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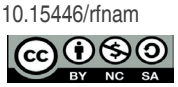

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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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EDITORIAL

Crónicas de un evento en tiempos de pandemia:
ISAE 2020 II Congreso Latinoamericano de Comportamiento y Bienestar Animal

Es muy grato recibir buenas noticias y más cuando son resultado del arduo trabajo y esfuerzo de un equipo de docentes y estudiantes de la propia Universidad Nacional de Colombia sede Medellín. El proyecto de este evento, que fue una realidad con mucha visibilidad, altura, calidad y alcance, comenzó en 2018 en la ciudad de Valdivia Chile, cuando de la reunión regional del ISAE se eligió a Colombia como sede del encuentro de 2020 y a nuestra Universidad como anfitrión de este magno evento. Desde entonces empezamos a contactar a los expertos, a difundir por todos los medios y a buscar patrocinio, que encontramos en empresas y entidades que creyeron en nosotros, además, por supuesto, del apoyo total de la Facultad de Ciencias Agrarias.

La pandemia estalló a principios de este año, dejándonos totalmente confundidos, el evento, que inicialmente sería presencial tendría que cancelarse y posponerse indefinidamente ante la incertidumbre de los acontecimientos mundiales como consecuencia del COVID 19. Estuvimos varios meses sin ningún avance, sin saber qué hacer y entonces, en junio, el grupo de docentes organizadores decidió tomar el reto, adaptarnos a la nueva situación mundial y realizar el evento de todos modos, tomando el riesgo de hacerlo virtual aun cuando nunca habíamos tenido la experiencia de organizar un evento de esta magnitud usando herramientas tecnológicas y videoconferencias en lugar de la tradicional charla en vivo y en directo. Hubo dificultades, pero también mucho apoyo, de todo el equipo organizador, del equipo logístico, de amigos fotógrafos que compartieron de forma gratuita su increíble trabajo para darle al evento una imagen de excelente calidad, hasta la ilustración de la portada, que muestra algunos animales emblemáticos de la fauna Colombiana fue una donación cariñosa y desinteresada, y un invaluable apoyo de nuestra Facultad de Ciencias Agrarias y de la Dirección Académica de la sede, además, los 18 conferencistas magistrales (9 nacionales y 9 internacionales) siguieron apoyándonos con su participación ahora de manera virtual y nuestros financiadores externos (Zoetis gran patrocinador y Agrosavia patrocinador) continuaron con el apoyo económico para que este evento pudiera ser posible.

Se realizó el primer llamado para las ponencias orales y no pensábamos tener la acogida y magnitud que tuvimos, se recibieron casi 200 trabajos de investigación de grupos de toda latinoamérica, la tarea de evaluar necesitó del apoyo de muchos expertos desde Argentina hasta Canadá y el resultado fueron 180 trabajos aceptados para su presentación en el evento. Las áreas elegidas para los tres módulos del ISAE 2020: Animales de Producción, Animales de Compañía y Fauna Silvestre, permitieron ampliar la acogida y logramos 681 personas inscritas como asistentes al congreso de 18 países que vale el esfuerzo mencionar: Argentina, Chile, Uruguay, Paraguay, Brasil, Venezuela, Bolivia, Perú, Ecuador, Mozambique, Panamá, México, España, Nicaragua, El salvador, Canadá, Estados Unidos y por supuesto, Colombia. De estas personas inscritas, la Facultad de Ciencias Agrarias aportó la beca de inscripción para 50 estudiantes de la Universidad Nacional de Colombia y la Open Philanthropy Project, las becas para inscripción de 100 asistentes de países en desarrollo, en este grupo tuvimos la fortuna de cruzar los mares y recibir una solicitud de beca de un investigador de Mozambique. Estas cifras nos llenan de orgullo por el trabajo bien hecho y nos demuestran que con todo y las dificultades, es posible pensar en estrategias diferentes para hacer las cosas, adaptarnos y seguir adelante.

Las memorias del evento, publicadas en el presente número de la Revista de la Facultad Nacional de Agronomía, quedarán como prueba de todo esto para la posteridad y sin duda sentarán un precedente sobre las ciencias del comportamiento y el bienestar animal en latinoamérica; lo que fortalece aún más a la Universidad Nacional de Colombia Sede Medellín, como una entidad de educación pública que fue la primera en el país en apostar por la enseñanza del bienestar animal en programas de producción animal, para lo cual, desde hace 10 años, incluyó en el pensum de zootecnia esta asignatura en el componente profesional obligatorio de nuestros estudiantes. Esperemos seamos una semilla que aporte para que a partir de ahora se estrechen los lazos de cooperación entre tantos grupos de investigación de tantos países que hicieron presencia con estudios de alta calidad.

El ISAE 2020 fue un éxito total, agradecemos a todas las personas que creyeron y apoyaron el evento de tantas maneras. El listón ha quedado bastante alto, el próximo ISAE regional tiene un reto enorme que esperamos pueda superar o al menos igualar la calidad de éste.

Gratitud total de parte del comité organizador.

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Research article

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Revista Facultad Nacional de Agronomía

Selection of potato genotypes *Solanum tuberosum* group Andigena by their tolerance to *Phytophthora infestans* (Mont.) of Bary

UNIVERSIDAD NACIONAL DE COLOMBIA

Selección de genotipos de papa *Solanum tuberosum* grupo Andigena según su tolerancia a *Phytophthora infestans* (Mont.) de Bary

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Keywords:
Late blight
Susceptibility
Tolerance

ABSTRACT

Phytophthora infestans is the most limiting biotic problem of potato crop in Colombia and the world. It is a pathogen that threatens the sustainability of the crop. Therefore, it is necessary to evaluate work collections of potato to identify genotypes that show disease tolerance. The objective of this work was to select genotypes of *Solanum tuberosum* group Andigena by their tolerance to *P. infestans*. This study was carried out under conditions of Pasto, in the South of Colombia at 2,820 masl. A total of 76 introductions of guata potatoes were evaluated under natural inoculum, including Capiro as a susceptible control, Betina as moderately tolerant and Pastusa Suprema as highly tolerant. The number of stems and stolons per plant was registered. Disease severity assessments and traits related to the area under the disease progress curve were made. At harvest stage, yield values per plant and its components were recorded. Principal Component and Classification analyses discriminated tolerant introductions of susceptible. Significant positive correlations were found between the yield with the number of stolons, tubers per plant and tuber weight, and negative correlations with severity and area under the relative disease progress curve. The selected introductions showed high yield per plant and mostly minor severities to the population, indicating an agronomic potential that must be evaluated in different environments to determine its adaptability and stability. UdenarStGua53, UdenarStGua61, UdenarStGua68, UdenarStGua73, UdenarStGua75, UdenarStGua77 y UdenarStGua78, coming from the International Potato Center (CIP) are confirmed as a source of tolerance to *P. infestans* and can be considered as parental within species improvement programs.

Palabras clave:
Tizón tardío
Susceptibilidad
Tolerancia

RESUMEN

Phytophthora infestans es uno de los problemas más limitantes de la papa en Colombia y en el mundo. Es un patógeno que amenaza la sostenibilidad del cultivo. Por lo tanto, es necesario evaluar colecciones de trabajo por su reacción ante el patógeno, con el fin de identificar genotipos que muestren tolerancia a la enfermedad. El objetivo de este trabajo fue seleccionar genotipos de *Solanum tuberosum* grupo Andigena acorde con su tolerancia a *P. infestans.* Esta investigación se realizó bajo condiciones del Altiplano de Pasto, Sur de Colombia a 2,820 msnm. Un total de 76 introducciones de papa guata fueron evaluadas bajo condiciones de inóculo natural, incluyendo Capiro como control susceptible, Betina como moderadamente tolerante y Pastusa Suprema como altamente tolerante. Se registró el número de tallos y estolones por planta y se hicieron evaluaciones de severidad y de variables relacionadas con el área bajo la curva de progreso de la enfermedad. En la cosecha, se consignaron los valores de rendimiento por planta y sus componentes. Los análisis de Componentes Principales y de Clasificación discriminaron introducciones tolerantes y susceptibles. Se encontraron correlaciones significativas positivas entre el rendimiento y el número de estolones, tubérculos por planta y peso de tubérculo y correlaciones negativas con severidad y área bajo la curva de progreso relativo de la enfermedad. Las introducciones seleccionadas mostraron alto rendimiento por planta y en su mayoría severidades menores a la población, indicando un potencial agronómico que debe ser evaluado en diferentes ambientes para determinar su adaptabilidad y estabilidad. UdenarStGua53, UdenarStGua61, UdenarStGua68, UdenarStGua73, UdenarStGua75, UdenarStGua77 y UdenarStGua78 provenientes del CIP se confirman como fuente de tolerancia a *P. infestans* y pueden ser considerados como parentales dentro de programas de mejoramiento de la especie.

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Potato cultivation is one of the most widespread agricultural activities in Colombia. Its production is distributed in 283 municipalities, mainly in the departments of Cundinamarca, Boyacá, Nariño, Antioquia, Santander, Norte de Santander, Cauca, Tolima, and Caldas, with a share of 3.3% in the national Gross Domestic Product. For the year 2017, 169,002 ha were reported with a production of 3,740,000 t. Its cultivation benefits 110,000 families and generates 75,000 direct jobs and 189,000 indirect jobs (Fedepapa, 2017). The 94% of the production is consumed mainly as a fresh product and 6% is used in industry (Agronet, 2019).

The potato is one of the most genetically diverse cultivated species, with diploid, triploid, tetraploid and pentaploid species, the vast majority is found in the Andean zone of South America. In the Andes, the genus is represented by eight cultivated species and about 200 wild ones. The rich diversity of the cultivated species is included in a polyploid series (2n=24, 36, 48, and 60 chromosomes), which includes 4,000 edible varieties, with high genetic potential for yield and broad adaptability to different climates, which has allowed it to become one of the most important crops of the world diet (Estrada, 2000).

However, the crop is subjected to different biotic and abiotic stresses that threaten its sustainable development. In this sense, one of the main biotic stress in the field, not only in Colombia but worldwide, is the late blight caused by *Phytophthora infestans* (Mont.) of Bary. It is a pathogen belonging to the Oomycete class, in the Chromista kingdom and phylogenetically related to Diatoms and brown algae. This fungus has a rapid development in high humidity conditions and can survive for days or even weeks, although its sporangia cannot withstand freezing temperatures. The main strategy adopted by farmers to control this pathogen is the application of fungicides, a practice that has represented between 10 and 29.9% of production costs (Juyó *et al*., 2011).

One of the alternatives for solving the biotic problems of crops is finding cultivars with genetic tolerance, which can be integrated into chemical control to reduce the use of fungicides, decrease the cost of production, and decline the damage to human health and the environment. Obtaining tolerant cultivars to late blight can provide additional protection to the crop over time, reducing the risk of losses from the disease and/or the costs of chemical control in each crop cycle (Muñoz *et al*., 2019).

In the case of *P. infestans*, researches have been carried out in various fields from conventional breeding, biotechnology, molecular marker assisted breeding to genetic transformation. Cristinzio and Testa (2019), used the technique of plant electrolyte exudation caused by the filtering of the pathogen culture to detect genetic tolerance under *in vitro* conditions of 10 potato cultivars to *P. infestans* with eight strains. Electrolyte leakage was used to screen leaf and tuber tissues with fungal culture filtrates. Under the leaf or tuber trials with almost all cultivars there were statistical differences in susceptibility, while leaf tolerance did not correlate with tuber tolerance. The Ajax variety was the least susceptible in the leaf and tuber tests, while Prima was the most susceptible in the tuber tests.

One of the indications of tolerance to *P. infestans* in potato cultivars is the presence of tolerant genes R1 and R2. Díaz *et al.* (2003) evaluated different genotypes of *S. tuberosum* group *Tuberosum* according to their tolerance to *P. infestans* and the molecular detection via PCR (Polymerase Chain Reaction) of R1 and R2 genes. The results showed a strong phenotypic and genotypic correspondence regarding the presence of R1 and R2 alleles in the different potato genetic materials containing one or another allele.

In the field of traditional breeding, in Southern and Central American countries, germplasm evaluation has been carried out and methods of inter- and intraspecific crosses, backcrossing and recurrent selection have been used to obtain more productive cultivars with tolerance to *P. infestans* (Gabriel *et al*., 2001; Barquero *et al*., 2005; Solano *et al.,* 2014). In Colombia, Rodríguez *et al.* (2009), obtained three new varieties of creole potato registered as Criolla Latina with yields between 18 to 20 t ha^{-1}, Criolla Paisa with yields of 22 to 25 t ha^{-1} and Criolla Colombia with yields of 13 to 15 t ha^{-1}. The first two were reported with moderate tolerance to *P. infestans*, while the latter was reported as sensitive to this pathogen.

According to the above mentioned authors, it is necessary to increase the probability of finding long-lasting tolerance and to do so, the genetic base of tolerance should be broadened, based on the evaluation of the available germplasm in breeding programs being developed by institutions such as the University of Nariño and the National University of Colombia; in addition, sources of tolerance should be sought in wild germplasm (Forbes and Huarte, 2014) in order to improve traditional varieties, which are generally susceptible to late blight. Therefore, the objective of this study was to select genotypes of guata potato *Solanum tuberosum* group Andigena by their tolerance to the natural inoculum of *Phytophthora infestans* (Mont.) of Bary, under conditions of Pasto, South of Colombia.

MATERIALS AND METHODS

Plant material

As genetic materials, 76 potatoes were used (Table 1), of which 43 were introduced from the CIP-Peru on July 18, 2016; 21 belong to the working collection of the University of Nariño (UDENAR), three were procured from the National University of Colombia, Bogotá (UNAL) and correspond to the improved varieties Betina, Única and Pastusa Suprema, and nine were obtained from the Central Colombian Collection under the responsibility of AGROSAVIA (Colombian Agricultural Research Corporation). Of these populations, the genetic material susceptible to late blight *P. infestans* was the variety Capiro (Monsalve-Fonnegra *et al.,* 2012), while Betina is moderately tolerant and Pastusa Suprema is highly tolerant (Ñustez, 2019). These varieties were used as controls of susceptibility and tolerance at field level.

Table 1. Population of 76 introductions of *Solanum tuberosum* group Andigena and their sources.

ID	Introduction	Source	ID	Introduction	Source
1	UdenarStGua07	UNAL-Col	39	UdenarStGua59	CIP-Peru
2	UdenarStGua12	Colombia	40	UdenarStGua60	CIP-Peru
3	UdenarStGua13	Colombia	41	UdenarStGua61	CIP-Peru
4	UdenarStGua20	UNAL-Col	42	UdenarStGua62	CIP-Peru
5	UdenarStGua21	Colombia	43	UdenarStGua63	CIP-Peru
6	UdenarStGua22	Colombia	44	UdenarStGua64	CIP-Peru
7	UdenarStGua23	Colombia	45	UdenarStGua65	CIP-Peru
8	UdenarStGua24	Colombia	46	UdenarStGua66	CIP-Peru
9	UdenarStGua25	Colombia	47	UdenarStGua67	CIP-Peru
10	UdenarStGua26	Colombia	48	UdenarStGua68	CIP-Peru
11	UdenarStGua27	Colombia	49	UdenarStGua69	CIP-Peru
12	UdenarStGua28	Colombia	50	UdenarStGua70	CIP-Peru
13	UdenarStGua29	Colombia	51	UdenarStGua71	CIP-Peru
14	UdenarStGua30	Colombia	52	UdenarStGua72	CIP-Peru
15	UdenarStGua31	UNAL-Col	53	UdenarStGua73	CIP-Peru
16	UdenarStGua33	Colombia	54	UdenarStGua74	CIP-Peru
17	UdenarStGua34	Colombia	55	UdenarStGua75	CIP-Peru
18	UdenarStGua35	Colombia	56	UdenarStGua76	CIP-Peru
19	UdenarStGua36	Colombia	57	UdenarStGua77	CIP-Peru
20	UdenarStGua38	Colombia	58	UdenarStGua78	CIP-Peru

Table 1. (continuation)

ID	Introduction	Source	ID	Introduction	Source
21	UdenarStGua39	Colombia	59	UdenarStGua79	CIP-Peru
22	UdenarStGua40	Colombia	60	UdenarStGua80	CIP-Peru
23	UdenarStGua41	Colombia	61	UdenarStGua82	CIP-Peru
24	UdenarStGua42	Corpoica	62	UdenarStGua83	CIP-Peru
25	UdenarStGua43	Corpoica	63	UdenarStGua84	CIP-Peru
26	UdenarStGua44	Corpoica	64	UdenarStGua85	CIP-Peru
27	UdenarStGua45	Corpoica	65	UdenarStGua86	CIP-Peru
28	UdenarStGua46	Corpoica	66	UdenarStGua87	CIP-Peru
29	UdenarStGua47	Corpoica	67	UdenarStGua89	CIP-Peru
30	UdenarStGua48	Corpoica	68	UdenarStGua90	CIP-Peru
31	UdenarStGua49	Corpoica	69	UdenarStGua91	CIP-Peru
32	UdenarStGua50	Corpoica	70	UdenarStGua93	CIP-Peru
33	UdenarStGua53	CIP-Peru	71	UdenarStGua94	CIP-Peru
34	UdenarStGua54	CIP-Peru	72	UdenarStGua95	CIP-Peru
35	UdenarStGua55	CIP-Peru	73	UdenarStGua97	CIP-Peru
36	UdenarStGua56	CIP-Peru	74	UdenarStGua99	CIP-Peru
37	UdenarStGua57	CIP-Peru	75	UdenarStGua100	CIP-Peru
38	UdenarStGua58	CIP-Peru	C	Control (C)	Colombia

Location

The experiment was carried out in Botana Experimental Farm of the University of Nariño, located at 2,820 masl, 1°09'28.3" NL and 77°16'29.5" WL, with a relative humidity of 82%, average temperature of 12 °C and a rainfall of 800 mm. The area is climatically classified as a low mountain rainforest, with sandy clayey Andisol soils (IDEAM, 2016).

Experimental arrangement

The evaluated genotypes were arranged in a total experimental area of 581 m^2, with a distance between furrows of 1.2 m and distance between plants of 0.4 m with previous application of lime and organic matter, for a planting density of 20,833 plants ha^{-1}. The area of the plot was 1.44 m^2. Each genotype was randomly located in each plot, which contained three plants. The Capiro introduction was established as a susceptible variety and it was the source of the inoculum. This variety was planted in each five plots. Every seven days, insecticides such as Tiametoxam+Lambdacihalotrina (2 $cm^3 L^{-1}$) and Profenofos+Cipermetrina (0.75 $cm^3 L^{-1}$) were applied. In addition, fungicides such as Carbendazim (1 $cm^3 L^{-1}$) and Difenoconazole (1 $cm^3 L^{-1}$) were applied, in order to protect plants from other pathogens and achieve uniformity.

The first edaphic fertilization was carried out at 20 days and the second one at 40 days after planting. It was applied 840 kg ha^{-1} of the formula 10-30-10 per plant. Additionally, periodic applications were made with foliar fertilizer in doses of 5 $cm^3 L^{-1}$.

Traits evaluated

In the vegetative phase, the number of stems and stolons per plant was recorded. Severity evaluations (SEV) of the disease caused by *P. infestans* were made every 14 days. At harvesting stage, yield values per plant and its components were recorded. The methodology for data collection of each of the traits is described below.

Number of stems per plant (NS). The number of stems per plant was counted and recorded for each plot.

Severity (SEV). SEV assessments were conducted from the first week of planting to 134 days of the crop cycle. In

each plant of the plot, a leaf from the middle third was randomly marked, in which the periodic SEV evaluations were made. The scale proposed by Clive (1971) was used for the qualification of the leaf area affected by the presence of the pathogen (Figure 1). The scale proposes values of 1, 10, 25, 50, 75 and 100%. The higher SEV values, the higher the susceptibility of the genotype evaluated.

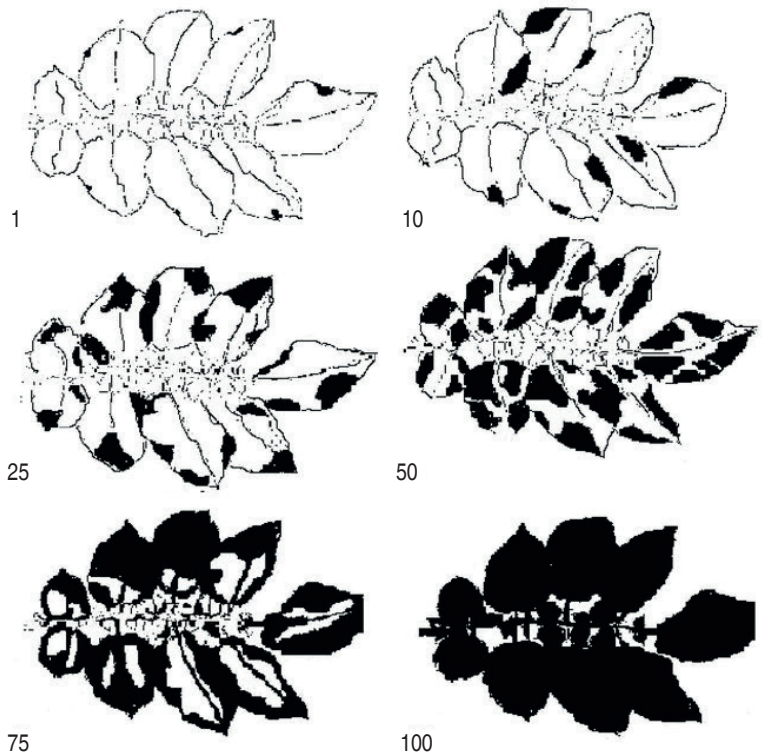

Figure 1. Clive's scale (1971) for the percentage of severity of *P. infestans* in *S. tuberosum* leaves, modified by Betancourth *et al.* (2008).

Area under the disease progress curve (AUDPC). The AUDPC is a variable that estimates the amount of disease throughout the crop cycle. It is calculated using Equation 1 proposed by Campbell and Madden (1990):

$$AUDPC = \sum_{i=1}^{n_i-1} \frac{(Y_i + Y_{i+1})}{2}(t_{i+1} - t_i) \qquad (1)$$

Where: t=time of each reading, Y_i=percentage of foliage affected in each reading and n=number of readings.

Relative area under disease progress curve (rAUDPC). The rAUDPC value indicates the proportion of infected tissue during the evaluation period, so that genotypes with severity values of 100% showed rAUDPC=1. Low rAUDPC values indicate a low percentage of infection during the evaluation period. These values correspond to the most tolerant genotypes (Pérez and Forbes, 2008). The rAUDPC was calculated using Equation 2:

$$rAUDPC = \frac{AUDPCX_i}{(t_f - t_i)} x100 \qquad (2)$$

Where: *rAUDPC*=relative area under disease progress curve, $AUDPCX_i$=area under the disease progress curve of the *i*-th genotype, t_f=time of the last evaluation and t_i=time of the first evaluation.

Rate of disease development (RD). The RD is a trait that measures the progress of the disease throughout the crop cycle. For its estimation, SEV reported in the first and last evaluation were taken as reference. The Equation 3 was used for the calculation of RD (Van Der Planck, 1963).

$$RD = \frac{1}{t_1 - t_0}\left(\log e\frac{X_0}{1-X_0} - \log e\frac{X_1}{1-X_1}\right) \quad (3)$$

Where: RD=rate of disease development, X_0=proportion of disease in the initial time, X_1=proportion of disease in the final time, t_0=initial time corresponding to the reading of X_0 and t_1=final time corresponding to the reading of X_1.

Decrease in disease (dAUDPC). For the calculation of dAUDPC in percentage, the Equation 4 proposed by Andrade *et al.* (2016) was applied.

$$dAUDPC = 1 - \frac{AUDPC_i}{AUDPC_t} x100 \quad (4)$$

Where: $AUDPC_i$=area under the disease progress curve of the *i*-th genotype and $AUDPC_t$=area under the disease progress curve of the control variety.

Genotype Susceptibility Scale (GSS). The GSS was calculated based on the resistance, tolerance and susceptibility to late blight scale proposed by Yuen and Forbes (2009) using the Equation 5, having a susceptible cultivar as reference.

$$GSS = \frac{rAUDPC_{Gn}}{rAUDPC_{GS}} x9 \quad (5)$$

Where: $rAUDPC_{Gn}$: relative area under disease progress curve of the potato genotype (Gn) and $rAUDPC_{Gs}$=area under the relative disease progress curve of the potato genotype with the highest susceptibility (Gs) and 9=value assigned to Gs.

Yield (Yld). Based on the weight of the plot harvested over the number of plants in the plot, the Yld was expressed in tuber weight (kg) per plant.

Number of tubers per plant (NTu). The average Ntu was obtained based on the plants in the plot.

Number of stolons per plant (NSt). The average NSt was determined based on the plants in the plot.

Tuber weight (TW). The TW corresponds to the ratio of Yld to NTu.

Data Analysis

The Path Analysis (PA) was performed, for which the correlations between the traits Yld, NTu, TW, NS, NSt, SEV, GSS, RD, dAUDPC, and rAUDPC were obtained. Based on the correlation coefficients, the traits were determined. Using the Excel program, the path coefficients were obtained to establish the direct and indirect effects on the association between the Yld and the other causal traits.

Likewise, the traits evaluated were subjected to Principal Component Analysis (PCA) and Hierarchical Classification, taking into account that only one of the two highly correlated traits was included in these analyses, in order to group the genotypes evaluated by discriminatory traits, such as yield and disease severity. To test the null hypothesis (Ho) that the group mean is equal to the original population mean, the *t-test,* proposed by Stiles (2000) was used (Equation 6):

$$t_c = \frac{\overline{Y}_i - \overline{Y}_j}{\sqrt{\frac{S_i^2 + S_j^2}{n_i + n_j - 2} x\left[\frac{n_i + n_j}{n_i \times n_j}\right]}} \quad (6)$$

Where: t_c=t calculated, $\overline{Y}_i$=average of the variable in the i-th group, $\overline{Y}_j$ =average of the variable in the j-th group, S_i^2 =variance of the i-th group, S_j^2=variance of the j-th group, n_i=number of individuals in the i-th group and n_j=number of individuals in the j-th group.

For the decision rule the t_c was compared with the t_t (t of the table with a α =0.05 and with degrees of freedom equal to $n_i + n_j$ -2). If $t_c < t_t$ Ho is accepted.

Subsequently, the best genotypes were selected based on a selection index (SI), whose weights were established according to the importance of the traits related to the Yld components and the disease. The first step was to standardize (S) the values of each of the traits that made up the SI (Lagos *et al*., 2015), using the Equation 7:

$$S = \frac{Y_{ij} - \overline{Y}}{SD} \quad (7)$$

Where: Y_{ij}=observation of the variable j-th in the introduction i-th, Y=overall average of variable j-th in the i-th

introductions and SD=standard deviation of variable j-th in the i-th introductions. Then, for each introduction (Y_{ij}) the SI was applied, given by the sum of the products of the standardized value of each variable by its weight (W), as follows: Yld(0.6), SEV(-0.3), rAUDPC(-0.1), dAUDPC(0.5), GSS(-0.05), RD(-0.05), NSt(0.08), NS(0.07), NTu(0.1) and TW(0.15).

RESULTS AND DISCUSSION

The Correlation Analysis for the 10 traits evaluated (Table 2), enabled to find a significant negative average association between Yld vs SEV (r=-0.50*) and Yld vs rAUDPC (r=-0.53*), indicating that the productive potential of the plants decreased due to the increase infection process caused by the pathogen. Yld vs NSt traits showed high positive association (r=0.70*) similar to Yld vs NTu (r=0.71*). This indicates that to improve Yld, it can be selected based on NSt and NTu.

For the Yld vs NS, the correlation was 0.46* (Table 2), however, the PA showed that the most of this dimension is explained by the indirect effect (outside the diagonal) through NTu with a value of 0.34 (Table 3). The association between Yld vs TW showed a value of association r= 0.70* (Table 2). Similarly, the PA showed that the direct effects (on the diagonal and in bold) explained the greater proportion of the dimensionality of the correlation, with a value of 0.62 (Table 3).

Table 2. Correlation analysis for traits related to yield and reaction components to disease caused by *P. infestans* in 76 introductions of *S. tuberosum* group Andigena.

Traits	SEV	rAUDPC	dAUDPC	GSS	RD	NSt	NS	NTu	TW
Yld	-0.50*	-0.53*	0.53*	-0.45*	0.07	0.70*	0.46*	0.71*	0.70*
SEV	1	0.59*	-0.59*	0.86*	-0.09	-0.50*	-0.41*	-0.49*	-0.18
rAUDPC		1	-1.00*	0.76*	-0.12	-0.59*	-0.36*	-0.53*	-0.24*
dAUDPC			1	-0.76*	0.12	0.50*	0.36*	0.53*	0.24*
GSS				1	-0.11	-0.43*	-0.34*	-0.45*	-0.18
RD					1	0.01	-0.07	0.04	0.05
NSt						1	0.66*	0.94*	0.15
NS							1	0.56*	0.13
NTu								1	0.10

*significant correlations (P<0.05)

Table 3. Path analysis (PA) for traits related to yield and disease response components caused by *P. infestans* in 76 introductions of *S. tuberosum* group Andigena.

Traits	SEV	rAUDPC	RD	NS	NTu	TW	rYld-y
SEV	**-0.08**	-0.01	0	0	-0.30	-0.10	**-0.50**
rAUDPC	-0.05	**-0.01**	0	0	-0.32	-0.20	**-0.53**
RD	0.01	0	**0.01**	0	0.02	0.03	**0.07**
NS	0.03	0.01	0	**0.01**	0.34	0.08	**0.46**
NTu	0.04	0.01	0	0	**0.60**	0.06	**0.71**
TW	0.01	0	0	0	0.06	**0.62**	**0.70**

SEV=severity (%), rYld-y=correlation between the Yld and the y variable

According to the PA in Table 3, the dimension of the correlations of Yld with SEV and rAUDPC is explained in greater proportion by the indirect effects through NTu (-0.30 and -0.32) than by the direct effects of SEV (-0.08) and rAUDPC (-0.01). These results differ from those found by Betancourth *et al.*(2008), who reported that the direct effect of SEV on Yld explains 50% of the magnitude of the correlation. Furthermore, the direct effect of the correlation between Yld and TW (0.70), is explained in greater proportion by the direct effect of TW (0.62) than by the indirect effects of the other variables of the PA. This result is useful to direct a selection by TW to achieve an increase in Yld.

Table 4 presents the PCA of the selected traits according to the correlation analysis related to the yield and reaction components to the disease caused by *P. infestans*. The first three components explain 82.70% of the total variance. Yld, NTu, TW, SEV, RD and rAUDPC were included in the PCA, generating six PCs (Núñez and Escobedo, 2014).

PCs accumulate a portion of the total variance and have greater importance and more accumulated variance the higher their eigenvalues. The PC1, whose eigenvalue is 2.90, has an accumulated variance (AV=48.4%). The first two PCs have an accumulated variance of 66.2% (Table 4). This procedure is used for quantitative data. The aim is to reduce the information, moving from one set of traits to another smaller set representing the former, without making any hypothesis about the meaning of the factors (David *et al.*, 2016).

Table 4. Principal Component Analysis (PCA) for traits related to yield and reaction to disease caused by *P. infestans* in 76 introductions of *S. tuberosum* group Andigena.

Traits	Principal Components (PC)		
	1	2	3
Yld	0.91	0.32	0.01
SEV	-0.74	0.34	-0.07
rAUDPC	-0.78	0.29	-0.03
RD	0.15	-0.26	-0.95
NTu	0.78	-0.27	0.22
TW	-0.55	0.79	-0.20
Eigenvalues	2.90	1.07	0.99
V	0.484	0.179	0.165
AV	0.484	0.662	0.827

V=proportion of the variance explained by the PC, AV=proportion of the cumulative variance explained by the PC.

For the selection of the PCs, the Kaiser Criterion was considered, which is based on the choice of PCs whose values or weights (Bernal *et al.*, 2019). Based on these considerations, the first three PCs that explain 82.7% were selected to explain the variance of the population studied (Table 4).

The traits Yld, SEV, rAUDPC and NTu define the PC1, with variable factor (rv-f) correlation values between -0.74 and 0.91. PC2 is related to the TW that generated the largest contribution to the total variance of the component with an rv-f of 0.79. Regarding PC3, the RD trait made the greatest contribution to the rv-f dimension with -0.95 (Table 4).

According to the Classification Analysis, four groups were identified (Figure 2 and Table 5). The first group (G1) consisted of 39 genotypes and the susceptible control Capiro identified by T1, T2, T3, T4, which corresponded to 49.37% of the population. This group is characterized by one of the highest percentages of SEV (86.90%) with respect to the general average of the population (A-Po) (53.96%). On the other hand, it showed a rAUDPC of 0.80, with 9.31 NTu and a TW of 54.11 g, which are

statistically lower than the average values of A-Po. The second group (G2) consisted of 33 genotypes, representing 36.71% of the total individuals studied (Figure 2). This group presented an Yld of 1.16 kg per plant which is higher than A-Po of 0.94 kg per plant. It also recorded a low SEV (19.33%), a RD of 0.06, values that differ statistically from population averages. Likewise, the RD (0.06) and the NTu (16.59) were higher than the A-Po (Table 5).

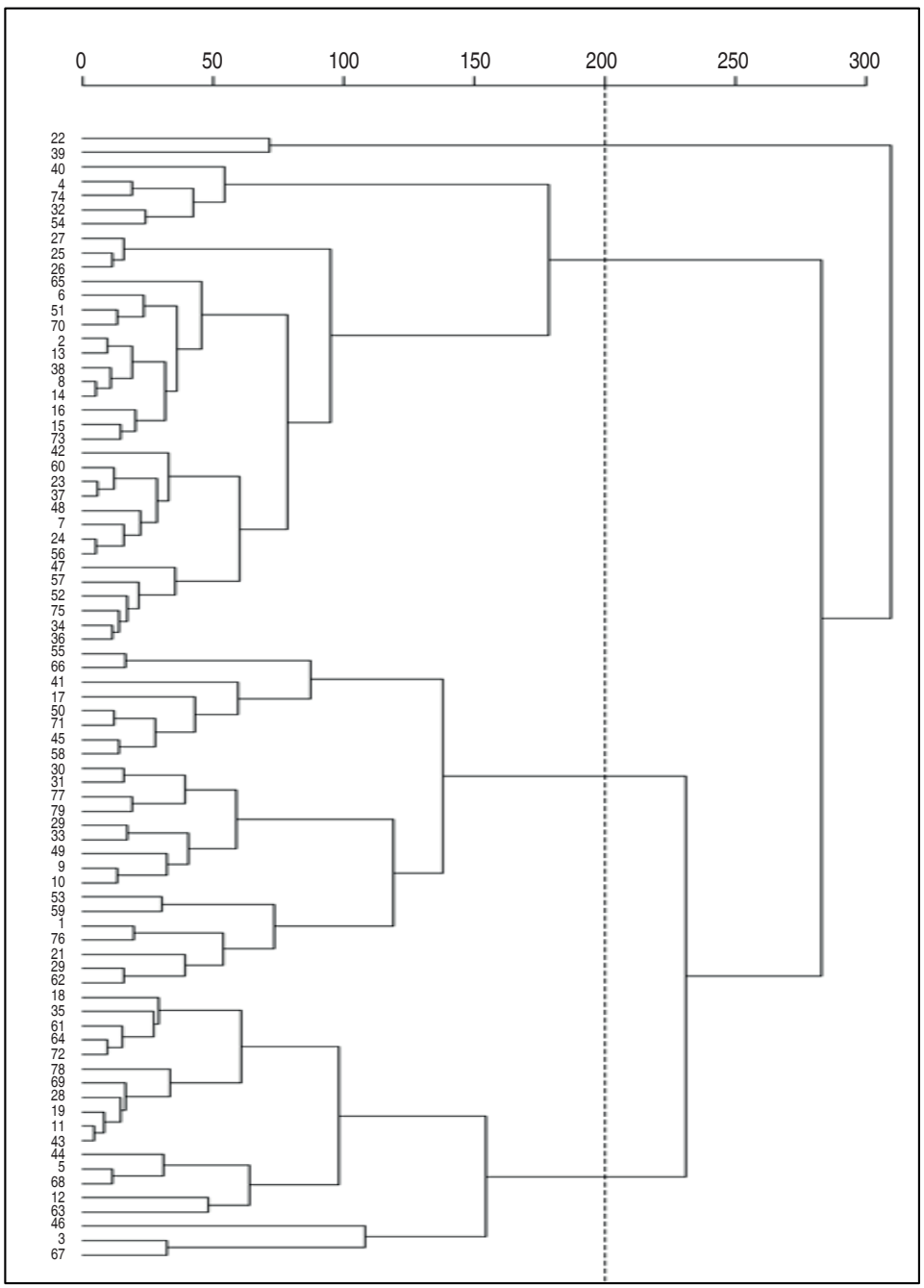

Figure 2. Dendrogram of Hierarchical classification analysis for 75 introductions of *S. tuberosum* group Andigena and one control sample evaluated for their reaction to disease caused by *P. infestans*.

Group three (G3) included five genotypes which are 6.33% of the total population. This group exhibited the highest Yld with 2.03 kg per plant higher than A-Po, a SEV (12.10%) lower than A-Po. The TW (164.84 g) recorded an average statistically higher than A-Po which showed an average of 72.40 g. Finally, group four (G4) included two genotypes, which presented the highest averages of TW and Yld with 272.29 g and 1.86 kg per plant, respectively, higher values than the overall average (Figure 2 and Table 5).

Table 5. Characteristics of four groups formed in the Classification Analysis according to six traits related to components of the disease *P. infestans* and yield in 76 genotypes of potato *Solanum tuberosum*.

Traits	A-Po N=76	SD-Po	A-G1 N=39	SD-G1	A-G2 N=33	SD-G2
SEV	53.96	36.84	86.90*	13.01	19.33*	13.28
rAUDPC	0.54	0.44	0.80*	0.32	0.29*	0.42
RD	0.03	0.17	0.01*	0.01	0.06*	0.26
NTu	12.47	6.79	9.31*	5.16	16.59*	6.86
TW	72.40	50.77	54.11*	32.49	67.89*	26.73
Yld	0.94	0.68	0.56	0.46	1.16*	0.59
Traits	**A-G3 N=5**	**SD-G3**	**A-G4 N=2**	**SD-G4**		
SEV	12.10*	11.72	87.50*	17.68		
rAUDPC	0.10*	0.10	0.74*	0.37		
RD	0.01*	0.00	0.02	0.01		
NTu	12.27	3.93	6.84*	1.18		
TW	164.84*	20.85	272.29*	3.84		
Yld	2.03*	0.71	1.86*	0.30		

*significant difference (P<0.05), A-Po=average of the population assessed, SD-Po=standard deviation of the population assessed, A-G=average group, SD-G=group standard deviation, N=number of individuals.

As it can be seen in the results described above, the introductions with the best behavior with regard to the disease are located in G3 with lower values of SEV and rAUDPC; However, in G4 are located the introductions of better behavior regarding the Yld, even under the pressure of the gradient of the disease, since in spite of reporting significant high values of SEV in comparison with the A-Po, they presented the highest values for the TW, for example, the genotype 40 that corresponds to the variety Red Huila M6 (UdenarStGua40) presented the greater TW with 275 g.

On the other hand, the selection index (SI) allowed the selection of 10 introductions that stood out from the original population, presenting a SI ranging from 1.90 to 3.63 with statistical differences regarding the population average (SI=0.00) (Table 6).

The introductions UdenarStGua53 (CIP 300046.22), UdenarStGua61 (CIP 391011.17), UdenarStGua68 (CIP 392557.171), UdenarStGua73 (CIP 393079.4), UdenarStGua75 (CIP 393280.82), UdenarStGua77 (CIP 393371.164) and UdenarStGua78 (CIP 393371.58) are from the CIP, where they are reported to be tolerant to attack by *P. infestans* (CIP, 2019). This behavior was similar in the present study, where they obtained values of SEV between 1.0 and 16.67% classified as tolerant and moderately tolerant, and with Ylds between 1.65 and 3.07 kg per plant, with significant differences with regard to the control varieties Capiro (0.05 kg per plant) and Betina (0.43 kg per plant).

Statistically, the Ylds of the 10 introductions surpassed the original population and the Betina and Capiro varieties, which are moderately tolerant and susceptible. With respect

Table 6. Selection index and mean traits of potato *S. tuberosum* group Andigena introductions chosen for high yield performance (Yld) and low severity levels (SEV) of *P. infestans* with susceptible and tolerant controls.

Genotype	Yld	SEV	rAUDPC	dAUDPC	GSS	RD	NSt	NS	NTu	TW	SI
UdenarStGua75	3.07	1.00	0.00	99.54	0.02	0.00	23.33	3.00	18.00	170.37	3.63
UdenarStGua61	2.24	5.33	0.06	92.76	0.32	0.01	12.00	4.00	11.33	197.94	2.71
UdenarStGua23	2.03	8.33	0.09	88.94	0.50	0.01	32.67	4.00	32.33	62.89	2.59
UdenarStGua73	2.15	10.00	0.11	86.31	0.61	0.01	22.00	1.67	20.67	104.03	2.33
UdenarStGua53	1.87	5.00	0.04	95.08	0.22	0.01	15.67	1.33	12.67	147.89	2.15
UdenarStGua77	1.65	10.00	0.05	93.12	0.31	0.01	25.00	4.00	22.33	73.88	2.09
UdenarStGua29	2.00	21.67	0.26	66.60	1.50	0.01	29.67	3.00	28.00	71.43	2.06
UdenarStGua12	1.90	16.67	0.20	74.28	1.15	0.01	26.67	2.33	25.67	74.03	2.00
UdenarStGua68	1.55	2.17	0.01	98.32	0.08	0.00	21.00	2.33	13.67	113.41	1.96
UdenarStGua78	1.78	15.00	0.17	78.56	0.96	0.01	24.67	2.33	20.33	87.70	1.90
Average (SG)	2.03	9.52	0.10	87.35	0.57	0.01	23.27	2.80	20.50	110.36	2.34
SD (SG)	0.42	6.63	0.09	10.92	0.49	0.00	6.12	0.97	6.87	46.74	0.53
Average Po (A)	0.94	53.96	0.54	30.76	2.77	0.03	15.08	2.03	12.47	72.40	0.00
SD Po (SD)	0.68	36.84	0.44	56.70	2.33	0.17	7.41	1.07	6.79	50.77	1.53
A+SD or A-SD	1.62	17.12	0·10	87.46	0.44	-0.14	22.49	3.11	19.26	123.17	1.53
SG-A	1.09	-44.44	-0.44	56.59	-2.20	-0.02	8.18	0.77	8.03	37.96	2.34
Capiro	0.05	95.00	0.78	0.25	4.47	0.02	2.83	1.25	2.58	22.02	-1.95
Betina	0.43	100.00	0.46	41.13	2.64	0.03	15.67	1.33	14.33	30.23	-0.84
P. Suprema	1.78	30.00	0.35	54.62	2.03	0.01	35.33	5.33	26.67	66.88	1.84

SG=selected genotypes. Po=population evaluated. SD = standard deviation. SI=selection index (estimated Yld). Underlined shows significant differences.

to the original population, these 10 selected introductions exceeded the population average by 1.09 kg per plant, which represents an increase in Yld of 53%. However, this gain is subject to factors, such as selection intensity, selection differential (SG-Po) and heritability, which define the true gain known as genetic gain (Nyquist, 1991).

CONCLUSIONS

Principal Components and Hierarchical Classification analyses allowed to discriminate between introductions of *Solanum tubersoum* group Andigena tolerant and moderately tolerant to the natural inoculum of *Phythopthora infestans*. As for the correlations of yield with severity and area under the relative disease progress curve, they showed a negative correlation.

The selected genotypes UdenarStGua75, UdenarStGua61, UdenarStGua23, UdenarStGua73, UdenarStGua53, UdenarStGua77, UdenarStGua12, UdenarStGua68 and UdenarStGua78 showed high yield values per plant and severity values below the population mean of 17.12%. Within the selected introductions, those from the International Potato Center-CIP (UdenarStGua75, UdenarStGua61, UdenarStGua73, UdenarStGua53, UdenarStGua77, UdenarStGua68 and UdenarStGua78) are considered as a potential source of tolerance to *P. infestans;* therefore they are recommended to be included in a breeding program that seeks to obtain tolerant varieties to the disease and should be considered for multi-environmental assessments to determine if there is differential behavior across contrasting conditions.

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Influence of burning and weed control on the soil fertility and vegetation cover of Brazilian Amazon pastures

Influencia de la quema y el control de malezas sobre la fertilizad del suelo y la cobertura de vegetal de los pastizales de la Amazonía brasileña

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ABSTRACT

In pastures it is common to use fire to control weeds, justified by the increase in soil fertility that ashes can generate. However, the benefits of this process is short-lived, and also increase the soil exposure. The permanence of weeds can contribute to the vegetation cover in areas of deficient pastures. This study aimed to evaluate the effect of prescribed burning and mowing on vegetation cover of *Urochloa brizantha (U. brizantha)* pastures and soil surface fertility in pastures located in the Brazilian Amazon. The study was conducted in Itupiranga, Pará state, Brazilian Amazon. Five pastures of *U. brizantha* cv. Marandu and a forest area were evaluated. The soil was a Ferralsol. Vegetation cover was estimated using a quadrat. Four composite soil samples were taken in each area, collected from 0–0.05 m depth. The percentage of vegetal cover of *U. brizantha*, weeds and bare soil was measured and fertility in soil samples (pH, Al^{3+}, Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, P, and H+Al) were determined. Three groups were identified in the evaluated pasture plots. Group I had the highest average percentage of bare soil (33.5%) and was associated with H+Al and P values. Group II had the highest average of *U. brizantha* cover (90%) and was associated with Ca^{2+}+Mg^{2+} and K^{+}. Group III had the highest average of weed cover (53.4%) and was associated with Al^{3+}. The higher percentage of *U. brizantha* in Group II was favored by the burning later. This group presented better levels of K^{+}, Mg^{2+} and sum of bases. The management adopting the least use of fire on the pastures, with longer time for the regeneration of forage, combined with the mechanical control of weeds, can be the best alternative for maintaining the soil cover and bringing benefits for quality surface layer of the soil.

Keywords:
Bare soil
Fire
Forest
Soil erosion
Urochloa brizantha

RESUMEN

En los pastos es común utilizar el fuego para controlar las malas hierbas justificado por el incremento de fertilidad en el suelo que pueden generar las cenizas. Sin embargo, los beneficios de este proceso son de corta duración, además de aumentar la exposición del suelo. La permanencia de las malezas puede contribuir a la cobertura vegetal en áreas de pastos deficientes. Este estudio tuvo como objetivo evaluar el efecto de la quema prescripta y corte de malezas en la cobertura vegetal de los pastos de *U. brizantha* y en la fertilidad de la superficie del suelo en pastos ubicados en la Amazonía brasileña. El estudio se realizó en Itupiranga, estado de Pará, Amazonía brasileña. Cinco pasturas de *U. brizantha* cv. Marandu y un área de bosque fueron evaluados. El suelo era un "Latosol Rojo-Amarillo" (Ferralsols). La cobertura vegetal se estimó utilizando un cuadrante. Se tomaron cuatro muestras de suelo compuestas en cada área, recolectadas de 0–0,05 m de profundidad. Se determinó el porcentaje de cobertura vegetal de *U. brizantha*, malezas y suelo expuesto y la fertilidad en muestras de suelo (pH, Al^{3+}, Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, P y H+Al). Se identificaron tres grupos entre las parcelas de pastos evaluadas. El grupo I tuvo el porcentaje promedio más alto de suelo expuesto (33,5%) y se asoció con los valores de H+Al y P. El grupo II tuvo el promedio más alto de cobertura de *U. brizantha* (90%) y se asoció con Ca^{2+} + Mg^{2+} y K^{+}. El grupo III tuvo el promedio más alto de cobertura de hierbas (53,4%) y se asoció con Al^{3+}. El mayor porcentaje de *U. brizantha* en el grupo II se vio favorecido por la quema posterior. Este grupo presentó mejores niveles de K^{+}, Mg^{2+} y suma de bases. El manejo adoptando el menor uso del fuego en los pastos, con un mayor tiempo de regeneración de forrajes, combinado con el control mecánico de malezas, puede ser la mejor alternativa para mantener la cobertura del suelo, trayendo beneficios a la calidad química de la capa superficial del suelo.

Palabras clave:
Suelo expuesto
Fuego
Bosque
Erosión del suelo
Urochloa brizantha

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The Brazilian legal Amazon has a total area of, approximately, 5.0 million km^2, corresponding to about 60% of the Brazilian territory. In 2018, it had a cumulative deforested area corresponding to 708,301 km^2, equivalent to about 17% of the entire Brazilian Amazon forest. Approximately 13% of the area is occupied by agriculture, with an estimated area of 43,092,115 ha occupied by pasture (Miranda *et al*., 2019).

In these pastures, it is common to use fire to control weeds and to stimulate pastures regrowth, arguing the ashes can increase the soil fertility. However, about 90 days after burning, soil fertility levels begin to decrease (Santana *et al*., 2013). Thus, the benefits of this process are short-lived, increasing soil exposure to erosive processes.

The conversion of Amazon rainforest to pasture through slash and burn, affects the physical, chemical, biological and even mineralogical properties of the soil. It is aggravated by the general characteristics of the soils formed in the region, which have extreme poverty in phosphorus available (P); high acidity; high aluminum saturation (Al^{3+}); low Cation Exchange Capacity (CEC); macro and micronutrient poverty and susceptibility to compaction and erosion (Vale-Júnior *et al*., 2011).

The effect of temperature caused by burning on soil chemical properties leads to an increase in pH, CEC, in addition to calcium (Ca), magnesium (Mg), potassium (K) and phosphorus available (P). The rise in temperature increase the pH value and decrease the CEC. Regarding nutrients, if the burning temperature rises in excess, Ca, Mg, K and P decrease (Giovannini *et al*., 1990).

The exposure of the soil to extreme temperatures (>600 °C) also causes auterations in mineralogy and texture of the soil, increasing the amount of sand and a decrease in silt and, mainly, clay. Regarding mineralogy, with increasing temperature, the peaks for gibbsite in the sand fractions can gradually decrease in intensity, and disappear completely at a temperature above 600 °C (Ketterings *et al*., 2000).

In pastoral systems, the topsoil is sensitive to changes in management, mainly due to the compacting of the surface caused by heavy animals grazing in extensive systems. Also, the microbial activity of the soil and the contributions of plant material affect the dynamics of soil properties on the surface in relation to the soil in depth (Boeni *et al*., 2014).

Studies about chemical changes in the superficial layer of the soil (0-0.05 m) under forest subjected to burning, showed an increase in the values of pH, electrical conductivity, organic carbon and exchangeable bases, caused by the addition of ash from the fires (Iglesias *et al*., 1997). Alterations may also occur in fertility, in which the soil has higher concentrations of P, Mg and K, after being subjected to high temperatures; however, a decrease in the density of microfungi could occur (Copogna *et al*., 2009). Other alterations in the superficial layer (0-0.05 m) can be observed in relation to the increase of the sand fraction and decrease of the silt and clay fractions (Ketterings *et al*., 2000).

Because of the bad aspects that fire can cause in soil properties, some alternatives can be used to suppress its use, including mowing (weed cutting practice). The permanence of plants considered weeds, can contribute to the vegetation cover in areas with deficient pastures, in order to protect the soil (Campos *et al*., 2019).

Weeds can protect to the soil in degraded pastures, as the plants intercept the direct splash of rain promoting infiltration, increasing water retention and dissipating runoff (Lewis *et al*., 2013). Studies show lower losses of nutrients, such as N, P and K, in cultivation systems in presence of weeds (Lenka *et al*., 2017).

Regarding soil quality, the non-removal of weeds contributed positively to Ca and CEC. In addition, it raises the total organic carbon content by 0–0.03 m (Araujo-Junior *et al*., 2011). Therefore, manual weed control in areas with deficient pasture can be an efficient alternative for maintaining soil quality without the use of fire, which can promote deleterious changes in edaphic characteristics.

Due to the high use of fire in pasture systems in the Amazon studies of the fertility levels of these soils are necessary. In this sense, this study aimed to evaluate the effect of prescribed burning and mowing on vegetation cover of *U. brizantha* pastures and soil fertility in pastures located in the Brazilian Amazon.

MATERIALS AND METHODS

Site description

The study was conducted in Itupiranga county (05°08'20" S, 49°19'25" W), Pará state, which is part of the Brazilian Amazon. The soil is a Latossolo Vermelho-Amarelo (Embrapa, 2009), Ferralsols (FAO, 2015). The regional climate is Am in the transition to Aw (Köppen and Geiger, 1954). The region has an average annual temperature of 26.35 °C and has a dry season between May and October, and a wet season between November and April (Lisbôa, 2017).

Five pasture plots were evaluated in two contiguous farms (A and B), as well as a native (original) forest fragment between them, as reference. In the areas where the pastures were located, a previous native forest was slashed and burned for pasture formation in 1993 and was seeded with *U. brizantha* cv. Marandu. The forest in question is classified as a land-based equatorial broadleaf forest. The pastures never received any type of improvement or fertilizer and only were subjected to slash-and-burn agriculture and mowing until the year 2015 (Table 1).

Table 1. Management applied in five areas of *U. brizantha* pasture in Itupiranga county, Pará state, Brazil.

Farm	Pasture plot	Area (ha)	Number of animals per hectare (animal ha^{-1})	Management applied
A	1	6.0	1.6	Burned in 2015, and mowing
A	2	6.0	1.6	Burned in 2014, and mowing
B	3	4.0	2.0	Burned in 2010, without mowing
B	4	6.5	2.0	Burned in 2010, and mowing
B	5	7.0	2.0	Burned in 2010, and mowing

Data were collected, and analyzed in 2015 during the dry season (July). Vegetation cover (*U. brizantha*, weeds, and bare soil, %) was estimated using a quadrat (Martha-Junior *et al.*, 1999), which consisted of 2.0 m^2 wooden square that contained a checkered mesh of string with 80 small squares. From each plot, 12 samples were recorded. Weeds were considered any other species than *U. brizantha*, including *Eupatorium* sp., *Cenchrus* sp., *Cynodon* sp., and *Crotalaria* sp.

Soil sampling and analysis

Soil samples were collected from 0–0.05 m under the soil surface. To cover as much as possible the entire area studied in each pasture area and in the forest area, four composite samples (obtained from three simple samples) were taken in each area (12 samples in each plot). Together with the pasture, soil samples from a natural (original) forest area that has never been altered were also collected, as a reference. In the laboratory, roots were manually removed from the soil samples before they were passed through a 2 mm sieve. The soil samples sieved were analyzed for: pH in water, in the ratio 1:2.5; Al^{3+}, Ca^{2+}, Mg^{2+} and Na^{+}, extracted with KCl at 1 mol L^{-1}, at the ratio 1:10; Al^{3+}, by titration with 0.025 mol L^{-1} NaOH; Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry; Na^{+} by flame photometry; K^{+} and P available by extraction with Mehlich-1 (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}) at the ratio 1:10; and H + Al, by Ca $(OAc)_2$ at 0.5 mol L^{-1}, adjusted pH 7.0, at the ratio 1:15, titrated with 0.0606 mol L^{-1} NaOH (Embrapa, 2011).

Statistical analysis

According to Monroe *et al.* (2016), Fontes *et al.* (2014), Rocha-Junior *et al.* (2014) and Lisbôa *et al.* (2016), the data was analyzed by ANOVA analysis. A randomized design with four replicates (four composite samples), which are considered as pseudo-replication in studies that involve data collection in the field. Each area (pastures and forest) was considered to be a fixed-effect treatment due to several sources of variation as manual control of weeds and burning.

The normality of the data was checked (Shapiro and Wilk, 1965). Also, a cluster analysis was conducted based on the vegetation cover data to identify groups in terms of pasture similarity. These data were subjected to analysis of variance (ANOVA) and a post hoc test (Tukey, $P<0.05$). The soil fertility data were combined based on the groups

obtained by the cluster analysis and compared with the reference area (forest) by ANOVA and a post hoc test (Tukey, $P<0.05$). A principal component analysis (PCA) was performed to analyze relationships among the vegetation cover variables and soils fertility data. All the analyses were performed using R, version 3.0.1 (R Core Team, 2013).

RESULTS AND DISCUSSION

Descriptive statistics of the vegetation cover variables are presented in Table 2.The highest percentages of *U. brizantha* cover were presented in pastures of the plot 4 (90.6%) and the plot 5 (89.4%); the highest percentage of bare soil occurred in pasture of the plot 1 (40.4%) and the highest percentage of weed cover was 53.4% in pasture of the plot 3.

The vegetation cover variables were subjected to a cluster analysis based on Euclidean distances by the complete method. Three groups were identified from the plots evaluated (Figure 1): Group I consisted of pasture plots 1 and 2 (Farm A), Group II contained pasture plots 4 and 5 (Farm B), and Group III only contained pasture plot 3 (Farm B).

Table 2. Vegetation cover and bare soil percentage in five pastures in Itupiranga county, Pará state, Brazil.

Pasture plot	*U. brizantha* (%)	Bare soil (%)	Weeds (%)
1	59.4±4.5	40.4±4.5	0.2±0.6
2	55.0±9.5	26.6±7.7	18.4±7.1
3	36.8±9.8	9.8±5.3	53.4±10.8
4	90.6±1.8	6.8±1.0	2.6±1.8
5	89.4±4.0	6.0±2.6	4.6±2.3

Vegetation cover data were combined based on their clusters and subjected to an ANOVA (Table 3). Group II had the highest average *U. brizantha* cover (90%), followed by Group I (57.2%) and III (36.8%). The absence of burning after 2010, and the practice of weed control, caused the high percentage of *U. brizantha* cover in Group II. The manual control of weeds in this group favored the domination by the species *U. brizantha*. The morphological and physiological characteristics of pastures such as a fibrous root system and C4 photosynthesis make them strong competitors, resulting in an important edaphic benefit protecting the soil surface and preventing moisture loss (Morris *et al*., 1993; Heringer and Jacques, 2002).

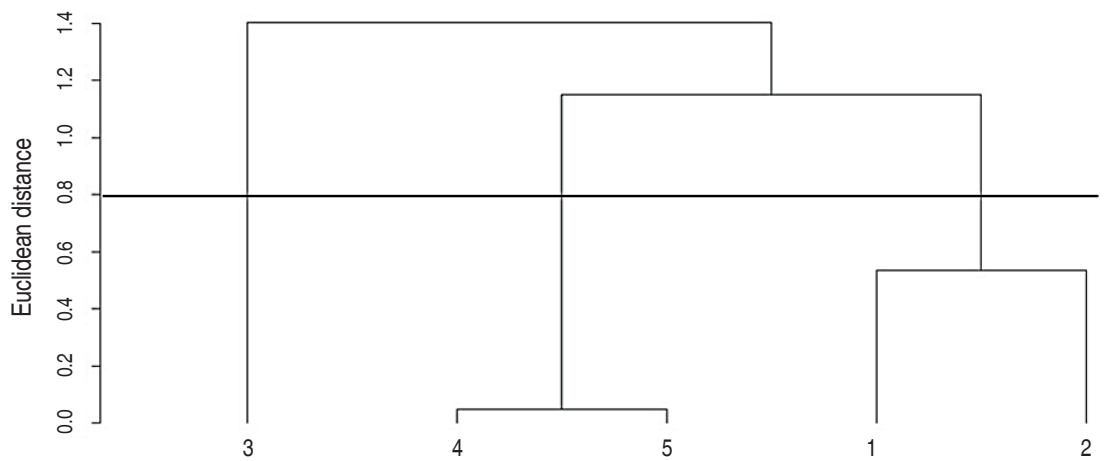

Figure 1. Cluster analysis of five *U. brizantha* pastures in Itupiranga county, Pará state, Brazil.

Group I had the highest average percentage of bare soil (33.5%), followed by Groups II (6.4%) and III (9.8%). Group I had the highest average percentage of bare soil because burning between 2014 and 2015 affected vegetation cover and caused an increase in bare soil. Burning is a quite common practice among Brazilian farmers since, theoretically, it increases soil fertility by adding nutrients from ash. However, this benefit is temporary because the soils are exposed to leaching by rainfall, triggering a low soil fertility and increase aluminum saturation, which affects plant growth (Heringer and Jacques, 2002; Santana *et al.*, 2013).

Group III had the highest average weed cover (53.4%), followed by Group I (9.3%) and II (3.6%). Group III (only contained pasture plot 3) had the highest average weed cover because it received no weed control, as opposed to Santos *et al.* (2019), who considered mowing as an inefficient activity for weed control. In this study, this method proved to be effective, since Group I and Group II showed less weeds compared to Group III.

Table 3. Vegetation cover variables averages (%) in three groups of *U. brizantha* pasture in Itupiranga county, Pará state, Brazil.

Group	*U. brizantha* (%)	Bare soil (%)	Weeds (%)
I	57.2[b]	33.5[a]	9.3[b]
II	90.0[a]	6.4[b]	3.6[b]
III	36.8[c]	9.8[b]	53.4[a]
[1]CV (%)	52.73	36.47	10.26

Different lower case letters in a column indicate a statistically significant difference (P<0.05) between the groups according to the Tukey test.
[1] Coefficient of variation.

Table 4 shows the fertility data of five pastures and forest plots in the areas studied. Soil pH varied from 3.5 to 4.0. P varied from 1.5 mg dm^{-3} (pasture 2) to 5.1 mg dm^{-3} (forest). K^+ varied from 120 mg dm^{-3} (forest) to 40 mg dm^{-3} (pasture 3 and 4). The highest value of Ca^{2+} was in forest soil (1.0 $cmol_c$ dm^{-3}), and the smallest value was in pasture 4 soil (0.1 $cmol_c$ dm^{-3}). The Mg^{2+} values varied from 0.2 $cmol_c$ dm^{-3} (pasture 4) to 0.9 $cmol_c$ dm^{-3} (forest and pasture 4). Al^{3+} was another element that presented values bellow 1.0 $cmol_c$ dm^{-3} and varied from 0.4 $cmol_c$ dm^{-3} (pasture 4) to 1.0 $cmol_c$ dm^{-3} (pasture 4). Higher values of H+Al were observed in forest soil (12.7 $cmol_c$ dm^{-3}).

Table 4. Soil fertility data of forest and pastures, in Itupiranga county, Pará state, Brazil.

	Forest	Pasture 1	Pasture 2	Pasture 3	Pasture 4	Pasture 5
pH	3.5±0.2	3.9±0.1	4.0±0.3	4.0±0.1	3.7±0.2	3.9±0.2
P	5.1±1.9	3.5±0.6	1.5±0.7	1.9±0.3	1.7±0.8	2.2±0.9
K^+	120.0±24.4	90.0±46.9	120±11.5	40.0±27.0	40.0±20.4	43.8±13.7
Na^+	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Ca^{2+}	1.0±0.2	0.5±0.2	0.4±0.2	0.3±0.2	0.1±0.1	0.4±0.2
Mg^{2+}	0.9±0.3	0.5±0.2	0.9±0.3	0.4±0.2	0.2±0.1	0.7±0.3
Al^{3+}	0.9±0.3	0.9±0.3	0.4±0.2	0.8±0.2	1.0±0.1	0.8±0.4
H+Al	12.7±2.1	7.7±1.4	4.2±2.1	7.0±1.0	5.9±1.6	7.3±1.5
SB	2.1±0.5	1.2±0.5	1.6±0.5	0.8±0.4	0.5±0.2	1.2±0.6

pH: hydrogen potential; P: phosphorus (mg dm^{-3}); K: potassium (mg dm^{-3}); Na: sodium (mg dm^{-3}); Ca: calcium ($cmol_c$ dm^{-3}); Mg: magnesium ($cmol_c$ dm^{-3}); Al: aluminium ($cmol_c$ dm^{-3}); H+Al: potential acidity ($cmol_c$ dm^{-3}); SB: Sum of Basis ($cmol_c$ dm^{-3}).

The soils of all studied areas presented low levels of fertility according to Prezotti and Guarçoni (2013) classification. Soil pH values were considered low and acid. The values of Al^{3+} were medium, whereas H+Al

values were high. The soils also presented low values for the sum of bases, except forest which had median values. The soil under the pastures presented low values of K^+, Ca^{2+}, and Mg^{2+}.

Zenero *et al.* (2016) found pH values in Amazon soils of 3.9, from 0 to 0.06 m of depth, which demonstrates this soil can be extremely acid. Soil pH is determined by the concentration of hydrogen ions (H^+), and it is influenced by acid (H^+, Al^{3+}, Fe^{2+}, or Fe^{3+}) and base-forming cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) in the soil. Acidic conditions occur in regions with higher amounts of precipitation. High precipitation causes leaching of base-forming cations and decreasing of soil pH. Natural acidic soils are commonly found in forest soils (McCauley *et al*., 2017).

The action of fire on the chemical and physical properties of the soil, combined with the contribution of the nutrients present in the ash, may have contributed to the increase in pH and H+Al in the pasture areas, regarding the soil forest (original) (Giovanni *et al*., 1990; Iglesias *et al*., 1997; Zanero *et al*., 2016). Regarding the Phosphorus content, the soil under forest showed better conditions in terms of Phosphorus availability for the crops, in relation to the soils under pasture (Prezotti and Guarçoni, 2013). These results are expected for Ferralsols, which present low fertility because of its source material, high weathering, removal of SiO_2, and accumulation of Al_2O_3 in its mineral phase, with a predominance of kaolinite and oxides of iron and aluminum (Araujo-Junior *et al*., 2011).

Relating to the availability of P in soils, fire can have a great influence on the bond between P and clay minerals. Considering that the Mehlich-I extraction is based on the principle of dissolution of minerals containing P (Corrêa, 1993), studies relate to the retention of P in mineral surfaces with temperature are important. With that in mind, Ketterings *et al.* (2000) evaluated the temperature caused by fire over mineralogy and soil texture. The authors observed that the temperature by the fire influenced the clay, decreasing its quantity, mainly, by 0-5 cm, including the reduction of clay minerals such as gibbsite ($Al(OH)_3$). This mineral is important in the adsorption of P in the soil, and the decrease in that nutrient (P) is related to the decrease in the mineral ($Al(OH)_3$). Despite this, a dense canopy cover, as in the forest area, protects soil against loss of P as runoff or leaching.

The soil analysis data were combined into their respective groups and compared to each other and the forest area (Table 5). Soil pH value was smaller in forest soil (3.5) than the other pasture soils. There were no differences in Al^{3+} values between the areas. Forest soil presented the highest values for H+Al, P (5.12 mg dm^{-3}), K^+ (120 mg dm^{-3}), Ca^{2+} (0.96 cmolc dm^{-3}), Mg^{2+} (0.85 cmolc dm^{-3}), and SB (2.12 cmolc dm^{-3}).

Table 5. Soil fertility data of forest soil and three pastures groups, in Itupiranga county, Pará state, Brazil.

	pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	SB
Forest	3.5 ^{b}	5.1 ^{a}	120.0 ^{a}	0.9 ^{a}	0.8 ^{a}	0.9 ^{a}	12.6 ^{a}	2.1 ^{a}
I	3.9 ^{a}	2.7 ^{b}	65.0 ^{b}	0.4 ^{b}	0.4 ^{ab}	0.8 ^{a}	7.3 ^{b}	1.0 ^{bc}
II	3.9 ^{a}	1.8 ^{b}	81.8 ^{ab}	0.4 ^{b}	0.7 ^{a}	0.6 ^{a}	5.7 ^{b}	1.4 ^{ab}
III	3.7 ^{ab}	1.7 ^{b}	40.0 ^{b}	0.1 ^{b}	0.2 ^{b}	0.9 ^{a}	5.8 ^{b}	0.5 ^{c}
CV (%)	5.3	42.2	50.7	47.7	47.6	41.8	25.7	41.9

Different lower case letters in a column indicate a statistically significant difference (P<0.05) between the groups according to the Tukey test. pH: hydrogen potential; P: phosphorus (mg dm^{-3}); K: potassium (mg dm^{-3}); Ca: calcium ($cmol_c$ dm^{-3}); Mg: magnesium ($cmol_c$ dm^{-3}); Al: aluminium ($cmol_c$ dm^{-3}); H+Al: potential acidity ($cmol_c$ dm^{-3}); SB: Sum of Basis ($cmol_c$ dm^{-3}). CV: Coeffcient of variation (%).

As it was demonstrated in Table 5, soil pH value was higher in pastures soils, along with ash deposition, which can contribute to raising pH values, this fact may be related to the loss of the OH groups of the clays, caused by the temperature rise by burning, which contributes to the formation of oxides of various elements derived from the rupture of carbonates (Giovanni *et al*., 1990). Also, the ashes from the fire, are mainly composed of Ca, Mg, K, Si, phosphates, and carbonates ($CaCO_3$). In spite of the short benefit of ash reported by the literature, the low solubility of this mineral allows its persistence for more than three years after the fire, keeping the pH moderately alkaline

in superficial horizons, in normally acidic soils (Iglesias *et al*., 1997).

The forest presented the highest values of macronutrients (P, K^+, Ca^{2+}, and Mg^{2+}). This fact demonstrates the important role that trees play absorbing available nutrients from lower depths and distribute it to the soil surface via litterfall. Besides, as in the forest area, it takes up available nutrients from lower depths and redistributes them to the soil surface vialitterfall, the decomposition of straw on the soil surface can increase the availability of nutrients, favoring plants(Campos et al., 2019). Kautz *et al.* (2013) explained the nutrient accumulation in the Ap horizon as a turnover and long-term accumulation of nutrients acquired from the subsoil and translocated in the shoot and root systems, predominantly as a result of litter mineralization in the Ap horizon. As the forest area has a huge diversity of tree species, they can have roots at different depths, which means greater access to nutrients compared to pasture.

As discussed before, the absence of fire after 2010 until 2015 and the practice of weed control caused a high percentage of *U. brizantha* cover in Group II, and this can benefit vegetation growth. Roots can reach high depth as in *U. brizantha*, and the high rhizosphere extension can assist them in the access to water and nutrients and provide greater shoot growth (Kautz *et al*., 2013).

The PCA results are presented in Figure 2. Vegetation cover from the forest area was not collected. The values of Ca^{2+} and Mg^{2+} were not included in this analysis to avoid multicollinearity, instead of that, they were summed as Ca^{2+}+Mg^{2+}, for the same reason, the sum of basis was not included (Hair *et al*., 2005).

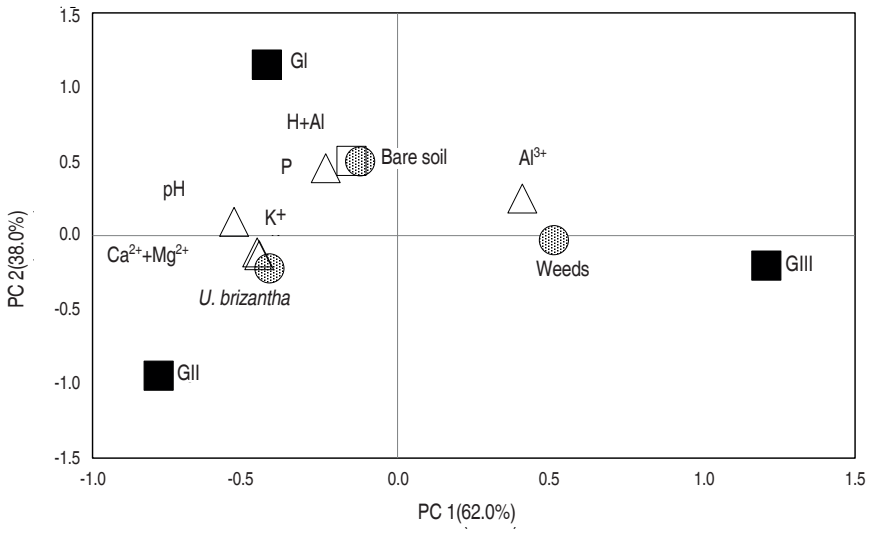

Figure 2. Principal component analysis of vegetation cover and soil fertility in *U. brizantha* pastures in Itupiranga county, Pará state, Brazil. GI: Group I; GII: Group II; GIII: Group III; P: phosphorus (mg dm^{-3}); K: potassium (mg dm^{-3}); Ca + Mg: calcium + magnesium (cmolc dm^{-3}); Al: aluminium (cmolc dm^{-3}); H+Al: potential acidity (cmolc dm^{-3}).

Principal component 1 (PC 1) explained 62.0% of the variability, and principal component 2 (PC 2) explained 38.0%. Group I, which had a high percentage of uncovered soil, was associated with the values of H+Al and P. This demonstrates that these soils have low natural fertility, which can be aggravated by burning pastures. With the most recent use of fire, 2014 and 2015, the low growth of forage in the pastures of this group indicates that, perhaps, this area has not yet recovered, with less soil coverage. In turn, the lack of cover can result in the worsening of soil quality, due to the lack of nutrient cycling, accumulation of nutrients and organic matter and leaching of nutrients. In addition, the lack of liming and soil fertilization, also aggravate the issue of acidity (Kautz *et al*., 2013; Santos *et al*., 2015).

Group II, where later burning occurred in 2010, there was the highest *U. brizantha* cover, and less weeds, was

better related to exchangeable soil bases (Ca^{2+}+Mg^{2+}, and K^{+}). According to Crespo *et al.* (2015), an important way of nutrients entry in pasture soils is the litter. The biomass of pasture roots can contribute to the nutrients deposition in these systems; after this, nutrients return to the soil, and subsequently recycled.

PCA results for Group III had a better association for weeds and Al^{3+}. This group presented the highest aluminum values and the lowest sum of base values. Under these conditions, cultivable plants have severe restrictions on their establishment, which may decrease their presence in the area, increasing the presence of weeds. In the absence of soil correction, with the lime application, acidification causes an increase in species regarded as agricultural weeds, and a reduction in productivity (Goulding, 2016). Weed species identified in the present study are commonly found in soils with low fertility and high acidity (Gazziero *et al.,* 2006; Brighenti *et al.,* 2010; Moreira and Bragança 2011; and Costa *el al*., 2011).

CONCLUSIONS

There was similarity between the pasture areas, reducing the five pastures to three groups. The highest percentage of uncovered soil was observed in Group I, which was associated with more recent pastures burning.

The highest percentage of *U. brizantha* was observed in Group II, which was favored by the later burning, in relation to the other pastures, and with the practice of mechanical weed removal. This group had better levels of K, Mg and sum of bases.

As expected, the highest percentage of weeds was observed in Group III, where there was no control for their proliferation. This group had a greater relationship with the high levels of Al^{3+} in the area.

Adopting the least use of fire on the pastures combined with the mechanical control of weeds, can be the best alternative for maintaining the soil cover and bringing benefits for quality surface layer of the soil.

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Short article

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Precision agriculture for rice crops with an emphasis in low health index areas

UNIVERSIDAD NACIONAL DE COLOMBIA

Agricultura de precisión para cultivos de arroz con énfasis en áreas con bajo índice de salud

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ABSTRACT

The delimitation of affected areas by low health index in crops is a useful tool that let farmers implement a differentiated rice-crop management. The objective of this research was to analyze and determine these areas in rice crops. For this purpose, health index maps were combined with RGB maps, digital elevation models (DEM), and tridimensional models to monitor this culture. The study was conducted in farms located in the regions of Tortí and Darién in Panama. Unmanned aerial vehicles (UAV) or drones with multispectral and RGB cameras were used to obtain orthomosaics (RGB, NIR). A specialized mapper software was used to generate the calibrated health map, and Google Earth and AgriYttium (mobile app) were used as crop visualization tools for farmers. The virtual tour above farm plot through visual and tridimensional models facilitated inspection of crop zones with difficult access. Map analysis allowed identifying areas with low health index, low plant density, weed problem, yellowing, competition with other crops and steep slopes. This information was presented to the producers through printed and digital reports with detailed information about the polygonal surfaces and the proportion of affected areas in crops and recommendations regarding areas for reseeding, land leveling, and corrections in pesticide/fertilizer applications. This tool led to saving agrochemical products since the applications can be made on precise sites.

Keywords:
Crop monitoring
Digital elevation models
Health index maps
NDVI
Multispectral images
Tridimensional models

RESUMEN

La delimitación de áreas afectadas a través de un índice bajo de salud en un cultivo es una herramienta útil que permite al agricultor un manejo diferenciado en sembrados de arroz. El objetivo de esta investigación fue analizar y determinar estas áreas en cultivos de arroz; para ello se combinaron mapas de índices de salud con mapas RGB, asimismo se usaron modelos de elevación (DEM) y tridimensionales como herramientas de monitoreo. El estudio se desarrolló en fincas ubicadas en las regiones de Tortí y Darién en Panamá. La captura de las imágenes se realizó con vehículos aéreos no tripulados (UAV) equipados con cámaras multiespectrales y RGB para obtener los ortomosaicos (RGB, NIR). Se utilizó un software especializado para generar los mapas calibrados de condición del cultivo y Google Earth y AgriYttrium (aplicación para Smartphone) para la visualización de los mismos. El recorrido virtual de la finca mediante los modelos visuales y tridimensionales facilitó la inspección de las zonas con difícil acceso. El análisis de los mapas permitió evidenciar zonas con bajo vigor vegetativo, baja densidad de plantas, presencia de arvenses, clorosis, competencia con otros cultivos y pendientes elevadas. Los productores recibieron un informe en formato digital e impreso con información detallada sobre superficies poligonales y proporción de las áreas afectadas y con las respectivas recomendaciones, como resiembra, nivelación del terreno y corrección en aplicación de pesticidas/fertilizantes. Esta herramienta conlleva al ahorro de agroquímicos ya que las aplicaciones se pueden hacer en los sitios precisos.

Palabras clave:
Monitoreo de cultivos
Modelos digitales de elevación
Mapas de índice de salud
NDVI
Imágenes multiespectrales
Modelos tridimencionales

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In Panama, rice crop (*Oryza sativa* L.) is important for agro-industrial development. Provinces of Chiriquí, Veraguas and Coclé have more than 60% of the rice production in the country (Barria, 2012). Since the 90s, producers of these regions are very known by their disposition to use emerging technologies, expecting to improve the yield of their crops. However, the Eastern region of the country, where Tortí and Darién are located, has emerged as an agricultural area (INEC, 2018). Precision Agriculture (PA) plays an important role in rice crop production since it provides techniques that allow optimal use of agricultural supplies (seeds, fertilizers, agrochemicals), regarding the quantification of the spatial and temporal variations of the agricultural cycle (Best and Zamora, 2008; Bongiovanni *et al*., 2006; IICA, 2014; Fedearroz, 2015).

The rice crop has three morphological phases: vegetative, reproductive, and maturation (Fedearroz, 2015; Serrano *et al*., 2018). Timely monitoring in the first two phases allows detecting the right place and time for the optimal physical, biological, and chemical treatments that improve the crop yield (Berrío *et al*., 2015; Brenes-González, 2016; Berger *et al*., 2019).

Unmanned aerial vehicles (UAV) use for the monitoring of rice and other crops is gaining worldwide acceptance, mainly because the satellite system limitations when capturing multispectral images are reduced (García and Martínez, 2010). UAV equipped with specialized cameras permits to obtain a higher image resolution and to conduct flights based on the climate changes of the study area (González-Betancourt and Mayorga-Ruíz, 2018), Barraza *et al*., (2019) presented a precision system to apply a herbicide on rice crop to reduce the negative ecological impact in Chiriquí, Panama. In Asia and Africa, these systems are employed in pest prevention, diseases and weed detection in crops (Goel *et al*., 2002; Berrío *et al*., 2015; Hernández, 2017). In Colombia, Fedearroz started promoting both systems (UAV and satellite image technology) for crop yield assessment (Lago *et al*., 2011; Berger *et al*., 2019) and irrigation planning to increase water use more efficient (Acosta and Mendoza, 2017; Norasma *et al*., 2018).

Crop health monitoring can be done through remote sensing and analysis of reflection coefficient or reflectance, which is the ratio between sunlight absorbed and the amount of solar energy reflected by the plants (Berger *et al*., 2019; Brenes-González, 2016). Reflectance varies according to plant cellular structures, physiological characteristics, and morphological phase. The parameter used to relate a crop reflectance with its health conditions is called vegetation index, which is a combination of reflectance values in different wavelengths, with high sensitivity to vegetation changes (Acosta and Mendoza, 2017). The index is used to detect healthy, harmed, or severely harmed plants (Berger *et al*., 2019; García and Martínez, 2010; Serrano *et al*., 2018; Goel *et al*., 2002).

The most used parameter is the Normalized Difference Vegetation Index (NDVI) (Baihua and Burgher, 2015), which measures the normalized difference between the spectral response in the nearby infrared and red of visible spectra bands. This index allows characterizing the crop through a scale of values from -1 to 1. Values closed to -1, are considered white surface as snow, ice, or clouds. Values lower than 0 are classified as water; values between 0 and 0.15 are considered as soil; values between 0.15 and 0.6 are considered mixed soil and vegetation, and between 0.6 and 1 covered vegetation. NDVI indicates if the plant has good vegetative vigor and if it is healthy (Goel *et al*., 2002; García and Martínez, 2010; Lago *et al*., 2011).

The information collected through NDVI also allows generating crop condition maps (Brenes-González, 2016), which are bidimensional and georeferenced plots, with a color scale for the visual representation of vegetation index in a region. A crop health map allows identifying problematic areas due to low plant density, water stress or leveling problems, for example. Other kinds of maps permit learning more about plant and soil conditions. Visual maps allow detecting low crop growth and weed distribution and are useful to study topographical conditions (Berrío *et al*., 2015). The analysis of these maps assists farmers in making better decisions (Lundström and Lindblomb, 2018).

Considering UAV remote sensing advantages in crop care and management, this research aimed to create NDVI-based health maps for Tortí and Darién regions through UAV, NIR and RGB technology in order to set improved agricultural plans for rice crops.

MATERIALS AND METHODS

Study area

This study was conducted in 14 farms located in two regions of Panama. The Tortí Province (8°55'30" N, 78°24'19.6" W, 96 masl) has warm weather and a temperature range of 21-33 °C; the rainy season lasts 11 months with an average annual precipitation of 190 mm; and the Darién Province (8°20'30" N, 77°54'51" W, 128 masl), in which the temperature varies from 17 to 35 °C and has average annual precipitation between 1700 and 2000 mm (Weather Spark, 2020). Table 1 shows the data of the three of the 14 studied farms.

In the three cases of study, the analysis was based on four maps: crop NDVI-based health map, visual map built as RGB orthomosaic to identify affected areas by weed presence, elevation map that informs on soil surface profile, and tridimensional map which is for virtual terrain inspections.

Table 1. Agricultural plot data involved in the study.

Case of study	Region	Farm plot area (ha)	Rice variety	Flights	Monitoring dates
1	Tortí Playa Chuzo Sector	12.61	Estrella 71	3	48 DAS 89 DAS 111 DAS
2	Darién Metetí Sector	27.30	Unknown	2	27 DAS 56 DAS
3	Tortí Santa Cruz sector	26.30	Estrella 71	3	20 DAS 41 DAS 62 DAS

DAS: Days after sowing

Equipment

The RGB images for this research were collected using the DJI Phantom 4 Pro drone with a 20 Mpx RGB camera. Multispectral images were taken with a DJI Inspire 1 V.2 and a 12 Mpx resolution Zenmuse X3 multispectral camera (DJI, 2020). The image pixel size varies depending on the height, obtaining values of 2 cm per pixel for flights at 80 m over ground level. These surveys were done at 3.5 cm per pixel of resolution. The software DroneDeploy was used for flight planning, taking an average of 425 images per flight with a 75% front and side overlap. The number of flights performed over each farm plot is presented in Table 1. Flight dates were set considering the rice cultivation cycle (vegetative, reproductive, and maturation): Case study 1: (48, 89 and 111 days after sowing (DAS)), Case study 2: (27 and 56 DAS), and Case study 3: (20, 41, and 62 DAS).

Data processing

NDVI was calculated according to equation 1 (García and Martínez, 2010).

$$NDVI=(NIR-R)/(NIR+R) \quad (1)$$

Where:
NDVI: Normalized Difference Vegetation Index
NIR: Reflectance in nearby infrared band measured at 850 nm.
R: Reflectance in the red band of the visible spectrum measured at 660 nm.

NDVI-based health maps

The software DroneDePloy was used to obtain RGB and NIR orthomosaics, the elevation model, and the 3D model. Additionally, the software YttriumMapper (Version 1) developed by the authors (Montilla, 2017) was used to calibrate the NIR camera using a calibration pattern, which was validated by obtaining NDVI maps. In each farm, the NDVI histogram for health map was calculated. YttriumMapper was also used for the superposition of RGB, digital elevation model (DEM), and 3D maps with the NDVI maps through a mechanism of commutation between both maps in geographical correspondence, which mixes two maps obtained by independent flights.

Google Earth (Version 7.7.3) was used as a visualization tool because it has simple graphic user interphase. This

software manages visual and NDVI-based health maps at a low resolution and allows visualizing polygons, traces limits of affected areas, and polygon measurement (areas and longitude). Moreover, a mobile App (AgriYttrium, Version 1) was used to visualize health maps and polygonal areas (Montilla, 2017). AgriYttrium allows navigating the crop and identifying low index areas and other reported issues. Maps used in AgriYttium were handled with 4K resolution and integrate along the polygons in a single KMZ format file to facilitate swift data management.

Report delivery

Results delivered to farmers included printed and digital format reports. Two access levels were provided, the most straightforward to farmer is a KMZ format file that can be directly visualized through the AgriYttrium mobile app and Google Earth. The reports provided a guide of the affected areas to perform agronomical management of these regions.

RESULTS AND DISCUSSION

Three study cases were analyzed. The analysis was based on four maps: NDVI-based health crop map, visual map built using RGB orthomosaics to identify affected areas by weed presence, elevation map to inform about the soil profile, and tridimensional map which was used for virtual terrain inspections.

Case of study 1

The processed images of the first flight showed three areas with crop health problems, which were delimited with white polygons (Figure 1A). The total problematic surface was 3.6 ha (28.2%) of the farm plot. The other part of the map shows colored shade areas with different range of NDVI values: (0.61-0.62), (0.54-0.60), and (0.49-0.53) in upper and lower zones of the farm. The most problematic areas were presented a red hue with the NDVI value ranging from 0.4 to 0.48. Areas in yellow and red hue indicate that canopy did not develop, and seedlings did not emerge, respectively. These values were measurement with the Zenmuse multispectral camera and adjusted to NDVI calibration panel (DJI, 2020). In this experience were not considered the maximum and minimum values of NDVI only mean values (Baihua and Burguer, 2015; Serrano *et al.*, 2018; Goel *et al.,* 2002), because in the plot the noisy data correspond to roads, others farms, and wild trees that could indicate high NDVI values based on the above range values of NDVI, 0.5 and 0.6 values were assigned to healthy plants.

Figure 1B shows a decrease of the red shades compared to those obtained from the first flight (48 DAS). The fading of the red zones is a evidence of the affected areas recovery (Figure 1A), although still shows health problems in 2.9 ha (23.23%) colored in yellow and light green ranging 0.46-0.55 NDVI values.

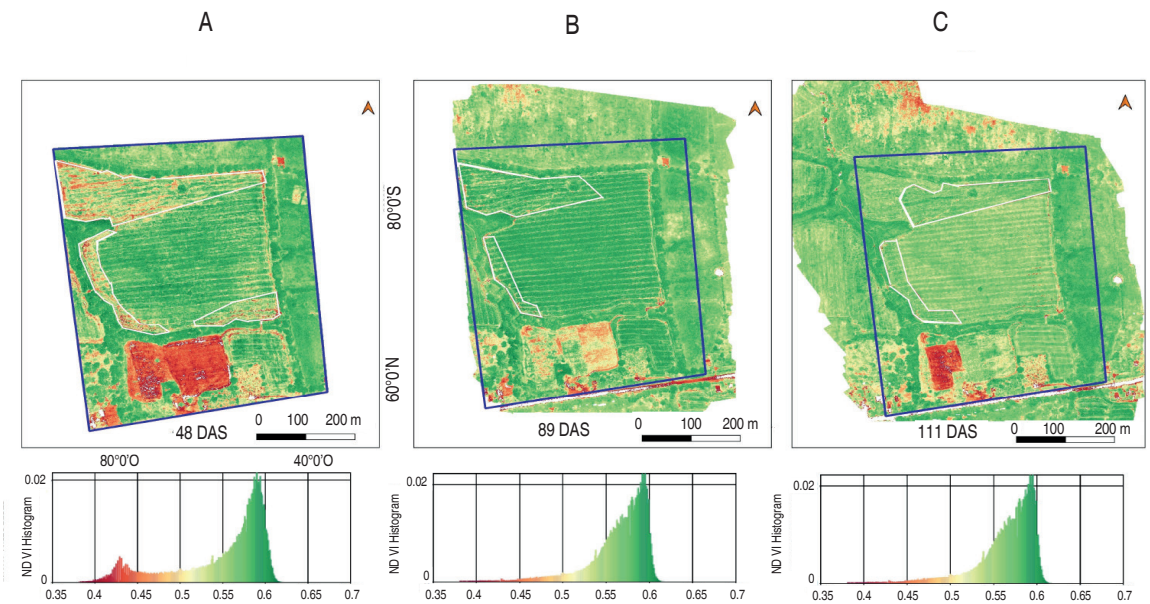

Figure 1. NDVI-based crop health maps for the Case of study 1, showing the studied area (blue polygon) and the affected areas (white polygons). A. 48 DAS; B. 89 DAS; C. 111 DAS.

By superposing orthomosaic 2D (Figure 2A) and NDVI-based plant health maps (Figure 1A), areas with no crop coverage were identified. Similarly, when the 2D map and DEM were compared, a slope was observed in the zone with no crop coverage (Blue-colored areas, Figure 2B). The elevation profile obtained from Google Earth showed a difference in the crop height between +20 m (+5%) and -23 m (-5.6%). This unevenness generates problems of irrigation distribution, causing a rice plant loss (González-Betancourt and Mayorga-Ruíz (2018); Berrío *et al.* (2015). Besides, there were also pest problems (birds) that ate part of the seeds in some areas, according to farmers. In this case, the solution was reseeding with pre-germinated seeds of short cycle to level the production.

The 3D map (Figure 2C) allowed identifying areas of interest where the farmer must conduct in site inspection (due to the perspective 3D presentation, the geometric forms of Figure 2C looks different to the rectangular shape of maps shown in Figures 2A and 2B, but it corresponds to same study area). The low plant density in the 3D map agree with the signaled areas in Figure 1A. Berrío *et al.* (2015) indicated that remote monitoring with UAV reduces cost (time and personal) because this equipment can explore large areas with difficult access.

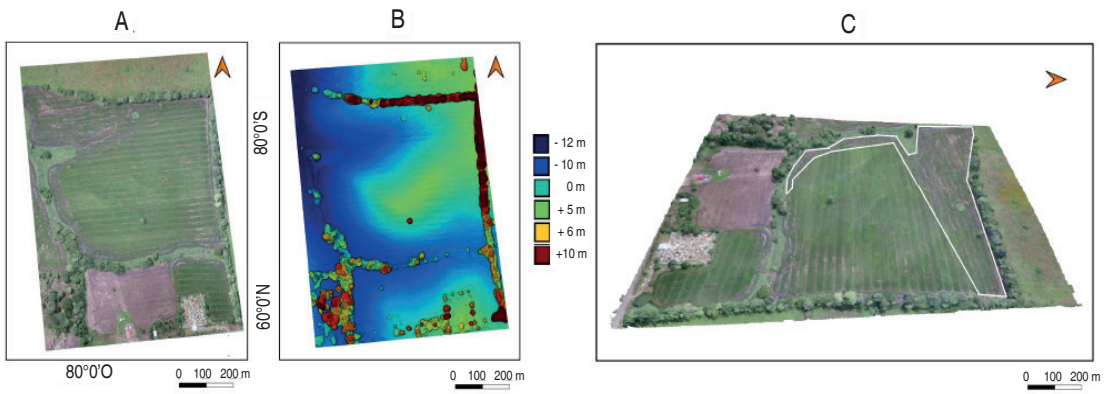

Figure 2. Visual maps for the case of study 1 on 48 DAS. A. 2D model; B. DEM; C. 3D model.

The low health index through the cultivation cycle is presented in Figure 3. It shows the differences between health crop maps and their change over time; this information helps farmers to take actions or modify conditions to improve the health of the affected plants. In this case, the affected area declined by about 5% from 48 to 89 DAS and 10% from 48 to 111 DAS. These data are important since a low health index is related to low yield. The high variability of colors in NDVI maps is associated health variability of crops.

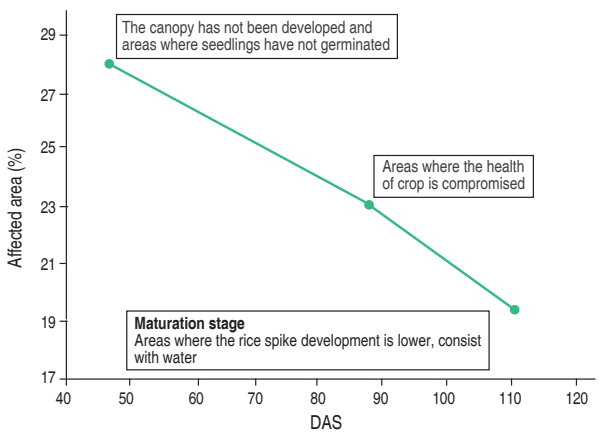

Figure 3. Percentage of the affected zone with low health index in the Case of study 1 along the evaluation period.

Figure 3 information can be considered in a cost analysis since it is related to the production losses and to the number of agrochemical applications to recover the plantation. Also, the involved areas can be used as an evolution quality measurement of the crop that is easy for farmers to understand. Two plantations mapped over the same involved area graph can be immediately compared and conclude which of the two plantations has a better possibility of high production yield. Therefore, the involved areas percentage curves can be used as an important and compact economical tool.

The cumulative NDVI was not considered because only three UAV flights were planned during the entire crop period; consequently, the temporal data is scarce to construct a cumulative NDVI (Baihua and Burgher, 2015; Serrano *et al.*, 2018; Goel *et al.*, 2002). Besides, the focus on the percentage of affected areas is easily understood by the rice producer.

Case of study 2

The processed image of the first flight showed low health index areas (Figure 4, 27 DAS), which were delimited by three white polygons – upper (1), middle (2), and the bottom section (3). The most problematic surface (bottom section) was 8.1 ha (41% of the farm plot) and showed colored shade areas with different range of NDVI values (0.4–0.55).

For the second flight (Figure 4, 56 DAS), the map was divided into four sections numbered from 1 to 4 (top-bottom order). Polygon 1 (4.8 ha, 24.2%) presented the greater health index and color uniformity with NDVI range of 0.55 and 0.60. Polygon 2 (6.6 ha, 33.4%) had lower NDVI values from 0.50 to 0.55. In polygon 3 (4.1 ha, 20.8%), crop recovery is evident in comparison with the first flight. However, the health index still low, and the lack of uniformity is noticeable, showing an NDVI range of (0.45–0.58). Polygon 4 (3.5 ha, 17.5%) evidenced a good crop recovery, but the health index remained low, and it also lacks uniformity with an NDVI range of 0.48–0.54.

DEM, 3D maps (Figures 5B and 5C), and the elevation profile obtained from Google Earth show a height difference of 12 m. The slope variations were over +5.4% and -5.1%

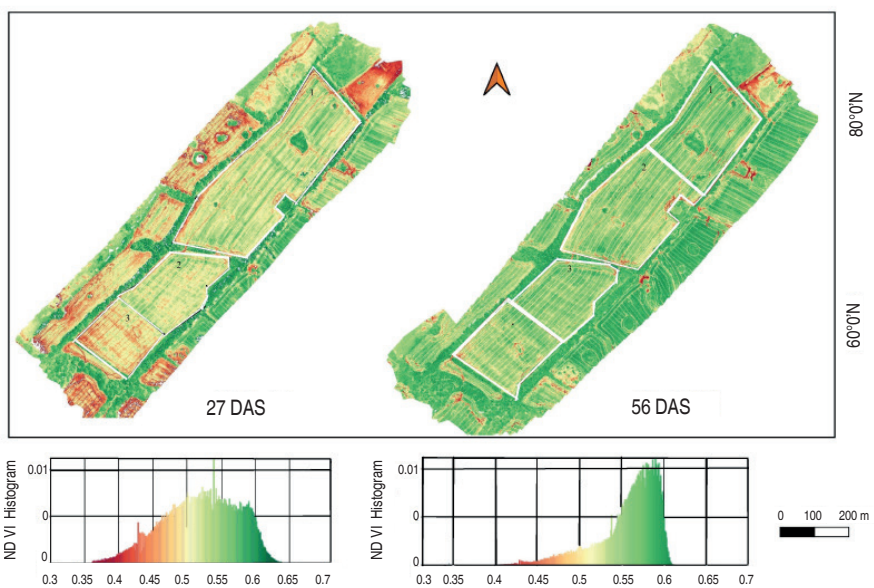

Figure 4. NDVI-based crop health maps for case study 2, showing the affected areas (white polygons).

in the area with no crop coverage. This elevation difference could wash away the seeds (Norasma *et al.*, 2018). In this case, it is recommended to level the area to improve drainage or replant the area with lower crop density.

Case of study 3

The processed image of the first flight (Figure 6A) shows a health map with little vegetation development. Yellow, orange, and red are predominant colors with 0.40–0.45

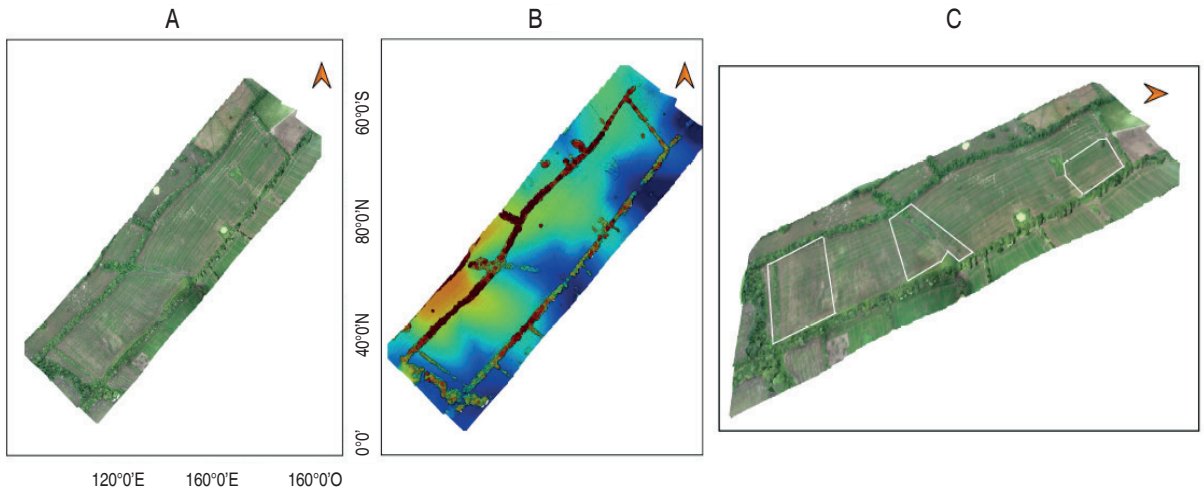

Figure 5. Visual maps for the Case of study 2 on 56 DAS. A. 2D model; B. Digital Elevation Model (DEM); C. 3D model.

NDVI values; few light yellow and light green areas with white polygons have 0.50–0.55 NDVI values. It does not necessarily correspond to a non-healthy crop zone because the flight was made in an early stage when the field did not have enough plants. Serrano *et al.* (2018) and Norasma *et al.* (2018) recommended the monitoring in this stage to detect plant phenological development and certificate if the growth level of the crop is according to reference patterns. The NDVI map shows a section of 2.5 ha (10.7%) with a health index over the normal range. In contrast, an area of 21.1 ha (89.3%) presented a low health index. These values are consistent with low vegetal development or low plant density in the vegetative stage of the crop (González-Betancourt and Mayorga-Ruíz, 2018).

For flight 2 (Figure 6B), the health index was not uniform. There was an increase in crop coverage regarding the first flight, although deficiencies in the crop health were still visible. Only the area shown on the bottom of the map (white polygon) presented an intense green; this sector has an area of 5.8 ha (24.6%) and NDVI values of 0.58–0.62. An area of 17.8 ha (75.4%) with 0.50-0.56 NDVI values was found as a poor healthy plot.

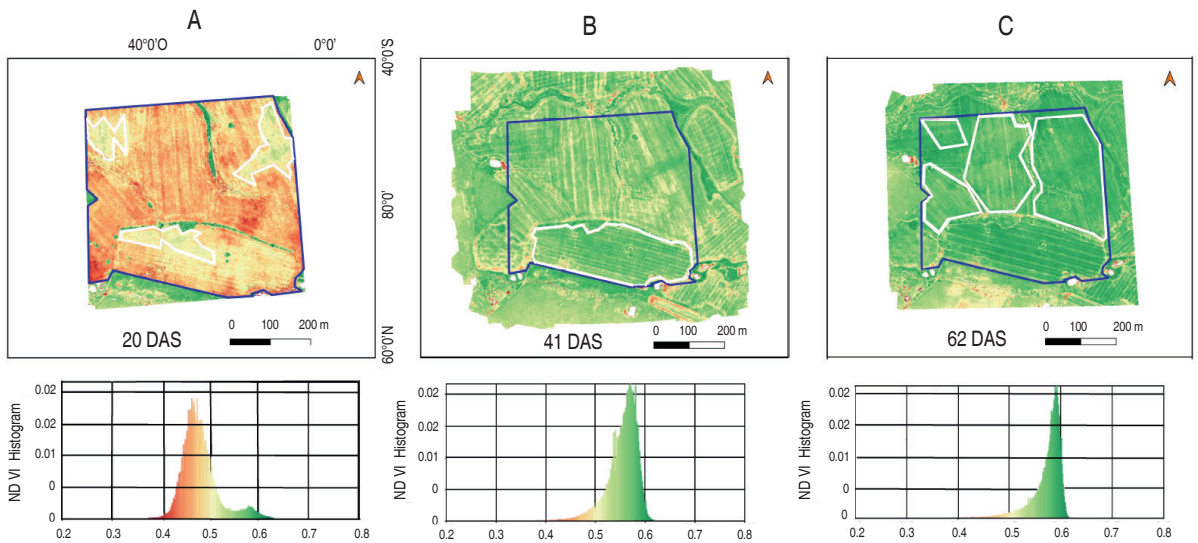

Figure 6. NDVI-based crop health maps for the case of study 3, showing the studied area (blue polygon) and the affected areas (white polygons). A. 20 DAS; B. 41 DAS; C. 62 DAS.

At 62 DAS, the map indicates a tendency to improve the health index in the rice crop, showing an intense green area (NDVI values of 0.58–0.62), although some irregular light green zones (low health index) of 12.05 ha (51.1%) and NDVI values of 0.50–0.56, were still present as in the first two flights. These zones, delimited by white polygons, could have weed growing, whereby it is recommended to conduct on-site revision to generate a robust model map. The zone with intense green corresponds to NDVI values of 0.58–0.62. The range of reported NDVI values for affected areas of this farm could also be related to water stress occasioned by terrain leveling; this can be explain through 2D, DEM, and 3D maps (Figure 7).

The 2D map (Figure 7A) obtained on 20 DAS allowed verifying the vegetation growth, which is scarce for its age. In DEM map (Figure 7B), the difference of level is approximately 13 m and extensive regions with abrupt slopes can be observed in the elevation profile with variations between +5.2% and -4.7%; these terrain conditions impact the rice crop development negatively because these slopes provoke strong runoffs that can wash away substrate, nutrients and seeds (González-Betancourt and Mayorga-Ruiz, 2018; Norasma et. al, 2018). In the 3D model (Figure 7C), low crop density areas were seen, as well as many hills and creeks, which indicates topographic variations.

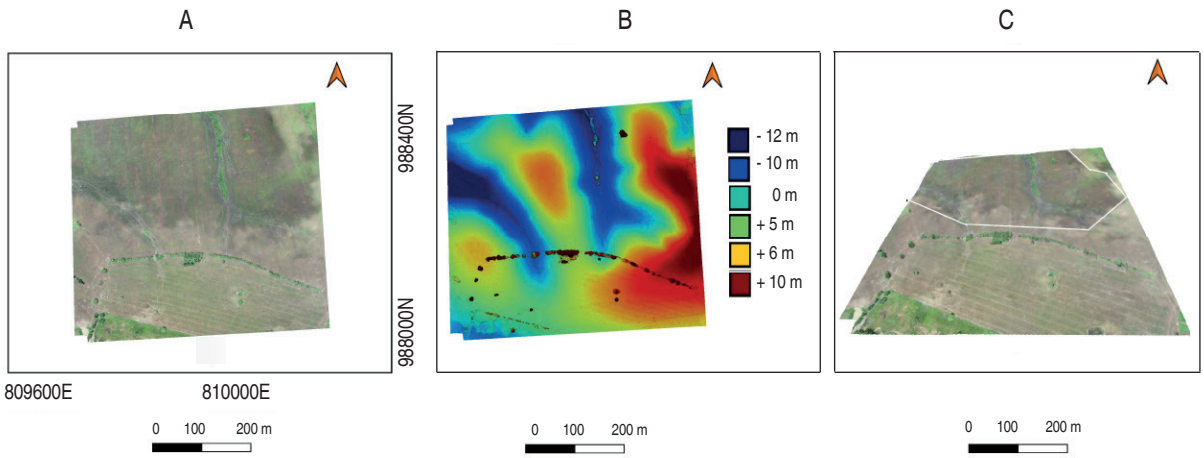

Figure 7. Visual maps for the Case of study 3. A. 2D model; B. DEM; C. 3D model.

The curve of the affected areas for this farm shows a lack of uniformity at 62 DAS of cultivation (Figure 8). The recovery percentage at 41 DAS of this farm was about 40% showing a gradually recovered.

Crop health monitoring

The health maps allowed identifying and measuring areas with low health index. The most common problems were identified, such as low uniformity in crop health, areas with little crop coverage, low vegetative growth and areas with severe deficiency due to pest damages. The maps were presented to producers in digital and printed format to familiarize them with the use of PA. However, the final corrective decisions were made by the producer based on their experience.

The rice crops are a new commodity in this production areas, previously intended for livestock; therefore, at the moment of the study, they were not equipped with good access to some locations, only by horse or tractor. However, some *in situ* inspections allowed validating the affected areas detected by NDVI, 2D, DEM, and 3D maps. Other map indexes were not generated, only the average NDVI was estimated for study areas. Micro-leveling of terrain was recommended to farmers for future crops, based on data from the DEM.

Lundström and Lindblomb (2018) carried out a similar experience focusing on the usability of a commercial application and the degree of acceptance of PA. The aim of showing this information to the farmers was to introduce the potential of the PA in the health map interpretation. The current farmers followed an empirical and traditional methodology; they expressed the benefits of the information provided, being able to know the real sowing or production area for the efficient use of inputs such as agrochemicals.

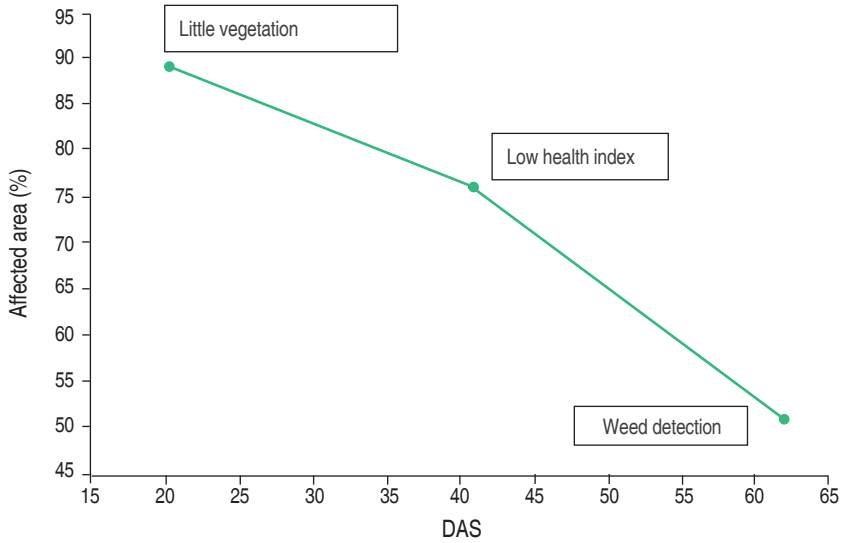

Figure 8. Percentage of the affected zone with low health index in the case of study 3 along the evaluation period.

Crop behavior

Maps generated from processed images, in the early stages of rice crop, showed areas of poor seed-germination and reduced vegetative development. The information of this study contrasts with other studies which tackling the behavior of the crops along time (Berguer *et al.*, 2019; González-Betancourt and Mayorga-Ruíz, 2018; Berrío *et al.*, 2015). The advantage of having several maps through the crop cycle is that it allows monitoring crop evolution to provide to the producer a decision-making tool based on the temporal variation of health indexes.

Weed detection

Weed presence in plots was associated with poor control of herbicide application and prevention practices. Weeds detection in the vegetative stage is difficult, but it is necessary to reduce operative cost according to Goel *et al.* (2002). In this research, agricultural experts analyzed and interpreted maps in the vegetative stage of rice crops using image processing to determined affected areas with weeds. For example, when the NDVI map showed zones with intense green color over 0.6, the superposition between the health map and RGB map through Ytmapper, allowed identifying the size of the most affected crop areas; if the conditions of clime and soil were propitious to vegetal development and the height rice plants were short, the expert inferred that this area was affected by weeds. The expert and farmers visited the affected area to validate this information.

The interpretation of the health map after the first flights allowed detecting the presence of dispersed weeds. The expert solutions are consistence with the reported by Goel *et al.* (2002) and Hernandez (2017), who indicated that the measurements and analysis of radiance and NDVI values were useful to discriminate weeds and soy crops because when crops mixed with weeds display higher radiance values between 6 to 11% than crops without weed due to the combined biomass. Similarly, Berrío *et al.* (2015) indicated that the use of remote sensing and PA allows analyzing crops with difficult access and detect affected areas by weeds or water stress.

Contribution of RGB, DEM and 3D maps

A recurring problem evidenced in the studied farm plots is the unevenness of soil surface. This fact is consistent with the regional topographic profile. Steep slopes cause seed displacement at the moment of sowing, washing of soil surface, nutrients and seeds during irrigation. Also, areas with water accumulation were found due to the soil unevenness. All the problems mentioned can be easily evidenced on the health map and corroborated with RGB, DEM and 3D maps, as is shown in similar studies (Goel *et al.*, 2002; Lago *et al.*, 2011; Brenes-González, 2016). RGB and health maps were useful to do fast inspections of farm productions, to validate the best practice in herbicide control, for crop density measurement and low health index zone inspections. Knowing the affected areas, the farmers

can save supplies and perform less invasive practice for crop monitoring. This method is efficient, economical and sustainable. DEM map is recommended to farmers for carrying out and verifying the leveling of land.

CONCLUSION

After evaluating three farms through NDVI-health, 2D, DEM, and 3D maps over the rice crop cycle, it was possible to identify and measure areas affected with low crop coverage, low vegetative development and areas with severe deficiency due to pest damages. The four maps combination generated relevant information that allowed a comprehensive virtual inspection of the land. The 2D and 3D models were useful to determine the real planting area of the farm. The benefits of PA maps were confirmed by the farmers of Tortí and Darien regions. In future works could be possible to make recommendations about replanting areas and application of necessary supplies in affected areas (nutrients or pesticides), and also, apply corrective methods to address problems, such as water stress or invasion by weeds or pests. PA proves to be an economical and reliable tool since a large amount of crop data can be acquired and processed in a short time compared to traditional monitoring methods.

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Effects of the sludge application at different concentrations on growth and production of rice (*Oryza sativa* L.) using a water channel underneath soil surface

Efectos de la aplicación de lodos a diferentes concentraciones sobre el crecimiento y producción de arroz (*Oryza sativa* L.) utilizando un canal de agua establecido por debajo de la superficie del suelo

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ABSTRACT

Indonesia's strategies are focus on improving food production using environmentally-friendly measures in its agricultural development. This research was designed in accordance with the national strategies on increasing the productivity of rice using efficient water while reducing methane emissions from rice fields. A low rice yield is obtained when the conventional anaerobic methods (flooded soil) are used. Additional to the water, sludge from palm oil factories was also applied. This research aimed to establish a proper concentration of sludge to increase the productivity of rice sown in aerobic condition with the water level at 5 cm below soil surface. The study was conducted by making experiments with 5 levels. Waste sludge from palm oil factories was applied on the soils tested with concentrations of 0 t ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1}, 20 t ha^{-1} and 25 t ha^{-1}. Height of the plant, total number of tillers, number of productive tillers, number of filled grains, percentage of filled grains, weight of 1000 grains and weight of filled grain per plot were studied. Application of 25 t ha^{-1} organic sludge showed a high level of productivity increasing the total number of tillers, the number of productive tillers and weight of filled grain per plot.

Keywords:
Aerobic
Anaerobic
Sludge
Rice
Water channel

RESUMEN

Las estrategias de Indonesia para desarrollar su agricultura se centran en mejorar la producción utilizando medidas respetuosas con el medio ambiente. Esta investigación fue diseñada de acuerdo con las estrategias nacionales para incrementar la productividad del arroz y un uso eficiente del agua mientras se reducen las emisiones de metano de los cultivos de arroz. El bajo rendimiento de arroz en los campos que emplean métodos convencionales se debe al suelo inundado (anaerobio). Además del uso eficiente del agua, también se aplicaron lodos residuales de las fábricas de aceite de palma. Esta investigación tiene como objetivo establecer una concentración adecuada de lodos para aumentar la productividad del arroz cultivado en condición aeróbica con el nivel de agua a 5 cm por debajo de la superficie del suelo. Se realizaron experimentos con 5 niveles. Los tratamientos que se probaron involucraron aplicación de lodos residuales de fábricas de aceite de palma con concentraciones de 0 t ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1}, 20 t ha^{-1} and 25 t ha^{-1}. Parámetros como la altura de la planta, número total de retoños, número de retoños productivos, número de granos llenos, porcentaje de granos llenos, peso de 1000 granos y peso de arroz molido seco fueron estudiados. La aplicación de 25 t ha^{-1} de lodos orgánicos mostró un alto nivel de productividad al incrementar el número total de retoños, el número de retoños productivos y el peso del arroz molido seco.

Palabras clave:
Aerobio
Anaerobio
Lodo
Arroz
Canal de agua

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Rice is a vital commodity in Indonesia as it is a basic food in the people diet. Food security in this country is highly dependent on the availability of this grain. Indonesia's population is in continuous growth, which implies to improve the rice productivity otherwise, the rice supply will be adversely affected. Currently, Indonesia is importing to meet its own rice supply. As a mandatory action, innovative cultivation techniques of rice must be considered to reduce the dependence on the other countries (Wardani *et al*., 2019).

Indonesia's strategy to improve the national agriculture is to incorporate a cleaner production considering the environmental issues. This strategy adopted by Indonesian government must be accompanied with efficient use of water as well as reduction of methane emission from rice fields (Leimona *et al*., 2015). Other measures include the use of organic fertilizers (Amjad *et al*., 2016).

Sludge is the waste of processes from wastewater treatment, which in this case is from palm oil factories. The process of waste treatment in palm oil factories consists in decomposition by bacterias. The bacterias can break down molecules and transform complex chemical structures into simpler ones and accelerate the absorption of nutrition in plants (Widiastuti *et al*., 2019). The sludge is stored in a waste pool and can be recommended for use as an organic fertilizer (Khairuddin *et al*., 2016).

Sludge derived from wastes of palm oil factories can be used as compost due to its humus and trace element contents. Application of sludge in soils can improve its fertile. Sludge taken from an anaerobic pool contains elements of nitrogen (0.047%), calcium (0.111%), phosphorus (0.004%), carbon (0.016%), magnesium (0.09%), and potassium (0.161%). Meanwhile, an aerobic pool contains elements of nitrogen (0.041%), calcium (0.145%), phosphorus (0.003%), carbon (0.012%), magnesium (0.083%) and potassium (0.131%). Fertilizers that have solidified from the liquid waste can be a supplement of an organic fertilizers and at the same time are environmentally friendly (Dwi *et al*., 2009). Pandapotan *et al*. (2017) evaluated the use of these sludge in an Ultisol. They found that a concentration of 21.25 t ha^{-1} can increase pH level of soil, C-organic, and the presence of phosphorus can improve its absorption in plants and the growth of the corn.

This research aimed to establish a proper concentration of sludge to increase the productivity of rice in aerobic using a water channel at 5 cm below soil surface.

MATERIALS AND METHODS

The research was conducted in rice fields of Parent Seed Center Institution in Padang Marpoyan, Pekanbaru city, Riau province, Indonesia. It has a temperature between 27–34 °C, is wet and has flat topography. This study brings together the previous research that was done in the field from June until October 2019.

The sludge chemical characterization indicated a total nitrogen 750 mg L^{-1}, phosphorus 18 mg L^{-1}, potassium 2,270 mg L^{-1}, magnesium 615 mg L^{-1} , calcium 439 mg L^{-1}, boron 7.6 mg L^{-1}, iron 46.5 mg L^{-1}, manganese 2.0 mg L^{-1} copper 0.89 mg L^{-1} and zinc 2.3 mg L^{-1} (Lang, 2007).

Preparation of experiment field

The experiment field was carried out in a muddy soil by and was prepared by a semi-mechanical method using tractor and manual work. The soil was broken down into smaller particles and was flooded with water about 21 days removing weed completely. The land was set up according to research plan setting the height of water at 5 cm below the soil surface with 2 m interval between channels.

Dosage treatment of organic sludge fertilizer

According to the 5 different levels of organic sludge from waste of palm oil factories, 15 squares (3 m^2 each) were prepared in the fields with 3 replications. G0=0 t ha^{-1}; G1=10 t ha^{-1}; G2=15 t ha^{-1}; G3=20 t ha^{-1}; G4=25 t ha^{-1} were the five levels evaluated.

Preparation of seeds and seed bed

Batang Piaman was the only variety of rice seeds used in the research, which is known for its great adaptability in low altitude areas. Seed bed was set up close to the experiment fields with an area of 5 m^2, 0.5 kg of compost was spread at 1 t ha^{-1}. It was kept in moisture conditions before the seedlings were sown. The seedling were soaked in water for 2 days before planting. The floating seeds indicated bad quality and were discarded while

ones that sank were used. The seeds were then cleaned and dried before they were sown and distributed evenly on the seed bed.

Planting seeds

The seedlings were transferred at 12 days after sowing, which were carefully removed to avoid to damage the roots. Then, these were planted in their respective plots about 2 cm deep. They were sown at 25 cm distance resulting 48 seedlings in each square.

Maintenance of rice plants

Weed control was performed twice at 20 days after planting (DAP) and at 40 DAP. Using a single application during planting phase of Curater 3G (17 kg ha^{-1}) pests were prevented.

The irrigation management was planned to keep the depth of the water channels at 5 cm below soil surface with 2 m between channels (Figure 1). The level of water in the channels remained stable under soil surface because the inlet and outlet sluice gate were conditioned.

Figure 1. Rice crop in field. Water channels set at 5 cm below ground surface.

The fields were drained upon entering the ripening of seedlings, which was about 2 weeks before harvesting stage.

Harvesting stage

When almost all of the rice turned yellow (80%) was the criteria used to harvest. It was carried out in each plot. Every treatment was done in triplicated i.e, each treatment had 3 plots.

After harvesting was concluded, filled grains were dried for 3 days or until the water content was less than 14%, then were weighed and analyzed.

Parameters of observation

- The height of plant was established by measuring the length of the plant from the surface of soil to the farthest tip of leaf. The measuring was taken once at the end of the vegetative phase.
- Total number of tillers; every stem in each unit of experiment was counted.
- Number of productive tillers: each tiller that produces panicles at harvesting time for each sample.
- The age of plant in each unit of experiment was carried out during harvesting time. It was done by counting the total number of growing days from the planting time to harvesting time.
- Number of filled grains per panicle was counted along with the counting of rice production from the same sample in each plot.
- Weight of 1000 grains (g) was done together with the counting of rice production. The weight of 1000 grains had approximately 14% water content. The sample was taken from every unit in the experiment.
- Weight of filled grain per plot with approximately 14% water content (kg).

Statistical analysis

Results were expressed as mean±standard deviation of three repetitions. The assays were compared by using one-way analysis of variance (ANOVA), followed by Duncans New Multiple Range Test post-hoc test,

with statistical significance (P<0.05). All statistical analyses were performed using IBM SPSS software.

RESULTS AND DISCUSSION

Application of organic sludge in different concentrations had an effect on the total number of tiller and productive tiller but it did not affect the height and age of the rice plants (P<0.05) (Table 1).

Application of 25 t ha^{-1} of organic sludge showed the highest plant height (116.45 cm); however, there was not significant differences with the other concentrations of organic sludge. The height of Batang Piaman variety was not affected by different concentrations of sludge fertilizer. This is probably because the fertility level of the soil is the same, causing few changes on the plant development. The characteristics of the lowland rice plant height of the Batang Piaman variety are not influenced by the relatively small differences in soil environment. Plant height could differ if the plant is planted in environments with different properties.The difference in soil environment caused by the treatment of various doses of organic sludge fertilizer can not significantly affect the growth of plant height because the different doses of organic sludge fertilizer applied do not cause significant changes in the soil environment. According to Huang *et al*. (1996) the genetic factor of Batang Piaman rice controls the height when the differences in the soil environment are not wide; the rice plants usually have 4-6 vertical nodes whose height is highly influenced by the qualitative gene and quantitative portrait loci. Also, Sasaki *et al*. (2002) stated that the length of internodes in rice plant is controlled by a gene that is expressed in every node, meaning that the lengthening of rice stem is coordinated in every node.

Table 1. Effects of the few changes on the plant development on height of plant, number of total tillers, number of productive tillers and harvesting age.

Concentration of organic sludge (t ha^{-1})	Height of plant (cm)	Number of total tillers	Number of productive tillers	Harvest age (days after planting)
0	115.84 a	22.76 d	10.93 b	106.66 a
10	115.94 a	23.60 cd	10.86 b	106.33 a
15	115.33 a	24.53 bc	11.66 b	105.66 a
20	116.20 a	25.20 b	13.26 ab	106.00 a
25	116.45 a	27.10 a	15.26 a	106.00 a
CV(%)	15.62	17.25	18.34	15.26

Numbers followed by the same lower case in the same column were not significantly different based on Duncan's New Multiple Range Test at 95% confidence level.

Application of 25 t ha^{-1} of organic sludge produced the highest number of tillers (27.10) and the highest number of productive tillers (15.26), which was different from the other treatments, being 25 t ha^{-1} the highest concentration. Organic sludge contains trace elements of nitrogen, calcium, phosphorus, carbon, magnesium, and potassium, all necessary components to improve the soil fertility. Macro elements found in sludge of palm oil factories can increase the number of productive tillers when these macro-elements are proportionately applied. The rise in the number of tillers after the use of organic sludge at 25 t ha^{-1}, showed that trace elements contribute to metabolism process, especially calcium which activates a number of enzymes in the process (Maryam and Amiri, 2014). The formation of tillers is influenced by the availability of nitrogen element. This substance is important given that stimulates their growth. In addition, nitrogen plays a crucial role in the vegetative growth as well as in the formation of productive tillers. Phosphorus-trace element is essential in rice plant particularly in its initial stage of growth. During this phase, phosphorus stimulates the formation of roots and multiplication of roots and tillers. Element phosphorus is fundamental in the growth of plants. This element supports plants in their flowering stage (De Groot *et al*., 2003). According to Enstone *et al*. (2003), a plant will grow well when all the necessary elements are available in enough quantities and in a state that can be absorbed by the plant itself.

Applying organic sludge in different concentrations did not affect the harvesting time of rice plant. The harvesting

period is influenced more by genetic factors rather than different environments (different concentrations of organic sludge). This also supports that the Batang Piaman rice takes between 105-117 days to harvest (Indonesian Agency for Agricultural Research and Development, 2003). Also, Islam *et al*. (2015) support this statament that heritability value of the harvesting stage of rice plants is 81.81, which is classified as high and indicates that the harvesting age is more determined by genetic rather than environment.

According to Table 2, application of organic sludge in different concentrations impacted the parameter of quantity of filled grains in each tiller and grain yield from each plot but it did not affect the percentage of filled grains and the weight of 1000 grains (P<0.05).

Table 2. Effects of the sludge applications at different concentrations on number of filled grains in each tiller, weight of 1000 grains and grain yield in each plot.

Concentration of organic sludge (t ha^{-1})	Number of filled grains in each panicles	Percentage of filled grains (%)	Weight of 1000 grains per plot (g)	Weight of filled grain per plot (kg)
0	102.62 c	81.29 a	26.82 a	2.40 c
10	103.04 c	84.54 a	27.04 a	2.88 bc
15	113.38 b	84.59 a	26.95 a	3.25 b
20	115.31 b	86.13 a	27.77 a	3.45 ab
25	125.69 a	88.89 a	27.45 a	3.99 a
CV(%)	18.87	15.68	16.05	19.45

Numbers followed by the same lower case in the same column were not significantly different based on Duncan's New Multiple Range Test at 95% confidence level.

The highest number of filled grains from each tiller resulting using 25 t ha^{-1} of organic sludge was 125.69 grains, which was different from other treatments. Filled grains are derived from a compound of assimilates resulting from the photosynthesis process that must be supported by the availability of trace elements in soil. These trace elements such as nitrogen, calcium, phosphorus, carbon, magnesium, and potassium come from organic sludge. Trace elements contained in organic sludge work simultaneously to form the higher filled grains from each tiller. Nitrogen synthesizes amino acid and protein in the rice plant. Phosphorus transports the results of metabolism process in the plant stimulating the flowering and pollination processes and the formation of roots and seeds. Potassium participates in the process of photosynthesis, transporting the results of assimilation process, enzymes and minerals including water. Calcium plays a role in the arrangement of chlorophyll, which is needed by enzymes in the metabolism of carbohydrate, and strengthen meristem cells. In the meantime, magnesium is a major component in the buildup of chlorophyll, which determines the speed of photosynthesis/ formation of carbohydrate runs as well as transporting Phosphate (Tränkner *et al*., 2018).

Different concentrations of organic sludge did not impact the percentage of filled grains in each tiller in the rice plants. The content of filled grains is determined by the result of photosynthesis (carbohydrate) in the stem and leaves which is then transferred and deposited inside the grains. It means, the percentage of filled grains did not show a response regard to the environment of the soil but it was heavily influenced by genetic factors. Manalu *et al*. (2017) stated that the score of the percentage filled grains is 70.6 (high) indicating a dominant influence by genetic factors rather than environmental factors.

The different concentrations of organic sludge did not show significant impact on the weight of 1000 grains. It was not influenced by environmental factors but more readily affected by genetic factors. This is in accordance to characteristic of Batang Piaman rice, in which the weight of 1000 grains is less than 27 g (Indonesian Agency for Agricultural Research and Development, 2003). Fan *et al*. (2006) reported that the weight of 1000 grains were mainly controlled by the Quantitative Trait Loci (QTL). Dou *et al*. (2016) further expressed that the weight characteristic of 1000 grains was influenced by each variety.

Application organic sludge at 25 t ha^{-1} resulted heavier in weight of filled grain per plot (3.99 kg) compared to the other treatments. The higher yield in each plot was due to some supporting factors such as the total number of tillers, number of productive tillers and number of grains for each tiller, which showed a high number applying 25 t ha^{-1} of organic sludge. According to Amjad (2016), to find the accurate portion of fertilizer, the concentration should establish based on the amount of trace element available in soil; therefore, the growth of rice plant could be optimal.

CONCLUSIONS

Based on the data, different doses of organic sludge fertilizer affected the total number of productive tillers and the productive ones, number of filled grains and seed weight in each plot. In general, the total number of tillers and the productive ones, number of filled grains and weight of seeds in each plot is proportional to the increase in the various doses of organic sludge fertilizer. The higher doses of the sludge, the higher increase in the total number of tillers and the productive ones, number of filled grains, and seed weight in each plot. The 25 t ha^{-1} of organic sludge fertilizer could increase the seed weight by 66.25% compared to that with no organic sludge fertilizer added.

Based on the result of the research, the researchers suggest that the cultivation of rice plant can develop in fields where the water channel is at 5 cm below soil surface using organic sludge at 25 t ha^{-1}. For future research, it is recommended to increase the doses of organic sludge fertilizer, in order to obtain optimal yields using different rice varieties in dissimilar soil environments.

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Digitaria insularis control by using herbicide mixtures application in soybean pre-emergence

Control de *Digitaria insularis* por medio de la aplicación de mezclas de herbicidas en la pre-emergercia de la soya

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Keywords:
Antagonism
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ABSTRACT

Sourgrass (*Digitaria insularis*) is one of the main weeds in the soybean crop. In order to control its growth, an increase of herbicide rates is required to simplify its management as it a plant with high vegetative capacity and seed production. It implies to select the herbicide-resistent Digitaria insularis biotypes. Nevertheless, some information is still contrasting the antagonist of synthetic auxinic herbicides, associated with glyphosate and ACCase inhibitors mixtures, for the control of weeds resistant or tolerant to herbicides. This study aimed to evaluate the *D. insularis* control, with a mixture of herbicides applied in soybean pre-emergence, with sequential application in soybean post-emergence, and to check possible antagonism between ACCase inhibitors herbicides with synthetic auxins and other latifolicides. The experiment was conducted in Palotina, Paraná (Brazil) and Corpus Christi, Canindeyú, (Paraguay.) The treatments consisted of associations of glyphosate, ACCase inhibitors (clethodim, haloxyfop), and latifolicides (2,4-D, triclopyr, dicamba, carfentrazone, saflufenacil, chlorimuron). A randomized block design was used. Only in Palotina, the weed control was satisfactory after sequential application in post-emergence. An antagonism for all synthetic auxins was observed with glyphosate+clethodim or haloxyfop mixtures, in both locations. As a result, in Palotina an efficacious control of perennial *D. insularis* was found in pre-emergence burndown for some mixtures such as glyphosate+ACCase inhibitor added to carfentrazone, saflufenacil, or chlorimuron. Antagonism was observed for all synthetic auxins, in both locations. In Corpus Christi, the herbicide associations were not effective, even with the post-emergence application in soybean of glyphosate+clethodim. With ineffective control for treatments composed with synthetic auxins, the post-emergence application in soybean increased the weed control with satisfactory final controls for all treatments.

Palabras clave:
Antagonismo
Resistencia a los herbicidas
Pasto amargo
Auxinas sintéticas
Malezas

RESUMEN

El pasto amargo (*Digitaria insularis*) es una de las principales malezas en el cultivo de soya. Para controlar su crecimiento, se requiere un aumento de las tasas de herbicida para simplificar su manejo al ser una planta con alta capacidad vegetativa y producción de semillas. Esto implica seleccionar los biotipos de Digitaria insularis resistentes a herbicidas Sin embargo, aún existe cierta información contrastante que considera el antagonismo de los herbicidas auxínicos sintéticos en las mezclas con inhibidores de ACCase y glifosato, para el control de malezas resistentes o tolerantes a los herbicidas. El estudio tuvo como objetivo evaluar el control de *D. insularis* con mezclas de herbicidas aplicados en la pre-emergencia y pos-emergencia del cultivo de soya y se verificó el posible antagonismo entre los herbicidas inhibidores de ACCase con auxinas sintéticas y otros latifolicidas. El experimento se realizó en Palotina, Paraná, (Brasil) y Corpus Christi, Canindeyú (Paraguay). Los tratamientos consistieron en mezclas de glifosato, inhibidores de ACCase (cletodim, haloxifop) y latifolicidas (2,4-D, triclopir, dicamba, carfentrazona, saflufenacil, clorimurón). Se utilizó un diseño de bloques al azar. El control de arvenses fue satisfactorio sólo en Palotina después de la aplicación secuencial en pos-emergencia. Allí se observó un control efectivo de *D. insularis* en la pre-emergencia, para algunas mezclas que presentaron glifosato+ACCase+carfentrazona, saflufenacil o clorimurón. Se observó antagonismo para todas las auxinas sintéticas en ambas ubicaciones. En Corpus Christi las mezclas de herbicidas no fueron efectivas, incluso con la aplicación de glifosato+cletodim en la pos-emergencia de soya. Aunque se obtuvo un control ineficaz para tratamientos compuestos con auxinas sintéticas, la aplicación en post-emergencia de soya aumentó el nivel de control, con resultados finales satisfactorios para todos los tratamientos.

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Sourgrass (*Digitaria insularis* [L.] Fedde), a Poaceae perennial weed, is infesting large areas across South America. Its dissemination is occurring because of the plant characteristic, such as tufted formation, rhizome structures, high seed production, and because of the pressure selection of plant biotypes with resistance to glyphosate and ACCase herbicides (Machado *et al*., 2008; Veldman and Putz, 2011; Melo *et al*., 2012; Gemelli *et al*., 2013; Gazola *et al*., 2019). All these characteristics avoid the easy control of this plant, genarating interference in crops. For instance, the coexistence of six plants m^{-2} of *D. insularis* with soybean crop is enough to reduce its yield in 40% (Gazziero *et al*., 2019).

The glyphosate has been used as the main herbicide in weeds management for many years, but its intensified use in weed pre-sowing control with non-tillage system and post-emergence generates glyphosate-tolerant transgenic crops (Green, 2018). *D. insularis* has a great vegetative propagation and a high seed production in a short time with seed germination across the year. Therefore, it is required to increase the rates of herbicides for effective control.

This grass presents cases of resistance to herbicides in Brazil, with resistance to glyphosate (Adegas *et al*., 2010; Carvalho *et al*., 2011) and ACCase inhibitors (haloxyfop and pinoxaden) (Takano *et al*., 2020).

There are many ways to manage the *D. insularis* resistant to glyphosate, including the herbicide application in the early stage of development, avoiding seed production, rotation of herbicides with different mechanisms of action or chemical groups, among other cultural practices including the burndown. It could be important for the effective management of *D. insularis*; its use must be implemented in advance of sowing and as complementary application to other products (Oliveira-Júnior *et al*., 2006; Canedo *et al*., 2019). The burndown immediately before sowing involves the application of one or more herbicides (usually systemic action), its choosing depends on the floristic composition of the site and infestation density (Oliveira-Júnior *et al*., 2006; Frisvold *et al*., 2020).

Particularly for *D. insularis*, there are few herbicides for chemical control. Paraquat, for example, with a single application is not enough to eradicate the whole plant causing re-growth (Zobiole *et al*., 2016). Besides, paraquat is being taken off the market in Brazil in September 2020 (ANVISA, 2020). Diquat is neither a great alternative in the control of grasses, generally with low efficacy improving when is associated with adjuvants; however still being unsatisfactory option to control it (Gitsopoulos *et al*., 2014). On the other hand, the use of ACCase herbicides, especially "fop" herbicides could lead to a rapid herbicide-resistant biotypes selection (Takano *et al*., 2020).

The use of herbicides of different mechanisms of action and with the same control spectrum is one strategy that must be used. Because of the presence of other weeds in the field, with infestation of grasses and broad-leaved, it is common the spray of glyphosate or ACCase mixed with synthetic herbicides. There are reports of the antagonist effect of 2,4-D on the action of ACCase inhibitors graminicides (Gomes *et al.,* 2020), due to the reduction of translocation and increase of herbicides metabolism from the ariloxifenoxipropionics group (Trezzi *et al*., 2007). Pereira *et al*. (2018) observed that synthetic auxinic (2,4-D and dicamba) associated with haloxyfop interfered negatively on *D. insularis* control.

There is still contrasting information that considers the antagonist action of 2,4-D and other synthetic auxinic herbicides (triclopyr and dicamba) mixtures with glyphosate and ACCase inhibitors. As *D. insularis* has few options of herbicides for chemical control, this study aimed to evaluate the control of *D. insularis*, with glyphosate plus ACCase inhibitors and latifolicides applied in pre-emergence and verify possible antagonism between ACCase inhibitors herbicides with synthetic auxins and other latifolicides.

MATERIALS AND METHODS

Design and experimental conditions

Two experiments were installed in a commercial area in 2018-2019 season. Palotina, Paraná (PR), Brazil, (24°2326.93"S 53°84'51.36"W) and Corpus Christi, Canindeyú, Paraguay (24°3'37.24"S 55°0'22.22"W) were the locations selected. The climate of both regions is classified as Cfa (humid subtropical with abundant rainfall, well distributed throughout the year), according to Köppen classification. Figure 1 (A,B) presents climate data during the period of experiment conduction.

Both areas had a high population of perennial sourgrass at flowering stage, with records of use of glyphosate, and

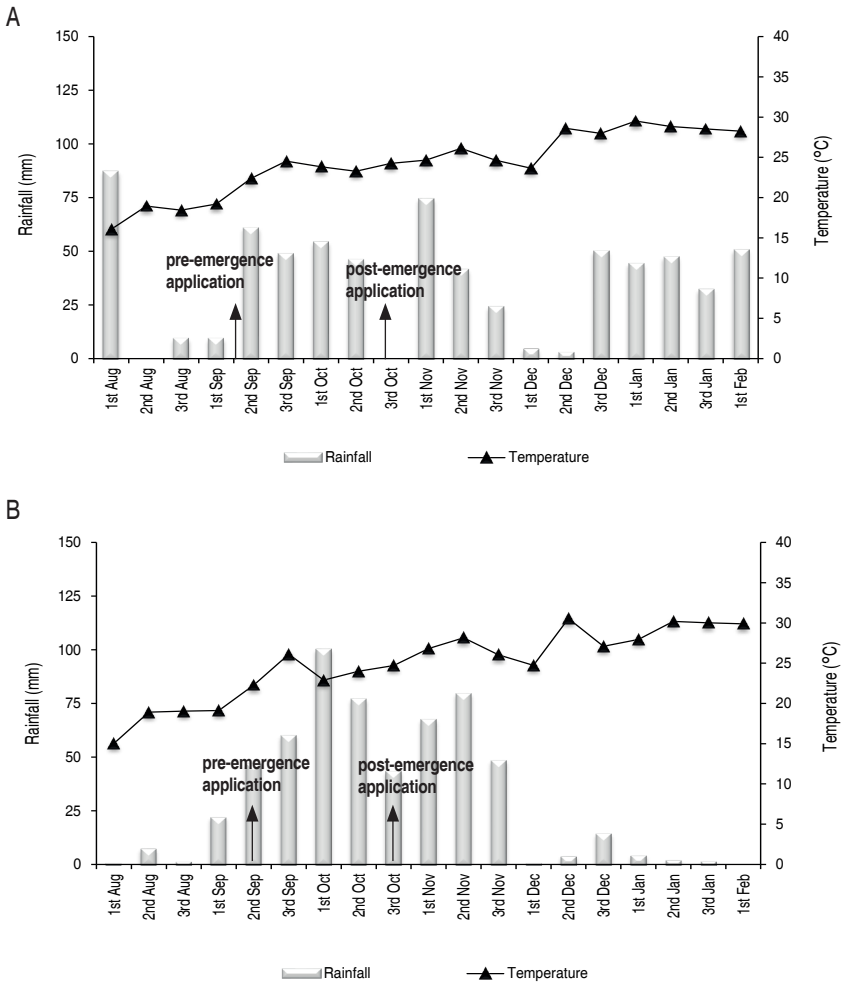

Figure 1. Rainfall representation average temperature for the experiment site. Aug – Nov 2018. A. Palotina, PR, Brazil. B. Corpus Christi, Canindeyú, Paraguay.

its loss of efficacy in recent years. Palotina assay had a populational density average of 1 to 2 tufts m^{-2}, meanwhile, in the Paraguay area, the populational density was higher, 2 to 4 tufts m^{-2}. The experiments were installed in a fallow field, prior to soybean sowing. Previously, the Palotina area had been cultivated with maize crop, and Corpus Christi was a fallow area since the soybean harvest in summer (without second season crop). A randomized block with four replications was used in the experimental design. The experimental plots were composed of 3x5 m^{-2}. The treatments are described in Table 1. The treatment applications were performed two days before soybean sowing. In Palotina the application occurred on September 11, 2018, at 29 °C, relative humidity of 60%, and wind speed of 6.5 km h^{-1}. While in Corpus Christi on September 15, 2018, the temperature was 23.9 °C, relative humidity 72.3%, and wind speed 6.8 km h^{-1}. The application of M1 in soybean post-emergence was performed 42 days after emergence (DAE) in both locations, with soybean plants at the V4-V5 stage (BBCH, 2001). This application was carried out in all treatments, except in the weedy control (without any application). In Palotina the application occurred on October 30, 2018, at temperature of 30 °C, relative humidity of 58%, and wind speed of 5.1 km h^{-1}. In Corpus Christi on November 03, 2018, the temperature was 26.9 °C, relative humidity 78%, and wind speed 6 km h^{-1}.

Table 1. Mixtures of herbicides application to control the *D. insularis*. 2018-2019 season.

Mixtures (M)	Herbicides[a]	Rates[b] (g)
	weedy control (without application)	–
1	glyphosate + clethodim[1]	1,080 + 192
2	glyphosate + haloxyfop[2]	1,080 + 120
3	glyphosate + clethodim[1] + 2,4-D	1,080 + 192 + 1,005
4	glyphosate + haloxyfop[2] + 2,4-D	1,080 + 120 + 1,005
5	glyphosate + clethodim[1] + triclopyr	1,080 + 192 + 960
6	glyphosate + haloxyfop[2] + triclopyr	1,080 + 120 + 960
7	glyphosate + clethodim[1] + dicamba	1,080 + 192 + 480
8	glyphosate + haloxyfop[2] + dicamba	1,080 + 120 + 480
9	glyphosate + clethodim[1] + carfentrazone	1,080 + 192 + 30
10	glyphosate + haloxyfop[2] + carfentrazone	1,080 + 120 + 30
11	glyphosate + clethodim[1] + saflufenacil	1,080 + 192 + 49
12	glyphosate + haloxyfop[2] + saflufenacil	1,080 + 120 + 49
13	glyphosate + clethodim[1] + chlorimuron	1,080 + 192 + 20
14	glyphosate + haloxyfop[2] + chlorimuron	1,080 + 120 + 20

Comercial product (common name) - Manufacturer

Roundup® Original (glyphosate) - Monsanto do Brasil Ltda, São Paulo, SP, Brazil. Select® 240 EC (clethodim) - Arysta Lifescience do Brasil S.A., São Paulo, SP, Brazil. Verdict® R (haloxyfop) - Dow Agrosciences Ltda, São Paulo, SP, Brazil. DMA® 806 BR (2,4-D) - Dow Agrosciences Ltda, São Paulo, SP, Brazil. Triclon® (triclopyr) - Volcano Agrociencia Ltda, São Paulo, SP, Brazil. Atectra® (dicamba) - Basf S.A., São Paulo, SP, Brazil. Aurora® 400 EC (carfentrazone) - FMC Química do Brasil Ltda, Campinas, SP, Brazil. Heat® (saflufenacil) - Basf S.A., São Paulo, SP, Brazil. Classic® (chlorimuron) - Du Pont do Brasil S.A., Barueri, São Paulo, SP, Brazil.

[1]Adjuvant use: Lanzar® 0.5% v/v (Arysta Lifescience do Brasil S.A., São Paulo, SP, Brazil); [2]Adjuvant use: Joint® Oil 0.5% v/v (Dow Agrosciences Ltda, São Paulo, SP, Brazil).

[a] Followed by application of glyphosate (1,000 g acid equivalent [a.e] ha^{-1}) + clethodim (108 g active ingredient [a.i] ha^{-1}), in soybean post-emergence (V4-V5), except for weedy control (without application).

[b] Rates in g a.e. ha^{-1} for glyphosate, haloxyfop, 2,4-D, and triclopyr; for the others, rates in g a.i. ha^{-1}.

CO_2-pressurized sprayer was used for all herbicide applications. It was equipped with six AIXR 110 015 spray nozzles spaced 0.5 m from each other, 2.5 kgf cm^{-2} calibrated pressure and speed of 1 m s^{-1}, resulting in a spray volume of 150 L ha^{-1}.

Evaluations and statistical analysis

At soybean pre-emergence, the visual evaluations of control were done at 7, 14, 21, 28, and 35 days after application (DAA) for both experiments. The control also was evaluated at 7, 14, and 21 DAA, in Palotina, and at 7 and 14 DAA in Corpus Christi, at soybean post-emergence. Percentage values from 0 up to 100% were assigned for the evaluation (0 no injuries, 100% plant death) with regard to weedy control (Velini *et al.*, 1995).

The data were submitted to analysis of variance (ANOVA) by F-test (P<0.05), according to Pimentel-Gomes and Garcia (2002). The means of treatments were grouped by Scott and Knott (1974) test (P<0.05). Sisvar 5.6 software was used for the analysis (Ferreira, 2011).

RESULTS AND DISCUSSION

All treatments with clethodim were more effective compared with haloxyfop treatments from 21 to 35 DAA (Palotina). At 7 DAA, there were not high levels of control, at most 39.8% (Table 2). On the other hand, the treatments with saflufenacil provided greater control, even 39.75% higher than other treatments. For 35 DAA, stands out that the best results were observed for treatments M13 or M9, with values up to 94.1%. After the post-emergence application of M1, it

was observed some differences between the treatments, there were lower values for some treatments with haloxyfop application, but all mixtures provided minimum control of 89.5%, at 21 DAA (Table 2).

Table 2. Control treatments to *D. insularis* at 7, 14, 21, 28, and 35 days after the application (DAA) of herbicides, at soybean pre-emergence, and at 7, 14, and 21 DAA at soybean post-emergence. 2018-2019 season, Palotina, PR, Brazil.

Mixtures (M)	Treatments	Pre-emergence application (DAA)					Post-emergence application (DAA)		
		7	14	21	28	35	7	14	21
		(%)							
	weedy control (without application)	0.0 d	0.0 e	0.0 g	0.0 f	0.0 h	0.0 e	0.0 d	0.0 c
1	gly + clethodim	18.8 c	81.5 a	91.9 a	94.9 a	83.0 b	80.8 b	86.5 a	95.8 a
2	gly + haloxyfop	16.9 c	65.8 b	65.8 d	68.9 c	39.8 f	51.0 d	85.5 a	90.9 b
3	gly + clethodim + 2,4-D	27.4 b	76.4 a	72.3 c	64.0 d	55.4 e	63.0 c	79.5 b	94.3 a
4	gly + haloxyfop + 2,4-D	19.4 c	43.0 d	45.1 f	42.3 e	28.9 g	47.0 d	85.5 a	92.8 a
5	gly + clethodim + triclopyr	22.5 c	82.1 a	82.9 b	79.6 b	68.0 d	75.0 b	92.8 a	95.3 a
6	gly + haloxyfop + triclopyr	19.4 c	52.3 c	53.5 e	45.8 e	34.6 g	49.5 d	74.8 b	90.0 b
7	gly + clethodim + dicamba	24.0 b	76.1 a	84.3 b	84.1 b	78.6 c	81.0 b	88.8 a	98.0 a
8	gly + haloxyfop + dicamba	13.9 c	54.0 c	45.8 f	44.3 e	34.6 g	43.8 d	68.5 c	89.5 b
9	gly + clethodim + carf	19.5 c	81.3 a	93.5 a	95.4 a	94.1 a	90.5 a	91.5 a	94.5 a
10	gly + haloxyfop + carf	19.9 c	55.4 c	60.8 d	66.3 c	53.1 e	57.0 c	74.3 b	92.8 a
11	gly + clethodim + saflufenacil	39.8 a	86.5 a	90.4 a	91.5 a	83.8 b	81.5 b	91.3 a	93.3 a
12	gly + haloxyfop + saflufenacil	33.8 a	77.3 a	58.3 e	59.8 d	40.1 f	45.0 d	71.0 c	89.5 b
13	gly + clethodim + chlorimuron	26.3 b	79.9 a	90.9 a	95.5 a	89.5 a	90.0 a	93.3 a	97.3 a
14	gly + haloxyfop + chlorimuron	16.4 c	43.6 d	57.4 e	71.0 c	51.4 e	60.3 c	90.0 a	93.5 a
	Mean	21.2	63.7	66.2	66.9	55.5	61	78.2	87.1
	CV (%)	10.1	9.4	6.6	6.9	9.8	6.8	6.2	3.6
	F	*	*	*	*	*	*	*	*

gly: glyphosate, carf: carfentrazone.
* Means followed by the same letters in the column do not differ by Scott and Knott (1974) test (P<0.05).

In Corpus Christi, the control was lower in general, probably due to the higher infestation of perennial plants. The higher averages on 28 DAA were reached by the treatment's M2, M12, M13, and M14 up 74.5%. Even after the post-emergence application at 14 DAA using M11, a *D. insularis* control up 66% was observed (Table 3).

M11 was effective in the control, especially in the first evaluations in Palotina. The application of glyphosate+saflufenacil has been reported effective especially to control eudicotyledon weeds, for example, *Conyza* spp. (Mahoney *et al.*, 2016). However, saflufenacil does not present high control on *Digitaria* spp. (Soltani *et al.*, 2014). Nevertheless, in this study, in Palotina, it could be an adjuvant on *D. insularis* control, when was mixed with clethodim (M11) or haloxyfop (M12). The addition of saflufenacil in the mixtures did not increase the control promoted by glyphosate+clethodim; however it did not have an antagonistic effect. Mixtures like this have a broad spectrum of action, being important in weed management in infested areas with *D. insularis* and other weeds (Roskamp *et al.*, 2012).

In Palotina, all treatments with clethodim were more effective compared with haloxyfop treatments from

Table 3. Control treatments to *D. insularis* at 7, 14, 21, 28, and 35 days after the application (DAA) of herbicides, at soybean pre-emergence, and at 7, 14, and 21 DAA at soybean post-emergence. 2018-2019 season, Corpus Christi, Canindeyú, Paraguay.

Mixtures (M)	Treatments	Pre-emergence application (DAA)					Post-emergence application (DAA)	
		7	14	21	28	35	7	14
					%			
	weedy control (without application)	0.0 d	0.0 f	0.0 e	0.0 e	0.0 d	0.0 c	0.0 c
1	gly + clethodim	10.3 c	19.3 d	62.0 c	64.5 b	46.8 b	52.0 b	55.0 b
2	gly + haloxyfop	8.8 c	18.8 d	58.0 d	69.8 a	55.0 a	55.5 a	65.0 a
3	gly + clethodim + 2,4-D	9.5 c	17.5 e	54.3 d	43.0 d	34.8 c	49.3 b	53.5 b
4	gly + haloxyfop + 2,4-D	8.8 c	15.0 e	55.3 d	51.5 c	41.8 b	48.0 b	51.5 b
5	gly + clethodim + triclopyr	10.0 c	25.5 c	63.5 c	52.5 c	48.0 b	51.3 b	54.5 b
6	gly + haloxyfop + triclopyr	10.5 c	22.0 d	61.8 c	51.3 c	47.0 b	52.0 b	61.0 a
7	gly + clethodim + dicamba	9.5 c	20.8 d	55.3 d	54.0 c	47.5 b	52.8 b	56.3 b
8	gly + haloxyfop + dicamba	10.3 c	21.3 d	59.0 d	52.0 c	46.8 b	48.8 b	60.3 a
9	gly + clethodim + carf	11.0 c	25.3 c	66.3 c	45.5 d	43.0 b	49.3 b	55.5 b
10	gly + haloxyfop + carf	11.0 c	27.3 c	71.8 b	59.8 b	45.0 b	53.5 b	56.3 b
11	gly + clethodim + saflufenacil	13.0 b	33.0 b	75.0 b	60.3 b	57.0 a	60.5 a	66.0 a
12	gly + haloxyfop + saflufenacil	17.5 a	44.0 a	80.8 a	73.0 a	58.8 a	61.0 a	63.3 a
13	gly + clethodim + chlorimuron	17.0 a	31.3 b	66.5 c	69.8 a	55.0 a	56.3 a	58.0 b
14	gly + haloxyfop + chlorimuron	15.5 a	29.8 b	63.0 c	74.5 a	57.8 a	61.5 a	61.3 a
	Mean	10.8	23.4	59.5	54.8	45.6	50.1	54.5
	CV (%)	16.5	12.7	7.5	10	8.5	10.8	8.2
	F	*	*	*	*	*	*	*

gly: glyphosate, carf: carfentrazone.
* Means followed by the same letters in the column do not differ by Scott and Knott (1974) test ($P<0.05$).

21 to 35 DAA. Zobiole *et al*. (2016) did not observe differences on *D. insularis* control between clethodim and haloxyfop mixed with glyphosate. Cassol *et al*. (2019) found similar efficacy of clethodim and haloxyfop in association with glyphosate on perennial *D. insularis* control and at soybean post-emergence weed control, but in the control of plants in the off-season, clethodim+glyphosate was more powerful than haloxyfop+glyphosate.

Other studies highlight the efficacy of clethodim and haloxyfop, at different mixtures, on *D. insularis* control (Barroso *et al*., 2014; Gilo *et al*., 2016). Nonetheless, it is not possible to determine which one is more effective on *D. insularis* control; their choice should consider several factors including the background of the use of these herbicides in the area. According to López-Ovejero *et al*. (2017), Takano *et al*. (2018), and Lucio *et al*. (2019) it is crucial to rotate the chemical groups in order to avoid herbicide-resistant *D. insularis* biotypes.

A reduction in weed control was found in both locations from 14 DAA regarding the synthetic auxins in association with ACCase inhibitors. In Palotina, at 28 DAA, 30.93, 15.30, and 10.85% of control losses were observed when 2,4-D (M3), triclopyr (M5), and dicamba (M7) were mixture with glyphosate+clethodim, respectively. In Corpus Christi at 28 DAA, the losses of effectiveness were 21.5, 17.25, and 10.5% for treatments with 2,4-D (M3), triclopyr (M5), and dicamba (M7) respectively, when compared with glyphosate+clethodim treatment. In relation to glyphosate+haloxyfop associations, the auxins 2,4-D, triclopyr and dicamba provided a reduction of 18.25, 18.5, and 17.75% in control effectiveness. At

the same time, the treatments with auxinics added to haloxyfop provided 42.25% (M3), 45.25% (M6), and 45.75% (M8) of weed control.

In this study, antagonism and reductions were verified by the association of ACCase inhibitors, as haloxyfop with 2,4-D (M4) and dicamba (M8). By Pereira *et al.* (2018), some cases more than 40% was observed for both. Clethodim+dicamba is also reported as an antagonist, with losses of 6 to 15%, in volunteer maize control in soybean (Underwood *et al.*, 2016). The losses due to antagonism were of 11.85% in Palotina and 10.5% in Corpus Christi treated with M7.

In the case of haloxyfop, the antagonism on these mixtures is possibly explained by the reduction of translocation. (Olson and Nalewaja, 1981). The clethodim+2,4-D antagonism was detected due to leaf necrosis in a few days after application (Gomes et al., 2020). This can result in less absorption and translocation triggering the less weed control. Mixtures of triclopyr with ACCase inhibitors also reduce its effectiveness on grasses control (Scherder *et al.*, 2005). About dicamba, it can be also explained for the possible reduction of translocation of the graminicide to the roots and the plant's rhizome (Aguero-Alvarado *et al.*, 1991).

The *D. insularis* control varied between the two locations, which could be explained by higher population density in Corpus Christi. While the lower efficacy of haloxyfop treatments (compared to clethodim) in Palotina, probably it is a risk warning for the selection of haloxyfop resistant biotypes. There are records of the *D. insularis* resistence to this herbicide and pinoxaden in Brazil (Takano *et al.*, 2020).

In a general context, the *D. insularis* control was satisfactory for treatments with triple combinations, only in the Palotina area. There, even with ineffective control for treatments composed with synthetic auxins, the post-emergence application in soybean increased the control level, with satisfactory final controls for all treatments. However, antagonism was observed for all synthetic auxins in both locations.

The coexistence of six plants m^{-2} of *D. insularis* with soybean crop is enough to reduce yield in 40% (Gazziero *et al.*, 2019). In Corpus Christi with a population of 2 to 4 tufts per m^2, the application was not effective. The anticipated control of *D. insularis* populations should be a priority to avoid crop losses due to weeds competition. The combination of ACCase inhibitors (haloxyfop, clethodim) and synthetic auxins (2,4-D, dicamba, and triclopyr) is not recommended. Another option is to use the auxin herbicides in sequential application. For instance, by Leal *et al.* (2020) haloxyfop must be applied at least 6 days before 2,4-D to control *Conyza* spp. and *D. insularis* when they are present simultaneously.

CONCLUSION

In Palotina, the perennial *D. insularis* control at soybean pre-emergence burndown was effective in some mixtures that presented glyphosate+ACCase inhibitor added to carfentrazone, saflufenacil, or chlorimuron, demonstrating the potential use of these associations for weed control. Even with ineffective control for treatments composed with synthetic auxins, the post-emergence application in soybean increased the control level, with satisfactory final controls for all treatments

In Corpus Christi, the herbicides combinations were not effective, even after the post-emergence application of glyphosate+clethodim.

Clethodim and haloxyfop had a reduction on the efficiency in combination with the synthetic auxins 2,4-D, triclopyr, and dicamba. Among synthetic auxins, dicamba showed the lowest antagonism.

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Yield and quality of winter wheat (*Triticum aestivum* L.) grain in relation to nitrogen fertilization

Rendimiento y calidad del grano de trigo de invierno (*Triticum aestivum* L.) con respecto a la fertilización nitrogenada

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ABSTRACT

Winter wheat is commonly cultivated in Ukraine. This crop has nutritional requirements that have been affected due to the climate changes that in the recent years the country has been suffered. In the spring season, the plant is found in growing stage, which requires a suitable nitrogen nutrition and it must be supplied due to the deficit of this element during the vegetative development. Within this framework, this study aimed to establish the optimal nitrogen rate for winter wheat that was applied in different configurations on leached black soil, and to determine the effectiveness of nitrogen application to the frozen soil surface in early spring as a basis for these fertilizations. The experiment was conducted at the Bila Tserkva Research-Selection Station (Ukraine) during 2017–2019. The soil of this place corresponds to a leached loamy black soil (Mollisol) with content of organic matter between 3.6-3.8%, mobile phosphorus (P_2O_5) 156-166 mg kg^{-1}, potassium (K_2O) 64-77 mg kg^{-1}, and pH_{KCl} 5.8–6.3. A randomized experimental design with four nitrogen rates (0, 60, 80, 110 kg ha^{-1}) with four replications as factorial arrangement was used. The results indicated that winter wheat yield was significantly affected by all nitrogen fertilizer rates (P<0.05). The highest average grain yield was obtained with 110 kg ha^{-1} nitrogen rate that combined application nitrogen to the frozen soil surface and the foliar feeding with 6.90 t ha^{-1}. In years of regular precipitation during the growing season (2018-2019), nitrogen fertilizers had a more pronounced effect on winter wheat yield than in a year of precipitation deficiency (2017). A significant increase in grain protein content was obtained in variants where foliar feeding of winter wheat with urea solution was a part of fertilization strategy; it was attributed to nitrogen rates of 80 and 110 kg ha^{-1}. The increase in protein content of the grain was less dependent on the weather conditions and was stable through the years of experiment.

Keywords:
Nitrogen
Productivity
Ukraine
Winter wheat

RESUMEN

El trigo de invierno se cultiva comúnmente en Ucrania. Este cultivo tiene requerimientos nutricionales que se han visto afectados por los cambios climáticos que en los últimos años ha sufrido el país. En la temporada de primavera, la planta se encuentra en etapa de crecimiento, la cual requiere una adecuada nutrición nitrogenada y debe ser suplida por el déficit de este elemento durante su desarrollo vegetativo. En este marco, este estudio tuvo como objetivo establecer la tasa óptima de nitrógeno para el trigo de invierno que se aplicó en diferentes configuraciones en suelo negro lixiviado, y determinar la efectividad de la aplicación de nitrógeno a la superficie del suelo congelado a principios de la primavera como base para estas fertilizaciones. El experimento se llevó a cabo en la estación de investigación y selección de Bila Tserkva (Ucrania) durante 2017-2019. El suelo de este lugar corresponde a un suelo negro arcilloso lixiviado (Mollisol) rico en materia orgánica 3.6-3.8%, fósforo móvil (P_2O_5) 156-166 mg kg^{-1}, potasio (K_2O) 64-77 mg kg^{-1} y pH_{KCl} 5,8–6,3. Se utilizó un diseño experimental aleatorio con un arreglo factorial, es decir, cuatro dosis de nitrógeno (0, 60, 80, 110 kg ha^{-1}) con cuatro replicas. Los resultados indicaron que el rendimiento del trigo de invierno se vio afectado significativamente por todas las dosis de fertilizantes nitrogenados (P <0,05). El mayor rendimiento promedio de grano se obtuvo con una tasa de nitrógeno de 110 kg ha^{-1} que combinó la aplicación de nitrógeno a la superficie del suelo congelado y foliar de 6,90 t ha^{-1}. En años de precipitación regular durante la temporada de crecimiento (2018-2019), los fertilizantes nitrogenados tuvieron un efecto más pronunciado en el rendimiento del trigo de invierno que en un año de deficiencia de precipitaciones (2017). Se obtuvo un aumento significativo en el contenido de proteína del grano en variantes donde la alimentación foliar de trigo de invierno con soluciones de urea fue parte de la estrategia de fertilización; se atribuyó a tasas de nitrógeno de 80 y 110 kg ha^{-1}. El aumento en el contenido de proteína del grano dependió menos de las condiciones climáticas y se mantuvo estable a lo largo de los años de experimentación.

Palabras clave:
Nitrógeno
Productividad
Ucrania
Trigo de invierno

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Winter wheat (*Triticum aestivum* L.) is the most common cereal in Ukraine with a cultivation area over 7.0 million ha with an annual grain harvest ranging 25-28 million t (APK-Inform Ukraine, 2020). It is grown in all soil-climatic zones of Ukraine, but the largest areas concentrated in the Forest-steppe and steppe zones, are the areas of highly productive black soils. Although the natural fertility of these soils remains quite high, significant climatic changes in the direction of increasing aridity have posed new challenges to the effectiveness of nutrients, especially nitrogen fertilizers under this crop.

The yield and quality of winter wheat grain strongly depends on the amount and application settings of nitrogen during the growing season. The greatest efficiency of nitrogen fertilizers for winter wheat is achieved when they are applied 3-4 times during the growing season in small doses about 20-60 kg ha^{-1} The nitrogen application is related to the most crucial phases of the plant growth and development such as sowing, mid-tillering, stem elongation, and grain filling (Abedi *et al*., 2011; Haile *et al*., 2012; Efretue *et al*., 2016). According to Haile *et al*. (2012) and Liu *et al*. (2019), the highest productivity of winter wheat is achieved when 120 kg ha^{-1} nitrogen rate was applied in three stages: 25% at sowing, 50% at mid-tillering, and 25% at anthesis.

However, there is not conclusive evidence of how suitable could be this technique with new challenges of climate warming. Krivenko (2018) and Gengalo *et al.* (2019) revealed that in the recent years winter wheat has been exposed to dry weather in autumn, leading in a poor tillering of the plants during that season; therefore, in spring, winter wheat requires fertilization with nitrogen in the earliest-term to accelerate regeneration processes. The efficient technique to provide winter wheat plants with accessibly consumable nitrogen in early spring is an application of ammonium nitrate or ammonium sulfate to the surface of frozen soil (Savchenko and Miroshnichenko, 2013). According to Efretue *et al.* (2016), early spring application of nitrogen to winter wheat in Ireland contributed to increased grain yields, yet with the first nitrogen treatment, the early stem elongation stage (BBCH 31-Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) was delayed to led to lower yields. Although the efficacy of early nitrogen treatment is evident, it is mainly based on practical experience with limited research data regarding optimal doses and forms of nitrogen application for this crop.

Other effective instruments to improve winter wheat yield and grain quality are the treatment through the leaves with amide nitrogen, which carried out in the stages of stem elongation and grain filling (Abedi *et al*., 2011; Efretue *et al*., 2016; Staugaitis *et al*., 2017; Liu *et al*., 2019). Under dry weather conditions, the foliar fertilization with the nitrogen can reduce the stress caused by climatic factors; therefore, some researchers consider it as an urgent and effective since this treatment influences plants favorably and increases the productivity of winter wheat despite the environmental change (Ahmed *et al*., 2011; Rahman *et al*., 2014; Mandic *et al*., 2015; Walsh *et al*., 2018). According to Wagan *et al.* (2017), foliar feeding of winter wheat with 5% urea solution at the stage of stem elongation contribute to the highest number of grains per spike, the highest value of seed index generating the highest grain yield. Improvement in grain filling stage is attributed to later urea foliar application on winter wheat, leading an effect on grain protein content, which depends on the amount of urea applied (Gholami *et al*., 2011).

Many opinions have been said concerning the optimal nitrogen rate for winter wheat (between 70 and 120 kg ha^{-1}) and the optimal method of appliance throughout the growing season (Abedi *et al*., 2011; Mandic *et al*., 2015; Rasmussen *et al*., 2015; Walsh *et al*., 2018; Yi *et al*., 2015). A study carried out in Serbia showed that nitrogen rate for winter wheat of 75 kg ha^{-1} maximized the profitability and minimized negative environmental impacts caused by nitrogen loss in the ecosystem, whereas the increase of nitrogen rate to 150 kg ha^{-1} did not lead to a significant cost-effective ratio (Mandic *et al*., 2015).

Soil fertility status and climate conditions have been changed during the last decades, affecting also, the way how the nitrogen application for winter wheat have been performed. Therefore, strategies for plant nutrition with nitrogen must be generated based on research results obtained from a local environment. This study aimed to

establish the optimal nitrogen rate for winter wheat that was applied in different configurations on leached black soil in Ukraine, and to determine the effectiveness of nitrogen application to the frozen soil surface in early spring as a basis for these fertilizations.

MATERIALS AND METHODS

The experiment was carried out in 2017–2019 at the Bila Tserkva Research-Breading Station of the Institute of Bioenergy Crops and Sugar Beet, Ukraine. A randomized experimental design was used with the main plot size: 33 m^2 drilling area, 25 m^2 harvested area (Dospekhov, 1985). All treatments were replicated four times. Winter wheat Yasochka cv was sown on September 26-2017, September 21-2018 and October 7-2019.

The soil of the experimental site was leached black soil (Mollisol), loamy texture. Soil samples were taken from 0–30 cm soil layer of every replication to analyze pH, organic matter, mobile phosphorus and potassium before winter wheat was drilled in September. Soil pH was determined in 1 N KCl extraction using a potentiometric method (DSTU ISO 10390-2007), organic matter by Tiurin (DSTU 4289-2004), mobile P_2O_5 and K_2O by Chirikov (DSTU 4115–2002). Agrochemical properties of the soil are presented in Table 1.

Table 1. The agrochemical properties of arable leached black soil layer (0–30 cm).

Soil properties	Year					
	2017		2018		2019	
	Value	GNCL	Value	GNCL	Value	GNCL
pH_{KCl}	5.9	V	5.7	V	6.3	V
Organic matter %	3.8	III	3.6	III	3.6	III
Mobile P_2O_5 (mg kg^{-1})	156	IV	159	IV	166	IV
Mobile K_2O (mg kg^{-1})	71	III	64	III	77	III

Soil indicators determined using 5 composite samples; groups of nutrient content level (GNCL): I – very low, II – low, III – medium, IV – high, V – very high.

Winter wheat was fertilized according to the following scheme: treatment 1. control, no nitrogen; phosphorus as superphosphate and potassium as chloride potassium at 60 kg ha^{-1} dose of each applied on ploughlands in autumn, treatment 2. nitrogen at a dose of 60 kg ha^{-1} as ammonium nitrate was applied lonely on frozen soil surface in early spring, treatment 3. nitrogen at dose 30 kg ha^{-1} as ammonium nitrate was applied on the frozen soil surface plus two foliar feeding with urea solution at BBCH 31-32 stage with 30 kg ha^{-1} nitrogen dose and at BBCH 75–77 with 20 kg ha^{-1} nitrogen dose, treatment 4. amultiple dose of nitrogen at 60 kg ha^{-1} applied on the frozen soil surface.

Within the years of the experiment, winter wheat was harvested in July. The plant samples were taken from four spots of each plot within 0.25 m^2 before harvesting to determine winter wheat productivity indicators. In each spot, 20 winter wheat plants were sampled to measure the number of productive stems, grain number per ear and 1,000-grain weight. Unhulled ears were detached from stems; the grains were hulled from ears, counted and weighed. The samples were taken in triplicate to assess grain quality indicators. Grain yield of winter wheat and straw yield was expressed under natural moisture. Total nitrogen content in plants was determined using the Kjeldahl method (DSTU 7169-2010), which then was transferred into crude protein content according to GOST 10846-91. Weather data was provided by the methodological station of Bila Tserkva district.

Statistical analysis

The yield and plant biometric data was submitted to an analysis of variance (ANOVA). Differences among the

treatments of the experiment assessed by the least significant difference limit (LSD, $P<0.05$). Correlation-regression analysis was performed to estimate the correlations between nitrogen rate and winter wheat productivity components using software Microsoft Excel, version 2013, (USA).

RESULTS AND DISCUSSION

The weather conditions during the years of research were predominantly favorable for winter wheat cultivation. In 2017–2019, during the growing season of winter wheat, mean daily temperature was significantly higher than long-term average. It exceeded the mean long-term value by 2.2 °C, 1.8 °C, and 3.0 °C in 2017, 2018, and 2019, respectively (Figure 1). Whereas, the rainfall for the growing season was less than long-term average, and an uneven distribution of rains during the vegetation months was presented. Total rainfall for four months (March-July) was 147 mm, 264 mm, and 252 mm in 2017, 2018, and 2019, respectively, with a long-term average over this period of 248 mm (Figure 2).

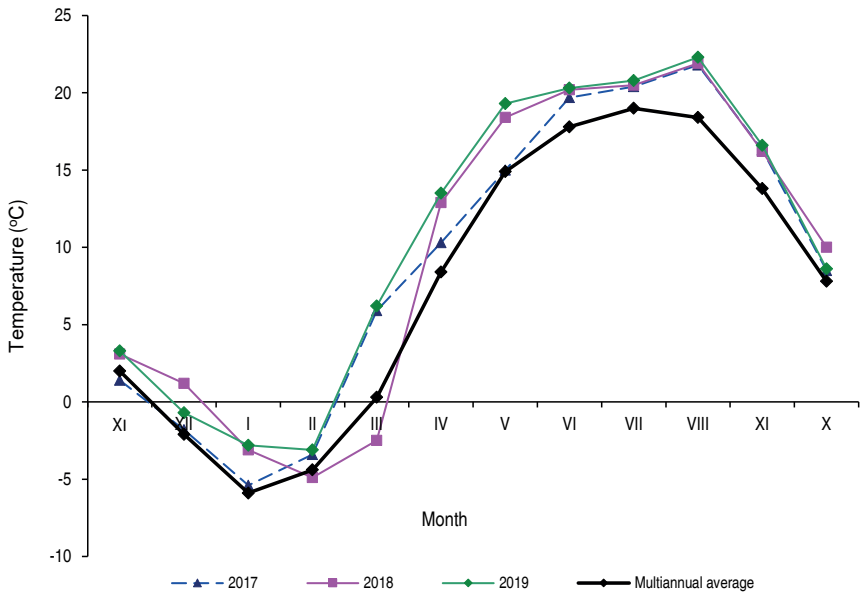

Figure 1. Mean monthly temperature values during winter wheat growing season (Bila Tserkva Meteorological Station).

Vegetation period in 2017 was warm and dry, followed with a decrease in precipitation of 101 mm. Particularly, dry weather was observed at the stages of tillering (March) and formation of the ear (April) of winter wheat. During these two dry months, a decrease in rainfall was presented regarding long-term average by 42 mm with an increase in mean daily temperature by 7.5 °C. Dry weather was also observed at the stage of maturity of winter wheat (June) with a deficiency of 36 mm and an increase in mean daily temperature of 2.1 °C. Under such dry and hot weather in 2017, the yield of winter wheat was the lowest, with a decrease of 1.5-2.0 t ha^{-1} regarding three-year average.

The year 2018 was unusual with a month later onset of frosts, which started in January, and heavy rainfall in December of 2017, which was twice higher than long-term average (91 mm); the extra raining had contributed to the accumulation of water in the soil. March of 2018 was unusually cold with a prevailing mean daily temperature of –2.5 °C, which led to the resumption of winter wheat tillering only in April. The growing season of year 2018 was warm and humid. April was accompanied by excessive rainfall (73 mm), exceeding long-term average by 44 mm. Within May-July, rainfall corresponded to long-term average (179 mm) with exceeding mean daily temperature by 7.4 °C.

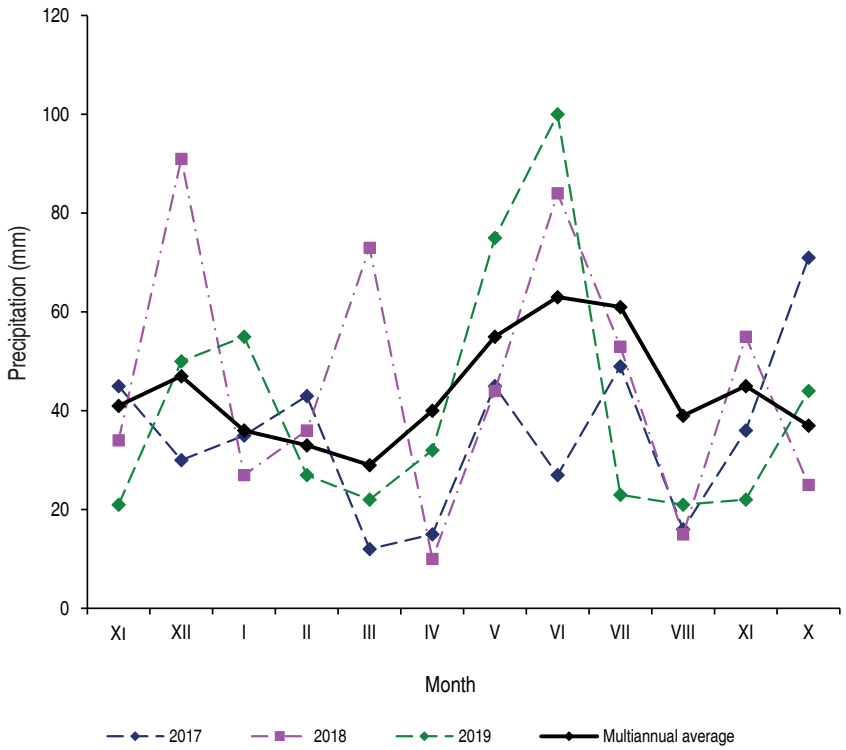

Figure 2. Rainfall values during winter wheat growing season (Bila Tserkva Meteorological Station)

Wet and hot weather in middle spring contributed to intensive tillering and development of winter wheat and provided the highest grain yield during the experimental years.

In 2019, the growing season was extra warm with regular total rainfall. At the time of intensive tillering and formation of the ear (March-April), weather was extremely warm with perennial rainfall of 54 mm the next two months (May-June); warm and humid with mean daily temperature increase of 3.8 °C, precipitation rate increase of 57 mm. Despite hot weather in March-June, winter wheat has developed and matured favourably, reaching grain yield of more than 7.5 t ha^{-1}. The high productivity of winter wheat can be due to the uniform and enough moisture supply of the plants during the growing season.

Investigation results showed that fertilization of winter wheat with nitrogen provided statistically significant increase in the components of its productivity, number of productive stems, number of grains per ear and 1000-grain weight that depended on the weather conditions, the doses and ways of nitrogen was applied.

Nitrogen used for winter wheat had a significant effect on the number of productive stems. According to the average data of 2017–2019, single fertilization of winter wheat with nitrogen at dose of 60 kg ha^{-1} applied on the frozen surface of the soil in early spring (treatment 2) increased the number of productive stems compared to control without nitrogen (treatment 1) from 435 to 484 per m^2 (11.3%); with a total nitrogen dose of 80 kg ha^{-1} applied in three steps (treatment 3) from 435 to 478 per m^2 (9.9%); with a total nitrogen dose of 110 kg ha^{-1} applied in three steps (treatment 4) from 435 to 501 per m^2 (15.2%). A statistically significant increase in the number of productive stems (P<0.05) was observed in all experimental years. Meanwhile, in the years of extra warm and humid spring (2018-2019), fertilization with the nitrogen had a greater effect on number of productive stems than in year 2017 when spring was overly dry. Maximum number of productive stems was achieved when nitrogen applied in split manner at rate

of 110 kg ha^{-1} that included nitrogen dose of 60 kg ha^{-1} on the frozen soil surface and two foliar feeding with nitrogen at BBCH 31–32 stage with dose of 30 kg ha^{-1} and BBCH 75–77 stage with dose of 20 kg ha^{-1}. This result showed that under changeable climate, winter wheat favorably responded to multiple treatment with nitrogen during the growing season, but the application of nitrogen on the frozen soil surface in early spring, as a part of this multiple application strategy, generated a strong foundation to ensure maximum quantity of the productive stems number through experimental years (Table 2).

Table 2. The effect of nitrogen nutrition on winter wheat productivity indicators.

No.	Treatment	Year				Increase
		2017	2018	2019		%
Number of productive stems per m^2						
1	(P60K60 under ploughing-Control)	381	496	427	435	-
2	(N60)	408	561	483	484	11.3
3	(N80)	393	555	486	478	9.9
4	(N110)	441	576	486	501	15.2
LSD (P<0.05)		26.1	30.5	36.2	32.3	
Number of grains per ear						
1	(P60K60 under ploughing-Control)	33	41	38	37	-
2	(N60)	33	42	39	38	2.7
3	(N80)	34	41	39	38	2.7
4	(N110)	34	44	39	39	5.4
LSD (P <0.05)		ns	2.4	ns	ns	
1000 grain weight (g)						
1	(P60K60 under ploughing-Control)	36.61	42.57	37.37	38.85	-
2	(N60)	36.84	44.94	43.74	41.84	7.70
3	(N80)	36.74	45.39	43.82	41.98	8.06
4	(N110)	36.94	45.66	44.78	42.46	9.29
LSD (P <0.05)		1.02	1.14	0.96	0.94	

ns: no significant

During this experiment, the evaluation of the impact of nitrogen fertilization on the number of grains per ear did not have a statistically significant difference in yield. The effect of the nitrogen had a not significant increase about 2.7-5.4%, compared to the control without nitrogen. However, the number of grains per ear significantly increased from 41 to 44 (7.3%) in 2018 with a total nitrogen dose of 110 kg ha^{-1} (treatment 4). That year, warm and regular humid in growing season was recorded and the nitrogen rate of 110 kg ha^{-1} applied in a split manner had an influence on this indicator value.

The weight of 1000 grains had a significant increase after the application of nitrogen during the years 2018-2019. In 2018, it increased with nitrogen treatments by 7.70-9.29%, including the treatment 2 from 42.57 to 44.94 g, the treatment 3 from 42.57 to 45.39 g, and the treatment 4 from 42.57 to 45.66 g. In 2019, an increase of 17.1-19.8% was registered in grain weight after treatment 4 with a maximum value of this indicator of 44.78 g and the control without nitrogen increased 7.41 g. This suggests that the effect of nitrogen fertilization on the weight of 1000 grains is variable and its effectiveness depends on climatic factor as well. In years when there was enough moisture supply during the growing season, nitrogen fertilizers had a significant effect on it.

The strategy of multiple fertilization of winter wheat with nitrogen based on the application of nitrogen to the frozen soil in early spring, can be an effective means to influence the crop productivity components, such as number of productive stems and weight of 1000

grains. Savchenko and Miroshnichenko (2013), Delin and Stenberg (2014) found similar results to this study. They consider that a high efficiency of nitrogen application in early spring, the period of cool weather and low temperatures, can be due to a low microbial activity in the soil, with an insignificant nitrogen loss through emission and leaching, promoting an increase in the nitrogen availability to the plants.

During experiment, the winter wheat yield ranged from 4.22 t ha^{-1} in 2017 (control without nitrogen), 8.04 t ha^{-1} in 2018 (nitrogen dose of 110 kg ha^{-1}) with an average yield of the grain of 6.30 t ha^{-1} during 2017-2019 (Table 3). According to the average data of three years, fertilization of winter wheat with nitrogen in all its configurations had a significant increase of grain yield compared to the control without nitrogen; 21.4% with a single application of nitrogen at 60 kg ha^{-1} on the frozen surface of the soil in early spring (treatment 2); 24.8% with split three times application of nitrogen at 80 kg ha^{-1} (treatment 3), and 30.4% with split three times application of nitrogen at 110 kg ha^{-1} (treatment 4). The maximum yield of winter wheat grain was obtained when nitrogen was applied under split multiple configuration with a rate of 110 kg ha^{-1}, including nitrogen at 60 kg ha^{-1} on the frozen surface of the soil in early spring as ammonium nitrate and two foliar feeding with urea solutions at BBCH 31-32 with nitrogen dose of 30 kg ha^{-1} and at BBCH 75-77 with nitrogen dose of 20 kg ha^{-1} (6.90 t ha^{-1}). In this treatment, yield of grain was higher 7.5% compared with the effect of single nitrogen fertilization in early spring (treatment 2) where no foliar nitrogen application was done (P<0.05). Both fertilizations with the nitrogen to the soil and foliar feeding contributed significantly to an increase in grain yield. Similar data was published by Krivenko (2018), whom optimized rate of nitrogen fertilizers applied to the soil and across the leaves increased the annual crop yield by 15.4–18.7%. The highest grain yield (10.16 t ha^{-1}) of winter wheat was reached in the treatment where foliar fertilizers were supplied in five applications and it agrees with Jankowski *et al.* (2016) whom noted the importance of multiple foliar fertilization with nitrogen.

Table 3. The effect of nitrogen nutrition on grain yield of winter wheat.

No.	Treatment	Year				Increase
		2017	2018	2019		%
1	(P60K60 under ploughing-Control)	4.42	6.16	5.28	5.29	-
2	(N60)	4.80	7.39	7.06	6.42	21.4
3	(N80)	4.84	7.66	7.30	6.60	24.8
4	(N110)	4.93	8.04	7.72	6.90	30.4
LSD (P<0.05)		0.21	0.37	0.22	0.25	

In years of regular precipitation during the growing season (2018-2019) nitrogen fertilizers had more effect on winter wheat yield than the year of deficient precipitation (2017). The studies demonstrated that configurations with nitrogen treatment at rate of 60-110 kg ha^{-1} increased grain yield compared to the control without nitrogen by 20.0-30.5% in 2018, by 33.7-46.2% in 2019, and only by 8.6-11.6% in 2017 when precipitation rate was the lowest.

Thus, maximum efficiency of nitrogen fertilizers that significantly increased winter wheat yield was associated with the years of favourable precipitation and with the strategy of split multiple fertilization, which was built on early term of nitrogen applied in spring. According to the research of Bobrecka-Jamro *et al.* (2013), Rasmussen *et al.* (2015), Yi *et al.* (2015), Walsh *et al.* (2018) the effectiveness of this fertilization is also attributed to the optimal rate of nitrogen for winter wheat ranging 90 to 120 kg ha^{-1}, which provides maximum economic and environmental benefit.

In all configurations, nitrogen application stimulated the accumulation of protein in winter wheat grain. Nevertheless, a significant increase in protein content in the grain was presented only using the split multiple application of the nitrogen at 80-110 kg ha^{-1}, which included foliar feeding of plants with urea solutions at BBCH 31-32 stages with nitrogen dose of 30 kg ha^{-1}

and at BBCH 75-77 with nitrogen dose of 20 kg ha^{-1} (treatment 3 and 4). According to the average data of 2017–2019, in these treatments compared to the control without nitrogen, the content of protein in grain was by 6.9–7.8% higher (P<0.05) with absolute value of the indicator of 12.4-12.5% (Table 4). Such an increase was stable through the years of experiment and did not depend on the variations of weather conditions. These data are consistent with a number of studies on the special role of foliar nitrogen treatments for protein accumulation in grain (Gholami *et al*., 2011; Litke *et al*., 2018; Wagan *et al*., 2017; Walsh *et al*., 2018), the importance of the last treatment (Gholami *et al*., 2011; Mandic *et al*., 2015) and the treatment from heading to flowering (Blandino *et al*., 2016) in increasing grain protein content, and the high efficiency of nitrogen feeding in reducing plant stress caused by hot weather (Mandic *et al*., 2015). Bhatta *et al.* (2017) found that the nitrogen rate at the flag leaf stage increased grain protein content by 1.5-8.1%. Thus, research data allows suggesting that feeding winter wheat with nitrogen through the leaves, as a complement application, could be efficient tool to improve quality of winter wheat grain under climate change.

Table 4. The effect of nitrogen nutrition on protein content in winter wheat grain.

No.	Treatment	Year				Increase
		2017	2018	2019		%
1	(P60K60 under ploughing-Control)	11.8	11.3	11.6	11.6	-
2	(N60)	12.4	11.7	12.1	12.1	4.3
3	(N80)	12.7	12.0	12.5	12.4	6.9
4	(N110)	12.8	12.1	12.7	12.5	7.8
LSD (P<0.05)		0.53	0.67	0.63	0.59	

Nitrogen fertilization increased the average of the straw yield by 27.5-39.1% compared to the control without nitrogen (Table 5). Such a significant increase was attributed to all configurations of nitrogen fertilization and was stable through the years of the experiment. Similar data was reported by Abedi *et al.* (2011) whom determined that enough nitrogen provision of winter wheat during growing season promotes an increase in grain and straw yield of the crop. After the correlation-regression analysis of data, significant correlation between rate of nitrogen fertilizer and yield of winter wheat was determined. This trend was observed in all years of the experiment (R^2=0.996-1.0) (Figure 3). The winter wheat had an increase in nitrogen rate and was capable to produce a higher grain yield in variable climate with extra warm and sometimes dry weather conditions.

Table 5. The effect of nitrogen nutrition on straw yield of winter wheat.

No.	Treatment	Year				Increase
		2017	2018	2019		%
1	(P60K60 under ploughing-Control)	6.1	7.7	7.0	6.9	-
2	(N60)	7.7	9.0	9.7	8.8	27.5
3	(N80)	8.0	9.5	10.3	9.3	34.8
4	(N110)	8.4	9.9	10.6	9.6	39.1
LSD (P<0.05)		0.46	0.61	0.67	0.54	

The study showed that with an increase of nitrogen rate, the grain protein content of winter wheat increased (Figure 4). The dependence of protein content on the rate of nitrogen fertilizers was strong (R^2=0.967-0.973).

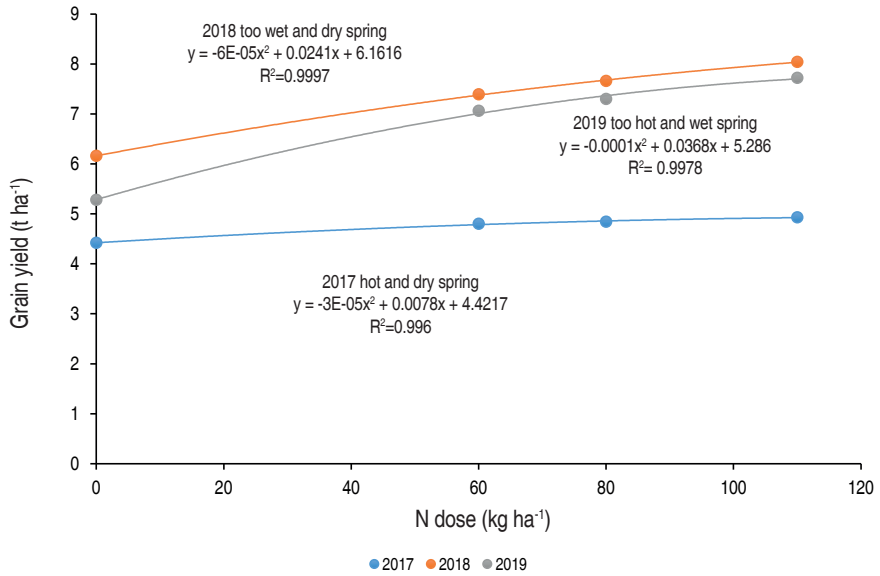

Figure 3. Winter wheat yield according to the annual N rate. 2017 weather was hot and dry spring. 2018 weather was wet and dry spring. 2019 weather was hot and wet spring.

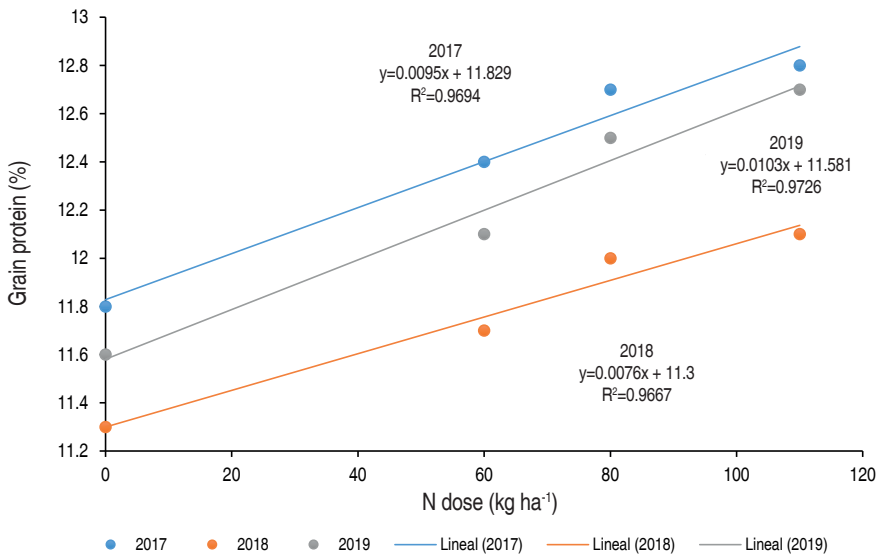

Figure 4. Grain protein content according to the annual N rate.

CONCLUSIONS

Nitrogen fertilizer has had a positive effect on winter wheat yield (60-110 kg ha^{-1}) (P<0.05). The highest average grain yield was obtained at a nitrogen rate of 110 kg ha^{-1} that combined application nitrogen at dose of 60 kg ha^{-1} on the frozen soil surface in early spring as ammonium nitrate and two foliar feeding with urea solutions at BBCH 31-32 with nitrogen dose of 30 kg ha^{-1} and BBCH 75-77 with nitrogen dose of 20 kg ha^{-1} – 6.90 t ha^{-1}. This nitrogen rate and configuration could be an application recommended in order to obtain a great grain yield. In years of regular precipitation during the growing season (2018-2019) nitrogen fertilizers had higher effect on winter wheat yield than the year of precipitation deficiency (2017). Significant increase in

grain protein content was obtained in variants where foliar feeding of winter wheat with urea solutions was a part of fertilization strategy; it was attributed to nitrogen rates of 80 and 110 kg ha^{-1}. The increase in protein content of the grain less depended on the weather conditions and was stable through the years of experiment. All configurations of winter wheat fertilization with nitrogen, with an increase in nitrogen rate from 60 to 110 kg ha^{-1}, contributed to the accumulation of protein in the grain. The correlation was evident in all years of the experiment and it had a low dependence on the variations in weather conditions.

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Biopriming of sweet pepper and tomato seeds with *Ascophyllum nodosum*

Acondicionamiento de semillas de pimiento y tomate con *Ascophyllum nodosum*

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ABSTRACT

Keywords:
Brown seaweed
Capsicum annuum
Germination
Solanum lycopersicum

Organic agriculture has been growing in recent years; however, one of the limitations in this area is the treatment of seeds with natural products and less aggressive to the environment. Seed biopriming with brown seaweed extract can be used to improve the physiological quality of seeds. This study aimed to evaluate the effect of seed biopriming with *Ascophyllum nodosum* extracts (algae) on pepper and tomato seed quality. Pepper seeds of All Big and Alegria cultivars (cvs), and tomato seeds of Cereja and Rio Grande cvs were used. The extract concentrations were 0, 125, 250, and 375 ppm. A completely randomized design in a 2×4 factorial scheme was used. The variables analyzed were percentage germination; germination speed index; root and shoot length; and root and shoot dry weight. Sweet pepper biopriming at 125 ppm enhanced germination in 16.5% for All Big cv; but it did not benefit Alegria cv. *A. nodosum* as a biopriming provided an increase of 50% in root length growth in Alegria cv; although, it had a negative effect on the growth of pepper seedlings of the All Big cv at 375 ppm. Tomato seed biopriming with *A. nodosum* at 125 ppm, enhanced root and shoot growth by 38 and 31% of Cereja cv; Nevertheless, it did not provide higher levels of germination. For Rio Grande cv, shoot growth was benefited at 125 ppm, with approximately 1.04 cm larger than the control. The effect of *Ascophyllum nodosum* priming depended on its concentration and the cultivar given that, different responses were obtained, also due to the compounds of the extract.

RESUMEN

Palabras clave:
Algas marrones
Capsicum annuum
Germinación
Solanum lycopersicum

La implementación de la agricultura orgánica ha tenido un desarrollo creciente los últimos años. Sin embargo, una de las limitaciones de esta área es el tratamiento de semillas con productos naturales y menos agresivos con el medio ambiente. El acondicionamiento de semillas con extracto de algas marrones se puede utilizar para mejorar la calidad fisiológica de las semillas. El objetivo de este estudio fue evaluar el efecto del acondicionamiento de semillas con extractos de *Ascophyllum nodosum* (alga) sobre la calidad de las semillas de pimiento cvs All Big y Alegria y las semillas de tomate de cvs Cereja y Rio Grande. Extractos de *A. nodosum* a 0, 125, 250 y 375 ppm fueron aplicados a las semillas. Como diseño experimental, se implementó un esquema factorial 2 × 4 completamente aleatorio. Las variables analizadas fueron porcentaje de germinación; índice de velocidad de germinación; longitud de raíz y brote; y el peso seco de la raíz y el brote. Los resultados obtenidos fueron sometidos a análisis de varianza y regresión. El acondicionamiento de semillas de pimiento con *A. nodosum* a 125 ppm, promovió incrementos en la germinación de semillas del 16,5% para el cv All Big; aunque, no benefició a los cv Alegria. El acondicionamiento proporcionó mayores niveles de crecimiento de raíces en un 50% en el cv Alegria, sin embargo, tuvo un efecto negativo en el crecimiento de las plántulas de pimiento del cv All Big a 375 ppm. El acondicionamiento de semillas de tomate con extracto de *A. nodosum*, a 125 ppm, afectó positivamente el crecimiento de cv Cereja mejorando el brote en un 31% y 38% la raíz; sin embargo, no proporcionó mayores niveles de germinación. Para el cv Río Grande, sólo se benefició el crecimiento de brotes a 125 ppm con un promedio 1,04 cm superior al control. El efecto del acondicionamiento de *Ascophyllum nodosum* dependió de su concentración y del cultivar dado que se obtuvieron diferentes respuestas, debido también a los compuestos del extracto.

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Peppers and tomatoes are among the most produced vegetables in Brazil. They are consumed commonly as raw vegetables and condiment. In 2016, Brazilian's production was 544,900 t of sweet peppers (CNA, 2017) and about 4.1 million t of tomatoes in 2018 (FAOSTAT, 2018).

About 82% of these crops correspond to family farming units (IBGE, 2017). The production of these vegetables has a high value-added per area and a fast-financial return compared to other activities. However, this activity requires a large investment, especially in the purchase of inputs, which creates an obstacle to the productive system (Vendruscolo *et al*., 2017). Different methodologies should be explored to improve the production process and the profitability.

In addition to this, the large number of pests and diseases is an important issue of these crops that could be mitigated by a seed treatment. This option may improve the physiological quality of plants such as growth and development while protects against pests and diseases (Sharma *et al*., 2015). According to Mauri *et al.* (2019), the absence of seed treatments can increase severity of attack by pests and diseases reflecting on a low germination and growth.

Organic agriculture system has modified the standard production dabbling sharply on the market without chemical treatments (fungicides and insecticides), which are not allowed in this kind of agriculture (Mauri *et al.,* 2019). Seed priming consists in a controlled hydration of the seeds, which can be used to improve the percentage germination and induce resistance to stress (Papparella *et al*., 2015; Waqas *et al*., 2019).

This technique is provided at the beginning of germination, with phases I (imbibition) and II (mobilization of reserves) without protrusion of the primary root. Traditionally, seed priming is performed with water under suitable conditions. However, some studies show that osmotic substances, bioactive molecules and secondary metabolites can be used. Species of vegetable such as lettuce, pepper and eggplant have been tested with this technique (Papparella *et al*., 2015; Forti *et al*., 2020). For instance, Delian *et al.* (2017) found that this technique can enhance germination, vigor and productivity of tomato seeds while promoting stress tolerance. Seed priming induces the antioxidant response and the DNA repair processes associated with the pre-germinative metabolism (Forti *et al.,* 2020).

Seaweed is another biopriming treatment that has showed a positive effect on improving growth, productivity, and stress tolerance. A wide range of seaweed species has been used in agriculture as biofertilizers and biostimulants (Madruga *et al*., 2020). The brown seaweed *Ascophyllum nodosum* is found in the Arctic seas and on the rocky shores of the Atlantic, contains substances such as cytokinins, auxins and gibberellic acid, which make it an alternative as bioregulators. They act in oxidative and metabolic processes and have macro and micronutrients that can assist during plant growth (Ali *et al*., 2019; Castro, *et al*., 2019).

Priming of chicory seeds with *A. nodosum* caused an increase in the percentage and seedling emergence speed in greenhouse conditions (Ferraz *et al*., 2019). Spinach, canola and barley were evaluated by Saeger *et al.* (2019) finding a positive impact on the germination and development. Sivritepe and Sivritepe (2016) observed that seaweed priming on tomato seed can increase the germination speed and uniformity. Souza *et al.* (2017) found a significant increase in plant height, number of leaves, stem diameter and length of roots at 0.9 mL L^{-1} using seaweed extract on tomato seeds.

In this context, the objective of this study was to evaluate the effect of *A. nodosum* extract on sweet pepper and tomato seed quality.

MATERIAL AND METHODS

The research was conducted in a Grain and Seeds laboratory at Federal University of Fronteira Sul, between February and October 2019. Pepper seeds of the All Big and Alegria cvs, and tomato seeds from Cereja and Rio Grande cvs were used. *A. nodosum* extract (brown alga) was used as conditioner at 0, 125, 250 and 375 ppm according to Sivritepe and Sivritepe (2016) for each species and cv. The experimental design was 2×4 factorial scheme (cv×concentration) completely randomized. To identify the pattern of solution absorption of the seeds, soaking curves were made with the different concentrations of the extracts.

Imbibition curve

It was performed with methodology adapted from Ferreira *et al.* (2013). Four replicates of 0.2 g of seeds for each treatment were soaked in the solutions in plastic Gerbox-

type boxes with metal plates and previously moistened Germitest papers (2.5 times their weight) at 25 °C, in a germination chamber until protrusion of the primary root. To determine the ratio water/solution absorbed, the seeds were removed from the Gerbox and dried using paper towels and weighed on a digital balance with an accuracy of 0.001 g at 60 min intervals after the first 12 h, every 3 h from 12 to 36 h, and every 6 h from 36 h; when the primary root protruded, the process was interrupted and the time was recorded in order to calculate later the appropriate time for seed priming procedure. After that, the water/solution absorption data were submitted to regression analysis to determine the imbibition period. This period was different for all species; for the pepper Alegria cv was 24 h, All Big cv was 15 h and for both tomato cvs was 18 h.

Seed priming was performed using a similar methodology to the soaking curve; however, the periods were determined by analyzing the results of the curve. Afterwards, the seeds were submitted to the germination analysis.

Germination test

Five replicates of 50 seeds were placed in plastic boxes of the Gerbox-type (11×11×3.5 cm), on two sheets of moistened Germitest paper, using distilled water by 2.5 times their mass. The boxes were kept in a germination chamber at 25 °C. The evaluations were performed at 7 and 14 days after sowing (DAS) according to Rules for Seed Analysis (MAPA, 2009).

Germination speed index. The number of germinated seeds was counted daily, during the 14 days of the germination test; the germination speed index was calculated by Maguire (1962).

Seedling length. It was determined using a methodology adapted from Nakagawa (1999). Randomly, 20 seedlings from each repetition were taken from the germination test at 14 DAS. These were measured with a graduated ruler and the values were recorded in cm.

Seedling dry mass. After determining the length of seedlings, they were put into Kraft® paper bags and placed in an oven with air circulation at 65 °C for 72 h. After this period, they were weighed to determine the dry mass. The results were recorded in mg per seedling.

The data were submitted to analysis of variance, Tukey multiple comparison test at 5% significance and regression in the Sisvar® software were done separately for each species.

RESULTS AND DISCUSSION

Sweet pepper seeds

The current study revealed a significant variation between cvs in response to priming with brown algae. The effect of seaweed extract concentration was found only for the All Big cv, in all variables except for root length, in which there was also a statistical difference with Alegria cv (Table 1).

Table 1. Effects on physiological quality of sweet pepper seed priming with seaweed extract.

Cultivar	Concentration (ppm)			
	0	125	250	375
	G (%)			
Alegria	76.0 aA*	70.4 aA	73.6 aA	78.0 aA
All Big	55.6 bA	64.8 aA	59.2 bA	40.0 bB
	GSI			
Alegria	75.6 aA	74.5 aA	78.6 aA	75.1 aA
All Big	71.2 bB	74.1 aAB	74.1 aAB	75.7 aA
	SL (cm)			
Alegria	2.14 aA	2.0 aA	2.0 aA	2.67 aA
All Big	1.90 aA	2.0 aA	1.97 aA	1.42 bA
	RL (cm)			
Alegria	1.6 aAB	1.5 aB	1.7 aAB	2.4 aA
All Big	1.2 aA	0.8 bA	1.1 aA	0.8 bA

G(%): averages of germination. GSI: germination speed index. SL: shoot length. RL: root length. *Averages followed by the same lower case letter in the column and upper case in the row do not differ by Tukey-test (P<0.05).

Seed germination of the All Big cv was reduced by the application of seaweed extract at 375 ppm. Although there was no statistical difference between concentrations, a 15.6% reduction in the germination of seeds submitted to seaweed extract at 375 ppm compared to the control was noticed. Also, Alegria cv had superior results compared to All Big cv at all concentrations, except at 125 ppm, where the germination was similar in both cultivars. The response of plants depends on several factors, including the species of plant and cv selected (Castro *et al.*, 2019).

Extracts of the *A. nodosum* seaweed may contain abscisic acid (ABA), auxins and gibberellins (GA). These compounds may have interfered with the hormonal levels of the seeds, causing a hormonal imbalance at the highest dose affecting the germination capacity (Ali *et al.*, 2019). Some authors have shown that ABA and GA antagonistically regulate many plant development processes, including dormancy and germination, root initiation and hypocotyl elongation (Shu *et al.*, 2018).

According to Castro *et al.* (2019), the composition of these extracts can be affected by several processes, such as time of alga collection and extraction method. Therefore, values for the composition of these phytohormones are variable.

As for the germination speed index, the results showed a positive effect after applying seaweed extract at 375 ppm to the All Big cv, compared to the control; however, there was not differences with the other treatments; for Alegria cv, there was no significant effect of seaweed extract dose.

In the All Big cv, the germination percentage was affected by application of the seaweed extract. The germination speed index was positively influenced at the highest concentration. These results are related to the composition of the extract, more specifically, to the hormonal balance. According to Shu *et al.* (2016), auxin affects the physiological effect of ABA on the plant, and high levels of auxin can decrease germination, inducing seed dormancy. However, auxin is one of the hormones responsible for growth, which may be related to an increase in the germination speed.

Regarding seedling growth, there were few differences between the treatments. As for the length of the shoot of seedlings, only at 375 ppm, Alegria cv was superior to All Big cv and there were no differences between the concentrations of the conditioner extract (Table 1).

Seedling root length increased 50% compared to the control in the Alegria cv at 375 ppm (Table 1). Similar results were obtained by Ali *et al.* (2019), with large increases in root length in pepper seedlings of Ikeda cv, obtained from seeds treated with seaweed extracts.

Marine algae are complex organisms, composed of several substances that could cause this increase in root length. Compounds such as auxins, micronutrients and some amino acids are growth inducers for plants (Castro *et al.*, 2019). Comparing the cultivars, Alegria showed better performance in relation to All Big at 125 and 375 ppm.

Regarding the dry mass of seedlings, the results showed differences between the concentrations and cvs, both roots and the shoot of seedlings (Figure 1). For the shoot dry mass of pepper seedlings, the Alegria cv showed a great increase in mass according to the increase in algae concentration, with an increase of 87% compared to the control at the maximum concentration (Figure 1A). In the All Big cv, a reduction in dry mass was observed with the use of seaweed extract, with no positive effect of treatment for this variable. Comparing the cvs, All Big showed superior performance at all concentrations, except 375 ppm.

The effect of algae extracts is related to gene expression and, therefore, differences in seed responses from different cvs are plausible (Castro *et al.*, 2019).

Regarding the root dry mass of pepper seedlings, the cvs responded differently to the concentration factor. The seeds of Alegria cv obtained dry mass gain with an increase in the concentration of brown alga extract, with greater performance at 230 ppm, according to the presented quadratic model. In the seeds of the All Big cv, there was no positive effect, since dry mass had a reduction while the dose increased (Figure 1B).

The biostimulant effect of brown seaweed extract can be related to many factors. Several studies report an increase in dry matter in seeds under the application of these compounds (Souza *et al.*, 2017; Ali *et al.*, 2019; Saeger *et al.*, 2019). The action of plant hormones mainly controls the dry mass accumulation process.

Therefore, these same studies indicate that the reason for these positive effects is the presence of compounds that interfere with the hormonal balance of plants, in addition to macro and micronutrients that have a biofertilizer effect. However, Sorgatto and Silva (2018) found a negative impact on the dry mass of seedlings following the application of seaweed extract to parsley seeds, which indicates the need for more specific studies on the action of these compounds on different plants and concentrations.

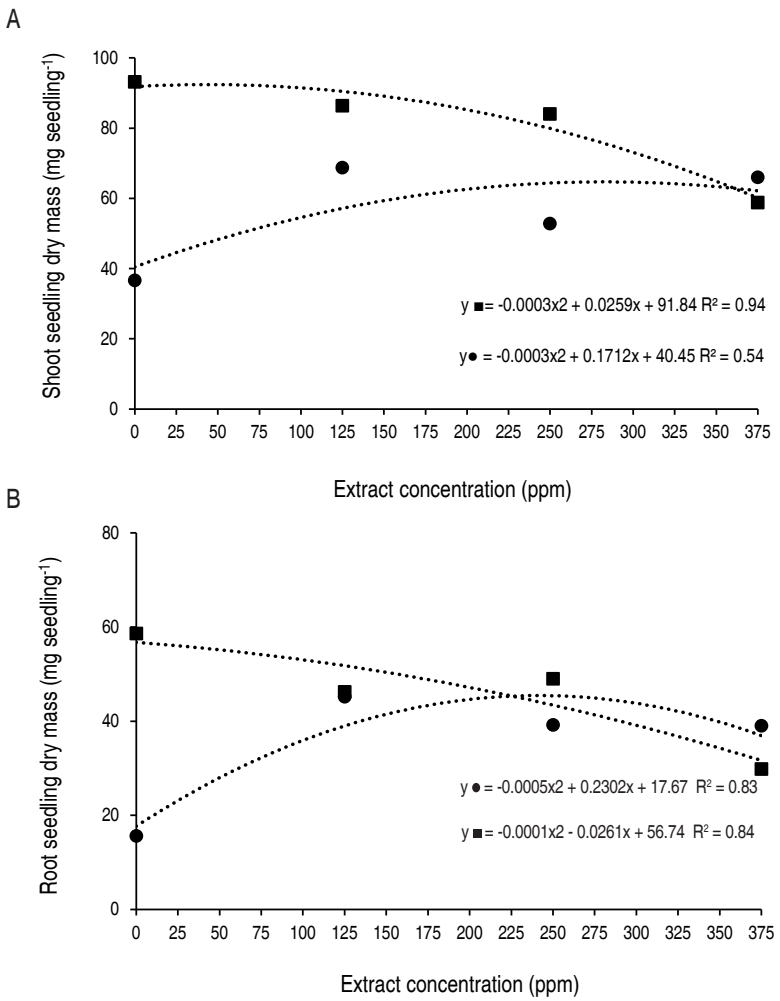

Figure 1. Averages of shoot (A) and root (B) pepper seedlings dry mass, Alegria (●) and All Big (■) cultivars, obtained from seeds conditioned with different concentrations of *Ascophyllum nodosum* extract.

Tomato seeds

In the conditioning stage of tomato seeds, an effect of treatments only on the variable length of seedling roots was observed (Table 2). Germination was not affected by the concentrations of *A. nodosum* extract (Table 2) and did not differ between the cvs used.

Some studies have demonstrated a positive effect of the application of *A. nodosum* on tomato germination, however at different doses, usually higher than the dose used in this experiment (Sivritepe and Sivritepe, 2016; Souza *et al.*, 2017; Delian *et al.*, 2017).

The germination speed index and shoot length of tomato seedlings did not show a significant difference between the concentrations of extract used for either cv (Table 2). Rio Grande presented earlier germination than Cereja.

Table 2. Effects on physiological quality of tomato seeds priming with seaweed extract.

Cultivar	Concentration (ppm)			
	0	125	250	375
	G (%)			
Cereja	81.6 aA*	85.2 aA	82.4 aA	82.4 aA
Rio Grande	85.2 aA	93.2 aA	87.6 aA	91.2 aA
	GSI			
Cereja	91.2 aA	91.1 bA	93.7 aA	92.0 aA
Rio Grande	98.2 aA	104.8 aA	97.6 aA	96.4 aA
	SL (cm)			
Cereja	2.55 bA	3.54 bA	2.68 bA	2.49 bA
Rio Grande	3.75 aA	4.79 aA	3.66 aA	3.70 aA
	RL (cm)			
Cereja	1.83 bAB	2.40 aA	1.69 bB	1.95 bAB
Rio Grande	2.73 abA	2.84 aA	2.43 aAB	2.13 aB

G%: averages of germination. GSI: germination speed index. SL: shoot length. RL: root length. *Averages followed by the same lower case letter in the column and upper case in the row do not differ by Tukey-test (P<0.05).

For the shoot length of seedlings, the Rio Grande cv exhibited better results compared to Cereja cv at all concentrations. This difference could be due to the genetic character of the cvs. According to Isla (2020a, 2020b), the Rio Grande and Cereja cvs present around 345 and 420 seeds per gram, respectively, which indicates that Cereja seeds are smaller and possibly have a lower content of reserve substances, compared to Rio Grande cv. According to Khan *et al.* (2012), seed weight has a strong effect on seedling vigor and growth. Still, the authors explain that there is a strong correlation between the vigor and the size and weight of the seeds, which could have a strong effect on the initial growth of the main root and the aerial part of seedlings. This positive effect of heavy seeds may be due to common genetic mechanisms that control these traits and also, to the high amount of reserve substances in larger seeds compared to small ones.

It is also worth mentioning that in the two cvs evaluated in this research, at 125 ppm the extract caused a significant increase in the length of the shoot, although there was no statistical difference. Ferraz *et al.* (2019) obtained results similar to these with application of extracts of *A. nodosum* on chicory seeds of the Lisa cv, with an increase of 0.9 cm at the lowest concentration, but with no effect at higher doses. The reason for this increase is related to the presence of plant regulators, such as auxins and cytokinins, which induce cell division and elongation.

Regarding seedling root length, in both cases, there was no significant increase in growth with the use of seaweed extract (Table 2); for the Cereja cv, the highest root growth was at 125 ppm, but no treatment caused improvements in relation to the control. In the Rio Grande cv, at 375 ppm the growth was reduced comparing to the control but at 125 ppm the seedling increased its lengh regarding the control.

In this study, the presence of seaweed extract resulted in an inhibitory effect at concentrations greater than 125 ppm on tomato seedling roots. There is a wide variation in the auxin content in *A. nodosum* extracts reported in the literature (Shukla *et al.*, 2016). It is known that auxins, like other plant hormones, act at low concentrations, and the balance between the various classes of hormones is what stimulates or inhibits a physiological process in plants. In addition, as the auxins, there are substances that at high concentrations can cause an inhibitory effect on growth (Taiz *et al.*, 2017).

It was possible to verify that the Cereja cv presented a better general performance with the treatment at 125 ppm; values higher than this, the alga extract causes some type of inhibition, which makes the performance equal or worse than the control. As for the Rio Grande cv, the effect of brown seaweed extract at the concentrations used is indifferent, but it was inhibitory in root length.

According to Saeger *et al.* (2019), *A. nodosum* extracts act on hormonal balance and regulate important processes in nutrient absorption and photosynthesis. However, the exact molecular basis of growth promotion caused by this application still needs to be elucidated, as it involves several processes in the plant (genome, enzyme activity and transcription, among others). For this reason, future studies must be carried out to discover the effect of these extracts on each metabolic process.

CONCLUSIONS

Biopriming of pepper seeds with *A. nodosum* extract at 125 ppm promoted increments in seed germination of the All Big cv. However, it did not benefit seed germination of the Alegria cv. Bio-conditioning with *A. nodosum* extract provided higher levels of growth in the Alegria cv, but it had a negative effect on the growth of pepper seedlings of the All Big cv at 375 ppm.

Biopriming of tomato seeds with *A. nodosum* extract at 125 ppm positively affected the root and shoot growth of Cereja cv. Yet, it did not provide higher levels of germination. Nevertheless, for Rio Grande cv only shoot growth was benefited by bio-conditioning at 125 ppm.

It was possible to verify the difference in the response of plants by the application of *Ascophyllum nodosum* extract, with great differences between cultivars in the variables analysed. According to these results, the use of *Ascophyllum nodosum* extract is an important tool for enhance germination levels of the pepper and tomato; however future studies should be done to better elucidate its effect on vegetable seeds.

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Effect of supplemental irrigation on bread wheat genotypes yield under Mediterranean semi-arid conditions of north-eastern Algeria

Efecto de la irrigación suplementaria sobre el rendimiento de trigo pan en condiciones mediterráneas semiáridas del noreste de Argelia

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Keywords:
Supplemental irrigation
Triticum aestivum L.
Genotype introduction
Grain yield
Agronomic traits

ABSTRACT

Different levels of supplemental irrigation regimes on four wheat (*Triticum aestivum* L.) genotypes were evaluated, two of which were introduced into Sétif region by ACSAD institution, during the growing season 2013-2014, in order to assess the effect of deficit irrigation pattern on yield traits performance and to determine most suitable genotype for local semi-arid conditions. On the basis of the experimentation data, it was found that supplemental irrigation improved the investigated genotypes yield, which ranged from 220.03 g m^{-2} for variety El-wifak in rainfed conditions to 368.3 g m^{-2} for variety Djanet (ACSAD899) with an increase of about 67%; just by applying two irrigations, the first at the jointing stage and the second at mid-flowering stage. This increase was related to the improvement of most agronomic traits that correlated significantly and positively with grain yield, in response to supplemental irrigation application. These findings indicated that Djanet (ACSAD899) was a genotype successfully introduced under irrigated conditions, while Hidhab (HD1220) with an average grain yield of 298.3 g m^{-2}, proved to be more stable and well adapted to the locally rainfed conditions.

Palabras clave:
Riego suplementario
Triticum aestivum L.
Introducción de genotipo
Rendimiento de grano
Caracteres agronómicos

RESUMEN

En esta experimentación, se evaluaron diferentes niveles de riego suplementarios en cuatro genotipos de trigo (*Triticum aestivum* L.), dos de los cuales fueron introducidos en la región de Sétif por ACSAD, durante la temporada 2013-2014, para evaluar el efecto de los distintos tratamientos de riego y para determinar el genotipo más adecuado para las condiciones semiáridas locales. Según los resultados obtenidos, el riego suplementario mejoró el rendimiento de los genotipos investigados, variando desde 220,03 g m^{-2} para la variedad El-wifak en condiciones de secano hasta 368,3 g m^{-2} para la variedad Djanet (ACSAD899) con un aumento de aproximadamente el 67% con sólo aplicar dos riegos, el primero en la etapa de primer nodo y el segundo en la etapa de floración. Este aumento se relacionó con la mejora de la mayoría de los caracteres agronómicos correlacionados positivamente con el rendimiento de grano, en respuesta a la aplicación de riego suplementario. Djanet (ACSAD899) tuvo buenos resultados bajo condiciones de riego, mientras que Hidhab (HD1220), con un rendimiento promedio de grano de 298,3 g m^{-2}, demostró ser más estable y bien adaptado a las condiciones locales de secano.

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In the northeastern high plateaus of Algeria, as well as the semi-arid plains of Sétif province, water resources are generally limited and a typical Mediterranean climate prevails, with rain mainly falling in winter and less in late spring. This irregular rainy period is followed by a hot and dry summer (Aissaoui, 2019). According to Guendouz *et al.* (2012) and Merouche *et al.* (2014), severe drought occurrence in later stages of bread wheat (*Triticum aestivum* L.) grown in these regions, shortens the grain filling and ripening duration, resulting in large annual fluctuations in grain yield. This situation discourages local farmers and disadvantages wheat production for subsequent seasons, reducing yield potential and complicating grain supply for bakery (Aissaoui and Fenni, 2018). It was therefore necessary to find reasonable practical solutions to face this water deficit of rainfed bread wheat production. The development of an appropriate irrigation strategy and/or introducing new drought-tolerant varieties could improve bread wheat yield in this region.

As a result, supplemental irrigation (SI), widely practiced in southern and eastern Mediterranean countries, appears to be an adequate practice for crop water supply. Oweis and Hachum (2012) defined supplemental irrigation as the application of a limited amount of water to rainfed crops when precipitation fails to provide necessary moisture for normal plant growth. This practice has shown a promising potential to mitigate the adverse effects of unfavorable rain patterns and thereby improves and stabilizes crops yields compared to the traditional irrigation practices **(**Oweis and Hachum, 2012; Khamssi and Najaphy, 2012, Sakumona *et al*., 2014; Meng *et al*., 2015). The objective for this research was to investigate grain yield and yield components performance for two sets of genotypes; locally cultivated and newly introduced, under both rainfed and irrigated conditions at critical development stages.

MATERIALS AND METHODS

Experimental location

The experiment was conducted in the Agricultural Research Station of the Technical Institute for Field Crops (ITGC) (36° 10'N, 5°2' E) localized 3 km south-west Sétif city, during the 2013-2014 growing season. The soil texture of the experimental site was silty clay with pH 8.1 and 1.2% organic matter content. The gravimetric soil water content of the top 60 cm soil, measured by pressure plate, was 24.6% at Pf 2.5 (-0.033 MPa) and 11.8% at Pf 4.2 (-1.5 MPa). The treatments included four bread wheat genotypes (G) and six water regimes (W). The plots were replicated three times in a randomized complete block design (RCBD). Each plot size was 1.20 m^2 (1.20 m×1.0 m).

Plant material

As shown in Table 1, plant material consisted of four genotypes of bread wheat (*Triticum aestivum* L.). Two commonly ameliorated varieties, Hidhab (HD1220) and El-wifak, grown locally in the plains of Sétif region and two newly introduced genotypes, Djemila (ACSAD969) and Djanet (ACSAD899), provided by ACSAD (The Arab Center for the Studies of Arid Zones and Dry Lands**)** institution. Crop development was categorized according to Zadoks scale (Z) (Zadoks *et al*., 1974).

Table 1. Pedigree and source of plant material.

Genotype	Pedigree	Source of material
Hidhab (HD1220)	HD1220/3*Kal/Nac CM40454	CIMMYT[1](Mexico)
El-wifak	K134/4/Tob/Bman/Bb/3/Cal/5/Bucc	CIMMYT(Mexico)
Djemila (ACSAD969)	Acsad529// prl4S4/ VEE's'	ACSAD (Syria)
Djanet (ACSAD899)	Acsad529/4/C182.24/C168.3/3/Cno*2/7C//CC/Tob-1s	ACSAD (Syria)

[1] CIMMYT: International Maize and Wheat Improvement Center.

Crop management

The seeds were planted in six rows with 17 cm row's interval on December 12, 2013 at a seed rate of 250 seeds m-2 and plots were harvested on June 22, 2014. Nitrogen application (total 80 kg ha^{-1} of urea) was split; a half at sowing and another half in the beginning of stem elongation stage. Phosphorus was applied at the sowing (Z0) as basal dressing in triple-superphosphate form (46% P_2O_5) at the rate of 70 kg ha^{-1}. Weed control was achieved both by application of post emergence herbicides and eventually by hand. Grain yield and other agronomic traits data were determined by the four center rows in each plot to avoid edge effects.

Irrigation application

Irrigation was applied to all targeted treatments to maintain the maximum allowable deficit (MAD) at 75% of field capacity (Wang *et al.*, 2013; Meng *et al.*, 2015). To ensure full coverage and uniform distribution of water on all plots, water was applied carefully along the six cropping rows.

Water regimes

Differing in amounts and timing (crop development stages) of application, six water regimes (W) were applied to the different plot. W0: Rainfed without irrigation; W1: Irrigated during the stem extension from the second node detectable in Z32-Z39 Zadok's stages; W2: Irrigated during two stages, jointing at the first node detectable (Z31) and flowering stage when yellow anthers are visible on 50% of the spikes (Z65-Z69); W3: Irrigated during three stages tillering (Z21-Z29), (Z32-Z39) and milky grain filling (Z70-Z79); W4: Irrigated during four stages (Z21-Z29), (Z32-Z39), (Z65-Z69) and (Z70-Z79); W5: Irrigated at five grow stages (Z21-Z29), (Z31), (Z32-Z39), (Z65-Z69) and (Z70-Z79).

Field measurements

Soil water content changes were gravimetrically monitored at 8 to 9 days intervals. A soil water balance approach based on measuring soil moisture content (θ) prior to / and after irrigation, which reflected both rainfall contribution and plant water depletion patterns was used. To determine actual crop water use (ETact, mm), measurements were made at an interval of 20 cm in the soil profile from the top layer to a depth of 60 cm where just below, a continuous hardpan limits root depth (Unpublished results). At maturity stage, data on number of spikes (NS, m^{-2}), number of grains per spike (NGS), number of grains (NG, m^{-2}) derived from NS and NGS, grains weight per spike (GWS, g $spike^{-1}$), were recorded on two intermediate rows designated arbitrarily then reported to unit area on each plot. Plant height (PH, cm) was measured over short lengths chosen randomly within intermediate rows from ground level to the top of spikes. Using a hand sickle, the above-ground biomass (BM, g m^{-2}) was determined by weighing the bunch of aerial plant mass of each plot, then related to its grain yield to derive the harvest index (HI, %). Grain yield (GY, g m^{-2}) was determined by immediate weighing of grains resulting from threshing of corresponding fully harvested plot. 500 grains were counted and weighed to deduce thousand grain weight (TGW, g).

Statistical analysis

The data were collected and presented as the means of three replicates, then statistically analyzed using SPSS package program (PASW Statistics Base Version 23.0) for a factorial design in blocks. An analysis of variance (ANOVA) was performed, and means were compared using LSD test and significant differences were considered (P<0.05)

RESULTS AND DISCUSSION

The mean annual rainfall at the station, over 20 years (1993-2013), was 356.4 mm, with around 279.6 mm from November to June, in coincidence with bread wheat growing season. In 2013-2014, 283.4 mm were recorded on the station as shown in Table 2 (ONM, 2014).

Table 2. Long term weather data for 1993-2013 and during the growing season in 2013-2014

Month	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
1993-2013										
Precipitation (mm)	46.9	29.9	34.0	38.1	39.7	38.5	27.4	42.4	40.4	19.1
Temperature (°C)	20.6	16.2	9.8	6.2	5.4	6.0	9.5	12.3	17.3	23.8
2013-2014										
Precipitation (mm)	28.9	49.3	22.4	31.0	37.7	16.9	74.0	2.2	60.8	38.4
Temperature (°C)	21.5	19.5	9.1	5.7	6.6	7.3	7.5	13.1	17.4	24.0

Although the cumulative rainfall for the long-term period 1993-2013 and during the growing season in 2013-2014 is almost equal, particularly in April, rainfall distribution suffers from a large deficit about 40.2 mm which was

considerably less than that required by late bread wheat development stages. Therefore, irrigation seemed more imperative for this crop in order to obtain a high yield.

Analysis of variance presented in Table 3 confirmed that there were significant differences among water regimes (W) for most parameters, particularly for GWS, NGS and BM (P<0.01). Besides, the effect of the genotype (G) was significant for most examined parameters; distinctly for HI and PH (P<0.001) and notably for GY and NS (P<0.01). Although the interactions (W×G) were not significant for all measured parameters indicating that genotype (G) had the same trend of the water regime (W).

Table 3. Analysis of variance of grain yield and yield related parameters.

SV	DF	Mean square								
		GY	TGW	NS	NGS	NG	GWS	BM	HI	PH
		(g m^{-2})	(g)	(NS m^{-2})	(unit)	(NG m^{-2})	(g)	(g)	(%)	(cm)
W	5	5,892.22*	36.59	1,471.75	202.80**	29,470,937*	0.47**	50,938.46**	4.89	30.39
G	3	14,663.89**	70.13*	17,668.16**	131.59*	38,839,680*	0.16	19,582.43	178.19***	377.43***
W×G	15	3,109.44	11.03	3,290.33	11.77	11,004,603	0.06	11,470.69	7.93	12.52
CV%		17.58	10.42	14.82	12.44	18.94	15.93	13.05	7.72	6.18
R^2		0.751	0.662	0.629	0.868	0.634	0.802	0.651	0.723	0.631

SV=Source of variation, DF=Degrees of freedom, GY=Grain yield, TGW=1,000 grain weight, NS=number of spikes per unit area, NGS=number of grains per spike, GWS=grain weight per spike, NG=number of grains per unit area, BM=above-ground biomass, HI=harvest index PH=plant height. CV (%): coefficient of variability, R^2: coefficient of determination *, ** and *** indicate the significance at 5, 1, and 0.1 % level, respectively.

Effect of water regimes on grain yield and yield components

Variations in grain yield and yield components across genotypes were affected by the gradual increase in number of irrigations with the application of supplemental irrigation water regimes (Table 4). Grain yield of the varieties increased gradually from 251.25 g m^{-2} in rainfed treatment to 310.83 g m^{-2} under the most irrigated regime (W5), improving their performance by 24% under the experimental conditions and by 74% relative to the mean grain yield for Sétif province, estimated around 178.2 g m^{-2} during 2014 (DSA, 2014).

Table 4. Mean grain yield and yield components scores by the water regimes.

Water regime	GY	TGW	NS	NGS	NG	GWS	BM	HI	PH
	(g m^{-2})	(g)	(NS m^{-2})	(unit)	(NG m^{-2})	(g)	(g)	(%)	(cm)
W0	251.25 b	41.83	349.5	42.3 c	14,697.5 c	1.965 b	810.00 b	30.96	68.30
W1	272.08 ab	43.99	326.1	50.4 ab	16,378.1 bc	2.168 b	893.50 ab	30.49	70.81
W2	287.92 ab	44.28	343.5	49.6 b	17,104.0 abc	2.197 b	920.17 a	31.06	69.15
W3	297.92 a	43.67	355.5	48.6 b	17,544.5 ab	2.147 b	913.50 a	32.31	72.47
W4	303.32 a	45.59	353.0	49.9 b	17,711.5 ab	2.217 b	983.83 a	30.86	71.49
W5	310.83 a	46.95	354.0	55.2 a	19,429.5 a	2.571 a	985.83 a	31.53	71.57
Mean	287.22	44.39	346.93	49.50	17,144.18	2.211	917.81	31.20	70.63
$LSD_{0.05}$	41.24	ns	ns	5.13	2,679.70	0.28	97.82	ns	ns

Similar letters are not significantly different at 5% probability level. ns: non-significant differences.

NGS, NG, GWS, and BM were highest in the most irrigated regime (W5) with 55.2 grains spike^{-1}, 19,429.5 grain m^{-2}, 2.57 g, and 985.83 g respectively, with an increase of 30.50%, 32.20%, 30.84% and 21.70% respectively, in comparison with rainfed treatment W0, (Table 4), while TGW, NS, HI and even PH were not statistically significant by increasing water regime levels.

Effect of genotype on grain yield and yield components

Differences in grain yield and most agronomic traits were observed for all genotypes across the different water regimes. The local varieties, El-wifak and Hidhab yielded 250 and 286.4 g m-^{2}, respectively. For the new introductions, Djanet dominated the grain yield and recorded the highest score with 319.17 g m^{-2} followed by Djemila with 293.3 g m^{-2} compared to the local cultivars (Table 5). Djanet, the most yielding genotype, had the highest NGS and HI with 53.6 grains per spike and 34.3%, respectively, while the local genotype El-wifak had the lowest scores for the same parameters. Conversely, El-wifak, the least yielding genotype, recorded the highest TGW and PH, while Djanet had the lowest (Table 5).

Table 5. Mean grain yield and yield components scores by genotypes.

Genotype	GY	TGW	NS	NGS	NG	GWS	BM	HI	PH
	(g m^{-2})	(g)	(NS m^{-2})	(unit)	(NG m^{-2})	(g)	(g)	(%)	(cm)
Hidhab	286.39 a	43.89 b	388.3 a	48.1 b	18,597.0 a	2.109	959.22	29.78 b	70.03 b
EL-wifak	250.00 b	47.20 a	352.4 b	48.2 b	16,958.4 ab	2.299	907.00	27.48 c	76.84 a
Djemila	293.33 a	43.86 b	316.3 c	48.2 b	15,183.0 b	2.153	880.56	33.24 a	69.88 b
Djanet	319.17 a	42.59 b	330.7 bc	53.6 a	17,838.3 a	2.282	924.45	34.31 a	65.79 c
Mean	287.22	44.39	346.93	49.50	17,144.2	2.211	917.81	31.20	70.63
$LSD_{0.05}$	33.66	3.06	34.65	4.19	2,187.96	ns	ns	1.61	2.91

Similar letters are not significantly different at 5% probability level. ns: non-significant differences.

Regarding spike parameters, the NS ranged from 316.3 spike m^{-2} for the newly introduced genotype Djemila, which showed lowest number of tillers (Unpublished results), to 388.3 spike m^{-2} for the local variety Hidhab which as well recorded the highest NG with 18,597.0 grains m^{-2}.

Water regimes by genotype interaction

For this experiment, the interaction between water regimes and genotypes (W×G) for grain yield and all agronomic traits was not statistically significant (*P*> 0.05) which is similar to what has already been found by Merouche *et al.* (2014) under similar conditions for winter durum wheat.

Correlation of yield and yield traits

Using a simple linear correlation between grain yield and yield traits, Table 6 indicated that positive correlation, shown by a significant *r* value, of GY was observed with BM (r=0.820***), HI (r=0.703***), NG (r=0.507***), NGS (r= 0.473***), GWS (r=0.390**) and NS (r=0.234*) which was consistent with the results of Tayyer (2008), where the grain yield was positively and significantly correlated with NGS and GWS, as well as with Guendouz *et al.* (2012) for NS and NG, while no significant correlation was observed between GY and TGW (r=0.057). In contrast, a negative significant correlation was reported between GY and PH (r = -0.232*), which agreed with the results of Siosemardeh *et al.* (2006); Khamssi and Najaphy (2012); Aissaoui and Fenni (2020).

It is evident that under the rainfed conditions, the distribution of rainfall over wheat growing season, furthermore, high daily temperatures and water stress in the spring time shortened grain filling period, leading to grain earlier maturity, which results in low grain yield as reported by Erekul *et al.* (2012). Therefore, GY in rainfed treatment (W0) was significantly lower than that under supplemental irrigation regimes even though, there were no significant differences (*P*>0.05) in TGW, NS or HI between water regimes for all treatments. These results are consistent with those obtained by Khamssi and Nadjaphy (2012); Guendouz *et al.* (2012); Wang *et al.*

(2013) and Sakumona *et al.* (2014). Oppositely, provide a permanent available soil water, as it was achieved with the most irrigated water regime (W5), significantly improved various agronomic traits such NGS, NG, GWS, and even BM, by 30.50%, 32.20%, 30.84% and 21.71%, respectively as shown in Table 4, which explains and confirms that improving agronomic yield (up to 24%) is strongly associated with the improvement of its components and agreed with the findings of Guendouz *et al.* (2012); Sakumona *et al.* (2014); Meng *et al.* (2015), when they confirmed that grain yield increased due to improvement in yield components under irrigation.

Table 6. Pearson's correlation coefficients between grain yield and agronomic traits.

Traits	GY	TGW	NS	NGS	GWS	NG	BM	HI	PH
GY	1								
TGW	0.057^{ns}	1							
NS	0.234*	0.221^{ns}	1						
NGS	0.473***	0.121^{ns}	-0.073^{ns}	1					
GWS	0.39**	0.636***	0.107^{ns}	0.767***	1				
NG	0.507***	0.259*	0.723***	0.629***	0.616***	1			
BM	0.82***	0.304**	0.405***	0.467***	0.504***	0.643***	1		
HI	0.703***	-0.280*	-0.103^{ns}	0.239*	0.038^{ns}	0.073^{ns}	0.176^{ns}	1	
PH	-0.232*	0.372**	0.107^{ns}	-0.207^{ns}	0.032^{ns}	-0.064^{ns}	0.100^{ns}	-0.514***	1

GY=Grain yield, TGW=1,000 grain weight, NS=Number of spikes per unit area, NGS=Number of grains per spike, GWS=Grain weight per spike, NG=Number of grains per unit area, BM=Above-ground biomass, HI=Harvest index, PH=Plant height.
^{ns} not significant. *.**. *** Significant at 5%. 1%. 0.1% probability levels.

It is evident that under the rainfed conditions, the distribution of rainfall over wheat growing season, furthermore, high daily temperatures and water stress in the spring time shortened grain filling period, leading to grain earlier maturity, which results in low grain yield as reported by Erekul *et al.* (2012). Therefore, GY in rainfed treatment (W0) was significantly lower than that under supplemental irrigation regimes even though, there were no significant differences (P>0.05) in TGW, NS or HI between water regimes for all treatments. These results are consistent with those obtained by Khamssi and Nadjaphy (2012); Guendouz *et al.* (2012); Wang *et al.* (2013) and Sakumona *et al.* (2014). Oppositely, provide a permanent available soil water, as it was achieved with the most irrigated water regime (W5), significantly improved various agronomic traits such NGS, NG, GWS, and even BM, by 30.50%, 32.20%, 30.84% and 21.71%, respectively as shown in Table 4, which explains and confirms that improving agronomic yield (up to 24%) is strongly associated with the improvement of its components and agreed with the findings of Guendouz *et al.,* 2012; Sakumona *et al.,* 2014; Meng *et al.,* 2015, when they confirmed that grain yield increased due to improvement in yield components under irrigation.

Thus, supplemental irrigation adoption has contributed to improve the grain yield of the tested genotypes, which varied from 220.0 g m^{-2} for variety El-wifak in rainfed treatment (W0) to 368.33 g m^{-2} for Djanet genotype under W2 water regime, as the highest grain yield score, with an increase of about 67% as shown in Figure 1.

This result was achieved just by applying two supplemental irrigations (W2); the first of 15 mm at the jointing stage corresponding to the first detectable node (Z31) and the second of 20 mm at the flowering stage Z65-Z69 (mid-way to complete anthesis), which has contributed to increase two major yield components; NG by 16.38% as well as GWS by 12% as plotted in Figure 2, in comparison to rainfed treatment W0, and thus improving the grain yield by 15%.

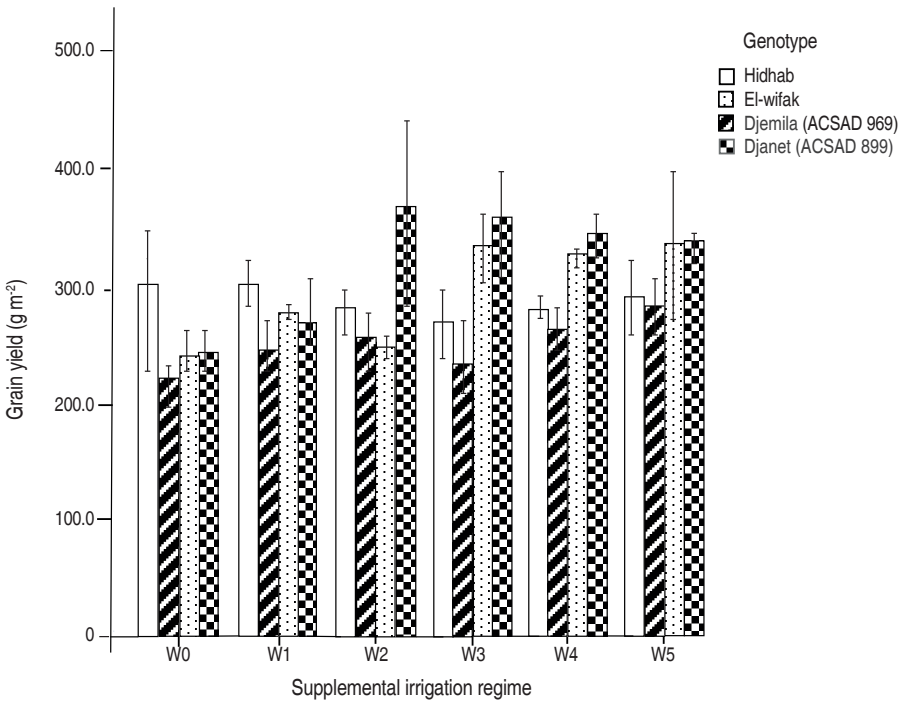

Figure 1. Grain yield of bread wheat genotypes under variable water regimes.
Rainfed treatment without any irrigation (W0), supplemental irrigation at Z32-Z39 (W1), supplemental irrigation at Z31 and Z65-Z69 (W2), supplemental irrigation at Z21-Z29, Z32-Z39 and Z70-Z79 (W3), supplemental irrigation at Z21-Z29, Z32-Z39, Z65-Z69 and Z70-Z79 (W4), most irrigated at the five stages Z21-Z29, Z31, Z32-Z39, Z65-Z69 and Z70-Z79 (W5). SI was applied to maintain MAD at 75% of field capacity. Vertical bars represent standard errors of the means.

In effect, improved GY with SI adoption was mainly associated with the increase of the number of grains NG (r=0.507, P<0.001) which was confirmed by the linear relationship (r^2= 0.924, n=72) between GY and NG (Figure 2). The positive relationship indicated that providing available soil-water content by multiplying number of irrigations has the potential to improve GY. Similarly, SI application was also associated with the increase of the grains weight per spike GWS (r=0.039, P<0.01) confirmed by the linear relationship (r^2=0.628, n=72) between GY and GWS, as presented in Figure 2.

Furthermore, it was found that applying SI during jointing at the first detectable node (Z31) has also led to enhance the accumulation of cellulose in the first leaves, the herbaceous tillers and the main stems as confirmed by Angus and Herwaarden (2001). This has contributed to improve BM production by 14% and 22%, for W2 and W5, respectively. These SI treatments, increased plant ability to head with more tiller survival and therefore improving GY, mainly correlated to the increase of the above-ground biomass BM (r=0.82, P<0.001). This effect was confirmed by the linear relationship (r^2= 0.906, n=72) between GY and BM (Figure 2), the positive relationship indicated that abundance of biomass with increasing number of irrigations has the potential to improve GY.

Moreover, providing adequate SI at or after anthesis gives plants an extra time, which transfer carbohydrate reserves toward the grain as reported by Zhang *et al.* (1998). In the same perspective, Aissaoui (2019) pointed out that ensuring an adequate availability of soil water during flowering stage stimulates actively photosynthesis in the post-anthesis phase and allows the assimilates, mainly carbohydrate reserves, to migrate to the newly pollinated florets forming the spikelets, which allows to conserve considerably the NGS and improve the GWS (Ozturk and Aydin, 2004 and Jalal *et al.*, 2009). These findings were also in agreement with Meng *et al.* (2015) where application of two irrigations, based on relative soil moisture contents rising to 75% of field capacity, at

jointing and anthesis stages increased grain yield for bread wheat cultivars.

On the other hand, genotypic differences in grain yield and yield components could be attributed to the four selected genotypes. The improved cultivar Hidhab (HD1220) is a late-maturing cultivar (192 days growing cycle), and benefited from a proper establishment since it dominated the number of germinated seeds (199.33), the number of survival tillers (658.67) (Unpublished results), the number of spikes (388.33) and the number of grains m^{-2} (1,8597.0). Probably, this variety has acquired an appreciable ability for adapting to local drought conditions of Setif region and a good stability

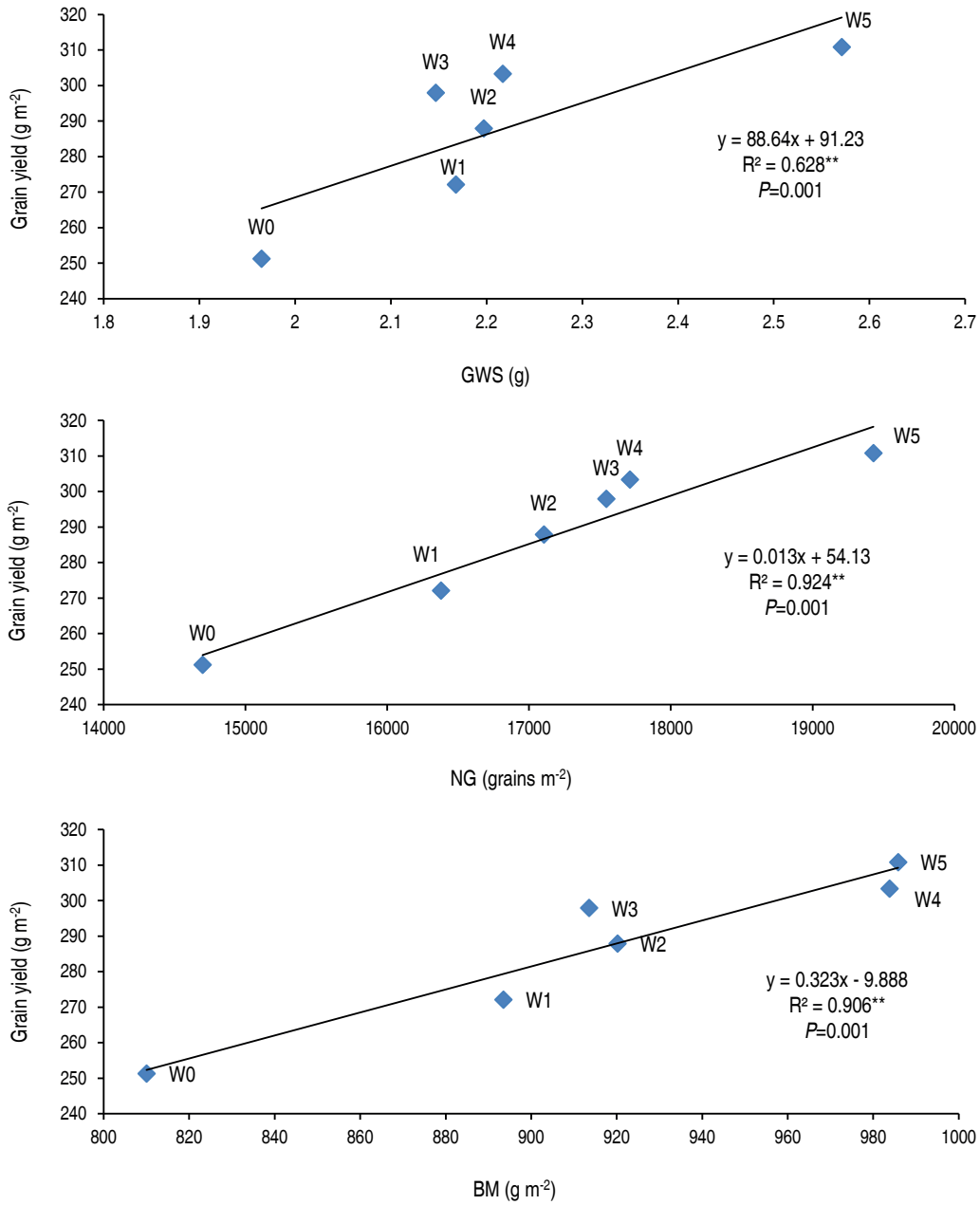

Figure 2. Scatter plots of grain yield vs. main grain yield components across water regimes.

of grain yield exposed to diverse water regimes (rainfed and SI). Similar conclusions for local varieties abilities to sustain drought stress were reported by Tayyar (2008) and Khamssi and Nadjaphy (2012). According to Sakumona *et al.* (2014), such adapted genotype possesses inherent potential or introgressed genes enabling it to control physiological mechanisms and to tolerate rigorous water stress conditions.

Oppositely, El-wifak genotype seems as an early-maturing cultivar with relatively shorter growing cycle (178 days), which allowed it to escape water stress at flowering stage likewise the sudden temperature rise of early May, and therefore ensuring a good remobilization of assimilates from vegetative tissues toward sink organs, retaining a considerable grain weight (47.2 g for TGW trait).

New introduced varieties Djanet and Djemila recorded better HI with 34.31% and 33.24%, respectively; confirming their genetic potential to produce higher grain/straw ratio regarding their relatively shorter PH compared to local genotypes (Table 4). This result is supported by Austin (1994) who pointed out that a high HI is solely obtained with short spikes under improved mineral nutrition at early stages of the plant. Likewise, Butler *et al.* (2005) confirmed that semi-dwarf stature genotypes achieved more favorable spike characteristics and produced more grain compared to straw due to increased partitioning of assimilates.

Among these four tested genotypes, cultivation of the semi-dwarf genotype Djanet presenting the lowest PH (65.79 cm) was correlated with the highest GY (319.17g m^{-2}) as well as with the highest HI (34.31%). Oppositely, El-wifak, the tallest genotype (76.84 cm), produced the lowest GY (250.0g m^{-2}) as well as with the lowest HI (27.48%), validating that bread wheat breeding with semi-dwarf stature improved GY, increasing harvest index, accoding to Aissaoui and Fenni (2020) and that semi-dwarf stature cultivation keeps preferable under the dry conditions occurring at the end of the wheat cycle (Rebetzke *et al.,* 2004).

CONCLUSION

The findings of this research showed that supplemental irrigation practice improves bread wheat production, particularly for the semi-dwarf genotype Djanet (ACSAD899), newly introduced in Sétif region. This genotype behaved as a high yielding cultivar (368.33 g m^{-2}) under limited watering conditions (W2), by supplying 35 mm total supplemental irrigation, provided in two applications; 15 mm at jointing stage corresponding to the first detectable node (Z31), and 20 mm at flowering stage on mid-complete anthesis when anthers are visible on 50% of the spikes (Z65-Z69). This improvement was associated with highest averaged scores of its agronomic traits; NGS (56.67 grain $spike^{-1}$), GWS (2.478 g), NG (21,494.0 grain m^{-2}), BM (1,034.67 g), HI (35.18%) and thus could be successfully cultivated under the W2 water regime conditions. However, Hidhab (HD1220) cultivar, which appeared more grain yielding stable (286.39 g m^{-2}) for this experiment, could be maintained as a relatively well adapted genotype under rainfed conditions of Sétif region.

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Effect of climate change on burley tobacco crop calendars

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del cambio climático en los calendarios de cultivo del tabaco burley

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ABSTRACT

In Colombia, tobacco crop is an important generator of employment and income for farmers; however it faces different problems as low crop yield compared to other countries; specifically, in the north of the country, where the climatic conditions are less favorable and the productivity is lower than other areas of the country due to the low mechanization. In order to improve the tobacco yield per hectare in the municipality of Ovejas, this research aimed to determine the water requirements of burley tobacco crop under conditions of climatic variability to obtain optimal information for crop calendars. Water requirements of burley tobacco were determined using the crop water requirement equation. This calculation method was programmed in Python to automate the generation of maps, developing a tool that allowed a detailed analysis per unit area per week. Based on the results obtained, weeks 17 and 18 of the year (last week of April and first week of May, respectively) are proposed as optimal planting times, since the cycles of crops planted in this period showed precipitation surplus in the initial phase of cultivation, which is a critical phase for their development. Climate change simulation showed that crops must be continuously monitored in order to adapt to new weather conditions.

Keywords:
Agricultural demand modeling
Climate change
Crop calendar
Water balance

RESUMEN

En Colombia, el cultivo de tabaco es un importante generador de empleo e ingresos para los agricultores. Sin embargo, se enfrenta a diferentes problemas, como el bajo rendimiento de los cultivos en comparación con otros países; específicamente, en el norte del país, donde las condiciones climáticas son menos favorables y la productividad es menor que en otras zonas del país debido a la baja mecanización. Con el fin de mejorar los rendimientos por hectárea del cultivo de tabaco en el municipio de Ovejas, el objetivo de esta investigación fue determinar los requerimientos hídricos del cultivo de tabaco burley bajo condiciones de variabilidad climática para obtener información óptima en las diferentes etapas del cultivo. La determinación de los requerimientos hídricos del cultivo de tabaco burley se llevó a cabo empleando la ecuación de requerimiento hídrico. Este método fue programado en Python para automatizar la generación de mapas, desarrollando una herramienta que permitió realizar un análisis detallado por unidad de área y por semana. A partir de los resultados obtenidos se proponen como épocas óptimas de siembra las semanas 17 y 18 del año (última semana de abril y primera semana de mayo, respectivamente), ya que los ciclos de cultivos sembrados en estas épocas presentaron superávit de precipitación en la fase inicial del cultivo, fase crítica para su desarrollo. La simulación de cambio climático mostró que los cultivos deben monitorearse continuamente para adaptarse a las nuevas condiciones climáticas.

Palabras clave:
Modelación de demanda agrícola
Cambio climático
Calendario de cultivo
Balance hídrico

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Agricultural activity is the basis of the economy in the municipality of Ovejas (Colombia). It represents approximately 60% of the use of countryside. Export-type black tobacco is noteworthy in the area, which is farmers' main source of income during the harvest as well for the municipality (Municipality of Ovejas, 2016). However, between 2002 and 2010, the production of black tobacco in the municipality of Ovejas decreased about 70%, creating economic instability in both the rural and urban sectors (Municipality of Ovejas, 2016). This can be attributed to a lack of climate and irrigation knowledge, generating low crop yields compared to other departments in the country where tobacco is grown, such as Boyacá, Huila and Norte de Santander (2.6, 2.6 and 3.2 t ha^{-1}, respectively) (DANE, 2016).

Water requirements refer to the amount of water the crop needs. In Colombia, these are supplied mainly by rainfall, because irrigation systems are not generally implemented. Most of the agricultural lands in Colombia are rainfed areas, reaching about 68% of the cultivated area (Allen *et al*., 2006; IICA, 2017). Therefore, the knowledge of the water requirements of tobacco crop is important since some evidences show that water stress can affect the quality and performance of it (Parker, 2009; Biglouei *et al*., 2010; Diez *et al.,* 2014, Liu *et al*., 2017). Water stress affects not only the yield of leaves, but also their maturity dynamics (Çakir and Cebi, 2010, Shtereva *et al*., 2017). Consequently, it is necessary to know the water requirements of the crop, the optimal sowing times and prevent the water stress, while increasing the tobacco yield.

Several international studies have reviewed irrigation scheduling and water stress, and their effect on crop yield and/or productivity (Assimi *et al*., 2004; Gao, 2006; Ju *et al.,* 2008; Çakir and Çebi, 2010; Jerie and Ndabaningi, 2011; Akinci and Lösel , 2012; Alameda *et al*., 2012; Liu *et al*., 2017; Ragab *et al*., 2017; Diez and Acreche, 2019). In the province of Guizhou, China, the response of tobacco crops to water deficit was studied (McNee *et al*., 1978; Peng *et al*., 2015), also the physiological response to water stress was evaluated (Muthappa *et al.,* 2010). Nevertheless, in Colombia, technology in agricultural areas remains deficient, as well as the knowledge about each crop which is considered unsustainable (Lecours, 2014). In the country, some authors have evaluated the crop in vast regions such as, Huila or Santander, to calculate the water demand (Barrientos *et al.,* 2012, Torrente *et al.,* 2008) and to organize fertilization programs (Hoyos and Plaza, 2014).

Those results show some variations due to the particular climatic and edaphic conditions of each analysis area. For that reason, the results obtained from a region cannot apply to other one with different conditions. Therefore, this research aimed to determine the water requirements of burley tobacco crops in the municipality of Ovejas, Sucre (Colombia), which would allow defining the optimal times for planting according to the current weather conditions in the studied area.

MATERIALS AND METHODS

Location of the study area

The municipality of Ovejas (Colombia) is located at the northern coast of Colombia. It is part of the Montes de María subregion, with a latitude of 09°31'48"N and longitude of 75°14 '01"W, and an average altitude of 265 masl (Figure 1). The municipality is located on the Magdalena Cauca macro-basins (Bajo Magdalena Cauca – San Jorge and Bajo Magdalena hydrographic sub-zone) and Caribe (Caribe Litoral hydrographic sub-zone). The study area presents a unimodal rainfall regime, with a very dry season that usually lasts from December to March (approximately 80 mm per month). The rainy season begins in April about by 155 mm of precipitation, gradually increasing its rain until to reach its maximum between September and October (monthly maximum about 240 mm of precipitation). The largest rainfall and lowest evapotranspiration occur in the northern part of the study area.

Collecting and processing data

Firstly, the input information was collected as time series (hydroclimatological variables) or as maps (location of crops, irrigation system if it exists, information on growth stage of crops). The input information was validated and the raster maps of precipitation, evapotranspiration, crop coefficients, and irrigation systems were obtained by GIS (Geographic Information Systems) (Figure 2). In addition, effective precipitation had to be defined according to the information on the soils in the study area. Each of the aforementioned variables were spatialized in each stage of the analysis. For the analysis, the modeler must select in advance the most appropriate spatial and temporal

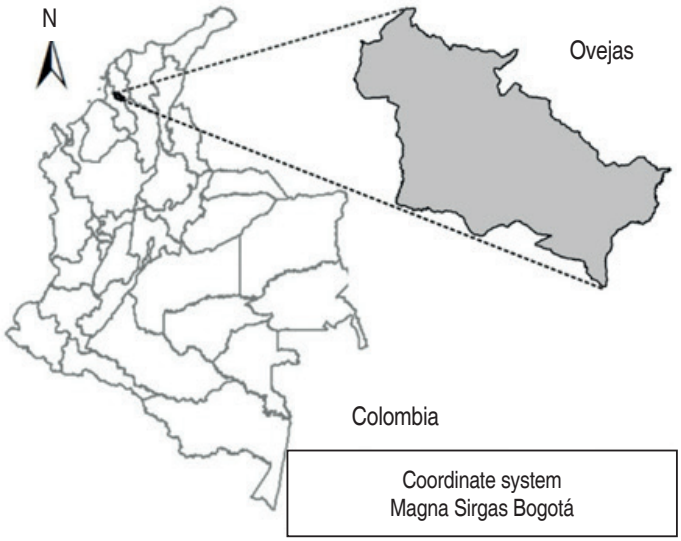

Figure 1. Geographical location of the municipality of Ovejas (Colombia).

resolution. Afterward, the Python tool was used to perform the calculations proposed in Equations 1 and 2, obtaining the results of crop water requirement and demand per analysis period and per area. In this case, the week was selected as the analysis period. Once the analysis for the base year is obtained (that is, one year under normal climatic conditions), future simulations such as climate change could be studied.

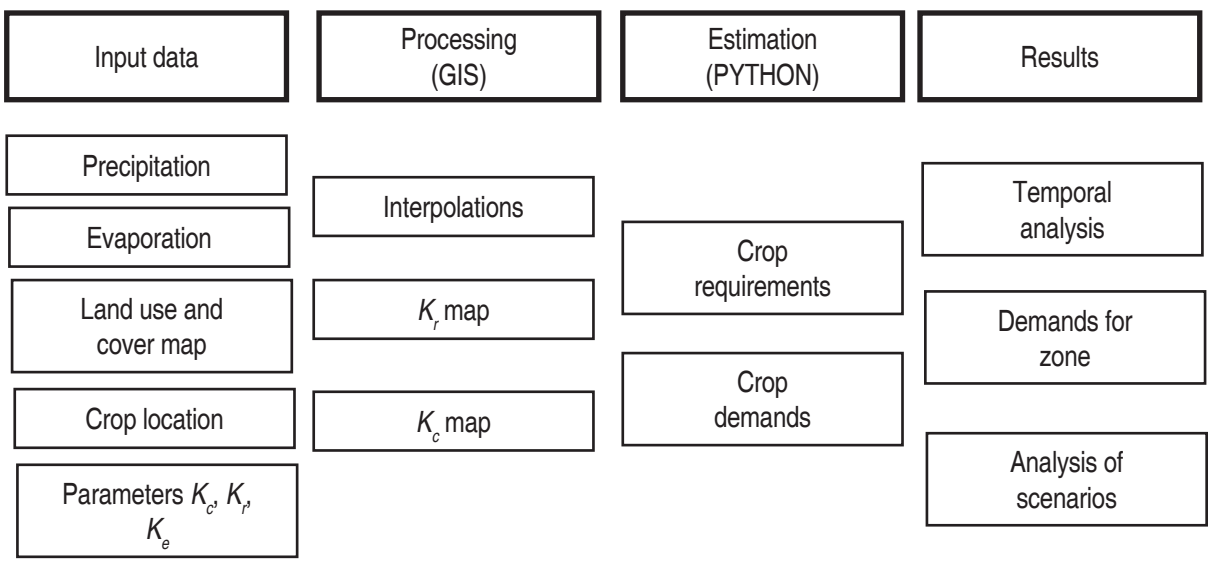

Figure 2. Outline of the proposed methodology for determining optimal planting schedules.

To collect the whole information, 28 hydroclimatological stations were identified, which were near the study area. Homogeneity, consistency and concurrency analysis tests were carried out in all of them (IDEAM, 2015). From these analyses, 10 precipitation stations and 3 evaporation stations were selected (Figure 3). These stations allowed to obtain precipitation and evapotranspiration series per week (52 weeks – January to December 1994). Precipitation and evapotranspiration series of the stations were interpolated to derive

weekly maps (30x30 m), applying the Inverse Distance Weighting method or IDW, since it is the most commonly used interpolation method (Hernández *et al*., 2013), and has no restrictions on the number of sampling points (Vargas *et al*., 2011). Maps with the tobacco farms locations were developed by the Agustín Codazzi Geographical Institute (IGAC) and available on the entity's website (Figure 3).

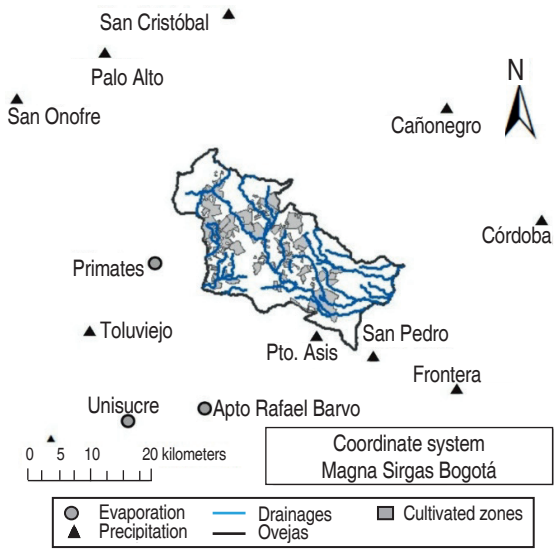

Figure 3. Location of tobacco crops and hydroclimatological stations used.

The crop coefficient was determined using the results of the study developed by the Faculty of Engineering of the Universidad Surcolombiana (Torrente *et al*., 2008). The tobacco-growing cycle consists of four phases (rosette stage, vigorous growth stage, flowering and ripening stage), with a total duration of 105 days. The analyses in this study were carried out weekly, so the duration of each phase of the cultivation was adjusted (Table 1).

Table 1. Coefficient of tobacco crop by the stage for the municipality of Ovejas

Crop phases	Week (days)	K_c
Rosette stage	Week 1 (1-7) Week 2 (8-14) Week 3 (15-21) Week 4 (22-28) Week 5 (29-35)	0.66
Vigorous growth stage	Week 6 (36-42) Week 7 (43-49) Week 8 (50-56) Week 9 (57-63)	0.83
Flowering stage	Week 10 (64-70) Week 11 (71-77) Week 12 (78-84)	0.92
Ripening stage	Week 13 (85-91) Week 14 (92-98) Week 15 (99-105)	0.87

The effective precipitation coefficient was calculated according to Villazón *et al.* (2019), who estimate the effective precipitation using the method of the United States Department of Agriculture (USDA), the AGLW-FAO method and the fixed percentage method for 20, 50 and 80% of the total precipitation. The results of the USDA and the AGLW-FAO methods offer effective precipitation values, similar to those values obtained by the fixed percentage method for 50 and 80% (Doorenbos and Pruitt, 1977). The areas of those studies present hydrological and soil characteristics similar to those considered in the municipality of Ovejas, allowing to select them for the study. *Kr* = 1 was the irrigation efficiency coefficient used, in order to analyze crop water requirement of tobacco and the optimal planting times without irrigation systems.

Determination of water requirements

Water requirements were estimated spatially and temporally by the crop water requirement equation (Allen *et al.*, 2006) (Equation 1) and the equation of agricultural demand for the crops (IDEAM, 2010, 2015) (Equation 2).

$$ET_c = K_c \times ET_o \quad (1)$$

Where ET_c is the evapotranspiration of the crop (mm day^{-1}), K_c is the dimensionless coefficient of the crop and ET_o is the reference evapotranspiration (mm day^{-1}).

$$D_a = 10\sum_{d=1}^{lp} \frac{[ET_c - (P \times K_e)]}{K_r} \times A \quad (2)$$

Where, Da is the additional crop water requirement or demand (m^3 ha^{-1}), 10 is the factor that applies to convert to m^3 ha^{-1}, *lp* is the length of the growth period, ET_c is the evapotranspiration of the crop (mm), *P* is the precipitation (mm), K_e is the runoff coefficient, K_r is the coefficient of irrigation efficiency and *A* is the planted area (km^2). Figure 4 show the model designed in the Python programming language implementing both equations, which was executed by ArcGis software.

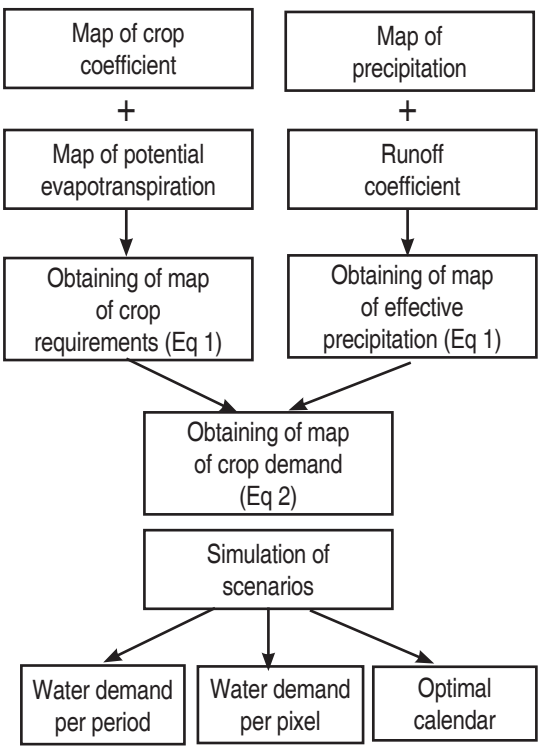

Figure 4. Flowchart of the algorithm in Python to obtain the crop requirements and demand simulations.

Model results

For the analysis time, 38 scenarios were considered where one scenario is a complete vegetative cycle of 15 weeks. First scenario started in week 1 of the year, and then each scenario was moved one week until the 38th to complete 52 weeks of the year.

Firstly, the model was executed by calculating the water requirements of the crop (Equation 1) and then the water demand (Equation 2). The variables required in each equation were transformed into raster maps, changing the conditions according to the modeling scenario. As results, the volume of required water for each hectare was obtained, and the irrigation volume if this was required. When the crop requires irrigation, the results are positive indicating that evapotranspiration exceeds precipitation.

Future simulations

Additional simulations were developed, taking into account the projection and climate trends in Colombia due to the incidences of climate change. These were performed based on the information of the Third National Communication on Climate Change, issued by IDEAM. According to the third national communication climate change simulations for Colombia (IDEAM *et al.*, 2016), between 2011-2100, the Caribbean region would present a decrease in precipitation of 10-40%, and the average annual temperature of the country could gradually increase by 0.9°C for the year 2040, 1.6°C for the year 2070 and 2.1°C for the year 2100. In order to analyze the incidence of climate change in the crop cycle, an increase in evaporation of 10% and a decrease in precipitation of 10% were considered (simulation CC1) as the most conservative scenario proposed by IDEAM (2016).

RESULTS AND DISCUSSION

Characterization of precipitation and evapotranspiration

The climate of the region in terms of precipitation exhibits a unimodal behavior, with values ranging between 1029 and 1999 mm. Spatial distribution of precipitation indicates that the highest annual precipitation occurs in the north, with records ranging between 1400 and 1500 mm, and the least amount of precipitation occurs in the southern center with values between 1200 and 1300 mm. Evapotranspiration presents annual mean values between 1422 and 1775 mm. Monthly records range from 103 mm (May in Primates station) to 179 mm (March in Rafael Bravo Airport station). According to the spatial distribution of annual evapotranspiration, the lowest values occur in the north ranging between 1660 and 1640 mm and the highest in the south with values ranging between 1660 and 1640 mm. The evaporation behavior shows a similar trend in the stations analyzed, registering the highest values from January to March and the lowest values in May, October, and November.

Crop water requirement

Water requirement of tobacco crop was obtained between 3059 and 2190 m^3 ha^{-1} by the model, corresponding to scenarios 1 and 34, respectively. Water requirements were much larger at the beginning of the year (Figure 5).

The scenario 1 began in the week 1 (January) and ended in the week 15 (April), the most significant water requirement occurred in week 6 (with values ranging between 254.60 and 277.80 m^3 ha^{-1} per week), which corresponds to the vigorous growth phase, especially in crops located in the southern of the town. Besides, in scenario 34, started in week 34 (August) and ending in week 48 (November), the highest water requirements were observed in week 9 (with values ranging between 177.80 and 188.90 m^3 ha^{-1} per week), also in the phase of vigorous growth. The results obtained through this analysis reflect the climatic conditions of the study area since the largest water requirements were found in the crop cycles planted in January, February and March, that correspond to the dry seasons in which the highest monthly evaporation values were reported.

Optimal planting periods

In the studied area for all the crop scenarios, irrigation was required in some weeks. Therefore, the optimal planting time was defined as those cycles that require the minimum amount of irrigation. The maximum was achieved because of the surplus of precipitation (9 weeks), 60% of the crop cycle time was covered with precipitation and 40% by irrigation. This was only achieved in scenarios 20 and 21, as shown in Figure 6, followed by scenarios 17, 18, 19, 22, 24 and 25, where excess of precipitation (surplus) occurred in 8 weeks of the crop cycle, that means, the requirements were fully covered at 53% of the time (these scenarios are highlighted in gray in Figure 6).

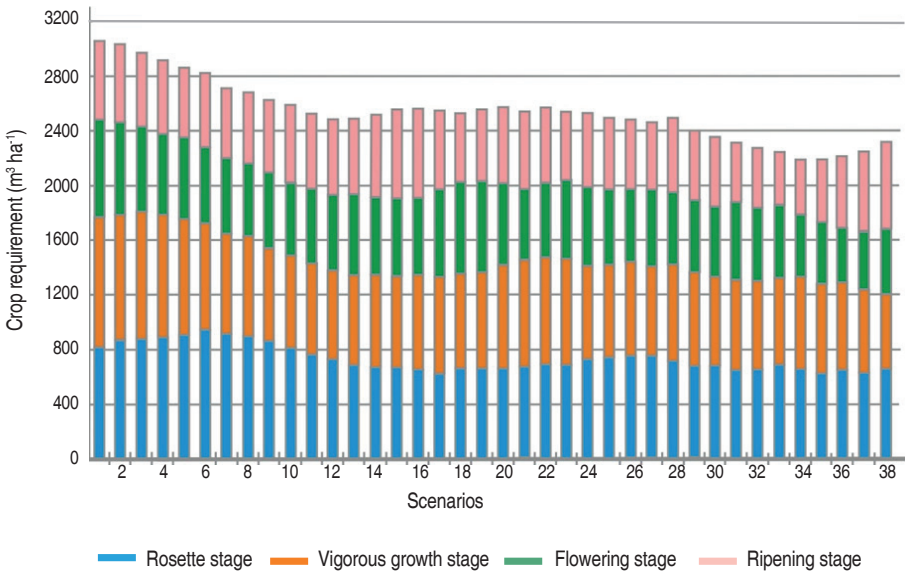

Figure 5. Water requirements of burley tobacco crop.

However, it is important to note that in the first phase of cultivation (rosette stage), there cannot be any water deficit, since once the crop is established, the cultivation of tobacco can adapt to land with water scarcity (Aguilera, 2013). A more detailed analysis of this phase demonstrated that in none of the scenarios, the maximum surplus values did not exceed 2.79 m^3 $week^{-1}$, while the maximum deficit values were presented in scenarios 2-6 with values of 2.14 m^3 $week^{-1}$, representing the greatest irrigation needs.

Moreover, it can be observed that the scenarios with the highest number of weeks with excess precipitation (20 and 21) presented irrigation needs in one week of the rosette stage. This same situation occurred in scenario 19, which presented irrigation needs in the last week of the rosette stage. Scenarios 22, 24, and 25 presented irrigation needs in at least two weeks of the first phase of the crop, while scenarios 17 and 18 did not present irrigation needs in the phase of rosette stage.

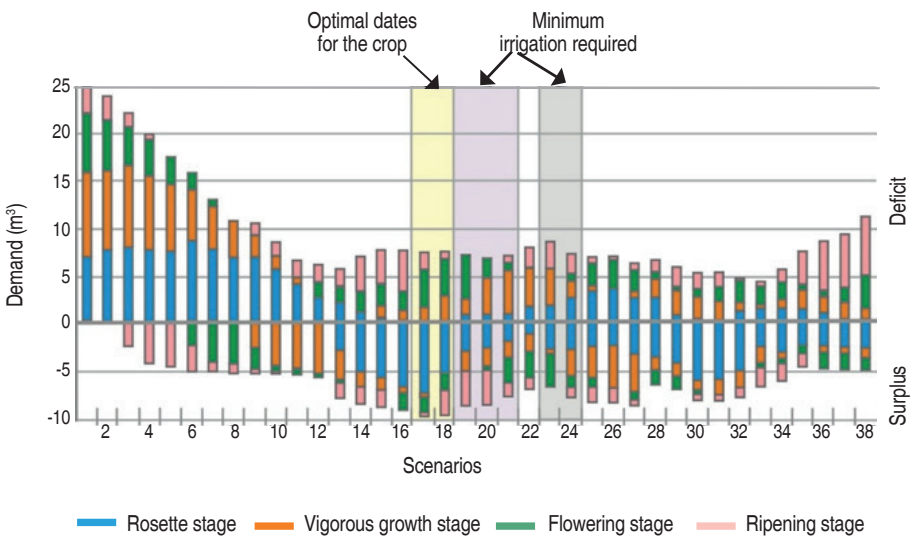

Figure 6. Water demand per cycle of burley tobacco crop in the base year (1994).

Since the irrigation needs occurred in all the cycles analyzed, the crop would not have an optimal development in any scenario. Therefore, scenarios 17 and 18 (week 17, April to week 31, August; and week 18, May to week 32, August, respectively) were considered the times with the best conditions for planting the tobacco crop (lower irrigation needs) given that they presented a surplus of precipitation in the initial phase of cultivation, although they presented weekly water deficits, being the highest of all the scenarios in week 11 and 12 of cultivation.

The recommended scenarios correspond to the months identified with the highest rainfall in the study area, confirming the results obtained from the analysis of this variable. In the cultivation cycles of scenarios 17 and 18, the crop water requirement was not totally satisfied by rainfall for 7 weeks, presenting irrigation needs that would cause low productivity of the cultivation.

The tobacco crop in the municipality of Ovejas would have a vegetative cycle between May and August with the best defined planting conditions. A second harvest can be planned, between 38 and 52 week. This scenario is proposed only as an alternative to optimize the production of land in the municipality of Ovejas through the implementation of a second harvest per year, and it was chosen because it is the cycle that presented the most rainfall surplus weeks (7 weeks) after completing the first recommended cycle of the crop. Nonetheless, at the slow growth phase there was a week with irrigation requirements. It is necessary to implement a system to supply the requirements of water at least during the rosette stage, if required. For the second harvest, other weeks were identified where the crop is not completely satisfied by rainfall, presented irrigation needs and possibly low productivity.

Effect of climate change on the crop – Simulation

The CC1 simulation showed that tobacco water requirements will increase by 14.3% regarding the base year of analysis (Figure 7). Additionally, the weeks with a surplus of precipitation were decreased from 9 to 7 weeks. It could be the scenarios that presented lower irrigation needs in the base year (17, 18, 19, 20, 21, 22, 24, and 25), according to the climate change scenario, two weeks of irrigation at least are demanded. Besides, the scenarios with the largest number of weeks with surpluses were18 in May, 31, 32, and 33 in August.

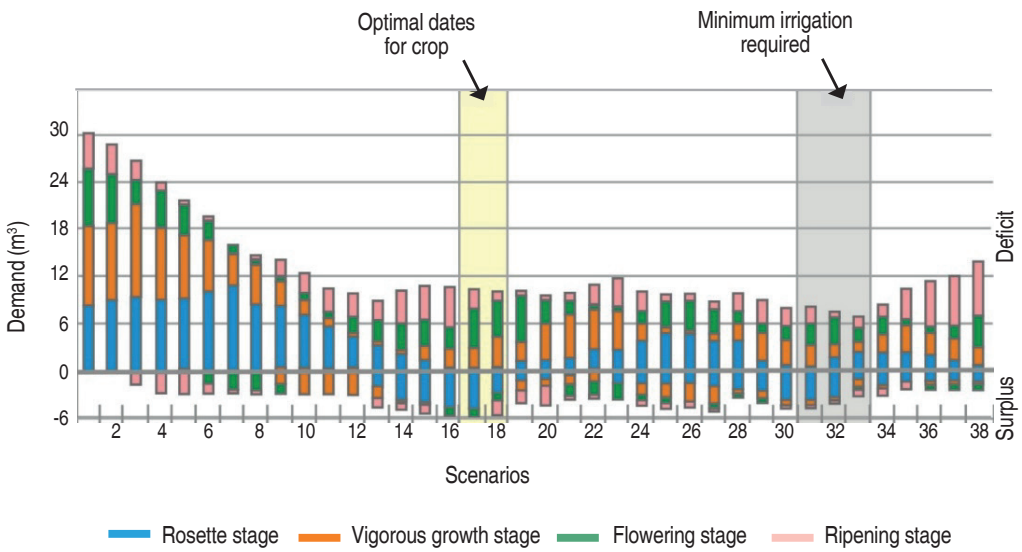

Figure 7. Water demand per cycle of burley tobacco crop by simulation CC1.

To determine the optimal planting times, it is suitable to start the crop cycle at week 17 or week 18, since the precipitation always exceeds evapotranspiration, presenting a surplus of water. These cycles would allow two harvests per year, starting the second harvest in week 32 or 33 (no optimal conditions), which correspond to scenarios 32 and 33, respectively. Therefore, the most significant weekly deficits were again presented in scenarios 2-6 with 2.45 m^3 $week^{-1}$.

The volumes of excess precipitation would be lower than those presented in the conditions of the base year (January to December 1994), evidencing decreases between 19.5 and 96.9%. Therefore, even considering storage for irrigation systems, the volume of available water is expected to be lower compared to what was estimated in the base year.

On the other hand, weekly volumes of unsatisfied requirements would be higher than those presented under the conditions of the base period, showing increases in weekly requirements between 14 and 1043%; this latter value was observed in week 5 of the scenario 38. The weekly demands are expected to be between 14-975%, with weekly averages per scenario ranging between 22-143% where the largest weekly increases would be observed in scenarios 12, 13, 16, 17, 18, 19 and 38 corresponding to March, April, May, and September (Figure 8).

For the same scenarios, the highest weekly maximums would be presented, indicating that these scenarios are very vulnerable to climate change. In general, deficits would increase remarkably from April to May and to a lesser extent from July to August. However, the surpluses that occurred in late February, March, May, and June could increase.

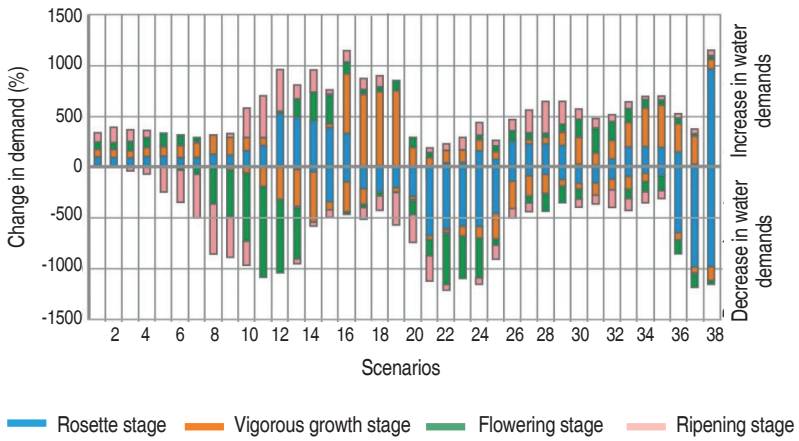

Figure 8. Changes in volumes not covered between simulation CC1 and base year.

In each growth phase, it was observed that the greatest changes would occur in the rosette and flowering phases, towards the surplus. In the rosette phase, the main increases in surpluses appeared from the end of April to the beginning of July, that is scenarios 15-25 and then considerable increases in surplus (up to 567%) were presented in scenarios 36-38 for the crops that started in September. In the flowering phase, the most significant changes were also presented in the surplus in May and June for scenarios 20-24, with increases of up to 325%. Moreover, in the rapid growth phase, the main changes appeared in the deficit increasing them, highlighting scenarios 16-19, which begin in April and May, with increases of up to 563%. All this information indicates the dates to start optimal crops are still scenarios 17 and 18. The irrigation requirements for these weeks would significantly increase, making the irrigation system less efficient.

CONCLUSIONS

It is not possible to meet the requirements of the crops in any cycle, applying the tool developed based only on the precipitation of the area. The implementation of irrigation is required for the sector to be fully productive. Therefore, the optimal planting times for tobacco cultivation that would require the least amounts of irrigation would be scenarios 17 and 18 (week 17, April to week 31, August and week 18, May to week 32, August, respectively), since they present a surplus of precipitation in the initial phase of cultivation, which is a critical phase for its development.

The results of the analysis of climate change show that there would be significant changes in the irrigation demand of crops, increasing water requirements and decreasing the volumes of excess rainfall. Regarding the amounts of unsatisfied needs, there is an increase concerning to the

base year, between 14 and 1043%. It was observed that the previously recommended optimal scenarios would be severely affected by decreases in rainfall and increases in evapotranspiration, presenting greater increases in deficit levels from April to May and to a lesser extent from July to August. Likewise, there is a high potential for water storage, since surplus levels increase very widely in late February, March, May to June. In addition to irrigation, it is important for farmers to adapt to new climatic conditions by modifying crop calendars so that they would be more efficient both in crop yields and in the operating costs of the irrigation districts.

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Extraction of anthocyanins from Mortiño (*Vaccinium floribundum*) and determination of their antioxidant capacity

Extracción de las antocianinas del Mortiño (*Vaccinium floribundum*) y determinación de su capacidad antioxidante

UNIVERSIDAD NACIONAL DE COLOMBIA

doi: 10.15446/rfnam.v74n1.89089

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ABSTRACT

Mortiño *(Vaccinium floribundum)* is a wild Andean berry, with a high content of bioactive compounds, especially anthocyanins, with protective effects against various diseases due to their antioxidant capacity. The aim of this study was to determine the best anthocyanin extraction conditions without affecting the mortiño antioxidant capacity. A completely randomized design with factorial arrangement 2^3 was applied in the extraction (solvent: ethanol and methanol; concentration: 20 and 60%; temperature: 30 and 60 °C). The anthocyanin content showed that the best extraction conditions were 20 and 60% hydroethanolic solution at 60 °C (P<0.05). For the antioxidant capacity, the treatments were analyzed in a factorial arrangement 2^2: ethanol and methanol 20%; 30 and 60 °C. The highest antioxidant capacity was found at 20% ethanol and 60 °C (19,653.3±256.62 µmol TE 100 g^{-1} FW (fresh weight)) by the FRAP method (Ferric Reducing Antioxidant Power) (P<0.05), and with the ABTS methodology, methanol and ethanol (20%), at 60 °C (8458.3±127.45 and 8,258.0±325.05 µmol TE 100 g^{-1} FW, respectively) (P<0.05). Extraction at 60 °C did not affect the antioxidant properties of anthocyanins. A high correlation was found between both methodologies by Pearson's Correlation (PC=0.898). In addition, the antioxidant capacity measured by the two methods was highly related to the anthocyanin content of the extracts (PC=0.973 and 0.952, respectively). Other berry species have been extensively investigated for their antioxidant compounds, but there is limited information about the optimization of anthocyanin extraction from mortiño and its high antioxidant capacity important for future industrial applications. As a result, ethanol at 20 and 60%, 60 °C for 4 h are the best conditions to extract and quantify the anthocyanins of the mortiño fruit.

Keywords:
ABTS
Andean berry
Antioxidants
Differential pH
FRAP

RESUMEN

Mortiño es una baya silvestre andina con un alto contenido de compuestos bioactivos, especialmente antocianinas, con efecto preventivo contra diferentes enfermedades por su capacidad antioxidante. Este estudio tuvo como objetivo determinar las mejores condiciones de extracción de antocianinas sin afectar su capacidad antioxidante. Se aplicó un Diseño Completamente al Azar con arreglo factorial 2^3 en la extracción (solvente: etanol y metanol; concentración: 20 y 60%; temperatura: 30 y 60 °C). El contenido de antocianinas mostró que las mejores condiciones de extracción fueron 20 y 60% como concentración de la solución hidroetanólica, a 60 °C (P<0.05). Para la capacidad antioxidante los tratamientos fueron dispuestos en un arreglo factorial 2^2: etanol y metanol 20%; 30 y 60 °C. Una alta capacidad antioxidante fue encontrada a 20% etanol y 60 °C (19,653.3±256,62 µmol ET 100 g^{-1} PF) por el método FRAP (P<0,05) y con la metodología ABTS: metanol y etanol 20% a 60 °C (8,458,3±127,45 y 8,258,0±325,05 µmol ET 100 g^{-1} PF, respectivamente) (P<0,05). 60 °C maximizó la extracción y no afectó las propiedades antioxidantes. Se encontró una alta correlación entre ambas metodologías (PC=0,898), además que la capacidad antioxidante medida por los dos métodos tuvo gran correlación con el contenido de antocianinas de los extractos (PC=0,973 y 0,952, respectivamente). Otras especies de bayas han sido ampliamente investigadas por sus compuestos antioxidantes, pero existe información limitada acerca de la optimización de la extracción de antocianinas a partir del mortiño y su alta capacidad antioxidante para futuras aplicaciones industriales. Como resultado, el etanol al 20 y 60%, 60 °C durante 4 h son las mejores condiciones para extraer y cuantificar las antocianinas del fruto mortiño.

Palabras clave:
ABTS
Baya andina
Antioxidantes
pH diferencial
FRAP

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Mortiño berry belongs to Ericaceae family and *Vaccinium* genus. This fruit is called Andean berry or wild berry because it grows spontaneously in the Andean region from 3,400 to 3,800 masl (Prencipe *et al*., 2014). There is a growing interest on Andean berries due to the useful bioactive phytonutrients that they contain; *Vaccinium floribundum* is also known as "superfruit" due to its high content of phenolic compounds, predominantly quercetin, hydroxycinnamic acids and cyanidant-3-glucosides (C3G) that support its great antioxidant power (Vizuete *et al*., 2016). *In vitro* studies showed an inhibitory effect on the accumulation of lipids, adipogenesis, and anti-inflammatory mediators of phenolic extracts from Mortiño (Schreckinger *et al*., 2010). On the other hand, a research with other berries has shown the positive effect of phenolic compounds also found in *Vaccinium floribundum*, for the prevention of diseases such as cancer, diabetes and hyperlipidemia *in vitro*, *in vivo* and epidemiological studies (Kianbakht *et al*., 2014).

The main effect of antioxidants is the capacity of scavenge free radicals. Compounds that are easier to oxidize are often the best antioxidants, since they can donate electrons or hydrogen atoms to reactive free radicals (Castañeda *et al*., 2009).

High concentrations of anthocyanins are found in the outer layer of mortiño (Skrovankova *et al*., 2015). These pigments, family of flavonoids are formed by an anthocyanidin aglycone and a sugar. The chemical form of the anthocyanins consists of two aromatic rings A and B linked by a 3-carbon chain; the variation of R1 and R2 groups in B ring originates different anthocyanins with different colors (Trouillas *et al*., 2016). An increase in hydroxylation produces a blue color while an increase in methoxylation turns to red. The most abundant anthocyanidins could be listed as: Pelargonidin (orange), Cyanidin (orange red), Delphinidin (red), Peonidin (orange red), Petunidin (red) and Malvidin (bluish-red) (Garzón, 2008).

Anthocyanins are soluble in alcohols, acetone, dimethyl sulfoxide and water, due to their polar character. Choosing an extraction method always seeks to maximize the recovery of the pigment, prevent its degradation and reduce the contaminants. Most of the time in aqueous solution anthocyanins could be found in three different states. In pH 1-3 the most abundant form is red flavylium cations, around pH 4-5 the specie is colorless, and in alkaline solution the tautomerized molecule generates a yellow color (Lila *et al*., 2016).

Anthocyanin rich extracts have been widely investigated for its use as natural dyes (Khoo *et al*., 2017; Mojica *et al.,* 2017). Furthermore, the food color market is expected to grow from 10 to 15% each year, and the mean trend in this industry is the use of natural colorants in order to produce “clean-labeled” food (Cortez *et al*., 2017). Antiproliferative and antioxidant properties of anthocyanins have also proved promising results for functional product development. However, anthocyanins are unstable and susceptible to the degradation in presences of factors such as pH, light, temperature, oxygen, solvents, metal ions, among others (Diaconeasa *et al*., 2015).

Due to the great number of industrial applications and health benefits of these colored compounds, despide the problems of stability derived from their obtaining and purification process. Therefore, the aim of this study was to identify the best conditions to extract anthocyanins from *Vaccinium floribundum* without affecting the antioxidant capacity of these polyphenols.

MATERIALS AND METHODS

Standards and Chemicals

TPTZ (PubChem CID: 77258; 98%) and Trolox (PubChem CID: 40634; 97%) were purchased from SIGMA; ABTS (PubChem CID: 16240279; 98%) was purchased from ROCHE; absolute ethanol (PubChem CID: 702) was purchased from PHARMCO; methanol (PubChem CID: 887; 99.9%), ferric chloride hexahydrate (PubChem CID: 24810; 100.5%), potassium chloride (PubChem CID: 4873; 99.8%) and sodium acetate trihydrate (PubChem CID: 23665404; 99.9%) were purchased from FISCHER SCIENTIFIC; potassium persulfate (PubChem CID: 24412; 98%) was purchased from LOBACHEMIE; hydrochloric acid (PubChem CID: 313; 37%) was purchased from MERCK.

Sample preparation

Mortiño (*Vaccinium floribundum)* was purchased at “La Kennedy Market” in Quito, Ecuador. The fruits were

chosen in good condition, without surface damage, and a maturity degree between four and five (fruits between purple and dark purple) (Buitrago *et al*., 2015). These berries were processed in Ultramaxx processor to form a paste, which was then stirred and homogenized using an Ultra-Turrax at 13,000 rpm.

Extraction and quantification of anthocyanins

Approximately 10 g of the paste were weighed in analytical balance (Mettler Toledo, Model AB204 S, 250±0.001) and placed in an Erlenmeyer flask (125 mL) (Oancea *et al*., 2012). 40 mL of the solvent were added and the mixture was stirred for 20 s using a Magic Clamp Universal Platform, and it was placed on the stove (Binder, Model ED 56), for 4 h at 30 °C and 60 °C according to the experimental design. Then it was filtered using ash-less filter paper (Macherey Nagel MN 640 w, with diameter 125 mm), to obtain the anthocyanin extract.

The treatments were arranged in a CRD with factorial model (2^3), corresponding to the combination of solvent (methanol and ethanol), concentration (20 and 60%) and temperature (30 and 60 °C). Three repetitions were performed, with a total of 24 experimental units. Ethanol and methanol were acidified with hydrochloric acid (37%) until reaching pH 1 (Garzón, 2008).

The treatments were: E1 (Ethanol 20%, 30 °C), E2 (Ethanol 20%, 60 °C), E3 (Ethanol 60%, 30 °C), E4 (Ethanol 60%, 60 °C), M1 (Methanol 20%, 30 °C), M2 (Methanol 20%, 60 °C), M3 (Methanol 60%, 30 °C), M4 (Methanol 60%, 60 °C).

The quantification of anthocyanins was performed according to the pH differential method (AOAC-2005.02, 2019). For absorbance, Genesys 10 UV Thermospectronic spectrophotometer was used. Samples at pH 4.5 and pH 1 were measured both 520 and 700 nm. A blank sample was also measured (each buffer without the addition of the extract) and final absorbance was calculated with Equation 1:

$$A = (A_{520\ nm} - A_{700\ nm})_{pH1.0} - (A_{520nm} - A_{700\ nm})_{pH4.5} \quad (1)$$

The concentration of monomeric pigments in the extract was expressed in mg of C3G per 100 g of mortiño (Kuskoski *et al*., 2005). It was calculated based on the volume of extract and sample weight (Equation 2):

$$AC=(A \times MW \times FD \times 100)/\varepsilon \quad (2)$$

Where:

AC: anthocyanins concentration; A: absorbance; MW: molecular weight; FD: dilution factor; ε: molar absorptivity (MW: 449.2 g mol^{-1}; molar absorptivity coefficient, ε:26,900).

Anthocyanin extracts were protected from light and frozen (-18 °C) for further analysis. To thaw the sample, it was placed in refrigeration at 4 °C for 24 h.

The data were subjected to analysis of variance (ANOVA) and means were assessed by Tukey test (P<0.05), with Minitab Statistical Software (2018).

Antioxidant capacity of mortiño

To determine the antioxidant capacity the FRAP method and the ABTS method were applied. A CRD with factorial model 2^2 (solvents: methanol and ethanol; temperatures: 30 and 60 °C) and 3 repetitions for each method. The concentration of both solvents was constant (20%). The data were subjected to analysis of variance (ANOVA) and means were assessed by Tukey test (P<0.05), with Minitab Statistical Software (2018).

The FRAP reagent was prepared by mixing 2.5 mL of TPTZ solution (0.0312 g of TPTZ compound with 40 mM HCl), 2.5 mL of 20 mM $FeCl_3$-$6H_2O$ (0.1352 g of $FeCl_3$-$6H_2O$ solubilized in 25 mL of distilled water) and 25 mL of 0.3 mM acetate buffer pH 3.6 (0.0061 g of sodium acetate in 200 mL of distilled water). The pH was adjusted to 3.6 with 40 mM HCl and the volume was completed up to 250 mL with distilled water.

The results were expressed in Trolox equivalent (µmol TE 100 g^{-1} FW), after elaborating a calibration curve of this compound (Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which is a water-soluble analogous of vitamin E widely used in the expression of antioxidant capacity studies (Ozgen *et al*., 2006). The absorbance was read at 570 nm using a MRX Microplate Reader equipment (Dynex Technologies).

The calibration curve by the FRAP method showed the following data: the regression equation y=0.0169X–0.0329; coefficient of determination R^2 =0.9990.

The treatments of this stage of the research were: FE1 (ethanol, 30 °C), FE2 (ethanol, 60 °C), FM1 (methanol, 30 °C), FM2 (methanol, 60 °C). The concentration of both solvents was constant (20%).

The cationic radical ABTS is a chromophore generated by an oxidation reaction of ABTS (2,2'-azino-bis-(3-ethyl benzthiazolin-6-sulfonate ammonium)) at a concentration 7 mM with potassium persulfate (2.45 mM) (1:1), incubated at room temperature (25 °C) and in the dark for 16 h. Once the radical $ABTS^{\bullet+}$ was formed, it was diluted with ethanol 96% v/v until to obtain an absorbance value of 0.700±0.100 at 730 nm (maximum absorption wavelength) (Cano *et al*., 2000).

The calibration curve by the ABTS method showed the following data: the regression equation y=1.6928X–0.5273; coefficient of determination R^2=0.9964.

For the determination of antioxidant capacity of anthocyanins extracts, 10 µL of the diluted sample with 96% ethanol (1:20) reacted with 990 µL of ABTS solution and the same procedure indicated above was followed. ABTS values were expressed as µmol TE 100 g^{-1} FW (Garzón *et al*., 2010).

The treatments were: AE1 (ethanol 20%, 30 °C), AE2 (ethanol 20%, 60 °C), AM1 (methanol 20%, 30 °C), AM2 (methanol 20%, 60 °C).

RESULTS AND DISCUSSION

The influence of the solvent type, concentration and temperature on the anthocyanin content determined by differential pH method is showed in Table 1. In the same way, the two-way interactions of the factors influenced the response variable (P<0.05). The coefficient of variation was 1.64%, less than 5%, which means a correct development of the experiment procedure and a good control of the extrinsic variation (Condo and Pazmiño, 2015).

Table 1. Analysis of variance (ANOVA) of the anthocyanin content of the treatments.

Sources of variation	DF	Sum Squares	Mean Squares	F Value
Total	23	83,797.7		
Treatments	7	83,500.9	11,928.7	643.07 *
Factor A (Solvent)	1	3,838.2	3,838.2	206.91 *
Factor B (Concentration)	1	2,380.6	2,380.6	128.34 *
Factor C (Temperature)	1	72,796.8	72,796.8	3,924.44 *
Interaction A x B	1	102.3	102.3	5.52 *
Interaction A x C	1	660.7	660.7	35.62 *
Interaction B x C	1	3,696.2	3,696.2	199.26 *
Interaction A x B x C	1	26.2	26.2	1.41 n.s.
Residue	16	296.8	18.5	

* Significant difference at 5% by the F test. n.s. not significant. DF: degree of freedom.

The highest concentration of anthocyanins (Table 2) was obtained (335.49 and 332.63 mg C3G 100 g^{-1} FW) using ethanol as solvent (20 and 60%) at 60 °C (treatments E2 and E4, respectively). There was no significant difference between the mentioned treatments (P<0.05). Other *Vaccinium floribundum* studies showed concentrations similar and higher than those obtained in this investigation. Vasco *et al*. (2009) reported 345 mg of C3G 100 g^{-1} FW after wetting 0.6 g of lyophilized sample and extracting four times with ethyl acetate (10 mL), and quantification by HPLC. In addition, Garzón *et al*. (2010) found 329.0±28.0 mg of C3G 100 g^{-1} FW by the differential pH method working with fresh berry powder sprayed with liquid nitrogen and frozen

at 70 °C and extracted with 100% methanol for three times until the solution became colorless. On the other hand, 376.2±49.9 mg of C3G 100 g^{-1} FW was analyzed by HPLC after the extraction of anthocyanins from the frozen mortiño, using dynamic maceration with 0.6 M HCl in methanol (Prencipe *et al*., 2014).

Table 2. Anthocyanin content obtained by different extraction treatments.

Treatment	Anthocyanin content (mg of C3G 100 g^{-1} FW) *
E2	335.49±5.24 a
E4	332.63±5.93 a
M2	301.75±0.23 b
M4	294.80±7.20 c
E3	238.90±4.47 c
M3	217.88±2.19 d
E1	187.94±2.71 e
M1	179.359±3.96 e

* E1 (ethanol 20%, 30 °C), E2 (ethanol 20%, 60 °C), E3 (ethanol 60%, 30 °C), E4 (ethanol 60%, 60 °C), M1 (methanol 20%, 30 °C), M2 (methanol 20%, 60 °C), M3 (methanol 60%, 30 °C), M4 (methanol 60%, 60 °C). Means followed by the same letter have not a statistically significant difference (P<0.05) by Tukey test.

Significantly, the temperature increased the amount of anthocyanins extracted from the berries, especially when the solvent was ethanol (Table 2). The best concentration of anthocyanins could be extracted at 60 °C, due to the heat damage of the cellular wall of mortiño outer layer, improving the pigment transference (Gavahian *et al*., 2018). The same tendency was reported by other studies (Marquez *et al*., 2014; Spigno *et al*., 2007). Also, the temperature increment accelerated the diffusion rate and improved the solubility of the required biomolecules.

On the other hand, the possible thermal degradation of anthocyanins can also occur. This effect was not found in this study because the protocol avoided extreme temperatures and times. The thermal degradation of anthocyanin is a first order reaction. Half-life time calculated in anthocyanin concentrates was 16.7 h at 60 °C (Wang and Xu, 2007). In the present research, 60 °C was the maximum temperature used for 4 h. Therefore, low thermal damage was expected. The best solvent in the extraction process was ethanol (Table 2). Other authors also reported the best anthocyanin extraction with ethanol solution at 70% (Fu *et al*., 2016; Pedro *et al*., 2016).

The amount of extracted compounds depends on the solvent polarity and the solubility of the substratum (Metrouh-Amir *et al*., 2015). The hydroethanolic solutions in different concentrations are the most popular options for anthocyanin extraction (Fu *et al*., 2016). As presented in Table 2, the treatments with ethanol at 20% (E2) and 60% (E4) concentration did not show a significant difference (P>0.05). At 60 °C, the concentration of ethanol did not significantly influence the extraction process. However, a higher concentration of anthocyanins was obtained using methanol at 20%.

The anthocyanin structures have different behaviors in the extraction medium. Flavonols and anthocyanins are soluble in polar solvents, while the glucosides are more soluble in water and the aglycones more soluble in alcohols (Pérez-Gregorio *et al*., 2010). The solubility of anthocyanins in polar solvents is due to the hydroxyl groups and sugars present in their structure; therefore, they are commonly extracted with methanol or ethanol or with a mixture (Welch *et al*., 2008). Ethanol has a polarity index of 4.3, methanol of 5.1 and water of 10. Consequently, a better extraction performance with ethanol (the best extraction treatments) could be explained by a lower polarity of the extraction medium (Gupta *et al*., 1997). In addition, the hydrophobic characteristic of anthocyanins is given by the polyphenolic structure allowing their solubility in organic solvents such as ethanol and methanol (Khoo *et al*., 2017).

The temperature and polarity of the solvent could affect the antioxidant capacity of the extracts. The difference in antioxidant activity due to the solvents could be explained by the different metabolites extracted depending on their polarity. Compounds with the same polarity are solubilized while the solvent penetrates through the solid phase (Muhamad *et al*., 2014).

Despite temperature accelerates mass transfer and free more antioxidant compounds, principally anthocyanins that increase the antioxidant power of the concentrate, temperatures higher than 50 °C possibly decompose the heat sensitive antioxidative compounds in more than

8 h of exposition (Spigno *et al*., 2007). Degradation of anthocyanidin with a 0-dihydroxyl substitution such as cyanidin, delphinidin and petunidin, which are the most susceptible to oxidation, can significantly reduce the antioxidant power (Castañeda-Ovando *et al*., 2009). However, in the present study, time and temperature combination avoided such problems.

The influence of the type of solvent, extraction temperature and interaction of both factors (P<0.05) on the antioxidant capacity of the extracts was observed with the two methodologies used (Table 3). For both cases, the coefficient of variation was less than 5%, which means a correct development of the experiment and a good control of the extrinsic variation.

Table 3. Analysis of variance of the antioxidant capacity of the treatments by FRAP and ABTS methods.

Sources of variation	DF	Mean Squares	
		FRAP	ABTS
Total	11		
Treatments	3	28,474,187 *	12,990,250 *
Factor A (Solvent)	1	6,380,033 *	781,137 *
Factor B (Temperature)	1	78,456,533 *	37,901,420 *
Interaction A x B	1	585,995 *	288,195 *
Residue	8	23,410	31,705

* significant difference (P<0.05) by the F test. DF: degree of freedom

In Table 4, the best antioxidant capacity (19,653.3±256.62 µmol TE 100g^{-1}) was determined with the FRAP method using 60 °C and ethanol at 20% as solvent (treatment FE2). This concentration was higher than 16,140 µmol TE 100 g^{-1} reported by Gaviria *et al*. (2009) and Moyer *et al*. (2002) with values between 12,000 and 15,000 µmol TE 100 g^{-1}. In the ABTS method, the treatments AM2 (methanol, 60 °C) and AE2 (ethanol, 60 °C) had the highest antioxidant capacity (8,458.3±127.45 and 8,258±325.05 µmol TE 100 g^{-1}, respectively), being significantly different from the rest of the treatments (P<0.05). Other study found similar results 8,694±435 µmol TE 100 g^{-1} (Gaviria *et al*., 2009). In addition, the higher extraction temperature did not affect the antioxidant capacity since at 60 °C higher values were obtained than at 30 °C (Table 4).

Table 4. Antioxidant capacity by FRAP and ABTS methods.

Treatments	FRAP µmol TE 100 g^{-1}*	Treatments	ABTS µmol TE 100 g^{-1}*
FE2	19,653.3±256.62 a	AM2	8,458.3±127.45 a
FM2	18,636.9±155.21 b	AE2	8,258.0±325.05 a
FE1	14,981.3±59.36 c	AM1	5,213.9±67.66 b
FM1	13,081.0±13.15 d	AE1	4,393.7±18.45 c

* FE1 (ethanol, 30 °C), FE2 (ethanol, 60 °C), FM1 (methanol, 30 °C), FM2 (methanol, 60 °C). The concentration of both solvents was constant (20%). Means followed by the same letter have not a statistically significant difference (P<0.05) by Tukey test

The amount of anthocyanin extracted from *Vaccinium floribundum* and its antioxidant capacity were greater than in other fruits of the same family, for instance, *Vaccinium corymbosum* (blueberry) most widely studied and available in other regions (Lee and Wrolstad, 2004). The inactivation of reactive oxygen species can occur by various routes. The application of different methodologies to evaluate antioxidant activity will explain the mechanisms

by which bioactive compounds such as anthocyanins act (Montoya *et al*., 2012). It is recommended to analyze the antioxidant capacity with at least two methods to achieve more reliable results (Boeing *et al*., 2014). According to this, in this work, the antioxidant capacity was determined by ABTS and FRAP methods, obtaining by this latter method a greater antioxidant capacity in all treatments. Pearson's correlation between the two methodologies in this study was 0.898 indicating a highly positive correlation. That is close to results found by other authors 0.94 (Dudonné *et al*., 2009) and 0.9877 (Montoya *et al*., 2012).

To analyze the relationship between antioxidant capacity and anthocyanin concentration, the Pearson coefficient was calculated, obtaining the following data: Pearson Coefficient of FRAP vs Anthocyanin Content: 0.973; Pearson Coefficient of ABTS vs Anthocyanin Content: 0.952. Both values represent high positive correlation between these two quantitative variables.

CONCLUSIONS

The highest content of anthocyanins was obtained under extraction conditions of 60 °C with 20 and 60% hydroethanolic solvent solution. The highest antioxidant capacity was also obtained when highest extraction temperature (60 °C) was used, indicating that the thermal effect after 4 h of extraction did not affect the antioxidant power of anthocyanins and this moderate temperature could increase the diffusion rate and solubility without damage of the compounds. The treatment extracted with hydroethanol solution (20%) presented the highest antioxidant capacity by the FRAP method, while by ABTS method with methanol and ethanol treatments at the same concentration (20%), there was not statistical difference between both treatments (AM2 and AE2). The highest temperature tested (60 °C) maximized the extraction of anthocyanins and did not affect the antioxidant activity. As a result, ethanol at 20 and 60%, 60 ºC for 4 h are the best conditions to extract and quantify the anthocyanins of the mortiño fruit.

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Edible coatings based on cassava starch, salicylic acid and essential oils for preservation of fresh-cut mango

Recubrimientos comestibles a base de almidón de yuca, ácido salicílico y aceites esenciales para la conservación de mango fresco cortado

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ABSTRACT

Mango has a short shelf-life after harvesting. The use of edible coatings on the elaboration of minimally processed mango is an alternative for its commercialization. In the present work edible coatings based on chitosan, starch-salicylic acid and starch-cinnamaldehyde-thymol were applied to fresh cut-mango. Weight loss, soluble solids, titratable acidity, color and microbiological analyses were studied along storage for 12 days at 8 °C and 90% relative humidity. Titratable acidity was the highest for mangoes coated with chitosan and the lowest was for starch-salicylic acid coating. Regarding instrumental texture, fruit coated with chitosan showed a higher penetration force compared to fruit coated with starch and uncoated samples. Microbiological results showed that all coated mangoes inhibited growing of fungi and yeast whereas uncoated samples showed an increase of both microorganisms along 12 days of storage period.

Keywords:
Chitosan
Cinnamaldehyde
Thymol
Tommy Atkins

RESUMEN

El mango se caracteriza por presenta un tiempo de vida útil corto luego de la cosecha. El uso de recubrimientos comestibles en la elaboración de mango mínimamente procesado es una alternativa para su comercialización. En este estudio, se aplicaron películas de quitosano, almidón de yuca–ácido salicílico o almidón de yuca–cinamaldehído–timol a mango Tommy Atkins cortado con posterior almacenamiento de 12 días a 8 °C y 90% de humedad relativa. Se analizó la pérdida de peso, sólidos solubles, acidez titulable, textura instrumental, color y análisis microbiológico del mango. Los resultados mostraron que la mayor y menor acidez titulable se obtuvo para las frutas recubiertas con quitosano y almidón-ácido salicílico, respectivamente. En cuanto a textura instrumental, la fruta recubierta con quitosano tuvo una mayor fuerza de penetración que la fruta tratada con almidón y el control. A nivel microbiológico, todas las películas inhibieron el desarrollo de hongos y levaduras mientras el control presentó un incremento durante 12 días de almacenamiento.

Palabras clave:
Quitosano
Cinamaldehído
Timol
Tommy Atkins

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Mango (*Mangifera indica* L.) is one of the five tropical fruits with the highest consumption worldwide (Caballero *et al*., 2015). Tommy Atkins is the most exported variety in Ecuador with 65% of total mango exports. Tommy Atkins mango has high resistance to handling; therefore, it has potential for the development of minimally processed products (Chiumarelli *et al.,* 2011).

Refrigerated minimally processed mango is a good option on the market; and it responds to the need of a modern world where less time is available to prepare food (González-Aguilar *et al.,* 2008). Industrialization of mango could contribute to the development of Ecuadorian agroindustry, through the creation of small and medium-sized enterprises (Cedeño and Cerón, 2018), and its contribution to GDP, which currently represents 7%, and is lower compared to Colombia (10%), Chile (13%), and Uruguay (12.4%) (Fiallo, 2017).

Postharvest mango losses vary between 20 and 50% (Dávila, 1998; Singh *et al.,* 2013). The most common problems of mango agroindustry are weight loss, mechanical damage and attack of bacteria and fungi. Physicochemical properties such as color, firmness, among others, are affected by metabolic disorders caused by cutting (Tovar *et al.,* 2001). Therefore, it is necessary to use techniques to preserve the quality attributes of minimally processed mango.

Fresh and minimally processed fresh-cut products are naturally contaminated by microorganisms of several sources, including the farm environment, post-harvest handling and processing (Abadias *et al.,* 2008). The microflora associated with raw fruits mostly includes yeasts and moulds (Burnett and Beuchat, 2000; Tournas, 2005).

Edible coatings are an alternative to preserve the quality and freshness of minimally processed products and prolong their shelf-life. The application of coatings creates a semipermeable gas and water vapor barrier that reduces the speed of respiration and dehydration of the coated products and creates conditions similar to foods subjected to modified atmospheres (Chiumarelli *et al.,* 2011). The most common polymers in the preparation of edible coatings are proteins, polysaccharides and lipids. Among the polysaccharides, cassava starch has been widely used due to its availability and relative low cost (Santacruz *et al*., 2015; Souza *et al*., 2012; Kampeerapappun *et al*., 2007; Flores *et al*., 2007). Edible coatings based on only polymers or in combination, have been applied to different fruits, e.g., cassava starch in fresh-cut pineapple (Bierhals *et al*., 2011), cassava starch and citric acid in fresh-cut Tommy Atkins mango (Chiumarelli *et al*., 2010), mixtures of starch and chitosan in guava (Bezerra *et al*., 2015), ascorbic acid and N-acetyl-cysteine in bananas (Palacín, 2012), and modified cassava starch in tomato (Hernández *et al*., 2011).

Chitosan has achieved considerable interest in the industry due to its biodegradability, biocompatibility and non-toxicity properties (Dash *et al*., 2011). Chitosan solutions exhibit good coating formation capacity and antimicrobial activity, making them potentially useful for antimicrobial biopolymer development (Dutta *et al*., 2009). The antimicrobial effect of chitosan could be the result of changes in cell permeability produced by the electric charge of chitosan (Devlieghere *et al*., 2004), molecular weight and degree of deacetylation (Zheng and Zhu, 2003). The pH and type of acid where chitosan is dissolved, as well as storage conditions may also influence antimicrobial properties (Begin and Van Calsteren, 1999; Leceta *et al*., 2013).

Edible coatings can be used with food additives acting against enzymatic browning, microbial growth and texture loss. The use of essential oils (EO) or active ingredients of essential oils such as carvacrol, carvone, cinnamaldehyde, citral, p-cimene, eugenol, limonene, menthol and thymol have been particularly prominent because they extend the shelf-life of food (Sung *et al*., 2013). Perdones *et al*. (2012) reported minimal changes in the physicochemical and microbiological characteristics of strawberries coated with chitosan and lemon EO for 15 days in storage at 4 °C compared to uncoated fruits or fruits coated only with chitosan. Another additive used together with edible coatings is salicylic acid. It delays the ripening of fruits, probably due to the inhibition or action of ethylene biosynthesis (Srivastava and Dwivedi, 2000). Salicylic acid has been used to control the aging by cooling of pears (Asghari *et al.,* 2007), strawberries (Babalar *et al.,* 2007), grapes (Asghari *et al.,* 2009)

and fresh-cut Sindrhi mangoes (Moradinezhad, 2020). There are no studies of the use of cassava starch with the addition of either cinnamaldehyde or thymol for the preservation of fresh-cut Tommy Atkins mango.

The present work aimed to study the use of edible coatings based on cassava starch together with salicylic acid, cinnamaldehyde and thymol, as well as chitosan, for the preservation of fresh-cut Tommy Atkins mango stored in refrigeration conditions. Analyses of instrumental texture (penetration force), titratable acidity, color and microbiological analysis (fungi and yeast) were performed on stored mango.

MATERIALS AND METHODS

The chitosan (molecular weight 149 kDa, deacetylation degree 95%) was donated by the Public University of Navarra (Pamplona, Spain). Tommy Atkins mangoes were purchased at a local market in the city of Manta, Ecuador. Mangoes with a degree of ripening of two (Báez, 1998) were selected according to the size and without damage.

The selected mangoes were washed, manually peeled and cut into 8.0x1.5 cm slices. These slices were immersed in the corresponding coating solution (chitosan, C; starch+salicylic acid, SSA; starch+cinamaldehyde+thymol, SCT) and dried at room temperature (approx. 25 °C). Mango samples with no coating were used as control samples. The mango slices (approx. 100 g) were then placed on polyurethane trays and coated prior to storage at 8 °C and 90% relative humidity.

Coating preparation

Coating based on either starch or chitosan were prepared by the casting technique. The chitosan coating was prepared using a chitosan solution 1% (w/v), using citric acid solution 1% (w/v) as solvent. Tween 20 at 1% (w/v), glycerol 0.5% (w/v) and glucose 0.5% (w/v) were added to the solution before homogenization with an ultraturrax (Politron, Switzerland) at 11,000 rpm for 4 min.The starch coating was prepared according to Santacruz *et al.* (2015). A solution of cassava starch 0.5% (w/v) was heated to 90 °C for 5 min. Tween 20 at 1% (w/v), glycerol 0.5% (w/v) and salicylic acid 2 mmol L^{-1} were added to the hot solution. Once the solution reached room temperature, glucose 0.5% (w/v), cinnamaldehyde 0.15% (w/v) and thymol 0.15% (w/v) were added. Finally, the solution was homogenized as described previously.

Physical-chemical characterization

Weight loss. It was calculated by weighting the fruit at 0 day and after each storage time. Measurements were performed in triplicate and the results were reported as percentage of weight.

Instrumental texture. Penetration analyses were performed according to Castro *et al.* (2014). Analyses were performed using a Shimadzu texturometer (EZ LX Model, Japan). A stainless-steel probe of 3 mm diameter and 8 cm length was used. The probe was introduced into the fruit at 15 mm depth with a velocity of 10 mm s^{-1}. The maximum force (penetration force) resulting from three measurements was reported.

Soluble solids. The fruit was disintegrated using a domestic blender, followed by a filtration on a piece of cloth. The filtered juice was analyzed by a digital refractometer (KRÜSS, Germany) according to the AOAC method (1990), the results of three measurements were reported as °Brix.

Titratable acidity. Titratable acidity was determined by titration with 0.01 M NaOH solution according to the AOAC method (1984), the results of three measurements were reported as percentage of citric acid.

Color analysis. The color of mango pulp was determined using a Konic Minolta (Japan) colorimeter in a L*, a*, b* scale. Color measurements were expressed based on the chromaticity parameters a* (green [-], red [+]) and b* (blue [-], yellow [+]). Measurements were made in triplicate.

Microbiological analysis. Fungi and yeasts counting were performed on mango samples at 0, 4, 8, and 12 days of storage. 10 g of sample were used to mix with 90 mL of KCl solution 0.1% (w/v). The inoculum was prepared by mixing 1 mL of the previous solution with 9 mL of distilled water. Counting of fungi and yeasts were made according to NOM-111-SSA1-1994 (Norma Oficial Mexicana, 1994). Three repetitions were performed for each sample.

Statistical analysis. The results were analyzed by means of ANOVA and a Tukey test, using a significance of 5% by the statistical package InfoStat, Professional Version 2016. Measurements of the previous analyses were performed in triplicate along 12 days of storage.

RESULTS AND DISCUSSION

Instrumental texture. The results of the instrumental texture revealed that the maximum penetration force decreased for coated and uncoated mango samples along the 12 days of storage with values ranging from 6.0 to 0.48 N (Figure 1).

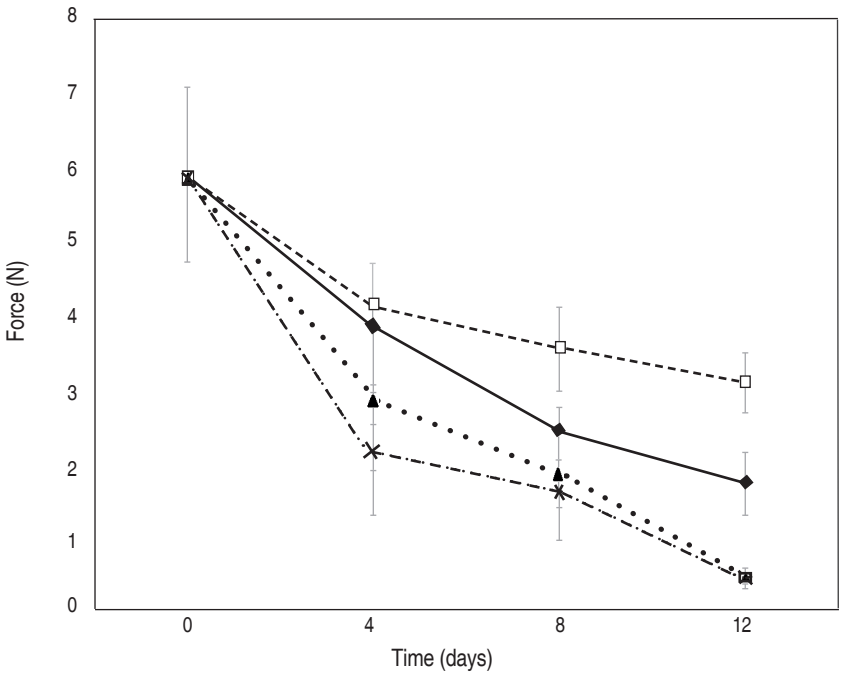

Figure 1. Maximum penetration force on fresh-cut Tommy Atkins mango with and without edible coating for 12 days at 8 °C and 90% relative humidity. □ chitosan (C), ▲ cassava starch+ salicylic acid (SSA), **X** cassava starch+cinamaldehyde+thymol (SCT) and ♦ uncoated

There was no statistically significant difference in penetration force between the samples at 4 day of storage. However, after 8 and 12 days of storage, penetration force was higher for samples coated with chitosan, while the lowest penetration force was recorded for mango samples coated with salicylic acid (SSA) and cinamaldehyde+thymol (SCT). The penetration force for the chitosan-coated (C) mango sample is maintained probably due to the reduced respiration rate (Cissé, 2015). Similar results were found by Zhu *et al.* (2008), using chitosan at different concentrations in 'Tainong' cv mangoes.

Weight loss. There was no difference on the weight loss of both samples coated with starch (SSA and SCT) during the whole storage time (P<0.05). Uncoated sample had the lowest weight loss followed by samples coated with chitosan. Castro *et al.* (2017) showed lower weight loss for papayas coated with chitosan and uncoated fruits compared to papayas coated with starch. The presence of compounds like cinnamaldehyde, thymol or salicylic acid into the coating, could accelerate a weight loss by osmotic dehydration (Vega *et al.,* 2007).

Soluble solids. The results showed no significant difference in soluble solids content between the uncoated and coated mango samples along storage (P<0.05). Samples had a soluble solids content of approximately 9.5 °Brix at 0 day and after 12 days of storage had a value of 8.5 °Brix (Table 1). A decrease in soluble solids content was found by Bueno *et al.* (2005), in minimally processed pineapple stored at 5 °C. The reduction of soluble solids is probably due to the respiratory process which may lead to high consumption of organic substrates, i.e. sugars (Kader *et al*., 2002).

Titratable acidity. The results showed that fruits coated with chitosan and uncoated samples presented a significant difference compared to samples treated with SSA and SCT (Table 1). Acidity values were higher for uncoated sample with value of 0.45% for 0 day, reaching 0.85% for 12 day,

followed by chitosan treatment with 0.45% for 12 day and 0.65% for 12 day of storage. The lowest values were for samples treated with salicylic acid, which presented values of 0.45% for 0 day and 0.49% for 12 day. Samples treated with cinamaldehyde+thymol, showed acidities of 0.45% for 0 day and 0.44% for 12 day of storage.

Table 1. Titratable acidity, soluble solids and weight of fresh-cut Tommy Atkins mango coated with either chitosan (C) or starch+salicylic acid (SSA), starch+cinnamaldehyde+thymol (SCT), stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days								
	4			8			12		
	Acidity	°Brix	Weight Loss	Acidity	°Brix	Weight loss	Acidity	°Brix	Weight loss
SCT	0.45^{a}	7.85^{a}	1.52^{a}	0.45^{a}	9.05^{a}	3.72^{a}	0.44^{a}	8.80^{a}	5.52^{a}
SSA	0.45^{a}	8.80^{a}	1.86^{a}	0.46^{a}	8.85^{a}	3.99^{a}	0.49^{a}	8.85^{a}	5.71^{a}
Uncoated	0.45^{a}	9.20^{a}	1.16^{b}	0.52^{b}	8.65^{a}	3.08^{b}	0.85^{c}	8.40^{a}	4.87^{b}
C	0.45^{a}	7.20^{a}	0.99^{c}	0.51^{b}	7.60^{a}	2.30^{c}	0.65^{b}	8.80^{a}	3.99^{c}

Acidity: percentage of citric acid. Weight loss: percentage of weight. The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05).

High values of titratable acidity of uncoated sample may be due to the production of organic acids by growing of microorganisms (Russo *et al.*, 2014). Similar behavior was found by Bueno *et al.* (2005) for minimally processed pineapple stored at 5 °C. Low values of titratable acidity for fruits treated with starch edible films is another indicator of ripening, as organic acids are also used during respiration (Freire *et al.*, 2005). Additionally, starch edible coating may reduce the level of O_2 inside the packages of mango slices, leading to minimal effect on the physical and chemical changes of mango during storage in low O_2 atmospheres (Freire *et al.*, 2005; Rathore *et al.*, 2007). The chitosan treatment showed greater values of acidity in relation to the other treatments throughout the storage period. Chitosan films are more selectively permeable to O_2 than to CO_2, maintaining the conditions of the coated fruit similar to an uncoated sample and promoting the production of metabolites that lead to an increase of acidity (Kweon *et al.*, 2001).

Color. Table 2 shows the results of color of mango slices during storage. There was not statistically difference for L*, a* and b* values between coated and uncoated samples. The only exception was the L* value of the sample treated with SSA after 12 days of storage, which was smaller and statistically different to the other samples. At those days of storage, L* value decreased by 10% for SSA samples and by 7.9% for SCT samples, while the uncoated sample increased 9%. The decrease in the L* value means that mango pulp became less bright during storage. There was not difference between a* and b* values among samples.

Table 2. Changes in L*, a* and b* values of fresh-cut Tommy Atkins mango coated with C, SSA or SCT, stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days								
	4			8			12		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
SCT	67.3^{a}	-3.4^{a}	60.6^{a}	63.4^{a}	-4.3^{a}	58.1^{a}	62.0^{b}	-2.9^{a}	55.6^{a}
SSA	67.3^{a}	-3.4^{a}	60.6^{a}	62.4^{a}	-2.1^{a}	62.1^{a}	60.6^{a}	-0.5^{a}	61.1^{a}
Uncoated	67.3^{a}	-3.4^{a}	60.6^{a}	68.5^{a}	-1.9^{a}	66.8^{a}	65.2^{b}	-2.8^{a}	59.2^{a}
C	67.3^{a}	-3.4^{a}	60.6^{a}	73.5^{a}	-4.9^{a}	62.6^{a}	73.4^{b}	-4.5^{a}	62.2^{a}

The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05).

Other authors (Robles *et al*., 2013) used antioxidant edible coatings for Kent mango cubes. They found at the end of storage that coated samples lost only 2.5% of the initial L* value compared to 7% loss in samples with no edible coating. Edible coatings based on polysaccharides and antioxidants have been used to delay browning in freshly cut apples maintaining L* values throughout storage (Lee *et al*., 2003; Fontes *et al*., 2008). Chiumarelli *et al.* (2011) reported that cassava starch coatings offer effective maintenance of color characteristics in cut mango samples due to the combined effect of the coating and citric acid. Although edible polysaccharide coatings such as starch are a good gas barrier (Dussan *et al*., 2014), in the present study, SSA or SCT did not achieve an effective browning delay.

Microbiological analysis. The results of the microbiological analysis (Table 3) show that all the treatments inhibited the development of fungi and yeasts in comparison with the uncoated samples, which showed an increase in the colonies from 1 log CFU g^{-1} at 0 day to 1.8 log CFU g^{-1} at 12 day of the study. Studies show chitosan at concentrations lower than 1% affects the sporulation of *Botrytis cinerea* and *Penicillium expansum* (Liu *et al*., 2007). The effect of chitosan on the germination of *Rhizopus stolonifer* spores has been previously reported at concentrations ranging from 1 to 2 mg mL^{-1} (Hernández *et al*., 2007; Hernández *et al*., 2008). Besides, Badawy and Rabea (2009) reported that chitosan applied in concentrations of 2 to 4 mg mL^{-1} can control *Botrytis cinerea* infections in tomato fruits. Bautista *et al.* (2003) showed that chitosan coatings control anthracnose in papaya fruits and inhibits the growth of fungi such as *Fusarium oxysporum*, *R. stolonifer, Penicillium digitatum* and *C. gloeosporioides* at 3% (Bautista *et al*., 2003; Bautista *et al*., 2004). The efficacy of cinamaldehyde in inhibiting the growth of fungi of the genera *Aspergillus* and *Penicillium* has been demonstrated by several authors. López *et al.* (2007a) found that *P. islandicum* and *A. flavus* were completely inhibited by cinamaldehyde-fortified in vapor phase. Tunc *et al.* (2007) found that cinamaldehyde is one of the strongest growth inhibitors of *Penicillium notatum*. Antimicrobial polypropylene films incorporating 2% cinamaldehyde also showed complete inhibition of *A. flavus, Penicillium comuna, P. expansum, Penicillium nalgiovense, Penicillium roqueforti, and P. islandicum* (López *et al*., 2007b). Plotto *et al.* (2003) reported that carvacrol, thymol, and citral compounds showed inhibition of mycelial growth of *Botrytis cinerea*, *Alternaria arborescens*, and *Rhizopus stolonifer*. Essential oils may affect stages of fungal development such as germination of spores and development of mycelium.

Table 3. Fungi and yeast growing on fresh-cut Tommy Atkins mango coated with C, SSA or SCT stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days			
	7	14	21	28
	(log CFU g^{-1})			
SCT	1.00[a]	nd	nd	nd
SSA	1.00[a]	nd	nd	nd
Uncoated	1.00[a]	nd	nd	nd
C	1.00[a]	1.2[a]	1.4[a]	1.8[a]

The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05). nd: not detected.

CONCLUSIONS

The use of edible chitosan-based coatings reduces textural and weight loss changes in mango slices stored in refrigeration, compared to coated samples with starch and uncoated samples. There was no difference in soluble solids between coated and uncoated samples during the whole storage, whereas differences in color were observed after the 8 day. The starch-based coatings reduce the changes of acidity during storage. Chitosan and starch-based coatings inhibits the growth of fungi and yeasts on mango slices. Further studies could examine the solubilization of chitosan in other

acids, as well as sensory analysis of coated samples for consumer acceptability

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POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

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INSTRUCCIONES A LOS AUTORES

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Los artículos deben ser enviados a través del Open Journal System en el Portal de Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Las unidades combinadas deben usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

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- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: (Gómez, 2000a, 2000b, 2000c)

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Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, se debe seguir el orden cronológico creciente; si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de publicación.

En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Libros: Autor(es). Año. Título del libro. Edición. Casa editora, ciudad de su sede. Páginas consultadas (pp. #-#) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, ciudad de su sede. Páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

Suplemento de revista: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Suppl. 2: 195-201.

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Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

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Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references. The maximum length must be 6000 words; excluding figures, tables, references. This type of articles is arbitrated and indexed.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

Articles must be submitted in accordance with the guidelines set forth in “Instructions to Authors”; those who violate the rules will not initiate the basic editorial process. Shall be filled the form “Authorization for Release of Works and Economic Rights Assignment”, which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

The Editorial Board, supported by a team of associate editors, will evaluate the scientific merit of the paper and will then submit it for evaluation under double-blind method- that is to say, strict anonymity in the review is kept- by two arbitrators specialized in the area, preferably one national and one international, who will give their report on the format provided by the Journal. The Editorial Board reserves the right to accept collaborations. The report, after the review process, can be: accepted for publication with no or few modifications; accepted for publication with major changes according to the comments of the evaluators; reconsidered for publication if it is substantially modified - in this case, it will be deemed as new material; rejected for publication. If articles are accepted, they will be returned to authors for correction and sent again to the Director of the Journal within 30 calendar days.

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INSTRUCTIONS TO AUTHORS

General guidelines

Papers must be sent b through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: “eight treatments”, “3,7 and 9 readings”, “15 kg”. Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter “a” and hyphen for growing seasons. Example period 2002 to 2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English and Spanish. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- If more than one date, they are separated by commas: Example: (Pérez, 1995, 1998, 2001).

- If there are two authors, they will be separated by the conjunction and. Example: (Gil and Ortega, 1993)

- If there are several works of an author published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: (Gómez, 2000a, 2000b, 2000c)

- For citations with three or more authors, it is necessary to mention in the text the surname of the first author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al.*, 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

- Organizations are cited by their initials; in case they do not have their full name is used. Example: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

References

Only bibliographical references cited in-text are listed in the references section. Lecture notes, articles in preparation, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications of the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

Gómez C. 2004. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín. Colombia. 78 p.

Citation of a citation, list the secondary source in your reference list: Example: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. # - #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Tropical Agriculture, http://agrotropical.edunet.es. 25 p.; accessed: November 2003.

Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

References

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

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