Timing of *Pistacia chinensis* Bunge. Rooting Using Morphological Markers Associated with Calendar Date and Degree Days

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Abstract. The objective of this study was to determine the most advantageous time to collect cuttings of Chinese pistache, a commonly recommended ornamental shade tree that is difficult to propagate by cuttings. In 1993, calendar date and degree days (daily mean temperature -7.2°C) were used to estimate an appropriate cutting time. The greatest percentage of rooted cuttings occurred in male cuttings harvested on 13 May 1993 (397 degree days) and treated with $17,500\text{ mg}\cdot\text{liter}^{-1}$ IBA or in male cuttings harvested on 20 May 1993 (482 degree days) and treated with either 8750 or $17,500\text{ mg}\cdot\text{liter}^{-1}$ IBA. In 1994, cutting time was associated with calendar days, degree days, and morphology. The most rooted cuttings (44%) were from green softwood cuttings taken on 9 May 1994, which was 380 degree days from orange budbreak using a threshold temperature of 7.2°C . Orange budbreak was characterized by separation of the outer bud scales such that the orange, pubescent inner bud scales were visible. Cuttings taken on 9 May 1994 and treated with $8750\text{ mg}\cdot\text{liter}^{-1}$ IBA produced the most primary and secondary roots and the longest primary roots per cutting. Male Chinese pistache cuttings should be collected from green softwood or red semi-softwood stems when about 380 to 573 degree days have accumulated after orange budbreak. Chemical names used: indolebutyric acid (IBA).

Chinese pistache is hardy in USDA hardiness zones 6 through 9 (Dirr, 1990), flourishes in full sun, and reaches a mature height of 9 to 12 m with a 4.2- to 9-m spread (Whitcomb, 1985). This tree is native to well-drained, alkaline soils and is tolerant of many soil conditions (Lee et al., 1976a). It is recommended for its drought hardiness, salt tolerance, and extreme heat and wind tolerance (Dewers, 1981; Behboudian et al., 1986). Furthermore, the tree endures winter temperatures to -26°C (Koller, 1978). As a street tree, it continues healthy growth even when planted on narrow spacings (Long, 1960). Most trees display shades of brilliant orange-red, although extreme diversity in coloration can exist even within the same tree (MacMillian Browse, 1988). Foliage is lush throughout the season and does not suffer any major insect or disease problems (Dirr, 1990; Whitcomb, 1985).

Currently, Chinese pistache trees are propagated from seed. Unfortunately, however, there is no dependable seed source for this species (MacDonald, 1986). Asexual reproduction of Chinese pistache would allow selection of cultivars having desirable characteristics such as reliable cold hardiness, vivid fall color, strong branching habit, and interesting bark texture. Male Chinese pistache cultivars could also be marketed for use in locations where fruitless trees are needed.

Adult cuttings of Chinese pistache are difficult to root. However, there has been some rooting success with cuttings from juvenile seedlings (Lee et al., 1976b; Pair and Khatamian, 1982). Attempts to root *Pistacia vera* L. cuttings from adult trees have been limited or unsuccessful with high concentrations of indolebutyric acid (IBA) (Al Barazi and Schwabe, 1982, 1984, 1985).

Juvenility in Chinese pistache seems to be lost after two years (Lee, et al., 1976b; Pair and Khatamian, 1982), making asexual propagation thereafter more difficult.

Proper timing is crucial to rooting success in many woody species. Methods for predicting optimum cutting time for various species include use of calendar date (Barnes and Lewandowski, 1991; Burd and Dirr, 1977), number of calendar days past budbreak (MacDonald, 1986; Whitcomb, 1982), degree day chilling-units (Major and Grossnickle, 1990), morphological condition of the cutting (Adams and Roberts, 1967), use of indicator plants (Congdon, 1965), and photoperiod (Butcher and Wood, 1984). Estimates of appropriate cutting time by degree day heat-units have not been documented, but they are commonly used to predict budbreak (Sparks, 1993; Spiers, 1976), leaf emergence (Eisensmith, et al., 1980), flowering (Anstey, 1966; Mainland, 1986), and harvest time (Addison, 1969; Fisher, 1962).

The objective of this study was to determine if a window of rootability exists for Chinese pistache which can be defined using calendar days, degree days, or morphological markers so that appropriate cutting dates could be determined regardless of geographic location.

Materials and Methods

Calendar timing. Cuttings were collected from two 34-year-old male and two 34-year-old female trees at 2-week intervals from 13 May 1993 through 24 Aug. 1993. Cuttings were immediately placed in 10C tap water, taken to a greenhouse and recut to 10 cm long. The basal 1 cm of the cuttings was dipped for 5 sec in $17,500\text{ mg}\cdot\text{liter}^{-1}$ IBA. The auxin solution was prepared by dissolving the appropriate amount of IBA in 50 ml of 70% isopropyl alcohol then using tap water to bring the solution to 100 ml. After auxin treatment, cuttings were placed in 12 cm wide $\times$ 36 cm long $\times$ 6 cm deep plastic rooting flats containing 1 peat : 4 perlite (by volume) and then kept in a polyethylene greenhouse under natural photoperiod with a maximum photosynthetic photon flux (PPF) of $845\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and maximum/minimum air temperatures of $36/13^{\circ}\text{C}$.

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Table 1. Observation, bud diameter, number of calendar days from budbreak, and degree days using a threshold temperature of 7.2C from budbreak of various morphological characteristics of Chinese pistache in 1994.

Date	Morphological marker	RHS color chart description of stem color ^z	Bud diam (mm) ^y	Calendar days from budbreak ^x	Degree days from budbreak ^x
18 Mar.	Dormant terminal bud	Greyed-Green 197D	4.6	---	---
24 Mar.	Orange budbreak	Greyed-Green 197D	4.8	---	---
04 Apr.	Green budbreak	Greyed-Green 197D	---	11	31
11 Apr.	Closed cone	Greyed-Green 197D	---	18	73
16 Apr.	Open cone	Greyed-Green 197D	---	23	123
20 Apr.	Bud completely open	Greyed-Green 197D	---	27	177
24 Apr.	Early shoot expansion	Yellow-Green 144B	1.7	31	249
09 May	Green softwood stem	Yellow-Green 144B	2.8	46	380
25 May	Red semi-soft stem	Greyed-Red 181B	3.6	62	573
07 June	Red semi-hard stem	Greyed-Orange 174B	4.0	75	799
06 July	Brown semi-hard stem	Greyed-Orange 164B	4.2	104	1371
05 Aug.	Brown hardwood stem	Greyed-Orange 164B	4.1	134	1937
16 Nov.	Dormant terminal bud	Greyed-Green 197A	4.3	237	---

^zRoyal Horticultural Society color charts.

^yMean of 10 replications. Buds measured were the third bud basipetal to the terminus.

^xBudbreak is considered to be when the outer bud scales open and the orange inner bud scales are showing.

Flats were placed on benches equipped with Flora Mist nozzles (A.H. Hummert, St. Louis) with an output of 32 liters/h placed 50 cm above the flats at 121-cm intervals. Mist cycles were adjusted as necessary to allow foliage to dry before misting, and averaged 2-sec duration every 8 min between 0800 and 1800 HR daily.

Degree days were calculated from 15 Mar. 1993 to the cutting date using the following formula: [(daily maximum temperature + minimum temperature)/2 - threshold temperature]. A threshold temperature of 7.2C was used (Baker and Brooks, 1994; Brown, 1952). Degree day calculations began 15 Mar. 1993 because this was the earliest date in which daily temperatures were consistently warm enough to accumulate degree day heat units using a 7.2C threshold. The 15 Mar. 1993 date was before budbreak.

A split-block design was used with 3 replications of 10 subsamples per treatment combination. Gender was the main plot and cutting date the subplot. Cuttings were evaluated 12 weeks after planting using the following rating scale: 0 = dead, 1 = no callus or roots, 2 = callus present, 3 = roots present. Statistical analysis was performed using analysis of variance (ANOVA) with mean separation by the protected least significant difference (LSD) procedure.

A second study was conducted using cuttings harvested on 13 or 20 May from the same male and female trees. Cuttings were treated with either 8750 or 17,500 mg-liter⁻¹ IBA. Treatment combinations were replicated three times with 10 subsamples per replication. The experimental design was a split plot with gender and IBA concentration as the main plots and cutting date as the subplot. Other conditions were identical to those described earlier.

Morphology and degree days. Morpho-

logical changes of one 17-year-old male Chinese pistache tree at the Oklahoma State Univ. Nursery Research Station, Stillwater, Okla. were documented from 18 Mar. 1994 through 16 Nov. 1994 (Table 1, Fig. 1). Cuttings were collected on dates which corresponded to the morphological conditions indicated in Table 1. Terminal cuttings from lateral shoots were harvested from the upper canopy. Cuttings were treated as described above except that they were recut to 9 cm long immediately below a reproductive bud then the basal 1 cm was dipped for 5 sec in a solution of 1) tap water-no auxin, 2) 8750 mg-liter⁻¹ IBA, 3) 17,500 mg-liter⁻¹ IBA, or 4) 35,000 mg-liter⁻¹ IBA. Cuttings were placed in 25 cm wide × 16 cm long × 8 cm deep plastic flats and placed under the environmental conditions described above, except that benches were equipped with DGT nozzles (A.H. Hummert) with an output of 2 liters/min and maximum/minimum air temperatures were 38/22C. Diameter of the third bud basipetal to the terminal was measured on one randomly selected cutting from each flat.

Visible morphological changes were recorded throughout the growing season. Stem color was based on Royal Horticultural

Fig. 1. Morphological changes in Chinese pistache cuttings from 18 Mar. through 4 Apr. (A) 18 Mar., dormant terminal bud; (B) 24 Mar., orange budbreak with outer bud scales open and pubescent orange inner buds closed; (C) 4 Apr., green budbreak, with pubescent orange inner bud scales open and green leaf tissue exposed.

Table 2. Visual root rating and percentage of cuttings rooted 12 weeks after planting male and female Chinese pistache cuttings that were harvested at 2-week intervals in 1993 and treated with 17,500 mg-liter⁻¹ IBA. Rating scale: 0 = dead, 1 = no callus, no roots, 2 = callus present, and 3 = roots present.

Cutting date	Degree days	Tree gender	Visual root rating	Percent rooted
13 May	397	Male	2.3	33.3
		Female	1.8	0.0
20 May	482	Male	2.5	50.0
		Female	2.0	3.3
1 June	642	Male	1.8	0.0
		Female	1.7	0.0
16 June	887	Male	1.8	10.0
		Female	2.2	16.7
2 July	1196	Male	1.4	0.0
		Female	1.5	0.0
13 July	1421	Male	0.2	0.0
		Female	1.3	0.0
27 July	1725	Male	1.0	0.0
		Female	1.0	0.0
10 Aug.	2006	Male	1.2	0.0
		Female	1.5	0.0
24 Aug.	2326	Male	1.0	0.0
		Female	1.0	0.0
Significance (LSD _{0.05})				
Gender for the same date			1.2	
Gender for different dates			3.6	

Society (RHS) color charts and recorded for five random cuttings per observation time (Table 1), with photographs illustrating the morphological condition from 18 Mar. through 4 Apr. (Fig. 1). Calendar days and degree days were calculated from orange budbreak (Table 1) to the date the morphological change was observed or cutting date. Degree days were calculated as described above.

A split-block design was used with 10 replications of 5 subsamples for each cutting date. IBA concentration was the main plot and cutting time was the subplot. Cuttings were evaluated 12 weeks after planting for primary root number, length of three longest primary roots, and number of secondary roots on three longest primary roots. Cuttings were rated using the previously described rating scale. Analysis of variance procedures (GLM) were performed on visual ratings using SAS statistical software (SAS Institute, Cary, N.C.). Protected LSD values were determined for visual ratings and means and standard errors were calculated for primary root number

and length and secondary root number on cuttings that rooted.

Results

Calendar timing. There was a significant interaction between cutting date in 1993 and tree gender for visual root rating (Table 2) with cuttings harvested from male trees on 13 or 20 May 1993 (397 or 482 degree days (DD), respectively) having higher root ratings and rooting percentages than cuttings harvested from either male or female trees on 27 July and 24 Aug. 1994 (1725 and 2006 DD). Regardless of gender, no rooting occurred on cuttings harvested on or after 2 July (1196 DD).

There was a significant interaction between cutting date, tree gender and IBA concentration for visual root rating (Table 3). Cuttings that were harvested 13 May (397 DD) from male trees and treated with 8750 mg-liter⁻¹ had a significantly lower visual root rating than those harvested on the same date from male trees and

Table 3. Visual root ratings and percentage of cuttings rooted 12 weeks after treating the basal 1 cm of male and female Chinese pistache cuttings with 8750 or 17,500 mg-liter⁻¹ IBA and planting on 13 and 20 May 1993. Rating scale: 0 = dead, 1 = no callus, no roots, 2 = callus present, and 3 = roots present.

Cutting date	Degree days	Tree gender	IBA concn (mg·liter ⁻¹)	Visual root rating	Percent rooted
13 May	397	Male	8,750	0.9	3.3
			17,500	2.3	33.3
		Female	8,750	1.5	3.3
			17,500	1.8	0.0
20 May	482	Male	8,750	2.4	40.0
			17,500	2.5	50.0
		Female	8,750	2.1	13.3
			17,500	2.0	3.3
Significance (LSD _{0.05}):					
Concentration and gender for the same date				1.1	
Concentration and gender for different dates				1.3	

Table 4. Sources of variation and significance for visual root rating in 1994. Treatment means are shown in Table 5.

Source of variation	df	Sum of squares
Block	9	10.87***
IBA (I)–linear (L)	1	1.82
I–quadratic (Q)	1	5.35**
I–cubic (C)	1	5.90**
Error A	27	14.99
Date (D)	7	564.98***
D × I–L	7	4.25
D × I–Q	7	9.99**
D × I–C	7	8.05**
Error B	252	107.16

treated with 17,500 mg·liter⁻¹ and those harvested on 20 May (482 DD) from male trees and treated with either IBA concentration. There was no significant difference in visual root rating of female

trees regardless of cutting date or IBA concentration.

Morphology and degree days. The interaction of IBA with cutting date was significant at $P \leq 0.01$ (Table 4). Green softwood cuttings taken on 9 May 1994, (380 DD after orange budbreak) and receiving 8750 mg·liter⁻¹ IBA had the highest root rating with the most rooted cuttings (44%) compared to cuttings harvested on all other dates, except 25 May (Table 5). The largest number of primary and secondary roots and longest primary roots occurred on cuttings harvested on 9 May and treated with 8750 mg·liter⁻¹ IBA. With the exception of one cutting on 6 July, rooting only occurred when cuttings were taken between orange budbreak and red semi-hardwood stems (0 to 799 DD). No rooting occurred when the parent plants had been exposed to more than 799 DD.

Discussion

Many cuttings, including *Acer palmatum* Thunb., *Betula nigra* L., *Betula pendula* Roth, *Fraxinus* spp. and cultivars, *Ulmus*

Table 5. Effect of cutting date and IBA concentration on visual root rating in 1994 using a scale of 0 to 3 in which 0 = dead, 1 = no callus, no roots, 2 = callus present, and 3 = roots present on Chinese pistache cuttings. The percentage of cuttings rooted was determined and the number and length of primary roots and number of secondary roots on rooted cuttings were evaluated.

Cutting date	Degree days ^z	IBA concn (mg·liter ⁻¹)	Visual root rating	Percent rooted	No. of primary roots (mean ± SE)	Length of primary roots (mm) (mean ± SE)	No. of secondary roots (mean ± SE)
18 Mar.	---	0	0.6	0.0			
		8,750	0.5	0.0			
		17,500	0.5	0.0			
		35,000	0.7	0.0			
25 Mar.	---	0	0.4	0.0			
		8,750	0.5	0.0			
		17,500	0.5	0.0			
		35,000	0.4	0.0			
9 May	380	0	1.9	4.0	0.0 ± 0.2	1.1 ± 5.9	0.0 ± 0.0
		8,750	2.4	44.0	1.1 ± 1.9	31.8 ± 60.2	1.9 ± 4.2
		17,500	1.9	12.0	0.2 ± 0.5	9.9 ± 36.0	0.44 ± 2.6
		35,000	1.8	6.0	0.1 ± 0.5	6.4 ± 31.8	0.2 ± 0.9
25 May	573	0	1.9	2.0	0.2 ± 1.6	1.3 ± 9.2	0.1 ± 0.4
		8,750	2.1	14.0	0.4 ± 1.6	4.4 ± 20.3	0.2 ± 1.2
		17,500	2.0	2.0	0.0 ± 0.1	0.2 ± 1.4	0.0 ± 0.0
		35,000	1.7	0.0			
7 June	799	0	0.8	0.0			
		8,750	1.4	0.0			
		17,500	1.1	0.0			
		35,000	0.7	0.0			
6 July	1371	0	0.0				
		8,750	1.1	2.0	0.0 ± 0.1	3.74 ± 26.4	0.34 ± 2.4
		17,500	1.1	0.0			
		35,000	1.0	0.0			
5 Aug.	1937	0	1.0	0.0			
		8,750	0.9	0.0			
		17,500	1.0	0.0			
		35,000	0.0	0.0			
16 Nov.	---	0	0.0				
		8,750	0.0	0.0			
		17,500	0.0	0.0			
		35,000	0.0	0.0			
Significance (LSD _{0.05})							
Date for the same concentration			0.6				
Date for different concentration			0.6				

^zBased on a threshold temperature of 7.2C (Baker and Brooks, 1944; Brown, 1952).

parvifolia Jacq., *Cotinus coggygia*, Scop. and *Cercis canadensis* L. (Barnes and Lewandowski, 1991), have a short (4–6 weeks) window of rootability. Burd and Dirr (1977) found May and early June cuttings had the highest rooting percentages for mature *Malus* L. Chinese pistache also appears to have a limited period of rootability during May.

In 1993, cuttings from male Chinese pistache trees consistently rooted more than cuttings from female trees. Similar results were reported on *Acer rubrum* L. trees (Snow, 1942). Gender did not affect primary or secondary root production, or primary root length, in other Chinese pistache studies (Dunn, 1995).

The degree day (heat-unit) system has been used in horticulture to predict phenological events such as budbreak (Sparks, 1993) and leaf emergence (Eisensmith et al., 1980). Use of degree days from budbreak to predict the most advantageous rooting time has not been previously reported. Major and Grossnickle (1990) used a method of accumulation of chilling units to determine collection time. Using *Juniperus*, they found that the chilling-unit method can be used to determine rooting ability for plants with a narrow window of opportunity for rooting. *Prunus serrulata* 'Kwanzan' Lindl. rooting declined more than 30 percent from mid-July to mid-August as stem hardening progressed (Barnes, 1989). In our studies, with the exception of one cutting, rooting was limited to cuttings with green soft stems or red semisoft stems (380 to 573 DD) regardless of IBA treatment. Schmidt (1989) and Barnes and Lewandowski (1991) stated that the status of the shoot harvested for rooting outweighs the effect of chemical treatments. In 1993 cuttings receiving 17,500 mg-liter⁻¹ IBA produced the highest visual root rating, and in 1994, 8750 mg-liter⁻¹ IBA produced the most roots per cutting and the most rooted cuttings.

In conclusion, Chinese pistache has a narrow window of rootability. The greatest potential for root formation occurred 20 May in 1993 and 9 May in 1994. The 20 May 1993 date corresponded to 482 DD while the 9 May date corresponded to 380 DD, using a threshold temperature of 7.2C. While the criteria for starting DD calculations differed somewhat by year, rooting potential essentially ended after accumulation of 573 DD each year. Morphologically, the highest percentage of rooted cuttings was from green softwood stems matched to yellow-green 144B on the RHS color chart, with buds about 2.8 mm in diameter.

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