.01.013

Tierno, R., A. López, P. Riga, S. Arazuri, C. Jarén, L. Benedicto, and J.I. Ruiz de Galarreta. 2016. Phytochemicals determination and classification in purple and red fleshed potato tubers by analytical methods and near infrared spectroscopy. J. Sci. Food Agric. 96(6), 1888-1899. Doi: 10.1002/jsfa.7294

Valiñas, M.A., M.L. Lanteri, A. ten Have, and A.B. Andreu. 2017. Chlorogenic acid, anthocyanin and flavan-3-ol biosynthesis in flesh and skin of Andean potato tubers (*Solanum tuberosum* subsp. *andigena*). Food Chem. 229, 837-846. Doi: 10.1016/j.foodchem.2017.02.150

Villa, M., L. Rodríguez, and M. Gómez. 2011. Effect of edaphic and foliar management of manganese on the yield of the Criolla Colombia cultivar. Agron. Colomb. 29(3), 447-454.

Waterhouse, A.L. 2002. Determination of total phenolics. Current Protoc. Food Analyt. Chem. 6(1), 1-8. Doi: 10.1002/0471142913.fai0101s06

Leaf area, chlorophyll content, and root dry mass in oil palms (*Elaeis guineensis* Jacq.) affected by the plumero disorder

Área foliar, contenido de clorofila, y masa seca de raíces en palmas de aceite (*Elaeis guineensis* Jacq.) afectadas por el disturbio del plumero

Martha Sofía España-Guechá[1], Daniel Gerardo Cayón-Salinas[2], Iván Ochoa-Cadavid[3], and Aquiles Enrique Darghan-Contreras[1]

ABSTRACT

The plumero disorder in oil palm is characterized by an abnormality in the development of the leaf area, yellowing of young leaves, and longitudinal chlorotic strips parallel to the central rib. In this research, the leaf area of leaf 17, the specific leaf area, chlorophyll contents, and root dry mass were evaluated in an oil palm (*Elaeis guineensis* Jacq.) plantation on the northern coast of Colombia to characterize the morphophysiological damage and quantify the severity of the disorder. For the statistical analysis, an ordinal regression model and analysis of variance tests were performed. The results indicated that the palm reduces its leaf area before the disorder is visually evident. Leaves became thicker and lower in chlorophyll content. There was also an increase in the tertiary and quaternary root dry mass in the initial grades. This variable decreased in the more severe grades of this disorder.

Key words: physiology, damage, disease severity, dry matter.

RESUMEN

El disturbio del plumero en palma de aceite se caracteriza por una anormalidad en el desarrollo del área foliar, el amarillamiento de las hojas jóvenes y el rayado clorótico longitudinal paralelo a la nervadura central. En esta investigación se determinó el área foliar de la hoja 17, el área foliar específica, los contenidos de clorofilas y la masa seca de raíces en una plantación de palma de aceite (*Elaeis guineensis* Jacq.) en la costa norte de Colombia con el objetivo de caracterizar los daños morfofisiológicos y cuantificar la severidad del disturbio. Para el análisis estadístico se realizó un modelo de regresión ordinal y pruebas de análisis de varianzas. Los resultados indicaron que la palma reduce su área foliar antes de que el disturbio sea evidente a nivel visual. Las hojas se vuelven más gruesas con menor contenido de clorofilas. También se presentó un aumento de la masa seca de raíces terciarias y cuaternarias en los grados iniciales. Esta variable disminuyó en los grados más severos del disturbio.

Palabras clave: fisiología, daño, severidad de enfermedad, materia seca.

Introduction

Since 2010, a disorder called plumero has been recognized in the oil palm (*Elaeis guineensis* Jacq.). This disorder is named after a Spanish word that refers to cleaning dusters because of the similarity between the leaf shape and this object. Symptoms of plumero are characterized by a visual reduction of the leaf area, yellowing of leaves, longitudinal chlorotic strips parallel to the central rib, drying of the tips of the leaflets in younger trees, and the presence of yellow strips usually located on one side of the central rib. The yellow strip may appear on one or several leaflets or one or several leaves (Arias *et al*., 2014). The causal agent or predisposing factors of this disorder are still unknown.

Plants show physiological responses to changes in environmental factors and the incidence of disease-causing organisms. In oil palms, research has been carried out to evaluate the physiological response of the plant to the attack of an abiotic agent. For example, the disease known as lethal wilt (*Candidatus* Phytoplasma asteris) generates increases in the internal temperature, decreasing the rate of photosynthesis and the ability to take nutrients from the oil palm that reduces the ability to produce sugars, the maintenance of leaves, and the production of new roots. During this process, the palm ends up decaying, preventing the leaves from opening their stomata to take up CO_2 and transpire (Cayón *et al*., 2007). The oil palm has a fasciculate radical system composed of primary, secondary, tertiary and quaternary roots (Reyes-Santamaría *et al*., 2000). Most of the absorption of nutrients is through the quaternary and absorbing tips of primary, secondary and tertiary roots (Tailliez, 1971). Ramírez *et al.* (2004) indicate that palms with lethal wilt produce very few roots, so they cannot

Received for publication: 23 February, 2020. Accepted for publication: 9 November, 2020. Doi: 10.15446/agron.colomb.v38n3.85309

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
[2] Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira (Colombia).
[3] Plantaciones Unipalma de los Llanos S.A., Cumaral, Meta (Colombia).
[*] Corresponding author: msespanag@unal.edu.co

take up the water required for nutrition and regulation of leaf temperature. In general, plants are often limited by several stressful factors that occur simultaneously, making it difficult to predict their geographical distribution based on physiological responses of an individual factor (Mittler, 2006; Fischer *et al*., 2016; Fischer and Melgarejo, 2020).

The negative impact of foliar diseases is directly expressed on yield components due to changes that pathogens induce in physiological processes responsible for crop productivity such as the increase in leaf area and the accumulation and distribution of dry mass in plant organs (Schierenbeck *et al*., 2014). The disease known as bud rot causes a decrease in photosynthesis, stomatal conductance, transpiration, efficient use of water and chlorophyll content, and increases the carotenoid content in oil palms.

The detailed description of some morphological and physiological aspects of a diseased plant allows knowing the infection processes, severity and damage of the disease (Rakib *et al*., 2019). So, the objective of this study was to characterize the morpho-physiological damages and to quantify the severity of the plumero disorder.

Materials and methods

Location

The work was carried out in an oil palm plantation located on the north coast of Colombia (9°56' N and 73°16' W, and altitude of 95 m a.s.l.). According to the Holdridge classification system, the plantation is located in the ecological formation tropical dry forest (Espinal and Montenegro, 1963). An average temperature of 27.5°C and average precipitation of 1300 mm were recorded.

Diagrammatic scale of the disorder

The diagrammatic severity scale proposed by Arias *et al.* (2014), indicates that palms affected by plumero disorder can be found in five grades: yellow strip, grade 1, grade 2, grade 3 and grade 4. Yellow-strip palms are characterized by the presence of at least one yellow strip in the canopy without visual reduction of the width of the leaflets, while grades 1 to 4 show a reduction in the width of the leaflets and sharp insertion of the leaflets in the rachis. In grade 1, the effect can be seen at the level of the 1^{st} leaf, grade 2 at the level of the 9^{th} leaf, grade 3 at the level of the 17^{th} leaf, and grade 4 when the affected leaves are seen underneath the level of the 17^{th} leaf.

To manage a scale with a smaller number of grades to facilitate the analysis and the diagramming of tables and figures, we used the same scale proposed by Arias *et al.* (2014), but grades 1 and 2 were combined into grade 1-2 and grades 3 and 4 were combined into grade 3-4. The combination of grades was confirmed by a multiple correspondence analysis of an exploratory nature from which two dimensions were extracted from the leaf area (LA) and specific leaf area (SLA) data. The planting year, the number of the lot or farm, the genetic origins, and the degrees of severity were included as factors of plumero, yielding an explained variability of 70.72%. This can be observed in Figure 1; the proximity between the points correspond to grades 1 and 2, as well as grades 3 and 4.

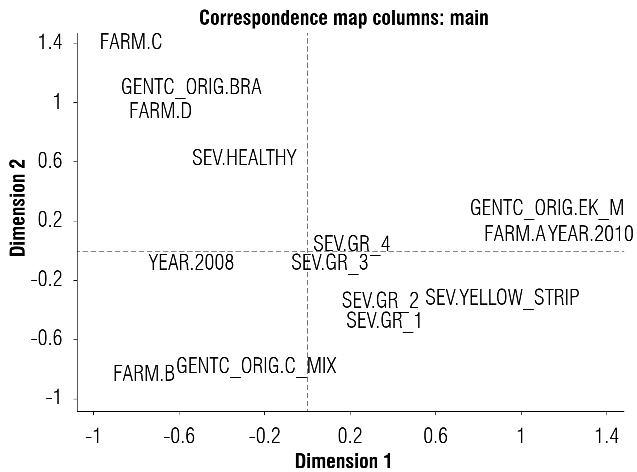


FIGURE 1. Multiple correspondence analysis of leaf area (LA) and specific leaf area (SLA). GENTC_ORIG.BRA: genetic origin Brabanta; GENTC_ORIG.C_MIX: genetic origin Congo Mixed; GENTC_ORIG.EK_M: genetic origin Ekona x Djongo * Mongana; YEAR.2008: planting year 2008; YEAR.2010: planting year 2010; SEV.HEALTHY: severity healthy; SEV.YELLOW STRIP: severity yellow strip; SEV.GR_1: severity grade 1; SEV.GR_2: severity grade 2; SEV.GR_3: severity grade 3; SEV.GR_4: severity grade 4.

Palm sampling

Samples were collected from 24 healthy palms and 24 palms from each of the severity grades of plumero, according to the multiple correspondence analysis listed in Table 1. The palms were randomly selected. The following variables were determined for each of the palms: leaf area (LA), specific leaf area (SLA), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t), primary root dry mass (PRDM), secondary root dry mass (SRDM), tertiary and quaternary root dry mass (TQRDM).

Leaf area and specific leaf area

The leaf area of leaf 17 (LA17) is an indicator of the total leaf area of the oil palm. LA17 has been used as a model for different measurements in the oil palm due to its average location within the total leaves of the palm and its stable nutrient content (Corley and Tinker, 2003). The formula proposed by Corley *et al.* (1971) was used to determine the leaf area of leaf 17 (LA) for the oil palms *Elaeis guineensis* (Eq. 1).

$$LA17 = 0.55 * [(LW) * n] \quad (1)$$

where LA17 is the leaf area of leaf 17(m^2), 0.55 is the correction factor for the palms *Elaeis guineensis*, L is the average length of the four largest central leaflets (m), W is the average width of the four longest central leaflets, and n is the total number of leaflets.

To determine the SLA the method of López *et al.* (2014) was used where a 30 cm long segment was taken from the middle part of two central leaflets of leaf 17. The width (w) and area of each segment was measured. Segments were then dried in an oven at 75°C for 72 h, and the dry weight was determined. Thus, the SLA ($cm^2\ g^{-1}$) was equal to the mean area of the leaf segments divided by their dry weight.

$$SLA = l\ w / p \quad (2)$$

where, l is the average length of the middle part of the leaflet that in this case takes the value of 30 cm, w is the mean mid-width of the leaflets, and p is the mean of the dry weight of the middle part of the leaflets.

Chlorophyll

The contents of Chl a, Chl b and Chl t were determined using the acetone extraction method (Flórez and Cruz, 2004). This consisted of collecting 10 leaf disks of 5 ml in diameter of non-veined plant tissue that were then preserved in Eppendorf tubes with 2 ml of 99% absolute alcohol. The tubes were labelled, covered with aluminum foil, and refrigerated. The leaf disks were macerated with 8 ml of a cold solution of 80% acetone and $CaCO_3$ (0.5 g L^{-1}). Absorbances of 645 nm and 663 nm were determined in the macerate using a spectrophotometer (BioMate 3, Madison, USA). The following equations were used to calculate the content of Chl a (Eq. 3), Chl b (Eq. 4) and Chl t (Eq. 5):

$$\text{Chl a} = \{[(12.7 \times D663) - (2.69\ D645)] \times V\} / (1000 \times W) \quad (3)$$

$$\text{Chl b} = \{[(22.8 \times D645) - (4.48\ D663)] \times V\} / (1000 \times W) \quad (4)$$

$$\text{Chl t} = \{[(20.2 \times D645) + (8.02\ D663)] \times V\} / (1000 \times W) \quad (5)$$

where D is the optical density, V the volume of the extract used in the determination of the optical density in ml, and W is the fresh mass in mg of the 10 leaf disks of plant tissue.

Root dry mass

Soil samples were collected at the four cardinal points of the base of the palm tree using a sharp-edged steel cylinder of known volume. The roots were then washed, separated into primary, secondary, tertiary and quaternary roots that were then dried in an oven at 75°C for 72 h. Since the weight of the roots was in grams and the volume of the soil sample was known, the data was extrapolated to dried roots per cubic meter of soil (kg m^{-3}).

Statistical analysis

An ordinal regression model with a degree of severity of *P* was applied as the response variable. The income variables were LA, SLA, Chl a, Chl b, Chl t, PRDM, SRDM, TQRDM in the statistical program Rstudio. Analysis of variance (ANOVA) and the test of minimum significant difference was performed with an alpha calculated using the Bonferroni correction (α_B) (Eq. 6).

$$\alpha_B = 1 - (1 - \alpha)^{1/n} \quad (6)$$

where n is the number of variables in this research; the number of significant variables in the model was n = 4, so the alpha with the Bonferroni correction used was $\alpha_B = 0.0127$ with $\alpha = 0.05$. A matrix of correlations between variables was performed to assess the relevance of using univariate analysis instead of multivariate analysis.

To facilitate the analysis, grade 1 was collapsed with grade 2 (labelled as grade 1-2) and grade 3 was collapsed with grade 4 (labelled as grade 3-4). This collapse was performed considering an analysis of multiple correspondences that was performed as data exploration. The proximity between the points corresponding to grades 1 and 2, as well as grades 3 and 4 descriptively validated the collapse of the categories. The veracity or falsity of atypical data was also determined using the box-plot graph of the RStudio software.

Results

All ordinal regression models evaluated reported the variables LA and SLA as significant ($P<0.05$) and indicated comparisons of degrees of severity: healthy with yellow strip, yellow strip with grade 1-2, and grade 1-2 with grade 3-4. However, the best fit model was model 4, where the variables LA, SLA, Chl t, TQRDM and the severity degree comparisons were reported as significant (healthy with yellow strip, yellow strip with grade 1-2; and grade 1-2 with grade 3-4). This model also reported an Akaike information criterion value of 279.38, the lowest among the various adjusted models (Tab. 1). The parameters of the best fit model are shown in Table 2. This model yielded 53.6% correct classifications; that is 53.6% of the palms observed at a certain degree of severity were correctly classified in that same degree of severity. The correlation matrix (Tab. 3) indicates that the variables LA, SLA, Chl t, and TQRDM were not correlated, so it was not applicable for performing multiple variance analysis.

TABLE 1. Adjusted ordinal models.

Variable	Model 1	Model 2	Model 3	Model 4
	Probability			
LA	<0.0001	<0.0001	<0.0001	<0.0001
SLA	0.0069	0.0058	0.0078	0.0063
Chl t	0.0450	0.0464	0.0601	0.0021
Chl b	0.1318	0.1324	0.1741	
PRDM	0.6483			
SRDM	0.3539	0.4075		
TQRDM	0.0010	0.0011	0.0004	0.0010
Healthy | Yellow strip	<0.0001	<0.0001	<0.0001	<0.0001
Yellow strip | grade 1-2	<0.0001	<0.0001	<0.0001	<0.0001
Grade 1-2 | grade 3-4	<0.0001	<0.0001	<0.0001	<0.0001
Ordinal statistical models				
Statistics	Model 1	Model 2	Model 3	Model 4
Akaike information criterion	271.17	269.38	268.08	267.94

LA - leaf area, SLA - specific leaf area, Chl b - chlorophyll b, Chl t - total chlorophyll, PRDM - primary root dry mass, SRDM - secondary root dry mass, TQRDM - tertiary and quaternary root dry mass.

TABLE 2. Estimated parameters for the reduced model 4.

Variable	Estimator	Standard error	t value	Probability
LA	-1.025	0.180	-5.684	<0.0001
SLA	-0.055	0.020	-2.730	0.0063
Chl t	-0.810	0.264	-3.069	0.0021
TQRDM	-0.699	0.213	-3.282	0.0010
Healthy | yellow strip	-14.948	2.239	-6.675	<0.0001
Yellow strip | grade 1-2	-13.764	2.180	-6.314	<0.0001
Grade 1-2 | grade 3-4	-11.925	2.087	-5.714	<0.0001

LA - leaf area, SLA - specific leaf area, Chl t - total chlorophyll, TQRDM - tertiary and quaternary root dry mass.

TABLE 3. Correlation matrix between physiological variables.

	SLA	Chl t	LA	TQRDM
SLA		-0.1113	0.2348	0.1421
Chl t	-0.1113		0.0742	-0.0791
LA	0.2348	0.0742		0.0443
TQRDM	0.1421	-0.0791	0.0443	

SLA - specific leaf area, Chl t - total chlorophyll, LA - leaf area, TQRDM - tertiary and quaternary root dry mass.

Univariate analyses indicated that the variables LA, SLA, Chl t and TQRDM were significant ($P<0.05$) which means that LA, SLA, Chl t and tertiary and quaternary root dry mass were different in healthy palms from those affected by plumero (Tab. 4).

TABLE 4. Anova of the variables leaf area (LA), specific leaf area (SLA), total chlorophyll (Chl t) and tertiary and quaternary root dry mass (TQRDM).

		LA			
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	59.3596	19.7865	17.89	<0.0001
Error	120	132.7284	1.106		
Total	123	192.088			
		SLA			
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	1368.9987	456.3329	4.99	0.0027
Error	120	10978.4834	91.4873		
Total	123	12347.4821			
		Chl t			
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	4.5253	1.5084	3.04	0.0316
Error	120	59.4762	0.4956		
Total	123	64.0016			
		TQRDM			
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	14.8904	4.9634	8.58	<0.0001
Error	117	67.666	0.5783		
Total	120	82.5565			

DOF - Degrees of freedom

Leaf area, specific leaf area

The leaves of palms affected by plumero (yellow strip, grade 1-2, and grade 3-4) had lower LA, SLA and Chl t than healthy palms (Fig. 2A-C). The roots of affected palms increased TQRDM at the initial stages (yellow strip and grade 1-2) but then decreased significantly in grade 3-4 (Fig. 2D).

Chlorophyll

The leaves of palms affected by plumero (yellow strip, grade 1-2, and grade 3-4) showed lower Chl t content than healthy palms (Fig. 2C).

Root dry mass

The dry matter of tertiary and quaternary roots increased in the initial degrees of severity (yellow strip and grade 1-2) compared to healthy palms, and it was significantly reduced in the last degrees (grade 3-4) (Fig. 2D).

Discussion

Leaf area and specific leaf area

LA progressively decreased as the degree of severity increased (Fig. 2A). This occurs long before a reduction in the width of the leaflet, as described by Arias *et al.* (2014) in the plumero severity scale. The decrease in LA caused by some pathogens drastically affects the interception of solar radiation and reduces the generation and distribution of biomass (Carretero *et al.*, 2009) because they accelerate the necrosis and senescence of leaves, causing fewer photoassimilates to be used in the synthesis of dry matter (Schierenbeck *et al.*, 2014).

The palms affected in grade 3-4 had thicker leaf laminae. The SLA indicated the variation in the relative thickness of the leaves as a consequence of leaf structural alterations, which make the leaf very sensitive to environmental and external factors (Reyes-Santamaría *et al.*, 2000), and a good indicator of crop productivity (Poorter and de Jong, 1999). The reduction of SLA indicated that the leaves have a thicker mesophyll due to the higher number and size of layers of palisade cells (Rodríguez and Cayón, 2008). This suggests that the number of photoassimilates in these leaves is greater (Ayala and Gómez, 2000) or photoassimilates are not efficiently transported from source to sink in response to nutritional deficiencies of elements such as boron (Wimmer and Eichert, 2013), magnesium (Verbruggen and Hermans, 2013) or potassium (Gerardeaux *et al.*,

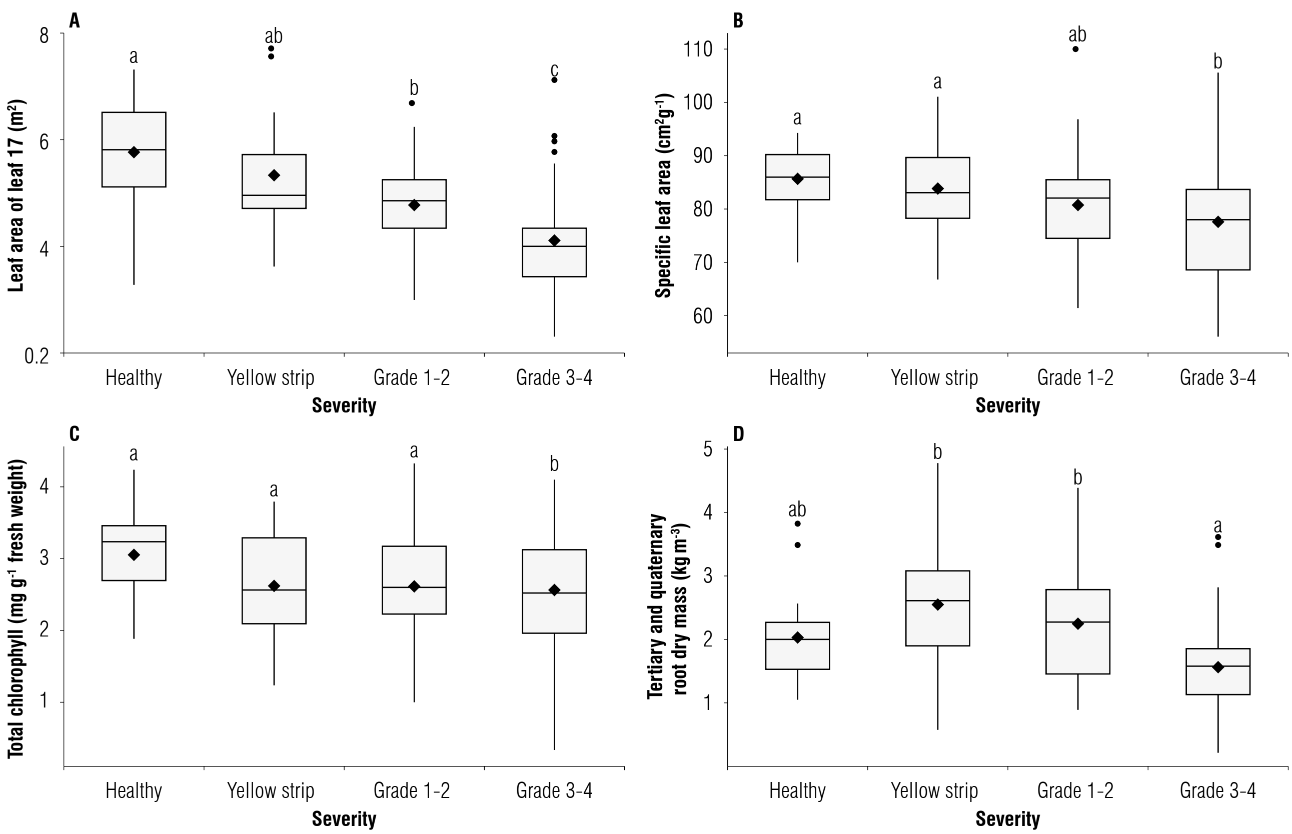


FIGURE 2. A) Leaf area of leaf 17 (m^2), B) specific leaf area, C) total chlorophyll and D) tertiary and quaternary root dry mass in healthy and affected palms by plumero. Different letters indicate differences in means with $P = 0.0127$.

2010). According to Nenova (2006), SLA is also increased due to iron deficiency and decreases due to excess iron, as observed in plants affected by plumero. However, palms affected by this disorder have a lower SLA because the leaf does not expand as the leaflets of healthy plants do. The expansion of the cells can be affected by stressful conditions such as temperature, evaporation, or water content of the soil, although the plant recovers quickly once the stress is over (Sadok *et al*., 2007). However, the elongation and final leaf area are affected in some cases when stress occurs in the last phase of leaf elongation (Granier and Tardieu, 2009).

Chlorophyll

Palms affected by plumero significantly reduced the chlorophyll content and root growth. A decrease in the chlorophyll content in response to abiotic and biotic stress is manifested by foliar yellowing, followed by wilting, affecting photosynthesis (Munné-Bosch, 2008) and consequently, reducing plant biomass (Casierra-Posada and Cutler, 2017; Sánchez-Reinoso *et al*., 2019). Mandal *et al*. (2009) stated that the chlorophyll content in diseased leaves of *Plantago ovata* affected by downy mildew was reduced by 24.39% in slightly chlorotic and 44.90% in severely chlorotic leaves as compared to healthy leaves, which appears to be one of the causes for the reduction of the photosynthesis process.

There are several alternatives to evaluate the establishment and development of a disease, such as estimating the chlorophyll content in the leaf (Uddling *et al*., 2007). Such estimation could provide a better alternative to evaluate disease severity in a plant (Chang *et al*., 2015) and be a good indicator for the degree of disease or infection and changes during pathogenesis (Rakib *et al*., 2019). The chlorophyll content in Ganoderma-infected oil palm seedlings declines as the infection progresses (Goh *et al*., 2016). Chang *et al*. (2015) also report that the chlorophyll content decreases as the disease progresses in different stages of cucumber growth. Chlorophyll content also decreases due to deficiencies of other nutrients including molybdenum (Agarwala *et al*., 1978) and boron. The latter generates oxidative damage in chloroplasts (Wimmer and Eichert, 2013). The reduction in chlorophyll content can also be caused by an attack of pathogens. In deciduous leaves attacked by phytoplasmas, chlorosis occurs as a form of cell death at the pathogen's entry points (Mittelberger *et al*., 2017).

The chlorophyll content is used to determine disease severity in some crops. In the case of fir decline, Oren *et al*. (1993) propose chlorophyll content and foliar nutrient analysis as a method to represent a range of severity for chlorosis to quickly identify the processes that occur in the soil of some areas where a decline occurs.

Root dry mass

In palms affected by plumero in the initial stages (yellow strip and grade 1-2) an increase in the development of tertiary and quaternary roots has been observed. This type of response could be associated with the response system of the plants to a sulfur deficiency with the slow initial growth of lateral roots and then with the rapid root growth (Hoefgen and Nikiforova, 2008; Gruber *et al*., 2013).

Conclusions

The plumero disorder compromises the physiological and productive performance of the affected palms by affecting the expansion of the leaf area and the development of roots. This characterization contributes to knowledge of the physiological damages caused by the plumero and is a fundamental tool for managing the disorder in the affected plantations.

Acknowledgments

Thanks to Unipalma de los Llanos S.A. for its support in the development of this research.

Literature cited

Agarwala, S.C., C.P. Sharma, S. Farooq, and C. Chatterjee. 1978. Effect of molybdenum deficiency on the growth and metabolism of corn plants raised in sand culture. Can. J. Bot. 56(16), 1905-1908. Doi: 10.1139/b78-227

Arias, N., D. Ibagué, and A. Ospino. 2014. Guía de bolsillo. Identificación y registro del Plumero en palma de aceite. Centro de Investigación en Palma de Aceite CENIPALMA, Bogota.

Ayala, I.M. and P.L. Gómez. 2000. Identificación de variables morfológicas y fisiológicas asociadas con el rendimiento en materiales de palma de aceite (*Elaeis guineensis* Jacq.). Rev. Palmas 21, 10-21.

Carretero, R., R.A. Serrago, M.O. Bancal, A.E. Perelló, and D.J. Miralles. 2009. Absorbed radiation and radiation use efficiency as affected by foliar diseases in relation to their vertical position into the canopy in wheat. Field Crops Res. 116, 184-195. Doi: 10.1016/j.fcr.2009.12.009

Casierra-Posada, C. and J. Cutler. 2017. Photosystem II fluorescence and growth in cabbage plants (*Brassica oleracea* var. capitata) grown under waterlogging stress. Rev. UDCA Act. Div. Cient. 20(2), 321-328. Doi: 10.31910/rudca.v20.n2.2017.390

Cayón, D.G., C.A. Avellaneda, and F. Rodríguez. 2007. Aspectos fisiológicos asociados a marchitez letal de la palma de aceite. Rev. Palmas 28(1), 373-382.

Chang, R.K., Y.H. Wang, X.T. Zhang, G.C. Tang, and Y. Wei. 2015. The research of disease detection method of greenhouse cucumber leaf based on chlorophyll fluorescence analysis. Univers. J. Agric. Res. 3(3), 76-80. Doi: 10.13189/ujar.2015.030302

Corley, R.H.V., J.J. Hardon, and G.Y. Tang. 1971. Analysis of growth of the oil palm (*Elaeis guineensis* Jacq.) I. Estimation of growth parameters and application in breeding. Euphytica 20, 307-315.

Corley, R.H.V. and P.B.H. Tinker. 2003. The oil palm. 4th ed. Blackwell Science, Oxford, UK.

Espinal, L. and E. Montenegro. 1963. Formaciones vegetales de Colombia: memoria explicativa sobre el mapa ecológico. Instituto geográfico Agustín Codazzi IGAC, Bogota.

Fischer, G. and L.M. Melgarejo. 2020. The ecophysiology of cape gooseberry (*Physalis peruviana* L.) - an Andean fruit crop. A review. Rev. Colomb. Cienc. Hortíc. 14(2), 76-89. Doi: 10.17584/rcch.2020v14i1.10893

Fischer, G., F. Ramírez, and F. Casierra-Posada. 2016. Ecophysiological aspects of fruit crops in the era of climate change. A review. Agron. Colomb. 34(2), 190-199. Doi: 10.15446/agron.colomb.v34n2.56799

Flórez, V. and R. Cruz. 2004. Guías de laboratorio de fisiología vegetal. 1st ed. Universidad Nacional de Colombia, Bogota.

Gerardeaux, E., L. Jordan-Meille, J. Constantin, S. Pellerin, and M. Dingkuhn. 2010. Changes in plant morphology and dry matter partitioning caused by potassium deficiency in *Gossypium hirsutum* (L.). Environ. Exp. Bot. 67(3), 451-459. Doi: 10.1016/j.envexpbot.2009.09.008

Goh, K.M., M. Dickinson, P. Alderson, L.V Yap, and C.V. Supramaniam. 2016. Development of an in planta infection system for the early detection of *Ganoderma* spp. in oil palm. J. Plant Pathol. 98(2), 255-264. Doi: 10.4454/JPP.V98I2.019

Granier, C. and F. Tardieu. 2009. Multi-scale phenotyping of leaf expansion in response to environmental changes: the whole is more than the sum of parts. Plant Cell Environ. 32, 1175-1184. Doi: 10.1111/j.1365-3040.2009.01955.x

Gruber, B.D., R.F.H. Giehl, S. Friedel, and N. von Wirén. 2013. Plasticity of the *Arabidopsis* root system under nutrient deficiencies. Plant Physiol. 163, 161-179. Doi: 10.1104/pp.113.218453

Hoefgen, R. and V.J. Nikiforova. 2008. Metabolomics integrated with transcriptomics: assessing systems response to sulfur-deficiency stress. Physiol. Plant. 132, 190-198. Doi: 10.1111/j.1399-3054.2007.01012.x

López, J. 2014. Caracterización fisiológica y morfológica de palmas de aceite Taisha (*Elaeis oleífera* HBK Cortes) y sus híbridos (*Elaeis oleífera* HBK Cortes x *Elaeis guineensis* Jacq.) en la región Amazónica del Ecuador. MSc thesis, Universidad Nacional de Colombia, Bogota.

Mandal, K., R. Saravanan, S. Maiti, and I.L. Kothari. 2009. Effect of downy mildew disease on photosynthesis and chlorophyll fluorescence in *Plantago ovata* Forsk. J. Plant Dis. Prot. 116(4), 164-168. Doi: 10.1007/BF03356305

Mittelberger, C., H. Yalcinkaya, C. Pichler, J. Gasser, G. Scherzer, T. Erhart, S. Schumacher, B. Holzner, K. Janik, P. Robatscher, T. Müller, B. Kräutler, and M. Oberhuber. 2017. Pathogen-induced leaf chlorosis: products of chlorophyll breakdown found in degreened leaves of phytoplasma-infected apple (*Malus x domestica* Borkh.) and apricot (*Prunus armeniaca* L.) trees relate to the pheophorbide *a* oxygenase/phyllobilin pathway. J. Agric. Food Chem. 65(13), 2651-2660. Doi: 10.1021/acs.jafc.6b05501

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11(1), 15-19. Doi: 10.1016/j.tplants.2005.11.002

Munné-Bosch, S. 2008. Do perennials really senesce? Trends Plant Sci. 13(5), 216-220. Doi: 10.1016/j.tplants.2008.02.002

Nenova, V. 2006. Effect of iron supply on growth and photosystem II efficiency of pea plants. Gen. Appl. Plant Physiol. Special issue, 81-90.

Oren, R., K.S. Werk, N. Buchmann, and R. Zimmermann. 1993. Chlorophyll-nutrient relationships identify nutritionally caused decline in *Picea abies* stands. Can. J. For. Res. 23(6), 1187-1195. Doi: 10.1139/x93-150

Poorter, H. and R. de Jong. 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytol. 143(1), 163-176. Doi: 10.1046/j.1469-8137.1999.00428.x

Rakib, M.R.M., A.H. Borhan, and A.N. Jawahir. 2019. The relationship between SPAD chlorophyll and disease severity index in *Ganoderma*-infected oil palm seedlings. J. Bangladesh Agricult. Univ. 17(3), 355-358. Doi: 10.3329/jbau.v17i3.43211

Ramírez, J., R. Bedoya, J. Guerrero, W. Valero, S. Otero, A. Erazo, and R. Bedoya. 2004. Resumen de actividades realizadas sobre la marchitez letal 1994-2004. Palmar del Oriente S.A.

Rodríguez, P.A. and D.G. Cayón. 2008. Efecto de *Mycosphaerella fijiensis* sobre la fisiología de la hoja de banano. Agron. Colomb. 26(2), 256-265.

Sadok, W., P. Naudin, B. Boussuge, B. Muller, C. Welcker, and F. Tardieu. 2007. Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. Plant Cell Environ. 30, 135-146. Doi: 10.1111/j.1365-3040.2006.01611.x

Sánchez-Reinoso, A.D., Y. Jiménez-Pulido, J.P. Martínez-Pérez, C.S. Pinilla, and G. Fischer. 2019. Chlorophyll fluorescence and other physiological parameters as indicators of waterlogging and shadow stress in lulo (*Solanum quitoense* var. *septentrionale*) seedlings. Rev. Colomb. Cienc. Hortíc. 13(3), 325-335. Doi: 10.17584/rcch.2019v13i3.10017

Schierenbeck, M., M.C. Fleitas, and M.R. Simón. 2014. Componentes ecofisiológicos involucrados en la generación de biomasa afectados por enfermedades foliares en trigo. Rev. Agron. Noroeste Argent. 34(2), 247-250.

Tailliez, B. 1971. The root system of the oil palm on the San Alberto Plantation in Colombia. Oleagineux 26(7), 435-447.

Uddling, J., J. Gelang-Alfredsson, K. Piikki, and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91, 37-46. Doi: 10.1007/s11120-006-9077-5

Verbruggen, N. and C. Hermans. 2013. Physiological and molecular responses to magnesium nutritional imbalance in plants. Plant Soil 368, 87-99. Doi: 10.1007/s11104-013-1589-0

Wimmer, M.A. and T. Eichert. 2013. Review: mechanisms for boron deficiency-mediated changes in plant water relations. Plant Sci. 2003-2004, 25-32. Doi: 10.1016/j.plantsci.2012.12.012

Narrow and twin-row plantings do not increase maize yield

Siembras en hileras estrechas y mellizas no incrementan el rendimiento del maíz

Luís Sangoi[1], Amauri Schmitt[1], Marcos Cardoso Martins Júnior[1]*, Hugo François Kuneski[1], and Antonio Eduardo Coelho[1]

ABSTRACT

Reducing row space and sowing in twin rows of maize (*Zea mays* L.) allow more equidistant plant distribution at the same density. The objective of this research was to evaluate the effect of these two management practices on the nitrogen content of the index leaf, the leaf area index at silking, and the grain yield of maize at different plant densities. The experiment was carried out in Lages, Santa Catarina State University, in southern Brazil during the growing seasons 2016-2017 and 2017-2018. A split-plot arrangement of a randomized complete block design was used. Two plant densities (7 and 9 plants m^{-2}) were distributed in the main plot, and five row spaces (0.4, 0.6, 0.8, 1.0 m and twin rows 0.6 m apart with 0.18 m between rows) were evaluated in split-plots. Physiological traits and grain yield were determined on the maize hybrid P30R50YH. The experiments were sown on 10/20/2016 and 10/21/2017. Kernel yields were higher at the plant density of 9 plants m^{-2} than at 7 plants m^{-2}. The row space did not affect the nitrogen content of the index leaf, the crop leaf area index at silking, and the maize grain yield. The increment of plant density was more effective than the use of narrow and twin rows to enhance P30R50YH hybrid grain yield.

Key words: grain yield, plant arrangement, plant density.

RESUMEN

La reducción del espacio entre líneas y la siembra en líneas dobles en maíz (*Zea mays* L.) permite una distribución más equidistante de las plantas con una misma densidad. El objetivo de este trabajo fue evaluar los efectos de dos prácticas de manejo sobre el contenido de nitrógeno en la hoja índice, el índice de área foliar del cultivo y el rendimiento de granos de maíz a diferentes densidades de plantas. El experimento fue realizado en Lages, en la Universidad del Estado de Santa Catarina, en el sur de Brasil, en los periodos de cosecha 2016/2017 y 2017/2018. Se utilizó un arreglo de parcelas divididas de un diseño experimental de bloques completos al azar. En la parcela principal se evaluaron dos densidades (7 y 9 plantas m^{-2}) y en las subparcelas se evaluaron cinco espacios entre líneas (0.4 m, 0.6 m, 0.8 m, 1.0 m y líneas dobles de 0.6 m entre cada una y 0.18 m entre líneas del par). Se evaluaron las características fisiológicas y el rendimiento de granos del maíz hibrido P30R50YH, sembrado en 20/10/2016 y 21/10/2017. Los rendimientos de grano fueron mayores en la densidad de 9 plantas m^{-2} que a 7 plantas m^{-2}. El espacio entre filas no afectó el índice de contenido de nitrógeno en la hoja, el índice de área foliar del cultivo en el espigamiento, y el rendimiento de granos. El incremento en la densidad de plantas fue más eficiente que la reducción del espacio entre líneas y la siembra en líneas dobles para incrementar el rendimiento del hibrido P30R50YH.

Palabras clave: rendimiento de granos, distribución de plantas, densidad de plantas.

Introduction

The adequate manipulation of plant arrangements is a very important management strategy for optimizing maize grain yield because it affects the leaf area index, the leaf insertion angle, and the crop efficiency for intercepting solar radiation at different canopy layers (Argenta *et al*., 2001; Sangoi *et al*., 2019).

The right choice of row spacing and plant density are two crucial decisions because they will determine the best use of abiotic factors, such as water, solar radiation, and soil nutrients, so that maize can express its physiological potential to produce grains (Penariol *et al*., 2003).

At the same plant density, a reduction in row spacing allows a greater distribution of plants per area. Therefore, the use of narrow rows may enhance the interception of solar radiation, improve water and nutrient uptake, and increase maize's ability to suppress weed growth. Furthermore, it can reduce water losses by evaporation and favor the use of the same row spacing for maize and soybean speeds up sowing. Narrow rows also permit better seedling distribution along the sowing row due to the low speed of the seeder (Balbinot Jr. *et al*., 2011; Boiago *et al*., 2017).

Received for publication: 6 April, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.86117

[1] Centro de Ciencias Agroveterinarias, Universidade do Estado de Santa Catarina, Lages, Santa Catarina (Brazil).
[*] Corresponding author: marcos.junior1@edu.udesc.br

Another strategy to change maize row arrangement is by using a cropping system with twin rows (TR). The planting concept with TR involves the sowing of two neighboring rows with reduced space (16-18 cm) apart from the next two neighboring rows at a greater distance (50-60 cm). The TR system also aims to improve plant distribution per area to decrease intraspecific competition, increase solar radiation interception, and provide more space for leaf and root development (Balem, 2013).

Several factors increased the grower's interest in reducing maize row spacing from the traditional 0.7-0.9 m to 0.4-0.5 m. Among these factors are the development of hybrids more tolerant to crowding, the offer of a great number of selective herbicides to control weeds after plant emergence, and the high agility of the industry to design agricultural equipment adapted for its use in narrow row maize crops (Sangoi & Silva, 2016).

The use of narrow rows does not always enhance maize yield. It also increases the production costs due to the need for adjusting the machine to harvest the crop. Furthermore, it hampers post emergence management practices and increases mechanical damages to the plants during these operations (Jeschke, 2014).

The benefits of reducing row spacing or using twin rows depend on several factors such as the kind of hybrid, the sowing date, plant density, and grain yield expectation. The advantages are more pronounced when the growers use hybrids with erect leaves, sown at the end of the winter with plant densities higher than 70,000 plants ha^{-1} and a yield perspective greater than 10,000 kg ha^{-1} (Strieder *et al.*, 2008; Sangoi *et al.*, 2010).

This work was carried out based on the hypothesis that the reduction of row distance and the use of twin rows are effective strategies for manipulating plant arrangements that enhance maize yield. The experiment had the objective of evaluating the effects of narrow and twin rows on the index leaf nitrogen content, the crop leaf area index, and the maize grain yield at different plant densities.

Materials and methods

This study was conducted in the municipality of Lages, state of Santa Catarina, in the highlands of southern Brazil, during the 2016-2017 and 2017-2018 growing seasons. The experimental site is located at 27°50'35" S, 50°02'45" W, and 849 m a.s.l. The climate of the region is Cfb according to the Köppen classification system (Kottek *et. al.*, 2006) with mild summers. The average temperature of the hottest month is below 22°C. The winters are cold, and there is adequate rainfall throughout the year. The soil at the experimental site was a Rhodic Kandiudox (United States Department of Agriculture, 2010).

A soil analysis performed in August, 2016 showed the following values: 470 g kg^{-1} of clay, water pH 5.2, 13.5 mg dm^{-3} of P, 232 mg dm^{-3} of K, 42 g kg^{-1} of organic matter, 6.1 $cmol_c$ dm^{-3} of Ca, 2.6 $cmol_c$ dm^{-3} of Mg, and 0.1 $cmol_c$ dm^{-3} of Al. The experimental area was in rotation with soybean (*Glycine max* L.) during the summer with the presence of black oat (*Avena strigosa* Schreb) preceding maize during the winter.

A randomized block design was used with treatments arranged in split plots. Two plant densities were tested in the main plots (7 and 9 plants m^{-2}), and five row space distances in the split plots (0.4 m, 0.6 m, 0.8 m, 1.0 m and TR). The distance between the rows inside each TR was 0.18 m and the distance between pairs of TR was 0.6 m. The split plots had five replicates and consisted of four rows, with a length of 7 m. All data were collected from the second and third row, excluding 0.5 m at the end of each row.

The experimental area was fertilized with a mixture of nitrogen, phosphorus and potassium on the day of sowing, based on the results of soil analysis and in accordance with the Sociedade Brasileira de Ciência do Solo - Comissão de Química e Fertilidade do Solo (2004) recommendations to achieve a grain yield of 18,000 kg ha^{-1}. The sources used to supply N, P, and K were urea (45% N), triple superphosphate (46% P_2O_5), and potassium chloride (60% K_2O), respectively. The rates applied at sowing were equivalent to 30 kg ha^{-1} N, 295 kg ha^{-1} P_2O_5, and 170 kg ha^{-1} K_2O. Fertilizers were distributed on the soil surface close to the plant rows. Nitrogen was side-dressed at a rate of 250 kg ha^{-1}, and split into three different applications, when the crop was at the V4, V8 and V12 growth stages, according to the scale proposed by Ritchie *et al.* (1993).

The experiment was hand-sown in a no-tillage system over a dead coverage of black oat on October 20, 2016 and October 21, 2017. The single-cross hybrid P30R50YH was used in both growing seasons. This is a very productive early hybrid recommended for the highlands of Southern Brazil. Three seeds per hill were used. The distance between neighboring hills per line varied according to the plant density and row spacing. Strings previously marked with the proper distance between hills were used to assure the right seed distribution on each treatment. According to Richie *et al.* (1993), the experiment was thinned when plants were at the V2 stage to adjust plant density to the desired value. Seeds were treated with thiamethoxam (140 g) + fipronil (12 g) and fludioxonil (25 g) + metalaxyl (10 g) per 100 kg of seeds to prevent damages caused by insects and diseases during the crop emergence and early growth stages.

Weeds were controlled with two herbicide applications. The first, immediately after sowing and prior to plant emergence, with a combination of atrazine (1,250 g ha^{-1}) and metolachlor (1,250 g ha^{-1}). The second, after corn emergence when the plants were at V4, with tembotrione (100 g ha^{-1}). Two applications of the fungicides azoxystrobin (60 g ha^{-1}) + cyproconazole, (25 g ha^{-1}) were performed when maize was at V10 and V18 growth stages to prevent leaf diseases.

Soil moisture was monitored with tensiometers. The experiment was irrigated when soil water tension was below -0.4 MPa, to maintain soil moisture close to field capacity. In the first growing season, pluvial precipitation during the crop cycle was 680 mm, and three irrigations were applied. In the second growing season, total rainfall reached 710 mm and the experiment was irrigated twice. Nearly 30 mm of water was provided by a sprinkler system at each irrigation.

Five plants were tagged on each split plot when the crop reached the R1 growth stage (silking), according to Ritchie *et al.* (1993). These plants were used to determine the nitrogen content of the index leaf (leaf attached to the stem node of the ear), according to procedures described by Vargas *et al.* (2012).

The same five plants were used to estimate leaf area (LA) and leaf area index (LAI) at the growth stages of R1 (silking) and 30 d after silking, when kernels were at R3 (milk stage). Both measurements followed the procedures used by Leolato *et al.* (2017). LA was estimated measuring the length (L) and largest width (W) of each photosynthetic active leaf (leaf with >50% green area). Leaf area per plant was calculated adding all the leaf measures and using the following equation:

$$LA = L \times W \times 0.75 \quad (1)$$

where 0.75 is a correction coefficient used because leaves do not have a rectangular area. LAI was determined dividing the leaf area per plant by the soil area occupied by each plant, according to the combination of plant density and row spacing.

The number of ears per plant was counted at harvest. The experiment was manually harvested on 04/20/2017 and 04/20/2018, when all leaves were senescent and kernel moisture ranged from 18% to 22%. The ears were shelled, and their grains were oven-dried using a drying oven with circulation/air exchange (MA035/1152, Marconi, Piracicaba, SP, Brazil) at 65ºC until the ears were at a constant weight. The number of kernels per ear, 1,000 grain weight and grain yield ha^{-1} were calculated and expressed at 13% grain moisture.

The data were statistically analyzed by analysis of variance using the SAS statistical software (SAS, 2016). The F test was performed in two ways: individually per growing season and collectively with data from both years. When F values for the main effects and interactions were significant at the 5% significance level ($P<0.05$), the means were compared by the Tukey's test ($P<0.05$).

Results and discussion

Significant statistical differences ($P<0.05$) were observed between the two growing seasons for the number of kernels per area, number of grains per ear, weight of 1,000 grains, and grain yield ha^{-1}. Data from these variables are presented separately for each experimental year. The data related to other variables are described collectively, considering the means of both growing seasons.

The nitrogen content of the index leaf ranged from 3.46% to 4.22%, depending on the plant arrangement (Tab. 1). The mean value for this variable in the experiment was 3.93%. The nitrogen content of the index leaf was affected by the plant density but not by row distance. The density of 7 plants m^{-2} showed a higher nitrogen percentage at the index leaf than the density of 9 plants m^{-2}. The lower N values observed at the higher density indicate that the competition for nitrogen was more intense in the crowded stand of maize. The same behavior was reported by Fontoura and Bayer (2009), who suggested an increase of 10 kg ha^{-1} in the nitrogen rate for each 5,000 plants added to plant populations above 65,000 plants ha^{-1}. Plants with adequate nitrogen supply will show a leaf nitrogen content ranging from 3.7% to 4.2% during maize flowering (Cantarella & Marcelino, 2008). Therefore, the results of the experiment show that the amount of nitrogen available in the soil was sufficient to fulfill the needs of the maize regardless of the plant arrangement.

TABLE 1. Nitrogen content of the index leaf of maize plants at silking in five row spaces and two plant densities. Mean values of two growing seasons (2016/2017 and 2017/2018).

Plant density (plants m^{-2})	Row distance (m)					Mean
	0.4	0.6	0.8	1.0	TR	
	---------- Index leaf nitrogen content (%) -------					
7	3.74	4.14	4.20	4.22	3.96	4.05 A
9	3.80	3.46	3.99	3.98	3.84	3.81 B
Mean	3.77 A	3.80 A	4.10 A	4.10 A	3.90 A	3.93

Means in the column followed by different uppercase letters are significantly different, and means in the row followed by the same letters are not significantly different according to the Tukey test (P<0.05). The distance between rows inside each twin row (TR) was 0.18 m and the distance between pairs of TR was 0.6 m. Coefficient of variance = 7.63% and 3.21% for plant density (main plot) and row space (split plot), respectively.

Leaf area index at silking and 30 d after silking and the senescent leaf area at maize flowering were significantly affected by plant density but not by row distance (Tab. 2). All row distances showed higher values at 9 plants m^{-2} than at 7 plants m^{-2}. In all treatments, LAI values measured at silking and 30 d after (kernels at milk stage) were greater than 5.0, considered by Andrade *et al.* (2002) and Robles *et al.* (2012) as the critical LAI required for the crop to intercept 95% of the incident solar radiation. The high LAI values, associated with the low senescent leaf area at flowering (less than 600 cm^2) indicate that maize plants had a favorable photosynthetic surface area for reaching high grain yield in the experiment.

Even the plant arrangement that combined high plant density (9 plants m^{-2}) and wide row space (1.0 m) did not speed up leaf senescence. The capacity to keep leaves photosynthetically active for a longer time during grain filling is an important feature in modern maize hybrids. It allows them a superior adaptation to environments with high intraspecific competition (Hammer *et al.*, 2009; Sangoi *et al.*, 2013).

The average grain yields were 15,440 and 16,570 kg ha^{-1} for the growing seasons of 2016-2017 and 2017-2018, respectively. During the first year grain yield ranged from 14,610 to 16,440 kg ha^{-1}, showing an amplitude of 1,830 kg ha^{-1} among the different plant arrangements (Tab. 3).

In the second year, grain yield varied from 15,650 to 17,570 kg ha^{-1}, having a range of 1,920 kg ha^{-1} among the different arrangements (Tab. 4). At both growing seasons, the best treatment (twin rows with a plant density of 9 plants m^{-2}) showed a 12% yield increase when compared to the worst

TABLE 2. Leaf area index (LAI) at silking, LAI 30 days after silking and senescent leaf area at silking of maize plants at five row distances and two plant densities. Mean values of two growing seasons (2016/17 and 2017/18).

Plant density (plants m^{-2})	Row distance (m)					Mean
	0.4	0.6	0.8	1.0	TR	
	------- LAI at silking ------					
7	6.6	6.2	6.0	6.1	6.7	6.3 B
9	7.5	7.4	7.4	7.0	7.7	7.4 A
Mean	7.0 A	6.8 A	6.7 A	6.6 A	7.2 A	6.8
	---- LAI 30 days after silking ----					
7	6.4	6.0	5.8	5.9	6.4	6.1 B
9	7.1	7.1	7.0	6.5	7.3	7.0 A
Mean	6.7 A	6.6 A	6.4 A	6.2 A	6.9 A	6.5
	--- Senescent leaf area at silking (cm^2 per plant) ---					
7	265	261	283	336	310	291 B
9	426	321	367	590	485	438 A
Mean	346 A	291 A	325 A	463 A	397 A	364

Means in the column followed by different uppercase letters are significantly different, and means in the row followed by the same uppercase letters are not significantly different according to the Tukey test (P<0.05). The distance between rows inside each twin row (TR) was 0.18 m and the distance between pairs of TR was 0.6 m. Coefficient of variance = 8.23%, 9.01% and 6.20% for plant densities (main plot) and 5.34%, 5.89% and 4.80% for row spaces (split plots) of the variables LAI at silking, LAI 30 days after silking, and senescent leaf area at silking, respectively.

treatment (row distance 0.4 m with a plant density of 7 plants m^{-2}). This low percentage difference indicates that there was a small effect of plant arrangement on maize yield. The environment interferes with the grain yield response of maize on the variations of plant density and row space. They can be pronounced according to the soil fertility, climate conditions, and management practices used during the crop growth and development (Schmitt, 2014).

TABLE 3. Grain yield (kg ha^{-1}) of maize plants at five row distances and two plant densities. 2016-2017.

Row distance (m)	Plant density (plants m^{-2})		Mean
	7	9	
0.4	14,610	15,630	15,120 A
0.6	15,620	15,780	15,700 A
0.8	15,050	15,400	15,220 A
1.0	14,810	15,730	15,270 A
TR	15,340	16,440	15,890 A
Mean	15,090 A	15,800 B	15,440

Means in the row followed by different uppercase letters are significantly different, and means in the column followed by the same uppercase letters are not significantly different according to the Tukey test (P<0.05). The distance between rows inside each twin row (TR) was 0.18 m, and the distance between pairs of TR was 0.6 m. Coefficient of variance = 6.40% and 4.86% for plant density (main plot) and row space (split plot), respectively.

TABLE 4. Grain yield (kg ha^{-1}) of maize plants at five row distances and two plant densities. 2017-2018.

Row distance (m)	Plant density (plants m^{-2})		Mean
	7	9	
0.4	16,130	17,570	16,850 A
0.6	16,000	17,400	16,700 A
0.8	15,820	16,640	16,230 A
1.0	15,650	16,410	16,030 A
TR	16,730	17,330	17,030 A
Mean	16,060 A	17,070 B	16,570

Means in the row followed by different uppercase letters are significantly different, and means in the column followed by the same uppercase letters are not significantly different according to the Tukey test (P<0.05). The distance between rows inside each twin row (TR) was 0.18 m and the distance between pairs of TR was 0.6 m. Coefficient of variance = 5.90% and 5.12% for plant density (main plot) and row space (split plot), respectively.

At both growing seasons, grain yield was affected by the main effect of plant density. The increase of plant population from 7 to 9 plants m^{-2} produced a yield increment of 4.7% and 6.3% in 2016-2017 and 2017-2018, respectively, on the mean of five row distances (Tabs. 3-4). The greater yield observed at the higher density was probably due to the favorable soil and climate conditions. The experiment was irrigated whenever necessary to keep soil moisture close to field capacity, and the soil fertility was high. The good management practices used during the crop growth and the high yield potential of hybrid P30R50YH also favored the response to crowding. Whenever the environmental conditions are suitable to high kernel yield, maize requires more plants per area to maximize grain yield (Jeschke, 2014; Sangoi *et al.*, 2019).

Row distance did not affect maize grain yield at both growing seasons, regardless of the plant density. This result shows that the reduction of row distance from 1.0 m to 0.4 m or the use of twin rows did not enhance maize yield. Therefore, the more even plant distribution promoted by narrow rows and TR was not an effective management strategy to increase kernel yield. Serpa *et al.* (2008), Novacek *et al.* (2013) and Robles *et al.* (2012) reported similar results in experiments carried out in Brazil and the United States of America, respectively.

The number of grains produced per area increased when plant density was raised from 7 to 9 plants m^{-2}, at both growing seasons (Tabs. 5-6). This yield component was not affected by row distance. On the other hand, the increase of plant density reduced the number of kernels per ear and the 1,000 grain weight, on the average of five row spaces. The number of ears per plant was close to 1 and did not change significantly with plant density and row distance. The yield component behavior proved the observations of Sangoi and Silva (2016) and Boiago *et al.* (2017), showing that the number of kernels per ear is the variable that has the largest impact on maize yield.

This study was carried out based on the hypothesis that the reduction of row distance and the use of twin rows are effective strategies to manipulate plant arrangement to enhance maize yield. This hypothesis is based on the premise that both management practices increase the distance between neighboring plants within the sowing row. The improvement in plant distribution may provide a better use of water, solar radiation, and nutrients (Boiago *et al.*, 2017).

The variables measured in the experiment did not confirm the theoretical expectation. The shrinkage of row distances from 1.0 m to 0.4 m or the row arrangement in TR did not significantly change the nitrogen content of the index leaf (Tab. 1) nor the crop LAI at silking and kernel at milk stage (Tab. 2). The absence of significant effects of a more even plant distribution derived from narrow and twin rows on these two physiological parameters was confirmed by the behavior of maize yield that was not affected by the five row distances tested in the trial (Tabs. 3-4).

TABLE 5. Yield components of maize plants at five row distances and two plant densities in 2016-17.

Plant density (plants m^{-2})	Row distance (m)					Mean
	0.4	0.6	0.8	1.0	TR	
	-------------------- Number of grains m^{-2} ---------------					
7	3946 (NS)	4002	3947	3869	3932	3940 B
9	4398	4359	4173	4356	4416	4341 A
Mean	4172 A	4181 A	4060 A	4113 A	4175 A	4140
	------------ Weight of 1,000 grains (g) ------------					
7	370	390	381	383	390	383 A
9	356	362	369	361	373	364 B
Mean	363 A	376 A	375 A	372 A	382 A	374
	----------- Number of grains per ear ----------					
7	574	566	581	544	549	563 A
9	488	515	488	516	492	500 B
Mean	531 A	541 A	535 A	531 A	521 A	532
	---------- Number of ears per plant ----------					
7	0.96	1.01	0.99	1.01	0.96	0.99 A
9	0.99	0.98	0.99	0.96	0.96	0.98 A
Mean	0.98 A	0.99 A	0.99 A	0.99 A	0.96 A	0.98

Means in the column followed by different uppercase letters are significantly different, and means in the row followed by the same uppercase letters are not significantly different according to the Tukey test ($P<0.05$). The distance between rows inside each twin row (TR) was 0.18 m and the distance between pairs of TR was 0.6 m. Coefficient of variance = 9.85%, 3.01%, 8.20% and 6.78 for plant density (main plot) and 8.34%, 2.89%, 7.87% and 5.85% for row space (split plot), for the variables number of grains m^{-2}, weight of 1,000 grains, number of grains per ear, and number of ears per plant, respectively.

TABLE 6. Yield components of maize plants at five row distances and two plant densities; Lages, SC, Brazil, 2017-2018.

Plant density (plants m^{-2})	Row distance (m)					Mean
	0.4	0.6	0.8	1.0	TR	
	--------------------- Number of grains m^{-2} ------------					
7	3734	3803	3734	3716	4090	3815 B
9	4414	4241	4153	4145	4405	4272 A
Mean	4074 A	4022 A	3943 A	3931 A	4248 A	4043
	------------- Weight of 1,000 grains (g) -------------					
7	432	421	424	422	410	422 A
9	398	411	401	396	394	400 B
Mean	415 A	416 A	412 A	409 A	402 A	411
	------------- Number of grains per ear -------------					
7	517	524	532	514	507	519 (A)
9	482	477	472	469	464	473 (B)
Mean	500 A	500 A	502 A	491 A	485 A	496
	----------- Number of ears per plant ------------					
7	0.98	0.99	1.03	1.03	1.05	1.02 (NS)
9	0.99	1.00	0.99	1.00	0.98	0.99
Mean	0.99	1.00 A	1.01 A	1.01 A	1.02 A	1.00

Means in the column followed by different uppercase letters are significantly different, and means in the row followed by the same uppercase letters are not significantly different according to the Tukey test ($P<0.05$). The distance between rows inside each twin row (TR) was 0.18 m and the distance between pairs of TR was 0.6 m. Coefficient of variance = 10.05%, 4.11%, 9.45% and 7.05% for plant density (main plot) and 8.85%, 3.49%, 8.05% and 6.15% for the number of grains m^{-2}, weight of 1,000 grains, number of grains per ear, and number of ears per plant, respectively.

According to Strieder *et al.* (2008) and Sangoi *et al.* (2010), the superior plant distribution derived from using narrow or twin rows has a higher probability of improving the maize grain yield when the crop is grown under conditions that favor high yields. For this reason, the experiments were conducted without water and nutrient restriction. The irrigation and the high soil fertility level provided conditions to attain grain productivity values that were nearly three times greater than the average Brazilian grain yield, which was 5,300 kg ha^{-1} at the growing season of 2019-2020 (CONAB, n.d.). However, even with yield ceilings above 15,000 kg ha^{-1}, no significant yield advantage of sowing in narrow or twin rows was found, regardless of the plant density (Tabs. 3-4).

The results shown in this research confirmed the observations by Andrade *et al.* (2002), Lee and Tollenaar (2007), and Jeschke (2014). These authors emphasized that narrow and twin rows have a greater potential for increasing maize grain yield under soil and climatic conditions that prevent the crop from reaching its critical LAI at flowering than when wide row distances are used. Under the environmental conditions of this experiment, the LAI at silking and 30 d after silking was closed to 6.0, even in the wider row spaces of 0.8 and 1.0 m. This shows that solar radiation interception was not compromised, because maize critical LAI ranges from 4.0 to 5.0, according to Andrade *et al.* (2002) and Robles *et al.* (2012).

Two other factors that probably contributed to the lack of response to the reduction of row distance were the soil fertility level and the leaf architecture of hybrid P30R50YH. Narrow rows can enhance nitrogen use efficiency (NUE) and increase maize capacity to uptake N from the soil (Barbieri *et al.*, 2008). The higher NUE may increase maize yield in nitrogen deficient environments (Barbieri *et al.*, 2000). The index leaf nitrogen content at silking measured in the trial (Tab. 1) was above the value considered adequate to meet maize requirements by Cantarella and Marcelino (2008). This indicates that there was no restriction in the nitrogen supply for the crop growth and development. The high N rates used in the trial, split over three top-dress fertilizations, contributed to assure an adequate nitrogen supply for the crop during its growth cycle.

The reduction of row distance and the sowing with twin rows have a superior possibility of improving maize yield when hyper-early hybrids, with erect leaves and short plants are grown. This kind of plant architecture allows a greater amount of solar radiation to reach different layers of the canopy. Furthermore, it also prevents the crop from achieving the critical LAI when low plant densities and wide row distances are used (Lauer *et al.*, 2012; Sangoi & Silva, 2016). The hybrid used in the experiment has an early cycle, produces tall plants (plant height ranging from 2.5 to 3.0 m), and has decumbent leaves. These traits allowed P30R50YH to reach the required leaf area index to intercept 95% of the incident solar radiation at all the plant arrangements evaluated in the trial.

Conclusions

The reduction of row distances or the use of twin rows do not change the nitrogen content of the index leaf, the crop leaf area index at silking, and the grain yield of maize hybrid P30R50YH, regardless of the plant density.

The increment of plant density from 7 to 9 plants m^{-2} is more efficient than the reduction of row distances or the sowing with twin rows to enhance grain yield of maize hybrid P30R50YH.

Acknowledgments

The authors would like to thank the Brazilian National Research Council (CNPq) for the scholarship granted to the first author. They would also thank FAPESC/PAP/UDESC for the financial support to carry out the experiment.

Author's contributions

LS and AS developed the methodology and designed the experiments, AS, MCMJr, HFK and AEC conducted the research and carried out the field and laboratory experiments, AS, MCMJr, HFK, LS and AEC applied the statistical techniques to analyze data, and LS, AS, MCMJr, HFK and AEC prepared the initial draft. All authors reviewed and edited the manuscript.

Literature cited

Andrade, F. H., Calviño, P., Cirilo, A., & Barbieri, P. (2002). Yield responses to narrow rows depend on increased radiation interception. *Agronomy Journal, 94*(5), 975–980. https://doi.org/10.2134/agronj2002.9750

Argenta, G., Silva, P. R. F., & Sangoi, L. (2001). Arranjo de plantas em milho: análise do estado-da-arte. *Ciência Rural, 31*(6), 1075–1084. https://doi.org/10.1590/S0103-84782001000600027

Balbinot Jr., A. A., Trezzi, M. M., & Vogt, G. A. (2011). Integration of practice for the weed management in corn. *Scientia Agraria, 12*(2), 81–87. https://doi.org/10.5380/rsa.v12i2.33724

Balem, Z. (2013). *Avaliação de espaçamento convencional e linhas gêmeas sob densidade populacional para cultura do milho.* [Master's thesis, Universidade Tecnológica Federal do Paraná]. UTFPR Repository. https://repositorio.utfpr.edu.br/

jspui/bitstream/1/528/1/PB_PPGAG_M_Balem%2c%20 Zenilson_2013.pdf

Barbieri, P. A., Saizs Rozas, H. R., Andrade, F. H., & Echeverría, H. E. (2000). Row spacing effects at different levels of nitrogen availability in maize. *Agronomy Journal, 92*, 283–288.

Barbieri, P. A., Echeverría, H., Saizs Rozas, H., & Andrade, F. H. (2008). Nitrogen use efficiency in maize as affected by nitrogen availability and row spacing. *Agronomy Journal, 100*(4), 1094–1100. https://doi.org/10.2134/agronj2006.0057

Boiago, R. G. F. S., Mateus, R. P. G., Schuelter, A. R., Barreto, R. R., Silva, G. J., & Schuster, I. (2017). Combinação de espaçamento entrelinhas e densidade populacional no aumento da produtividade em milho. *Revista Brasileira de Milho e Sorgo, 16*(3), 440–448. https://doi.org/10.18512/1980-6477/rbms.v16n3p440-448

Cantarella, H., & Marcelino, R. (2008). Fontes alternativas de nitrogênio para a cultura do milho. *Informações Agronômicas*, (122), 12–14.

CONAB. (n.d.). Serie Histórica das safras: Milho total (1ª, 2ª e 3ª safra). Retrieved September 18, 2020, from https://www.conab.gov.br/info-agro/safras/serie-historica-das-safras?start=20

Fontoura, S. M. V., & Bayer, C. (2009). Adubação nitrogenada para alto rendimento de milho em plantio direto na região Centro-Sul do Paraná. *Revista Brasileira de Ciência do Solo, 33*, 1721–1732. https://doi.org/10.1590/S0100-06832009000600021

Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., Zinselmeier, C., Paszkiewicz, S., & Cooper, M. (2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. Corn belt? *Crop Science, 49*(1), 299–312. https://doi.org/10.2135/cropsci2008.03.0152

Jeschke, M. R. (2014). *Is the future of corn production in narrow rows?* Pioneer. https://www.pioneer.com/us/agronomy/corn_production_narrow_rows.html

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrif, 15*(3), 259-263. https://doi.org/10.1127/0941-2948/2006/0130

Lauer, S., Hall, B. D., Mulaosmanovic, E., Anderson, S. R., Nelson, B., & Smith, S. (2012). Morphological changes in parental lines of Pioneer Brand maize hybrids in the U.S. Central Corn Belt. *Crop Science, 52*(3), 1033–1043.

Lee, E. A., & Tollenaar, M. (2007). Physiological basis of successful breeding strategies for maize grain yield. *Crop Science, 47*(S3), 202–215. https://doi.org/10.2135/cropsci2007.04.0010IPBS

Leolato, L. S., Sangoi, L., Durli, M. M., Panison, F., & Voss, R. (2017). Growth regulator and maize response to the increase in plant density. *Pesquisa Agropecuária Brasileira, 52*(11), 997–1015. https://doi.org/10.1590/s0100-204x2017001100005

Novacek, M. J., Mason, S. C., Galuscha, T. D., & Yaseen, M. (2013). Twin rows minimally impact irrigated maize yield, morphology and lodging. *Agronomy Journal, 105*(1), 268–276. https://doi.org/10.2134/agronj2012.0301

Penariol, F. G., Filho, D. F., Coicev, L., Bordin, L., & Farinelli, R. (2003). Comportamento de cultivares de milho semeadas em diferentes espaçamentos entre linhas e densidades populacionais, na safrinha. *Revista Brasileira de Milho e Sorgo, 2*(2), 52–60.

Ritchie, S. W., Hanway, J. J., & Benson, G. O. (1993). *How a corn plant develops.* Iowa State University of Science and Technology.

Robles, M., Ciampiatti, I. A., & Vyn, T. J. (2012). Response of maize hybrids to twin-row spatial arrangement at multiple plant densities. *Agronomy Journal, 104*(6), 1747–1756. https://doi.org/10.2134/agronj2012.0231

Sangoi, L., Silva, P. R. F., & Argenta, G. (2010). *Estratégias de manejo do arranjo de plantas para aumentar o rendimento de grãos de milho.* Udesc.

Sangoi, L., Zanin, C. G., Schmitt, A., & Vieira, J. (2013). Leaf senescence and response of maize hybrids commercially released in different times to crowding. *Revista Brasileira de Milho e Sorgo, 12*(1), 21–32.

Sangoi, L., & Silva, P. R. F. (2016). Estratégias de manipulação do arranjo de plantas e desempenho agronômico do milho. In J. A. Wordell Filho, & L. A. Chiaradia (Eds.), *A cultura do milho em Santa Catarina* (pp. 85–121). Epagri.

Sangoi, L., Schmitt, A., Durli, M. M., Leolato, L. S., Coelho, A. E., Kuneski, H. F., & Oliveira, V. L. (2019). Management of plant arrangement to optimize grain productivty in maize. *Revista Brasileira de Milho e Sorgo, 18*(1), 14–29. https://doi.org/10.18512/1980-6477/rbms.v18n1p47-60

SAS. (2016). Statistical Analysis System. SAS/INSIGHT User's guide. SAS Institute Inc.

Schmitt, A. (2014). *Arranjo de plantas para maximizar o desempenho agronômico do milho em ambientes de alto manejo.* [Doctoral dissertation, Universidade do Estado de Santa Catarina]. UDESC Repository. https://www.udesc.br/arquivos/cav/id_cpmenu/1370/tese_amauri_15706374988225_1370.pdf

Serpa, M. S., Jandrey, D., & Silva, P. R. F. (2008, July 15–17). *Redução do espaçamento entre-linhas no milho na época de semeadura do cedo da Depressão Central do Rio Grande do Sul, Brazil.* [Conference presentation abstract]. 53ª Reunião Técnica Anual de Milho. Pelotas, Brazil. CD-ROM.

Sociedade Brasileira de Ciência do Solo - Comissão de Química e Fertilidade do Solo (2004). Milho. In Manual de adubação e de calagem para os estados do Rio Grande do Sul e de Santa Catarina, (10), 140-143.

Strieder, M. L., Silva, P. R. F., Rambo, L., Sangoi, L., Silva, A. A., Endrigo, P. C., & Jandrey, D. B. (2008). Crop management systems and maize grain yield under narrow row spacing. *Scientia Agricola, 65*(4), 346-353.

United States Department of Agriculture (2010). *Keys to soil taxonomy* (11th ed). United States Department of Agriculture, Natural Resources Conservation Service.

Vargas, V. P., Sangoi, L., Ernani, P. R., Siega, E., Carniel, G., & Ferreira, M. A. (2012). Leaf attributes are more efficient than soil mineral N to evaluate the availability of this nutrient to maize. *Bragantia, 71*(2), 245–255. https://doi.org/10.1590/S0006-87052012000200014

Efficacy of imazapic/imazapyr and other herbicides in mixtures for the control of *Digitaria insularis* prior to soybean sowing

Efectividad de imazapic/imazapyr y otros herbicidas en mezclas para el control de *Digitaria insularis* en pre-siembra de soya

Alfredo Junior Paiola Albrecht[1], Leandro Paiola Albrecht[1], André Felipe Moreira Silva[2]*, Romulo Augusto Ramos[3], Everson Pedro Zeny[3], Juliano Bortoluzzi Lorenzetti[4], Maikon Tiago Yamada Danilussi[4], and Arthur Arrobas Martins Barroso[4]

ABSTRACT

Herbicide mixtures, use of multiple sites of action, and other weed management practices are necessary to avoid cases of biotype resistance. The aim of this study was to evaluate the efficiency of imazapic/imazapyr and other herbicides in mixtures to control *Digitaria insularis* at burndown before soybean sowing. This field research was conducted in Umuarama, State of Parana (PR), Brazil, in the 2018/19 soybean season. The experiment was conducted in a randomized block experimental design with four replicates and 11 treatments composed of the application of glyphosate, clethodim, haloxyfop, imazapic/imazapyr, glufosinate, 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, triclopyr, and saflufenacil, in mixtures. Weed control was evaluated as well as soybean injury and yield. An analysis of variance and F-test were performed, and the treatment means were compared by the Scott-Knott test. All treatments showed great control over the weed and low crop injury rate while maintaining soybean yield. The application of imazapic/imazapyr in mixtures with other herbicides was effective in controlling glyphosate-resistant *D. insularis* in burndown before soybean sowing and with sequential application of haloxyfop + glyphosate at V3 stage of soybean. This chemical management was also selective for soybean.

Key words: *Glycine max*, acetolactate synthase (ALS) inhibitors, weed, crop injury, chemical weed control.

RESUMEN

Las mezclas entre herbicidas, el uso de múltiples sitios de acción y otras prácticas de manejo de malezas son necesarias para evitar otros casos de resistencia de biotipos. El objetivo de este estudio fue evaluar la eficiencia de imazapic/imazapyr y otros herbicidas en mezclas para controlar *Digitaria insularis* en la desecación antes de la siembra de soya. Esta investigación de campo se realizó en Umuarama, Estado de Paraná (PR), Brasil, en la cosecha de soya de 2018/19. El experimento se realizó en un diseño experimental de bloques al azar, con cuatro repeticiones y 11 tratamientos, compuestos por la aplicación de glifosato, cletodim, haloxifop, imazapic/imazapir, glufosinato, ácido 2,4-diclorofenoxiacético (2,4-D), dicamba, triclopir y saflufenacil, en mezclas. Se evaluó el control de malezas, así como el daño y el rendimiento de la soya. Se realizaron un análisis de varianza y test F, y se compararon las medias de los tratamientos con el test de Scott-Knott. Todos los tratamientos mostraron un gran control sobre la maleza y una baja tasa de daño a los cultivos mientras se mantenía el rendimiento de la soya. La aplicación de imazapic/imazapir en mezclas con otros herbicidas fue eficaz en el control de *D. insularis* resistente al glifosato, en desecación antes de la siembra de soya y con aplicación secuencial de haloxifop + glifosato en la etapa V3 de la soya. Este manejo químico también fue selectivo para la soya.

Palabras clave: *Glycine max*, inhibidores de acetolactato sintasa (ALS), hierba, daño al cultivo, control químico de malezas.

Introduction

The weed *Digitaria insularis,* known as sourgrass, is native to tropical and subtropical areas of America. It can be found in pasture lands, coffee plantations, orchards, crop fields, roadsides, and in abandoned fields. It is referred to as rhizomatous and produces seeds throughout the summer, growing in clumps. These traits allow them to aggressively compete against cultivated crops (Moreira and Bragança, 2011; Lorenzi, 2014). There are four known *D. insularis* herbicide-resistant biotypes, all found in South America, from which three are glyphosate-resistant and one is resistant to haloxyfop and fenoxaprop. Weed resistance is a problem across the globe, and Brazil is ranked fifth with 50 registered resistance cases to EPSPs, ALS, ACCase, PSII, PSI, PPO, and auxins (Heap, 2020).

Received for publication: 21 October, 2019. Accepted for publication: 9 November, 2020. Doi: 10.15446/agron.colomb.v38n3.83046

[1] Universidade Federal do Paraná, Palotina, Parana (Brazil).
[2] Crop Science Pesquisa e Consultoria Agronômica, Parana (Brazil).
[3] Basf S.A., Santo Antônio de Posse, São Paulo (Brazil).
[4] Universidade Federal do Paraná, Curitiba, Parana (Brazil).
* Corresponding author: afmoreirasilva@alumni.usp.br

In addition to resistance cases, as a perennial grass *D. insularis* is hard to control, especially during flowering development (Zobiole *et al*., 2016; Canedo *et al*., 2019). A study by Gazziero *et al*. (2019) shows that a cohabitation of six *D. insularis* plants m^{-2} among soybean crops reduces its yield by 40%. Thus, appropriate managing practices including different control methods are shown to be important to have an efficient outcome.

The association of different herbicides, rotation of sites of actions, preventive methods, among other practices are essential to manage and prevent new weed biotypes resistant to herbicides (Green, 2018; Heap and Duke, 2018; Neve *et al*., 2018; Frisvold *et al*., 2020). However, in the literature, there are reports of the antagonist effect of 2,4-D on the action of ACCase inhibitor graminicides, due to the translocation reduction and increase in the metabolism of herbicides from the ariloxifenoxipropionics group (Trezzi *et al*., 2007). Thus, there is a need for studies on the different mechanisms of control of *D. insularis*.

Pre-emergent herbicides used before sowing are emphasized as an important tool to weed management, especially in crop fields (Byker *et al*., 2013; Belfry *et al*., 2015). In this sense, ALS inhibitors are highlighted for weed control as pre-emergence herbicides used in soybean fields (Braz *et al*., 2017; Underwood *et al*., 2017). Imidazolinone herbicides are part of the group of the acetolactate synthase (ALS) (also called acetohydroxyacid synthase [AHAS]) inhibiting herbicides which hamper the synthesis of the branched-chain amino acids valine, leucine, and isoleucine. After their absorption, the herbicides are translocated to meristems and apexes that are actively growing areas, inhibiting the growth of susceptible plants. Chlorosis happens to sensitive plants followed by death in 7 to 14 d after treatment (Oliveira Júnior, 2011; Shaner and O'Connor, 2017).

These herbicides are applied at pre- and post-emergence, controlling a range of monocotyledons and dicotyledons in cereal and soybean crops and nonagricultural areas (Oliveira Júnior, 2011; Rodrigues and Almeida, 2018). Piasecki and Rizzardi (2016) report that imazapic/imazapyr (premix formulation) is efficient for controlling volunteer corn at soybean pre-emergence. Likewise, studies by Melo *et al*. (2017) show the efficiency of imazapic/imazapyr on the control of *D. insularis*.

It is believed that the imazapic/imazapyr is effective in controlling *D. insularis* when applied at pre-sowing burndown. Thus, this study aimed to evaluate the efficiency of imazapic/imazapyr (premix formulation) and other herbicides in mixtures to control *D. insularis* at burndown before soybean sowing.

Materials and methods

Conditions and experimental design

This field research was conducted in Umuarama, State of Parana (PR), Brazil (23°50'25.23" S, 53°13'45.70" W) during the 2018/19 crop season. The soil was classified as sandy (11% clay, 6.5% silt, and 82.5% sand) with the following chemical properties on the 0-20 cm layer: pH ($CaCl_2$) of 4.4, 1.33% organic matter, and 5.78 $cmol_c$ dm^{-3} cation exchange capacity. Under the Köppen classification, the climate is classified as Cfa, and Figure 1 shows the weather conditions during the time of the research.

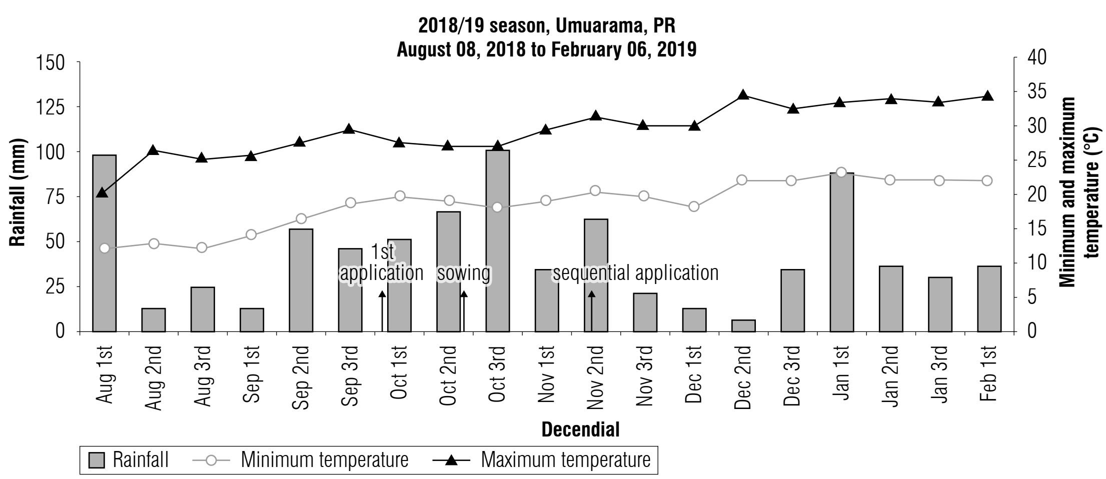


FIGURE 1. Representation of rainfall and minimum and maximum temperature for the study site. 2018/19 season, Umuarama, PR, Brazil.

The trial site was infested by *D. insularis,* identified as indicative of glyphosate resistance. Before application, the plants were at the flowering stage with 3.5 clumps m^{-2}. The area was previously cultivated with maize. A no-till sowing system was used for soybean, using 0.45 cm row spacing. The cultivar used was Monsoy® 6410 IPRO (Monsanto Co. do Brasil, São Paulo, SP, Brazil). The experiment was conducted in a randomized block experimental design, with four replications and 5x3 m plots. For the useful area, the four central lines were used, discarding the first and last meter of the plot. The treatments are described in Table 1. The commercial products used in the trial are presented in Supplementary material 1.

The first application was performed on October 5th, 2018. The soybean was sown on October 18th, 2018, and the sequential application was carried out on November 16th, 2018 when soybean plants were at the V3 growth stage (Fehr *et al*., 1971). One hundred mm of rainfall was reported in the area between the first application and seeding.

Both applications were performed using a CO_2 backpack sprayer at 3.6 km/h with AIXR 110.015 spray nozzles pressured at 2.5 kgf cm^{-2} with a volume of 150 L ha^{-1}. The weather conditions were a temperature of 23.1°C, 62.3% relative humidity, and wind speed of 6.8 km/h. The sequential application had the following conditions: temperature of 23.1°C, 60.1% relative humidity, and wind speed of 2.2 km/h.

Evaluations

Weed control was visually evaluated at 7, 14, 21, and 35 d after application (DAA) and on the 14th and 28th d after the sequential application. Soybean crop injury was evaluated at 7, 14, 21, 28, and 35 d after sowing (DAS). Percentage grades from 0% (absence of symptoms) to 100% (death of the plant) were given, based on the apparent symptoms related to the plant growth stage (Velini *et al*., 1995).

By the time of harvest, at the R8 stage (full maturation) of soybean plants (Fehr *et al*., 1971), the yield was calculated using only the plot's two central lines of 4 m long each. The grain moisture was corrected to 13% and the results converted to kg ha^{-1}.

Data analysis

An analysis of variance and the F-test ($P \leq 0.05$) were performed as described by Pimentel-Gomes and Garcia (2002). The means of treatments were grouped together using the Scott-Knott (1974) test ($P \leq 0.05$). For this purpose, the software Sisvar 5.6 was used (Ferreira, 2011).

Results

There was little or no control observed in the first application at 7 DAA (all below 35%); however, at 14 DAA all treatments associated with auxinic herbicides and saflufenacil showed inferior control compared to the others. At 21 DAA, the best results were observed for treatments with the application of ACCase inhibitors (haloxyfop and clethodim). Control superior to 80% was reported at 28 and 35 DAA by all herbicide combinations. It is important to highlight that at 35 DAA only the treatments involving glufosinate and 2,4-D showed lower performance (Tab. 2).

After the sequential application of glyphosate + haloxyfop at 14 DAA, weed control of at least 83.3% was observed. Besides that, the treatments involving auxinic herbicides and

TABLE 1. Treatments consisting of herbicide mixtures for the control of *D. insularis.* 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	Rates[2]
Control (without herbicide application)	-
Glyphosate + clethodim[3]	1,080 + 192
Glyphosate + haloxyfop[4]	1,080 + 120
Glyphosate + imazapic/imazapyr[5]	1,080 + 78.75/26.25
Glufosinate + imazapic/imazapyr[5]	500 + 78.75/26.25
Glyphosate + clethodim[3] + imazapic/imazapyr	1,080 + 192 + 78.75/26.25
Glyphosate + haloxyfop[4] + imazapic/imazapyr	1,080 + 120 + 78.75/26.25
Glyphosate + 2,4-D + imazapic/imazapyr[5]	1,080 + 670 + 78.75/26.25
Glyphosate + dicamba + imazapic/imazapyr[5]	1,080 + 288 + 78.75/26.25
Glyphosate + triclopyr + imazapic/imazapyr[5]	1,080 + 480 + 78.75/26.25
Glyphosate + saflufenacil + imazapic/imazapyr[5]	1,080 + 35 + 78.75/26.25

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

[2]Rates at g a.e. ha^{-1} for glyphosate, haloxyfop, 2,4-D and triclopyr. Rates at g active ingredient (a.i.) ha^{-1} for other herbicides. Addition of adjuvants: [3]Lanzar® 0.5% v/v; [4]Joint® 0.5% v/v; [5]Dash® HC 0.5% v/v.

the ones involving glufosinate were considered inferior as grass regrowth was frequently observed (≥89%). However, at 28 DAA efficient *D. insularis* control was observed by all the tested combinations of herbicides, computing a rate control of at least 94.8%. The application of glufosinate + imazapic/imazapyr is highlighted for its control levels of 85% at 35 DAA and 96.3% after the sequential application at 28 DAA (Tab. 2), indicating the importance of adding sequential applications to weed management practices.

Lower crop injury by all evaluations was also observed. In general, symptoms were characterized by small chloroses and yellowing of leaves. The application of glyphosate with clethodim or haloxyfop did not cause symptoms (Tab. 3).

TABLE 2. Control (%) of *D. insularis* under application of herbicides mixtures. 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	After 1st application					After 2nd application	
	7	14	21	28	35	14	28
	DAA						
Control (without application)	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c	0.0 c	0.0 b
Glyphosate + clethodim	28.3 b	43.0 a	88.8 a	94.3 a	92.0 a	93.8 a	94.8 a
Glyphosate + haloxyfop	26.5 b	43.3 a	85.3 a	90.0 a	91.3 a	91.3 a	97.0 a
Glyphosate + imazapic/imazapyr	24.8 b	40.0 a	76.8 b	88.5 b	92.0 a	93.0 a	96.0 a
Glufosinate + imazapic/imazapyr	20.8 b	38.8 a	64.3 c	83.5 b	85.0 b	86.3 b	96.3 a
Glyphosate + clethodim + imazapic/imazapyr	34.8 a	42.5 a	88.8 a	92.5 a	94.0 a	93.3 a	97.5 a
Glyphosate + haloxyfop + imazapic/imazapyr	28.0 b	41.5 a	82.3 a	92.8 a	94.0 a	93.0 a	97.0 a
Glyphosate + 2,4-D + imazapic/imazapyr	25.3 b	30.0 b	74.5 b	83.0 b	83.8 b	83.8 b	96.0 a
Glyphosate + dicamba + imazapic/imazapyr	27.0 b	32.3 b	76.3 b	87.0 b	89.8 a	89.0 b	96.3 a
Glyphosate + triclopyr + imazapic/imazapyr	31.8 a	34.8 b	73.3 b	86.3 b	89.3 a	86.5 b	96.3 a
Glyphosate + saflufenacil + imazapic/imazapyr	26.5 b	35.0 b	77.0 b	91.8 a	91.3 a	92.8 a	97.0 a
Mean	24.9	34.6	71.5	80.9	82.0	82.0	87.6
CV (%)	14.2	12.3	8.8	4.3	3.0	4.5	1.4
F	*	*	*	*	*	*	*

DAA: days after application; CV: coefficient of variation.

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott (1974) test ($P \leq 0.05$).

TABLE 3. Crop injury (%) and yield (kg ha^{-1}) of soybean plants under application of herbicide mixtures, for the control of *D. insularis*. 2018/19 season, Umuarama, PR, Brazil.

Treatments[1]	Crop injury				Yield
	14	21	28	35	
	DAS				
Control (without application)	0.0 a	0.0 a	0.0 a	0.0 a	825 b
Glyphosate + clethodim	0.0 a	0.0 a	0.0 a	0.0 a	2,432 a
Glyphosate + haloxyfop	0.0 a	0.0 a	0.0 a	0.0 a	2,444 a
Glyphosate + imazapic/imazapyr	3.8 b	5.0 b	2.8 b	2.5 b	2,506 a
Glufosinate + imazapic/imazapyr	3.5 b	4.5 b	2.0 b	2.0 b	2,450 a
Glyphosate + clethodim + imazapic/imazapyr	3.8 b	5.3 b	2.5 b	2.8 b	2,432 a
Glyphosate + haloxyfop + imazapic/imazapyr	3.5 b	4.3 b	2.5 b	2.0 b	2,379 a
Glyphosate + 2,4-D + imazapic/imazapyr	3.3 b	5.0 b	2.3 b	2.0 b	2,516 a
Glyphosate + dicamba + imazapic/imazapyr	4.0 b	5.8 b	3.5 c	3.3 b	2,292 a
Glyphosate + triclopyr + imazapic/imazapyr	4.5 b	6.3 b	4.0 c	2.8 b	2,433 a
Glyphosate + saflufenacil + imazapic/imazapyr	3.3 b	4.3 b	2.0 b	2.3 b	2,415 a
Mean	2.7	3.7	2.0	1.8	2,284
CV (%)	11.7	11.6	8.3	16.8	10.8
F	*	*	*	*	*

DAS: days after sowing; CV: coefficient of variation.

[1]Sequential application in all herbicide treatments of haloxyfop (66 g acid equivalent (a.e.) ha^{-1}) + glyphosate (720 g a.e. ha^{-1}), at post-emergence (V3) of soybean.

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott (1974) test ($P \leq 0.05$).

The treatment glyphosate + triclopyr + imazapic/imazapyr showed the greatest values of injury, with 6.3% at 21 DAS, followed by the treatment glyphosate + dicamba + imazapic/imazapyr (5.8%). However, from 21 DAS the symptoms of all treatments started to decrease, and all percentages were considered acceptable and not harmful to the crop. Despite the symptoms, there was no change in yield (Tab. 3).

Discussion

Less than 30 d passed between the application of imazapic/imazapyr and the sowing of soybean, in accordance with the label recommendations (Rodrigues and Almeida, 2018). Additionally, about 100 mm of precipitation were observed in this period, also a necessary volume according to the label (Rodrigues and Almeida, 2018). This precipitation combined with the sandy texture of the soil may have favored the leaching of the product, which favored the selectivity for soybean.

Every herbicide mixture performed in this study showed efficient performance on *D. insularis* control with minimum injury while maintaining soybean yield. The use of imazapic/imazapyr and other herbicides in mixtures was potentially selective at burndown before soybean sowing. Melo *et al.* (2017) observed 100% of *D. insularis* control at 35 DAA using imazapic/imazapyr (52.5/17.5 g a.i. ha^{-1}) at postemergence. Francischini *et al.* (2012) also noted imazapic/imazapyr efficiency controlling *D. insularis* along with other weeds that is similar to that verified in the present study.

Besides successfully controlling *D. insularis* in different mixtures as shown in this study, imazapic/imazapyr is also efficient in the control of volunteer corn (Piasecki and Rizzardi, 2016) and is selective to soybean. Additionally, post-application of imazapic/imazapyr was also reported as efficient in controlling different grasses and broadleaved weeds, such as eudicotyledons *Bidens pilosa* and *Raphanus raphanistrus* (Santos *et al.*, 2012) and monocotyledons *Hymenachne amplexicaulis* (Silva *et al.*, 2012), *Oryza sativa*, *Echinochloa crus-galli* and *Cyperus esculentus* (Helgueira *et al.*, 2018).

Regarding effectiveness, the glyphosate + imazapic/imazapyr mixture is highlighted when combined with auxinic herbicides, especially triclopyr and dicamba. This mixture is equivalent to that of glyphosate + imazapic/imazapyr with ACCase inhibitors (haloxyfop or clethodim). The application of these mixtures shows a broad control spectrum considering the effectiveness of auxins for the control of eudicotyledons combined with the control of *D. insularis* verified in this study.

High levels of infestation of weeds such as *D. insularis* and other grasses require ACCase inhibitor herbicides such as haloxyfop and clethodim. However, applying synthetic auxins such as 2,4-D and dicamba is not always an option because of the antagonism that can be created between these herbicides and ACCase inhibitors (Trezzi *et al.*, 2007; Pereira *et al.*, 2018; Gomes *et al.*, 2020). The antagonism between these mixtures is possibly explained by the reduction of translocation of these herbicides to their action sites, compared to the cases when herbicides are pulverized alone (Scherder *et al.*, 2005). Thus, eudicotyledon control is hampered and glyphosate or some other herbicide is used as an option for chemical management. There are few studies in the literature that provide information on antagonism, additive effect, or synergism of imazapic/imazapyr in a mixture with synthetic auxins, glufosinate, or saflufenacil, so the present work is unprecedented.

The mixture of saflufenacil, glyphosate, and imazapic/imazapyr was effective on the control of *D. insularis* (≥91.3%) at 28 and 35 DAA. In general, saflufenacil does not show high control of *Digitaria* spp. (Soltani *et al.*, 2014). However, in this study, it was identified as a tool for *D. insularis* control when used in mixtures with glyphosate and imazapic/imazapyr since mixtures like this have a broad spectrum of action. Dalazen *et al.* (2015) observe synergism for saflufenacil + glyphosate, and Datta *et al.* (2013) observe it for imazapic + saflufenacil. It is noteworthy that these treatments had slower action, compared to treatments with ACCase inhibitors (haloxyfop and clethodim). Bianchi *et al.* (2020) observe a synergistic effect of clethodim + glyphosate for the control of *D. insularis* at 21 DAA. In the present study, treatments without ACCase inhibitors achieved greater levels of control after 28 DAA.

The results of this study showed that glufosinate + imazapic/imazapyr application as a burndown technique prior to sowing, and when applied along with glyphosate (only at soybean post-emergence) associated with haloxyfop they were efficient for controlling *D. insularis* at a 96.3%. Studies by Everman *et al.* (2007), Melo *et al.* (2012), Gemelli *et al.* (2013), and Silva *et al.* (2017) also emphasize the use of glufosinate in the control of *D. insularis* in different chemical weed management programs.

In this sense, it is important to emphasize the four biotype resistance cases of *D. insularis* in the world. Three of these biotypes are resistant to glyphosate and one to haloxyfop and fenoxaprop (Heap, 2020). Therefore, other mechanisms of action are useful to manage this grass; thus, imazapic/imazapyr (ALS inhibitors) and glufosinate (GS inhibitor) can be used to control *D. insularis* offering soybean selectivity as verified in this study.

Conclusion

The application of imazapic/imazapyr in mixtures with other herbicides was effective in controlling glyphosate-resistant *D. insularis* in burndown before soybean sowing and with sequential application of haloxyfop + glyphosate at the V3 stage of soybean. This chemical management was also selective for soybean.

Literature cited

Belfry, K.D., N. Soltani, L.R. Brown, and P.H. Sikkema. 2015. Tolerance of identity preserved soybean cultivars to preemergence herbicides. Can. J. Plant Sci. 95, 719-726. Doi: 10.4141/CJPS-2014-351

Bianchi, L., V.M. Anunciato, T. Gazola, S.M. Perissato, R.C. Dias, L. Tropaldi, C.A. Carbonari, and E.D. Velini. 2020. Effects of glyphosate and clethodim alone and in mixture in sourgrass (*Digitaria insularis*). Crop Prot. 138, 105322. Doi: 10.1016/j.cropro.2020.105322

Braz, G.B., R.S. Oliveira Júnior, L.H.S. Zobiole, R.S. Rubin, C. Voglewede, J. Constantin, and H.K. Takano. 2017. Sumatran fleabane (*Conyza sumatrensis*) control in no-tillage soybean with diclosulam plus halauxifen-methyl. Weed Technol. 31(2), 184-192. Doi: 10.1017/wet.2016.28

Byker, H.P., N. Soltani, D.E. Robinson, F.J. Tardif, M.B. Lawton, and P.H. Sikkema. 2013. Control of glyphosate-resistant Canada fleabane [*Conyza canadensis* (L.) Cronq.] with preplant herbicide tankmixes in soybean [*Glycine max*. (L). Merr.]. Can. J. Plant Sci. 93, 659-667. Doi: 10.4141/cjps2012-320

Canedo, I.F., L.S. Araújo, L.G.B. Silva, M.D.S. Valente, M.A.M. Freitas, and P.C.R. Cunha. 2019. Differential susceptibility to *glyphosate* herbicide and re-growth capacity of different populations of sourgrass. Rev. Ceres 66(1), 18-25. Doi: 10.1590/0034-737x201966010003

Dalazen, G., N.D. Kruse, S.L.O. Machado, and A. Balbinot. 2015. Synergism of the glyphosate and saflufenacil combination for controlling hairy fleabane. Pesqui. Agropecu. Trop. 45, 249-256. Doi: 10.1590/1983-40632015v4533708

Datta, A., R.E. Rapp, J.E. Scott, L.D. Charvat, J. Zawierucha, and S.Z. Knezevic. 2013. Spring-applied saflufenacil and imazapic provided longer lasting *Euphorbia esula* L. control than fall applications. Crop Prot. 47, 30-34. Doi: 10.1016/j.cropro.2012.12.006

Everman, W.J., I.C. Burke, J.R. Allen, J. Collins, and J.W. Wilcut. 2007. Weed control and yield with glufosinate-resistant cotton weed management systems. Weed Technol. 21(3), 695-701. Doi: 10.1614/WT-06-164.1

Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11(6), 929-931. Doi: 10.2135/cropsci1971.0011183X001100060051x

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. Cienc. Agrotecnol. 35(6), 1039-1042. Doi: 10.1590/S1413-70542011000600001

Francischini, A.C., G. Santos, J. Constantin, H. Ghiglione, G.F. Velho, N. Guerra, and G.B.P. Braz. 2012. Efficacy and selectivity of herbicides from the imidazolinone group applied in post-emergence of monocotyledon weeds on CL sunflower culture. Planta Daninha 30(4), 843-851. Doi: 10.1590/S0100-83582012000400019

Frisvold, G.B., J. Albright, D.E. Ervin, M.D. Owen, J.K. Norsworthy, K.E. Dentzman, T.M. Hurley, R.A. Jussaume, J.L. Gunsolus, and W. Everman. 2020. Do farmers manage weeds on owned and rented land differently? Evidence from US corn and soybean farms. Pest Manage. Sci. 76(6), 2030-2039. Doi: 10.1002/ps.5737

Gazziero, D.L.P., F.S. Adegas, A.F. Silva, and G. Concenço. 2019. Estimating yield losses in soybean due to sourgrass interference. Planta Daninha 37(4), e019190835. Doi: 10.1590/s0100-83582019370100047

Gemelli, A., R.S. Oliveira Júnior, J. Constantin, G.B.P. Braz, T.M. Campos Jumes, E.A. Gheno, F.A. Rios, and L.H.M. Franchini. 2013. Estratégias para o controle de capim-amargoso (*Digitaria insularis*) resistente ao glyphosate na cultura milho safrinha. Rev. Bras. Herb. 12(2), 162-170. Doi: 10.7824/rbh.v12i2.201

Gomes, H.L.L., V.C. Sambatti, and G. Dalazen. 2020. Sourgrass control in response to the association of 2,4-d to ACCase inhibitor herbicides. Biosci. J. 36(4), 1126-1136. Doi: 10.14393/BJ-v36n4a2020-47895

Green, J.M. 2018. The rise and future of glyphosate and glyphosate-resistant crops. Pest Manage. Sci. 74(5), 1035-1039. Doi: 10.1002/ps.4462

Heap, I. and S.O. Duke. 2018. Overview of glyphosate-resistant weeds worldwide. Pest Manage. Sci. 74(5), 1040-1049. Doi: 10.1002/ps.4760

Heap, I.M. 2020. The international herbicide-resistant weed database. URL: http://www.weedscience.org (accessed 01 April 2020).

Helgueira, D.B., T. D'Ávila Rosa, L. Galon, D.S. Moura, A.T. Martini, and J.J.O. Pinto. 2018. Weed management in rice under sprinkler and flood irrigation systems. Planta Daninha 36(2), e018177637. Doi: 10.1590/s0100-83582018360100141

Lorenzi, H. 2014. Manual de identificação e controle de plantas daninhas: plantio direto e convencional. Instituto Plantarum, Londrina, Brazil.

Melo, M.S.C., L.J.F.N. Rocha, C.A.C.G. Brunharo, D.C.P. Silva, M. Nicolai, and P.J. Christoffoleti. 2017. Alternativas de controle químico do capim-amargoso resistente ao glyphosate, com herbicidas registrados para as culturas de milho e algodão. Rev. Bras. Herb. 16(3), 206-215. Doi: 10.7824/rbh.v16i3.556

Melo, M.S.C., L.E. Rosa, C.A.C.G. Brunharo, M. Nicolai, and P.J. Christoffoleti. 2012. Alternativas para o controle químico de capim-amargoso (*Digitaria insularis*) resistente ao glyphosate. Rev. Bras. Herb. 11(2), 195-203. Doi: 10.7824/rbh.v11i2.145

Moreira, H.J.C. and H.N.P. Bragança. 2011. Manual de identificação de plantas infestantes. FMC Agricultural Products, Campinas, Brazil.

Neve, P., J.N. Barney, Y. Buckley, R.D. Cousens, S. Graham, N.R. Jordan, A. Lawton-Rauh, M. Liebman, M.B. Mesgaran, M. Schut, J. Shaw, J. Storkey, B. Baraibar, R.S. Baucom, M. Chalak, D.Z. Childs, S. Christensen, H. Eizenberg, C. Fernández-Quintanilla, K. French, M. Harsch, S. Heijting, L. Harrison, D. Loddo, M. Macel, N. Maczey, A. Merotto Jr., D. Mortensen, J. Necajeva, D.A. Peltzer, J. Recasens, M. Renton, M. Riemens, M. Sønderskov, and M. Williams. 2018. Reviewing research priorities in weed ecology, evolution and management: a horizon scan. Weed Res. 58(4), 250-258. Doi: 10.1111/wre.12304

Oliveira Júnior, R.S. 2011. Mecanismos de ação dos herbicidas. pp. 141-192. In: Oliveira Júnior, R.S., J. Constantin, and M.H. Inoue (eds.). Biologia e manejo de plantas daninhas. Omnipax, Curitiba, Brazil.

Pereira, G.R., L.H.S. Zobiole, and C.V.S. Rossi. 2018. Resposta no controle de capim-amargoso a mistura de tanque de glyphosate e haloxifope com auxinas sintéticas. Rev. Bras. Herb. 17(2), e606. Doi: 10.7824/rbh.v17i2.606

Piasecki, C. and M.A. Rizzardi. 2016. Herbicidas aplicados em pré-emergência controlam plantas individuais e touceiras de milho voluntário RR® F2 em soja? Rev. Bras. Herb. 15(4), 323-331. Doi: 10.7824/rbh.v15i4.497

Pimentel-Gomes, F. and C.H. Garcia. 2002. Estatística aplicada a experimentos agronômicos e florestais: exposição com exemplos e orientações para uso de aplicativos. FEALQ, Piracicaba, Brazil.

Rodrigues, B.N. and F.S. Almeida. 2018. Guia de herbicidas. Editing authors, Londrina, Brazil.

Santos, G., A.C. Francischini, J. Constantin, R.S. Oliveira Júnior, H. Ghiglione, G.F. Velho, and A.M. Oliveira Neto. 2012. Use of the new Clearfield® system in sunflower culture to control dicotyledonous weeds. Planta Daninha 30(2), 359-365. Doi: 10.1590/S0100-83582012000200015

Scott, A.J. and M. Knott. 1974. A cluster analysis method for grouping means in the analysis of variance. Biometrics 30(3), 507-512. Doi: 10.2307/2529204

Shaner, D.L. and S.L. O'Connor. 2017. The imidazolinone herbicides (1991). CRC press, Taylor & Francis, Boca Raton, USA.

Scherder, E.F., R.E. Talbert, and M.L. Lovelace. 2005. Antagonism of cyhalofop grass activity by halosulfuron, triclopyr, and propanil. Weed Technol. 19(4), 934-941. Doi: 10.1614/WT-03-177R2.1

Silva, K.S., S.L.O. Machado, L.A. Avila, E. Marchesan, M.V.P. Alves, and L.J.K. Urban. 2012. Sensitivity of west Indian marsh grass to herbicides. Planta Daninha 30(4), 817-825. Doi: 10.1590/S0100-83582012000400016

Silva, W.T., D. Karam, L. Vargas, and A.F. Silva. 2017. Alternatives of chemical control for sourgrass (*Digitaria insularis*) on maize crop. Rev. Bras. Milho Sorgo 16(3), 578-586. Doi: 10.18512/1980-6477/rbms.v16n3p578-586

Soltani, N., C. Shropshire, and P.H. Sikkema. 2014. Sensitivity of dry bean to dimethenamid-p, saflufenacil and dimethenamid-p/saflufenacil. Am. J. Plant. Sci. 5(21), 3288. Doi: 10.4236/ajps.2014.521343

Trezzi, M.M., D. Mattei, R.A. Vidal, N.D. Kruse, M.S. Gustman, R. Viola, A. Machado, and H.L. Silva. 2007. Antagonistic action of clodinafop-propargyl associated with metsulfuron-methyl and 2,4-D in the control of Italian ryegrass (*Lolium multiflorum*). Planta Daninha 25(4), 839-847. Doi: 10.1590/S0100-83582007000400021

Underwood, M.G., N. Soltani, D.E. Robinson, D.C. Hooker, C.J. Swanton, J.P. Vink, and P.H. Sikkema. 2017. Weed control, environmental impact, and net revenue of two-pass weed management strategies in dicamba-resistant soybean. Can. J. Plant Sci. 98, 370-379. Doi: 10.1139/cjps-2017-0147

Velini, E.D., R. Osipe, and D.L.P. Gazziero. 1995. Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. SBCPD, Londrina, Brazil.

Zobiole, L.H.S., F.H. Krenchinski, A.J.P. Albrecht, G. Pereira, F.R. Lucio, C. Rossi, and R.S. Rubin. 2016. Controle de capim-amargoso perenizado em pleno florescimento. Rev. Bras. Herb. 15(2), 157-164. Doi: 10.7824/rbh.v15i2.474

SUPPLEMENTARY MATERIAL 1. Commercial products used.

Commercial products	Herbicides
Roundup® Original	Glyphosate
Select® 240 EC	Clethodim
Verdict® R	Haloxyfop
Amplexus™	Imazapic/imazapyr
Finale®	Glufosinate
DMA® 806 BR	2,4-D
Atectra®	Dicamba
Triclon®	Triclopyr
Heat®	Saflufenacil

Relationship between chemical fertilization in sorghum and *Melanaphis sacchari/sorghi* (Hemiptera: Aphididae) populations

Relación entre la fertilización química en sorgo y las poblaciones de *Melanaphis sacchari/sorghi* (Hemiptera: Aphididae)

José Arturo Schlickmann-Tank[1]*, Oscar Morales-Galván[1], Joel Pineda-Pineda[1], Gonzalo Espinosa-Vázquez[2], María Teresa Colinas-León[3], and Mateo Vargas-Hernández[1]

ABSTRACT

The aphid *Melanaphis sacchari/sorghi* is considered the most important pest of sorghum cultivation in Mexico. It can cause losses in production of up to 100%. This research was conducted at the Universidad Autonoma Chapingo during 2018 and 2019 with the objective of determining the relationship between chemical fertilization in sorghum and *M. sacchari/sorghi* (Hemiptera: Aphididae) populations. Different levels of nitrogen (125, 250 and 500 kg ha^{-1}), phosphorus (19.5, 39 and 78 kg ha^{-1}) and potassium (210 and 420 kg ha^{-1}) were supplied to sorghum plants planted in polyethylene pots with a capacity of 6 L under greenhouse conditions. The plants were infested with a total of 15 third-instar nymphs within 60 d of crop emergence. A total of 6 samplings were carried out at 7 d intervals, starting at 7 d after the infestation. Quantifications of total soluble proteins, total soluble sugars, reducing sugars, and levels of nitrogen, phosphorus, potassium and magnesium in leaf tissue were also performed. We observed that higher doses of nitrogen and phosphorus increased the aphid population, while increasing the potassium dose considerably decreased the aphid population. We also found a positive correlation between the aphid population and the total soluble protein concentrations, reducing sugars and nitrogen levels, while the correlation was negative with potassium levels in leaves.

Key words: nitrogen, phosphorus, potassium, sugarcane aphid.

RESUMEN

El pulgón amarillo *Melanaphis sacchari/sorghi* es considerado la plaga más importante del cultivo de sorgo en México, ya que puede provocar pérdidas de hasta 100% de la producción. La presente investigación se realizó en la Universidad Autónoma Chapingo durante los años 2018 y 2019 con el objetivo de determinar la relación entre la fertilización química en sorgo y las poblaciones de *M. sacchari/sorghi* (Hemiptera: Aphididae). Se suministraron diferentes niveles de nitrógeno (125, 250 y 500 kg ha^{-1}), fósforo (19.5, 39 y 78 kg ha^{-1}) y potasio (210 y 420 kg ha^{-1}) a plantas de sorgo sembradas en macetas de polietileno de 6 L de capacidad en condiciones de invernadero. Las plantas fueron infestadas con un total de 15 ninfas de tercer instar a los 60 d de emergido el cultivo. Se realizó un total de 6 muestreos a intervalos de 7 d, iniciando 7 d después de la infestación. También se realizaron cuantificaciones de proteínas solubles totales, azúcares solubles totales, azúcares reductores, y niveles de nitrógeno, fósforo, potasio y magnesio en tejido foliar. Se observó que las dosis más altas de nitrógeno y fósforo incrementaron la población del pulgón, mientras que al incrementar la dosis de potasio la población del pulgón disminuyó considerablemente. También se constató una correlación positiva entre la población del pulgón y las concentraciones de proteínas solubles totales, azúcares reductores y niveles de nitrógeno, mientras que con los niveles de potasio en hoja la correlación fue negativa.

Palabras clave: nitrógeno, fósforo, potasio, pulgón amarillo del sorgo.

Introduction

The sugarcane aphid *Melanaphis sacchari/sorghi* (Zehntner, 1897) (Hemiptera: Aphidididae) is native to the African continent and has become an invasive pest attacking sorghum cultivation in the Americas (Zapata *et al*., 2016). In 2013, it arrived in Mexico causing losses between 30 and 100% (Rodríguez-del-Bosque & Terán, 2015; Knutson *et al*., 2016). It is considered a "super-clone" due to its low genetic diversity, but it has great potential for adaptation to various geographical and climatic regions (Nibouche *et al*., 2018; Perales-Rosas *et al*., 2019). This aphid feeds on phloem causing direct damage to the plant, and like most aphids it excretes honeydew. Honeydew is the result of excess sugar

Received for publication: 15 May, 2020. Accepted for publication: 11 December, 2020.

Doi: 10.15446/agron.colomb.v38n3.87308

[1] Maestría en Ciencias en Protección Vegetal, Universidad Autónoma Chapingo, Chapingo, Estado de Mexico (Mexico).
[2] Colegio de Posgraduados, Montecillo, Estado de Mexico (Mexico).
[3] Departamento de Fitotecnia, Universidad Autónoma Chapingo, Chapingo, Estado de Mexico (Mexico).
* Corresponding author: al17131713@chapingo.mx

in the aphid's diet and is used as a food source by many other organisms. This results in the formation of black sooty mold that can reduce the photosynthetic capacity of the sorghum plant (Peña-Martínez *et al*., 2018).

Different strategies have been implemented for the management of this pest, such as the delimitation of sowing dates, conservation of natural enemies, use of biological control, elimination of alternate hosts, etc. (Knutson *et al*., 2016; De Souza & Davis, 2019). However, chemical control has become the primary control measure (Bowling *et al*., 2016; Knutson *et al*., 2016). Crop nutrition as a management tactic for *M. sacchari/sorghi* has not had much relevance, due to the scarce information on the subject. It is well-known that the availability of numerous nutrients affects the development and survival of herbivorous insects, since their biomass generally contains much higher concentrations of elements compared to plants (Boswell *et al*., 2008). Nitrogen (N) fertilization strongly influences aphid population growth parameters; increased nitrogen is associated with higher nymph growth rates and, thus, increases the fecundity of many aphid species (Hosseini *et al*., 2010; Chow *et al*., 2011; Gash, 2012). Increased nitrogen fertilization can improve the nutritional quality and plant attractiveness for aphids by improving the dietary parameters of the phloem with an increase in the level of amino acids and nitrates in the host plants (Douglas, 2006; Fallahpour *et al*., 2015). Recent studies have shown a marked increase in the population parameters of *M. sacchari/sorghi* with increased nitrogen fertilization (Lama *et al*., 2019; Wilson *et al*., 2020).

To date, no work has been reported on the relationship between potassium and phosphate fertilization with the population dynamics of *M. sacchari/sorghi*. However, it is known that potassium (K)-induced changes in plant metabolite concentrations are multiple and include potassium dependence on enzyme metabolism (including those associated with jasmonic acid and salicylic acid), photosynthesis, and long-range transport (Amtmann *et al*., 2008). According to Venter *et al*. (2014), the application of potassium phosphate to wheat crops induces a certain degree of tolerance to the attack of the Russian wheat aphid (*Diuraphis noxia*). Phosphorus (P) can act as a host plant susceptibility modifier by changing secondary metabolites such as phenols and terpenes. When this occurs, the accumulation of phenolic compounds (tannins, lignin) acts as a barrier that has deterrent or directly toxic effects on herbivorous insects (Facknath & Lalljee, 2005). In some cases, such as those reported by Sinha *et al*. (2018), P produced a repressive effect on the insect population, where the mustard aphid (*Lipaphis erysimi*) considerably decreased its population by applying high doses of this element.

The present study was carried out under the hypothesis that the nutritional imbalance in the sorghum crop influences the population density of *M. sacchari/sorghi*. Based on this hypothesis, the objective of this research was to determine the relationship between N, P and K and the populations of *M. sacchari/sorghi* (Hemiptera: Aphididae).

Materials and methods

Location and preparation of host plants

The experiment was conducted in a greenhouse of the Department of Agricultural Parasitology at the Universidad Autonoma Chapingo. In the establishment of the crop, seeds of hybrid sorghum UPM-219 were used, which has a high susceptibility to *M. sacchari/sorghi* attack. So, it was possible to observe the relationship between fertilization and the aphid population without the interference of other factors such as weather, natural enemies, etc.

Sorghum seeds were sown in polyethylene bags with a capacity of 6 L, which were filled with soil from the experimental field. This soil has a clay loam structure, with a content of 3.4% organic matter, 6.99 mg kg^{-1} of phosphorus Bray, 363 mg kg^{-1} of potassium and 5.33 mg kg^{-1} of nitrogen as nitrate. Although N and P levels are considered low to medium for this type of soil, the K levels are considered medium to high. Five sorghum seeds were placed in each bag; 5 d after emergence the plants were thinned out, keeping the two most vigorous and largest plants. The irrigation kept the soil at levels close to field capacity, to maintain the optimal development of the crop.

Evaluated treatments

Five applications of fertilizer were carried out, the first at the time of sowing and the remaining every 7 d. This was done with the intention of minimizing the toxic effects of fertilizers on plants. The fertilizers were weighed with an Ohaus Adventurer® precision analytical balance and mixed in individual plastic bags and were applied manually in diluted water (solution). The fertilizers used were urea (46% N) as a source of N, $Ca_3(PO_4)_2$ (calcium phosphate) as a source of P, and KNO_3 (potassium nitrate) as a source of K, applied in three levels, based on previous analysis:

- Low level: half of the required dose.
- Medium level: optimal dose.
- High level: double the required dose.

The treatments consisted of combinations of the three nutrients mentioned above. For N and P three levels of fertilization were used (high, medium and low), whereas for K only two levels were used (high and medium), this because of the concentrations of the nutrient found in the soil. As an experimental unit, a 6 L plastic bag with two sorghum plants was used. For the calculation of the nutrient dose, the nutritional needs for a production of 8000 kg ha^{-1} and a density of 180,000 plants ha^{-1} were taken into account as proposed by Fontanetto *et al*. (2009). Regarding the final dose of fertilizers, the pre-existing concentrations of nutrients in the soil used for the study were considered, adding the necessary amounts of fertilizer needed to reach the stable dose. The experimental design used was a randomized whole block design with 12 treatments and six replicates. The treatments are described in Table 1.

TABLE 1. Fertilization rates provided to evaluate relationship between N, P and K fertilization and *M. sacchari/sorghi* population.

Treatments	Nitrogen	Phosphorus	Potassium
		kg ha^{-1}	
T1	125	19.5	210
T2	125	39	210
T3	125	78	210
T4	250	19.5	210
T5	250	39	210
T6	250	78	210
T7	500	19.5	210
T8	500	39	210
T9	500	78	210
T10	125	39	420
T11	250	39	420
T12	500	39	420

Plant infestation

The plants were infested with 15 nymphs of *M. sacchari/sorghi* in the third instar (the number of nymphs was determined based on preliminary trials, where 15 nymphs were an optimal number for the development of the population) within 60 d of emergence of the crop. The nymphs used for the infestation were obtained from a purification brood; five adult females (obtained from an infested sorghum crop) were placed on 5 cm diameter circles of sorghum leaves, which were previously placed in Petri dishes with agar to maintain the turgidity of the leaves. These boxes were taken to a bioclimatic breeding chamber for 3 d (25 ± 1°C, R.H. 60 ± 5% and a 12:12 photoperiod). Twenty-four hours after the beginning of breeding, the adult females were removed and when the infestation of the plants took place, the breeding was homogenized leaving 15 third-instar nymphs in each circle.

Estimation of the density of *M. sacchari/sorghi* in the different treatments

The rapid monitoring scale proposed by Bowling *et al*. (2015) was used to estimate the aphid population. This consists of a diagrammatic scale that allows us to determine approximate numbers of aphids per leaf through images and averages. One lower and one upper leaf of each plant per pot were evaluated, performing a total of six samples at seven-day intervals, starting 7 d after infestation.

Biochemical components of sorghum

Two samples of leaf tissues were collected, one at the vegetative stage and the other at full bloom. Total soluble sugars were measured by the anthrone method proposed by Witham *et al*. (1971). Absorbance was measured at 600 nm with a Spectrophotometer (Bausch & Lomb, Rochester, NY, USA), and reducing sugars were measured by Somogyi's method (Somogyi,1952), measuring absorbance at 540 nm in the spectrophotometer. Total soluble proteins were quantified using Bradford's method (Bradford, 1976) measuring absorbance at 595 nm in the spectrophotometer. Nitrogen, phosphorus and potassium were also analyzed. For N, the microKjeldahl method (Bremner, 1965) was used. For P, the molybdenum blue method (Murphy & Riley, 1962) for color development was used and absorbance was measured at 420 nm in a Spectrum-20 Spectrophotometer (Bausch & Lomb, Rochester, NY, USA). Potassium was determined by a flammometry Photometer Corning 400 (Corning Inc., NY, USA), and Mg content was measured by atomic absorption in Phyllis Pye SP9 Spectrophotometer (Pye Unicam Ltd. Cambridge, England).

Data analysis

The population data of *M. sacchari/sorghi* were subjected to an analysis of variance and means comparison test with the Tukey's method (α = 0.05). The statistical analyses were performed using a generalized linear model using the GLIMMIX procedure. Since aphids have a contagion distribution, a Poisson distribution was considered as a link function to the natural logarithm. For the parameters of N, P, K, Mg, total soluble proteins, total soluble sugars and reducing sugars, the general model was used with the General Linear Model procedure. These analyses were performed with the SAS® software (Statistical Analysis System) version 9.4.

Results

Relationship between N, P and K fertilization with the *M. sacchari/sorghi* population

Figure 1 shows that the sugarcane aphid population is a function of the different levels of applied macroelements

N, P and K. Nitrogen was the element that had a significant effect ($P<0.0001$) on the increase of the sugarcane aphid population, showing a positive correlation; in other words, the more nitrogen applied, the greater the density of aphids. This behavior has an arithmetical trend, given that when the N goes from 125 to 259 kg ha^{-1}, the aphid density almost doubles. However, after 259 kg ha$^{-1,}$ the density of the sugarcane aphid showed a slight reduction that may be due to intraspecific competition (Applebaum & Heifetz, 1999). According to Honěk (1993), as the insect population increases, nutritional resources decrease, which results in a drop in fertility due to the smaller size of the females.

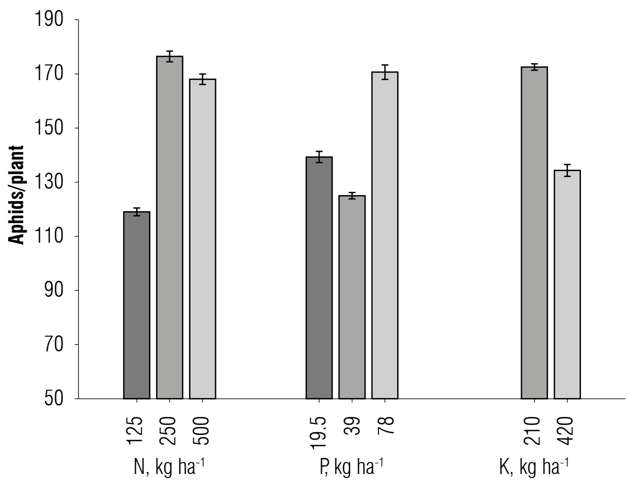


FIGURE 1. Relationship between N, P and K fertilization and *M. sacchari/sorghi* population. Bars indicate the standard error (SE).

In the case of phosphorus, a positive relationship with the sugarcane aphid density is observed, given that when the dose of P is increased, the density of the pest increases ($P<0.0001$), that is, when the population increases by 36% from 39 kg ha^{-1} to 78 kg ha^{-1}.

Contrary to the two previous elements, when the dose of potassium is doubled, a considerable reduction is observed in the number of aphids estimated per plant. Figure 2 shows the evolution of the sugarcane aphid population in response to different levels of fertilization, where a gradual increase in the upper levels of N and P fertilization is evident (Fig. 2 A-B), while the opposite was observed in the case of K (Fig. 2 C).

Effect of N, P and K fertilization on some biochemical components of sorghum leaves

In Tables 2 and 3, the results of the foliar analyses showed that N doses had a positive correlation with leaf N concentration. Similar results were obtained with P concentrations in response to P doses and K concentrations in response to K doses on both assessment dates. K, Mg, total soluble proteins, and total soluble sugar concentrations showed significant differences in the different doses of nitrogen applied, with a positive correlation with magnesium and total soluble proteins, and vice versa with K and total soluble sugars. However, for P and reducing sugar concentrations, nitrogen applications were not significant.

In both evaluations, phosphorus applications were not significant for N, K, total soluble protein and reducing sugar concentrations; however, the addition of phosphorus to the crop generated a significant effect on Mg and total soluble sugar concentrations, with a positive correlation in both cases.

Potassium applications to the crop showed a significant effect on the concentrations of reducing sugars and total soluble sugars, where a marked decrease in these can be observed with the increase of the dose of potassium.

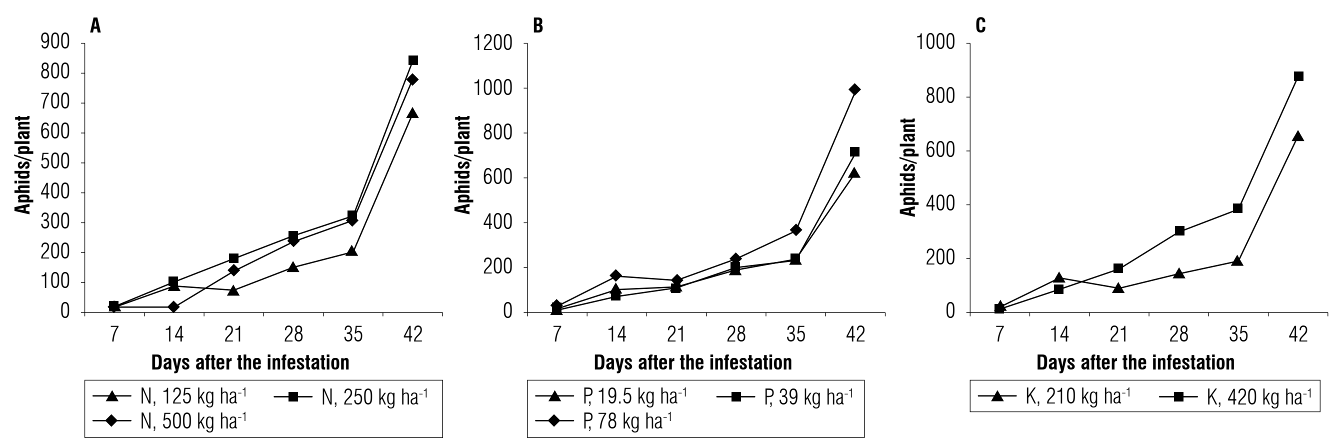


FIGURE 2. Population dynamics of *M. sacchari/sorghi* in response to different levels of N, P and K.

As for total soluble protein concentrations, Mg, N and P concentrations, potassium application was not significant (Tabs. 2 and 3). It can also be seen that in the second evaluation, all the biochemical components considered showed a decrease in their concentrations.

Relationships between the *M. sacchari/sorghi* population and biochemical components in sorghum leaves

Figure 3 summarizes all the relationships between the sugarcane aphid populations and the biochemical components of sorghum (N, P, K, Mg, total soluble proteins, total

TABLE 2. Effect of N, P and K on biochemical components at the vegetative stage of sorghum.

	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Total soluble proteins (%)	Total soluble sugars (g/100 g of fresh tissue)	Reducing sugars (mg of glucose/100 g of fresh tissue)
Fertilization levels							
Nitrogen levels							
125 kg ha^{-1}	2.376	0.366	4.386	0.487	14.848	13.166	6.052
250 kg ha^{-1}	2.600	0.382	4.331	0.597	16.251	12.195	6.215
500 kg ha^{-1}	2.775	0.292	3.690	0.736	17.345	11.260	6.325
S. E. (±)	0.330	0.073	0.457	0.170	2.063	1.700	0.412
Phosphorus levels							
19.5 kg ha^{-1}	2.559	0.284	4.119	0.490	15.993	10.667	6.361
39 kg ha^{-1}	2.566	0.342	4.284	0.676	16.036	12.618	6.340
78 kg ha^{-1}	2.644	0.417	4.056	0.586	16.528	12.925	6.803
S. E. (±)	0.330	0.073	0.457	0.170	2.063	1.700	0.412
Potassium levels							
210 kg ha^{-1}	2.628	0.350	4.105	0.643	16.427	12.770	6.034
420 kg ha^{-1}	2.450	0.336	4.229	0.798	15.313	10.618	6.903
S. E. (±)	0.330	0.073	0.457	0.170	2.063	1.700	0.412

S. E. = Standard Error of the mean.

TABLE 3. Effect of N, P and K on biochemical components at the reproductive stage of sorghum.

	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Total soluble proteins (%)	Total soluble sugars (g/100 g of fresh tissue)	Reducing sugars (mg of glucose/100 g of fresh tissue)
Fertilization levels							
Nitrogen levels							
125 kg ha^{-1}	2.077	0.302	4.226	0.492	12.979	10.837	5.417
250 kg ha^{-1}	2.689	0.309	3.942	0.495	16.807	8.681	5.525
500 kg ha^{-1}	2.832	0.305	3.364	0.688	17.701	9.591	5.636
S. E. (±)	0.433	0.053	0.667	0.081	2.707	0.879	0.341
Phosphorus levels							
19.5 kg ha^{-1}	2.399	0.234	3.874	0.478	14.997	9.519	6.281
39 kg ha^{-1}	2.571	0.313	4.180	0.555	16.066	8.942	6.060
78 kg ha^{-1}	2.590	0.362	4.141	0.646	16.188	11.410	6.504
S. E. (±)	0.433	0.053	0.667	0.081	2.707	0.879	0.341
Potassium levels							
210 kg ha^{-1}	2.636	0.300	3.610	0.560	15.847	10.041	5.796
420 kg ha^{-1}	2.524	0.320	4.547	0.552	15.774	8.688	3.517
S. E. (±)	0.433	0.053	0.667	0.081	2.707	0.879	0.341

S. E. = Standard Error of the mean.

soluble sugars and reducing sugars). The sugarcane aphid population was positively correlated with N (r = 0.6935), total soluble protein (r = 0.7797) and reducing sugar (r = 0.5602) concentrations, but it was negatively correlated with K (r = -0.7692) content in sorghum leaves.

Discussion

Fertilization is one of the most important pillars in agricultural production, and sorghum is no exception. The essential nutrients N, P and K that are applied to sorghum in different fertilizer forms maintain soil fertility and prevent deficiencies of these nutrients from limiting the yield of this crop. The most commonly used nutrients in the cultivation of sorghum is N followed by P. Normally the recommendations for the application of the N, P, and K are at rates of 190, 40, and 0 kg ha^{-1}, respectively (Díaz de León *et al*., 2008), but sometimes farmers exceed these levels with the expectation of obtaining a higher yield.

In this study, nitrogen fertilization showed an important impact on the sugarcane aphid population, with a significant increase in the population as the dose of N to the crop increased. Similar results were observed in the first report

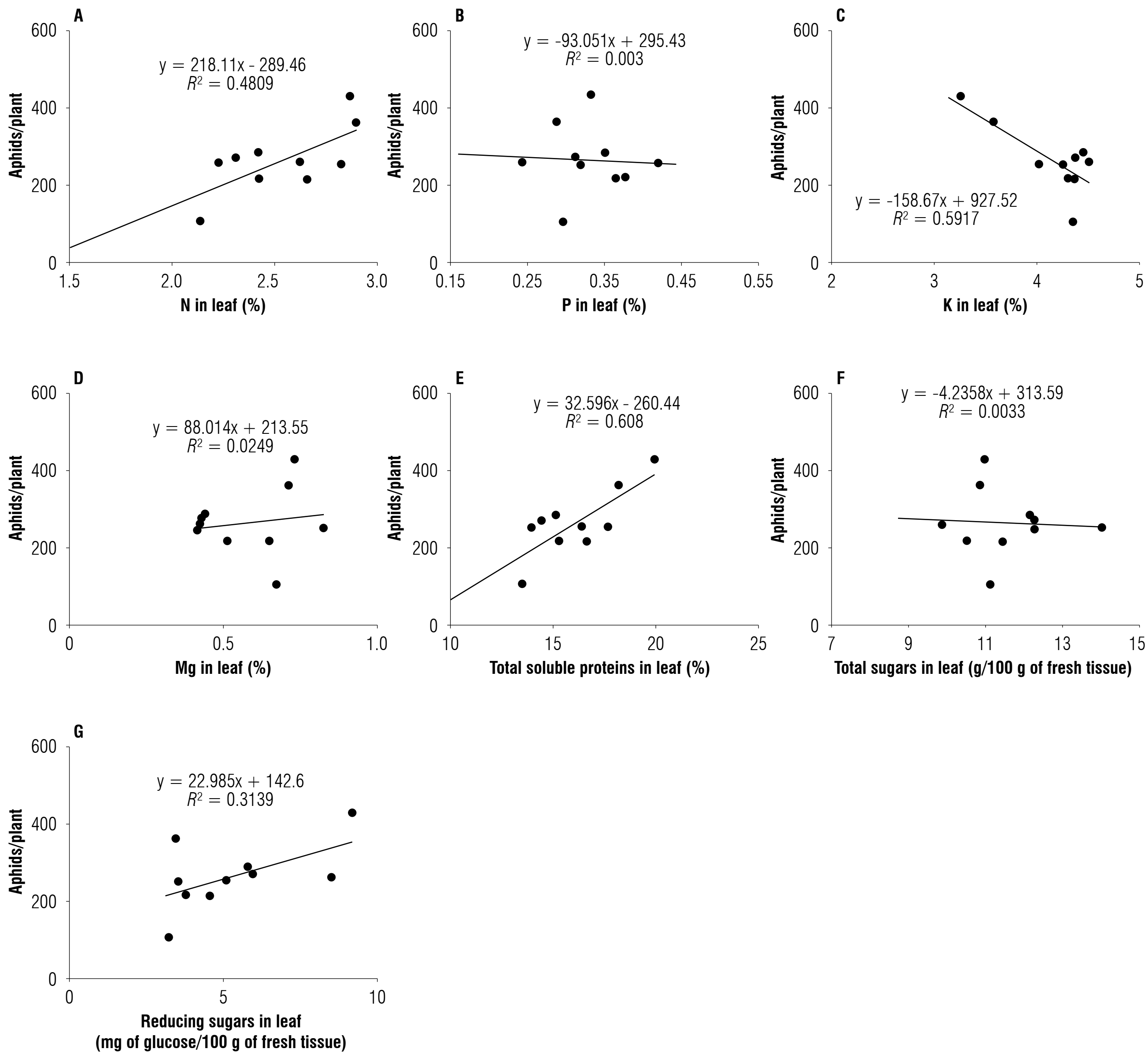


FIGURE 3. Relationships between the *M. sacchari/sorghi* population and biochemical components: nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), total soluble proteins, total soluble sugar content and reducing sugar content in sorghum plants.

on the influence of nitrogen fertilization on the sugarcane aphid by Lama *et al.* (2019). Several studies report that excessive nitrogen fertilization causes an increase in the aphid fertility rate, stimulating an excessive increase in the population (Aqueel & Leather, 2011; Rostami *et al.*, 2012; Hosseini *et al.*, 2015; Aziz *et al.*, 2018; Dong *et al.*, 2018). According to Bala *et al.* (2018), excessive doses of N result in green, succulent plants that attract pest populations. Plants with excessive nitrogen fertilization increase their dry weight, leaf area as well as their leaf chlorophyll content and grain yield. Together with the above, the increase in N dosage promotes the accumulation of proteins, free amino acids, and sugars that can attract insects. Contrasting results were obtained in the present study, where the content of total soluble proteins and reducing sugars showed a positive correlation with the sugarcane aphid population; these metabolites, in turn, had an increase directly proportional to that observed in the dose of N. Behmer (2009) proposes that the balance of nutrients in the diets of the animals is the basis of most parts of their physical condition, and he calls this the "geometrical framework". This theory assumes that animals eat an optimal amount of food to meet their demand. Depending on the composition of the feed, this optimal amount determines their "target feed intake", and as the individual moves away from the actual intake, the more significant the fitness costs will be. This theory contrasts with the results of our study, where a lower development of low-dose N in the sugarcane aphid population was observed. This theory suggests that aphids fed until their optimal N requirements were met, but in turn had an excess intake of carbohydrates (Tabs. 2 and 3). All this excess carbohydrate requires a fitness cost for disposal.

When increasing the N dose to double the plant requirement, a slight decrease in the sugarcane aphid population is noted. Sauge *et al.* (2010) observe that the population of *Myzus persicae* remained stable at low N levels and decreased slightly at high N levels, showing a parabolic response to the N level. The most considered hypotheses for this phenomenon are toxicity due to an excessive content of metabolites in the phloem of the plant and the decrease in fertility of females due to a decrease in size due to the scarcity of nutritional resources (Honěk, 1993).

Opposite results to those obtained in this study are reported by Choudhary *et al.* (2001) who observed a significant increase in the population of *Myzus persicae* in canola evasively fertilized with N. Instead, many research studies reinforce the findings obtained in our study. In a study with *Aphis craccivora* in ornamentals, Hosseini *et al.* (2015) observe that increasing nitrogen fertilization levels dramatically increased the aphid population. In a study conducted by Sinha *et al.* (2018), an increase in the population of the aphid *Lipaphis erysimi* is reported with high doses of N in mustard. Similar results are obtained by Fallahpour *et al.* (2015) with the same pest in canola.

In our study, P showed a significant effect on the sugarcane aphid population, with an increase in the population being observed when high doses of P were administered to the crop. In a compilation of studies on the effects of plant nutrition on the order Hemiptera, Singh and Sood (2017) find that in 50% of the cases phosphorus fertilization has a positive correlation with the insect population, either increasing it or improving some biological parameter. In the other half of cases, the effect was reversed. Supporting the results of the present study, Rashid *et al.* (2014) find that raising the P dose in rice markedly increases the population of *Nilaparvata lugens*. Similar results are reported by Dash *et al.* (2007) on the same pest. El-Zahi *et al.* (2012) find that the application of P decreases the incidence of *Bemisia tabaci* in cotton. Supporting these results, Sinha *et al.* (2018) and Pandey (2010) observe a decrease in the population of the mustard aphid at high doses of P. Facknath and Lalljee (2005) mention that high concentrations of P decrease the attractiveness of the host to insect pests through the accumulation of phenolic compounds (tannin and lignin), which act as a barrier with deterrent or direct toxic effects on insects. Bala *et al.* (2018) mention that an excessive phosphorus fertilization can cause an exuberant growth of plants which is more attractive for some insects. In the case of the present study, this attraction can be explained by an increase in the total proteins generated by the increase in the P dose (Tab. 2), since a positive correlation was observed between the concentration of total leaf proteins and the sugarcane aphid population (Fig. 3).

It is now well understood that K influences several physiological and biochemical processes that are relevant to the susceptibility of plants to insects (Amtmann *et al.*, 2008). In our study, the increase of the K dose had a negative effect on the sugarcane aphid population. These results can be attributed to the fact that with high doses of K, N uptake by the plant decreased considerably, and a negative correlation with the concentration of reducing sugars was also observed (Tab. 4). Wyn-Jones and Pollard (1983) show that more than 60 enzymes depend on K activity, and many of them are involved in sugar and nitrogen metabolism. Low doses of K in crops result in an accumulation of soluble sugars, especially leaf reducers (Huber, 1984; Bednarz & Oosterhuis, 1999; Pettigrew, 1999). Potassium also plays the role of osmoregulator and long-distance transport of metabolites, being indispensable for the mobilization of primary metabolites in the plant (Amtmann *et al*., 2008), which may explain the accumulation of proteins in the leaves in our study.

Recent studies reinforce the strong influence of K on the development of herbivorous insects. Sinha *et al*. (2018) report that potassium fertilization reduces the attack of the mustard aphid *Lipaphis erysimi* on the crop. According to results obtained by Venter *et al*. (2014), the application of potassium to the wheat crop can induce a certain degree of tolerance to the attack of the Russian wheat aphid (*Diuraphis noxia*).

Conclusions

The results obtained in our study clearly showed that fertilization with N, P and K generated remarkable effects on the main nutritional parameters of sorghum and these, in turn, on the susceptibility of the crop to attack by *M. sacchari/sorghi*. The most notorious effect was observed in the total soluble proteins, reducing sugars, and concentrations of N and K in the leaf. Understanding the complex interactions that exist between the plant, the insect and the nutrients is the key to including crop nutrition in the management plan. The results obtained in this study can be used as a tactic to be considered for an integrated management of the pest in the sorghum crop.

Acknowledgments

Our sincere thanks to the Universidad Autonoma Chapingo for providing the necessary facilities for this study, and to the government of Mexico for the financial assistance provided. We also thank all the staff of the multiple use laboratory of the Department of Phytotechnology and the chemistry laboratory of the Department of Soils of the Universidad Autonoma Chapingo.

Author's contributions

JAST and OMG formulated the research goals and aims, JAST, OMG, JPP, GEV and MTCL developed the methodology, JAST, OMG and MVH verified the overall replication/reproducibility of the results/experiments, OMG and MVH applied the statistical techniques to analyze study data, JAST conducted the research and investigation process, JPP, GEV and MTCL provided the study materials, JAST prepared the initial draft, JAST and OMG carried out the critical review, commentary and revision of the manuscript, JPP, OMG, GEV, MVH and MTCL created the visualization/data presentation, and JAST and OMG managed and coordinated the research activity planning and execution.

Literature cited

Amtmann, A., Troufflard, S., & Armengaud, P. (2008). The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum*, *133*(4), 682–691. https://doi.org/10.1111/j.1399-3054.2008.01075.x

Aqueel, M. A., & Leather, S. R. (2011). Effect of nitrogen fertilizer on the growth and survival of *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) (Homoptera: Aphididae) on different wheat cultivars. *Crop Protection*, *30*(2), 216–221. https://doi.org/10.1016/j.cropro.2010.09.013

Applebaum, S. W., & Heifetz, Y. (1999). Density-dependent physiological phase in insects. *Annual Review of Entomology*, *44*(1), 317–341. https://doi.org/10.1146/annurev.ento.44.1.317

Aziz, S. M., Akter, T., Ali, M., Nasif, S. O., Shahriar, S. A., & Nowrin, F. (2018). Effect of nitrogen, phosphorus and potassium (NPK) application on insect pests infesting transplanting Aman rice (*Oryza sativa* L .). *Asian Research Journal of Agriculture*, *9*(3), 1–15. https://doi.org/10.9734/ARJA/2018/42953

Bala, K., Sood, A. K., Singh, V., & Thakur, S. (2018). Effect of plant nutrition in insect pest management: A review. *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 2737–2742.

Bednarz, C. W., & Oosterhuis, D. M. (1999). Physiological changes associated with potassium deficiency in cotton. *Journal of Plant Nutrition*, *22*(2), 303–313. https://doi.org/10.1080/01904169909365628

Behmer, S. T. (2009). Insect herbivore nutrient regulation. *Annual Review of Entomology*, *54*(1), 165–187. https://doi.org/10.1146/annurev.ento.54.110807.090537

Boswell, A. W., Provin, T., & Behmer, S. T. (2008). The relationship between body mass and elemental composition in nymphs of the grasshopper *Schistocerca americana*. *Journal of Orthoptera Research, 17*(2), 307–313. https://doi.org/10.1665/1082-6467-17.2.307

Bowling, R. D., Brewer, M. J., Knutson, A., Way, M., Porter, P., Bynum, E., Allen, C., & Villanueva, R. (2015). *Monitoreo de pulgón amarillo en sorgo* [Brochure]. https://cdn-ext.agnet.tamu.edu/wp-content/uploads/2016/03/sugarcane-aphid-guide-images-Espa%C3%B1ol_lista.pdf

Bowling, R. D., Brewer, M. J., Kerns, D. L., Gordy, J., Seiter, N., Elliott, N. E., Buntin, G. D., Way, M. O., Royer, T. A., Biles, S., & Maxson, E. (2016). Sugarcane aphid (Hemiptera: Aphididae): A new pest on sorghum in North America. *Journal of Integrated Pest Management*, *7*(1), 1–13. https://doi.org/10.1093/jipm/pmw011

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Bremner, J. M. (1965). Inorganic forms of nitrogen. In A. G. Norman (Ed.), *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* (pp. 1179–1237). American Society of Agronomy. https://doi.org/10.2134/agronmonogr9.2

Choudhary, A. K., Ramesh, L., & Sharma, V. (2001). Incidence of mustard aphid (*Lipaphis erysimi* Kalt and *Myzus persicae* sulzer) in *Brassica* species at varying fertility levels in the mid-hill zone of Himachal Pradesh. *Insect Environment*, *7*(2), 58–59.

Chow, A., Chau, A., & Heinz, K. M. (2011). Reducing fertilization: a management tactic against western flower thrips on roses. *Journal of Applied Entomology*, *136*(7), 520–529. https://doi.org/10.1111/j.1439-0418.2011.01674.x

Dash, D., Rath, L. K., & Mishra, B. K. (2007). Studies on nutrient status in rice foliage and its relationship with leaf folder and brown planthopper incidence. *Indian Journal of Plant Protection*, *35*(2), 243–247.

De Souza, M. F., & Davis, J. A. (2019). Determining potential hosts of *Melanaphis sacchari* (Hemiptera: Aphididae) in the Louisiana agroecoscape. *Environmental Entomology, 48*(4), 929–934. https://doi.org/10.1093/ee/nvz072

Díaz de León, T. J., Mejía, Á. C., & Hurtado, G. B. (2008). *Recomendaciones de fertilización para mejorar la nutrición* [Brochure]. Brócoli, INIFAP, Campo Experimental Bajío.

Dong, Y. C., Han, P., Niu, C. Y., Zappalà, L., Amiens-Desneux, E., Bearez, P., Lavoir, A. V., Biondi, A., & Desneux, N. (2018). Nitrogen and water inputs to tomato plant do not trigger bottom-up effects on a leafminer parasitoid through host and non-host exposures. *Pest Management Science*, *74*(3), 516–522. https://doi.org/10.1002/ps.4750

Douglas, A. E. (2006). Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany*, *57*(4), 747–754. https://doi.org/10.1093/jxb/erj067

El-Zahi, E. S., Arif, S. A., Jehan, B. A., & Madeha, E. H. E. (2012). Inorganic fertilization of cotton field-plants in relation to sucking insects and yield production components of cotton plants. *Journal of American Science*, *8*(2), 509–517.

Facknath, S., & Lalljee, B. (2005). Effect of soil-applied complex fertiliser on an insect-host plant relationship: *Liriomyza trifolii* on *Solanum tuberosum*. *Entomologia Experimentalis et Applicata*, *115*(1), 67–77. https://doi.org/10.1111/j.1570-7458.2005.00288.x

Fallahpour, F., Ghorbani, R., Nassiri-Mahallati, M., & Hosseini, M. (2015). Demographic parameters of *Lipaphis erysimi* on canola cultivars under different nitrogen fertilization regimes. *Journal of Agricultural Science and Technology*, *17*(1), 35–47.

Fontanetto, H., Keller, O., Belotti, L., Negro, C., & Giailevra, D. (2009). Efecto de diferentes combinaciones de nitrógeno y azufre sobre el cultivo de sorgo granífero. *Informaciones Agronómicas*, (46), 21–23. http://www.ipni.net/publication/ia-lacs.nsf/0/997A673515052C9F8525798400580E8B/$FILE/21.pdf

Gash, A. F. J. (2012). Wheat nitrogen fertilisation effects on the performance of the cereal aphid *Metopolophium dirhodum*. *Agronomy*, *2*(1), 1–13. https://doi.org/10.3390/agronomy2010001

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, *66*(3), 483–492. https://doi.org/10.2307/3544943

Hosseini, M., Ashouri, A., Enkegaard, A., Goldansaz, S. H., Mahalati, M. N., & Hosseininaveh, V. (2010). Performance and population growth rate of the cotton aphid, and associated yield losses in cucumber, under different nitrogen fertilization regimes. *International Journal of Pest Management*, *56*(2), 127–135. https://doi.org/10.1080/09670870903248827

Hosseini, A., Hosseini, M., Goldani, M., Karimi, J., & Madadi, H. (2015). Effect of nitrogen fertilizer on biological parameters of the cowpea aphid and associated productivity losses in common globe amaranth. *Journal of Agricultural Science and Technology*, *17*, 1517–1528.

Huber, S. C. (1984). Biochemical basis for effects of K-deficiency on assimilate export rate and accumulation of soluble sugars in soybean leaves. *Plant Physiology*, *76*(2), 424–430. https://doi.org/10.1104/pp.76.2.424

Knutson, A., Bowling, R., Brewer, M. J., Bynum, E., & Porter, P. (2016). *The sugarcane aphid: management guidelines for grain and forage sorghum in Texas*. Texas A&M AgriLife Extension and Research, Texas A&M University.

Lama, L., Wilson, B. E., Davis, J. A., & Reagan, T. E. (2019). Influence of sorghum cultivar, phenological stage, and fertilization on development and reproduction of *Melanaphis sacchari* (Hemiptera: Aphididae). *Florida Entomologist*, *102*(1), 194–201. https://doi.org/10.1653/024.102.0131

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nibouche, S., Costet, L., Holt, J. R., Jacobson, A., Pekarcik, A., Sadeyen, J., Amstrong, J. S., Peterson, G. C., McLaren, N., & Medina, R. F. (2018). Invasion of sorghum in the Americas by a new sugarcane aphid (*Melanaphis sacchari*) superclone. *PLosOne*, *13*(4), Article e0196124. https://doi.org/10.1371/journal.pone.0196124

Pandey, A. K. (2010). Effect of nitrogen, phosphorus and potash on mustard aphid and yield attributing characters of mustard in cold arid region (Ladakh). *Indian Journal of Entomology*, *72*(2), 117–121.

Peña-Martínez, R., Lomeli-Flores, R., Bujanos-Muñiz, R., Muñoz-Viveros, A. L., Vanegas-Rico, J. M., Salas-Monzón, R., Hernández-Torres, O. E., Marín-Jarillo, A., & Ibarra-Rendón, J. (2018). *Pulgón amarillo del sorgo, (PAS), Melanaphis sacchari (Zehntner, 1897), interrogantes biológicas y tablas de vida*. Fundación Guanajuato Produce.

Perales-Rosas, D., Hernández-Pérez, R., López-Martínez, V., Andrade-Rodríguez, M., Alia-Tejacal, I., Juárez-López, P., Perdomo-Roldán, F., & Guillén-Sánchez, D. (2019). Evaluación de la antibiosis, antixenosis, y tolerancia de *Melanaphis sacchari/sorghi* en híbridos de sorgo. *Southwestern Entomologist, 44*(3), 763–770. https://doi.org/10.3958/059.044.0321

Pettigrew, W. T. (1999). Potassium deficiency increases specific leaf weights and leaf glucose levels in field-grown cotton. *Agronomy Journal*, *91*(6), 962–968. https://doi.org/10.2134/agronj1999.916962x

Rashid, M., Jahan, M., Islam, K., Bari, M., & Haque, S. (2014). Effect of nutrient management on population growth of brown planthopper, *Nilaparvata lugens* (Stål). *Bangladesh Rice Journal*, *17*(1–2), 38–48. https://doi.org/10.3329/brj.v17i1-2.20900

Rodríguez-del-Bosque, L. A., & Terán, A. P. (2015). *Melanaphis sacchari* (Hemiptera: Aphididae): a new sorghum insect pest in Mexico. *Southwestern Entomologist*, *40*(2), 433–434. https://doi.org/10.3958/059.040.0217

Rostami, M., Zamani, A., Goldasteh, S., Shoushtari, R., & Kheradmand, K. (2012). Influence of nitrogen fertilization on biology of *Aphis gossypii* (Hemiptera: Aphididae) reared on *Chrysanthemum Iindicum* (Asteraceae). *Journal of Plant*

Protection Research, *52*(1), 118–121. https://doi.org/10.2478/v10045-012-0019-2

Sauge, M. H., Grechi, I., & Poëssel, J. L. (2010). Nitrogen fertilization effects on *Myzus persicae* aphid dynamics on peach: vegetative growth allocation or chemical defence? *Entomologia Experimentalis et Applicata*, *136*(2), 123–133. https://doi.org/10.1111/j.1570-7458.2010.01008.x

Singh, V., & Sood, A. K. (2017). Plant Nutrition: a tool for the management of hemipteran insect-pests - A review. *Agricultural Reviews*, *38*(4), 260–270. https://doi.org/10.18805/ag.R-1637

Sinha, R., Singh, B., Rai, P. K., Kumar, A., Jamwal, S., & Sinha, B. K. (2018). Soil fertility management and its impact on mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae). *Cogent Food and Agriculture*, *4*(1), Article 1450941. https://doi.org/10.1080/23311932.2018.1450941

Somogyi, M. (1952). Notes of sugar determination. *Journal of Biological Chemistry*, *195*(1), 19–23. https://doi.org/10.1016/S0021-9258(19)50870-5

Venter, E., Mansoor, C. V., Sibisi, P., & Botha, A.-M. (2014). Potassium phosphate induces tolerance against the Russian wheat aphid (*Diuraphis noxia*, Homoptera: Aphididae) in wheat. *Crop Protection*, *61*, 43–50. https://doi.org/10.1016/j.cropro.2014.03.015

Wilson, B. E., Reay-Jones, F. P. F., Lama, L., Mulcahy, M., Reagan, T. E., Davis, J. A., Yang, Y., & Wilson, L. T. (2020). Influence of sorghum cultivar, nitrogen fertilization, and insecticides on infestations of the sugarcane aphid (Hemiptera: Aphididae) in the southern United States. *Journal of Economic Entomology*, *113*(4), 1850–1857. https://doi.org/10.1093/jee/toaa121

Witham, F. H., Blaydes, D. F., & Devlin, R. M. (1971). *Experiments in plant physiology*. Van Nonstrand Reinhold Company.

Wyn-Jones, R. G., & Pollard, A. (1983). Proteins, enzymes and inorganic ions. In A. Läuchli, & R. L. Bieleski (Eds.), *Encyclopedia of Plant Physiology* (pp. 528–562). Springer.

Zapata, S. D., Villanueva, R., Sekula, D., Esparza-Díaz, G., Duke, K., & Mutaleb, M. (2016, February 6–9). *The economic impact of the sugarcane aphid on sorghum production* [Conference presentation]. Southern Agricultural Economics Association's Annual Meeting, San Antonio, Texas, United States. https://ageconsearch.umn.edu/record/229992. https://doi.org/10.22004/ag.econ.229992

Impact of biochar use on agricultural production and climate change. A review

Impacto del uso del biocarbón sobre la producción agrícola y el cambio climático. Una revisión

Sandra Moreno-Riascos[1, 2] and Thaura Ghneim-Herrera[1]*

ABSTRACT

Biochar is a solid material obtained from the thermal decomposition of biomass of diverse biological origins through a process called pyrolysis. Biochar has great potential for reducing greenhouse gas emissions, sequester carbon in the soil, rehabilitate degraded soils, and reduce dependence on chemical fertilizers in crops. It also improves the physical, chemical, and biological properties of the soil and has a positive effect on plant growth. Given these attributes, there is a growing interest for adopting its use in agriculture, soil and land reclamation, and climate change mitigation. The effects of biochar application can be neutral or positive and will be determined mainly by factors such as the origin of the raw materials, carbonization conditions, frequency of applications, the method of application and dosage. In this review, we offer a detailed examination of the origins of biochar and the technologies used for its production. We examine the various materials that have been used to produce biochars and how they affect their physico-chemical characteristics, and we describe their applications in agriculture and climate change mitigation. Finally, we list the guides that describe the standards for the production, characterization, and commercialization of biochar that seek to guarantee the quality of the product and the essential characteristics for its safe use.

Key words: pyrolysis, carbon, biomass, amendment.

RESUMEN

El biocarbón es un material sólido obtenido a partir de la descomposición térmica de biomasa de diverso origen biológico mediante un proceso llamado pirólisis. El biocarbón tiene un gran potencial para reducir las emisiones de gases de efecto invernadero, secuestrar el carbono en el suelo, rehabilitar los suelos degradados y reducir la dependencia de los fertilizantes químicos en los cultivos. También mejora las propiedades físicas, químicas y biológicas del suelo y tiene un efecto positivo en el crecimiento de las plantas. Teniendo en cuenta estos atributos, existe un interés creciente en adoptar su uso en la agricultura, la recuperación de suelos y tierras, y en la mitigación del cambio climático. Los efectos de la aplicación del biocarbón pueden ser neutros o positivos y estarán determinados principalmente por factores como el origen de la materia prima, las condiciones de carbonización, la frecuencia de las aplicaciones, el método de aplicación y la dosis. En este artículo ofrecemos un examen detallado de los orígenes del biocarbón y las tecnologías utilizadas para su producción. Examinamos los diversos materiales que se han utilizado para producir biocarbones y cómo éstos afectan a sus características fisicoquímicas, y describimos sus aplicaciones en la agricultura y la mitigación del cambio climático. Por último, enumeramos las guías que describen las normas de producción, caracterización y comercialización del biocarbón, las cuales tratan de garantizar la calidad del producto y las características esenciales para su uso seguro.

Palabras clave: pirólisis, carbono, biomasa, enmienda.

Introduction

Biochar is a solid, porous, carbon-enriched compound, produced by high temperature biomass thermal degradation under an inert atmosphere (absence of oxygen) through a thermochemical process known as pyrolysis (Itskos *et al.*, 2016; Basu, 2018; Baskar *et al.*, 2019). This process consists of simultaneous, successive and serial reactions that include dehydration, depolymerization, isomerization, aromatization, decarboxylation, and carbonization (Lee *et al.*, 2019; Xiao *et al.*, 2020). These stages are intertwined (Kan *et al.*, 2016) and cover a complex set of reactions that involve the formation of radical groups (Odinga *et al.*, 2020). Although it is widely considered as an independent biomass conversion technology, pyrolysis actually constitutes the first stage of the gasification and combustion processes (Levin *et al.*, 2016). The main stages of pyrolysis have been generally described as: i) initial evaporation of free moisture, ii) primary decomposition, and iii) secondary reactions (oil cracking and repolymerization) (Uddin *et al.*, 2018; Chen *et al.*, 2019).

Received for publication: 18 May, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.87398

[1] Departamento de Ciencias Biológicas, Universidad ICESI, Cali (Colombia).
[2] Center for Bioinformatics and Photonics-CIBioFi, Universidad del Valle, Cali (Colombia).
* Corresponding author: tghneim@icesi.edu.co

The complexity of the pyrolysis process is strongly related to the conditions of manufacturing such as the maximum process temperature, the particle size, the residence time (Zhang *et al.*, 2019), the temperature/heating time relationship, pressure, etc. (Itskos *et al.*, 2016; Leng & Huang, 2018; Szwaja *et al.*, 2018; Sato *et al.*, 2019). During this process, large chains of polymers and inorganic compounds, mainly made up of carbon, hydrogen and oxygen, decompose into smaller molecules in the form of gases, condensable vapors (tars and oils) and solid carbon (Kan *et al.*, 2016). The diversity in composition of the biomass, depending on its origin, added to the conditions described above, will determine the speed and degree of decomposition of each of the components (Tabakaev *et al.*, 2019) as well as their performance and quality (Intani *et al.*, 2016).

Through direct and indirect effects, biochar can improve the physicochemical properties and quality of the soil, increase the productivity of crops, or help mitigate climate change (Cheng *et al.*, 2018; Diatta *et al.*, 2020). That is why biochar has managed to position itself as a key input within the wide range of alternative practices for more sustainable development, thanks to its versatility and potential in different fields such as agriculture or the environment. Some effects derived from the application of biochar to the soil are a reduction of greenhouse gas emissions and the promotion of carbon sequestration in the soil, making possible the rehabilitation of degraded soils (Bis *et al.*, 2018; Gupta *et al.*, 2020). Additionally, biochar reduces dependence on chemical fertilizers in crops by improving the physical, chemical and biological properties of the soil (pH, surface area, cation exchange capacity (CEC), particle density, humidity, and conductivity) (Burrell *et al.*, 2016; Zwart, 2020), reducing the leaching of nutrients, or making a direct or indirect contribution of nutrients that exerts a promoting effect on plant growth and increasing crop yield (Ouyang *et al.*, 2014; Sun *et al.*, 2019). Biochar not only improves the physico-chemical properties of the soil, but it can also have a positive impact on its biological properties, such as an increase in the abundance and diversity of beneficial soil microorganisms, since it provides a micro habitat that serves as protection against other pathogenic microorganisms (Liao *et al.*, 2016; Ajema, 2018).

However, it is important to note that the type and magnitude of the effect on soil properties are associated not only with the chemical and physical properties of biochar (Weber & Quicker, 2018), but they can also vary remarkably depending on the type of soil where it is added. Therefore, the characteristics of the biochar and the type of soil are two important factors to consider before its use.

In this review, we provide detailed information about the different technologies, process conditions, and starting materials employed for biochar production and their influence on the final physico-chemical characteristics and practical applications of biochar. We also analyze the impact of biochar use in various applications, in areas such as the mitigation of climate change and agriculture, as well as the international regulations that govern biochar use.

A brief history of biochar

The origin of biochar goes back to the black earths, anthropogenic dark earths (ADE), or *Terra Preta de Indio,* as they were known by the indigenous populations that inhabited the Brazilian Amazon region (De Oliveira *et al.*, 2020). These lands exhibit completely different properties to most soils in that region (Glaser & Birk, 2012). These soils are widely distributed throughout the region and are characterized by having a high content of nutrients such as carbon (C), phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn) and organic matter (Barbosa *et al.*, 2020). Its organic component does not come strictly from the accumulation of vegetal cover, but rather it has originated as a result of ancestral human practices as a method for the use of plant and animal bone remains, cooking coal, and waste from fires that were made to replace forests with farming lands (Costa *et al.*, 2017; Macedo *et al.*, 2017). The use of coal for promoting agronomic productivity has been known for centuries (Ogawa & Okimori, 2010), since it retains its stability and properties for hundreds of years. Also, it has proven to be an excellent soil amendment (Ye *et al.*, 2020).

The term biochar was used for the first time in 1999 to describe an activated carbon prepared from sorghum grain and deployed in a process known as reverse-burn gasification (the ChemChar process) (Bapat *et al.*, 1999). Later, it was incorporated into scientific literature on bioenergy to describe charcoal prepared from various crop residues to be used as fuel. The idea of using biochar as an alternative to mitigate climate change was conceived by different authors since 1993, long before the term was born (Paustian *et al.*, 2016). However, it was not until the beginning of the 21st century that Johannes Lehmann associated biochar with the concept of climate change as a way to reduce greenhouse gas emissions (GHG); thus, biochar started to be considered as a soil amendment (Lehmann *et al*., 2006; Hyland *et al*., 2010).

Influence of pyrolysis process parameters and type of biomass on biochar characteristics

Biochar can be produced from a variety of biological, raw materials such as crop residues, decaying organic matter of animal origin (livestock manure, pigs, poultry, etc.) (Tag *et al*., 2016; Pariyar *et al*., 2020; Tomczyk *et al*., 2020). Regarding crop residues, the raw biomass is constituted mainly by carbohydrates formed from photosynthesis; the structures and compounds are very diverse depending on the type of plant. These characteristics also influence and significantly determine biochar properties (Li *et al*., 2020). The yield, ash contents, development and size of the pore, surface area, and even pH are just some of the characteristics that change considerably between the different types of starting material (Tan *et al*., 2017; Jafri *et al*., 2018).

Several types of biomass have been used to produce biochar with different purposes (Tab. 1).

TABLE 1. Different raw materials used to produce biochar and some areas of study and potential application.

Application	Type of raw material	Reference
Agriculture	Cow manure	(Zhao *et al.*, 2013)
	Pig manure	(Jin *et al.*, 2016)
	Shrimp waste	(Kazemi *et al.*, 2019)
	Cocoa shell	(Oyedeji *et al.*, 2018)
	Corn Sewage sludge	(Shareef *et al.*, 2018) (Chen *et al.*, 2020)
	Pine tree	(Lyu *et al.*, 2016)
	Corn cobs	(Shaheen & Turaib Ali Bukhari, 2018)
	Corn stem Bagasse Coconut fiber	(Liu *et al.*, 2013)
	Rice husk Almond shell Tree bark Algae Soybean husk	(Lee *et al.*, 2013)
	Peanut shell Grass	(Zhao *et al.*, 2013) (Ahmad *et al.*, 2012)
	Wheat straw Sawdust	(Laghari *et al.*, 2016; Muhammad *et al.*, 2017)
	Brazilian pepper Bamboo	(Yao *et al.*, 2012; Huang *et al.*, 2020)
Climate change	Oak wood Hard wood Pine tree chips	(Stewart *et al.*, 2013; Nelissen *et al.*, 2015)
	Peanut husk	(Cheng *et al.*, 2016)
	Rice husk	(Nguyen *et al.*, 2020b)
	Organic waste	(Kammann *et al.*, 2017)
	Peanut shell	(Lan *et al.*, 2019)
	Orange peel	(Ahmad *et al.*, 2012)
	Cow manure	(Nguyen *et al.*, 2020a)
Immobilization and pollutant removal	Wood, sewage sludge, agriculture waste	(Zhao *et al.*, 2019)
	Pruning waste	(Park *et al.*, 2015)
	Spent mushroom substrate	(Wu *et al.*, 2019)

Lignocellulose, the principal component of plant biomass, is mainly constituted of three organic polymers: cellulose (40-60% w/w), hemicellulose (20-40% w/w) and lignin (10-25% w/w) (Yang *et al*., 2007). Each one of these polymers react differently during the heat treatment (Weber & Quicker, 2018), due to their different thermal stabilities that influence not only the required treatment temperature, but also the yield regardless of the chosen conditions (Tag *et al*., 2016; Uddin *et al*., 2018).

A large number of studies have shown that there are several interactions between the main components of biomass (Yu *et al*., 2017); therefore, it is not possible to predict the pyrolysis results simply based on the individual thermal behavior of the three components (Yaashikaa *et al*., 2019). The interaction between hemicellulose and lignin promotes the production of phenols, while it hinders the generation of hydrocarbons (Cao *et al*., 2018). Lignin also interacts significantly with cellulose by limiting the polymerization of levoglucosan which reduces the formation of biochar, while the interaction between cellulose and hemicellulose has less effect on the formation and distribution of pyrolysis products (Kan *et al*., 2016).

Low heating rates and long residence times are usually used to produce biochar. At higher temperatures, the carbon content in the final material increases. However, for vegetal residues high temperatures are not ideal due to the increase in ash contents (Rafiq *et al*., 2016). As temperature increases, water evaporation rises. Volatile compounds are then released, which, in turn, enlarges the relative fixed carbon ratio of the solid compared to the raw biomass (Jouhara *et al*., 2018). At the same time, there are variations in the carbon, hydrogen, and oxygen contents that lead to some physicochemical changes such as polarity reduction, aromaticity increase, and biochar hydrophobicity (Lehmann *et al*., 2009; Gray *et al*., 2014) 500°C, and 620°C. The surface area as well as pore volume also increase slightly due to the release of volatiles during carbonization. This process reconfigures the structure of lignocellulose compounds, giving rise to the formation of structures shaped as canals (Sigmund *et al*., 2017).

The thermal decomposition of the organic components in the biomass begins between 350-550°C with the formation of two structural fractions composed by the stacking of crystalline sheets of poly-aromatic graphene that grow laterally, and by an amorphous fraction of randomly organized aromatic structures (Weber & Quicker, 2018). Both of these fractions are associated with ring shaped carbon bonds of benzene types with oxygen or hydrogen (Kwiatkowski & Kalderis, 2020), that give rise to layers, forming the structure of a lattice or slit that originates multiple spaces corresponding to the pores. These bonds between aromatic structures of C-O and C-H determine the stability of biochar and are used as a parameter to measure the degree of aromaticity (Choudhary *et al*., 2019). They also contain oxidized and aliphatic carbon structures that are easily degradable.

The dominant process is carbonization, that occurs at between the 600-800°C, and causes the removal of most non-carbonaceous atoms such as calcium (Ca), magnesium (Mg), and potassium (K) (Zhang *et al*., 2017; Chatterjee *et al*., 2020). Hydrogen (H), oxygen (O), phosphorus (P), and sulfur (S) are mainly located within aromatic rings as heteroatoms. The presence of heteroatoms contributes greatly to the reactivity of biochar and the chemical heterogeneity of its surface (Cheng & Li, 2018).

As a result, biochar with low H/C ratios, that corresponds to a higher degree of carbonization, contain fewer functional groups and more aromatic structures than biochar produced at low temperatures. O/C ratios are much higher than those present in mineral coal that also originates from biomass, but that is formed by geological processes in periods that include geological scales (Bakshi *et al*., 2020).

The pH of biochar also depends on the production parameters and type of biomass, which results in products with a wide range of pH values (Tag *et al*., 2016). When biochar is produced at temperatures below 400°C, it reaches a pH below 7, but at temperatures above 500°C, the pH can vary from 7 to more alkaline (Tomczyk *et al*., 2020).

Biochar production technologies

Within the diverse thermochemical processes that transform biomass into biochar, four methods are used: slow pyrolysis, fast pyrolysis, flash pyrolysis, and gasification (Novak *et al*., 2010; Ippolito *et al*., 2012).

Slow pyrolysis, or carbonization, is characterized by long residence times (hours to days) at relatively low temperatures (~300-700°C) and materials with different particle sizes (5-50 mm) (Claoston *et al*., 2014). In this type of pyrolysis, the thermal decomposition of biomass occurs at a very low heating rate (temperature/time), using enough time to maximize the solid yields thanks to repolymerization reactions. The previous method is used mainly to produce the solid fraction or coal, although it can also be gasified to obtain hydrogen-rich gas.

Fast pyrolysis generally involves high heating rates (>10-200°C sec^{-1}) and short residence times (0.5-10 sec) (Claoston *et al.*, 2014). It is used to obtain a high yield of liquid products, such as bio-oil, in relation to the dry biomass base, which can be as high as 50-70% by weight.

Finally, the pyrolysis process known as flash is characterized by very high heating rates (10^3-10^4°C sec^{-1}) and much shorter residence times (<0.5 sec), resulting in very high bio-oil yields that can reach up to 75-80% by weight (Gilbe *et al.*, 2008; Gao *et al.*, 2011; Enders *et al.*, 2012). In comparison to residual biomass, these materials offer greater chemical and biological decomposition resistance (Zimmerman, 2010) as well as a greater number of aromatic structures (Baldock & Smernik, 2002).

Biochar quality standards

In order to guarantee the safety of the use of biochar as a soil amendment to the market and especially consumers, the members of the International Biochar Initiative (IBI) in conjunction with research centers, scientists, farmers, and producers worldwide, developed guidelines and policies that provide the rules for the characterization of biochar products. All the standards are based on a series of guiding principles and the follow-up of guidelines consistent with the best practices for the development of standards such as the International Standards Organization (ISO), ASTM International, and the Institute of Electrical and Electronics Engineers (IEEE). Compliance with these standards by manufacturers is not mandatory. However, the IBI has implemented a certification program to expand the biochar industry since 2013. Through this program the producer can certify that the product complies with quality standards and that it is safe for use in soils. In addition, the IBI Certified TM quality seal is awarded, which is recognized worldwide (International Biochar Initiative, 2015).

At the same time, Europe has the European Biochar Certificate (EBC) that applies a system for the sustainable production of biochar based on the latest and most innovative research and practices. These practices aim to guarantee the production control and quality of biochar with processes that have scientific and legal support and that are economically viable and practically applicable. Unlike the IBI standards, the European certificate is mandatory for all producers (European Biochar Foundation, 2016). Currently, there are two documents approved by the members of the IBI and EBC that contain the technical programs, policies, and guidelines: IBI Biochar standards (version 2.0) and European biochar certificate guidelines (version 6.4E).

Biochar in agriculture

Great interest has been generated on biochar and its application to soils due to the similarity between biochar particles and those found in *Terra Preta de Indio* (Lehmann *et al.*, 2011). Several studies have shown that the physical and chemical properties of biochar favor adsorption thanks to its high specific surface area compared to other organic amendments, thus improving the availability of nutrients in the soil (Bonanomi *et al.*, 2017; Yadav *et al.*, 2019).

The pH is one of the most important soil parameters because of its great influence on biological, chemical, physical, and geological processes, and because it is related to soil fertility (Neina, 2019). Changes in pH trigger a series of modifications in the soil environment, affecting the availability of nutrients for plant growth and microbial activity, or accelerating biogeochemical processes in the rhizosphere (Ducey *et al.*, 2015). The addition of biochar as a soil amendment has become a common practice, as it improves soil quality by reducing soil acidity. Due to its porous matrix structure, it can also improve soil structure by forming micro aggregates, increase water retention, improve solubilization, nutrient retention and transport, enhance the soil quality index and reduce nutrient leaching (Martinsen *et al.*, 2014; Wang *et al.*, 2014; Pratiwi *et al.*, 2016; Jeffery *et al.*, 2017; Oladele, 2019).

Some properties of biochar, such as high surface charge density, large surface area, internal porosity, and the presence of polar and non-polar surface sites play an important role in the liming effect (Shetty & Prakash, 2020). The effect of pH on acidic soils is probably due to their alkalinity and high buffering capacity (Juriga & Šimanský, 2019). The basic cations (e.g., K, Ca, Mg and Si) that are contained in biochar as carbonates or oxides can reduce the acidity by increasing the reaction of the exchangeable basic cations. This process is carried out through the functional groups on the surface of biochar as COO^- and O^- with the H^+ or Al^{+3} ions in the soil, which would cause an increase in the soil pH (Hansen *et al.*, 2016; Dai *et al.*, 2017; Zong *et al.*, 2018).

Another physicochemical property of the soil associated with its fertility is cation exchange capacity (CEC), which refers to the capacity of soils to retain cations interchangeably through adsorption (Durães *et al.*, 2018; Zhang *et al.*, 2018). The CEC of biochar is mainly a consequence of the temperature conditions at which it is produced (Tan *et al.*, 2017; Leng & Huang, 2018). The carboxylic groups formed in the bridges of the aromatic nuclei of biochar are responsible for the increase of the CEC and reactivity

(Zhang *et al.*, 2018). After biochar is added to the soil, the charges of its functional groups are generally positive, but over time the functional groups on the surface oxidize and generate more negative charges relative to the positive ones, increasing the CEC. Adsorption of highly oxidized organic matter on biochar surfaces is another process that affects CEC (Tomczyk *et al.*, 2020). When biochar is exposed to oxygen and water, more functional groups can be generated on the surface through oxidation, thus increased CEC is attained (Hue, 2020). However, CEC is highly variable depending on the surface chemistry of biochar and tends to change once it is incorporated due to interactions with the environment (Hailegnaw *et al.*, 2019).

These processes can directly or indirectly influence the improvement of other physical and chemical soil properties (Karimi *et al.*, 2020). A positive effect of biochar on the productivity of the crops has been shown (Jin *et al.*, 2016; He *et al.*, 2017; Masud *et al.*, 2020). The most significant changes have been observed in acid soil (Diatta *et al.*, 2020), which suggests that its yield effect is similar to that produced by liming, accompanied by better water retention (Ahmed *et al.*, 2016). The water retention capacity is mainly due to two factors: the large internal surface and the high number of residual pores in the biochar, where water is retained by capillarity, improving soil aggregation and structure. This increases the general porosity of the soil and the water content, leading to a decrease in the mobility of water, reducing water stress in plants (Batista *et al.*, 2018).

Biochar improves the structure, porosity and aggregation of the soil, facilitating tillage (Du *et al.*, 2018) and favoring the availability of nutrients (Karimi *et al.*, 2020). Nutrient composition and availability from biochar depend on biochar raw material and pyrolysis conditions (Purakayastha *et al.*, 2019). In addition, various factors affect the nutrient status in biochar-treated soils including the soil and feedstock type, pyrolysis temperature, and addition rate (Yu *et al.*, 2019). Biochar can directly provide higher nutrient content such as P, K, Ca, Mg (Gao *et al.*, 2017). Also, it can influence soil microbial activity due to changes in labile carbon and soil properties. The activity of soil microbes can significantly affect soil organic matter decomposition and nutrient cycling (Yu *et al.*, 2019). The soil microbial biomass and enzyme activities have major impacts on the soil nutrient status and crop productivity. Another promoted benefit of biochar application to soil is reduced nutrient leaching (Zhao *et al.*, 2019; Karimi *et al.*, 2020).

Therefore, biochar does not act by means of a single effect. It influences different, interconnected soil physicochemical and biological properties. A highly variable response has been shown in the soil biota, reporting increases, decreases (Kolton *et al.*, 2011; Li *et al.*, 2018), or no significant effects (Lehmann *et al.*, 2011). These interactions between microorganisms and biochar are also controlled by multiple environmental factors such as types and rates of biochar amendment, soil type, land use, and vegetation types (Gorovtsov *et al.*, 2020).

Biochar as a matrix carrier for plant growth-promoting microorganisms

Peat is the most widely used inoculum carrier in the world; however, its availability is limited (Sahu & Brahmaprakash, 2016). Exploration of other matrix alternatives generated by more sustainable processes and that compete biologically and economically with the materials currently used could be beneficial to the biofertilizer industry (Herrmann & Lesueur, 2013; Flores-Félix *et al.*, 2019; Saeid & Chojnacka, 2019). Biochar has the potential to be used as an alternative vehicle in the development of new biofertilizers. Its use as a carrier of plant growth-promoting microorganisms could favor both the proliferation of the microorganism of interest immobilized in it and the abundance of indigenous microorganisms (Ajema, 2018). However, it is important to consider that the raw material, the pyrolysis temperature, the degree of oxidation, and the size of the pores obtained during the production of biochar affect the characteristics of the final product and, therefore, its effect on microorganisms. The effects of inoculated biochar also vary according to the environmental conditions that directly affect the viability of microorganisms (Palansooriya *et al.*, 2019).

The effects of biochar incorporation on soil microorganisms can be direct through the contribution of labile organic matter that provides a source of energy and nutrients. They can also be indirect, through changes in some soil properties, such as pH or porosity (Gorovtsov *et al.*, 2020). The porous structure of biochar can act as a habitat for microorganisms. It can also increase the soil surface, which is able to retain water and nutrients favoring the growth of soil biota and rhizosphere microbiome (Jenkins *et al.*, 2017; Husna *et al.*, 2019).

The addition of biochar can increase mineralization through its contribution of labile organic matter. In general, biochar obtained through slow pyrolysis contains a greater amount of labile organic matter and tends to cause a greater increase in soil biota than more recalcitrant biochar (Cross & Sohi, 2011; Hardy *et al.*, 2019).

Hale *et al.* (2014) show that survival but not abundance of *Enterobacter cloacae* (UW5), a well-studied bacterium that produces indole acetic acid (IAA) in a tryptophan-dependent manner, increases in soils amended with any of five different types of biocarbon (palm leaves, pine wood, coconut shell, pistachio shell, and fruit pits) when compared to peat and vermiculite. Ghazi (2017) evaluates the effect of *Rhizobium*-based formulations developed with biochar from rice husks on bean seeds, which were compared with peat and vermiculite materials. The biochar-based treatment records a maximum carrier survival population after 180 d of inoculation. In addition, a slight decrease in pH was recorded at the end of the storage period.

Biochar derived from acacia wood and inoculated with *Azospirillum lipoferum* (AZ 204) was tested at different dose levels (0, 5, 10, 15, 20, 25 t ha^{-1}) and its effect on maize growth was evaluated. A significant increase was observed in *Azospirillum* and other diazotroph populations in the rhizosphere after the application of biochar at all the stages of crop growth. Furthermore, a significant increase of native mycorrhizal colonization was observed in response to biochar-*Azospirillum* application (Saranya *et al.*, 2011).

Additionally, combinations of bacteria have been evaluated in the formulation of biofertilizers based on biochar, with the aim of finding sets of microorganisms that enhance plant growth through an interactive and harmonious work. The microbial consortium formed by endophytic bacteria (*Brevibacillus, Enterobacter, Kytococcus, Pantoea, Pseudomonas, Serratia,* and *Stenotrophomonas*) and fungi (*Cutaneotrichosporon, Mucor,* and *Wickerhamomyces*) isolated from rapeseed (*Brassica napus*) and barley (*Hordeum vulgare*) were inoculated into a biochar derived from processed wood waste to evaluate its potential as a carrier of plant growth promoting microorganisms (PGPM) and its growth effect on barley seedlings. A synergistic effect of the microbial consortium with the biochar on root growth was observed. A decrease in urease activity in the soil was also found, without causing a negative effect on seedling development during the study (Vecstaudza *et al.*, 2017).

Biochar has been recognized as a carrier matrix not only for microorganisms with a plant growth-promoting effect, but also as an alternative for the immobilization of microorganisms used in bioremediation. For example, biochar was used as a carrier of bacterial consortia in soils contaminated with cypermethrin, an insecticide for agricultural use (Liu *et al.*, 2017).

Biochar can also play an important role as a carrier of bacteria used in biological control. In sugarcane, this strategy showed positive results for the control of the pathogen *Macrophomina phaseolina* by the antagonistic rhizobacterium *Paenibacillus illinoisensis* RH-3 (Shahjahan *et al.*, 2018). That study demonstrated that biochar derived from sugarcane bagasse is a better carrier of *P. illinoisensis* RH-3, compared to peat and lignite. The neutral pH of biochar together with its porosity and high availability of nutrients seemed to be determinants of its positive effect (Shahjahan *et al.*, 2018).

All research on the potential of biochar as a carrier of microbial inoculum supports its use as an alternative to classical chemical fertilization. However, further studies are needed to elucidate the synergistic effects of biochar and soil microorganisms on plant growth promotion, their mechanisms of action, and the interactions between microorganisms and their microhabitats. Finally, such effects must also be validated under field conditions.

Biochar in climate change mitigation

Biochar was first recognized for its potential as a tool to counteract climate change in 2009 (Lehmann *et al.*, 2009). Its porous structure and high internal surface area give it the capacity to absorb soluble organic compounds, gases, some inorganic compounds, and different pollutants thanks to the diversity of functional groups on its surface (Mendes *et al.*, 2018). Biochar has been evaluated for the removal of heavy metals in contaminated waters such as lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) (Shim *et al.*, 2015; Wang *et al.*, 2019; Zhao *et al.*, 2019). The type of biomass and the temperature of the process are very influential for the absorption capacity of biochar. For example, materials produced at high temperatures result in materials with a larger surface area, higher carbon content and microporosity, leading to increased efficiency in removing compounds such as trichloroethylene (TCE), benzene (C_6H_6) and nitrobenzene ($C_6H_5NO_2$) (Zhou *et al.*, 2010; Ahmad *et al.*, 2012).

The application of biochar to soils has been proposed as a strategy to reduce the concentration of CO_2 in the atmosphere, as it would serve as a long-term carbon sink. Its recalcitrant nature, i.e., its resistance to degradation due to all the carbon present in its structure, gives it the ability to remain in soils for hundreds or thousands of years (Woolf *et al.*, 2010) and to reduce the rate at which carbon fixed by photosynthesis returns to the atmosphere. It has also been suggested that carbon retention can be much greater when soil carbon stocks increase (Ding *et al.*, 2018; Ventura *et al.*, 2019). However, some studies have found that many of the practices used to sequester carbon in the soil are often offset by increased greenhouse gas emissions (Shen *et al*., 2014; Zhang *et al*., 2019) and that soils show a low potential for carbon accumulation (Smith, 2016; Sharma, 2018; Gupta *et al*., 2020). However, among the possible strategies to remove CO_2 from the atmosphere, biochar stands out in this area and currently represents the most promising alternative.

Biochar application can also reduce the emissions of nitrous oxide (N_2O) and sometimes, the flow CO_2 in soil, generating an additional tool to mitigate their effects (Woolf *et al*., 2010). However, the results have not always been positive in terms of CO_2 reductions (Spokas *et al*., 2010).

Regarding the potential of biochar to reduce nitrous oxide (N_2O) emissions, several hypotheses have been put forward on how biochar could interact with denitrification, by directly stimulating or suppressing the amount of N that passes into the gaseous form. These hypotheses relate this effect to several different biochar properties, soil type, and environmental conditions such as temperature and precipitation (Dicke *et al*., 2015; Lan *et al*., 2019). Although to date the impact of biochar on denitrification has not yielded conclusive results (Cayuela *et al*., 2014; Ameloot *et al*., 2016; Kammann *et al*., 2017), it appears that the magnitude and mechanisms associated with biochar inhibition of N_2O depend on the dose and application frequency (Liu *et al*., 2020).

Biochar-induced changes in the composition or activity of the microbial community not only affect nutrient cycles and plant growth but also the soil organic matter cycle (Ouyang *et al*., 2014; Hardy *et al*., 2019; Gorovtsov *et al*., 2020). While research under controlled conditions has shown that biochar can affect N_2O emissions from the soil (Taghizadeh-Toosi *et al*., 2011; Schimmelpfennig *et al*., 2014; Kammann *et al*., 2017), laboratory results cannot be extrapolated to what is expected in the field. In field trials, often no statistical differences are observed between biochar and control treatments. Despite the extensive literature published on the subject in recent years, it is still very unpredictable whether a type of biochar will be effective in mitigating N_2O emissions in a field with crops. Factors such as application rate, biochar characteristics, soil type, environmental conditions, less homogeneous particle distribution, and greater soil (and plant) heterogeneity in the fields result in a high variability in N_2O flows (Hüppi *et al*., 2015). Therefore, most research efforts are now directed towards achieving the greatest reductions in N_2O emissions by selecting the most efficient biochars and analyzing the mechanisms involved.

Conclusions

The addition of biochar to the soil provides different benefits derived from changes in the physical, chemical, and biological characteristics of the soil, which in turn, promote plant growth and productivity by increasing the availability of nutrients and pH. The porosity of biochar, along with water and nutrient retention, can promote the establishment and increase the abundance and diversity of microbiota which, in turn, promote plant growth.

The potential of biochar for carbon sequestration and its ability to reduce greenhouse gas emissions make it a very attractive alternative to counteract the adverse effects of climate change. However, further research is needed in this field to elucidate its long-term effects.

The wide availability of biochar as well as the varied responses in crops that will depend not only on the crop species but also on the type of soil, make it difficult to establish standard parameters regarding the dose and form of biochar application. For this reason, it is necessary to expand research in this field, since the wide availability of raw materials and the different technologies available for pyrolysis offer a wide range of opportunities for the production, marketing, and profitable use of biochar in Colombian agriculture.

Acknowledgments

SMR MSc studies were financially supported by a grant from Universidad Icesi and the CIBioFi Project, funded by the Colombian Science, Technology and Innovation Fund - General Royalties System (Fondo CTeI- SGR), Gobernación del Valle del Cauca, and Ministerio de Ciencia, Tecnologia e Innovación (Minciencias), under contract No. BPIN 2013000100007. We also kindly thank Franklin Gerardo Ojeda for his revision of the manuscript.

Author's contributions

SMR carried out the writing of the original draft and TGH conducted the critical review and revision of this manuscript.

Literature cited

Ahmad, M., Lee, S. S., Dou, X., Mohan, D., Sung, J. K., Yang, J. E., & Ok, Y. S. (2012). Effects of pyrolysis temperature on soybean stover- and peanut shell-derived biochar properties and TCE adsorption in water. *Bioresource Technology*, *118*, 536–544. https://doi.org/10.1016/j.biortech.2012.05.042

Ahmed, A., Kurian, J., & Raghavan, V. (2016). Biochar influences on agricultural soils, crop production, and the environment:

a review. *Environmental Reviews*, *24*(4), 495–502. https://doi.org/10.1139/er-2016-0008

Ajema, L. (2018). Effects of biochar application on beneficial soil organism review. *International Journal of Research Studies in Science, Engineering and Technology*, *5*(5), 9–18. https://doi.org/10.13140/RG.2.2.15186.66247

Ameloot, N., Maenhout, P., De Neve, S., & Sleutel, S. (2016). Biochar-induced N_2O emission reductions after field incorporation in a loam soil. *Geoderma*, *267*, 10–16. https://doi.org/10.1016/j.geoderma.2015.12.016

Bakshi, S., Banik, C., & Laird, D. A. (2020). Estimating the organic oxygen content of biochar. *Scientific Reports*, *10*(1), Article 13082. https://doi.org/10.1038/s41598-020-69798-y

Baldock, J. A., & Smernik, R. J. (2002). Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Organic Geochemistry*, *33*(9), 1093–1109. https://doi.org/10.1016/S0146-6380(02)00062-1

Bapat, H., Manahan, S. E., & Larsen, D. W. (1999). An activated carbon product prepared from milo (*Sorghum vulgare*) grain for use in hazardous waste gasification by ChemChar cocurrent flow gasification. *Chemosphere*, *39*(1), 23–32. https://doi.org/10.1016/S0045-6535(98)00585-2

Barbosa, J. Z., Motta, A. C. V., Corrêa, R. S., Melo, V. de F., Muniz, A. W., Martins, G. C., Silva, L. de C. R., Teixeira, W. G., Young, S. D., & Broadley, M. R. (2020). Elemental signatures of an Amazonian Dark Earth as result of its formation process. *Geoderma*, *361*, Article 114085. https://doi.org/10.1016/j.geoderma.2019.114085

Baskar, G., Kalavathy, G., Aiswarya, R., & Abarnaebenezer Selvakumari, I. (2019). Advances in bio-oil extraction from nonedible oil seeds and algal biomass. In K. Azad (Ed.), *Advances in eco-fuels for a sustainable environment, a volume in Woodhead Publishing series in energy* (pp. 187–210). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-102728-8.00007-3

Basu, P. (2018). Biomass gasification, pyrolysis and torrefaction, practical design and theory (3rd ed.). Academic Press. https://doi.org/10.1016/B978-0-12-812992-0.00005-4

Batista, E. M. C. C., Shultz, J., Matos, T. T. S., Fornari, M. R., Ferreira, T. M., Szpoganicz, B., de Freitas, R. A., & Mangrich, A. S. (2018). Effect of surface and porosity of biochar on water holding capacity aiming indirectly at preservation of the Amazon biome. *Scientific Reports*, *8*(1), Article 10677. https://doi.org/10.1038/s41598-018-28794-z

Bis, Z., Kobyłecki, R., Ścisłowska, M., & Zarzycki, R. (2018). Biochar - potential tool to combat climate change and drought. *Ecohydrology & Hydrobiology*, *18*(4), 441–453. https://doi.org/10.1016/j.ecohyd.2018.11.005

Bonanomi, G., Ippolito, F., Cesarano, G., Nanni, B., Lombardi, N., Rita, A., Saracino, A., & Scala, F. (2017). Biochar as plant growth promoter: better off alone or mixed with organic amendments? *Frontiers in Plant Science*, *8*, Article 1570. https://doi.org/10.3389/fpls.2017.01570

Burrell, L. D., Zehetner, F., Rampazzo, N., Wimmer, B., & Soja, G. (2016). Long-term effects of biochar on soil physical properties. *Geoderma*, *282*, 96–102. https://doi.org/10.1016/j.geoderma.2016.07.019

Cao, W., Li, J., Martí-Rosselló, T., & Zhang, X. (2018). Experimental study on the ignition characteristics of cellulose, hemicellulose, lignin and their mixtures. *Journal of the Energy Institute*, *92*(5), 1303–1312. https://doi.org/10.1016/j.joei.2018.10.004

Cayuela, M. L., van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., & Sánchez-Monedero, M. A. (2014). Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. *Agriculture, Ecosystems and Environment*, *191*, 5–16. https://doi.org/10.1016/j.agee.2013.10.009

Chatterjee, R., Sajjadi, B., Chen, W. Y., Mattern, D. L., Hammer, N., Raman, V., & Dorris, A. (2020). Effect of pyrolysis temperature on physicochemical properties and acoustic-based amination of biochar for efficient CO_2 adsorption. *Frontiers in Energy Research*, *8*, Article 85. https://doi.org/10.3389/fenrg.2020.00085

Chen, C., Liu, G., An, Q., Lin, L., Shang, Y., & Wan, C. (2020). From wasted sludge to valuable biochar by low temperature hydrothermal carbonization treatment: insight into the surface characteristics. *Journal of Cleaner Production*, *263*, Article 121600. https://doi.org/10.1016/j.jclepro.2020.121600

Chen, W. H., Wang, C. W., Ong, H. C., Show, P. L., & Hsieh, T. H. (2019). Torrefaction, pyrolysis and two-stage thermodegradation of hemicellulose, cellulose and lignin. *Fuel*, *258*, Article 116168. https://doi.org/10.1016/j.fuel.2019.116168

Cheng, F., & Li, X. (2018). Preparation and application of biochar-based catalysts for biofuel production. *Catalysts*, *8*(9), Article 346. https://doi.org/10.3390/catal8090346

Cheng, N., Peng, Y., Kong, Y., Li, J., & Sun, C. (2018). Combined effects of biochar addition and nitrogen fertilizer reduction on the rhizosphere metabolomics of maize (*Zea mays* L.) seedlings. *Plant and Soil*, *433*, 19–35. https://doi.org/10.1007/s11104-018-3811-6

Cheng, Q., Huang, Q., Khan, S., Liu, Y., Liao, Z., Li, G., & Ok, Y. S. (2016). Adsorption of Cd by peanut husks and peanut husk biochar from aqueous solutions. *Ecological Engineering*, *87*, 240–245. https://doi.org/10.1016/j.ecoleng.2015.11.045

Choudhary, T. K., Khan, K. S., Hussain, Q., Ahmad, M., & Ashfaq, M. (2019). Feedstock-induced changes in composition and stability of biochar derived from different agricultural wastes. *Arabian Journal of Geosciences*, *12*, Article 617. https://doi.org/10.1007/s12517-019-4735-z

Claoston, N., Samsuri, A. W., Ahmad Husni, M. H., & Mohd Amran, M. S. (2014). Effects of pyrolysis temperature on the physicochemical properties of empty fruit bunch and rice husk biochars. *Waste Management and Research, 32*(4), 331–339. https://doi.org/10.1177/0734242X14525822

Costa, A. da R., Silva Júnior, M. L., Kern, D. C., Ruivo, M. de L. P., & Marichal, R. (2017). Forms of soil organic phosphorus at black earth sites in the Eastern Amazon. *Revista Ciência Agronômica, 48*(1), 1–12. https://doi.org/10.5935/1806-6690.20170001

Cross, A., & Sohi, S. P. (2011). The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology and Biochemistry*, *43*(10), 2127–2134. https://doi.org/10.1016/j.soilbio.2011.06.016

Dai, Z., Zhang, X., Tang, C., Muhammad, N., Wu, J., Brookes, P. C., & Xu, J. (2017). Potential role of biochars in decreasing soil acidification - A critical review. *Science of The Total Environment*, *581–582*, 601–611. https://doi.org/10.1016/j.scitotenv.2016.12.169

De Oliveira, E. A., Marimon-Junior, B. H., Marimon, B. S., Iriarte, J., Morandi, P. S., Maezumi, S. Y., Nogueira, D. S., Aragão,

L. E. O. C., da Silva, I. B., & Feldpausch, T. R. (2020). Legacy of Amazonian Dark Earth soils on forest structure and species composition. *Global Ecology and Biogeography*, *29*(9), 1458–1473. https://doi.org/10.1111/geb.13116

Diatta, A. A., Fike, J. H., Battaglia, M. L., Galbraith, J. M., & Baig, M. B. (2020). Effects of biochar on soil fertility and crop productivity in arid regions: a review. *Arabian Journal of Geosciences*, *13*(14), Article 595. https://doi.org/10.1007/s12517-020-05586-2

Dicke, C., Andert, J., Ammon, C., Kern, J., Meyer-Aurich, A., & Kaupenjohann, M. (2015). Effects of different biochars and digestate on N_2O fluxes under field conditions. *Science of The Total Environment*, *524–525*, 310–318. https://doi.org/10.1016/j.scitotenv.2015.04.005

Ding, F., Van Zwieten, L., Zhang, W., Weng, Z. H., Shi, S., Wang, J., & Meng, J. (2018). A meta-analysis and critical evaluation of influencing factors on soil carbon priming following biochar amendment. *Journal of Soils and Sediments*, *18*, 1507–1517. https://doi.org/10.1007/s11368-017-1899-6

Du, Z., Xiao, Y., Qi, X., Liu, Y., Fan, X., & Li, Z. (2018). Peanut-shell biochar and biogas slurry improve soil properties in the North China Plain: a four-year field study. *Scientific Reports*, *8*, Article 13724. https://doi.org/10.1038/s41598-018-31942-0

Ducey, T. F., Novak, J. M., & Johnson, M. G. (2015). Effects of biochar blends on microbial community composition in two coastal plain soils. *Agriculture, 5*(4), 1060–1075. https://doi.org/10.3390/agriculture5041060

Durães, N., Novo, L. A. B., Candeias, C., & da Silva, E. F. (2018). Distribution, transport and fate of pollutants. In A. C. Duarte, A. Cachada, & T. Rocha-Santos (Eds.), *Soil pollution, from monitoring to remediation* (pp. 29–57). Academic Press. https://doi.org/10.1016/B978-0-12-849873-6.00002-9

Enders, A., Hanley, K., Whitman, T., Joseph, S., & Lehmann, J. (2012). Characterization of biochars to evaluate recalcitrance and agronomic performance. *Bioresource Technology*, *114*, 644–653. https://doi.org/10.1016/j.biortech.2012.03.022

European Biochar Foundation. (2016). *Guidelines for a sustainable production of biochar.* European Biochar Foundation (EBC). https://doi.org/10.13140/RG.2.1.4658.7043

Flores-Félix, J. D., Menéndez, E., Rivas, R., & Velázquez, M. de la E. (2019). Future perspective in organic farming fertilization: management and product. In S. Chandran, M. R. Unni, & S. Thomas (Eds.), *Organic farming global perspectives and methods* (pp. 269–315). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-813272-2.00010-0

Gao, L., Wang, R., Shen, G., Zhang, J., Meng, G., & Zhang, J. (2017). Effects of biochar on nutrients and the microbial community structure of tobacco-planting soils. *Journal of Soil Science and Plant Nutrition*, *17*(4), 884–896. https://doi.org/10.4067/S0718-95162017000400004

Gao, X., & Wu, H. (2011). Biochar as a fuel: 4. Emission behavior and characteristics of PM_1 and PM_{10} from the combustion of pulverized biochar in a drop-tube furnace. *Energy & Fuels*, *25*(6), 2702–2710. https://doi.org/10.1021/ef200296u

Ghazi, A. A. (2017). Potential for biochar as an alternate carrier to peat moss for the preparation of *Rhizobia* bio inoculum. *Microbiology Research Journal International*, *18*(4), 1–9. https://doi.org/10.9734/MRJI/2017/30828

Gilbe, C., Öhman, M., Lindström, E., Boström, D., Backman, R., Samuelsson, R., & Burvall, J. (2008). Slagging characteristics during residential combustion of biomass pellets. *Energy & Fuels*, *22*(5), 3536–3543. https://doi.org/10.1021/ef800087x

Glaser, B., & Birk, J. J. (2012). State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (*terra preta de índio*). *Geochimica et Cosmochimica Acta*, *82*, 39–51. https://doi.org/10.1016/j.gca.2010.11.029

Gorovtsov, A. V., Minkina, T. M., Mandzhieva, S. S., Perelomov, L. V., Soja, G., Zamulina, I. V., Rajput, V. D., Sushkova, S. N., Mohan, D., & Yao, J. (2020). The mechanisms of biochar interactions with microorganisms in soil. *Environmental Geochemistry and Health*, *42*, 2495–2518. https://doi.org/10.1007/s10653-019-00412-5

Gray, M., Johnson, M. G., Dragila, M. I., & Kleber, M. (2014). Water uptake in biochars: the roles of porosity and hydrophobicity. *Biomass and Bioenergy*, *61*, 196–205. https://doi.org/10.1016/j.biombioe.2013.12.010

Gupta, D. K., Gupta, C. K., Dubey, R., Fagodiya, R. K., Sharma, G., A, K., Noor Mohamed, M. B., Dev, R., & Shukla, A. K. (2020). Role of biochar in carbon sequestration and greenhouse gas mitigation. In J. S. Singh & C. Singh (Eds.), *Biochar applications in agriculture and environment management* (pp. 141–165). Springer International Publishing. https://doi.org/10.1007/978-3-030-40997-5_7

Hailegnaw, N. S., Mercl, F., Pračke, K., Száková, J., & Tlustoš, P. (2019). Mutual relationships of biochar and soil pH, CEC, and exchangeable base cations in a model laboratory experiment. *Journal of Soils and Sediments*, *19*, 2405–2416. https://doi.org/10.1007/s11368-019-02264-z

Hale, L., Luth, M., Kenney, R., & Crowley, D. (2014). Evaluation of pinewood biochar as a carrier of bacterial strain *Enterobacter cloacae* UW5 for soil inoculation. *Applied Soil Ecology*, *84*, 192–199. https://doi.org/10.1016/j.apsoil.2014.08.001

Hansen, V., Müller-Stöver, D., Munkholm, L. J., Peltre, C., Hauggaard-Nielsen, H., & Jensen, L. S. (2016). The effect of straw and wood gasification biochar on carbon sequestration, selected soil fertility indicators and functional groups in soil: an incubation study. *Geoderma*, *269*, 99–107. https://doi.org/10.1016/j.geoderma.2016.01.033

Hardy, B., Sleutel, S., Dufey, J. E., & Cornelis, J. T. (2019). The long-term effect of biochar on soil microbial abundance, activity and community structure Is overwritten by land management. *Frontiers in Environmental Science*, *7*, Article 110. https://doi.org/10.3389/fenvs.2019.00110

He, Y., Zhou, X., Jiang, L., Li, M., Du, Z., Zhou, G., Shao, J., Wang, X., Xu, Z., Hosseini-Bai, S., Wallace, H., & Xu, C. (2017). Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis. *GCB Bioenergy*, *9*(4), 743–755. https://doi.org/10.1111/gcbb.12376

Herrmann, L., & Lesueur, D. (2013). Challenges of formulation and quality of biofertilizers for successful inoculation. *Applied Microbiology and Biotechnology*, *97*(20), 8859–8873. https://doi.org/10.1007/s00253-013-5228-8

Huang, L. Q., Fu, C., Li, T. Z., Yan, B., Wu, Y., Zhang, L., Ping, W., Yang, B. R., & Chen, L. (2020). Advances in research on effects of biochar on soil nitrogen and phosphorus. *IOP Conference*

Series: Earth and Environmental Science, *424*, Article 012015. https://doi.org/10.1088/1755-1315/424/1/012015

Hue, N. (2020). Biochar for maintaining soil health. In B. Giri & A. Varma (Eds.), *Soil health* (pp. 21–46). Springer International Publishing. https://doi.org/10.1007/978-3-030-44364-1_2

Hüppi, R., Felber, R., Neftel, A., Six, J., & Leifeld, J. (2015). Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize cropping system. *Soil*, *1*(2), 707–717. https://doi.org/10.5194/soil-1-707-2015

Husna, N., Budianta, D., Munandar, M., & Adipati, N. (2019). Evaluation of several biochar types as inoculant carrier for indigenous phosphate solubilizing microoorganism from acid sulphate soil. *Journal of Ecological Engineering*, *20*(6), 1–8. https://doi.org/10.12911/22998993/109078

Hyland, C., Hanley, K., Enders, A., Rajkovich, S., & Lehmann, J. (2010, August 1–6). *Nitrogen leaching in soil amended with biochars produced at low and high temperatures from various feedstocks* [Conference presentation]. 19th world congress of soil science, soil solutions for a changing world. Brisbane, Australia. https://www.iuss.org/19th%20WCSS/Symposium/pdf/0742.pdf

International Biochar Initiative. (2015). Standardized product definition and product testing guidelines for biochar that is used in soil. International Biochar Initiative. https://www.biochar-international.org/wp-content/uploads/2018/04/IBI_Biochar_Standards_V2.1_Final.pdf

Intani, K., Latif, S., Kabir, A. K. M. R., & Müller, J. (2016). Effect of self-purging pyrolysis on yield of biochar from maize cobs, husks and leaves. *Bioresource Technology*, *218*, 541–551. https://doi.org/10.1016/j.biortech.2016.06.114

Ippolito, J. A., Laird, D. A., & Busscher, W. J. (2012). Environmental benefits of biochar. *Journal of Environment Quality*, *41*(4), 967–972. https://doi.org/10.2134/jeq2012.0151

Itskos, G., Nikolopoulos, N., Kourkoumpas, D. -S., Koutsianos, A., Violidakis, I., Drosatos, P., & Grammelis, P. (2016). Energy and the Environment. In S. G. Poulopoulos & V. J. Inglezakis (Eds.), *Environment and development, basic principles, human activities, and environmental implications* (pp. 363–452). Elsevier. https://doi.org/10.1016/B978-0-444-62733-9.00006-X

Jafri, N., Wong, W. Y., Doshi, V., Yoon, L. W., & Cheah, K. H. (2018). A review on production and characterization of biochars for application in direct carbon fuel cells. *Process Safety and Environmental Protection*, *118*, 152–166. https://doi.org/10.1016/j.psep.2018.06.036

Jeffery, S., Abalos, D., Prodana, M., Bastos, A. C., Van Groenigen, J. W., Hungate, B. A., & Verheijen, F. (2017). Biochar boosts tropical but not temperate crop yields. *Environmental Research Letters*, *12*(5), Article 053001. https://doi.org/10.1088/1748-9326/aa67bd

Jenkins, J. R., Viger, M., Arnold, E. C., Harris, Z. M., Ventura, M., Miglietta, F., Girardin, C., Edwards, R. J., Rumpel, C., Fornasier, F., Zavalloni, C., Tonon, G., Alberti, G., & Taylor, G. (2017). Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. *Gcb Bioenergy*, *9*(3), 591–612. https://doi.org/10.1111/gcbb.12371

Jin, Y., Liang, X., He, M., Liu, Y., Tian, G., & Shi, J. (2016). Manure biochar influence upon soil properties, phosphorus distribution and phosphatase activities: a microcosm incubation study. *Chemosphere*, *142*, 128–135. https://doi.org/10.1016/j.chemosphere.2015.07.015

Jouhara, H., Ahmad, D., van den Boogaert, I., Katsou, E., Simons, S., & Spencer, N. (2018). Pyrolysis of domestic based feedstock at temperatures up to 300°C. *Thermal Science and Engineering Progress*, *5*, 117–143. https://doi.org/10.1016/j.tsep.2017.11.007

Juriga, M., & Šimanský, V. (2019). Effects of biochar and its reapplication on soil pH and sorption properties of silt loam haplic luvisol. *Acta Horticulturae et Regiotecturae*, *22*(2), 65–70. https://doi.org/10.2478/ahr-2019-0012

Kammann, C., Ippolito, J., Hagemann, N., Borchard, N., Cayuela, M. L., Estavillo, J. M., Fuertes-Mendizabal, T., Jeffery, S., Kern, J., Novak, J., Rasse, D., Saarnio, S., Schmidt, H. P., Spokas, K., & Wrage-Mönnig, N. (2017). Biochar as a tool to reduce the agricultural greenhouse-gas burden - knowns, unknowns and future research needs. *Journal of Environmental Engineering and Landscape Management*, *25*(2), 114–139. https://doi.org/10.3846/16486897.2017.1319375

Kan, T., Strezov, V., & Evans, T. J. (2016). Lignocellulosic biomass pyrolysis: a review of product properties and effects of pyrolysis parameters. *Renewable and Sustainable Energy Reviews*, *57*, 1126–1140. https://doi.org/10.1016/j.rser.2015.12.185

Karimi, A., Moezzi, A., Chorom, M., & Enayatizamir, N. (2020). Application of biochar changed the status of nutrients and biological activity in a calcareous soil. *Journal of Soil Science and Plant Nutrition*, *20*, 450–459. https://doi.org/10.1007/s42729-019-00129-5

Kazemi, R., Ronaghi, A., Yasrebi, J., Ghasemi-Fasaei, R., & Zarei, M. (2019). Effect of shrimp waste–derived biochar and arbuscular mycorrhizal fungus on yield, antioxidant enzymes, and chemical composition of corn under salinity stress. *Journal of Soil Science and Plant Nutrition*, *19*, 758–770. https://doi.org/10.1007/s42729-019-00075-2

Kolton, M., Harel, Y. M., Pasternak, Z., Graber, E. R., Elad, Y., & Cytryn, E. (2011). Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Applied and Environmental Microbiology*, *77*(14), 4924–4930. https://doi.org/10.1128/AEM.00148-11

Kwiatkowski, M., & Kalderis, D. (2020). A complementary analysis of the porous structure of biochars obtained from biomass. *Carbon Letters*, *30*, 325–329. https://doi.org/10.1007/s42823-019-00101-4

Laghari, M., Hu, Z., Mirjat, M. S., Xiao, B., Tagar, A. A., & Hu, M. (2016). Fast pyrolysis biochar from sawdust improves the quality of desert soils and enhances plant growth. *Journal of the Science of Food and Agriculture*, *96*(1), 199–206. https://doi.org/10.1002/jsfa.7082

Lan, Z. M., Chen, C. R., Rezaei Rashti, M., Yang, H., & Zhang, D. K. (2019). Linking feedstock and application rate of biochars to N_2O emission in a sandy loam soil: potential mechanisms. *Geoderma*, *337*, 880–892. https://doi.org/10.1016/j.geoderma.2018.11.007

Lee, J. W., Hawkins, B., Li, X., & Day, D. M. (2013). Biochar fertilizer for soil amendment and carbon sequestration. In J. W. Lee (Ed.), *Advanced biofuels and bioproducts* (pp.

57–68). Springer Science and Business Media. https://doi.org/10.1007/978-1-4614-3348-4_6

Lee, J., Sarmah, A. K., & Kwon, E. E. (2019). Production and formation of biochar. In Y. S. Ok, D. C. W. Tsang, N. Bolan, & J. M. Novak (Eds.), *Biochar from biomass and waste, fundamentals and applications* (pp. 3–18). Elsevier. https://doi.org/10.1016/B978-0-12-811729-3.00001-7

Lehmann, J., Gaunt, J., & Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems - A review. *Mitigation and Adaptation Strategies for Global Change*, *11*, 403–427. https://doi.org/10.1007/s11027-005-9006-5

Lehmann, J., & Joseph, S. (2009). Biochar for environmental management: an Introduction. In J. Lehmann, & S. Joseph (Eds.), *Biochar for environmental management, science, technology and implementation* (pp. 1–12). Taylor & Francis Group.

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota - A review. *Soil Biology and Biochemistry*, *43*(9), 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022

Leng, L., & Huang, H. (2018). An overview of the effect of pyrolysis process parameters on biochar stability. *Bioresource Technology*, *270*, 627–642. https://doi.org/10.1016/j.biortech.2018.09.030

Levin, A. A., Shamansky, V. A., & Kozlov, A. N. (2016). A model of pyrolysis in a staged scheme of low-grade solid fuel gasification. *Journal of Physics: conference Series*, *754*, Article 022006. https://doi.org/10.1088/1742-6596/754/2/022006

Li, X., Chen, X., Weber-Siwirska, M., Cao, J., & Wang, Z. (2018). Effects of rice-husk biochar on sand-based rootzone amendment and creeping bentgrass growth. *Urban Forestry and Urban Greening*, *35*, 165–173. https://doi.org/10.1016/j.ufug.2018.09.001

Li, Y., Xing, B., Ding, Y., Han, X., & Wang, S. (2020). A critical review of the production and advanced utilization of biochar via selective pyrolysis of lignocellulosic biomass. *Bioresource Technology*, *312*, Article 123614. https://doi.org/10.1016/j.biortech.2020.123614

Liao, N., Li, Q., Zhang, W., Zhou, G., Ma, L., Min, W., Ye, J., & Hou, Z. (2016). Effects of biochar on soil microbial community composition and activity in drip-irrigated desert soil. *European Journal of Soil Biology*, *72*, 27–34. https://doi.org/10.1016/j.ejsobi.2015.12.008

Liu, H., Li, H., Zhang, A., Rahaman, M. A., & Yang, Z. (2020). Inhibited effect of biochar application on N_2O emissions is amount and time-dependent by regulating denitrification in a wheat-maize rotation system in North China. *Science of the Total Environment*, *721*, Article 137636. https://doi.org/10.1016/j.scitotenv.2020.137636

Liu, J., Ding, Y., Ma, L., Gao, G., & Wang, Y. (2017). Combination of biochar and immobilized bacteria in cypermethrin-contaminated soil remediation. *International Biodeterioration and Biodegradation*, *120*, 15–20. https://doi.org/10.1016/j.ibiod.2017.01.039

Liu, N., Sun, Z., Wu, Z., Zhan, X., Zhang, K., Zhao, E., & Han, X. (2013). Adsorption characteristics of ammonium nitrogen by biochar from diverse origins in water. *Advanced Materials Research*, *664*, 305–312. https://doi.org/10.4028/www.scientific.net/AMR.664.305

Lyu, H., He, Y., Tang, J., Hecker, M., Liu, Q., Jones, P. D., Codling, G., & Giesy, J. P. (2016). Effect of pyrolysis temperature on potential toxicity of biochar if applied to the environment. *Environmental Pollution*, *218*, 1–7. https://doi.org/10.1016/j.envpol.2016.08.014

Macedo, R. S., Teixeira, W. G., Corrêa, M. M., Martins, G. C., & Vidal-Torrado, P. (2017). Pedogenetic processes in anthrosols with pretic horizon (Amazonian Dark Earth) in Central Amazon, Brazil. *PLOS One*, *12*(5), Article e0178038. https://doi.org/10.1371/journal.pone.0178038

Martinsen, V., Mulder, J., Shitumbanuma, V., Sparrevik, M., Børresen, T., & Cornelissen, G. (2014). Farmer-led maize biochar trials: effect on crop yield and soil nutrients under conservation farming. *Journal of Plant Nutrition and Soil Science*, *177*(5), 681–695. https://doi.org/10.1002/jpln.201300590

Masud, M. M., Abdulaha-Al Baquy, M., Akhter, S., Sen, R., Barman, A., & Khatun, M. R. (2020). Liming effects of poultry litter derived biochar on soil acidity amelioration and maize growth. *Ecotoxicology and Environmental Safety*, *202*, Article 110865. https://doi.org/10.1016/j.ecoenv.2020.110865

Mendes, K. F., Júnior, A. F. D., Takeshita, V., Régo, A. P. J., & Tornisielo, V. L. (2018). Effect of biochar amendments on the sorption and desorption herbicides in agricultural soil. In S. Edebali (Ed.), *Advanced sorption process applications* (pp. 87–103). IntertechOpen. https://doi.org/10.5772/intechopen.80862

Muhammad, N., Aziz, R., Brookes, P. C., & Xu, J. (2017). Impact of wheat straw biochar on yield of rice and some properties of Psammaquent and Plinthudult. *Journal of Soil Science and Plant Nutrition*, *17*(3), 808–823. https://doi.org/10.4067/S0718-95162017000300019

Neina, D. (2019). The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science, 2019*, Article 5794869. https://doi.org/10.1155/2019/5794869

Nelissen, V., Ruysschaert, G., Manka'Abusi, D., D'Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W., & Boeckx, P. (2015). Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. *European Journal of Agronomy*, *62*, 65–78. https://doi.org/10.1016/j.eja.2014.09.006

Nguyen, B. T., Trinh, N. N., & Bach, Q.-V. (2020a). Methane emissions and associated microbial activities from paddy salt-affected soil as influenced by biochar and cow manure addition. *Applied Soil Ecology*, *152*, Article 103531. https://doi.org/10.1016/j.apsoil.2020.103531

Nguyen, T. K. P., Khoi, C., Ritz, K., Sinh, N., Tarao, M., & Toyota, K. (2020b). Potential use of rice husk biochar and compost to improve P availability and reduce GHG emissions in acid sulfate soil. *Agronomy*, *10*, Article 685. https://doi.org/10.3390/agronomy10050685

Novak, J. M., Busscher, W. J., Watts, D. W., Laird, D. A., Ahmedna, M. A., & Niandou, M. A. S. (2010). Short-term CO_2 mineralization after additions of biochar and switchgrass to a Typic Kandiudult. *Geoderma*, *154*(3–4), 281–288. https://doi.org/10.1016/j.geoderma.2009.10.014

Odinga, E. S., Waigi, M. G., Gudda, F. O., Wang, J., Yang, B., Hu, X., Li, S., & Gao, Y. (2020). Occurrence, formation, environmental fate and risks of environmentally persistent free radicals in biochars. *Environment International*, *134*, Article 105172. https://doi.org/10.1016/j.envint.2019.105172

Ogawa, M., & Okimori, Y. (2010). Pioneering works in biochar research, Japan. *Australian Journal of Soil Research*, *48*, 489–500. https://doi.org/10.1071/SR10006

Oladele, S. O. (2019). Changes in physicochemical properties and quality index of an Alfisol after three years of rice husk biochar amendment in rainfed rice - maize cropping sequence. *Geoderma*, *353*, 359–371. https://doi.org/10.1016/j.geoderma.2019.06.038

Ouyang, L., Tang, Q., Yu, L., & Zhang, R. (2014). Effects of amendment of different biochars on soil enzyme activities related to carbon mineralisation. *Soil Research*, *52*(7), 706–716. https://doi.org/10.1071/SR14075

Oyedeji, S., Animasaun, D. A., Ademola, O. I., & Agboola, O. O. (2018). Growth performance of cowpea in spent oil-contaminated soils ameliorated with cocoa shell powder and biochar. *Journal of Biological and Environmental Sciences*, *12*(36), 105–112.

Palansooriya, K. N., Wong, J. T. F., Hashimoto, Y., Huang, L., Rinklebe, J., Chang, S. X., Bolan, N., Wang, H., & Ok, Y. S. (2019). Response of microbial communities to biochar-amended soils: a critical review. *Biochar*, *1*, 3–22. https://doi.org/10.1007/s42773-019-00009-2

Pariyar, P., Kumari, K., Jain, M. K., & Jadhao, P. S. (2020). Evaluation of change in biochar properties derived from different feedstock and pyrolysis temperature for environmental and agricultural application. *Science of the Total Environment*, *713*, Article 136433. https://doi.org/10.1016/j.scitotenv.2019.136433

Park, J. H., Ok, Y. S., Kim, S. H., Kang, S. W., Cho, J. S., Heo, J. S., Delaune, R. D., & Seo, D. C. (2015). Characteristics of biochars derived from fruit tree pruning wastes and their effects on lead adsorption. *Journal of the Korean Society for Applied Biological Chemistry*, *58*(5), 751–760. https://doi.org/10.1007/s13765-015-0103-1

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, *532*, 49–57. https://doi.org/10.1038/nature17174

Pratiwi, E. P. A., Hillary, A. K., Fukuda, T., & Shinogi, Y. (2016). The effects of rice husk char on ammonium, nitrate and phosphate retention and leaching in loamy soil. *Geoderma*, *277*, 61–68. https://doi.org/10.1016/j.geoderma.2016.05.006

Purakayastha, T. J., Bera, T., Bhaduri, D., Sarkar, B., Mandal, S., Wade, P., Kumari, S., Biswas, S., Menon, M., Pathak, H., & Tsang, D. C. W. (2019). A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: pathways to climate change mitigation and global food security. *Chemosphere*, *227*, 345–365. https://doi.org/10.1016/j.chemosphere.2019.03.170

Rafiq, M. K., Bachmann, R. T., Rafiq, M. T., Shang, Z., Joseph, S., & Long, R. (2016). Influence of pyrolysis temperature on physico-chemical properties of corn stover (*Zea mays* L.) biochar and feasibility for carbon capture and energy balance. *PLOS One*, *11*(6), Article e0156894. https://doi.org/10.1371/journal.pone.0156894

Saeid, A., & Chojnacka, K. (2019). Fertlizers: need for new strategies. In S. Chandran, M. R. Unni, & S. Thomas (Eds.), *Organic farming, global perspectives and methods* (pp. 91–116). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-813272-2.00004-5

Sahu, P. K., & Brahmaprakash, G. P. (2016). Formulations of biofertilizers - approaches and advances. In D. P. Singh, H. B. Singh, & R. Prabha (Eds.), *Microbial inoculants in sustainable agricultural productivity* (pp. 179–198). Springer. https://doi.org/10.1007/978-81-322-2644-4_12

Saranya, K., Kumutha, K., & Krishnan, P. S. (2011). Influence of biochar and *Azospirillum* application on the growth of maize. *Madras Agricultural Journal*, 98(4/6), 158–164.

Sato, M. K., de Lima, H. V., Costa, A. N., Rodrigues, S., Pedroso, A. J. S., & de Freitas Maia, C. M. B. (2019). Biochar from acai agroindustry waste: study of pyrolysis conditions. *Waste Management*, *96*, 158–167. https://doi.org/10.1016/j.wasman.2019.07.022

Schimmelpfennig, S., Müller, C., Grünhage, L., Koch, C., & Kammann, C. (2014). Biochar, hydrochar and uncarbonized feedstock application to permanent grassland-Effects on greenhouse gas emissions and plant growth. *Agriculture, Ecosystems and Environment*, *191*, 39–52. https://doi.org/10.1016/j.agee.2014.03.027

Shaheen, A., & Turaib Ali Bukhari, S. (2018). Potential of sawdust and corn cobs derived biochar to improve soil aggregate stability, water retention, and crop yield of degraded sandy loam soil. *Journal of Plant Nutrition*, *41*(20), 2673–2682. https://doi.org/10.1080/01904167.2018.1509092

Shahjahan, M., Inam-ul-Haq, M., Mukhtar, T., & Khalid, A. (2018). Biochar as a carrier of antagonistic rhizobacteria suppressing *Macrophomina phaseolina*. *Transylvanian Review*, *26*(28), 7469–7476.

Shareef, T. M. E., Zhao, B., & Filonchyk, M. (2018). Characterization of biochars derived from maize straw and corn cob and effects of their amendment on maize growth and loess soil properties. *Fresenius Environmental Bulletin*, *27*(5A), 3678–3686.

Sharma, S. P. (2018). Biochar for carbon sequestration: bioengineering for sustainable environment. In D. Barh & V. Azevedo (Eds.), *Omics technologies and bio-engineering volume 2: towards improving quality of life* (pp. 365–385). Academic Press. https://doi.org/10.1016/B978-0-12-815870-8.00020-6

Shen, J., Tang, H., Liu, J., Wang, C., Li, Y., Ge, T., Jones, D. L., & Wu, J. (2014). Contrasting effects of straw and straw-derived biochar amendments on greenhouse gas emissions within double rice cropping systems. *Agriculture, Ecosystems and Environment*, *188*, 264–274. https://doi.org/10.1016/j.agee.2014.03.002

Shetty, R., & Prakash, N. B. (2020). Effect of different biochars on acid soil and growth parameters of rice plants under aluminium toxicity. *Scientific Reports*, *10*(1), Article 12249. https://doi.org/10.1038/s41598-020-69262-x

Shim, T., Yoo, J., Ryu, C., Park, Y. K., & Jung, J. (2015). Effect of steam activation of biochar produced from a giant *Miscanthus* on copper sorption and toxicity. *Bioresource Technology*, *197*, 85–90. https://doi.org/10.1016/j.biortech.2015.08.055

Sigmund, G., Hüffer, T., Hofmann, T., & Kah, M. (2017). Biochar total surface area and total pore volume determined by N_2 and CO_2 physisorption are strongly influenced by degassing temperature. *Science of the Total Environment*, *580*, 770–775. https://doi.org/10.1016/j.scitotenv.2016.12.023

Smith, P. (2016). Soil carbon sequestration and biochar as negative emission technologies. *Global Change Biology*, *22*(3), 1315–1324. https://doi.org/10.1111/gcb.13178

Spokas, K. A., Baker, J. M., & Reicosky, D. C. (2010). Ethylene: potential key for biochar amendment impacts. *Plant and Soil*, *333*(1–2), 443–452. https://doi.org/10.1007/s11104-010-0359-5

Stewart, C. E., Zheng, J., Botte, J., & Cotrufo, M. F. (2013). Co-generated fast pyrolysis biochar mitigates green-house gas emissions and increases carbon sequestration in temperate soils. *GCB Bioenergy*, *5*(2), 153–164. https://doi.org/10.1111/gcbb.12001

Sun, H., Zhang, H., Shi, W., Zhou, M., & Ma, X. (2019). Effect of biochar on nitrogen use efficiency, grain yield and amino acid content of wheat cultivated on saline soil. *Plant, Soil and Environment*, *65*(2), 83–89. https://doi.org/10.17221/525/2018-PSE

Szwaja, S., Poskart, A., & Zajemska, M. (2018, June 26–29). *A new approach for evaluating biochar quality from biomass thermal processing* [Conference presentation]. 3rd International Conference on Smart and Sustainable Technologies (SpliTech), Split, Croatia. https://ieeexplore.ieee.org/document/8448316

Tabakaev, R., Kanipa, I., Astafev, A., Dubinin, Y., Yazykov, N., Zavorin, A., & Yakovlev, V. (2019). Thermal enrichment of different types of biomass by low-temperature pyrolysis. *Fuel*, *245*, 29–38. https://doi.org/10.1016/j.fuel.2019.02.049

Tag, A. T., Duman, G., Ucar, S., & Yanik, J. (2016). Effects of feedstock type and pyrolysis temperature on potential applications of biochar. *Journal of Analytical and Applied Pyrolysis*, *120*, 200–206. https://doi.org/10.1016/j.jaap.2016.05.006

Taghizadeh-Toosi, A., Clough, T. J., Condron, L. M., Sherlock, R. R., Anderson, C. R., & Craigie, R. A. (2011). Biochar incorporation into pasture soil suppresses *in situ* nitrous oxide emissions from ruminant urine patches. *Journal of Environment Quality*, *40*(2), 468–476. https://doi.org/10.2134/jeq2010.0419

Tan, Z., Lin, C. S. K., Ji, X., & Rainey, T. J. (2017). Returning biochar to fields: a review. *Applied Soil Ecology*, *116*, 1–11. https://doi.org/10.1016/j.apsoil.2017.03.017

Tomczyk, A., Sokołowska, Z., & Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Bio/Technology*, *19*, 191–215. https://doi.org/10.1007/s11157-020-09523-3

Uddin, M. N., Techato, K., Taweekun, J., Rahman, M. M., Rasul, M. G., Mahlia, T. M. I., & Ashrafur, S. M. (2018). An overview of recent developments in biomass pyrolysis technologies. *Energies, 11*(11), Article 3115. https://doi.org/10.3390/en11113115

Vecstaudza, D., Senkovs, M., Nikolajeva, V., Kasparinskis, R., & Muter, O. (2017). Wooden biochar as a carrier for endophytic isolates. *Rhizosphere*, *3*, 126–127. https://doi.org/10.1016/j.rhisph.2017.04.002

Ventura, M., Alberti, G., Panzacchi, P., Vedove, G. D., Miglietta, F., & Tonon, G. (2019). Biochar mineralization and priming effect in a poplar short rotation coppice from a 3-year field experiment. *Biology and Fertility of Soils*, *55*, 67–78. https://doi.org/10.1007/s00374-018-1329-y

Wang, X., Li, X., Liu, G., He, Y., Chen, C., Liu, X., Li, G., Gu, Y., & Zhao, Y. (2019). Mixed heavy metal removal from wastewater by using discarded mushroom-stick biochar: adsorption properties and mechanisms. *Environmental Science: processes and Impacts*, *21*(3), 584–592. https://doi.org/10.1039/C8EM00457A

Wang, Y., Yin, R., & Liu, R. (2014). Characterization of biochar from fast pyrolysis and its effect on chemical properties of the tea garden soil. *Journal of Analytical and Applied Pyrolysis*, *110*, 375–381. https://doi.org/10.1016/j.jaap.2014.10.006

Weber, K., & Quicker, P. (2018). Properties of biochar. *Fuel*, *217*, 240–261. https://doi.org/10.1016/j.fuel.2017.12.054

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications*, *1*, Article 56. https://doi.org/10.1038/ncomms1053

Wu, Q., Xian, Y., He, Z., Zhang, Q., Wu, J., Yang, G., Zhang, X., Qi, H., Ma, J., Xiao, Y., & Long, L. (2019). Adsorption characteristics of Pb(II) using biochar derived from spent mushroom substrate. *Scientific Reports*, *9*(1), Article 15999. https://doi.org/10.1038/s41598-019-52554-2

Xiao, R., Yang, W., Cong, X., Dong, K., Xu, J., Wang, D., & Yang, X. (2020). Thermogravimetric analysis and reaction kinetics of lignocellulosic biomass pyrolysis. *Energy*, *201*, Article 117537. https://doi.org/10.1016/j.energy.2020.117537

Yaashikaa, P. R., Senthil Kumar, P., Varjani, S. J., & Saravanan, A. (2019). Advances in production and application of biochar from lignocellulosic feedstocks for remediation of environmental pollutants. *Bioresource Technology*, *292*, Article 122030. https://doi.org/10.1016/j.biortech.2019.122030

Yadav, V., Karak, T., Singh, S., Singh, A. K., & Khare, P. (2019). Benefits of biochar over other organic amendments: responses for plant productivity (*Pelargonium graveolens* L.) and nitrogen and phosphorus losses. *Industrial Crops and Products*, *131*, 96–105. https://doi.org/10.1016/j.indcrop.2019.01.045

Yang, H., Yan, R., Chen, H., Lee, D. H., & Zheng, C. (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, *86*, 1781–1788. https://doi.org/10.1016/j.fuel.2006.12.013

Yao, Y., Gao, B., Zhang, M., Inyang, M., & Zimmerman, A. R. (2012). Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere*, *89*(11), 1467–1471. https://doi.org/10.1016/j.chemosphere.2012.06.002

Ye, L., Camps-Arbestain, M., Shen, Q., Lehmann, J., Singh, B., & Sabir, M. (2020). Biochar effects on crop yields with and without fertilizer: a meta-analysis of field studies using separate controls. *Soil Use and Management*, *36*, 2–18. https://doi.org/10.1111/sum.12546

Yu, H., Zou, W., Chen, J., Chen, H., Yu, Z., Huang, J., Tang, H., Wei, X., & Gao, B. (2019). Biochar amendment improves crop production in problem soils: a review. *Journal of Environmental Management*, *232*, 8–21. https://doi.org/10.1016/j.jenvman.2018.10.117

Yu, J., Paterson, N., Blamey, J., & Millan, M. (2017). Cellulose, xylan and lignin interactions during pyrolysis of lignocellulosic biomass. *Fuel*, *191*, 140–149. https://doi.org/10.1016/j.fuel.2016.11.057

Zhang, C., Liu, L., Zhao, M., Rong, H., & Xu, Y. (2018). The environmental characteristics and applications of biochar. *Environmental Science and Pollution Research*, *25*(22), 21525–21534. https://doi.org/10.1007/s11356-018-2521-1

Zhang, C., Zeng, G., Huang, D., Lai, C., Chen, M., Cheng, M., Tang, W., Tang, L., Dong, H., Huang, B., Tan, X., & Wang, R. (2019). Biochar for environmental management: mitigating greenhouse gas emissions, contaminant treatment, and potential negative impacts. *Chemical Engineering Journal*, *373*, 902–922. https://doi.org/10.1016/j.cej.2019.05.139

Zhang, H., Chen, C., Gray, E. M., & Boyd, S. E. (2017). Effect of feedstock and pyrolysis temperature on properties of biochar governing end use efficacy. *Biomass and Bioenergy*, *105*, 136–146. https://doi.org/10.1016/j.biombioe.2017.06.024

Zhao, J., Shen, X. J., Domene, X., Alcañiz, J. M., Liao, X., & Palet, C. (2019). Comparison of biochars derived from different types of feedstock and their potential for heavy metal removal in multiple-metal solutions. *Scientific Reports*, *9*, Article 9869. https://doi.org/10.1038/s41598-019-46234-4

Zhao, L., Cao, X., Mašek, O., & Zimmerman, A. (2013). Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. *Journal of Hazardous Materials*, *256–257*, 1–9. https://doi.org/10.1016/j.jhazmat.2013.04.015

Zhou, Z., Shi, D., Qiu, Y., & Sheng, G. D. (2010). Sorptive domains of pine chars as probed by benzene and nitrobenzene. *Environmental Pollution*, *158*, 201–206. https://doi.org/10.1016/j.envpol.2009.07.020

Zimmerman, A. R. (2010). Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science and Technology*, *44*(4), 1295–1301. https://doi.org/10.1021/es903140c

Zong, Y., Wang, Y., Sheng, Y., Wu, C., & Lu, S. (2018). Ameliorating soil acidity and physical properties of two contrasting texture Ultisols with wastewater sludge biochar. *Environmental Science and Pollution Research*, *25*, 25726–25733. https://doi.org/10.1007/s11356-017-9509-0

Zwart, K. (2020). Effects of biochar produced from waste on soil quality. In E. Meers, G. Velthof, E. Michels, & R. Rietra (Eds.), *Biorefinery of Inorganics*: *recovering mineral nutrients from biomass and organic waste* (pp. 283–299). John Wiley & Sons Ltd. https://doi.org/10.1002/9781118921487.ch5-7

A comparison of two open-source crop simulation models for a potato crop

Comparación de dos modelos de simulación de cultivo de código abierto para un cultivo de papa

Diego Quintero[1]* and Eliécer Díaz[1]

ABSTRACT

An open-source model is a model that makes it possible to modify the source code. This tool can be a great advantage for the user since it allows changing or modifying some of the background theory of the model. World Food Studies (WOFOST) and AquaCropOS open-source crop models were compared using field recorded data. Both models are free open-source tools that allow evaluating the impacts of climate and water on agriculture. The objective of this research was to assess the model's efficiency in simulating the yield and above-ground biomass formation of a potato crop on the cundiboyacense plateau. WOFOST simulates biomass accumulation in the crop organs using partitioning of assimilates to establish the biomass fraction that turns into yield. AquaCropOS simulates total above-ground biomass accumulation using crop water productivity (WP) and considers the Harvest Index (HI) to calculate yield formation. Crop modules for both models were built using information recorded in previous studies by other authors; those works performed a physiological and phenological characterization of some potato varieties. It was found that the WOFOST model simulates yield formation better than AquaCropOS; despite that, AquaCropOS simulates total above-ground biomass better than WOFOST. However, AquaCropOS was as efficient as WOFOST in simulating yield formation.

Key words: crop modelling, crop yield, agrometeorology, *Solanum tuberosum* L.

RESUMEN

Un modelo de código abierto permite modificar el código fuente. Esto puede ser una gran ventaja para el usuario, pues permite modificar o cambiar parte de la teoría en la que se sustenta el modelo. Los modelos de código abierto WOFOST y AquaCropOS fueron comparados usando información medida en campo. Ambos modelos son herramientas gratuitas de código abierto que permiten evaluar los impactos del clima y el agua en la agricultura. El objetivo de esta investigación fue evaluar la eficiencia de los modelos para simular el rendimiento y la formación de acumulación de biomasa sobre el suelo para un cultivo de papa en el altiplano cundiboyacense. WOFOST simula la acumulación de biomasa en los órganos del cultivo utilizando la partición de asimilados para establecer la fracción de biomasa que va al rendimiento. AquaCropOS simula la acumulación total de biomasa sobre el suelo usando la productividad de agua del cultivo (PA) y tiene en cuenta el índice de cosecha (IC) para calcular la formación del rendimiento. Los módulos de cultivo para ambos modelos fueron construidos usando información recolectada en estudios previos hechos por otros autores; estos trabajos hicieron una caracterización fisiológica y fenológica de algunas variedades de papa. Se encontró que el modelo WOFOST simula la formación del rendimiento mejor que AquaCropOS; a pesar de esto, AquaCropOS simula la acumulación total de biomasa sobre el suelo mejor que WOFOST. Sin embargo, AquaCropOS fue tan eficiente como WOFOST simulando la formación del rendimiento.

Palabras clave: modelización de cultivos, rendimiento de cultivos, agrometeorología, *Solanum tuberosum* L.

Introduction

Crop modeling is increasingly being used to describe agricultural systems, helping scientists to incorporate their understanding of the interactions among components in predicting performance of agricultural systems for better goal achievement of farmers and society (Wallach *et al.*, 2014). Predicting the performance of agricultural systems has also been used as a tool for decision support in crop production, involving topics such as sowing dates, irrigation amounts and fertilization management (Graeff *et al.*, 2012). Crop model calibration is necessary for using crop models since the quality of the simulation depends on the quality of the parameters of the crop. Although this study is not about model calibration, it is about crop parameter estimation. For crop parameter assessment, we used data recorded in the field by other authors in previous studies (Ñústez *et al.*, 2009; Valbuena *et al.*, 2010).

Received for publication: 25 March, 2019. Accepted for publication: 23 July, 2020. Doi: 10.15446/agron.colomb.v38n3.82525

[1] Departamento de Geociencias, Facultad de Ciencias, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: diaquinteropu@unal.edu.co

Teh (2006) defines crop modeling as describing and translating a real agricultural system into a mathematical form, finding the patterns in the behavior or action of the crop system and translating those patterns into an equation or set of equations. A system is a limited part of reality that contains interrelated elements, and a model is a simplified representation of a system where the variables that govern the system are described. These variables may be interacting with each other. Simulation is the building of mathematical models, and the study of their behavior in reference to that of the system they represent (Boogaard *et al.,* 2014).

According to Boogaard *et al.* (2014), a mathematical model may be a descriptive model or an explanatory model. A descriptive model usually describes the behavior of a system in a relatively simple manner and reflects little or none of the mechanisms that are the cause of that behavior. An explanatory model consists of a quantitative description of the main processes involved. Within an explanatory model, the system processes are related to each other based on comprehension of their interaction.

A descriptive model describes processes that govern crop growth and yield development in a wide way; for example, a direct relation between some weather indicators such as total incoming radiation and yield formation. An explanatory model describes a process in detail; for example, a relation between the total assimilated CO_2 and yield, where the total assimilated CO_2 depends on the photosynthetic rate, the total incoming radiation, and the canopy cover (Marcelis e*t al.,* 1998). A simple relation may involve many sub processes that are interacting with each other. A crop model must take weather, soil, crop and crop management information and use those data for solving the equations of the model.

AquaCropOS and WOFOST models are computer programs that compile equations that then describe the behavior of the water-soil-crop-atmosphere continuum. AquaCropOS and WOFOST are made up of multiple modules, and each of those simulates a specific process. Simulation of a specific process may consist of the interaction of several subprocesses (Raes *et al.,* 2018; De Wit *et al.*, 2019). AquaCropOS and WOFOST are open-source models, which means that the user of the model can modify the source code. Therefore, all the equations, theory, and relations that describe the crop's system can be modified. Open-source models are generally executed on the terminal. This characteristic could be harder for beginner users, but executing a model that way is also a great benefit since the user can make several simulations without spending too much time comparing it to other models that must be executed using a graphic user interface (GUI) (Foster *et al.,* 2017; De Wit, 2018a; De Wit, 2018b).

In WOFOST, crop growth is simulated based on ecophysiological processes such as growth and phenological development with a fixed time step of one day. The potential production in the model is limited only by radiation, temperature, atmospheric CO_2 concentration, and crop features. WOFOST growth limiting factors are related to water and/or nutrient limitation. Growth-reducing factors are associated with weeds and pollutants. The major processes simulated by the model are phenological development, leaf development, and light interception, CO_2 assimilation, root growth, transpiration, respiration, partitioning of assimilates to the various organs, and dry matter formation (De Wit *et al*., 2019).

AquaCropOS is a recent model based on the previous AquaCrop model. This tool simulates crop growth based on crop water productivity. Water productivity expresses the above-ground dry matter (kg or g) produced per unit of land area (m^2 or ha) per unit of transpired water (mm) (Foster *et al.,* 2017; Raes *et al.,* 2018). The potential production in the model is only limited by crop transpiration and atmospheric CO_2. AquaCrop growth limiting factors are the same used by WOFOST. Growth-reducing factors are related to weeds and soil salinity. As WOFOST, this model uses a fixed time step of one day.

Condori *et al.* (2016) summarized some of the most relevant works on potato crop modeling in Latin America. They found that the most frequent topic in publications on modeling is evaluating varieties and their calibration in different simulation models. Crop calibration is often focused on fertilizer and irrigation management, as well as the study of the effects of pests and diseases on the potato crop. Almost all crop modeling works collected by Condori *et al.* (2016) were about decision support systems for agrotechnology transfer (DSSAT) and Agro models, but none of them were about AquaCrop or WOFOST.

There are not many studies on the comparison between the WOFOST and AquaCrop models. However, a recent project aims to improve agricultural models, based on their intercomparison and evaluation. The agricultural model intercomparison and improvement project (AgMIP) (Rosenzweig *et al*., 2015) was founded in 2010 and consists of a group of experts in crop modeling and agricultural economy. Despite this, AgMIP does not report any research that compares the two models that are the subject of this work.

Todorovic *et al.* (2009) compared the AquaCrop, CropSyst, and WOFOST models in the simulation of sunflower growth. They found that although AquaCrop requires less input information than CropSyst and WOFOST, it performed similarly to them in simulating both the total above-ground biomass and yield at harvesting. Furthermore, Huang *et al.* (2017) used the WOFOST and AquaCrop models for a multiple crop model ensemble. They emphasized that each of the models has a specific target parameter for simulation of growth; therefore, instead of using only one of the models, it is better to use both as an ensemble. The weight of each model in the ensemble would depend on the climate characteristics of the location.

Therefore, the objective of this research was to study and compare two open-source crop simulation models for a potato crop, under the agrometeorological conditions of the Cundiboyacense plateau.

Materials and methods

A potato crop cycle was simulated with limiting water conditions during the second semester of 2004. The simulation period and weather data provider station were selected according to details found on Ñústez *et al.* (2009) about the location and date of execution of the experiments. Observed data were recorded at a farm located in the municipality of Zipaquira (5°0.133' N and 73°59.529' W, and altitude of 2580 m a.s.l.).

The observed data recorded by Ñústez *et al.* (2009) and a phenological description performed by Valbuena *et al.* (2010) were used to estimate some crop parameters of the WOFOST and AquaCropOS models. Both authors collected their data in the field for various potato varieties at the Cundiboyacense plateau. Some parameters cannot be estimated using those data, and for that reason, default potato crop parameters were adopted for both models. For this work, the potato variety selected was Diacol Capiro. Both models were run for the same period when data was recorded. The results obtained for the two models were compared to the observed data, using the root-mean square error (RMSE) and an efficiency coefficient of a model.

The results for the two models were compared to the observed data. RMSE, Pearson correlation, and an efficiency index were obtained for both models. The efficiency index of the model was calculated according to Equation 1, which was proposed by Confalonieri *et al.* (2009) for assessing the efficiency of crop models. Model efficiency (EF) ranges from negative infinity to one. Negative values of EF indicate that the average value of all observations is a better estimator than the model. If the EF value equals one, it means that the model simulates almost perfectly that system.

$$EF = 1 - \frac{\sum_{i=1}^{n}(D_i)^2}{\sum_{i=1}^{n}(O_i - \overline{O})^2} \qquad (1)$$

where: D_i is the model's residual for observation i, O_i is the value of observation i, Õ is the average of observations, and n is total number of observations. The efficiency of the model (EF) ranges from negative infinity to one. Negative values of EF indicate that the average value of all observations is a better estimator than the model. If the EF value is one, it means that the model simulates almost perfectly that system.

RMSE (Eq. 2) is indicates the mean difference between simulated values by the model and observed values.

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n}(E_i - O_i)^2\right]^{1/2} \qquad (2)$$

where: E_i is the estimated value, O_i is the observed value and n is total number of observations.

The AquaCropOS model was executed using GNU Octave version 4.2.2, and the WOFOST model was executed in Python 2.7.15. The Python crop simulation environment library (PCSE) was used for running the WOFOST model. PCSE (De Wit, 2018a) is a Python package for building crop simulation models, in particular the crop models developed at Wageningen University (Netherlands).

The model can distinguish an entire crop system in three subsystems, crop, soil and atmosphere. When it comes to the crop subsystem, AquaCropOS simulates it by using four different submodules: roots, canopy cover, phenology, yield, and biomass. All these submodules are affected by stress coefficients at any step (Raes *et al.*, 2018). On the other hand, WOFOST uses nine submodules for simulating plant growth and yield: phenology, radiation fluxes, assimilation rates, maintenance respiration, dry matter partitioning, carbon balance check, senescence, net growth, and root growth (De Wit, 2018a).

Either for AquaCropOS or WOFOST it is necessary to define some parameters that will determine soil, plant, and atmosphere behavior and interactions. According to this, it is required to define soil, crop, and weather parameters which vary according to the location, crop species and variety, soil texture, etc.

Crop: WOFOST crop file included information about crop phenology, assimilation and respiration characteristics, and partitioning of assimilates to plant organs. Phenology and partition of assimilates parameters were estimated from the studies by Ñústez *et al.* (2009) and Valbuena *et al.* (2010).

Assimilation and respiration parameters were obtained from De Wit (2018b), who had calibrated the WOFOST model for a potato crop under the conditions of central Europe. Although the assimilation and respiration parameters defined by De Wit (2018b) were obtained for a different variety, they were used due to the difficulty in obtaining those parameters for local potato varieties since it requires years of research and field experiments under very controlled environments. AquaCropOS crop file included information about crop phenology and crop water productivity. AquaCropOS crop parameters were obtained from Cortés *et al.* (2013), who estimated crop parameters based on Ñústez *et al.* (2009).

Soil: Soil texture was defined using a general soil study for the province of Cundinamarca carried out by the Instituto Geográfico Agustin Codazzi (IGAC, 2000). The defined soil texture was sandy clay loam. Hydrodynamic soil parameters, such as field capacity and permanent wilting point moisture contents, were estimated using the RETC sofwtware (Van Genuchten *et al.*, 1998). RETC uses pedo-transfer functions to compute hydrodynamic soil parameters from soil texture.

Atmosphere: Meteorological data for the second half of 2004 was obtained from the Instituto de Meteorología, Hidrología y Estudios Ambientales (IDEAM) database. "La cosecha" station was selected for requesting meteorological data. This station is located at 74.0012° W and 4.989° N and was selected because it is the nearest station to the place where data was collected (approximately 1.8 km away). Figure 1 depicts the locations of the weather station and the experiments from Ñústez *et al.* (2009). The weather and soil conditions can be considered as equal for both locations.

FIGURE 1. Detailed location of the experiments from Ñústez *et al.* (2009) and the weather station that was used as data source.

Table 1 shows the principal characteristics of both models; some of those characteristics are about required data.

TABLE 1. Main characteristics of WOFOST and AquaCropOS crop models (adapted from Raes *et al.* (2018) and De Wit *et al.* (2019)).

Model characteristic	Model	
	WOFOST	**AquaCropOS**
Developer	Timothy Foster (University of Manchester)	Allard De Wit (Wageningen University & Research)
Kind of model	Explanatory	Descriptive
Programming language	Python / FORTRAN	MATLAB / OCTAVE
Installation and operation difficulty	High (It requires medium - advanced knowledge of the programming language)	Medium (It requires basic knowledge of programming)
Crop Calendar	Growing degree days (GDD)	GDD / calendar days
Hydrodynamic soil parameters required	Soil humidity and soil hydraulic conductivity as a function of soil matrix potential	Soil humidity at field capacity, permanent wilting point and saturation. Saturated hydraulic conductivity
Crop management	Irrigation, fertilizer application	Mulches, irrigation
Meteorological data required	Maximum and minimum temperature, total incoming radiation/total sunshine hours, average vapor pressure, wind speed and precipitation	Maximum and minimum temperature, reference evapotranspiration, precipitation
Minimum files required for its execution	3	16
Yield calculation	Partitioning of assimilates	Harvest Index
Main crop parameters required	Parameters for: Crop phenology (growing degree days), CO_2 assimilation, conversion efficiency of assimilates, respiration, assimilate partitioning, leaf death rates, root depth, water, temperature and nutrient stress	Water productivity index, canopy growth parameters, crop yield index, root growth parameters, water and temperature stress parameters

Results and discussion

Tuber biomass simulated by both models was very well adjusted to the observed data (yield or harvestable biomass, Fig. 2). Both models achieved a quite good approximation to tuber biomass through crop development. Simulated final tuber biomass by WOFOST and AquaCropOS was 10.48 t ha^{-1} and 10.73 t ha^{-1}, respectively, whereas the observed final tuber biomass was 10.45 t ha^{-1}. The simulated total above-ground biomass by the two models did not strictly follow the observed data (Fig. 3). However, AquaCropOS exhibited a better fit. Simulated final total above-ground biomass by WOFOST and AquaCropOS was 17.92 t ha^{-1} and 12.90 t ha^{-1}, respectively, whereas the observed final total above-ground biomass was 12.29 t ha^{-1}.

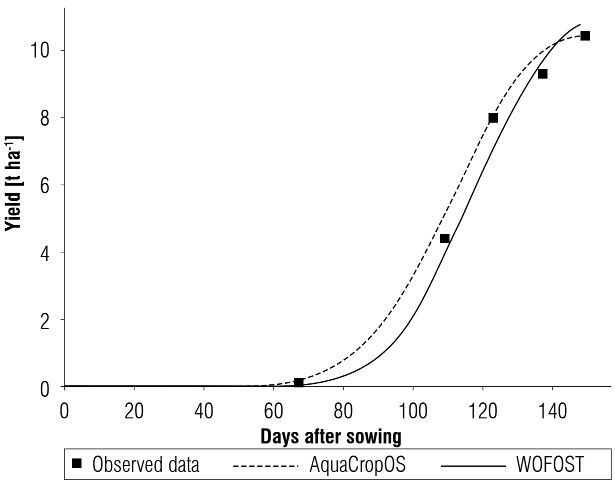


FIGURE 2. Tuber biomass (yield) simulated by AquaCropOS and WOFOST models, and observed tuber biomass.

Table 2 shows the efficiency of the model (EF) and RMSE values for the simulation of yield and total above-ground biomass for the two models. The obtained results agree with Todorovic *et al.* (2009), who concluded that both models are a good approximation to real yield, although WOFOST showed the best performance. Both RMSE and EF values show that WOFOST is the best model at simulating yield. The efficiency index of both models suggests that the two of them can simulate yield formation with a very high precision. However, WOFOST simulated yield formation with higher accuracy and with a lower error. On average, WOFOST and AquaCropOS error at simulating yield formation was 0.391 t ha^{-1} and 0.614 t ha^{-1}, respectively. The efficiency index of the model in simulating total above-ground biomass was higher for AquaCropOS. Nevertheless, Figure 3 shows that in the last stages before the peak of biomass, WOFOST simulation fits better the observed values.

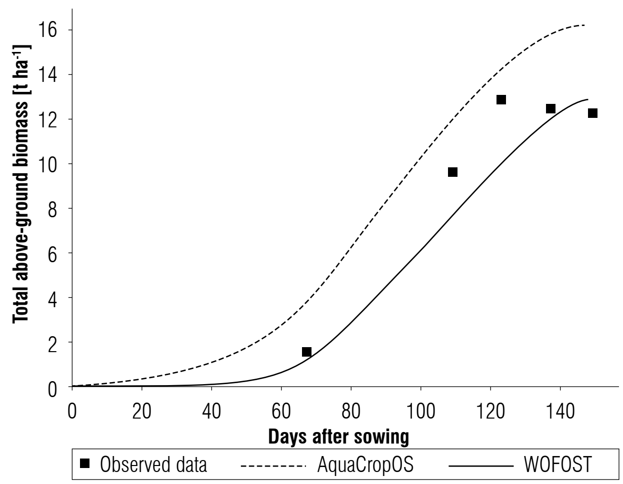


FIGURE 3. Total above-ground biomass simulated by the AquaCropOS and WOFOST models and observed total above-ground biomass.

WOFOST, as a physiological model that considers processes like photosynthesis and biomass partition in detail, showed a better performance than AquaCropOS. The former considers crop transpiration and atmospheric CO_2 as the only yield-defining factors. Despite this, the performance of WOFOST for total above-ground biomass was poor in comparison to AquaCropOS. This discrepancy between observed and simulated above-ground biomass for both models was due to the model's inability of simulating drop of leaves. WOFOST can simulate the death of leaves; however, even though leaves undergo senescence, they are not falling. In the model, the process of leaf death only implies that they are not contributing to physiological processes such as photosynthesis and transpiration anymore. AquaCropOS

TABLE 2. Efficiency of the models, root-mean square error (RMSE), and correlation (r^2) in yield and total above-ground biomass simulated by AquaCropOS and WOFOST models.

	Model	Efficiency	RMSE (t ha^{-1})	Correlation
Harvestable biomass (Yield)	AquaCropOS	0.974	0.614	0.990
	WOFOST	0.991	0.391	0.997
Total above-ground biomass	AquaCropOS	0.828	1.778	0.951
	WOFOST	0.615	2.658	0.979

Highlighted cells show the best result.

simulates canopy cover decline, but it is not associated with a biomass loss due to death and drop of leaves.

Conclusions

Although WOFOST shows the best performance in simulating tuber biomass, AquaCropOS's performance is nearly as good. Due to its lower complexity and the smaller number of parameters, AquaCropOS is the best option for simulating the yield of the potato crop. AquaCropOS is better in simulating above-ground biomass. Less efficiency in simulating above-ground biomass for the two models is due to difficulty at simulating the death of leaves and their consequential fall.

Crop modeling is a powerful tool to develop data-driven and climate-smart platforms in agriculture. Studies on the calibration and validation of crop models result in an important advance in the endless effort to achieve a more sustainable and efficient agricultural production. To represent local conditions for different crop varieties, it is necessary to perform new calibration works.

Literature cited

Boogaard, H., C. Van Diepen, R. Rötter, J. Cabrera, and H. Van Laar. 2014. WOFOST Control Centre 2.1 and WOFOST 7.1.7. Wageningen University and Research Centre, Wageningen, Netherlands.

Condori, B., A. de la Casa, A. Mazetti, R. Peres, S. Olarte, E. Jerez, N. Clavijo, D. Rodríguez, B. Gómez, I. Trebejo, F. Vilaró, C. García, L. Sarmiento, J. Rodríguez, and M. van den Berg. 2016. Modelación de la papa en Latinoamérica: estado del arte y base de datos para parametrización. Publications Office of the European Union, Luxembourg. Doi: 10.2788/520167

Confalonieri, R., Acutis, M., Bellocchi, G. and Donatelli, M. 2009. Multi-metric evaluation of the models WARM, CropSyst, and WOFOST for rice. Ecol. Model. 220(11), 1395-1410. Doi: 10.1016/j.ecolmodel.2009.02.017

Cortés, C., J. Bernal, E. Díaz, and F. Méndez. 2013. Uso del modelo AquaCrop para estimar rendimientos para el cultivo de papa en los departamentos de Cundinamarca y Boyacá. FAO, Colombia.

De Wit, A. 2018a. PCSE: the Python crop simulation environment. URL: https://pcse.readthedocs.io/en/stable/ (accessed 5 February 2019).

De Wit, A. 2018b. WOFOST Crop Parameters. URL: https://github.com/ajwdewit/WOFOST_crop_parameters (accessed 24 January 2019).

De Wit, A., H. Boogaard, D. Fumagalli, S. Janssen, R. Knapen, D. van Kraalingen, I. Supit, R. van der Wijngaart, and K. van Diepen. 2019. 25 years of the WOFOST cropping systems model. Agric. Syst. 168, 154-167. Doi: 10.1016/j.agsy.2018.06.018

Foster, T., N. Brozović, A.P. Butler, C.M.U. Neale, D. Raes, P. Steduto, E. Fereres, and T.C. Hsiao. 2017. AquaCrop-OS: an open source version of FAO's crop water productivity model. Agr. Water Manage. 181, 18-22. Doi: 10.1016/j.agwat.2016.11.015

Graeff, S., J. Link, J. Binder, and W. Claupein. 2012. Crop models as decision support systems in crop production. pp. 3-27. In: Sharma, P. (ed.). Crop production technologies. InTech, Shangai, China. Doi: 10.5772/28976

Huang, X., G. Huang, C. Yu, S. Ni, and L. Yu. 2017. A multiple crop model ensemble for improving broad-scale yield prediction using Bayesian model averaging. Field Crops Res. 211, 114-124. Doi: 10.1016/j.fcr.2017.06.011

IGAC. 2000. Estudio general de suelos y zonificación de tierras del Departamento de Cundinamarca. IGAC, Bogota.

Marcelis, L.F., E. Heuvelink, and J. Goudriaan. 1998. Modelling biomass production and yield of horticultural crops: a review. Sci. Hortic. 74(1-2), 83-111. Doi: 10.1016/s0304-4238(98)00083-1

Ñústez, C., M. Santos, and M. Segura. 2009. Acumulación y distribución de materia seca de cuatro variedades de papa (*Solanum tuberosum* L.) en Zipaquirá, Cundinamarca (Colombia). Rev. Fac. Nac. Agron. Medellín 62(1), 4823-4834.

Raes, D., P. Steduto, T. Hsiao, and E. Fereres. 2018. Chapter 3 - Calculation procedures. AquaCrop Version 6.0-6.1 Reference manual. FAO, Rome.

Rosenzweig, C., J. Jones, J. Hatfield, J. Antle, A. Ruane, and C. Mutter. 2015. The agricultural model intercomparison and improvement project: phase I - Activities by a global community of science. pp. 3-24. In: Ronsenzweig, C. and D. Hillel (eds.). Handbook of climate change and agroecosystems. Imperial College Press, London. Doi: 10.1142/9781783265640_0001

Teh, C. 2006. Introduction to mathematical modeling of crop growth: how the equations are derived and assembled into a computer model. Brown Walker Press, Boca Raton, USA.

Todorovic, M., R. Albrizio, L. Zivotic, M. Abi Saab, C. Stöckle, and P. Steduto. 2009. Assessment of AquaCrop, CropSyst, and WOFOST models in the simulation of sunflower growth under different water regimes. Agron. J. 101(3), 509-521. Doi: 10.2134/agronj2008.0166s

Valbuena, R., G. Roveda, A. Bolaños, J. Zapata, C. Medina, P. Almanza, and P. Porras. 2010. Escalas fenológicas de las variedades de papa Parda Pastusa, Diacol Capiro y Criolla "Yema de Huevo" en las zonas productoras de Cundinamarca, Boyacá, Nariño y Antioquia. Corpoica, Produmedios, Bogota.

Van Genuchten, M.T., J. Simunek, F.J. Leji, and M. Sejna. 1998. RETC, version 6.0. Code for quantifying the hydraulic functions of unsaturated soils. US Salinity Laboratory, USDA, Riverside, USA.

Wallach, D., D. Makowski, J. Jones, and F. Brun. 2014. Working with dynamic crop models: methods, tools and examples for agriculture and environment. 2nd ed. Elsevier, San Diego, USA. Doi: 10.1016/C2011-0-06987-9

Influence of some environmental factors on the feijoa (*Acca sellowiana* [Berg] Burret): A review

Efecto de algunos factores ambientales sobre la feijoa (*Acca sellowiana* [Berg] Burret): Una revisión

Gerhard Fischer[1]* and Alfonso Parra-Coronado[2]

ABSTRACT

Climatic alterations affect the physiology, growth and production of the feijoa, a native plant to the higher zone between Brazil, Uruguay, Paraguay and Argentina. In Colombia, optimal growth temperatures are between 13 and 21°C (16°C). Very high temperatures (>32°C) affect pollination and fruit set, but low temperatures down to -4°C in adult plants do not cause significant damage. Thus, feijoa is a well-adapted plant to cold conditions since 3.04°C has been found as the base (minimum) temperature for the phase between flower bud and fruit set, while 1.76°C was measured as the base temperature for fruit development. The plant requires a minimum of 1000 hours of direct sunlight/year (optimum≥1500); the pyramidal form of the tree favors the entry of light into the crown organs. In the crown of a feijoa tree, trained with three branching levels of horizontal bent primary laterals, the outer middle quadrant produces the largest fruits, compared to those that grow in the upper quadrant. The reduced fruit growth is due to the excessive incidence of light (especially UV) and heat on these fruits on the periphery of the tree. In Colombia, altitudes between 1800 and 2700 meters above sea level (m a.s.l.) are adequate for growth and production of this fruit, while lower elevations favor the incidence of fruit flies. Precipitations between 700 and 1200 mm/year (max. 2000 mm) benefit the vegetative and reproductive performance of the tree, with an important drier season at the beginning of the reproductive season (flowering and fruit set). Due to the strength of its branches and the small, thick leaves, the tree is relatively wind resistant.

Key words: growth temperature, base temperature, cold adaption, solar radiation, altitude, wind.

RESUMEN

Las alteraciones del clima afectan la fisiología, crecimiento y producción de la feijoa, planta originaria de la zona alta entre Brasil, Uruguay, Paraguay y Argentina. En Colombia encuentra temperaturas óptimas entre 13 y 21°C (16°C), mientas que las temperaturas muy elevadas (>32°C) afectan la polinización y el cuajado de frutos, en tanto que las temperaturas hasta -4°C en plantas adultas no causan daños significativos. Así, la feijoa es una planta bien adaptada a condiciones de frío debido a que se registró 3.04°C como la temperatura base (la mínima) para la fase entre botón floral y cuajado del fruto y solamente 1.76°C como la temperatura base para el desarrollo del fruto. La planta exige mínimo 1000 horas de brillo solar/año (óptimo≥1500) y la formación piramidal del árbol favorece la entrada de luz a los órganos de la copa. En una copa de feijoa, formada en tres pisos con ramas laterales horizontales, el cuadrante medio externo produce los frutos más grandes, comparado con los que crecen en el cuadrante superior de la copa. El crecimiento reducido se debe a la excesiva incidencia lumínica (especialmente UV) y de calor sobre estos frutos en la periferia del árbol. En Colombia altitudes entre 1800 y 2700 msnm son adecuadas para el crecimiento y la producción, mientras elevaciones más bajas favorecen la incidencia de la mosca de la fruta. Las precipitaciones entre 700 y 1200 mm/año (máx. 2000 mm) benefician el desempeño vegetativo y reproductivo del árbol, siendo un tiempo más seco importante al comienzo de la época reproductiva (floración y cuajado de frutos). Por la estructura fuerte de sus ramas y hojas pequeñas y gruesas el árbol es relativamente resistente al viento.

Palabras clave: temperatura de crecimiento, temperatura base, adaptación al frío, radiación solar, altitud, viento.

Introduction

Environmental imbalances generated by deforestation and the increase in greenhouse gases alter crop growth and productivity significantly affecting global food security (Dhankher & Foyer, 2018). Traditional cultivation methods of crops will no longer provide optimal growing conditions, and they will increase production costs (Fischer & Melgarejo, 2020). The climatic changes that are taking place at different altitudes because of increases in temperature and radiation require additional research in order to understand the new growth conditions that will

Received for publication: 8 July, 2020. Accepted for publication: 18 November, 2020. Doi: 10.15446/agron.colomb.v38n3.88982

[1] Independent consulter, Emeritus researcher of Colciencias, Bogota (Colombia).
[2] Department of Civil and Agricultural Engineering, Faculty of Engineering, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: gerfischer@gmail.com

modify the phenological phases, maturation, quality and performance of crops (Ramírez & Kallarackal, 2015; Fischer *et al*., 2016; Ramírez & Kallarackal, 2018a). The physiological behavior of plants and the quality of their fruits depend on the characteristics of each species and variety as well as environmental conditions and crop management (Fischer *et al*., 2018; Fischer *et al*., 2020).

Therefore, studying plant ecophysiology is of utmost importance for knowing how abiotic and biotic factors of the environment affect the physiology of cultivated plants (Fischer *et al*., 2016) and how physiological mechanisms interact with these physicochemical and biotic factors (Lambers *et al*., 2008).

The feijoa (*Acca sellowiana* [O. Berg] Burret) belongs to the Mirtaceae family, known for its botanical richness and represented by some 121 genera with 5800 species, many of these with a high agro-industrial potential for their aromatic fruits (Farias *et al*., 2020). The characteristics of feijoa are similar to those of the guava (*Psidium guajava*) and the bayberry (*Mircia acuminata*), but with a different phenotype (Perea-Dallos *et al*., 2010; Fischer & Melgarejo, 2021).

The feijoa is native to South America, in the southern zone of Brazil and Uruguay, where it is known as "goiabeira-serrana" and "guayabo del país" (Puppo *et al*., 2014; Donazzolo *et al*., 2015). It is also known from the upper region of western Paraguay and northeast Argentina (Pachón & Quintero, 1992; Parra-Coronado & Fischer, 2013; Borsuk *et al*., 2015). It was introduced to other countries in the world, where it is known as feijoa or pineapple guava (Moretto *et al*., 2014). The most extensive production is found in Colombia, New Zealand, Georgia, Ukraine, but also in the United States, Australia, Turkey, China and with an increasing area of cultivation in Brazil (Sachet *et al*., 2019; Sánchez-Mora *et al*., 2019). This distribution in such varied countries and climates demonstrates the great potential for the plasticity that this species possesses for adaptation to very diverse environmental conditions (Donazzolo *et al*., 2019). Ouafaa *et al*. (2019) not only highlight the fruit's easy adaptation to subtropical climates but also the sweet aromatic characteristics of the feijoa fruit that favor its distribution in so many countries. However, Phan *et al*. (2019) state that this fruit has remained relatively unknown to many people in the world until today.

Two different populations of feijoa have been described (Ducroquet *et al*., 2000). One population is found in the higher altitude regions of the basalt plateau of Southeastern Brazil. This population has long and hard seeds, often with a bitter pulp and hard peel fruits, called "Brazil group" (Cabrera *et al*., 2018). The other population is found on the acid soils of Uruguay and the south of the Rio Grande do Sul state in Brazil. These fruits (called "Uruguay group") are sweet and contain small, soft seeds that have been distributed in many countries (Schotsmans *et al*., 2011). Ducroquet *et al*. (2000), describing these two groups and zones where the feijoas were found, mentioned that these were sites with a medium temperature of 16°C. However, the researchers also affirmed that they had no information of naturally occurring feijoas in Paraguay which were mentioned by other authors (e. g. Pachón & Quintero, 1992).

Feijoa germplasm banks are mainly concentrated in three centers: 1) the Feijoa Active Germplasm Bank (BAG) in São Joaquim-SC (Brazil), with 313 accessions, 2) the Feijoa National Center (CENAF) in La Vega (Cundinamarca, Colombia), with some 1500 accessions, and 3) the Nikitsky Botanical Garden (Crimean peninsula) that has 400 accessions (Sánchez-Mora *et al*., 2019). In Colombia, Parra-Coronado *et al*. (2016) reported commercial varieties such as clone 41 (Quimba), clone 8-4, Mammouth, Apollo, Gemini, Triumph, Rionegro, Tibasosa and some others.

In tropical areas, the tree can produce throughout the year, while in subtropical areas, production predominates at a unique time of year (Quintero, 2012). In Colombia, feijoa is a very promising fruit due to its adaptation to areas between 1800 and 2700 m a.s.l. (Quintero, 2012). Feijoa production in Colombia reached 9290 t in the first half of 2019, surpassing fruits such as cape gooseberry and pitaya (DANE, 2020), with the most important cultivation in the Cundinamarca-Boyaca mountain valleys.

Feijoa is a subtropical shrub or small tree with a maximum height of 3.5 to 5 m. It is a long-lived perennial fruit species that can produce, in technically controlled crop systems, 20 t ha^{-1} or more (Fischer, 2003; Quintero, 2012). The species was previously considered to be an ornamental plant (Pachón & Quintero, 1992). In highly controlled cultivation, the feijoa grows erectly with a central leader (Omarova *et al*., 2020), forming between three and four levels of horizontal bent laterals (Quintero, 2012). The plant has elliptical to oval shaped leaves, bright green on the upper side and 6×4 cm whitish color on the underside (Fischer, 2003).

The hermaphrodite flowers of 3-4 cm are composed of four greenish-gray sepals and a corolla formed of four white petals. In the center, there are 60-120 stamens of red filaments with white anthers, according to the particular cultivar (Fischer, 2003; Ramírez & Kallarackal, 2017). In general, *A. sellowiana* has barriers to self-fertilization such as the dichogamy through protogyny and self-incompatibility (Stewart & Craig, 1987), and Finatto *et al.* (2011) found a late-acting self-incompatibility in this species.

In Colombian plantations, the authors of this review observed that the flowers develop mainly 1) in the leaf axils of shoots of the same year, 2) on the branches bent horizontally (up to several years), 3) on the thin and mostly hanging branches in the inner crown and 4) on stump sprouts emerging after cuttings in the lower and middle part of the crown. In a study near Bogota (Colombia), Ramírez and Kallarackal (2018b) characterize two feijoa cultivars, with six different phenological stages according to the scale of the BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie): 1) bud development, 2) leaf development, 3) stem development, 4) flower emergence, 5) flowering and 6) fruit development.

Many cultivars are self-fertile, but they also allow cross-pollination by insects or birds (Ramírez & Kallarackal, 2017). The most important ornithological agent in Colombia is the blackbird (*Turdus fuscater*), *Turdus merula* in New Zealand and *Acridotheres tristis*, and *Turdus* spp., *Thraupi*s spp. and *Tangar*a spp. in Brazil. All these species eat the petals, which increase their sugar content immediately before and during the opening of the flower (Duarte & Paull, 2015). This fact is very important for the control of *Botryti*s in petals during flowering (Quintero, 2012).

For humans, the flower petals are edible and contain, according to the variety, high concentrations of anthocyanins, flavonoids, total phenols, increased antioxidant activity and high soluble solids content (Amarante *et al.*, 2019). Magri *et al.* (2020) found that the petals attain the highest peak of polyphenols, anthocyanins and ascorbic acid in the F2 state, when the petals continue to open and the anthers and filaments have a dark red color, according to the BBCH scale (Ramírez & Kallarackal, 2018b).

The fruit is a berry, mainly ovoidal in shape and with a bright green or grayish green color, smooth to rough skin. Fruit dimensions are 3-5 cm in diameter, 4-10 cm in length, weight between 20 and 250 g and contains between 20 and 40 seeds (Fischer, 2003; Schotsmans *et al.*, 2011). Apart from fresh, un-processed consumption (with a very sweet and aromatic flavor), fruits can be processed for juices, jellies, drinks, ice creams and yogurts (Mitra, 2010; González-García *et al.*, 2018).

Zhu (2018) highlights the effects of the fruit on health as an important source of polyphenols, vitamin C, fiber, sugars and minerals, mainly potassium. It also has medicinal value for its antibacterial, antioxidant and antiallergic activity, with a higher content of antioxidants, such as flavonoids and ascorbic acid in the fruit skin rather than in the pulp (Phan *et al.*, 2020).

Sachet *et al.* (2019) confirm that ecophysiological studies are necessary to improve the cultivation of feijoa. Furthermore, Germanà and Continella (2004) clearly state that ecophysiological evaluation is important for understanding the possibilities of acclimatization and the diffusion of species, including feijoa. The objective of this review is to present the results of the literature on ecophysiological topics of the feijoa, including temperature, light, altitude, water, and wind. These data should be very useful for researchers and plant breeders as well as for fruit growers.

Ecophysiological factors and their effect on feijoa

Some research exists describing the influence of temperature, solar radiation, altitude, water (precipitation, drought) and wind factors on this crop. It should be understood that in the different areas of cultivation there is not a single climatic factor that acts alone. It is the interaction of several factors that influence the plant, causing stress conditions such as drought, waterlogging, very high and low temperatures or ultraviolet light that can be detrimental to plant performance (Mittler, 2006).

Temperature

In temperate or subtropical zones, feijoa adjusts its physiology to the seasonal temperatures. Low winter temperatures inhibit the sprouting of branches and flowers are reactivated with the increase of temperatures in spring (Fischer, 2003). In temperate zones, when soil temperatures reach 8-10°C in spring, root growth begins and continues until autumn when soil temperatures drop below 8°C (Thorp, 2008). Morley-Bunker (1999) reports that feijoa in temperate zones produce larger fruits and yields in the sites with higher temperatures compared to those with lower winter temperatures.

Flowering and ripening of the fruit can be advanced by eight weeks in warmer climates compared to cold climates (Duarte & Paull, 2015). Furthermore, climate change impacts tree phenology (Ramírez & Kallarackal, 2017). However, fruits produced in cold weather are supposedly of better flavor than those produced in hot climates

(California Rare Fruit Growers, 1996). This was confirmed in a study carried out in Colombia by Parra-Coronado *et al.* (2015a) for feijoa fruits from clone 41 (Quimba), where they found that the fruits produced at higher altitudes (cold weather) are sweeter than those produced at low altitudes (warm weather). Barrero (1993) reports that adequate temperatures for feijoa production in Colombia are between 13 and 21°C (average 16°C).

In temperate areas some authors report that feijoa requires a certain number of chilling hours (temperatures below 7 or 7.2°C according to author) to increase flower production, and this would be deficient in areas with less than 50 chilling hours (Fischer, 2003). Sharpe *et al.* (1993) indicated that the feijoa needs between 100 and 200 chilling hours below 7°C to sprout. For this situation, Schotsmans *et al.* (2011) summarize that feijoa reacts not only to changes in temperature that characterize the season in subtropical and temperate zones, but they also arise from the changes in the pattern of rainfall and as a reaction to pruning, as in Colombia (Quintero, 2012).

According to Rom (1996), the effects of temperature on a fruit tree should not be isolated from other agroecological factors, especially water and light stress. Temperature tolerance depends on the concentration of carbohydrates stored in the tissues and the level of nutrient ions in the plant. The feijoa plant is highly susceptible to very high temperatures (>32°C), combined with low relative humidity during pollination and fruit set (Duarte & Paull, 2015). In general, Quintero-Monroy (2014) observes that high temperatures in Colombia cause damage to apical and juvenile sprouts, causing a folding of the leaves. As the authors of this review presume, the fruit development time of the feijoa will decrease due to global warming (Duarte & Paull, 2015). This can affect the fruit quality increasing the concentration of sugars and decreasing that of acids (Ubeda *et al.*, 2020). This causes the fruit to be tasteless, without a good sugar/acid ratio; therefore, varieties that do not show this behavior should be selected (Parra-Coronado & Fischer, 2013).

Feijoa is considered a subtropical fruit tree that tolerates winter frosts. It tolerates temperatures down to -10°C that can occur in the Bogota High Plateau (Quintero-Monroy, 2014) and temperatures down to -4°C do not cause significant damage in adult plants (Pachón & Quintero, 1992). In the Bogota High Plateau, temperatures lower than -4°C for more than 1 h cause losses of flowers and juvenile regrowth (Quintero-Monroy, 2014). Therefore, the authors of this review do not recommend pruning feijoa trees for 1-2 months before the frost season to avoid new (tender) shoots. Fischer (2003) observed that frost can cause abortion of leaves and small fruits, but frost also damages the fruit during its ripening, with premature browning of the affected tissues (Schotsmans *et al.*, 2011). In a study with five feijoa varieties, Stanley and Warrington (1984) found that trees are damaged by frost with temperatures below -3°C in the summer season and below -8°C in the winter season, and the temperature can be lethal to the plant still at 4-6°C lower than these values mentioned.

Adult plants are more resistant to low temperatures (Tocornal, 1988), while juveniles (<1 year) can die during longer frosts. In Tenjo (Cundinamarca, Colombia) in a frost that reached -10°C, Quintero-Monroy (2014) observed that half the 1-year-old plants lost all their leaves and resprouted only at the base of the stem, while the 2-year-old plants showed slight damage and the 10-year-old plants only had some flowers burned.

In a study carried out in Cundinamarca (Colombia), Parra-Coronado *et al.* (2015b) found that temperature influences the growth and quality of feijoa fruits (clone 41 'Quimba'). Thus, in San Francisco de Sales with a registered average temperature of 18.3°C (1800 m a.s.l.), fruit development advanced much faster (155 d) than in Tenjo (180 d) with a registered average temperature of 12.3°C (2580 m a.s.l.). This was similar to that observed by Fischer *et al.* (2007) in cape gooseberry with 66 d at 17.0°C compared to 12.5°C and 75 d in the neighboring department of Boyaca. Interestingly, Parra-Coronado *et al.* (2016) recorded much less growing degree days (GDD) (Eq. 1) for the fruit at 12.3°C (1972 GDD) rather than at 18.3°C (2677 GDD). This can be seen as an adaptation to these colder conditions. Also, a temperature of 18.3°C produced smaller fruits (38 g) than those that grew at 12.3°C (69 g), indicating that feijoa is rather a cold-weather crop of cool Andean highland conditions. It should not be forgotten that in this study lower temperature was accompanied by higher cumulative radiation (11082 W m^{-2}) during the development of the fruit rather than at the lower elevation and, thus, hotter site (8918 W m^{-2}) (Parra-Coronado *et al.*, 2015a).

$$GDD = \sum_{i=1}^{n} T_i - nBt \tag{1}$$

where GDD are the growing-degree days (°C) accumulated during n days of fruit development, Ti is the average daily temperature (°C) for day i and Bt is the base temperature for fruit growth (1.76°C). Ti = (Tmax + Tmin) / 2.

Parra-Coronado *et al.* (2015b) also studied the base temperature (Bt) in 'Quimba', that is, the minimum for the growth of the reproductive organs of feijoa, and they find that feijoa is a plant well-adapted to cold conditions. They register 3.04°C Bt for the flower bud phase before anthesis and from anthesis to fruit set, while the Bt for the phase from fruit set until its harvest is only 1.76°C, lasting 189 d from flower bud until the fruit harvest at 2651 GDD. Also, in other fruit trees from Andean areas, Bt was calculated for fruit development as low as that for cape gooseberry with 1.9°C (Salazar *et al*., 2008) and 0.01°C for that of curuba (banana passion fruit) (Mayorga *et al*., 2020). Parra-Coronado *et al*. (2015b) recommend that the prediction of a time course based on the growing-degree days (in this case from flower bud to fruit maturity) can be applied to schedule harvest and crop management practices related to the phenological phases of the plant.

Solar radiation

Reproductive stages like flowering, pollination, fruit set, and fruit fillings are favored in free exposure to solar radiation (≥1500 h of direct sunlight per year), since they adapt well to full luminosity, as long as there are no dry and high temperature conditions (Fischer, 2003; Fischer *et al*., 2020). According to Quintero-Castillo (2003), in sites where there are only 1000 h of direct sunlight/year, but with very favorable agro-ecological and crop management conditions (irrigation, fertilization, pruning, pollination), it is possible to obtain a good production. Parra-Coronado *et al*. (2015a) harvested 44% larger fruits at higher altitudes (2580 m a.s.l.), at which the accumulated solar radiation before harvest was 20% higher than at the lower site (1800 m a.s.l.).

Despite the fact that feijoa tolerates partial shade and at sites with <1500 h of direct sunlight/year, Duarte and Paull (2015) recommended training the tree canopy to a central leader (in a pyramid shape) to take full advantage of the light. Quintero (2012) also highlights the advantages of forming pyramidal-shaped tree crowns with three branching levels of horizontal bent primary laterals, allowing a better incidence of light on the flower buds and higher photosynthetic rates of the leaves, compared to a tree using straighter branches or a free growth like a bush. Martínez-Vega *et al*. (2008) harvested smaller fruits in the inner and outer crown of the basal part of the feijoa tree, where only 35 and 54% of the external solar radiation falls on a full sunny day (Fig. 1).

Times of high and prolonged solar radiation that commonly occur with excessive heat and drought (as during the "El Niño Phenomenon" in northern South America) can cause fruit burning when not protected by the foliage (Fischer, 2003).

Martínez-Vega *et al*. (2008) studied the effect of fruit position on 6-year-old feijoa trees, cv. Quimba, trained in three branching levels with horizontal bent laterals (Fig. 1), in an orchard located at 2350 m a.s.l. in the municipality of La Vega (Cundinamarca, Colombia). The physiologically ripe fruits harvested in the outer middle quadrant (Fig. 1) showed higher fresh weight, while fruit color was more intense green at the base and inside the crown. This confirms that the fruits growing in the outer middle quadrant stand out for good overall features. The fruits of the upper quadrant develop a higher maturity ratio (total soluble solids/total titratable acidity) but with a lower weight. This confirms the large effects of the differential microclimates on the tree canopy and on the quality of the fruits, taking into account that the leaves on the upper periphery of the feijoa tree have a greater number of stomata (Naizaque *et al*., 2014). However, trees are exposed to elevated solar radiation (especially UV) in the high tropics and must adapt to stress conditions due to very high temperatures that can cause situations of stomatal closure and photoinhibition that also limit fruit filling (Fischer & Orduz-Rodríguez, 2012). On cloudy days, Martínez-Vega *et al*. (2008) recorded differences in incident radiation in the different parts of the canopy that are much less pronounced than on a sunny day (Fig. 1).

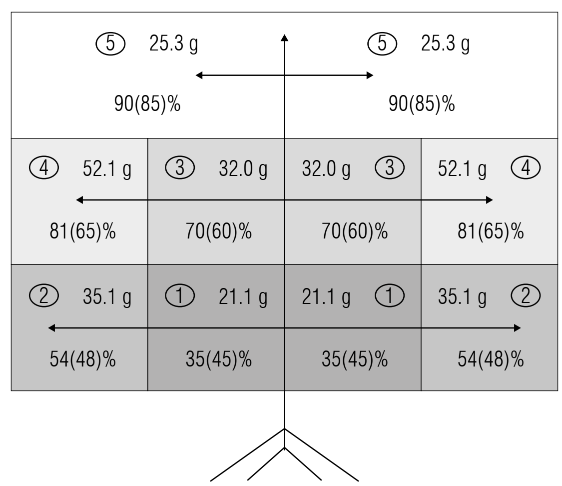


FIGURE 1. Distribution of the quadrants in the crown of the feijoa tree: 1) internal base, 2) external base, 3) internal medium, 4) external medium, and 5) superior, including the fresh weight of the fruits (g) and the percentages of the incident radiation on a sunny day (on average 1920 μmol m^{-2} s^{-1}) and (in parentheses) on a cloudy day (on average 348 μmol m^{-2} s^{-1}). Modified from Martínez-Vega *et al*. (2008) with permission of the Revista Colombiana de Ciencias Hortícolas.

Altitude

Since the tropics are areas with thermal uniformity (i.e., without seasons of temperature), thermal zones are formed with a reduction in temperature as altitude increases, in which the only contrast is between day and night. Some authors describe for the high tropical zones the daytime as summer and the nighttime as winter (Fischer & Orduz-Rodríguez, 2012). These authors define the climatological changes that occur with increasing altitude as a reduction in temperature of 0.6°C on average per 100 m, a decrease in the partial pressure of gases such as O_2, CO_2, N_2 and water vapor as well as decreased precipitation (from 1300-1500 m a.s.l.), while UV, visible and infrared radiation, and wind increase with tropical elevation.

The adaptation of the feijoa to the tropical highland climate comes from its area of origin (Jackson & Looney, 1999) at about 1000 m a.s.l. in southern Brazil. In Colombia, commercial feijoa plantations adapt well to a range between 1800 and 2700 m a.s.l. (Parra-Coronado *et al.*, 2019), as do deciduous fruit trees and strawberries (Fischer & Orduz-Rodríguez, 2012). The optimal range between 2100 and 2600 m a.s.l. is apparently the best for feijoa (Duarte & Paull, 2015). Quintero-Monroy (2014) mentions that plantations >2700 m a.s.l. lack economic profitability because plants grow too slowly and show inferior production and fruit quality.

For Barrero (1993), the ideal altitudinal zone for commercial feijoa production in Colombia lies between 2000 and 2400 m, while areas lower than 1800 m are not consistent for correct crop behavior. Pachón and Quintero (1992) point out that at altitudes below 1600 m (e.g. the Colombian coffee zone) the growth of the plant is limited by the *Anastreph*a sp. fruit fly. However, Fischer (2003) reports that the fruit fly has been found up to altitudes of 2600 m in Tibasosa (Boyaca). In general, Quintero (2012) states that the efficient control of fruit flies (the most restrictive pest of feijoa) is easier in orchards in tropical highland areas above 2000 m a.s.l. and recommends the production of organic feijoas at altitudes higher than 2500 m.

The adaptation to these relatively high altitudes favors the thick cuticle and the small size of the leaves of the feijoa (Schotsmans *et al.*, 2011), which reduces transpiration in dry seasons and also filters UV radiation, avoiding its mutagenic effects and favoring their phytosanitary status (Fischer & Orduz-Rodríguez, 2012).

Although the cool Andean highland conditions postpone the harvest date (Mayorga *et al.*, 2020), this results in larger and better-quality fruits compared to lower growing sites (Parra-Coronado *et al.*, 2015a). Fischer and Orduz-Rodríguez (2012) report that apples from plantations in high altitude tropical regions (2400-2700 m a.s.l.) stand out for their juiciness, coloration, aroma, compact pulp and firm texture.

Water

Precipitation between 700 and 1200 mm/year favors the production of feijoa, and the fruit tolerates up to about 2000 mm if there is good light and low relative humidity (RH) (Duarte & Paull, 2015) of around 70% (Pachón & Quintero, 1992). Fischer (2003) points out that the pattern of rainfall during the course of the year is important for flowering and fruit set. Therefore, a season without rain favors flowering, which is still improved if there is irrigation at these stages at the beginning of the reproductive phase and during fruit filling (Fischer *et al.*, 2012). Quintero (2012) states that in commercial plantations an adequate and regular water supply is essential for meeting the requirements of the tree at its full vegetative and reproductive growth, allowing two harvests per year in areas where a bimodal rainfall regime prevails (Duarte & Paull, 2015). Feijoa shows certain characteristics of drought tolerance due to its thick leaf and fruit cuticles. Intense and prolonged dry periods can generate leaf, flower and fruit fall (Jackson & Looney, 1999), but they can also interrupt plant development and delay fruit ripening (Fischer, 2003). In turn, Tocornal (1988) indicates that feijoa has a very fibrous and superficial root system that increases its sensitivity to water stress, so that additional irrigation during dry seasons favors production.

Germanà and Continella (2004) compared feijoa ecophysiological behavior with that of avocado and custard apple and found that feijoa showed low water use efficiency (WUE) and high respiration rates, primarily during flowering, when the energy demand of this tree is very high. Feijoa leaves mainly showed higher transpiration rates than the other two species, due to their low stomatal resistance that causes a WUE of only 1/3 compared to avocado and custard apple (Germanà & Continella, 2004).

Quintero-Monroy (2014) states that the most productive crops of feijoa are found in areas where the full flowering season coincides with the dry season, but if the flowering peak coincides with high rainfall, the flowers fall due to the incidence of *Botrytis cinerea*, because these organs form small concavities, in which water accumulates and causes rot. Also, elevated rainfall makes it difficult to pollinate flowers, because the avian pollinating agents do not fly much in rainy seasons to avoid wetting their plumage or the negative effects of falling water on the leaves of the plant (Quintero-Monroy, 2014). The same author points out that poorly pollinated flowers could be aborted prior to fruit set and, in this case, subsequent fruits that are formed are low-caliber and asymmetric.

Drier conditions favor floral induction in feijoa (Peña-Baracaldo & Cabezas-Gutiérrez, 2014) confirmed in plants of cv. Tibasosa (3 years old) where a deficit irrigation of only 25% for 406 d induced the highest number of flower buds compared to irrigation with 100, 75, 50 and 0% of water. A dry season clearly benefits flowering in this species, while a wet one decreases it (Quintero-Monroy, 2014). However, due to climate change, there are no longer well-defined dry seasons, because dry seasons now occur at different times of the year. To have good feijoa production, pruning should be carried out about 60 d before full blooming and this should be during the dry season. But due to the uncertainty of the climate, it is possible to increase flowering with the application of phosphorus, using KH_2PO_4 (0.5%), as found by García *et al.* (2008) in cultivar 41.

Water stress can limit the accumulation of dry matter (DM) in feijoa leaves. Therefore, Omarova *et al.* (2020) recommend selecting varieties that are more resistant to water deficiency and that do not decrease their concentration of leaf DM under the conditions of water stress. These authors registered varieties such as 'Sentiabraskaja' that, during water stress, accumulated a higher leaf DM than others, and the dry season coincides with the time of fruiting. Likewise, Omarova *et al.* (2020) observe that, in periods of high thermohydric stress, the studied varieties increase the content of the osmoprotector proline in leaves between 1.5 and 2.8 times. These authors also stated that the resistance of plants to adverse environmental factors is highly determined by the activation of the enzymatic system of antioxidants that can inhibit the damaging effect of oxidative stress.

In general, feijoa thrives well in regions that have a RH of around 70% (Pachón & Quintero, 1992) and RH >70% for prolonged periods increase the incidence of diseases, mainly *Botrytis* during flowering. Therefore, Duarte and Paull (2015) highlight dry conditions in combination with high luminosity as the most important for avoiding this disease and guaranteeing a high percentage of well-curdled fruits. Feijoa resists high RH during short periods (Fischer, 2003). In areas where these humid conditions are regularly prolonged, the formation of the cone-shaped (pyramidal) tree is the most productive, despite the incidence of *Botrytis* compared to trees in free growth or with an excess of branches (Quintero-Castillo, 2003). In addition, high RH favors the incidence of epiphytes on the trunk and branches of the crown as well as mosses and lichens that secrete substances affecting plant health (Quintero-Monroy, 2014).

Studies in Suba (Cundinamarca) by Galvis *et al.* (1999) and in Tibana (Boyaca) by Naizaque *et al.* (2014) show that leaf transpiration of the feijoa is greater in the upper stratum of the crown than in the lower, with more stomata in the upper periphery (91/mm^2) than in the lower part (78/mm^2) of the plant (Naizaque *et al.*, 2014). These authors observe that the transpiratory rate increases according to increases in temperature and solar radiation in the crown but decreases with the increase of RH in the tree (Fig. 2).

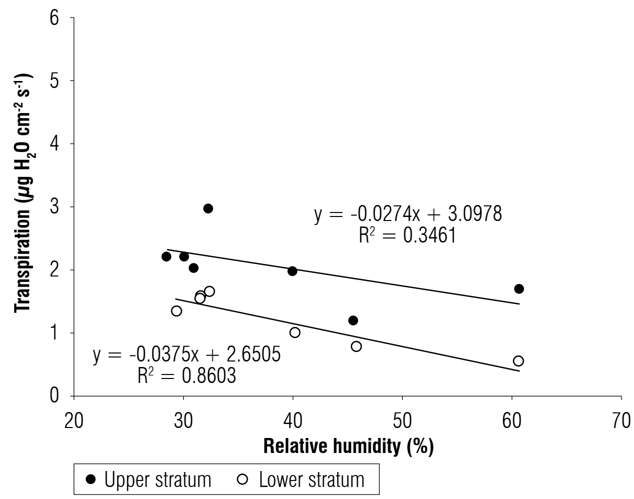


FIGURE 2. Effect of the increase in relative humidity on leaf transpiration in the upper and lower stratum of the canopy of feijoa trees (Naizaque *et al.*, 2014). With permission of Revista U.D.C.A Actualidad & Divulgación Científica.

The feijoa plant shows some tolerance to salinity in the soil and irrigation water, but it reduces tree growth and production (Duarte & Paull, 2015). However, Casierra-Posada and Rodríguez (2006) do not observe differences in dry mass distribution and tree production when they add 0, 20, 40, 60 and 80 mM NaCl to the substrate (corresponding to an electrical conductivity (EC) of 2, 8, 4.6, 6.1, 8.4 and 11.0 dS m^{-1}), increasing the level weekly until reaching this concentration of salts. They only record reduced evapotranspiration with increasing salinity. The authors of this review observed leaf fall and low flowering on feijoa trees in a plot in Mosquera (Cundinamarca) with an EC between 4 and 6 dS m^{-1}. Due to the lack of research on this topic, more studies are necessary.

Wind

Feijoa trees, due to the strong structure of branches and their small and thick leaves, are relatively resistant to the wind (including salty wind from the sea), for which they are used mixed with other species in live barriers against this element (Duarte & Paull, 2015). Gentle winds are important for drying the plant after a rain, avoiding fungal diseases, cooling the leaves on hot days, and renewing the CO_2 concentration in the crown (Fischer & Orduz-Rodríguez, 2012).

Conclusions

The study of environmental effects - now altered by climate change - is of utmost importance for finding feasible growing sites for promising fruit species. The feijoa plant in Colombia adapts to climatic conditions between medium and cold climate (1800-2700 m a.s.l.), but high temperatures affect the beginning of fruiting. Due to its site of origin in the highlands, the plant resists temperatures below 0°C and has a base temperature of 3.04°C for the initial phases of its reproductive stage. It is well-adapted to a great number of direct sunlight hours, but extremely high solar radiation intensities decrease the size and can burn the fruit. The greater radiation accumulated during fruit development produces larger fruits. For floral induction, flowering and fruit set, drier environmental conditions and a relative humidity around 70% are favorable. There exists no concluding information on the tolerance to salinity and waterlogging. The strong trunk and branch structure of feijoa favors its good wind resistance.

Author's contributions

The two authors declare that each one contributed effectively to the design of the document, the obtaining of the bibliographic material, the writing and editing of this review article.

Literature cited

Amarante, C. V. T., Souza, A. G., Beninca, T. D. T., Steffens, C. A., & Ciotta, M. N. (2019). Physicochemical attributes and functional properties of flowers of Brazilian feijoa genotypes. *Pesquisa Agropecuária Brasileira, 54,* Article e00445. https://doi.org/10.1590/S1678-3921.pab2019.v54.00445

Barrero, F. (1993). La ecotecnología en el contexto de la extensión y el desarrollo rural. *Agro-Desarrollo, 4*(1–2), 104–117.

Borsuk, L. J., Saifert, L., Otálora, J. M., Sánchez, F. D., & Nodari, R. O. (2015). Phenotypic variability in feijoa fruits [*Acca sellowiana* (O. Berg.) Burret] on indigenous lands, quilombolas communities and protected areas in the south of Brazil. *Revista Brasileira de Fruticultura, 39*(1), Article e-699. https://doi.org/10.1590/0100-29452017699

Cabrera, D., Vignale, B., & Pritsch, C. (2018). *Acca sellowiana* Berg Burret. Instituto Interamericano de Cooperación para la Agricultura (IICA).

Casierra-Posada, F., & Rodríguez, S. Y. (2006). Tolerancia de plantas de feijoa (*Acca sellowiana* [Berg] Burret) a la salinidad por NaCl. *Agronomía Colombiana, 24*(2), 258–265.

California Rare Fruit Growers. (1996). *Feijoa*. https://crfg.org/wiki/fruit/feijoa/

DANE. (2020). Encuesta Nacional Agropecuaria (ENA), primer semestre 2019. Boletín Técnico. Departamento Administrativo Nacional de Estadística.

Dhankher, O. P., & Foyer, C. H. (2018). Climate resilient crops for improving global food security and safety. *Plant, Cell and Environment, 41*(5), 877–884. https://doi.org/10.1111/pce.13207

Donazzolo, J., Ornellas, T. S., Bizzocchi, L., Vilperte, V., & Nodari, R. O. (2015). O armazenamento refrigerado prolonga a viabilidade de sementes de goiabeira-serrana. *Revista Brasileira de Fruticultura, 37*(3), 748–754. https://doi.org/10.1590/0100-2945-179/14

Donazzolo, J., Turra, E. L. C., Voss, L. C., Danner, M. A., Citadin, I., & Nodari, R. O. (2019). Reproductive biology and flowering of feijoa (*Acca sello*wiana (Berg) Burret) in areas of marginal occurrence. *Journal of Agricultural Science, 11*(8), 156–164. https://doi.org/10.5539/jas.v11n8p156

Duarte, O., & Paull, R. E. (2015). *Exotic fruits and nuts of the new world*. CABI Publishing.

Ducroquet, J. P. H. J., Hickel, E. R., & Nodari, R. O. (2000). *Goiabeira-serrana* (*Feijoa sellowiana* Berg). Série Frutas Nativas, 5. FUNEP.

Farias, D. P., Neri-Numa, I. A., Araújo, F. F., & Pastore, G. M. (2020). A critical review of some fruit trees from the Myrtaceae family as promising sources for food applications with functional claims. *Food Chemistry, 306*, Article 125630. https://doi.org/10.1016/j.foodchem.2019.125630

Finatto, T., Dos Santos, K. L., Steiner, N., Bizzocchi, L., Holderbaum, D. F., Ducroquet, J. P. H. J., Guerra, M. P., & Nodari, R. O. (2011). Late-acting self-incompatibility in *Acca sellowiana* (Myrtaceae). *Australian Journal of Botany, 59*(1), 53–60.

Fischer, G. (2003). Ecofisiología, crecimiento y desarrollo de la feijoa. In G. Fischer, D. Miranda, G. Cayón, & M. Mazorra (Eds.), *Cultivo, poscosecha y exportación de la Feijoa (Acca sellowiana* Berg.*)* (pp. 9–26). Produmedios.

Fischer, G., Ebert, G., & Lüdders, P. (2007). Production, seeds and carbohydrate contents of cape gooseberry (*Physalis peruviana* L.) fruits grown at two contrasting Colombian altitudes. *Journal of Applied Botany and Food Quality, 81*(1), 29–35.

Fischer, G., & Orduz-Rodríguez, J. O. (2012). Ecofisiología en frutales. In G. Fischer (Ed.), *Manual para el cultivo de frutales en el trópico* (pp. 54–72). Produmedios.

Fischer, G., Ramírez, F., & Casierra-Posada, F. (2016). Ecophysiological aspects of fruit crops in the era of climate change. A review. *Agronomía Colombiana, 34*(2), 190–199. https://doi.org/10.15446/agron.colomb.v34n2.56799

Fischer, G., Melgarejo, L. M., & Cutler, J. (2018). Pre-harvest factors that influence the quality of passion fruit: A review. *Agronomía Colombiana, 36*(3), 217–226. https://doi.org/10.15446/agron.colomb.v36n3.71751

Fischer, G., & Melgarejo, L. M. (2020). The ecophysiology of cape gooseberry (*Physalis peruviana* L.) - an Andean fruit crop. A review. *Revista Colombiana de Ciencias Hortícolas, 14*(1), 76–89. https://doi.org/10.17584/rcch.2020v14i1.10893

Fischer, G., & Melgarejo, L. M. (2021). Ecophysiological aspects of guava (*Psidium guajava* L.). A review. *Revista Colombiana de Ciencias Hortícolas, 15*(2), Article e12355. https://doi.org/10.17584/rcch.2021v15i2.12355

Fischer, G., Parra-Coronado, A., & Balaguera-López, H. E. (2020). Aspectos del cultivo y de la fisiología de la feijoa (*Acca sellowiana* [Berg] Burret). Una revisión. *Ciencia y Agricultura, 17*(3), 11–24. https://doi.org/10.19053/01228420.v.17.n3._2020.11386

Galvis, J. A., Hernández, M. S., & Fischer, G. (1999). Transpiración de la feijoa (*Acca sellowiana* Burrett) en la Sabana de Bogotá. *Revista Comalfi, 26*(1–3), 56–61.

García, O. J., Dueñez, E. Y., Fischer, G., Chaves, B., & Quintero, O. C. (2008). Efecto del nitrato de potasio, fosfato de potasio y ethephon en la inducción floral de la feijoa o goiabeira serrana (*Acca sellowiana* [O. Berg] Burret). *Revista Brasileira de Fruticultura, 30*(3), 577–584.

Germanà, C., & Continella, A. (2004). Physiological behaviour of some subtropical species in Mediterranean area. *Acta Horticulturae, 632*, 117–123. https://doi.org/10.17660/ActaHortic.2004.632.15

González-García, K. E., Guerra-Ramírez, D., del Ángel-Coronel, O. A., & Cruz-Castillo, J. G. (2018). Physical and chemical attributes of feijoa fruit in Veracruz, Mexico. *Revista Chapingo Serie Horticultura, 24*(1), 5–12. https://doi.org/10.5154/r.rchsh.2017.01.006

Jackson, D. I., & Looney, N. E. (1999). *Temperate and subtropical fruit production.* CABI Publishing.

Lambers, H., Chapin III, F. S., & Pons T. L. (2008). *Plant physiological ecology*. Springer. https://doi.org/10.1007/978-0-387-78341-3

Magri, A., Adiletta, G., & Petriccione, M. (2020). Evaluation of antioxidant systems and ascorbate-glutathione cycle in feijoa edible flowers at different flowering stages. *Foods, 9*(1), Article 95. https://doi.org/10.3390/foods9010095

Martínez-Vega, R. R., Fischer, G., Herrera, A., Chaves, B., & Quintero, O. C. (2008). Características físico-químicas de frutos de feijoa influenciadas por la posición en el canopi. *Revista Colombiana de Ciencias Hortícolas, 2*(1), 21–32. https://doi.org/10.17584/rcch.2008v2i1.1170

Mayorga, M., Fischer, G., Melgarejo, L. M., & Parra-Coronado, A. (2020). Growth, development and quality of *Passiflora tripartita* var. *mollissim*a fruits under two environmental tropical conditions. *Journal of Applied Botany and Food Quality, 93*(1), 66–75. https://doi.org/10.5073/JABFQ.2020.093.009

Mitra, S. K. (2010). Important *Myrtaceae* fruit crops. *Acta Horticulturae,* (849), 33–38. https://doi.org/10.17660/ActaHortic.2010.849.2

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science, 11*(1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002

Morley-Bunker, M. (1999). Feijoas. In D. I. Jackson, & N. E. Looney (Eds.), *Temperate and subtropical fruit production* (2nd ed., pp. 267–269). CABI Publishing.

Moretto, S. P., Nodari, E. S., & Nodari, R. O. (2014). A introdução e os usos da feijoa ou goiabeira serrana (*Acca sellowiana*): A perspectiva da história ambiental. *Fronteiras Journal of Social, Technological and Environmental Science, 3*(2), 67–79. https://doi.org/10.21664/2238-8869.2014v3i2.p67-79

Naizaque, J., García, G., Fischer, G., & Melgarejo, L. M. (2014). Relación entre la densidad estomática, transpiración y las condiciones ambientales en feijoa (*Acca sellowiana* [O. Berg] Burret). *Revista U.D.C.A Actualidad y Divulgación Científica, 17*(1), 115–121. https://doi.org/10.31910/rudca.v17.n1.2014.946

Omarova, Z., Platonova, N., Belous, O., & Omarov, M. (2020). Evaluation of the physiological state of feijoa (*Feijoa sellowiana* Berg) in subtropical Russia. *Potravinarstvo Slovak Journal of Food Sciences, 14*, 286–291. https://doi.org/10.5219/1290

Ouafaa, A. F., Maryama, E., Abakar, A. H. A., & Mohamed, B. (2019). Chemical composition of isoflavones compounds and antioxidants from *Feijoa sellowiana* leaves. *GSC Biological and Pharmaceutical Sciences, 9*(1), 120–124. https://doi.org/10.30574/gscbps.2019.9.1.0157

Pachón, G., & Quintero, O. (1992). La feijoa (*Feijoa sellowiana* Berg.) fruta promisoria para Colombia. *Acta Horticulturae, 310*, 239–248. https://doi.org/10.17660/ActaHortic.1992.310.29

Parra-Coronado, A., & Fischer, G. (2013). Maduración y comportamiento poscosecha de la feijoa (*Acca sellowiana* (O. Berg) Burret). Una revisión. *Revista Colombiana de Ciencias Hortícolas, 7*(1), 98–110. https://doi.org/10.17584/rcch.2013v7i1.2039

Parra-Coronado, A., Fischer, G., & Camacho-Tamayo, J. H. (2015a). Development and quality of pineapple guava fruit in two locations with different altitudes in Cundinamarca, Colombia. *Bragantia, 74*(3), 359–366. https://doi.org/10.1590/1678-4499.0459

Parra-Coronado, A., Fischer, G., & Chaves-Cordoba, B. (2015b). Tiempo térmico para estados fenológicos reproductivos de la feijoa (*Acca sellowiana* (O. Berg) Burret). *Acta Biológica Colombiana, 20*(1), 167–177. https://doi.org/10.15446/abc.v20n1.43390

Parra-Coronado, A., Fischer, G., & Camacho-Tamayo, J. H. (2016). Growth model of the pineapple guava fruit as a function of thermal time and tropical altitude. *Ingeniería e Investigación, 36*(3), 6–14. https://doi.org/10.15446/ing.investig.v36n3.52336

Parra-Coronado, A., Fischer, G., & Camacho-Tamayo, J. H. (2019). Influencia de las condiciones climáticas de cultivo en la calidad en cosecha y en el comportamiento postcosecha de frutos de Feijoa. *Tecnología en Marcha, 32*, 86–92. https://doi.org/10.18845/tm.v32i7.4264

Peña-Baracaldo, F. J., & Cabezas-Gutiérrez, M. (2014). Aspectos ecofisiológicos de la feijoa (*Acca sellowiana* Berg) bajo condiciones de riego y déficit hídrico. *Revista U.D.C.A Actualidad y Divulgación Científica, 17*(2), 381–390. https://doi.org/10.31910/rudca.v17.n2.2014.241

Perea-Dallos, M., Fischer, G., & Miranda-Lasprilla, D. (2010). Feijoa. *Acca sellowiana* Berg. In M. Perea-Dallos, L. P. Matallana-Ramírez, & A. Tirado-Perea (Eds.), *Biotecnología aplicada al mejoramiento de los cultivos de frutas tropicales* (pp. 330–349). Editorial Universidad Nacional de Colombia.

Phan, A. D. T., Chaliha, M., Sultanbawa, Y., & Netzel, M. E. (2019). Nutritional characteristics and antimicrobial activity of Australian grown feijoa (*Acca sellowiana*). *Foods, 8*(9), Article 376. https://doi.org/10.3390/foods8090376

Phan, A. D. T., Chaliha, M., Bicknel, R., Sultanbawa, Y., & Netzel, M. E. (2020). Nutritional characteristics of Australian grown feijoa (*Acca sellowiana*) and its antimicrobial activity. *Proceedings, 36*(1), Article 100. https://doi.org/10.3390/proceedings2019036100

Puppo, M., Rivas, M., Franco, J., & Barbieri, R. L. (2014). Propuesta de descriptores para *Acca sellowiana* (Berg.) Burret. *Revista Brasileira de Fruticultura, 36*(4), 957–970. https://doi.org/10.1590/0100-2945-393/13

Quintero-Castillo, O. (2003). Selección de cultivares, manejo del cultivo y regulación de cosechas de feijoa. In G. Fischer, D. Miranda, G. Cayón, & M. Mazorra (Eds.), *Cultivo, poscosecha y exportación de la Feijoa* (*Acca sellowiana* Berg.) (pp. 49–71). Produmedios.

Quintero, O. C. (2012). Feijoa (*Acca sellowiana* Berg). In Fischer, G. (Ed.), *Manual para el cultivo de frutales en el trópico* (pp. 443–473). Produmedios.

Quintero-Monroy, O. C. (2014, April 8-10). *La feijoa en Colombia* [Conference presentation]. VI Encontro sobre Pequenas Frutas e Frutas Nativas do Mercosul, Pelotas, RS, Brazil.

Ramírez, F., & Kallarackal, J. (2015). *Responses of fruit trees to global climate change*. Springer Briefs in Plant Science, Springer International Publishing. https://doi.org/10.1007/978-3-319-14200-5

Ramírez, F., & Kallarackal, J. (2017). Feijoa [*Acca sellowiana* (O. Berg) Burret] pollination: A review. *Scientia Horticulturae, 226*, 333–341. https://doi.org/10.1016/j.scienta.2017.08.054

Ramírez, F., & Kallarackal, J. (2018a). *Tree pollination under global climate change*. Springer Briefs in Agriculture, Springer International Publishing. https://doi.org/10.1007/978-3-319-73969-4

Ramírez, F., & Kallarackal, J. (2018b). Phenological growth stages of feijoa [*Acca sellowiana* (O. Berg) Burret] according to the BBCH scale under tropical Andean conditions. *Scientia Horticulturae, 232*, 184–190. https://doi.org/10.1016/j.scienta.2017.12.059

Rom, C. R. (1996). Environmental factors regulating growth: light, temperature, water and nutrition. In K. M. Maib, P. K. Andrews, G. A. Lang, & K. Mullinix, (Eds.), *Tree fruit physiology: growth and development - A comprehensive manual for regulating deciduous tree fruit growth and development* (pp. 11–30). Washington State Fruit Commission and Good Fruit Growers.

Sachet, M. R., Citadin, I., Guerrezi, M. T., Pertille, R. H., Donazzolo, J., & Nodari, R. O. (2019). Non-destructive measurement of leaf area and leaf pigments in feijoa trees. *Revista Brasileira de Engenharia Agrícola e Ambiental, 23*(1), 16–20. https://doi.org/10.1590/1807-1929/agriambi.v23n1p16-20

Salazar, M. R., Jones, J. W., Chaves, B., Cooman, A., & Fischer, G. (2008). Base temperature and simulation model for nodes appearance in cape gooseberry (*Physalis peruviana* L.). *Revista Brasileira de Fruticultura, 30*(4), 862–867.

Sánchez-Mora, F. D., Saifert, L., Ciotta, M. N., Ribeiro, H. N., Petry, V. S., Rojas-Molina, A. M., Lopes, M. E., Lombardi, G. G., Santos, K. L. D, Ducroquet, J. P. H. J., & Nodari, R. O. (2019). Characterization of phenotypic diversity of feijoa fruits of germplasm accessions in Brazil. *Agrosystems, Geosciences and Environment, 2*(1), Article 190005. https://doi.org/10.2134/age2019.01.0005

Schotsmans, W. C., East, A., Thorp, G., & Woolf, A. B. (2011). Feijoa (*Acca sellowiana* [Berg] Burret). In E. M. Yahia (Ed.), *Postharvest biology and technology of tropical and subtropical fruits. Volume 3: Cocona to mango* (pp. 115–133). Woodhead Publishing.

Sharpe, R. H., Sherman, W. B., & Miller, E. P. (1993). Feijoa, history and improvement. *Selected Proceedings of the Florida State Horticultural Society, 106*, 134–139.

Stanley, J., & Warrington, J. (1984). Seasonal frost tolerance of feijoa (*Feijoa sellowiana*). *New Zealand Journal of Experimental Agriculture, 12*(4), 315–317.

Stewart, A. M., & Craig, J. L. (1987) Factors affecting pollinator effectiveness in *Feijoa sellowiana*. *New Zealand Journal of Crop and Horticultural Science, 17*, 145–154. https://doi.org/10.1080/01140671.1989.10428023

Tocornal, G. (1988). La feijoa. In Frutales no tradicionales: Kaki, feijoa, níspero, zarzaparrilla. *Publicaciones Misceláneas Agrícola No. 20* (pp. 125–153). Universidad de Chile, Facultad de Ciencias Agrarias y Forestales.

Thorp, G. (2008). Feijoa *Acca sellowiana* (Berg) Burret, Myrtaceae. In Janick, J., & Paull, R. E. (Eds.), *Encyclopedia of fruit and nuts* (pp. 526–533). CAB International.

Ubeda, C., Hornedo-Ortega, R., Cerezo, A. B., García-Parrilla, M. C., & Troncoso, A. M. (2020). Chemical hazards in grapes and wine, climate change and challenges to face. *Food Chemistry, 314*, Article 126222. https://doi.org/10.1016/j.foodchem.2020.126222

Zhu, F. (2018). Chemical and biological properties of feijoa (*Acca sellowiana*). *Trends in Food Science and Technology, 81*, 121–131. https://doi.org/10.1016/j.tifs.2018.09.008

Study of the effects of glyphosate application on Collembola populations under controlled conditions

Estudio de los efectos de la aplicación de glifosato sobre poblaciones de Colémbolos en condiciones controladas

Felipe Torres-Moya[1] and Mónica Dotor-Robayo[1]*

ABSTRACT

Glyphosate is the most widely used herbicide worldwide. However, the effects of this molecule on non-target populations are still a subject of study. The objective of this research was to determine the effect of the application of different glyphosate doses on variation in collembolan (springtail) populations. To accomplish this goal, samples of organic substrate that contained different collembolan populations were collected. Samples were taken to the laboratory and acclimatized for 48 h. Glyphosate C^{14} was then applied to the samples in doses equivalent to 0 L ha^{-1}, 2 L ha^{-1}, and 4 L ha^{-1} under a completely randomized experimental design with three treatments and five replicates. Population counts were performed by implementing the flotation method at 0, 4, 7 and 11 d after application (DAA). We found that individuals were distributed in the families Isotomidae and Entomobryidae and divided into species of the genus *Proisotoma* (Börner), *Lepidocyrtus* (Bourlet) and *Seira* (Lubbock). A decrease in the number of arthropods between 40% and 60% was reported for the treatments with herbicide application at 4 and 7 DAA, showing a drop in the size of the community in those treatments in which the herbicide was applied compared to the control. However, no differences were observed between herbicide doses. Additionally, the presence of glyphosate C^{14} was demonstrated in dead individuals. This confirms a possible effect of the herbicide on some biological systems that led to a decrease in the size of the population.

Key words: bioindicator, ecotoxicology, herbicide, springtails.

RESUMEN

El glifosato es el herbicida más utilizado a nivel mundial. Sin embargo, los efectos de esta molécula sobre poblaciones no objetivo aún es tema de estudio. La presente investigación tuvo como objetivo determinar el efecto de la aplicación de diferentes dosis de glifosato sobre la variación de las poblaciones de colémbolos. Para esto, se recolectaron muestras de un sustrato orgánico que contenía diferentes poblaciones de colémbolos. Las muestras se llevaron al laboratorio y se aclimataron durante 48 h. Después se les aplicó glifosato C^{14} en dosis equivalentes a 0 L ha^{-1}, 2 L ha^{-1}, y 4 L ha^{-1} bajo un diseño experimental completamente al azar, con tres tratamientos y cinco repeticiones. Se realizaron conteos poblacionales implementando el método de flotación a los 0, 4, 7 y 11 d después de aplicación (DDA). Se encontró una distribución de individuos en las familias Isotomidae y Entomobryidae, divididos en especies de los géneros *Proisotoma* (Börner), *Lepidocyrtus* (Bourlet) y *Seira* (Lubbock). Se reportó una disminución entre el 40% y 60% en el número de artrópodos para los tratamientos donde se utilizó el herbicida a los 4 y 7 DDA, mostrando una disminución del tamaño de la comunidad en aquellos tratamientos en los cuales se aplicó el herbicida en relación con el testigo. Sin embargo, no se observaron diferencias entre las dosis de herbicida. Adicionalmente se demostró la presencia de glifosato C^{14} en los individuos muertos; esto confirma un posible efecto del herbicida sobre algunos sistemas biológicos que llevo a una disminución del tamaño de la población.

Palabras clave: bioindicador, ecotoxicología, herbicida, colémbolos.

Introduction

The use of herbicides in agricultural systems can have effects on non-target organisms (Santos *et al*., 2010), affecting certain edaphic populations. This probably implies an alteration of the diversity, structure, and function of ecosystems (Casabé *et al*., 2007). Lins *et al*. (2007) suggest that undisturbed ecosystems have greater macro- and mesofaunal biodiversity compared to agricultural systems that have been subjected to monocultural practices. This can be observed in that portion of the mesofauna that is in greater abundance, where mites and collembolans are found (Rusek, 1998; Johnston, 2000; Gómez-Anaya *et al*., 2010). The Collembola (Hexapoda:Collembola), commonly known as springtails, are hexapods of sizes between 250 μm and 10 mm. They are wingless, have ametabolous development and entognathous mouthparts. Springtails are considered a very important functional unit among the soil's

Received for publication: 9 March, 2020. Accepted for publication: 18 November, 2020. Doi: 10.15446/agron.colomb.v38n3.85626

[1] Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogota (Colombia).
* Corresponding author: mydotorr@unal.edu.co

mesofauna since they contribute to the decomposition of the soil organic phase through the mechanical disaggregation of plant materials (Lins *et al*., 2007). They also promote nutrient mineralization (Al-Assiuty & Khalil, 1996) and the formation and maintenance of the soil structure (Lins *et al*., 2007), having a direct influence on soil formation and fertility and on the levels of energy production of an ecosystem (Gómez-Anaya *et al*., 2010).

Palacios-Vargas and Castaño (2014) state that this group is of interest as an ecological indicator of the conservation or alteration of a given environment.

Several methods have been proposed for determining the reasons behind the presence or absence of this species in specific habitats. One of these methods seeks to explain the effect of changes in land use and diverse agricultural practices on collembolan populations (Palacios-Vargas & Castaño, 2014). This method compares different species in altered agricultural habitats to those in one or more unaffected environments in order to analyze the effect of agricultural practices (Ponge *et al*., 2003). In addition, monitoring springtail populations on site before and after an alteration (such as the application of fertilizers, amendments, pesticides, or crop planting, etc.) can also serve this purpose (Palacios-Vargas & Castaño, 2014). All these practices can be performed to detect changes in the structure of communities and predict the effects of a habitat's alterations. In agriculture, it is possible to use edaphic entomofauna as a bioindicator (Pereira *et al*., 2018). In this context, springtails stand out since they have the capacity to colonize practically any agricultural environment, are easy to breed in the laboratory, have a short life cycle and, therefore, can respond quickly to environmental changes. All these characteristics make them a group of great importance as a bioindicator of environmental quality in edaphic systems (Palacios-Vargas & Castaño, 2014).

Ecotoxicological studies in springtails usually use one or several species onto which the toxic substance is sprayed on the substrate or the base diet. The model species *Folsomia candida* (Willem, 1902) is frequently used to perform standardized tests of acute toxicity by lethality and chronic toxicity of reproduction (Hopkin, 2002; Santos *et al.,* 2012). These tests can be complemented with an avoidance test, in which the arthropod's migration into a contaminated medium is analyzed. All these tests offer advantages such as greater speed, economy, sensitivity, and environmental reliability since springtails can flee from the sources of pollution in real-life scenarios (Palacios-Vargas & Castaño, 2014).

Glyphosate is the most popular herbicide used around the world. It is an organic compound formed by a fraction of glycine and an amino phosphate radical that can be found in acid form or be used commercially as an isopropylamine salt of N-(phosphonomethyl) glycine. It behaves as a non-selective, systemic herbicide of foliar action (Duke & Powles, 2008). In plants, it acts on the shikimic acid pathway by competitive inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Niemeyer *et al*., 2018). The lack of control in catalysis due to the enzyme's inhibition reduces the biosynthesis of amino acids and compounds such as tetrahydrofolate, ubiquinone, and vitamin K (Salazar-López & Madrid, 2011). Although glyphosate is applied foliarly, a great amount of this herbicide reaches the soil where it is strongly adsorbed by clay minerals and the organic fraction. There, it can be degraded by edaphic microorganisms into aminomethylphosphonic acid (AMPA) and sarcosine, depending on the soil type, nutrient concentration, pH, temperature and humidity. Nevertheless, this herbicide can be a residue for beneficial soil invertebrates (Niemeyer *et al.,* 2018). Although the metabolic pathway in which glyphosate acts has not been reported in animal cells, there are multiple reports of harmful effects on non-target organisms such as insects (Schneider *et al*., 2009; Benamú *et al*., 2010; Santos *et al*., 2010; Santos *et al*., 2012; Al-Daikh *et al*., 2016). As glyphosate can affect different arthropods, we formulate the hypothesis that this herbicide may have a negative impact on the population structure of springtails.

The objective of this research was to determine the effect of two different glyphosate doses on the changes in collembolan populations in an organic substrate under laboratory conditions. Changes in the abundance and diversity of these beneficial arthropods have been identified as a contribution to an understanding of the agricultural system as a whole.

Materials and methods

The study was performed in two phases: a field phase related to the procurement of the living material and a laboratory phase in which the test was performed.

Field collected and samples preparation

In order to obtain a considerable number of springtails for the experiment, organic substrate was collected from the composter at Universidad Nacional de Colombia, Bogota campus (4°38'10.5" N, 74°05'22.5" W). The soil contained enough hexapods for taking samples to expose them to different treatments. Samples were collected in pitfall traps and supplemented by taking the organic phase located between 0 and the first 10 cm of the pit's depth.

A plastic container with dimensions 27.2 cm x 21 cm x 14.5 cm was filled to 60% of its capacity with Pindstrup® plus Orange substrate (blonde peat with low nutrient content): (23 g Mg Om^{-3}, 240 g K_2Om^{-3}, 50 g $NH_4^+m^{-3}$, 70 g $NO_3^-m^{-3}$, pH 6, and electrical conductivity 0.8 dS m^{-1}). The remaining 30% was filled with the different collected samples, and an empty space of 10% was left for air circulation. Subsequently, the peat and organic matter with springtails were mixed and homogenized to obtain a similar number of samples in each treatment. Collembolans were bred until there was a population greater than 1000 individuals m^{-2}. These were observed under a VanGuard stereo microscope (1122SP, VEE GEE Scientific, Vernon Hills, IL, USA) taking samples of the substrate of a known area. The peat was moistened with water and sprayed with an atomizer to increase the relative humidity. The sample was left in a drying chamber at 22°C ± 2 to allow springtails to acclimatize to the new conditions before applying the treatments.

Experimental procedure

The sample was divided into 15 Fido jars of 125 ml, guaranteeing homogeneous experimental units (EU) with 80 g of the substrate added with the help of a digital scale. Different treatments were applied in these jars. Population counts were made 1 d before application (DBA), the day of application (day 0), and at 4, 7, and 11 d after application (DAA), by taking 2.5 g of substrate sample with a closed base plastic cylinder with 1.5 cm radius × 1.5 cm height for each count. Subsequently, population densities were projected in m^2. Counting was carried out by placing the samples from the plastic cylinder into another plastic container of greater volume. Subsequentially, 10 ml of water was added to bring all the arthropods to the surface, using the flotation technique for sampling and collection (Álvarez *et al*., 2001). Live individuals were collected for counting with a fine tip brush. These individuals were returned to the substrate to avoid a population decrease in the following sample. Dead individuals were collected in Eppendorf tubes to subsequently quantify the possible amount of herbicide present.

The amount of herbicide was determined in order to establish the interaction between glyphosate and the arthropods, based on the collection of dead individuals. Dead collembolans were processed in biological oxidizer OX600 and taken for quantification performed in a Tri-carb liquid scintillation counter (2910TR, PerkinElmer, Waltham, MA, USA), adding aliquots of the analyte to 5 ml vials containing a liquid scintillation cocktail.

Data analysis

To run the experiment two treatments with radio labeled herbicide + one control treatment were established using a completely randomized statistical design (CRSD). Each treatment had five replicates for a total of 15 EU. The treatments were as follows: T0 - absolute control without glyphosate application, adding 10 ml of water in the same amount as in the other treatments; T1 - treatment with glyphosate application (commercial formulation Roundup®) at a rate of 2 L ha^{-1} (1.76 µl); T2 - treatment with glyphosate application (Roundup®) at a rate of 4 L ha^{-1} (3.52 µl). For the treatment with glyphosate, a solution of the commercial glyphosate formulation Roundup® and 0.2671 µl Ci of C^{14} glyphosate was used. This solution was applied with a micropipette dividing the total area of the container into five equal parts. The data obtained were analyzed in R® 3.5, with tests of analysis of variance and Tukey's comparison of means (α = 0.05). The assumption of normality was confirmed with the Shapiro-Wilk test (α = 0.05).

Mounting process and taxonomic Identification

For taxonomic identification, individuals were randomly collected from three samples taken from the original container with peat and organic matter using the previously described flotation method. Samples were processed using a stereomicroscope, and then transferred to plastic test tubes containing 70% alcohol. A total of 30 slides were examined to identify each specimen to the genus level. The methodology proposed by Palacios-Vargas and Mejía (2007) was followed for slides that consisted of transferring the arthropods to 10% KOH on a concave slide for two minutes. Subsequently, the specimens were placed in lactophenol that was heated with a lighter until steam was released. Hoyer's solution was used as a mounting medium, and each slide was covered and placed on a griddle plate for drying at 70°C for a week. The mounts were covered and marked with a label with the geographical coordinates, collecting method, and collector's name.

Taxonomic keys were used to identify individuals to genus level (Bellinger *et al*., 1996-2019) using a Nikon E600 phase contrast optical microscope (Nikon, Japan). A photographic record was performed to support the results, using Image Pro Insight® (Media Cybernetics, Inc. Rockville, USA).

Results

After counting the population, there was a greater abundance of collembolans of the family Isotomidae with 283 individuals corresponding to 86% of the total number where the genus *Proisotoma* was found. The family Entomobryidae contributed to the remaining 14% with 47 individuals divided into the genus *Seira* and *Lepidocyrtus,* in a 6:1 ratio (Fig. 1). So, considering a reference measurement such as m^2, there were up to 10,500 individuals m^{-2}.

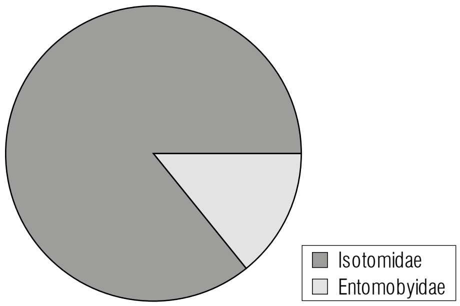


FIGURE 1. Percentage of individuals per family with percentage distribution. Values correspond to the total individuals found in the four samples.

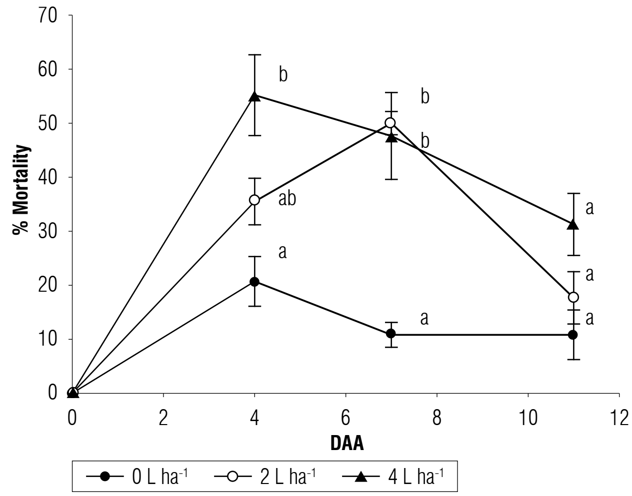


FIGURE 2. Mortality percentages of springtails with the different treatments. Each value on the X axis represents the sample number starting from the first sample (0 days after application (DAA)) in which the treatments were applied. Letters indicate significant differences between treatments over time. Means denoted by the same letter do not significantly differ at $\alpha = 0.05$ according to the Tukey's test.

Exposure to the herbicide

Population counts were performed at 0, 4, 7 and 11 DAA to measure the effect of the herbicidal application on the collembolan populations. In the first sample, populations of all treatments were kept at similar levels without showing significant differences and with values between 8000 and 9000 individuals m^{-2}. A significant population decrease was observed in the treatments with glyphosate at 4 and 7 DAA, showing values between 5000 and 6000 individuals m^{-2}, whereas the control exceeded 10,000 individuals m^{-2}. These values were reflected in mortality rates of 40% and 57.4% for treatments with 2 L ha^{-1} and 4 L ha^{-1} at 4 DAA, and 60% and 44.6% for treatments with 2 L ha^{-1} and 4 L ha^{-1} at 7 DAA (Fig. 2). No statistically significant differences were found between treatments with herbicide application.

According to Figure 2, a representative change on the population structure was observed compared to these values of the control treatment, especially at 4 and 7 DAA. A negative effect of the application of the herbicide on the mortality percentage at 4 DAA was observed for the treatment with 4 L ha^{-1}, decreasing with time. The treatment with 2 L ha^{-1} showed a slower effect and increased progressively with time until 7 DAA. At 11 DAA there were no significant differences between treatments with a low mortality percentage that did not exceed 23%.

With the treatment of glyphosate application at an equivalent dose of 2 L ha^{-1}, we observed a reduction in the number of individuals throughout the different samples (Figs. 2-3). A considerable decrease in the number of living springtails and an increase in the number of dead individuals was evident at 4 and 7 DAA compared to the base population at 0 DAA (Fig. 3). However, the last sample date (11 DAA) showed a different behavior compared to 7 and 4 DAA, since more live arthropods than dead ones were found. In addition, an increase in the number of live individuals was highlighted compared to 7 and 4 DAA, considering that the mortality percentage was lower at this sampling date (Fig. 2).

Figures 2 and 3 allow the variation in the number of individuals in the treatment with glyphosate to be apparent at a rate of 4 L ha^{-1}. There was a reduction in the number of arthropods throughout the different samples. Although this treatment showed the highest initial average of living individuals, it displayed a significative decrease compared to the control at 4 DAA. In addition, this sampling date was the only one with a greater number of dead individuals than live ones. The numbers of live springtails at 7 and 11 DAA were higher, whereas the number of dead arthropods decreased on those days, showing a possible trend towards a population recovery.

Figure 4 shows the relative values accumulated throughout the four samples. The herbicide application caused a decrease in the population size and indicated that exposure to the herbicide generated a moderate population detriment that is not exclusively quantifiable with dead arthropods.

With the result of the quantification of radiolabeled glyphosate present in the dead springtails collected in the different treatments, we showed that an exposure to the herbicide

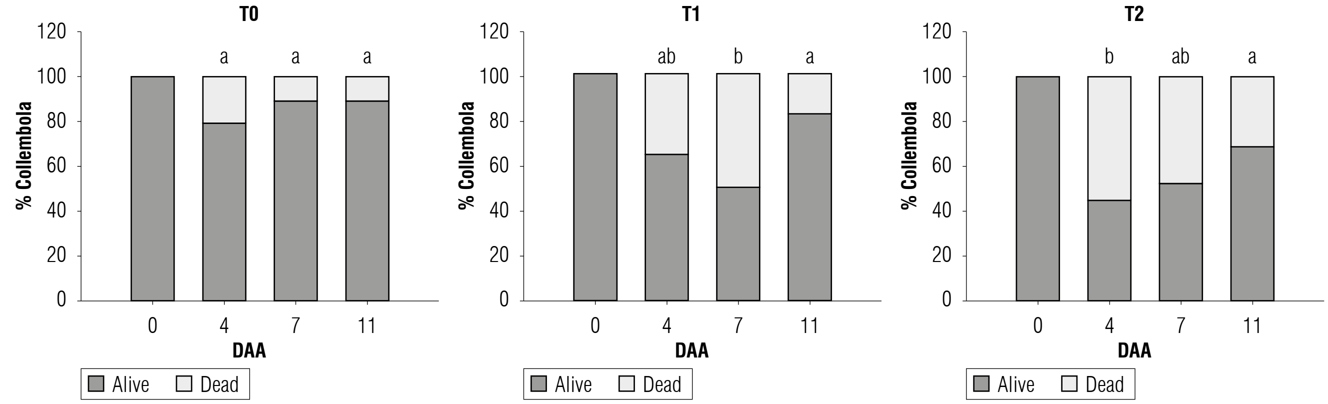


FIGURE 3. Relative values of the collembolan population changes with the different treatments T0: 0 L ha^{-1}, T1: 2 L ha^{-1}; T2: 4 L ha^{-1}. Each series of bars on the X-axis represents the sample number starting from the first sampling (0 days after application (DAA)) in which the treatments were applied. Letters indicate significant differences between treatments over time. The means denoted by the same letter do not significantly differ at $\alpha = 0.05$ according to the Tukey's test.

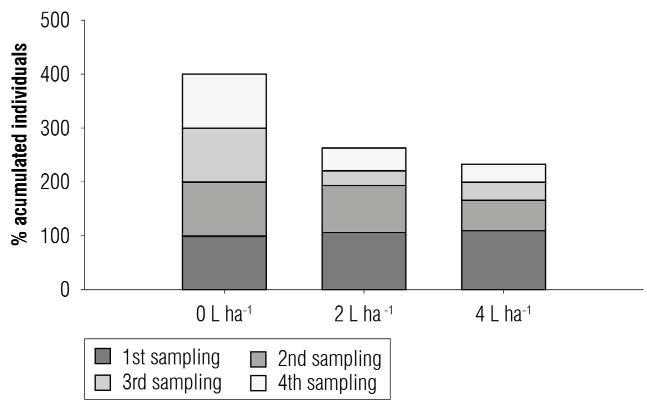


FIGURE 4. Cumulative relative values of the springtail population size in the different treatments over time.

close to 0.0059% and 0 .0071% for 2 L ha^{-1} and 4 L ha^{-1} of the total applied dose (Tab. 1) has a possible direct effect on the decrease of the springtail population.

TABLE 1. C^{14} glyphosate (Roundup®) quantified in dead arthropods. The reported concentration indicates the total herbicide quantified in dead springtails collected at the end of the experiment.

	Herbicide dose	
	4 L ha^{1}	2 L ha^{1}
C^{14} glyphosate (μCi)	0.2671	0.2671
Applied glyphosate (μl)	3.52	1.76
Total glyphosate quantified in dead springtails (μl)	2.0776E-06	1.2465E-06
% of glyphosate quantified in dead springtails	0.00590219	0.00708263

Discussion

Springtails along with mites are common organisms found in compost and usually represent more than 90% of the arthropod cluster (Palacios-Vargas & Mejía, 2007). Their abundance is related to the composting and decomposition processes of the organic phase through mechanical-enzymatic fractionation and disintegration (Rusek, 1998), nutrient mineralization (Al-Assiuty & Khalil, 1996), the formation of humic substances (Rusek, 1998; Arbea & Blasco-Zumeta, 2001), and the stimulation of fungal and bacterial activity (Robles *et al*., 2012). For this reason, it is not uncommon to find thousands of springtails in the relatively small area of m^2. This behavior can be seen in the data about springtails per m^2 that was around 10,000 individuals on day 0 before any application. Similar values were found in diversity samplings in composters by Palacios-Vargas and Mejía (2007) and Robles *et al.* (2012).

The great success of springtails as colonizers of this environment is due to the compost being a medium rich in resources that allow their reproductive success. It is a medium that allows the establishment of bacteria and fungus from which springtails can feed (Castaño-Meneses *et al*., 2004). Also, it is a favorable habitat for the development of the biological and physiological processes that maintain the average temperatures above 20°C and the high humidity. These variables were reflected in high populations before the application of treatments on 0 DAA.

Figure 1 shows that only specimens of the families Isotomidae and Entomobryidae were found in percentages of 86% and 14%, respectively. However, this result does not represent the totality of possible families and genera that may exist at the sampling site. These results agree with Cutz-Pool (2008) and Palacios-Vargas and Castaño (2014), who showed the dominance of three families in composters in Mexico. Two of those families were those found in this study. In Colombia, Arango and Macías (2004) identified only the family Entomobryidae in composters.

Regarding population changes in Collembola, the results agree with field and laboratory tests in which this herbicide was used on springtail populations or species (Santos *et al*., 2010; Reimche, 2014; Al-Daikh *et al*., 2016; Mohammed *et al*., 2017; Vinod & Sanal-Kumar, 2017; Pereira *et al*., 2018) and other arthropods such as isopods, Neoptera, Coleoptera and spiders (Niemeyer *et al*., 2006; Schneider *et al*., 2009; Benamú *et al*., 2010; Santos *et al*., 2010; Al-Daikh *et al*., 2016). The results indicate an effect on the decrease of the population size with treatments of glyphosate (Figs. 2-4). This effect is confirmed by the presence of the herbicide in dead arthropods demonstrated by radiolabeled quantification (Tab. 1). It is important to clarify that currently it is not clear how the herbicide could directly affect these populations, since the herbicidal site of action does not exist in these arthropods, and it is exclusive to autotrophic organisms (Niemeyer *et al*., 2018; Pereira *et al*., 2018). However, both the metabolism of the herbicide in animals and the compounds accompanying the molecule's formulation must be taken into account, since there are often unknown inert substances that act as surfactants and can interact with arthropods (De Aguiar *et al*., 2016; Niemeyer *et al*., 2018).

In the case of herbicides, it is common to find that methyl, ethyl, and thiocyanate groups can negatively influence the functioning of some biological systems (Vinod & Sanal-Kumar, 2017). Therefore, the herbicide or the accompanying molecules may have suffered this type of degradation, and their derivatives may have become toxic to the springtails, with a possible effect on the reproductive or respiratory systems. Regarding respiration in springtails, we know that the process is carried out through the cuticle. The cuticle in springtails is very thin, and it may allow the accumulation of the chemical groups already mentioned in the integumentary system of these animals. This might interfere in the gas exchange with the possibility of a subsequent diffusion towards the hemolymph of the arthropods (Al-Assiuty & Khalil, 1996; Mohammed *et al*., 2017; Vinod & Sanal-Kumar, 2017). There may be an indirect effect on reproduction through abnormalities of the reproductive system, especially in the ovaries. Additionally, an alteration of reproductive hormones, a decrease in the number of ovipositions, and an increase in the development time of the instars can be seen (Schneider *et al*., 2009; Benamú *et al*., 2010; Mohammed *et al*., 2017; Vinod & Sanal-Kumar, 2017; Abdullahi & Gbarakoro, 2019). Moreover, *in vitro* studies in animals classified glyphosate as an endocrine disruptor that increases the mortality of placental cells. Hypothetically a similar pathway could be occurring in these arthropods (Schneider *et al.,* 2009) which may be related to a reduction in fecundity and fertility. It is also possible that there is an effect of the herbicide on other food source organisms or endosymbiont bacteria, since some of these microorganisms have different degrees of sensitivity to the herbicide, depending on their population density (Bórtoli *et al*., 2012).

As shown in the different figures that illustrate the population variations, it is important to highlight that there were no significant changes between the doses used. This result could be explained by the possible effect of the treatments even at lower doses (Santos *et al*., 2010). These authors observed a significant decrease in the number of adults and juveniles from 900 individuals in the control to values of 100 individuals under 1, 1.5 and 2 mg glyphosate kg^{-1} of soil. Although the herbicide could affect springtail populations at a moderate level, these arthropods were never entirely eliminated. Of the total population on the last sampling date, herbicide treatments obtained 42% (for T1) and 46% (for T2) fewer individuals than the absolute control treatment and a percentage of mortality of less than 23%. Under field conditions, populations might increase their density and recover from the negative impacts of a pesticide as observed by Frampton (2000) and Vinod and Sanal-Kumar (2017). Additionally, we underline that the number of individuals increased from 7 d to 11 d for treatments where glyphosate was used. This fact may be related to the degradation of the pesticide in the substrate and the reduction of harmful radicals for springtails.

Although the role of springtails on soil health has been amply documented and recognized (Hopkin, 2002), the controversy over the effects of glyphosate on the development, physiology, behavior, biochemical and immunological alterations of non-target organisms is still ongoing; and practices to preserve beneficial arthropods in the agriculture are receiving little attention.

Aspects such as the dosage, form, and frequency of application must be considered in order to allow the recovery of the population. Thus, marginal effects on fitness and a considerable reduction in the population growth for the next generations (Schneider *et al.,* 2009) would be avoided and, thus, a negative effect on the physicochemical properties of soils. In conclusion, the results found in this study provide evidence of the negative effects of the herbicide glyphosate on these arthropods.

Conclusions

The predominant families in the trial were Isotomidae and Entomobryidae, in which the genera *Proisotoma*, *Seira* and *Lepidocyrtus* were found. This study is the first report on Collembola present in a composter in Bogota. The sensitivity of Collembola to external stress factors, such as the application of this herbicide at both doses, led to a reduction of the population size that was measurable on the first sampling date, and that tended to recover at the end of the experiment. However, there were no significant differences between treatments. The presence of radiolabeled herbicide in dead individuals is highlighted, confirming that the application of Roundup® had a detrimental effect on the springtail population. This study contributes to understanding the impact of the most widely used herbicide on non-target organisms that perform a vital function in the soil. It is necessary to carry out other trials, such as migration and field tests, to analyze the behavior of this phenomenon in an agroecosystem.

Acknowledgments

This research was supported by the Universidad Nacional de Colombia [Project number: 44515]. The authors would like to thank the Malherbology Laboratory at the Universidad Nacional de Colombia, Bogota, the UNAB entomological museum, and the International Atomic Energy Agency (IAEA) for their collaboration.

Author's contributions

MDR and FTM designed the experiments, FTM carried out the field and laboratory experiments, FTM contributed to the data analysis, MDR and FTM wrote the article. All authors reviewed the manuscript.

Literature cited

Al-Assiuty, A., & Khalil, M. (1996). Effects of the herbicide atrazine on *Entomobrya musatica* (Collembola) in field and laboratory experiments. *Applied Soil Ecology*, *4*(2), 139–146. https://doi.org/10.1016/0929-1393(96)00107-2

Abdullahi, M., & Gbarakoro, T. (2019). Effects of herbicides on soil microarthopods richness and distribution in tropical soils in Nigeria. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, *5*(4), 125–133.

Al-Daikh, E., El-Mabrouk, A., & El Roby, A. (2016). Effect of glyphosate herbicide on the behavior of soil arthropods in non-organic tomato system. *Advance in Agriculture and Biology*, *5*(1), 14–19. https://doi.org/10.15192/PSCP.AAB.2016.5.1.1419

Álvarez, T., Frampton, G., & Goulson, D. (2001). Epigeic Collembola in winter wheat under organic, integrated and conventional farm management regimes. *Agriculture, Ecosystems and Environment*, *83*(1–2), 95–110. https://doi.org/10.1016/S0167-8809(00)00195-X

Arango, G., & Macías, K. (2004). Mesofauna de los colémbolos en el compost de la Corporación Universitaria Lasallista. *Revista Lasallista de Investigación*, *1*(1), 102–104.

Arbea, J., & Blasco-Zumeta, J. (2001). Ecología de los Colémbolos (Hexapoda, Collembola) en Los Monegros (Zaragoza, España). *Boletín de la Sociedad Entomológica Aragonesa*, *28*(2001), 35–48.

Benamú, M., Schneider, M. I., & Sánchez, N. E. (2010). Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory. *Chemosphere*, *78*(7), 871–876. https://doi.org/10.1016/j.chemosphere.2009.11.027

Bellinger, P., Christiansen, K., & Janssens, F. (1996–2019). *Checklist of the Collembola of the world*. https://www.collembola.org/taxa/collembo.htm

Bórtoli, P., Verdenelli, R., Conforto, C., Vargas-Gil, S., & Meriles, J. M. (2012). Efectos del herbicida glifosato sobre la estructura y funcionamiento de comunidades microbianas de dos suelos de plantaciones de olivo. *Ecología Austral*, *22*, 33–42.

Casabé, N., Piola, L., Fuchs, J., Oneto, M., Pamparato, L., Basack, S., Giménez, R., Massaro, R., Papa, J., & Kesten, E. (2007). Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments 7*(4), 232–239. https://doi.org/10.1065/jss2007.04.224

Castaño-Meneses, G., Palacios-Vargas, J., & Cutz-Pool, L. (2004). Feeding habits of Collembola and their ecological niche. *Anales del Instituto de Biología serie Zoología*, *75*(1), 135–142.

Cutz-Pool, L. (2008). Primeros registros sobre colémbolos de composta en México. In E. G. Estrada-Venegas, A. Equihua-Martínez, J. Padilla-Ramírez, & A. Mendoza-Estrada (Eds.), *Entomología mexicana* (pp. 808–812). Sociedad Mexicana de Entomología - Colegio de Postgraduados.

De Aguiar, L., Figueira, F., Gottschalk, M. S., & da Rosa, C. (2016). Glyphosate-based herbicide exposure causes antioxidant defense responses in the fruit fly *Drosophila melanogaster*. *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology*, *185*, 94–101. https://doi.org/10.1016/j.cbpc.2016.03.006

Duke, S., & Powles, S. (2008). Glyphosate: once-in-a-century herbicide. *Pest Management Science*, *64*(4), 319–325. https://doi.org/10.1002/ps.1518

Frampton, G. (2000). Recovery responses of soil surface Collembola after spatial and temporal changes in long-term regimes of pesticide use. *Pedobiologia*, *44*(3–4), 489–501. https://doi.org/10.1078/S0031-4056(04)70066-9

Gómez-Anaya, J., Palacios-Vargas, J., & Castaño-Meneses, G. (2010). Abundancia de colémbolos (Hexápoda: Collembola) y parámetros edáficos de una selva baja caducifolia. *Revista Colombiana de Entomología*, *36*(1), 96–105.

Hopkin, S. (2002). *Biology of the springtails* (Insecta: Collembola). Oxford University Press.

Johnston, J. (2000). The contribution of microarthropods to aboveground food webs: a review and model of belowground transfer in a coniferous forest. *The American Midland Naturalist*, *143*(1), 226–238. https://doi.org/10.1674/0003-0031(2000)143[0226:TCOMTA]2.0.CO;2

Lins, V., Santos, H., & Gonçalves, M. (2007). The effect of the glyphosate, 2, 4-D, atrazine e nicosulfuron herbicides upon the edaphic collembola (Arthropoda: Ellipura) in a no tillage system. *Neotropical Entomology*, *36*(2), 261–267. https://doi.org/10.1590/S1519-566X2007000200013

Mohammed, A., Umeozor, O., & Gbarakoro, T. (2017). The effects of glyphosate and multrazine on the abundance and diversity of soil microarthropods at the University Park, University of Port-Harcourt, Nigeria. *European Journal of Experimental Biology*, *7*(1–2), 1–5. https://doi.org/10.21767/2248-9215.100002

Niemeyer, J., Santos, V., Rodrigues, J., & Da-Silva, E. (2006). Comportamento de *Cubaris murina* Brandt (Crustacea: Isopoda) em solo com glifosato: testes de fuga em laboratório. *Journal of the Brazilian Society of Ecotoxicology*, *1*(1), 13–16.

Niemeyer, J., de-Santo, F. B., Guerra, N., Filho, A. M., & Pech, T. M. (2018). Do recommended doses of glyphosate-based herbicides affect soil invertebrates? Field and laboratory screening tests to risk assessment. *Chemosphere*, *198*, 154–160. https://doi.org/10.1016/j.chemosphere.2018.01.127

Palacios-Vargas, J., & Mejía, B. (2007). *Técnicas de colecta, montaje y preservación de microartrópodos edáficos*. Universidad Nacional Autónoma de México, Facultad de Ciencias.

Palacios-Vargas, J., & Castaño, G. (2014). Los colémbolos (Arthropoda: Hexapoda) como bioindicadores. In C. A. González, A. Vallarino, J. C. Pérez, & A. M. Low Pfeng (Eds.), *Bioindicadores: guardianes de nuestro futuro ambiental* (pp. 291–307). El Colegio de la Frontera Sur (ECOSUR).

Pereira, J., Araujo, T., Rodrigues-Silva, N., Silva, A., & Picanco, M. (2018). Edaphic Entomofauna variation depending on glyphosate application in roundup ready soybean crops. *Planta Daninha*, *36*, Article e018171328. https://doi.org/10.1590/s0100-83582018360100110

Ponge, J., Gillet, S., Dubs, F., Fédoroff, E., Haese, L., Sousa, J., & Lavelle, P. (2003). Collembolan communities as bioindicators of land use intensification. *Soil Biology and Biochemistry, 35(*6), 813–826.

Reimche, G. (2014). *Herbicidas dessecantes na fauna edáfica e genotoxicidade*. [Doctoral dissertation, Universidade Federal de Santa Maria]. UFSM Repository. http://repositorio.ufsm.br/handle/1/3238

Robles, F., Salvador, I., Juárez, C., Montiel, D., Mejía, B., & Palacios-Vargas, J. (2012). Colémbolos (Hexápoda) asociados a lombricomposta de cultivo de Alfalfa (*Medicago sativa*) en la Magdalena Contreras, D.F. In A. Equihua-Martínez, E. G. Estrada-Venegas, J. A., Acuña-Soto, M. P. Chaires-Grijalva, & G. Durán Ramírez (Eds.), *Entomología mexicana* (pp. 487–491). Sociedad Mexicana de Entomología - Colegio de Postgraduados.

Rusek, J. (1998). Biodiversity of Collembola and their functional role in the ecosystem. *Biodiversity Conservation*, *7*(9), 1207–1219. https://doi.org/10.1023/A:1008887817883

Salazar-López, N., & Madrid, M. (2011). Herbicida glifosato: usos, toxicidad y regulación. *BIOtecnia*, *13*(2), 23–28.

Santos, M., Soares, A., & Loureiro, S. (2010). Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembola *Folsomia candida*. *Chemosphere*, *80*(9), 1021–1030. https://doi.org/10.1016/j.chemosphere.2010.05.031

Santos, M., Ferreira, M., Cachada, A., Duarte, A., & Sousa, J. (2012). Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of *Folsomia candida* and *Eisenia andrei. Ecotoxicology*, *21*(8), 2113–2122.

Schneider, M., Sánchez, N., Pineda, S., Chi, H., & Ronco, A. (2009). Impact of glyphosate on the development, fertility and demography of *Chrysoperla externa* (Neuroptera: Chrysopidae): ecological approach. *Chemosphere*, *76*(10), 1451–1455.

Vinod, P., & Sanal-Kumar, M. (2017). A study on the effect of two agrochemicals on the fecundity of a soil collembolan *Cryptopygus thermophilus. International Journal of Pure and Applied Researches*, *4*(1), 34–41.

Resource-use efficiency in maize production: the case of smallholder farmers in Ghana

Eficiencia en el uso de recursos en la producción de maíz: el caso de pequeños agricultores en Ghana

Frank Osei Danquah[1], He Ge[1]*, Lady Nadia Frempong[1], and Bright Asiamah Korankye[1]

ABSTRACT

This study aimed to evaluate the efficiency with which smallholder maize farmers use their input resources such as land, labor, capital, fertilizers, herbicides, pesticides, and improved seed in maize production. The study used the multi-stage sampling technique to collect cross-sectional data from 600 smallholder maize producers from the northern, Brong-Ahafo, eastern, and central regions of Ghana. This study employed the marginal value product (MVP) to marginal factor cost (MFC) ratio (MVP/MFC) of the input resources. The results revealed an increased return to scale, meaning smallholder maize farmers in the pooled sample and the studied regions should enlarge their production scale by about 3.2%, 2.2%, 7.6%, 6.8%, and 2.8%, respectively, to maximize productivity. The results also revealed that resource inputs like fertilizer, herbicides, pesticides, improved seed, and land were underutilized. Therefore, these resources need to be increased if smallholder maize farmers in the pooled sample want to be efficient in their production. Labor and capital were over-utilized and needed to be reduced to increase efficiency in the farmers' maize production.

Key words: pesticides, fertilizers, herbicides, improved seeds.

RESUMEN

El objetivo de este estudio consistió en evaluar la eficiencia con la que los pequeños agricultores de maíz utilizan sus insumos tales como la tierra, mano de obra, capital, fertilizantes, herbicidas, plaguicidas y semilla mejorada en la producción del maíz. Para ello se empleó una técnica de muestreo de multietapas con el fin de recolectar datos seccionales cruzados de 600 productores de maíz de las regiones del norte, Brong-Ahafo, oriente y centro de Ghana. Se empleó también el cociente entre el producto de valor marginal (PVM) y el factor de costo marginal (FCM) (PVM/FCM) de los recursos de ingreso. Los resultados obtenidos revelaron un aumento en el ingreso de retorno, lo que significa que los pequeños agricultores de maíz de la muestra y en las regiones mencionadas deberían aumentar su escala de producción en cerca de 3.2%, 2.2%, 7.6%, 6.8% y 2.8%, respectivamente, con el fin de maximizar la productividad. Los resultados mostraron también que los insumos tales como los fertilizantes, herbicidas, pesticidas, semillas mejoradas y la tierra fueron subutilizados. Por lo tanto, estos insumos deben incrementarse si los pequeños agricultores de maíz en la muestra quieren ser eficientes en su producción. El trabajo y el capital resultaron sobreutilizados y por lo tanto hay una necesidad de reducirlos con el fin de hacer más eficiente la producción de maíz por parte de los agricultores.

Palabras clave: pesticidas, fertilizantes, herbicidas, semillas mejoradas.

Introduction

Agricultural development is one of the most powerful tools for ending extreme poverty, boosting shared prosperity, and feeding a projected 9.7 billion people by 2050. Growth in the agricultural sector is two to four times more effective for raising incomes among the poorest compared to other sectors (World Bank, 2019). In Africa, agriculture remains the most feasible option for promoting economic growth, overcoming poverty, and improving food security. As a result, a very significant factor is needed to help sustain and increase agricultural productivity with improved agricultural production technologies and ensure good soil management. Agriculture is an important element in Ghana's economy; in the third quarter of 2019 agriculture contributed 7638.80 million Ghana cedis to the gross domestic product, an increase from the previous quarter (second quarter) that recorded 6464.36 million Ghana cedis (Ghana Statistical Service, 2018).

Maize is considered a vital food for about 1.2 billion people worldwide and is the most-produced cereal in the world. In

Received for publication: 10 February, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.85069

[1] College of Management, Sichuan Agricultural University, Chengdu (China).
* Corresponding author: hege@sicau.edu.cn

2014, over 1,022 million t of maize were produced in more than 170 countries on about 181 million ha of land (Food and Agriculture Organization of the United Nations, 2016). The top producers were the United States of America with 361 million t, China with 216 million t, Brazil with 80 million t, and Argentina and Ukraine with 33 and 28 million t, respectively (Food and Agriculture Organization of the United Nations, 2016). In Africa, maize is graded as the cereal grain of greatest economic importance with wheat and rice ranking second and third, respectively (Thobatsi, 2009). In Ghana, maize accounts for over 50% of the total cereal (that includes maize, rice, sorghum, and millet) production, making it the most important staple crop (Ministry of Food and Agriculture, 2012). It is widely cultivated and serves as a major source of food and cash income for many people in Ghana (Tachie-Obeng, *et al.*, 2010). Maize is the number one crop in terms of planted area (Ministry of Food and Agriculture, 2014). Total maize production in Ghana is carried out by about 70% of smallholder farmers (Ministry of Food and Agriculture, 2014). It is assumed that almost every household in Ghana's farming communities is directly or indirectly involved in maize or rice cultivation. Maize is used to prepare different kinds of food in Ghana; for example, porridge, *kenkey*, *banku*, and *tuo zaafi*. *Kenkey* is a traditional food mostly consumed by all the tribes and regions in Ghana. It is predominantly consumed by the Gas who live in the capital city of Accra. *Banku* is mostly eaten by the Fantes and the Ashantis, and *tuo zaafi* is the main food for the people in the northern region of Ghana.

Maize production in Ghana has increased since the introduction of the fertilizer subsidy program (FSP) in 2008 as an intervention policy to counter the high fertilizer costs in 2007. Maize production in Ghana increased from 442 thousand t in 1969 to 2,760 thousand t in 2019, growing at an average annual rate of 8.33% (World Data Atlas, 2019). Despite the comprehensive cultivation and the importance of maize in the country, production is still low due to inefficient and inappropriate use of improved agricultural technologies. Due to the significance of this crop in Ghana, through the Ministry of Food and Agriculture (MOFA), the government has promoted modern technologies in agriculture in several ways (Nyamadzawo *et al.*, 2013).

This study analyzed smallholder maize farmers' input resource-use efficiency in the study areas (i.e. northern, Brong-Ahafo, eastern, and central regions). Unlike the study by Abatania *et al.* (2012) that examined the resource and technical efficiency of one agro-ecological zone, this research covered all the agro-ecological areas of Ghana (Northern, Brong-Ahafo, Eastern and Central regions) in order to analyze the efficient use of input resources.

Materials and methods

Primary study area

The study was carried out in Ghana. Researchers collected primary data for this study from household surveys conducted in villages/communities selected from four regions (northern, Brong-Ahafo, eastern, and central regions) in Ghana from July to September 2019. These regions were purposively selected to cover all the agro-ecological zones and their maize production performance for the 2017-2018 farming seasons in Ghana.

The capital of the northern region is Tamale. In 2018, it had a population of about 3.576 million people and was ranked the fourth highest populous region in Ghana (EUROSTAT, 2018). More than 75% of the economically active population work in agricultural and agricultural-related jobs in this region. Maize, rice, sorghum, and millet, are mostly grown in the northern region.

The Brong-Ahafo region is located in the southern part of Ghana. The capital of the Brong-Ahafo region in Sunyani. The Brong-Ahafo region has a total population of 2,310,983, with an average growth rate of 2.2% compared to the national average of 2.4%. The area is ranked sixth in terms of population with 9.33% (EUROSTAT, 2018). Agriculture is the predominant activity, and many of the region's population are engaged in this sector. The main food crops are corn, yams, cassava, and some other root crops.

Koforidua is the capital of the eastern region. The eastern region has a population of about 2,633,154, and is ranked as the third most populous area of Ghana with 10.68% of the country's total population (EUROSTAT, 2018). The eastern part covers 19,323 km^2, about 8.1% of Ghana's total land area. Crops produced in this region include grains, such as wheat, corn, and barley. Additionally, field crops, such as cotton and tobacco, vegetables, fruits and nuts, and horticultural specialties, such as flowers and ornamental plants, are grown in this region.

Cape Coast is the capital of the central region. The central area has a population of about 2,201,863, and it is ranked the eighth most populous region in Ghana, recording a 8.93% of the country's total population (EUROSTAT, 2018). The main economic activity of the region is fishing and farming, and maize is the primary cereal grown here.

The central region contains many tourist attractions such as castles, forts, and beaches stretched along the region's coastline.

Sample size determination

This study used both primary and secondary data. The primary data was obtained through a cross-sectional survey conducted in Ghana's four regions (northern, Brong-Ahafo, eastern, and central regions) (Supplementary material 1). The study used the sample size formula (below) (Hashim, 2010) to determine the appropriate sample size (Eq. 1).

$$n = \frac{t^2 \times p \times q}{d^2} \tag{1}$$

where n is the sample size, t is the value for the selected alpha level of 0.025 in each tail, which is = 1.96, p is the proportion of the population engaged in maize production, q is the ratio of the people not involved in maize production, and d is the acceptable margin of error for the proportion being estimated = 0.05 (error researchers are willing to accept).

The Ghana Living Standard Survey reported that 49.1% of farmer households that cultivated staple and or cash crops were maize farmers (Ghana Statistical Service, 2018). Assuming a 95% confidence level and 5% margin of error, the sample size was calculated as follows:

$$n = \frac{1.96^2 \times 0.491 \times 0.509}{0.05^2} \tag{2}$$

The study followed Salkind and Rainwater (2003) recommendation of oversampling by 40%-60% to account for a low response rate. Therefore, this study's sample was increased by 56.2%, resulting in a sample size of 600 (Salkind & Rainwater, 2003).

Sampling technique and size

A multi-stage sampling technique was used in this study. The first was to select four regions (northern, Brong-Ahafo, eastern and central regions) in Ghana to cover all the agro-ecological zones. After that, four districts/municipalities were randomly selected from each of the four selected regions. In the next stage, three villages or communities were randomly selected from each of the four districts/municipalities. For the last step, a random selection was performed by picking every K^{th} (sampling interval) farmer in a list, where k was obtained by dividing the population of smallholder maize farmers in the village by the sample size.

Method for estimating the efficiency of resource use in maize production

For maize farmers to be efficient in their use of production resources, these resources should be used in a manner such that their marginal value product (MVP) is equal to their marginal factor cost (MFC) under perfect competition, according to Tambo and Gbemu (2010). Consequently, the resource use efficiency parameter was calculated using the ratio between the MVP of inputs and the MFC. According to Fasasi (2006) and Goni *et al.* (2007), the efficiency of resource use can be calculated as follows:

$$RE = \frac{MVP}{MFC} \tag{3}$$

where RE is the resource efficiency coefficient, MVP is the marginal value product, and MFC is the marginal factor cost of inputs.

$$MFC = P_x \tag{4}$$

where P_x is the unit price of the input, say X.

$$MVP_x = MPP_x \times P_y \tag{5}$$

where y is the mean value of output, x is the mean value of input employed in the production of a product, MPP_x is the marginal physical product of input X, and P_y is the unit price of maize output.

Taking into consideration the translog production function (Eq. 5)

$$\beta_x = \frac{\delta 1nY}{\delta 1nX} = \frac{\delta Y}{\delta X} \times \frac{X}{Y} \tag{6}$$

$$MPP_x = \frac{\delta Y}{\delta X} = \beta_x \frac{Y}{X} \tag{7}$$

where MPP_x is the marginal physical product of input X and is a measure of input X's technical efficiency, and β_x is the output elasticity of input X.

Hence,

$$MVP = \frac{\delta Y}{\delta X} \times P_y = \beta_x \frac{Y}{X} \times P_y \tag{8}$$

where Y represents the value of the output, X represents the value of the input and P_y represents the unit price of the output.

The MVP of a particular input resource is consequently computed by the product of output elasticity of that input, the ratio of mean output to mean input values, and the unit output price. The MFC of input was attained from the data composed on that input's unit price.

To decide whether or not an input is used efficiently, we used the following convention. If $r=1$, it implied the input was used efficiently, $r>1$ meant the input was underutilized, so the output would be increased if more of that input were employed. Finally, $r<1$ means that the input is over-utilized, so both the production and profit would be maximized if less input were utilized (Eze & Okorji, 2003).

Results and discussion

Socio-economic characteristics of farmers

Table 1 below shows that the pooled sample revealed that most maize farmers in the study area are males (449) representing 74.8%, compared to 151 females representing 25.2%. Male dominance in maize farming runs through all the four regions of the study. These results can be attributed to the fact that most Ghana people perceive farming to be an occupation for men and not women. This result was in agreement with the work of Sadiq *et al.* (2013), who found a dominance of male maize farmers of 67% compared to 33% of female maize farmers in the studies of profitability and production efficiency of small-scale maize production in the Niger State of Nigeria. The result also showed the average age of 46 years with a minimum age of 18 and a maximum of 79 years (Tab. 1). The majority of the farmers were between 18 and 40 years, while very few farmers were above 60. This result means most maize farmers in Ghana are young, a fact that may affect productivity. This result was consistent with the studies by Ojiako and Ogbukwa (2012) who found a mean age of 44.8 years for farmers (Tab. 1).

Most of the maize farmers had a junior high school and senior high school education. The average number of schooling for maize farmers was five years. This result can be attributed to the fact that most educated youths wish to work in offices and see farming as a job for school dropouts in Ghana. This result agrees with the studies by Oladejo and Adetunji (2012) who also found that most maize farmers in the Oyo State of Nigeria (82.3%) had received formal education. The studies revealed that many maize farmers (56.9%) did not belong to farmer associations. This result was probably obtained because maize farmers in Ghana do not see the benefits of such farmer associations or because there are no farmer groups in the study areas.

The average household size of maize farmers in Ghana was seven people (Tab.1) with a range of 1 to 37 people. These results were consistent with the study of Oladejo and Adetunji (2012), who found an average household size of 8 among maize farmers in the Oyo state of Nigeria. The average years of experience for maize farmers in the pooled sample was 14 years, meaning farmers interviewed in the study areas have spent much time in maize cultivation. The studies show that a slight majority of farmers had no access to extension with a percentage of 54.5% compared to 45.5% of farmers who had access to extension services, and an average number of visits of 3 (Tab. 1). Generally, there was poor extension contact with maize farmers, and this could affect their adoption of improved farming practices.

Our results revealed that smallholder maize farmers who were not beneficiaries of non-governmental organizations (NGOs) were slightly fewer compared to those who enjoyed some benefits: 48.2% for non-beneficiaries against 51.8% beneficiaries in Ghana. Also, our results revealed that very few maize farmers benefited from government subsidies with a percentage of 28.3% compared to 71.7% non-beneficiaries. For instance, the northern region recorded 63.9% of smallholder maize farmers enjoying government subsidies against 36.1% of the smallholder maize farmers who do not enjoy government subsidies. This result could be traced to the fact that the standard of living in the northern region of the country is low compared to other areas. For that matter, most smallholder maize farmers in this region are less able to afford to purchase improved production technologies to boost their productivity. Therefore, the government helps smallholder maize farmers in this region by subsiding enhanced production technologies. A meagre percentage recorded for maize farmers who had access (26.3%) as compared to 73.7% with no access to credit for the pooled sample. This trend runs through all the study regions. Low percentages of farmers have access to credit recording, 22.2%, 30.6%, 15.4%, and 37.2% for the northern region, Brong-Ahafo region, eastern region, and central region, respectively.

Resource use efficiency by maize farmers in Ghana

The return to scale parameters in Table 2, calculated as the sum of individual production input elasticity, showed an increase. This means that maize production in Ghana (northern, Brong-Ahafo, eastern and central regions) was a production function. The return to scale values for the pooled sample and the northern, Brong-Ahafo, eastern, and central regions were recorded as 3.268, 2.229, 7.594, 6.804, and 2.841, respectively (Tab. 2). These results suggest that smallholder maize farmers in the pooled sample (northern,

TABLE 1. Descriptive statistics of the characteristics of the interviewed maize farmers.

Variables	Pooled sample				Northern region				Brong-Ahafo region				Eastern region				Central region			
	Min	Max	M	SD	Min	Max	M	SD	Min	Max	M	SD	Min	Max	M	SD	Min	Max	M	SD
Age (years)	18	79	45.84	11.24	18	79	47.13	10.62	22	73	44.70	12.16	19	78	46.1	11.07	25	70	45.41	17.67
Education (years)	0	18	6.20	4.76	0	16	4.10	6.03	0	18	7.21	3.76	0	17	9.08	4.01	0	16	4.426	1
Experience (years)	1	54	13.68	11.81	1	54	19.41	12.34	1	48	11.8	11.27	1	45	8.81	7.45	1	50	14.73	2.01
Household size	1	37	7.14	5.32	1	37	8.92	5.76	1	29	6.67	5.18	1	22	6.90	3.81	1	18	6.08	2.01
Extension visit	0	17	2.84	4.56	0	6	1.31	1.61	0	11	2.83	2.12	0	10	1.01	2.41	0	17	6.24	0
	Freq.		**%**		**Freq.**		**%**		**Freq.**		**%**		**Freq.**		**%**		**Freq.**		**%**	
Gender																				
Male	449		74.8		109		75.7		101		70.1		131		84.0		108		69.2	
Female	151		25.2		335		24.3		43		29.9		25		16.0		48		30.8	
Total	600		100		144		100		144		100		156		100		156		100	
Member of farmer's group																				
Yes	180		30.0		62		43.1		46		31.9		25		16.0		47		30.1	
No	420		70.0		82		56.9		98		68.1		131		84.0		109		69.9	
Total	600		100		144		100		144		100		156		100		156		100	
Government subsidy																				
Yes	170		28.3		92		63.9		30		20.8		27		17.3		21		13.5	
No	430		71.7		52		36.1		114		79.2		129		82.6		135		86.5	
Total	600		100		144		100		144		100		156		100		156		100	
Beneficiary of NGOs																				
Yes	289		48.2		98		68.1		74		51.4		48		30.8		69		44.2	
No	311		51.8		46		31.9		70		48.6		108		69.2		87		55.8	
Total	600		100		144		100		144		100		156		100		156		100	
Access to credit																				
Yes	158		26.3		32		22.2		44		30.6		24		15.4		58		37.2	
No	442		73.7		112		77.8		100		69.4		132		84.6		98		62.8	
Total	600		100		144		100		144		100		156		100		156		100	

NGO_s - non-governmental organizations, Min - minimum, Max - maximum, M - mean, SD - standard deviation, Freq. - frequency.

Brong-Ahafo, eastern, and central regions) should enlarge their production scale by about 3.2%, 2.2%, 7.6%, 6.8%, and 2.8%, respectively, because of the scale elasticity recorded by them for maximizing productivity given their disposable resources (Tab. 2). The results revealed that smallholder maize farmers in Ghana could increase their maize output using more of the mentioned resources (fertilizer, pesticides, herbicides, improved seed, land, labor, and capital). This result agrees with Saura-Calixto *et al.* (2007), who report that farmers could increase their output by increasing the quantity of fertilizer, seed, labor, and cultivated land size. The increasing returns to scale finding agree with those of Uchegbu (2001) and Ajibefun (2002), even though they contradict the conclusions of Obasi (2007).

TABLE 2. Input elasticity.

Variables	Elasticity				
	Pooled sample	Northern region	Brong-Ahafo region	Eastern region	Central region
Fertilizer	0.476	0.529	0.587	0.669	0.933
Herbicides	0.178	0.436	0.745	0.660	0.019
Pesticides	0.004	0.003	0.007	0.002	0.012
Improved seed	0.724	0.019	1.251	0.162	0.921
Land	1.146	0.134	3.554	4.245	0.122
Labor	0.246	0.894	0.587	0.765	0.743
Capital	0.494	0.214	0.424	0.301	0.031
Scale of elasticity	3.268	2.229	7.594	6.804	2.841

Table 3 presents the marginal productivities that revealed that maize farmers in the pooled sample and all the regions (northern, Brong-Ahafo, eastern and central regions) used land more efficiently than other resources (fertilizer, herbicides, pesticides, improved seed, labor, capital). This result implied that if maize farmers had cultivated more land, it would have likely led to a rise in maize output by 1103 kg, 4369 kg, and 1408 kg among maize farmers in the pooled sample and the Brong-Ahafo and eastern regions, respectively (Tab. 3). Similarly, maize farmers in the northern region were more efficient in using herbicides to control weeds in their maize crops compared to the other regions. The results also revealed that maize farmers in the central region were more efficient in using labor in their maize production than the other regions. The results of the northern region suggest that if maize farmers had used more herbicides, the maize output would have increased by 157 kg (Tab. 3). The output of maize in the central region would have also increased by 40.3 kg if maize farmers had increased their use of labor in maize production (Tab. 3). The results revealed an inefficient use of capital in the pooled sample and all the four regions (northern, Brong-Ahafo, eastern, and central regions). It recorded the lowest MPP of 2.53, 0.69,1.99,1.04, and 0.34, respectively (Tab. 3). These results also corroborate the research work conducted by Saura-Calixto *et al.* (2007).

Considering the technologies available to maize farmers and the inputs and output prices, resource use efficiency would be achieved at a level where the MVP is equal to the MFC. The resource can only be used efficiently if the compared MVP and the MFC are the same. The results revealed that the ratios of the MVP to the MFC for maize farmers in the pooled sample were greater than one for the use of fertilizer, herbicides, pesticides, seeds, and land with values of 2.7, 9.5, 7.0, 18.2, and 35.6, respectively (Tab. 3). The ratios for the use of labor and capital were found to be less than one with values of 0.8 and 0.2, respectively (Tab. 3). These results imply that maize farmers in Ghana were not efficient in allocating their resources (fertilizer, herbicides, pesticides, seed, land, labor, and capital). The above means that maize farmers in Ghana underutilized fertilizer, herbicide, pesticide, seed, and land, while labor and capital were over-utilized. In general, maize output could have been increased if more underutilized inputs like fertilizer, herbicides, pesticides, seed, and land were used.

TABLE 3. Marginal value product to marginal factor cost ratio across the various regions in Ghana.

Variable	Pooled sample				Northern region				Brong-Ahafo region				Eastern region				Central region			
	MPP	MVP	MFC	R	MPP	MVP	MFC	R	MPP	MVP	MFC	R	MPP	MVP	MFC	R	MPP	MVP	MFC	R
Fertilizer	3.9	3.8	1.4	2.7	2.6	1.94	1.6	1.2	5.4	4.8	1.4	3.4	5.6	6.3	0.6	10.5	10.4	10.6	0.87	12.2
Herbicide	94.5	91.8	9.7	9.5	157	124	10.4	12.0	642	584	8.3	70	140	165	8.4	19.6	11.8	11.9	11.4	1.04
Pesticide	74.6	72.5	10.4	7.0	76	60.4	0.7	86.2	166	151	0.9	168	54.1	63.7	0.114	559	10.5	10.6	0.3	35.3
Seed	54.4	52.7	2.9	18.2	1.50	1.29	3.8	0.3	118	109	2.4	45	7.65	9.1	2.9	3.1	32	32.3	2.9	11.1
Labor	9.9	9.6	12	0.8	26.4	21	7.3	2.9	32	28.4	18.9	1.5	18.4	21.8	10.4	2.1	40.3	40.6	11.6	3.5
Land	1103	1078	30.3	35.6	117	92.1	14.2	6.5	4369	3975	47.3	84	1408	1669	28.27	59.0	21.3	21.5	32.4	0.66
Capital	2.53	2.46	12.8	0.2	0.69	0.57	0.05	13.8	1.99	1.9	12.9	0.1	1.04	1.06	13.39	0.08	0.34	0.35	13.7	0.02

MPP - marginal physical product, MVP - marginal value product, MFC - marginal factor cost, R - efficiency coefficient.

In contrast, labor and capital could have been reduced. This result agrees with the work of Chiedozie *et al.* (2010), who report similar results in their study that fertilizer, land, and pesticides were underutilized.

Table 4 shows MVPs adjustments for optimal resource utility (% divergence) by maize farmers in Ghana. The result from the pooled sample indicates that for resources to be efficiently utilized, there should be an increase of more than 70.4%, 89.7%, 86.3%, 94.6%, and 97.4% for fertilizer, herbicide, pesticide, seed, and land, respectively, to ensure a higher maize output (Tab. 4). However, labor and capital were over-utilized. Therefore, these inputs need to be decreased by 20.9% and 81.9%, respectively, for efficiency in maize productivity to be ensured (Tab. 4). In the northern region, for optimal resource utilization by farmers to be achieved, there should be an increase in fertilizer, herbicides, pesticides, labor, land, and capital of 21.8%, 91.9%, 99.4%, 66.5%, 84.7%, and 94.5%, respectively. In comparison, the quantity of seed would be expected to be reduced by 66.8% to ensure optimization (Tab. 4). The optimal resource use adjustment by maize farmers in the Brong-Ahafo region also revealed that for an optimal resource to be achieved, there should be an increase of 73.3% of fertilizer, 98.7% of herbicide, 99.6% of pesticide, 97.8% of seed, 33.4% labor, and 98.8% of land. In comparison, capital needs should be reduced by 85.9% for optimal output levels to be achieved (Tab. 4). For maize farmers in the eastern region, an increase of 90.5%, 95%, 99.8%, 68.9%, 52.4%, 99.3%, and 98.3% for fertilizer, herbicide, pesticide, seed, labor, and land, respectively, as well as a decrease in capital input of 90.8% would be required for optimal resource use to be achieved (Tab. 4). Optimal adjustment regarding optimal use of resources by maize farmers in the central region requires an increase of 91.9%, 3.9%, 99.3%, 91.8%, and 71.4% in fertilizer, herbicides, pesticides, seed, and labor, respectively. In comparison, land and capital need to be decreased by 33.8% and 98.4%, to ensure resource-use efficiency optimization for increasing maize production (Tab. 4). This result agrees with the studies of Chiedozie *et al.* (2010) and Wongnaa *et al.* (2012), who obtain similar results in their studies on resource use efficiency. Our results suggest a significant divergence from the optimal levels of pesticides (underutilized) in all the agro-ecological zones compared to any other input resources. The divergence of pesticide use from the optimal levels was greater in the eastern region, whereas that of land was greater in the Brong-Ahafo region of Ghana (Fig. 1).

TABLE 4. Adjustments in marginal value products (MVP) for optimal resource use (% divergence).

Variable	Pooled sample		Northern region		Brong-Ahafo region		Eastern region		Central region	
	EG	% D	EG	% D	EG	% D	EG	% D	EG	% D
Fertilizer	2.7	70.4	0.43	21.8	3.6	73.3	5.8	90.7	9.67	91.9
Herbicide	82.3	89.7	113.9	91.9	574.8	98.7	155.7	95.2	0.46	3.9
Pesticide	62.4	86.3	59.8	99.4	149.3	99.6	63.4	99.7	10.8	99.3
Seed	49.8	94.6	2.42	66.8	104.8	97.8	6.4	68.8	29.7	91.8
Labor	2.5	20.9	13.8	66.5	9.45	33.4	11.31	52.5	28.8	71.4
Land	1037.8	97.4	78.1	84.7	3927.92	98.8	1630.8	98.2	10.89	33.8
Capital	10.64	81.9	11.4	95.4	10.99	85.7	12.6	90.9	13.56	98.4

EG - efficiency gap, D - divergence from optimal level.

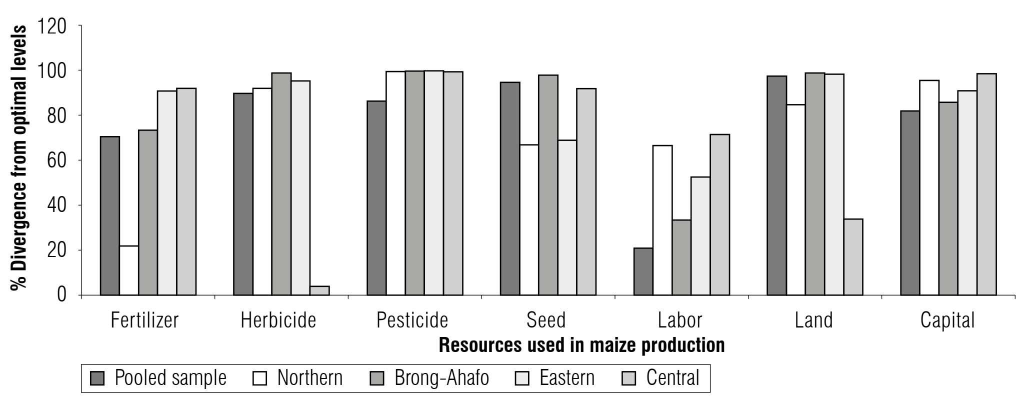


FIGURE 1. Adjustment in marginal value of products for optimal resource use.

In the northern region, the results for the ratios of the MVP and MFC were greater than one for the use of fertilizer, herbicide, pesticide, labor, land, and capital with values of 1.2, 12.0, 86.2, 2.9, 6.5 and 13.8, respectively, while the use of seed was found to be less than one with a value of 0.3 (Tab. 3). This result means that fertilizer, herbicide, pesticide, labor, land, and capital were underutilized by maize farmers in this region, while seed was over-utilized. The maize output in the northern region could be increased if more underutilized inputs like fertilizer, herbicide, pesticide, labor, land, and capital were used. At the same time, the quantity of seed should be reduced.

In the Brong-Ahafo Region, the ratios of the MVP and MFC were greater than 1 for fertilizer, herbicide, pesticide, seed, labor, and land with values of 3.4, 70, 168, 45, 1.5, and 84, respectively, while the only input with a ratio less than one was capital 0.1 (Tab. 3). The implication of this is that fertilizer, herbicide, pesticide, seed, labor, and land were underutilized, while only capital was over-utilized. Therefore, maize farmers in the Brong-Ahafo region could have increased their outputs if inputs like fertilizer, herbicide, pesticide, seed, labor, and land were increased while capital was reduced.

In the eastern region, the MVP and MFC ratio was greater than one for fertilizer, herbicides, pesticides, seed, labor, and land with values of 10.5, 19.6, 559, 3.1, 2.1, and 59.0, respectively. The capital ratio of MVP and MFC was less than one (0.08) (Tab. 3). This means capital was over-utilized while fertilizer, herbicides, pesticides, seed, labor, and land were underutilized by maize farmers in this region. Therefore, if maize farmers had increased their use of fertilizer, herbicides, pesticides, seed, labor, and land and decreased the amount of capital, then it is likely that their maize output would have increased.

The MVP and MFC ratios calculated for maize farmers in the central region were greater than one for fertilizer, herbicide, pesticide, seed, and labor, recording 12.2, 1.04, 35.3, 11.1, 3.5, respectively. Simultaneously, land and capital were found to be less than one, recording 0.66 and 0.02, respectively (Tab. 3). This result indicates that fertilizer, herbicide, pesticide, seed, and labor were underutilized, while land and capital were over-utilized. Maize farmers in the central region of Ghana could have increased their outputs if more of such inputs like fertilizer, herbicide, pesticide, seed, and labor were used while land and capital were reduced. The studies of Chiedozie *et al.* (2010) also reported similar results.

Conclusion

For maize farmers in Ghana to be efficient in their use of resources, fertilizer, herbicides, pesticides, improved seed, and land should be increased because of these resources' underutilization. On the other hand, labor and capital need to be decreased since they were overutilized. In the northern region, we found that for maize farmers to be optimal in their productivity levels, there should be an increment in fertilizer, herbicide, pesticide, labor, land, and capital. At the same time, the quantity of seed should be decreased. For maize farmers in the Brong-Ahafo region to be optimal in their maize production, fertilizer, herbicides, pesticides, seed, labor, and land need to be increased while capital should be reduced. All the other resource inputs, including fertilizer, herbicide, pesticide, improve seed, labor, and land should be increased for optimal productivity levels in the eastern region. Finally, in the central region, inputs such as fertilizer, herbicide, pesticide, seed, and labor should be increased to ensure optimal resource use, whereas land and capital should be reduced. Based on the results obtained, the researchers recommend that the government and other stakeholders subsidize the price of input resources like fertilizer, herbicides, pesticides, and improved seed to enable farmers to increase their use. Extension offices should also encourage farmers to use the underutilized resources and advise them to reduce over-utilized resources like capital.

Author's contributions

FOD formulated the research goals and aims, created the visualization, wrote the original draft, and reviewed and edited the manuscript, HG provided all the resources needed for this study and also supervised all the writing, LNF applied the statistical techniques and software to analyze data and BAK developed the methodology for this study and was responsible for data curation.

Literature cited

Abatania, L. N., Hailu, A., & Mugera, A. W. (2012, February 7–10). *Analysis of farm household technical efficiency in Northern Ghana using bootstrap DEA* [Conference presentation]. 56th annual conference of the Australian Agricultural and Resource Economics Society, Fremantle, WA, Australia. https://doi.org/10.22004/ag.econ.124211

Ajibefun, I. A. (2002, July 7–12). *Analysis of policy issues in technical efficiency of small scale farmers using the stochastic frontier production function: With application to Nigerian farmers* [Conference presentation]. 13th International Farm Management Congress, Wageningen, The Netherlands. https://doi.org/10.22004/ag.econ.7015

Chiedozie, E. C., Blessing, A., & Oliver, N. (2010). Resource use efficiency in arable crop production among smallholder farmers in Owerri agricultural zone of Imo State, Nigeria. *Researcher*, *2*(5), 14–20.

EUROSTAT. (2018). *Population structure and aging*. EUROSTAT Statistics Explained. https://ec.europa.eu/eurostat/statistics-explained/index.php/Population_structure_and_ageing

Eze, C., & Okorji, E. (2003). Cocoyam production by women farmers under improved and local technologies in Imo State, Nigeria. *African Journal of Science, 5*(1), 113–116.

Fasasi, A. R. (2006). Resource use efficiency in yam production in Ondo State, Nigeria. *Agricultural Journal*, *1*(2), 36–40.

Food and Agriculture Organization of the United Nations. (2016). FAOSTAT. Countries by commodity. http://www.fao.org/faostat/en/#rankings/countries_by_commodity

Ghana Statistical Service. (2018). Ghana Demographic and Health Survey 2018. Ghana statistical service (GSS), and ICF Macro.

Goni, M., Mohammed, S., & Baba, B. A. (2007). Analysis of resource use efficiency in rice production in the Lake Chad area of Borno State, Nigeria. *Journal of Sustainable Development in Agriculture and Environment*, *3*(2), 31–37.

Hashim, Y. A. (2010). Determining sufficiency of sample size in management survey research activities. *International Journal of Organisational Management & Entrepreneurship Development*, *6*(1), 119–130.

Ministry of Food and Agriculture. (2012). *Agriculture in Ghana: facts and figures 2012*. Ministry of Food and Agriculture - Statistics, Research and Information Directorate (SRID). https://nobowa.com/wp-content/uploads/sites/26/2017/01/Mofafactsandfigures2012.pdf

Ministry of Food and Agriculture. (2014). *Agricultural production survey (Minor season) 2013, second round*. Ministry of Food and Agriculture - Statistics, Research and Information Directorate (SRID). https://www2.statsghana.gov.gh/nada/index.php/catalog/87/related_materials

Nyamadzawo, G., Wuta, M., Nyamangara, J., & Gumbo, D. J. S. (2013). Opportunities for optimization of in-field water harvesting to cope with changing climate in semi-arid smallholder farming areas of Zimbabwe. *SpringerPlus*, *2*(1), 100. https://doi.org/10.1186/2193-1801-2-100

Obasi, P. (2007). Farm size-productivity relationships among arable crops farmers in Imo State, Nigeria. *International Journal of Agriculture and Rural Development, 9*(1), 91–99. https://doi.org/10.4314/ijard.v9i1.2673

Ojiako, I. A., & Ogbukwa, B. C. (2012). Economic analysis of loan repayment capacity of smallholder cooperative farmers in Yewa North Local Government Area of Ogun State, Nigeria. *African Journal of Agricultural Research*, *7*(13), 2051–2062.

Oladejo, J., & Adetunji, M. (2012). Economic analysis of maize (*Zea mays* L.) production in the Oyo state of Nigeria. *Agricultural Science Research Journals*, *2*(2), 77–83.

Sadiq, M. S., Yakasai, M. T., Ahmad, M. M., Lapkene, T. Y., & Abubakar, M. (2013). Profitability and production efficiency of small-scale maize production in Niger State, Nigeria. *IOSR Journal of Applied Physics*, *3*(4), 19–23.

Salkind, N. J., & Rainwater, T. (2003). *Exploring research*. Prentice-Hall Upper Saddle River.

Saura-Calixto, F., Serrano, J., & Goñi, I. (2007). Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry*, *101*(2), 492–501. https://doi.org/10.1016/j.foodchem.2006.02.006

Tachie-Obeng, E., Gyasi, E., Adiku, S., Abekoe, M., & Zierrogel, G. (2010, August 16–20). *Farmers' adaptation measures in climate change scenarios for maize production in semi-arid zones of Ghana* [Conference presentation]. 2nd International conference on climate sustainability and development in semi-arid regions, Fortaleza, Brazil.

Tambo, J., & Gbemu, T. (2010, September 14–16). *Resource-use efficiency in tomato production in the Dangme West District, Ghana* [Conference presentation]. Conference on international research on food security, natural resource management, and rural development, Zurich, Switzerland.

Thobatsi, J. T. (2009). *Growth and yield responses of maize (Zea mays L.) and cowpea (Vigna unguiculata L.) in an intercropping system* [Master's thesis, University of Pretoria]. Cite Seer X. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.918.9252&rep=rep1&type=pdf

Uchegbu, B. (2001). *Comparative analysis of small and large scale food crops farmers in Imo State* [Unpublished Master's thesis]. Imo State University.

Wongnaa, C. A., & Ofori, D. J. A. (2012). Resource-use efficiency in cashew production in Wenchi Municipality, Ghana. *Agris on-line Papers in Economics and Informatics*, *4*(2), 73–80. https://doi.org/10.22004/ag.econ.131364

World Bank. (2019). World development report: Agriculture for development.

World Data Atlas (2019). *Ghana - Maize production quantity 2019*. https://knoema.com/atlas/Ghana/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production

SUPPLEMENTARY MATERIAL 1. Survey questionnaire.

Farmers Interview Schedule.

I am FRANK OSEI DANQUAH from Sichuan Agricultural University, China. I am currently researching how improving production technology enhances maize farmers' economic efficiency in Ghana. The information provided will be treated with a high level of confidentiality.

Questions are addressed to farmers, preferably the household heads or decision-makers in the household.

A. IDENTIFICATION

A1. Enumerator's Name:____ Phone No.____

A2. Respondent's Name: ____ Phone No.____

A3. Date of interview: ___ /___/

A4. Region (Please tick)
1. Northern region
2. Brong-Ahafo region
3. Eastern region
4. Central region

A5. Which district/Municipality is the farmer located in ____

A6. Area Operated (Farm Location) ____

A7. Description of operational area
Urban = 0 Rural = 1

B. DEMOGRAPHIC CHARACTERISTICS AND SOME SOCIAL CULTURAL PRACTICES.

B1. Gender:____ Male = 1 Female = 0

B2. Age of the respondent (years)
18-45 years = 1; 46-60 years = 2;
above 60 years = 3

B3. Educational Level:
No formal education=1; Primary education=2
Secondary education =3; Tertiary education = 4

B4. Actual number of years spent in school (if formally educated)____

B5. Marital status:____
Single = 0 Married = 1

B6. How many people do you have in your household ____

B7. How many years of farming experience ____

B8. Approximately how many non-formal trainings have you attended in the past five (5) years:
None = 1; 1-5 times = 2; 6-10 times = 3;
more than 10 times = 4

B9. What is the total farm size you own (acres)____

B10. How many plots of lands do you own____

B11. How much of your land is used for maize cultivated (acres)____

B12. Are you currently a member of any farmer's group or local association in the village?
No = 0 Yes = 1

B13. Are you a beneficiary of any subsidies from the government?
Yes = 1 No = 0

B14. Do you hold a formal land title or registration to the whole or part of your land?
Yes = 1 No = 0

B15. How did you get access to the land you are cultivating?
1. I bought
2. I inherited it from my parents
3. Its parts of my family properties
4. Other (Specify) ____

B16. If yes, what kind of subsidy was that____

B17. Are you a beneficiary of any NGO program?
Yes = 1 No = 0

B18. If yes, what is the name of the NGO ____

B19. What was the form of benefits given to you?
Farming =1; Paying of your ward school fees = 2; Construction of house for you = 3; Financial Support = 4; Others (Please specify) = 5

B20. Has any agricultural extension officer visited you?
No = 0 Yes = 1

B21. If yes, how many times? 1 = last year; 2 = two years ago; 3 = three years ago; 4 = beyond three years.

B22. Have you received any credit for your farming?
Yes = 1 No = 0

B23. What was your total capital at the beginning of the production season? Gh¢____

B24. What was the amount of credit you accessed last year in any Gh¢____

B25. Have you engaged in other income generation activities apart from maize farming previous year?
Yes = 1 No = 0

B26. If yes, which of the following were you engaged in (Select only one)
1. Artisan (carpentry, fitting, dressmaking, etc.)
2. Trading
3. Food processing
4. By day labourer
5. Public servant
6. Others (please specify)

B27. Which of the improved production technologies would you like to adopt
1. Fertilizer use
2. Pesticides use
3. Herbicides use
4. Improved seed use
5. Combination of all the technologies

B28. Which one of the following groups of improved production technologies did you employ in your maize production last year? Please tick.
1. Improved seeds + fertilizer use + pesticide use + herbicide use + other soil fertility management practices
2. Some of the technologies mentioned in (1) above but not all
3. Only one of the technologies mentioned in (1) above

B29. Did you use fertilizer on your maize farm?
Yes / No

B30. If yes, why did you apply it on your maize farm ____

B31. Did you use Pesticides on your maize farm?
Yes / No

B32. If yes, why did you apply it on your maize farm ____

B33. Did you use Herbicides on your maize farm?
Yes / No

B34. If yes, why did you apply it on your maize farm ____

B35. Did you use the improved seed in your maize farm?
Yes / No

B36. If yes, why did you apply it on your maize farm ____

B37. Approximately how many minutes does it take you from your house to your farm? ____

B38. Around how many kilometres do your cover from your house to your farm?____

B39. Why did you plant the variety of maize seeds you planted last year?
1. It was very cheap
2. It was the only one available
3. It was the only known variety to me
4. That was what customers preferred
5. It is high yielding
6. Others (please specify)

B40. What is your source of labour for your maize farming?
1. Family members
2. Hired Labour
3. Friends
4. Others

C. OUTPUT OF MAIZE

C1. The output of maize for all land cultivated and the selling price.

Year	Season	Total maize farm size (Acres)	Total output		Selling price (Gh¢/Bag)
			No. of bags	Weight (kg)	
2016	Major				
	Minor				
2017	Major				
	Minor				
2018	Major				
	Minor				

C2. Did you make a profit by selling your maize?
Yes = 1 No = 0

C3. What do you think about the level of yield on your farm for the past three farming seasons?
1 = Increased 2 = Decreased 3 = No change

C4. If there is an increase, what might be the cause ____

C5. What do you perceive to be the cause for the decrease, if any____

C6. According to you, what is the food security status in your household?
1 = Not sufficient 2 = Sufficient

D. RESOURCE OR INPUT USED IN PRODUCTION OF MAIZE

D1. Indicate whether the following input was used in your maize farm last year and indicate the quantity used, cost per unit, and the total cost per acre.

No.	Variable input	Quantity used	Unit cost (GH¢)	Total cost (GH¢)
1	**Fertilizer (Kg)**			
2	**Pesticides (L)**			
3	**Herbicides (L)**			
4	**Improved seed (kg)**			
Labour (person-days)				
a	Land clearing			
b	Sowing			
c	Falling of trees			
d	Others (indicate)			

E. SUMMARY OF UNIT PRICE OF THE KEY PRODUCTION ITEMS

No.	Production variable	Cost/price GH¢
	The average price of maize per kg	
	The average cost of rented land per hectare	
	The average price of seed per kg	
	Average price per fertilizer per kg	
	The average price of herbicides per litre	
	The average price of pesticides per litre	
	The average price of labour per person-day	

Technical and economic assessment of two harvesting tools for young *Elaeis oleifera* x *E. guineensis* oil palms

Evaluación técnica y económica de dos herramientas para la cosecha de palmas de aceite *Elaeis oleifera* x *E. guineensis* jóvenes

Elizabeth Ruiz Álvarez[1], Jhon Banguera[2], Wilson Pérez Toro[1], Juan Hernández Hernández[1], Javier Arévalo[2], and Mauricio Mosquera Montoya[1]*

ABSTRACT

The harvest of oil palm fruit bunches represents 25% of the total cost of production of one metric ton of fresh fruit bunches (FFB), and nine-tenths of the costs of this process (i.e., harvest) are labor costs. This study was undertaken to analyze and compare the labor productivity and harvesting costs of young oil palm trees of the species *Elaeis oleifera* x *E. guineensis* (OxG) using two different cutting tools. The first tool was a chisel, and the second was a mechanized oil palm cutter. From a methodological perspective, we conducted a time and motion study. The results show that the average number of palms harvested by a worker using a chisel was 320 per workday (on average 291 fresh fruit bunches were cut per workday). A worker using a mechanized oil palm cutter (MOPC) harvested 546 palms per workday (on average 551 fresh fruit bunches were cut per workday). Finally, the cost per ton of fresh fruit bunches (FFB) harvested decreased by 15% following the MOPC; in other words, the implementation of this novel technology is cost-effective.

Key words: cutting tools, palm cutter, time and motion study, process, labor productivity, economic efficiency, OxG hybrid.

RESUMEN

La labor de cosecha de racimos de palma de aceite representa el 25% de los costos totales de producción de una tonelada de racimos de fruto fresco (RFF), y nueve décimas partes de los costos de este proceso (es decir, la cosecha) son costos laborales. El estudio se realizó con el objetivo de analizar y comparar los rendimientos laborales y los costos de cosecha de palmas jóvenes de la especie *Elaeis oleifera x E. guineensis* (OxG), utilizando dos herramientas de corte. La primera herramienta fue el palín malayo y la segunda fue el cortador mecanizado para palma de aceite. Desde el punto de vista metodológico se realizó un estudio de tiempos y movimientos. Los resultados muestran que el número promedio de palmas cosechadas por un trabajador usando el palín malayo fue 320 por día de trabajo (en promedio se cortaron 291 RFF por jornada). De otro lado, un trabajador empleando el cortador mecanizado cosechó 546 palmas (en promedio se cortaron 551 RFF por jornada). Finalmente, el costo por tonelada de racimos de fruto fresco cosechada disminuyó en 15% con el uso del cortador mecanizado; en otras palabras, la implementación de esta novedosa tecnología es rentable.

Palabras clave: herramientas de corte, cortador mecanizado, estudio de tiempos y movimientos, proceso, productividad laboral, eficiencia económica, híbridos OxG.

Introduction

When one evaluates the long-term trends in the real price of crude palm oil (CPO) worldwide in US dollars, a decreasing rate of -1.6% per year is seen for the period from 1950 to 2010. This obviously negatively impacts the income of oil palm growers and the profitability of their businesses (Mesa, 2016). Additionally, the Colombian palm agroindustry is considered a price taker when exporting crude palm oil (CPO) to foreign markets. The latter responds to the fact that Malaysia and Indonesia together represent more than 90% of the global CPO exports (Fedepalma, 2017). Finally, comparative analyses aimed at estimating production costs in different countries indicate that CPO production in Colombia is 20% more expensive than in Malaysia, mostly due to the economies of scale at the mill, land's opportunity costs, and higher wages (Mosquera *et al.*, 2017a). Therefore, alternatives that contribute to increasing labor productivity, specifically in processes that are more labor-intensive, such as the harvest, must be pursued (Ruiz *et al.*, 2017; Mosquera *et al.*, 2017b). This study focuses on improving labor productivity of oil palm harvesters.

Received for publication: 27 February, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.85303

[1] Agricultural Economics and Biometrics, Corporación Centro de Investigación en Palma de Aceite (Cenipalma), Bogota (Colombia).
[2] Palmeiras Colombia S.A., Cali (Colombia).
* Corresponding author: mmosquera@cenipalma.org

The harvest is of critical importance for the production of palm oil because the quality of the final product depends upon the opportune cutting of the fresh fruit bunches (FFB) and bunches need to be cut at the right time to maximize the oil content in the fruits. In consequence, having properly trained workers is a must (Aramide *et al*., 2015). Given the fact that FFB do not reach the optimal moment to be cut together, it is necessary to visit the same plots every 7 to 15 d (Corley and Tinker, 2015). Therefore, harvest in oil palm crops is a labor-intensive task. In fact, 90% of the harvesting costs are represented by labor payment (Mosquera-Montoya *et al*., 2019).

The harvest of oil palm is carried out by searching for ripe bunches, cutting them off, collecting FFB from the ground, and gathering FFB at collecting points in the field (Mosquera, 2017a; Sinambela *et al*., 2020). These operations may be performed by harvest teams (division of labor) or by individual workers, depending on plantation yields and the availability of machinery.

The equipment commonly used to harvest young palms includes a chisel to cut bunches off, a cart pulled by an animal (water buffaloes or bulls) in which the harvester places the FFB, and the picked loose fruit, and a machete to cut off the peduncles from the FFB (Castillo *et al*.,2017). The average labor productivity for the harvest of young oil palms in plantations in Colombia oscillates between 1.5 t FFB/workday and 2.5 t FFB/workday (Mosquera *et al*., 2009). When the harvest is performed by a single worker and mechanized equipment is not used, lower labor productivity results (Castiblanco and Mosquera, 2010). When the harvest is divided into two operators (one specialized in cutting off FFB and the other specialized in filling the containers either mechanically with a grabber or manually) and there are devices such as a cableway to help to evacuate FFB from the field, the highest labor productivity results (Castiblanco and Mosquera, 2010; Munévar, 2020).

It is easier to mechanize the process of fruit evacuation from the field than cutting off FFB from oil palm trees. Although research has been conducted with the aim of increasing labor productivity during the harvest over the period from 1995 to 2008, achievements are moderate (Jelani *et al*., 2008). The lack of substantial achievements may be because oil palm is a perennial crop in which every palm is an individual different from others. Additionally, bunches are ready to harvest at different times, depending on each palm; therefore, the same plot must be regularly harvested. Finally, the ability to access bunches of oil palms is not a straightforward process since harvesters must cut the frond holding the bunch and probably some other fronds that impede access to the bunch.

Palm cutters, such as the Cantas cutters, are among the novel technologies developed for harvesting oil palms and are recommended by Jelani *et al*. (2008) to help reduce workers effort and increase their productivity. The use of a Cantas cutter for harvesting oil palm trees with bunches located at a height less than 5 m yielded a 40 to 50% increase in the number of bunches cut, and a 30% reduction in the cost of harvesting a ton of FFB. Another device evaluated was the C-kat, a palm cutter suited for palms whose bunches are located at a of height less than 1.5 m (Jelani *et al*., 2008). Field trials showed that workers using the C-kat may be able to increase their labor productivity by 10% to 20% compared to workers using a chisel (Jelani *et al*., 2008).

Jelani *et al*. (2018) surveyed literature on the mechanization of cutting oil palm bunches and concluded that the use of mechanized alternatives instead of a traditional chisel (in young palms) or a long-armed sickle (in tall palms) (Jelani *et al*., 2018) results in an average 50% reduction in the cost of harvesting a metric ton of FFB. Additionally, cutting was conducted with greater efficiency, which allowed for a timely harvest and required fewer workers to complete the task.

Most studies analyzing the mechanized harvest of oil palm refer to crops planted with *Elaeis guineensis* (Jelani *et al*., 2003; Jelani *et al*., 2008; Alfonso *et al*., 2009; Khalid and Shuib, 2014; Aramide *et al*., 2015). To our knowledge, no reports have described the benefits of using these alternatives in crops planted with crosses of *Elaeis oleifera x E. guineensis* (OxG). OxG crops have been used as an alternative crop by Colombian growers interested in staying in the oil palm business in areas that have experienced outbreaks of bud rot (BR) because of their partial resistance to BR. OxG crosses are more robust than *E. guineensis* cultivars, and, thus, a study aiming to determine whether these mechanized tools can be used to harvest OxG oil palms that feature thicker fronds and petioles is of great interest.

This manuscript reports the results of a study conducted to assess the implementation of a mechanized oil palm cutter (MOPC) that is suited for harvesting young OxG oil palm (i.e., bunches at a maximum height of 3 m). The use of this device is compared to the use of a chisel in terms of labor productivity and production costs.

The use of a MOPC is expected to improve the performance of harvesters in the field. The MOPC reduces the physical effort by the worker when performing his job. The MOPC is equipped with an engine that converts fuel energy into an oscillatory movement that eases the cutting process through traction. When the worker uses a chisel to cut bunches, he must use his strength to repeatedly strike the frond holding the bunch in order to cut it. Then, he must strike the peduncle of the fresh fruit bunch to cut it. In contrast, when the worker uses the MOPC, he does not need to strike the base of the bunch multiple times as the cutter only needs to be operated once.

Regarding production costs, when cultivating OxG crosses in Colombia, one of the processes responsible for most of the investment in labor is the harvest (together with pollination), and in terms of production costs it occupies the second place, after fertilization, with 18.4% in the share of total costs (Mosquera *et al*., 2020). In consequence, these results provide insights into mechanized alternatives for increasing labor productivity and reducing production costs in crops planted with OxG oil palms.

Materials and methods

Location

This research was conducted at an oil palm plantation in Colombia located in the municipality of Tumaco (southwest of Colombia) known as Palmeiras. This plantation contains 559 ha planted with OxG crosses of oil palm. These oil palms were planted in 2008 and in 2009, and the data used by the authors were collected when these oil palms were 8 and 9 years old.

Harvesting teams

There are workers responsible for cutting fronds and bunches (cutters) and other workers (pickers) in charge of picking up FFB from the ground, placing the FFB in wheeled containers (pulled by water buffaloes) and locating the fronds the plates. The process of covering the oil palm plates with fronds is conducted at the plantation where the study is performed (i.e., Palmeiras) because of the presence of an insect known as *Sagalassa valida* Walker (*S. valida*) that feeds on oil palm tree roots and lays their eggs on oil palm plates. An effective strategy for controlling *S. valida* consists of using cut fronds to form a barrier that prevents this insect from laying their eggs close to the oil palm roots (Corredor *et al*., 2016). Table 1 shows the number of workers required to harvest a plot.

TABLE 1. Harvest teams according to the cutting tool used.

	Chisel	Mechanized oil palm cutter
Harvesting teams	1 cutter	1 cutter
	1 picker	2 pickers
	1 water buffalo	2 water buffaloes
	1 wheeled container (capacity: 1t)	2 wheeled containers (capacity: 1t)
	1 chisel	1 mechanized oil palm cutter

Figure 1 illustrates the methods assessed and Table 2 shows the main features of each of the tools used for harvesting young OxG oil palms.

FIGURE 1. Cutting methods for oil palm trees tested in the present study. A) Chisel vs. B) Mechanized oil palm cutter.

TABLE 2. Main features of the cutting tools evaluated.

	Chisel	Mechanized oil palm cutter
Features	Reach: 3 m Weight: 3 kg Metal bar adapted to a chisel No engine is required. Energy required for cutting depends on the strength of the operator Brand: Herragro, Colombia	Reach: 3 m Weight: 8 kg Iron bar adapted to a chisel Engine: 25.4 cc, power 0.95 Kw Manual fuel pump at the carburetor Starting system: ElastoStar Brand: Stihl Model PC70, Brazil
Extra tools required	Whetstone	Whetstone Lubricant: grease Gasoline: fuel oil (3:1)
Tools	 Chisel	 Chisel
		 Engine
	 Metal bar	 Metal bar

Time and motion study

We performed a time and motion study to compare the outcomes of using either the chisel or the MOPC to cut FFB from young OxG oil palms, based on the method proposed by Sánchez *et al.* (2010). Time and motion studies require a first stage that consists of establishing the sequence of processes required to complete the task, in this case cutting off bunches (harvest). The result of this stage is the process flow diagram for each cutting tool being assessed.

Flow diagram of the process of cutting FFB

The process of cutting oil palm bunches was observed for a period of two weeks. The goal was to characterize not only the processes involved in cutting bunches but also the demand for inputs, tools and machinery required to complete this task. We used the symbols proposed by the American society of mechanical engineers (ASME) (Tab. 3).

TABLE 3. Symbols used for flow diagrams.

Symbol	Process	Activity
●	Operation	Something is produced or a task gets carried out
➡	Transport	Switching of placement, implies movement
■	Inspection	Verification of quality or quantity
◗	Delay	Interference or unexpected stop of the process
▼	Storing	Keeping or protecting something
◆	Deciding	Decide on continuing or switching activity

Source: Sánchez *et al.*, 2010.

Estimation of time length for each subprocess and for labor productivity

The second stage builds flow diagrams for the processes and consists of measuring time for each process. Data on time records must suffice to encompass the observed variability on time length for each process. Additionally, the frequency and the best measure of central tendency (mean or median) must be considered according to the distribution of the collected time data for each process. The result of this stage is the standard time required to complete the task of cutting FFB in OxG young oil palms, which results from the sum of the mean/median times of each process multiplied by their frequency.

We used the information recorded by a GIS system available at Palmeiras known as Click Palm. Click Palm allows data to be collected from the field in real time because every oil palm tree is tagged; thus, the worker records any process performed at any specific palm using a cell phone. Data related to cutting fruit bunches was used in the present study. Click Palm enables an assessment of the daily performance at the worker level. The analysis is possible because each worker is provided with a cell phone equipped with the application and the information is uploaded to the database. The system records the time, enabling an estimate of the amount of time a task requires.

We analyzed the time required to conduct this time and motion study to accomplish the harvest process by four workers that had at least six months of experience using the MOPC. Regarding the chisel, which is the traditional method, specific expertise was not required, but workers must have used the chisel. We analyzed harvest data from a six-month period. Since we are focused on the processes carried out during the harvest, we considered palms with zero bunches to cut as palms harvested, as well as those with one or more bunches to cut. The latter means that each harvested palm has its corresponding work cycle. A work cycle is defined as the set of processes carried out in order to harvest an oil palm tree, and that are repeated many times during a workday (i.e., processes with the greater frequency). We refer to the number of harvested palms considered for the statistical analysis according to the number of records (Tab. 4).

TABLE 4. Number of records per worker (palms harvested).

Worker	Cutting tool	Number of palms harvested
A	MOPC	57,384
B	MOPC	74,288
C	MOPC	48,391
D	MOPC	51,445
E	Chisel	14,271
F	Chisel	7,333
G	Chisel	9,927
H	Chisel	1,338

MOPC - Mechanized oil palm cutter.

The data from each worker were analyzed using descriptive statistics in order determine their distribution. Subsequently, the proper measure of the central trend representing the data was chosen. These measures were also used to determine records that were too long, which tended to correspond to non-productive times or actions that are not part of the necessary subprocesses, such as resting, maintaining hydration, and attending to personal needs, etc. These activities must be accounted since they are considered non-effective working time that is part of a workday. Additionally, one must consider that people are unable to work for an entire day at the same pace. In fact, fatigue plays a key role in explaining the decrease in labor productivity during a workday. This explains why it is best to account for reliable data for the entire workday, instead of carrying out work sampling during the workday. We evaluated time data from entire workdays, collected by means of Click Palm.

We considered two different indicators, the number of oil palms harvested per workday and the number of fresh fruit bunches cut per workday, to determine the labor productivity according to the tools used. These indicators were calculated using Click Palm records for each of the workers mentioned above.

Cost efficiency analysis of both harvesting methods

We estimated the total costs of cutting one ton of oil palm bunches by considering fixed and variable costs using a previously described method (Edwards, 2015) (Fig. 2). We asked for the market prices of tools and machinery to estimate the fixed costs since they correspond to the initial capital outlay. In other words, the fixed costs represent the expenditure the grower incurs to buy tools and machinery. Additionally, we considered the life span and scrap value of tools and machinery. Then, we estimated the depreciation using the straight-line depreciation method. Regarding variable costs, we considered operating costs such as wages, repair, and maintenance (of tools and machinery), fuel and lubricant oil. We considered the frequencies of payments the grower incurred.

We used information recorded by the plantation (Palmeiras) regarding payments for labor and other expenses, such as caring for the buffaloes (feed and veterinary costs), fuel, grease, and equipment repair. We only considered expenses related to the harvesting process. We estimated these FFB harvest costs in terms of COL$ t^{-1}.

Results and discussion

Flow diagram of the process of cutting FFB

Figure 2 displays the flow diagrams for both FFB cutting processes (chisel and MOPC); the work cycle corresponds to processes 40-70. The work cycle of the process of cutting bunches includes: 1) walking along oil palm rows searching for ripe FFB, 2) confirming that the FFB meets the harvest criteria, 3) cutting the leaf holding the FFB and cutting the FFB, and 4) recording the number of FFB cut from the harvested palm (using Click palm).

The process of cutting FFB using the MOPC requires two extra processes compared to the process using the chisel: starting the engine at the plot before beginning to search for ripe bunches and fueling the engine as required. The latter occurs a maximum of twice daily, and thus, it does not substantially alter the standard time estimation.

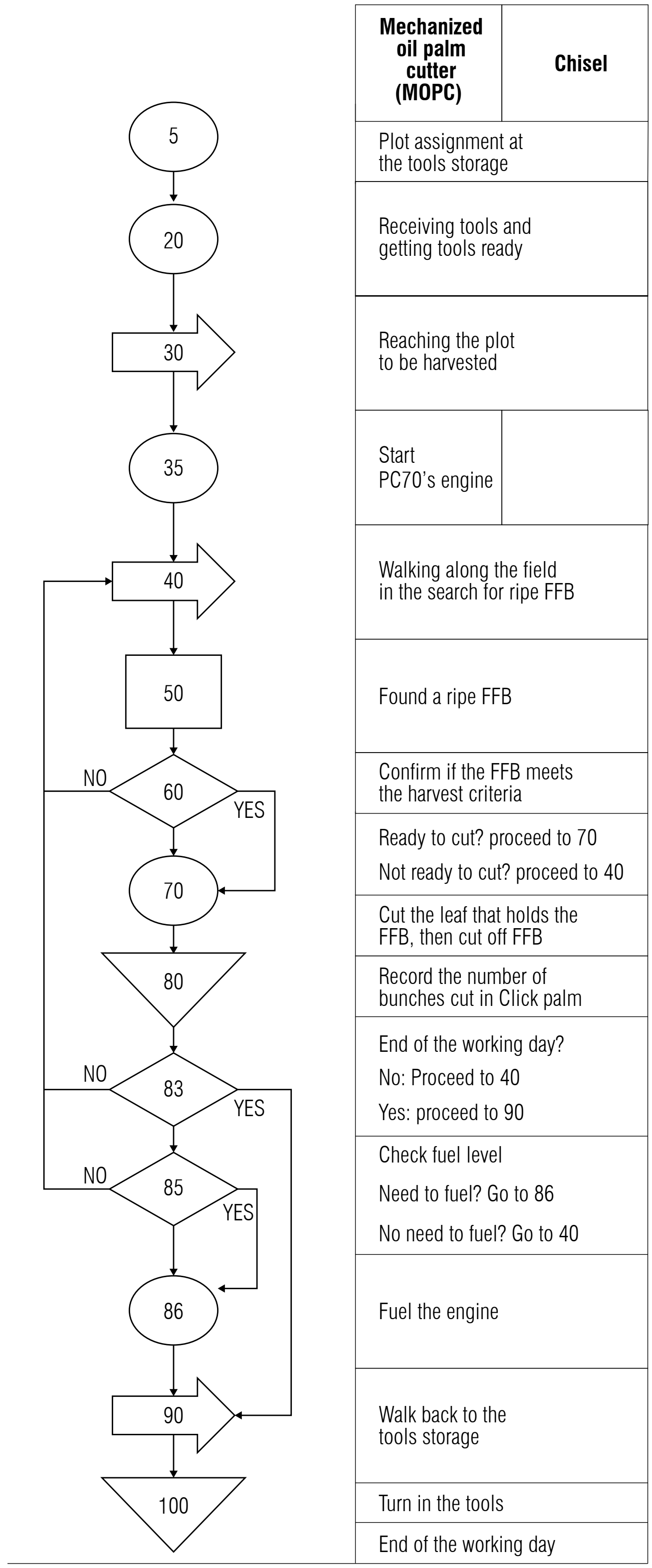


FIGURE 2. Flow diagram for the process of cutting fresh fruit bunches (FFB).

The MOPC eases the process of cutting fronds and, in consequence, cost savings are incurred for pruning oil palm trees. The staff from Palmeiras states that they are paying for pruning once a year when the MOPC is used, while they must pay for pruning twice a year when bunches are cut using chisels.

Estimated time required for processes involved in cutting bunches

Importantly, 30% of the work cycle corresponds to zero FFB identified, 40% to one FFB cut, 24% to two FFB cut and, 4% to three FFB cut (Tab. 2). By analyzing the data, the median time was the central tendency measured used because the frequency distribution is biased to the right (Figs. 3 and 4). Table 5 contains synthetized information on the median time per work cycle, i.e., the median value of the time elapsed while performing processes 40 to 70 (from the process flow diagram), according to the number of bunches to cut.

The time elapsed per work cycle is greater when the worker uses a chisel to cut fronds and FFB than when the worker

TABLE 5. Median of time elapsed per work cycle* (sec).

Number of bunches cut per cycle	Workers using the mechanized oil palm cutter (median of time elapsed per work cycle in seconds)				
	A	B	C	D	Median
0	21	26	17	23	22
1	40	47	42	29	41
2	52	65	61	45	57
	Workers using chisel (median of time elapsed per work cycle in seconds)				
	E	F	G	H	Median
0	16	14	15	15	15
1	23	23	26	26	25
2	30	33	36	37	35

*A work cycle comprehends processes from 40 to 70.

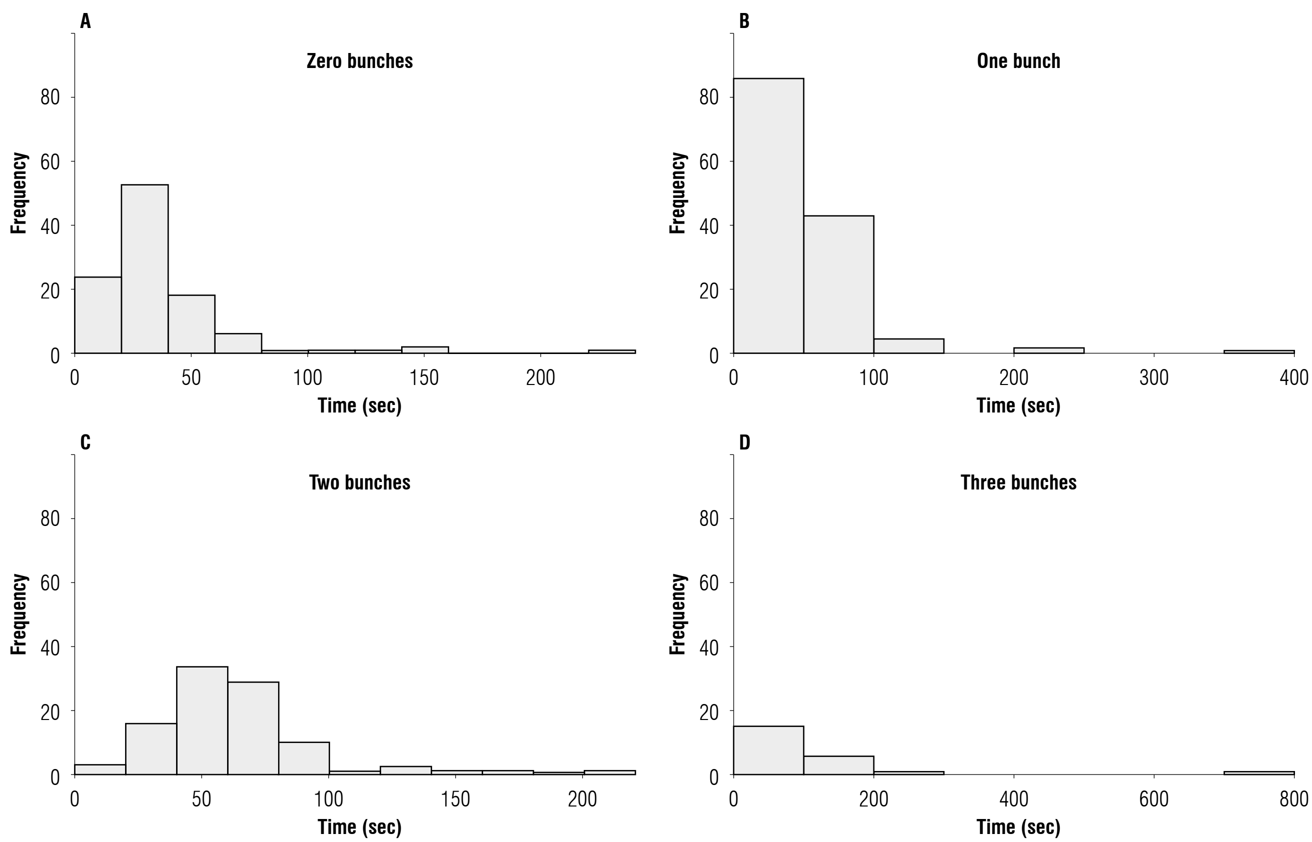


FIGURE 3. Distribution of time elapsed per work cycle according to number of bunches cut using the chisel.

uses the MOPC (Tab. 5). It is likely that a shorter time elapses when no bunches are cut because no fronds interfere with the view of bunches when the palm cutter is used. When palms with ripe FFB to be cut are identified, the time elapsed per work cycle was noticeably shorter when the MOPC was used than the time spent by workers using chisels (Tab. 5).

As shown in Figures 3 and 4, some work cycles are longer than the median value. This increase in work cycles occurs when workers perform actions that lead to non-productive time. These actions are not suggested to be unnecessary and do not indicate that the workers are not committed to the accomplishment of their job (resting, remaining hydrated, addressing personal needs, receiving instructions from the harvest supervisor, eating a snack or lunch, moving through drainage channels or other obstacles, etc.). The existence of non-productive time was assessed using the median value plus a standard deviation. Actions leading to non-productive time tended to increase throughout the workday and totaled 1.4 h per day (Tab. 6).

TABLE 6. Non-productive time along a workday, according to the cutting tool.

Hour of the workday	Time (sec)	
	Chisel	Mechanized oil palm cutter
7	129	587
8	159	552
9	516	264
10	918	398
11	580	421
12	1127	1363
13	1310	1212
14	233	146

Number and weight of bunches cut per workday (labor productivity)

Cutters using the MOPC averaged 546 palms harvested per workday (approximately 4.7 ha), while cutters using chisels averaged 320 palms harvested per workday (approximately 2.76 ha). The latter implies an increase of 71% in the number of oil palm trees harvested.

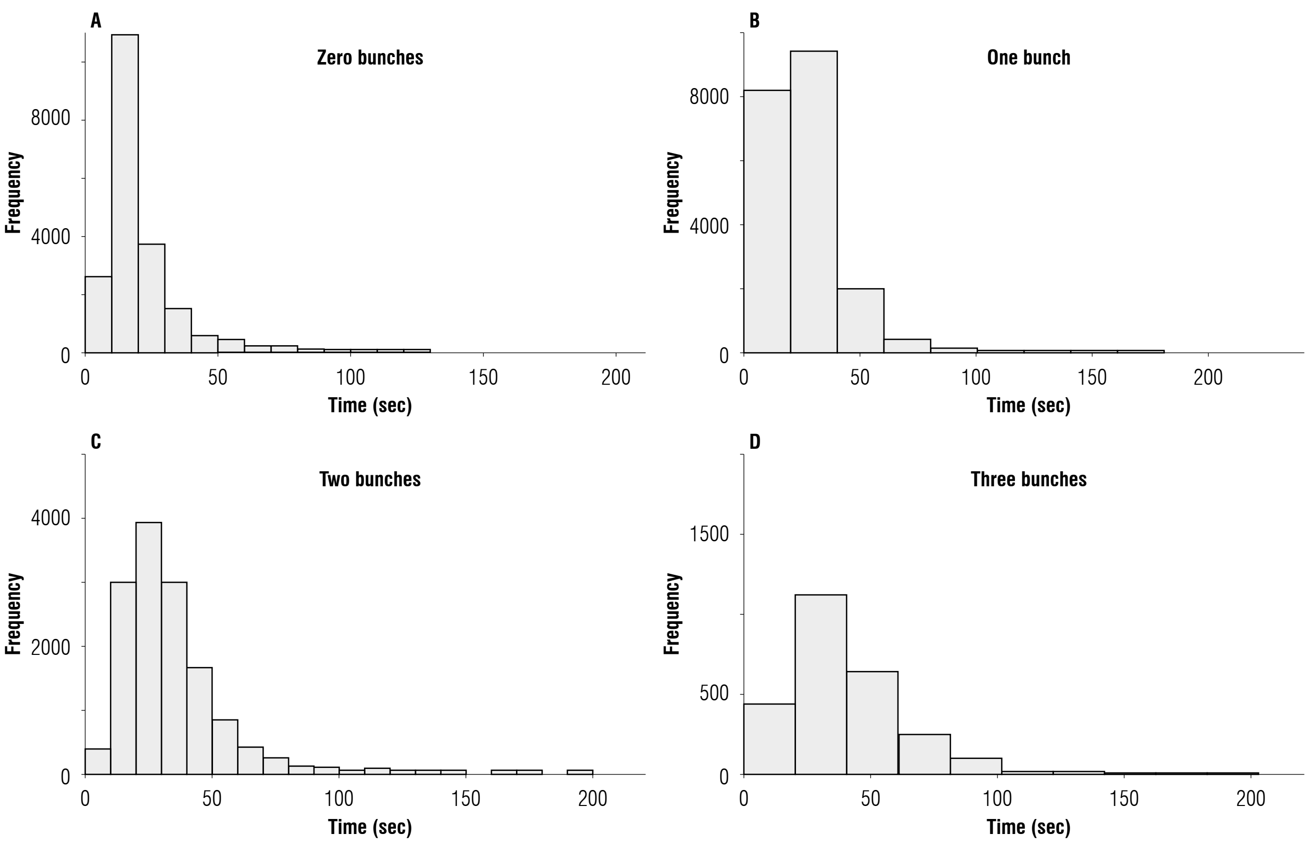


FIGURE 4. Distribution of time elapsed per work cycle according to number of bunches cut using the mechanized oil palm cutter.

Regarding the number of bunches cut per workday, cutters using the MOPC cut an average of 551 FFB per workday (3.3 t FFB/workday), while workers using chisels cut an average 291 FFB per workday (2.5 t FFB/workday). Thus, the labor productivity increased 90% when workers used a MOPC palm cutter. During the workday, labor productivity tended to decrease due to physical fatigue experienced when performing the job (Fig. 5). The latter was observed for both harvest tools.

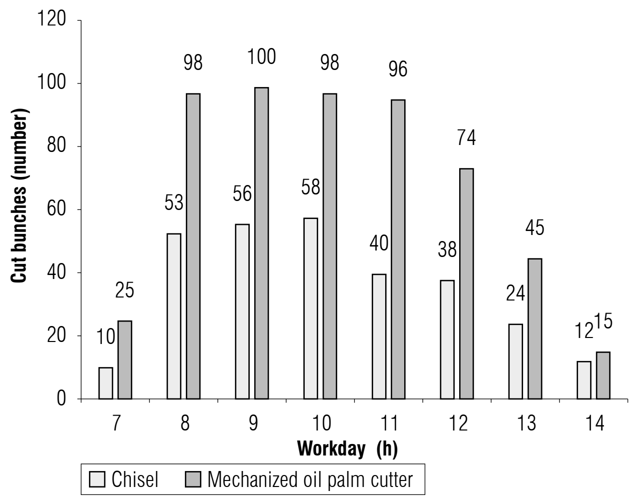


FIGURE 5. Number of FFB cut off along the workday, according to the tool used by the cutter.

Cost efficiency analysis

The use of the palm cutter implies the need for an extra worker to pick up the bunches and to accommodate fronds, compared to the situation in which the cutter uses a chisel for cutting bunches (Tab. 7). Finally, we considered the number of tons cut to estimate the costs per ton of FFB harvested (Tab. 7).

TABLE 7. Need of workers, tools and equipment per harvesting team and labor productivity.

	Chisel	Mechanized oil palm cutter
Cutting tool (number/team)	1	1
Cutter (number/team)	1	1
Pickers (number/team)	1	2
Buffaloes (number/team)	1	2
Wheeled containers (number/team)	1	2
Labor productivity (t FFB/workday)	2.5	3.3

Fixed costs

We used market prices for equipment, tools and animals required to complete the harvest. The price of a cutting tool was COL$45,000 for the chisel (6-month lifespan) and COL$1,600,000 for the MOPC (1-year lifespan). The price of a trained buffalo was COL$4,000,000 (10-year lifespan). Finally, the price of a wheeled container was COL$5,000,000 (3-year life span). Calculations were performed to estimate the cost per ton of FFB harvested (Tab. 8).

Variable costs

The estimated costs of the harvest when using the MOPC was COL$ 18,531 t^{-1} FFB harvested, while the costs of the harvest when using the chisel was COL$ 21,816 t^{-1} FFB. This means the harvest costs decrease 15%, and the total costs of producing a ton of FFB decreases 2.4% (Tab. 8).

Cutting FFB using the MOPC implies that the harvesting team must be rearranged. The increase in the number of cut bunches implies that one cutter will require two pickers, instead of requiring one picker for each cutter, as when using the chisel. This allows for a cost decrease in labor that suffices to offset the extra costs incurred with the use of the MOPC (depreciation, maintenance, fuel and fuel oil).

The results of this study are consistent with findings from previous reports showing that mechanized processes represent an alternative for lowering unit costs (Castiblanco and Mosquera, 2010; Fontanilla *et al.*, 2010; Alfonso and Castiblanco, 2013). Additionally, given the fact that there is a scarcity of labor in rural areas, this is a significant contribution from using the MOPC.

TABLE 8. Fixed and variable costs involved in the harvesting process according to the cutting tool used.

	Mechanized oil palm cutter	Chisel
Labor productivity (t FFB/workday)	10.0	5.2
Fixed costs (COL$$t^{-1}$ FFB)		
Cutting tool	541	58
Buffaloes	126	122
Wheeled container	901	866
Variable costs (COL$$t^{-1}$ FFB)		
Fuel	379	
Lubricant oil	124	
Cutting tool repair	69	
Wheeled container repair	338	325
Buffaloes (feed and veterinary care)	429	413
Labor	15,625	20,032
Total	18,531	21,816

Conclusion

The switch from using a chisel to using a MOPC implies that the strength needed to cut off bunches is no longer provided by the cutter but by the engine. Naturally, a learning curve must be considered. At Palmeiras, the company where this study was conducted, harvesters (cutters) required approximately one year to master the use of the MOPC. During the adjustment to the use of the MOPC, some minor damage to the equipment occurred since workers typically forgot that they no longer needed to strike the palms.

However, the benefits of using these mechanized tools justify the costs associated with the training process since labor productivity increased by 90%. The costs of harvest decreased because the MOPC facilitates the completion of the job by cutters and increases labor productivity, which offsets not only the greater price of the tool but the expenses related to the operation and repair of the MOPC.

Additionally, one of the main results of this study is the development of a method to use information collected by an information system, such as Click Palm, as if it was data collected for a time and motion study. This approach is very interesting since the use of software for controlling cropping processes is a trend that may improve labor productivity studies.

Finally, there is little to no literature associated to OxG crossings, specifically when it comes to the efficiency of harvesting methods. These crossings have been grown at an industrial scale for only 13 years in Colombia, so there are a few references to harvesting this type of palms that differ from *E. guineensis* in the sense that leaves are thicker and more difficult to cut off. In this regard, this work fills a gap in the existing knowledge.

Literature cited

Alfonso, O.A. and J.S. Castiblanco. 2013. Análisis comparativo de costos para la aplicación de fertilizantes en palma de aceite. Rev. Palmas 34(1), 31-38.

Alfonso, O.A., R. Botero, and H.M. Romero. 2009. La mecanización del cultivo de la palma de aceite. Rev. Palmas 30(4), 21-29.

Aramide, B.P., O.K. Owolarafe, and N.A. Adeyemi. 2015. Comparative evaluation of the performance of motorized and pole and Knife oil palm fruit bunch harvester. Agric. Eng. Int: CIGR J. 17(4), 165-172.

Castiblanco, J.S. and M. Mosquera. 2010. Evaluación económica de dos sistemas de evacuación de fruta: alce manual y cable vía. Rev. Palmas 31(1), 33-42.

Castillo, E.G., L.F. Rodríguez, and A.F. Páez. 2017. Evaluation of two harvesting procedures for oil palm (*Elaeis guineensis* Jacq.) fruits. A case study. Agron. Colomb. 35(1), 92-99. Doi: 10.15446/agron.colomb.v35n1.58524

Corley, R.H.V., and P.B.H. Tinker. 2015. The oil palm. 5th ed. Wiley Blackwell, Oxford, England.

Corredor, J.E., M. Mosquera, C.A. Fontanilla, and E. Ruíz. 2016. Sagalassa valida Walker y el paradigma de los platos limpios. Rev. Palmas 37(4), 123-131.

Edwards, W. 2015. Estimating farm machinery costs. Ag Decision Maker File A3-29.

Fedepalma. 2017. Oferta y consumo aparente. Anuario estadístico 2017.

Fontanilla, C.A., S. Pachón, J.S. Castiblanco, M. Mosquera, and A.C. Sánchez. 2010. Referenciación competitiva a los sistemas de evacuación y alce de fruta. Boletín Técnico Número 25. Fedepalma - Cenipalma, Bogota.

Jelani, A.R., A.R. Shuib, A. Hitam, J. Jamak, and M.M. Noor. 2003. Hand-held mechanical cutter. MPOB Information Series No. 180. Malaysian Palm Oil Board, Kuala Lumpur.

Jelani, A.R., A. Hitam, J. Jamak, A. Mohamed, Y. Gono, F. Ismail, and M.M. Noor. 2008. Motorized chisel (Ckat^{tm}) for short palm harvesting. MPOB Information Series No. 409. Malaysian Palm Oil Board, Kuala Lumpur.

Jelani, A.R., M.R. Ahmad, M.I.H. Azaman, Y. Gono, Z. Mohamed, S. Sukawai, A. Aduka, A. Aziz, A. Bakri, A. Mohamed, M.B. Selamat, A. Ismail, M.S. Deraman, A.R. Shuib, N. Kamarudin, and K.A. 2018. Development and evaluation of a new generation oil palm motorised cutter. J. Oil Palm Res. 30(2), 276-288. Doi: 10.21894/jopr.2018.0015

Khalid, M.R. and A.R. Shuib. 2014. Field evaluation of harvesting machines for tall oil palms. J. Oil Palm Res. 26(2), 125-132.

Mesa, J. 2016. La productividad un compromiso gremial. Rev. Palmas 37(4), 56-65

Mosquera, M., C. Fontanilla, C. Martínez, A. Sánchez, and W. Alarcón. 2009. Identifying oil palms with ripe bunches before harvesting, IRBBH: a strategy for increasing labor productivity. pp. 993-1006. Proceedings of the global economics and marketing (GEM) conference: PIPOC 2009 International Palm Oil Congress: palm oil - balancing ecologics with economics, 2009, November 9-12, Kuala Lumpur. Malaysian Palm Oil Board, Kuala Lumpur.

Mosquera, M., E. Ruiz, C.A. Fontanilla, M. Valderrama, D.F. López, and L.E. Castro, and M.A. González. 2017a. Costos de producción para el cultivo de la palma de aceite en Colombia. pp. 427-435. In: Moreno Y. and E. Mantilla (eds.). Mejores prácticas agroindustriales del cultivo de la palma de aceite en Colombia. Cenipalma, Bogota.

Mosquera, M., E. Ruiz, and E. Mesa. 2017b. Economic assessment of technology adoption in oil palm plantations from Colombia. Int. J. Financ. Res. 8(3), 74-84. Doi: 10.5430/ijfr.v8n3p74

Mosquera, M., E. Ruiz, D. Munévar, L. Castro, L. Díaz, and D. López. 2020. Costos de producción 2019 para la palmicultura colombiana. Estudio de referenciación competitiva entre empresas que han adoptado mejores prácticas. Rev. Palmas 41(4), 3-16.

Mosquera-Montoya, M., D. López, E. Ruíz, M. Valderrama, and L. Castro. 2019. Mano de obra en cultivos de palma aceitera de Colombia: participación en el costo de producción y demanda. Rev. Palmas 40(1), 46-53.

Munévar, D., E. Ruiz, W. Díaz, D. Báez, J.S. Hernández, O. Salamanca, and M. Mosquera. 2020. Cosecha en cultivos de palma de aceite mediante el uso del *grabber*: caso de estudio en una plantación de Colombia. Rev. Palmas 41(2), 13-26.

Ruiz, E., E. Mesa, M. Mosquera, and J. Barrientos. 2017. Technological factors associated with oil palm yield gaps in the Central region in Colombia. Agron. Colomb. 35(2), 256-264. Doi: 10.15446/agron.colomb.v35n2.61894

Sánchez, C., C. Fontanilla, and M. Mosquera. 2010. Métodos para el desarrollo de estudios de tiempos y movimientos para labores del cultivo en palma de aceite. Cenipalma, Bogota.

Sinambela, R., T. Mandang, I.D.M. Subrata, and W. Hermawan. 2020. Application of an inductive sensor system for identifying ripeness and forecasting harvest time of oil palm. Sci. Hortic. 265, 109231. Doi: 10.1016/j.scienta.2020.109231

Ginkgo biloba L. mini-cuttings: indole butyric acid, substrates, and biochemical composition of the mother plants

Miniestacas de *Ginkgo biloba* L.: ácido indolbutírico, substratos y composición bioquímica de las plantas madre

Renata de Almeida Maggioni[1], Leandro Porto Latoh[1], Leandro Marcolino Vieira[1]*, Emilio Romanini Netto[2], and Katia Christina Zuffellato-Ribas[1]

ABSTRACT

The objective of this study was to evaluate the viability of the *Ginkgo biloba* mini-cutting technique, as well as the influence of substrates and different concentrations of indole butyric acid (IBA) on adventitious rooting in addition to the protein and sugar content in the mini-cutting. Mini-cuttings were 4 ± 1 cm in length, with the bases immersed in solutions of 0, 1000, 2000, and 3000 mg L^{-1} IBA. They were then planted in polypropylene tubes using two substrates (vermiculite and Tropstrato®) and maintained under greenhouse conditions for 60 d. The experiment was carried out with a 2 × 4 factorial scheme (substrates × IBA). There was no influence of IBA application on the promotion of rhizogenesis in *Ginkgo biloba* mini-cuttings. The rooting percentages were higher than 55% regardless of the treatment used. The vermiculite substrate showed a higher number of roots (4.94) and lower mortality (11.60) of mini-cuttings than Tropstrato®. We conclude that the mini-cutting technique is feasible for *Ginkgo biloba*, and the use of IBA is not necessary. We found that the induction of adventitious rooting depended on the biochemical composition of the mother plants, due to the translocation of non-reducing sugars and leaf proteins for root formation.

Key words: vegetative propagation, medicinal plants, plant growth regulators, medicinal plant cloning, rooting.

RESUMEN

El objetivo de este estudio fue evaluar la viabilidad de la técnica de miniestacas de *Ginkgo biloba*, así como la influencia de diferentes sustratos y diferentes concentraciones de ácido indolbutírico (IBA) en la formación de raíces adventicias además del contenido de proteínas y azúcares en las miniestacas. Las miniestacas tenían 4 ± 1 cm de longitud, y sus bases se sumergieron en soluciones de 0, 1000, 2000 y 3000 mg L^{-1} IBA. Luego las miniestacas se plantaron en tubos de polipropileno usando dos sustratos (vermiculita y Tropstrato®), bajo condiciones de invernadero durante 60 d. El experimento se llevó a cabo en un esquema factorial 2 × 4 (sustratos × IBA). No hubo influencia de la aplicación de IBA en la promoción de la rizogénesis de las miniestacas de *Ginkgo biloba*. Los porcentajes de enraizamiento fueron superiores al 55%, independientemente del tratamiento utilizado. El sustrato de vermiculita mostró un mayor número de raíces (4.94) y menor mortalidad (11.60) de miniestacas que el Tropstrato®. Por lo tanto, se puede concluir que la técnica de miniestaca es factible para *Ginkgo biloba*, y que el uso de IBA no es necesario. Se encontró que la inducción de raíces adventicias depende de la composición bioquímica de las plantas madre, debido a la translocación de azúcares no reductores y proteínas de la hoja para la formación de las raíces.

Palabras clave: propagación vegetativa, plantas medicinales, reguladores del crecimiento, clonación de plantas medicinales, enraizamiento.

Introduction

Ginkgo biloba L. (Ginkgoaceae) is a deciduous species from Asia whose extracts, leaves, and fruits have been used in the treatment of mental illnesses for more than 2000 years (Zhang *et al.*, 2011). It is one of the most studied and popular medicinal plants with contents of gincolids and flavonoids, free radical scavenging properties, and a proven effect on the human cardiovascular system, particularly in cerebral circulation (Lin *et al.*, 2008; Van Beek & Montoro, 2009; Song *et al.*, 2010; El-Ghazaly *et al.*, 2015). Besides its medicinal potential, gingko also has a high ornamental potential, with fan-shaped leaves, lush color that turns greenish-brown in autumn and yellowish in winter (Tommasi & Scaramuzzi, 2004; Bitencourt *et al.*, 2010).

Because it is a dioecious species, seedling production requires plants of both sexes, and this hinders species

Received for publication: 15 May, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.86430

[1] Programa de Pós-graduação em Agronomia (Produção Vegetal), Universidade Federal do Paraná (UFPR), Curitiba, Parana (Brazil).
[2] Programa de Pós-graduação em Meio Ambiente e Desenvolvimento, Universidade Federal do Paraná (UFPR), Curitiba, Parana (Brazil).
* Corresponding author: leandro.marcolino@ufpr.br

propagation (Singh *et al*., 2008). In addition, vegetative propagation of the species requires plant growth regulator application and, even so, the produced plants have low vigor of (Acharya *et al*., 2001; Valmorbida & Lessa, 2008; Bitencourt *et al*., 2010). Based on this, it is necessary to develop and evaluate vegetative rescue techniques to optimize asexual propagation.

Vegetative propagation of plants has been one of the most important methods of clonal forestry (Martins *et al*., 2011) in recent decades, representing significant gains for the industry and society (Steffens & Rasmussen, 2016) and ensuring uniformity in planting, higher productivity, and low cost and allowing the selection of desirable characteristics (Xavier *et al*., 2009; Wendling *et al.,* 2016). Mini-cutting is a vegetative propagation technique that can be considered a specialization of conventional cutting. This technique basically consists of the use of seedlings propagated by seeds or rooted cuttings that will constitute a mini garden (Alfenas *et al*., 2009). The mini-cutting success is mainly related to cell and tissue rejuvenation that favors meristem differentiation to rooting formation (Ferriani *et al*., 2010).

The rooting and survival of mini-cuttings can be influenced by several factors such as physiological conditions, type and position of propagules, collection season, juvenility, substrate, and the use of plant growth regulators for rooting induction (Carpanezzi *et al*., 2001; Rezende, 2007). The level of endogenous auxin is considered a critical factor in adventitious rooting in many tree species, requiring the use of exogenous auxins to induce rooting (Wendling *et al*., 2015).

The objective of this study was to evaluate the viability of the *Ginkgo biloba* mini-cutting technique, testing different substrates and concentrations of indole butyric acid (IBA). In addition, the contents of sugars and proteins related to the rhizogenesis of the species will verify the relationship between adventitious rooting and the biochemical composition of cuttings.

Materials and methods

Experiment I - *Gingko biloba* mini-cuttings

Gingko biloba shoots were collected on October 16, 2017 from ~30 mini-stumps conducted in a mini-garden system (Fig. 1A) at the Forest species propagation laboratory (Embrapa Forests, Colombo-PR, 25°19' S and 49°09' W, 950 m a.s.l.). After collection, the plant material was kept in styrofoam boxes with water to avoid dehydration. The material was then transported to the Macropropagation Laboratory at the research and study group on cuttings (GEPE), located at the Federal University of Paraná (UFPR), in Curitiba - PR (25°44' S and 49°23' W, 920 m a.s.l.). From the shoots of the mini-stumps, stem mini-cuttings were obtained with 4 ± 1 cm length, with a bevel cut in the base and straight cut at the tip, maintaining a leaf in the apical portion (Fig. 1B).

After preparation, mini-cutting bases were immersed in the indole butyric acid (IBA) plant growth regulator in

FIGURE 1. A) Mini-stumps, B) mini-cutting, and C) experiment installed in the greenhouse.

50% hydroalcoholic solution for 10 sec (Bitencourt *et al.*, 2010), according to the treatments 0 mg L^{-1}, 1000 mg L^{-1}, 2000 mg L^{-1} and 3000 mg L^{-1}. The planting was carried out in polypropylene tubes with 53 cm^3 capacity, using two substrates separately: vermiculite of fine granulometry and the commercial substrate Tropstrato®. The cuttings were planted at about ⅓ the depth of the base and maintained for 60 d in a greenhouse with intermittent mist (temperature of 24°C ± 2°C and 80% relative humidity) (Fig. 1C), located in the Biological Sciences Department of the Federal University of Paraná.

The experiment was arranged in a completely randomized design with a 2 × 4 factorial scheme (substrates × IBA concentrations) for a total of 8 treatments with 4 replicates and 14 mini-cuttings per experimental unit, totaling 448 mini-cuttings.

After 60 d, the following variables were evaluated: percentage of rooted mini-cuttings (mini-cuttings that emitted roots of at least 1 mm in length) (Fig. 2A), number of roots per mini-cutting, average length of three major roots per mini-cutting (cm), percentage of mini-cuttings with calluses (mini-cuttings alive, without roots, with formation of undifferentiated cell mass in the base) (Fig. 2B), percentage of live mini-cuttings (mini-cutting without calluses and without roots) (Fig. 2C), percentage of dead mini-cuttings (mini-cuttings with necrotic tissues); percentage of mini-cutting shoots (live mini-cuttings with or without roots and callus, showing shoots of new leaves) (Fig. 2A), and percentage of mini-cuttings that retained their original leaves (live mini-cuttings, with or without roots and callus, which retained the original leaves at the time of evaluation) (Fig. 2A).

Experiment II - Biochemical composition of *Gingko biloba* mini-cuttings

For the biochemical analysis, ten *Ginkgo biloba* mini-cuttings were used at the time of the rooting and ten mini-cuttings at the time of evaluation at 60 d. For the quantification of the total protein content, the method described by Bradford (1976) was used and the total soluble sugar contents were determined by the phenol-sulfuric method described by Dubois *et al.* (1956).

Statistical analysis

For both experiments, the treatment variances were tested for homogeneity using the Bartlett's test ($P<0.05$) and normality test [Shapiro-Wilk (W)]. Subsequently, the variables were subjected to analysis of variance (ANOVA) and those with significant differences in relation to the F test had their means compared with the Tukey test ($P<0.01$ and $P<0.05$). Only the variables live mini-cuttings, percentage of mortality, callus formation, and percentage of mini-cuttings that retained their original leaves did not show normality but were homogeneous with the Bartlett test.

Results and discussion

Experiment I - *Gingko biloba* mini-cuttings

There was no significant interaction ($P<0.05$) between the substrate × IBA concentrations for all the evaluated

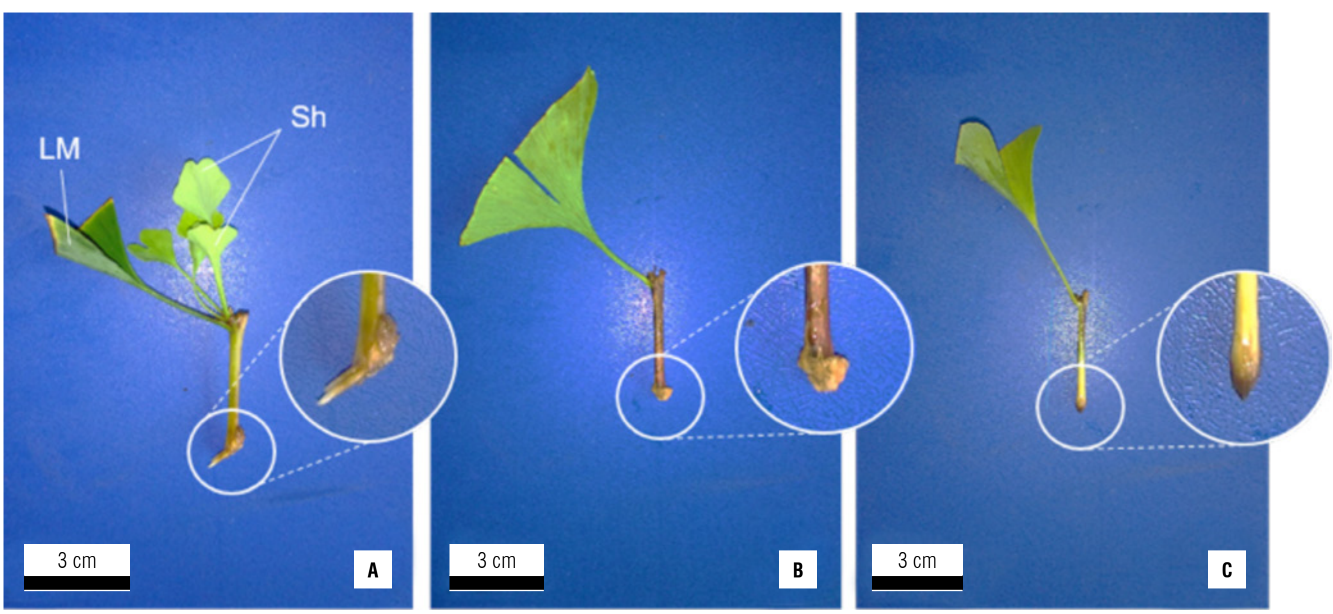


FIGURE 2. A) Rooted mini-cutting, B) mini-cutting with callus and C) live mini-cutting. LM - leaf maintenance; Sh - shoots of new leaves.

variables, indicating that these factors can act alone in the adventitious rooting of the species. The concentrations of IBA did not influence the induction and development of *Ginkgo biloba* roots (Tab. 1). In a previous conventional cutting study of the species, Valmorbida and Lessa (2008) observed that the IBA influenced adventitious rooting formation, when compared to the treatment without the use of this plant growth regulator, with rooting percentage results up to 80.55%. Bitencourt *et al.* (2010) found statistical differences with higher concentrations of IBA (4000 and 8000 mg L^{-1}) bound in talc on ginkgo cutting rooting (45.00 and 46.25% rooting percentages, respectively).

Thus, since the effect of IBA on the rooting of the species has not been observed, the results obtained in this study are promising considering the high rooting rates (mean of 62.05%). This result shows a positive effect of the rejuvenation of the plant material in the adventitious rooting of *Ginkgo biloba* and indicates the potential of the mini-cutting technique for the species, resulting in a reduction in the cost of production since plant regulators are not used for root induction.

One of the advantages of the mini-cutting technique is precisely the reduced need for exogenous auxin application due to the reinvigoration of parent plants (Stuepp *et al.*, 2015; Stuepp *et al.*, 2017b). Similarly, Stuepp *et al.* (2017a) confirm the efficiency of the mini-cutting technique for the species, resulting in a high rooting index (92.5%). The low percentages of live and dead mini-cuttings in all treatments are a consequence of the high levels of rooting with fast formation, demonstrating the adaptation of the material to greenhouse conditions. The high potential of rhizogenesis of *Ginkgo biloba* mini-cuttings validates the efficiency of the mini-cutting technique for the species.

Regarding the variable mini-cuttings with callus, the Tropstrato® substrate promoted a higher percentage of callus that differed statistically from the vermiculite substrate. The treatment without IBA application and with 1000 mg L^{-1} showed a higher percentage of callus when compared to the others; however, these treatments did not have an effect on the rooting of cuttings. The treatments that showed the highest percentage of calluses may be promising, indicating that a longer permanence of the mini-cuttings in the greenhouse could result in root induction. This fact is justified since the treatments with higher percentages of calluses were the same with lower percentages of rooting (Tab. 1). This inverse relationship between the percentage of callus and adventitious root formation has already been reported by other authors who observed that the formation of adventitious roots in *Ginkgo biloba* can occur from the callus tissue formed at the base of cuttings or mini-cuttings. This suggests that root induction could result from a longer permanence of mini-cuttings in the greenhouse (Valmorbida & Lessa, 2008; Bitencourt *et al.*, 2010; Stuepp *et al.*, 2017a).

There was a significant difference between the concentrations of IBA in the percentage of shoots, with the highest percentages observed in the treatments without IBA application and in the concentration of 3000 mg L^{-1} (Tab. 1). According to Moubayidin *et al.* (2010), root growth occurs when, in the apical meristem, cell division prevails over differentiation. This occurs when there is a greater concentration of auxins promoting cell division, in contrast to the cytokinin concentrations that promote cellular differentiation.

The auxin:cytokinin ratio regulates tissue morphogenesis. While a high auxin:cytokinin ratio stimulates

TABLE 1. Rooting percentage (R), number of roots per mini-cutting (NR), average length of three major roots per mini-cutting (LR), live mini-cuttings (A), percentage of mortality (M), callus formation (C), percentage of mini-cutting shoots (Sh), percentage of mini-cuttings that retained their original leaves (LM), subjected to substrates and concentrations of indole butyric acid (IBA) in *Ginkgo biloba* mini-cuttings.

Substrates	R (%)	NR	LR (cm)	A (%)	M (%)	C (%)	Sh (%)	LM (%)
Vermiculite	68.30 ^{a}	4.94 ^{a}	1.04 ^{a}	13.80 ^{a}	11.60 ^{b}	6.68 ^{b}	33.92 ^{a}	84.37 ^{a}
Tropstrato®	55.80 ^{a}	3.73 ^{b}	1.02 ^{a}	12.50 ^{a}	21.42 ^{a}	11.27 ^{a}	29.91 ^{a}	74.10 ^{a}
IBA								
0 mg L^{-1}	58.03 ^{a}	3.78 ^{a}	1.12 ^{a}	16.96 ^{a}	15.17 ^{b}	12.71 ^{a}	42.85 ^{a}	80.35 ^{a}
1000 mg L^{-1}	59.82 ^{a}	4.84 ^{a}	1.11 ^{a}	6.25 ^{b}	24.10 ^{a}	9.82 ^{ab}	24.99 ^{b}	74.10 ^{a}
2000 mg L^{-1}	60.71 ^{a}	4.56 ^{a}	0.97 ^{a}	16.90 ^{a}	14.28 ^{b}	8.03 ^{b}	26.78 ^{b}	83.03 ^{a}
3000 mg L^{-1}	69.64 ^{a}	4.15 ^{a}	0.93 ^{a}	12.50 ^{ab}	12.49 ^{b}	5.35 ^{b}	33.03 ^{ab}	79.46 ^{a}
CV(%)	31.70	29.75	42.93	53.02	32.92	37.72	34.58	17.96

Means followed by the same letter in each variable do not differ according to the Tukey test at 5% probability. CV: coefficient of variation.

the formation of adventitious roots, a low ratio leads to higher growth of the shoot (Taiz *et al*., 2017). According to Agulló-Antón *et al.* (2011) and Agulló-Antón *et al.* (2014), exogenous auxin supplementation in cuttings affect the endogenous cytokinin concentration, influencing adventitious rooting. Souza and Miranda (2006) found in an *in vitro* study that the proliferation of shoots and root formation in *Gerbera jamesonni* depends on the balance between auxin and cytokinin. Although a greater number of shoots was observed in the treatment without synthetic auxin application, adventitious rooting was not influenced, indicating that the presence of leaves induced an auxin-favorable balance.

It is possible to verify a close relationship between the rooting percentage and adventitious root development by maintaining mini-cutting leaves in all treatments (Tab. 1). The importance of leaf maintenance for these two variables has been reported in the literature, especially related to the presence of certain leaf compounds, such as carbohydrates, auxins and rooting cofactors, which can be translocated by phloem to the base of the cuttings, thus stimulating root formation (Bona & Biasi, 2010; Fragoso *et al*., 2015).

Experiment II - Biochemical composition of *Gingko biloba* mini-cuttings

Adventitious rooting is influenced by the biochemical composition of propagules (Yan *et al*., 2017). Some studies have demonstrated the correlation between rooting and carbohydrate content (Aslmoshtaghi & Reza-Shahsavar, 2010; Ragonezi *et al*., 2010; Denaxa, *et al.,* 2012).

The carbohydrates translocated by phloem are non-reducing sugars such as sucrose, because they are less reactive than reducing sugars (Taiz *et al*., 2017). This fact can be seen in Figure 3, where it is possible to verify a decrease in the non-reducing sugar concentration at the time of mini-cutting evaluation (60 d after experimental set up). Additionally, it is possible to observe a high level of non-reducing sugars at the moment of root evaluation.

These carbohydrates translocated by phloem can be used as an energetic resource in root induction and development (Aslmoshtaghi & Reza-Shahsavar, 2010; Souza *et al*., 2015) and as a carbon source for biosynthesis of amino acids and nucleic acids (Fachinello *et al*., 2005). They can also act in the regulation of gene expression (Wang & Ruan, 2013). Thus, the reduction of endogenous carbohydrate levels indicates that these sugars were used during root emission and growth (Husen & Pal, 2007), as demonstrated in the present study.

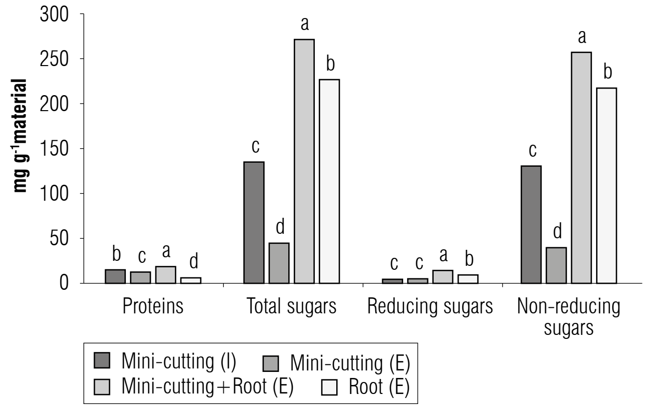


FIGURE 3. Biochemical composition of different organs of *Ginkgo biloba* and collection time. I - Installation; E - evaluation. Means followed by the same letter do not differ according to the Tukey test ($P<0.05$).

The quantity of protein in the mini-cuttings observed at the moment of evaluation was also lower than that observed at the moment of setting up the experiment (Fig. 3). Proteins are biochemical compounds associated with rooting, that can help in the induction, formation and development of adventitious roots (Taiz *et al*., 2017), and may be involved in the signaling and biosynthesis of auxins (Franklin *et al.,* 2011; Hornitschek *et al.,* 2012; Zhang *et al*., 2017).

When evaluated separately from cuttings, the reduction in the levels of non-reducing sugars and proteins during rooting that is associated with the high contents of these compounds in the roots (Fig. 3), indicates an ideal redirection of the source of drain energy. This fact reassures the importance of the nutritional status of the stock plant since it is directly related to the formation of the root system (Hartmann *et al*., 2011), as confirmed by the high rooting indexes in the present study. Furthermore, these results reinforce the importance of the nutritional status of the stock plant that directly affects the mini-cutting rooting and survival, and that is justified by the high rooting results observed in the present study.

Conclusion

The mini-cutting technique is feasible for the production of *Ginkgo biloba* seedlings regardless of the substrate used. Additionally, we concluded it is not necessary to use a plant growth regulator to induce rooting.

The induction of adventitious roots is dependent on the biochemical composition of the mother plants.

Acknowledgments

The authors would like to acknowledge the GEPE (Research and Study Group on Cuttings) and UFPR (Federal

University of Paraná) for providing the infrastructure used in this research and the Coordination for the Improvement of Higher Education Personnel (CAPES), by financial support of the research and scholarships granted.

Author's contributions

RDAM, LPL, LMV, and KCZ formulated the research goals and aims, RDAM, LPL, LMV, and ERN developed the methodology, RDAM, LMV, and KCZ conducted the research and investigation process, RDAM prepared the initial draft and created the visualization/data presentation, LPL implemented the software, LPL and LMV applied the statistical techniques to analyze study data, ERN and KCZ carried out the critical review, commentary, and revision of the manuscript, KCZ verified the overall replication/reproducibility of the results/experiments, provided the study materials, oversaw, managed and coordinated the research activity planning and execution, and acquired the financial support for the project.

Literature cited

Acharya, M., Ghosh, T. C., Acharya, R., & Acharya, K. (2001). *Ginkgo* propagation by simple cutting. *Indian Forester*, *127*(7), 827–828.

Agulló-Antón, M. Á., Sánchez-Bravo, J., Acosta, M., & Druege, U. (2011). Auxins or sugars: what makes the difference in the adventitious rooting of stored carnation cuttings? *Journal of Plant Growth Regulation*, *30*(1), 100–113. https://doi.org/10.1007/s00344-010-9174-8

Agulló-Antón, M. Á., Ferrández-Ayela, A., Fernández-García, N., Nicolás, C., Albacete, A., Pérez-Alfocea, F., Sánchez-Bravo, J., Pérez-Pérez, J. M., & Acosta, M. (2014). Early steps of adventitious rooting: morphology, hormonal profiling and carbohydrate turnover in carnation stem cuttings. *Physiologia Plantarum, 150*(3), 446–462. https://doi.org/10.1111/ppl.12114

Alfenas, A. C., Zauza, E. A. V., Mafia, R. G., & Assis, T. F. (2009). *Clonagem e doenças do eucalipto* (2nd ed.). Editora UFV.

Aslmoshtaghi, E., & Reza-Shahsavar, A. (2010). Endogenous soluble sugars, starch contents and phenolic compounds in easy- and difficult-to-root olive cuttings. *Journal of Biological and Environmental Sciences, 4*(11), 83–86.

Bitencourt, J., Zuffellato-Ribas, K. C., & Koehler, H. S. (2010). Estaquia de *Ginkgo biloba* L. utilizando três substratos. *Revista Brasileira de Plantas Medicinais*, *12*(2), 135–140. https://doi.org/10.1590/S1516-05722010000200002

Bona, C. M., & Biasi, L. A. (2010). Influence of leaf retention on cutting propagation of *Lavandula dentata* L. *Revista Ceres*, *57*(4), 526–529. https://doi.org/10.1590/S0034-737X2010000400014

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Carpanezzi, A. A., Tavares, F. R., & Sousa, V. A. (2001). Estaquia de Corticeira-do-Banhado (*Erythrina crista-galli* L.). *Embrapa Florestas-Comunicado Técnico 64*.

Denaxa, N. K., Vemmos, S. N., & Roussos, P. A. (2012). The role of endogenous carbohydrates and seasonal variation in rooting ability of cuttings of an easy and a hard to root olive cultivars (*Olea europaea* L.). *Scientia Horticulturae, 143*, 19–28. https://doi.org/10.1016/j.scienta.2012.05.026

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analitycal Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

El-Ghazaly, M. A., Sadik, N. A., Rashed, E. R., & Abd-El-Fattah, A. A. (2015). Neuroprotective effect of EGb761® and low-dose whole-body γ-irradiation in a rat model of Parkinson's disease. *Toxicology and Industrial Health*, *31*(12), 1128–1143. https://doi.org/10.1177/0748233713487251

Fachinello, J. C., Hoffmann, A., & Nachtigal, J. C. (2005). *Propagação de plantas frutíferas*. EMBRAPA informação tecnológica.

Ferriani, A. P., Zuffellato-Ribas, K. C., & Wendling, I. (2010). Miniestaquia aplicada a espécies florestais. *Revista Agroambiente On-line, 4*(2), 102–109. https://doi.org/10.18227/1982-8470ragro.v4i2.363

Fragoso, R. O., Witt, N. G. P. M., Obrzut, V. V., Valério, S., Zuffellato--Ribas, K. C., & Stuepp, C. A. (2015). Maintenance of leaves and indole butyric acid in rooting of juvenile Japanese Flowering Cherry cuttings. *Revista Brasileira de Ciências Agrárias, 10*(1), 97–101. https://doi.org/10.5039/agraria.v10i1a5111

Franklin, K. A., Lee, S. H., Patel, D., Kumar, V., Spartz, A. K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J. D., Wigge, P. A., & Gray, W. M. (2011). Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proceedings of the National Academy of Sciences*, *108*(50), 20231–20235. https://doi.org/ 10.1073/pnas.1110682108

Hartmann, H. T., Kester, D. E., Davies Jr, F. T., & Geneve, R. L. (2011). *Hartmann and Kester's plant propagation: principles and practices* (8th ed.). Prentice Hall.

Hornitschek, P., Kohnen, M. V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J. M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I., & Fankhauser, C. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *The Plant Journal*, *71*(5), 699–711. https://doi.org/10.1111/j.1365-313X.2012.05033.x

Husen, A., & Pal, M. (2007). Effect of branch position and auxin treatment on clonal propagation of *Tectona grandis* Linn. f. *New Forests, 34*(3), 223–233. Doi: https://doi.org/10.1007/s11056-007-9050-y

Lin, L. Z., Chen, P., Ozcan, M., & Harnly, J. M. (2008). Chromatographic profiles and identification of new phenolic components of *Ginkgo biloba* leaves and selected products. *Journal of Agricultural and Food Chemistry, 56*(15), 6671–6679. https://doi.org/10.1021/jf800488x

Martins, F. B., Soares, C. P. B., Leite, H. G., Souza, A. L., & Castro, R. V. (2011). Índices de competição em árvores individuais de eucalipto. *Pesquisa Agropecuária Brasileira, 46*(9), 1089–1098. https://doi.org/10.1590/S0100-204X2011000900017

Moubayidin, L., Perilli, S., Ioio, R. D., Mambro, R. D. M., Costantino, P., & Sabatini, S. (2010). The rate of cell differentiation controls the *Arabidopsis* root meristem growth phase. *Current Biology*, *20*(12), 1138–1143. https://doi.org/10.1016/j.cub.2010.05.035

Ragonezi, C., Klimaszewska, K., Castro, M. R., Lima, M., Oliveira, P., & Zavattieri, M. A. (2010). Adventitious rooting of conifers: influence of physical and chemical factors. *Trees, 24*(6), 975–992. https://doi.org/10.1007/s00468-010-0488-8

Rezende, A. A. (2007). *Enraizamanto de estacas de candeia (Eremanthus erythropappus (DC.) MacLeish).* [Master's thesis, Universidade Federal de Lavras]. UFLA Repository. http://repositorio.ufla.br/bitstream/1/2714/1/DISSERTA%C3%87%-C3%83O_Enraizamento%20de%20estacas%20de%20candeia%20(Eremanthus%20erytropappus%20DC.)%20Mac%20Leish.pdf

Singh, B., Kaur, P., Gopichand, Singh, R. D., & Ahuja, P. S. (2008). Biology and chemistry of *Ginkgo biloba*. *Fitoterapia, 79*(6), 401–418. https://doi.org/10.1016/j.fitote.2008.05.007

Song, J., Fang, G., Zhang, Y., Deng, Q., & Wang, S. (2010). Fingerprint analysis of *Ginkgo biloba* leaves and related health foods by high-performance liquid chromatography/electrospray ionization-mass spectrometry. *Journal of AOAC International, 93*(6), 1798–1805.

Souza, C. M., & Miranda, R. M. (2006). Otimização do balanço entre auxina e citocinina para multiplicação *in vitro* de *Gerbera jamesonni* var. 'Ornela'. *Revista Agronomia*, *40*(1–2), 66–72.

Souza, E. R., Lenk, F. L., Ono, E. O., & Rodrigues, J. D. (2015). Conteúdo de carboidratos em estacas de videira do porta-enxerto cv. IAC 572. *Brazilian Journal of Applied Technology for Agricultural Science, 8*(2), 7–15. https://doi.org/10.5935/PAeT.V8.N2.01

Steffens, B., & Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology*, 170, 603–617. https://doi.org/10.1104/pp.15.01360

Stuepp, C. A., Zuffellato-Ribas, K. C., Koehler, H. S., & Wendling, I. (2015). Rooting mini-cuttings of *Paulownia fortunei* var. *Mikado* derived from clonal mini-garden. *Revista Árvore, 39*(3), 497–504. https://doi.org/10.1590/0100-67622015000300010

Stuepp, C. A., Fragoso, R. O., Maggioni, R. A., Zuffellato-Ribas, K. C., & Wendling, I. (2017a). Vegetative rescue and *ex vitro* plants production system for *Ginkgo biloba* by cuttings and mini-cuttings. *Revista Brasileira de Plantas Medicinais*, *19*(2), 300–303.

Stuepp, C. A., Wendling, I., Koehler, H. S., & Zuffellato-Ribas, K. C. (2017b). Successive mini-cuttings collection in *Piptocarpha angustifolia* mini-stumps: Effects on maturation, adventitious root induction and root vigor. *Acta Scientiarum Agronomy, 39*(2), 245–253. https://doi.org/10.4025/actasciagron.v39i2.31059

Taiz, L., Zeiger, E., Møller, I. A., & Murphy, A. (2017). *Fisiologia e Desenvolvimento Vegetal* (6th ed.). Artmed.

Tommasi, F., & Scaramuzzi, F. (2004). *In vitro* propagation of *Ginkgo biloba* by using various bud cultures. *Biologia Plantarum*, *48*, 297–300. https://doi.org/10.1023/B:BIOP.0000033460.75432.d1

Valmorbida, J., & Lessa, A. O. (2008). Enraizamento de estacas de *Ginkgo biloba* tratadas com ácido indolbutírico e ácido bórico. *Ciência e agrotecnologia, 32*(2), 398–401. https://doi.org/10.1590/S1413-70542008000200008

Van Beek, T. A., & Montoro, P. (2009). Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. *Journal of Chromatography*, *1216*(11), 2002–2032. https://doi.org/10.1016/j.chroma.2009.01.013

Wang, L., & Ruan, Y. (2013). Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Science*, *4*, 1–9. https://doi.org/10.3389/fpls.2013.00163

Wendling, I., Brooks, P. R., & Trueman, S. J. (2015). Topophysis in *Corymbia torelliana* × *C. citriodora* seedlings: adventitious rooting capacity, stem anatomy, and auxin and abscisic acid concentrations. *New Forests*, *46*, 107–120. https://doi.org/10.1007/s11056-014-9451-7

Wendling, I., Stuepp, C. A., & Zuffellato-Ribas, K. C. (2016). Araucaria clonal forestry: types of cuttings and mother tree sex in field survival and growth. *Cerne*, *22*(1), 19–26. https://doi.org/10.1590/01047760201622012105

Xavier, A., Wendling, I., & Silva, R. L. (2009). *Silvicultura clonal: princípios e técnicas* (2nd ed.). Editora UFV.

Yan, S. P., Yang, R. H., Wang, F., Sun, L. N., & Song, X. S. (2017). Effect of auxins and associated metabolic changes on cuttings of hybrid aspen. *Forests*, *8*(4), Article 117. https://doi.org/10.3390/f8040117.

Zhang, L., Lam, W. P., Lü, L., Wang, Y. X., Wong, Y. W., Lam, L. H., Tang, H. C., Wai, M. S., Mak, Y. T., Wang, M., & Yew, D. T. (2011). How would composite traditional chinese medicine protect the brain - an example of the composite formula "Pien Tze Huang". *Current Medicinal Chemistry*, *18*(23), 3590–3594. https://doi.org/10.2174/092986711796642535

Zhang, W., Fan, J., Tan, Q., Zhao, M., & Cao, F. (2017). Mechanisms underlying the regulation of root formation in *Malus hupehensis* stem cuttings by using exogenous hormones. *Journal of Plant Growth Regulation, 36*(1), 174–185. https://doi.org/10.1007/s00344-016-9628-8

SCIENTIFIC NOTE

Optimization of the extraction of antioxidant compounds from quinoa (*Chenopodium quinoa* Willd.)

Optimización de la extracción de compuestos antioxidantes a partir de quinua (*Chenopodium quinoa* Willd.)

Julia Luisetti[1]*, Héctor Lucero[1], and María Cristina Ciappini[1]

ABSTRACT

Argentina contains a great biodiversity of natural foods such as quinoa that can be included in the human diet because of their nutritional characteristics and content of bioactive compounds. Among other properties, these bioactive have an antioxidant capacity that protects biomolecules from oxidant damage. Bioactive compounds contribute beneficially to diverse antimicrobial, anti-inflammatory and anti-carcinogenic physiological activities. The objective of this study was to optimize the parameters for the extraction of antioxidant compounds from quinoa: drying temperature of the grain, liquid/solid ratio (L/S), and ethanol concentration in the extraction solvent, based on an experimental design of three variables at three levels. A face-centered central composite design was used. The proposed levels were 40°C, 60°C and 80°C; 20:1, 30:1 and 40:1, and 30%, 50%, and 70% v/v of ethanol. Antioxidant capacity was determined by the capture of the DPPH free radical. The values obtained were from 16.3 mg to 161.5 mg of equivalent trolox (ET) 100 g^{-1} of quinoa. The maximum antioxidant capacity was obtained for the L/S ratio of 28:1, and the drying temperature of the grain was 58°C and 39% v/v of ethanol in the extraction solvent. The ethanol concentration was the most influential variable in the antioxidant compound extraction.

Key words: hydroalcoholic extraction, bioactive compounds, face-centered central composite design.

RESUMEN

Argentina posee una gran biodiversidad de alimentos tales como la quinua que pueden ser incluidos en la dieta humana por sus características nutricionales y su contenido de compuestos bioactivos. Estos compuestos, entre otras propiedades, presentan una capacidad antioxidante que protege a las biomoléculas frente al daño oxidativo. Los compuestos bioactivos contribuyen benéficamente a diversas actividades fisiológicas antimicrobianas, antinflamatorias y anticancerígenas. El objetivo de este estudio fue optimizar los parámetros para la extracción de compuestos con capacidad antioxidante de la quinua: temperatura de secado del grano, relación liquido/sólido (L/S) y concentración de etanol en el solvente de extracción, basado en un diseño experimental de tres variables a tres niveles. Se uso un diseño central compuesto centrado en las caras. Los niveles propuestos fueron 40°C, 60°C y 80°C; 20:1, 30:1 y 40:1, y 30%, 50%, 70% v/v de etanol. La capacidad antioxidante se determinó mediante la captura del radical libre DPPH. Se obtuvieron valores desde 16.3 a 161.5 mg de trolox equivalente (TE) 100 g^{-1} de quinua. La máxima capacidad antioxidante se obtuvo para la relación L/S de 28:1, 58°C de temperatura de secado de grano y 39% v/v de etanol en el solvente de extracción. La variable de mayor influencia fue la concentración de etanol en el solvente.

Palabras clave: extracción hidroalcohólica, compuestos bioactivos, diseño central compuesto centrado en las caras.

Introduction

Quinoa is an annual plant from the Andean region of South America, cultivated from Colombia to Argentina and Chile. In recent years, there has been a progressive increase in quinoa crops, especially in Bolivia, Peru, and Ecuador, which have been the main producers (FAO-ALADI, 2014).

Quinoa exhibits multiple abilities to adapt itself to solar radiation, temperature, water availability, and atmospheric CO_2 concentration, allowing its cultivation in different agroecological zones (Zurita-Silva *et al*., 2014; Melo, 2016; Reguera *et al*., 2018). It is a plant with additional notable agronomic adaptations to different adverse weather conditions like drought, high salinity, and frosts (Ruiz *et al*., 2014; Ruiz *et al*., 2016).

Quinoa is considered a pseudocereal because of its particular composition, making it a grain of special interest as a human food. Its grains are exceedingly nutritious (López *et al*., 2011; Padrón Pereira *et al*., 2015). Quinoa seeds are rich in lipids, carbohydrates, polyphenols, fiber, and proteins

Received for publication: 22 April, 2020. Accepted for publication: 11 December, 2020. Doi: 10.15446/agron.colomb.v38n3.86520

[1] Centro de Investigación y Desarrollo en Tecnología de los Alimentos (CIDTA), Facultad Regional Rosario, Universidad Tecnológica Nacional, Santa Fe (Argentina).
* Corresponding author: jluisetti@frro.utn.edu.ar

(Padrón-Pereira *et al*., 2015; Tang *et al*., 2015; Fischer *et al*., 2017; Vidaurre-Ruiz *et al*., 2017; Jaikishun *et al*., 2019). The protein contents range between 12% and 16% depending on the quinoa cultivar. They have high nutritional value because of the presence of essential amino acids such as leucine and isoleucine. These seeds contain proteins without gluten, and they are currently appearing in diets free of this component (Álvarez-Jubete *et al*., 2009).

Argentina has great diversity of foods that protect biomolecules from oxidative damage by different mechanisms. There are two fundamental strategies to protect the organism from free radicals: the enzymatic and non-enzymatic (endogenous and exogenous) (Wu, 2015). The endogenous strategy requires external support, but exogenous antioxidants are recommended as they can capture free radicals from oxygen and chelate transition metals. Exogenous antioxidants can generate reactive oxygen species (ROS) by the Fenton/Haber-Weiss reaction in its free ionic state. This phytochemical benefit is present in quinoa, but it varies in different cultivars of the same plant (Tang *et al*., 2015).

In addition to being incorporated into the daily diet, concentrate extracts can be considered as possible sources of antioxidant compounds that can be used for the enrichment of other products with antioxidant compounds. The extraction process is regulated by different variables like extraction temperature, the nature of the extraction solvent, and its concentration, treatment time, and the state of aggregation of the substrate. Engineering aims to develop different optimal processes to meet the variable values and costs.

In this research, the optimal values of the pretreatment parameters for the extraction of antioxidant compounds were studied. These were the drying temperature of quinoa grains, liquid/solid ratio, and the ethanol concentration in the extraction solvent.

Materials and methods

Quinoa was provided by the Quinoa Real company, whose crops are located in Yavi, Jujuy, Argentina (22°7'47" S, 65°27'44" W). The cultivar used was Real Blanca. Quinoa was preserved in glass flasks of 5 L with hermetic covers and stored in the dark until used. The trials were carried out in a uniform batch material.

Obtaining antioxidant compounds

Following the wet de-saponification treatment of quinoa, the grains were dried in a forced convection laboratory dryer (Tecnodalvo Model CHC/F/I, Argentina) until reaching a moisture content of 12%. This process was required to avoid the deterioration caused by microorganisms. De-saponified and dried quinoa grains were ground in a mill (IKA, Germany) to pass through a 40-mesh sieve.

The flour obtained was added to a mixture of water and ethanol for 1 h in an orbital shaker (OS-20 Orbital Shaker, Boeco Germany, Germany) at 25°C. This solution was filtered and conserved at 4 ± 1°C in a caramel-colored container, until subsequent tests. Water-ethanol mixtures were used as extractants because of their efficiency, selectivity, low cost, low toxicity, and ease of removal.

Antioxidant capacity determination

The compound 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable radical that has an intense violet color and absorbs at 517 nm. The method proposed by Brand-Williams *et al*. (1995) evaluates the antioxidant capacity by measuring the reduction of the absorbance. It translates into a DPPH concentration decrease because of the scavenging effect.

One ml of the quinoa water-ethanol solution was reacted with 5 ml of 100 μM DPPH solution prepared at the moment of use. The mixture was conserved at room temperature in the dark for 50 min. Then, absorbance at 517 nm was recorded by using a UV-1800 spectrophotometer (UV-1800 Spectrophotometer, Shimadzu, Japan). The results were expressed as Equivalent Trolox (ET) 100 g^{-1} quinoa on dry bases (d.b.). A calibration curve was obtained using a Trolox standard solution.

The linear equation was as follows:

$$y = -108.6x + 103 \qquad (1)$$

with $R^2 = 0.996$, where y = mg de ET/L of solution and x = absorbance.

Experimental design and statistical analysis

The optimization of the extraction process of compounds with antioxidant capacity was performed based on an experimental design of three variables and three levels; therefore, a face-centered central composite design was used. The parameters and their levels were as follow: grain drying temperature (T) of 40°C, 60°C and 80°C, liquid/solid extractant ratio (L/S): 20:1, 30:1 and 40:1, and ethanol concentration in the solvent (% Et): 30%, 50%, and 70% v/v.

Table 1 shows codified values of the levels (variables) used in the experimental design and their corresponding real values for the entry factors.

TABLE 1. Relationship among codified variables and real values of independent variables.

	Temperature T (°C)	Ethanol concentration % Et	Liquid/solid ratio L/S
Level -1	40	30	20
Level 0	60	50	30
Level +1	80	70	40

Eighteen experiences in a group of 8 were required to check the two-level design (-1, 1) to the three factors (2^k, k = 3); Four replicates were used on the central point to evaluate the pure error, and the remaining six trials to test faced-centered values (star points).

Linear interaction and quadratic coefficients were evaluated by an ANOVA (*P*<0.05). F-test and probability (p) were determined to analyze the significant statistical contribution of all the terms. The results of the experimental design were processed by applying multiple regression analyses. The model's goodness of fit was checked by the coefficient of determination (R^2) and the significance of the model terms was established with a confidence level of 95%. The process conditions were optimized by response-surface graphics. All the analyses were performed using the free version of the Minitab 18 software (Minitab, Pennsylvania, USA).

Results and discussion

The values of the antioxidant capacity obtained for the different test conditions are shown in Table 2.

The antioxidant capacity values were coincident with the range published by Vollmannová *et al.* (2013), who evaluates the antioxidant capacity of five cultivars of quinoa by the DPPH method. They report values between 65.08 mg and 310.36 mg ET 100 g^{-1} quinoa. Valencia *et al.* (2017), using 24 Peruvian quinoa accessions by the DPPH method achieve values between 121.66 mg and 299.28 mg ET 100 g^{-1} of quinoa. It would be possible that the antioxidant capacity differs between plant varieties and among the parts of the same plant and the maturation phase of each plant organ (Sawa *et al.*, 1999). Factors such as soil characteristics, climatic conditions, and storage conditions could modify the content of antioxidant capacity compounds (Naczk & Shahidi, 2006).

TABLE 2. Antioxidant capacity response values.

Temperature T °C	Ethanol concentration % Et	Liquid/solid ratio L/S	Antioxidant capacity mg equivalent Trolox 100 g^{-1} quinoa
40	30	20	107.4 ± 1.9
80	30	20	101.8 ± 0.2
40	70	20	70.8 ± 0.7
80	70	20	63.3 ± 0.5
40	30	40	105.1 ± 4.8
80	30	40	97.0 ± 5.3
40	70	40	16.3 ± 1.1
80	70	40	25.0 ± 0.6
60	50	30	156.3 ± 7.3
60	50	30	158.9 ± 0.3
60	50	30	157.5 ± 5.2
60	50	30	161.5 ± 3.3
40	50	30	100.9 ± 4.3
80	50	30	61.7 ± 3.2
60	30	30	104.1 ± 0.3
60	70	30	51.7 ± 3.8
60	50	20	102.9 ± 3.1
60	50	40	30.9 ± 0.7

A quadratic equation (Eq. 2) was obtained by the software. It showed the effects of each factor, and its interactions according to the antioxidant capacity.

$$\text{mg TE 100 g}^{-1}\text{ d.b.} = -425.5 + 6.48\ T(°C) + 5.03\ \%Et + 20.40\ L/S - 0.0562\ T(°C)*T(°C) - 0.0647\ \%Et*\%Et - 0.3687\ L/S*L/S \quad (2)$$

The statistical analysis (Tab. 3) showed that the percentage of ethanol and the L/S ratio had a significant effect (*P*<0.05) on the antioxidant capacity as well as the quadratic interaction of temperature, percentage of ethanol, and the L/S ratio. A second-degree polynomial model could be used to represent the relationship between the selected parameters. The percentage of ethanol in the solvent showed the most influence.

TABLE 3. Antioxidant capacity values of extracts of quinoa seeds for different conditions of drying temperature (°C), percentage of ethanol (% Et) and L/S ratio.

Source of variation	T	%Et	L/S	T(°C) x T(°C)	%Et*%Et	L/S*L/S	Model
***P*-value**	0.321	0.000	0.006	0.041	0.022	0.003	0.000
Model summary	S	15.6537	R^2	93.26%	R^2 (adj)	88.54%	

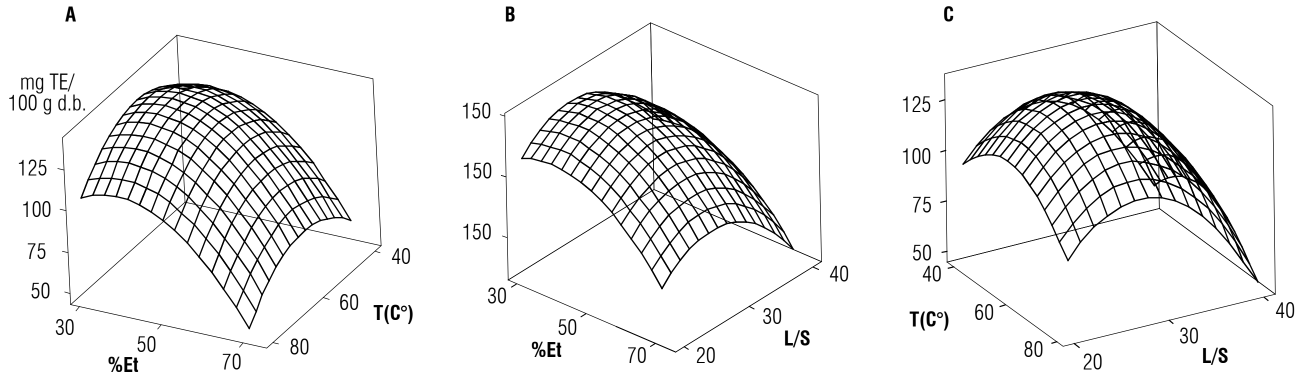


FIGURE 1. Antioxidant capacity response surface, expressed as mg Trolox equivalent of 100 g^{-1} of quinoa, according to the process variables: drying grain temperature (T), % of ethanol, and liquid/solid ratio (L/S). A) Setting values of the L/S ratio = 30:1; B) Setting values of T = 60°C; C) Setting values of % Et = 50 % v/v.

Figure 1 shows the behavior of the antioxidant capacity according to the three studied variables. When setting the L/S ratio (Fig. 1A), the antioxidant capacity increased at temperature values near 60°C. Maintaining a constant temperature, the antioxidant capacity was the maximum when alcohol concentration in the solvent was closed to 40% (Fig. 1B). Setting the ethanol concentration (Fig. 1C), the highest antioxidant capacity value was obtained when the L/S ratio was near 30:1, and it was lower for 20:1 and 40:1.

The values of process variables that maximize the antioxidant capacity response were 58°C grain drying temperature, 39% v/v ethanol concentration in the extractant, and a L/S ratio of 28:1.

Miranda *et al.* (2010) evaluate the impact of different drying temperatures on nutritional properties, the content of total phenolic compounds, and the antioxidant capacity of quinoa grains. They find that drying temperatures between 60°C and 80°C lead to degradation of total phenolic compounds. Vidaurre-Ruiz *et al.* (2017) find that phenolic compounds, flavonoids and betalamic pigments of Negra Collana, and Pasankalla varieties of quinoa decrease significantly after the drying process, while other compounds with higher antioxidant capacity are formed.

The optimum L/S ratio used in the extraction will depend on two basic considerations: the maximum content of the solvent is limited in practice by the consequences of the dilution of the substrates of interest and the performance of the extract according to the compounds of interest is directly related to the content of the solid from the starting material. The optimum value will result from the implementation of these considerations (Carciochi, 2014).

Regarding the solvent, the increase of the antioxidant capacity for extractant mixtures with 39% of ethanol indicates a higher presence of bioactive compounds of hydrophilic nature in quinoa (Repo-Carrasco-Valencia & Serna 2011; Stikic *et al.*, 2012; Abderrahim *et al.*, 2015; Fischer *et al.*, 2017; Valencia *et al.*, 2017). Since we propose that the extractant can be used in the food industry, the use of non-toxic solvents is crucial. Particularly, water-ethanol mixtures are most commonly used to obtain antioxidant extracts, due to their efficiency, selectivity, low cost, low toxicity and easy removal (Silva *et al.*, 2007; Wang *et al.*, 2008; Zhang *et al.*, 2011; Gong *et al.*, 2012). By changing the proportion of water and ethanol, solvent polarities change, modifying the nature of the extract, according to the different solubility of phenolic compounds present in the original matrix (Galvan d'Alessandro *et al.*, 2012).

Conclusions

In this preliminary study, optimal values of grain drying temperature, ethanol percentage in the extractant mixture, and the L/S ratio to the extraction process of antioxidant compounds from quinoa were established (Chenopodium quinoa Willd.). The optimal processing values were a L/S ratio of 28:1, 58°C and 39% v/v of ethanol in the extraction solvent. The ethanol concentration was the most influential variable in the extraction of the antioxidant compound. Further research and a consideration of other processing variables, such as temperature and time of extraction, need to be carried out.

Author's contributions

JL conducted the investigation and formal analysis and prepared the initial draft. HL developed the methodology, provided the study materials, reviewed and edited the manuscript, and oversaw the research activity planning and execution. MCC reviewed and edited the manuscript, managed and coordinated the research activity planning and execution, and acquired the financial support for the project.

Literature cited

Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., González, M., & Condezo-Hoyos, L. (2015). Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa* Willd.) from Peruvian Altiplano. *Food Chemistry, 183*, 83–90. https://doi.org/10.1016/j.foodchem.2015.03.029

Álvarez-Jubete, L., Arendt, E. K., & Gallagher, E. (2009). Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *International Journal of Food Sciences and Nutrition, 60*(S4), 240–257. https://doi.org/10.1080/09637480902950597

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Carciochi, R. A. (2014). *Obtención de ingredientes alimenticios con capacidad antioxidante mejorada por aplicación de distintos procesos a semillas de quinoa (Chenopodium quinoa).* [Doctoral dissertation, Universidad de Buenos Aires]. Core. https://core.ac.uk/download/pdf/299812718.pdf

FAO-ALADI. (2014). Tendencias y perspectivas del comercio internacional de quinua. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Asociación Latinoamericana de Integración. http://www.fao.org/3/a-i3583s.pdf

Fischer, S., Wilckens, R., Jara, J., Aranda, M., Valdivia, W., Bustamante, L., Graf, F., & Obal, I. (2017). Protein and antioxidant composition of quinoa (*Chenopodium quinoa* Willd.) sprout from seeds submitted to water stress, salinity and light conditions. *Industrial Crops and Products, 107*, 558–564. https://doi.org/10.1016/j.indcrop.2017.04.035

Galvan d'Alessandro, L., Kriaa, K., Nikov, I., & Dimitrov, K. (2012). Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and Purification Technology, 93*, 42–47. https://doi.org/10.1016/j.seppur.2012.03.024

Gong, Y., Hou, Z., Gao, Y., Xue, Y., Liu, X., & Liu, G. (2012). Optimization of extraction parameters of bioactive components from deffated marigold (*Tagetes erecta* L.) residue using response surface methodology. *Foods and Bioproducts Processing, 90*(1), 9–16. https://doi.org/10.1016/j.fbp.2010.12.004

Jaikishun, S., Li, W., Yang, Z., & Song, S. (2019). Quinoa: In perspective of global challenges. *Agronomy, 9,* 176. https://doi.org/10.3390/agronomy9040176

López, L., Capparelli, A., & Nielsen, A. (2011). Traditional post-harvest processing to make quinoa grains (*Chenopodium quinoa* var. *quinoa*) apt for consumption in Northern Lipez (Potosí, Bolivia): ethnoarchaeological and archaeobotanical analyses. *Archaeological and Anthropological Sciences, 3*, 49–70. https://doi.org/10.1007/s12520-011-0060-5

Melo, D. (2016). Studio di adattabilità colturale della quinoa (*Chenopodium quinoa* Willd.) in Italia settentrionale. [Doctoral dissertation, Università Cattolica del Sacro Cuore]. Sistema Bibliotecario d'Ateneo DocTA. http://tesionline.unicatt.it/handle/10280/35878.

Miranda, M., Vega-Gálvez, A., López, J., Parada, G., Sanders, M., Aranda, M., Uribe, E., & Di Scala, K. (2010). Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd). *Industrial Crops and Products, 32*(3), 258–263. https://doi.org/10.1016/j.indcrop.2010.04.019

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523–1542. https://doi.org/10.1016/j.jpba.2006.04.002

Padrón-Pereira, C. A., Oropeza-González, R. A., & Montes-Hernández, A. I. (2015). Semillas de quinua (*Chenopodium quinoa* Willdenow): composición química y procesamiento. Aspectos relacionados con otras áreas. *Revista Venezolana de Ciencia y Tecnología de Alimentos, 5*(2), 166–218.

Reguera, M., Conesa, C., Gil-Gómez, A., Haros, C., Pérez-Casas, M., Briones-Labarca, V., Bolaños, L., Bonilla, I., Álvarez, R., Pinto, K., Mujica, Á., & Bascuñán-Godoy, L. (2018). The impact of different agroecological conditions on the nutritional composition of quinoa seeds. *PeerJ*, *14*(6), Article e4442. https://doi.org/10.7717/peerj.4442

Repo-Carrasco-Valencia, R. A. M., & Serna, L. A. (2011). Quinoa (*Chenopodium quinoa* Willd.) as a source of dietary fiber and other functional components. *Ciência e Tecnologia de Alimentos, 31*(1), 225–230. https://doi.org/10.1590/S0101-20612011000100035

Ruiz, K. B., Biondi, S., Oses, R., Acuña-Rodríguez, I. S., Antognoni, F., Martínez-Mosqueira, E. A., Coulibaly, A., Canahua-Murillo, A., Pinto, M., Zurita-Silva, A., Bazile, D., Jacobsen, S. E., & Molina-Montenegro, M. A. (2014). Quinoa biodiversity and sustainability for food security under climate change. A review. *Agronomy for Sustainable Development, 34*(2), 349–359. https://doi.org/10.1007/s13593-013-0195-0

Ruiz, K. B., Biondi, S., Martínez, E. A., Orsini, F., Antognoni, F., & Jacobsen, S. E. (2016). Quinoa - a model crop for understanding salt-tolerance mechanisms in halophytes. *Plant Biosystems. Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana, 150*(2), 357–371. https://doi.org/10.1080/11263504.2015.1027317

Sawa, T., Nakao, M., Akaike, T., Ono, K., & Maeda, H. (1999). Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor promoter effect of vegetables. *Journal of Agricultural and Food Chemistry, 47*(2), 397–402. https://doi.org/10.1021/jf980765e

Silva, E. M., Rogez, H., & Larondelle, Y. (2007). Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology, 55*(3), 381–387. https://doi.org/10.1016/j.seppur.2007.01.008

Stikic, R., Glamoclija, D., Demin, M., Vucelic-Radovic, B., Jovanovic, Z., Milojkovic-Opsenica, D., Jacobsen, S., & Milovanovic, M. (2012). Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. *Journal of Cereal Science, 55*(2), 132–138. https://doi.org/10.1016/j.jcs.2011.10.010

Tang, Y., Li, X., Zhang, B., Chen, P. X., Liu, R., & Tsao, R. (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. *Food Chemistry, 166*(1), 380–388. https://doi.org/10.1016/j.foodchem.2014.06.018

Valencia, Z., Cámara, F., Ccapa, K., Catacora, P., & Quispe, F. (2017). Compuestos bioactivos y actividad antioxidante de Semillas de quinua peruana (*Chenopodium quinoa* W.). *Revista de la Sociedad Química del Perú, 83*(1), 16–29. https://doi.org/10.37761/rsqp.v83i1.100

Vollmannová, A., Margitanová, E., Tóth, T., Timoracká, M., Urminská, D., Bojňanská, T., & Čičová, I. (2013). Cultivar influence on total polyphenol and rutin contents and total antioxidant capacity in buckwheat, amaranth, and quinoa seeds. *Czech Journal of Food Science, 31*(6), 589–595. https://doi.org/10.17221/452/2012-CJFS

Vidaurre-Ruiz, J. M., Días-Rojas, G., Mendoza-Llamo, E., & Solano-Cornejo, M. A. (2017). Variación del contenido de Betalaínas, compuestos fenólicos y capacidad antioxidante durante el procesamiento de la quinua (*Chenopodium quinoa* W.). *Revista de la Sociedad Química del Perú, 83*(3), 319–330.

Wang, J., Sun, B., Cao, Y., Tian, Y., & Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry, 106*(2), 804–810. https://doi.org/10.1016/j.foodchem.2007.06.062

Wu, G. (2015). Nutritional properties of quinoa. Industrial crops and products. In K. Murphy, & J. Matanguihan (Eds.), *Quinoa: improvement and sustainable production* (pp. 193–210). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118628041.ch11

Zhang, G., He, L., & Hu, M. (2011). Optimized ultrasonic-assisted extraction of flavonoids from *Prunella vulgaris* L. and evaluation of antioxidant activities in vitro. *Innovative Food Science and Emerging Technologies, 12*(1), 18–25. https://doi.org/10.1016/j.ifset.2010.12.003

Zurita-Silva, A., Fuentes, F., Zamora, P., Jacobsen, S. E., & Schwember, A. R. (2014). Breeding quinoa (*Chenopodium quinoa* Willd.): potential and perspectives. *Molecular Breeding, 34*(1), 13–30. https://doi.org/10.1007/s11032-014-0023-5

Editorial policy

Agronomía Colombiana is a scientific and technical publication of the agricultural sector, edited by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia - Bogota campus. It is directed to agricultural science researchers, extension workers and to all professionals involved in science development and technological applications for the benefit of agricultural producers and their activity.

Issued as a triannual publication, this journal is intended to transfer research results in different areas of Agronomy in the tropics and subtropics. Original unpublished papers are, therefore, accepted in the following areas: plant physiology, crop nutrition and fertilization, genetics and plant breeding, entomology, phytopathology, integrated crop protection, agroecology, weed science, environmental management, geomatics, soil science, water and irrigation, agroclimatology and climate change, post-harvest and agricultural industrialization, rural and agricultural entrepreneurial development, agrarian economy, and agricultural marketing.

The authors of the manuscripts submitted to *Agronomía Colombiana* must be aware of and avoid scientific misconduct (code of conduct by Committee on Publication Ethics, COPE) related to: scientific fraud in all or part of the data of the study and data falsification and manipulation; dishonesty due to fictitious authorship or gifting or exchange of co-authorship, duplicate publications, partial or complete, in different journals and self-plagiarism by reusing portions of previous writings; citation omission, citation copying without consultation and excessive self-citation, among others. The authors have the following rights: fair and impartial evaluation of articles done in a reasonable amount of time, correspondence taken seriously and requests for changes and corrections respected, manuscripts subject to review by the peer reviewers, and articles remained unaltered.

Agronomía Colombiana uses a double-blind peer review process anticipated by the previous quick preliminary review. The manuscripts must be submitted according to the rules established in the instructions to authors. If the articles fulfill the minimum criteria established for the preliminary review in terms of language and scope, these are sent to three or more expert reviewers in the specific area of knowledge to obtain two or three evaluations; two of the experts are external to the Universidad Nacional de Colombia, and the third one belongs to the research and teaching staff of the Universidad Nacional de Colombia. When the manuscript obtains two approval evaluations, the manuscript can be considered for publication by the editors; if two of the reviewers consider that the scientific level of the manuscript is not acceptable for publication, the manuscript will be rejected. Once the peer-review process is completed, the manuscript is sent back to the author(s) who must introduce the suggested corrections and answer all the questions obtained from the reviewers or the Editor. Finally, the Editor-in-Chief or the Editorial Committee reserves the right to accept or reject the submitted manuscripts.

For additional information, correspondence, subscriptions and journal swap, you may contact Universidad Nacional de Colombia, Facultad de Ciencias Agrarias, Journal *Agronomía Colombiana*, Bogota campus; P.O. Box 14490, Bogota-Colombia; phone numbers: (571) 316 5355 / 316 5000 extension 10265; e-mail: agrocol_fabog@unal.edu.co; the digital version of the journal is available at http://www.scielo.org.co and http://www.revistas.unal.edu.co/index.php/agrocol

Instructions to authors

The manuscripts submitted for publication to the Editorial Committee must be unpublished. In consequence, those that have been previously published in other journals, technical or scientific publications will be rejected. Contributions to the study and the submitted manuscript and any conflict of interest must be declared by the authors in the sections created for these purposes.

To submit manuscripts, the authors must be registered in our platform (https://revistas.unal.edu.co/index.php/

agrocol/about/submissions#onlineSubmissions) and follow the submission instructions. Corresponding authors will be required to use their ORCID Id when submitting a manuscript. After manuscript submission, papers will be screened for plagiarism using a specialized software. In case of finding a significant level of duplication, the manuscript will be rejected.

At the preliminary review stage, two aspects, English grammar and scope, will be assessed in order to ensure the minimum requirements of any manuscript to be peer-reviewed. If the manuscript is rejected during the preliminary review because of the English grammar aspect, authors are encouraged to edit their manuscript for language using professional services and submit the manuscript again. When the reason for preliminary reject is a mismatch of the scope between the manuscript and the journal interests, resubmission should be avoided.

Agronomía Colombiana accepts the following three types of original articles:

- **Scientific and technological research papers**: Those documents presenting detailed original research results. The most generally applied structure has four main parts: introduction, materials and methods, results and discussion, and conclusions.
- **Review articles**: Published only at the invitation of the Editor-in-Chief and with the approval of the Editorial Committee of *Agronomía Colombiana*. The review article should present an unbiased summary of the current understanding of a topic considered as a priority by the Editorial or Scientific Committee of *Agronomía Colombiana*.
- **Scientific notes**: Brief document introducing original preliminary or partial results of a scientific or technical research, which usually needs immediate publication.

Format and organization of the text

Research article length should not exceed 5,200 words, whereas scientific notes should have no more than 4,000 words. As review articles contain a large amount of detailed information, their length may be greater than research articles but should not exceed 8,000 words, or 10,000 words including the list of references. For review articles, the list of references (Literature cited section) should include at least 50 references. Tables and figures, that is to say, diagrams, drawings, schematic and flow diagrams, pictures, and maps should be consecutively numbered (Table 1 ...Table n; Figure 1... Figure n, etc.).

Texts and tables should be prepared using the MS Word® processor. Manuscripts including tables as embedded images will not be published. All text should be double-spaced including table headers, figure captions and cited literature. All pages must be numbered consecutively. Line numbering on each page is mandatory. Tables and diagrams of frequency (bar and circular diagrams) should be included in the mentioned Word file as well as in their original MS-Excel® or other graphic formats but maintaining a high resolution. Other figures, including photographs and drawings should be submitted in digital JPG (or JPEG) compression format, with a minimum resolution of 300 dpi.

As a general rule, tables and figures should only be submitted in black and white, except for those intended for the cover page of the Journal or for those cases in which it is absolutely necessary to present them in color (at the judgment and discretion of the Editor).

Languages, units, and style

The journal's official language is English. Regarding measurement units, the metric system (SI) should be consistently used through the manuscript, unless the need is seen to apply any specific units that are of frequent use by the scientific community. Multiplication followed by negative superscript (e.g., kg ha^{-1}) can only be used with SI units. The slash (/) is a mathematical operation symbol that indicates "divided by". Anyway, in sciences it is used as a substitute for the word "per", and it is used to indicate rates. Use the slash to connect SI to non-SI units (e.g., 10°C/h or 10 L/pot).

Decimal fractions should be separated by a point (.), not a comma (,).

All abbreviations should be explained in full length when first mentioned in the manuscript.

With regards to the tenses, the most commonly used ones are the past, for the introduction, procedures and results; and the present, for the discussion.

Title and authors

The title in English, as well as its corresponding Spanish translation, shall not exceed 15 words. The scientific names of plants and animals shall be italicized and lowercased, except for the first letter of the genus (and of the species author), which must be uppercased.

The authors (including first and second names) shall be listed in order of their contribution to the research and preparation of the manuscript, in completely justified text format (filling the whole line, or, if necessary, the next one below) under the translated version of the title. At the bottom of the article's first page, only the name and city location of the employer or supporting institution(s), and the e-mail address of the corresponding author should be included.

Abstract, resumen, and key words

The Abstract should be written in English with Spanish translation for the "Resumen". Both texts should contain brief (no longer than 200 words in a single paragraph) and accurate descriptions of the paper's premise, justification, methods, results and significance. Both language versions shall be mandatorily provided with a list of (maximum six) key words that have not appeared in the title or abstract, and included in the Agrovoc thesaurus by Agris (FAO).

Introduction

The introduction must include the delimitation and current status of the problem, the theoretical or conceptual basis of the research, the literature review on the topic, and the objectives and justification of the research. Common names must be accompanied by the corresponding scientific ones, plus the abbreviation of the species author surname when mentioned for the first time.

Materials and methods

Besides a clear, precise and sequential description of the materials used for the research (plant or animal materials, plus agricultural or laboratory tools), this section illustrates the procedures and protocols followed, and the experimental design chosen for the statistical analysis of the data.

Results and discussion

Results and discussion can be displayed in two different sections or in a single section at the authors' convenience. The results shall be presented in a logical, objective, and sequential order, using text, tables (abbreviated as Tab.) and figures (abbreviated as Fig.). The latter two should be easily understandable and self-explanatory, in spite of having been thoroughly explained in the text. The charts should be two-dimensional and prepared in black and white, resorting to a tone intensity degradation to illustrate variations between columns. Diagram curves must be prepared in black, dashed or continuous lines (- - - - or ———), using the following conventions: ▪, ▲, ⬩, ●, □, ◊, ○. The tables should contain a few columns and lines.

Averages should be accompanied by their corresponding Standard Error (SE) values. The discussion shall be complete and exhaustive, emphasizing the highlights and comparing them to the literature data.

This section should briefly and concisely summarize the most important findings of the research.

Conclusion (optional)

A short conclusion section is useful for a long or complex discussion. It should provide readers with a brief summary of the main achievements from the results of the study. It can also contain final remarks and a brief description of future complementary studies that should be addressed.

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.

Conflict of interest statement

All manuscripts that are submitted to and published in *Agronomía Colombiana* must be accompanied by a conflict of interest disclosure statement by the authors. Please, include such a statement or declaration at the end of your manuscript, following any acknowledgments and prior to the references, under the heading 'Conflict of interest statement'.

Example: The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

This information is mandatory for *Agronomía Colombiana* from 2020 onwards. In order to describe each of the authors' contribution, please follow the CRediT taxonomy and use the following roles as a guide:

Contributor roles

Conceptualization: AAA formulated the overarching research goals and aims.

Data curation: AAA carried out activities to annotate scrub data and maintain research data for initial use and later re-use.

Formal analysis: AAA applied statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.

Funding acquisition: AAA obtained the financial support for the project leading to this publication.

Investigation: AAA conducted the research and investigation process, specifically performing the experiments or data/evidence collection.

Methodology: AAA developed or designed the methodology; created the models.

Project administration: AAA managed and coordinated the research activity planning and execution.

Resources: AAA provided the study materials, reagents, laboratory samples, instrumentation, computing resources, or other analysis tools.

Software: AAA implemented the computer code and supporting algorithms/software.

Supervision: AAA oversaw and led the research activity planning and execution.

Validation: AAA verified the overall replication/reproducibility of results/experiments and other research outputs.

Visualization: AAA prepared, created, and/or presented the published work and oversaw its visualization/data presentation.

Writing – original draft: AAA wrote/translated the initial draft.

Writing – review & editing: AAA carried out the critical review, commentary, or revision of the manuscript.

Authors have to keep in mind the CRediT taxonomy is not useful to determine who is eligible as author, but to state the contribution of each author in the study or the article. More information about the CRediT taxonomy is available in: https://casrai.org/credit/

Example: MYD and AFT designed the experiments, AFT carried out the field and laboratory experiments, AFT contributed to the data analysis, MYD and AFT wrote the article. All authors reviewed the manuscript.

Citations and literature cited

The system (author(s), year) will be consistently applied to all citations intended to support affirmations made in the article's text. When the cited reference has three or more authors, the citation shall only mention the name of the first author, accompanied by the Latin expression et al. (which means 'and others'), italicized and followed by a period, and separated from the year by a comma: (García *et al.*, 2003). Alternatively, you can leave just the year in parenthesis: García *et al.* (2003). In case of references with only two authors, citations should include both names separated by '&': (García & López, 2012) or García and López (2012).

Tables and figures should be cited in parenthesis as follows: (Tab. 1), (Tab. 2), (Tab. 3), etc., or (Fig. 1), (Fig. 2), (Fig. 3), etc. In the text, each table or figure must be referred to using a capital T or F, for example: ...as shown in Table 1, Table 2, Table 3, etc., or in Figure 1, Figure 2, Figure 3, etc.

The complete list of cited references in alphabetical order, according to the authors' surnames, must be included at the end of the article. When the list includes various publications of the same author(s), they shall be listed in chronological order. When they correspond to the same year, they must be differentiated with lower case letters: 2008a, 2008b, etc.

Agronomía Colombiana has adopted the American Psychological Association (APA) standards (https://apastyle.apa.org/about-apa-style) to elaborate the final list of references cited in the text ("Literature cited" section). This standard will be required for new manuscripts received from March 1st, 2020 onwards.

However, a transition period has been established for 2020, during which manuscripts received and processed before March 2020 may be published with either of the two reference styles, APA or Agronomía Colombiana style.

Basic information about the use of APA for the list of references is available here: https://apastyle.apa.org/style-grammar-guidelines/references. In order to illustrate these standards, authors can check some examples about how to create each item of the list of references, keeping in mind the type of publication cited as follows (click on each option to open APA web information):

Journal article

Example: García-Arias, F., Sánchez-Betancourt, E., & Núñez, V. (2018). Fertility recovery of anther-derived haploid cape gooseberry (*Physalis peruviana* L.) plants. *Agronomía Colombiana*, *36*(3), 201–209. https://doi.org/10.15446/agron.colomb.v36n3.73108

Published dissertation or thesis references

Example: Franco, C. V. (2012). *Efecto de la colchicina sobre el número cromosómico, número de cloroplastos y características morfológicas del fruto en ecotipos de uchuva (*Physalis peruviana *L.) Colombia, Kenia y Perú* [Undergraduate thesis, Universidad Francisco de Paula Santander]. UFPS Library. http://alejandria.ufps.edu.co/descargas/tesis/1610259.pdf

Whole book

Example: Suescún, L., Sánchez, E., Gómez, M., García-Arias, F. L., & Núñez Zarantes, V. M. (2011). *Producción de plantas genéticamente puras de uchuva*. Editorial Kimpres Ltda.

Edited book chapter

Example: Ligarreto, G., Lobo, M., & Correa, A. (2005). Recursos genéticos del género *Physalis* en Colombia. In G. Fischer, D. Miranda, W. Piedrahita, & J. Romero. (Eds.), *Avances en cultivo, poscosecha y exportación de la uchuva* Physalis peruviana *L. en Colombia* (pp. 329–338). Universidad Nacional de Colombia.

For other types of references such as technical reports, conference presentations or proceedings, magazine articles or preprints see https://apastyle.apa.org/style-grammar-guidelines/references/examples. Archival documents, letters, collections and unpublished documents can be referenced using some standards users can find here: https://apastyle.apa.org/style-grammar-guidelines/references/archival. In this link (http://shorturl.at/xGIPZ) authors can find a summary of the APA style for references created by Mendeley.

Authors can also consider the use of some APA list of reference generators such as Reference Management (Mendeley, https://www.mendeley.com/reference-management/mendeley-desktop), Scribbr (https://www.scribbr.com/apa-citation-generator/#/) or MyBib (https://www.mybib.com/).

Whenever possible, please provide a valid DOI for each reference in the literature cited.

AUTHOR INDEX OF *AGRONOMÍA COLOMBIANA* VOLUME 38, 2020

Continued

Author name	Article title	Location
Cayón-Salinas Daniel Gerardo	Leaf area, chlorophyll content, and root dry mass in oil palms (*Elaeis guineensis* Jacq.) affected by the plumero disorder	No. 3, pp. 335-341
Chaparro-Giraldo Alejandro	Freedom to operate analysis, design and evaluation of expression cassettes that confer tolerance to glyphosate	No. 2, pp. 161-170
Ciappini María Cristina	Optimization of the extraction of antioxidant compounds from quinoa (*Chenopodium quinoa* Willd.)	No. 3, pp. 436-441
Coelho Antonio Eduardo	Sowing date and maize response to the splitting of nitrogen side-dressing fertilization	No. 3, pp. 316-324
Coelho Antonio Eduardo	Narrow and twin-row plantings do not increase maize yield	No. 3, pp. 342-349
Colinas-León María Teresa	Relationship between chemical fertilization in sorghum and *Melanaphis sacchari/sorghi* (Hemiptera: Aphididae) populations	No. 3, pp. 357-366
Cordero-Cordero Carina Cecilia	Characterization of eggplant producers in the Caribbean region of Colombia: socio-economic aspects and local production technology	No. 1, pp. 120-132
Correa-Álvarez Ender Manuel	Characterization of eggplant producers in the Caribbean region of Colombia: socio-economic aspects and local production technology	No. 1, pp. 120-132
Costa e Melo Isabel Giovanna	Biomass of *Crotalaria juncea* as a function of plant densities in the semiarid region of Northeastern Brazil	No. 1, pp. 148-155
Cruz Anna Bárbara de Souza	Effect of the use of pre- and post-emergence herbicides on nodulation and production of cowpea (*Vigna unguiculata* L.) in the Amazonian savannah	No. 2, pp. 280-286
Cruz Diego Lima de Souza	Effect of the use of pre- and post-emergence herbicides on nodulation and production of cowpea (*Vigna unguiculata* L.) in the Amazonian savannah	No. 2, pp. 280-286
Cruz-Gutiérrez Nelson Alirio	Effect of gibberellic acid-3 and 6-benzylaminopurine on dormancy and sprouting of potato (*Solanum tuberosum* L.) tubers cv. Diacol Capiro	No. 2, pp. 178-189
Cuervo-Andrade Jairo Leonardo	Impact of dry sludges and sludge biochar on height and dry matter of *Solanum lycopersicum* L.	No. 2, pp. 242-252
Danilussi Maikon Tiago Yamada	Efficacy of imazapic/imazapyr and other herbicides in mixtures for the control of *Digitaria insularis* prior to soybean sowing	No. 3, pp. 350-356
Danquah Frank Osei	Resource-use efficiency in maize production: the case of smallholder farmers in Ghana	No. 3, pp. 406-417
Darghan-Contreras Aquiles	Impact of dry sludges and sludge biochar on height and dry matter of *Solanum lycopersicum* L.	No. 2, pp. 242-252
Darghan-Contreras Aquiles Enrique	Leaf area, chlorophyll content, and root dry mass in oil palms (*Elaeis guineensis* Jacq.) affected by the plumero disorder	No. 3, pp. 335-341
De Oliveira Gustavo Hugo Ferreira	Path correlation and Bayesian analysis on popping expansion components in popcorn hybrids	No. 1, pp. 3-8
De Oliveira Tâmara Rebecca Albuquerque	Path correlation and Bayesian analysis on popping expansion components in popcorn hybrids	No. 1, pp. 3-8
Díaz Eliécer	A comparison of two open-source crop simulation models for a potato crop	No. 3, pp. 382-387
Dionello Rafael Gomes	Immediate and latent damages of drying temperature in the quality of black oat (*Avena strigosa* Schreb.) seeds	No. 2, pp. 226-233
Dotor-Robayo Mónica	Study of the effects of glyphosate application on Collembola populations under controlled conditions	No. 3, pp. 398-405
Escobar Alexander	Evaluation of Phytoseiidae mites and *Chrysoperla carnea* (Stephens) on the control of *Tetranychus urticae* in *Carica papaya* L.	No. 1, pp. 101-109
España-Guechá Martha Sofía	Leaf area, chlorophyll content, and root dry mass in oil palms (*Elaeis guineensis* Jacq.) affected by the plumero disorder	No. 3, pp. 335-341
Espinosa-Vázquez Gonzalo	Relationship between chemical fertilization in sorghum and *Melanaphis sacchari/sorghi* (Hemiptera: Aphididae) populations	No. 3, pp. 357-366
Ferreira Lais Tomaz	Selection of soybean (*Glycine max* (L.) Merril) genotypes for cultivation on the Brazilian Brejo Paraibano	No. 2, pp. 171-177
Figueiredo Francisco Romário Andrade	Chlorophyll *a* fluorescence and development of zucchini plants under nitrogen and silicon fertilization	No. 1, pp. 45-52
Fischer Gerhard	Influence of some environmental factors on the feijoa (*Acca sellowiana* [Berg] Burret): A review	No. 3, pp. 388-397
Fornaguera-Espinoza Federico	Effect of gibberellic acid-3 and 6-benzylaminopurine on dormancy and sprouting of potato (*Solanum tuberosum* L.) tubers cv. Diacol Capiro	No. 2, pp. 178-189
Franco-Flórez Adriana	Fruit quality attributes of ten Colombian blackberry (*Rubus glaucus* Benth.) genotypes	No. 1, pp. 9-18
Franco-Lara Liliana	Can *Amplicephalus funzaensis* Linnavuori 1968 (Hemiptera: Cicadellidae) transmit phytoplasms to strawberry?	No. 1, pp. 73-84

Continued

Continued

Continued

Continued

Continued

AGRONOMIA COLOMBIANA

VOLUME XXXVIII, No. 3 SEPTEMBER-DECEMBER 2020 ISSN (print): 0120-9965 / ISSN (online): 2357-3732

AGRONOMIA COLOMBIANA

P.O. Box 14490, Bogota-Colombia

Phones: (571) 316 5498 / (571) 316 5000 ext. 19095

Fax: (571) 316 5176

E-mail: agrocol_fabog@unal.edu.co

http://www.scielo.org.co

http://www.revistas.unal.edu.co/index.php/agrocol

http://agronomia.unal.edu.co