



DOI: <https://doi.org/10.29298/rmcf.v10i51.183>

Article

Propagación por estacas y calidad de planta en *Acer negundo* L.

Propagation by cuttings and plant quality in *Acer negundo* L.

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Resumen:

La propagación vegetativa de *Acer negundo* representa una opción para conservar características fenotípicas deseadas y propagarla en períodos cortos. En un diseño experimental completamente al azar con tres repeticiones, en el que los factores de interés evaluados fueron el tipo de estaca (dura, blanda), el enraizador (con y sin enraizador) y la fertilización (alta=150-60-120, baja=100-50-80), se plantaron 480 estacas en charolas de polietileno, con 54 cavidades de 200 mL; después de cuatro meses se observó su morfología. Los resultados indican que las estacas duras fueron superiores en el diámetro del cuello del brote (2.68 mm), la longitud del brote a la yema (71.85 mm) y la longitud hasta la punta de las hojas (214 mm). La aplicación de enraizador produjo mayores valores en longitud del brote a la yema (69.4 mm) y longitud hasta la punta de las hojas (214.3 mm), así como en los pesos de las hojas, fresco (3.78 g) y seco (0.72 g). La interacción enraizador*fertilizante tuvo efectos sobre las variables longitud del brote hasta la yema y hasta la punta de la hoja. Hubo significancia en la interacción de los tres factores en el peso fresco de raíz, con el mayor (1.56 g) para estaca dura, fertilización alta con enraizador. El valor más alto del índice de Dickson ocurrió en estacas duras (3.12), en las que se propició el enraizamiento y los brotes por el posible mayor contenido de carbohidratos y el AIB (enraizador).

Palabras clave: Arce, indicadores de calidad de planta, enraizamiento, propagación asexual, viveros forestales.

Abstract:

The vegetative propagation of *Acer negundo* represents an option to preserve desired phenotypic characteristics and propagate it in short periods of time. In a completely randomized experimental design with three replications, in which the factors of interest evaluated were the cutting type (hard, soft), the rooting (with and without root starter) and fertilization (high = 150-60-120, low = 100-50-80), 480 cuttings were planted in polyethylene trays, with 54 cavities of 200 mL each; after four months its morphology was observed. The results indicate that the hard cuttings were superior in the diameter of the shoot (2.68 mm), the length of the shoot to the bud (71.85 mm) and the length to the tip of the leaves (214 mm). The root starter application produced higher shoot lengths to the bud (69.4 mm) and length to the tip of the leaves (214.3 mm), as well as leaf weights, fresh (3.78 g) and dry (0.72 g). The interaction root starter* fertilizer had effect on the variables lengths of the shoot to the bud and to the tip of the leaf. There was significance in the interaction of the three factors in the fresh root weight, with the highest (1.56 g) for hard cutting, high fertilization with root starter. The highest value of the Dickson index occurred in hard cuttings (3.12), in which the rooting and sprouts were favored by the possible higher carbohydrate content and the AIB (root starter).

Key words: Maple, plant quality indicators, rooting, asexual propagation, forest nurseries.

Fecha de recepción/Reception date: 26 de enero de 2018

Fecha de aceptación/Acceptance date: 7 de diciembre de 2018

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Introduction

Acer negundo L., commonly known as *negundo*, *acezintle*, or Mexican maple, is typical of deciduous and pine-oak forests, and of riparian vegetation, and is used as an urban tree in various regions. In Mexico, it is distributed at altitudes ranging between 1 800 and 2 300 masl, on all soil types, except for alkaline soils. It thrives in dry, poor soils. It requires a temperate humid climate, but tolerates minimum temperatures of 0 °C to -15 °C and seasonal droughts. It is utilized as an ornamental tree due to its rapid growth, attaining a height of up to 20 m, and has a broad crown that provides shade; furthermore, it is easily managed once it has acclimated to the plantation site (Conafor-Conabio, 2010).

In the asexual or vegetative propagation, the utilization and culture of vegetal tissues that preserves the potential for multiplying and for cell differentiation in order to generate individuals that are similar to the parent trees (donor plants) from the vegetative parts of the plants (Vázquez-Yanes and Cervantes, 1993). In ornamental species for urban environments, the vegetative propagation may be an option to obtain a good source of clonal material (Ramos *et al.*, 2012).

In Mexico, very little research has been done on the quality of plants resulting from vegetative propagation. The plant quality influences, to a great extent, the willingness of producers to plant new trees. Only if the plants have a high quality is it deemed worthwhile to prepare the ground, transport the trees to the field, and plant and maintain them (Wightman and Cruz, 2003).

According to Ritchie *et al.* (2010), the quality of the plant consists of the quantifiable traits of an individual produced in the nursery that can be used as indicators for its performance once it has been established in the plantation site; i.e. meeting certain standards is likely to increase the survival and growth of the plant.

Within this context, the objective of the present research was to assess the effect of the cutting type, the application of a root starter and the level of fertilization on the morphological attributes of the quality of nursery grown *Acer negundo* plants. The

hypothesis is that, with the application of a root starter and fertilizer, hard cuttings will produce plants with better morphological indicators of quality.

Materials and Methods

Gathering of cuttings

The cuttings were gathered on December 16, 2016, at the facilities of the *Universidad Autónoma Chapingo* (Chapingo Autonomous University), from branches of the middle and lower parts of the crown –according to their accessibility– of healthy adult *Acer negundo* trees. Only the material of the first three knots from the tip to the base of each branch was used. The cuttings had an average length of 15 cm, and were collected in a plastic ice box; they were kept moist and shaded until they were placed in the substrate.

Establishment of the experiment

The experiment was installed in *Tepetlaoxtoc*, Mexico State; black polyethylene trays with 54 cavities and a volume of 200 mL per cavity were utilized. The substrate was a mixture of peat moss, agrolite, and medium texture vermiculite, in a proportion of 40, 20 and 40 %, respectively. Controlled release fertilizer (*Osmocote*®) was added in a dose of 1 kg m⁻³ of substrate. The seedlings were irrigated with running water.

The factors taken into account were: cutting type, application of a root starter, and fertilization level. The cuttings were divided into two levels, the first of which is known as “soft cutting” and corresponds to the material gathered from the buds in the branches, with diameters of 4.5 to 8.5 mm, a slightly flexible consistency, and a light green color. The second level corresponded to “hard cuttings” from the base of the branch, with diameters ranging between 9 and 16 mm, with a rigid consistency, and a dark green to brown color.

The levels of root starter (*Radix 10 000*®, with 1 % indolebutyric acid (IBA) and 99 % inert material) were: with and without application. The utilized dose of the soluble

fertilizer, with the formula 20-20-20 (20 % N, 20 % P₂O₅, 20 % K₂O, the rest of inert material), was rated as high (150-60-120) or low (100-50-80) (N, P, K, ppm).

480 cuttings were placed in the trays, one per cavity, with a total of 60 cuttings for each of the eight treatments. At the moment of planting, the application of root starter was assigned at random, the cuttings that required it were moistened at the base and impregnated with *Radix* 10 000[®], the excess of which was removed before they were planted in the substrate. In order to seal the exposed incision, white acrylic paint was used, mixed with *Captán*[®] fungicide, in a dose of 1 g L⁻¹ of paint.

After the transplant, the trays were placed in a tunnel with a white polyethylene cover for greenhouses measuring 1.5 m in width, 3 m in length, and 1.5 m in height, with both ends covered with shade mesh, and a first irrigation was applied, adding 1 g L⁻¹ of *Captán*.

After eight weeks of the establishment of the experiment, at the start of the emission of adventitious roots, *Peters Professional*[®] soluble fertilizer for general use, convenient for the rapid growth stage in the nursery (20 – 20 – 20), began to be administered in the doses mentioned before, with irrigation every other day in order to maintain the moisture of the substrate.

After fourth months, the rooting percentage (in regard to the total number of cuttings) was registered, and the cuttings were considered to be rooted when side roots were observed. A sample of 40 cuttings was then obtained, in which the shoot diameter, the length of the shoot from the base to the bud, the length of the shoot from the base to the leaf tip, the root length, the (average) height from the base at which the roots emerged, the fresh weight of the leaves, the root, the aerial part, and the underground part, and the dry weight of the leaves, root, aerial part, and underground part were measured, using a plastic ruler calibrated in centimeters, a Truper[®] digital vernier caliper, and a *OHAUS Scout*[®] *Pro* digital scale.

The leaves, roots, aerial and subterranean parts were dried inside craft paper bags, in a (Ríos Rocha H-41) drying oven at 80 °C until they attained a constant weight (generally after 72 h).

Furthermore, the shoot/root ratio was estimated based on the relationship between the dry weights of each of one of the parts and Dickson's quality index, which is estimated dividing the total dry weight of the plant (g) by the sum of the slenderness coefficient (the ratio of the height of the plant, in cm, to its diameter, in mm) and the shoot/root ratio (Birchler *et al.*, 1998).

In this study, neither the dry or fresh weights nor the quality indicators included the weight of the cuttings.

Experimental design and statistical analysis

The experimental design was completely random and constituted a 2 × 3 factorial experiment. For the statistical analysis of the rooting, the percentage of rooted cuttings was transformed with the arc sine function of the square root of this percentage expressed as per-unit. The PROC ANOVA procedure of the SAS® software (*Statistical Analysis System*), V. 9.4, was utilized, and the means were compared using the Tukey's test.

The following statistical model was utilized:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + E_{ijk}$$

Where:

μ = Overall mean

α_i = Effect of the i^{th} level of the cutting factor

β_j = Effect of the j^{th} level of the root starter factor

γ_k = Effect of the k^{th} level of the fertilization factor, the combinations of letters indicate the interactions between the levels of the involved factors

E_{ijk} = Experimental error

Results and Discussion

The statistical analysis of the rooting, based on each individual factor, obtained statistical significance exclusively for the application of the root starter ($P \leq 0.05$) (Table 1), and the means comparison indicated the best result for the cuttings with root starter (20.5 % of rooting) (Table 1). Relatively common response; thus, Palanisamy and Subramanian (2001) register significant effects for the application of (1000 ppm) indolebutyric acid in *Tectona grandis* L. f. cuttings with rooting percentages of 74 to 100 %.

Table 1. Statistical analysis of the cutting rooting percentage.

P values					
Cutting type		Application of root starter		Level of fertilization	
0.5262		0.0071		0.4950	
Tukey's test					
Cutting		Root starter		Fertilization	
Hard	17.5a	With	20.48a	High	17.5a
Soft	15.0a	Without	12.08b	Low	15.0a

Means with a different letter in the same column indicate significant differences according to Tukey's test, $P \leq 0.05$.

The statistical analysis of the variables measured in the laboratory show that there were significant effects of four factors or their interactions on six studied variables: shoot diameter, shoot lengths to the bud and to the leaf tip, fresh weights of leaves and root, and leaves dry weight (Table 2).

Table 2. Results of the statistical analysis (P values) for variables assessed in the laboratory.

Source	SD	SLB	SLLT	RL	RSH	FLW	FRW	DLW	DRW
M	0.0569	0.0232*	0.0290*	0.1231	0.8698	0.1259	0.2079	0.1114	0.5607
C	0.0142*	0.0104*	0.0227*	0.3849	0.5561	0.3791	0.5377	0.1285	0.3000
R	0.8559	0.0338*	0.0212*	0.4155	0.8081	0.0371*	0.1527	0.0276*	0.1246
F	0.4106	0.5405	0.3851	0.4200	0.8486	0.5714	0.4488	0.9724	0.7092
C*R	0.2596	0.6978	0.8062	0.0947	0.6900	0.4481	0.7543	0.7110	0.5011
C*F	0.0967	0.9266	0.2677	0.0538	0.7028	0.5091	0.2599	0.7933	0.8302
R*F	0.0989	0.0179*	0.0386*	0.2010	0.2861	0.0578	0.4348	0.0477	0.2083
C*R*F	0.3074	0.6458	0.8521	0.1957	0.2938	0.2025	0.0289*	0.3949	0.9772

M = Model; C = Cutting; R = root starter; F = Fertilization; SD = shoot diameter; SLB = Stem length to the bud; SLLT = Shoot length to the leaf tip; RL = Root length; RSH = Root-shoot height; FLW = Fresh leaf weight; FRW = Fresh root weight; DLW = Dry leaf weight; DRW = Dry root weight. * = Significant values with $P \leq 0.05$.

Response to the cutting type

According to the means comparison, the hard cuttings had superior results for shoot diameter, shoot length from the base to the bud, and shoot length from the base to the leaf tip (Table 3).

Table 3. Response to the cutting type.

Cutting	SD (mm)	SLB (mm)	SLLT (mm)	RL (mm)	RSH (mm)	FLW (g)	FRW (g)	DLW (g)	DRW (g)
Hard	2.68a	71.85a	214a	104.1a	13.62a	3.43a	0.97a	0.68a	0.2a
Soft	2.23b	45.45b	165.60b	93.1a	11.92a	2.94a	0.83a	0.52a	0.13a

Means with a different letter in the same column indicate significant differences according to Tukey's test, $P \leq 0.05$.

The hard cuttings were taken from the part closest to the stem; therefore, they may contain a larger amount of carbohydrates than soft cuttings. In this regard, Veierskov (1988) points out a rising gradient in the concentration of these reserve products toward the stem base. According to Hartmann and Kester (1991), the thicker cuttings from the basal portion of the branches accumulate more reserve carbohydrates. This pattern is adjusted to, and accounts for the greater response in the variables mentioned above. This result is similar to that obtained by Liao *et al.* (2012), who attempted the vegetative reproduction of *Myrciaria dubia* (Kunth) McVaugh and obtained the best average for the shooting of leaves with large diameter cuttings. Nevertheless, different responses are to be expected in different species. dos Santos *et al.* (2011) conclude that *Cestrum laevigatum* Schldl. and *Salix humboldtiana* Willd. can be propagated with any cutting type; however, woody cuttings, which tend to be even thicker, work best for species like *Ficus adathodigifolia* L. and *F. citrifolia* Mill.

Response to the application of a root starter

The root starter (IBA) produced a tendency with a higher value in the variables stem length, both from the base to the bud and from the base to the leaf tips; it also influenced the fresh and dry leaf weight (Table 4). This agrees with the findings of Boschini and Rodríguez (2002), who, after applying different doses of IBA to *Morus alba* cuttings, registered statistically significant differences in the overall shooting of buds and roots between the plants on which IBA was used. Likewise, Maldonado *et al.* (2017) achieved the rooting of *Malphigia mexicana* A. Juss. cuttings, and Quintero *et al.* (2008), that of *Symphoricarpos microphyllus* H. B. K. stakes, using root starters.

Table 4. Response to the application of a root starter.

R	SD (mm)	SLB (mm)	SLLT (mm)	RL (mm)	RSH (mm)	FLW (g)	FRW (g)	DLW (g)	DRW (g)
W	2.47a	69.4a	214.3a	103.7a	13.13a	3.78a	1.07a	0.72a	0.21a
WO	2.44a	47.9b	165.3b	93.45a	12.43a	2.59b	0.74a	0.49b	0.12a

R = Root starter; W = with; WO = Without; Means with a different letter in the same column indicate significant differences according to Tukey's test, $P \leq 0.05$.

Hartmann and Kester (1991) state that the forming of adventitious roots may be due to certain inherent, non-translocable factors determined by the genotype of the individual tissue cells, as well as to the endogenous rooting factors present in each individual; furthermore, the lack of response to the application of synthetic auxin is due to the fact that several species have enough natural auxin to promote rooting. In the study by dos Santos *et al.* (2011), conducted on cuttings of 20 Brazilian forest species, *C. laevigatum* and *S. humboldtiana* formed roots without a root starter, but 11 taxa —e.g. *Dendropanax cuneatus* (DC). Decne. & Planch., *Erythrina falcata* Benth. and *Casearia sylvestris* Sw.— did not exhibit roots with any of their treatments.

Response to the level of fertilization

No significant differences were obtained in the mean comparison analysis for the levels of fertilization (Table 5), which may be due to the fact that “low fertilization” is sufficient to meet the nutritional demands of the plant, at least in terms of its morphological characteristics. The results were similar to those registered by Bualó *et al.* (2006) for *Calibrachoa linearis* (Hook.) Wijsman and *P. kleinii* L. B. Sm. & Downs., which were subjected to two levels of fertilization; furthermore, their growth and morphological characteristics did not differ significantly between treatments.

Table 5. Response to the level of fertilization.

F	SD (mm)	SLB (mm)	SLLT (mm)	RL (mm)	RSH (mm)	FLW (g)	FRW (g)	DLW (g)	DRW (g)
H	2.53a	61.65a	198.7a	103.7a	13.05a	3.34a	0.99a	0.61a	0.18a
L	2.39a	55.65a	180.9a	93.5a	12.50a	3.02a	0.82a	0.61a	0.15a

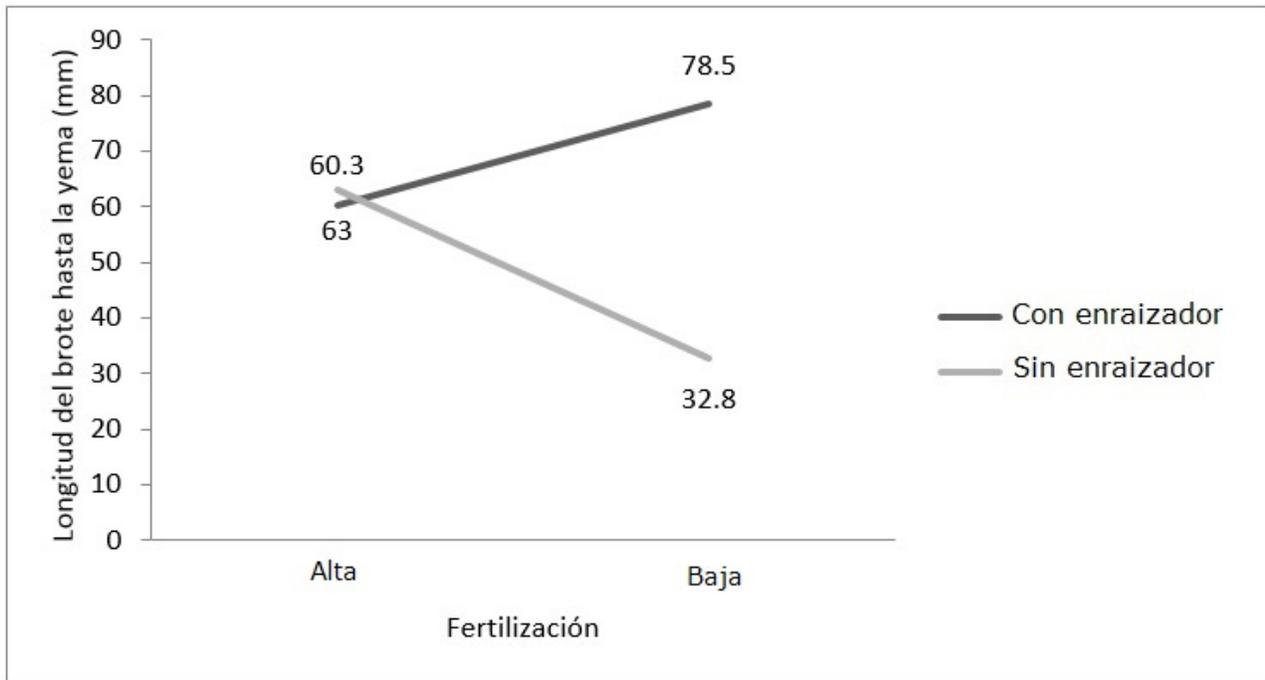
F = Fertilization; H = High; L = Low; Means with a different letter in the same column indicate significant differences according to Tukey’s test, $P \leq 0.05$.

After applying a root starter ($3\ 000\ \text{gL}^{-1}$ of IBA) during 5 s, Caetano *et al.* (2010) tried various fertilizers and a control for rooting semi-woody olive tree (*Olea europaea* L.) cuttings, and they point out that the largest number of roots was produced with a bio fertilizer containing 0.1 % K, 0.34 % Zn, 0.02 % Cu, 0.04 % Mn, 0.18 % B, 0.43 % Mo, 0.12 % S, 0.64 % Ca, and 0.27 % organic C.



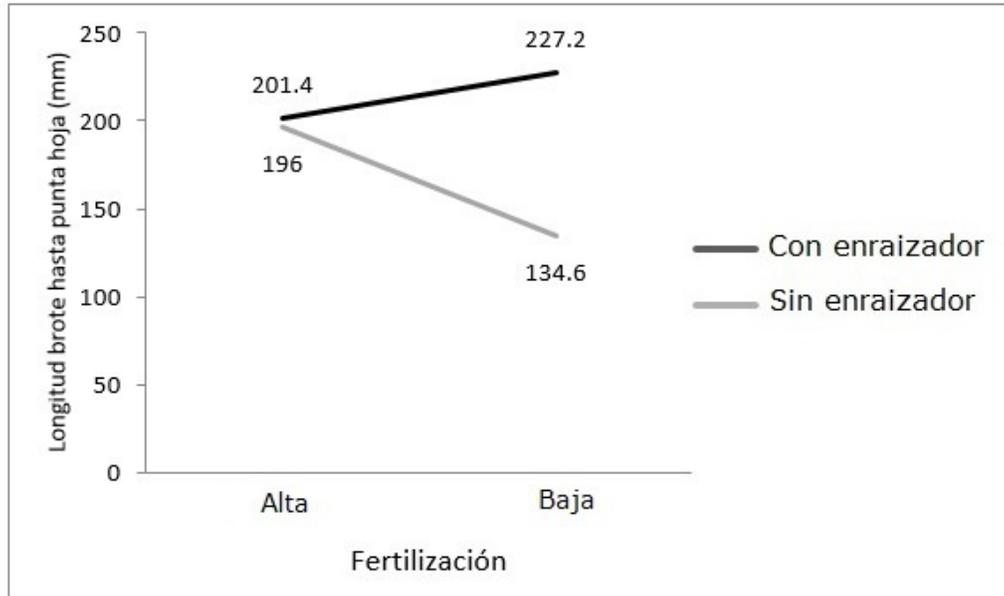
Root starter–fertilizer interaction

The interaction between the root starter and the fertilizer had effects on the length of the shoot from its base to the bud and to the leaf tip (Table 2). The highest shoot length values were registered with the application of the low dose of fertilizer, and diminished with the high dose. When “high fertilization” was applied, the value for shoot length was similar, regardless of whether or not a root starter was used. Without the latter, the shoot length increased with a higher dose of fertilizer (figures 1 and 2).



Longitud del brote hasta la yema = Length of the shoot from the base to the bud;
Fertilización = Fertilization; *Alta* = High; *Baja* = Low; *Con enraizador* = With root starter;
Sin enraizador = Without root starter.

Figure 1. Effect of the root starter * fertilizer interaction on the length of the shoot from the base to the bud.

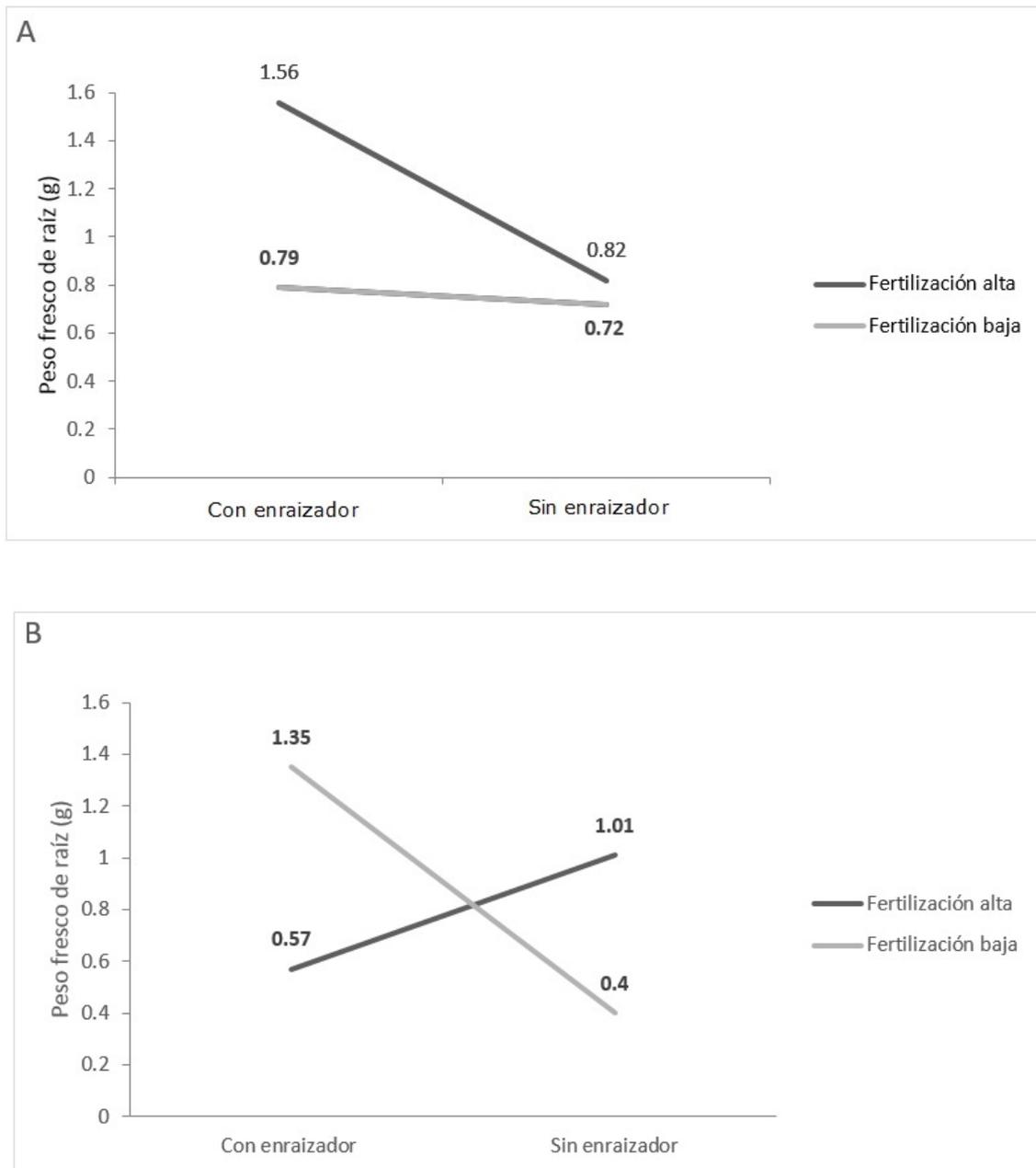


Longitud brote hasta punta hoja = Length of the shoot from the base to the leaf tip
Fertilización = Fertilization; *Alta* = High; *Baja* = Low; *Con enraizador* = With root starter; *Sin enraizador* = Without root starter.

Figure 2. Effect of the root starter * fertilizer interaction on the length of the shoot from the base to the leaf tip.

Triple interaction

Although the use of cuttings with various levels of hardness is advisable for different groups of species, those of hard wood tend to contain more carbohydrates than those of soft wood (Hartman and Kester, 1991). Hence, regardless of the level of fertilization, IBA on hard cuttings favored the accumulation of the fresh root weight. This tendency was also present in soft cuttings with "low fertilization", and was even more pronounced; however, the highest value was attained using "high fertilization" without a root starter (Figure 3).



Peso fresco de raíz = Fresh root weight; *Fertilización alta*= High fertilization; *Fertilización baja*= Low fertilization Low; *Con enraizador* = With root starter; *Sin enraizador* = Without root starter.

A) Hard cuttings; B) Soft cuttings.

Figure 3. Effect of the interaction between the cutting type, the use of a root starter, and fertilization on the fresh root weight.

The greatest response for fresh root weight in the hard wood cuttings with “high fertilization” may be partly related to K, as supplementation with this nutrient stimulates root growth, induced by the auxins. Likewise, K is very important for the emission of adventitious roots, since it influences the cellular expansion, the turgor pressure, the moisture content of the cell, and the stomatal action, and therefore modulates the effects of hydric stress in the cuttings due to their initial absence of roots. This was observed in hard wood cuttings of *Junipers virginiana* L., with a correlation between K and the percentage of rooting (Henry *et al.*, 1992).

High fresh weights occurred in soft cuttings with the application of a root starter and “low fertilization”, or without a root starter and “high fertilization”; this tendency can be associated with the concentration of nutrients in the cuttings when they are cut, which influences the redistribution of K and differs between cutting types (Henry *et al.*, 1992).

The concentration of nutrients may be expected to be lower in harder, more lignified cuttings; this caused the soft cuttings, which initially contain more K, to inhibit the accumulation of fresh weight when extra K was added and a root starter was applied. Similar interactions have been documented in other species. For example, in semi-hard *Dalbergia sissoo* Roxb. wood cuttings, fertilization (with NPK) favored rooting and shooting; furthermore, the indolebutyric acid–fertilizer interaction proved to be significant (Bakshi, 2008).

Other plant quality indicators

Dry shoot weight / dry root weight ratio

With this indicator, a ratio equal to one means that the aerial biomass is equal to the underground mass. However, if the ratio is below one, then the underground biomass exceeds the aerial mass; the opposite is true when the value is above one. In the present study, all the values were above one; therefore, the root exhibited little development in relation to the aerial part, which impacts the plant’s resistance to hydric stress in the field (Rodríguez, 2008; Prieto *et al.*, 2009), although it was not statistically significant (Table 6). Similar values to those obtained when assessing the

leaves and roots of *Acer negundo* (Table 7) have been cited for *Tabebuia rosea* (Bertol.) DC. and *Swietenia humilis* Zucc., both of which were rated as low-quality plants (Sáenz *et al.*, 2014). This indicator was developed for seed-produced individuals; therefore, it is advisable to carry out more tests on cuttings of other broadleaves and in plantations in an urban or rural environment.

Table 6. P values of the statistical analysis of the plant quality indices, for two indicators of quality.

	Shoot dry weight / root dry weight	Dickson's index
	P values	
Model	0.5883	< 0.0001
Cutting	0.1617	< 0.0001
Root starter	0.4252	0.3800
Fertilizer	0.3597	0.3423
Cutting*Root starter	0.6220	0.1540
Cutting*Fertilizer	0.7789	0.5409
Root starter*Fertilizer	0.4881	0.8019
Cutting*Root starter*Fertilizer	0.2708	0.6501



Table 7. Tukey's test for two indicators of plant quality.

Treatments	Dry shoot weight / Dry root weight	Dickson's index
Cutting type		
Hard	9.08 a	3.12a
Soft	4.31 a	0.66b
Root starter		
With	8.04 a	2.03 a
Without	5.35 a	1.75 a
Fertilization		
High	8.24 a	2.04 a
Low	5.15 a	1.74 a

Means with a different letter in the same column indicate significant differences according to Tukey's test, $P \leq 0.05$.

Dickson's index

Dickson's index is considered as one of the best parameters to indicate the quality of the plant, as it expresses balance between the distribution of the mass and the robustness of the plant, preventing the selection of out of proportion plants and the rejection of shorter but more vigorous specimens (García, 2007). In Mexico, the plant quality is generally categorized according to Dickson's index. Plants with values of 0.20 or under are rated as low-quality; medium quality plants have values of 0.45 to 0.20, and high-quality plants are those with a value of 0.5, or above if they are conifers (Sáenz *et al.*, 2014); the higher the value, the higher the quality (Basave *et al.*, 2017).

The variance analysis evidenced statistical significance ($P < 0.05$), influenced by the cutting factor (Table 6). Tukey's test indicated the best results for this index in hard cuttings (Table 7), which may be directly related to a higher root production (Guzmán *et al.*, 2012).

Conclusions

Acer negundo improved its rooting with indolebutyric acid. Because hard cuttings have more reserve carbohydrates, they produced more vigorous shoots, with larger diameters, and greater lengths from the base to the bud and to the leaf tip. The two latter variables are also favored by indolebutyric acid, which induces the generation of photosynthetic surface; therefore, this substance has a positive impact on the dry and fresh weight of the leaves. "High fertilization" is not affected by the root starter; however, with "low fertilization", this substance promotes shoot length, whereas the low dose of fertilizer reduces it. Finally, the triple interaction between cutting type, fertilization and root starter maximizes the fresh root weight. The indolebutyric acid used as the root starter and, especially, the additional potassium of "high fertilization" in the hard cuttings, facilitate the accumulation of water in the tissues.

Acknowledgements

To *Conacyt* for the grant given to the first autor to study Forest Sciences Master at the *Universidad Autónoma Chapingo* (UACH). To the UACH, for the support provided to accomplish the actual work. To Mr. Gerardo Mendoza Ángeles for his help in the collection of twigs and laboratory work.

Conflict of interests

The authors declare no conflict of interests.



Contribution by author

Alina González Pulido: proposal of the topic, field, nursery and laboratory work, analysis and writing of the manuscript; Dante Arturo Rodríguez Trejo: planning, field work, supervision of nursery, laboratory work, statistical analysis and correction of the manuscript; Alejandro Corona-Ambriz: statistical analysis and correction of the manuscript; José Amando Gil Vera-Castillo: general advise and correction of the manuscript.

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