



DOI: <https://doi.org/10.29298/rmcf.v10i52.414>

Article

Inyecciones de glucosa en *Jacaranda mimosifolia* D. Don en áreas urbanas de Texcoco de Mora

Glucose injections in *Jacaranda mimosifolia* D. Don in urban areas of Texcoco de Mora

Luis Manuel Morales Gallegos¹, Tomás Martínez Trinidad^{1*}, Armando Gómez Guerrero¹, Ramón Razo Zarate² y Javier Suárez Espinoza³

Resumen

Los árboles de jacaranda son comunes en áreas urbanas del valle de México y se desarrollan frecuentemente bajo condiciones de estrés nutricional o hídrico, lo que restringe el crecimiento del arbolado. El suministro de carbohidratos a través del sistema vascular recientemente se ha recomendado como una opción para mejorar el crecimiento y vitalidad. Por lo anterior, el objetivo del trabajo fue evaluar el efecto de la glucosa sobre crecimiento y vitalidad de árboles de *Jacaranda mimosifolia* inyectada al tronco en cuatro concentraciones: 0 (testigo), 30, 55 y 80 g L⁻¹. Se midieron incrementos en diámetro (cm) y altura (m), se monitoreó la condición de copa y producción de follaje y además se evaluó el contenido de carbohidratos en brotes, troncos y raíces, así como la fluorescencia de la clorofila (Fv/Fm). Se encontraron diferencias significativas ($P \leq 0.05$) en el incremento en diámetro y altura con la aplicación de 80 g L⁻¹ de glucosa. La condición de copa mostró valores significativamente más altos en densidad y bajos en transparencia, así como una buena producción de follaje, con la concentración más alta del carbohidrato (80 g L⁻¹). Su contenido presentó diferencias significativas ($P \leq 0.05$) en tronco, no así en brotes, raíces, ni en valores de fluorescencia de la clorofila. Las inyecciones de glucosa al tronco tienen un efecto significativo en el crecimiento de árboles de esta especie y afectan en menor medida la vitalidad del arbolado.

Palabras clave: Árboles urbanos, azúcares solubles, clorofila, estrés, fenología, *Jacaranda mimosifolia* D. Don.

Abstract

Jacaranda trees are common in urban areas of *Valle de Mexico* and often grows under nutritional and water stress conditions, which affects its growth. The supply of carbohydrates through the vascular system has recently been recommended as an option to improve growth and vitality. Therefore, the objective of this work is to assess the effect of glucose injected to the trunk of *Jacaranda mimosifolia* trees in for concentrations: 0 (control), 30, 55 and 80 g L⁻¹. Diameter (cm) and height (m) increments were determined, the canopy condition and foliage production were monitored and the content of carbohydrate in shoots, trunks and roots as well as the chlorophyll fluorescence (Fv/Fm). Significant differences were found ($P \leq 0.05$) in the increase in diameter and height with the application of 80 g L⁻¹ glucose. The crown condition showed significantly higher values in density and lower in transparency, as well as an adequate foliage production, with the highest concentration (80 g L⁻¹). Carbohydrate content showed significant differences ($P \leq 0.05$) in trunk, not in buds, roots, or chlorophyll fluorescence. The injections of glucose to the trunk have a significant effect on the growth of *Jacaranda* trees and affecting vitality to a lesser extent.

Key words: Urban trees, soluble sugars, chlorophyll, stress, phenology, *Jacaranda mimosifolia* D. Don.

Fecha de recepción/Reception date: 14 de septiembre de 2018

Fecha de aceptación/Acceptance date: 22 de febrero de 2019

¹Posgrado en Ciencias Forestales. Colegio de Postgraduados. México. Correo-e: tomtz@colpos.mx

²Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo. México.

³Posgrado en Estadística. Colegio de Postgraduados. México.

Introduction

Urban trees under optimal healthy conditions provide ecosystem services such as shade, recreation, carbon capture, interception of air pollutants and positive impact on human well-being (Moser *et al.*, 2018; Scholz *et al.*, 2018). However, in many cases, the environment is not favorable for the growth of trees (Martínez *et al.*, 2010, Moser *et al.*, 2017). due to space limitations, nutritional deficiencies, soil compaction, lack of water, extreme temperatures, pollution and vandalism (Stojnic *et al.*, 2016, Allen *et al.*, 2017). This stresses the tree and results in poor growth that has an impact on vitality and high mortality rates (Koeser *et al.*, 2014).

From photosynthesis, the synthesis of carbohydrates is essential for the tree, since these compounds maintain their metabolism, accumulate reserves and promote the growth of new tissues (Maselli and Silveira, 2017). If the production of sugar depends on the availability of minerals in the soil and environmental factors (Gamboa and Marín, 2012, Valenzuela *et al.*, 2013), then, environmental stress conditions, derived from the urban environment, cause a reduction in the tree's carbohydrate reserves (Martínez *et al.*, 2010; Koeser *et al.*, 2014; Moser *et al.*, 2018). Vitality is directly related to the quantity of reserves, so vigorous individuals are able to resist in stressful situations (Johnstone *et al.*, 2013; Callow *et al.*, 2018; Ramírez *et al.*, 2018).

The exogenous supply of carbohydrates to the tree vascular system has a positive effect on its development, due to the increase in energy reserves (Percival *et al.*, 2004; Martínez *et al.*, 2009; Martínez *et al.*, 2013). Although injections to the trunk were first used for the chemical treatment of diseases, fungi and pests (Costonis, 1981; Perry *et al.*, 1991; Dal Maso *et al.*, 2014; Acimovic *et al.*, 2015), They have recently been used as the most viable option to improve the state of urban trees (Martínez *et al.*, 2009; Percival and Sacre, 2014; Suryanto *et al.*, 2018). The advantage of the injection to the trunk is that the applied compound is used in its entirety when incorporated into the vascular system (Costonis, 1981; Wise *et al.*, 2014; Acimovic *et al.*, 2016) and it is distributed throughout the tree (Kobza *et al.*, 2011).

The jacaranda tree (*Jacaranda mimosifolia* D. Don), is an arboreal species belonging to the Bignoniaceae family (Sharma *et al.*, 2016). Its ornamental use in urban spaces has increased in recent decades (Miyajima *et al.*, 2013; Zaouchi *et al.*, 2015) because it is a medium-sized species with abundant and colorful flowering (Miyajima *et al.*, 2013). It is important to improve the vitality condition of jacarandas that grows in environments that are not always favorable. Therefore, the objective of this work was to evaluate the effect of glucose injected into the trunk on the growth and vitality of *Jacaranda mimosifolia* trees located.

Materials and Methods

The work was carried out in the trees of the *Texcoco-Chapingo* Boulevard (19°30'04.19" N and 98°53'00.85" W) in *Texcoco de Mora* municipality, State of Mexico. The area has an average altitude of 2 240 m, a temperate semi-dry climate, an average annual temperature of 15.9 °C and an average precipitation of 686 mm per year; the soil is clay-textured Vertisol (Gutiérrez and Ortiz, 1999). A total of 36 *Jacaranda mimosifolia* trees with a normal diameter of 27 cm and a height of 7 m on average were selected, aligned with a 6 m spacing.

The application of glucose was made through macro injections at the base of the trunk of each tree (Costonis, 1981). Drillings of 4.4 mm in diameter were made at the base of the tree at a depth of approximately 25.4 mm (Acimovic *et al.*, 2016) perpendicular to the trunk; a 550 rpm cordless drill DW130V (DeWalt Industrial Tool Co.) and a manual pump (H. D. Hudson Industries™) were used at a constant pressure of 0.13 MPa, to apply a total of 10 L of solution per tree in July 2017.

To know the growth of the trees 11.5 months after the application of the treatments, the trunk diameter (cm) was measured at the beginning and at the end of the experiment at a height of 10 cm above the ground with a diametric tape 283D (Forestry suppliers Inc.); by the frequent bifurcation of the trunk below 1.30 m in height and the measurement point was marked with indelible ink. The total height

(m) was measured with a clinometer (Brunton™). From the above variables, the increase of 11.5 months in diameter (ID) and in height (IA) was estimated.

To determine the vitality of the trees, information was recorded on the crown condition of each tree through the crown density (Dnc) and crown transparency (Trc); this was carried out visually by two people located at a horizontal distance proportional to the height of each tree, it was determined with a scale divided into 5 % classes (Westfall *et al.*, 2009; Saavedra *et al.*, 2016). The amount of foliage production of each tree was assessed by the 6-class method used to evaluate infestation by mistletoe (Hawksworth, 1977). The crown of each tree was divided into three thirds, each third was evaluated separately assigning a grade of 0, 1 or 2, for each amount of foliage, where: 0 is non-visible or low production, 1 is an adequate production and 2 is a high production. In the actual study, the assessment of the amount of foliage produced in the different treated trees was made.

The content of carbohydrates, total sugars and reducing sugars in tree tissues was calculated. Samples were taken at random from five branches of the lower crown of each tree; In addition, with an increment hammer (Haglof Company Group TM), five samples were taken near the base of the trunk (4mm × 100 mm) (Martínez *et al.*, 2009). These were macerated in liquid nitrogen and stored at -20 °C until processing in the laboratory. Two alcoholic extractions of sugars from the harvested tissues were performed, each with 40 mL of 80 % ethanol (v/v) for 20 min at 100 °C. The result of the extraction was diluted in 10 mL of distilled water for the quantitative determination (Quentin *et al.*, 2015); samples were worked in triplicate.

The concentration of total sugars was determined by the anthrone method (Witham *et al.*, 1971); a Genesys™10S Vis & UV-Vis spectrophotometer was used to read the absorbance at 600 nm; total sugars were quantified with a standard glucose curve at a concentration of 20 to 200 µg mL⁻¹. For the reducing sugars, the method of Nelson (1944) and Somogyi (1952) was used; absorbance was recorded on the spectrophotometer at 540 nm and a standard curve containing glucose of 15 to 150 µg mL⁻¹ was used. The results were expressed in mg of glucose per gram of dry weight (mg g⁻¹ ps⁻¹).

Analogously, the content of starch in roots was quantified; for this, approximately 5 g of tissue was collected below the base of the trunk, and the precipitate of the alcohol extraction process described above was hydrolyzed with the diastase enzyme (SIGMA) (Palevitz and Newcomb, 1970). The anthrone method was applied to the resulting hydrolyzate based on the glucose content present in the sample; the result was expressed in $\text{mg g}^{-1} \text{ ps}^{-1}$.

Finally, chlorophyll fluorescence (F_v/F_m) was recorded with a Pocket PEA portable fluorometer (Hansatech Instruments Ltd.), with a detection time of 1 s and emitting light at a wavelength of 650 nm, with an intensity of $3500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Zhang *et al.*, 2016). Measurements were made after adapting to the dark for 10 minutes with pocket PEA clips a total of 10 randomly chosen leaves taken from the outside of the low crown of each tree (Martínez *et al.*, 2009).

The experimental design was completely random; the treatments consisted of four glucose concentrations: 0 (control), 30, 55 and 80 g L^{-1} ; the control only contained purified water. The distribution of treatments was random with nine repetitions for each treatment (Percival *et al.*, 2004; Martínez *et al.*, 2009).

The assumptions of normality were checked with Shapiro-Wilk ($n \leq 50$ and $\alpha = 0.05$) and the homogeneity of variances (Bartlett test). A multivariate analysis (MANOVA) was performed on those variables that fulfilled the assumptions of normality and homogeneity ($P \leq 0.05$), we proceeded with the comparisons of means with the DSH test (significant honest difference of Tukey) and $\alpha = 0.05$. In those variables that did not meet the assumptions ($P > 0.05$), the nonparametric method of Kruskal-Wallis was used and the means were compared with the sum of the Wilcoxon ranges. It was analyzed statistically with the RStudio Team® program (R Core Team, 2015).

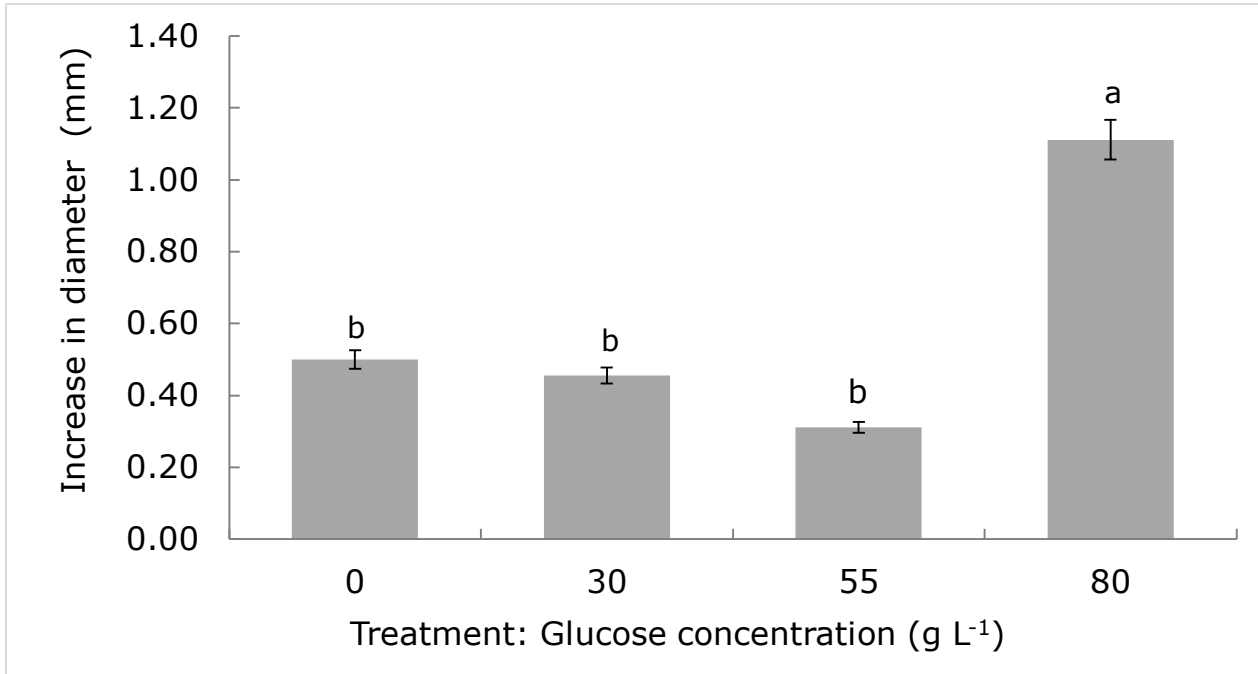


Results and Discussion

Increase in diameter

Significant differences ($P \leq 0.05$) in the increase in diameter of the jacaranda trees were recorded after 11.5 months of the application of the treatments. The highest concentration of glucose (80 g L^{-1}) showed average growth values 200 % higher than those of the specimens injected with the control (Figure 1). However, the application of carbohydrate doses lower than 80 g L^{-1} of glucose had no effect on growth in diameter, possibly due to the demand of large amounts of carbohydrates (Piper and Fajardo, 2016). Therefore, low glucose concentrations are insufficient to promote growth and its effect is probably diluted by being mobilized to cover the energy requirements of other physiological processes such as photosynthesis, respiration, transpiration, absorption and translocation (Martínez *et al.*, 2013; Ramírez *et al.*, 2018). In some cases, sugars are used to store carbohydrate reserves, all at the expense of vegetative growth (Piper and Fajardo, 2016). Previous studies have shown that the use of carbohydrates has positive effects on the diameter growth of trees such as oak (*Quercus virginiana* P. Miller), poplar (*Populus nigra* (Moench) Koehne), English oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) (Martínez *et al.*, 2009; Percival and Sacre, 2014), a positive effect has also been observed in the diameter of Ceylon oak branches (*Schleicera oleosa* Merr.) (Suryanto *et al.*, 2018).





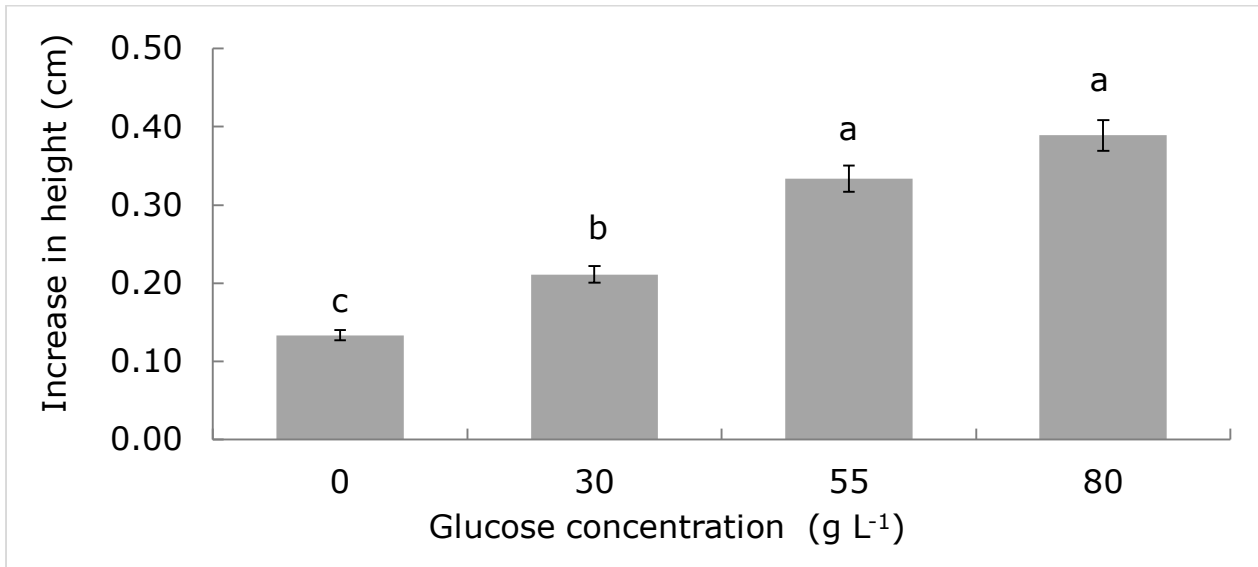
The bars indicate the standard error. Equal letters mean statistically equal means (Tukey: $P \leq 0.05$).

Figure 1. Average increase in basal diameter (mm) of *Jacaranda mimosifolia* D.Don trees injected with four glucose concentration.

Increase in height

The increase in height presented significant differences ($P \leq 0.05$) to the glucose injection; the height of the trees showed higher average values for the two highest concentrations of glucose (55 and 80 g L⁻¹), compared to the low concentrations (0 and 30 g L⁻¹) (Figure 2). This may indicate that the former stimulate growth in height (Martínez *et al.*, 2013; Percival and Sacre, 2014). In certain broadleaf species such as willows (*Salix* sp.), poplar trees (*Populus* sp.), birches (*Betula* sp.) and maples (*Acer* sp.). The growth of the apical bud and flowering is manifested long before the vegetative growth (Percival and Sacre, 2014). Initial stages of vegetative reproduction depend exclusively on the reserves that the tree accumulated in the previous growth cycle (Percival and Sacre, 2014; Ramírez *et al.*, 2018). This supports

the idea that the exogenous application of carbohydrates stimulates growth in urban trees (Martínez *et al.*, 2013).



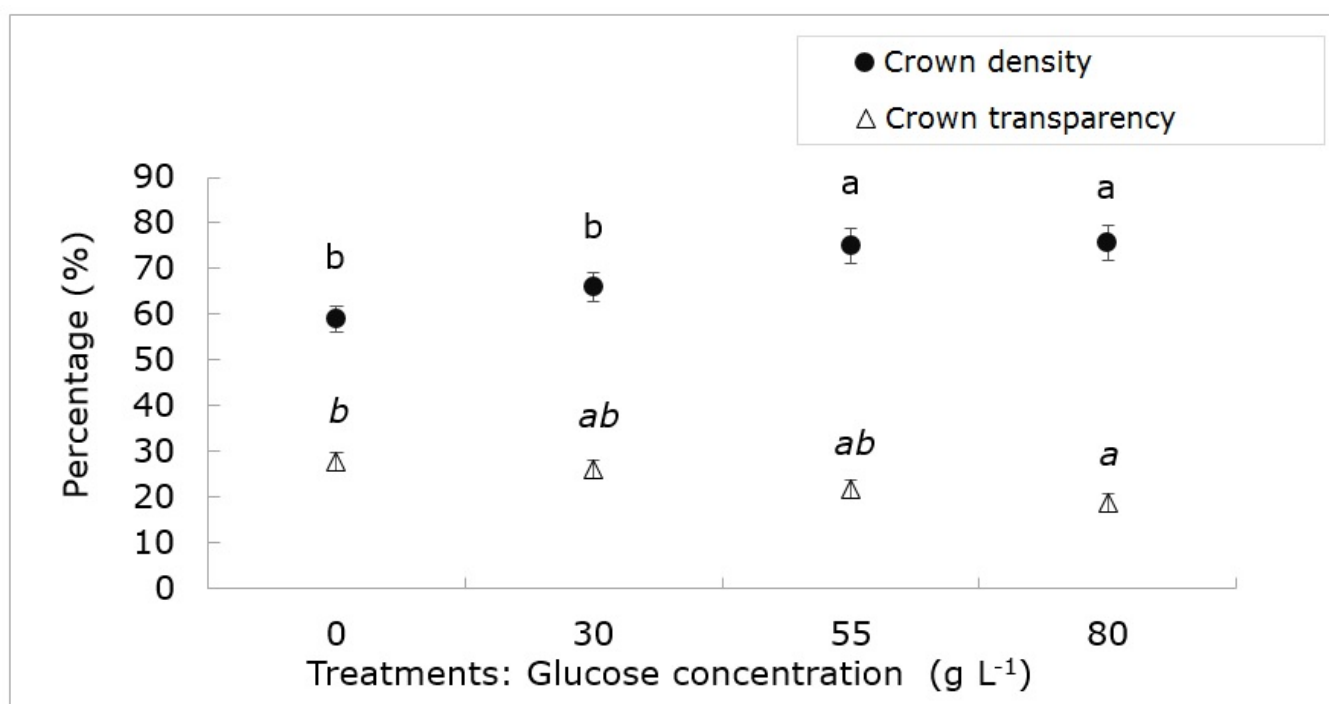
The bars indicate the standard error. Equal letters mean statistically equal means (Tukey: $P \leq 0.05$).

Figure 2. Increase in height (cm) of *Jacaranda mimosifolia* D.Don trees injected with four glucose concentrations.

Crown condition

The average values of crown density (Dnc) were significantly different among glucose concentrations ($P \leq 0.05$). The highest average value in Cnd occurred at 80 and 55 g L⁻¹ (75 % and 76 %, respectively), while the lowest average was observed in the control (0 g L⁻¹) with 59 % (Figure 3). More than 50 % of the evaluated trees developed 70 % in crown density, which indicates a good amount of foliage (Saavedra *et al.*, 2016). It is well-known that above 75 % is unusual for most urban tree species, unless they grow in open spaces (Westfall *et al.*, 2009). Dnc stand out represent a high potential of growth, and, therefore, of survival, in comparison with those mature specimens with less amount of foliage (Zaragoza *et al.*, 2014; Maselli and Silveira, 2017). Healthy trees are considered to be those with a crown density greater than

50 % (Westfall *et al.*, 2009; Saavedra *et al.*, 2016), while if they are less than 30 % they have a high probability of dying in a year (Steinman, 1998). The assessed jacaranda trees have a better canopy density than other species located in urban spaces such as the *San Juan de Aragón* Park, *Alameda Norte*, South and the Eastern Mexico City with averages less than 70 % (Zaragoza *et al.*, 2014; Saavedra *et al.*, 2016).



The bars indicate the standard error. Equal letters mean statistically equal means ($P \leq 0.05$) using the sum of Wilcoxon ranges.

Figure 3. Density and transparency of the crown of *Jacaranda mimosifolia* D. Don trees subjected to four glucose concentration treatments.

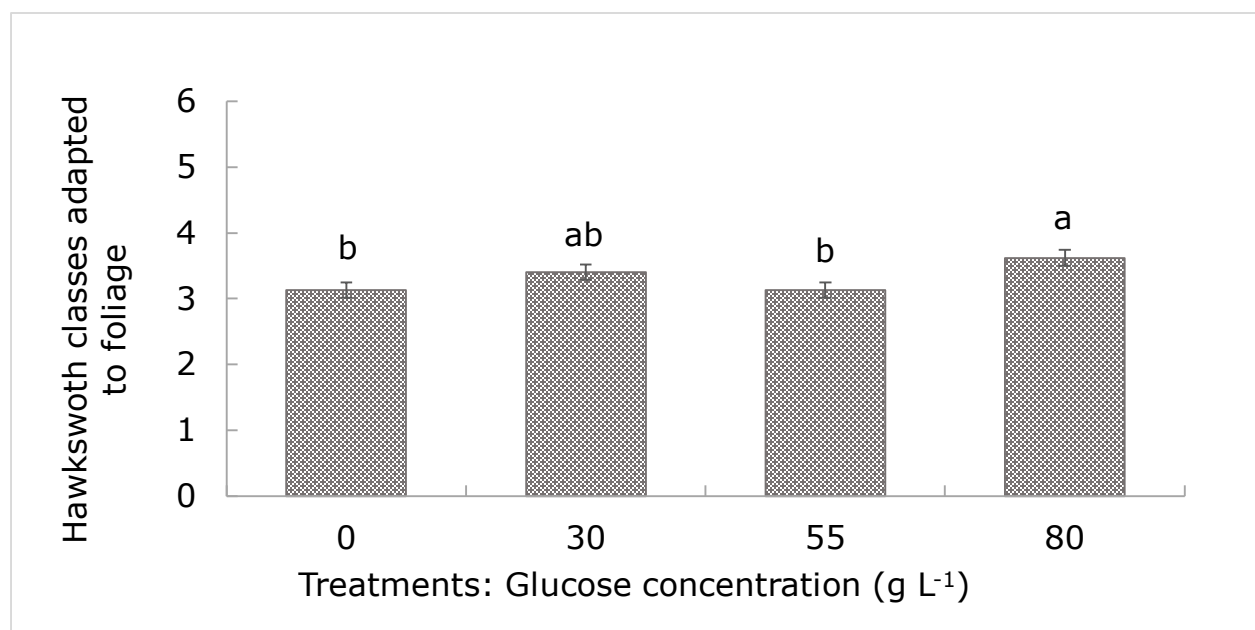
The average canopy transparency values (Trc) were significantly different ($P \leq 0.05$). The highest for this variable was shown by the control trees (0 g L⁻¹), with 28 %, while the lowest corresponds to the treatment with 80 g L⁻¹ of glucose with an average of 19 % (Figure 3). It was also observed that at least 50 % of the studied individuals presented an average of 25 % Trc, which suggests that the amount of foliage in the

trees is sufficient to carry out fundamental processes such as photosynthesis and respiration; however, the crown is not dense enough to block the passage of light (Zaragoza *et al.*, 2014).

High Trc values are directly related to stressors, such as pest attack, drought, pruning activities, air pollution, soil compaction or vandalism (Schomaker *et al.*, 2007; Zaragoza *et al.*, 2014); however, the evaluated trees showed compaction and pruning, mainly. Figures below 30 % of this variable in the trees indicate a healthy condition (Saavedra *et al.*, 2016). In general, for the assessed trees in the *Texcoco-Chapingo* Boulevard are below the mentioned limit so as not to be considered in a state of decline or stress (Figure 3).

The level of foliage production was influenced by the highest glucose concentration ($P \leq 0.05$). Despite this, this variable, independently of the glucose concentration, was located between classes 3 and 4 (Hawksworth, 1977); therefore, satisfactory values are considered. The production of foliage is directly related to the Dnc variable described, so it was observed that the higher the value of Dnc, the foliage also increased, as shown by the results for the concentration of 80 g L^{-1} (Figure 4). This amount of leaves allows the trees to survive and perform their functions correctly (Zaragoza *et al.*, 2014).





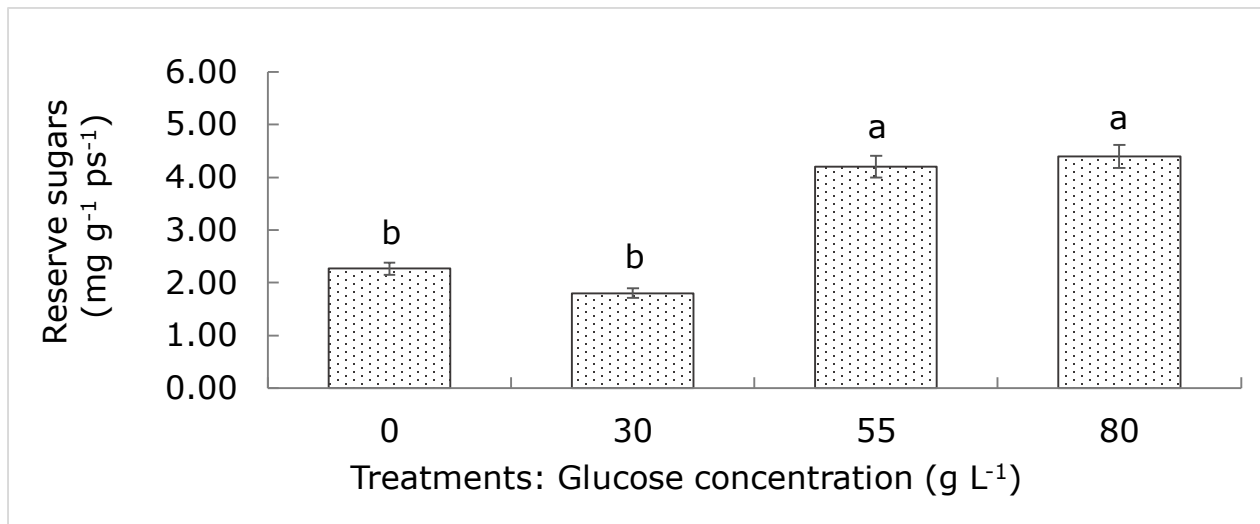
The bars indicate the standard error. Equal letters mean statistically equal means (Tukey: $P \leq 0.05$).

Figure 4. Hawksworth classes adapted to evaluate foliage production in *Jacaranda mimosifolia* D.Don trees subjected to four glucose concentration treatments.

Total Sugars and Reducing Sugars

The analysis of carbohydrates showed that both total and reducing sugars in shoots, trunk and starch in roots registered an annual variation, with maximum values in the autumn period, and that decreased gradually in the winter, to finally reach their lowest values during the spring, shortly after the foliage emergence period. This fluctuation is due to the fact that sugars are used in processes such as respiration during winter and the production of new tissues in spring (Valenzuela *et al.*, 2013; Ramírez *et al.*, 2018). This annual variation has been recorded in carbohydrate levels in species such as: semievergreen oak (*Quercus virginiana* P. Miller), orange (*Citrus sinensis* L.), tangerine (*Citrus reticulata* Blanco) and mango (*Mangifera indica* L.) (Martínez *et al.*, 2009; Gamboa and Marín, 2012; Laskowski, 2014). All the variables presented variations related to the phenological stages of the tree (Laskowski, 2014).

No significant differences were observed ($P > 0.05$) in total sugars and reducers in shoots, nor in starch from the roots of jacaranda trees. The total sugars in the trunk presented them ($P < 0.05$) in the treatment of 80 g L^{-1} , while the reducing sugars, did not show statistical evidence that indicated an alteration of this variable with the injection of glucose to the trunk; previous studies revealed an increase in reducing sugars, as an indicator of the increase in vitality (Martínez *et al.*, 2009; Laskowski, 2014), which was not confirmed in the current study. The determination of total sugars includes reducing sugars and fractions of polysaccharides such as starch, an important reserve carbohydrate in different organs of the tree (root, stem and branches) used for situations of energy deficiency (Martínez *et al.*, 2013; Piper and Fajardo, 2016). Therefore, it is possible to indirectly estimate the carbohydrate reserve of the trees with a simple difference between the values of these sugars. In this sense, statistical evidence was found ($P < 0.05$) indicating that trees injected with high glucose concentrations maintain more than twice the amount of reserves in the trunk than those treated with low glucose concentrations (Figure 5).



The bars indicate the standard error. Equal letters mean statistically equal means (Tukey: $P \leq 0.05$).

Figure 5. Concentration of soluble sugars in the trunk of *Jacaranda mimosifolia* D.Don trees subjected to four treatments of glucose concentration.

In another study, Ramírez *et al.* (2018) determined that urban trees such as maple (*Acer platanoides* L.) have a higher carbohydrate reserve than trees growing under natural conditions; this characteristic improves their ability to face periods of imbalance in carbohydrate levels related to abiotic factors.

Chlorophyll fluorescence

The fluorescence of chlorophyll showed no significant differences in any of the treatments ($P > 0.05$). This variable kept constant throughout the assessment with average F_v/F_m values of 0.787 for the concentrations of 0 g L⁻¹, 0.810 for 30 g L⁻¹, 0.804 for 55 g L⁻¹, and 0.811 for 80 g L⁻¹ glucose. Trees with F_v/F_m values between 0.78 and 0.85 are considered healthy and free from any type of stress (Johnstone *et al.*, 2013; Uhrin and Supuka, 2016; Callow *et al.*, 2018). Based on this variable, the trees did not exhibit evident signs of stress, as they remained in a condition of constant vitality throughout the experiment. The opposite occurs in the evergreen oak (*Quercus virginiana*), the sycamore maple (*Acer pseudoplatanus* L.) and the kusum (*Schleicheria oleosa* Merr), in which the chlorophyll fluorescence was stimulated by the injection of carbohydrates or altered by abiotic factors typical of urban environments such as water stress, compaction or mechanical damage (Martínez *et al.*, 2009; Uhrin and Supuka, 2016; Suryanto *et al.*, 2018).

Conclusions

The injection of 80 g L⁻¹ glucose into the trunk of jacaranda trees positively stimulated the growth in diameter and height. The crown condition kept a profuse density and foliage production, and indicates a good health of the trees; which coincided with a larger amount of reserve carbohydrates. No evidence of an alteration of the vitality condition was found with the use of chlorophyll fluorescence. Therefore, the application of glucose to the trunk promotes vegetative growth and to a lesser extent stimulates the vitality of the trees of the species *Jacaranda mimosifolia* in urban areas of Texcoco de Mora city.

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Acknowledgements

The authors thank the *Consejo Nacional de Ciencia y Tecnología* (National Council of Science and Technology) (Conacyt) for financing the original project and the municipality of *Texcoco de Mora* city for allowing to carry out this project in their urban green areas.

Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Luis Manuel Morales Gallegos: installation of the project in the field, data collection in the field, writing of the manuscript; Tomás Martínez Trinidad: project management, planning and follow-up, data analysis and manuscript revision; Armando Gómez

Guerrero: project planning and follow-up, as well as review of the manuscript; Ramón Razo Zárate: follow-up of the project and review of the manuscript; Javier Suárez Espinoza: data analysis and review of the manuscript.