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Effect of cutting age and substrate temperature on rooting of *Taxus globosa*

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Abstract *Taxus globosa* (Mexican yew) is a conifer endemic to México and Central America. It produces a substance known as taxol, which is useful in treatment of ovarian cancer. Because seed production for this dioecious tree is limited, and seed germination is extremely difficult, the use of cuttings could facilitate propagation of this species. With the intention of massively propagating individuals selected for taxol content, two trials were established in which the effect of substrate temperature (average temperatures 18 and 23°C), age (i.e., young vs. old shoots) and management of cuttings, as well as clone variation in rooting capacity, were evaluated. Low temperature favored rooting (53 vs. 34% on average for the two trials); younger shoots rooted three times (61 vs. 23%) more than mature ones, while basal wounding did not affect rooting capacity. A wide variation was found in rooting capacity of clones (8–76%), which could be associated with genetic or physiological differences among donor trees.

Keywords Age of cuttings · Cloning · Genetic variation · Rooting capacity · Substrate temperature · Yew

Introduction

Taxus globosa Schltdl. (Mexican yew) is endemic to Mexico and Central America, and is subjected to special protection because of its restricted natural distribution (SEMARNAT

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2001). It inhabits protected isolated areas, humid ravines, and steep slopes coexisting with elements of cloud and pine forests (Zavala-Chavez et al. 2001). Though rarely, it is used as an ornamental plant, as well as for timber, tannins or charcoal production (Zamudio-Ruiz 1992). However, it has been found that this conifer has a high potential for pharmaceutical use. As other *Taxus* species, the tree produces a chemical substance known as taxol, a drug that has been found to be useful in the treatment of ovarian and breast cancer. The highest concentrations of the substance are found in the bark, although certain amounts are present in the foliage (Ramos-Lobato et al. 2003; Shemluck et al. 2003).

Little is known about the biology of the Mexican yew, especially in subjects such as reproduction and propagation (Zavala-Chavez et al. 2001). Apparently, the natural recruitment process is by seed (Nicholson and Munn 2003), although this method of propagation is rarely used for seedling production purposes because of the seeds' dormancy, low levels of germination and slow seedling growth, along with the disadvantage of variation among descendants (Hartmann et al. 2001). In natural populations, individuals with a notable production of new shoots close to the stem base have been observed, thus suggesting potential vegetative propagation (Zavala-Chavez et al. 2001). For most woody plant species, the use of cuttings is the most efficient method of vegetative propagation in terms of time and cost (Hartmann et al. 2001).

Rooting capacity depends, among other factors, on the age of donor plants and cuttings, environmental conditions and use of promoter substances or hormones (Mesén et al. 1997; Berhe and Negash 1998; Negash 2002). After genetic traits, age of cuttings is the most limiting rooting factor for woody species; mature tissues take a longer time to form roots and develop fewer roots than younger ones (Steele et al. 1990; McGranahan et al. 1999; Mitchell et al. 2004; Krakowski et al. 2005). According to Mitchell et al. (2004), maturation of tissues could be related to the age of the donor plant (cyclophysis), to the position of the propagule within the plant (topophysis), or to the health and physiological status of the plant (periphysis). Within a given donor tree, maturation of cuttings can also be related to chronological ageing of individual branch segments (i.e., age of shoot growth); in most tree species, rooting ability of cuttings varies from apical to basal sections of shoots (Hartmann et al. 2001; Ruiz-García et al. 2005; Kaul 2008).

In trying to enhance rooting capacity of woody species, several treatments have been proposed; for instance, wounding the base of the cuttings might promote rooting by inducing callus formation and internal hormonal changes within the cutting itself (Dirr and Heuser 1987). Substrate temperature can also affect rooting capacity by modifying various physiological processes including cell division and movement of auxins towards the base of the cuttings, along with the rate of transpiration and respiration (Iglesias-Gutiérrez et al. 1996; Prat et al. 1998).

Although there are studies that have shown that vegetative propagation of different species of *Taxus* is possible (Doede et al. 1993; Mitchell 1997; Kaplow and Scher 1998; Kaul 2008), including a previous study on *T. globosa* (Nicholson and Munn 2003), the results indicate great differences in rooting capacity. Thus, it is possible that different factors influence the success of vegetative propagation of this species. This study was conducted to identify some of these factors and to develop a protocol for effective cloning of *T. globosa*. The specific objectives of the study were (1) to evaluate the effects of cutting age (age of shoot growth), wounding the base of the cuttings, and substrate temperature on the rooting capacity of *T. globosa*, and (2) to compare the rooting capacity of cuttings obtained from different donor trees (clones) of this species.

Materials and methods

Preparation of cuttings and trial set up

The study included two trials established under similar conditions, using cuttings from different mother trees to evaluate the repeatability of treatments. The trials were established in the forest nursery of the Colegio de Postgraduados, Montecillo, State of Mexico. A chamber in which relative humidity and air and substrate temperature could be monitored and controlled was used. Yew cuttings were collected in October 2006 in Santa María Yavesía, Ixtlán, Oaxaca (17°11′03.0″N and 96°22′44.5″W) from 21 healthy, vigorous adult (mature) trees; age of trees was not determined, but stem diameter at breast height was over 5 cm. From each of these trees, branches having at least the last 3 years of shoot growth (under 10 mm in diameter at the base of the branch and 60 cm in length) were selected from the lower part of the crown. Identification of each donor plant (clone) was maintained both during and after the establishment of the trials.

The substrate used was a mixture of peat moss, agrolite and vermiculite in a ratio of 2:1:1 (volume). In both trials, substrate temperature, age of cuttings and wounds in the base of cuttings were considered. To evaluate the effects of substrate temperature, the cuttings were established under two conditions: one at room temperature (18 ± 5°C), and the other about 5°C higher (23 ± 5°C) on average (Fig. 1) by using electrical resistances at the bottom of the bed. Substrate temperature at 3–4 cm depth was recorded during the trials at 1-h intervals using digital sensors with data storage capacity (data loggers) inserted in the substrate. The sensors were moved systematically at weekly intervals across the bed to register any spatial variation within each temperature environment; most variation in substrate temperature within each environment was related to a daily (24-h) cycle (Fig. 1), with minimal variation among blocks. In both environments relative humidity was maintained above 85% using a sensor connected to an automatic misting system.

Two different (but correlated) criteria were used to separate cuttings of different age within a given branch. In the first trial, cuttings were classified based on stem diameter: cuttings having less than 5 mm in diameter (age-1 cuttings, coming from 1-year-old shoots) and cuttings with diameter between 5 and 10 mm (age-2 cuttings, coming from

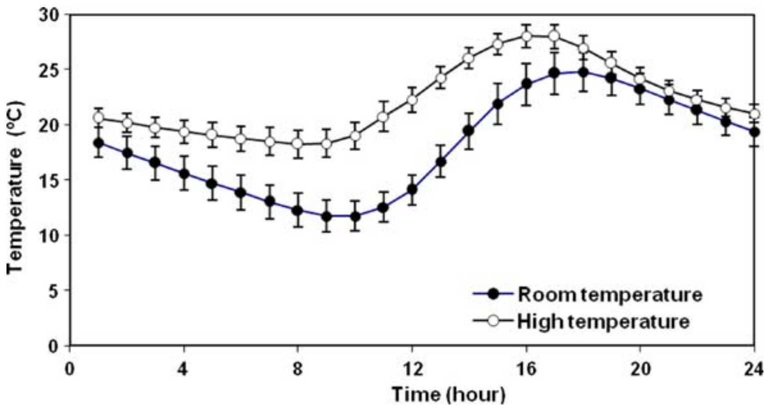

Fig. 1 Daily average temperature curve of the substrate, with standard deviation, in the two rooting environments

2-year-old shoots). In the second trial, the classification criterion for cuttings of different age was based on their position on the branch: the first two internodes from the branch tip were considered as age-1 and the following two internodes as age-2. Two 1-cm-long lengthwise cuts were performed from the basal end through the bark using a thin blade on half of the cuttings of both ages to evaluate the effect of wounding on callus and root formation.

Length of cuttings ranged from 12 to 15 cm, and in both trials the basal cut was made in the node of the shoot. The foliage was eliminated within the first 4 cm from the basal end of cutting, and in the upper part leaves were cut at midpoint to reduce transpiration and to prevent dehydration. Subsequently, a powder mixture of 10,000 ppm IBA and Benomyl® (fungicide with 50% active ingredient) in a ratio of 5:1 (IBA:Benomyl) was applied to the base of all the cuttings (with or without wounds) to induce formation of adventitious roots, accelerate initiation and increase the number and quality of roots produced. The cuttings were then shaken to remove excess of powder mixture applied and immediately inserted 3–4 cm in the substrate inside the propagation chamber, following the pre-established experimental design. After inserting the cuttings, slight mist irrigation was applied to the substrate and then the relative humidity within the chamber was controlled with the sensor.

Experimental design and traits evaluated

The two experimental designs were similar: two substrate temperature conditions and four treatments in each temperature environment were tested, resulting from the factorial combination of two cutting ages and two wounding conditions. Treatments were replicated four times for each temperature environment. A randomized complete blocks design was used, with donor trees (clones) handled as blocks, either individually or in groups, including 16 and five trees, respectively, in the first and second trial. A total of 80 cuttings were used for each treatment (20 in each experimental unit), with all clones equally represented within and across temperature environments. After 5 months in the rooting chamber, the cuttings were counted as alive or dead to determine the percentage of survival for each temperature environment and treatment. Number of visible shoots and roots present on the cuttings were also assessed. With this information, the percentage of cuttings with elongated shoots and roots (relative to the initial number of cuttings) was calculated for each experimental unit and clone, as well as the number of shoots and roots per cutting. Some cuttings elongated shoots before dying, so shooting percentage was higher than survival percentage for some treatments and clones.

With the average values by experimental unit, a statistical analysis was performed to test the main effects and interactions of the three factors under study, following the GLM procedure from SAS (SAS Institute 1998), with all factors considered as fixed-effects. Traits expressed in percentage were transformed with the arcsin function before the analysis of variance, and afterwards the average values were transformed back into the original units. For variables where significant differences were found for any of the factors, the Tukey's test ($P \leq 0.05$) was used to compare treatment means. Given that significant differences were found among blocks (clones or groups of clones), a second analysis was done to compare the average rooting capacity of clones across all treatments, separately for each trial; only the effects of clone, temperature, and clone × temperature interaction were considered in this model.

Results and discussion

Effect of substrate temperature

Substrate temperature had a significant effect ($P \leq 0.05$) on percentage of cuttings with visible shoot elongation (shooting) in both trials; in the second trial, substrate temperature also affected cutting survival, rooting capacity and number of roots formed (Table 1). Shooting was similar for both trials, occurring in nearly 50% of the cuttings initially established, and the effect of temperature on this trait was also consistent, with around 20% more cuttings with shoots under low temperature conditions (58.4 vs. 39.7% in the first trial and 58.1 vs. 35.6% in the second trial). Although there was no significant effect of temperature on rooting capacity (46.6 vs. 38.4%, Table 2) in the first trial, rooting percentage was twice as much in the low temperature than in the other environment (59.1 vs. 28.8%) in the second trail (Table 2). The number of roots formed was also greater in the low temperature environment with an average of 4.8 roots per cutting (Table 2).

The rooting capacity found in both trials was lower than that reported for this species by Nicholson and Munn (2003), who obtained 89.5% root formation in cuttings taken from trees collected in Northern Mexico using a substrate temperature of 24°C during a rooting period of 5 months. The differences between the two studies could be due to several causes, including a population effect, tree age, cutting age or environmental conditions of trials, among others. Therefore, a detailed analysis to explain these differences is not possible. However, the results look more favorable when compared with data obtained in other species of *Taxus*. For instance, an average of 50% rooting capacity was obtained after 2 years in cuttings of *T. brevifolia* Nutt., with substrate temperature of 20°C and high relative humidity (Mitchell 1997). Kaplow and Scher (1998) also obtained a similar rooting capacity for *T. brevifolia* cuttings, using substrate temperatures of 15–21°C. The rooting capacity of *T. globosa* in this trial is also consistent with the findings in a previous trial done under similar conditions, in which 50–65% rooting was obtained during a 4.5-month period (Soto-Hernández, Pers. Comm.).

According to the results obtained in the two trials, an increase in substrate temperature above 20°C was not favorable for rooting of *T. globosa* cuttings, as lower percentages were

Table 1 Significance of factors included in the rooting trials (first and second) for traits related to survival and shoot and root formation in *Taxus globosa* cuttings after 5 months in the rooting chamber

Source of variation	Survival		Shooting		Shoots (No.)		Rooting		Roots (No.)	
	First	Sec.	First	Sec.	First	Sec.	First	Sec.	First	Sec.
Blocks	*	*	*	*	ns	*	*	*	ns	*
Temperature	ns	*	*	*	ns	ns	ns	*	ns	*
Block × temp.	–	–	–	–	–	–	–	–	–	–
Age	*	*	*	*	ns	ns	*	*	ns	ns
Wounding	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Temperature × age	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Age × wound	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Temperature × wound	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Temp. × age × wound	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Significant with $P \leq 0.05$; *ns* non significant

Table 2 Tukey's test comparison of means for each trait and factor evaluated on the rooting capacity in two trials of *Taxus globosa* cuttings, after 5 months in the rooting chamber

Factor	Survival (%)	Shooting (%)	Shoots (No.)	Rooting (%)	Roots (No.)
First trial					
Temperature (°C)					
18	49.4 a	58.4 a	3.8 a	46.6 a	5.1 a
23	41.6 a	39.7 b	4.4 a	38.4 a	5.4 a
Age					
Age 1	71.0 a	72.4 a	3.9 a	66.3 a	5.5 a
Age 2	18.2 b	25.0 b	4.3 a	17.0 b	5.0 a
Wounding					
Yes	40.6 a	46.7 a	4.2 a	38.3 a	5.0 a
No	46.7 a	50.3 a	4.1 a	42.3 a	5.6 a
Average	43.7	48.5	4.1	40.3	5.3
Second trial					
Temperature (°C)					
18	63.8 a	58.1 a	3.0 a	59.1 a	4.8 a
23	34.7 b	35.6 b	3.1 a	28.8 b	3.4 b
Age					
Age 1	67.3 a	60.6 a	3.0 a	55.3 a	4.2 a
Age 2	30.5 b	29.3 b	3.1 a	29.2 b	4.1 a
Wounding					
Yes	44.7 a	38.6 a	3.1 a	42.8 a	4.2 a
No	53.0 a	50.8 a	3.0 a	41.1 a	4.1 a
Average	48.9	44.7	3.1	42.0	4.2

Mean values within each factor followed by same letter are not significantly different ($P \leq 0.05$); shooting (%), percent of initial cuttings with visible shoot growth; shoots (No.), number of elongated shoots (>1 cm) per cutting; rooting (%), percent of initial cuttings with visible root growth; roots (No.), number of visible roots (>5 mm) per cutting

obtained. It is possible that heat in the substrate is beneficial for rooting evergreen species only during the first days in which the primordial roots of the cuttings are formed (de la Iglesia 1992). Prat et al. (1998) found that increasing the substrate temperature has no positive effect on the rooting capacity of jojoba [*Simmondsia chinensis* (Link) Schneider] clones, except for that with the lowest rooting percentage, so there might be some genetic or physiological interactions involved. For further studies it is recommended that *T. globosa* cuttings be placed in substrate temperature between 15 and 18°C, with relative humidity above 80%.

Effect of cutting age

The age of cuttings had significant effects ($P \leq 0.05$) on survival, shooting and rooting capacity in both trials. Consistently, younger shoots had higher survival rates and better rooting capacity than the older ones (Table 2). In the first trial, younger shoots had a rooting capacity equivalent to almost four times that of the older ones (66.3 vs. 17%). In the second trial, rooting capacity of younger shoots was almost twice of that obtained for the older ones (55.3 vs. 29.2%). However, average number of roots per cutting was similar for both ages of shoots tested (Table 2).

In other species of *Taxus*, similar shoot age effects have been observed, with chronologically juvenile cuttings (1–2 years of shoot growth) having higher rooting capacity. For example, Eccher (1988) reported that cuttings obtained from the apical part of *Taxus baccata* L., *T. cuspidata* Siebold & Zucc. and *T. x media* (hybrid between *T. baccata* and *T. cuspidata*) reached a higher rooting percentage than cuttings taken from the base of the branch. Kaul (2008) found that rooting response in *Taxus wallichiana* Zucc. was the highest in 1- and 2-year-old shoot cuttings, as compared to older ones. Doede et al. (1993) also showed that by using cuttings of the second year of growth, a rooting percentage of 43% was obtained for *T. brevifolia*. However, Kaplow and Scher (1998) found no significant differences among rooting capacity for *T. brevifolia* cuttings taken from their second and third year of growth.

Reduction in rooting capacity of older shoots could be due to either genetic regulation (i.e., irreversible tissue maturation) or physiological status (nutrients, carbohydrates, water, etc.) of shoots (Mitchell et al. 2004). For instance, the lower shooting and rooting capacity observed in older shoots of *T. globosa* might be due to substances which inhibit root formation, such as the presence of phenolics (Dirr and Heuser 1987; Hartmann et al. 2001), a reduction in levels of endogenous root-promoting substances, or an increased lignification (Kaul 2008). Generally, younger shoots produce roots more easily or with less exogenous addition of auxins than more lignified ones, as in the case of *Gmelina arborea* Roxb., whose apical cuttings showed higher rooting capacity (71.8%) than basal cuttings (43.7%), and needed a lower dosage of exogenous IBA (Ruiz-García et al. 2005). Likewise, Santiago-Gregorio and Vargas-Hernández (1999) found that *Tilia mexicana* Schltdl. cuttings which came from mature branches had a lower survival rate, as well as lower shooting and rooting capacity, than cuttings taken from chronologically young branches. It was found that *Chamaecyparis lawsoniana* Parl. cuttings taken from the apical portion of branches also had higher percentages of root formation than those taken from the basal end (Stumpf et al. 2001). In the present study, chronologically mature cuttings had lower rooting capacity than that reported in the literature for other species of *Taxus*. Therefore, use of chronologically young cuttings, from the last or second to last year of growth, with diameters around 5 mm or less, with at least two nodes, and free of disease and other visible damage is recommended for rooting of *T. globosa*.

Effect of wounding at the base of the cuttings

Consistently, wounding at the base of the cuttings had no significant effect on any of the traits evaluated (Table 1). In both trials, the average survival of cuttings with or without wounds was 45% and rooting capacity was around 40–43% (Table 2). When wounds are made at the base of the cuttings, combined with the application of a rooting substance (IBA, for example), a higher quantity and quality of roots per cutting is commonly expected (Macdonald 1990; Hartmann et al. 2001). Mateo-Sánchez et al. (1998) showed that by wounding the base of the cuttings of certain conifer species, the number of roots formed per cutting could be increased up to 80–90%, as in the case of *Thuja orientalis* L. and false cypress (*C. lawsoniana*). In *T. globosa*, even though the number and length of roots were not significantly affected by the presence of wounds, it was observed that in the wounded cuttings most roots were originated from the wounds.

Interaction among factors

The different interactions between factors did not significantly affect rooting capacity of cuttings in any of the trials, except in the case of the interaction temperature × wounding in the second trial, in which a significant effect on root number (Table 1) was found. As a result of this interaction, the temperature effect on the number of roots was more evident in cuttings without wounds at the base: the amount of roots formed at lower temperature was almost double of that found at the other environment (data not shown). In contrast, there were no differences in number of roots between the two temperature environments in cuttings that were wounded. Apparently, wounding did not stimulate formation of roots when cuttings were subjected to the low substrate temperature; it did, however, when cuttings were rooted at higher (23 ± 5°C) temperature.

Variation among clones

Differences among clones were found in all rooting traits of cuttings in both trials (Table 3), while the interaction temperature x clone was only significant in survival percentage and rooting capacity in the second trial (Table 3). In the first trial, survival percentage of clones varied from 14.6 to 88.7%, and in the second it varied from 33.8 to 80.4%. Likewise, rooting capacity varied from 8.0 to 75.8% in the first trial, and from 24.9 to 58.9% in the second. Considering both trials, at least eight clones had a rooting capacity greater than 50% of the initial cuttings, but in three clones it was below 20%. The average number of roots per cutting varied among clones from 2.3 to 7.9 in the first trial, and from 2.7 to 5.9 in the second.

These results indicate that a wide variation in rooting capacity exists in cuttings taken from different *T. globosa* trees (from 8 to 75%), similar to the findings of Mitchell (1992) in *T. cuspidata* clones, (from 1.3 to 49.1%), and Mitchell (1997) among *T. brevifolia* clones (from 14.5 to 87.5%). Variation in rooting capacity among clones of different *Taxus* species is similar to that found in many other woody species (Prat et al. 1998; McGranahan et al. 1999; Almehdi et al. 2002; Krakowski et al. 2005). Given that the *T. globosa* trees from which cuttings were taken for our study were growing in natural stands and were of different sizes, it is not possible to determine whether the differences in rooting capacity among them is due to genetic, ontogenetic (i.e., age of mother trees) or physiological factors.

The interaction clone × temperature influenced only survival percentage and rooting capacity in the second trial (Table 3). The results of the interaction clone × temperature on survival of cuttings showed that, although for most clones better results were obtained when cuttings were placed in the lower substrate temperature (18 ± 5°C), clone 18 had better survival and rooting at the higher temperature. On the other hand, the high

Table 3 Significance of variance analysis for clone effect on survival and shoot and root formation in *Taxus globosa* cuttings after 5 months in the rooting chamber, first and second trials

Source of variation	Survival		Shooting		Shoots (No.)		Rooting		Roots (No.)	
	First	Sec.	First	Sec.	First	Sec.	First	Sec.	First	Sec.
Clone	*	*	*	*	*	*	*	*	*	*
Temperature × clone	ns	*	ns	ns	ns	ns	ns	*	ns	ns

* Significant with $P \leq 0.05$; *ns* non significant

temperature had a marked effect in reducing cutting survival and rooting of three clones, suggesting that some clones were relatively sensitive to the increase in substrate temperature.

Conclusions

Results obtained in this study indicate that it is possible to obtain consistent results for rooting capacity of *T. globosa* cuttings if the main environmental factors are controlled during the process and cuttings are placed in a favorable rooting medium. For both trials a higher rooting capacity was achieved when cuttings were placed in substrate with a temperature of 18 ± 5°C. A higher rooting capacity was also obtained when young cuttings, from the first and second year of shoot growth were used. However, it is possible to obtain between 15 and 20% rooting capacity even with older and lignified cuttings (up to 10 mm in diameter). On the other hand, wounding the base of the cutting did not significantly affect rooting capacity in any of the trials. Except for the effect of temperature and wounds on the number of roots formed, there was no significant interaction among factors on rooting capacity in any of the trials.

A wide clonal variation in rooting capacity, from less than 10% to almost 80%, was found. At least 8 of the 21 clones used in both trials had a rooting capacity greater than 50%; hence, it is possible to consider the use of cuttings in a vegetative propagation program of the species. Unfortunately, it was not possible to distinguish which factors were responsible for clonal differences in rooting capacity.

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