

## PROPAGATION OF THREE *BURSERA* SPECIES FROM CUTTINGS

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**Abstract:** Vegetative propagation of three species of *Bursera* (*B. glabrifolia*, *B. copallifera* and *B. linanoe*) was studied. Cuttings were collected during the dry season, when trees were leafless and dormant. In a first trial, the effects of species, indol-butyric acid concentration (control, 4,000, and 9,000 ppm), and relative age of stockplants (young vs. mature trees) on rooting success were evaluated. Percentage of rooted cuttings and number of roots per cutting differed significantly among species, indol-butyric acid, and age of the stockplants. The three species studied showed increased rooting when indol-butyric acid was applied compared to the control, but sensitivity to applied indol-butyric acid varied relative to the type of cutting. Cuttings from young stockplants attained higher rooting percentages but were less responsive to indol-butyric acid application. In a second trial performed later in the dry season, one species (*B. linanoe*) showed a significant increase in rooting success. Overall, rooting percentages higher than 70% were attained when the sources of cuttings were young stockplants and when indol-butyric acid was applied. These results show that propagation of these species from cuttings is feasible. Rooted cuttings may be used for the restoration of degraded secondary tropical dry forests, where they are usually absent, for commercial plantations, or in agroforestry.

**Keywords:** Burseraceae; indolbutyric acid; restoration; rooting; tropical dry forest.

**Resumen:** Se estudió la propagación vegetativa de tres especies de *Bursera* (*B. glabrifolia*, *B. copallifera* y *B. linanoe*). Las estacas se recolectaron durante la temporada seca, cuando los árboles se encontraban en estado de reposo y sin hojas. En un primer ensayo se evaluó el efecto de la especie, la concentración de ácido indol-butírico (control, 4,000 y 9,000 ppm) y la edad relativa de las plantas de origen (árboles jóvenes vs. maduros) en el enraizamiento. El porcentaje de estacas con raíces y el número de raíces por estaca difirió significativamente entre especies y en respuesta a la concentración de ácido indol-butírico y la edad de las plantas de origen. El porcentaje de enraizamiento aumentó en todas las especies cuando se aplicó ácido indol-butírico, pero la sensibilidad al ácido indol-butírico varió con el tipo de estaca. Las que provinieron de plantas juveniles alcanzaron mayores porcentajes de enraizamiento y respondieron menos a la aplicación del ácido indol-butírico. En un segundo ensayo, realizado más tarde durante la temporada seca, se presentó un aumento considerable en el éxito de enraizamiento de una especie (*B. linanoe*). En conjunto, cuando se usaron estacas de árboles jóvenes y se aplicó ácido indol-butírico, se alcanzaron porcentajes de enraizamiento superiores a 70%. Estos resultados muestran que la propagación de estas especies a partir de estacas es posible. Las estacas enraizadas pueden usarse para la restauración de bosques tropicales secos degradados, en donde normalmente están ausentes, para establecer plantaciones comerciales o en agroforestería.

**Keywords:** ácido indolbutírico; bosque tropical seco; Burseraceae; enraizamiento; restauración.

There are around 100 tree species in the genus *Bursera* (Burseraceae), which has a broad distribution in America. Its highest diversity is found in Mexico, especially along the Pacific coast and the Balsas river basin. *Bursera* species are dioecious, deciduous trees and shrubs, often dominant or subdominant in many tropical dry forests (McVaugh and Rzedowski, 1965; Rzedowski *et al.*, 2004). The fruit is a drupe with an orange (yellow to red) pericarp, and is consu-

med by a wide range of animals, including birds, which act as dispersal agents (Scott and Martin, 1984).

Knowledge on propagation techniques for native species of trees of the tropical dry forest is scarce, and it is becoming increasingly necessary to broaden the spectrum of species that can easily be propagated (Bonfil and Trejo, 2010), considering that extensive areas of these forests have been severely disturbed by frequent forest fires, cattle raising and

wood extraction (Murphy and Lugo, 1986; Janzen, 1988; Trejo and Dirzo, 2000; Miles *et al.*, 2006; Sánchez-Azofeifa and Portillo-Quintero, 2011). *Bursera* trees usually become important in late successional stages in Mexican tropical dry forests (Rzedowski and Kruse, 1979; Romero-Duque *et al.*, 2007; Lebrija-Trejos *et al.*, 2008; Williams-Linera *et al.*, 2011), and therefore their propagation and reintroduction in disturbed sites could be an effective strategy to accelerate succession and restore them. Since *Bursera* trees are frequently used as living fences, their production in local nurseries could enhance the conservation value of agricultural landscapes. A few species have been overexploited because of their economic importance, resulting in significant population decline, and their propagation could promote the establishment of agroforestry plantations. This is the case for the three species whose vegetative propagation we studied: *B. linanoe* (La Llave) Rzedowski, Calderón & Medina, *B. glabrifolia* (H.B.K.) Engel., and *B. copallifera* (Sessé & Moc., ex D. C.) Bullock.

The wood and seeds of *B. linanoe* (commonly known as linaloe) are used in traditional woodcrafting, essential oil extraction and traditional medicine. As a consequence of over-harvesting, trees are either absent or scarce in the vicinity of the villages where traditional crafts are manufactured (Hersch-Martínez and Glass, 2006; Fuentes-López, 2009). This reduced availability is also found in the areas where *alebrijes* (a popular handcraft from Oaxaca) are made from the soft wood of *B. glabrifolia* (Purata *et al.*, 2004; Hernández-Apolinar *et al.*, 2006). Resin of *B. copallifera* is collected from natural populations and sold in many regional markets as copal, a kind of incense used in traditional and religious ceremonies (Linares and Bye, 2008). All these traditional uses have decimated natural populations, and thus it is becoming increasingly important to establish enrichment and agroforestry plantings to fulfill regional demand and restore degraded forests.

Several studies have shown poor seed germination in a number of *Bursera* species, and therefore plant production from seed is unreliable (Johnson, 1992; Maradiga *et al.*, 2000; Andrés and Espinosa, 2002; Bonfil-Sanders *et al.*, 2008). Vegetative propagation by cuttings is a low-tech option which could allow the production of large numbers of plants in a short period of time (Landis *et al.*, 1999). Also, vegetatively propagated plants could keep the favorable features of the parent trees and they could be selected for their domestication as agroforestry crops (Simons and Leakey, 2004). In addition, the potential use of stakes and cuttings has been put forward as a promising tool for the restoration of tropical dry forests (Ray and Brown, 1995; Zahawi, 2005). Some *Bursera* species are propagated by local farmers using large stakes (~1.5 m long), not only for *B. simaruba* in Mexico and the Caribbean, but also for *B. grandifolia*, *B. lancifolia*, *B. glabrifolia*, and *B. linanoe* in many tropical dry forests areas of Mexico (Hersch-Martínez *et al.*, 2004; Zahawi and

Holl, 2009). However, the use of large branches limits not only the number of new plants that can be obtained, but is also relatively expensive and may have negative impacts on small populations of *Bursera*.

Preliminary studies on the propagation of several species of *Bursera* from cuttings showed differences among species in rooting ability, as well as response to indol-butyric acid (IBA), and suggested that a refinement of the technique could improve the success of plant propagation by cuttings (Bonfil-Sanders *et al.*, 2007). Thus, the current investigation was aimed at evaluating the effect of: (a) species, (b) IBA concentration, and (c) age of the stockplants, on rooting success of cuttings of *Bursera glabrifolia*, *B. copallifera*, and *B. linanoe* collected during the dry season. This first trial identified appropriate experimental conditions, which were then used in a second trial to confirm its efficiency and to propagate larger numbers of plants to test their field performance (not reported in this paper, but see Castellanos-Castro and Bonfil, 2010).

## Materials and methods

The research was carried out between March and August 2007. Cuttings were collected from leafless individuals in different localities of the state of Morelos (central Mexico), during March and May. Period of collection was chosen following traditional knowledge on vegetative propagation of these species from large tree branches, which are used for living fences. Shoot cuttings of *Bursera copallifera* and *B. glabrifolia* were collected from vigorous individuals at the archaeological site of Xochicalco (18° 48' N, 99° 17' W, altitude 1,200-1,350 m), while cuttings from *B. linanoe* were collected at Chimalacatlán, Sierra de Huautla (18° 27' N, 99° 5' W, altitude 1,000 m). Both sites are part of national protected areas having secondary tropical dry forests in which *Bursera* trees are dominant. Forests are not managed in Xochicalco, while in the area of Chimalacatlán seeds of *B. linanoe* are collected for oil extraction. The climate in the region is warm subhumid, with a rainy season in summer (June to October), and a dry season that usually extends from December to May (García, 1988); annual mean temperature is 22.9 °C in Xochicalco and 29.2 °C near Chimalacatlán, while average annual precipitation is 1,055 and 900 mm, respectively.

The first trial was performed on cuttings collected in March, and evaluated the effect of species, IBA concentration and relative age (*i.e.*, maturity) of the stock trees on the rooting ability of cuttings. Individual source trees were growing in natural forests in the vicinity of agricultural or cattle grazing areas. They were categorized as mature reproductive trees ( $\geq 5$  m tall and a well-defined single trunk) or young non-reproductive saplings ( $\leq 2$  m tall with one or more basal branches). For each plant species, 75 cuttings were collected from five adult trees (15 per tree), and 27-

45 cuttings were collected from three to five young trees or saplings (~9 per sapling). There were fewer cuttings from young stockplants of *Bursera glabrifolia* and *B. copallifera* than from mature trees due to their lower availability in the field. From this sample 25 mature and 8-15 young cuttings were randomly assigned to one of the following solutions: control (water), 4,000 ppm IBA, and 9,000 ppm IBA. The number of cuttings per stock individual was the same in all treatments. The 4,000 ppm solution was available in the market (name AIB 0.4% solution), while the 9,000 ppm solution was obtained by dissolving 35% IBA tablets (name Radix 35% TB) in water, as indicated by the manufacturer (Intercontinental Import Export, S. A. for both products). In a second trial, the protocol achieving the best results in March (IBA concentration + age of stockplants) was applied to 100 cuttings per species collected in the dry season, in May 2007, to confirm its efficiency and to propagate a larger number of plants to test their field performance (not reported in this paper). For this trial five stock individuals were used for *B. linanoe* and six for *B. copallifera* and *B. glabrifolia*. The source individuals used in each collection date were not the same, but in all cases the same number of cuttings per stock tree was assigned to each treatment, to reduce the possibility of an individual stockplant effect.

All cuttings were taken from lateral twigs (hardwood corresponding to the last two growing seasons) growing in full sun. As they were collected in the middle of the dry season, all plants were leafless and relative humidity was low. Twigs were collected in the morning to avoid high irradiance and heat; the basal and proximal ends of each twig were distinguished by different angles of cut to keep their polarity; they were tied in groups according to their stockplant. On the same day of collection the twigs were taken to the nursery (at Jardín Etnobotánico INAH, Cuernavaca, Morelos) in closed coolers, in order to keep them in shaded, fresh conditions. At the nursery the cuttings were pruned to their final size and measured (mean length  $\pm$  s.d. = 25.1  $\pm$  3.6 cm and mean basal diameter  $\pm$  s.d. = 12.4  $\pm$  3.2 mm; 2-3 nodes per cutting); no more than two cuttings were obtained from each twig; one from the apex and the next just below it. Two longitudinal, superficial cuts of ~2 cm long were made at the basal end of each cutting (just deep enough to take off the bark, approximately 2-3 mm), and they were immediately immersed in the IBA solution for 1 min, after which they were sown (~5 cm depth) in black polyethylene bags filled with a mixture of black soil and agrolite (1:1 V/V). The sieved organic soil (obtained from fertile oak forest areas in the region) was purchased from local markets, while the agrolite (purchased from nursery suppliers) is a substrate of mineral origin that increases porosity and drainage, favoring root development and avoiding waterlogging from irrigation.

The nursery walls and ceiling were covered with white plastic film and water was applied by an automatic intermittent mist system activated twice a day for ~5 min; addi-

tionally cuttings were watered manually when necessary. Temperature and humidity were not controlled and daily fluctuations were registered with a datalogger during the rooting period (Hobo H8 Pro Temp/Rh). Average temperature and humidity ( $\pm$  s.d.) from 00:00 to 8:00 h were 18.5 °C ( $\pm$  0.3) and 93.9% ( $\pm$  3.7), from 8:00 to 16:00 they were 30.6 °C ( $\pm$  0.9) and 63.6% ( $\pm$  6.6), and from 16:00 to 24:00 h they were 23.3 °C ( $\pm$  0.9) and 78.2% ( $\pm$  9.5). Maximum and minimum values during the rooting period were 43.9 °C and 14.5 °C for temperature, and 100% and 26% for humidity. Photosynthetically active radiation (PAR) on a clear summer day (between 12:00 – 14:00 h) inside the nursery was 303.25  $\mu\text{mol seg}^{-1} \text{m}^{-2}$ , 23% of the full radiation on a clear day.

In the first trial, bud and leaf development were registered during the rooting period (three months, from March to June) as an indirect measure of plant health and possible root development. Rooting response evaluations were undertaken 11 and 15 weeks after setting the cuttings into the rooting medium, in order to allow for root growth and avoid early uprooting, which can cause wilting (personal observation). The variables registered were presence of callus and roots, number of roots and number of leaves and leaf buds per cutting. Root data were obtained by splashing water on the rooting medium and gently removing remains of the medium from the roots. Once the information was registered, the cuttings were returned to the bag. For the collection conducted in May, the number of roots and the diameter and length of the largest root per cutting were recorded in July and September 2007.

**Data analysis.** A generalized linear model was used to analyze the effects of species, age of stockplant, and IBA concentration on rooting of cuttings collected in March. As the response variable was the number of rooted and non-rooted cuttings, a binomial error and a logit link function were used. The effects of the same variables on the number of roots per cutting were analyzed by means of a factorial ANOVA, previously transforming data ( $\log N+1$ ) to achieve normality and homocedasticity. The relationship between final number of leaves + buds and number of roots in cuttings of each species was evaluated using Fisher exact test.

In cuttings collected in May, the number of roots per species was compared using a Kruskal-Wallis test, while length and diameter of the longest root were analyzed using one factor ANOVA and Tukey post-hoc tests when appropriate.

## Results

Eleven weeks after the first experiment was set, cuttings of all three species had initiated roots and the proportions recorded did not increase four weeks later, at the second root assessment. Callus formation was frequent only in the control treatment: 19.4% of cuttings in *Bursera glabrifolia*,

37.5% in *B. copallifera*, and 40% in *B. linanoe*. Most of the cuttings that developed noticeable calli failed to produce roots by the time of the final evaluation.

To analyze the proportion of rooted cuttings, a generalized linear model that included all main factors (species, type of cutting, and IBA concentration) and its interactions was initially used. However, as the fitting of this model was not significantly ( $P = 0.73$ ) better than the one with only second grade interactions, the latter, simpler model was used for the analysis. All main factors had significant effects on the proportion of rooted cuttings: species (deviance 53.4, df 2,  $P < 0.0001$ ), IBA concentration (deviance 42.4, df 2,  $P < 0.0001$ ) and age of stockplant (deviance 15.6, df 1,  $P < 0.0001$ ). The interaction age  $\times$  IBA was also significant (deviance 8.37, df 2,  $P = 0.015$ ), while remaining interactions were not (species  $\times$  age  $P = 0.11$  and species  $\times$  IBA  $P = 0.18$ ).

*Bursera glabrifolia* attained the highest mean rooting percentage (58.9% of all cuttings) and *B. linanoe* the lowest (9.2%). Higher proportions of rooted cuttings were observed when using IBA concentrations of 4,000 and 9,000 ppm than in the control (Table 1), and in cuttings from young trees than in those from mature trees. The significant interaction between age of stock tree and IBA is explained by a larger response to auxin application (*i.e.* increase in the proportion of rooted cuttings) in cuttings from mature trees than in those from younger trees. This was especially conspicuous in *B. glabrifolia*, in which there was no noticeable response to applied IBA when using young cuttings, but it was evident among cuttings from mature trees (Table 1).

**Root development.** The effect of species ( $F = 54.9$ , df 2,  $P < 0.0001$ ), age of stock tree ( $F = 21.2$ , df 1,  $P < 0.0001$ ), applied IBA ( $F = 29.8$ , df 2,  $P < 0.0001$ ), and the interactions species  $\times$  age ( $F = 7.04$ , df 2,  $P = 0.0007$ ), species  $\times$  IBA ( $F = 6.2$ , df 4,  $P < 0.0001$ ), and species  $\times$  age  $\times$  IBA ( $F = 2.7$ , df 4,  $P = 0.03$ ) on number of roots per cutting were significant. Mean number of roots per cutting was largest in *Bursera glabrifolia* and smallest in *B. linanoe* (Table 2). In *B. copallifera* and especially in *B. glabrifolia* this num-

**Table 1.** Effects of two IBA concentrations and types of stockplants on percentage of rooted cuttings of three species of *Bursera*.

Stock trees		IBA concentration (ppm)		
		0	4,000	9,000
<i>B. glabrifolia</i>	Mature	9.4	61.5	57.7
	Young	75.0	75.0	75.0
<i>B. copallifera</i>	Mature	0.0	20.8	44.4
	Young	12.5	87.5	71.4
<i>B. linanoe</i>	Mature	0.0	17.0	16.0
	Young	7.4	7.4	16.7

**Table 2.** Mean number of roots ( $\pm$  s.e.) per cutting in response to two IBA concentrations and types of stockplants in three species of *Bursera*. Different letters indicate significant differences in both columns and rows ( $P \leq 0.05$ ).

Stock trees		IBA concentration (ppm)		
		0	4000	9000
<i>B. copallifera</i>	Mature	0.4 $\pm$ 0.9 <sup>b</sup>	6.3 $\pm$ 0.9 <sup>a</sup>	10.0 $\pm$ 0.9 <sup>a</sup>
	Young	1.5 $\pm$ 1.6 <sup>b</sup>	7.5 $\pm$ 1.6 <sup>a</sup>	8.0 $\pm$ 1.6 <sup>a</sup>
<i>B. glabrifolia</i>	Mature	0	0.7 $\pm$ 0.9 <sup>b</sup>	2.0 $\pm$ 0.9 <sup>b</sup>
	Young	0.1 $\pm$ 1.6 <sup>b</sup>	8.5 $\pm$ 1.6 <sup>a</sup>	7.3 $\pm$ 1.7 <sup>a</sup>
<i>B. linanoe</i>	Mature	0	0.3 $\pm$ 0.9 <sup>b</sup>	0.5 $\pm$ 0.9 <sup>b</sup>
	Young	0.1 $\pm$ 0.9 <sup>b</sup>	0.4 $\pm$ 0.9 <sup>b</sup>	0.7 $\pm$ 0.8 <sup>b</sup>

ber increased in cuttings derived from young stockplants relative to mature ones, but this difference was not quite as important in *B. linanoe*, which accounts for the significant interaction species  $\times$  age. Not all species showed the same response to IBA application, as differences among cuttings in the control treatment and those with IBA were much larger in *B. glabrifolia* and *B. copallifera* than in *B. linanoe*. Overall, differences among cuttings derived from younger and mature stockplants were small without IBA (control), but increased when the auxin was applied, particularly in *B. glabrifolia* (Table 2).

A significant positive association was found between presence of buds and leaves, and the presence of roots in *Bursera glabrifolia* and *B. copallifera* (Fisher exact test  $P = 1.6 \times 10^{-17}$  and  $P = 2.2 \times 10^{-14}$  respectively). These observations allowed us to establish that cuttings with the best root development were also the ones that had the largest number of leaves, while the ones that did not develop leaves usually had no roots, though they could have a few leaf buds. Buds were recorded in a high proportion of cuttings 11 days after sowing, while fully expanded leaves were observed ten days later. Overall, correlations between the initial size (length and diameter) of the cuttings and the number of roots per cutting were not significant in *B. glabrifolia* nor in *B. copallifera*, except for length of the cutting in the former species ( $r = 0.37$ ,  $P = 0.005$ ). The low number of rooted cuttings of *B. linanoe* precluded these analyses.

Although it was not our main goal to evaluate differences in rooting ability in cuttings obtained from different individual source trees, we noticed that most trees produced high proportions of rooted cuttings, but in one or a few of them this proportion was much lower; this pattern was observed in all species and in cuttings of both ages.

In the second collection made later in the dry season (in May, approximately eight weeks later), only the IBA concentrations producing the best results in cuttings from young trees were tried: 9,000 ppm in *Bursera linanoe* and

4,000 ppm in *B. glabrifolia* and *B. copallifera*. Rooting percentages were high and similar to those obtained in March for two species: *B. glabrifolia* 75.8% (cuttings from young stockplants in March 75%, see Table 1) and *B. copallifera* 70.6% (71–87% in March, Table 1), but in *B. linanoe* there was a noticeable increase in the percentage of rooted cuttings (81.4% in May vs. 16.7% in March). Comparisons among species on the number of roots per cutting and length and diameter of the longest root showed significant differences among species in the three variables (number of roots Kruskal-Wallis  $H = 26.7$ ,  $P < 0.001$ ; length and diameter of the largest root  $F = 3.69$ ,  $df 2$ ,  $P = 0.04$  and  $F = 4.05$ ,  $df 2$ ,  $P = 0.02$  respectively). *Bursera glabrifolia* and *B. copallifera* had more roots per cutting than *B. linanoe*, but the latter had a larger main root than the other two species (Table 3).

## Discussion

Root development was successful in cuttings of the three *Bursera* species studied, and it was strongly influenced by IBA treatment and age of stockplants. The great majority of rooted cuttings developed buds, leaves, and roots by the time of the first evaluation, eleven weeks after sown, indicating that this period is appropriate to evaluate final root development under the temperature and humidity conditions found in the nursery.

There was no association between external callus and root formation. In cuttings of *Bursera simaruba* a high proportion of callus formation (0.93) and a low rooting proportion (0.04) has been reported (García-Orth, 2002), and in other *Bursera* species cuttings with conspicuous calluses frequently lack roots (Bonfil-Sanders *et al.*, 2007). Although there is a general belief that callus tissue that develops as part of the wounding response is a precursor to the development of adventitious roots (Landis *et al.*, 1999), it has been reported that recalcitrant species form callus without subsequent root formation (Hartmann *et al.*, 1997), and a similar response might have occurred in untreated (control) cuttings. Callus formation without rooting has been reported to increase with ageing and related to unfavorable metabolic conditions for the development of roots (Husen and Pal, 2006).

A positive response of rooting to IBA application was observed in most cases, as in many other tropical species

where this auxin induced root development, especially in leafy cuttings (Aminah *et al.*, 1995; Mesén *et al.*, 1997; Eganathan *et al.*, 2000; Negash, 2002, 2003; Tchoundjeu *et al.*, 2002; Husen and Pal, 2007a). No signs of toxicity due to the high concentrations of IBA used were observed, although negative effects on survival with similar and lower concentrations have been reported both on leafy and leafless cuttings (Ofori *et al.*, 1996; Puri and Verma, 1996; Negash, 2002). However, full identification of optimal, suboptimal, and supraoptimal concentrations of the hormone will be achieved in future studies.

The higher root development found in cuttings from young rather than from mature trees has been reported in other species, such as *Prosopis cinerea* (Arya *et al.*, 1994), *Inga feuillei* (Brennan and Mudge, 1998), *Juniperus procera* (Negash, 2002), *Argania spinosa* (Nouaim *et al.*, 2002), *Robinia pseudoacacia* and *Grewia optiva* (Swamy *et al.*, 2002), *Backhousia citriodora* (Kibbler *et al.*, 2004a) and *Ulmus villosa* (Bhardwaj and Mishra, 2005). Ontogenetic juvenility has been associated with a number of factors, such as optimum levels of auxins, sugars and carbohydrates, and low nitrogen levels (Bhardwaj and Mishra, 2005). Young plants also have less lignified tissues and lower production of rooting inhibitors than older plants (Leopold and Kriedemann, 1975; Hartmann *et al.*, 1997). Other differences are related to higher peroxidase activity during root development in young cuttings (Husen and Pal, 2007b), and differences in fiber and vessel elements of the stem (Husen and Pal., 2006; Amissah *et al.*, 2008).

In this study, juvenility refers to ontogenetic ageing, and differences in physiological juvenility were controlled by collecting young twigs (*i.e.*, grown during the last two growing seasons) from both types of stockplants. It is not possible to establish which of the above mentioned factors associated with juvenility were more relevant in this case, but cuttings from young trees of all species have less lignified tissues than those from mature trees (as was evident by the different strength needed to collect them) and the bark is thinner in the former than in twigs from large reproductive trees. Endogenous auxin levels were probably not optimal even in cuttings from young trees, as they responded strongly to IBA application (except for *Bursera glabrifolia*). The significant interaction between age of stockplant and IBA also supports the hypothesis of metabolic differences between young and mature stockplants, as has been observed on cuttings of *Dalbergia megaloxylon* (Amri *et al.*, 2010).

The young trees used as stock material were not as juvenile as smaller-sized or continuously hedged stockplants, but their morphology suggests that most of them had been coppiced and had resprouted. Additionally, these young trees allowed us to obtain more cuttings per individual than smaller, younger plants. The use of cuttings from young shoots on managed stockplants is recognized as the best vegetative propagation strategy (Landis *et al.*, 1999; Negash, 2004),

**Table 3.** Number of roots (mean  $\pm$  s.e.), length (L) and diameter (D) of the longest root (measured 1 cm below the basal end of the cutting), and sample size (N), of rooted cuttings of three species of *Bursera*. Different letters in the same column indicate significant differences ( $P \leq 0.05$ ).

Species	Root number	L (cm)	D (cm)	N
<i>B. glabrifolia</i>	8.1 $\pm$ 0.7 <sup>a</sup>	14.9 $\pm$ 1.4 <sup>b</sup>	1.4 $\pm$ 0.11 <sup>b</sup>	52
<i>B. copallifera</i>	8.3 $\pm$ 1.1 <sup>a</sup>	12.9 $\pm$ 2.2 <sup>b</sup>	1.5 $\pm$ 0.17 <sup>ab</sup>	21
<i>B. linanoe</i>	3.5 $\pm$ 0.7 <sup>b</sup>	19.4 $\pm$ 1.6 <sup>a</sup>	1.9 $\pm$ 0.12 <sup>a</sup>	42

and future research in a larger set of *Bursera* species may help to establish the morphological and physiological characteristics of the shoots that encourage root development, as well as the best management strategy for the stockplants (Leakey, 2004).

Although the effect of different IBA concentrations on the percentage of rooted cuttings was analyzed using a small sample size in some cases (*i.e.*, young cuttings of *Bursera glabrifolia* and *B. copallifera*, see methods section), the results were supported by the high percentage of rooted cuttings obtained later in the dry season, when the protocols rendering the best results in the first experiment were used. The only noticeable difference between results from these two trials was an increase in the proportion of rooted cuttings of *B. linanoe* in May. In species growing in highly seasonal environments, such as tropical dry forests, the increase in temperature during spring, when bud dormancy ends, induces carbohydrate mobilization and enzymatic activity, favoring root development in leafless hardwood cuttings (Puri and Verma, 1996; Bhardwaj and Mishra, 2005; Kibbler *et al.*, 2004b, Haile *et al.*, 2011). In this case, good results were obtained when leafless cuttings were completely dormant, prior to the beginning of bud swelling and development of flowers and leaves, which occurs early in May, before the onset of the rainy season, in *B. copallifera* and *B. glabrifolia*, (Velázquez-Herrera, 2011). *Bursera linanoe* grows in relatively drier conditions than the other two species (Hernández-Pérez *et al.*, 2011), and buds may become active a few weeks later. In other tropical dry species an increase in rooting potential has also been found when cuttings are collected towards the end of the dry season but before bud breaking (Danthu *et al.*, 2002; Haile *et al.*, 2011).

When using the number of roots per cutting as a response variable to the experimental factors the same general trend was shown: all main factors had a very significant effect, as well as the interaction species  $\times$  IBA. The positive effect of IBA on rooting percentage and on root number has been observed in other assessments of vegetative propagation (Amri *et al.*, 2010), supporting its overall positive effect. We advise the use of young stockplants and IBA application when propagating cuttings of *Bursera*, considering differences regarding the best concentration for each species. It is worth noticing that even if young cuttings of *B. glabrifolia* did not need IBA to develop roots, the number of roots increased significantly when the hormone was applied to cuttings of both ages. A good root system is important for tree stability and growth (Asaah *et al.*, 2010).

Shoot growth can reduce rooting as a result of internal competition for resources in leafy softwood cuttings (Ofori *et al.*, 1996; Mesén *et al.*, 1997; Kibbler *et al.*, 2004b), but a positive relationship between the development of both types of tissues is common in leafless hardwood cuttings and has been considered an indicator of metabolic activity (Tchigio and Duguma, 1998, Dick *et al.*, 1998). The positive

association found between bud and leaf development and root formation in two of three species of *Bursera* supports the second statement, and the fact that cuttings having the largest number of leaves were also the ones that developed more roots suggests the benefit of current assimilates to supplement the mobilization of stored carbohydrates in leafless cuttings from dormant shoots, and the absence of competition in this situation between the two types of tissues. Root development probably started a few weeks after the appearance of fully expanded leaves (three to four weeks), so a period of six weeks may be adequate to make an initial evaluation of root development and assess the speed of rooting in future studies.

It is likely that efforts to propagate other species of *Bursera* from young, dormant cuttings will be successful. Leafy cuttings can probably also be propagated under good propagation conditions, and a comparison of the rooting behavior of both types of cuttings will be useful to draw sound conclusions on the best procedure to propagate trees of this diverse and important genus. The propagation of large numbers of plants of *Bursera* species from small cuttings will certainly contribute to the restoration of degraded tropical dry forests of America and will also allow the establishment of productive plantations and agroforests in regions experiencing high demand of fruits, resin, or wood for scents and handicrafts.

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