Revista Mexicana de Biodiversidad 83: 809-816, 2012
DOI:10.7550/rmb.21610

# Vegetative propagation of native species potentially useful in the restoration of Mexico City's vegetation

## Propagación vegetativa de especies nativas potencialmente útiles en la restauración de la vegetación de la ciudad de México

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**Abstract.** Hardwood and softwood cuttings of *Buddleja cordata* HBK, *Dodonaea viscosa* Jacq and *Senecio praecox* D.C. were tested to know their ability to form adventitious roots. Cuttings were prepared in 2 different seasons (wet and dry) and treated with different microclimatic conditions and auxin concentrations (IBA and NAA). Hardwood and softwood cuttings of *B. cordata* rooted during the dry and wet season, whilst hardwood and softwood cuttings of *D. viscosa* rooted only in the wet season, cuttings of *S. praecox* rooted only in wet season with a higher rooting for hardwood than softwood cuttings. Low hormone concentrations (10 - 100 ppm) favored the rooting percentage more than high concentrations (1000 - 10 000 ppm). However, high hormone concentrations favored number, length of roots and number of developed shoots. Natural regeneration of these species is limited and the species are difficult to propagate from seeds. Vegetative propagation of these species could be an alternative to get clonal planting stock for reforestation programs in some Mexico City areas.

Key words: auxins, *Buddleja cordata*, *Dodonaea viscosa*, *Senecio praecox*, rooting cuttings.

**Resumen.** Se probó la habilidad de estacas lignificadas y suaves de *Buddleja cordata* HBK, *Dodonaea viscosa* Jacq y *Senecio praecox* D.C. para formar raíces adventicias. Las estacas se prepararon en las estaciones húmeda y seca; se trataron con diferentes concentraciones de auxinas (IBA y NAA) y en diferentes condiciones microclimáticas. Las estacas de madera lignificadas y madera suave de *B. cordata* enraizaron durante la estación seca y húmeda, mientras que las de *D. viscosa* lo hicieron sólo en la época húmeda, las estacas de *S. praecox* sólo en la estación seca teniendo las estacas lignificadas un mayor enraizamiento que las de madera suave. Las concentraciones bajas de hormonas (10 - 100 ppm) favorecieron más el porcentaje de enraizamiento que las altas concentraciones (1000 - 10 000 ppm). ). Sin embargo, las concentraciones altas de hormonas favorecieron el número y la longitud de las raíces, además del número de yemas. La regeneración natural de estas especies es muy limitada y son difíciles de propagar por semillas. La propagación vegetativa de estas especies puede ser una alternativa para obtener una fuente de material clonal adecuado para los programas de reforestación en algunas áreas de la ciudad de México.

Palabras clave: auxinas, *Buddleja cordata*, *Dodonaea viscosa*, *Senecio praecox*, enraizamiento de estacas.

## Introduction

Exotic species have long been used for reforestation, but in many cases such species have produced undesirable ecological effects, because they can become invasive plants (D´Antonio and Meyerson, 2002). This situation and the alarmingly current deforestation rates have caused a shift towards the use of native species for reforestation and restoration purposes (Vázquez-Yanes and Batis, 1996). However, since there is a lack of studies about most native species, it is necessary to increase our knowledge about plant propagation from seeds or cuttings to introduce them to the ecosystem instead of exotic plants. Because the first life stages are crucial for the establishment showing a high seedling mortality, it is very important to improve the use of native species vegetatively propagated, which have the advantage of avoiding these critical phases (Weber and Stoney, 1986; Arriaga et al., 1994; Leakey et al., 1994).

The Pedregal de San Angel Reserve (PSAR) was an extensive area to the south of Mexico City comprising 4000 ha, and considered as a place of great floristic richness (Lot and Cano-Santana, 2009). Due to urban development its surface has been drastically reduced in spite of its importance as a recharge area for the basin aquifers (Ezcurra et al., 1999). Before 1989 this area endured severe

Recibido: 10 diciembre 2012; aceptado: 09 abril 2012

disturbances caused by non-regulated urban settlements (Soberón et al., 1991). Although some of the originally reported species are no longer found, new species to the reserve have been reported, due to introduction of propagules in soil from other sites. Local extinction of taxa is associated with urbanization and illegal extraction of ornamental species. In this area the harsh conditions on the basaltic rock make seed germination and seedling and plant survival extremely difficult (Vázquez-Yanes et al., 1999; Mendoza-Hernández, 2002).

One of the most conspicuous and abundant species in the reserve was *Senecio praecox*, an endemic species to the Pedregal, for this reason the vegetation of the Pedregal was called *Senecionetum praecoxis* by Rzedowski (1954). However, its relative abundance has been reduced due to competition with other species. This change could be due to the low seed germination of *S. praecox* (5%, Pérez and Franco, 2000) or poor recruitment (R. Pérez, personal communication). On the other hand, *Dodonaea viscosa* and *Buddleja cordata* are common pioneer tree species in the Basin of Mexico, frequently found in disturbed habitats (Rzedowski and Rzedowski, 2001) which have been proposed for restoration of the disturbed areas in the Mexico Valley (Soberón et al., 1991).

In this way, *Buddleja cordata*, *Dodonaea viscosa*, *Senecio praecox*, and other Pedregal species could be reintroduced into the PSAR and other areas, at the same time this approach could improve the microenvironment for the establishment of the same and other plant species (Arriaga et al., 1994; Barradas, 2000a). This strategy could be used in the first steps of restoration programs, followed by the substitution of the vegetatively propagated individuals by saplings to avoid the reduction of genetic variability linked to vegetative propagation. *Buddleja cordata,* an evergreen species considered as pioneer, is well adapted to eroded places, so it is potentially useful in the recovery process of degraded zones (González and Camacho, 1994). *Dodonaea viscosa*, is an evergreen species growing in forest gaps, ravines and in altered land with irregular surface. It has also been considered a good species for reforestation due to its resistance to water deficit (Degollado, 2000). *Senecio praecox*, a deciduous shrub or tree, is greatly appreciated for its landscape value due to its conspicuous flowers, which appear during the dry season. These species are all native to, and dominant in, the PSAR.

Additionally, plants in the urban environment play an important role by mitigating extreme microclimates, collecting dust and gases from the air, and contributing to the recharge of aquifers (Sanders, 1986; McPherson and Nowak, 1993; Barradas et al., 1999; Johnson et al., 1999; Barradas, 2000b). Therefore, these species, mainly *S. preaecox*, can also be re-introduced into parks and streets where the original lava substratum still remains.

In order to improve the use of native plants propagated by cuttings in restoration programs, rooting capacity from *Buddleja cordata*, *Dodonaea viscosa* and *Senecio praecox* was tested in hard and soft cuttings collected during the dry and/or the wet season in the PSAR to determine their potential for the restoration and/or rehabilitation of the southern part of Mexico City. We also determined optimum hormone types and concentrations to root the cuts of the studied species, which can be used as a basis for other studies.

## Materials and methods

*Study site.* The PSAR is situated in the southeastern part of Mexico City, 2 300 m a.s.l., between 19º20'22"-19º13'25" N, 99º08'26"- 99º14'3" W (Rzedowski, 1954; Rojo, 1994). The Reserve's substrate is basaltic, and its topography very irregular (Rzedowski, 1954). The mean accumulated rainfall is 803 mm, 93% of which occurs during the rainy season (May to October). Mean annual temperature is 15.1º C, the maximum average temperature is 29.5º C and the minimum -1.1º C in January (Sánchez, 1990; Barradas et al., 1999).

The PSAR is characterized by a xerophilous shrubland vegetation growing on the shallow soils on a deep lava substratum and it is believed that the original number of plant species was about 350, but now it is possible to find only 226 species (Valiente-Banuet and de Luna García, 1990). Although some of the originally reported species are no longer found, new species have been reported, due to introduction of propagules in soil from other sites, and the probable diaspore dispersion from oak forests.

*Species studied, and selected donor plants*. Three plant species growing in the PSAR were selected for this study: *Buddleja cordata* H.B.K. Loganiaceae, *Dodonaea viscosa* Jacq. Sapindaceae, and *Senecio praecox* D.C. Compositae.

The donor individuals from each species were selected from areas outside the Reserve but with similar vegetation and environment, along the central road dividers, sidewalks, gardens and streets of the UNAM Campus. Only those individuals that looked anatomically and morphologically healthy and vigorous were selected. Sixty individuals of each species were chosen and numbered according to their location in the area. These individuals were used to obtain the stem cuttings for all treatments. Plants were upright stem, mature trees 2-3 m high with similar morphological and environmental features for each species.

*General procedures.* Cuttings were taken from the selected donor plants, and a layer of sealing paint Curabien (Agroquímica S.A. de C. V., México, D.F.) was applied to each tip to prevent dehydration. They were then labeled and placed in moist paper bags. Foliose softwood cuttings were made from lateral branches at 2 m from the soil surface. Cuttings were 20 cm length, 3-3.5 cm diameter, with 4 to 5 nodes and 6 to 20 leaves and coppiced. Leaves of cuttings were cut when there were more than 5 leaves in order to reduce evaporation. Cuttings were hormone treated and sown immediately after cutting.

The hormones used were: pure indolebutyric acid (IBA) (Sigma Chemical CO., MO, USA), commercial IBA in talc (Radix, Diseño y Control Electroquímico, S.A., México, D.F.) and commercial naphtalenacetic acid (NAA) in talc (Rootone, Green Light Co., Texas, U.S.A.). Pure IBA was placed in 250 ml of alcohol and diluted with distilled water to get the concentrations required for each treatment, then cuttings were exposed to IBA immersing the tip in 4 ml of solution, for the control group 4 ml of distilled water was used. The cuttings were treated by IBA placing the base of the cuttings in the aqueous solution for 24 hours or in distilled water for the control; they were then placed on the substrate prepared before the cuttings had been cut. The cuttings with commercial IBA (Radix) and NAA (Rootone) in talc were placed inside damp paper bags to be placed on the substrate at the same time as the other cuttings.

The substrate consisted of a mixture of peat and agrolite in a 1:1 (v:v) proportion and sterilized at 90 ºC in an autoclave for 3 days. The substrate was then placed inside 24 x 24 cm black polyethylene bags, filling only 75%. The cuttings were individually set in the substrate at approximately 10 cm deep, watered with a very fine sprayer and placed on shelves in a nursery. They were watered near to field capacity and checked every third day. A 'shade net' was placed above them to reduce the maximum photosynthetic active radiation to 325 µmol $m^{-2}$ $s^{-1}$, at 13:00 hours during the month of May. After 5 months, the cuttings were removed from the bags and assessed for: a) The percentage of cuttings that had formed roots (at least one), b) the number of roots (NR), c) length of primary roots (LR), d) number of developed buds (NS) and e) survival of rooted cuts.

*Experiments*. Hormone treatments were combined with 4 experiments as shown in Table 1. The objective of the first 2 was to test the rooting capability of cuttings from hardwood stems collected during the wet and dry seasons and placed in a nursery. The third and fourth experiments were done only in the wet season, one with hardwood cuttings with leaves placed in a non-mist-poly-propagator similar to that recommended by Longman and Wilson (1993) and Vázquez-Yanes et al. (1997); and the other with softwood cuttings with new leaves placed in a mist-propagation nursery with a 60 min burst frequency during the day (Table 1. For each kind of cut, the propagation place was established according to Longman and Wilson (1993) and Hartmann et al. (1997).

Experimental design was for the first and second experiments: 4 pure IBA concentrations x 1 commercial IBA concentration x 2 temperatures x 4 replicates x 30 cuttings, each. The third experiment design was 4 pure IBA concentrations x 2 commercial IBA concentrations x 2 temperatures x 4 replicates x 30 cuttings; and 4 pure IBA concentrations x 2 commercial IBA concentrations x 1 NAA concentration x 2 temperatures x 4 replicates x 10 cuttings for the fourth experiment. Control for each experiment was 2 temperatures x 4 replicates x 30 cuttings. Information on each experiment and species was analyzed by Kruskal-Wallis tests and visually compared with box-and-whiskers plots (Tukey, 1977; Statgraphics, ver. 5.0, Statistical Graphics Co., Rockville, MD, USA). The probability of rooting was calculated by logistic regression analysis using JMP ver. 4 (SAS Institute, Inc., Cary, NC, USA).

## Results

Probability of rooting, overall, was below 30% (except in *S. praecox*, in several treatments) (Figs. 1a, 2a, 3a, 4a). The use of different hormonal concentrations of IBA or the use of commercial products (Radix and NAA) induced significant differences in cuts rooting. The effect of each one of them depended on the species and the experiment. *B. cordata* rooted in 3 of the 4 experiments, but always had low rooting (5-15%); best results were obtained using radix 1500 and IBA 100 and 10 ppm in hardwood cuts collected in the wet season and sown with or without leaves (Figs 1a, 3a). *D. viscosa* only rooted in the fourth experiment, the best probability of rooting was obtained using NAA 20 ppm (25%, Fig. 4b). All cuts of all the species that rooted survived. Cuts of *S. praecox* rooted also in 3 of the 4 treatments, being the species with the highest probabilities. The best results were reached with hard cuts, independent of the collection season or the environment of propagation; in the second and third experiments rooting was higher than 50% and it was induced by IBA 100 and 10 ppm, respectively (Figs. 2a, 3a). In this species softwood cuts reached the highest rooting with IBA 100 ppm (30%, Fig. 4c).

In *B. cordata*, as in the other species, the best development of roots did not coincide with the treatments that had the highest rooting probabilities. The high length and number of roots were reached in experiments 1 and 4. In the first one with IBA 10 ppm and in the fourth one IBA 100 ppm produced a higher number of longer roots (Figs. 1b, c; 4d, g). In *D. viscosa* the longest and the highest

**Table 1.** Summary of treatments and environmental conditions when the experiments were made

| Experiment | | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Season | | Dry | Wet | Wet | Wet |
| Month of collection | | April | August | September | October |
| Control | 0 | 720 hardwood cuts from 60 donors, 120 cuttings per treatment (4 replications of 30 cuttings each). Nursery | | 700 foliose hardwood cuttings; 50 donors, 100 cuts per treatment (4 replications of 30 cuttings each) Propagator | 320 foliose softwood cuttings, from 10 donors; 40 cuts per treatment (4 replications of 10 cuttings each). Nursery |
| Pure IBA (ppm) | 10 | | | | |
| | 100 | | | | |
| | 1 000 | | | | |
| | 10 000 | | | | |
| Commercial IBA (Radix ppm) | 1 500 | | | | |
| | 5 000 | ------- | ----- | | |
| Commercial NAA (Rootone, ppm) | 20 | ------- | ----- | ---------- | |
| Temperature (°C) | Nursery | 24.5 | 21.1 | ---------- | 20 |
| | Propagator | ---------- | ---------- | 22.3 | ---------- |
| | Rooting media | 21.3 | 19 | 20.5 | 18 |
| Watering | | Every day | Every day | Every day | Every third day |

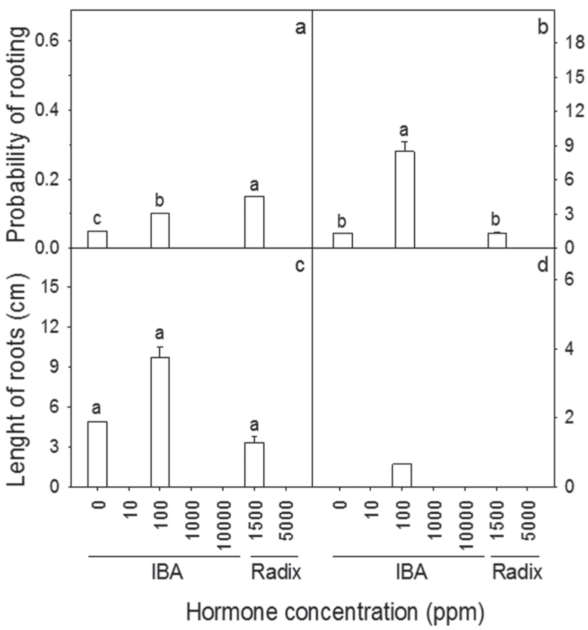


**Figure 1.** Probability of rooting (a), number of produced roots (b), length of roots (c) and number of buds (d) of hardwood cuttings of *Buddleja cordata* collected in the wet season and placed in the nursery in different hormone concentrations during experiment 1. Statistical analysis for each variable was performed among hormonal treatments. A column represents the mean value and bars the standard error.

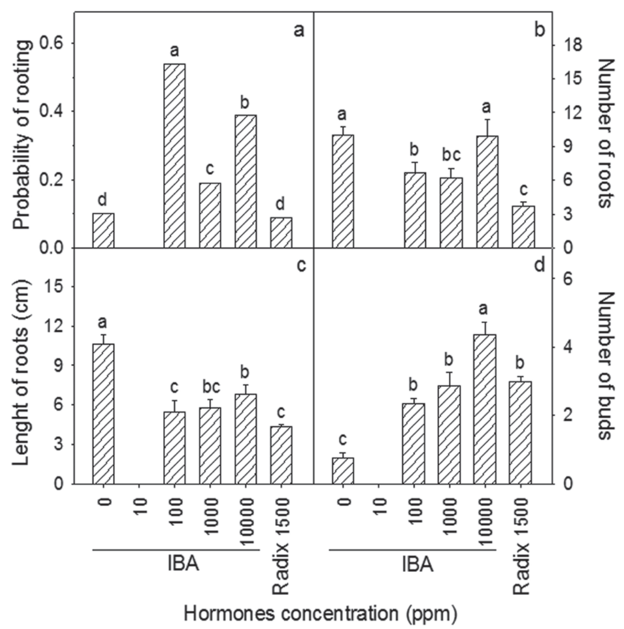


**Figure 2.** Probability of rooting (a), number of produced roots (b), length of roots (c) and number of buds (d) of hardwood cuttings of *Senecio praecox* collected in the dry season and placed in the nursery in different hormone concentrations during experiment 2. Statistical analysis for each variable was performed among hormonal treatments. A column represents the mean value and bars the standard error.

number of roots were reached with the NAA treatment (Fig. 4e, h). With *S. praecox* a good root development was reached in the 3 experiments where the species rooted; IBA, Radix and NAA produced good results in cuts of soft and hardwood (Figs. 2b, c, 3b, c, 4f, i). For the number of roots, the best result was obtained in the fourth experiment with IBA 1000 ppm, but this result did not differ significantly from others (Figs. 4f, i).

In the fourth experiment *Buddleja cordata* produced mainly buds which showed a very low rooting. The best

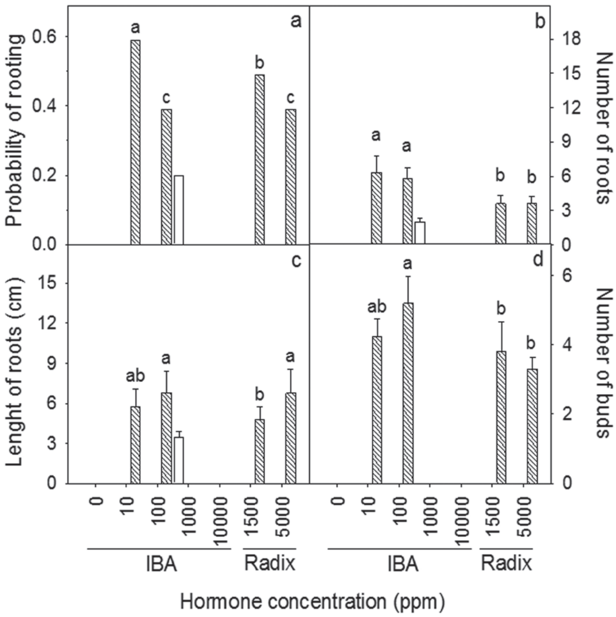


**Figure 3.** Probability of rooting (a), number of produced roots (b), length of roots (c) and number of buds (d) of hardwood foliose cuttings of *Senecio praecox* (empty columns) and *Buddleja cordata* (filled columns) collected in the wet season and placed in a non-mist-propagator in different hormone concentrations during experiment 3. Statistical analysis for each variable was performed among hormonal treatments. A column represents the mean value and bars the standard error.

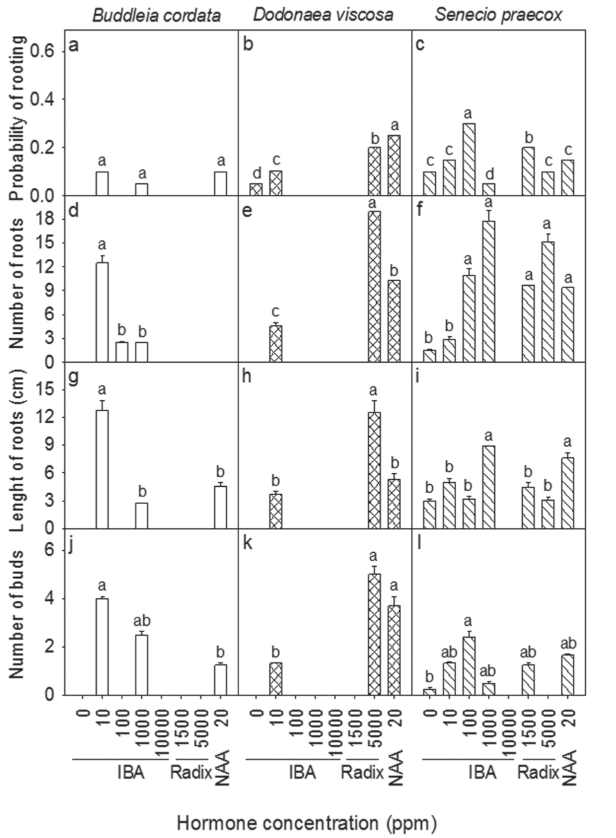


**Figure 4.** Probability of rooting (a, b, c), number of produced roots (d, e, f), length of roots (g, h, i) and number of buds (j, k, l) of softwood cuttings with new leaves of *Senecio praecox* (a, d, g, h), *Dodonaea viscosa* (b, e, h, k) and *Buddleja cordata* (c, f, I, l) collected in the wet season and placed in a mist nursery in different hormone concentrations during experiment 4. Statistical analysis for each variable was performed inside each species and among hormonal treatments. A column represents the mean value and bars the standard error.

hormonal treatment was IBA 10 and 1 000 ppm (Fig. 4j). In the first experiment produced with IBA 100 ppm a low number of buds in relation to the buds produced in several treatments in the fourth experiment (Fig. 1d). *D. viscosa* developed more buds in the same treatments where the development of roots was best, that is with IBA 20 ppm and NAA. *S. praecox* produced also buds in the 3 experiments where rooting occurred; bud development was best in the second and third experiment at 10 000 and 100 ppm of IBA, respectively.

## Discussion

The rooting process in the 3 species showed considerable variation in the different experiments, and the rooting percentage of the species depended on species, season or month of collection and hormone level. *Buddleja cordata*, the species with the hardest wood, had low rooting probabilities (≤ 0.2) in 3 of the 4 experiments (cuts of hard and softwood). Nevertheless, variables that are related to the health of the rooted cut as number and length of these and production of buds (de Andres et al., 1999), were best when the cuts were of softwood produced during the wet season (summer), this could be expected for woody species (Stankova and Panetsos, 1997; Agbo and Obi, 2007). In spite of the reduced rooting probability (0.1), the health of cuts and the low percentages of IBA required for rooting, make vegetative propagation an economic method that may favor the success of planting in the field. Because number of roots and root length indicate the cutting ability to assimilate nutrients, survive in the soil, have structural support, and develop buds to ensure the future $CO_2$ assimilation of the plant, these also indicate the acclimatization for future planting, which may increase survival in reforestation efforts (de Andres et al., 1999; 2004).

*Dodonaea viscosa* also had low rooting probabilities (≤ 0.25), but in contrast with *B. cordata*, only the soft summer leafy cuttings rooted; the health of the cuts was good in most of the hormonal treatments, the best results were induced by NAA and Radix (5 000 ppm). Soft leafy cuttings as well as the collection season, adequate for *B. cordata* and *D. viscosa* propagation are also key to the propagation of other tree species such as *Robinia pseudoacacia*, *Grewia optiva* (Swamy et al., 2002) and *Olea europea* (Negash, 2003). It is possible that the low rooting capacity of *B. cordata* and *D. viscosa* may be due to the hardness of their lignified tissues. It has been reported for hardwood species that the use of cuttings from juvenile plants is more appropriate than the use of the low lignified soft cuts from mature plants (Swamy et al., 2002; Pignatti and Crobeddu, 2005; Agbo and Obi, 2007). The soft cuts were collected in the wet season, but in the future it will be necessary to take into account that the season of the year is relevant to the physiological condition of plants, therefore it could be necessary to harvest the soft cuts when lignification has started as had been suggested by Stankova and Panetsos (1997). This has been related to the hormonal and nutritional stage of plants, which ought to be adequate to produce new structures (Hartmann et al., 1997).

In contrast with the other 2 species, the non woody *S. praecox* reached higher rooting probabilities than the woody species and rooting and health of the cuttings was also good in hard cuttings (mature tissues), collected during the dry and the wet seasons. The percentages of rooting of hardwood cuttings reached more than the 50% considered as economically convenient for mass production in nurseries (Pignnatti and Crobeddu, 2005). Soft cuttings also had high rooting probabilities, but lower than hard cuttings (experiment 3). This occurs in some woody species, where a slight lignification is required for rooting (Stankova and Panetsos, 1997) as occurs in the mature tissues of *S. praecox*, which contain more initial carbohydrate reserves and a hormonal condition adequate to cope with the dry season, during which plants are deciduous but flowering occurs. Further, the stems of *S. praecox* are succulent, as they retain moisture, which probably influenced the rooting capability and the good development of the other evaluated traits. This is common among succulent plants (Baldini, 1992).

Low IBA concentrations (10 and 100 ppm) or Radix induce high rooting and good cutting health, which is important from the commercial point of view. Radix and low hormone concentrations favor rooting in other species such as *Colutea istrea*, *Dalbergia sissoo*, *Pachecoa venezuelensis* and *Tilia mexicana* (Díaz et al., 1995; Sunil and Verma, 1996; Santiago and Vargas, 1999; de Andrés, 2004). *Senecio praecox* reached the highest rooting probabilities in the nursery and the poly-propagator without a moisture supply, which reflect its adaptation to dry environments. Differing from the other studied species, it is deciduous, has superficial roots and stores water in the stems, while the others only avoid low water availability by having deeper roots (Ramos-Vázquez and Barradas, 1998; Corona, 1999; Degollado, 2000; Barradas et al., 2004). The influence of plant susceptibility to water stress in rooting capability has been reported for *Colutea arborescens* (de Andrés, 1999).

For *S. praecox* and *B. cordata* rooting occurred in the non mist nursery or in the propagator, although it is known that mist-propagators favor rooting (Hartmann et al., 1997). This is considered a low tech system suitable for rural areas (McDick et al., 1996). Differing from the latter, *D. viscosa* required a mist propagator and very low rooting percentages were reached. Cuts from juvenile plants at higher substrate temperatures (20 - 33 ºC are recommended) than used (20 - 24 °C) could improve rooting in *D. viscosa* and in *B. cordata* (Iglesias et al., 1996; Leakey, 2004). Vegetative propagation of trees from the 3 studied species can improve restoration programs in the southern portion of Mexico City and other areas. Vegetative propagation is recommended to avoid the high mortality of seedlings and saplings and increase the success of restoration programs (Zahawi, 2005; 2008). Negative aspects of the use of vegetatively propagated plants can be overcome by using a large number of plant donors to increase genetic variability and by using these plants only for the first restoration steps before using those propagated from seeds.

Rooting capacity varied according to species and collection season. The highest rooting capability in *B. cordata* took place in hard tissue cuttings collected during the dry season, which might be due to a favorable eco-physiological state. In *D. viscosa*, only the softwood cuttings with leaves achieved any rooting, so the type of cutting and the collection season are key elements in this species propagation. In *S. praecox*, the type of cutting and the turgidity of its tissues greatly promoted higher rooting capability of the cuttings collected during the wet season.

In spite of the low rooting response of 2 of the studied species, rooting can be effective ways of reforesting altered sites of the area known as PSAR as well as other areas still have the original volcanic substrate and/or other sites where these species are dominant. However, follow-up research is needed to refine techniques to increase rooting percentage in all the studied species.

**Acknowledgements**

This study was supported by a grant from the Consejo Nacional de Ciencia y Tecnología, Mexico, CONACYT (GOO11-N).

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