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AGRO PRODUCTIVIDAD

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In vitro conservation of *Vanilla planifolia* hybrids in minimal growth conditions

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ABSTRACT

Objective: To maintain minimal growth in *in vitro Vanilla planifolia* hybrids.

Design/Methodology/Approach: Explants of seven interspecific hybrids of *V. planifolia* with different origin parents were used. The treatments consisted of different doses of mannitol and sucrose in the culture medium which varied from 0, 5, 10, 15, 20, 25 and 30 g L^{-1}. The number of nodes, shoots and roots was recorded every 30 d for six months.

Results: 30 g L^{-1} mannitol and no sucrose in the culture medium allowed minimal growth in most of the hybrids. The higher the mannitol and lower the sucrose content, the length, number of between nodes, shoots and roots of the explants was lower ($P \leq 0.05$).

Limitations of the study/implications: There is a differential behavior between the biological material and the used culture medium, particularly in hybrids, due to their new genetic combinations. Therefore, for their conservation, the culture medium components must be adjusted.

Conclusions: 30 g L^{-1} mannitol without sucrose in in vitro culture medium significantly reduces growth during 180 d in vanilla hybrids.

Keywords: *Ex-situ* conservation, *in vitro* culture, vanilla, mannitol.

INTRODUCTION

Vanilla (*Vanilla planifolia*) is a species of the Orchidaceae family (Hagsater *et al*., 2005; Freuler, 2007). Soto (2003), Bouétard *et al*. (2010), and Gigant *et al*. (2011) reported that there are around 110 species in the Vanilla genus distributed throughout the tropical areas of the world. This number keeps increasing due

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to reports of new species, such as *V. rivasii* in Colombia (Molineros *et al*., 2014), *V. soto arenasii* M. Pignal in Costa Rica (Azofeifa *et al*., 2017) and *V. yanesha* in Peru (Damián, 2019). In Mexico, there are ten reported vanilla species (Soto, 2003). The assessment of the morphometric variation of fruits and seeds in four species of the *Vanilla* genus by Reyes *et al*. (2014) proposed the separation of three clones within *V. planifolia*. Flores *et al*. (2017) report seven species distributed throughout nine states in Mexico, taking into account georeferenced data from national and international herbaria and data from the vanilla germplasm bank; These authors mention that, in Mexico, the number of species in the Vanilla genus may increase, because not all the areas where *Vanilla* species possibly exist in the wild have been assessed, and therefore taxonomic studies are necessary to identify species and clones within species. Ramos-Castellá *et al*. (2016) separated accessions of *V. planifolia* and wild cultivars from Oaxaca and Quintana Roo, Mexico, using molecular techniques. These last three studies indicate that the vanilla diversity in Mexico may be more extensive. The cultivation of vanilla in Mexico presents technical, organizational, environmental and ecological problems that limit its production (Hipolito-Romero, 2010; López-Juárez *et al*., 2019; Santillan *et al*., 2019). *Vanilla planifolia* is the species with the largest planted area worldwide and the most demanded in the food industry for its aromatic qualities (Bory *et al*., 2008; Greule *et al*., 2010). FAO (1995) considers it as a species with a high degree of genetic erosion. In recent years in Mexico the sown area has been reduced and low yields are recorded (Luis-Rojas, 2020); In addition, it is considered a secondary economic activity with elderly producers and in a smallholding situation (Santillán-Fernández, 2019). The NOM-059-SEMARNAT-2010 standard considers it as a species subject to special protection. Faced with this situation, it is necessary to create technological strategies for both *in situ* and *ex situ* conservation that can help to stop the loss of genetic diversity of the *Vanilla* genus in Mexico and worldwide. In this regard, Azofeifa *et al*. (2014) mention that the conservation of the genus *Vanilla* must be under an integrated strategy, that sustainably allows its perpetuity, conserves the greatest possible genetic variability and significantly reduces the vulnerability of some of its species.

Among the different methods of *ex situ* conservation is *in vitro* conservation, which has advantages such as space and labor saving, development of healthy plants, high multiplication rates, constant supply of plants to producers and maintenance of the genetic fidelity of the material (García-Águilar, 2007; Engelmann and González, 2013; Bonilla *et al*., 2015). The minimal growth of explants in vitro has allowed successful medium and long-term conservation (Divakaran *et al*., 2006; Rayas *et al*., 2013; Bello *et al*., 2015). For this technique development, components have been used in the culture medium that retard the growth of the explants to avoid subcultures in short periods. Osmotic regulators such as mannitol and sorbitol (Montalvo *et al*., 2007; Hassan *et al*., 2014) and growth inhibitors such as abscisic acid (ABA) (Pence *et al*., 2002; Sarasan *et al*., 2006; Barrueto and Carvalho, 2008) and paclobutrazol (Ziv, 2000) have been used. Ávila and Salgado (2006) used sucrose, mannitol and sorbitol in the *in vitro* conservation of water yam (*Dioscorea alata*) and white yam (*Dioscorea rotundata*). Rayas *et al*. (2013) studied the effect of mannitol and silver nitrate for *in vitro* conservation of malaga (*Xanthosoma* spp.). Muñoz *et al*. (2019) reported that mannitol and sorbitol significantly reduce *in vitro* growth in three native Chilean potato (*Capsicum* sp.) genotypes. López-Puc (2013) developed an *in vitro* slow growth protocol for the conservation of the orchid *Epidendrum chlorocorymbos* SHLTR, where 1 % sorbitol and MS at half its ionic strength proved to be effective. In vanilla, Divakaran *et al*. (2006) used mannitol in the culture medium for minimal growth in several species of the genus *Vanilla* from India, allowing their conservation for more than seven years. In Mexico, Bello *et al*. (2015) used mannitol and poliethylenglycol, abscisic acid and paclobutrazol for *V. planifolia* conservation in slow-growth conditions, prolonging the period between subcultures every 180 days, without affecting the viability and phenotype of the plants.

The studies in Mexico for minimum growth in vanilla has focused only on *V. planifolia* and not on its interspecific hybrids. Such information is necessary for a genetic improvement program of vanilla, given that the crosses generate genetic combinations different from the parents. Also, each hybrid behavior may be different in the culture media used for the conservation of materials with high agronomic potential. Therefore, the objective of this work was to evaluate the effect of mannitol and sucrose, both individually and jointly, at different doses in a culture medium for in vitro conservation for minimal growth conditions.

MATERIALS AND METHODS

The biological material used was seven single-cross hybrids obtained from ten accessions of *V. planifolia* from

Chiapas, Puebla, Quintana Roo and Veracruz, Mexico, from the vanilla germplasm bank of the Benemérita Universidad Autónoma de Puebla. The crosses were manually carried out during March and April 2013. The fruits were harvested 60 d after pollination. The in vitro F1 seedlings were obtained in the in vitro and cryopreservation laboratory of the National Center for Genetic Resources (CNRG) in Tepatitlán, Jalisco, Mexico. *In vitro* culture was carried out in four stages: disinfection, sowing, incubation, and multiplication.

For the maintenance and multiplication of the *in vitro* plants, the culture medium proposed by Murashige and Skoog (1962) was used, supplemented with 30 g L^{-1} sucrose, 1 g L^{-1} activated carbon and 8.5 g L^{-1} agar. The pH was adjusted to 5.7 with a potentiometer (HANNA®), the medium was then sterilized in a Yamato model SM200 vertical autoclave during 15 min at 121 °C. The plants were incubated at 18 °C, in a 16 h light and 8 h darkness photoperiod, with a 68 μmol m^{-2} s^{-1} light intensity, using white fluorescent lamps. Twenty protocorms from each cross were grown to a height of 12-15 cm. This procedure lasted 2 years (2015 to 2016).

For the *in vitro* culture under minimum growth conditions, 15 plants of each cross were used, from which, 1 cm length axillary buds were cut inside a laminar flow hood. These buds were then sown in 22 × 220 mm test tubes containing 15 mL culture medium. The medium was MS with (0.22 % (w/v)) Gelrite™ (Sigma®) as a gelling agent, mannitol and sucrose were added as a carbon source at concentrations of 0, 5, 10, 15, 20, 25 and 30 g L^{-1}. The explants were incubated at 18 °C during 16 h light and 8 h dark photoperiod, with a light intensity of 68 μmol m^{-2} s^{-1}, using white fluorescent lamps. The different the treatments were the concentrations of mannitol and sucrose (Table 1), which were distributed in a completely randomized experimental design with three repetitions, and 10 explants were used per treatment. The assessed variables were the length of the explant, number of internodes, and the number of shoots and roots. Five evaluations took place every 30 days, for six months. An analysis of variance and means comparison was performed using the Tukey test ($P \leq 0.05$), a simple linear regression analysis considering the explants growth and the time of evaluation with the SAS v9 software (SAS Institute, 2002).

Table 1. Mannitol and sucrose concentration in culture media for minimal growth of *Vanilla planifolia* hybrids.

Treatment	Mannitol (g L^{-1})	Sucrose (g L^{-1})
T1	0	0
T2	0	30
T3	5	25
T4	10	20
T5	15	15
T6	20	10
T7	25	5
T8	30	0

RESULTS AND DISCUSSION

Treatment T8, with 30 g L^{-1} mannitol and without sucrose in the culture medium, allowed the shortest explants length, with 11.42 mm; although it has no statistical differences with T7, T6, T5 and T1, had no roots and allowed lower values in its between nodes and number of shoots (Table 2). The data suggest that the higher the mannitol concentration the slower the explants growth is and similar to T1. This behavior was due to the fact that in T1 the culture medium was not supplemented with sucrose and mannitol. Similar results are reported by Rukundo (2012) who found slow growth in banana seedlings when he used mannitol as a carbon source. Fortes and Scherwinski (2001), and Espinosa *et al*. (1986) reported that, in potato (*Solanum tuberosum*), mannitol reduces growth and the number of shoots per explant. Borges *et al*. (2003) reported a minimal growth effect in buds of *D. alata* when using

Table 2. Effect of mannitol and sucrose on *in vitro* growth of interspecific hybrids of *Vanilla planifolia* in minimal growth conditions.

Treatment	Longitude mm	Number Internodes	Number shoots	Number roots
T1	11.60[c]	0.16[f]	0.93[b]	0.03[e]
T2	26.56[a]	2.39[a]	1.09[a]	1.28[a]
T3	21.36[b]	1.91[b]	1.[11a]	0.94[ab]
T4	19.05[b]	1.78[bc]	1.10[a]	0.63[bc]
T5	15.10[c]	1.32[cd]	1.12[a]	0.46[cd]
T6	14.36[c]	1.09[de]	1.0[ab]	0.37[cde]
T7	13.09[c]	0.69[e]	0.98[ab]	0.11[de]
T8	11.42[c]	0.11[f]	0.98[ab]	0.0[e]
DMS	3.71	0.46	0.14	0.40

Means with the same letter in columns are statistically equal (Tukey, 0.05).

mannitol at higher than 5.5 % concentrations. Starisky *et al*. (1985) observed that mannitol in culture medium significantly reduces the growth of shoots of *Colocasia esculenta* and *Xanthosoma brasiliense*. The mannitol concentration in the culture medium are variable, since it depends on the species or plant variety, because this compound can be naturally produced in various plant species such as olives, celery, carrot, parsley, coffee, pumpkin, bean, pea etc. (Stoop *et al*., 1996).

In relation to sucrose as a source of energy and carbon, T2 with 30 g L^{-1} allowed the highest length values, number of internodes and roots in the explants with 26.56 mm, 2.39 and 1.28, respectively; this due the added sucrose in T2 which is recommended to obtain good growth of the explants. Menchaca *et al*. (2011) used 20 g L^{-1} of sucrose in culture medium, and reported good results in for seed germination *in vitro* of a hybrid of *V. planifolia* and *V. pompona*. Others, like Gatjens *et al*. (2018), used 30 g L^{-1} sucrose in the culture medium for massive propagation and formation of vanilla protocormic callus from root tips.

The lowest values were recorded on culture medium with no sucrose. These were 11.42 mm in the explant length and 0.11, 0.98 and 0.0 number of internodes, shoots and roots, respectively. The other treatments showed intermediate growth, since when sucrose decreased and mannitol increased, the explant growth was minimal. Lemoine (2013) remarks that the sugars synthesis, transport and metabolism are important in plants that grow in the field, since they determine their growth. However, in *in vitro* culture conditions, the explants are not sufficiently autotrophic, because the conditions are not very suitable for photosynthesis, and the addition of sugars to the culture medium is essential to satisfy the energy and carbon demand for the adequate development of explants (Pierik, 1990; Ertola *et al*., 1995; Rukundo, 2012). In a research on *V. planifolia*, Divakaran *et al*. (2006) managed to conserve shoots *in vitro* in MS culture medium supplemented with 15 g L^{-1} sucrose.

The *in vitro* behavior of the interspecific vanilla hybrids in the different treatments with sucrose and mannitol were statistically different ($P \leq 0.05$). H6 allowed the shortest length, with 13.40 mm, and the lowest number of internodes, 0.66. H4 allowed the greatest length, 19.18 mm, and 1.61, 0.88, and 0.0 for the number of internodes, shoots and roots, respectively. The other hybrids displayed an intermediate behavior, presenting an interval between 15.16 to 18.34 mm in length and 0.96 to 1.61 knots on the stems (Table 3).

The H4 was the hybrid with the lowest number of shoots (0.88), while H2, registered the highest number of roots (1.03). H4 and H5 had no roots during the six conservation months. The differentiated behavior recorded in the hybrids may be due to their genetic condition as a result of the crosses with different origin parents (Figure 1). Raya *et al*. (2009) found that the *in vitro* rooting of the Freedom and Saltereck vine rootstocks varied between genotypes and also depended on the source and concentration of sugar, as well as the osmotic potential in the medium. This shows that the conditions to induce rooting are defined by each genotype and should not be generalized. In this regard, Gianni and Sottile (2015) stated that, in the *in vitro* conservation of plum germplasm under slow growth the capacity of the sprouts was closely related to the genotypes that generate new combinations in the F1 explants. Márquez (1988) remarks that when a cross is made, the hybrid vigor is taken advantage of, this phenomenon that occurs in progeny with genetic characteristics, they tend to be better than the parents.

Table 3. Behavior of *Vanilla planifolia* hybrids in minimal growth conditions.

Hybrid (Parents)	Longitude mm	Number Internodes	Number shoots	Number roots
H1 (27×27)	16.57^{ab}	0.96^{cd}	1.07^{a}	0.41^{c}
H2 (195×20)	18.34^{ab}	1.44^{ab}	1.15^{a}	1.03^{a}
H3 (111×39)	17.90^{ab}	1.34^{abc}	1.04^{a}	0.86^{ab}
H4 (111×7)	19.18^{a}	1.61^{a}	0.88^{b}	0^{d}
H5 (112×21)	15.16^{bc}	1.09^{bc}	1.05^{a}	0^{d}
H6 (124×27)	13.40^{c}	0.66^{d}	1.04^{a}	0.45^{c}
H7 (124×28)	15.90^{abc}	1.15^{bc}	1.06^{a}	0.62^{bc}
DMS	3.40	0.42	0.13	0.37

Means with the same letter in column between treatments are statistically equal (Tukey, 0.05); mm = millimeters.

According to the simple linear regression analysis on the length of explants over time (Table 4), T8 had the lowest regression coefficient, 0.34, followed by T1 and T7 with 0.40 and 0.75, respectively. T2 and T3 registered the highest regression coefficients, with 3.97 and 2.76, each. T4, T5 and T6 presented intermediate regression coefficients (Figure 2) since it is observed that during the six months that the hybrids were in growth, T8 was the one that showed minimal growth compared to the other treatments.

Overall, the behavior of mannitol and sucrose together in the culture medium turned out to be effective to reduce *in vitro* growth of hybrids of *V. planifolia*. Few similar works carried out for the species, like that of Bello *et al*. (2015) where two osmotic agents (mannitol and poliethylenglycol) and two plant growth inhibitors (abscisic acid and paclobutrazol) were used for their conservation under slow-growth conditions, the length of the explants tended to be greater compared to those obtained in the present work in a similar period.

The information obtained in this study has been essential for the vanilla genetic improvement program at the Benemérita Universidad Autónoma de Puebla, since the hybrids that are currently being developed in fields with good agronomic characteristics (Figure 3), must be conserved *in vitro* under minimal growth for later use massively by producers in Mexico.

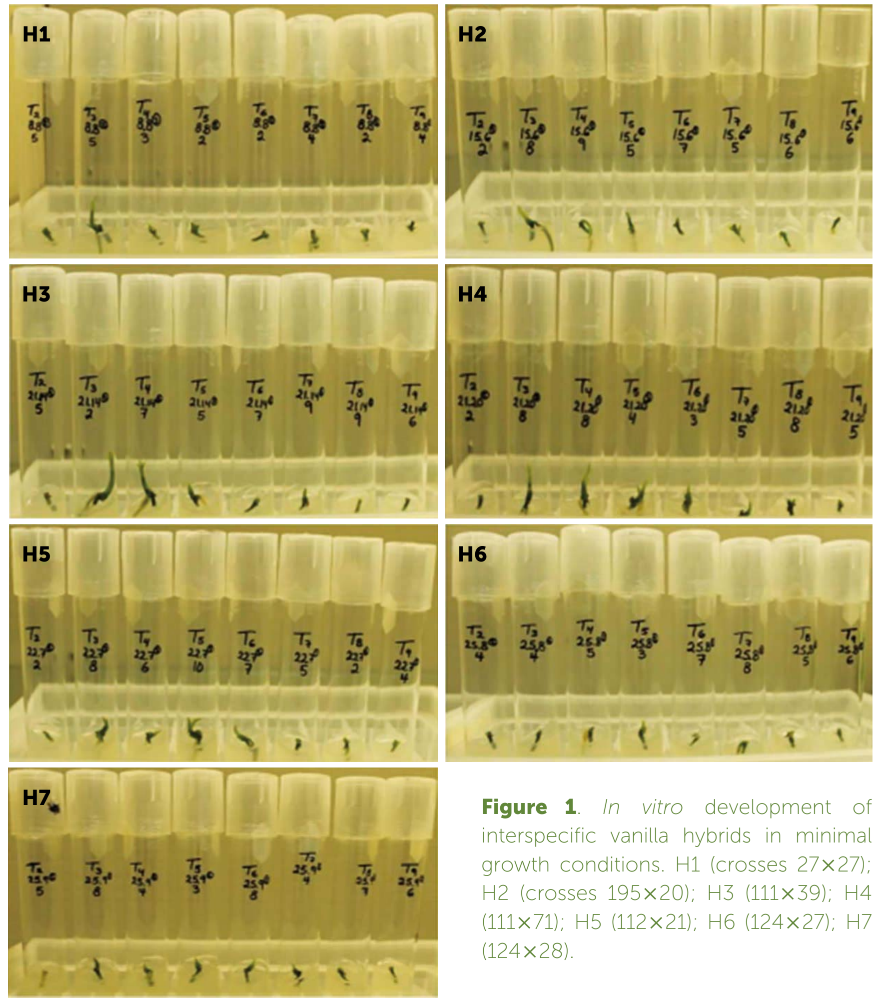

Figure 1. *In vitro* development of interspecific vanilla hybrids in minimal growth conditions. H1 (crosses 27×27); H2 (crosses 195×20); H3 (111×39); H4 (111×71); H5 (112×21); H6 (124×27); H7 (124×28).

CONCLUSIONS

Mannitol and sucrose significantly reduce the explant growth of interspecific hybrids of *V. planifolia* when developed in a culture medium with concentrations of 30 g L^{-1} mannitol without sucrose. The differences on the behavior of *V. planifolia* hybrid explants at the different concentrations of mannitol and sucrose could be due to the new genetic combinations generated when making the crosses between progenitors of different origin.

Table 4. Linear regression coefficients in eight treatments for growth of seven interspecific crosses of *Vanilla planifolia* under minimal growth conditions.

Treatment	Intersect	Regression coefficient	R^2	RMSE
1	9.58	0.394 **	0.995	0.05
2	5.06	3.97 **	0.957	1.53
3	6.95	2.76 **	0.980	0.56
4	7.99	1.99 **	0.997	0.16
5	7.6	1.78 *	0.801	1.62
6	8.89	1.08 **	0.996	0.13
7	9.16	0.75 **	0.987	0.16
8	9.64	0.34 **	0.973	0.10

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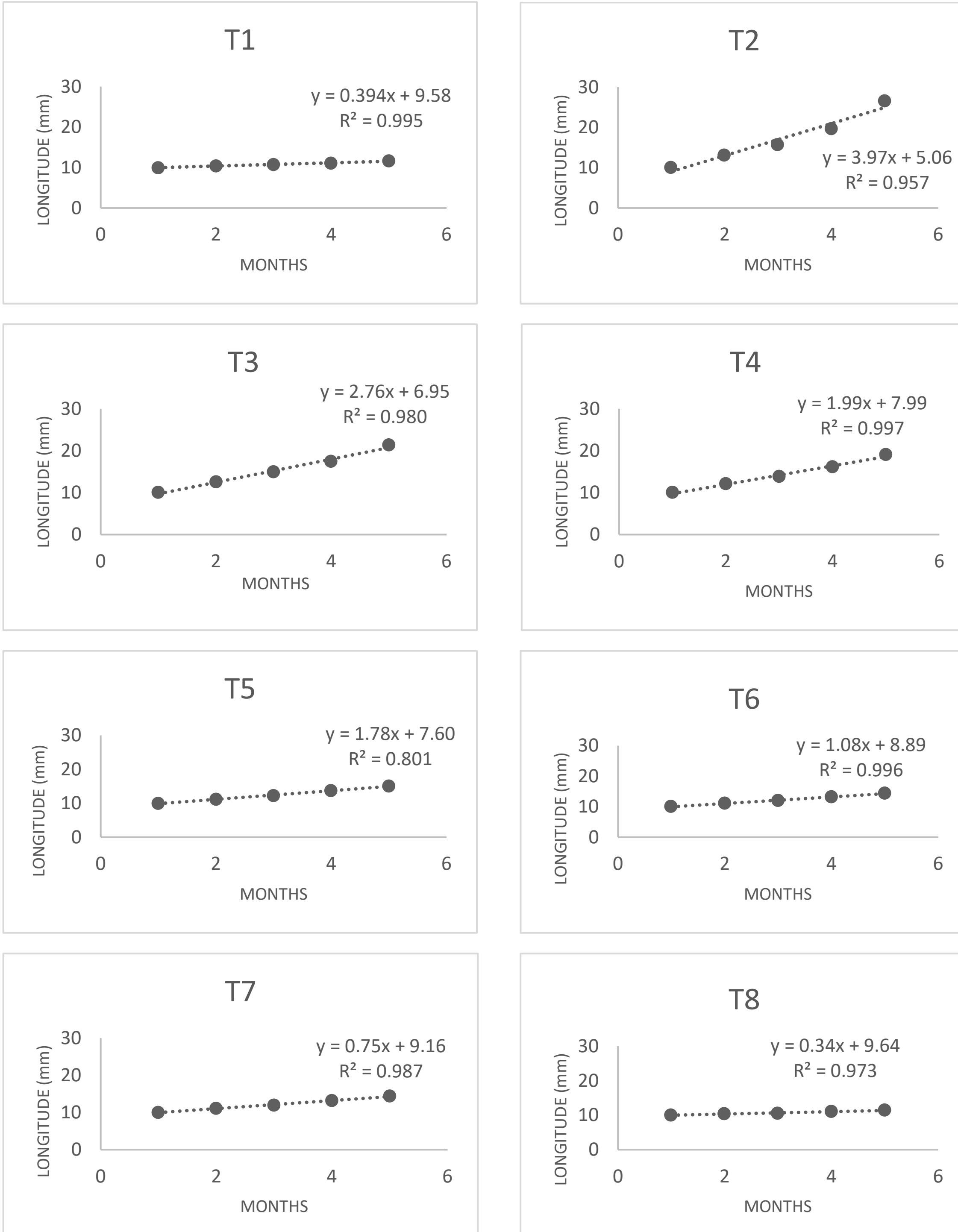

Figure 2. Growth behavior of hybrids of *V. planifolia* in different concentrations of mannitol and sucrose in *in vitro* culture medium. mm = millimeters. T1, T2, T3, T4, T5, T6, T7 and T8 = Treatments.

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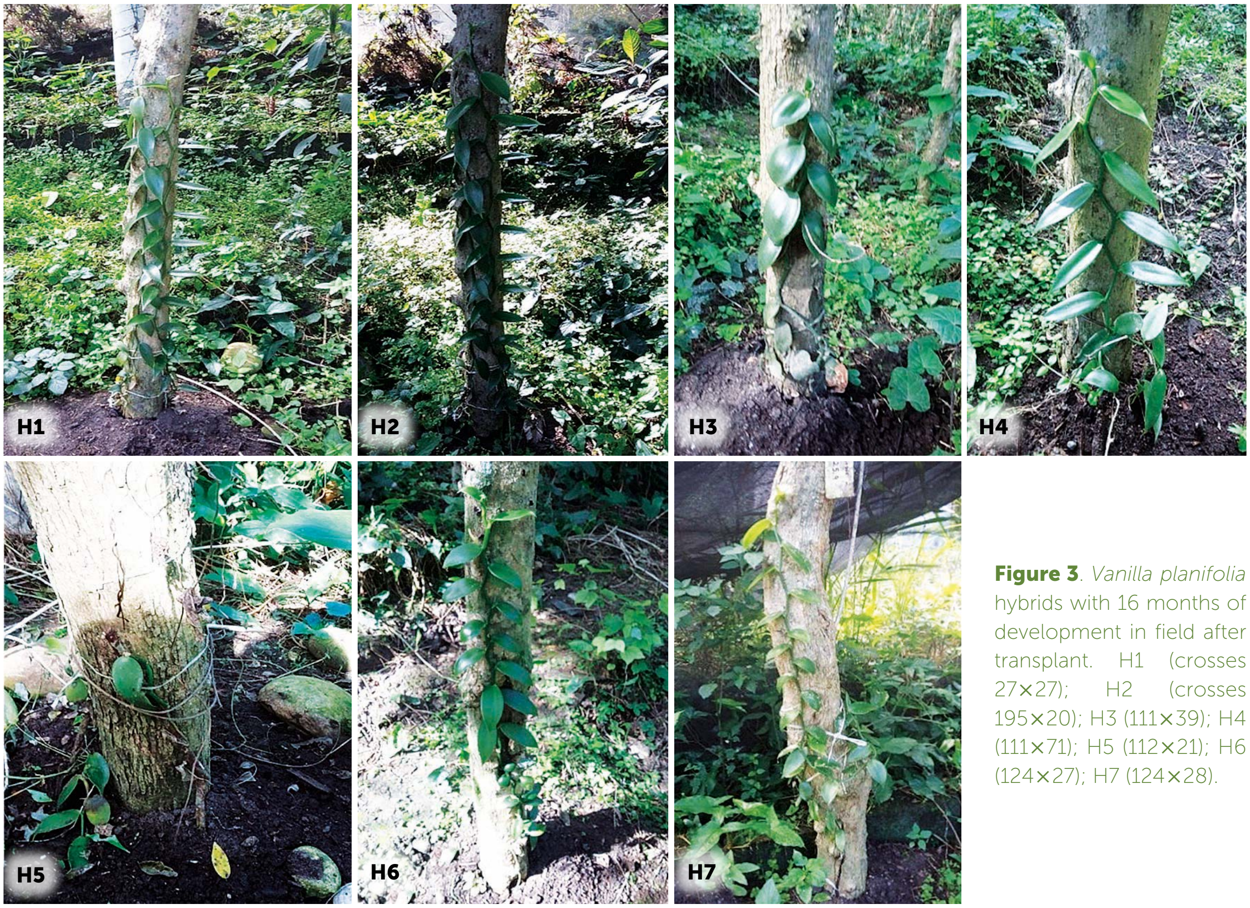

Figure 3. *Vanilla planifolia* hybrids with 16 months of development in field after transplant. H1 (crosses 27×27); H2 (crosses 195×20); H3 (111×39); H4 (111×71); H5 (112×21); H6 (124×27); H7 (124×28).

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Nature-based tourism as an alternative for sustainable development in the Altas Montañas de Veracruz region, Mexico

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ABSTRACT

Objective: Determine the current state of nature-based tourism in the High Mountains Region of Veracruz, Mexico, by the analysis of participatory workshop results with most of the municipal tourism managers of the region.

Design/methodology/approach: A participatory workshop took place, the "1st. Nature-based Tourism Forum of the Altas Montañas de Veracruz Region", for which an evaluation instrument was elaborated. The questionnaire, called "Technical Sheet for the Diagnosis and Inventory of Touristic Initiatives", was filled by the participants. During this participatory workshop, the region was divided into five subregions and participants worked in separate tables by subregion.

Results: 106 consolidated projects were registered, 38 in process and 130 elements with tourism potential were identified; 53 problems that affect tourism in the region were also identified. A total of 123 people participated: 61 of them representing 32 municipalities, 16 were from seven municipalities, 12 represented five institutions and 34 people were organizers.

Limitations of the study/implications: The greatest limitation faced by this project was the participation of all municipal authorities throughout the whole region.

Findings/conclusions: Out of the 57 municipalities in the region 32 attended (more than 56%). The number of consolidated projects (106) is high, considering 44% of the municipalities were not present, which highlights that the majority are of the community and private management. However, most of the projects in process are organized by municipalities, which indicates their interest in tourism. Regard the elements with touristic potential in the region (natural and historical sites and traditions), the number registered was high (130). This is a consequence of the great biocultural wealth in the area, which can be developed and conserved through ecotourism.

Keywords: Forum; Ecotourism; Rural tourism; Biocultural wealth.

INTRODUCTION

Nature-based tourism refers to those trips that aim to carry out recreational activities in direct contact with nature and the cultural expressions that contextualize them, especially with an attitude and commitment to know, respect, enjoy and participate in the conservation of natural and cultural resources. These trips are grouped according to the interest of the tourist in three main modalities:

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"Ecotourism", "Adventure Tourism" and "Rural Tourism" (SECTUR, 2017). Thus, in recent years, nature-based tourism has come to occupy an important place in Mexico, being used as a sustainable development option for rural and indigenous groups in the country (López-Ojeda *et al*., 2019). The implementation of tourism in rural areas not only in Mexico but throughout Latin America has promoted high expectations as a promoter of social, economic and ecological change, considering this type of tourism as the solution to the problems of the rural environment (Kieffer, 2018). However, although this tourism represents a sustainable alternative for natural and cultural resource usage, it depends on how it is planned and developed so that it becomes a viable and real option.

The Altas Montañas de Veracruz region, Mexico, is one of the 10 regions in which the state is divided (INAFED, 2010) and is made up of 57 municipalities, including two of the largest cities in the state: Córdoba and Orizaba and their respective conurbation areas. The largest altitudinal gradient in Mexico is found in this region, which ranges from approximately 70 to 5,636 meters above sea level (Rivera-Hernández *et al*., 2018) in a strip of around 100 km wide on average, which gives it remarkable characteristics. The objective of this research was to develop an inventory of the tourism projects of the region and to carry out an exchange of experiences, to have a scenario of the state in which they are located in the area to form a tourism network in the region.

MATERIALS AND METHODS

In order to achieve the current state of nature-based tourism at the Altas Montañas de Veracruz region, Mexico, we carried out a participatory method consisted of a workshop where the municipal authorities of the 57 municipalities were invited to attend. This workshop was organized through the Master's Program in Landscape and Rural Tourism of the Colegio de Postgraduados Campus Córdoba; the workshop called: "1st. Regional Forum of Nature Tourism of the Altas Montañas de Veracruz region", to assess first-hand, the tourism initiatives that the municipal governments are carrying out and supporting.

To publicize the event, a poster was printed with relevant information about this workshop (Figure 1) and an official letter was sent to the authorities of each of the 57 municipalities in the region, specifying the information about the event and its objectives. An evaluation instrument in the form of a questionnaire, called "Technical Sheet for Inventory and Diagnosis of Tourism Initiatives" was also prepared and distributed amongst the participants, so that it would be filled out before the reunion to reduce the filling time during the event (Table 1). The poster and the information of the event were published on social media one month in advance so that doubts of potential participants were addressed, both via social networks and email. The workshop was held on April 18, 2018, from 9:00 a.m. to 2:30 p.m. To develop a structured work, work tables were organized, where the region was divided into five subregions: 1) Huatusco-Tlaltetela, 2) Pico de Orizaba, 3) Acultzingo-Orizaba, 4) Zongolica-Tezonapa and 5) Córdoba-Center (Figure 2). Each worktable systematized the technical inventory sheets, their analysis, and doubts clarification. For those municipalities that had not previously sent the required data, the information was obtained from these tables.

Tourism projects classification

The projects in the technical sheets were codified, classified, and defined as follows:

Community management. It refers to the management of touristic projects through the local population, who control and manage their common welfare,

Figure 1. Poster of the 1st. Regional Forum on Nature Tourism in the Altas Montañas de Veracruz region.

Table 1. Technical inventory sheet and diagnosis of tourism initiatives at the Altas Montañas de Veracruz region, Mexico.

List and mention the tourist projects that exist in your municipality
What localities are included in your municipality? (If you like, you can draw a picture of the municipality to locate them geographically)
What kind of tourism servies do yo offer? (cabins, zip line, trails, tours, swimming pools, archaeological site, festivities, etc.) Please describe
Who is the responsible of the tourism services mentioned? By the communities, individuals, or the municipal government?
In case your municipality does not have a tourism project. Could you mention some places that have the potential to undertake tourism projects? Could you mention why they have potential?
Would your municipality like to be joined in a tourism network seeking inter community support and benefit? If you say yes, could you add your main reasons?
Your municipality has problems or any problem that avoid the establishment or the prosperity of tourism project? What would these problems be?
Your municipality would be interested in training for the development of tourism projects? Why?
This space is free if you would like to add something missing of your municipality, any aspect regarding tourism issues is welcome

based on the organizational figures of their community structure (Del Barco-Quiroga, 2010).

Private management. It refers to management by people who do not belong to any type of community organizational figure, call it a community or "ejido". It can be a single person or a group of organized people.

Municipality management. Projects which management comes from the municipal authorities in charge of tourism development.

Rural tourism. Tourism whose purpose is the interaction of visitors with a receiving rural community, in activities of their typical social, cultural, and productive expressions (SECTUR, 2004).

Ecotourism. It refers to tourism whose main characteristic is been responsible for the visited natural and cultural environment, visitors who respect the environment stand out (SECTUR, 2004).

Adventure tourism. In Mexico, this tourism is defined as that where trips are made to carry out recreational activities related to challenging conditions by air, land, or water while interacting with nature (SECTUR, 2004).

Historical/religious tourism. It relates to an interest in the cultural, social, or historical relevance at the visited site (García Muñoz-Aparicio *et al*., 2017).

Gastronomic tourism. It is the type of tourism where gastronomy is the main reason to travel to a certain place (OMT, 2017).

Both historical/religious tourism and gastronomic tourism are included in this study, since, in the assessed region, these are mainly developing in rural areas, thus forming part of rural tourism.

Others. Includes other types of tourism not considered in those specified above.

Problems and limitations

The technical sheet also consulted about the problems with nature-based tourism. The classification of the problems was shared with the workshop participants so that they all maintained the same criteria. The problems were classified as follows:

Environmental. These are those problems that affect tourism that relate to the natural environment.

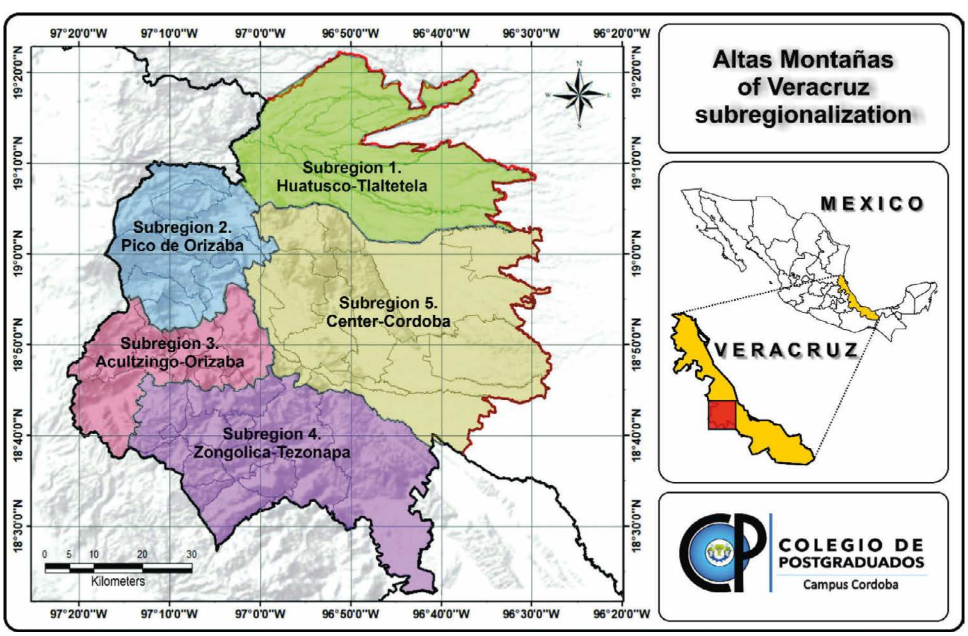

Figure 2. Subregionalization map of the Altas Montañas de Veracruz region, Mexico.

Social. Those problems where the impact on tourism comes from the social environment that surrounds it.

Economical. Those effects on tourism where the main element relates to a lack of economic resources.

Technical. Those effects whose main element is the lack of technical training on tourism.

For the analysis of the obtained data, descriptive statistics were used, graphs and tables to better illustrate the results obtained were developed.

RESULTS AND DISCUSSION

The workshop was attended by 123 people, 61 were from 32 municipal councils, 16 individuals from seven municipalities and 12 representatives from five institutions: Faculty of Biological and Agricultural Sciences, Universidad Veracruzana, Servus, AC, Colegio de Postgraduados Campus Veracruz, Universidad Tecnológica del Centro de Veracruz and Direction of the Cofre de Perote National Park, National Commission of Protected Natural Areas (CONANP). The Colegio de Postgraduados Campus Córdoba participated with 34 organizers (Table 2).

Consolidated projects

In the region, 106 consolidated projects were registered. A consolidated project refers to a touristic product made up of tangible and intangible elements and attractive resources, which already have services, equipment and infrastructure, have planned leisure or recreational activities and has an identifiable image in the market (SECTUR, 2003). Most of these projects are managed by individuals and communities and are of ecotourism type (Table 3; Figure 3).

Projects in process

In total, 38 projects were registered as in process in the region. In this case, those developed by municipalities stand out, the majority of which are ecotourism in nature (Table 4, Figure 4).

Elements with touristic potential

A total of 130 elements with tourist potential were registered for the region. This group of elements refers to those sites (waterfalls, rivers, lagoons, caves, mountains, flora and fauna, etc.), traditions (patron saint festivities, ethnic festivities, "jaripeos", festivals, etc.) or historical remains ("ex-haciendas", archaeological remains, etc.), which currently have no touristic use, but

Table 2. Total number of participants and municipalities participating in the 1st. Regional Forum on Nature Tourism in the Altas Montañas de Veracruz region by subregion. A = Attendees and O = Organizers.

Subregion	A	O	Total	Participant Municipalities
1. Huatusco-Tlaltetela	10	7	17	6 (Comapa, Tlaltetela, Zentla, Huatusco, Tenampa y Tlacotepec de Mejía)
2. Pico de Orizaba	12	7	19	6 (Alpatláhuac, Calcahualco, Coscomatepec, Ixhuatlancillo, La Perla y Tomatlán)
3. Acultzingo-Orizaba	21	9	30	6 (Magdalena, Huiloapan de Cuauhtémoc, Maltrata, Ixtaczoquitlán, Río Blanco y Ciudad Mendoza)
4. Zongolica-Tezonapa	7	6	13	2 (Zongolica y Tezonapa)
5. Centro-Cordoba	39	5	44	12 (Amatlán de los Reyes, Atoyac, Camarón de Tejeda, Carrillo Puerto, Coetzala, Fortín de las flores, Ixhuatlán del Café, Naranjal, Omealca, Rafael Delgado, Tepatlaxco y Yanga)
Total	89	34	123	32

Table 3. Existing consolidated projects in each subregion by management type and type of tourism at the Altas Montañas de Veracruz region, Mexico. Management type: C = Community, P = Private, M = Municipal. Tourism type: E = Ecotourism, A = Adventure, H / R = Historical / Religious, G = Gastronomic.

Subregion	Consolidated projects	Type of management			Type of tourism					
		C	P	M	R	E	A	H/R	G	Other
1. Huatusco-Tlaltetela	20	10	5	5	0	6	2	7	2	3
2. Pico de Orizaba	32	22	6	4	1	11	0	15	1	4
3. Acultzingo-Orizaba	23	5	12	6	0	18	0	5	0	0
4. Zongolica-Tezonapa	13	5	4	4	0	9	1	2	1	0
5. Centro-Cordoba	18	1	16	1	0	11	3	3	1	0
Totals	106	43	43	20	1	55	11	27	5	7

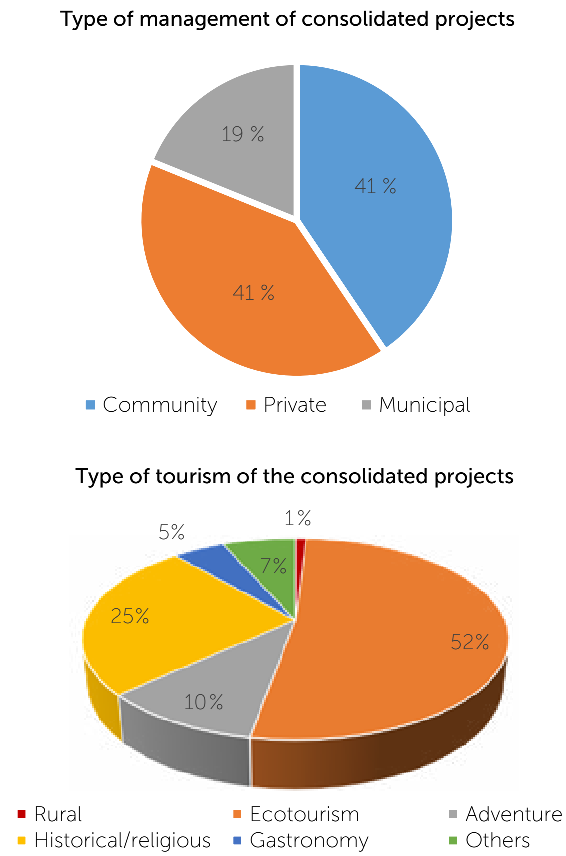

Figure 3. Percentages of management types and types of tourism in consolidated projects in the Altas Montañas de Veracruz region, Mexico.

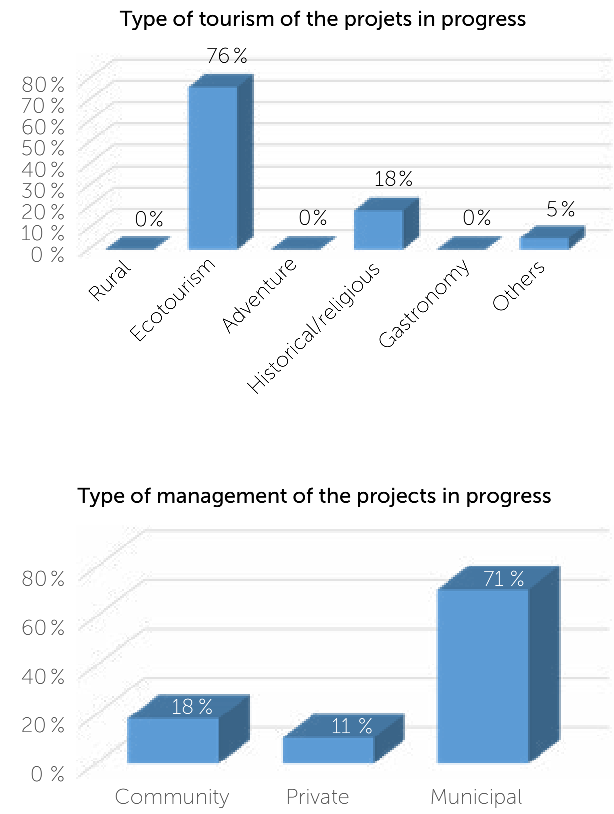

Figure 4. Percentages of management types and types of tourism in projects in process at the Altas Montañas de Veracruz region, Mexico.

Table 4. Existing projects in progress (PP) at each subregion, by management and tourism type at the Altas Montañas de Veracruz region, Mexico. Management type: C = Community, P = Private, M = Municipal. Tourism type: E = Ecotourism, A = Adventure, H / R = Historical / Religious, G = Gastronomic.

Subregion	Projects in progress	Type of management			Type of tourism					
		C	P	M	R	E	A	H/R	G	Other
1. Huatusco-Tlaltetela	7	3	0	4	0	4	0	1	0	2
2. Pico de Orizaba	7	0	1	6	0	5	0	2	0	0
3. Acultzingo-Orizaba	7	0	0	7	0	7	0	0	0	0
4. Zongolica-Tezonapa	0	0	0	0	0	0	0	0	0	0
5. Centro-Cordoba	17	4	3	10	0	13	0	4	0	0
Totals	38	7	4	27	0	29	0	7	0	2

which, according to the participants (most of them in charge of tourism in their municipalities), have potential use. In this case, the elements with touristic potential with community management stand out, followed by those of municipal management. Regard their tourism type, the ecotourism ones stand out, followed by the historical / cultural (Table 5; Figure 5).

In total, 53 problems were registered (Table 6; Figure 6). In this subject, economic problems stand out, mainly emphasizing the lack of resources for tourism and, consequently, the lack of infrastructure for it, among other relevant aspects. It is followed by technical problems, related to a lack of training of the municipalities for proper development of tourism in their territories.

The results obtained are encouraging, taking into account that, from the 57 existing municipalities in the region, 32 of them were present at the workshop (more than 56%). Also, it is worth highlighting the interest shown by the

Table 5. Elements with potential (EP) in each subregion by management and tourism type in the Altas Montañas de Veracruz region, Mexico. Management type: C = Community, P = Private, M = Municipal. Tourism type: E = Ecotourism, A = Adventure, H / R = Historical / Religious, G = Gastronomic.

Subregion	EP	Type of management			Type of tourism					
		C	P	M	R	E	A	H/R	G	Other
1. Huatusco-Tlaltetela	20	4	3	13	0	7	4	8	1	0
2. Pico de Orizaba	31	16	2	13	0	16	0	12	2	1
3. Acultzingo-Orizaba	32	11	5	16	0	14	2	12	1	3
4. Zongolica-Tezonapa	6	6	0	0	0	6	0	0	0	0
5. Centro-Cordoba	41	17	18	6	1	14	6	14	1	5
Totals	130	54	28	48	1	57	12	46	5	9

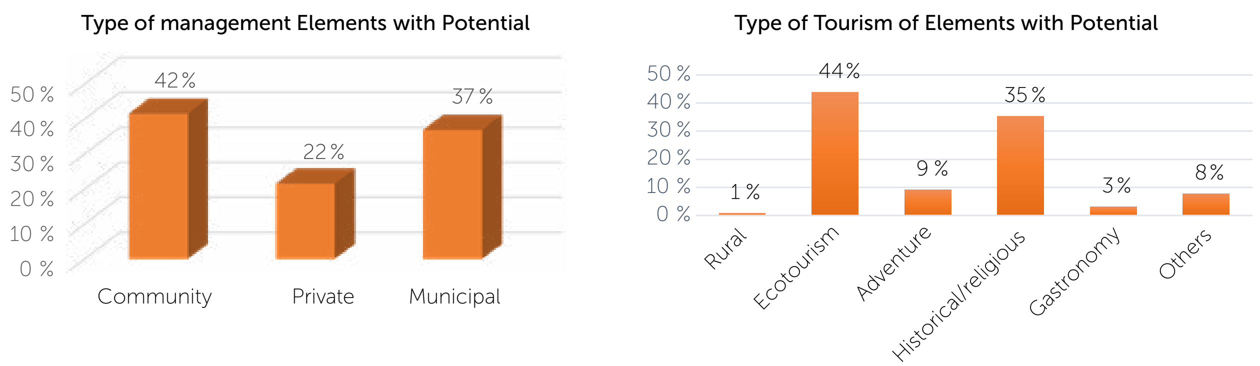

Figure 5. Percentages of management types and types of tourism of elements with touristic potential for development in the Altas Montañas de Veracruz region, Mexico.

participants, working hard with the organizing team, to combine the information presented here.

On the other hand, the number of consolidated projects, 106 for 32 participating municipalities, that is, 3.31 consolidated projects on average per municipality, is high if we consider that information was obtained from little more than half of the municipalities in the region, therefore a higher number is to be expected for the entire region. It also stands out that most of the projects are community and private, however, most of the projects in process are initiatives of current municipal authorities. These speak of the rebound and interest that municipalities are having in the tourism issue, considering that those municipalities took possession in December 2017 and the workshop was held in April 2018. The elements with identified tourist potential had a high number of records (130), which stands out if we take into account that only a little more than half of the municipalities in the region participated, so this number will likely increase for the region as a whole. It also to be noted that most

Table 6. Identified problems related to nature-based tourism in the Altas Montañas de Veracruz region, Mexico.

Subregion	Problematic	Ambiental	Social	Economical	Technical
1. Huatusco-Tlaltetela	10	1	1	6	2
2. Pico de Orizaba	12	1	2	4	5
3. Acultzingo-Orizaba	15	5	3	5	2
4. Zongolica-Tezonapa	1	0	1	0	0
5. Centro-Cordoba	15	1	5	5	4
TOTALS	53	8	12	20	13

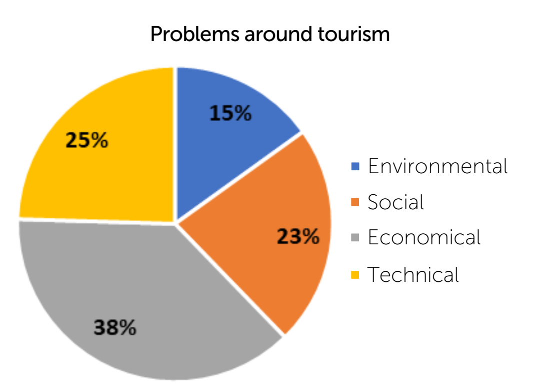

Figure 6. Percentages of problems related to tourism in the Altas Montañas de Veracruz region, Mexico.

of the elements with touristic potential are of community or municipal management, which would facilitate their use by rural communities in the region. Finally, most of these elements are ecotourism and historical / cultural, which is consistent with the biocultural richness of the region.

At all times during the workshop process, the total willingness of the participating municipal authorities to collaborate with the Colegio de Postgraduados Córdoba Campus of the and in strengthening nature-based tourism in the region. Likewise, most participants showed interest and willingness to continue collaborative work, to develop an ecotourism network and receive training in different topics related to it, to create new projects, improve those they already have, and overall, to have a better touristic offer, integrated and shared by all or several participants of this network, in the form of different tourist routes.

CONCLUSIONS

Finally, this exercise was important to assess a considerable number of those involved in ecotourism in the region to initiate a collaborative process among all interested parties. As already mentioned, a large number of elements with touristic potential is a highlight of the region, as a consequence of the great biocultural wealth in the area. These can be used and conserved through nature-based tourism, that is, tourism can be a tool for sustainable use of biocultural richness, as well as being an excellent means of promotion and thus, valued for its proper measure.

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Effects of flowering and production inducers in the Mexican lime (*Citrus aurantifolia* Swingle)

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ABSTRACT

Objective: To evaluate new hormonal and biostimulant products to induce flowering of Mexican lime (*Citrus aurantifolia* Swingle) for winter production at the state of Guerrero, Mexico.

Design/methodology/approach: Prohexadione calcium (PHD-Ca), cytokinins, paclobutrazol, biuretic urea, granulated urea (46%) and a control were evaluated.

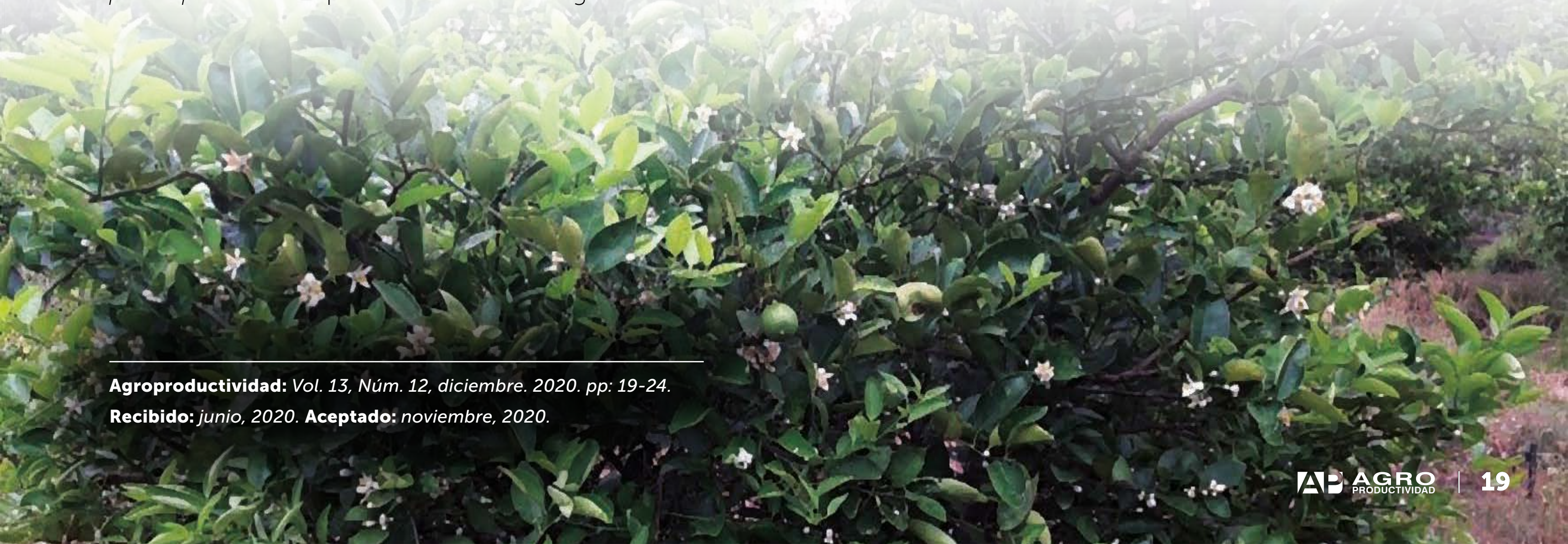
Results: There are differences between treatments for the number of flowers and number of fruits (Pr>0.0411 and 0.048); regard the "number of flowers" variable, paclobutrazol, prohexadione calcium and granulated urea (46%) stood out; while, prohexadione calcium, paclobutrazol and cytokinins favor the "number of fruits" variable. The yields show statistical differences (Pr>0.0332) and the cytokinins and paclobutrazol stood out.

Findings/conclusions: The usage of cytokinins for induction and winter production of Mexican lime is suggested.

Keywords: Citrus, new inducers, biostimulants, phytohormones.

INTRODUCCION

The main citrus fruits produced in Mexico are oranges, Mexican lemons, Persian lemons, grapefruits and mandarins, important due to their economic, social and cultural impact. During 2018, the citrus fruits established area was 572,033 ha, which produced 7.8 million tons of fruit (SADER, 2019). For this reason, Mexico ranks fifth in world citrus production (FAOSTAT, 2019). The Mexican *per capita* consumption of lime is 14.0 kg.



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The environmental conditions at Guerrero, Colima, and Oaxaca, coastal areas in Mexico, are similar regard their production areas, and significantly differ from those at Michoacán, production areas; currently, these areas distribute at altitudes from 0 to 1000 m, with 860 mm average annual rainfall and temperatures ranging between 25 and 28 °C, in plains and hills, and soils with pH values between 5 and 7. Their average obtained yields low (Ariza *et al*., 2004). Persian and Mexican limes produced in similar conditions reported average yields between 14.33 and 14.15 t ha^{-1} respectively (SADER, 2019). Their production is scarce from December to April, when it reaches its highest prices and their cultivation is profitable (Ariza *et al*., 2004). In this context, cultural practices can control the flowering season, a technique to improve the crop's profitability (Ruiz, 2001); such as pruning, girdling and the application of chemical substances (Ariza *et al*., 2004).

Knowledge on the reproductive biology of these plants has considerably increased in recent years, it is, therefore, necessary to understand more about the effects growth regulators have on citrus plants and their fruits since it is a complex and critical phenomenon, which depend on external and internal factors of the plants (Iglesias *et al*., 2007). Some studies mention the physiological effects of flowering induction in citrus fruits, such as hydric stress, pruning and girdling with periodic urea and Biofol applications (Lovatt *et al*., 1988; Ariza *et al*., 2004); as well as applying biostimulants, such as urea and Biofol in Mexican lime (Ariza *et al*., 2015) and Persian lime (Ambriz *et al*., 2018).

The applications of chemical substances favor flowering induction, a widely practiced agronomic activity (Ruiz, 2001). Authors such as Lugo *et al*. (2009) have reported that naphthaleneacetic acid application favors flowering at Morelos, Mexico, therefore this product's effect requires evaluation at different production times. Ariza *et al*. (2015) mention that a 4% urea application favors flowering in Mexican lime. The harvest seasonality continues to prevail in the production areas from May to September, so their prices are low. Because of it, there is an increasing interest in new alternatives to produce flowers and fruits during the low production seasons. This situation occurs in Oaxaca and Guerrero states, where fruits commercialization during that period is difficult due to high production, to such a degree that exports and consequently assets decreased. The objective here was to assess the effects of new inducers and growth regulators on flowering induction of Mexican lemons, to increase productivity and competitiveness of production and quality of their fruits during winter.

MATERIALS AND METHODS

Location and description of the study area. The test took place at Los Playones, Acapulco de Juárez, Guerrero, Mexico (km 7.0 scenic highway La Venta to Acapulco Diamante). The plantation is five years old. On it, trees are set 6 m between rows and 4 m between plants within rows and 417 plants per hectare density. The assessed products, doses and application methods are listed in Table 1. Their application occurred during the second quarter of September during the rainy season onset.

The trees were pruned by removing 30 cm from the treetop canopy. To apply the evaluated chemical substances, commercial adherent was used in doses of 1.5 ml L^{-1} of water.

Agronomic management. The evaluated Mexican lime orchard was chemical fertilized with 120-60-60 (N-P-K); Polychel multi was applied to the foliage in a 3 L ha^{-1} dosage. Also, imidacloprid + cyfluthrin (300 ml ha^{-1}), azadirachtin + citroline (250 ml ha^{-1}) and

Table 1. Evaluated products, doses and times of applications to induce flowering in *Citrus aurantifolia* Swingle during the higher profitability season at Los Playones, Acapulco de Juárez, Guerro, Mexico.

Product	Concentration	Application Method	Evaluation application periods (days)^{Z}
Citocininas (4000 ppm)	2.4 ml L^{-1} water	Sprinkled to foliage	0, 15 and 30
Prohexadiona de calcio	1.5 g L^{-1} water	To foliage	0 and 30
Urea 46%	7.5 g L^{-1} water	To foliage	0
Urea biurética 44%	1.2 g L^{-1} agua	To foliage	0 and 30
Paclobutrazol 1%	1.5 ml L^{-1} agua	Drained at the base of the stem or to the foliage	0
Control	No aplication		0

^{Z} Indicates applications at 0, 15 and 30 days.

mineral oil (2 L ha^{-1}) were applied for fruit plow mite control (*Phyllocoptruta oleivora*), insects such as aphids (*Aphys gossypi* and *A. spiraecola*) and diaphorina (*Diaphorina citri* Kuwayama). The presence of stem-end rot (*Lasiodiplodia* sp.) was controlled with matalaxyl + chlorothalonil (450 g) in 200 L^{-1} of water. The orchard was irrigated with a micro-sprinkler system, at a 100 to 120 L d^{-1} rate.

Table 2. Analysis of variance (ANOVA) of the number of flowers and number of fruits regard the flowering induction treatments (*Citrus aurantifolia* Swingle).

Variation source	Number of flowers		Number of fruits regard	
	Fc	P>F	Fc	P>F
Model	2.16	0.0006	1.25	0.047
Sampling date (A)	6.51	0.0001	1.61	0.037
Treatments (B)	2.6	0.0411	1.57	0.028

Evaluated variables. The effect of the treatments on the Mexican lime trees was evaluated at a 3.0 m height and 3.0 m within the tree crown diameter, as well as the number of flowers and fruits with "marble" size in selected branches of 75 cm length at 1.6 m in height from ground level, at each cardinal point in the tree. These evaluations were carried out every fifteen days, after treatment application, from October to December 2011. The fruit yield per tree was determined from the harvests during January and February, obtained from the total product weight of each tree with a 10 kg capacity mechanical scale, assessed every month and determined as kg ha^{-1}. A randomized complete block design was used, with five repetitions, a tree as an experimental unit. The statistical analysis was performed in the Statistical Analysis System (Anonymous, 2015) version 9.3 software, for means separation the Tukey test at 0.05 % probability of error.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for the numbers of flowers (Table 2) showed differences (P≤0.05), between sampling dates and products applied for the flowering induction.

The number of registered flowers with paclobutrazol was statistically similar to those produced in control trees and most of the treatments, except for biuret urea, which was higher (Figure 1). It should be noted that the number of flowers produced in the control trees was statistically equal to the number of flowers produced in all treatments (Figure 1).

To assess the effects, the treatments had on the number of flowers, the sum of the sampling dates was made. For the October 11, 2018 sampling, the treatment effects were already shown. Overall, paclobutrazol and prohexadione calcium stood out (Figure 2). Flowering in plants is shown in Figure 3. The Figure 2 shows the numerical superiority of the accumulated flowers during the sampling dates, achieved with paclobutrazol, followed by prohexadione calcium, compared with trees treated with granulated urea, cytokinins and the control.

Flowering relates to the summer season, not to the physiological age of the shoot, so that the Mexican lime flowers are in its lateral shoots (Hittalmani, 1977), while in Persian lime occurs in the terminal shoots (Hittalmani *et al*., 1977). Naturally, flowering in Mexican limes peaks

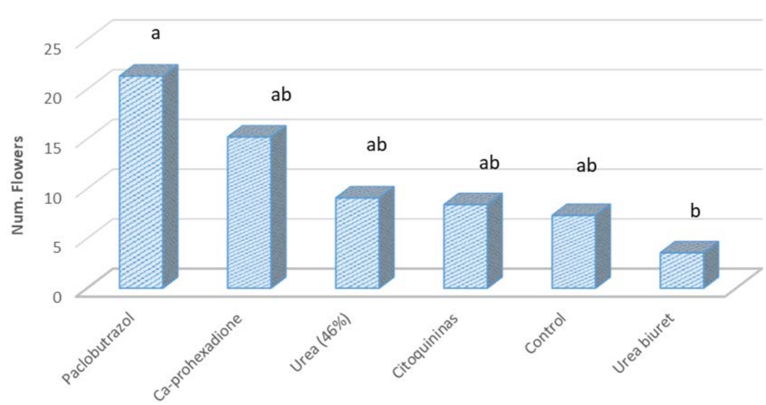

Figure 1. Effects of flowering inducing products. Treatments with the same letter are statistically equal (Tukey, $\alpha \leq 0.05$) and DMS = 16.25.

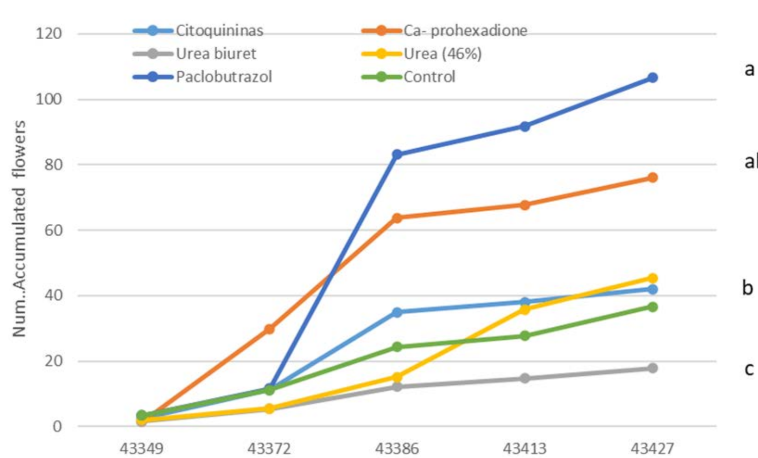

Figure 2. Number of flowers accumulated per tree (*Citrus aurantifolia* Swingle) for the three evaluation dates. Treatments with the same letter are statistically equal with the mean comparison test (Tukey, $\alpha \leq 0.05$) and a DMS = 16.25.

Figure 3. Induction to flowering and fruits in *Citrus aurantifolia* Swingle with prohexadione calcium.

during May and June (Ariza *et al*., 2004). However, with flowering inducers such as urea (46%), their maximum peak is obtained by December (Ariza *et al*., 2015), in some instances, register two flowering flows, from December to January and from July to August (Athani *et al*., 1998), as shown in a study with a similar trend at the end of November. Tripathi and Dhakai (2005) applied paclobutrazol in *C. aurantifolia* during the second half of July and a flowering induction response was obtained 70 d later; which does not coincide with the test, given that at 50 d flowering increased in acid lime.

Some studies in 'Persian' lime, record that pruning + urea + ringing and pruning + Biofol® + ringing in September quantified an average of 38 and 28 flowers each, which presented between 80 and 50% more flowers respect the other treatments (Ambriz *et al*., 2018). The application of urea + light pruning in Mexican lime and 'Persian' lime trees significantly favors flowering, while the application of urea and Biofol® favors higher flower production during winter (Ariza *et al*., 2004, 2015; Almaguer *et al*., 2011). The flowering increase is due to urea to ammonium conversion, which reduces growth because of the ethylene synthesis which induces flowering (Lovatt *et al*., 1988).

The results here presented were higher than those recorded by Ariza *et al*. (2015), given that in that study 6% urea and Biofol treatments applied to Mexican lemon stood out. Ambriz *et al*. (2018) report that 6% urea plus pruning more ringed and pruning plus Biofol plus ringed applied in Persian lime, induced higher flowering and fruiting. Therefore, this study has shown satisfactory results in the field. The same applies to the number of fruits.

The analysis of variance (ANOVA) for the number of fruits (Table 2) showed differences (P≤0.05) between treatments. With the difference that the trees treated with prohexadione calcium showed a higher number of fruits, followed by the paclobutrazol treatment compared to the control trees (Figure 4). In such a way, the differences between treatments are appreciated in Figure 5. Mahalle *et al*. (2010) report with the application of cycocel at 1000 mg kg^{-1} flowering of the acid lime in August, in addition to a greater number of fruits during September and October. This can vary between production regions, as it occurs in the Coast of Guerrero state. Devi *et al*. (2011) reported a higher number of fruits with paclobutrazol. Likewise, this trial shows a greater

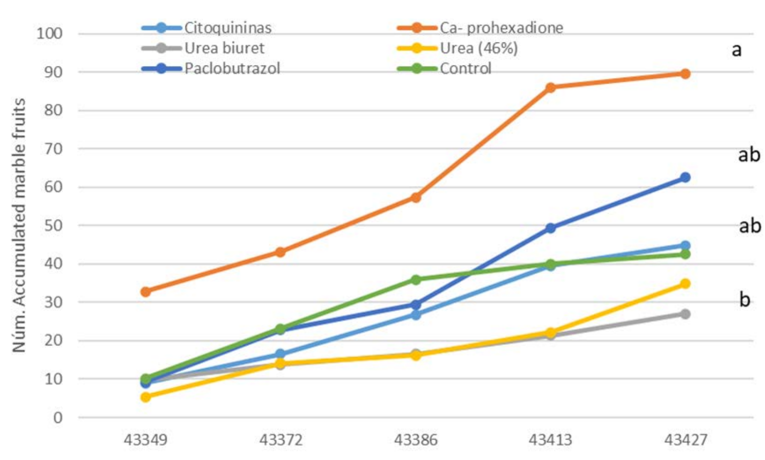

Figure 4. Effect of flowering inducers on the number of accumulated marble fruits of (*Citrus aurantifolia* Swingle). Treatments with the same letter are statistically equal with the mean comparison test (Tukey, $\alpha \leq 0.05$) and a DMS = 28.30.

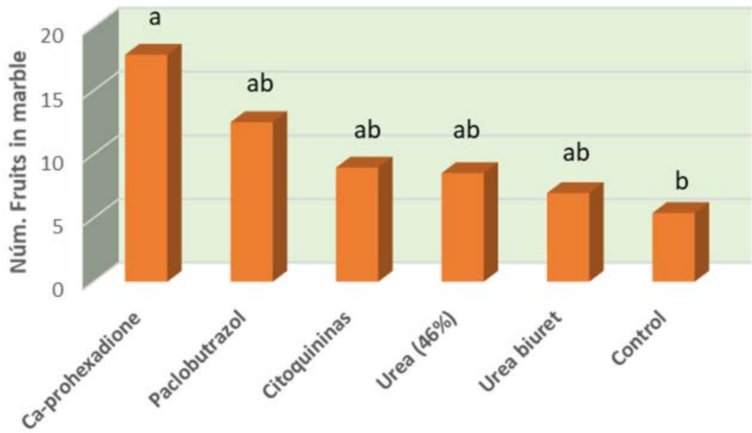

Figure 5. Effects of flowering inducing products on *Citrus aurantifolia* Swingle. Treatments with the same letter are statistically similar (Tukey, $\alpha \leq 0.05$ DMS = 11.873).

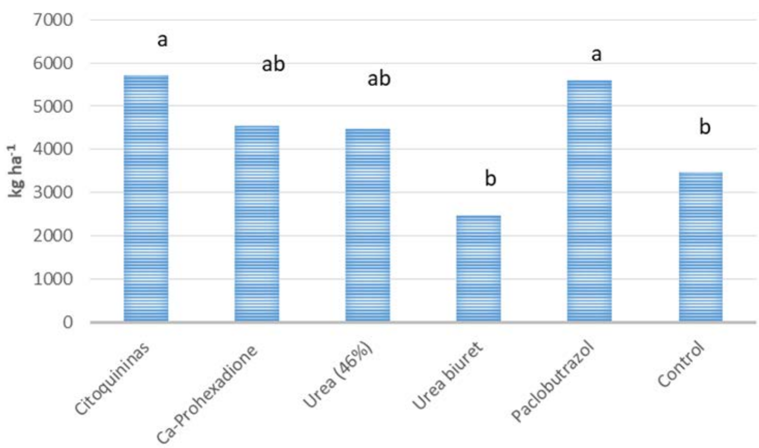

Figure 6. Effects of flowering inducers on yield (kg ha^{-1}) of Mexican lime (*Citrus aurantifolia* Swingle). Treatments with the same letter are statistically similar (Tukey, $\alpha \leq 0.05$, DMS = 2254).

number of fruits with prohexadione calcium, with 17 fruits on average and 92 accumulated fruits, which coincides with those obtained by Devi *et al*. (2011), followed by paclobutrazol, cytokinins and the rest of the treatments, during the sampling period.

Prohexadione calcium shows effects on flowering induction and production in apples (Kiessling *et al*., 2008) and oranges (Garner *et al*., 2010). In Mexican lime it has satisfactory shown results; however, they are not similar to those reported by Ariza *et al*. (2015), since Biofol® and granulated urea showed excellent fruit production in acid limes.

Regard their yield, registered differences between treatments ($\alpha \geq 0.05$) with paclobutrazol and cytokinins had a production of 5.8 and 5.6 t ha^{-1}, respectively; followed by prohexadione calcium and granulated urea, while the lowest values were with biuret urea and the control (Figure 5).

In Mexico, the national yield average is 14 t ha^{-1}, although in some states such as Yucatán and Colima yields are 20 t ha^{-1}. In this study, only the yield obtained in the harvest months of January to April was assessed, which is low, but shows a higher trend than that of the granulated urea biostimulant. With the application of Biofol® and granulated urea biostimulants, the flowering and production of Mexican lime is induced, yield was twice as high during the same period (Ariza *et al*., 2015) and in 'Persian' lime with granulated urea (Almaguer *et al*., 2011). Flowering biostimulants do not generate wear for plants (Ariza *et al*., 2015), as recorded with cultural practices such as girdling (Ambriz *et al*., 2018). Paclobutrazol at 2.0 ml L^{-1} was not effective in Mexican lime (Ariza *et al*., 2015); however, this trial showed a better result, since the application formation drained at the base of the stem produced satisfactory results.

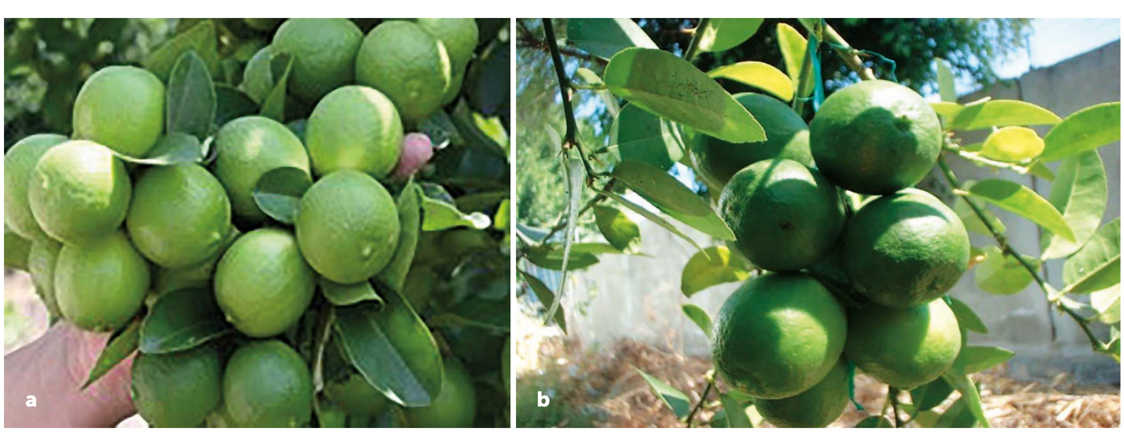

Figure 7. Response of flowering inductors for production of Mexican lime (*Citrus aurantifolia* Swingle) in Guerrero, Mexico. a. Cytokinins, b. Calcium prohexadione.

CONCLUSIONS

With the biostimulant products of prohexadione calcium, paclobutrazol and cytokinins, flowering is induced and has effects on the formation of fruits. Cytokinins and paclobutrazol showed high yields and favored winter production. For this reason, they outperformed granulated urea in acid lime production.

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Chemical Composition and Digestibility of Six Species of Legumes (Fabaceae)

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ABSTRACT

Objectives: To determine the chemical composition and *in vitro* digestibility of *Acacia angustissima*, *Dalea* spp., *Desmodium* spp., *Leucaena leucocephala*, *Phaseolus vulgaris* and *Tephrosia vicioides* (Fabaceae).

Design/Methodology/Approach: Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude lignin (CL), cellulose (Cel), hemicellulose (Hcel), cellular content (CC) and *in vitro* dry matter digestibility (IVDMD) were determined. The design was completely random with three replicates. The variables were correlated by pairs and the means of the species were compared with the Tukey's test ($P<0.05$).

Results: *Dalea* spp. had the highest crude protein (17.7%), followed by *A. angustissima* (15.9%) and *L. leucocephala* (14.1%). *A. angustissima* (73.2%), *Dalea* spp. (74.9%) and *P. vulgaris* (77.5%) showed the highest IVDMD. *L. leucocephala*, *Tephrosia vicioides* and *Desmodium* spp. showed the lowest values of CP (14.1, 11.8 and 12.3%, respectively) and IVDMD (70.4, 70.2 and 64.9%, respectively). *Desmodium* spp. showed high levels of NDF (59.2%), ADF (41.4%), CL (17.5%), Cel (29.7%) and Hcel (17.8%) ($P<0.05$). The IVDMD showed positive correlation with CC and negative correlations with NDF, CL, and Hcel ($P<0.05$).

Study Limitations/Implications: *Desmodium* spp. showed high content of CL and low values of CP and IVDMD, therefore supplements should be added when used this legume in animal feed.

Findings/Conclusions: *Dalea* spp. showed low levels of lignin and high levels of protein and digestibility, making possible to use it as feed for ruminants.

Keywords: Fabaceae, crude protein, neutral detergent fiber, *in vitro* digestibility.

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INTRODUCTION

Forages are the main source of food for livestock, they can grow naturally, be sown and established for direct grazing, and they can be acquired through manual or mechanical cutting (Santos *et al*., 2015). The challenge for livestock producers lies in improving production in order to cover the demand and conserve natural resources and the environment (Giraldo, 1999). An important factor in animal production is the composition and quality of the forages that make up the diet (Rojas *et al*., 2005). Therefore it is important to know the forage resources available in a specific region, in order to develop systems that are more sustainable and productive (Lascano and Ávila, 1991). Legumes have nutritional quality and are cultivated as a protein bank in strips or in association with grasses (Poaceae) (Hess and Lascano, 1997). Since they have a short growing cycle, they can be used as emergency crops of high quality (Reta *et al*., 2013). Moreover, forage Fabaceae are of better quality than grasses due to better digestibility, which is related to lower fiber content, and most of their digestible energy comes from soluble cell constituents, rather than from fiber (Buxton *et al*., 1996). Although Fabaceae have higher protein content, there is little information in the literature reviewed about its nutritional value to justify including it in a diet, as either a principal or secondary component, in order to improve its efficiency for use by livestock (González and Cáceres, 2002; García and Medina, 2006). The objective was to determine the chemical composition and *in vitro* digestibility of six Fabaceae forage species native to the state of Morelos, Mexico.

MATERIALS AND METHODS

Six species of the Fabaceae family were used, and they were collected during the same time of year in several municipalities in Morelos, Mexico. Leaves, stems and fruits (aerial part) were collected in order to carry out a botanical identification up to genus (Table 1).

The samples were dried at a constant weight in an oven at 55 °C, and then each sample was analyzed in triplicate in order to determine the crude protein (CP), ether extract (EE) and ash (Ash) content or proximate analysis (AOAC, 1990), and the Van Soest *et al*. (1991) analysis: neutral detergent fiber (NDF), acid detergent fiber (ADF), crude lignin (CL), cellulose (Cel), hemicellulose (Hcel), cellular content (CC) and *in vitro* dry matter digestibility (IVDMD) with the methodology described by Giraldo *et al*. (2007). The study was carried out in the Animal Nutrition Laboratory of the Animal Science Department, in Texcoco, Mexico.

The variables of chemical composition and IVDMD were subjected to an analysis of variance (ANOVA) with the completely randomized experimental design. The means were compared with the Tukey's test ($P<0.05$). Also, a correlation analysis was carried out by pairs of variables ($P<0.05$) using the SPSS (2011) software.

RESULTS AND DISCUSSION

The results are expressed in dry basis (Table 2 and 3), and Table 4 shows the correlation between pairs of variables studied. *Desmodium* spp. showed the lowest IVDMD, CP and CC. Low CP values are associated with low levels of CC, and low CC levels are associated with low IVDMD. Inversely, Dalea spp. showed the highest IVDMD associated with elevated CP content. *Acacia angustissima* showed similar IVDMD values as *Dalea* spp., and slightly lower CP levels. Based on these results, it was inferred that with higher CP content there is higher IVDMD. These results can be explained by the following positive correlations: IVDMD *vs* CC and CC *vs* CP (Table 4). The positive correlation (0.96) between IVDMD (65.3%) and CP (22.5%) has been demonstrated ($P<0.01$) in vetch by Gezahagn *et al*. (2014).

Desmodium spp. showed higher content of all fibrous components (NDF, ADF, CL, Cel and Hcel; Table 2) and lower IVDMD (Table 3). *Dalea* spp. showed the lowest

Table 1. Species and collection sites in the state of Morelos, Mexico.

Species	Municipality	Collection site
Acacia angustissima	Ticuman	Tlaltizapan-Yautepec Highway, 5 km from Ticuman.
Dalea spp.	Tlalquiltenango	At 200 m from the community of San Pablo, Tlalquiltenango
Desmodium spp.	Nepoapulco	Next to the bridge "El Vigía".
Leucaena leucocephala	Tepoztlán	"El Puente" place, in the community of Santa Catarina, Tepoztlán
Phaseolus vulgaris	Nepoapulco	Before the bridge "El Vigía", towards Mexico City.
Tephrosia vicioides	Totolapan	Tlayacapan-Atlatlahuacan highway, after the deviation towards Mexico City

Table 2. Fibrous components of six species of legumes (Fabaceae) from Morelos, Mexico.

Species	% Dry basis				
	NDF	ADF	CL	Cel	Hcel
Acacia angustissima	42.9^{c}	29.0^{c}	15.6^{ab}	13.4^{b}	13.9^{b}
Dalea spp.	50.4^{b}	41.3^{a}	11.6^{c}	29.7^{a}	9.1^{c}
Desmodium spp.	59.2^{a}	41.4^{a}	17.5^{a}	29.7^{a}	17.8^{a}
Leucaena leucocephala	50.5^{b}	30.9^{c}	18.8^{a}	12.0^{b}	19.6^{a}
Phaseolus vulgaris	49.5^{b}	38.1^{b}	12.7b^{c}	25.4^{a}	11.4^{c}
Tephrosia vicioides	59.0^{a}	40.0a^{b}	13.4b^{c}	26.6^{a}	19.0^{a}
Standard error of mean	1.38	1.22	0.69	1.96	0.98

$^{a, b, c}$ Different letters in the same column indicate significant differences ($P<0.05$). NDF: neutral detergent fiber, ADF: acid detergent fiber, CL: crude lignin, Cel: cellulose, Hcel: hemicellulose.

Table 3. *In vitro* digestibility and non-fibrous components of six species of legumes (Fabaceae) from Morelos, Mexico.

Species	% Dry basis				
	IVDMD	CP	CC	EE	Ash
Acacia angustissima	73.2^{ab}	15.9^{b}	57.1^{a}	2.7^{d}	4.2^{d}
Dalea spp.	74.9^{ab}	17.7^{a}	49.6^{b}	3.6^{b}	6.7^{a}
Desmodium spp.	64.9^{c}	12.3^{d}	40.8^{c}	3.0^{c}	4.7^{c}
Leucaena leucocephala	70.4^{b}	14.1^{c}	49.5^{b}	4.2^{a}	6.7^{a}
Phaseolus vulgaris	77.5^{a}	11.7^{d}	50.5^{b}	3.7^{b}	6.0^{b}
Tephrosia vicioides	70.2^{b}	11.8^{d}	41.0^{c}	2.8c^{d}	6.1^{b}
Standard error of mean	1.04	0.55	1.38	0.15	0.23

$^{a, b, c, d}$ Different letters in the same column indicate significant differences ($P<0.05$). IVDMD: *in vitro* dry matter digestibility, CP: crude protein, CC: cellular content, EE: ether extract, Ash: ashes.

value of CL and Hcel (Table 2 and 3), associated with high levels of IVDMD. *Acacia angustissima* showed low amounts of NDF, ADF, Cel and Hcel, and higher IVDMD than *Desmodium* spp. This indicates a negative correlation between IVDMD and NDF, CL and Hcel (Table 4). These negative correlations by pairs ($P<0.05$) between fibrous components and IVDMD were reported by Ortíz-Domínguez *et al*. (2017) in pods of different Fabaceae (previously known as legumes) for IVDMD *vs* NDF (−0.848) and for IVDMD *vs* CL (−0.957).

Tephrosia vicioides showed similar NDF, ADF, CL, Cel and Hcel levels (Table 2) as *L. leucocephala* and its IVDMD values were also similar (Table 3). The elevated levels of IVDMD showed by *P. vulgaris* are probably due to its low values of CL and Hcel (Table 2). The negative correlations in Table 4 indicate that lower CL *vs* higher IVDMD (−0.601), and lower Hcel *vs* higher IVDMD (−0.713). At higher levels of NDF, there were high levels of ADF, Hcel or Cel. With higher levels of the fiber variables (NDF, CL or Hcel), there are lower values of IVDMD, CP or CC. Therefore, *Desmodium* spp., which resulted with high levels of NDF, ADF, CL, Cel and Hcel, produced the lowest values of IVDMD, CP and CC. At low values of CL and Hcel, there were high levels of IVDMD; therefore, *Dalea* spp., which produced low values of CL and Hcel, produced high levels of IVDMD.

Comparison of CP and IVDMD values of this study with those from the literature

Acacia angustissima (CP: 15.9%, IVDMD: 73.2%). Ncube *et al*. (2017) reported 23.4% of CP in *A. angustissima*. Aganga *et al*. (1997) found 12.52%, 17.14%, 13.67% and 12.74% of CP for *A. robusta*, *A. nigrescens*, *A. karoo*, and *A. rehmanniana*, respectively. Castrejón *et al*. (2017)

Table 4. Significant correlations between the response variables.

	IVDMD	CP	CC	EE	NDF	ADF	CL
IVDMD	1						
CC	0.587*	0.585*	1				
Ash				0.75*			
NDF	−0.587*	−0.585*			1		
ADF			−0.725**		0.725**	1	
CL	−0.601*						1
Cel			−0.579*		0.579*	0.884**	−0.79**
Hcel	−0.713**	−0.526*	−0.513*		0.513*		0.662**

The non-fibrous components are IVDMD: *in vitro* dry matter digestibility, CP: crude protein, CC: cellular content, EE: ether extract, Ash: ashes. The fibrous components are NDF: neutral detergent fiber, ADF: acid detergent fiber, CL: crude lignin, Cel: cellulose, Hcel: hemicellulose. (*): $P<0.05$, (**): $P<0.01$.

reported 16.8% of CP and 64.3% of IVDMD for *Acacia farnesiana*. Goel *et al*. (2015) found IVDMD values between 56.9% and 65.7% for *Acacia nilotica*. Ortíz-Domínguez *et al*. (2017) found IVDMD values of 44.5% for *Acacia pennatula*.

***Dalea* spp. (CP: 17.7%, IVDMD: 74.9%)**. Huang *et al*. (2015) reported 13.3% (N×6.25) of CP in *Dalea purpurea*, and Peng *et al*. (2020) reported 70.5% of IVDMD in *Dalea purpurea*.

***Desmodium* spp. (CP: 12.3%, IVDMD: 64.9%)**. Tessema and Baars (2006) reported CP values of 23.1% in *Desmodium uncinatum*, and Chamorro *et al*. (2005) reported an average of 76.4% of IVDMD in *Desmodium barbatum*.

***Leucaena leucocephala* (CP: 14.1%, IVDMD: 70.4%)**. Gutiérrez *et al*. (2005), found 25% of CP in *Leucaena leucocephala*. Castrejón *et al*. (2017) reported 17.6% of CP and 74.9% of IVDMD for *Leucaena leucocephala* in Paso del Toro, Veracruz, six weeks after regrowth. Ortíz-Domínguez *et al*. (2017) found 46.4% of IVDMD for *Leucaena leucocephala*.

***Phaseolus vulgaris* (CP: 11.7%, IVDMD: 77.5%)**. Alatorre-Hernández *et al*. (2018) found 11.7% of CP and 49.4% of IVDMD for *Phaseolus acutifolius*. Vojtíšková and Kráčmar (2013), found 41.0% of IVDMD in *Phaseolus vulgaris*.

***Tephrosia vicioides* (CP: 11.8%, IVDMD: 70.2%)**. Ojo *et al*. (2012) reported 13.6% of CP for *Tephrosia bracteolata* 20 weeks after sowing, and Chamorro *et al*. (2005) reported 80.5% of IVDMD in *Tephrosia cinerea*, similar and superior when compared to the values reported in this study.

In general, the majority of the values of the variables in this study were 10% higher or lower than those in the literature consulted. For example, Tessema and Baars (2006) found values of NDF (46.50%) and ADF (35.4%) in *Desmodium uncinatum*, which are lower than those reported in this study (Table 2). For the Ash variable, Ncube *et al*. (2017) found values of 4.3% in *Acacia angustissima*, and Ojo *et al*. (2012) found values of 5.3% in *Tephrosia bracteolata*, similar to those in this study (Table 3). Ojo *et al*. (2012) reported 13.6% of CL and 2.2% of EE in *Tephrosia bracteolata*. García *et al*. (2008) reported ADF levels of 25.4% for *Leucaena* spp., while in this study 30.9% was obtained (Table 2). In most cases the values of CL and Ash in the present study were similar; those of EE, CP and NFD were lower, and ADF and IVDMD were higher than those reported in the literature for the same species of Fabaceae.

CONCLUSIONS

Dalea spp. was the best forage species. With *Desmodium* spp. high supplementation with protein and non-fibrous carbohydrates should be used; and with *Dalea* spp. supplementation could be less or none. For the rest of the species that were not in the extreme positions, moderate supplementation could be used.

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Prediction of Milk Production per Cow Lactation in the Mexican Tropics

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ABSTRACT

Objective: To evaluate non-linear and linear mathematical models used to estimate milk production per lactation, at different frequencies of milk weighing from records of Holstein (Ho), Brown Swiss (BS) cows and their crosses with Zebu (Z).

Design/Methodology/Approach: The models evaluated were: Wood, Wilmink and Linear Interpolation. Daily records of milk production from 471 lactations of 72 cows were used; 1,884 records were created with frequencies of weekly, biweekly and monthly milk production. The following were included in the statistical model: the genotype (Ho×Z and BS×Z), birth season (rainy and dry), and number of lactation (1 and 2) with double and triple interactions. The statistical analyses were performed with GLM from MINITAB v17. The means were compared with Tukey's test.

Results: No differences were found (P≥0.05) between the models for the average milk production per lactation in kg, obtained from daily measurements or estimated from weekly, biweekly and monthly data, although for the factors of birth season, number of lactation, and genotype they showed differences (P≤0.05) in milk production per lactation.

Study Limitations/Implications: Daily records of milk production are necessary to obtain production per lactation; the models applied predict milk production in a similar way in different frequencies of weighing in Holstein, Brown Swiss cows and their crosses with Zebu.

Findings/Conclusions: The models used allow predicting the milk production per cow in a similar way in different frequencies of weighing.

Keywords: Wood, Wilmink, Linear Interpolation, Prediction of milk production.

INTRODUCTION

A common characteristic of most milk production systems in Mexico, particularly in tropical zones with double-purpose cattle is the absence of production records. An adequate analysis is difficult to make without accurate information of the milk production records. Keeping track of the

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daily or weekly production in each milking per cow is tedious and costly, and for this reason, an alternative is to use production records or controls at regular intervals during the lactation, with which the total production is calculated or estimated (Van Raden *et al*., 1999; Geary *et al*., 2010). In Mexico there is a lack of information generated in the double-purpose systems. The mathematical models applied to the dairy industry constitute analysis tools that contribute to understanding the dynamic of the systems based on static information (Fernández and Saad de Schoos, 1999). One of the estimators to evaluate the productivity of a herd is the average of milk production per cow and lactation, which helps to establish programs of genetic and productive improvement. The implementation of a methodology that leads to monthly measurements or even less frequently would allow predicting the milk production per lactation, with the information generated through the use of non-linear and linear models (Van Raden *et al*., 1999). This results in a more economical activity that in addition can promote the use of more productive records, with which the cattle can be evaluated and genetic and productive programs could be developed in the livestock herds.

Mechanistic models have been developed (Pollott, 2000; Wood, 1967) or polynomial empirical models (Schaeffer *et al*., 2000) which allow predicting milk production or of its components, as function of time. The implementation of these models has allowed advances in the productivity of dairy cattle in many countries (Camerón, 1997). There is no perfect or complete model, since the behavior can depend on details of the population under study and the data used, and can vary by race, number of birth and lactation, which is why continuous or multiple ways need to be tested. The objective was to compare and evaluate different mathematical models that estimate milk production per lactation, based on different frequencies of records in the weight of milk in the Holstein (Ho), Brown Swiss (BS) breeds and their crosses with Zebu (Z), in Mexico's tropical zone.

MATERIALS AND METHODS

This study was carried out in the tropical dairy module of the "La Posta" Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias), in Paso del Toro, Veracruz, Mexico (Km 22.5 Veracruz-Córdoba highway, 19° 00′ 49″ N and 96° 08′ 19″ W, at 12 m of altitude). The climate is tropical sub-humid Aw_2, with average relative humidity of 77.4% and annual precipitation of 1461 mm (Vidal, 2005). The module has 25.4 ha, of which 16.4 ha are pastures established with African stargrass (*Cynodon plectostachyus*); 8.0 ha were destined to sorghum (*Sorghum bicolor* L.) fodder production and 1.0 ha for the facilities of the module.

The cows were managed in nocturnal rotational grazing and daytime stabling. They were milked mechanically twice per day, at 6:00 and at 16:00 h. In each milking the dairy production was measured and recorded daily. During milking, the cows were complemented with 2 kg of a concentrate elaborated in the La Posta Experimental Field, with 16% of Raw Protein and 70% of Total Digestible Nutrients. Sorghum ensilage was offered in the pens (20 to 25 kg in Humid Base), mineral salts, and free access to fresh and clean water every day during the research period.

The variables of study were the following:

- Milk production per lactation (kg days^{-1} in lactation).
- Milk production per lactation, obtained from daily measurements.
- Milk production per lactation, estimated taking weekly data.
- Milk production per lactation, estimated taking biweekly data.
- Milk production per lactation, estimated taking monthly data.

The evaluation of the different prediction models of milk per lactation was made by comparing their estimations with those obtained from the daily milk production records from 1998 to 2004 of Ho, BS cows and their crosses with Z. The records were refined by eliminating cows of unknown genotype and incomplete lactations. The database finally included 471 lactations of 72 cows and a total of 1,884 records.

The explanatory factors included were: genotype, birth season, number of lactation, mathematical model and frequency of data recording. The effect of the genotype had two levels: Level 1 of Ho×Z bred cows, and level 2 of BS×Z bred cows. The birth date was classified into two seasons: dry season and rainy season; the lactations were grouped into first and second or more lactations; the models included were Wood (1976), Wilmink (1987) and linear interpolation (Sargent *et al*., 1968).

The Wood model is described with the following equation:

$$y=ax^{b}\,e^{-cx}$$

where y = daily milk production; x = days in lactation; a = production at the beginning of the lactation; b = parameter that explains the rate of increase before the peak of lactation; c = parameter that explains the rate of decrease after the peak of lactation; e = base of natural logarithm.

In the model proposed by Wilmink, the milk production estimated was calculated as:

$$y=a_o+a_1t=a_2e^{(-0.05t)}$$

where y = milk production in a time interval; a = coefficients (parameters) to be estimated; t = time.

In the Linear Interpolation Model (Sargent *et al*., 1968), milk productions per lactation were calculated through the formula:

$$y=\sum_{i=1}^{n}\left[\left(INT_i-1\right)*\,y_i+\left(INT_i+1\right)*\,y_{i+1}\right]/2$$

where: y = milk production; y_i = i-th production (daily, weekly, biweekly or monthly); INT_i = interval in days between productions (daily, weekly, biweekly or monthly) y_i and $y_i + 1$; n = total number of productions (daily, weekly, biweekly or monthly).

The weekly, biweekly and monthly milk production records were obtained from daily weighing. The total milk production of each cow per lactation (average of 300 d) was obtained from daily weighing. For the models by Wood and Wilmink, the intermediate days between measurements were simulated, to add and obtain the results from the estimations of the total milk productions per lactation. These were run with the Scientist® software, using the Powell algorithm and for the linear method the Excel software was used. The parameters were determined by cow, and this way equations were obtained with their respective coefficients and later the milk production was estimated per lactation per cow for both models proposed, using the simulation module of the software. The statistical parameters were obtained, such as the Model Selection Criterion (MSC) and the coefficient of determination (R) of the statistical model of the same software.

The linear model for the statistical analysis was the following:

$$Y_{ijklmo} = \mu + G_i + L_j + E_k + M_l + F_m + G_iM_l + G_iF_m + E_kM_l + E_kF_m + L_jM_l + L_jF_m + M_lF_m + G_iM_lF_m + E_kM_lF_m + L_jM_lF_m + \xi_{(ijklmo)}$$

Y_{ijklm} = o-th observation of the milk weighing; μ = population mean; G_i = effect of the i-th genotype (i = 1 and 2); L_j = effect of the j-th number of lactation (j = 1 and 2); E_k = effect of the k-th birth season (k = 1 and 2); M_l = effect of the l-th estimation method (l = 1, 2 and 3); F_m = effect of the m-th frequency (m = 1, 2, 3 and 4); GM_{il} = interaction between the genotype and the estimation method; GF_{im} = interaction between genotype and frequency of milk weighing ; EM_{kl} = interaction between birth season and estimation method; EF_{km} = interaction between season and frequency of weighing; ML_{lj} = interaction between estimation method and number of lactation; LF_{jm} = interaction between number of lactation and frequency of weighing; MF_{lm} = interaction between estimation method and frequency of weighing; GMF, EMF and LMF are triple interactions; and $\xi_{(ijklm)}$ = experimental error $N(0,\sigma 2)$.

The data were analyzed with the statistical package MINITAB version 17, with the GLM routine (General Linear Model). The means comparison was performed with Tukey's test, with α of 0.05.

RESULTS AND DISCUSSION

Milk production per lactation of Ho×Z cows, fed in a tropical dairy system, was 3,130 kg, which was higher in 26% ($P\leq0.05$) than BS×Z cows with 2,489 kg. Likewise, in milk production starting with the second lactation (2,967 kg), they showed higher production, outperforming the cows from the first lactation in 12% (2,652 kg; $P<0.05$), and these values agree with what was reported by Cañas *et al*. (2011) and Carvajal-Hernández *et al*. (2002), who reported that first birth cows are less productive than cows with 2 to 5 births. During the rainy season, the highest milk production per lactation was found, of 2,951 kg *versus* 2,268 kg, which was the production of cows during the dry season, this being a significant difference ($P\leq0.05$). A higher milk production is favored during the rainy season, which is related to the absence of heat stress, an increase of food consumption and an increase of blood flow toward the gastrointestinal tract that increased the nutrient flow toward the mammary gland and therefore increases the quality of milk (Ponce, 2009; West, 2003).

Table 1 shows the milk production means per lactation of Ho and BS cows crossed with Z, for simple purposes, prediction model and recording frequency or milk weight. A difference was not observed (P≥0.05) in the prediction of milk production per lactation, between the Wood, Wilmink and Linear models, which agrees with what was reported by Stanton *et al*. (1992) and Silvestre *et al*. (2009) who used the Wood model to predict milk, fat and protein production per lactation of the cows. Regarding the effect of frequencies of milk weighing, it was observed that there is no difference (P≥0.05), which is why weighing the milk monthly during the whole lactation and covering the entire curve, that is, from the first month until the end of the lactation, would be enough, assuming this does not include incomplete lactations. Therefore, the prediction of milk production per lactation of Ho×Z and BS×Z cows would have reliability higher than or equal to 95% with the three models studied. This agrees with what was reported by López *et al*. (1991), where they conclude that the Wood model predicts milk production per lactation reliably in pure Holstein cows, in a monthly frequency of weighing.

The results (Figure 1a) with the prediction models in genotypes (Ho×Z and BS×Z) and the birth season (rainy and dry) evidenced how the three models estimate the milk production per lactation similarly (P≥0.05) in cows

Table 1. Effect of the model and frequency of milk weighing (kg) in the prediction of milk production per lactation, of Holstein and Brown Swiss cows crossed with Zebu, in a Tropical Dairy system.

Milk Prediction Models				
Wood	Wilmink		Linear Interpolation	SEM
2806.6[a]	2804.7[a]		2818.5[a]	59.3
628	628		628	N
Milk Weighing Frequency				
Daily	Weekly	Biweekly	Monthly	SEM
2814.7[a]	2811.9[a]	2792[a]	2820.7[a]	68.5
471	471	471	471	N

SEM = Standard Error of the Mean; N = observations. Different literals in the same column indicate significant difference, Tukey (P≤0.05).

with genotype of the Ho×Z crosses and productions higher than 3,000 kg and similar behavior with cows of the BS×Z genotype, with productions close to 2,500 kg. Although there is a statistical difference (P≤0.05) between the genotypes for each model, it was because of the genotype effect and not the model; that is, the models predict the milk production independently of the genotype. Figure 1b shows the same behavior, without difference (P≥0.05) in the milk production by birth season, and the three models predict the milk production, although there is statistical difference from the effect of the birth season.

Figure 2 presents the graphs of the prediction models in the number of lactation (a) and the frequency of milk weighing (b). Figure 2a indicates how the three models similarly predict milk production in lactation one and two

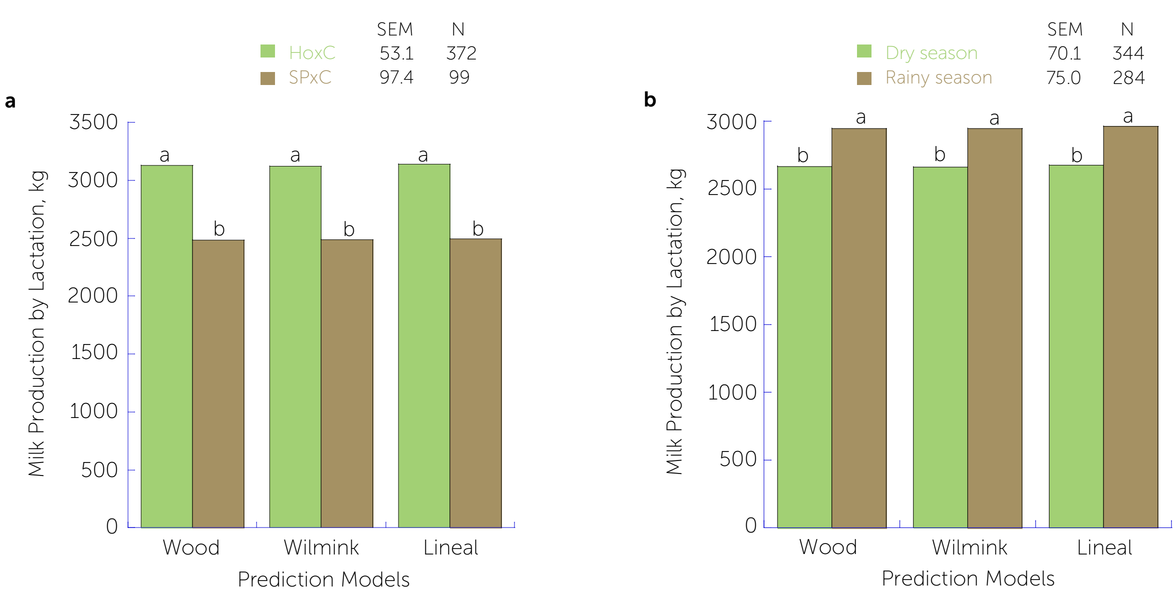

Figure 1. a) Prediction models in the Ho×Z and BS×Z genotypes, b) Prediction models in the birth season (rainy and dry).

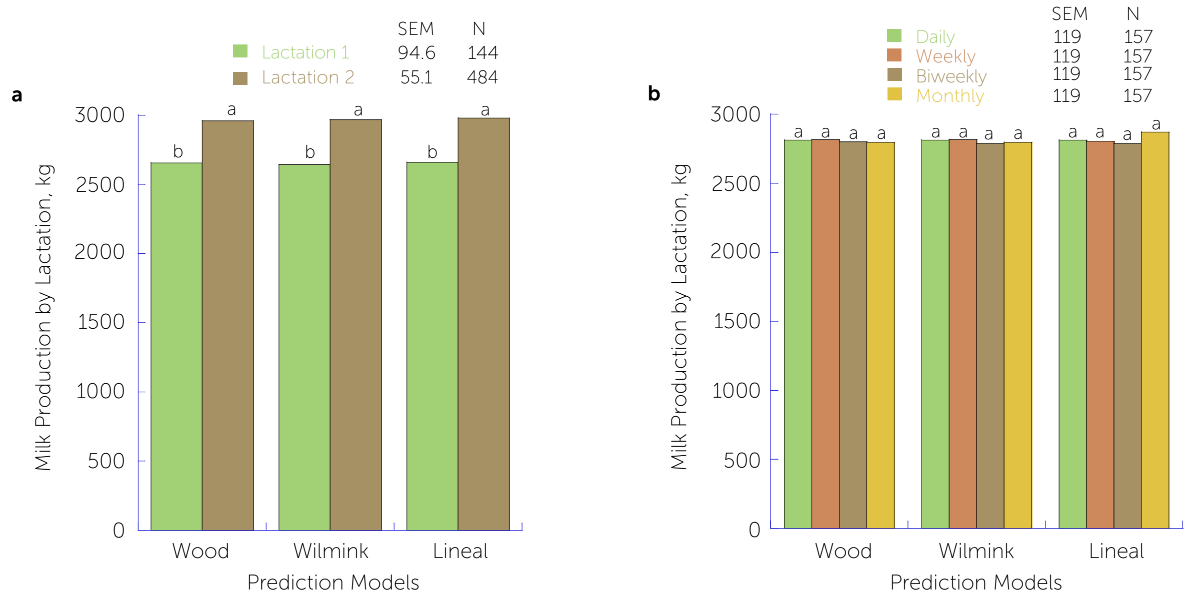

Figure 2. a) Prediction models in the number of lactations, b) Prediction models in the frequencies of weighing.

(P≥0.05). Figure 2b shows that the frequency of milk weighing does not have a significant effect on the three models or between them (P≥0.05), which confirms what was found when the simple effects were analyzed both by model or by frequency; that is, the three models can be used to predict the milk production per lactation of Ho×Z and BS×Z bred cows in a tropical dairy system, weighing the milk monthly.

Table 2 shows the comparison of the Wood, Wilmink and Linear Interpolation models in the milk production per number of lactation in the different frequencies of weighing of the milk of bred cows, highlighting that there is no significant difference (P≥0.05); that is, the non-linear (Wood and Wilming) and linear (Linear Interpolation) models predict the milk production of Ho×Z and BS×Z bred cows in the tropical dairy system with a certainty of 95%, regardless of the number of lactation and frequency of weighing.

Table 3 shows the comparison of the Wood, Wilmink and Linear Interpolation models in milk production per genotype in different frequencies of milk weighing from bred cows, highlighting that there is no significant difference (P≥0.05); that is, the non-linear (Wood and Wilming) and linear (Linear Interpolation) models predict the milk production of Ho×Z and BS×Z bred cows in the tropical dairy system with an accuracy of 95%, regardless of the number of genotype and frequency of weighing.

Table 4 shows the comparison of the Wood, Wilmink and Linear Interpolation models in the milk production per birth season (rainy and dry) in the different frequencies of milk weighing of the bred cows, highlighting that there is no significant difference (P≥0.05); that is, the non-linear (Wood and Wilmink) and linear (Linear Interpolation) models predict the milk production of Ho×Z and BS×Z bred cows in the tropical dairy system with certainty of 95%, regardless of the birth season and frequency of weighing.

Table 2. Milk production (kg) per lactation of Ho×Z and BS×Z bred cows, with different prediction models, number of lactation and frequencies of milk weighing.

Frequency	Milk Prediction Models					
	Wood		Wilmink		Linear Interpolation	
	Lactation		Lactation		Lactation	
	1	2	1	2	1	2
Daily	2657[a]	2972[a]	2657[a]	2972[a]	2657[a]	2972[a]
Weekly	2659[a]	2974[a]	2665[a]	2968[a]	2650[a]	2955[a]
Biweekly	2649[a]	2953[a]	2601[a]	2977[a]	2621[a]	2954[a]
Monthly	2655[a]	2933[a]	2645[a]	2951[a]	2707[a]	3032[a]
N	121	36	121	36	121	36
SEM	189	110	189	110	189	110

SEM = Standard Error of the Mean; N = observations. Equal literals in the same column indicate that there is not significant difference, Tukey (P≤0.05).

One of the important criteria to define whether a model adjusts well to the data, allowing with this to make a good prediction, is the Model Selection Criterion (MSC), which is none other than a modification to the Akaike

Table 3. Milk production (kg) per lactation, of crossed cows with different prediction models, genotypes and frequencies of milk weighing.

Frequency	Milk Prediction Models					
	Wood		Wilmink		Linear Interpolation	
	Genotype		Genotype		Genotype	
	Ho×Z	Bs×Z	Ho×Z	Bs×Z	Ho×Z	Bs×Z
Daily	3135[a]	2494[a]	3135[a]	2494[a]	3135[a]	2494[a]
Weekly	3158[a]	2475[a]	3146[a]	2488[a]	3136[a]	2469[a]
Biweekly	3115[a]	2487[a]	3082[a]	2496[a]	3114[a]	2461[a]
Monthly	3105[a]	2484[a]	3124[a]	2472[a]	3177[a]	2561[a]
N	124	33	124	33	124	33
SEM	106	195	106	195	106	195

SEM = Standard Error of the Mean; N = observations. Equal literals in the same column indicate that there is not significant difference, Tukey (P≤0.05).

Table 4. Milk production (kg) per lactation, of Ho×Z and BS×Z bred cows with different prediction models, birth season (rainy and dry) and frequencies of milk weighing.

Frequency	Milk Prediction Models					
	Wood		Wilmink		Linear Interpolation	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Daily	2677[a]	2952[a]	2677[a]	2952[a]	2677[a]	2952[a]
Weekly	2691[a]	2942[a]	2683[a]	2951[a]	2668[a]	2937[a]
Biweekly	2659[a]	2943[a]	2639[a]	2939[a]	2631[a]	2944[a]
Monthly	2637[a]	2951[a]	2658[a]	2939[a]	2727[a]	3012[a]
N	71	86	71	86	71	86
SEM	140	150	140	150	140	150

SEM = Standard Error of the Mean; N = Observations. The same letters in the same column indicate that there are no significant differences, Tukey (P≤0.05).

Table 5. Model Selection Criterion (MSC) and coefficient of determination (R) in the Wood and Wilmink models, in weekly, biweekly, and monthly frequency.

Milk Prediction Models	MSC	R	SEM
Wood	0.9364[a]	0.6632[a]	0.02286
Wilmink	0.8972[a]	0.6478[a]	0.02286
Milk Weighing Frequency			
Weekly	0.9546[a]	0.6280[a]	0.02802
Biweekly	0.8819[a]	0.6611[a]	0.02802
Monthly	0.9139[a]	0.6774[a]	0.02793

The same letters in the same column indicate that there is no significant statistical difference. Tukey's means test (P≥0.05). SEM = Standard Error of the Mean.

criterion. It is observed that there is no statistical difference (P≥0.05), on the Wood model (0.93) and the Wilmink model (0.90) in the different intervals of weekly, biweekly and monthly intervals, which indicates that both models are adjusted similarly to the data and help describe the lactation curve of Ho×Z and BS×Z bred cows in a tropical dairy system with reliability higher than or equal to 95% (Table 5). In the coefficient of determination R, it is an estimator that helps us establish together with the MSC the adjustment of the data to the models. The results did not indicate difference (P≥0.05) between the Wood and Wilmink models. Regarding the frequencies of milk weighing, no significant difference was found (P≥0.05) (Table 5).

CONCLUSIONS

The Wood, Wilmink and Linear Interpolation models can estimate similarly the milk production per lactation, in Holstein and Brown Swiss cows and their crosses with Zebu, whether during rainy season or dry season, and in different frequencies of milk weighing. The frequency of milk weighing from the cows analyzed can be carried out weekly, biweekly, or monthly, using any of the models: Wood, Wilmink and Linear Interpolation.

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AGRO PRODUCTIVIDAD

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Characterization of subsistence sheep farming: The case of three communities of the municipality of Tlahuapan, Puebla, Mexico

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ABSTRACT

Objective: to characterize household units for sheep production at three localities in the Santa Rita Tlahuapan municipality, Puebla.

Design/methodology/approach: semi-structured interviews conducted with 38 producers. Variables of the producer profile, crop production and herd management were analyzed using descriptive statistics, cluster analysis and variance analysis.

Results: four groups of producers were identified; most of them (92%) were classified as small producers, with 24 to 36 sheep and low production of crop forage. The producer's average age was 55 years, with an average family size of four. Economic savings is the main objective for this production and family labor is used exclusively in sheep farming activities. The animals are housed in rustic pens, with no difference in their age, sex or physiological stage. Their main food source is pastures forage and cultivation areas; mostly supplemented (92%) with mineral blocks and common salt.

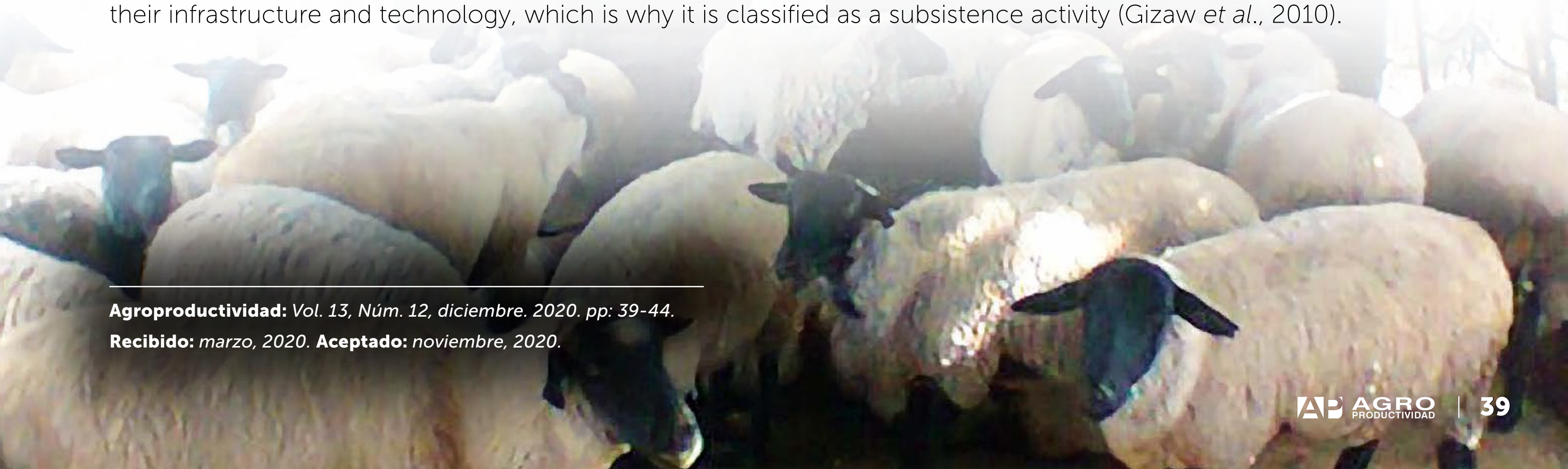
Limitations/Implications: the lack of producer records and social mistrust to some degree to provide information.

Findings/conclusions: the assessed productive units are for subsistence, having sheep farming as a secondary activity, with low productive parameters. The poor housing practices, the scarcity of own grazing lands, and the animal's nutritional stress are identified as the main factors that stagnate sheep farming in the study area.

Keywords: sheep, subsistence producers, production systems, typology.

INTRODUCTION

Worldwide, sheep production makes marginal contributions to the global meat and milk market; however, its products are basic for rural families with limited economic resources in various regions, mainly in developing countries (Alonso *et al*., 2010). In this regard, low purchasing power producers use sheep as a source for savings, income, and as a genetic resource inherent to family food security (Tesfay and Kumar, 2014). However, it is considered a secondary activity, since minimum inputs are invested, nor in their infrastructure and technology, which is why it is classified as a subsistence activity (Gizaw *et al*., 2010).



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Productivity, in most cases, is markedly low due to genetic, environmental, and institutional factors (Gizaw *et al*., 2010), with the main critical points being the scarcity of grazing land, poor nutrition and scarce suitable housing infrastructure (Legesse *et al*., 2008; Lakew *et al*., 2017). Due to poor management, animals are generally prone to infectious diseases, uncontrolled reproduction and conception rates, as well as the lamb's births set to non-strategic or forage-scarce seasons, resulting in high mortality.

According to Kechero *et al*. (2013), if the main critical points of subsistence sheep production are identified and characterized, it is possible to improve and increase the potential of the system. Examples from this in Africa (Legesse *et al*., 2008; Gizaw *et al*., 2010), have suggested selection criteria and genetic improvement strategies, local forage production, supplementation and veterinary medicine plans, practices that may result in positive changes in the herds.

In the temperate zones of Mexico, in the state of Puebla, at the municipality of Santa Rita Tlahuapan, sheep farming is a traditional and deeply rooted practice, of which very little has been documented about the identification, characterization and documentation of their production system. Therefore, for the region, critical points and management plans that could be established for production improvement to increase the economic income of the producers are unknown. Based on the above, the objective of this study was to describe the productive characteristics of sheep farming, its management practices, as well as to identify and prioritize the limitations that impact the development of production, to propose possible strategies and opportunities for herd improvement.

MATERIALS AND METHODS

Study area. The evaluation took place at the municipality of Santa Rita Tlahuapan, Puebla, Mexico (19° 15′ 36″ and 19° 27′ 54″ LN and 98° 29′ 18″ and 98° 40′ 06″ LW), 2,640 m altitude, with temperate and semi-cold subhumid climate.

The communities of La Preciosita, Santa Cruz Moxolahuac and San Juan Cuauhtémoc were selected for having a greater sheep number. The sampling frame was made through guided visits and participatory meetings with the producers, and a Neyman stratified random sample (Olayiwola *et al*., 2013) of 38 family units was obtained.

Sheep farming characterization. A questionnaire was applied with variables on the profile of the producer, agricultural resources availability, social and economic aspects, herd management and facilities, among others. Direct coproparasitoscopic tests were performed on 400 sheep (≥4 months of age, indirectly estimated by the dentition, including all males and stallions). An eggs per gram counting from the feces was performed following the McMaster technique, using the total count per 100/number of chambers. These animals were also blood sampled to test for Brucella abortus using the card method (Official Mexican Standard NOM-041-ZOO-1995).

Statistical analysis. According to the methodology described by Köbrich *et al*. (2010), the production units were characterized, and the producers were typified utilizing a cluster analysis. With the obtained data, an analysis of variance was conducted using the PROC GML procedure and a means comparison with the Tukey test with α=0.05 in the SAS (2002) statistical software.

RESULTS AND DISCUSSION

Producers profile: Many producers (89.5%) have agriculture as their main activity, 5.2% stated to be merchants and 5.3% dedicated exclusively to livestock activities. The average number of members per family was four.

Agricultural crops: The main crops for family sustenance are corn, beans, oats, and wheat, produced during the rainy season. From the corn and beans, 70% of the production is used for self-sustenance and 30% for sale. As for oats and wheat, 65% is used for livestock fodder and 35% for sale. Land tenure for agricultural activities is mainly communal (84%), the rest is private.

Sheep farming characteristics: The herds are mainly managed by men (84.9%), women have little participation (10.6%). The average time that a producer has dedicated himself to this activity is 19.5 years. In addition to sheep, there are also goats, poultry, horses, and donkeys to a lesser extent. The average size of the herds was 37 sheep mainly composed of "vientres" and "primalas". The breeds are mixed-race type, with some crosses with Suffolk-Dorper and Hamshire-Dorper breeds.

Animal nutrition: It is based on pasture grazing (44.7%) and post-harvest agricultural areas (28.9%) mainly, and some other crops such as wheat (13.1%), barley (2.6%), and alfalfa (7.8%). Out of the crop residues, the most used is corn stubble, used all year round due to its high availability. The average time spent grazing is 3 hours with an interval of 2-4 hours. Of the producers, 92.1% supplement the entire herd, some only the stallions (2.6%) during the breeding season and others do not carry out this activity (5.3%). The most common supplements are mineral building blocks, common salt, vitamins, and some forage grains.

Housing: The housing pens are mostly rustic, made with sheet roofs (cardboard or galvanized), wooden fences, wire, mesh and dirt floors, with feeders and drinkers. The herds are usually kept in a single enclosure pen where animals from different physiological stages coexist, most lack other types of pens (Figure 1), such as pens for sick animals, handling pens, births corrals, or even feedlots.

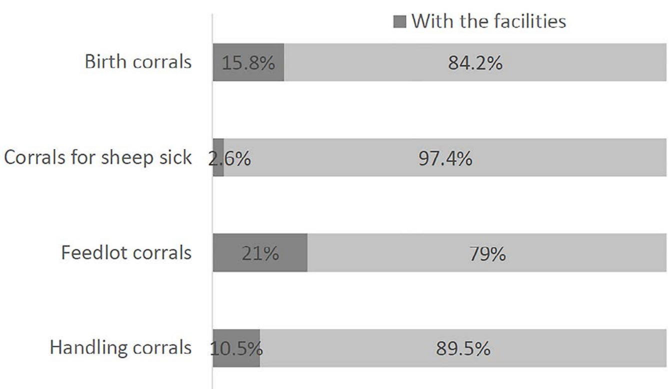

Figure 1. Percentage and type of facilities within the sheep production units at Santa Rita Tlahuapan, Puebla, Mexico.

Table 1. Gender and the average quantity of eggs (± standard error) per gram of sheep feces in the herds of the assessed communities.

Genus	Average	Minimum	Maximum
Trichuris spp	28.7 ± 9.0	0	200
Eimerias spp.	472.8 ± 85.0	10	2350
Nematodos spp.	660 ± 65.2	50	1600
Toxacara spp.	11.4 ± 5.8	0	150

Health: Most of the producers mentioned that they perceive the health of their herds to be between bad (44.7%) and fair (39.7%); only 15.6% perceive it as good. Although they mentioned these had been treated for parasites, high abundances of internal parasites were detected, mainly Nematodes and *Eimeirias* genera (Table 1). *Brucella* was not detected.

Mortality: Mortality is less frequent in adult animals than in lambs under one year of age (Figure 2). The main causes are poor nutrition, the poor conditions of the animal housing areas as they provide poor protection against the weather and the lack of preventive medicine.

Producer's typology: Four groups were identified (Figure 3 and Table 2):

1) Small producers of advanced age: the most prevalent (19 production units, 50% of the sample), made up of older producers (65 years on average), low schooling (only primary school), little animal inventory (24 sheep on average and an average three from other ruminants) and fewer crops for sheep feeding (two).
2) Young small producers made up of 16 production units (42.1% of the sample), of younger producers (44 years old).
3) Producers with the high sheep number and sales made up of two production units (5.2% of the sample). The difference between this group and the previous two is the greater number of sheep in the herds (182 animals' average) and the highest-selling.
4) Producers with forage crops: one production unit (3% of the sample). The particularity of this unit is that it has the same number of sheep as group 1 and 2; however, in the unit, the producer sows different forage crops (12) to feed their sheep.

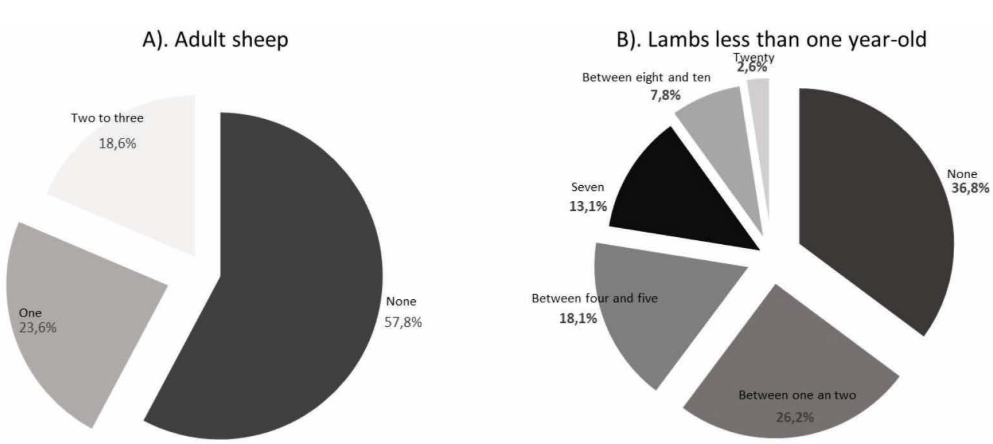

Figure 2. Mortality of animals per herd in sheep production units in three communities of Santa Rita Tlahuapan, Puebla. A) In adult animals; B) in lambs less than one-year-old.

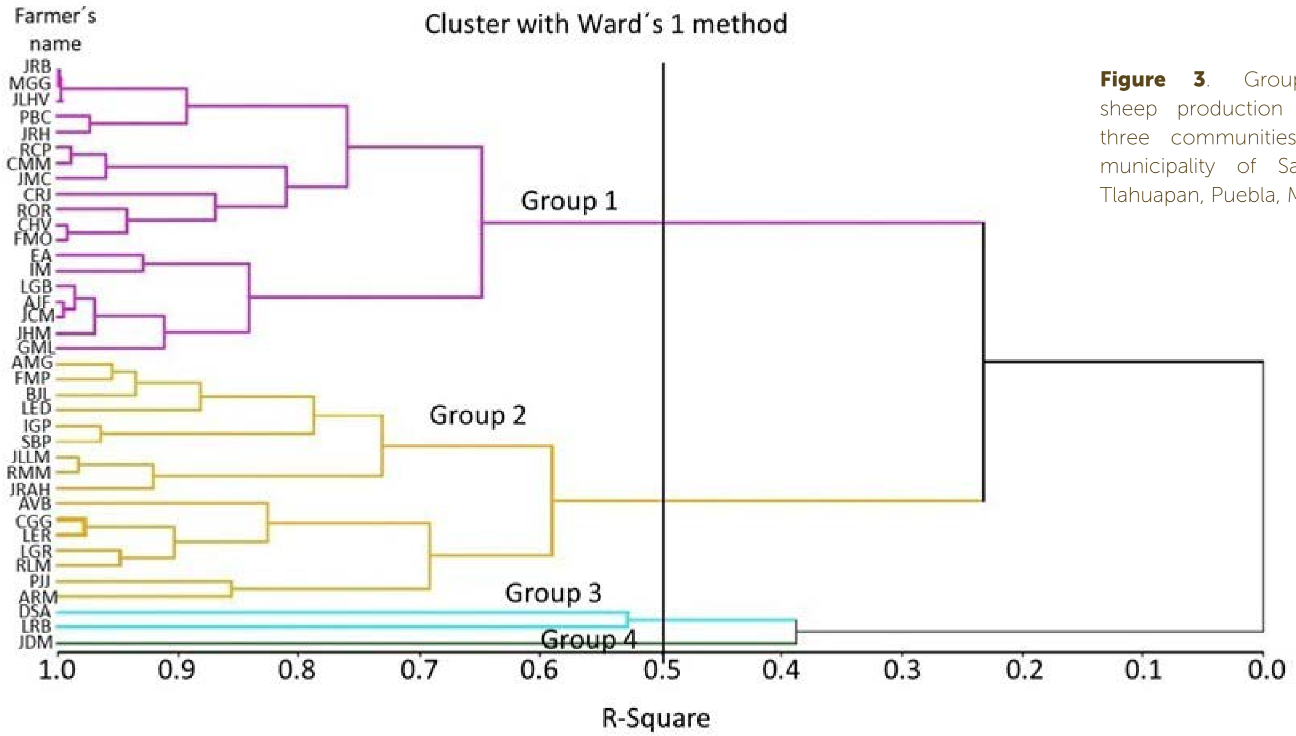

Figure 3. Grouping of sheep production units of three communities at the municipality of Santa Rita Tlahuapan, Puebla, Mexico.

Table 2. Number of animals, number of forage crops and number of sales made in each group of producers.

Variates	Groups				Average
	1	2	3	4	
Age	65a	44b	54a	42b	55.0
Years of school	2b	7a	8a	6a	4.0
Sheep	24b	35b	182a	36b	37.0
Other ruminats	3b	2b	0b	120a	6.0
Loss of animals	3c	4c	15b	22 a	4.5
Forage crops	2b	4b	3b	12 a	2.8
Sales	4c	3c	30a	15b	4.8

a, b Means in each row with the same letters are not statistically different (Tukey, α=0.05).

Characteristics of the production units

The producer grouping analysis indicated that the majority of the assessed sheep farmers (95%) are "small-scale", classified within what Gizaw *et al*. (2010) and Tesfay *et al*. (2014) call subsistence production systems. These systems are characterized by having few dietary inputs, little infrastructure in the production units and, overall, low livestock productivity, kept as a source of economic savings (Legesse *et al*., 2008).

Producer's profile: Following the classification by Iniesta-Arandia *et al*. (2014), the producer's age in the present study is still in productive age. However, at this age (55 years), limitations likely arouse when adopting new management practices and herd augmentation. Morris *et al*. (2017) discusses that older agricultural producers with low education may have greater limitations to access and adopt new technologies, mainly due to the gaps made by rapid progress, their reduced social network and changes in perspective when entering old age. Likewise, according to Legesse *et al*. (2008) sheep producers with higher education (9-11 years) tend to improve herd management practices, generate specific production objectives, and seek to improve the profitability of their subsistence sheep farming. Based on this, the older age and the low average schooling (5 years) of the evaluated sheep breeders may be reasons for these production units to be marginalized.

Land tenure and herd size: Similar results regard land tenure are reported by Tilahun *et al*. (2006) and Kechero *et al*. (2013), who points out that most of the producers lack their own grazing lands, the available agricultural areas are mainly for basic crops sowing, and there is a reduced area of communal pastures. This helps to explain the low animal number (37 sheep) for each production unit in the current study, factors that, according to Legesse *et al*. (2008) and Tesfay and Kumar (2014) conditions the herd size increase. Smaller herd sizes (9-11 sheep per unit) than the current investigation are reported by Tilahun *et al*. (2006); while Hernández *et al*. (2019) report similar size to that of the current study

(30-33 sheep), a number linked to the scarcity of own grazing lands.

Housing: Tilahun *et al*. (2006), Kechero *et al*. (2013) and Lakew *et al*. (2017) indicate in their studies that most sheep producers (85-88%) house their animals in rustic pens, together with other ruminants (goats and cattle); with little or no and few cleanings. According to Kechero *et al*. (2013), with this, diseases, infections, and parasites (internal and external) of economic importance are increased, a similar situation found in the present study. This may be aggravated by the lack of clinical knowledge of the producers, the low veterinary coverage in the study area, coupled with the price of possibly expensive treatments.

The fact that the herds are not separated by batches, according to Tilahun *et al*. (2006), Tesfay and Kumar (2014) and Lakew *et al*. (2017) induces early and uncontrolled reproduction in females, with extreme inbreeding cases, low lamb weights at birth, and low growth rates. In addition to this, the birth season generally occurs during unfavorable times (*i.e*., during drought or forage shortages) for newborn lambs. In these cases, likely, the females will not reach their maximum colostrum and milk production levels, which in turn, lead to poor lamb nutrition and high lamb mortality (34-57%) before weaning. The latter could explain the higher mortality of under one year of age lambs compared to adults in the current study.

Feeding: Grazing in degraded rangelands and with low nutritional value forage species and post-harvest crop areas is the main source of food for sheep in several regions of the world. It can contribute in large proportions to sheep feeding in family productive units (Gizaw *et al*., 2010). However, Legesse *et al*. (2008) and Gizaw *et al*. (2010) mention that forage in these sites is seasonal, and when scarce, it has low crude protein and digestibility levels. In this way, by not meeting the nutritional requirements, weight gains and body condition are negatively affected. Therefore, sheep must be supplemented; still, it is only done with sporadic applications of vitamins and mineral salts supply. This was found in the present study and coincides with that reported by Tilahun *et al*. (2006) where producers supplement the sheep with common salt and some other local minerals from the region, but even so, a good diet is not achieved.

CONCLUSIONS

Sheep farming in the study area is a subsistence mixed production system (crops-livestock). The main limiting factors, directly from the producers, that can stagnate production are age and schooling, which have certain implications for technological changes. The scarce availability of forages, the nutritional stress of the animals and the deficient sanitation and housing practices are also limiting.

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Egg Production of Hens Fed Homemade or Commercial Feed in a Cage-Free System

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ABSTRACT

Objective: To evaluate two types of feed (homemade or commercial), in egg production (EP, %), egg weight (EW, g), and egg mass (EM, g bird^{-1} d^{-1}), of hens in a cage-free system.

Methodology: Sixty hens aged 37 weeks (Rhode Island Red and Barred Plymouth Rock) were allocated to two treatments: COM, 150 g of commercial feed bird^{-1} d^{-1} and CAS, 150 g of homemade feed bird^{-1} d^{-1}. The birds were managed in a cage-free system with access to a paddock of white clover (*Trifolium repens* L.). EP, EW and EM were evaluated for 11 weeks.

Results: EP was different between treatments ($P<0.05$) in the last four weeks of observation. In this time, the COM birds laid 17 to 24% more than CAS birds. The EW produced by birds from the COM group (59.1 to 60.7 g) was greater ($P<0.05$) than that of the CAS birds (55.0 to 57.0 g). In the second half of the study period, a lower EM ($P<0.05$) was observed in the CAS treatment (24.7 to 31.8 g bird^{-1} d^{-1}) compared to the COM treatment (39.7 to. 41.8 g bird^{-1} d^{-1}).

Study Implications: The results obtained are only valid for the types of concentrate evaluated and under the specified experimental conditions.

Conclusions: The homemade feed reduces the productive performance of hens in a cage-free system in terms of EP, EW, and EM, when compared to the commercial feed.

Keywords: homemade feed, egg production, cage-free system.

INTRODUCTION

In Latin America, backyard poultry farming produces up to 70% of the eggs and poultry meat consumed by rural families (Soler and Fonseca, 2011), signaling its importance (Mottet and Tempio, 2017) and function as a tool for reducing poverty (Sonaiya, 2008; Abebe and Tesfaye, 2017). In poultry farming, animal feed can represent 60 to 90% of the production costs (Gunaratne,

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2013). Industrial poultry feed is made primarily with corn and soybean meal as energy and protein sources, respectively. However, poultry farming in developing countries oftentimes depends on the import of these ingredients (Ravindran, 2013a). In contrast to intensive production, birds in a backyard system look for feed themselves in the areas surrounding a house (food waste, insects, forage) and sometimes receive a supplement (maize, commercial feed, etc.) (Ravindran, 2013b; Sonaiya, 2008; Gerber *et al*., 2013). This suggests that their diet is not balanced and constant in time, which contrasts with the fact that a balanced diet is prerequisite to optimal productive performance of birds (Ravindran, 2013b). In the type of mentioned poultry production, access to materials used for birds' feed in an industrial production system represents an economical and logistical challenge. Therefore, practical and economical diets are needed, based on locally-available feed sources that ensure an optimal productive performance of the birds (Chadd, 2008).

The Food and Agriculture Organization of the United Nation's (FAO) Special Programme for Food Security (PESA) has strongly promoted backyard poultry production in Mexico. Through this program, close to 117,500 poultry projects were promoted nationally between 2007 and 2012 (UTN-FAO, 2013). The PESA recommended the use of homemade feed based on easily accessible ingredients in rural projects (PESA, 2005; PESA, 2007; PESA, 2010). Although there is precedent for egg production based on homemade feed (Aganga *et al*., 2003; Cahuec-Maas, 2017; Mutayoba *et al*., 2012), to our knowledge, the respective information is scarce in Mexico. A recommendation of homemade feed should be based on the information about the nutritional needs of the birds, the local availability of ingredients, their nutritional quality (Chadd, 2008), and a systematic evaluation of their effect on the productive performance of the animals (Soler and Fonseca, 2011). The objective of the present study was to evaluate the effect of the type of feed (commercial and homemade) on egg production, egg weight, and egg mass of hens in a cage-free system.

MATERIALS AND METHODS

The experiment was conducted from April to June of 2019, in the Experimental Poultry Farm of the Department of Zootechnics at Chapingo Autonomous University (km 18.5 of the Texcoco-Lechería highway, Texcoco, State of Mexico, at an altitude of 2,278 m, 19° 29' 13.7'' N and 98° 53' 48.0'' W). According to García (2004), the climate is sub-humid temperate with summer rains (C(wo)(w)b(i').

Sixty, 37-week-old hens were used [30 Rhode Island Red (RIR) and 30 Barred Plymouth Rock (BPR)] with an average weight of 2536±39 g. The birds were managed in a cage-free system: 15 h of confinement every 24 h (6:00 pm to 9:00 am) and 9 h of free access to a white clover paddock (*Trifolium repens* L.) every 24 h (9:00 am to 6:00 pm). During confinement, the hens were kept in an ambient-temperature poultry house with lateral curtains. The interior of the poultry house had eight 1.8 m^2 pens, with wood chip beds. Each pen had an automatic bell water dispenser (15 cm $bird^{-1}$), a hopper feeder (13 cm $bird^{-1}$), and nesting boxes (4 birds $nest^{-1}$). For the grazing period, the white clover paddock (established one year prior) was divided into sub-paddocks (SP) with a 1.2 m high plastic mesh and held 7 to 8 birds, considering 5 m^2 of meadow per animal. Each SP had a 5 L barrel-type water dispenser that was refilled with fresh water every 3 h. Every SP also had a usage period of 7 d, after which new SP were enabled, allowing a resting period of 21 d for every recently-used meadow area. The hens received 150 g of feed $bird^{-1}$ d^{-1}, divided in two rations of 75 g each. The water was offered *ad libitum*. The birds were subject to a lighting program of 16 h light: 8 h darkness.

Treatments and Data

Two treatments were evaluated: COM, 150 g of commercial feed $bird^{-1}$ d^{-1}, and CAS, 150 g of homemade feed $bird^{-1}$ d^{-1}. The 60 birds were randomized to form eight experimental units, which were randomly assigned one of the two treatments. The randomization was restricted so that each genotype (RIR and BPR) was equally represented among the treatments (15 birds of each genotype per treatment) and replicates. Therefore, each treatment had four replicates (two replicates of seven and two replicates of eight birds each, totaling 30 birds per treatment). According to factory values, the composition of commercial feed for adult laying hens (brand GRANJERO, CONCENTRA Consorcio Agroindustrial, S.A. de C.V., Hidalgo, Mexico), was the following: 14% minimum protein, 2% minimum fat, 5% maximum fiber, 12% maximum ash, 12% maximum moisture, 55% nitrogen-free extract.

The homemade concentrate was made based on the PESA recommendations (PESA, 2007). The composition

(%) of the concentrate was the following: ground corn (80.000), toasted and ground black bean (15.000), dehydrated guaje (*Leucaena leucocephala*) leaves (3.333), ground and toasted egg shell (0.833), sugar (0.500), and common salt (0.334). Based on these proportions and information from the literature (Mateos and Sell, 1980; Ravindran and Blair, 1992; NRC, 1994; Teguia and Fru, 2007; Hassan 2015), the nutritional composition of the homemade concentrate was estimated: 3220 kcal of metabolizable energy (ME) kg of feed^{-1}, 11% of crude protein (CP), 0.49% of lysine and 0.18% of methionine. All the eggs from each replicate and treatment were collected daily (11:00 am, 3:00 pm and 6:00 pm) to determine the values of the following variables: egg production (EP, %), egg weight (EW, g), and egg mass (EM, g bird^{-1} d^{-1}). In each replicate, the EP was calculated by dividing the number of eggs between the number of hens in the production day, and the quotient was multiplied by 100. The EW was determined, in the laying day, by individually weighing each egg in an OHAUS scale with a maximum capacity of 15 kg and a resolution of 0.001 kg (RC31P15 model, USA), and later calculating the average per replicate. Finally, the EM was estimated by multiplying the EP by the corresponding EW. Once the data for variables per production day were obtained, weekly values were then estimated.

The EP, EW and EM data were analyzed under a randomly experimental design with repeated measurements, using the SAS V.9.3 MIXED procedure (SAS, 2011). The differences between treatments were considered significant at P≤0.05.

RESULTS AND DISCUSSION

Table 1 shows means of variables associated with the productive performance of hens from 37 to 47 weeks of age. The EP varied from 61.6 to 68.7% in birds with the COM treatment, while hens from the CAS group showed values of 43.6 to 71.3% during the study period. The EP was different between treatments (P<0.05) in the last four weeks of observation, the birds in the COM treatment laid 17 to 24% more than the CAS birds.

In 11 weeks of study, the weight of eggs produced by the COM group hens (59.1 to 60.7 g) was greater (P<0.05) than that of the hens that received the homemade feed (55.0 to 57.0 g). In the end, the type of feed given to the hens had an effect on the EM of the second half of the study period and lower values were observed (P<0.05) in the flocks with the CAS treatment (24.7 to 31.8 g bird^{-1} d^{-1}) compared to the COM group (39.7 to 41.8 g bird^{-1} d^{-1}). Nevertheless, even in the weeks when the differences were not significant (P≥0.05), the birds fed with the homemade concentrate tended to have a lower EM.

When the hens were fed with homemade feed, recommended by an official agency, the flock produced fewer eggs and with lower weight, compared to birds fed with commercial feed, and thus the daily productivity per bird was also reduced. The birds with the CAS treatment tended to have a lower EP 27.3% of the experiment time. In addition, this disadvantage, when compared to the hens treated with COM was significant in 36.4% of the study period. The lowest EP in the hens treated with CAS coincides with the findings by Aganga *et al*. (2003) and Cahuec-Maas (2017), who observed that non-industrial birds fed with homemade feed (13.5 and 11% CP, respectively) reached only 20 and 86% of the production observed in birds that received commercial feed, for each case. In contrast, Mutayoba *et al*. (2012) did not find differences in the number of eggs produced when local birds were fed with commercial feed (14.6% CP/2604 kcal EM kg^{-1}) or homemade feed (15.5 %

Table 1. Adjusted means of variables associated with productive performance of Rhode Island Red and Barred Plymouth Rock hens, in a cage-free system and fed two types of feed [commercial (COM) or homemade (CAS)].

Age (weeks)	% Egg production†		Egg weight (g)‡		Egg mass (g hen^{-1} d^{-1})§	
	COM	CAS	COM	CAS	COM	CAS
37	67.9	71.3	59.1^a	56.2^b	40.2	40.1
38	63.4	66.9	59.8^a	55.0^b	38.1	36.8
39	64.8	68.1	60.1^a	56.5^b	39.1	38.5
40	65.3	57.3	60.1^a	56.8^b	39.3	32.5
41	61.9	53.0	60.3^a	56.4^b	37.4	29.9
42	65.4	56.0	60.6^a	56.7^b	39.7^a	31.8^b
43	61.6	61.6	60.6^a	56.7^b	37.3	34.9
44	68.7^a	50.9^b	60.7^a	57.0^b	41.8^a	29.2^b
45	67.1^a	50.1^b	60.2^a	56.2^b	40.3^a	28.3^b
46	67.9^a	43.6^b	60.5^a	56.5^b	41.1^a	24.7^b
47	66.4^a	48.9^b	60.7^a	55.9^b	40.3^a	27.4^b

†SE = 4.7; ‡SE = 0.6; §SE = 2.8. a, b Means of each productive variable, with different letter within each row, are significantly different (P<0.05). SE = standard error of the mean.

CP/3620 kcal EM kg^{-1}). A balanced diet is requisite for an optimal productive performance in hens (Ravindran, 2013b). The commercial feeds are formulated with precision so that they contain the quantity of nutrients that meets the birds' needs (Chadd, 2008). Based on the estimated nutritional content of the homemade concentrate evaluated in this study, this feed differs from reference values (NRC, 1994) in terms of EM, CP, lysine, and methionine. In particular, the concentrations of the last three nutrients were less than those recommended. Lysine and methionine are two essential amino acids for birds and a marginal deficiency in them decreases egg production (Cuca-García *et al*., 2016), which explains the results observed in the EP variable.

The eggs produced by the hens fed homemade feed weighed less than the eggs from the hens fed with the commercial feed, and this difference between treatments varied from 4.9 to 8.0%. This result agrees with what was reported by Aganga *et al*. (2003), who observed that the eggs of local hens fed with homemade feed (13.5% CP) were 5% lighter than those produced by birds fed with commercial feed (16.0% CP). Authors like Mutayoba *et al.* (2012) did not find differences in egg weight (38.9 to 39.3 g) of local hens fed with commercial feed (14.6% CP/2604 kcal EM kg^{-1}) or homemade feed (15.5 % CP/3620 kcal EM kg^{-1}). As indicated, the homemade feed evaluated in the present study was found to be deficient in terms of CP, lysine, and methionine. The results observed in terms of EW can be explained by feed composition (Cuca-García *et al*., 2016), thus the amino acid content of the CAS diet was considered to be lower for protein synthesis in the magnum (Penz and Jensen, 1991; Gomez and Angeles, 2009; Silva *et al*., 2015).

During the entire evaluation period, the EM of birds with homemade feed was numerically lower when compared to the birds that received commercial feed. This difference was significant during 45.5% of the trial time. There is no precedent for evaluation of EM with homemade feeds; however, experiments with commercial birds have shown that the increase in EM occurs when increasing amounts of essential amino acids are administered in the diet (Gómez and Ángeles, 2009; Bonekamp *et al.*, 2010). The lowest EM observed in the CAS treatment is explained by the afore-mentioned deficiency in CP, lysine, and methionine, and the function of these nutrients in the EP and the EW (Cuca-García *et al*., 2016).

Based on the results obtained, it is necessary to design homemade feeds that consider the nutritional requirements of the birds and the nutritional characteristics of the ingredients to be used. Likewise, and before being recommended for their use, the feeds need to be systematically evaluated in order to know their effect in the productive performance of birds.

CONCLUSIONS

The use of homemade feed based on corn, toasted and ground black bean, guaje leaves flour, toasted and ground eggshells, sugar, and common salt, reduced the productive performance of hens in a cage-free system, in terms of egg production, egg weight, and egg mass, when compared to the use of a commercial feed.

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Fodder and Grain Yield in Native Maize (*Zea mays* L.)

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ABSTRACT

Objective: To quantify the grain yield, biomass, crop stubble, and leaf:stalk index in seven varieties of maize (*Zea mays* L.), as well as the relationship between biomass, stubble, and plant height.

Design/Methodology/Approach: The process consisted in planting seven genotypes of native maize (*Zea mays* L.). The experimental design comprised randomized blocks with four repetitions. The plant's height, total biomass, its accumulation in stalk, leaf, husk, cob, grain, and stubble (biomass of stalk+leaf+husk+cob) were evaluated. A variance analysis, the Tukey means comparison test, and a regression analysis were applied.

Results: Significant differences were found between the native maize genotypes. Notable cases were the Ixtenco Yellow, followed in terms of biomass and stubble by the Texcoco White-Wide, then the Ixtenco White and Red. The plant height determined biomass in 67% and stubble in 77%.

Study Limitations/Implications: The tendencies found could vary depending on the native cultivars studied and their management.

Findings/Conclusions: In the study region, there are differences between native maize cultivars for the agronomic characteristics evaluated. With respect to the yield of grain, biomass, and stubble, the Ixtenco Yellow cultivar was outstanding, followed by the Texcoco White-Wide, and Ixtenco White and Red. The plant height determined biomass in 67%, and stubble quantity in 77%.

Keywords: Dry matter, stalk, leaf, grain, stubble.

INTRODUCTION

In Mexico, native or Creole maize (*Zea mays* L.) is cultivated principally by producers that employ traditional agriculture with multiple uses, such as grain production, fodder, tortillas, tamale wrapping, butter, container and artisanal products (Ávila-Bello *et al*., 2016). The INEGI (2014) observes that 82% of the maize-cultivated surface area in Mexico consists of Creole or native varieties, which in addition to being adapted to the producers' climate and technological conditions, possess characteristics

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that respond to the culinary tastes of populations and cultures. The sowing of Creole maize seeds has generated a highly biodiverse phytogenetic resource, with more than 50 recognized native varieties (Kato *et al*., 2009). Escalante *et al*. (2013) report genetic variability in stubble production, total biomass, yield, and specific leaf area in native maize cultivars. Escalante and Rodríguez (2016) report a biomass (dry matter) of 608 g m^{-2} and a yield of 209 g m^{-2}, with 2.45 m height, in Blue San Miguel Tlaixpan native maize sown under 330 mm rainfall. Aguilar *et al*. (2016) report that the native maize Michoacán 21, cultivated in Montecillo, Mexico, during the rainy season, showed a biomass of 860 g m^{-2} and a grain yield of 236 g m^{-2}. At the maize's physiological maturity, the grain is harvested and the rest of the plant (stubble) is used as fodder for livestock (Hellin *et al*., 2013; Reyes-Muro *et al*., 2013; Beuchelt *et al*., 2015). From the maize stubble, the leaves are the most digestible for ruminants due to their lower lignin content compared to the stalk (Williams *et al*., 1997). For every kilogram of grain produced, an estimated 1 kg of byproduct is obtained. The stubble represents an average of 50% of the total aerial biomass (Dhugga, 2007). The leaf:stalk index (proportion of dry matter in leaves with respect to the stalk), indicates the quantity and quality of leaf fodder. Another byproduct is the olote or cob, that can be used as a mulching substrate for humidity retention (Rodríguez-Martínez *et al*., 2016), to develop nitrogenous fertilizers with prolonged or slow-release action (Kabel *et al*., 2007; Córdoba *et al*., 2013), or else for ethanol production (García and Garza, 2016). The studies on production of grain, biomass, stubble, and agronomic characteristics of native maize are uncommon, therefore the objective of this study was to determinate in native maize cultivar: a) the grain yield, biomass production, stubble quantity, leaf:stalk index, and agronomic characteristics; and b) the relationship between the production of biomass, stubble, and the plant's height.

MATERIALS AND METHODOLOGY

The study was conducted in Montecillo, Texcoco, State of Mexico (19° 29' N, 98° 53' W, at 2250 m altitude), with a temperate climate, light rainfall, mean annual temperature of 14.6 °C, and 558.5 mm precipitation (García, 2004), in clay soil with pH of 7.8. The treatments consisted in sowing seven cultivars (horticultural varieties, cvs) of native maize (*Zea mays* L.) of different origins, sown by the farmers close to the region of Montecillo, Texcoco, Mexico (Table 1), at a density of 4.16 plants m^{-2} (80×30 cm) and with 100-100-00 of NPK. The experimental unit was four 5 m long furrows.

The experimental design was in random blocks with four repetitions. The phenological stages were registered, as well as maximum (Tmax) and minimum (Tmin) temperature, and rainfall (PP, mm) during the crop's development. At harvest, 15 plants were taken from the useful plot to register the plant height (ALT, cm), total biomass of the aerial part (TB, dry matter g m^{-2}), its accumulation (DMA) and distribution in the stalk, leaves, husks, cob, grain (yield, GR), stubble (TB-GR), and the leaf:stalk index (accumulated biomass in leaf/accumulated biomass in stalk). The study variables were analyzed with the SAS package, version 9.0 (SAS, 2003), with variance analysis (ANOVA), and for treatments with significant differences, the means comparison test (Tukey α=0.05) was used. A regression analysis was carried out to determine the relationship between total biomass and stubble production and the plant height, and between biomass production and stubble.

RESULTS AND DISCUSSION

Phenological phases occurred 9 d after sowing (das) until emergence, flowering (F) between 70 and 80 das, and harvesting at 140 d. During the crop's development, the average Tmax was 27 °C and the Tmin, 8 °C, and the PP added up to 350 mm. For the plant height, significant differences were observed between cultivars (cvs). The

Table 1. Characteristics of the location of origin of the materials.

Location	Landrace maize	Coordinates	Height above sea level (m)	Mean annual temperature (°C)	Annual precipitation (mm)
Ixtenco Tlaxcala*- Temperate climate (Cw)	1) Yellow 2) White 3) Red 4) Black 5) Pepitilla	19° 15' N, 97° 53' O	2542	11.1-16.1 °C	647
Texcoco Edo. México. Temperate climate (Cw)	1) White-Wide 2) Blue	19° 29' N, 98° 53' O	2250	12.3-18.2 °C	610

References:*http://siglo.inafed.gob.mx/enciclopedia/EMM29tlaxcala/municipios/29016a.html; García (2004).

Yellow cultivar (cv) showed plants with greater height at 231 cm, followed by White (215 cm), and Red (214 cm). The lowest height corresponded to the White-Wide (200 cm) and Pepitilla (180 cm) (Table 2).

Table 2 shows the results for total biomass (TB) and its accumulation in stalks, leaves, husks, cobs, and grains. The highest TB (977 g m^{-2}) corresponded to the Yellow genotype due mostly to a greater accumulation of DM (DMA) in the stalk, husks (totomoxtle) and grain; followed by the White genotypes (845 g m^{-2}), Red (856 g m^{-2}), White-Wide (842 g m^{-2}), Blue (831 g m^{-2}), and Black (781 g m^{-2}). The lowest TB was registered in Pepitilla (573 g m^{-2}). TB production, with the exception of the Black genotype, exceeds the maximum reported for other native varieties, such as the Labrador (447 g m^{-2}), Blue (608 g m^{-2}), and Michoacán 21 (680 g m^{-2}) for similar study conditions (Escalante *et al*., 2013; Escalante and Rodríguez, 2016; Aguilar *et al*., 2016, respectively).

In terms of the DMA in leaves, White-Wide and Black presented the greatest value with 144 and 110 g m^{-2}. The lower values corresponded to the rest of the genotypes that were statistically equal. With respect to the stalk, the White and Yellow genotypes were the ones with the greatest DMA with 312 and 285 g m^{-2}, respectively. The inferior value corresponded to Pepitilla with 245 g m^{-2}. Concerning the husks, the Yellow and White-Wide genotypes presented the highest values with 172 and 153 g m^{-2}. Regarding the cob, the Red maize presented the heaviest cobs (79 g m^{-2}), followed by the Black, Blue, Yellow and White (52, 37, 39 and 36 g m^{-2}, respectively). The lowest DMA corresponded to the Blue and Pepitilla maize (37 and 21 g m^{-2}). The highest GR was presented by Yellow and Red maize with 394 and 377 g m^{-2}, which surpassed the GR of other native types (Escalante and Rodríguez, 2016; Aguilar *et al*., 2016), followed by the White, Black, and Blue maize. The inferior values corresponded to White-Wide and Pepitilla maize with 234 and 160 g m^{-2}, respectively. Notably, the genotypes that stand out for TB production and GR were Yellow, White, Red, Blue and Black (Table 2). Generally, 34% of DMA was in the stalk, 12% in leaves, 13% in husks, 5% in cobs, and 36% in grain.

Stubble Production and Leaf:Stalk Index

White-Wide and Yellow maize showed the highest production of stubble (609 and 583 g m^{-2}, respectively), compared to other evaluated varieties, followed in stubble by Blue and White maize, then Black and Red, and the low value corresponded to Pepitilla (Table 3). On average, stubble represented 65% of TB, which exceeds that indicated by Dhugga (2007) by 50%. Regarding leaf DMA with regards to the stalk (leaf:stalk) (Table 3), the

Table 3. Leaf:stalk index and amount of stubble (g m^{-2}) in landrace maize cultivars from different locations. Montecillo, Texcoco State of Mexico. Mexico. Summer 2018.

Cultivar	Leaf:stalk	Stubble (g m^{-2})
Yellow	0.30 bc	583 a
White	0.27 c	538 b
Red	0.36 b	479 c
Black	0.44 ab	483 bc
Pepitilla	0.35 b	413 d
White-Wide	0.53 a	609 a
Blue	0.28 bc	556 ab
Probability of F.	**	**
Tukey 0.05	0.11	55

** P>0.01. In columns values with similar letter are statistically equal according to Tukey 0.05.

Table 2. Distribution of dry matter (DM, gm^{-2}) in landrace maize cultivars from different locations. Montecillo, Municipality of Texcoco State of Mexico. Mexico. Summer 2018.

Cultivar	Height (cm)	Stem	Leaf	Bracts	Cob	Grain	Total
Yellow	231 a	285 a	87 b	172 a	39 b	394 a	977 a
White	215 b	312 a	85 b	103 b	36 b	309 ab	845 b
Red	214 bc	252 b	92 b	56 c	79 a	377 a	856 b
Black	207 bc	252 b	110 ab	69 c	52 b	298 b	781c
Pepitilla	180 d	245 b	87 b	60 c	21 c	160 d	573 d
White-Wide	200 bcd	271 ab	144 a	153 a	40 bc	234c	842 b
Blue	210 bc	306 a	86 b	127 ab	37b	275 b	831b
Probability of F.	**	**	**	**	**	**	**
Tukey 0.05	15	48	43	57	20	66	59

** P>0.01. In columns values with similar letter are statistically equal according to Tukey 0.05.

outstanding maize varieties were White-Wide (0.53) and Black (0.44), with values exceeding those reported by Amador and Boschini (2000) for Creole maize (0.37), followed by Red (0.36), Pepitilla (0.35), and the inferior values were from Yellow (0.30), Blue (0.28), and White (0.27) maize. This suggests that if the principal use is for fodder, the most appropriate varieties would be White-Wide and Yellow for their higher proportion of leaves compared to the stalk, which implies more palatability for livestock.

Biomass and Stubble Relationship with Stalk Height

After performing a regression analysis for biomass and stubble with respect to plant height, it was found that the plant height (cm) presented a determination coefficient (R^2) of 0.67 and 0.77, respectively, which indicates that the changes in TB and stubble depend on 67 and 77%, respectively, with changes in plant height. The equations that can estimate biomass and stubble in function of the plant height are shown in Figures 1 and 2. Similar tendencies in relation to TB and plant height have been reported in other native maize varieties by Escalante *et al*. (2017), which indicates that the plant height can be a variable to be included in prediction models for TB and stubble.

Stubble-to-Biomass Ratio

Figure 3 shows that the changes in stubble were determined in 69% by changes in biomass. This indicates that plants with higher TB are required to obtain a greater amount of stubble. The Figure 3 equation indicates that for every gram of biomass, 0.69 g would correspond to stubble for the studied maize cultivars.

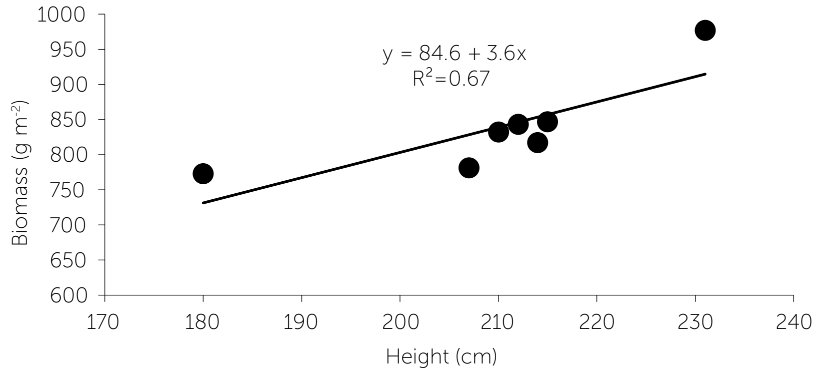

Figure 1. Relationship between biomass (g m^{-2}) and plant height (cm) in landrace maize. Montecillo, Texcoco Mexico, Mexico. Summer 2018.

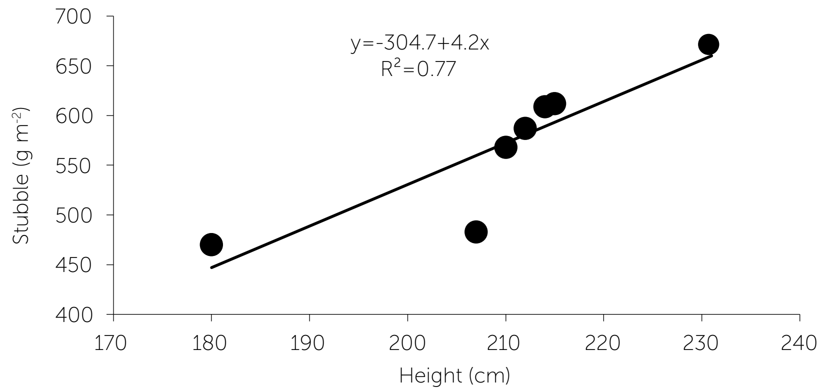

Figure 2. Relationship between stubble (g m^{-2}) and plant height (cm) in landrace maize. Montecillo, Texcoco Mexico, Mexico. Summer 2018.

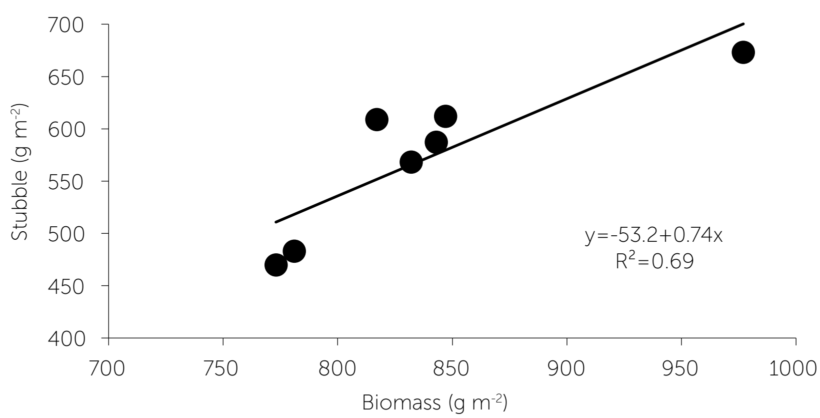

Figure 3. Relationship between stubble (g m^{-2}) and biomass (g m^{-2}) in landrace maize. Montecillo, Texcoco Mexico, Mexico. Summer 2018.

CONCLUSIONS

There are differences between native maize varieties in terms of grain yield, plant height, biomass production, accumulation in plant structures, stubble, and leaf:stalk ratio. The highest grain yield corresponded to the Ixtenco Yellow and Red, followed by the Ixtenco White and Black, and the Texcoco Blue. The lowest yield was found in Texcoco White-Wide and Ixtenco Pepitilla. With respect to grain yield, biomass and stubble, the Ixtenco Yellow cultivar was the most outstanding, followed by the Texcoco White-Wide, and Ixtenco White and Red. The highest value in leaf:stalk index corresponded to the Texcoco White-Wide maize, followed by the Ixtenco Yellow, Red, and Black. Based on their coefficient of determination (R^2), stubble and biomass quantity was acceptable in relation to plant height. This variable could be used in prediction models for biomass and stubble.

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Flowering Dynamics in Alfalfa (*Medicago sativa* L.) Based on Heat Units

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ABSTRACT

Objective: To describe the sequential pattern of reproductive development of *Medicago sativa* L. (flowering dynamic) using the relation between principal floral stem nodes and accumulated growing degree day (GDD) in different environmental conditions, and to determine the use of this relationship as a predictor of the seed harvesting date.

Design/Methodology/Approach: It was carried out in random blocks and factorial arrangement of 2×5, with two repetitions. Each repetition was made up of 30 plants.

Results: The flowering dynamic followed the same pattern between varieties, with a linear relation between GDD and number of floral nodes. The year A0 was different from the years A1 and A2. Therefore, A1 with cutting, and A2 with and without cutting presented less GDD during the flowering stage. In contrast, A0 showed more GDD during this period. In the different environmental conditions, the linear curves of flowering dynamic turned erratic after the tenth floral node (300 GDD) due to a low rate of plants with more than 10 floral nodes along the principal stem.

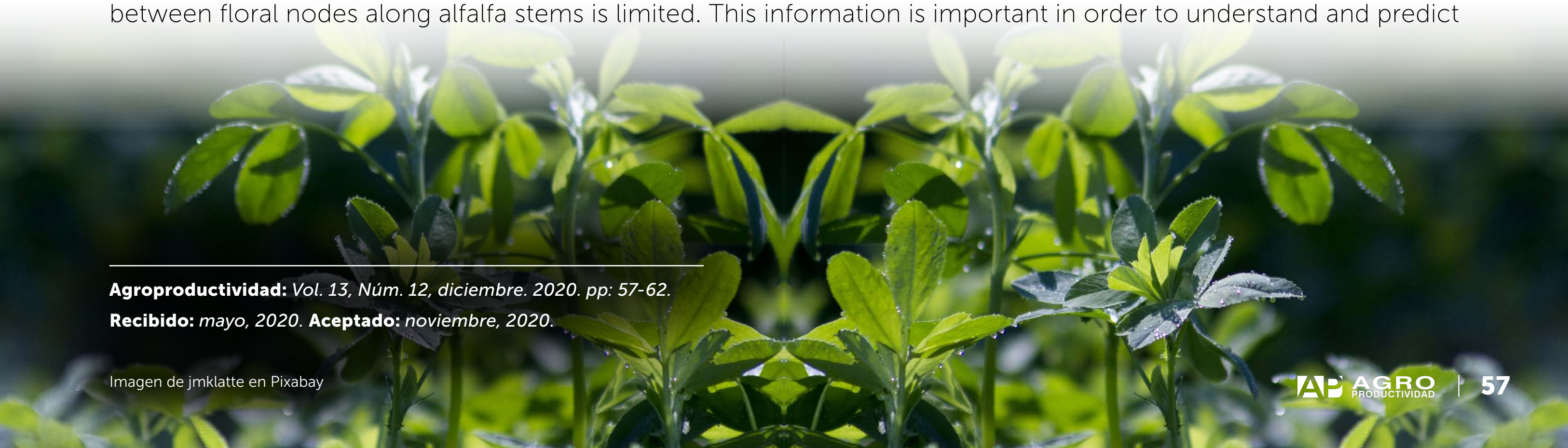
Study Limitations/Implications: It is necessary to have a meteorological station near the alfalfa seed production site, in order to specify the GDD required for predicting the correct moment to harvest seeds.

Findings/Conclusions: The reproductive development of *M. sativa* presents a linear relation between the number of floral nodes and GDD, which allows the prediction of the optimal seed harvesting date, in any environmental condition present.

Key words: Growth, accumulated growing degree days, flowering.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) develops a group of erect thin primary stems of up to one meter in height with branching. Each stem is composed of several vegetative and floral nodes. This branching pattern gives rise to a certain growth habit with indefinite sequential flowering between the different nodes along each stem (Lesins and Lesins, 1979). The information available describing the pattern of the flowering dynamic between floral nodes along alfalfa stems is limited. This information is important in order to understand and predict



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the variations of seed production and its distribution along the canopy.

The flowering dynamic has been described in other Fabaceae species to understand the variations in grain yield between plants, such as in soy (*Glycine max* L.) (Nico *et al*., 2016), fava bean (*Vicia faba* L.) (Daur *et al*., 2011), and lupin (*Lupinus angustifolius* L.) (Lagunes-Espinoza *et al*., 2000). In alfalfa it has been shown that the competition for nutrients between densely sowed plants increases and the rate of flowering decreases (Baldissera *et al*., 2014). This allows assuming that the selection of fodders normally carried out in isolated plants could generate erroneous information on the flowering dynamic and seed production, since there is not high competition for nutrients between plants, as seen in dense canopies which are the normal conditions of fodder growth.

Taking into account that the environmental temperature is correlated to plant development, the concept of accumulated growing degree days (GDD) could be used as a tool to predict the development of flowering toward a specific phenological stage (Krishna-Jagadish *et al*., 2016). In order to achieve this, it is important to know the basal temperature (Tb) of the crop of interest. The Tb is that which minimizes the variability of the required GDD necessary for a plant to achieve a specific phenological stage (Jungers *et al*, 2016). Years ago, the minimal temperature for the growth of alfalfa leaves was considered to be 5 °C (Sanderson *et al*., 1994). A basal temperature of 5 °C has been more frequently used in the development of prediction equations in alfalfa seed production for all stages of development (Bolaños-Aguilar and Huyghe, 2005). The objective was to understand the sequential pattern of reproductive development between the different floral nodes along the primary stem, in function of the accumulated growing degree days (GDD) with the aim of determining if that pattern varies with the environmental conditions of crop growth and with the variety, in order to know the feasibility of predicting the optimal date of seed harvesting associated with the relation between reproductive development and GDD.

MATERIALS AND METHODS

The study was carried out in field conditions during two years in the Center of Genetic Improvement of Forage Plants in Lusignan (46.26° N; 0.07° E) of the National Institute of Agricultural Research (INRA) in France. European and Magali varieties of alfalfa were studied, the former known for its resistance to cold and lodging and the latter for adapting better to Mediterranean climate and less resistance to lodging. In both varieties the sowing density was 10 kg ha^{-1}, established in a design of random blocks in factorial arrangement of 2×5 with two repetitions. Each repetition had 30 plants. The area of each plot or repetition was 18.75 m^2, formed by four lines of 7.5 m long by 0.41 m between lines. For this study, central lines were used, eliminating the two external lines in order to avoid the border effect. Sowing was done in each line with the *chorrillo* technique. The present study was carried out in five different environmental conditions presented in Table 1.

For the creation of these environmental conditions, plants were evaluated in sowing year (A0), plants cut one year after sowing (A1 with cut), plants cut two years after sowing (A2 with cut), and plans without cutting two years after sowing (A2 without cut). Cutting was done at a height of 6 cm above ground level, beginning evaluations at the beginning of the month of May. Climate data were obtained from the same Research Center for every year. To record flowering dynamics, 30 primary stems (one per plant) were randomly selected by variety and repetition before the beginning of flowering.

The distance between the stems was approximately 0.20 m, to ensure that they did not belong to the same plant. The start of flowering of each variety was registered the moment when 20% of the stems selected presented one open flower in the inflorescence inserted on the first floral node of the primary stem of each plant selected. A node was considered floral when it developed at least one completely open flower (anthesis) in its inflorescence. As

Table 1. Treatments and sowing dates of *Medicago sativa* L.

Variety	Treatments	
	Environmental condition	Sowing date of treatments
Europa	Y0-1997	April 1997
	Y0-1998	April 1998
	Y1 with cutting	April 1997
	Y2 with cutting	April 1995
	Y2 without cutting	April 1995
Magali	Y0-1997	April 1997
	Y0-1998	April 1998
	Y1 with cutting	April 1997
	Y2 with cutting	April 1995
	Y2 without cutting	April 1995

A0: Sowing year; 1997 and 1998: Consecutive sowing years; A1: Regrowth year of sowing from 1997; A2: With cutting and without cutting: Regrowth year of sowing from 1995.

of the first open flower from the first floral node, every third day the opening of the first flower of each floral node along the primary stem was registered and tagged.

The progress of the number of alfalfa floral nodes was described in linear functions based on accumulated growing degree days (GDD) from the beginning of flowering until seed maturity (period of reproductive development). With this data the average number of floral nodes was calculated for each treatment. The duration of the reproductive period was based on the sum of GDD. The GDD were calculated by

$$GDD = \sum (T - Tb)$$

where T is the average daily temperature (maximum temperature + minimum temperature/2), and Tb is the baseline temperature, established at 5 °C for alfalfa (Sharrat *et al.*, 1989). This Tb of 5 °C was applied in all of the environments evaluated.

The linear regressions for the flowering dynamics from their start until seed maturity, for all treatments, were done using the REG procedure from SAS (SAS, 2010). The slope of each regression curve was used in order to estimate the flowering dynamics along the primary stem expressed as the GDD necessary for the sequential flowering of floral nodes on the primary stem. The regression model was

$$y = a + bx$$

where y = number of floral nodes, a = regression constant, x = accumulated growing degree days (Tb = 5 °C), and b = regression slope.

RESULTS AND DISCUSSION

The pattern of flowering dynamics (relation between the number of flower nodes and GDD) along the primary stem for the European and Magali varieties in each environmental condition is represented in Figures 1a and b, respectively. It was observed that in both varieties the flowering dynamics follow the same linear pattern, increasing the number of floral nodes with an increase in the GDD. This linear relation between the number of open flowers and the GDD was also observed in field conditions in bean cultivation (Jenni *et al.*, 2000; Kakon *et al.*, 2019). The flowering dynamics varied between different environmental conditions (Table 2).

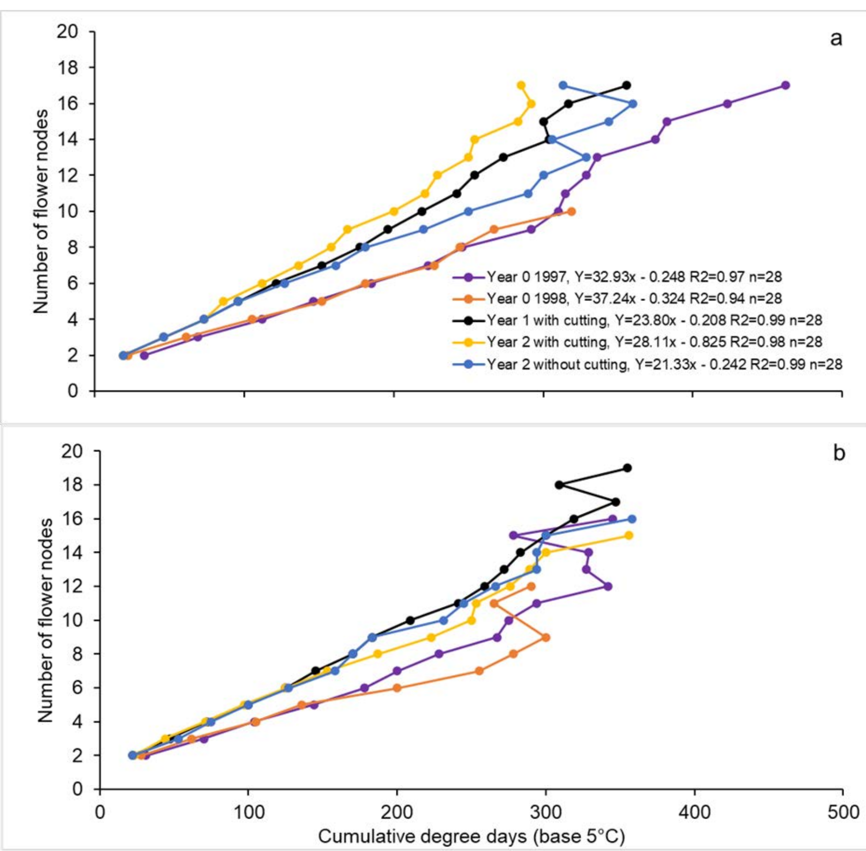

Figure 1. Relation between the number of flower nodes and accumulated growing degree days (GDD) in European (a) and Magali (b) varieties of *Medicago sativa* L., in different environmental conditions.

Independently of the variety, the flowering dynamics was slower in A0 than in A1 and A2, because an increased number of GDD were required in A0 for sequential flowering of floral nodes in the primary stem. Table 2 also shows that between A1 and A2 without cutting there was no significant difference in flowering dynamics. The lower flowering dynamics seen in A0 could be consequence of the differences of source-demand relationships in the plant. The former explanation is based on Khaiti and Lemaire (1992), who demonstrated that there is a more efficient use of solar radiation in the formation of biomass in alfalfa for regrowth years (A1 and A2) than for the sowing year (A0). This is explained by the higher leaf area index and by higher stem density per plant in regrowth years. This was corroborated by Bolaños-Aguilar and Huyghe (2005) and Nico *et al.* (2016) in

Table 2. Environmental condition, number of flower nodes and flowering dynamics in accumulated growing degree days (GDD) with 5 °C basal temperature for two varieties of *Medicago sativa* L.

Varieties	Condition environmental	Average number of flower nodes[1]	Flowering dinamics in CDD
Europa	Y0-1997	5.88 a	32.93 c
	Y0-1998	5.64 a	37.24 d
	Y1 with cutting	4.23 b	23.80 a
	Y2 without cutting	4.53 ab	21.33 a
	Y2 with cutting	6.71 a	28.11 b
Magali	Y0-1997	6.53 a	30.41 c
	Y0-1998	6.29 a	34.81 d
	Y1 with cutting	6.26 a	22.52 a
	Y2 withput cutting	6.21 a	24.67 ab
	Y2 with cutting	6.08 a	26.39 b

Means with the same letters between lines in the variety are not statistically different (P>0.05). A0, A1, A2; sowing year and two years after regrowth, respectively. [1]Each average is the result of 60 registered products of two repetitions with 30 stems each.

alfalfa, when observing that at higher leaf area and number of stems induces a higher accumulation of dry matter at the time of seed harvesting, with higher seed production seen in regrowth years.

Similarly, in another study by Bolaños-Aguilar *et al.* (2002) on alfalfa seed production carried out in 12 different environmental conditions, they observed that lower seed productions took place in the sowing year (A0), than in subsequent growing periods showing a genetic correlation of 0.79 between seed production and dry matter accumulation present at the time of seed harvesting. These results were explained as a consequence of lower dry matter production in A0, which was subsequently seen in other studies (Brown *et al.*, 2006; Otero and Castro, 2019).

The European and Magali alfalfa varieties showed similar flowering dynamics in the different environmental conditions evaluated, even though the original environment was different for each of them. However, there were differences ($p<0.05$) in the flowering dynamics between growing environmental conditions for each variety. Thus, the GDD required to pass from one floral node to the other in the European variety was 21.33 to 37.24 GDD, which corresponded to A2 conditions without cutting and to A0-1998, respectively, and for the Magali variety of 22.52 to 34.81 GDD with regard to the A1 conditions without cutting and A0-1998. Considering that between the two varieties there was similar flowering dynamics in any of the environmental conditions evaluated, the GDD required for seeds to reach maturity and be ready for harvesting was calculated by combining the results of the two varieties (Table 3).

The A2 conditions with and without cutting had a total of GDD during the blooming period lower than the rest of the treatment. The response was the opposite in A0 conditions, where the total GDD was higher than the rest of the treatments. However, the total number of accumulated development days between the five different environmental conditions evaluated was similar (Table 3). This result indicated that the GDD parameter is an important tool in order to correct the variations of plant growth related to temperature differences that occur on different days.

In both varieties, the curves that represent the flowering dynamics in different environmental conditions become erratic after the tenth flowering node, that is

Table 3. Period of reproductive development, accumulated days, accumulated growing degree days, and accumulated rain, in each environmental condition of plant growth.

Environmental Condition	Reproductive Development Period[1]	Cumulative Days	Reproductive Development Period (CDD)	Cumulative Rain (mm)
	Year 1997			
Y2: Without cutting	May 16 – June 25	40	454 a	74
Y2: With cutting	June 6 – July 17	41	491 a	143
Y0: Without cutting	July 1 – August 11	41	621 b	83
	Year 1998			
Y0: Without cutting	June 26 – August 7	42	583 b	73
Y1: With cutting	June 5 – July 19	44	550 a	84

Means with the same letters between lines within each year are not statistically different (P>0.05). A0, A1, A2; sowing year and two years after regrowth.
[1]Period lapsed from the beginning of flowering until seed maturity.

to say, once 300 GDD were reached (approximately 25 d of reproductive development) counted from the beginning of flowering (Figures 1a and b). This behavior was the consequence of the decrease in the number of primary stems with more than ten flowering nodes in all the environmental conditions evaluated, as shown by Huyghe *et al.* (2001) who studied vertical (between different flowering nodes along the primary stem) and horizontal (at the same level of the flowering node, but between primary stems) distribution in the production of alfalfa seeds.

The number of flowering nodes by stem varied from 14 (A0) to 17 (A1 and A2 with and without cutting) for both varieties. The number of stems with more than ten floral nodes was also lower in A0 than in the other environmental conditions. The duration of the reproductive development period was calculated based on the sum of GDD. These results deduce that the first ten floral nodes are important in the definition of seed production at the end of 300 GDD, counted from the beginning of flowering. Authors such as Bolaños and Huyghe (2005) previously observed in the European and Magali varieties that during the first 600 GDD of the reproductive period, the accumulation of dry matter was primarily assigned to reproductive organs rather than stems and roots. Considering that in alfalfa the time required to go from "open flower" in an inflorescence to another on the same primary stem was between 24 and 48 hours, as previously demonstrated by Genter *et al*. (1997), depending on the GDD; and that the seed development model can be measured by GDD from pollination, then it is possible to develop a model that explains alfalfa seed production. Determining flowering dynamics in varieties of broad use in different alfalfa production regions in Mexico would be of great use in order to predict the moment of seed harvesting.

CONCLUSIONS

The flowering dynamics along the primary alfalfa stem is described as a linear function based on accumulated growing degree days (GDD) during the reproductive period, with basal temperature of 5 °C. The pattern followed by the flowering dynamics (linear relation) does not vary between varieties, but depending on the environmental condition, the necessary GDD varies in order for alfalfa to reach seed maturation and for seed harvesting.

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Productive behavior of sheep fed with soy (*Glycine max* L.) forage

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ABSTRACT

Objective: To evaluate the productive behavior of Creole sheep in the tropics fed a whole food diet that includes soybean (FS, *Glycine max*) fodder at different phenological stages.

Methodology: The treatments T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d), T5: 30% (SF-110 d) were assigned randomly to 30 male Creole sheep (15.51±1.6 kg LW). The experiment lasted 56 d. The variables evaluated were: dry matter intake (DMI), daily weight gain (DWG) and feed conversion (FC). The experimental design was completely randomized and the averages of the treatments were compared using the Tukey test.

Results: Differences ($p<0.05$) were found in DMI, DWG and FC. The DMI was lower ($p<0.05$) in the control group. The DWG was higher in the animals that consumed soybean fodder compared to the control group (0.205 *vs* 0.121 kg d^{-1}). The FC decreased ($p<0.05$) 51% in T3 (SF 68 d), with respect to the control group.

Study Limitations: The nutrient contribution of soybean fodder is based on the phenological stage of the plant. Likewise, producers in the tropical region have scarce knowledge about how to incorporate it in the feeding of small ruminants.

Conclusions: The inclusion of 30% of soybean fodder at 68 d of age in a whole food diet improves the productive performance of the lambs because it increases daily weight gain and decreases feed conversion.

Keywords: *Glycine max*, sheep, soybean fodder, tropics.

INTRODUCTION

At present, the elevated cost of animal feed due to the use of grains and oilseeds drive up production costs (Khan *et al*., 2015) and reduce the profitability of sheep farming. In general, worldwide sheep production is developed in grazing production systems (Palma, 2005; Partida *et al*., 2013; Vélez *et al*., 2016). This situation represents an economic advantage since farmers save on animal feed production costs, and these systems generate the best cost/benefit relation and also improve the nutritional quality of the meat

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(Pirela *et al*., 2010). The Mexican tropics, due to their climatic, edaphic and topographic characteristics, are known for their great biological diversity in terms of natural resources such as plant, trees and fodder shrub species (Palma, 2005). These species produce fruit rich in digestible energy, protein and minerals which can be incorporated in sheep feed during the dry season (Zamora *et al*., 2001). These fruits are an alternative for sheep and can be used as a strategy to decrease the dependence on commercial concentrates in ruminant production systems (Garcia *et al*., 2008; Clavero, 2013; Delgado *et al*., 2014). On the other hand, soy [*Glycine max* (L.) Merr.] is considered a legume with forage potential due to its high production of dry matter (Tobia and Villalobos, 2004; Vargas-Bello-Pérez *et al*., 2008) and elevated protein content, therefore it represents an alternative to increase the protein content in the animal's diet (Dias *et al*., 2016). This favors a reduction in the use of protein supplements and, therefore, decreases the costs of animal feed in tropical production systems (Rigueira *et al*., 2015). It is important to mention that soybean fodder which produces the most seeds is the best variety, since this is the part of the plant that contains the greatest percentage of fat and protein (Tobía *et al*., 2006). In this sense, studies on soybean fodder (*Glycine max*) are centered on agronomic studies and have reported soybean fodder as a protein-energy alternative for ruminants' feed in the tropics (Tobia and Villalobos, 2004; Tobía *et al*., 2006; Orozco *et al*., 2015). In the revised literature in Mexico, there was no information on its use in *in vivo* studies in small ruminants. Therefore, the objective of the present study was to evaluate the productive behavior of Creole sheep in the tropics, fed with a whole food diet which included soybean (*Glycine max*) fodder in different phenological stages.

MATERIALS AND METHODS

Location

This study was carried out in the Experimental Farm of the Agricultural Baccalaureate Center No. 102 and Animal Nutrition Laboratory of the School Veterinary Medicine and Zootechnics No. 2 of the Universidad Autónoma de Guerrero, both located in Cuajinicuilapa, Guerrero (16° 18′ 58″ N, 98° 43′ 44″ W, at 50 masl). The region's climate is warm sub-humid with summer rains, monsoonal rainfall, and average annual temperature of 27 °C (CONAGUA, 2019).

Animals and Management

Thirty Creole male lambs were used (15.51±1.6 kg LW), held in individual cages (2 m^2) equipped with a feeder and drinking trough. Before the experimental phase they received prophylactic treatment with an anti-parasitic (Ivomec®, Ivermectina, 200 μg kg^{-1} LW) and antibacterial (Bobact® 8, 2.0 mL $lamb^{-1}$). The lambs adapted to the diets (Table 1) for 10 days and the evaluation time was 56 days. The feed was offered at 07:00 and 15:00 hours and water *ad libitum*.

Table 1. Ingredients and chemical composition of the experimental diets elaborated with soybean fodder at different phenological stages.

Ingredients (g kg^{-1} DM)	T1	T2	T3	T4	T5
Urea	1	0.25	0.50	0.75	1
Corn grain	38	41	41	41	41
Bypass fat	3	3	3	3	3
Coconut meal	18	15.75	15.50	15.25	15
Cane molasses	7	7	7	7	7
Mineral premix†	2	2	2	2	2
Calcium carbonate	1	1	1	1	1
Corn silage	30	0	0	0	0
Soy forage	0	30	30	30	30
Chemical composición (%)					
Dry matter	61.65	95.82	94.74	93.88	93.92
Crude protein	12.4	13.1	13.0	13.3	13.2
Neutral detergent fiber	25.83	23.24	25.63	24.09	27.78
Acid detergent fiber	13.53	14.68	16.08	14.90	18.87

T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d), T5: 30% (SF-110 d).
†Superbayphos® each 100 g contains: P 10 %; Ca 12 %; Fe 0.5 %; Mg 0.1 %; Cu 0.15 %; Zn 0.12 %; Mn 0.055 %; Co 0.05 %; I 0.02 %; Se 200 ppb; Vitamin A 50 000 UI.

Treatments

Soybean fodder (SF) was manually harvested (56, 68, 85 and 110 days after sowing) in forage banks of that specific species, established in tropical zones. Afterwards it was sun dried on cement floors, and turned over many times during the day in order to get homogeneous drying and avoid fungi growth. Once dried, it was ground in a hammer mill (Azteca, No. 16) with a ½″ diameter sieve and then mixed together with the other ingredients in order to make the feed (Table 1). The experimental diets (Table 1) were formulated based on the nutritional requirements for finishing lambs, for a gain of 200 g d^{-1} (NRC, 2007) (Table 1). The treatments evaluated were T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d) and T5: 30% (SF-110 d).

During the experimental phase, 3 samples were collected of each experimental diet in order to determine its chemical composition (Table 1); the following were determined: dry matter (DM; method 930.15), ash (method 942.05), crude protein (CP; method 984.13) and ether extract (EE; method 954.02) in accordance to the methodology described by AOAC (2005). The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined with the method proposed by Van Soest *et al*. (1991).

Table 2. Dry matter intake (kg d^{-1}) of lambs fed with a whole food diet elaborated with soybean fodder at different phenological phases.

Day	T1	T2	T3	T4	T5
14	0.670^{Cb}	0.670^{Bb}	0.715^{Ca}	0.589^{Cc}	0.593^{Bc}
28	0.740^{BCc}	0.881^{Aab}	0.860^{Bbc}	0.873^{Bb}	0.890^{Aa}
42	0.825^{ABc}	0.917^{Ab}	0.915^{Bb}	0.926^{ABb}	0.989^{Aa}
56	0.897^{Ab}	0.990^{Aab}	1.019^{Aab}	1.070^{Aa}	0.990^{Aab}

T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d), T5: 30% (SF-110 d). [a,b,c] Means with different letters in a row are different (Tukey; $p \leq 0.05$); [A,B,C] Means with different letters in a column are different ($p \leq 0.05$).

Production Variables

The intake of DM (DMI, kg d^{-1}) was calculated by the difference between the feed offered and the feed rejected each day. Daily weight gain (DWG, kg d^{-1}) was measured by weighing the lambs at the start of the experiment and every 14 days (07:00 h). Feed conversion (FC) was calculated as the DMI/DWG relation.

Experimental Design and Statistical Analysis

The experimental design was completely random with five treatments and six repetitions per treatment. Data were analyzed with PROC GLM (SAS, 2011) and the treatment averages were compared with the Tukey test ($p<0.05$).

RESULTS AND DISCUSSION

Including soybean fodder in different phenological phases in the whole food diet affected ($p<0.05$) the production behavior variables (DMI, DWG and FC) in the lambs (Table 2). The intake of DM (DMI) in this study was 10.8% higher in lambs fed with soybean fodder in the diet, compared to the control treatment (0.87 *vs* 0.78 kg^{-1} DM d^{-1}). It should be noted that at the end of the experimental period, the most DMI was found in lambs that consumed soybean fodder diet at 85 d of the phenological phase (T4) (Table 2).

The former can be related to the fact that as the lambs' weight increases, so does their DMI, due to the higher nutrient requirements and a greater digestive capacity (Pérez-Gil *et al*., 2011). The nutritional value of soybean fodder in the tropics can be compared to alfalfa in early flowering (Orozco *et al*., 2015), and in some situations it can be used to replace corn or sorghum grain in the fattening process. On the other hand, soybean fodder meadows can also be grazed or harvested from the flowering phase almost until maturity for its use as high quality hay (Kökten *et al*., 2014). Reséndiz *et al*. (2013) evaluated a whole food diet for sheep with 30% alfalfa, and found a greater DMI (36%), this difference is attributed to the fact that the lambs used had higher LW (22.1 *vs* 15.51 kg) when compared to the lambs used in the present study. On the other hand, Protes *et al*. (2018) did not find differences in DMI in lambs fed with silage made from soybean and sorghum fodder. However, Lima *et al*. (2013) reported higher DM intake in lambs fed with silage made from soybean and sugarcane tips.

In other studies, when up to 30% *Enterolobium cyclocarpum* (Fabaceae) was included in the whole food diet for lambs (Peralta *et al*., 2004; Álvarez *et al*., 2003), lower DMI values were reported (633 *vs*. 864 gr) than those in the present study. Similarly, Velázquez *et al*. (2011) reported lower DMI (620.2 g) when including 10, 20, 30 and 40% of *Acacia farnesiana* (Fabaceae) in whole food diets for lambs, which is similar to results in this study when the DMI was 647 g at 14 days. The differences could be due to different forage contents included in the diet, physiological age and race (Patiño and Van, 2010); also, environmental and management factors could affect the animal response. On the other hand, it could also be attributed to the palatability of the diet, protein content and intake, elevated intake of DM, better digestibility of the diet, and nitrogen use (Obeidat *et al*., 2020).

Daily weight gain (DWG) of the lambs fed with the whole food diet prepared with soybean fodder is shown in Table 3. As the evaluated days lapsed, the DWG increased, obtaining the highest values at 42 days (0.196 kg d^{-1}); however, at the end of the trial period there was a decrease (188 kg d^{-1}). This could be due to a compensatory growth effect that the lambs could experience because of better nutrition (Patiño and Van, 2010). On the other hand, it could be related to the fact that as the phenological phase of the forage species changes, their nutritional quality is also affected as the regrowth age increases (López and Briceño, 2016), which could result in changes in DWG. These changes were seen in the lambs that consumed diets with soybean fodder at phenological phases of 68 and 85 d (0.217 and 0.214 kg d^{-1}) in comparison to the control group (0.121 kg d^{-1}); this effect could be due to higher content of metabolizable energy in soybeans, since the whole plant was used when making the animal feed.

In this sense, Reséndiz *et al.* (2013) found 30% higher DWG when evaluating lambs fed with a whole food diet with 30% alfalfa, compared to the results of this study, which could also be due to higher live weight of the lambs (22.1 *vs* 15.51 kg) compared to those in this study. In other studies, which used *Enterolobium cyclocarpum* (Fabaceae) pods as supplement for lambs, lower DWG values were reported (0.149 kg^{-1} d^{-1}) (Peralta *et al.*, 2004; Álvarez *et al.*, 2003). However, it should be mentioned that even though Creole lambs were used in this study, the DWG obtained were satisfactory for a production system in the tropics. The DWG is consistent with that reported in other studies with lambs fed with diets with high grain content (Vicente-Pérez *et al.*, 2015). It should be noted that the diets used in this study allowed the animals to express their genetic weight gain potential. It is important to point out that the feed conversion

Table 3. Daily weight gain (kg d^{-1}) of lambs fed with a whole food diet elaborated with soybean fodder at different phenological phases.

Day	T1	T2	T3	T4	T5
14	0.107^{Cb}	0.170^{Cab}	0.245^{Aa}	0.197^{Bab}	0.202^{Bab}
28	0.120^{Bb}	0.187^{Aab}	0.221^{Ba}	0.219^{ABa}	0.189^{Bab}
42	0.130^{Ab}	0.193^{Aab}	0.205^{Ba}	0.223^{Aa}	0.231^{Aa}
56	0.130^{Ab}	0.181^{Bab}	0.199^{Ba}	0.219^{ABa}	0.211^{Ba}

T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d), T5: 30% (SF-110 d).
^{a,b,c} Means with different letters in a row are different (Tukey; p≤0.05); ^{A,B,C} Means with different letters in a column are different (p≤0.05).

Table 4. Feed conversion ratio (DMI/DWG) of lambs fed with a whole food diet elaborated with soybean fodder at different phenological phases.

Day	T1	T2	T3	T4	T5
14	6.070^{Ca}	4.910^{Bab}	3.100^{Db}	3.490^{Bb}	3.060^{Bb}
28	5.870^{Ca}	4.660^{Cab}	3.950^{Cb}	4.110^{Bb}	4.980^{Aab}
42	6.640^{Aa}	5.120^{Bb}	4.451^{Bb}	4.293^{ABb}	4.301^{Ab}
56	6.590^{ABa}	5.760^{Ab}	5.150^{Ab}	5.010^{Ab}	4.750^{Ab}

T1: control, T2: 30% (SF-56 d), T3: 30% (SF-68 d), T4: 30% (SF-85 d), T5: 30% (SF-110 d).
^{a,b,c} Means with different letters in a row are different (Tukey; p≤0.05); ^{A,B,C} Means with different letters in a column are different (p≤0.05).

(FC) reported here was 41% lower in animals that received the diet made with soybean fodder (FC; 4.44), compared to animals in the control group (FC; 6.29). Protes *et al.* (2018) reported a FC of 3.9 in lambs fed with 35% soybean fodder silage; in contrast, Lima *et al.* (2013) found higher values (6.17) in lambs fed with 30% soy silage. This productive response could be due to better nutrient utilization when soybean fodder is added at 68 days after sowing.

CONCLUSIONS

Adding 30% of soybean fodder at 68 days after sowing to the whole food diet improved the productive behavior of the lambs, because it increased the daily weight gain and optimized the food conversion as a result of more efficient nutrient use.

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Response of tomato varieties (*Solanum lycopersicum* L.) to water stress

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ABSTRACT

Background and Objectives: Water scarcity is limiting for tomato (*Solanum lycopersicum* L) production, due to its sensitive to drought in the different phases of development, so irrigation should be done in an optimal way; because of this, the objective was to evaluate four levels of irrigation in greenhouse tomato varieties.

Methods: A completely randomized experimental design with factorial arrangement was used and various agronomic variables, fruit quality and biomass were measured as response variables.

Results: Tomatoes were significantly affected ($P \leq 0.05$) by variety factors (V), irrigation (R), and their interaction. 100% of variety P presented fruits of greater weight (122 g); while weight decreased to 84, 90, 34 and 18 g when reducing water up to 25%, in varieties Cid, P, E and C, respectively. However, in terms of yield and leaf area, 100% of the Cid variety presented the highest values, around 3.3 kg/plant and 8.6 m^{-2}.

Conclusions: Variety c was tolerant to water stress and also does not present apical rotting (calcium deficiency) in the fruit.

Keywords: leaf area, apical rotting, yield, *Solanum lycopersicum* L.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is of paramount economic importance worldwide, and adverse climate conditions generate abiotic stress which is one of the principal limiting factors for production (Grayson, 2013). Drought affects 64% of the global land surface (Mittler, 2006). Tomato crops demand 23 to 30 liters of water per kilogram of fresh fruit (Medrano *et al*., 2007).

During its different development phases, this crop is sensitive to water stress, duration, severity and environmental factors which provoke it (Florido and Bao, 2014). Regarding stress severity and duration, the plants activate defense mechanisms at a molecular, morphological, physiological and cellular level, which can result in higher stomatal resistance (Witcombe *et al*., 2008; Peleg *et al*., 2011). Apical rotting is a common physiological disorder in fruits, which can reduce commercial yield by up to 50% (Taylor *et al*., 2004), and it is related to diverse factors such as temperature, transpiration, relative humidity and low calcium content (Matthew *et al*., 2004). Based on the aforementioned, four varieties of tomato were studied with four levels of irrigation during greenhouse plant growth.

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MATERIALS AND METHODS

The study was carried out in a polyethylene greenhouse at the Superior Technological Institute in Guasave, Sinaloa, located at 25° 52’ N and 108° 37’ W at an altitude of 15 m. Two varieties of Roma tomato were studied (E=1001 and P=1007), one Bola tomato (C=1006) from the company Mar-seed®, and the Cid control (T) F1 (Harris Moran®).

The four varieties were evaluated in four water humidity regimes in substrate during 150 days after transplanting. These irrigation regimes were based on information from Flores *et al*. (2007), who reported that water consumption of tomatoes ranges from 0.2 L per plant in initial seedling phases to 1.5 L in the adult phase with maximum water demand in substrate. For this experiment, a minimum of 0.3 L was used (25% = 300 mL d^{-1}) for treatment 1 (T1); T2 (50% = 600 mL d^{-1}); T3 (75% = 900 mL), and a maximum of 1.2 L (100% = 1200 mL d^{-1}) of water per plant for T4. Irrigation started 30 days after transplantation. In order to achieve this, two drip irrigation systems were installed, one with Steiner solution at 100% three times concentrated (Steiner, 1961). To avoid confusion, the same amount of nutrients and water levels were applied to the plants in all four treatments and water was added to complement the amounts in each watering, except for T1 which did not receive any additional water.

In order to compare treatments (four levels of irrigation × four varieties of tomatoes), a completely random experimental design was used with factorial arrangement, with four repetitions (one plant per repetition). The sowing of seeds took place on August, 25, 2019, in polystyrene trays with 200 cavities of 9 mL with peat. After 30 days, the seedlings were transplanted in 40 × 36 black polyethylene bags which contained 10 L of river sand with a diameter of 2-5 mm. The plants were managed at one stem and strung individually with raffia.

The agronomic varieties evaluated during the cycle were: 1) plant height (m), measured with a flex meter from the plant’s base to the apex, 2) leaf area (m^2) was determined in all fresh leaves, sampled with a portable laser leaf area meter (Licor, Inc. Lincoln, NE, USA), 3) number of fruits, 4) fruit weight (g), and 5) fruit yield (kg/plant), which were weighed in each cut and added to obtain the total weight.

The fruit quality variables measured in four fruits randomly selected from each treatment were: 1) number of locules, 2) total soluble solids (%) measured with a digital refractometer ATAGO PR-100® (Japan) (A.O.A.C., 1990), and 3) number of fruits with apical rotting counted by sampling.

The biomass variable was performed with a random destructive sample 150 days after transplant; two plans were taken from each experimental unit. The plants and each organ were dissected in the laboratory in a stove (Riossa®, Mexico) at 70 °C for 72 hours in order to measure total dry matter, until constant weight.

All of the variables were subjected to a variance analysis through a completely random design with factorial arrangement of two factors, varieties by regimes, and a means test using Tukey’s method (P≤0.05). The analyses were carried out with the SAS statistics software (version 9.0) and the tables with Microsoft Excel 2010® software.

RESULTS AND DISCUSSION

The results show that the tomato varieties were significantly (P≤0.05) affected by the factors variety (V) and irrigation (I), and by the interaction V × I in all the variables studied: agronomic, biomass and fruit quality (Tables 1 and 2). However, the interaction between variables is different.

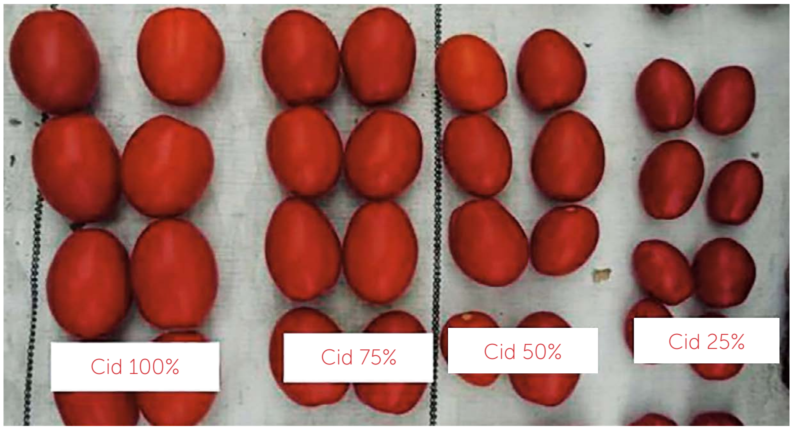
Of the agronomic variables (Table 1), only the number of fruits had an effect on V with 77% compared to the total variation due to treatments. Meanwhile, I caused more of an effect on plant height (57%), leaf area (61%), fruit weight (54 %) and

Table 1. Sum of squares of the agronomic variables measured in tomato plants cultivated in greenhouses with four levels of irrigation in Guasave, Sinaloa, Mexico.

FV	GL	Plant Height (m)	Leaf area (m^2)	Number of fruits	Weight Per Fruit (g)	yield (kg/plant)
		Sum of squares				
Trats	15	7.9**	339**	6008**	60712**	43.1**
V	3	2.7**(34)	105**(31)	4630**(77)	15108**(25)	1.4**(3)
R	3	4.5**(57)	207**(61)	953**(16)	32486**(54)	28.5**(66)
V × R	9	0.7**(9)	25**(8)	423**(7)	13117**(21)	13.2**(31)
Error	48	0.13	0.6	192	1080	0.4
Total	63	8.03	339.6	6200	61792	43.5

**: Statistically significant with P≤0.01; FV: source of variation; GL: degrees of freedom, Trats: treatments, V: variety, R: Irrigation, V × R: variety × irrigation. (Initials based on Spanish terms).

yield (66%). For its part, the interaction between V and I had slightly significant effects of 1 to 9%, although in fruit weight (21%) and yield (31%) it was high. Fruit quality (Table 2) affected by V were: fruits with calcium deficiency (76%) and number of locules (99%). Meanwhile, total biomass and total soluble solids were affected by I (64 and 54 %). Also a slight significance was seen in the V × I interaction, which oscillated between 1 and 17% in all the variables evaluated.



The Cid variety.

Plant height decreased as the availability of water for the plants was reduced, so that the lowest were the ones that received the least volume of irrigation. The Cid variety (control) with 100% and 75% had the tallest plants, and with complete irrigation (100%), the C variety was lower. The plants that received 25% water treatment, E and C varieties, were less affected with 0.4 and 0.3, respectively; Cid and P lost 1 m of height. The leaf area of Cid at 100% (8.6 m^{-2}) is 60% of E variety at 100% (3.3 m^2); in the four varieties water reduction generated a decrease in leaf area such that Cid, P, C and E at 25% treatment had leaf area decreased by 6.3, 6.7, 3.6 and 2.6 m^2/plant with the E variety being the least affected (Table 3). In terms of number of fruits, the highest amount was shown by the C variety in contrast with the P variety. Reduction in irrigation by 25% caused a reduction in number of fruits: Cid (6), P (9), E (4) and C (18). The P variety at 100% had the heaviest fruits and the C variety at 100% the lightest; with 25% treatment, weight of the fruits decreased by 109 and 9 g in C and P, respectively, when compared to 100% irrigation.

The Cid variety at 100% produced more yield compared to the E variety at 100% (Table 3). A decrease in irrigation (25%) caused less production in the four varieties, Cid, C, E and P, in different magnitudes (2.8, 1.8, 1.0 and 2.3 kg/plant) when compared to the 100% treatment, such that the E variety was the least affected in yield by water stress. In total biomass the control accumulated 407 g (Cid 75%) when compared to the E variety with 242 g at 100% treatment, which indicates that it had 37% more than the E variety. The regime affected in greater proportion the P and control varieties with stops, and E and C were less affected in biomass accumulation.

The C variety had fewer locules (6.5) because of its round shape when compared to Cid with 2 locules; related to this characteristic, drought had no effect (Table 4). With complete irrigation (100%) the content of total soluble solids in the fruits varied from 5 to 6 between the varieties. The reduction in water to 25% caused an increase in total soluble solids in 52, 45, 38, and 34% for Cid, P, E and C, respectively.

It should be noted that the quantity of fruits with apical rotting highlights the C variety which did not have any fruit with this physiological disorder; in contrast to P, E and control varieties, which presented fruits with this physiological plant pathology in both 100% irrigation and in all water reduction levels.

The results found in this study indicate that the characteristics of the variables evaluated presented significant differences (Table 1 and 2). The reduction in water

Table 2. Sum of squares of total biomass and fruit quality of tomato plants cultivated in greenhouses with four irrigation regimes in Guasave, Sinaloa, Mexico.

Variation source	Degrees of freedom	Biomass (g/ planta)	Number of locules	Total soluble solids (%)	Blossom end rot
		Sum of squares			
Tratamiento	15	413759**	172**	129**	2842**
V	3	115177**(27)	172**(100)	37**(29)	2167(76)**
R	3	263955**(64)	0(0)ns	70**(54)	392(14)**
V × R	9	34627**(8)	0(0)ns	22**(17)	283(10)**
Error	48	2664	18	4	58
Total	63	416423	1163	133	2900

***: Statistically significant with P≤0.05 and 0.01; ns not significant, R: irrigation, V: variety, V × R: variety × irrigation. (Initials based on Spanish terms).

affected physiological functions and therefore the whole plant. The variables: number of fruits, number of locules, and number of fruits with apical rotting showed characteristics specific to each variety. On the other hand, water stress had a greater effect on fruit weight, yield, total biomass and total soluble solids, which agrees with Cui *et al*. (2020) who mentioned that irrigation is the most important source of water for tomatoes and affects both yield and fruit quality. The plants exposed to water deficit presented alterations in physiological and metabolic processes, such as a reduction in photosynthesis rates, a decrease in total protein synthesis and in growth rates (Chaves *et al*., 2009).

Table 3. Means comparison in plant quality in tomatoes cultivated in greenhouses with four levels of irrigation (25, 50, 75 and 100 %).

Treatment	Plant height (m)	Leaf area (m^2 / plant)	Number of fruits	Weight per fruit (g)	Yield (kg/plant)
Cid 25	1.3 h	2.1 h	25.3 efg	22.4 j	0.6 g
Cid 50	1.8 cd	3.9 e	34.7 b	45.5 hg	1.6 g
Cid 75	2.3 a	2.8 g	33.0 bc	95.8 bc	3.1 a
Cid 100	2.3 a	8.4 a	32.0 bcd	106.1 b	3.4 a
C 25	1.2 i	0.9 j	29.3 cde	34.5 hij	1.0 h
C 50	1.4 g	2.0 h	46.0 a	44.4 ghi	2.0 ef
C 75	1.6 e	3.7 e	46.5 a	47.9 fg	2.2 de
C 100	1.7 d	4.5 d	47.5 a	52.4 fg	2.4 cd
E 25	1.1 i	0.7 j	27.2 edf	53.4 fg	1.0 f
E 50	1.3 h	1.6 i	29.5 cde	58.3 ef	2.0 ef
E 75	1.5 f	2.7 g	30.5 bcd	70.3 de	1.9 f
E 100	1.6 e	3.3 f	31.0 cbd	87.1 c	1.9 f
P 25	1.3 h	1.4 i	13.3 h	32.8 ji	0.4 i
P 50	1.8 d	3.6 e	16.3 h	72.7 d	1.2 h
P 75	2.0 c	5.8 c	21.5 g	124.3 a	2.6 bc
P 100	2.2 b	8.1 a	22.3 fg	122.2 a	2.7 b

E (1001), C (1003), P (10001). Means with different letters indicate significant differences ($p<0.05$).

The commercial Cid variety had a height of 2.3 m, reached 150 days after transplantation (Table 4). This coincides with Núñez *et al*. (2012), where the maximum height of 2.8 m was reached at 180 days with a Bola Beatrice variety. In this context, Osakabe *et al*. (2013) mentioned that prolonged water stress decreases the hydric potential of leaves and stomatal opening, reduces leaf size, and limits growth and plant productivity.

Table 4. Means comparison of variables of fruit quality in tomato plants cultivated in greenhouses with four irrigation regimens (25, 50, 75 and 100 %).

Treatment	Biomass (g/planta)	Number of locules	Total soluble solids (%)	Blossom end rot
Cid 25	205 gh	2.2 cb	10.2 a	14.0 bc
Cid 50	275 d	2.0 b	7.3 d	13.0 cd
Cid 75	407 a	2.3 cb	5.9 ef	5.0 f
Cid 100	390 ab	2.0 c	5.3 fg	2.8 fg
C 25	200 h	6.5 a	8.4 c	0 g
C 50	272 d	6.3 a	5.8 ef	0 g
C 75	314 c	6.5 a	5.5 fg	0 g
C 100	316 c	6.5 a	5.2 fg	0 g
E 25	126 j	3.3 b	8.6 bc	2.2 fg
E 50	221 fg	3.3 b	5.7 ef	1.3 g
E 75	231 ef	3.3 b	5.6 fg	1.5 g
E 100	242 e	3.2 b	4.8 g	1.2 g
P 25	175 i	3.0 bc	9.3 b	20.3 a
P 50	250 e	3.0 bc	7.2 d	16.5 b
P 75	374 b	3.0 cb	6.5 de	11.0 d
P 100	379 b	2.7 cb	6.0 ef	8.0 e

E (1001), C (1003), P (10001). Means with different letters indicate significant differences ($p<0.05$).

Leaf area ranged between 8.4 and 3.3 m^2, in Cid and E with 100% irrigation, while in those submitted to stress (25%) this decreased from 1.2 to 0.7 m^2 (Table 3). Such loss of leaf area is important since leaves are a fundamental organ for photosynthesis, where energy from sunlight is captured by chlorophyll and utilized for the synthesis of water and carbon components (Fischer *et al*., 2012; Wang *et al*., 2014).

The number of fruits varied between 22 and 47 with 100% treatment in P and C varieties (Table 3), compared to 25% treatment which decreased markedly the amount of fruits in P (13) and C (29), respectively. This demonstrated that drought affects each variety of tomato. Pervez *et al*. (2009) determined that drought significantly reduces the number of fruits, plant height and number of leaves.

The fruit weight ranged from 52 to 122 g in the C and P varieties with irrigation (100%) and with water stress (25%), the weight of the same varieties decreased to 34 and 32 g (Table 3). This indicated that C variety is tolerant to drought in terms of fruit size. According to Kinet and Peet (1997), the final fruit size is closely related to dominant environmental conditions during the fruit's growth phase.

The highest yield was obtained from the Cid variety with complete irrigation (100%) with 3.4 kg/plant, compared to C plants that only produced 2.4 kg/plant, which represents a 1 kg decrease (Table 3). However, the 25% treatment with C variety produced 1000 g, which is more than the 600 g produced by the control per plant. According to Nuruddin (2001), water deficit affects negatively the fruit and is reflected in the yield due to water and nutrient deficiencies.

In terms of total biomass, the control accumulated the most (390 g) in the 100% treatment 37 % than the E variety (242 g), and the 25% water treatment decreased 185 and 116 g, where the least affected was the E variety. Heuvelink (1995) and Link (2000) mentioned that 70% of total biomass is destined to fruits. The production of biomass in any crop is strongly determined by the amount of water available (Medrano *et al*. 2007).

The number of locules (Table 4) obtained were similar to that reported by Raana (2019), who mentioned that tomatoes varied in number from 2 to 10 locules. In this study the highest number of locules (6) was seen in the C variety. The amount of total soluble solids in terms of fruit quality shows that it can vary with water stress and during fruit development (Table 4), because the flow of water to the fruit decreases and causes stress from salts (osmosis), which induces the accumulation of active solutes. According to Sakamoto *et al*. (1999), tomato fruits under stress accumulate mainly ions and organic molecules (fructose and glucose). The results of total soluble solids in 100% irrigation agree with Bui *et al*. (2010), who indicated that tomatoes should have between 4.5 and 6.25 % soluble solids.

The calcium deficiency was present in Cid, P and E in all treatments, although the 25% treatment had the most number of fruits with this physiological disorder (Table 4). Its emergence is attributed to alterations in the absorption and transport of calcium from the roots to the fruits, especially in its distal part and the factors which accelerate this are high temperature, high radiation and low relative humidity (Cardona *et al*., 2005). The disorder starts in the immature fruit since only 3% of calcium makes it to the fruit, despite the fact that fruits represent 90% of the crop's growth and the least susceptible varieties are those that have a stronger xylema network (Ho *et al*., 1993). The C variety does not present this physiological plant pathology which is clear evidence of its tolerance and immunity.

CONCLUSIONS

The C variety was shown to tolerate water stress and also did not present apical rotting (calcium deficiency) in the fruit. With water stress at 25%, the E variety significantly exceeded the hybrid, with a 400 g difference per plant.

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Forage yield of *Urochloa* cultivars in a warm sub-humid environment

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ABSTRACT

Objective: To evaluate for 10 weeks the growth of five *Urochloa* cultivars: Camello, Cobra, Cayman, Convert 330, Mavuno and Xaraés; during the North Winds (NS), Dry (DS) and Rainy (RS) seasons in a warm sub-humid environment.

Design/Methodology/Approach: The variables were crop growth rate (CGR; kg ha^{-1} d^{-1}) and total dry matter yield (TDM; t ha^{-1}); which were analyzed under a completely randomized design (Tukey; p=0.05).

Results: During NS, Mavuno and Xaraés presented, on average, the highest values of CGR ($p<0.05$), 23.7 and 19.4 kg ha^{-1} d^{-1}, respectively. In DS the cultivars Xaraés, Convert 330, Mavuno, and Cayman presented the highest values, with 20.7 and 26.8 kg ha^{-1} d^{-1}, in week 2 and 4, respectively. During RS, in the second and fourth week of regrowth, the Xaraés and Mavuno cultivars presented the highest CGR ($p<0.05$), 119.8 and 144.7 kg ha^{-1} d^{-1}, on average. In the NS, the Mavuno hybrid presented the highest yields ($p<0.05$) of TDM, 0.886, 1.553 and 2.156 t ha^{-1} in week 6, 8 and 10, respectively. For RS, the cultivars Camello, Cayman, Mavuno, and Xaraés presented similar yields of TDM; which, on average, were 4.652, 5.312 and 6.278 t ha^{-1}, at 6, 8 and 10 weeks, respectively.

Findings/Conclusions: The cultivars Xaraés, Mavuno and Cayman presented good forage potential in a warm sub-humid environment; mainly for showing greater growth and accumulation of total dry matter.

Key words: *Brachiaria* hybrid, *Urochloa* hybrid, growth rate.

INTRODUCTION

The state of Hidalgo dedicates nearly 11% of its territory (233,000 ha) to livestock production; where the area destined for cattle, sheep and goats is 218,000, 10,000 and 5,000 ha, respectively (INEGI, 2018). On the other hand, the state of Veracruz dedicates about 3.4 million ha (48% of the state territory) to livestock, where 99% of the surface is for bovine

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production (INEGI, 2018). Both states have a livestock vocation, and in addition, they share a territorial limit and therefore the climatic conditions in the northeast of Hidalgo and north of Veracruz are similar. In this region, the climate is semi-warm with abundant rains in summer (>1,200 mm per year) and temperatures above 15 °C (INEGI, 2017), conditions that allow tropical forage grasses to present high growth rates, which is reflected in higher forage yield (Santos *et al*. 2014). However, animal production is affected by the variation in forage yield during the year, since there is a shortage during the dry season and surpluses in the rainy season.

In the humid tropical region there are three well-defined seasons: the rainy season, when the precipitation is greater and together with the temperature they contribute to the active growth of forages; the dry season, when the yield is drastically affected by hydric stress due to lack of water in the soil; and the north winds season, when there is high cloudiness and a decrease in temperature, so the growth of tropical species is affected (Hernández *et al*., 1990; Martínez *et al*., 2008). Photosynthesis and the accumulation of dry matter are closely associated with photosynthetically active radiation (Mishra *et al*., 2010), so that cloudiness could affect these processes; in addition to this, the minimum threshold temperature of most tropical grasses is 15 °C (Berone, 2016), although some *Urochloa* cultivars such as Xaraés and Insurgente have lower minimum threshold temperature, 12.4 and 10.5 °C, respectively (Silva *et al*., 2012).

The forage yield of *Urochloa* cultivars ranges between 11 and 15 t ha^{-1} year^{-1} and said production is not constant throughout the year, since it depends mainly on environmental conditions (Garay *et al*., 2017); therefore, the annual forage yield is presented at 72, 18 and 10%, during the rainy, north winds and dry seasons, respectively (Martínez *et al*., 2008). It has been reported that the most recent hybrids of the genus *Urochloa* present greater potential for forage production and higher nutritional value (protein and digestibility) (Pizarro *et al*., 2013; Vendramini *et al*., 2014). It is important that before introducing a new forage material to any production system, the productive behavior should be evaluated in a controlled manner to determine if it is an option for livestock production in the region. Therefore, the objective of this study was to evaluate the dynamics of forage growth and production of Urochloa cultivars in the north winds, dry and rainy seasons in a warm sub-humid environment.

MATERIALS AND METHODS

Location of the Experimental Site and Climatic Characteristics

The study was carried out in seasonal conditions from November 2018 to August 2019, at the Huejutla Technological Institute, located on the Huejutla-Chalahuiyapa Highway, km 5.5. Huejutla de Reyes, Hidalgo, Mexico. The geographical coordinates are 21° 9′ 19.66″ N and 98° 22′ 11.02″ W, at 112 meters above sea level. The climate is classified as warm sub-humid [Ax_2(x′); García, 2004] with abundant summer rains mean annual temperature that ranges between 24 and 26 °C (INEGI, 2017). In this place there are three well-defined seasons: dry, rainy, and north winds (Figure 1; SMN, 2020).

Treatments and Management of the Experimental Plots

The evaluated treatments (cultivars) were five hybrids of *Urochloa*: Camello, Cobra, Cayman, Convert 330 and Mavuno, and one variety (*U. brizantha* cv. Xaraés). They were evaluated for 10 weeks during the north winds, dry and rainy seasons. These cultivars were sown with botanical seed (05/11/2018) in streams in rows at 0.3 m distance in experimental plots of 16 m^2 (4×4 m).

Prior to sowing, a germination test was carried out and the sowing dose was determined for each treatment (4 kg ha^{-1} of viable pure seed); for this purpose, representative samples of the seed were taken from each

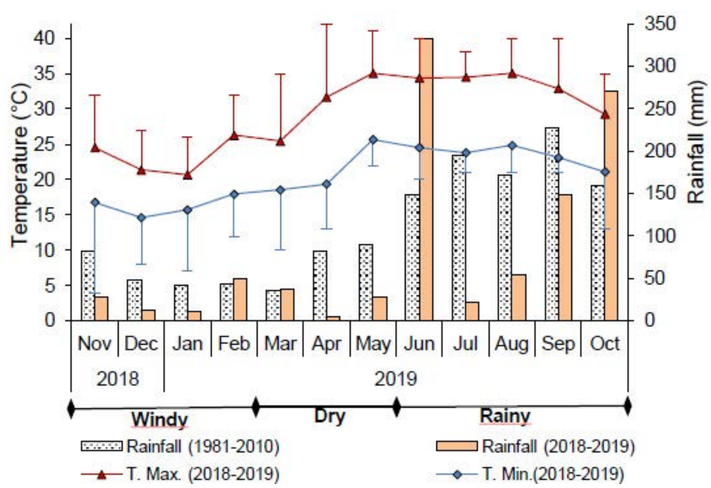

Figure 1. Monthly accumulated precipitation (Preci.) and maximum (Max. T.) and minimum (Min. T.) temperature during the period 2018-2019 (18-19), in Huejutla de Reyes, Hidalgo. Average information of 29 years (1981-2010). Source: SMN (2020).

genotype, and a trial was set up with four repetitions of 100 seeds for each genotype, which were put on newspaper, moistened daily and the germinated seeds were counted, with which the germination percentage was calculated. There was an establishment period of 6 months, in which two uniformity cuts were made at 15 cm above ground level to stimulate clustering and a rapid establishment of the meadows. Before starting with the sampling in each season, a uniformity cut was made at 15 cm above the ground and then five samplings were carried out every two weeks. At the center of the furrow, the forage present was harvested periodically in a linear meter (experimental unit). The harvested forage was weighed and a 200 g subsample was taken, later it was placed in a forced air stove at 65 °C for 72 h to dry. The subsamples were weighed before and after drying to determine the dry matter content.

Variables Evaluated and Statistical Analysis

With the values of the dry matter content, calculations were carried out to determine the accumulation of total dry matter (TDM; t ha^{-1}) using the formula proposed by Hunt (1990) to estimate the CGR (kg ha^{-1} d^{-1}). The variables were analyzed with the GLM procedure by SAS (2003) in a completely randomized design with four repetitions, in an arrangement of measurements repeated over time. The means comparison was carried out using the Tukey test (p=0.05).

$$Y_{ijk}=\mu+C_i+\delta_{i(j)}+S_k+(CS)_{ik}+\varepsilon_{ijk}$$

Where: Y_{ijk}=response variable; μ=general mean; C_i= effect of the cultivar; $\delta_{i(j)}$=error associated with cultivars; S_k=effect of the sampling week; $(CS)_{ik}$=interaction between the cultivar and the sampling week; ε_{ijk}=experimental error associated with the sampling week.

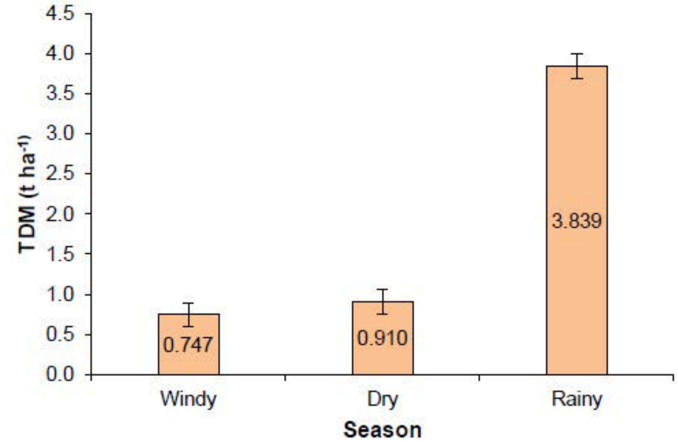

Figure 2. Total dry matter yield (TDM) during the north winds, dry and rainy seasons in Huejutla de Reyes, Hidalgo. The bars represent the least significant difference (Tukey; p=0.05).

RESULTS AND DISCUSSION

It was observed (Figure 2) that the forage yield was higher (p<0.05) during the rainy season, 3,849 t ha^{-1}, on average. In contrast, yields were lower (p>0.05) during NS and DS (0.747 and 0.910 t ha^{-1}, respectively).

During the NS (November-January) it was observed that the accumulated precipitation was 45 mm, which was 74% lower (Figure 3), compared to the average accumulation of 29 years (1981-2010) for the same season (173 mm; SMN, 2020). During this season it was observed that the average maximum and minimum

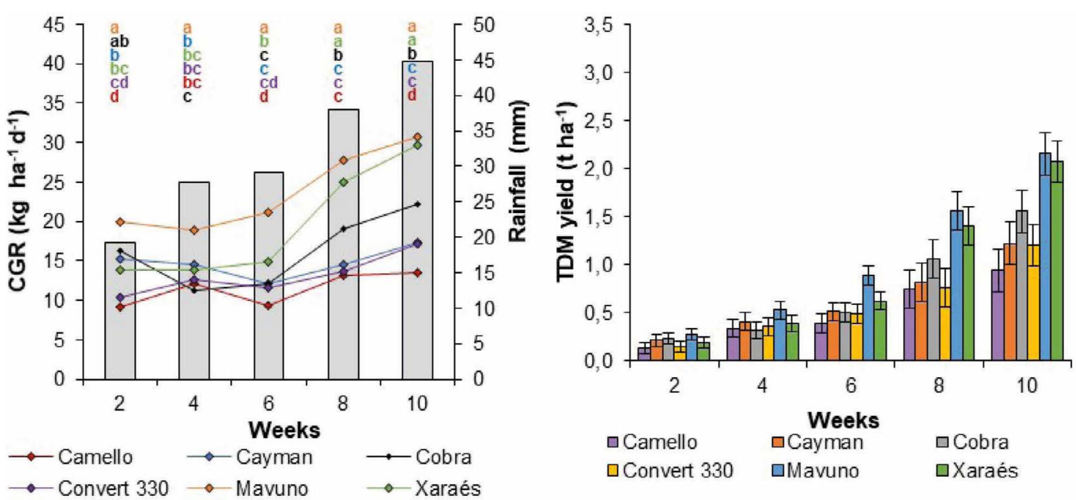

Figure 3. Crop growth rate (CGR) and total dry matter yield (TDM) of cultivars of the genus *Urochloa* during the north winds season in Huejutla de Reyes, Hidalgo. Different letters between cultivars (a, b, c, d) and the bars in each regrowth age indicate a statistically significant difference (Tukey; p=0.05).

temperatures were 22±6 and 16±9 °C, respectively (Figure 1). In this season, the evaluated cultivars presented different growth dynamics, where Mavuno and Xaraés presented on average the highest values of TCC ($p<0.05$), 23.7 and 19.4 kg ha^{-1} d^{-1}, respectively. In contrast, the Camello, Convert 330 and Cayman cultivars had the lowest CGR, with 11.5, 13.1 and 14.8 kg ha^{-1} d^{-1}, respectively. The average CGR during the NS was 14.1, 13.9, 13.5, 18.9 and 21.8 kg ha^{-1} d^{-1} at 2, 4, 6, 8 and 10 weeks, respectively (Figure 3).

The average yield of total dry matter (TDM) during the NS was 0.198, 0.389, 0.567, 1.057, 1.525 t ha^{-1} at 2, 4, 6, 8 and 10 weeks, respectively. Differences were only observed between the cultivars from the sixth week, where the hybrid Mavuno presented the highest yields ($p<0.05$) of TDM, 0.886, 1.553 and 2.156 t ha^{-1} in week 6, 8 and 10, respectively. The Xaraés cultivar only equaled Mavuno at the eighth and tenth weeks. Meanwhile, the hybrid Cobra presented similar yields of TDM that Xaraés. The cultivars that presented the lowest TDM yields during the NS were Cayman, Camello and Convert 330 (Figure 3).

During the DS (March-May) the accumulated precipitation was 35 mm, which was below 83% (Figure 4) of the average reported for the same season in the study area (207 mm; SMN, 2020). During this season it was observed that the average maximum and minimum temperatures were 31±9 and 21±6 °C, respectively (Figure 1). During this time, the CGRs were 15.4, 21.8, 19.2, 18.9, 26.5 kg ha^{-1} d^{-1} at 2, 4, 6, 8 and 10 weeks, respectively. There were only differences between the cultivars evaluated in the second and fourth week of regrowth ($p<0.05$), when the Xaraés, Convert 330, Mavuno and Cayman cultivars presented the highest values (on average), with 20.7 and 26.8 kg ha^{-1} d^{-1}, respectively (Figure 4). On the other hand, the Camello cultivar presented the lowest CGR ($p<0.05$) in the same cutting dates, 5.3 and 8.5 kg ha^{-1} d^{-1}, respectively. From the sixth to the tenth week, all cultivars presented similar ($p>0.05$) CGR (Figure 4). In this season, only significant differences ($p<0.05$) were observed between the cultivars for the TDM variable in the fourth, eighth and tenth weeks. The most outstanding were Cayman (meaning week 10), Cobra (meaning week 4), Convert 330, Mavuno and Xaraés (Figure 4), which on average presented 0.685, 1.145 and 1.935 t ha^{-1} at 4, 8 and 10 weeks, respectively. In contrast, the Camello hybrid was the one that presented the lowest TDM values, 0.237, 0.618 and 1.469 t ha^{-1}, respectively, for the aforementioned regrowth ages. The TDM yield for this season was 0.216, 0.610, 0.808, 1.057 and 1.857 t ha^{-1} at 2, 4, 6, 8 and 10 weeks, respectively (Figure 4).

During the rainy season (June-August) the accumulated precipitation was 304 mm, which was 41% lower, compared to the average reported for the same season (517 mm) in the place where the evaluation was carried out (SMN, 2020). Likewise, it was observed that the average maximum and minimum temperatures were 35±5 and 24±4 °C, respectively (Figure 1). During this season the highest growth dynamics was observed in the

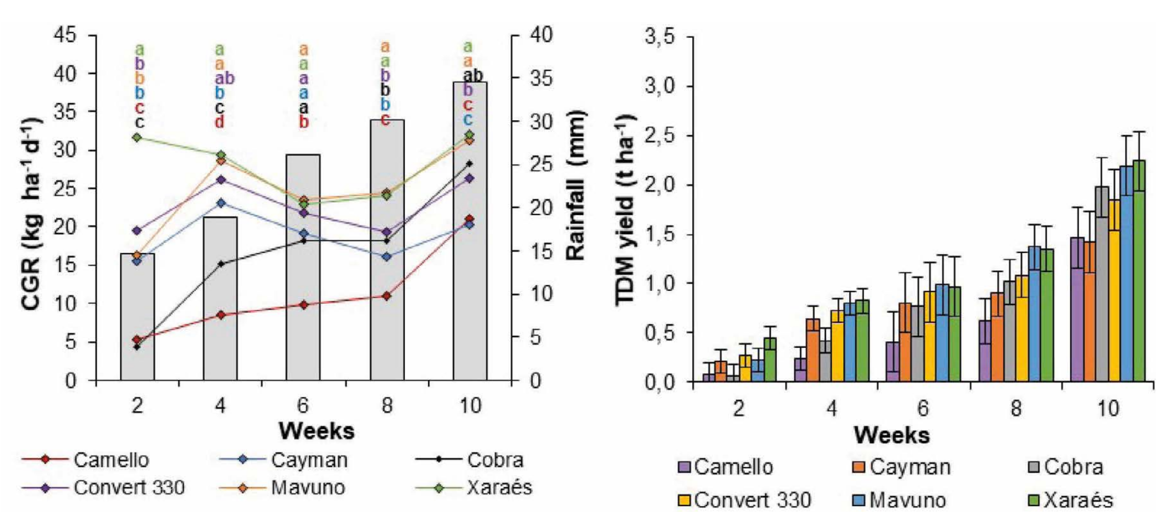

Figure 4. Crop growth rate (CGR) and total dry matter yield (TDM) of cultivars of the *Urochloa* genus during the dry season in Huejutla de Reyes, Hidalgo. Different letters between cultivars (a, b, c, d) and the bars at each regrowth age indicate a statistically significant difference (Tukey; p=0.05).

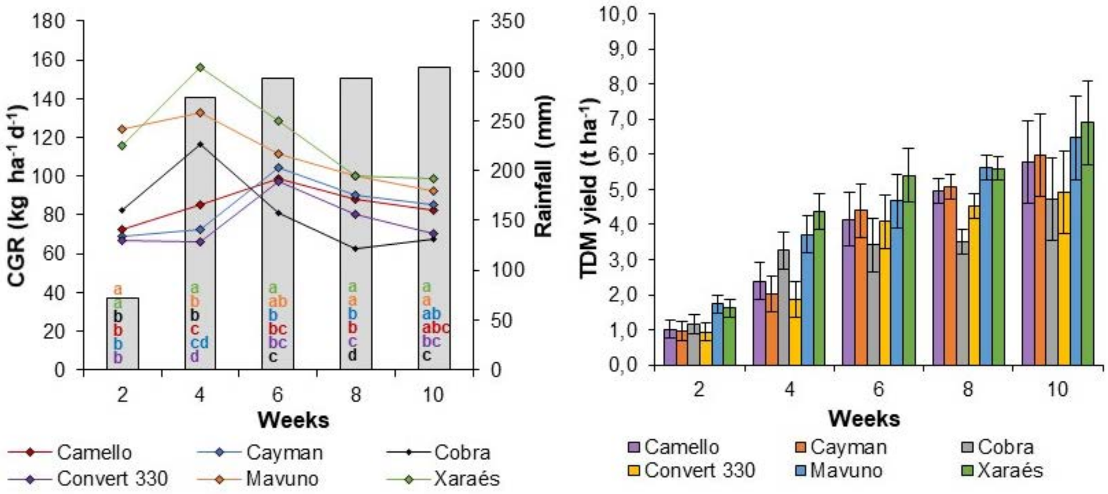

Figure 5. Crop growth rate (CGR) and total dry matter yield (TDM) of cultivars of the *Urochloa* genus during the rainy season in Huejutla de Reyes, Hidalgo. Different letters between cultivars (a, b, c, d) and the bars at each regrowth age indicate a statistically significant difference (Tukey; p=0.05).

grasses evaluated. In this sense, the CGRs were 88.4, 105.0, 103.6, 87.1, 82.7 kg ha^{-1} d^{-1} at 2, 4, 6, 8 and 10 weeks, respectively. In the second and fourth week of regrowth, the Xaraés and Mavuno cultivars presented the highest CGRs ($p<0.05$), which on average were 119.8 and 144.7 kg ha^{-1} d^{-1}, respectively (Figure 5).

On the contrary, Camello, Convert 330 and Cayman, presented the lowest values in the CGR ($p<0.05$), which, on average were 69.5 and 74.7 kg ha^{-1} d^{-1}, respectively, for the aforementioned regrowth ages (Figure 5). It should be noted that the Cobra cultivar, during the first two regrowth ages (2 and 4 weeks), presented CGR similar to those of Xaraés and Mavuno; however, from the sixth to the tenth week, these values decreased considerably, ranging between 62.7 and 81.2 kg ha^{-1} d^{-1}. The greater growth dynamic observed during the rainy season was reflected in the greater accumulation of TDM. In this sense, yields of 1,238, 2,939, 4,350, 4,880 and 5,790 t ha^{-1} were obtained at 2, 4, 6, 8 and 10 weeks, respectively. At 2 and 4 weeks of regrowth, the Xaraés and Mavuno cultivars presented the highest TDM yields ($p<0.05$), with values (on average) of 1,677 and 4,051 t ha^{-1}, respectively. From the sixth week on, the Camello, Cayman, Mavuno and Xaraés cultivars presented similar TDM yields, which, on average, were 4,652, 5,312 and 6,278 t ha^{-1}, respectively (Figure 5).

The CGR or absolute growth rate (AGR), is the increase in the dry matter of the plant or of each of its organs per unit area that it occupies in the soil, per unit of time (Hunt, 1990), behavior which was observed in Figures 3 to 5. The reduction in CGR during NS and DS was the result of the low availability of moisture (45 and 35 mm, respectively). In addition to this, during the NS there were minimum temperatures that were below 10 °C (Figure 1), which affected the growth of the grasses. It has been documented that in tropical grasses, as is the case of the grasses evaluated in this study, when the temperature falls below 15 °C, respiration and photosynthesis are interrupted, thus inevitably stopping plant growth (Durán *et al*., 2011). In contrast to the two seasons mentioned above, during the rainy season there was greater growth dynamics. This was mainly influenced by the availability of moisture (304 mm of precipitation) and the relatively high temperatures, which were 24 and 35 °C, average minimum and maximum temperatures, respectively; these conditions favored active growth of the forage (Cruz *et al*., 2011; Martínez-López *et al*., 2014; Santos *et al*., 2014).

Temperature is a factor that directly affects the growth of plants. The minimum threshold temperature of most tropical grasses has been reported to be 15 °C (Durán *et al*., 2011; Berone, 2016). However, it has been reported that the Xaraés cultivar presents a minimum threshold temperature of 12.4 °C (Silva *et al*., 2012), a characteristic that allowed it to have higher CGR and accumulation of TDM during NS. The growth of the *Urochloa* cultivars was affected by the conditions that were present during the north winds season; mainly, a decrease in temperature and high cloudiness (Hernández *et al*., 1990; Martínez *et al*., 2008). The latter directly affects the photosynthesis process, since it is closely related to photosynthetically active radiation (Mishra *et al.*, 2010), which was reflected in the lower accumulation of CGR. The highest growth dynamics occurred during the RS, as a result of the active growth of the grass (Castro *et al.*, 2013). Likewise, during this season there was an increase in the temperature, which accelerates the growth of the plant and this can cause senescence of the forage to be earlier (Santos *et al.*, 2014). In addition, during this season, there was greater availability of humidity, and also temperatures above 18 °C, which allowed the growth of the grasses. It has been indicated that in order not to affect the growth of forage grasses, in addition to the humidity present in the soil, there should be temperatures above 15 °C, which is the minimum threshold temperature of most tropical grasses (Mendonça and Rassini, 2006; Durán *et al.*, 2011). The forage production of cultivars of the *Urochloa* genus was variable during the year, since it depended on the environmental conditions present during each season. It has been reported that the annual forage production of this genus is distributed as follows in the three seasons: from 13 to 22% in the NS, from 8 to 12% in the DS, and from 66 to 79% in the RS (Martínez *et al.*, 2008; Cab *et al.*, 2008); which are similar to the yields reported in this study.

CONCLUSIONS

In the conditions where the experiment was carried out there are difficulties for forage production, mainly during NS and DS. The Xaraés, Mavuno and Cayman cultivars showed greater growth and accumulation of total dry matter; therefore, they can be an option for the livestock production that is carried out in warm sub-humid conditions. It is worth mentioning that this evaluation was only carried out during the period of one year, which was atypical compared to the average values of the climatological events for the place of study.

ACKNOWLEDGMENTS

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Critical Nitrogen and Nutritive Index in Cultivars of *Cenchrus purpureus* (Schumach) Morrone

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ABSTRACT

Objective: To determine and compare the parameters of the nitrogen curve and the nutritive nitrogen index of cultivars of *Cenchrus purpureus* with and without nitrogenous fertilization.

Design/Methodology/Approach: Five cultivars of *C. purpureus* with or without fertilization were evaluated, and the dose was 141N-43P-20K. The grass was harvested every 21 d with production of dry matter (DM) and nitrogen (N). Growth rates and Critical Nitrogen (Nc) were obtained. The negative power equation with its parameters Nc and the Nitrogen Decrease Coefficient (Cd) were used. The Nutritive Nitrogen Index (NNI) was obtained with the Nc parameter of each curve, divided by the N content of the cultivar in each sampling. The data were analyzed with a completely randomized design in a factorial arrangement of five (cultivars) and two levels of fertilization.

Results: Nc of 2.6 and a Cd of 0.65 fertilized, and 1.14 of Nc and 0.68 of Cd without fertilization, and in turn all of them supplemented the amount of N for their growth from soil. The NNI was 0.8 for the fertilized cultivars, with classification of excess and sufficient for the first 56 d.

Study Limitations/Implications: Variables to determine the availability of water and the balance of soil-plant N could further expand the knowledge of these tools.

Findings/Conclusions: Parameters obtained such as Nc and NNI help to determine nutritional status of the fodders.

Keywords: Nitrogenous Fertilization, Tropical Fodders, Model Curves.

INTRODUCTION

The genus *Pennisetum* (Poaceae) has about 140 species, most of them native to Africa and Central and South America. However, species of *Pennisetum* may be found in any country in the tropics and subtropics. It was introduced to Mexico in 1950 (Ramos-Sánchez, 1985), to the state of Veracruz, where it was introduced together with a wide collection of Poaceae for evaluation and selection; one of them was the *Pennisetum merkeri*, a product of

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that evaluation. It was recommended for use as cut fodder and released as Zacate Merkeron, which spread throughout the country's southeast. Mexico has a broad diversity of environments, in which the production of perennial Poaceae is highly feasible. These offer high potential for biomass production per surface unit, since it has been documented that the intensely fertilized species *P. purpureum* (Schumach), syn. *Cenchrus purpureus* (Schumach) Morrone, Chemisquy *et al*. (2010), produces large amounts of biomass that can exceed 50 t of dry matter ha^{-1} and year (Calzada *et al*., 2014; Rueda *et al*., 2016; Reyes-Castro *et al*., 2018). Given that the species *C. purpureus* has a wide potential for biomass production, its preferential use is for cattle feed, whether in grazing, cut, or else as fodder ensilage. In recent years, various cultivars of *C. purpureus* have been introduced to Mexico, from different origin and with scarce technical information, such as: Taiwan, King Grass, CT-115, CT-169, Maralfalfa, Mott, Vruckwona, OM-22, Roxo or Purple, and Clone 69, among others. However, these new cultivars are unknown regarding their management, particularly fertilization, and therefore, they require high amounts of nutrients, specifically nitrogen. Nitrogen (N) is essential for crop growth (Tillman *et al*., 2002) and is considered a limiting factor after water for forage production in meat and dairy production systems in the tropics. The practice of fertilization provides enough N to reach the potential allowed in appropriate climatic conditions, so that the producers apply amounts that are high or less than that required by the plant to obtain the maximum forage production to ensure that this potential is reached every year, although it is known that excess N reduces the efficiency and can cause environmental problems (Jeuffroy and Meynard, 1997). The management of N in soil and in fertilizer is an important point for the profitability and sustainability of extensive meat and dairy production systems in the tropics, and for this purpose, methods are required that estimate the critical concentration of N in the fodders, in order to adjust the dose of N and the frequency of applications according to their growth potential. Authors such as Justes *et al*. (1994) developed the methodology to know the nitrogen status during the growth and production of biomass considering the term critical nitrogen (Nc), defined as the minimum concentration of nitrogen required to obtain the maximum growth rate, expressed as g N per 100 g in relation to the accumulated dry matter (DM) (t DM ha^{-1}). This curve is a negative power function that is described as follows:

$$\%N = a\ DM^{-b}$$

Both are specific physiological coefficients where: *a*, is the concentration of *%N* in the plant when the fodder produces a t DM ha^{-1} and *b*, is the concentration of Nitrogen in the plant when it decreases.

Derived from the previous function, the nutritional status of the fodders is defined and the term Nutritional Nitrogen Index is obtained by its acronym NNI (Colnenne *et al*., 1998), which can be used as a basis to make decisions about the need to fertilize with N and its frequency in applications (Lemaire *et al*., 2008). It is described next:

$$NNI = N/Nc$$

where *N* is the current nitrogen concentration of the fodder in percentage of DM and *Nc* is the critical nitrogen calculated with the negative power function equation.

For N management in fodders, the NNI threshold has been defined as follows: Values greater than 1 indicate that N is in excess, values between 1 and 0.8 indicate that the nutrition is satisfactory, and values below 0.8 indicate N deficiency, so growth and production are limited (Duru *et al*., 1997). It has been observed that lower concentrations of N than critical nitrogen (Nc) indicate that growth is restricted by a deficit of N, while higher values of N than Nc indicate that the contribution of N is in excess (Greenwood *et al*., 1990). Forage production is related to NNI (Lemaire and Gastal, 1997; Ziadi *et al*., 2008; Agnusdei *et al*., 2010). The concept of Nc and NNI has been confirmed to be an effective tool for agronomic analysis and interpretation (Lemaire *et al*., 1997; Lemaire and Meynard, 1997; Gonzalez-Dugo *et al*., 2005). The objective was to determine and compare the parameters of the nitrogen curve and the nutritive nitrogen index of cultivars of *C. purpureus* with and without nitrogenous fertilization, to know the nitrogen status in the fodder.

MATERIALS AND METHODS

The study was carried out in the experimental field "La Posta" in Paso del Toro, Veracruz, located at coordinates 19° 00' 49'' N and 96° 08' 19'' W at 12 m altitude (INEGI, 2009). The area's climate corresponds to Aw_2 sub-humid tropical (Vidal Zepeda, 2005), with rainfall of 1461 mm, and relative humidity of 77.4%. The average, maximum and minimum temperatures are 25, 35 and 15 °C. The soils are Vertisol, acidic pH (5.4), clay-sandy crumb texture and with organic matter content of 2.6%.

Treatment Design

The evaluated cultivars were: CT-115, Elefante, King-grass, Roxo and Taiwan, all with and without nitrogenous fertilization; fodder samples were carried out every 21 d for six months in the rainy season. The land was prepared with subsoiling, fallow, double raking and plowing at 80 cm. Two cuttings or stakes of three nodes were used, which were planted every 80 cm on the embankment of the furrow, ensuring that two of the three nodes were buried at a 45° angle in relation to the surface. The establishment period was July-August. The sampling area at each cutting date was 5.76 m^2, equivalent to three rows, 2.4 m wide by 2.4 m long, and eight cuts were made.

At the beginning of the experiment, a uniformity cut was made at 25 cm. The fertilization dose was 141N-43P-20K (N200 kg of Urea (46%), 50 kg of the mixture 18N-46P-00K, and K200 kg of the mixture 20N-10P-10K) which was dosed in two applications; in the first, eight days after the uniformity cut, the composition was 100 kg of Urea, 25 kg of the 18-46-00 mixture and 100 kg of the 20-10-10 mixture; in the second, 60 d after the first application, the rest of the nitrogen, phosphorus and potassium were added. The fodder (green matter) was harvested at a height of 25 cm from the ground, and every 21 d of growth.

During sampling, dry matter (DM) production was recorded of the complete plant in each plot, from which a subsample (300 g) was taken to dry at 55 °C until reaching constant weight using a forced air oven, and later it was ground. With the registered weights the crop's growth curves and rates were obtained. The amount of nitrogen in the fodder was determined by the Kjeldhal method, in samples of 0.5 g of complete plants, in the Animal Nutrition Laboratory of CIRGOC-INIFAP, in Campo La Posta, Veracruz. The first cut was made at 14 days of growth and then every 21 d, until 161 d, in the unfertilized plots every 21 d until 168 d of age.

Response Variables

The Nc parameters of the different cultivars of *C. purpureus* were obtained with a non-linear regression analysis using the equation (Justes *et al.*, 1994):

$$\%N = a\ DMP^{-b}$$

where $\%N$ = is the percentage of nitrogen in the total plant in DM; a = is the critical nitrogen parameter, expressed in g N 100 g of DM; b = is the parameter that indicates the dilution of nitrogen as the fodders grow and PDM = is the dry matter production of the fodders at each point or sampling time, and these in turn were adjusted with the Gompertz model to obtain the estimated data in t MS ha^{-1}.

The observed N percentages of each time in the fodder's growth were adjusted with the Levenberg-Marquart algorithm, and the Kaleidagraph® v3.0 software was used. The NNI of the fodders at each time of growth was determined for each repetition, dividing the amount of nitrogen observed at each time of growth or sampling and the parameter of Nc determined with the equation defined above.

The growth rate was calculated in kg DM ha^{-1} d^{-1}, with the DM production data estimated with the Gompertz model in the fodder's ages, with the following equation:

$$TC = \frac{MSP_{t2} - MSP_{t1}}{T2 - T1}$$

where TC = Growth rate in kg DM ha^{-1} d^{-1}, MSP_{t2} = Dry matter present in time 2, kg DM ha^{-1}, MSP_{t1} = Dry matter present in time 1, kg DM ha^{-1}, $T2$ = end time, $T1$ = initial time.

Experimental Design and Statistical Analysis

The parameters obtained from the nitrogen curves of each fodder by repetition a = Nc and b = Cd, as well as the coefficient of adjustment, were analyzed in an ANOVA in a completely randomized design in a factorial arrangement of 5×2:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$$

where factor A was the five fodders, factor B the application or not of nitrogen fertilizer, and AB the interaction. The data were analyzed with the MINITAB software version 17 in the GLM procedure. The means comparison was carried out with Tukey's test at an alpha of 0.05%.

RESULTS AND DISCUSSION

Table 1 shows the results of the data adjustment of the nitrogen percentages in the plant and DM production, during growth every 21 d and up to 168 d of age of the *C. purpureus* cultivars, with the negative power function equation of the parameters Nc, Cd and R^2. The Nc values did not show a difference (P≥0.05) between the studied cultivars; these are found in an interval between 1.85 and 2.22 g of N per 100 g DM, necessary to produce 1.0 t DM ha^{-1}. In relation to the coefficients of N, no differences (P≥0.05) were found between the cultivars; however, CT-115 and King Grass showed high negative rates of nitrogen dilution through growth with 73.5%, while the Roxo cultivar presented negative rates of less than 57.6%, that is, its decrease is very fast, which indicates that the percentage of N decreases drastically in these fodders. Nc values of 2.5 and 2.78 g of N per 100 g DM have been reported for *Brachiaria humidicola* and *Brachiaria humidicola* × *Brachiaria ruziziensis* (Juárez, 2005). In this regard, Duru *et al*. (1997) determined that the Nc for tropical fodders under non-limiting conditions of water and nitrogen is 3.6 g of N per 100 g DM and a nitrogen coefficient of 34%; in turn, Lemaire and Salette (1984 a, b) and Lemaire *et al*. (1985) demonstrated that tropical fodders decrease Nc as DM production increases.

Nitrogenous Fertilization Effect

Table 2 shows the effect of fertilization on the parameters of the nitrogen curve in *C. purpureus* cultivars, which presented differences (P≤0.05), for Nc, but not for Cd. Nc values of 2.63 g N per 100 g DM represent the minimum amount necessary to produce one t DM ha^{-1} for fodders that were fertilized with doses of 141 kg of N ha^{-1}, whereas for unfertilized fodders, it was 1.42. This decrease is possibly due to the fact that the organic matter content in the soil was not sufficient and would be limited by the lack of water. Juárez *et al*. (2004) reported a concentration of Nc of 2.9 with a fertilization dose of 200 kg of N ha^{-1} and 2.6 for the unfertilized, for species of *Brachiaria* sp., in rainfed conditions and soil rich in organic matter.

Table 1. Effect of the cultivar on the parameters and goodness of fit of the nitrogen curve during its growth, up to 168 days of five fodder genotypes.

Factor A	N	Nc	SEM	Cd	SEM	R^2	SEM
CT-115	6	2.080[a]	0.308	−0.735[a]	0.057	0.965[a]	0.011
Elephant	4	2.220[a]	0.377	−0.656[a]	0.070	0.979[a]	0.013
King grass	5	1.851[a]	0.344	−0.736[a]	0.064	0.977[a]	0.012
Roxo	6	2.001[a]	0.308	−0.576[a]	0.057	0.986[a]	0.011
Taiwan	5	1.961[a]	0.344	−0.623[a]	0.064	0.979[a]	0.012

N = number of observations; Nc = Critical Nitrogen; Cd = Nitrogen Coefficient; R^2 = Goodness of fit; SEM = Standard error of the mean; Different literals are statistically significant (Tukey; P≤0.05).

Table 2. Effect of fertilization on the parameters of the nitrogen curve during growth of five fodder genotypes.

Factor B	N	Nc	SEM	Cd	SEM	R^2	SEM
Fertilized	14	2.627[a]	0.204	-0.645[a]	0.038	0.977[a]	0.007
No Fertilized	12	1.419[b]	0.222	-0.686[a]	0.041	0.977[a]	0.008

N = number of observations; Nc = Critical Nitrogen; Cd = Nitrogen Coefficient; Goodness of fit R^2; SEM = Standard error of the mean; Different letters are statistically significant (Tukey; P≤0.05).

Growth Rate and Nitrogen Curves of Cultivars

Figures 1, 2, 3, 4, and 5 show the growth rate behavior and the nitrogen curve of cultivars *C. purpureus* with and without fertilization.

Cultivar CT-115

It is observed that the effect of nitrogenous fertilization on the maximum growth rate was 112 kg DM ha^{-1} day^{-1} and was reached at 35 d of age, with the production of 3,920 kg DM (Figure 1). In addition, with the adjustment of the nitrogen curve, an Nc content of 2.62 g of N per 100 g DM necessary to produce 1.0 t of DM ha^{-1} was obtained, for a DM production interval of 1 to 8 t of DM ha^{-1} (Figure 1). On the contrary, without fertilization, this cultivar showed a growth rate three times lower, of 44 kg DM d^{-1}, presenting its peak at 42 d of age and requiring 1.54 g of N per 100 g DM ha^{-1} day^{-1} to produce only 1,800 kg DM ha^{-1} at 42 d. In other words, this cultivar showed rapid growth by only providing 65% of the nitrogenous fertilization at the beginning, plus the soil content, requiring 102 kg of N ha^{-1} to produce 4 t of DM, with an efficiency of 72 % of the fertilizer; this suggests that the second portion of the nitrogenous fertilization (35% remaining) had no effect on the maximum growth rate, possibly due to the efficiency resulting from the effect of the cultivar. In this case, a fertilization frequency appropriate to its growth is suggested.

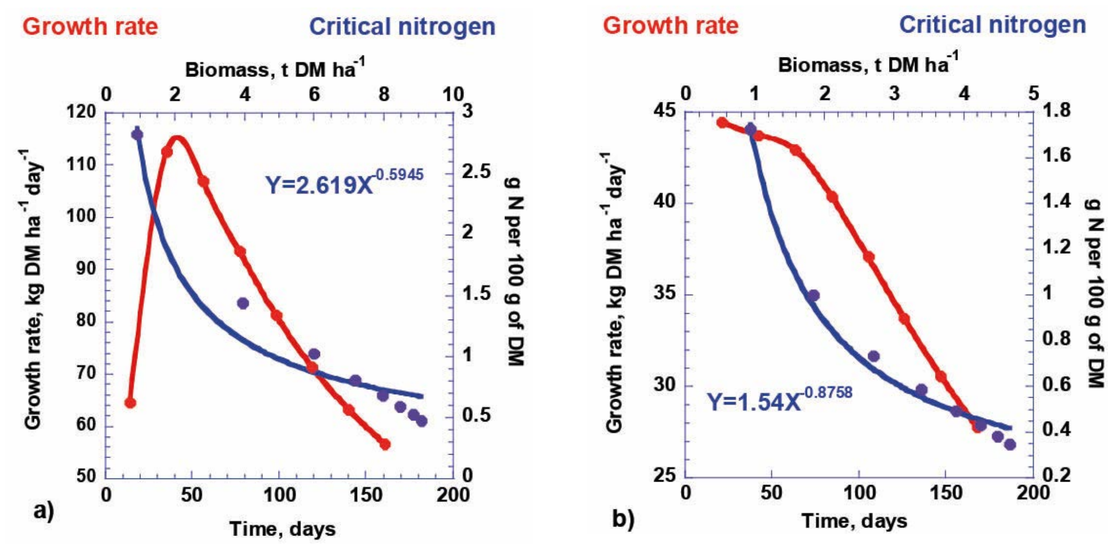

Figure 1. Growth rate and Nc of cultivar CT-115: a) Fertilized b) Not Fertilized.

Cultivar Elefante

The fertilized cultivar Elefante used 2.6 g of N per 100 g of DM to produce 1.0 t of DM ha^{-1} similar to cultivar CT-115, but required 149 kg of N ha^{-1} to produce 5,700 kg of DM at its maximum peak at 56 d of age, with a growth rate of 102 kg of DM ha^{-1} day^{-1} (Figure 2a). A similar situation occurred with the unfertilized control in CT-115, with only 1,828 g of N per 100 g DM per t of DM ha^{-1}, resulting in this cultivar extracting nitrogen from the soil, making the latter poorer at the expense of increasing production of DM ha^{-1} and with an efficiency of nitrogen use of 87% (Figure 2b).

Cultivar King Grass

The growth rate of fertilized King Grass was 56 kg DM ha^{-1} day^{-1} against 27.7 kg DM ha^{-1} day^{-1} without nitrogenous fertilization (Figure 3b). A maximum peak of production of 4,300 kg DM ha^{-1} was observed at 77 d of age and 1,749 kg DM ha^{-1} at 63 d of age, respectively. With Nc values of 2.62 and 1.08 g of N per 100g of DM, for fertilized and unfertilized, respectively. For fertilized King Grass, 113 kg of N ha^{-1} were required, and for this same cultivar without fertilization only 18.9 kg of N ha^{-1} to obtain its maximum DM production. In both cases, the percentage of nitrogen decreases at a rate of 69% in the fodder. On the other hand, with the contribution of 100% of nitrogen in two applications as fertilizer, it seems that there was an effect on the production peak, since the second application of nitrogen (35% of the 141 kg) was carried out after 56 d of regrowth. The efficiency of nitrogen use against the soil content was 83.4%.

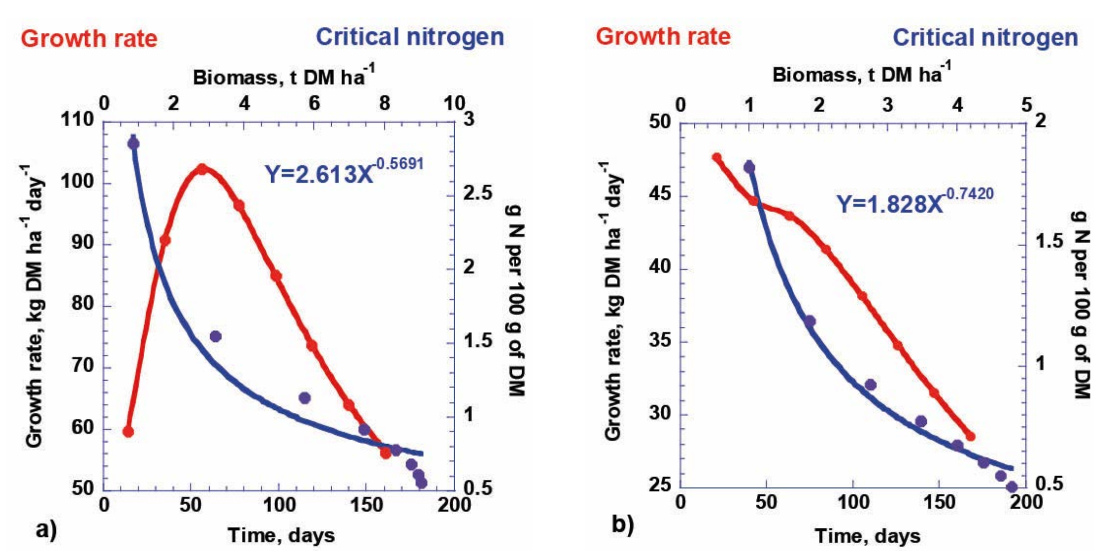

Figure 2. Growth rate and Nc of cultivar Elefante: a) Fertilized b) Not Fertilized.

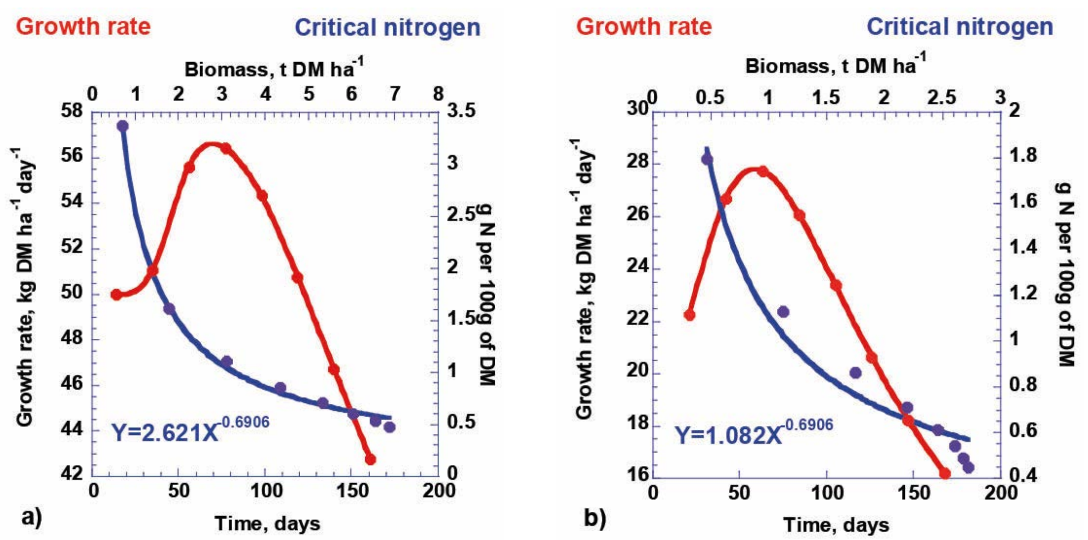

Figure 3. Growth rate and Nc of the cultivar King Grass: a) Fertilized b) Not Fertilized

Cultivar Roxo

The cultivar Roxo presented a higher growth rate compared to the cultivar King Grass of 76 kg DM ha^{-1} day^{-1} when fertilized and 38.5 kg DM ha^{-1} day^{-1} without fertilizing, at 77 and 63 d of age respectively. The Nc values were 2.65 and 1.35 g of N per 100g of DM, fertilized and unfertilized, respectively; therefore, it required a greater amount of nitrogen (155 kg N ha^{-1}) to produce 5,844 and 2,425 kg of DM respectively. The efficient use of nitrogen was close to 78%, so the fertilization dose was not enough, since nitrogen was extracted from the soil.

Cultivar Taiwan

The fertilized cultivar Taiwan presented the highest production of DM ha^{-1} compared to the previously mentioned cultivars. With production of 6,314 kg of DM ha^{-1} at 77 d, in which it reached its maximum growth rate with 82.72 kg of DM ha^{-1} day^{-1}. The Nc was similar to the cultivars with 2.6 g of N per 100 g of DM in a production of 1.0 t DM ha^{-1}, but when this cultivar did not receive nitrogenous fertilization, its production decreased by 73% (1,700 kg of DM ha^{-1}). Its growth rate was reduced to half (40.5 kg DM ha^{-1} day^{-1}) and its Nc content was 1.29 g of N per 100 g of DM. The efficiency of the use of nitrogen was 86% and showed balance in the nitrogen consumption of the soil, that is, the dose of nitrogenous fertilization was sufficient to grow and obtain these fodder productions.

N, Nc, NNI Parameters in Cultivars

In general, when the evaluated cultivars are fertilized with doses of 141 kg of N ha^{-1} in two applications and

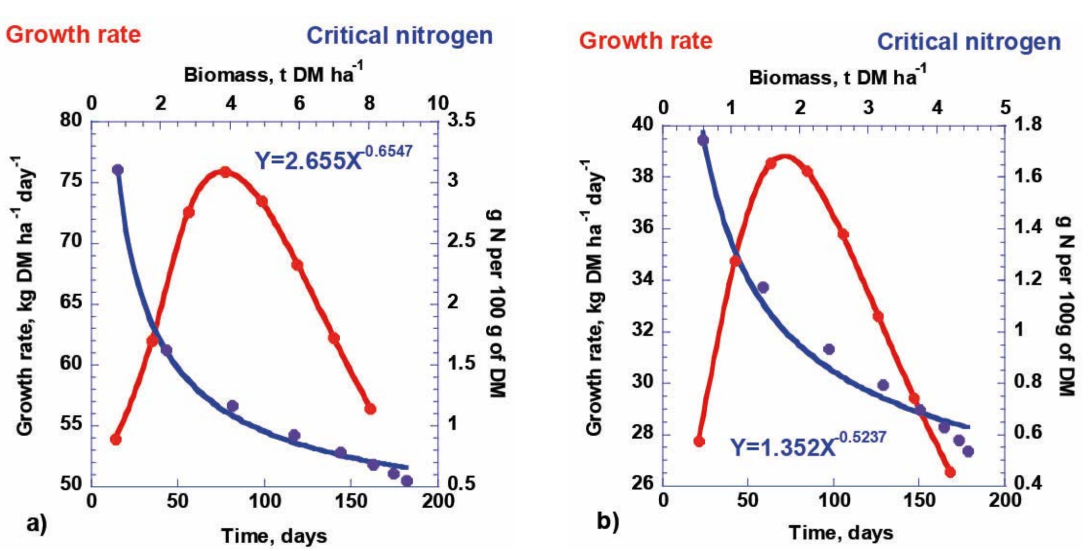

Figure 4. Growth rate and Nc of the Roxo cultivar: a) Fertilized b) Not Fertilized.

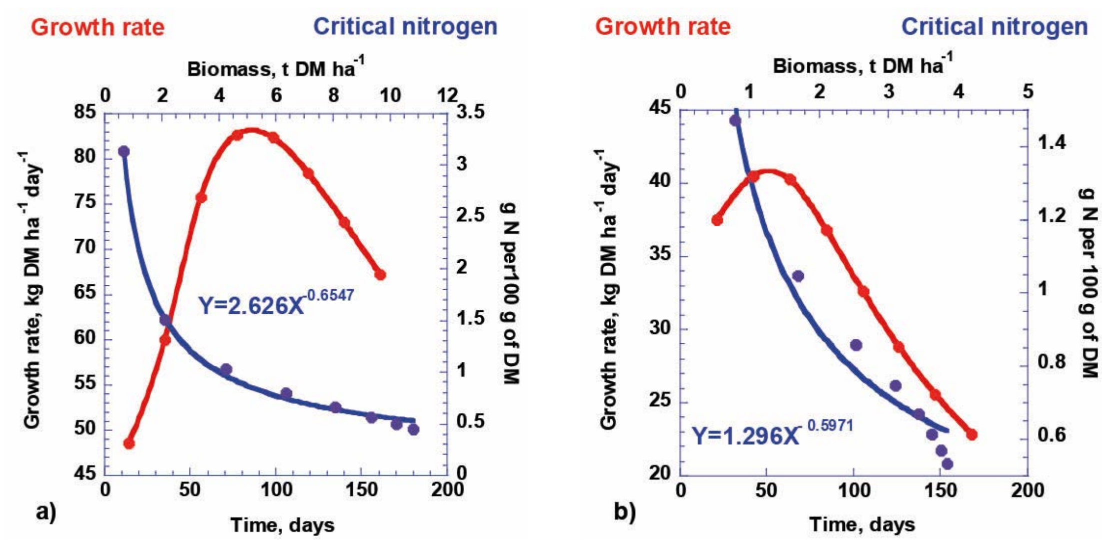

Figure 5. Growth rate and Nc of the cultivar Taiwan: a) Fertilized b) Not Fertilized.

in the rainy season in the Mexican tropics, they have Nc of 2.6 g of N per 100 g of DM in t MS ha^{-1}. The Nc values of this study were lower than those reported by Lemaire and Gastal (1997) who mentioned that the Nc values are 3.6 g of N per 100 g of DM in tropical fodders. These differences may be due to factors, such as the high DM production of these cultivars of *C.pupureus*, the deficiency or lack of nitrogen, water stress, among other factors. They also presented a decrease in the N content, with an average of 59% in the fodder, which caused that in all cultivars, except Taiwan, more nitrogen was extracted from the soil due to the deficit in the dosage or contribution of mineral nitrogen.

Regarding the growth rates, cultivars CT-115 and Elefante registered their maximum growth before applying 35% of the rest of the nitrogen, that is, it was before 56 d of age, in contrast to King Grass, Roxo and Taiwan whose maximum growth was recorded at 77 d of age. These results suggest applying the fertilization dose before its maximum growth occurs. In antecedents to the thresholds established by Duru *et al*. (1997) for the management of the NNI of fodders, Figure 6a shows where the NNI was higher than 1.0 in the first 14 d, which indicates that the studied cultivars had excess N for their growth. However, from 35 d of age the NNI of cultivars King Grass, CT-115, Elefante, Roxo and Taiwan, these values were located in the classification of nitrogen deficient (NNI<0.8). With the results obtained, the application of nitrogen can be planned to obtain the highest efficiency; since, as it was observed, at the beginning of the growth nitrogen is present in excess and after day 35 of growth the deficiencies appear.

Figure 6b shows the NNI behavior of cultivars CT-115, Elefante, King Grass, Roxo and Taiwan, during growth without application of the fertilizer. The NNI data are less than 0.8, therefore in accordance with the threshold described by Duru *et al*. (1997), it is in deficiency. This is demonstrated by the low Nc value of 1.14 g of N per 100 g of DM and low growth rates and production of dry matter. Although the NNI data in the cultivars studied during the first 35 d of age are sufficient, the dose used did not reach the fodder requirements, since they extracted N from the soil to complement growth and DM production.

CONCLUSIONS

The critical nitrogen content of the cultivars of *Cenchrus purpureus* fertilized was 2.6 g of N per 100 g of DM to produce 1.0 t DM ha^{-1} and growth rates up to 112 kg of DM per day in 77 d. The NNI values were higher than 0.8 during the first 56 d, which is considered good nutritional status, but considering the data of the unfertilized group it is considered that these cultivars require a greater amount of N in order not to extract the complement from the soil. This demonstrates the usefulness of these agronomic tools such as Nc and NNI to help diagnose the N requirements of said fodders, and thus be able to make adjustments to fertilization and its frequency of application.

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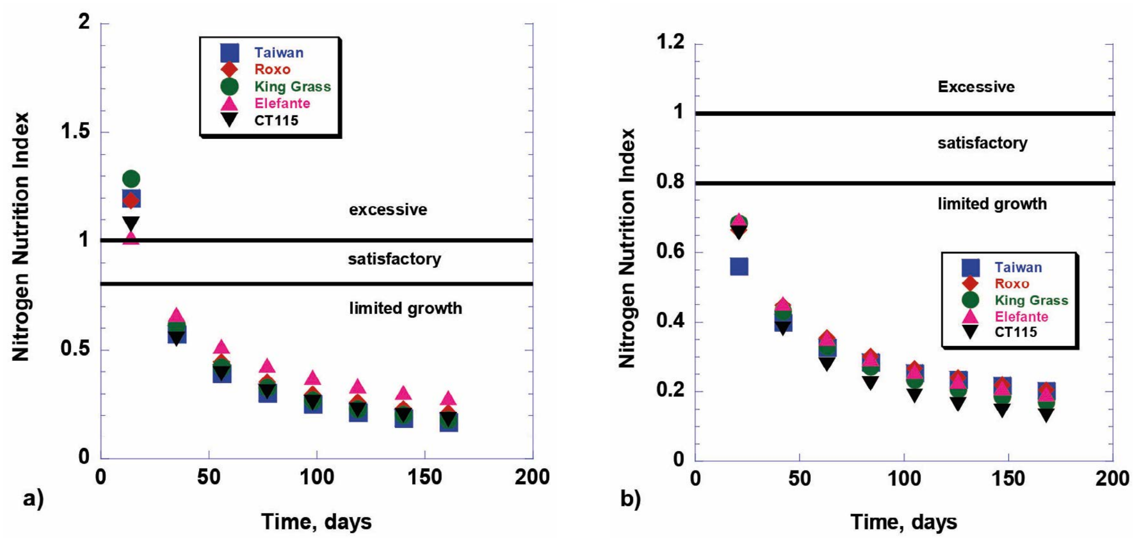

Figure 6. NNI of cultivars of *Cenchrus purpureus* during growth: a) Fertilized b) Not Fertilized.

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Dark cutting in large ruminants: Effect of management and environmental factors

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ABSTRACT

Objective: This review analyzes the scientific findings on the main management and environmental factors that increase the incidence of dark cuts in the carcass of water buffalo and bovine of the *Bos* genus.

Design/methodology/approach: Scientific articles were obtained from CAB Abstracts, Pubmed, Scopus and Google Scholar databases. The primary search was carried out on the following keywords: *Antemortem* stress and handling, dark cut, transport, *antemortem* rest, fasting prior to sacrifice. Detailed searches were subsequently carried out on each species.

Results: There are various stressors in handling and pre-slaughter procedures in ruminants. Stress in animals causes bodily injury and affects the quality of the meat, increasing dark cutting in the carcass.

Findings/conclusions: Management practices must be improved in the pre-slaughter stages, animal welfare must be ensured, and stressors must be avoided or reduced. These benefits will help to increase the level of welfare of the animals and will avoid economic losses, due to the presence of dark cutting in the carcass.

Keywords: *Bubalus bubalis*, *ante-mortem* handling, river buffalo, dark cutting, transport, lairage, beef.

INTRODUCTION

The water buffalo (*Bubalus bubalis*) and cattle of the genus *Bos* are important food sources for the human population. The end development of these animals can occur through fattening in feedlot or grazing. When the animals reach mature body weight, they are shipped, transported, and taken to the slaughterhouse, during slaughter the animals suffer from stress caused by various external factors thus affecting meat quality (Mota-Rojas *et al*., 2010a,b).

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Water buffaloes are valued for their production, disease resistance, longevity, fertility, and meat quality (Macedo, 1999; Sheikh, 2002; Mora-Medina *et al*., 2019; Bertoni *et al*., 2019; Hoogesteijn and Hoogesteijn, 2008; Guerrero-Legarreta *et al*., 2020). These animals have better rearing advantages than *Bos* cattle, for example, they are adapted to the hot and humid regions of muddy and swampy lands of the tropics (Sheikh, 2002; Bertoni *et al*., 2019; Guerrero-Legarreta *et al*., 2019a; Mota-Rojas *et al*., 2019a, b). Although, the appreciation of buffalo meat varies between countries and regions. In some Asian and European countries, buffaloes are marketed at lower prices than cattle, livestock companies implement sales prices and consequently, the productive interest of farmers is limited (Hoogesteijn and Hoogesteijn, 2008; Guerrero-Legarreta *et al*., 2019b).

The handling and transport of finished animals for meat production, commonly suffer from stress, detracting from their welfare and inducing causes of shrinkage of the carcass, presence of the dark cut in the meat and damage to the carcass due to injuries or bruises (Chandra and Das, 2001ab; Guerrero-Legarreta *et al*., 2002; Alarcón-Rojo and Duarte-Atondo, 2006; Guerrero-Legarreta and Totosaus, 2006; Mota-Rojas *et al*., 2010a, b; Guerrero-Legarreta *et al*., 2019b). In reference to beef (*Bos*), a pH of 6.0 in 24 h (pH_{24}) after slaughter, causes a poor quality of meat. Therefore, becoming undesirable for consumption creating economic losses (Mach *et al*., 2008). For example, the meat industry in Spain penalizes the price of carcasses with discounts between 30 and 60%, when the pH of the meat is greater than 5.8 (Mach *et al*., 2008). Dark firm dry meat (DFD) occurs when the final pH (measured in 12-48 h) post-mortem is ≥6 (Apple *et al*., 2005; Warris, 2000). The term DFD is most commonly used in pork meat, so when it occurs in beef, the term assigned is "dark cutting" (Prince *et al*., 1994; Southern *et al*., 2006). Beef with a pH_{24} greater than 6.0 has a dark red color (Mach *et al*., 2008; Pérez-Linares *et al*., 2006), there is deterioration in tenderness, the water holding capacity increases (dry) and lower palatability with firmer texture, increases light absorption, microbial growth, unpleasant odor and less uptake of curing salts (Grandin, 1997; Mach *et al*., 2008; Prince *et al*., 1994; Alarcón-Rojo and Janacua-Vidales, 2010).

The consumer classifies dark cutting as old meat (Pérez-Linares *et al*., 2008; Guerrero-Legarreta *et al*., 2020; Cruz-Monterrosa *et al*., 2020). The increase in pH_{24} is due to the decrease in glycogen pre-slaughter, caused by the physical and psychological stress that occurs in the animals before slaughter. The various stressors involved are the long transport times given from the farm to the slaughter plant, the time the animal waits to enter the slaughterhouse, the climatic conditions and the social breakdown (Schaefer *et al*., 2001; Önenç, 2004; Ferguson and Warner, 2008; Mach *et al*., 2008). However, glycogen levels also vary widely at slaughter, depending on the amount of feed consumed, body weight, nutritional status, muscle type, fiber type, muscle buffer capacity, gender, breed type and temperament (Ferguson and Warner, 2008; Mach *et al*., 2008). Additionally, the handling of the buffalo is more difficult than the cattle, it requires more personnel and time for the activities of loading, confinement, herding, movement, unloading and in the waiting pens for slaughter. Management of water buffalo must have an adequate balance in the truck to avoid injuries (Chandra and Das, 2001).

Most meat-producing countries report annual frequencies of dark cutting meat from 3 to 22% (Pérez-Linares *et al*., 2008). The UK reports a 10% incidence of dark cutting. The United States reports 4% (Warris, 2003, Pérez-Linares *et al*., 2006). Spain (Mach *et al*. 2008) and northern Mexico (Pérez-Linares *et al*. 2006) report incidences of dark cutting of 13 and 9%, respectively. The causes of stress are associated with high ambient temperatures, body efforts and high arousal after slaughter (Prädl *et al*., 1994).

This review analyzes the environmental and management factors that influence dark cutting in water buffalo and cattle (Figure 1, 2). The fasting period, duration of transport, transport conditions and environmental factors, among others, are described.

Environmental and management factors that influence the presence of dark cutting

Buffalo and cattle can be stressed by hunger, thirst, fatigue and injuries (Ferguson and Warner, 2008; Grandin, 1997; Mach *et al*., 2008; Mota-Rojas *et al*., 2019a,b,c). The presence of dark cutting implies animal stress and inadequate conditions before and during slaughter (Grandin, 1997; Mota-Rojas *et al*., 2010a,b). Factors that induce chronic stress include long periods without food consumption (prolonged fasting), fatigue caused by long periods of transport, fights between animals from different herds when boarding the truck (Silva *et al*., 1999; Grandin, 1997; Warris, 2000; Alarcón-

Rojo and Janacua-Vidales, 2010; Pérez-Linares *et al*., 2008) and/or poor handling during unloading. All these factors cause physical exhaustion (Kent and Ewbank, 1983; Grandin, 1997; Mounier *et al*., 2006; Ferguson and Warner, 2008; Jeleníková *et al*., 2008; Pérez-Linares *et al*., 2008) and have a negative effect in meat quality (Broom, 2003; Mounier *et al*., 2006; Alarcón-Rojo and Janacua-Vidales, 2010).

Effect of fasting

Perhaps fasting in ruminants has lower adverse effects than in other species, because the rumen acts as a reservoir of nutrients and the production of volatile fatty acids is a constant source of energy (Gallo and Tadich 2005). However, the loss of energy reserves causes depletion of liver and muscle glycogen, inducing problems of browning of the meat in the postmortem period (Ferguson and Warner, 2008). Hargreaves *et al*. (2004) mentions an increase in dark cutting, when the steers were transported without rest for 24 h while fasting. Animal transport must be programmed, from the moment they arrive at the truck until they are housed in the pens of the slaughterhouse, considering rest periods during the trip. Rest activities for 16 h and fasting for 24 h in animals induces a decrease in β-HBA (Tadich *et al*., 2005; Gallo and Tadich, 2005). Food deprivation in animals must be managed properly to prevent adverse effects on meat quality. The cited authors conclude that good quality meat can be achieved when resting times are given, before slaughtering the animals.

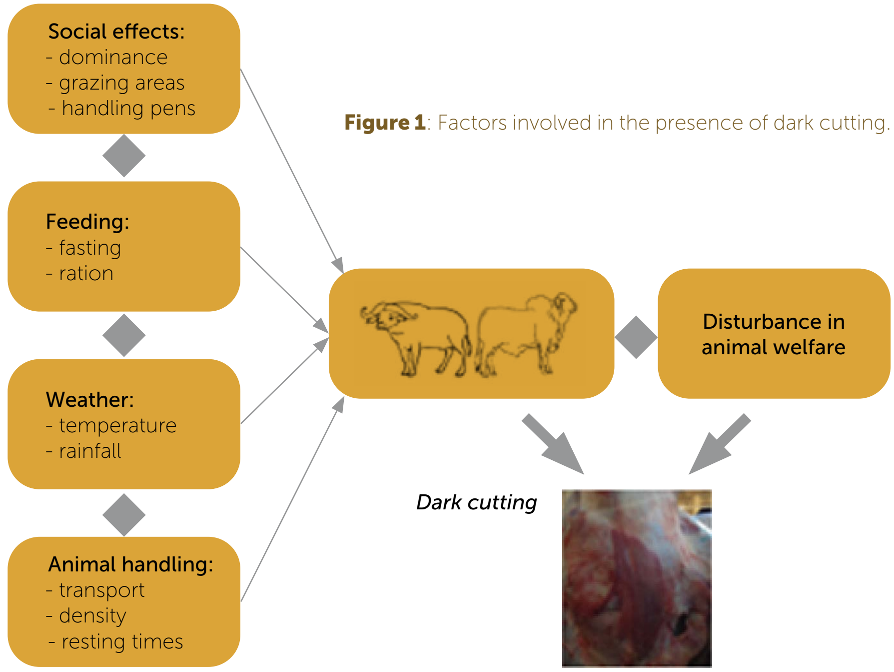

Figure 1: Factors involved in the presence of dark cutting.

Effect of climatic conditions

Adverse seasonal conditions enhance stress in meat-producing animals, cattle are more sensitive to a hotter environment than to cold temperatures, especially when there are sudden changes in temperature (Kadim *et al*., 2004). Kreikemeier *et al*. (1998) cite that the dark cutting percentages were more common in the months of October (0.43%) to February (0.69%). Likewise, Grandin (1992 and 1993) reported a higher percentage of dark cutting in animals raised in very cold climates and with high rainfall, due to loss of body heat and shivering.

Effect of transport

Animal stress caused by transport is due to the lack of resting times, lack of food or water, exaggerated noises, fatigue, sudden changes in temperature, accelerated movements of the truck and space restrictions (Ferreira

Figure 2: Water buffalo (*Bubalus bubalis*) and its commercial meat.

et al., 2006; Gallo, 1994; Hargreaves *et al*., 2004; Gallo and Tadich 2005).

The time of transfer of animals from the place of breeding to the slaughterhouse should be from 1 to 48 h; if the time is longer, the quality of the carcass and the meat is affected. Losses are particularly caused in live weight, carcass yield and quality grade, appearing dark cutting (Schaefer *et al*., 2001). Gallo and Tadich (2005) report that the muscular pH of the steers was not affected when the transport of the animals was less than 16 h from the corral to the slaughterhouse, and when resting periods before slaughter were less than 16 h. In the case of the water buffalo, Zava (2011) mentions that transport for more than 700 km causes a loss of 7 and 9% of their body weight. Also Pérez-Linares *et al*. (2006) indicate that the arrival of the animals to the truck for more than 35 minutes, with temperatures above 16 °C, caused the damage of dark cutting.

Effect of charge density

Animal load density during transport affects the levels of some hematic variables (Knowles, 1999; Gallo and Tadich 2005). Tadich *et al*. (2003b) evaluated the densities of 400 *vs*. 500 kg/m^2 on some blood metabolites, indicating that the higher density increased cortisol, glucose and creatine kinase. Cortisol and CK levels remained high until 12 h of animal rest, while the glucose level did not change.

The effect of prolonged transport stress and fasting in sheep also increased blood cortisol content, unlike animals with shorter transportation times (Gallo and Tadich 2005). Similar results in cattle report high glucose and CK when they were transported in the truck without offering feed on arrival (Tadich *et al*., 2003b). In Chile, the main risk factors that induce dark cutting is the relationship between fasting and transportation times (Amtmann *et al*., 2004, Gallo and Tadich 2005). Consequently, the animals should avoid prolonged transportation and long waits until slaughter.

Behavior of the water buffalo during transport

Chandra and Das (2001a) cite previous routines that should be considered before transporting water buffalo to the slaughterhouse. In Asia, non-productive animals are transferred to the place-sale, then butchers buy the animals and transport them by trucks to the slaughterhouse (Bertoni *et al*., 2019). On the other hand, a study carried out with 100 buffalos, divided into ten different transport routes with distances of 20 and 40 km for 30 minutes. The researchers recorded the activities of the animals on the moving truck. The results of clinical signs that buffaloes presented during the trip were: Ocular congestion, tearing, nasal discharge, signs of stress and fear in buffalo. During the transport route, some animals changed their orientation and placed themselves in a parallel, perpendicular or diagonal position inside the truck. Subsequently, 14 buffaloes changed their initial orientation from parallel to perpendicular or diagonal, there was loss of balance in 80 animals, the curves of the road and speed of the truck caused stress in the animals. The same authors reported ocular congestion lesions (52 animals), nasal discharge (13 animals) and salivation (4 animals). During the trip, urination of the animals was observed in 55 animals, only 20 animals urinated twice, 6 animals three times and 21 buffalo defecated. The high frequency of urination and defecation recorded in animals is an indicative sign of fear (Chandra and Das, 2001a).

Presence of injuries and well-being of the buffalo during transport

Chandra and Das (2001b) reported injuries to water buffalo during short movements in India. Bruises were considered injuries of interest in the body, sometimes there is blood secretion in the surrounding areas, due to the rupture of blood vessels. During slaughter, the authors used the Australian scoring system to classify animal carcass bruises. They reported 244 bruises of different categories in the 100 buffalo. Small-deep bruises were the most common (59.0%), then medium bruises (19.3%), small (9.8%), medium-deep (6.1%) and intense (5.7%). Many bruises were observed in the hind limbs (43.4%), the abdomen and upper region (21.3%), the shoulder, neck and back (16.0%) and the perianal region (11.1%). The skin was opened in 10% of traumatic injuries, while the muscle tissues were affected in 90%. Consequently, the economic losses were considerable due to trauma, with the removal of the damaged tissue in the post-mortem canal. Most of the damaged tissues were attributed to improper handling of the animals during loading and unloading and the fall of the animals, caused by mishandling the sudden braking of the truck.

Rest times in the animals

The resting places should be the least stressful for the animals. The stress factors cause physiological disorders in the animals and the carcass with high pH limits vacuum packaging, there is rapid deterioration, the carcass yields and the price of meat decreases (Almonacid, 2003; Gallo and Tadich

2005). Pipek *et al*. (2003) mentioned that the incidence of DFD can be reduced when the animals are housed in individual pens, improving the quality of the meat (pH_{24} and reflectance), contrary to when several animals are put in a pen. Pérez-Linares *et al*. (2008) observed that resting the animals inside the pen for 12 and 18 h was not enough to reduce stress. On the other hand, Hargreaves et al. (2004) mentioned benefits with the increase of resting time, reducing stress and the pH of the meat. The cited authors suggest resting times should be greater than 24 h in the pen. This condition improves glycogen reserve, there is less muscle glycolytic activity, decreases post-mortem lactic acid formation (Hargreaves *et al*., 2004), and undesirable characteristics in meat are prevented (Prince *et al*., 1994).

CONCLUSION

The appearance of dark cutting is directly related to the amount of muscle and liver glycogen thus decreasing the quality of the meat. Animals cannot remain for a long time without eating food and must be handled properly during the antemortem period. Stress periods in buffaloes and cattle must be kept to a minimum, avoiding the use of energy reserves and the increase in pH of the meat. Good practices of handling training on ruminant welfare must be applied to avoid suffering prior and during to slaughter.

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Postharvest management of *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics

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ABSTRACT

Objetive: Evaluate different techniques to prolong the postharvest life of *Heliconia psittacorum* x *H. spathocircinata* cv. Tropics.

Design/methodology/approach: Two experiments were carried out; in the first it was evaluated the effect of five pulse solutions [(T1 = control (water); T2 = Hydraflor® 100 (0.5 g L^{-1}); T3 = Hydraflor® 100 (0.5 g L^{-1}) + sucrose (5% w/v); T4 = salicylic acid (1 mM) and T5 = salicylic acid (1 mM) + sucrose (5% w/v)] prior to storage at 11 °C and 85% RH for 10 d. In the second experiment, a wax coating was applied to the heliconia bracts and then maintain at 13 °C and 84% RH for 10 d; after storage the stems were placed in water or salicylic acid solution (1 mM). The results were analyzed by a completely randomized design, 10 replicates were used per treatment, one stem was one experimental unit. An ANOVA and a means comparison test (Tukey, α=0.05) were performed with the SAS version 9.4.

Results: The anatomy of heliconia stems determines their vase life, since they have wide xylem vessels susceptible to cavitation, for which the absorption of water is limited, also and non-functional stomata in the bracts, which increase the loss of moisture from the tissue. The use of pulse solutions or preservatives has little effect in prolonging the life of heliconia stems. Therefore, the application of wax creates a physical barrier that maintains the turgor of the stems, reduces oxidation, maintaining the bracts quality and increased the total postharvest life for 6 d more than the control stems.

Study limitations/implications: No limitations were found in this study.

Findings/conclusions: The postharvest life of heliconia is conditioned by the loss of turgor and low water absorption, which leads to an early wilting of the inflorescences. The application of pulse solutions or preservatives have little effect in prolonging the life of the vase, so the most suitable technique to preserve the quality of the stems is waxing.

Palabras clave: cold storage, water absorption, waxing, pulse solution, preservative solution, vase life.

INTRODUCTION

The postharvest life of ornamentals is linked to the interaction between genetics and the anatomical and physiological characteristics of each species (Castro *et al.*, 2015). The shelf life of cut flowers depends on the absorption of water, respiration, and transpiration; and biochemical changes such as enzymatic activity, lipid peroxidation, permeability of the

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cell membrane, associated with oxidative stress (Abri *et al*., 2013). Then the turgor and fresh weight of the cut stems, depend on their ability to absorb water, which is determined by the water conductivity and characteristics of the vascular bundles of the stems (van Meeteren and van Gelder, 1999). Likewise, transpiration is essential to regulate water absorption, however if the stomata do not respond adequately to environmental factors, a negative water balance develops, which implies greater loss of water than that absorbed by the stem, which leads to accelerated wilting (van Doorn, 2012).

The use of pulse or preservative solutions help to maintain physiological processes, due to their low pH, and content of sucrose and biocides that reduce the microbial load of the water, improving the water absorption and supplying the necessary substrate for respiration (Pun and Ichimura, 2003). In tropical species such as *Zingiber spectabile*, the use of Hydraflor® 100 (Floralife Co.) (16 mL L^{-1}) increased the vase life (Coelho *et al*., 2012). The use of salicylic acid (AS) solution in cut flowers of anthurium (*Anthurium andraeanum* cv. Sirion) delayed the spathe browning, associated with a low polyphenol oxidase activity and the delay of electrolyte leakage (Aghdam *et al*., 2016). On the other hand, wax coatings on tropical flowers have been proven to increase longevity by lowering the transpiration and respiration and in addition improving their appearance.

Like other fruit and vegetable products, cut flowers are highly perishable, for that reason must be handled and stored properly. To maintain its value, quality, and prolong the vase life, postharvest techniques are needed (Folha *et al*., 2016). However, in *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics little information is available for its postharvest handling, so the aim of this work was to determine an adequate treatment to prolong its shelf life.

MATERIALES Y MÉTODOS

Plant material

Inflorescences of *H. psittacorum* × *H. spathocircinata* cv. Tropics were obtained from a commercial plantation located in Ixtaczoquitlan, Veracruz (18° 52' 18.90'' N and 97° 01' 34.84'' W, altitude 998 m). The stems were transferred to the laboratory at room temperature (18-21 °C). Inflorescences with two to three open bracts were selected, cleaned (removing the flowers from inside the bracts), washed and standardized stem length of 100 ± 1 cm.

Anatomical description

Histological preparations of the stem and bracts were made and observed under a scanning electron microscope in order to describe the stomatal (type and stomatal frequency), and stem vascular system characteristics. Transpiration test was performed with a gravimetric method, based on the weight loss of 10 stems in a controlled environment (24 °C, 60% RH and 12 h photoperiod).

Experiment 1

One hundred stems were harvested and divided into five batches, then placed in a pulse solution (12 h) prior to storage: T1 = control (water); T2 = Hydraflor® 100 (0.5 g L^{-1}); T3 = Hydraflor® 100 (0.5 g L^{-1}) + sucrose (5% w/v); T4 = salicylic acid (1 mM) and T5 = salicylic acid (1 mM) + sucrose (5% w/v). Later they were stored at 11 °C and 85% RH for 10 d. Half of the stems per treatment were stored in water (wet condition) and the other half in dry (in plastic bags). After storage the variables the stems were place in water and variables: fresh weight (FW), water consumption (WC), percentage of cell membrane integrity (CMI) and vase life (VL) were evaluated.

Experiment 2

Sixty stems were selected and divided into two groups: 1) control (30 stems) and 2) waxed (30 stems). The bracts were waxed (Lustr 227 F®, Decco) based on carnauba-shellac, dried for 3 h. They were handled under two conditions: room temperature (22 °C, 68% RH) and cold storge (13 °C and 84% RH) for 10 d in a dry condition; after storage, the stems were placed in water or salicylic acid solution (1 mM). Then treatments were: Control = stems in water at room temperature; WW = stems waxed placed in water at room temperature; RW = stems cold stored and placed in water; RSA = stems cold stored and placed in salicylic acid (1 mM); RWW = stems waxed and cold stored and placed in water; RWSA = waxed stems and cold stored and placed in salicylic acid solution (1 mM). Each treatment had 10 repetitions, each stem in one replicate. During the VF, the solution was renewed every 5 d and a cutout of the base of the stem of 2-3 cm.

Variables evaluated

Fresh weight (FW %) and water or solution consumption (WC or SC): The fresh weight of the heliconia stem was recorded with a digital scale. The results were expressed as a percentage of fresh weight loss related to the original weight. Solution consumption was evaluated as the difference between the initial and final volume consumed by each flower stem. The results were expressed as mL of solution per gram of fresh weight (mL g^{-1}).

Cell Membrane Integrity (CMI %): Samples of three discs (10 mm in diameter) were taken from the bract tissue of the second bract. The method of Mangave *et al*., (2013) was followed.

Vase life (VF) and total vase life (TVL): Vase life was evaluated as the number of days elapsed since they left storage until the flower stems showed visible signs of deterioration. To evaluate the visual quality of the inflorescences, an A - D scale was designed (Figure 1), where grade D was considered the end of the vase life of the stems. The total postharvest life was determined as the sum of the days of storage plus the days of vase life.

Statistical analysis

In both experiments the variables results were analyzed by a completely randomized design, 10 replicates were used per treatment, one stem was one experimental unit. Analysis of variance (ANOVA) was performed for all data and the values were analyzed with the mean comparison test (LSD, 0.05). All statistical analyzes were performed with SAS® version 9.4.

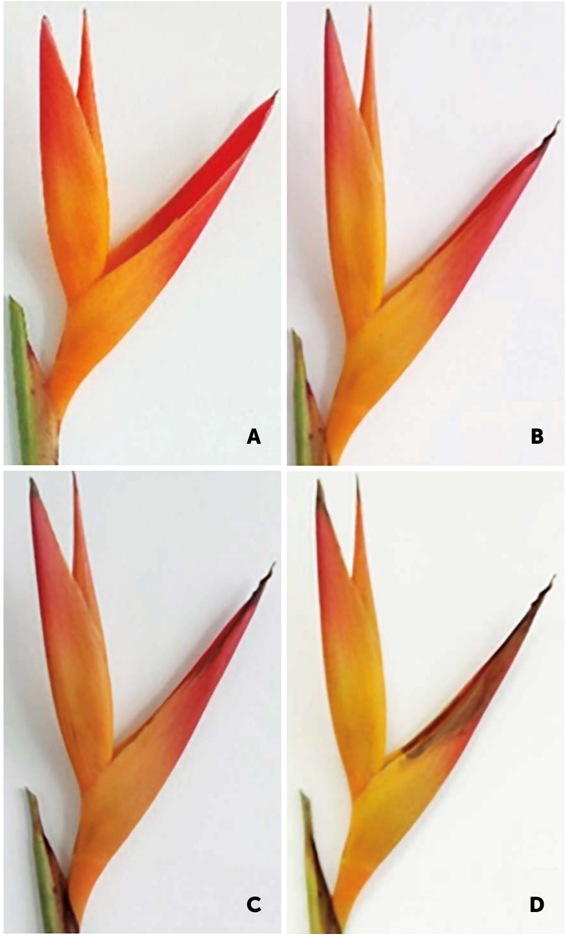

Figure 1. Wilt scale in inflorescences of *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics.

RESULTS AND DISCUSION

Anatomical description

H. psittacorum × *H. spathocircinata* cv. Tropics is an ornamental plant native to South America. It has a musoid growth habit, that is, with upright leaves and very long petioles. Its height can reach 2 m with leaves blade of 37 to 60 cm long, the inflorescence is terminal and erect, bracts type cimbiform, distic, and range in number from 3 to 7 per inflorescence (Whistler, 2000; Arriechi & Sanabria, 1995). The bracts have paracytic stomata, characterized by presenting two attached cells at the same level as the epidermal ones, arranged parallel to the major axes of the occlusive cells. The stomatal frequency were 56.71 and 13.46 mm^{-2} stomata in the abaxial (Figure 2A) and adaxial (Figure 2B) epidermis respectively, and the length of the stomata is around 31.30 μm.

We found low response of these stomata to environmental factors, which suggests non-functional type, which do not respond to temperature, light, or RH, that induce dehydration or water stress, leading to the development of a negative water balance and consequently the end of the vase life (van Doorn, 2012). Elibox and Umaharan (2008) report that the stomata of non-foliar organs such as stem, bracts and petals are not always functional.

The bracts contain air channels, fiber bundles distributed very closely to each other on the periphery of the abaxial region (Figure 2C), and vascular bundles of a length of about 234.60 μm, aligned between the fiber bundles and the air channels. (Figure 2D). In the pseustem in the outer layer, fiber bundles were observed in great quantity in the periphery, a spongy aerenchyma covering a large part of the surface (Figure 2E); while the central part is made up mainly of vascular bundles with an average length of 418.02 μm (Figure 2F). Stomata are the main channels for the vapor exchange between the plant and atmosphere (transpiration), and their functionality contributes or not to the water loss in the flowers (Huang *et al*., 2018). An opposite behavior occurs in cut flowers such as *Rosa hybrida* cv. Akito with a good correlation between stomatal functionality and fresh flower weight and vase life (Woltering and Paillart, 2018).

Experiment 1

The stem water balance involves the absorption, transport, transpiration, and the capacity of the tissues

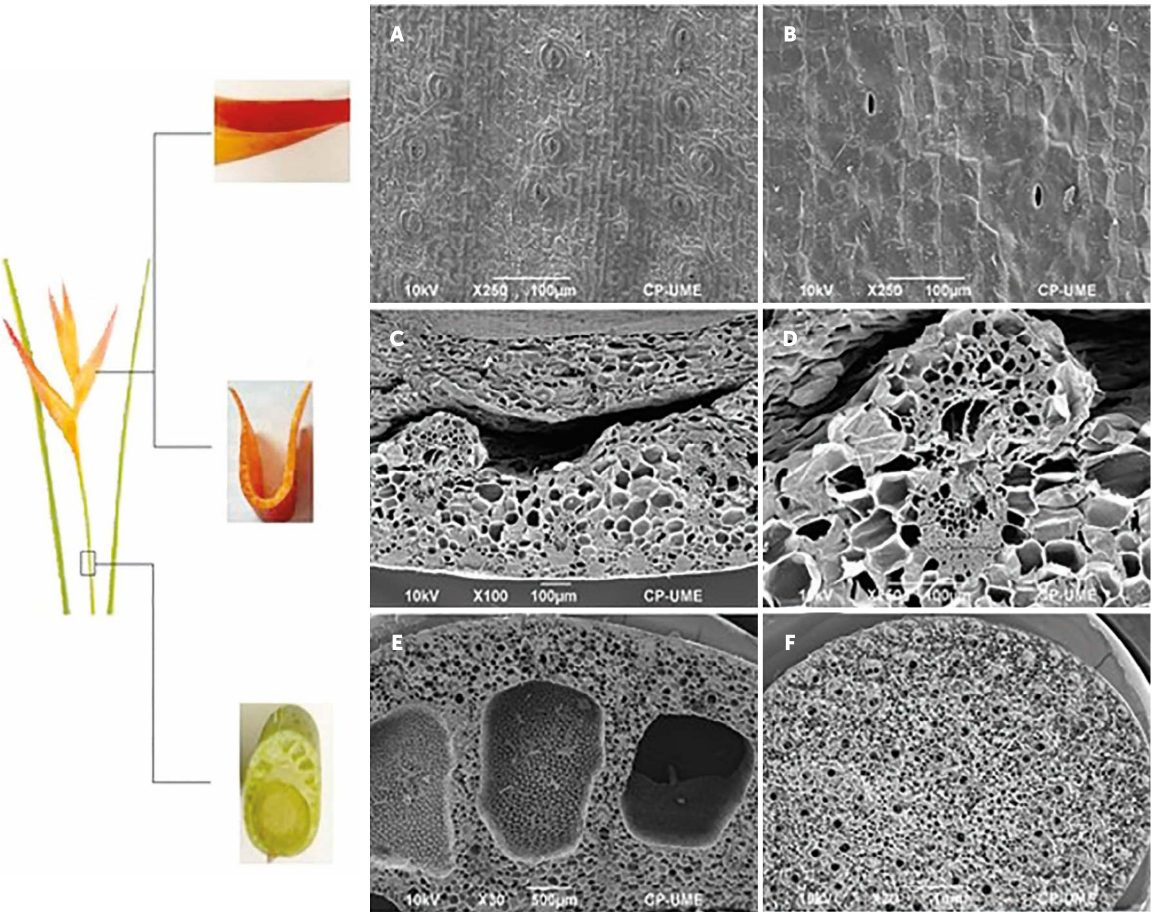

Figure 2. Micrograph of the stem of *H. psittacorum* × *H. spathocircinata* cv. Tropics. A) abaxial and B) adaxial of the epidermis of the bracts; C) Fiber bundles, air channels; D) vascular bundle of a cross section of the bract; E) cross section of the pseudostem: fiber bundles, vascular bundles and aerenchyma and F) vascular bundles of the central part.

in water to retain, all these factors contributed to keep the turgor of cut flowers and consequently the vase life (Moraes-Dias, 2016; Bañuelos-Hernández *et al.*, 2016).

In general, the fresh weight two days after cold storage was significantly higher ($P \leq 0.0001$) in the stems that were kept in water than those maintained in dry. The stems that preserved the FW were those treated with pulse solution T3 (Hydraflor® 100 + sucrose) with 98% of the FW after storage (Figure 3A). The stems with the greatest weight loss were those in which sucrose was applied and kept dry, however, they were the stems that had the highest water consumption (Figure 3B). Similar results have been reported in species such as *Eustoma grandiflorum* "ABC Purple" (Ahmad *et al.*, 2012), *Rosa* sp. and *Gerbera jamesonii* (Berlingieri-Durigan and Mattiuz, 2009; Mosqueda-Lazcares *et al.*, 2012) where flowers stored dry had a higher water consumption during their vase life than those stored wet. The WC of all treatments was around 0.08 to 0.11 mL g^{-1} (two days after VF), being the stems of T1 (control) and T4 (salicylic acid) and those that presented the highest WC (Fig. 3 B).

It is interesting to note that the CMI was significantly lower ($P \leq 0.0001$) in the stems stored humid than in those kept dry. The control (dry) treatment was the one that maintained a very similar percentage (81.20%) to the initial value, followed by T2 (Hydraflor® 100) and T4 (salicylic acid) (78%), of dry storage; while T3 (Hydraflor® 100 + sucrose) was the treatment that presented the greatest damage to the membrane in both wet (68.03%) and dry storage (74.59%) (Figure 3C). Membrane dysfunction is one of the adverse impacts of cold stress on tropical crops. At low temperatures, the transition of crystalline liquid to a rigid gel provoked to a decrease in the selective permeability of the membrane leads oxidation, which decreases the commercial value of flowers (Aghdam *et al.*, 2016).

The VF of heliconia at room temperature was 7 d, their senescence was characterized by wilting at the apex of

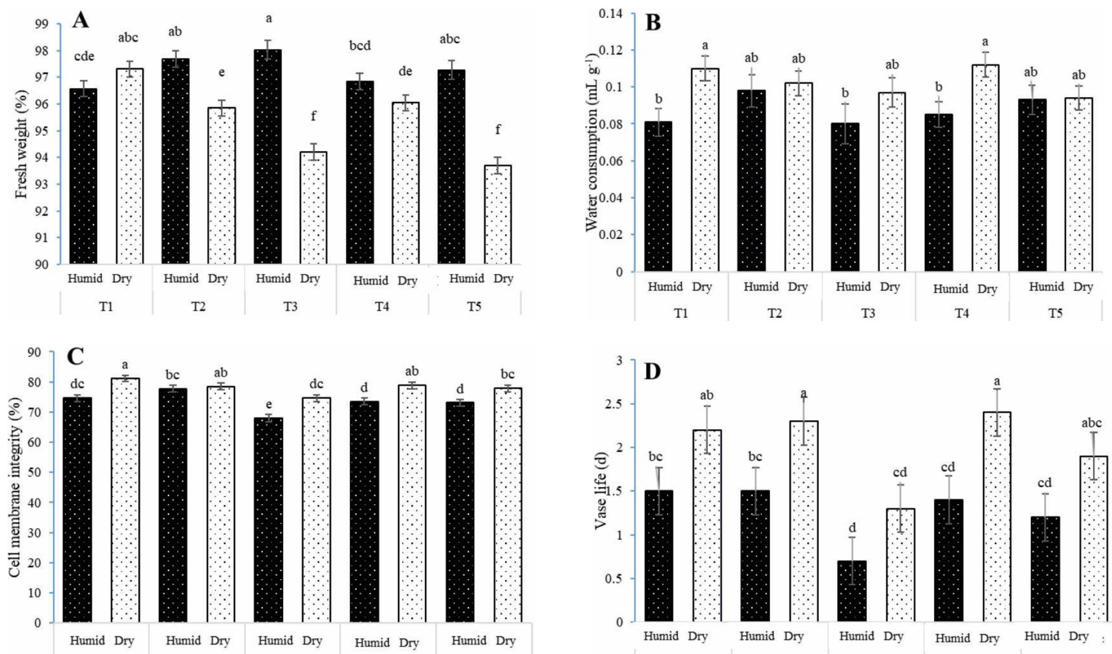

Figure 3. Postharvest variables of *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics two days after cold storage (10 d, at 11 °C and 85% RH) with a previous pretreatment with pulse solutions. A) Fresh weight (%), B) Water consumption (mL g^{-1}), and C) Cell membrane integrity (%) and D) Vase life. Each bar represents the mean of ten observations + standard error. T1 = control (water); T2 = Hydraflor® 100 (0.5 g L^{-1}); T3 = Hydraflor® 100 (0.5 g L−1) + sucrose (5% w v); T4 = salicylic acid (1 mM) and T5 = salicylic acid (1 mM) + sucrose (5% w/v). Values with equal letters are not statistically different (P≤0.05).

the bracts and a necrotic tissue. The stems that were stored in humid cold storage, the VF was not greater than 1.5 d, also 60% of the stems of T3 (Hydraflor®100 + sucrose) at the end of storage showed browning in the bracts. All the stems that were stored dry, had about two days of VF (Figure 2D). In heliconia, it has been reported that bract wilt and stem thinning are the typical symptoms of senescence (Leyva-Ovalle *et al.*, 2011). In anthurium (*Anthurium andreanum*), a delayingd browning of the spathe was associated with high values of CMI in treatments with salicylic acid (Aghdam *et al.*, 2016). We can highlight that the low water absorption of heliconia stem is related to their wide xylem vessels of the stem, so the application of pulse solutions does not contribute significantly improve its vase life.

Experiment 2

The WL was significantly lower for the stems that were maintained under room temperature (control and WW); while the stems in cold storage had higher values particularly those without wax (RW, RSA), with around 30 to 50% more WL (Table 1). This can be attributed to the influence of wax on gas exchange without the effect of SA that in previous reports showed the increased of vase life by promoting stomatal closure (Villanueva-Couoh *et al.*, 2009).

The SC had an inverse behavior to WL, and it was significantly higher (p≤0.006) in the stems of the control and WW treatments and it was reduced by 50 - 60% on the cold stored stems (RW, RSA, RWW and RWSA). Regarding the preservative solution of salicylic acid (1 mM) and water, no clear effect was observed (Table 1). Leyva-Ovalle *et al.*, (2011) mention that in stems of heliconia and roses, both species maintain better turgor when they are kept in water than in hydrogel. Additionally, heliconia stems conduct water and nutrients at a much lower speed than roses, due to the fact that they are thicker stems and mostly made of spongy tissue.

The end of the vase life of the cold stored stems was characterized by dark spots with a burnt appearance of the bracts (Figure 4). After cold storage, the vase life compared to the control stems at room temperature was reduced by approximately 5.5 d in the stems without wax (RW and RAS) and by around 4.3 d for the wax stems (RWW and RWSA). Regarding to the TVL, the treatments with the best results were those waxed, with around 15 d of postharvest life, with no significant difference (P≤0.0001) between the use of water or SA as preservative solution (Table 1). Since the loss of turgor is a consequence of water stress, in heliconia it is essential to avoid the loss of water from the tissues, which was achieved with the application of wax (van Doorn, 2012).

Bañuelos-Hernández *et al.* (2016) evaluated different conditions of storage of *Heliconia* cv. Tropics from 12 to 26 °C and RH between 37 and 90%, been the best temperature at 15 °C with 63% RH, with VL of 6.6 d and a TVL of 16.6 d. In the present study, the VL of waxed stems at room temperature was 13.81 d and at 13 °C and 84% RH and a VPT of 15.87 d.

Table 1. Postharvest variables evaluated two days after storage in stems of *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics kept at room temperature (22 ± 2 °C and 69% RH) and cold storage (13 °C and 84% RH; 10 d).

Treatment	% WL	SC (mL g^{-1})	VL (d)	TVL (d)
Control	2.90 c	0.150 a	9.93	9.93 c
W_W	2.86 c	0.119 b	13.81	13.81 b
R_W	3.73 ab	0.077 cd	4.40	14.40 b
R_{SA}	4.40 a	0.079 c	4.30	14.30 b
RW_W	3.35 bc	0.075 cd	5.87	15.87 a
RW_{SA}	3.21 bc	0.062 d	5.47	15.47 a
CV (%)	36.57	29.44		11.35

Means with equal letter in the same column are not statistically different (P≤0.05). Control: water at room conditions; WW = wax + water at room conditions; RW = Cold storage and placed in water; RSA = Cold storage and placed in salicylic acid solution; RWW = wax and cold storage and placed in water; RWSA = wax, cold storage and placed in salicylic acid. Where: WL = weight loss; SC = Solution consumption; VL: vase life; TVL = total vase life (including 10 d of storage).

The solution consumption is very low (0.15 mL g^{-1} PF) compared to species such as rose (*Rosa hybrida* L. cv. Black magic) with around 4 mL g^{-1} PF (Juárez *et al.*, 2008) or lisianthus (*Eustoma grandiflorum* L.) with 0.55 mL g^{-1} PF (Ahmad *et al.*, 2012). This low absorption rate may be due to the size of the vascular bundles, because heliconia stems are thick and mostly made of spongy tissue; for this reason, the low response to the application of pulse or preservative solutions (Leyva-Ovalle *et al.*, 2011). Similar results have been reported for other tropical species such as *Alpinia purpurata* "Red Ginger" (Ferreira *et al.*, 2008) or *Zingiber spectabile* (Lessa *et al.*, 2015) where the use of just water can maintain the quality and postharvest life of stems.

Figure 4. *Heliconia psittacorum* × *H. spathocircinata* cv. Tropics five days after cold storage. Control: room temperature; WW = wax + water at room temperature; RW = Cold storage and placed in water; RSA = Cold storage and placed in salicylic acid solution; RWW = wax, cold storage and placed in water; RWSA = wax, cold storage and placed in salicylic acid.

Given the low absorption capacity of heliconia, the only available technique to avoid the loss of water is the use of coatings, as reported in *Anthurium andraeanum* cut flowers (Mujaffar and Sankat, 2003) or in *Heliconia bihai* cv. Halloween (Bañuelos-Hernández *et al.*, 2017) where the application of wax and chitosan, respectively, maintain the quality by limiting the weight loss. In this study, the waxing treatments maintained the heliconia stems quality by increasing the vase life.

CONCLUSIONS

Stems of *H. psittacorum* × *H. spathocircinata* cv. Tropics maintain better quality if they store dry in cold temperatures, due the rotting reduction of the base stem and improves water absorption after storage. The use of pulse or preservative solutions does not improve postharvest quality due to the low absorption rate of heliconia stems (associated with their anatomical characteristics). The application of wax to the bracts proved to be the best option for preserving the turgor of flower stems, delaying senescence and reducing oxidation of the tissue.

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Yield, Agronomic Characteristics and Chemical Composition of Silage Fodder (*Sorghum bicolor* L.) With and Without Fertilization

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ABSTRACT

Objective: To evaluate the yield and structural characteristics of sorghum fodder, as well as the chemical quality of the fodder and the silage with and without fertilization.

Design/Methodology/Approach: The evaluated variables were, in the fodder: dry matter yield (DMY, kg ha^{-1}), leaf:stalk ratio (L:S, g g^{-1}), plant height (H, cm) and intercepted radiation (IR, %); and in the silage: dry matter (DM, %) crude protein (CP, %), neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), lactic acid (LA, %) and pH. There were two treatments, with fertilization and without fertilization.

Results: The agronomic characteristics L:S, H and IR improved with fertilization ($P<0.05$). The DMY with fertilization was 7060 kg DM ha^{-1} and without fertilization, it was 5472 kg DM ha^{-1}. The values of DM, CP, NDF, ADF and LA of the silage with fertilization were 33.60, 7.83, 51.06, 28.51 and 4.51%, respectively, and the pH was 4.3, and without fertilization these values were 47.67, 5.83, 43.36, 25.21 and 2.86%, respectively, and the pH was 4.66; all values were different when comparing with and without fertilization ($P<0.05$).

Study Limitations/Implications: These quality parameters should continue to be studied in sorghum silages and with different fertilization doses to expand the decision outlook.

Findings/Conclusions: According to the results obtained, it is concluded that the sorghum fodder crop should be fertilized for the ensilage, since higher dry matter yield, better agronomic, and nutritional characteristics are obtained.

Keywords: sorghum, sowing, fertilization, ensilage, bromatology analysis.

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INTRODUCTION

Sorghum fodder is very important in the elaboration of balanced feed for animals (Nichols *et al.*, 1997). It is of forage quality and requires less water than maize, so it adapts to rainfed management. In addition, it can be cultivated in the tropics with productive response and nutritional value similar to maize (Contreras *et al.*, 2011; Rebora *et al.*, 2018).

Sorghum fodder uses less water than maize in grain production, in addition to its dense and ramified roots, and the lower growth speed of the leaf area, making the use of nitrogen of the soil more efficient (Bolaños *et al.*, 2018) al emplear plantas con concentraciones promedio de 300 g kg^{-1} de MS. Se estudiaron tres variedades de grano (Solarius, Aralba y Topsilo. Nitrogen is the most restrictive element for plant growth and therefore for production (Diez, 2015). In recent decades its use has increased to raise the productivity of the crops (Espinosa *et al.*, 2002).

It is important to understand the yield and nutritional composition of sorghum since it is an alternative in the Mexican tropics due to the irregularity of rainfall (Rojas *et al.*, 2018). This irregularity leads to times of scarcity and abundance of fodder. In times of abundance, the excess fodder must be turned into silage to be used in times of scarcity. Ensilage is a fodder conservation method with high moisture content (60-70%). This method consists in compaction of the fodder, air expulsion and fermentation, which allow bacterial acidification of the silage until obtaining pH values below 5 (Fernández *et al.*, 2017).

In Mexico, Ramírez *et al.* (2019) obtained in average 27.7% of dry matter and 11500 kg MS ha^{-1} with different varieties of sorghum in the tropics, at the time of ensilage and in the silage pH values of 3.7 and raw protein of 8.4%. However, there are few studies about the effect of fertilization on the quality of sorghum silages in Mexico. Therefore, the objective of this study was to evaluate the yield and the chemical quality of the fodder and the silage, with and without fertilization.

MATERIALS AND METHODS

Location

The field research was carried out in 2018 in Rancho los Laureles, located on Km 7 of the Cuajinicuilapa highway, Guerrero-Santo Domingo, Oaxaca, and the chemical studies were done in the Animal Nutrition Laboratory of the Veterinary and Zootechnics School No. 2 of the Universidad Autónoma de Guerrero, on Km 197 of the Acapulco-Pinotepa Nacional highway, in Cuajinicuilapa, Guerrero, Mexico; coordinates 16° 08' N and 98° 23' W 50 masl, average temperature 29 °C and accumulated rainfall of 1200 mm.

The climate is Aw, dry tropical according to the Köppen-Geiger classification (García, 2004). The climate data were obtained from CONAGUA's agro-meteorological station located in the municipality of Cuajinicuilapa, 10 km from the experimental plots. The maximum temperature, 36 °C, was reached on the month of November, while the minimum, 17 °C, in August and September; the accumulated rainfall was 950 mm (Table 1).

Management of the Plots

Sowing was carried out in the rainy season, on August 1st, and the ensilage on November 1st, 2018, in Rancho los Laureles, using sorghum from the company DEKALB® variety BRS-72. Six hectares of sorghum were sown; three of them fertilized and three without fertilization. Sowing was carried out with an agricultural tractor (New Holland®, Mexico) in furrows of 80 centimeters of separation, placing the seed by continuous string and at a depth of 3 cm; at 14-16 seeds per linear meter, the sowing density was 8 kg ha^{-1} of pure viable seed. The soil, of clay-sandy texture, pH 4.8 to 5.0, was deficient in organic matter.

At the time of sowing, 150 kg ha^{-1} of DAP fertilizer (18-46-00) and 100 kg ha^{-1} of ammonium sulfate (12-61-00) were applied for the treatment with fertilization, together with the seed with the sower's hopper. At 40 days after sowing (das), fertilization was carried out again with 150 kg ha^{-1} of urea (46-00-00). Weed control was performed manually with a hoe.

Table 1. Maximum, minimum, mean (°C) temperature and precipitation (mm) during the study period in 2018.

Month	Maximum	Minimum	Average	Precipitation	Irrigation
August	33	17	25	269	No
September	33	17	25	290	No
October	35	18	26	282	No
November	36	18	27	109	No

At 90 das, fodder cutting was performed with an agricultural tractor (New Holland®, Mexico) and the ensilage with a silo (Twin 623600®, SWISSMEX). The fodder chopping was in 2 to 3 cm, depositing it directly in trailers to be transported to the silo (cake) that was uncovered after 90 days (180 das).

Evaluated Variables

Dry Matter Yield

At 90 days after sowing (das), the plants were ensilaged in the milky-pasty stage of panicle. One day later two squares of 1 m^2 per ha^{-1} were harvested at 10 cm height from the ground. The harvested fodder was washed and placed in paper bags. The samples were dried in a forced-air stove at 55 °C until constant weight, and their weight was recorded.

Plant Height

Twenty readings per repetition were taken randomly one day before harvesting the fodder for ensilage. For this purpose, a ruler measuring in cm was used, which was placed randomly in the plots so that the lower part of the ruler was at ground level and the posterior part was in contact with the flag leaf.

Leaf:Stalk Ratio

After harvesting the fodder for ensilage, the weights of the leaves and stalks of the plants were recorded in dry base and with them the leaf:stalk ratio was estimated ($g\ g^{-1}$). Drying was done at 55 °C until constant weight in a forced air stove.

Intercepted Radiation

Five radiation readings per experimental plot were taken randomly the day prior to the harvest, with the method of wooden meter described by Rojas *et al*. (2016).

Chemical Variables

In the plant components, when finalizing the ensilage process which lasted 90 days, the following variables were determined in the ensilage: dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lactic acid (LA), and pH (Van Soest *et al*., 1991; AOAC, 2005). Three samples were taken randomly in the middle part of each ensilage.

Statistical Analysis

The treatments were assigned to experimental units according to a design of random blocks with three repetitions, and each repetition was one ha^{-1}. The data obtained were organized and analyzed with PROC GLM of SAS (SAS, 2011), the means were compared with Tukey's test ($P<0.05$).

The experimental unit for the yield was a sample taken from each one of the two squares, from each one of the three hectares with and without fertilization; in total, six experimental units with fertilization and six without fertilization.

RESULTS AND DISCUSSION

The fodder yield with fertilization was 7060 kg MS ha^{-1} and without fertilization 5472 kg MS ha^{-1} ($P<0.05$). The plant height, leaf:stalk ratio, and intercepted radiation were also higher with fertilization ($P<0.05$) than without fertilization, 1.77 cm, 0.44 $g\ g^{-1}$ and 95 % *vs*. 1.62 cm, 0.37 $g\ g^{-1}$ and 89%, respectively.

Table 2. Yield of sorghum fodder and agronomic characteristics with and without fertilization.

Variable	With fertilization	No fertilization	SEM
DM kg ha^{-1}	7060a	5472b	110
Height (cm)	1.77a	1.62b	0.42
Leaf: stem ratio ($g\ g^{-1}$)	0.44a	0.37b	0.05
Intercepted radiation (%)	95a	89b	0.12

ab Means with different literals in the same row are statistically different ($p<0.05$), DM = dry matter yield; SEM = standard error of the mean.

Boschini and Elizondo (2005), when evaluating different cutting ages in sorghum fodder, obtained in average 6303 kg MS ha^{-1} that are very similar to results in this study. On the other hand, Ibarguren *et al*. (2020) reported in sorghum hybrids a height that ranges from 1.54 to 2.81 by applying fertilization (18-46-00), similar to results in this study. Espinosa *et al*. (2002) reported in sorghum at different physiological ages that height and yield increase from nitrogenous fertilization. Diez (2015) reported a higher amount of leaf and panicle, up to 20%, when nitrogenous fertilization is carried out in sorghum crop destined to ensilage. The height and intercepted radiation are important for a good harvest in the milky-pasty stage of the panicle; in tropical fodders, 95% of intercepted radiation is considered optimal and it is reached with fertilization (Da Silva and Nascimento, 2007; Rojas *et al*., 2018).

There was a difference in the DM obtaining a higher percentage from the sorghum plant fertilized with 37.66 % and a lower one in non-fertilized sorghum of 33.60 % ($P<0.05$). In the CP of the panicle, stalk and leaf of the sorghum plant there were differences between the fertilized and non-fertilized sorghum, obtaining the highest percentage when fertilized with 2.49, 4.89 and 15.72% of protein (P<0.05). The neutral detergent and acid detergent fibers in the components of the sorghum plant are quite variable depending on whether it is fertilized or not.

For their part, Boschini and Elizondo (2005) obtained a similar behavior to this study's when evaluating different frequencies of black sorghum fodder in the structures of the plant obtained in average, harvesting sorghum at 133 days after sowing, dry matter of 23.3%, crude protein of 13.88%, and neutral and acid detergent fiber with 70.39 and 38.32%, respectively, in the leaf's component. In contrast, in the stalk the crude protein content was lower, with 4.79%, although higher in the neutral and acid detergent fibers with 80.91 and 53.28, respectively. However, in this study it was harvested 90 days after sowing since it is when the best conditions were found for ensilage with the panicle in milky-pasty stage; this depends on the variety and soil-climate elements of the region (Ibarguren *et al*., 2019).

Compared to silage without fertilization, the sorghum silage with fertilization produced higher values (P<0.05) of CP, NDF, ADF and LA (7.83, 51.06, 28.51 and 4.51%, respectively) and lower value (P<0.05) of pH (4.3). The advantage of the sorghum silage without fertilization was its higher DM (47.67%), which in the fertilized sorghum silage was 33.60%. These results were similar to those reported by Corral *et al*. (2011) who reported higher values of CP and LA from nitrogenous fertilization.

Table 3. Nutritional analysis of the components of sorghum plants with and without fertilization for ensilage.

	With fertilization	No fertilization	SEM
DM	33.60b	37.66a	3.19
PCP	2.49a	1.68b	0.25
PCT	4.89a	3.11b	0.52
PCH	15.72a	14.48b	0.60
FDNP	85.01b	89.08a	1.87
FDNT	61.74b	71.31a	2.19
FDNH	61.55b	67.50a	1.39
FDAP	21.54b	24.55a	2.33
FDAT	39.19b	44.53a	1.30
FDAH	33.77a	33.76a	0.53

ab, means with different literals in the same row are statistically different (p<0.05); SEM = standard error of the mean; DM = dry matter of the plant; PCP = crude panicle protein; PCT = crude stem protein; PCH = crude leaf protein; FDNP = neutral detergent fiber of panicle; FDNT = stem neutral detergent fiber; FDNH = neutral detergent fiber of the leaf; FDAP = acid detergent fiber of the panicle; FDAT = acid detergent fiber from the stem; FDAH = neutral detergent fiber of the leaf.

Table 4. Chemical analysis of the sorghum silage with and without fertilization.

	With fertilization	No fertilization	SEM
DM	33.60b	47.67a	3.19
PC	7.83a	5.83b	0.64
FDN	51.06a	43.36b	1.87
FDA	28.51a	25.21b	0.84
AL	4.51a	2.86b	0.37
pH	4.3b	4.66a	0.08

ab Means with different literals in the same row are statistically different (p<0.05); DM = dry matter; PC = crude protein; NDF = neutral detergent fiber; FDA = acid detergent fiber; AL = lactic acid; pH = hydrogen potential; SEM = standard error of the mean.

Dávila *et al*. (2016), when evaluating sorghum silages associated with *Tithonia diversifolia*, found similar values of DM (27%) to those of this study, and higher of CP (13.26%); this higher value of CP is probably due to the association of sorghum with *T. diversifolia*.

CONCLUSIONS

The sorghum fodder crop should be fertilized to ensile since higher yield of dry matter, and better agronomic and chemical characteristics are obtained. It is suggested to perform studies with ensilages with more varieties of sorghum, other levels of fertilization, other frequencies of cutting, and associations with other species.

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AGRO PRODUCTIVIDAD

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Productive Performance of Alfalfa (*Medicago sativa* L.) at Different Age of Resprout in the Spring Season

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ABSTRACT

Objective: To determine the growth and productive performance curves, to obtain the optimal cutting moment in alfalfa, depending on the age of resprouting, in the Spring season.

Design/Methodology/Approach: The treatments were cuts at different age of the plant and the variables evaluated: Dry Matter Yield (DMY) Botanical and Morphological composition (BMC), Plant Height (PH), Leaf/Stem Ratio (L/SR), and Intercepted Radiation (IR). The stastical analysis was with the PROC GLM procedure, of the SAS software, and the adjusted curves were obtained with the Curve Expert Professional 2.0 software.

Results: There was an increase in the DMY, leaf, stem, PH, and IR, as the resprouting age advanced, but not, the L/SR which had an inverse behavior. The maximum DMY was obtained (4,768 kg DM ha^{-1}) in week seven. There was a greater amount of leaf with average 52%, followed by the stem (36%), weeds (7%), detritus (4%) and inflorescence (1%). The PH was higher in week seven with 53 cm. Likewise, the highest IR in week tree with 86%. However, the L/SR was higher in week one with 2.4. The IR and L/SR presented the lowest R2 (0.90 and 0.93, respectively). In contrast, DMY and PH presented a positive relationship (R^2 of 0.98 and 0.97, respectively).

Study Limitations/Implications: There were no limitations

Findings/Conclusions: The productive performance of Premium variety alfalfa was variable depending on the resprouting age, in which the botanical and morphological characteristics changed, with better characteristics in the fifth week.

Keywords: *Medicago sativa* L., resprouting age, botanical-morphological composition.

INTRODUCTION

The performance in seasonal production of dry matter in fodder species is useful to detect seasons of high and low availability of forage (Rojas *et al*., 2019). With the determination of the growth curve of a species, the management of defoliation can be established, which will make the production of fodder efficient and reduce the deterioration of meadows (Hernández

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and Martínez, 1997). Likewise, the productivity of a meadow is in function of management, influencing the production of kg DM ha^{-1}, and changes in the population, density and size of stems, which determine the rate of appearance, elongation and half-life of the leaf (Rojas *et al*., 2016). On the other hand, the analysis of a fodder's growth in a specific climate and place is a tool that determines the moment of biomass production of higher quality, considering the morphological and botanical composition, and in addition predicts the yield of leaf, stem, detritus, and inflorescence and thus establishes an adequate crop management (Wilson *et al*., 2017). Also, the studies consider that the quality of the fodder is related with productive variables such as the specific leaf area and the height of the crop, which at the same time is related with the intercepted radiation. The latter can be used as an indicator for an adequate harvest (Berone, 2016; Da Silva and Nascimento, 2007; Da Silva and Hernández, 2010). Therefore, it is important to determine the optimal moment for cutting, so that a producer can advance or delay its harvest, depending on the needs for fodder. As a result, the objective of this study was to determine growth and productive performance curves in order to obtain the optimal moment of harvesting for alfalfa (*Medicago sativa* L.) variety Premium, in function of the age of harvest in the Spring season.

MATERIALS AND METHODS

Study Area: An alfalfa meadow (*Medicago sativa* L.) was used, Premium variety, in its first year of establishment, in the experimental area "El Bajío" of the Antonio Narro Agrarian Autonomous University, in Saltillo, Coahuila, Mexico (25° 23' latitude North and 101° 00' longitude West), at an altitude of 1783 m. The climate is semi-dry temperate, with average temperature of 18 °C and some days with temperatures below zero °C, with mean annual precipitation of 340 mm (Climate-Data-org, 2010).

Plot Management: The meadow was established on February 4, 2019. The experimental area was divided into 21 plots of 9 m^2 (3×3 m). At the beginning a standardization cut was made (May 4, 2019), at an average height of 5 cm, manually with a sickle. Weekly samples were continued for seven times, randomly selecting three plots and each one was considered a repetition. Irrigation was applied at field capacity every 15 days, when necessary, through an irrigation system by underground drip using a band caliber 6000, buried at 25 cm of depth, and 60 cm of separation.

Evaluated and Calculated Variables

Dry Matter Yield: By repetition, two quadrants of 0.25 m^2 (50×50 cm) were harvested randomly at a height of 5 cm from the ground. The biomass obtained was deposited in paper bags and dried in a forced air stove Model POM-246F, at a temperature of 55 °C for 72 h, until constant weight, and the values were expressed in kg DM ha^{-1} cut^{-1}.

Botanical and Morphological Composition: A sub-sample of the harvested fodder was used (approximately 10%) to estimate the dry matter yield. The sub-sample was separated into leaf, stem, detritus, inflorescence and weeds. The components were dried together with the samples to estimate dry matter. The contribution of the components to the yield in percentage and kg DM ha^{-1} was determined.

Plant Height: Twelve random measurements were taken, by repetition, with a wooden ruler of 100 cm, of 1 mm precision, and an average value by repetition was obtained.

Leaf:Stem Ratio: It was determined with the leaf and stem values of the botanical and morphological composition, when dividing kg DM ha^{-1} of leaf by the stem.

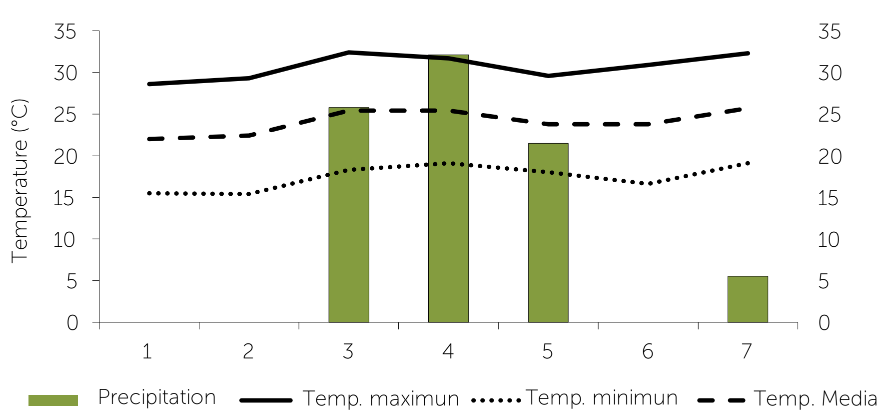

Figure 1. Mean, maximum and minimum temperature, weekly and accumulated precipitation during the study period (February 4 to June 21, 2019).

Intercepted Radiation: It was calculated from the light data received (readings on the canopy) and negotiated (under the canopy), taken with a quantum sensor of 70 cm length, model PS-100, Apogee Inst, Utah, USA, between 13:00 and 14:00 h. In order to determine the percentage of light intercepted, the formula used was:

$$\%LI = ((LT - LR) * 100) / LR$$

where $\%LI$ = Percentage of light intercepted, LR = Light received, and LT = Light transmitted through the canopy.

Statistical Analysis: The statistical analysis was carried out with the PROC GLM procedure, from SAS (2004), for a completely random design with three repetitions. A means comparison was performed using Tukey's test ($p<0.05$). The adjusted growth curves with their respective models and coefficients were obtained with the Curve Expert Professional 2.0 software (Curve Expert Computer Software. Vers 2.0 N.p. D.d. Web).

RESULTS AND DISCUSSION

Dry Matter Yield: The weekly increase in dry matter yield, as the plant's age increased, is shown in Figure 2, where a positive trend was observed as the resprouting age increases. The production of dry matter increased during resprouting with R^2 of 0.98, describing a linear curve, because the change in fodder production was constant through time. In this regard, Rojas *et al*. (2019) show similar trends in red clover (*Trifolium pratense* L.), with positive trends in growth with R^2=0.98, adjusting to a linear equation. Likewise, in cereals, the R^2 found are >0.93, but the adjustment is an exponential model (Wilson *et al*., 2017). The values ranged from 488 kg DM ha^{-1} in the first week, to 4,768 kg DM ha^{-1} in week 7, with an average production of 2,603 kg DM ha^{-1}, so they presented differences between weeks of resprouting ($p<0.05$), showing a positive growth up to week six. For the state of Oaxaca, Montes *et al*. (2016) reported alfalfa yields at 49 days of 2,794 kg DM ha^{-1}, in the same Spring season. In turn, during an evaluation of cutting intervals, Gaytán *et al*. (2019) obtained yields of 6,844 kg DM ha^{-1}, in a 4-week interval. In this study the values are different, since according to Álvarez *et al*. (2018), the conditions of climate and soil are determining factors in the production of fodder.

Botanical and Morphological Composition: The changes in botanical and morphological composition of alfalfa, harvested at different resprouting ages, are presented in Figure 3. From the first week and until week 7, the contribution of the components of dry matter production (kg DM ha^{-1}) increased. The leaf was what contributed most with 52% average, followed by the stem, weeds, detritus and inflorescence, with 36, 7, 4 and 1%, respectively. The leaf reached its maximum percentage of contribution (59%) in week 2, and it decreased to 41% in week seven. In the stem, an increase of 21% was observed in the first week, until 38% in week seven, with its maximum contribution in week five (41%). In the detritus and weeds, the values ranged between 0 and 11% and 2 and 19%, respectively, without having a clear trend. In turn, the inflorescence emerged in the last three weeks of sampling, which reflects the physiological maturity of the plant. Therefore, it is recommended to harvest alfalfa in the fifth week of resprouting, where an acceptable proportion of leaf is found, compared to the rest of the components and in addition reflected a higher amount of fodder, at the beginning of flowering (Figure 3). However, Mendoza *et al*. (2010) established that the highest distribution of leaf, regardless of the season of the year, is found in cutting intervals of three to four weeks. For their part, Gaytan *et al*. (2019) found that alfalfa harvested four weeks after resprouting show the highest leaf productions in meadows one and two years of established, with yields of 5,856 and 3,900 kg DM leaf ha^{-1}.

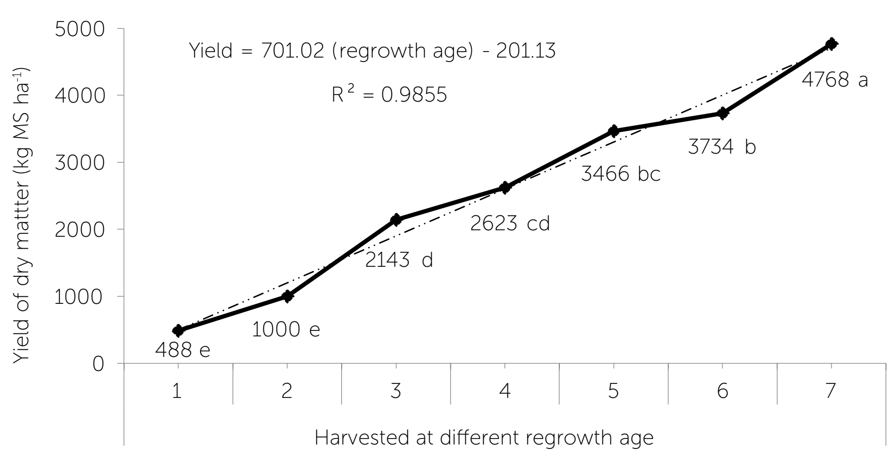

Figure 2. Dry matter yield (kg DM ha^{-1}) of alfalfa (*Medicago sativa* L.), harvested at different resprouting age. Different lowercase letters between cuts indicate statistical differences (Tukey $p<0.05$).

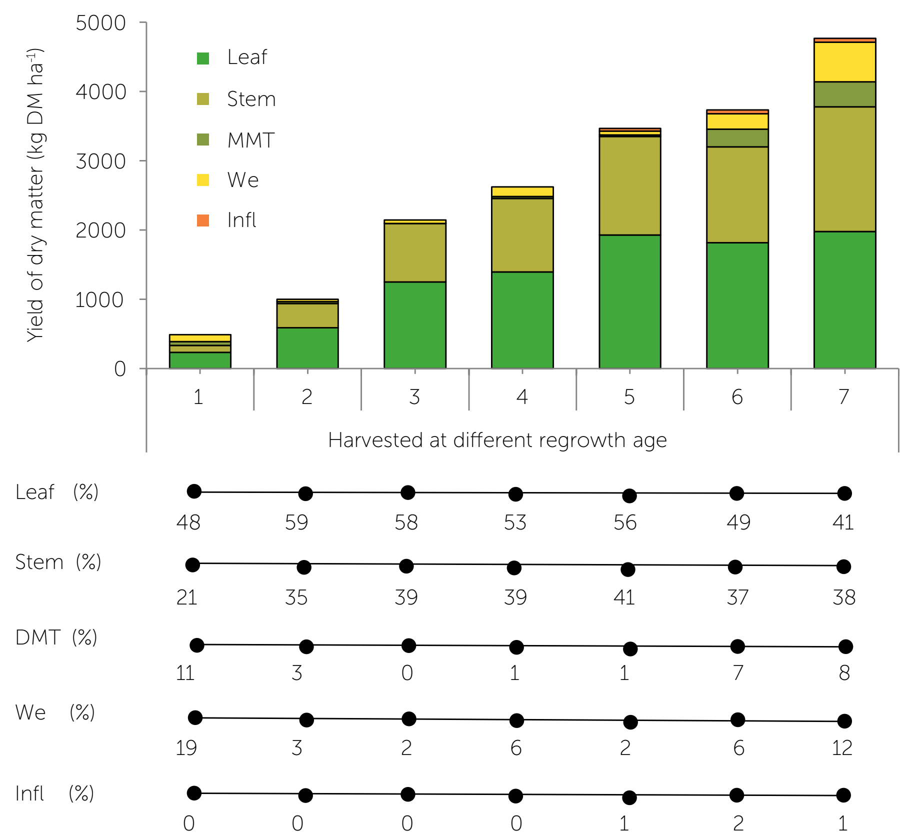

Figure 3. Botanical and Morphological Composition (BMC) of lucerne harvested at different regrowth age. DMT = Dead material, We = Weed, Infl = Inflorescence.

Plant Height: The changes in plant height of the alfalfa (*Medicago sativa* L.), Premium variety, are shown in Figure 4. A progressive increase was seen in plant height, from week one to week three, with values of 14 to 44 cm, respectively ($p<0.05$). Starting on week three and until week seven, no statistical differences were found ($p>0.05$), reaching their highest value in the last week with 53 cm. The performance of plant height adjusted better to a polynomic model, with R^2 of 0.97. According to Gaytán *et al*. (2019), the highest values of plant height were obtained in week four, in one-year meadows established with values of 37 cm, and they were higher in a defoliation frequency of three weeks with 28 cm. The data obtained in this study are different from those reported, since under certain experimental and environmental conditions (Figure 1), the variety managed could determine the parameters of growth and yield of alfalfa (Mustafa *et al*., 2010).

Leaf:Stem Ratio: The trend that alfalfa presented during seven weeks of resprouting is shown in Figure 5. As the age of resprouting increases, the leaf:stem ratio decreased. The values ranged from 2.4 in week one to 1.4 in week four. This value was maintained from week four to six, ending in a minimum value in week seven of 1.1 ($p<0.05$). The value of R^2 is acceptable with 0.93. The values higher than one show that the leaf component was always higher in proportion than the stem, which is why according to Rojas *et al*. (2019) it should be taken as reference for the quality of fodder. For their part, Zaragoza *et al*. (2009) report average values in alfalfa of 1.1, in week one and 0.5 in week eight, similar trend to the one found in this study. Likewise, Rojas *et al*. (2019) found values of 0.94 in the fourth week of resprouting in five varieties of alfalfa. However, in meadows of one and three years of established, when comparing frequencies of defoliation of four and five weeks, they report values of 2.8 and 3.4, respectively (Gaytán *et al*., 2019). However, in other studies the lowest leaf:stem ratio has been obtained in the resprouting ages where the highest dry matter yield is presented (Rivas *et al*., 2005), which is why it is important to evaluate the weight and height of the stems in relation to the leaf. Concerning this, Sun *et al*. (2011) reported a greater height in correspondence to a lower

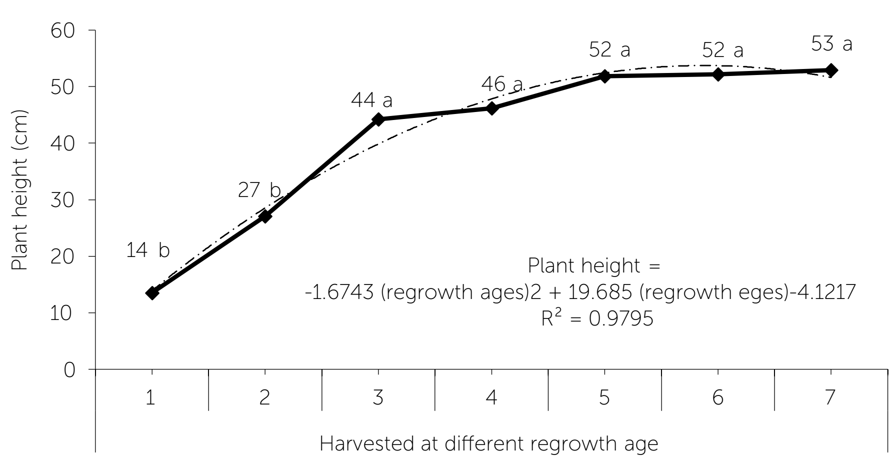

Figure 4. Plant height (cm) of alfalfa (*Medicago sativa* L.) harvested at different resprouting age. Different lowercase letters between cuts indicate statistical differences (Tukey $p<0.05$).

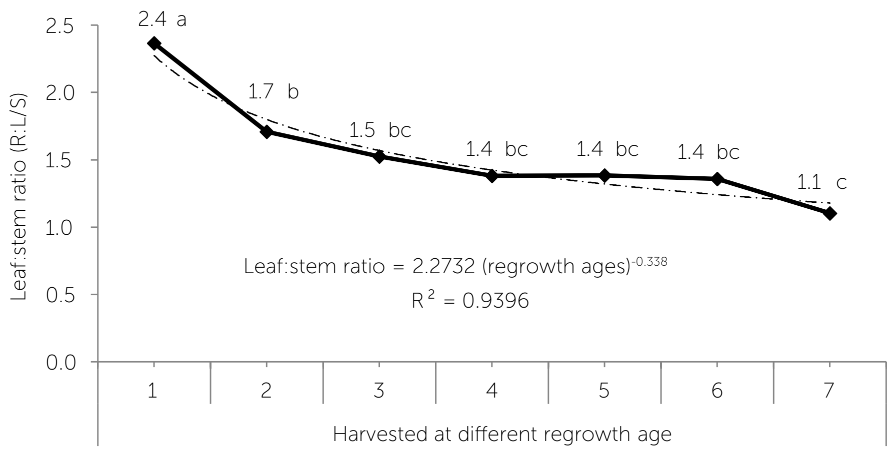

Figure 5. Leaf:stem ratio (R:H/T), alfalfa (*Medicago sativa* L.), harvested at different age of resprouting. Different lowercase letters between cuts indicate statistical differences (Tukey $p<0.05$).

amount of leaf, decreasing the leaf:stem ratio when approaching the flowering stage.

Intercepted Radiation: The behavior that the variable of intercepted radiation followed is shown in Figure 6, where R^2 of 0.90 was obtained, positive correlation as the age of the plant was higher. In general, a progressive increase in the percentage of light intercepted was found, from week one to week three ($p<0.05$), and continuing with constant values until week seven ($p>0.05$). The maximum percentage of light intercepted was reached in week three and four with 86% and the lowest in week one with 37% ($p<0.05$). In the Spring season, Rojas *et al*. (2016) reported a maximum value of intercepted radiation of 93% in the Jupiter variety, which in both studies is below the 95% value, percentage found for an optimal harvest (Montagner *et al*., 2012), as long as the plant density is competitive. In this regard, at differences between plants of 10, 15, 20, 25 and 30 cm, in Spring it is possible to obtain 95% of intercepted light, while in Summer and Winter they are only reached at 10 and 15 cm of distance between plants (Mattera *et al*., 2013). Despite this, Baldissera *et al*. (2014) mention that the lower the separation between plants, the higher the radiation of intercepted light, fodder yield, and leaf area index. However, in the Summer season, 95% of the intercepted light was found in the Jupiter variety (Rojas *et al*., 2016), which is why it is advisable to study the Premium variety, evaluated in this study, in the rest of the seasons of the year, since differences have been reported between times of the year (Mendoza *et al*., 2010; Rojas *et al*., 2012).

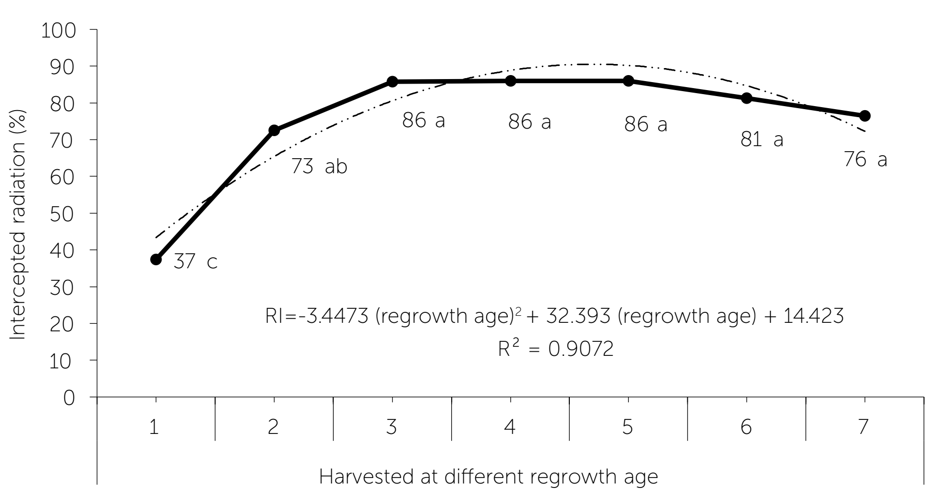

Figure 6. Intercepted Radiation (RI = %) of alfalfa (*Medicago sativa* L.), harvested at different age of resprouting. Different lowercase letters between cuts indicate statistical differences (Tukey $p<0.05$).

CONCLUSIONS

The performance of the growth curve of alfalfa (*Medicago sativa* L.), Premium variety, was variable in function of the resprouting age of the species. As the date of harvest increased, the morphological and botanical characteristics changed, obtaining a balance between quality and quantity, between the third and fourth week of evaluation, with a higher percentage of leaf, compared to the stem in the Spring season. It is recommended to continue evaluating this species in a longer time in other seasons of the year.

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Effect of a Fungi Complex in Nine Ecotypes of *Cenchrus purpureus* (Schumach) Morrone

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ABSTRACT

Objective: The objective of this study was to evaluate the response of different concentrations of a fungal consortium on the growth and yield of *Cenchrus purpureus* ecotypes under storm conditions.

Design/Methodology/Approach: An experiment was established under a completely randomized design with a 9×2×4 factorial arrangement. The factors were 9 ecotypes of *Cenchrus purpureus*, two seasons of the year (Summer-Fall and Winter-Spring), and four levels of mycorrhizal consortium. The variables evaluated were: number of buds, number of leaves, height of the bud, leaf length, total biomass, and leaf-stalk ratio.

Results: The response of the ecotypes had a differential effect on the variables NB, LLL, TB and L/SR, while the season affected the variables LN, PH, LLL, MLL, SLL, TB and L/SR. Low inoculation levels increased the variables NB, PH, LLL, MLL and TB.

Study Limitations/Implications: The evaluated factors indicate that the level of inoculant and the season determine the growth and yield of *Cenchrus purpureus*.

Findings/Conclusions: The mycorrhizal consortium dose and season of the year mainly determined the growth and yield of *C. purpureus*. Ecotypes respond differently to changes in the season and in mycorrhizal consortium dose. The evaluated factors indicate that the mycorrhiza dose and the season of the year determine the growth and yield of *C. purpureus*. Two growth strategies of the ecotypes are visualized: 1) many buds, with few small leaves and 2) few buds with many large leaves.

Keywords: Growth, Forage, Mycorrhiza, Elephant grass, Yield.

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INTRODUCTION

The distribution of forage yield throughout the year in conditions of natural and induced pastures, and established pastures, show a decrease in the dry matter yield in the dry season, affecting importantly the bodily condition and yield of animals (Amamou *et al*., 2018). A strategy for this problem is the establishment of perennial fodders, with capacity for adaptation to the deficiency in water resources, as long as there are favorable conditions in the soil (Paredes, 2018). Among the species introduced there are grasses of the *Cenchrus* genus, which are characterized by adapting to various adverse situations, and therefore have a broad distribution. Their resistance to conditions of water stress and the disposition of their germplasm stand out, due to their presence in different genomic banks (Pattanashetti *et al*., 2016). In addition, they provide ecosystem services since they prevent erosion (Hendrickson and Sanderson, 2017), and their roots have the capacity to increase the presence of beneficial microorganisms in the soil (Crotty *et al*., 2015).

When the levels of fertility in the soil are not adequate for the development and survival of these fodder species, mycorrhizal fungi have been used that strengthen the capacity to withstand environmental stress, when improving the radicular development and the exchange of phytoregulators, as well as minerals that allow a better plant performance (Lenoir *et al*., 2016). Mycorrhizal fungi participate to a large extent in environmental conservation and they can be used in the regeneration of soils and in reforestation processes (Rocha *et al*., 2015). The mycorrhizae have been isolated and used as inoculants, although their use in agriculture is limited and is slightly developed (Goss *et al*., 2017). It has been shown that some fungi such as *Funneliformis mosseae* have effects on the growth of plants by improving phosphorus absorption, and increases up to 60% the appearance of sprouts (Jiang *et al*., 2016). Likewise, it gives resistance to water stress (Bernardo *et al*., 2019) and tolerance to contaminated soils with pesticides (Rivera-Becerril *et al*., 2016). When there is excess water it also helps to retain nutrients in the soil avoiding its lixiviation (Köhl and Van der Heijden, 2016). *Diversispora ebúrnea* creates an environment of competition of growth between inoculated plants (Shi *et al*., 2016). Hernández-Zamudio (2017) reported a high survival and resistance of this mycorrhiza in arid and semiarid ecosystems. On the other hand, *Rhizophagus fasciculatus* increases the density in roots and aerial part of the plant in soils of low fertility (Channabasava *et al*., 2015). Rożek *et al*. (2019) report this species in temperate forests. Tarraf *et al*. (2017) report that *Septoglomus viscosum* increases significantly the biomass of plants, and also gives excellent quality, which is why Pellegrino and Bedini (2014) recommend the inoculation to increase the absorption of nutrients in the soil, which allows improving the yields in the harvests. However, it is important to consider different variables, such as the biochemical conditions of the soil and the climatic variation, which influence their degree of effectiveness (Garzón, 2016). Therefore, the objective of this research was to evaluate the effect of a mycorrhizal consortium made up of four species in the growth and yield of ecotypes of *C. purpureus* under conditions of two seasons in a year.

MATERIALS AND METHODS

The experiment was established in June, 2017, and ended in June, 2018, in the Technological Institute of Valle de Oaxaca (ITVO) located in the municipality of Santa Cruz Xoxocotlán, Oaxaca. The coordinates are 17° 01′ 16′′ N and 96° 45′ 51′′ W, with predominant Vertisol soils (INEGI 2010). According to the National Meteorological Service, the closest station to the experiment is No. 20354 – Zaachila, which is located 8 km away in a straight line from the experimental place. With average temperature of 20.6 °C, maximum of 23.1 °C in the month of May, minimum of 17.5 °C in the months of January and December. The average annual precipitation is 709 mm; the month of June is the most rainy month with 146 mm, and January and December are the driest months with 1.5 and 3.2 mm, respectively (CONAGUA, 2015).

Nine ecotypes of the Cenchrus purpureus species were used, which were: Elephant, Maralfalfa, CT-115, Roxo, Vruckwona, Taiwan, Merkeron, Mott and King Grass. These materials were donated by the Experimental Agricultural Field "La Posta" of the INIFAP, Veracruz Unit.

A compound mycorrhizal consortium of four mycorrhizae species was used: *Diversispora ebúrnea*, *Funneliformis mosseae*, *Rhizophagus fasciculatus* and *Septoglomus viscosum* provided by the Sierra Juárez University (UNSIJ), obtained from an agroecosystem of granadilla (*Passiflora ligularis*) from the community of San Antonino el Alto, Zimatlán, Oaxaca, Mexico, through isolation and its consequent reproduction. Four levels of inoculation were used: 0, 5, 7.5 and 10 g per plant, which were applied at the time of establishment, directly with the stake in the ground. The experiment was carried out in a period of 12 months, and it was divided into two seasons, the rainy season, during the months of July to December, 2017 (Summer-Fall, S-F) and January to June, 2018 (Winter-Spring, W-S). In each season the plants were registered six times every 30 days. Soil preparation was carried out with farming tasks, through plowing and trawling with a tractor. The sowing method was using stakes at 40° distributed in squares using the nine ecotypes of *C. purpureus* where two cuts are performed.

Variables

The following response variables were evaluated. Number of buds per plant (NB), considering the buds on the main axes of the plant. Number of leaves per bud (NL); the total number of leaves on each bud was counted. Plan height (PH), measured level on the ground up to the top plant tissue, for this variable a metric tape of 1 m was used. Leaf length, large leaf (LLL), medium (MLL), small (SLL), for each bud the largest, medium and small leaf were selected, which were measured with the metric tape from the ligule to the apex. Total biomass (TB), which was determined through dry matter six months after establishment of the crop, and for this the method of the square (1 m^2) was used, cutting the plant five cm from the ground level and kept in paper bags; they were put in the Riossa brand Model H-33 drying stove, at 55 °C for 96 h, and finally the weighing of each bag was carried out to obtain the total biomass. Leaf/stalk ratio (L/SR), obtained through the separation of the leaf and the stalk from the samples of total biomass, which were weighed separately to later divide the value of the leaf by that of the stalk.

The experiment was established in a completely randomized design (CRD), with factorial arrangement 9×2×4, where: A is the factor that corresponds to the nine ecotypes; B is the factor that corresponds to the two seasons of the year; and C is the factor that corresponds to the four levels of inoculation. In total there were 72 treatments with four repetitions. The data were analyzed to estimate the effect of the inoculant on the ecotypes of the Elephant grass, the means were compared with the Tukey test ($p \leq 0.05$) and SAS for Windows version 9.3 was used (SAS Institute, 2011).

RESULTS AND DISCUSSION

The results obtained from the factorial analysis show that there are highly significant differences ($p<0.05$) between ecotypes of *C. purpureus* in all the variables evaluated. Regarding the seasons, the variables with higher significance were number of leaves, height of the bud, and length of the small leaf. These results are related to the precipitations, since in most of the fodders there is an increase in the elongation of leaves during the rainy season (Cruz *et al*., 2017a). As consequence, a maximum growth is reached in a short time, as reported by Pérez *et al*. (2004), obtaining a maximum growth at four months in a period of six months. For the level of inoculation of mycorrhizae, the variables were highly significant except in the number of leaves and length of the small leaf. In the interaction ecotype-level, there were highly significant differences for the number of buds and height of bud, for the ecotype-season interaction all the variables were highly significant, in the season-level interaction there was no significance in any of the variables, in the ecotype-season-level interaction it was determined that in the variable of number of bud there were significant differences (Table 1). The effects that are observed between the interactions of the factors are positive, showing differences in the plant's organs; these results agree with Calzada-Marin *et al*. (2018) who observed that the morphological composition in these ecotypes varies between different ages.

Table 1. Variance analysis considering the levels or factors.

Variable	Factor a	Factor b	Factor c	Inter. a*c	Inter. a*b	Inter. b*c	Inter. a*b*c	Rep.	C. V.
NB	2856.55 **	0.35 ns	1402.36 **	216.87 **	764.06 **	119.61 ns	213.24 **	316.13 ns	65.82
NH	89.78 **	4830.47 **	42.52 ns	24.20 ns	77.93 **	43.93 ns	22.07 ns	39.61 ns	66.77
AB	9767.38 **	347864.7 **	5929.50 **	1232.25 **	2967.24 **	774.30 ns	559.86 ns	5101.73 **	50.47
LHG	5767.78 **	887.45 ns	3662.80 **	501.01 *	884.24 **	100.81 ns	295.61 ns	2639.23 **	43.21
LHM	1376.18 **	341.86 ns	874.20 **	130.45 ns	250.60 **	79.93 ns	60.44 ns	747.96 **	44.13
LHC	77.20 **	921.12 **	14.29 ns	17.49 ns	83.00 **	11.50 ns	17.89 ns	27.17 ns	92.74

**=Highly significant; *=significant; ns=no significant; Factor a=ecotypes of *C. purpureus*.; Factor b=Season; Factor c=level; Inter=Interaction; Rep=Repetition; C.V.=coefficient of variation; NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Ecotype factor

As indicated in Table 2, when the means comparison is made, the results show that for the NB, the ecotype Elephant was the one that showed the highest value (21.9), producing 82% more stalks compared to the ecotypes CT-115, Merkeron, Mott and King Grass, which were the ones that evidenced the lowest numbers of resprouts (p>0.05), and not different between one another (p>0.05), which produced in average 12 stalks. Meanwhile, in the variable number of leaves, with the exception of the ecotype Elephant, the rest of the ecotypes did not show differences, with the ecotype Maralfalfa showing the highest number of leaves per stalks (7.07). In plant height, the ecotype Merkeron was the one that showed the highest value (54 cm), exceeding in 70% the ecotypes Elefante, Maralfalfa, Roxo and King Grass (p>0.05), which were the lowest ecotypes, respectively.

This is similar to what was observed by Calzada-Marín *et al*. (2014) who explain that Maralfalfa is characterized by a constant increase in its growth. This makes it an alternative for zones with similar characteristics to the medium where it was established (Uvidia, 2013). The efficiency of Elephant in some studies reaches a maximum growth in a short time (Vivas-Quila *et al*., 2019), with an efficient production in quality and quantity of fodder (González *et al*., 2011). The results obtained are affected primarily by the capacity for adaptation that Elephant, Maralfalfa and King Grass have in comparison to the remaining ecotypes and to environmental conditions (Sterling and Guerra, 2010).

Season factor

As indicated in Table 3, the means comparison of the variables showed that the variable NB during the two seasons was the same, producing in average 13 buds. In the variables NL, PH, LLL, MLL and SLL, differences were observed (p<0.05) during the two seasons, with higher values in the S-F season. The lower development of Cenchrus purpureus in W-S is adjudicated to the absence of the water resource which was lower compared to the S-F season. Likewise, these fodders have adapted to environmental conditions of precipitation and temperature (Rojas *et al*., 2011), reaching a higher average per cut of 3.38 t ha^{-1} in rainy periods (Álvarez *et al*., 2013). On the other hand, Reyes-Castro *et al*. (2018) reported an increase in yield of the ecotype Moott in the rainy season compared to the dry season. For their part, Cruz *et al*. (2017b) reported a higher number of buds in the rainy season. Therefore, Murillo *et al*. (2014) recommend sowing Elephant grass in seasonal conditions, specifically in the rainy season, since a maximum development of

Table 2. Comparison of variables considering the ecotypes of *C. purpureus*.

Ecotypes	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
Elefante	21.89 a	4.84 c	34.42 c	32.42 cd	16.86 cd	4.03 c
Maralfalfa	7.3 d	7.07 a	33.95 c	25.68 cd	14.04 ef	5.59 a
CT-115	12.57 c	6.29 ab	48.65 ab	41.56 a	21.23 a	5.02 abc
Roxo	16.68 b	5.8 abc	34.83 c	29.36 de	14.98 de	4.04 bc
Vruckwona	13.03 c	6.75 ab	43.89 b	36.01 bc	18.28 bc	4.61 abc
Taiwan	13.95 bc	6.58 ab	46.47 b	36.73 abc	18.35 bc	4.31 abc
Merkeron	12.26 c	6.69 ab	54.46 a	38 ab	19.81 ab	5.35 ab
Mott	11.46 c	6.8 ab	44.63 b	33.94 bcd	17.59 bc	4.13 bc
King Grass	11.97 c	5.45 bc	28.59 c	22.79 f	11.83 f	3.72 c

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Table 3. Means comparison taking into consideration the season.

Station of the year	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
Summer - Autumn (rains)	13.6 a	7.94 a	56.01 a	33.87 a	17.54 a	3.77 b
Winter- Spring (dry)	13.52 a	4.36 b	24.74 b	32.13 b	16.62 b	5.4 a

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH= number of leaves; AB=regrowth height; LHG= large blade lenght; LHM= medium blade lenght; LHCh= blade length small.

the plant is shown (Pilco and Pérez, 2017). Likewise, there is an increase in the appearance of leaves (Ramírez *et al*., 2010).

Level of inoculant factor

This factor did not affect (P>0.05) the variables NL and SLL; the variables NB, PH, LLL and MLL were higher (P<0.05) with the doses of 0 and 5 g of the consortium than with the others (Table 4).

Ojeda *et al*. (2018) reported that arbuscular mycorrhizae increased the yield of biomass, raw protein, and mineral extractions of the soil, with mycorrhizal efficiency of 100% in *Rhizoglomus intraradices*, which is why it is an option for fertilization. For that purpose, the use of mycorrhizae is important, allowing the plant to capture, translocate and transfer nutrients, in addition to adopting a lower dependency to chemical fertilizers (Beltrán and Fiallos, 2016). When comparing with chemical fertilization, these ecotypes have high rates of production and fodder yield (Vivas-Carmona *et al*., 2019). Fodders under conditions of good fertility and moisture represent forage potential (Ramos-Trejo *et al*., 2012).

Total Biomass and Leaf/Stalk Ratio

The variance analysis of total biomass (TB) showed significant differences (p<0.05) between the three factors (Table 5). The ecotype, the season and the level of inoculant had a significant effect on the increase of total biomass. In this sense, Karti *et al*. (2018) showed similar results when inoculating with mycorrhiza, they observed an increase of 30% in the production of dry weight in *P. purpureum*. Likewise, Rao *et al*. (1985), when inoculating arbuscular mycorrhizae in *Pennisetum* reporte dan increase of 41.7 % of dry matter. Meanwhile, for the leaf/stalk ratio (L/SR) there was no significance for the factor of level of mycorrhiza inoculated. For the interactions that were performed solely in ecotype-season, there were highly significant differences only for the variable leaf/stalk ratio. These performances can be caused by environmental conditions of precipitation and temperature that were present during the experiment.

In the TB variable, there are differences (p<0.05) between the means of the ecotypes studied, where Merkeron, Taiwan, Ct-115, Vruckwona and Elephant predominate with a higher yield (1.73, 1.40, 1.38, 1.23 and 1.18 t MS ha^{-1}, respectively) (Table 6). This agrees with what was reported by Goyes-Vera *et al*. (2018), that the ecotype that better adapts to the absence of chemical fertilizers is Elephant, showing higher amounts of biomass.

The ones with lower performance were Maralfalfa and King Grass (0.55 and 0.40 t DM ha^{-1}). In the seasons, there is difference (p<0.05), where Summer-Fall was higher in yield in 100% compared to the other season. In the levels of inclusion of the inoculant, they present significant differences, where the control and the level of inoculation of 5 % presented the highest yield of total biomass. Mujica and Molina (2017) found that mycorrhizae increase yield. In the variable Leaf/Stalk Ratio differences were observed (p<0.05). The ecotypes had similar performance, except for King Grass, Elephant and Mott with averages of 1.57, 1.41 and 1.18, respectively; compared to Cuba CT-115

Table 4. Means comparison considering the level of inoculants.

Inoculants (g)	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
0	14.36 ab	6.12 a	43.82 a	35.17 a	18.02 a	4.75 a
5	15.59 A	6.45 A	45.72 a	36.57 a	18.83 a	4.67 a
7.5	11.12 C	6.55 A	37.81 b	30.29 b	15.76 b	4.41 a
10	13.19 B	5.85 A	37.25 b	30.03 b	15.55 b	4.33 a

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Table 5. Variance analysis of biomass and leaf/stalk ratio.

Factor / Variable	Factor a	Factor b	Factor c	Inter. a*b	Inter. a*c	Inter. a*b*c	Inter. b*c	Rep.	C, V.
BT	48935.08 **	247089.60 **	86899.40 **	23615.14 ns	12767.21 ns	7607.73 ns	26140.61 ns	39675.59 **	79.99
RH/T	1.57 **	26.01 **	0.30 ns	2.57 **	0.41 ns	0.51 ns	0.49 ns	0.45 ns	34.98

**=Highly significant; *=significant; ns=no significant; Factor a=ecotypes of *C. purpureus*.; Factor b=Season; Factor c=level; Inter=Interaction; Rep=Repetition; C.V.=coefficient of variation; BT=total biomass; RH/T=leaf/stem ratio.

with 1.95, so they are different ($p<0.05$) (Table 6). Considering the season, there were differences in the means of both, being higher in the W-S season. It can be observed that the accumulation of fodder, the growth and development of *Cenchrus* will be conditioned to the age of resprout and the climatic conditions, which are determined by the time or season of the year (Calvano, 2011; Fortes *et al*., 2015). Taking into consideration the levels of inoculation, no significant differences were observed, with a similar performance in the four levels.

Table 6. Means comparison of total biomass and leaf/stalk ratio.

Factor	Variable	BT (t MS ha^{-1})	RH/T
Ecotype	Merkeron	1.73 a	1.75 ab
	Taiwan	1.40 ab	1.77 ab
	CT-115	1.38 ab	1.95 a
	Vruckwona	1.23 abc	1.7 ab
	Elefante	1.18 abc	1.41 bc
	Mott	0.82 bcd	1.18 c
	Roxo	0.78 bcd	1.73 ab
	Maralfalfa	0.55 cd	1.76 ab
	King grass	0.40 d	1.57 abc
Season	Summer - Autumn	1.38 a	1.32 b
	Winter- Spring	0.73 b	1.98 a
Inoculation levels (g)	0	1.28 a	1.6 a
	5	1.51 a	1.67 a
	7.5	0.74 b	1.58 a
	10	0.71 b	1.7 a

Values with different letters in the same column are significantly different according to the test of Tukey ($P<0.05$); BT=total biomass; RH/T=leaf/stem ratio.

CONCLUSIONS

The response of the ecotypes had a differential effect on the variables NB, LLL, TB and L/SR. The season affected the variables NL, PH, LLL, MLL, SLL, TB and L/SR. The low levels of inoculation increased the variables of NB, PH, LLL, MLL and TB. The factors evaluated indicate that the level of inoculant and the season determine the growth and the yield of *Cenchrus purpureus*.

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AP AGRO PRODUCTIVIDAD

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Size, imbibition, and viability of seeds of two creole melon (*Cucumis melo* L.) from the state of Guerrero, Mexico

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ABSTRACT

Objective: To determine the physical and physiological characteristics of Creole melon seeds from the Costa Chica of Guerrero.

Design/Methodology/Approach: It was established under a completely randomized design, and Student's t-test (α=0.05) and correlation with Pearson's test were performed. Viability and imbibition were carried out using the methodologies described by the International Seed Testing Association (ISTA).

Results: Creole seeds of the two varieties presented a significant statistical difference (95% confidence level) in the physical quality variables. The imbibition ended at 18 and 32 h in V_2 and V_1, respectively, after being submerged in water. The humidity percentage was higher in V_1 (7.19); while, V_2 presented a higher percentage of germination and viability (96 and 90%, respectively). There is a positive association between the humidity and the physical dimensions of the seed and the germination and viability (r^2=0.954) that is highly significant (P=0.003).

Study Limitations/Implications: Morphological and taxonomic classification studies of the Creole genotypes of the Costa Chica region of Guerrero are required.

Findings/Conclusions: There was a positive correlation between the physical and physiological quality of the Creole melon seeds.

Keywords: Correlation, Germination, Seeds, Tetrazolium.

INTRODUCTION

Melon (*Cucumis melo* L.) is a species in the Cucurbitaceae family and is originally from Asia and Africa (Mehra *et al*., 2015). This family includes more than 900 species, among which 10 genera are of economic importance, mainly including cucumbers and melons (*Cucumis* sp), squashes and zucchinis (*Cucurbita* sp.), and watermelons (*Citrullus lanatus* L.) (Paris *et al*., 2017). Melon is considered one of the most diverse

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species within the *Cucumis* genus due to its extensive genotypic and phenotypic variation (Farcuh *et al*., 2019). The 16 recognized melon groups have merged, five of them being divided into the subspecies *agrestis* and 11 into the subspecies melo, including the *cantalupensis* (cantaloupe), *reticulatus* (muskmelon) and *inodorus* (honeydew) groups (Assis-Dantas *et al*., 2015).

Commercially, melon is one of the most important horticultural crops in México due to the surface area farmed and production volume (Monge-Pérez, 2013). In Guerrero, melon farming is economically important: in 2019, 99,862 t were produced with a yield of 29.66 Ton ha^{-1} (SIAP, 2019). Additionally, traditional production systems subsist in the state where native varieties are conserved *in situ* and gathered species are also used (Vera-Sánchez *et al*., 2016).

The main method of melon propagation is by seed, which generates high demand for quality seeds that should have different attributes including genetic, physiological, physical, and sanitary quality (Basra, 1995; Copeland and McDonald, 1995). For this purpose, characteristics such as fidelity to the variety, germination percentage, purity, vigor, appearance, and absence of disease are considered, since they contribute to a higher productive varietal efficiency and the ability to sprout quickly and uniformly under different environmental conditions (Finch-Savage and Bassel, 2016). Germination and viability tests have been widely used in seed evaluation (Villa *et al*., 2019). Therefore, the objective of the present study was to collect seeds in the municipality of Florencio Villareal in the state of Guerrero to determine the physical and physiological characteristics of Creole melon (*Cucumis melo* L.) seeds.

MATERIALS AND METHODS

The collection site was in the region of Costa Chica, in the municipality of Florencio Villareal, located in the southern part of the state of Guerrero (16° 43′ 26′′ LN y 99° 07′ 24′′ LW). The varieties were classified according to the fruit's shape; V_1 were round, corrugated or segmented fruits, and V_2 were elongated and smooth fruits, commonly known as cucumber-melons. After collecting, the seeds were washed to eliminate pulp residues and placed on drying paper to eliminate water excess. Later, they were sent in hermetically sealed, labeled containers to the Multidisciplinary Laboratory of the Faculty of Biological and Agricultural Sciences at the University of Colima, located in the municipality of Tecomán, Colima.

Physical Characterization

The physical quality of the seeds was determined by their size (Pérez-Mendoza *et al*., 2016). The variable studied was the weight of 1000 seeds (WTS), which was estimated by weighing 100 seeds in eight repetitions and calculating the average, typical deviation, and variation coefficient that resulted in ≥3.1%, then the average was multiplied by 10 (ISTA, 1996). The seed length (SL), width (SW), and thickness (ST) were measured with a digital caliper (Truper®), and the ratio between seed length and width (LWR) was calculated. The humidity content was determined with the stove method (ISTA, 2014) at 70 °C for 72 hours, and 25 complete seeds were used with three repetitions. The weight was recorded in an analytical balance (Sartorius®, BP221S), and calculations were made based on the fresh weight (Poulsen, 2000; Lezcano *et al*., 2007). The humidity content (H_C) was calculated with Eq. 1 and expressed in percentage, were H_C = humidity content, W_1 = weight of the container expressed in g, W_2 = initial container and seed weight (g) and W_3 = final container and seed weight (g).

$$H_C = (W_1 - W_3) * \frac{100}{(W_1 - W_2)} \qquad \text{Eq. 1}$$

Imbibition Kinetics

Using an analytical balance (Sartorius®, BP221S), 25 g of seeds of each variety were weighed. Later, they were submerged in distilled water for 36 h at room temperature (23±2 °C). The increase in weight was registered every two h and the amount of water absorbed was expressed through Eq. 2, where W_{ad} = water adsorbed, W_i = initial weight, W_f = final weight, and H_i = humidity content (Domínguez-Domínguez *et al*., 2007).

$$W_{ad} = \frac{W_f - W_i}{W_i\left(1 - \frac{H_i}{100}\right)} \qquad \text{Eq. 2}$$

Physiological Quality in the Laboratory

The standard germination test (SGT) was carried out according to the ISTA rules (2014) with modifications. Fifty seeds were distributed in five rows in a transparent polyethylene clamshell with a layer of cotton previously moistened with distilled water. These were then placed inside a germination chamber at temperature of 25±2 °C. Counting started from the first day and germination was calculated using Eq. 3 and expressed as a percentage, where *GP* = germination percentage, *ni* = total germinated seeds, and *N* = total seeds sampled.

$$GP(\%) = \frac{ni}{N} * 100 \qquad \text{Eq. 3}$$

The germination speed index (GSI) was obtained through the methodology proposed by Maguire (1962) and Martínez-Solís *et al*. (2010). Germinated seeds were counted daily, seeds with sprouted radicles were considered, and Eq. 4 was applied, where *GSI* = germination speed index, *Ti* = time in hours passed between the test start and the end of the interval, and *Ni* = number of germinated seeds within consecutive time intervals.

$$GSI = \sum \frac{Ni}{Ti} \qquad \text{Eq. 4}$$

The coefficient of germination speed (CGS) is a distribution measure based on the number of germinated seeds through time and the number of germinated seeds per day (González-Zertuche and Orozco-Segovia, 1996), and was determined by Eq. 5, proposed by Kotowski (1926), where *CGS* = coefficient of speed, *n* = number of seeds germinated per day *i*, and *t* = number of days since planting.

$$CGS = \frac{\sum ni}{\sum (niTi)} * 100 \qquad \text{Eq. 5}$$

The viability analysis (V) was done with the technique involving tetrazolium chloride, described by ISTA (2014) and Maldonado-Peralta *et al*. (2016). One-hundred seeds were placed in an uncovered jar with distilled water, and this was put in a water bath at a temperature of 35 °C for 14 hours. Later, a 1% tetrazolium chloride solution was added, and the jar of seeds was put in a water bath at a temperature of 35 °C for 4 h. Finally, the seeds were rinsed with distilled water and examined under a stereoscopic microscope (LEICA, EZR®). The embryos were classified according to color intensity: 1) alive with high vigor, when they were completely dyed with an intense red color, 2) alive with low vigor, when their coloration was a pale red, and 3) not viable, when they remained colorless. This was expressed as the percentage of viable and unviable embryos.

Data Analysis

The data obtained for each measured variable in percentages was transformed with the *Arcsine* $\sqrt{x/100}$ formula and the averages were compared in pairs using the Student's t-test (α=0.05). For all the physical and physiological characteristics evaluated in the seeds, a correlational analysis was done with the Pearson test (α=0.05), using the MINITAB 18 statistical software.

RESULTS AND DISCUSSION

Physical Characterization

The analysis demonstrated statistical differences (P<0.0001) in seed size among varieties, except in the length/width ratio, which was statistically equal (P=0.427). Concerning the weight of 1000 seeds, V_1 was statistically different (P<0.0001) from V_2 (Table 1). Mansouri *et al*. (2017) reported average values of 7.75, 3.50, and 1.00 mm in length, width, and thickness, respectively. The weight of 1000 seeds fluctuated between 40.66 and 49.57 g, similar to the weight of V_2 in this study.

Karayel *et al*. (2004) evaluated the uniformity of a seeder with different vacuum pressures on different seeds. The averages found in melon seeds were 11.44 mm in length, and 4.62 and 2.35 mm in width and thickness, respectively, while the weight of 1000 seeds without coats (testa) was 36.70 g. The physical attributes of seeds are important for calculating the surface area and volume of the grains to model the storage and design of separation equipment (Sologubik *et al*., 2013; Mansouri *et al*., 2017), as well as agricultural implements (Karayel *et al*., 2004).

Table 1. Average values for physical quality variables of Creole melons (*Cucumis melo* L.).

Variety	SL (mm)	SW (mm)	TS (mm)	LWR	W1000s (g)
V_1	10.97±0.21^a	5.06±0.04^a	1.74±0.05^a	2.16±0.05^a	172.3±13^a
V_2	6.26±0.11^b	2.82±0.05^b	1.38±0.02^b	2.21±0.08^a	41.27±1.27^b
p value	<0.0001	<0.0001	<0.0001	0.427	<0.0001
C.V.	1.95	1.37	2.66	3.36	8.66

Means ± standard deviation with different literals is statistically different with a confidence level of 95% (Student's t). SL = seed length. SW = seed width. TS = thickness of seeds. LWR = length / width ratio. W1000s = weight of one thousand seeds. C.V. = coefficient of variation.

Imbibition Kinetics

Figure 1 shows the imbibition curve of Creole melon seeds, in which within the first two hours, the water adsorbed by the seed increased in weight by approximately 56% in V_1 and by 42% in V_2, which constitutes the first phase of imbibition (Suárez and Melgarejo, 2010). Domínguez-Domínguez *et al*. (2007), for their part, found the first phase in hibiscus seeds (*H. sabdariffa* L.) between the first six and 10 h. These authors explain that, since they are irregular geometric bodies, the speed of water transference to the interior cannot solely be explained by geometric variables, such as the volume to surface area ratio.

Teixeira Pinheiro *et al*. (2016) report that melon seeds induced to saline stress in different imbibition times, increase enzymatic activity and decrease germination rates. In the end, V_1 seeds stopped water absorption after 32 h, while V_2 after 18 h, at which time the biochemical activity begins and the radicle appears (Maynard, 2007).

Physiological Quality

Table 2 shows that the percentage of humidity and germination were statistically different between V_1 and V_2 ($P=0.010$ and $P=0.030$, respectively); viability, speed index and coefficient were statistically equal ($P \geq 0.065$). Barros-Torres and Marcos-Filho (2003) evaluated the humidity content in different lots of two melon hybrids and noted it fluctuated between 7.0 and 7.3%, while in this study, V_2 was reported to have a lower content (5.77%). These same authors indicate that the variation in humidity content of the seeds is probably due to their size. Casenave and Toselli (2010) reported that in 'Honeydew' melon seeds subjected to different conditions of water stress, germination was ≤94%, and they indicate that by modifying the water potential, the percentage of germination was reduced in the seeds.

Nery *et al*. (2007) reported that in seeds of five watermelon cultivars (*C. lanatus* L.), germination values were 39% in a triploid hybrid and from 89 to 99% in diploids. Of viable seeds, the triploid hybrid presented 83% and the diploids >98%, similar values to those obtained in this study. The quality of *C. Lanatus* L. seeds could be low in hybrids (triploids compared to diploids) due to the time they take to germinate and that they present embryonic malformation problems from chromosomal alterations necessary to avoid seed formation (Souza *et al*., 1999; Grangem *et al*., 2003; Nery *et al*., 2007). The speed index, reported by these same authors, was >2.24; while in V_1 melon seeds, it was 2.24, and in V_2 it was 2.08. Concerning the speed coefficient, 44% and 48% of V_1 and V_2 seeds, respectively, germinated per day. In *Arachis hypogaea* L. genotypes, Caroca *et al*. (2016) found that GSI varies from 2.94 to 20.84 due to the effect of temperature, and they note that seeds exposed to high temperatures (>32 °C) are negatively affected in germination speed.

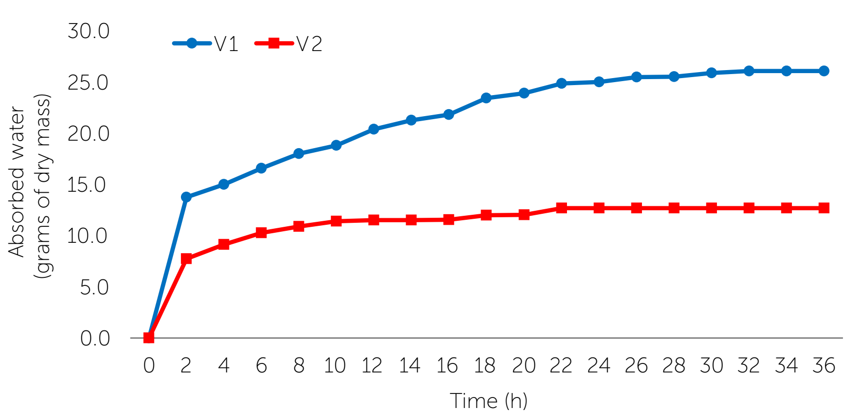

Figure 1. Imbibition curves of two varieties of Creole melon (*Cucumis melo* L.) seeds from the Costa Chica region of Guerrero, Mexico.

Regarding the viability analysis, the results obtained showed that 78% and 90% (Table 2) of V_1 and V_2 seeds, respectively,

Table 2. Average values for physical quality variables in Creole melons (*Cucumis melo* L.).

Variedades	H (%)	GP (%)	V (%)	GSI	CGS
V_1	7.19±0.27^a	84.0±6.0^b	78.0±8.0^a	2.24±0.18^a	0.44±0.03^a
V_2	5.77±0.46^b	96.0±2.0^a	90.0±2.0^a	2.08±0.07^a	0.48±0.01^a
p value	0.010	0.030	0.065	0.230	0.227
C.V.	5.89	4.97	6.94	5.37	5.98

Means ± standard deviation with different literals is statistically different with a confidence level of 95% (Student's t). H = humidity. GP = germination percentage. V = viability. GSI = germination speed index. CGS = coefficient of germination speed. CV = coefficient of variation.

presented complete staining of the embryo and cotyledons (Figure 2A and 2C), indicating that they were alive; while Figure 2B and 2C show the unviable seeds that were not stained by the tetrazolium.

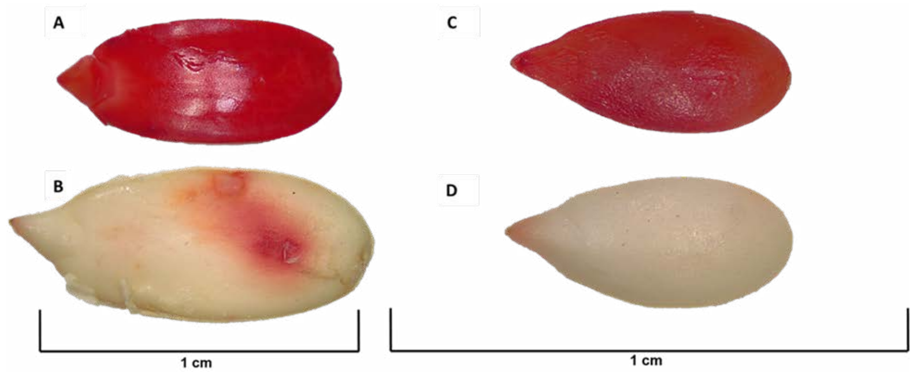

Figure 2. Viability trial in Creole *Cucumis melo* L. seeds. A and C) Viable seeds of V_1 and V_2. B and C) Unviable seeds of V_1 and V_2.

Barone *et al*. (2016) report that the greatest difficulty to ensure good interpretation of results is finding an adapted protocol; although it has been used in different species, there are limited reports on melon seeds. However, Inácio-Barros *et al*. (2005) showed that the tetrazolium test is efficient to determine the viability of squash seeds, finding between 59 and 100% of viable seeds among different batches. Another factor that influences viability is the age of the seed. Enríque-Peña *et al*. (2004) reported that seeds of *Taxodium mucronatum* (Ten.) presented an average reduced viability of 63% after 21 months in storage.

Correlation Analysis

A high positive correlation (r^2>0.859) was found between humidity and length, width, and thickness, and P1000s (Table 3). Concerning the germination percentage, it presented r^2=0.954 with high significance (P=0.003), where a trend or increase was observed between germination and viability, and with these results, the yield potential of the studied varieties can be predicted (Panwar *et al*., 2018). Likewise, the physical characteristics of the seeds showed positive correlations, indicating that weight is influenced by the seed dimensions (Table 3). Nuraini *et al*. (2018) suggested that associations in seed quality, specifically seed weight, are useful for improving the existing varieties and developing new genotypes.

A negative correlation (r^2=−0.868) was found between humidity and germination, where it was observed that the higher the humidity content, the lower the germination percentage. This could be explained with Table 1, where

Table 3. Correlational analysis of physical and physiological variables in Creole melon (*Cucumis melo* L.) seeds.

	H (%)	GP (%)	GSI	CGS	V (%)	w1000s (g)	SL (mm)	AS (mm)	TS (mm)
PG (%)	−0.868*								
	0.025**								
GSI	0.591	-							
	0.217	-							
CGS	-0.574	-	−0.998						
	0.234	-	0.001						
V (%)	-	0.954	-	-					
	-	0.003	-	-					
W1000s (g)	0.895	−0.865	0.516	−0.517	-				
	0.016	0.026	0.294	0.293	-				
LS (mm)	0.903	−0.828	0.581	−0.586	-	0.987			
	0.014	0.042	0.227	0.221	-	0.001			
AS (mm)	0.921	−0.858	0.554	−0.555	-	0.996	0.996		
	0.009	0.029	0.254	0.253	-	0.001	0.001		
TS (mm)	0.859	−0.743	0.512	−0.525	-	0.969	0.990	0.979	
	0.028	0.091	0.299	0.285	-	0.001	0.001	0.001	

* Pearson's correlation coefficient (α=0.05). ** p values with a confidence level of 95%. GP = germination percentage. V = viability. GSI = germination speed index. CGS = coefficient of germination speed. SL = seed length. SW = seed width. TS = thickness of seeds. LWR = length / width ratio. W1000s = weight of one thousand seeds. C.V. = coefficient of variation.

seeds of V_1 showed a greater humidity percentage and a lower germination percentage compared to V_2, which was the inverse. The physical characteristics showed a significant negative association with the germination percentage. The speed index and coefficient presented $r^2=-0.998$ with $P=0.001$ as an inverse value; that is, the higher the germination average per day, the lower the value obtained for the Kotowski index (speed coefficient) (González-Zertuche and Orozco-Segovia, 1996).

CONCLUSIONS

The physical and physiological characteristics among varieties showed differences in seed dimensions and weight. Concerning the physiological variables, V_2 presented >90% in germination and viability, which are tools used for genetic improvement and increased yield in the field.

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AGRO PRODUCTIVIDAD

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Effect of plant growth-promoting bacteria and inoculation media in the yield on cooksfoot yield

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ABSTRACT

Objective: To determine the effect of three PGPB bacteria (*Ewingella americana*, *Bacillus simplex* and *Microbacterium ginsengiterrae*) and three inoculation media (digestate, compost and cornstarch) on plant height, leaf temperature, stalk density, morphological composition, and dry matter yield of orchard grass.

Design/Methodology/Approach: A completely randomized design was used, with a factorial arrangement 4×3, the experimental units being a pot with five initial stalks.

Results: The dry matter did not show difference between treatments with bacteria (P>0.05), but the control the values increased up to 50%. Compost and digestate media outperformed cornstarch by 22%. The inoculation media had an effect (P<0.05) on the yield and leaf variables, while the bacteria promoted the formation of new stalks and the production of biomass, and reduced the leaf temperature.

Findings/Conclusions: Compost and digestate as inoculation media can notably favor the beneficial effect of *M. ginsengiterrae*, which was the one that registered the best yields.

Keywords: *Dactylis glomerata*, *Ewingella americana*, *Bacillus simplex*, *Microbacterium ginsengiterrae*.

INTRODUCTION

Plant growth-promoting bacteria (PGPB) are a group of microorganisms that, under specific conditions, activate direct and indirect mechanisms for improving radicular development or modifying the metabolism of plants in order to increase plant growth and productivity (Gragueda-Cabrera *et al*., 2012). The positive effect of bacterial inoculation in crop production has been widely demonstrated, because in direct association with the plant, the bacteria create favorable conditions for the exchange of beneficial metabolites for the plant and vice

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versa, in addition to improving soil structure, reducing compaction, increasing porosity, improving water filtration, and intervening in nutrient cycles (Pérez-Montaño *et al*., 2014; Menna *et al*., 2017; Singh *et al*., 2017). In this way, they have been considered to be an agricultural alternative to increase the yield of crops while conserving or even increasing the functional biodiversity of the soil (Alori *et al*, 2017; Planes-Leyva *et al*., 2004).

Several studies have shown that inoculation of grasses with *Azospirillum* spp. promotes the yield of dry matter, resulting in an increase in sprouting percentage, plant height, biomass, and grain yield in different proportions. For example, sorghum grain yield has been reported to increase up to 55% compared to the same crop fertilized with 240 kg N ha^{-1} (Rangel *et al*., 2014).

In Mexico, information on the effect of plant growth-promoting bacteria in forage production is scarce. Therefore, the objective of this study was to evaluate the effect of different inoculation media and PGPB bacteria on height, SPAD units, leaf temperature, botanical composition, and dry matter yield of orchard grass (*Dactylis glomerata* L.) under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions in the Center for Research in Applied Biotechnology (CIBA) of the Instituto Politécnico Nacional (IPN), located in Tepetitla de Lardizábal, Tlaxcala (19° 16′ 55.17″ N, 98° 21′ 57.59″ W; 2222 masl). The inoculation media used were prepared in the following manner:

Compost. This substrate was sterilized in an autoclave three times every other day. The necessary inoculum was added to 40 grams of compost to adjust to a concentration of 1×10^8 CFU per g^{-1}. It was divided into aliquots of one gram in sterile 1.5 mL tubes and one aliquot was used per pot. The control consisted of one gram of compost without inoculum.

Corn Starch. During 5 days, 40 mL of bacterial inoculum were lyophilized with a concentration of 1×10^8 CFU. Afterwards, 0.04 g of lyophilized inoculum were taken and mixed with 0.1 g of corn starch. This mixture was applied to the soil of each experimental unit.

Digestate. This medium was diluted to 50% with distilled water and 20 g L^{-1} of sucrose was added as a source of carbon, previously sterilized in an autoclave. Later, 40 mL of the necessary inoculum was mixed in to a concentration of 1×10^8 CFU per mL of digestate. The mixture was distributed in aliquots of 1 mL, to apply one aliquot to each pot. The control was sterilized digestate without bacteria.

The bacterial strains used in this study were previously identified through 16s RNAr sequencing and correspond to the species *Ewingella americana*, *Bacillus simplex* and *Microbacterium ginsengiterrae*.

The media and substrates were sterilized in a Prado brand autoclave, model AH-80170.

The orchard grass seeds (*Dactylis glomerata* L.) were donated by the forage laboratory of Colegio de Postgraduados. The inoculation media used were: 1) digestate, donated by the Universidad Autónoma Chapingo; 2) compost, donated by the Zacatenco Composting Unit of the Instituto Politécnico Nacional (IPN); 3) commercial-brand corn starch, sterilized in an autoclave; and a sandy Fluvisol soil was used as a substrate in the experimental units, obtained from the experimental plot of the Center for Research in Applied Biotechnology (CIBA) of the IPN Tlaxcala Unit.

The treatments and experimental units were distributed according to a completely random design with a 4×3 factorial arrangement, where the factors were: inoculum (3 strains of bacteria and the control) and inoculation medium (digestate, corn starch, and compost). The values obtained in the experimentation phase were graphed in SigmaPlot V.10.0 (2015) and the statistical analyses were carried out using the GLM procedure of the SAS® Version 9.0 for Windows® (2002) statistical software. The treatment means were compared using the Tukey test at a significance level of 5%.

The control treatments were: one gram of compost and 0.1 g of corn starch and digestate; all without bacterial inoculation.

The experimental unit was a pot with 1.5 kg of soil, containing five stalks of orchard grass, each 45 days old. At the experiment start, the stalks were uniformly cut at a height of 5 cm to reduce the effect of covariables, and the solid media were inoculated with the PGPB bacteria and directly applied to the rhizosphere zone.

Evaluated Variables

Plant Height. Once 70 days passed since the homogenizing cut, the height of each grass cluster per pot was measured with a 30 cm graduated ruler at precision of 0.1 cm, with the ruler positioned vertically from the base of the plant to the top youngest leaf (Castillo *et al*., 2009).

Leaf Temperature. The datum was recorded from the upper leaves with differentiated ligule, with a Spectrum Technology Inc® brand infrared thermometer.

Morphological Composition. The harvested forage was separated into its morphological components: leaf (from the base of the ligule to the leaf apex), and stalk (the pseudostalks and leaf veins were included).

Dry Matter Yield. The harvested forage was weighed fresh and then dried in a forced air oven at 70 °C for 48 hours. Finally, the dried samples were weighed in an analytical balance.

Density or Number of Sprouted Stalks. Before the final cut, the stalks of each pot were counted using a plastic ring to identify them, and the initial amount of 5 stems per pot was subtracted from the total count.

RESULTS AND DISCUSSION

Concerning plant height, the control presented the lowest value ($P<0.05$) compared to the rest of the treatments. The inoculation medium did not affect ($P>0.05$) plant height (Table 1). After analyzing the results per treatment in detail, differential performances were observed. While *Bacillus simplex* and *Ewingella americana* promoted greater plant height when inoculated with compost, *Microbacterium ginsengiterrae* showed better performance when inoculated with digestate (Figure 1D). The leaf temperature with *M. ginsengiterrae* was lower ($P<0.05$) compared to *Bacillus simplex*, *Ewingella Americana* and the control (Table 1). The inoculation medium did not affect this variable ($P>0.05$). Likewise, it was observed that without inoculation (control) and with corn starch, the leaf temperature was higher ($P<0.05$) compared to the digestate, which showed the lowest values (Figure 1E).

Morphological Composition. The weight of the dry stalks was different ($P<0.05$) with the control; however, there was no difference between the bacterial treatments ($P>0.05$), although quantitively the highest value was seen in treatments with *Microbacterium* and *Ewingella*. The inoculation medium did not have a major effect ($P>0.05$). By treatment, the *Bacillus simplex* inoculated with the corn starch medium showed the maximum values for dry weight of stalks (Figure 1A).

Concerning the dry weight of leaves, there were differences as a result of bacteria and the cultivation medium ($P<0.05$). The maximum weight was obtained with *M. ginsengiterrae* and *B. simplex* and the minimum,

Table 1. Variables evaluated in orchard grass inoculated with different PGPB bacteria and inoculation methods.

Factor	Stem accumulated	Stem (g DM pot^{-1})	Dry matter (g DM pot^{-1})	Leaf (g DM pot^{-1})	Leaf Temp. °C	Height (cm)
Bacterias						
M. ginsengiterrae	7.8 A	1.81 A	3.07 A	1.26 A	23.3 B	7.2 AB
B. simplex	7.7 A	1.5A AB	2.61 A	1.03 AB	24.9 A	7.5 A
E. americana	5.4AB	1.62 A	2.62 A	1.0 BC	25.4 A	7.5 A
Control	4.1 B	1.07 B	1.84 B	0.77 C	26.5 A	6.1 B
Inoculation method						
Lyophilized	5.3	1.38	2.2 B	0.83 B	24.7	6.6
Digestate	7.3	1.67	2.75 A	1.1 A	24.4	6.9
Compost	6.2	1.45	2.64 A	1.19 A	25.2	7.5
Significance						
Strain	**	**	**	**	**	**
Méthod	NS	NS	**	**	NS	*
C * M	NS	NS	**	**	NS	NS

Different capital letters in columns are statistically different Tukey ($P<0.05$). Significance; NS=Non-significant; *=0.05; **=0.01.

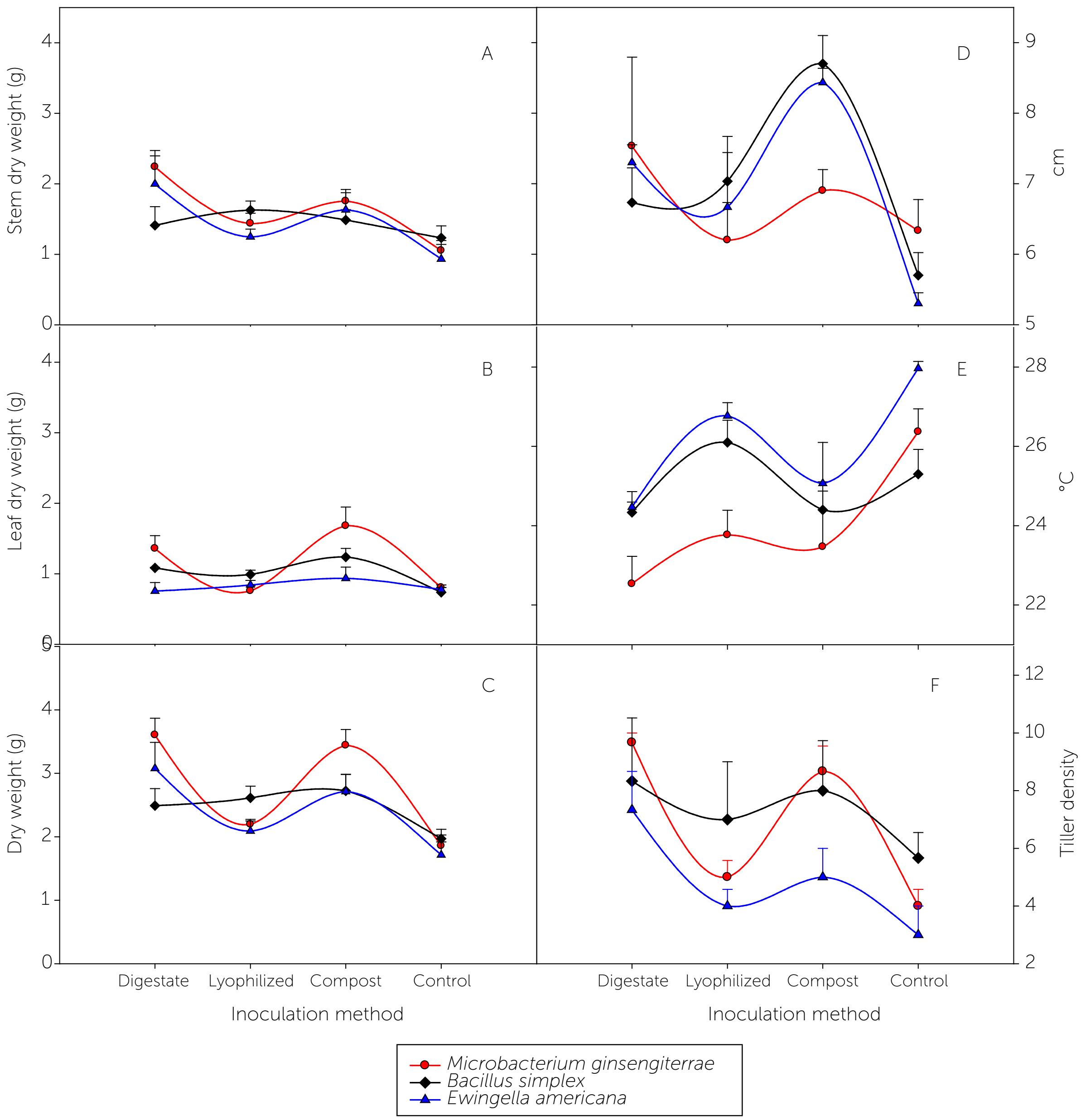

Figure 1. Dry weight of stalk or pseudo-stalk (A), dry weight of leaf (B), total biomass (C), plant height (D), leaf temperature (E), SPAD units (F) of orchard grass inoculated with PGPB bacteria and different inoculation media.

with the control; although there was no difference with compost and digestate, the minimum value was obtained with corn starch (Figure 1B). In addition, a significant effect was observed in the interaction between bacteria and inoculation medium (Table 1).

The total dry weight demonstrated that, except for the control, there was no difference in the rest of the treatments with bacteria ($P<0.05$). An increase of up to 50% more of total dry matter was achieved with the bacteria, compared to the control; while the compost and digestate surpassed corn starch by 22% ($P<0.05$). The effect of the principal factors (Strain and Method) and their interaction was highly significant ($P<0.01$) (Table 1). Figure 1C shows that, when using compost and digestate, *M. ginsengiterrae* and *E. Americana* produced a greater amount of dry matter than when using corn starch.

Accumulated Stalks. The values obtained show that the clusters inoculated with *M. ginsengiterrae* and *B. simplex* showed different values ($P<0.05$), achieving up to 150% more stalks ($P<0.05$) compared to the initial population (5 stems per pot). With *E. americana* and the control, up to 90% more stalks were obtained, without differences ($P>0.05$) between these treatments (Figure 1F). As a main factor, the inoculation media did not show any effect on this variable (Table 1).

Forage grasses have demonstrated that their growth depends on the interaction of climate factors as well as the management of the frequency and severity of defoliation (Hernández-Garay *et al*., 1997; McKenzie *et al*., 1999). In the present experiment, orchard grass was evaluated in the same environmental conditions, modifying only a small proportion of soil microorganisms. Therefore, it is evident that the bacteria and the inoculums had a significant effect on the evaluated variables.

The differential reactions in the yield of orchard grass can be attributed to the fact that the inoculated microorganisms that colonized the rhizosphere incentivized the development of plants through complex interactions, such as those that occur between the root exudate derived from photosynthesis and other physiological processes of the plant, soil, microbiome, and population dynamics of the stalks (Singh *et al*., 2017). According to other studies, the bacteria act through a wide variety of mechanisms. Within these, the mineralization of organic material is accelerated, which facilitates mineral availability (Tilvikiené *et al*., 2018). With respect to this, we can speculate that the improved yield, achieved when using digestate and compost as inoculation media, can be partially attributed to the organic matter content of these media.

The results evidenced in this experiment coincide with those reported by Lopes *et al*. (2018), who observed a significant increase in the yield of *Brachiaria brizanta* under a silvopasture system, when inoculated with PGPR bacteria. Nonetheless, the changes noted in orchard grass yields are less than those reached by other grasses, according to descriptions by Rangel *et al*. (2014), who indicated that after inoculating wheat with PGPB bacteria, the achieved yield surpassed the control crop by up to 55%.

The optimal temperature range for the growth of grasses in temperate climate is between 16-24 °C (Yao *et al*., 2011); however, in this study the leaf temperature values found with the infrared thermometer were above 25 °C; this condition could have caused stress to the crop and affected the yield.

The inoculation with bacteria promoted plant growth and an increase in number of stalks when compared to the control, and both variables are correlated with the yield (Hernández-Garay *et al*., 1997). In parallel, the accumulation of forage is correlated with the accumulation or storage of non-structural carbohydrates in the root, which constitute an important source of carbon for bacteria and for re-sprouting after defoliation (Benot *et al*., 2019).

The bacteria evaluated in this experiment have been reported as PGPB bacteria by different authors, and their benefits as phosphate solubilizers, nitrogen fixers, and siderophore synthesizers have been mainly evaluated in growth media and *in vitro* conditions (Rashid *et al*. 2012). Although techniques to measure in vitro microbial activity were not used in this work, the results found can be partially attributed to the effect of the bacteria and the inoculation medium. It is important to underline that the effect of PGPB bacteria varies considerably depending on the species, type of soil, plant species, environmental conditions, and soil microbiome with which the inoculated bacteria commonly compete (Armenta-Bojórquez *et al*., 2010).

CONCLUSIONS

The use of compost and digestate as inoculation media catalyze the effect of PGPB bacteria on the production of dry matter in orchard grass, and the inoculation of *Microbacterium ginsengiterrae* on the yield of orchard grass.

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