Secondary Branch Angles and Potential Topophytic Effects of Vegetatively Propagated Clones of *Taxodium*[1]

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Abstract

Baldcypress [*Taxodium distichum*] branch angles were measured between the primary leader (1st order) and second order branches at three different positions (low, medium, high) in the canopy of 828 four-year-old, field-grown seedlings. Seedlings represented twenty-three open-pollinated families collected from Central Mexico to Alabama. Based on these measurements, six different representative families (three families with the most acute branch angles and three families with the most obtuse branch angles) were chosen to test 1) whether topophysis significantly affects the orientation and/or growth of ramets and 2) if branch angle divergence from the trunk of the ortet impacts the degree of topophysis observed on the ramets from rooted shoot tips. Cuttings from the six seedlings were harvested in June 2008 from basal (more horizontally oriented branches) and terminal (more erect upper branches) portions of the canopies in order to test topophytic effects. Rooted cuttings were oriented vertically upon transplanting and grown for approximately 8 months. Measurements included initial and final height and stem diameter and the final angle between the primary leader and the substrate surface. Branch angle data collected from field-grown seedlings revealed a significant interaction ($P \leq 0.0001$) between family and location within the canopy. Most families measured had branch angles that were most obtuse at the bottom of the canopy becoming progressively more acute toward the tree apex. MX6D, TX3D, TX6D and EP7D were families that exhibited minimal to no branch angle differences in regard to position within canopy. Data analysis showed no significant main effect or interactions of cutting location within the canopy on vertical orientation during container production. A significant main effect ($P \leq 0.0001$) of clone was observed for all measurements except initial stem diameter. Clone MX2MC31 cuttings displayed the greatest mean angle of divergence from vertical at 48° while cuttings of MX6MC14 diverted ≈12° from vertical. In this study neither initial branch angles on the ortet nor position within the canopy from which cuttings were taken was an accurate significant predictor of cutting orientation relative to vertical. Tendencies for clonal differences in vertical growth however, suggest the potential for selection of baldcypress clones that would have a reduced need for staking during production.

Index words: *Taxodium distichum*, topophysis, orthotropic, plagiotropic, ortet, ramet.

Species used in this study: *Taxodium distichum* var. *distichum*, *Taxodium distichum* var. *mexicanum*.

Significance to the Nursery Industry

This study documents the apparent absence of topophysis (phenomenon that propagules maintain the same orientation to vertical that they had on the ortet) related to the canopy location of cuttings in the *Taxodium* observed in this study. However, a genetic component to the vertical growth of rooted cuttings was observed. This finding could lead to future selections of baldcypress based on vertical growth habit which would lessen or negate the need for staking and training. The absence of topophysis in baldcypress also allows propagators freedom to harvest cuttings or scions from any portion of the ortet.

Introduction

Topophysis is defined by Olesen (5) as ‘the phenomenon that scions, buddings and cuttings for some time after grafting, budding or rooting maintain the branch-like growth habit they had as shoots on the ortet’. This phenomenon has been documented in a number of different species, particularly in conifers (2, 6, 7). Black (2) discovered that the branch position in the crown from which a cutting was harvested made a significant difference in rooting percentages of Douglas-fir, *Pseudotsuga menziesii*, cuttings. Varied response in timing for rooted cuttings to reorient from a plagiotropic (away from vertical) to an orthotropic (vertical) growth habit was also observed. Smith et al. (7) emphasized topophysis in a vegetative propagation study of black cottonwood (*Populus trichocarpa*) and Carolina poplar (*Populus canadensis* var. *eugenei*). Cuttings were taken from three different positions on each donor plant. Significant correlations between original orientation of the cuttings on the ortet with both survival and growth of ramets were revealed. Mean vertical growth measurements for both species were 30.5 cm (12 in) for cuttings from leaders, 20.3 cm (8.0 in) for cuttings from primary branches next to the leader and 11.4 cm (4.5 in) for cuttings from secondary branches. Power et al. (6) studied topophysis in coast redwood (*Sequoia sempervirens*) cuttings. Cuttings taken from terminal branches predominantly grew in an orthotropic manner, while primary and secondary branches grew progressively in a more plagiotropic habit. It was also noted that more mature donor plants rendered a cutting that was more inclined to exhibit the negative effects of topophysis. No literature was found concerning topophysis in baldcypress.

Baldcypress, *Taxodium distichum*, is a highly adaptable tree of ecological importance in the southeastern United States (1). In previous studies baldcypress seed from twenty

[1]Received for publication December 19, 2012; in revised form February 4, 2013. This study was included as part of a thesis written in partial fulfillment of the requirements for the M.S. degree by A.R. King. This work was supported in part by funds from Texas A&M AgriLife Research. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the authors, Texas A&M University, or Texas A&M AgriLife Research and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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three open-pollinated families with provenances including Mexico, Texas, Louisiana, Mississippi, and Alabama were collected, germinated and field-grown for four years (3). These baldcypress seedlings were used in the current research.

The objective of the current research was to determine 1) whether or not topophysis significantly affects the orientation and/or growth of ramets in baldcypress and 2) if branch angle divergence from the trunk of the ortet impacts the degree of topophysis observed on the ramets from rooted shoot tips.

Materials and Methods

Documenting field level variation in branch angles. Branch angle measurements were taken from 4-year-old baldcypress seedlings grown in a research plot in College Station, TX (lat. 30°37'645' N long. 96°22'319' W). Twenty-three open-pollinated families were collected from provenances that ranged from central Mexico to Alabama as described by Denny (3). Seedlings were planted in 18 east to west oriented rows containing two randomized replications of each of 23 families per row (828 total trees). Identification codes were assigned to each seedling. The first two characters in the identification code for each clone indicate eastern (EP), central Texas (TX) or south Texas to Mexican (MX) provenances. The third character is a family number within the provenance and the fourth character designates the botanical variety (D = *T. distichum* var. *distichum*, I = *T. distichum* var. *imbricarium*, or M = *T. distichum* var. *mexicanum*). In subsequent text and figures these codes followed by the letter C and a one or two digit number indicate individual clones within families. Branch angle measurements were determined by measuring the angle with a large protractor between a randomly-selected first order secondary branch and the primary leader derived directly from the apical meristem. The protractor was oriented in such a way that a measurement of 90 degrees was perpendicular to the bole of the tree. Measurements were taken on branches which were perpendicular to the row (oriented north or south) in an effort to negate any effects of canopy shading by adjacent trees. Branch angles were measured at three different locations on the tree: a secondary branch near the terminus of the central leader (but below any succulent immature growth); a secondary branch near the middle of the canopy height; and a secondary branch within the bottom 30% of the canopy. Three similar measurements were taken on opposite sides of each tree. These six measurements were used as means for comparison of the general habit of the trees. Possible interactions between open-pollinated family, location in canopy (high, middle, low) and cardinal direction of branch (north or south) were analyzed using the General Linear Models Procedure in SAS and means were compared using least squared means comparisons (SAS 9.1 for Windows, Institute, Cary, NC) From this analysis the three families with the most acute branch angles on average and the three families with the most obtuse branch angles on average were determined. A representative tree from each of these six families was selected. Clones MX6MC14, MX4MC17, and EP7DC29 were selected for collection of cuttings with obtuse branch angles and clones MX2MC31, MX3MC13 and EP6DC14 were selected for acute branch angles.

Determining topophytic effects during container nursery production. For each of the selected clones from the obtuse (MX6MC14, MX4MC17 and EP7DC29) and acute (MX2MC31, MX3MC13 and EP6DC14) branch angle families, 30 cuttings were taken from the upper portion of the canopy, near the terminal branch (more upright growth), and 30 cuttings were taken from the basal portion of the canopy (more horizontal growth). Cuttings of each of the six families were made on June 18, 2008, and kept overnight in a cooler at 8.7C (47.6F) in plastic bags partially filled with water to ensure hydration. Cuttings were planted the following day, June 19, 2008. Cuttings were 12.5 cm (4.9 in) long on average. The basal end of each cutting was submerged 5 cm (2.0 in) deep into a 7,500 mg·liter^{-1} (31.1 mM) solution of K-IBA (potassium salt of indole-3-butyric acid, Sigma-Aldrich Chemical, St. Louis, MO) for five seconds. Cuttings were then placed in 10 × 36 × 51 cm (4 ×14 × 20 in) black plastic nursery flats (Dyna-flat™, Kadon Corp., Dayton, OH) containing a 1:1 (by vol) substrate of peat moss:perlite. Cuttings were placed in a modified randomized completed block design with three blocks containing 10 cuttings from each canopy cutting location and clone. Cuttings were placed in a greenhouse at the Texas A&M University Horticultural Gardens (College Station, TX) under natural photoperiods. Reverse osmosis water was misted intermittently through high pressure mist nozzles for a period of 10 sec on a 16 min cycle during daylight hours. Cuttings were allowed to root for approximately 12 weeks.

Successfully rooted cuttings were transplanted into #1 (4.4 liter) black plastic containers (Nursery Supplies Inc., Fairless Hills, PA and Orange, CA) on September 11, 2008. Care was taken during transplanting to ensure that cuttings were oriented as vertically as possible in the containers after transplanting from the propagation flats. Vertical status was confirmed on September 15, 2008, after the substrate settled from initial watering. Initial height (from soil surface to apex) and trunk diameter [5 cm (2.0 in) above soil surface] of rooted cuttings were measured. Rooted cuttings were maintained in a gravel-floored nursery at Texas A&M University Horticultural Gardens (lat. 30°37'45' N, long. 96°20'34' W). Plants were grown for approximately 8 months. At cessation of the experiment, the angle that the trunk made with the substrate surface and the overall orientation of the plant was determined with a protractor. Ending height (from soil surface to apex) and trunk diameter [5 cm (2.0 in) above soil surface] were measured and recorded. The protractor was oriented such that a measurement of 90 degrees was vertical. The angle that the plant made with the substrate was then subtracted from 90 degrees. This calculation made it such that a value of 90 degrees would be perpendicular to vertical in both the field and container studies.

Possible interactions between clones propagated and cutting location in the canopy (high or low) and main effects of clones propagated and cutting location in the canopy (high or low) were analyzed using the General Linear Models Procedure in SAS and means were compared using least squared means comparisons (SAS 9.1 for Windows, SAS Institute Inc, Cary, NC). Possible correlations among growth parameters and angle deviation from vertical were analyzed using the PROC CORR function in SAS (SAS 9.1 for Windows, Institute, Cary, NC).

Results and Discussion

Documenting field level variation in branch angles. The three-way interaction among families, canopy location, and

Table 1. Levels of significance for factors in the analysis of variance for branch angles in field.

Source of variation	Angle
Family	****[z]
Canopy location	****
Family × canopy location	****
Cardinal direction	****
Family × cardinal direction	ns
Canopy location × cardinal direction	ns
Family × canopy location × cardinal direction	ns

[z]Significant at $P \leq 0.05, 0.01, 0.001, 0.0001$ = *, **, ***, ****, respectively; ns = not significant at $P \leq 0.05$.

cardinal direction of the branch and the two-way interactions among families and cardinal direction, and canopy location and cardinal direction were not significant ($P \leq 0.05$) for branch angles in the field (Table 1). The two-way interaction among open-pollinated families and location in the canopy (low, middle, high) along with the main effect of cardinal direction in which measurements were taken (north or south) were highly significant ($P \leq 0.0001$) (Table 1).

The significant ($P \leq 0.0001$) two-way interaction among open-pollinated families and location in the canopy was because of a trend of branch angles to be more acute toward the apical portion of the canopy (Fig. 1). Branch angles toward the bottom of the canopy were more obtuse with branches in the middle of the canopy growing at intermediate angles on average. While this general trend was apparent for most families, a few families such as MX6M, TX3D, TX6D, and EP7D exhibited minimal or no branch angle differences related to canopy position (Fig. 1). This result is consistent with the moderate heritability of branch angle reported in Haapanen et al. (4) and Velling and Tigerstedt (8) for Scots Pine (*Pinus sylvestris* L.), another coniferous tree.

There was a significant ($P \leq 0.0001$) main effect of cardinal direction of branches (north or south). Branches oriented toward the south grew approximately one degree more acutely than those oriented toward the north (Fig. 2), likely a within canopy shading effect. Although of minimal practical concern, due to the large sample size this slight one degree difference proved to be highly significant statistically.

Determining topophytic effects during container nursery production. Due to rooting inconsistencies, only five of the six clones initially propagated were used in the experiment and sample sizes were uneven for each of the remaining clones. Three clones (MX4MC17, MX6MC14, EP7DC29) represented families with the most obtuse branch angles observed in the field study while only two clones (MX2MC31, MX3MC13) represented those families with the most acute branch angles (Fig. 1). Clone EP6DC14 was excluded from the experiment due to poor rooting percentages and a high mortality rate for successfully rooted cuttings.

No significant interactions ($P \leq 0.05$) were found among the clones propagated and the location of the shoots for rooted cuttings (ramets) on the original tree (ortet) for stem angle deviation from vertical, initial height at transplant from the mist bench, final height or final trunk diameter (caliper) after nursery production (Table 2). The main effects of canopy location were also not significant ($P \leq 0.05$) for any of these parameters. A main effect of clone was highly significant ($P \leq 0.0001$) for all parameters measured, with the exception of initial caliper at transplant from the mist bench (Table 2).

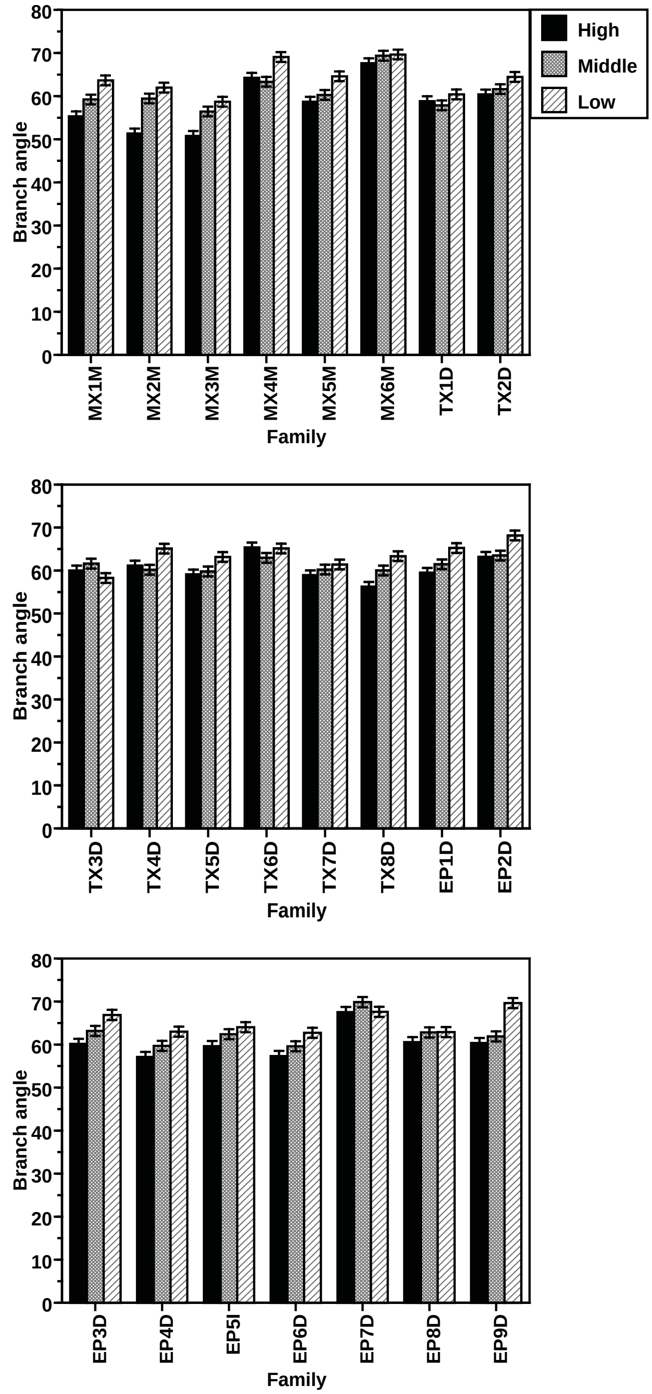

Fig. 1. Means (± standard errors) for family by canopy location effects on branch angles of field-grown trees across all families and positions in the canopy measured, n = 108.

A main effect of clone was significant ($P \leq 0.0001$) for the final stem angle of the ramets at the end of nursery production (Table 2). No significance ($P \geq 0.05$) was found for the cutting position in the canopy. These results confirm the non-quantified observations of clonal significance with coast redwood [*Sequoia sempervirens*] by Power et.al. (6); however, they contradict other results of the same research in which topophysis significantly affected the stem angle of ramets. Results of the current research indicate that in baldcypress, genetic effects (clonal variation) are the most

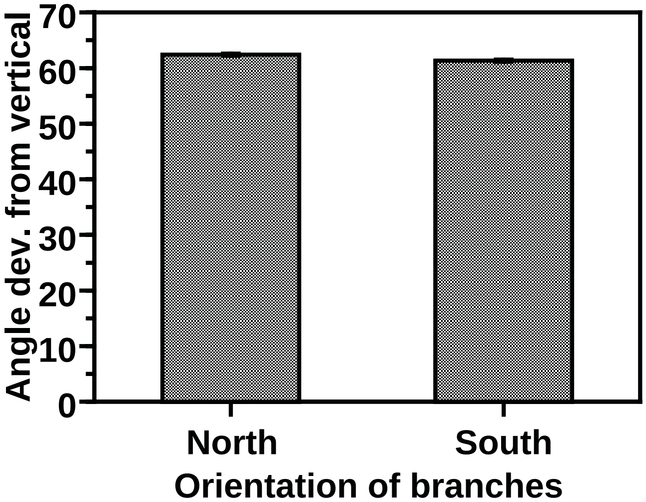

Fig.2. Means (± standard errors) for main effect of cardinal direction on angles of branches oriented to the north or south in field-grown baldcypress, n = 108.

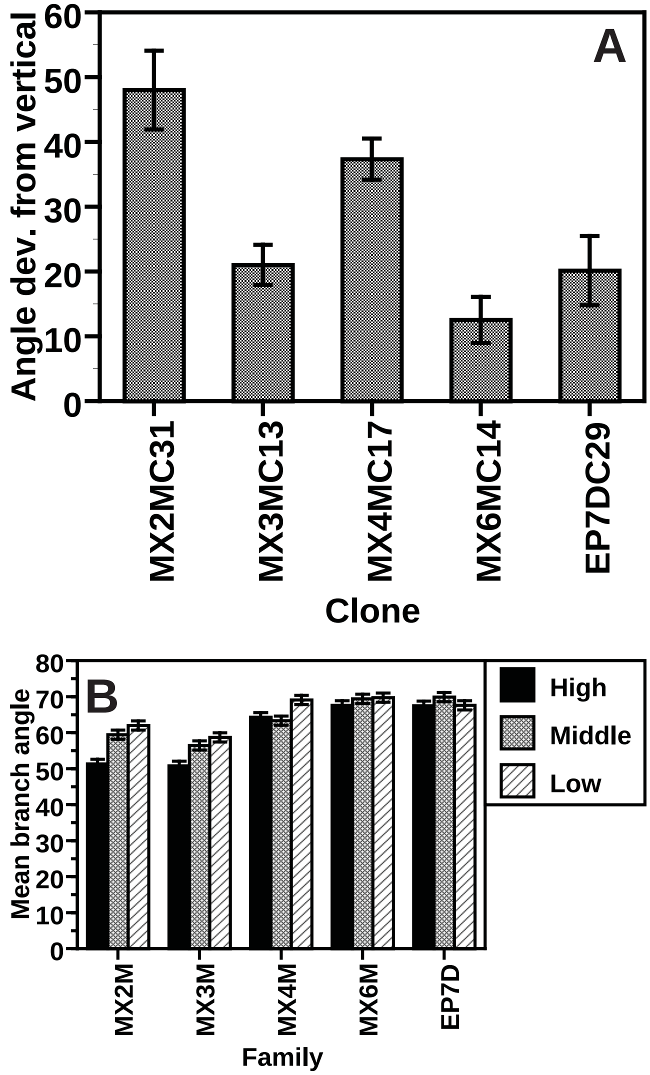

Fig. 3. Means (± standard errors) for the angle at which the cuttings (ramets) grew in a container (A) and the interaction of clones by canopy location on branch angles observed in the field (B) for five families (ortets) tested for topophytic and cyclophytic response, n = 8–29 and n = 72, respectively.

important factors influencing orthotropic and plagiotropic growth habits in the ramets. Topophytic responses of ramets relative to the location of cuttings on the ortet were not observed. These results however do not exclude the possibility of baldcypress displaying topophytic responses when more mature ortets or different clones are tested. Power et al. (6) reports the tendency of increased topophytic response with increasing maturity of the ortet in coast redwood.

The significance of the clonal effects on divergence from vertical growth suggests that it would be possible to select clones with increased chances of producing rooted cuttings with vertical leaders that would not require as much staking during production (Fig. 3A). It also suggests, at least for these clones and age of materials tested, that the location in the canopy from which cuttings are harvested would not alter staking requirements in the nursery. The initial angle of the branches (acute versus more obtuse, Fig. 3B) on the ortet in the field was not a good predictor of the tendency of ramets to grow vertically or not when rooted and grown in the nursery (Fig. 3A). This suggests the factors controlling branch angle divergence from the main trunk and tendencies for topophytic responses are under different mechanisms of control.

Main effects of clones propagated were also significant ($P \leq 0.0001$) for both initial and final height of the ramets (Table 2). Since the initial heights were measured immediately after transplanting from the mist bench this measurement gives insight into the amount of growth that occurred during the 12-week propagation period. Clone MX4MC17 grew to just over 9 cm (above the substrate) on average while clone EP7DC29 averaged approximately 4.1 cm per cutting (Fig. 4A). This difference of approximately 5 cm (2.0 in) is likely caused by the planting depth of the cutting (determined by the height of the uppermost adventitious root regenerated which was planted just below the substrate surface), but may also represent small differences in shoot elongation during rooting in the mist bench. Upon final height measurements, clone MX3MC13 had grown to approximately 40 cm while clone MX6MC14 had reached only 24 cm (9.4 in) in height, a difference of over 33% (Fig. 4B). The final height of the ramet was positively correlated with the initial height ($R^2 = 0.42$, $P \leq 0.0001$) indicating that the same clones which showed increased vigor in the mist bench were generally the most vigorous during the remainder of the experiment.

A significant ($P \leq 0.0001$) main effect of clone propagated was observed for the final trunk diameter of the ramets in the nursery. The initial trunk diameter measurements of the

Table 2. Levels of significance of factors in analysis of variance for stem angle and growth parameters in containers for rooted cuttings from clones with obtuse versus acute branch angles originating from upper versus basal portions of the ortet canopy.

Source of variation	Stem angle	Initial height	Final height	Initial caliper	Final caliper
Clone	****[z]	****	****	ns	****
Location	ns	ns	ns	ns	ns
Clone × location	ns	ns	ns	ns	ns

[z]Significant at $P \leq 0.05$, 0.01, 0.001, 0.0001 = *, **, ***, ****, respectively; ns = not significant at $P \leq 0.05$.

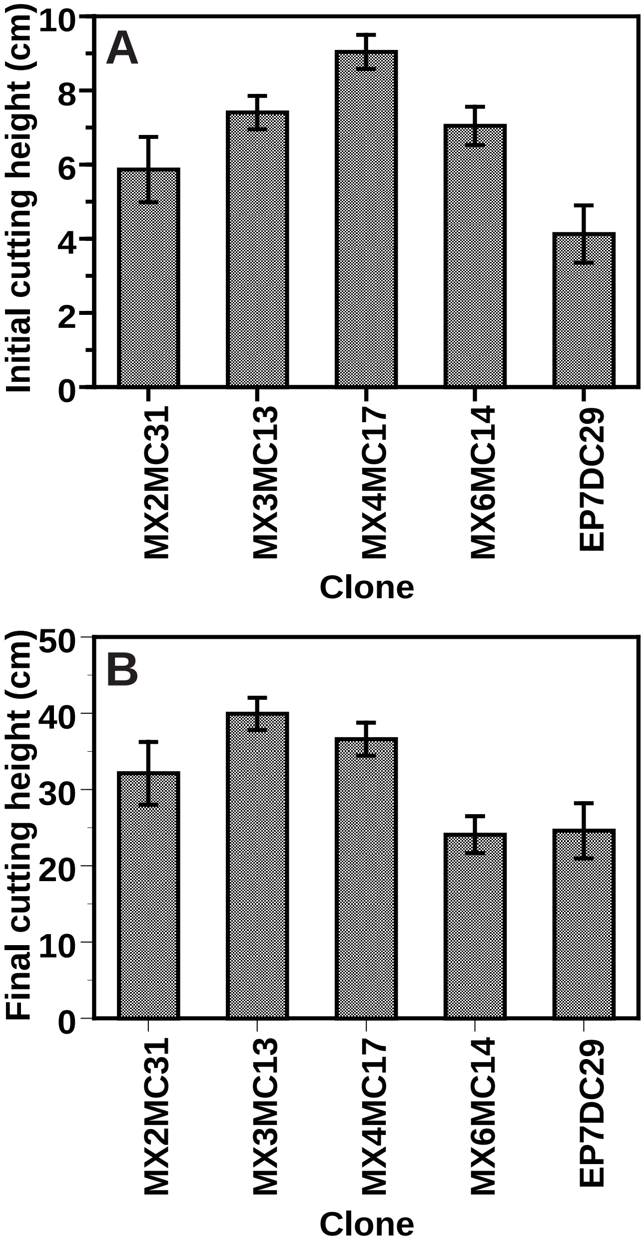

Fig. 4. Means (± standard errors) for the main effects of clones on the initial (A) and final (B) cutting heights in containers, n = 8–29.

plants were not significant (Table 2). This non-significance in the mist bench was likely due to the care taken to make the cuttings as uniform as possible across clones and the relatively slow rate of trunk girth growth in the mist bench when compared to height. Upon measurement of the final trunk diameter, clone MX3MC13 had grown to a trunk diameter of approximately 5.2 mm (0.20 in) while clone MX2MC31 was approximately 2.8 mm (0.11 in) (Fig. 5). This difference of approximately 2.5 mm (0.10 in), though relatively small, is quite significant in light of the fact that the difference between the greatest and smallest initial trunk diameter was 0.37 mm (0.015 in). This confirms Denny's (3) previous reports of differential growth responses among these families in the nursery and field. Final trunk diameter was also positively correlated with both final ($R^2 = 0.86$, $P \leq 0.0001$) and initial height ($R^2 = 0.39$, $P \leq 0.0001$) measurements, respectively.

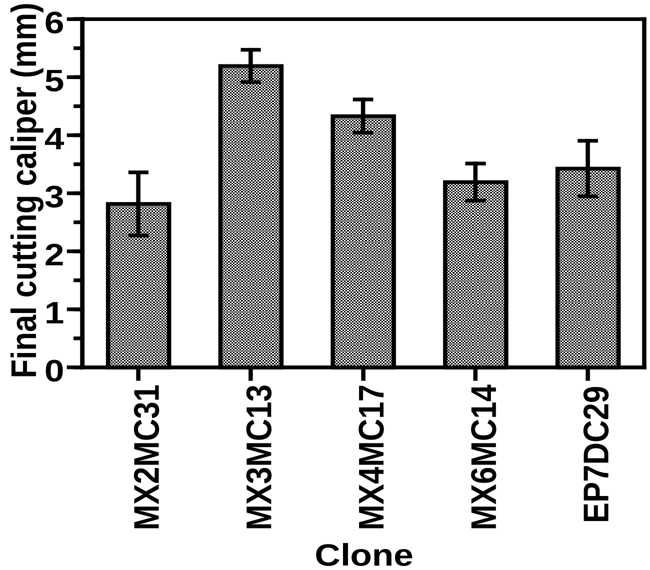

Fig. 5. Means (± standard errors) for the main effects of clone on trunk diameter (caliper) of cuttings at the end of the experiment revealed significantly different ($P \leq 0.05$) growth rates, n = 8–29.

In this study, branch angles of the ortet in the field did not correspond with the stem angle that the ramets developed relative to the substrate surface during container production (Fig. 3). The location in the canopy of the ortet from which cuttings were taken (high, low) proved to be not significant ($P \leq 0.05$) for divergence of stems from vertical, whereas the clonal effect was highly significant ($P \leq 0.0001$) (Table 2). These results suggest that clones can be selected which will grow more vertically and present less problems with topophytic responses during nursery production. Branch angle of the ortet was not a good predictor of this response.

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