VEGETATIVE PROPAGATION OF

CHINESE PISTACHE

By

DIANE ELAINE DUNN

Associate of Applied Science
Oklahoma State University
Oklahoma City, Oklahoma
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Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
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Thesis Approved:

Janet C. Cole
Thesis Adviser

Michael Smith Jr.

Douglas C. Head

James D Ownby

Thomas C. Collins
Dean of the Graduate College

PREFACE

Chinese pistache (*Pistacia chinensis*, Bunge.) is a commonly recommended ornamental shade tree in the nursery and landscape industry. Currently, Chinese pistache trees are propagated commercially from seed, resulting in highly variable branch habit and fall color. Mature Chinese pistache, have proven difficult to root, graft, or bud successfully. This study was initiated to investigate the effect of various timing, auxin, bottom heat, and bud position treatments on root formation of cuttings. It also investigated the potential of mound layering and tissue culture as alternative vegetative propagation methods for producing genetically identical clones of superior mature Chinese pistache trees.

I appreciate the research assistantship given me by the Horticulture department. I want to thank my major professor Dr. Janet Cole for accepting me as her graduate student, advising me in many areas of course work and research, and editing this thesis and associated research papers. I appreciate the support she has given me, especially this last year. I couldn't have done it without her. I thank Dr. Mike Smith for serving on my committee and for all his statistical expertise. I appreciate his advice and the time spent writing programs on my behalf. I also thank Dr. Doug Needham and Dr. James Ownby for serving on my committee. Their knowledge of plant propagation and plant physiology served as a comforting safety

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"*For there is hope for a tree--if it's cut down it sprouts again, and grows tender new branches. Though its roots have grown old in the earth, and its stump decays, it may sprout and bud again at the touch of water, like a new seedling.* Job 14:7-9 (LB)

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INTRODUCTION

Pistacia chinensis Bunge. is an ornamental member of the cashew family Anacardiaceae (Stoffey, 1981). *Pistacia chinensis*, commonly known as Chinese pistache, is a dioecious, deciduous tree with alternate, pinnately compound leaves (Copeland, 1955). From a taxonomic point of view, members of the family Anacardiaceae have been described by Hutchinson (1926) and Rendle (1938). Wood anatomy of members of this family was described by Record (1939). The wood is characterized as having lactiferous or resin canals in the vascular bundles of the stems, roots, leaves, and fruits. The wood of Chinese pistache is valued for its toughness, being used as boat rudders for Chinese junks. It is also used in China as fuel for heating (Wilson, 1905), and young shoots are eaten as a vegetable (Crockett, 1972). Chinese pistache occurs in abundance in China, being native to thirteen of the eighteen provinces, but it has never been found growing naturally outside of China (Whitehouse, 1957). Height of mature trees in China often reaches 18.5 meters (Whitehouse, 1957), but in Kansas 10-15 meters (Pair and Khatamian, 1982), and in Oklahoma 6-12 meters (Whitcomb, 1985) are the normal heights obtained. *Pistacia* spp. naturally grow in a well drained soil with a high lime content (Whitehouse, 1957).

Chinese pistache is in the same genus as the edible pistachio nut, *Pistacia vera* L. (Joley, 1969). Among others in the genus *Pistacia* are *P. terebinthus* L. which yields turpentine and tannins, and *P. lentiscus* L. which yields mastic (Whitehouse, 1957). The pistachio is the only commercially acceptable edible nut of some 12 to 15 *Pistacia* species. Whitehouse (1957) stated that pistachio nut cultivars propagated on *P. chinensis* have been short lived compared to those on other species, and extreme scion-rootstock incompatibility occurs. He also noted *P. atlantica* Desf. grows twice as fast as *P. chinensis.* Interspecific hybridization readily occurs among *P. vera, P. atlantica, P. terebinthus, P. palaestina* L., *P. integerima* J.L. Steward ex Brandis, and *P. chinensis* (Crane, 1986). Seed availability is affected by pronounced alternate bearing in *Pistacia* species (Crane and Nelson, 1971). Chinese pistache was specifically noted as an undependable seed source by MacDonald (1986). *Pistacia* spp. are wind pollinated and the viable pollen may drift more than half a mile (Joley and Opitz, 1971), causing seedling diversity.

Copeland (1955) did extensive research on the reproductive structures of Chinese pistache. He found clusters of flower buds of both sexes appear in February or March, in advance of the foliage. Flower buds are in axillary positions on twigs of the previous year.

Root regenerating potential of one-year-old Chinese pistache was studied by Lee and Hackett (1976). They proposed that at least two factors are required for root regeneration of *Pistacia chinensis.* One was the presence of physiologically non-dormant buds which provide auxin to stimulate cambial activity and xylem

differentiation. The other controlling factor was the availability of carbohydrates which influence phloem differentiation. Lee, et al. (1976a), studying bare root seedlings and rooted cuttings from 2-year-old Chinese pistache, showed the highest number of roots were regenerated in media at 75% Ca saturation and 20% air filled porosity. Paul and Leiser (1968) found Ca saturation of sphagnum peat greatly increased rooting percentages in *Euonymus fortunei*' Colorata' Rehd., *Hebe salicifolia* (G. Forst.) Penn., and *Pyracantha* M.J. Roem. Root pruning of Chinese pistache has been studied (Harris, et al., 1971) to improve root problems common to the species.

The influence of staking on Chinese pistache trunk development was studied by Neel (1968). He observed that staking may actually alter the internal structure of the wood by inhibiting the formation of reaction wood.

When a cutting is made, living cells at cut surfaces are injured and exposed, and the wound healing response begins (Cline and Neely, 1983). The origin of wound-induced *de novo* adventitious roots in stems of *Pistacia* spp. is the phloem area close to the cambium (Jackson, 1986). Anatomical changes that occur in *de novo* wound roots consists of 1) cellular dedifferentiation followed by 2) formation of root initials, 3) development of these initials into root primordia, and 4) growth and emergence of the new roots (Girouard, 1967; Davies et al., 1982). Histological studies showed root initials were formed in the cambial area near the phloem in *Pistacia* (Al Barazi and Schwabe, 1984). Division of the first root-initial cells is triggered by either applied or endogenous auxin (Haissig, 1972). Difficulty in rooting *Pistacia* may involve the fate of endogenous and exogenously applied auxin and its

relation to oxidizing systems in the tissue (Al Barazi and Schwabe, 1984). They postulated that the application of IBA at high concentrations is required initially to trigger the process of root initiation, and indole acetic acid-oxidase (IAA-O) activity was temporarily reduced. The involvement of the enzyme polyphenol-oxidase (PPO) in the rooting process was proposed by Haissig (1974). Haissig also proposed that lack of adventitious root formation in response to auxin may be due to 1) lack of essential enzymes to synthesize root inducing auxin-phenol conjugates, 2) presence of enzyme inhibitors, 3) lack of enzyme activators, 4) lack of substrate phenolics, and 5) physical separation of enzyme reactants. IBA treatments may control endogenous auxin levels of cuttings either through direct regulation of the IAA-O system or indirectly through transport of auxin protectors (Mato and Vieitez, 1986).

Calendar Time, Degree Days, and Cutting Morphology

Burd and Dirr (1977) found May and early June cuttings had the highest rooting percentages for mature *Malus* L. Juvenile pecan (*Carya illinoensis* Koch.) cuttings root at higher percentages when taken in February, June, and August, while adult cuttings root best from 15 Aug. cuttings (Smith and Chiu, 1980). The best rooting in *Quercus palustris* Muenchh. is in early July, while *Tilia cordata* Mill. roots better when cuttings are taken during late June (Chapman and Hoover, 1981). When *Rhododendron* L. cuttings were evaluated on a morphological time scale, rooting capacity decreased with increasing tissue age (Adams and Roberts, 1967). They also found their morphological time scale to be superior to calendar dating for predicting shoot rootability. Seasonal rooting changes in *Malus* hardwood cuttings

was important (Bassuk and Howard, 1981). Seasonal changes influenced rooting of both juvenile and adult (difficult-to-root) *Ficus pumila* L. cuttings; however, treating juvenile cuttings with IBA overcame seasonal fluctuations (Davies, 1984). Stoutemyer (1942) found difficult-to-root *Chionanthus retusus* Lindl. and Paxt., will root in high percentages if obtained during a one week period in May. A very narrow window of rootability was also shown in native *Rhododendron* L. (Nienhuys, 1980), *Syringa* L. (Wedge, 1977; Mezitt, 1978) and *Myrtus communis* L. (Pokorny and Dunavent, 1984). Concomitant with successful timing is the increase/decrease of root promoters/inhibitors, carbohydrate and nutritional balances/imbalances, cutting softness/hardness, and physical barriers (Dirr and Heuser, 1987).

Many methods have been investigated to document the most advantageous cutting time for various species. In addition to calendar days, morphological condition of the cutting and chilling-units based on mean temperatures have been investigated. Morphological condition of the cutting tissue is used as a factor to divide cuttings into three general categories, 1) softwood, 2) semi-hardwood, and 3) hardwood. Softwood cuttings are selected from the current season's growth before extensive lignification has occurred (MacDonald, 1986). Additional characteristics associated with softwood cuttings include: 1) stems that bruise easily; 2) tendency to wilt readily; 3) gradation in leaf size from terminal to base; and 4) requirement for high humidity (mist) to ensure rooting (Barns and Lewandowski, 1991). Semi-hardwood (semi-ripe) cuttings can be subdivided into two groups, soft and firm, based on the degree of lignification. With soft semi-ripe cuttings summer dormancy

has not begun, thus the shoot is still growing; however the lower region of the stem is becoming lignified. With firm, semi-ripe cuttings the shoot has virtually stopped growing following the onset of summer dormancy, and the whole stem is undergoing varying stages of lignification (MacDonald, 1986). Hardwood cuttings are usually cut in the fall or winter when the previous season's growth flush is complete, terminal bud is present, stems are firm and woody, plants have usually been exposed to at least one frost, and leaves of deciduous plants have abscised or can be pulled off easily without tearing bark (Barnes and Lewandowski, 1991).

Reaumur (1735) found the mean daily air temperatures for 91 days during the months of April, May, and June in his locality and determined the sum to be a nearly constant value for the development of any plant from year to year. He assumed this summation of temperatures, known as Reaumur's thermal constant of phenology, expressed the amount of heat required for a plant to reach a given stage of maturity. This idea was the beginning of the heat unit (or degree-days) system used today. Linsser (1867, 1869) concluded that a given plant species reaches the same stage of vegetative development yearly at the time that the same mean daily temperature is reached. Arnold (1960) devised a method of estimating accumulated heat units by means of daily maximum and minimum temperatures and a specified threshold. This degree days system has been used in horticulture to predict phenological events such as budbreak (Sparks, 1993; Spiers, 1976), leaf emergence (Eisensmith, et al. 1980), flowering (Anstey, 1966; Mainland, 1986), and harvest time (Addison, 1969, Fisher, 1962, Gilmore and Rogers, 1958). In plant propagation, Major and Grossnickle

(1990) found chilling units effective to determine collection time for *Juniperus* L. species.

Auxins

Thimann and Went (1934) reported the root promoting hormone indole-3-acetic acid promoted rooting. Thimann and Poutasse (1941) and Kogl, et al. (1934) demonstrated synthetic indoleacetic acid was active in root promotion. It was also demonstrated that naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) were active in adventitious root formation (Thimann and Koepfli, 1935; Zimmerman and Wilcoxon, 1935). IBA and NAA are the most widely used root promoting chemicals used today. Although IBA is the most commonly used, NAA has been shown to be superior in some species (Dirr, 1986; Morini and Isoleri, 1986). Hitchcock and Zimmerman (1939) found higher concentrations of hormones were required later in the season than earlier to promote equal rooting in *Rhododendron* L. Effectiveness of chemicals has been found to be dependent on formulation, too. Dirr (1986) states that liquid formulations of IBA or NAA are superior to the talcs simply because the hormones are in solution and can be readily absorbed. The alcohol acts as a carrier and penetrant facilitating increased movement of hormones into the cutting. Howard (1974) discussed factors such as treatment duration, moisture tension, depth of dipping and epidermal run off. Meahl and Lanphear (1967) reported a quick dip was equal or superior to powder in the promotion of rooting. They noted 8,000 $mg \cdot liter^{-1}$ IBA powder was not as effective as 8,000 $mg \cdot liter^{-1}$ IBA in solution. Gray (1959) suggested the quick dip method be used for hard-to-root species. A 5 sec dip

was as effective as a 160 sec dip in root promotion. A shallow dip was suggested by Howard and Nahlawi (1970) to give better results. Successful rooting of adult *Pistacia vera* utilized the shallow 5 sec quick dip (Al Barazi and Schwabe 1982, 1985). Extremely concentrated quick dips of 20,000 to 40,000 mg·liter^{-1} IBA or NAA will often cause phytotoxicity at the base of the cutting; however, rooting may occur in the untreated region just above the injured tissue (Chong, 1981; Dirr and Heuser, 1987). This rooting above an injured basal area was noted by Al Barazi and Schwabe (1982) with *Pistacia vera*.

Lee, et al., (1976b) found rooting of stem cuttings of *Pistacia chinensis* is greatly promoted by dipping in H_2SO_4 (sulfuric acid) prior to applying IBA. Acid pretreatment promoted rooting of plants native to alkaline soil, suggesting a short exposure to acid may break acid-labile linkages (Ca bridge) in cell walls of calciphilous plants, loosen cell walls, increase permeability (Uhrstrom, 1974), and facilitate absorption of applied auxin and/or emergence of root initials. Lee et al. (1976b) also found pre-treatment reduced loss of foliage in woody species in which root initiation takes more than 6 weeks. While formation of callus and formation of roots are independent of each other (Hartmann, et al., 1990), origin of adventitious roots from callus tissue has been associated with difficult-to-root species (Bhella and Roberts, 1975; Davies, et al., 1982).

Bottom Heat

The optimum media temperature for propagation is 18C to 25C for temperate climate species and 7C higher for warm climate species (Dykeman, 1976; Kester,

1970). Dykeman (1976) found a high temperature of 30C resulted in more rapid root initiation, shorter emergence time, and more roots per *Forsythia* Vahl. cutting. However, subsequent root development including elongation, diameter, root hair formation, and secondary branching, occurred more readily at 25C. Burholt and Vant Hoff (1970) suggested that the rate of cell division increases to a maximum between 30C and 35C. Scott (1972) showed that auxin activity in roots is greater at higher temperatures. The beneficial influence of high temperature on initiation may be due to its effect on translocation of supportive factors (carbohydrates) on the related increase in respiration (Ooishi, et al. 1978) and in catabolism of simple sugars stored in starch at lower temperatures (Veierskov and Andersen, 1982). Bottom heat ranging from 25C to 30C has been used in successful rooting of *Pistacia vera* (Al Barazi and Schwabe, 1982, 1985).

Benomyl

Treatment with a fungicide has been shown to protect newly formed roots from fungal attack, increase survival, and increase overall quality of the rooted cuttings (Wells, 1963; Hansen and Hartmann 1968). It has been reported that some systemic fungicides, such as benomyl, improved rooting of woody cuttings (Fiorino et al. 1969). McGuire and Vallone (1971) found better rooting was obtained with a combination of IBA and benomyl in clones that are normally difficult to root. Hoitink and Schmitthenner (1970) reported a decrease in time required to obtain a good root system when benomyl was combined with auxin.

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Bud Position and Retention

The relationship between buds and root formation has been reported as both inhibitory and advantageous. Van der Lek (1925) was one of the first to report that position of the basal cut was important, noting the difference between wound roots and morphological roots. He pointed out that preformed root initials or the formation of root initials were most abundant in the first one-half inch below the node and that roots were stronger and more capable of supporting the cutting than wound roots. Van der Lek (1934) was also one of the first to report that buds on cuttings promoted rooting. Fadl and Hartmann (1967), obtained 75% rooting on *Pyrus* L. cuttings that contained 4 buds per cutting, while only 15% of the cuttings that had the buds removed rooted. They also found that the buds that promoted rooting in summer, inhibited rooting in the winter. Spiegel (1954) found that the presence of the lowest bud on *Vitis* L. cuttings promoted rooting. Such an influence was also noted by Harada and Nakayama (1957), working with *Camellia sinensis* L. cuttings. They found that rooting was highest in cuttings with 2 or 3 buds and lowest in those without buds. In cuttings with only one bud, roots appeared directly below that bud. Went and Thimann (1937) showed that added auxin would not substitute for buds as a stimulus to root formation. Kemp (1948) suggested that flower buds inhibited the rooting of *Rhododendron* spp. shoots, and DeBoer (1953) increased rooting in this species by removing flower buds. O'Rourke (1940) increased rooting percentages of *Vaccinium* spp. by removing flower buds. Cahlahjan and Nekrasova (1962, 1964), found that *Prunus persica* (L.) Batsch. cuttings bearing sprouting buds

produced no roots; whereas, cuttings with dormant buds rooted satisfactorily. When sprouting buds were removed, root formation was encouraged. Leopold (1964) found auxin and kinetin production associated with actively growing tissues, such as expanding buds. Johnson and Roberts (1968) established that removal of potential flower buds significantly enhances leaf rooting in *Rhododendron* spp. Reproductive bud removal significantly increased the rooting percentage of *Dahlia* Cav. cuttings from 42% to 77% in disbudded cuttings. Flower buds form a stronger "sink" than vegetative buds (Biran and Halevy, 1973).

Gender

Snow (1942) reported that cuttings from male *Acer rubrum* L. trees rooted at higher percentages than those taken from female trees. Edgerton (1944) found that cuttings from male *Acer rubrum*, or females producing little or no seed, rooted more readily than cuttings from more fruitful females.

Mound Layering

Hackett (1985) discussed a biological basis for juvenile and mature growth phase changes. Interactions of hormonal cellular isolation were proposed. Phenolics decrease in some plants with age. This may affect rooting response since phenolics are believed to function as cofactors with auxin in the rooting process (Hess, 1961, 1962, 1963). According to Hess (1969) these cofactors are naturally occurring and are more concentrated in easy-to-root species. Fortainier and Jonkers (1976) state invigoration treatments such as severe pruning can change the status of food reserves, the carbon/nitrogen ratio, auxin distribution, inhibitor/promoter balance,

and levels of cytokinins, gibberellins, and rooting cofactors in the remaining shoot(s). Sax (1962), working with 10-, 50-, and 90-year-old *Cryptomeria* D. Don., found rooting potential was not consistent, indicating strong genetic controls. Roberts and Moeller (1978) concluded ontogenetical aging, particularly flowering, is not a valid explanation for loss of cutting rooting potential with increasing chronological age in Douglas Fir (*Pseudotsuga menziesii* Mirb.).

In an attempt to return *Ulmus americana* L. to a juvenile state, Schreiber and Kawase (1975) pruned 12-year-old elms to four different heights ranging from 23 to 200 cm. Cuttings were collected from sprouts and rooted, with most successful rooting (83%) resulting from the 23 cm stumps. Hoogendoorn (1984) maintains a seedling stock block and successfully roots (60%) of *Acer griseum* (Franch.) Pax. from June cuttings. Maintenance of the juvenile state in *Pinus radiata* D. Don is successful by growing stock plants in hedge rows kept continually pruned (Libby and Hood, 1976; Menzies, 1985). A stool bed or hedge can be used for 15 to 20 years providing it is maintained in a vigorous condition (Hartmann, et al., 1990). Howard, et al. (1988) stated severe pruning, micropropagation and the induction of adventitious shoots, produce plants which are juvenile-like in appearance and vigorous in growth. Propagation of the cashew (*Anacardium occidentale* L.) is accomplished by pruning the whole plant and mound layering vigorous juvenile shoots.

Tissue Culture

Based upon his studies on callus formation and wound healing, Sachs (1880), suggested that plants contain organ-forming substances. Tissue culture later began in earnest in the 1930s when serious attempts were made by researchers to obtain continuously growing cultures from a variety of explants. These included *Acer pseudoplatanus* L., *Sambucus nigra* L., and *Salix capraea* L. In 1955 the discovery of cytokinins as regulators of cell division (Miller et al. 1955a, 1955b) established the importance of growth regulators. Skoog and Miller (1957) discovered that the relative concentrations of an auxin and a cytokinin controlled the morphogenic response of tobacco (*Nicotiana tabaccum* L.) tissue in culture. Cytokinins are routinely used in combination with auxins for the initiation as well as for maintenance of callus cultures. When used at appropriate concentrations cytokinins can induce shoot regeneration from callus in woody angiosperms (Bonga, 1987). Apart from the direct effects of growth regulators, cellular uptake of various organic and inorganic nutrients from the culture medium is also affected (Giladi, et al. 1971; Saftner and Wyse, 1984). Micropropagation of *Pistacia vera* and other *Pistacia* species has been documented (Abousalim and Mantell, 1992; Barghchi and Alderson, 1983a, 1983b, 1985; Martinelli, 1987, 1988; Pontikis, 1984; Picchioni and Davies, 1990, Parfitt et al., 1990, and Parfitt and Almedhi, 1991, 1992). Martinelli (1987) found during the multiplication phase *Pistacia vera, P. integerima,* and *P. atlantica* behaved differently in relation to shoot production, but different clones of the same species behaved similarly. Synthetic cytokinin N^6-benzyladenine or 6-

benzylaminopurine (BA) is more effective than naturally occurring forms at promoting *Pistacia* shoot growth (Barghchi and Alderson, 1983a, 1985).

Objectives of the Research

Chinese pistache is a commonly recommended ornamental shade tree in the nursery and landscape industry. A cultivar with reliable characteristics would be a welcome addition to these industries across the United States. Results of a successful rooting technique may encourage more propagation and production of Chinese pistache in Oklahoma. Three of the top eight nurseries in sales dollars have their headquarters or growing fields in Oklahoma (Brantwood, 1987).

Vegetative propagation of Chinese pistache would offer many benefits. Asexual reproduction in general, preserves desirable genetic traits by producing genetically identical clones. Benefits would include establishment of cultivars for marketing in the nursery industry. These cultivars would be propagated from superior, mature trees that have been evaluated for vigor, vivid fall color, desirable branching habit, dense canopy, attractive bark texture and color, and disease and insect resistance. Establishment of cultivars based on fruit bearing would also be beneficial where fruit would be a problem. Along with eliminating seedling variability, the selection of superior cultivars could eliminate problems such as weak crotch angles (Whitehouse, 1957), crooked trunks and multiple leaders (Dirr, 1990), root defects (Harris, et al., 1971), and problems with transplanting (Lee, et al. 1976a; Lee and Hackett, 1976).

Because of the stated variabilities and advantages that establishment of proven cultivars would bring, asexual propagation would be desirable. Mature cuttings have proven difficult to root (Joley, 1960); however, some rooting success has been obtained with cuttings from seedlings (Pair and Khatamian, 1982; Lee et al. 1976b). This is typical of the difficult to root *Pistacia* genus. Attempts to root *Pistacia vera* cuttings from mature trees have been unsuccessful (Joley and Opitz, 1971) or limited with very high concentrations of indolebutyric acid (IBA) (Al Barazi and Schwabe, 1982, 1984, 1985). Maturity in Chinese pistache seems to be reached after two years (Pair and Khatamian, 1982; Joley, 1960; Lee, et al., 1976b), making asexual propagation more difficult. Inconsistencies in grafting and budding Chinese pistache have also been encountered (Joley, 1960; Long, 1960; Hall, 1975), regardless of budding technique.

These findings suggest that techniques for propagation of Chinese pistache need to be investigated further. Due to the complexity of the interacting rooting factors, a systematic process of establishing optimum requirements for rooting is needed. Many asexual reproduction techniques, such as mound layering and micropropagation, have not been tested on Chinese pistache. Treatments that have been investigated were done several years ago and need to be updated due to new techniques and chemicals now available.

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Chapter II

Cutting Propagation of *Pistacia chinensis* I. Calendar Date, Degree Days, and Morphology

Diane E. Dunn, Janet C. Cole, and Michael W. Smith
Department of Horticulture and Landscape Architecture
Oklahoma State University
Stillwater, OK 74078

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Abstract. **Chinese pistache (*Pistacia chinensis*, Bunge.) is a commonly recommended ornamental shade tree. Currently, Chinese pistache trees are propagated from seed, resulting in highly variable branching habit and fall color. Adult Chinese pistache, like other *Pistacia* are difficult to root, graft, or bud successfully. For many species, the time of the year that cuttings are taken can be critical to rooting success. The objective of this study was to determine the most advantageous time to collect cuttings of Chinese pistache. Methods to document the most advantageous cutting time have included calendar date, days from budbreak, chill-units, and morphological condition. Degree days have not been used to document cutting time, but are commonly used to predict other phenological events. In 1993, calendar date was used to document cutting time with the highest number of rooted cuttings coming from softwood cuttings taken on 20 May and given 17,500 mg·liter^{-1} IBA. In 1994, cutting time was correlated with calendar days, degree days, and morphological change. The most rooted cuttings (44%) were from green softwood cuttings taken on 9 May, which was 46 calendar days, and 380 degree days**

from orange budbreak based on a threshold temperature of 7.2C. The interactions of IBA and IBA by cutting date were significant at $P \leq 0.001$. Cuttings taken on 9 May and treated with 8,750 mg·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 before 799 degree days have accumulated after orange bud break.

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 30 to 40 feet with a 20 to 30 foot spread (Whitcomb, 1985). It develops an oval, umbrella-like crown providing generally light-textured shade throughout the growing season. Chinese pistache is native to well-drained, alkaline soils, but is tolerant to many soil conditions (Lee et al., 1976a). In California, it is recommended for its xerophilous qualities and salt tolerance (Crockett, 1972). It also endures extreme heat and drying winds (Dewers, 1981; Behboudian et al., 1986; Spiegel-Roy et al., 1977). It survives winter temperatures to -26C (Koller, 1978), but is not adapted where spring frost occurs after budbreak. As a street tree, it continues healthy growth even when planted on narrow spacings (Long, 1960). Fall color is variable, ranging from dark red to yellowish-green. Most trees display shades of brilliant orange-red, yet extreme diversity 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 (Crockett, 1972; Whitcomb, 1985; Dirr, 1990).

Currently, Chinese pistache trees are propagated from seed; however, MacDonald (1986) specifically noted Chinese pistache as an undependable seed source. *Pistacia* are wind pollinated with viable pollen drifting more than half a mile (Joley and Opitz, 1971). Vegetative propagation of Chinese pistache offers many benefits. Asexual reproduction allows selection of cultivars with desirable characteristics such as reliable cold hardiness, vivid fall color, strong branching habit, interesting bark texture and color, dense canopy, and disease and insect resistance. Male Chinese pistache cultivars could be marketed for use in locations reserved for fruitless trees.

Adult cuttings are difficult-to-root (Joley, 1960); however, there has been some rooting success with cuttings from juvenile seedlings (Pair and Khatamian, 1982; Lee et al. 1976b). This is typical of the difficult to root *Pistacia*. Attempts to root *Pistacia vera* L. cuttings from adult trees have been limited or unsuccessful with high concentrations of indolebutyric acid (IBA) (Joley and Opitz, 1971; Al Barazi and Schwabe, 1982, 1984, 1985). Juvenility in Chinese pistache seems to be lost after two years (Pair and Khatamian, 1982; Joley, 1960; Lee, et al., 1976b), making asexual propagation more difficult.

Before the introduction of auxin, proper timing was crucial to rooting success. Methods for documenting cutting time for various species include use of calendar date (Burd and Dirr, 1977; Smith and Chiu, 1980; Chapman and Hoover, 1981), number of calendar days past budbreak (Whitcomb, 1982; MacDonald, 1986), degree day chilling-units (Major and Grossnickle, 1990), morphological condition of the

cutting (Adams and Roberts, 1967), use of indicator plants (Congdon, 1965) and number of hours of sunlight (Butcher and Wood, 1984). Estimates of appropriate cutting time by degree day heat-units has not been documented, but they are commonly used to predict budbreak (Spiers, 1976; Sparks, 1993), leaf emergence (Eisensmith, et al., 1980), flowering (Anstey, 1966; Mainland, 1986), and harvest time (Fisher, 1962; Addison, 1969). Another indicator that is used is flowering time. Leach (1965), stated that it is not the time of year which is important, but the condition of the plant tissues, as they mature under the influence of rainfall and temperature, and their individual species differences.

The objective of this study was to determine if a window of rootability exists for Chinese pistache and to document it using calendar days, degree days, and 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 two-week intervals from 27 Jan. 1993 through 24 Aug. 1993. Cuttings were immediately placed in 10C tap water, taken to a greenhouse and re-cut to 10 cm. Cuttings were dipped 5 sec in the following IBA treatments: cuttings taken 27 Jan. to 4 May received 35,000 mg·liter^{-1}, those taken 13 May to 24 Aug. received 8,750 mg·liter^{-1} or 17,500 mg·liter^{-1}, and cuttings taken 16 June through 24 Aug. received 17,500 mg·liter^{-1} or 35,000 mg·liter^{-1}. After the auxin treatment, cuttings were placed in 12 cm wide x 36 cm long x 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and maximum/minimum air temperatures of 36/13C. Flats were placed on benches equipped with Flora Mist nozzles (Hummerts, St. Louis, Mo.) with an output of 32 liters per hour (LPH) 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 HR and 1800 HR daily.

A split-block design was used with 3 replications of 10 subsamples per treatment interaction. Cutting time was the main plot and IBA concentration and gender were the sub-plots. Cuttings were evaluated at 12 weeks using the following rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots. Statistical analysis was performed using analysis of variance (ANOVA) with mean separation by the protected least significant difference (LSD) procedure.

Morphology and degree days. Morphological changes of one 17-year-old male Chinese pistache tree at the Oklahoma State University Nursery Research Station, Stillwater, Okla. were documented from 18 Mar. 1994 through 16 Nov. 1994 (Table 2.1 and Fig. 2.1). Cuttings were collected on the following dates and corresponded to the morphological conditions indicated: 18 Mar., dormant terminal bud before bud break; 25 Mar., orange terminal bud; 9 May, green softwood stem; 25 May, red semi-

soft stem; 7 June, red semi-hard stem; 6 July, brown semi-hard stem; 5 Aug., brown hardwood stem, and 16 Nov., dormant terminal bud after leaf drop. (Table 2.1). Terminal cuttings from lateral shoots were harvested from the upper canopy. Cuttings were placed in 10C tap water, taken into the greenhouse, then re-cut to 9 cm long immediately below a reproductive bud. The basal 1 cm was dipped for 5 sec in a solution of 1) tap water-no auxin, 2) 8,750 mg·liter^{-1} IBA, 3) 17,500 mg·liter^{-1} IBA, or 4) 35,000 mg·liter^{-1} IBA. The auxin solution was prepared by dissolving IBA in 50 ml of 70% isopropyl alcohol. Tap water was used to bring the solution to 100 ml. Cuttings were placed in 25 wide x 16 long x 8 deep cm plastic flats containing 1 peat : 4 perlite (by volume) in a polyethylene greenhouse under natural photoperiod with a maximum PPF of 845 μmol·m^{-2}·s^{-1} and maximum/minimum air temperatures of 38/22C. Flats were placed on benches equipped with DGT nozzles (Hummert, St. Louis, Mo.) with an output of 2 LPM placed 50 cm above flats at 121 cm intervals. Mist was applied for 2 sec every 2 min between 0800 HR and 1800 HR. Mean diameter of the reproductive bud was measured on the third bud basipetal to the terminal on one randomly selected cutting from each flat.

Visible morphological changes were documented throughout the growing season. Stem color was based on Royal Horticultural Society (R.H.S.) color charts and documented on five random cuttings per observation time, (Table 2.1, Fig. 2.1).

Calendar days and degree days were calculated from orange budbreak to the date the morphological change was observed, or from cutting date. Degree days were calculated using the following formula: [(daily maximum temperature +

minimum temperature) /2] - threshold temperature. A threshold temperature of 7.2C was used.

A split-block design was used with 10 replications of 5 subsamples for each cutting date. Cutting time was the main plot and IBA concentration was the sub-plot. Cuttings were evaluated 12 weeks after collection for primary root number, mean length of three longest primary roots, and mean number of secondary roots on three longest roots. Analysis of variance procedure (GLM) was performed using SAS statistical software (SAS Institute, Cary, N.C.). Cuttings were rated using the scale: 0 = dead, 1 = no callus/no roots, 2 = callus present/no roots, 3 = roots present/ no callus formed, 4 = roots and callus, roots originating from the callus, or 5 = roots and callus present but in different locations.

Results

Calendar Timing. A significant interaction between tree gender and cutting date occurred for ratings of hardwood cuttings taken 27 Jan. through 4 May and given 35,000 mg·liter^{-1} IBA (Table 2.2). Ratings indicated that cuttings were alive after twelve weeks, but no roots were apparent. The interaction of cutting date by tree gender was also significant, with male cuttings harvested on 20 May producing the largest visual root rating (Table 2.3). The tree gender by IBA interaction was significant with male softwood cuttings treated with 17,500 mg·liter^{-1} producing more roots than cuttings from female plants at either IBA concentration (Table 2.4). The IBA by gender by cutting date interaction was significant for cuttings taken from 16 June through 24 Aug. As with cuttings taken in May no rooting was apparent (Table

2.5). There was a curvilinear relationship between concentration and date for visual ratings. Cuttings taken 13 May through 24 Aug. and treated with 17,500 mg·liter^{-1} IBA had a significant curvilinear response (Table 2.6). The highest ratings, (the most rooting), occurred on cuttings taken from male trees on 20 May and given 17,500 mg·liter^{-1} IBA.

Morphology and degree days. The interaction of IBA quadratic and IBA cubic by cutting date was significant at $P \leq 0.001$ (Table 2.7). Green softwood cuttings taken on 9 May, which was equivalent to 380 degree days (based on a threshold of 7.2C), that received 8,750 mg·liter^{-1} IBA produced the most rooted cuttings (44%) (Table 2.7). Of the 47 rooted cuttings, 45 had roots associated with callus. This suggests that the presence of callus is important for root formation. Hardwood cuttings taken on 18 Mar. and 25 Mar. resulted in a large number of dead cuttings. Cuttings collected on 9 May and 25 May produced more callused cuttings than other dates. The largest number of primary and secondary roots and longest primary roots occurred on cuttings harvested on 9 May and receiving 8,750 mg·liter^{-1} IBA. With the exception of one cutting on 6 July, rooting was limited to cuttings taken between orange budbreak and red semi-hardwood stems. No rooting occurred when the parent plants had been exposed to more than 799 degree days.

Discussion

Many cuttings taken from trees have a short (4-6 weeks) window of rootability, including *Acer palmatum* Thunb., *Betula nigra* L., *Betula pendula* Roth, *Fraxinus* spp. and cvs., *Ulmus parvifolia* Jacq., *Cotinus coggygria,* Scop. and *Cercis*

canadensis L. (Barnes and Lewandowski, 1991). Burd and Dirr (1977) found May and early June cuttings had the highest rooting percentages for mature *Malus* L. Juvenile pecan (*Carya illinoensis* Wangenh. C. Koch) cuttings root at higher percentages when taken in Feb., June, and Aug., while adult cuttings root best from 15 Aug. cuttings (Smith and Chiu, 1980). The best rooting in *Quercus palustris* Muenchh. is in early July, while *Tilia cordata* Mill. root more when cuttings are harvested during late June (Chapman and Hoover, 1981). In 1993, Chinese pistache cuttings taken 27 Jan. through 4 May and given 35,000 mg·liter^{-1} IBA, showed a significant relationship between tree gender and cutting date (Table 2.2). Chinese pistache also appears to have a limited period of rootability during May. Cuttings taken on 13 May and 20 May treated with 8,750 mg·liter^{-1} IBA produced more roots than those treated with 17,500 mg·liter^{-1} (Table 2.4). The most root production was in cuttings taken from male trees on 20 May and given 17,500 mg·liter^{-1} IBA.

In 1993, male Chinese pistache trees consistently provided more rooted cuttings than female trees. Similar results were reported on *Acer rubrum* L. trees (Snow, 1942). Effect of gender was not significant to primary or secondary root production, or primary root length, in other Chinese pistache studies (Chapter III).

In 1994, cuttings collected on 9 May, 380 degree days from orange budbreak using a threshold of 7.2C, and receiving 8,750 mg·liter^{-1} IBA produced 44% rooted cuttings (Table 2.7). The importance of callus formation to rooting was apparent since only one cutting rooted without callus, and one cutting rooted with callus in a different location than roots. On these two cuttings roots appeared to emerge

through the lenticels. Although callus formation and root formation are independent processes, callus formation is apparently a precursor to root formation in some species. The origin of adventitious roots from callus tissue has been associated with difficult-to-root species (Davies, et al., 1982; Hiller, 1951).

While 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* species, they found that the chilling-unit method can be used to determine rooting ability for plants with a narrow window of opportunity for rooting.

Cuttings collected on 9 May and receiving 8,750 mg·liter^{-1} IBA produced more primary and secondary roots and longer primary roots per cutting than any other date and treatment interactions. Rooting of cuttings given 8,750 mg·liter^{-1} IBA decreased by 30% from 9 May to 25 May (Table 2.7). *Prunus serrulata* 'Kwanzan' Lindl. cuttings declined more than 30 percent from mid-July to mid-August as stem hardening progressed (Barnes, 1989). Still and Zanon (1991) found that *Amelanchier laevis* Wieg. cuttings taken in May and early June rooted better than those taken in July. With the exception of one cutting, rooting was limited to cuttings collected between orange budbreak and red semi-hard stem which was equivalent to 799 degree days, 75 calendar days, 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, 8,750 mg·liter^{-1} IBA produced the most roots per cutting and the most rooted cuttings.

In 1993 rooting was limited to May softwood cuttings. In 1994, the two cutting dates producing rooted cuttings were morphologically described as green softwood cuttings and red semi-softwood cuttings. Considering the narrow window of rooting in both years, and that roots appeared to emerge from the callus or through lenticels, it is suggested that Chinese pistache may have an anatomical barrier to root formation. Beakbane (1961) proposed a correlation between the degree of sclerification of the primary phloem and rooting capacity suggesting that rooting of difficult subjects might be facilited by using very young shoots taken before the cells of the primary phloem lose their living protoplasts. He stated that it may be possible to forecast rooting capacity from the structure of young stems. This study shows that rooting capacity for Chinese pistache can be predicted by the morphology of the stem cutting. When Adams and Roberts (1967) evaluated *Rhododendron* cuttings on a morphological time scale, rooting capacity decreased with increasing tissue age. This agrees with results from these studies.

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 9 May date corresponds to 46 days from orange budbreak, and 380 degree days, with threshold temperature of 7.2C. Rooting potential ended at the accumulation

of 799 degree days (threshold of 7.2C), or 75 calendar days. Morphologically, the highest percentage of rooted cuttings were green softwood stems matched to yellow-green 144B on the R.H.S. color chart, with reproductive buds approximately 2.8 mm in diameter.

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Table 2.1. Observation or cutting date, reproductive bud size, number of calendar days and degree days from bud break of various morphological characteristics of Chinese pistache.

Date	Cut	Morphological marker	R.H.S. color chart description of stem color[z]	Diameter of reproductive bud size(mm)[y]	Calendar days from budbreak[x]	Degree days from budbreak[x]
						Celsius 7.2
18 Mar.	Yes	Dormant terminal bud	Greyed-Green 197D	4.6	---	--
24 Mar.	Yes	Orange budbreak	Greyed-Green 197D	4.8	---	--
04 Apr.	No	Green budbreak	Greyed-Green 197D	---	11	31
11 Apr.	No	Closed cone	Greyed-Green 197D	---	18	73
16 Apr.	No	Open cone	Greyed-Green 197D	---	23	123
20 Apr.	No	Bud completly open	Greyed-Green 197D	---	27	177
24 Apr.	No	Early shoot expansion	Yellow-Green 144B	1.7	31	249
09 May	Yes	Green softwood stem	Yellow-Green 144B	2.8	46	380
25 May	Yes	Red semi-soft stem	Greyed-Red 181B	3.6	62	573
07 June	Yes	Red semi-hard stem	Greyed-Orange 174B	4.0	75	799
06 July	Yes	Brown semi-hard stem	Greyed-Orange 164B	4.2	104	1371
05 Aug.	Yes	Brown hardwood stem	Greyed-Orange 164B	4.1	134	1937
16 Nov.	Yes	Dormant terminal bud	Greyed-Green 197A	4.3	237	----

[z]Royal Horticultural Society color charts.
[y]Mean of one randomly chosen subsample from each repetition of each treatment.
Reproductive bud measurements were from the third bud behind the terminus.
[x]Budbreak is considered to be when the outer bud scales break and orange inner bud scales are showing.

Table 2.2. Visual ratings 12 weeks after planting male and female Chinese pistache cuttings taken at two week intervals and treated with 35,000 mg·liter^{-1} IBA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6= ≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Cutting date	Visual root rating[z]	
	Male	Female
27 Jan.	1.4	0.1
09 Feb.	0.1	0.2
23 Feb.	1.2	0.3
10 Mar.	0.4	0.0
24 Mar.	0.1	0.3
06 Apr.	0.0	0.0
21 Apr.	0.0	0.0
04 May	0.0	0.0
Significance:		
Date (D)		
Linear (L)	***	
Quadratic (Q)	NS	
Cubic	NS	
Gender (G)	*	
G*DL	*	
G*DQ	NS	
G*DC	NS	

[z]Mean of 30 cuttings.
NS,*,*** Nonsignificant or significant at $P \leq 0.05$, or 0.001, respectively.

Table 2.3. Visual ratings 12 weeks after planting male and female Chinese pistache cuttings harvested and planted on 13 May and 20 May 1993. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Cutting date	Tree gender	Visual root rating [z]
13 May	Male	1.42
	Female	1.39
20 May	Male	2.63
	Female	2.07
ANOVA		
Date		**
Gender		***
Date*Gender		***
Concentration		**
Date*Concentration		NS
Gender*Concentration		**
Date*Gender*Concentration		NS
Significance ($LSD_{0.05}$)		
Cutting date for same gender		1.7
Gender for the same or different date		1.3

[z]Mean of 30 cuttings.
NS,**,*** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Table 2.4. Visual ratings 12 weeks after planting male and female Chinese pistache cuttings cut on 13 May and 20 May 1993 and treated with 17,500 or 35,000 mg·liter^{-1} IBA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Tree gender	IBA concn. (mg·liter^{-1})	Visual rating[z]
Male	8,750	2.27
	17,500	3.73
Female	8,750	1.93
	17,500	1.92
ANOVA		
Date		**
Gender		***
Date*Gender		***
Concentration		**
Date*Concentration		NS
Gender*Concentration		**
Date*Gender*Concentration		NS
Significance ($LSD_{0.05}$)		
IBA concn. for the same gender		2.0
Gender for the same or different IBA concn.		2.0

[z]Mean of 30 cuttings.
NS,**,*** Nonsignificant or signinificant at $P \leq 0.01$, or 0.001, respectively.

Table 2.5. Visual rating 12 weeks after planting male and female Chinese pistache cuttings taken at two week intervals and treated with 17,500 or 35,000 mg·liter IBA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6= ≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Cutting date	IBA concn. (mg·liter^{-1})	
	17,500	35,000
	Male	
16 June	1.9	2.0
2 July	1.4	1.4
13 July	0.2	0.8
27 July	1.0	1.4
10 Aug.	1.2	1.2
24 Aug.	1.0	1.0
	Female	
16 June	2.9	1.4
2 July	1.5	1.3
13 July	1.3	0.5
27 July	1.0	1.3
10 Aug.	1.5	1.2
24 Aug.	1.0	1.0
ANOVA		
Date (D)-linear (L)	**	
D - quadratic (Q)	**	
D - cubic	*	
Gender	NS	
Gender*Date-L	NS	
Gender*Date-Q	NS	
Gender*Date-C	NS	
Concentration (Conc)	NS	
Conc*Date-L	*	
Conc*Date-Q	*	
Conc*Date-C	NS	
Conc*Gender	***	
Conc*Gender*Date-L	*	
Conc*Gender*Date-Q	NS	
Conc*Gender*Date-C	NS	

[z]Mean of 30 cuttings.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.6. Visual rating 12 weeks after planting male and female Chinese pistache cuttings taken at two week intervals and treated with 17,500 mg·liter^{-1} IBA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6= ≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Cutting date	Visual Rating[z]	
	Male	Female
13 May	2.6	1.8
20 May	4.3	2.0
01 June	1.8	1.7
16 June	1.9	2.9
02 July	1.4	1.5
13 July	0.2	1.3
27 July	1.0	1.0
10 Aug.	1.2	1.5
24 Aug.	1.0	1.0
Significance:		
Date (D)		
Linear (L)	***	
Quadratic (Q)	NS	
Cubic	*	
Gender (G)	NS	
G*DL	**	
G*DQ	**	
G*DC	NS	

[z]Mean of 30 cuttings.
NS,*,**,*** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 2.7. Number of Chinese pistache cuttings receiving 0, 8,750, 17,500, or 35,000 mg·liter^{-1} IBA and cut on 18 Mar., 25 Mar., 9 May, 25 May, 7 June, 6 July, 5 Aug. or 16 Nov. and rated on a scale of 0 to 5 with 0= dead, 1=no callus/no roots, 2= callus present/no roots, 3=roots present/no callus formed, 4=roots and callus, roots originating from callus, 5= roots and callus present but in different locations. Primary and secondary roots were counted, primary root length was measured, and percentage of rooted cuttings per treatment was calculated.

Cutting date and degree days[z]	IBA (mg·liter^{-1})	Number of cuttings Visual rating 0	1	2	3	4	5	Primary root number[y]	Root length (mm)[x]	Secondary root number[w]	Rooted cuttings (%)
18 Mar.	0[v]	25	22	3	0	0	0	0.00	----[u]	----[u]	0
	8,750	27	20	3	0	0	0	0.00	----	----	0
(NA)	17,500	27	22	1	0	0	0	0.00	----	----	0
	35,000	18	30	2	0	0	0	0.00	----	----	0
25 Mar.	0	31	19	0	0	0	0	0.00	----	----	0
	8,750	24	26	0	0	0	0	0.00	----	----	0
(NA)	17,500	24	26	0	0	0	0	0.00	----	----	0
	35,000	28	22	0	0	0	0	0.00	----	----	0
9 May	0	0	6	42	0	2	0	0.04	1.12	0.00	4
	8,750	0	0	28	0	21	1	1.08	31.76	1.94	44
(380)	17,500	3	5	36	1	5	0	0.16	9.94	0.44	12
	35,000	0	14	33	0	3	0	0.10	0.40	0.16	6
25 May	0	0	8	41	0	1	0	0.22	1.30	0.06	2
	8,750	0	1	42	0	7	0	0.40	4.44	0.20	14
(573)	17,500	0	0	49	0	1	0	0.02	0.20	0.00	2
	35,000	0	14	36	0	0	0	0.00	----	----	0
7 June	0	18	22	10	0	0	0	0.00	----	----	0
	8,750	13	3	34	0	0	0	0.00	----	----	0
(799)	17,500	21	1	28	0	0	0	0.00	----	----	0
	35,000	29	5	16	0	0	0	0.00	----	----	0
6 July	0	0	45	5	0	0	0	0.00	----	----	0
	8,750	0	46	3	0	1	0	0.02	3.74	0.34	2
(1371)	17,500	1	43	0	0	0	0	0.00	----	----	0
	35,000	1	49	0	0	0	0	0.00	----	----	0

Table 2.7 continued

5 Aug.	0	0	50	0	0	0	0	0.00	----	----	0
	8,750	3	47	0	0	0	0	0.00	----	----	0
(1937)	17,500	6	39	5	0	0	0	0.00	----	----	0
	35,000	4	46	0	0	0	0	0.00	----	----	0
16 Nov.	0	3	46	1	0	0	0	0.00	----	----	0
	8,750	2	42	6	0	0	0	0.00	----	----	0
(NA)	17,500	0	43	7	0	6	0	0.00	----	----	0
	35,000	2	33	15	0	0	0	0.00	----	----	0
Significance:											
Time								***			
Block								NS			
IBA (L)								NS			
IBA (Q)								**			
IBA (C)								***			
IBA (L) x Date								NS			
IBA (Q) x Date								***			
IBA (C) x Date								***			

L= Linear, Q= Quadratic, C= Cubic

NS, **, ***,Nonsignificant or significant at $P \leq 0.01$, 0.001, respectively.

[z] Based on a Celsius threshold temperature of 7.2.

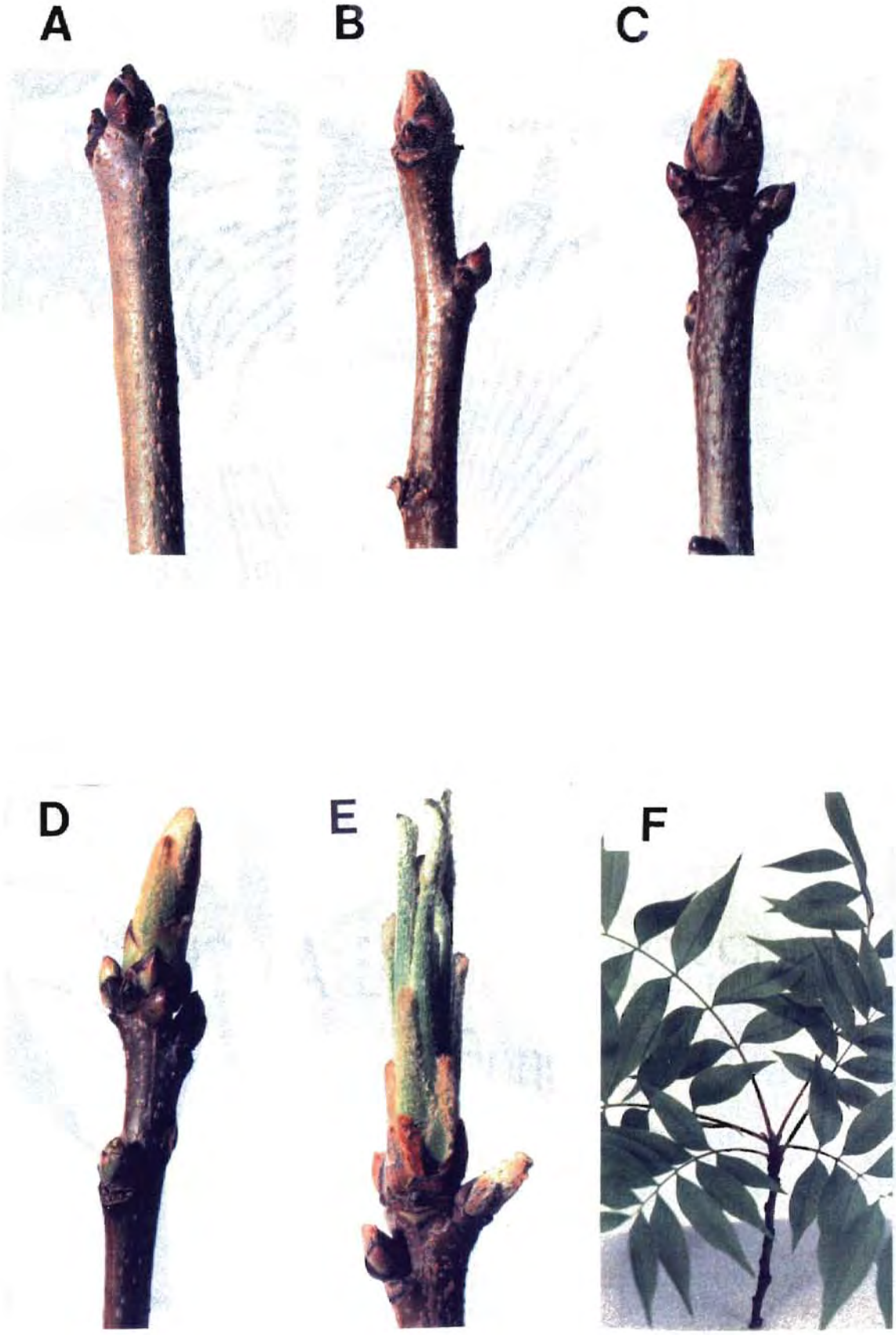
[y] Mean number of roots per cutting.

[x] Mean root length of the 3 longest roots per cutting.

[w] Mean number of secondary roots per longest primary root per cutting.

[v] 50 observations per treatment.

[u] Due to the low number of observations, statistical analysis was not appropriate.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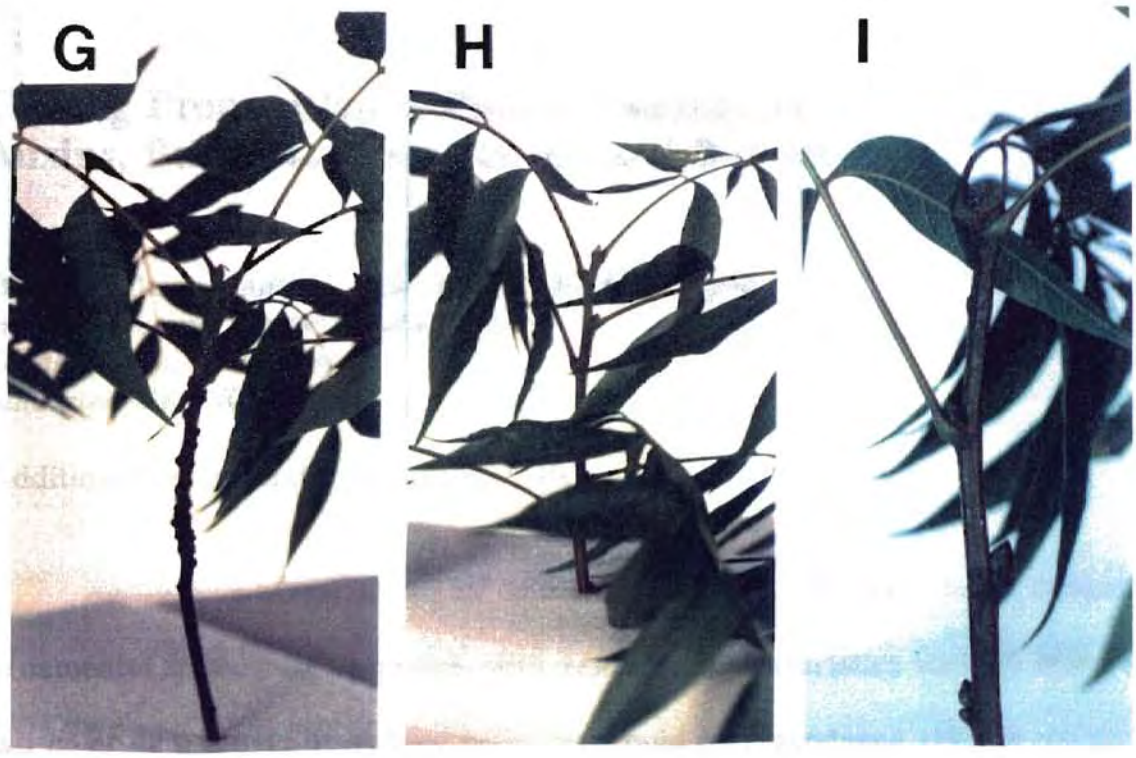
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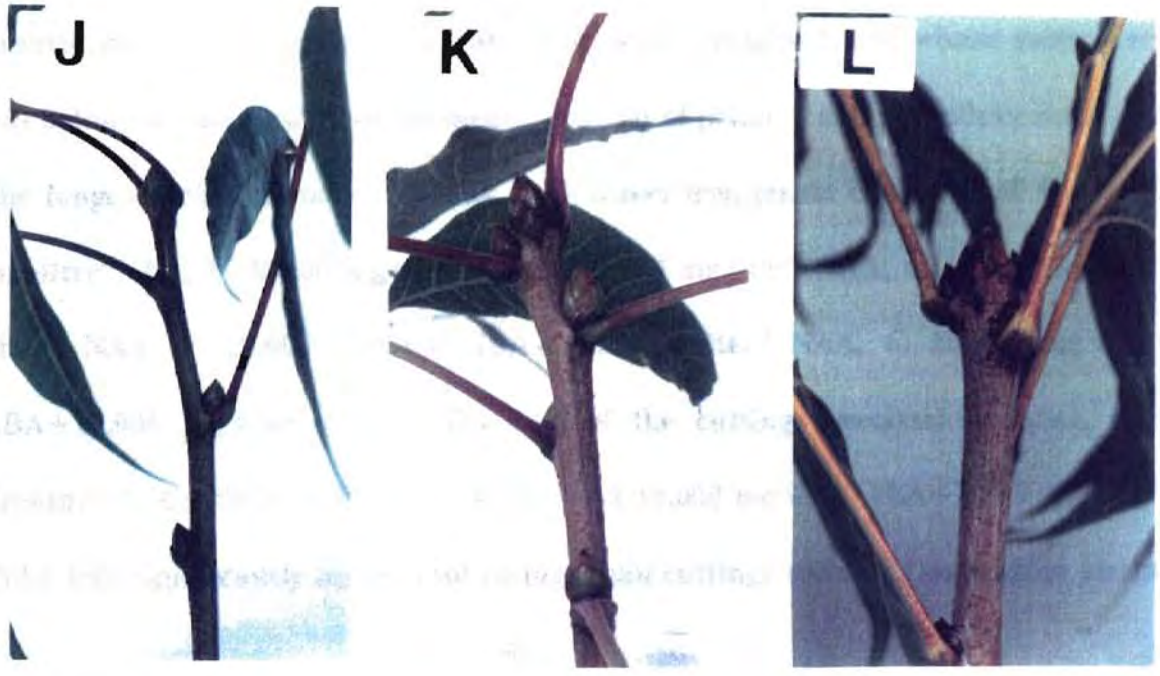
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Chapter III

Cutting Propagation of *Pistacia chinensis* II. Auxins, Bud Retention, Gender, and Bottom Heat

Diane E. Dunn, Janet C. Cole, and Michael W. Smith
Horticulture and Landscape Architecture
Oklahoma State University,
Stillwater, OK 74078

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Abstract. **Chinese pistache (*Pistacia chinensis*, Bunge.) is a desirable ornamental shade tree. A cultivar with desirable characteristics such as attractive fall color or a strong branching habit that could be reproduced reliably would be extremely marketable. Like other members of *Pistacia*, cuttings of Chinese pistache have proven difficult to root. Investigating the effect of cutting time, gender, IBA concentration, bud retention, and cut position, cuttings taken on 16 May, which were treated with 15,000 mg·liter IBA, whose buds were retained, and whose stems were cut below the bud, produced the largest number of primary and secondary roots and the longest primary roots. In May 1993, auxin treatments consisted of 1) 10,000 mg·liter^{-1} IBA, 2) 20,000 mg·liter^{-1} IBA, 3) 2,500 mg·liter^{-1} NAA, 4) 10,000 mg·liter^{-1} NAA, 5) 10,000 mg·liter^{-1} IBA+2,500 mg·liter^{-1} NAA, 6) 20,000 mg·liter^{-1} IBA+10,000 mg·liter^{-1} NAA. One-half of the cuttings received a H_2SO_4 pre-treatment. Cuttings receiving no H_2SO_4 and 10,000 mg·liter^{-1} IBA+2,500 mg·liter^{-1} NAA had significantly higher root ratings than cuttings receiving no acid/no auxin.**

On 9 May and 18 May 1994, auxin treatments consisted of 0; 5,000; 10,000; and 15,000 mg·liter^{-1} IBA, or 0; 5,000; and 10,000 mg·liter^{-1} NAA alone or in IBA/NAA factorial combination. Cuttings receiving 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA produced more rooted cuttings (38%) than other treatments. The IBA by NAA by cutting time interaction for root number was significant at $P \leq 0.01$. Cuttings collected on 16 May and 23 May were dipped in 0; 7,500; 15,000; or 22,500 mg·liter^{-1} IBA and cut either above or below the bud, with remaining buds either retained or removed. Cuttings harvested on 16 May and given 15,000 mg·liter^{-1} IBA with buds retained and stems cut below a bud produced more primary and secondary roots with longer primary roots than the other treatments. Bottom heat used on hardwood cuttings did not promote rooting.

Chemical names used: sulfuric acid (H_2SO_4); indolebutyric acid (IBA); naphthaleneacetic acid (NAA)

Vegetative propagation of Chinese pistache offers certain benefits. Asexual reproduction would allow marketing of cultivars with desirable characteristics such as improved cold hardiness, vivid fall color, desirable branching habit, an attractive bark texture or color, dense canopy, or disease and insect resistance. Chinese pistache, like other members of *Pistacia*, have proven difficult to root, graft, or bud (Joley, 1960; Long, 1960; Hall, 1975).

Cuttings of other difficult to root species have been manipulated in several ways to increase rooting. Application of NAA and IBA has increased adventitious root formation (Thimann and Koepfli, 1935; Zimmerman and Wilcoxon, 1935). The most widely used root promoting chemicals used today are IBA and NAA. Although IBA is the most commonly used to induce rooting, NAA is superior for some species (Dirr, 1986; Morini and Isoleri, 1986).

Van der Lek (1934) was one of the first to report that buds on cuttings promoted rooting. The relationship between the presence of buds near the base and root formation has been reported as both inhibitory (Kemp, 1948; DeBoer, 1953; O'Rourke, 1940; Johnson and Roberts, 1968) and advantageous (Fadl and Hartmann, 1967; Spiegel, 1954; Harada and Nakayama, 1957). Fadl and Hartmann (1967) found high levels of root promoting activity in extracts of easy-to-root 'Old Home' pear buds and basal segments during the period of maximum rooting. High inhibitor concentrations occurred in the buds during their rest period. Leopold (1964) found auxin production associated with actively growing tissues, such as expanding buds.

Another treatment that has proven successful to increase rooting is bottom heat. The optimal medium temperature for propagation is 18C to 25C for temperate species and 7C higher for tropical species (Dykeman, 1976; Kester, 1970). Dykeman (1976) found 30C resulted in more rapid root initiation, shorter emergence time, and more roots per forsythia cutting. Burholt and Vant Hoff (1970) suggested that the rate of cell division increased to a maximum between 30C and 35C. Bottom heat

ranging from 25C to 30C with 35,000 mg·liter^{-1} IBA was successful for rooting of *Pistacia vera* (Al Barazi and Schwabe, 1982, 1985).

The objectives of these studies were to 1) determine the effect of IBA and NAA on rooting of Chinese pistache cuttings, 2) investigate the effect of gender and the presence and position of reproductive buds on rooting of Chinese pistache softwood cuttings, and 3) determine if bottom heat would increase rooting of hardwood cuttings.

Materials and Methods

Auxin solutions in this study consisted of the given amount of auxin dissolved in 50 ml of 70% isopropyl alcohol and then brought to final volume of 100 ml with tap water.

May auxins 1993. On 19 May, softwood terminal cuttings from lateral shoots, 14 cm long, were taken from the upper canopy of 34-year-old Chinese pistache trees located at the Oklahoma State University Nursery Research Station in Stillwater, Okla. Cuttings were placed into 10C water, taken to the greenhouse, and then re-cut to 10 cm long. The basal 1 cm was dipped 5 sec in one of the following auxin treatments: 1) 10,000 mg·liter^{-1} IBA, 2) 20,000 mg·liter^{-1} IBA, 3) 2,500 mg·liter^{-1} NAA, 4) 10,000 mg·liter^{-1} NAA, 5) 10,000 mg·liter^{-1} IBA + 2,500 mg·liter^{-1} NAA and 6) 20,000 mg·liter^{-1} IBA + 10,000 mg·liter^{-1} NAA. One-half of the cuttings received a 1 molar sulfuric acid (H_2SO_4) pre-treatment for 5 sec prior to auxin application. Control treatments included alcohol, water, H_2SO_4, and H_2SO_4 followed by alcohol. Cuttings were placed in 12 cm wide x 36 cm long x 6 cm deep plastic

flats filled with 1 peat: 4 perlite (by volume) and placed in a polyethylene greenhouse on benches equipped with Flora-Mist nozzles (Hummerts, St. Louis, Mo.) with an output of 32 liters per hour (LPH) placed 50 cm above the flats and spaced at 121 cm intervals. Mist cycles were adjusted for changing environmental conditions as needed, but averaged 4 sec duration every 4 min between 0800 HR and 1800 HR daily. Cuttings were exposed to natural photoperiod at a maximum photosynthetic photon flux (PPF) of 374 μmol·m^{-2}·s^{-1} with maximum/minimum air temperatures of 31/13C.

June auxins 1993. Semi-softwood cuttings were harvested on 15 June and treated as described for the May experiment with the following exceptions: Auxin application rates were 1) 15,000 mg·liter^{-1} IBA, 2) 25,000 mg·liter^{-1} IBA, 3) 5,000 mg·liter^{-1} NAA, 4) 12,500 mg·liter^{-1} NAA, 5) 15,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA, and 6) 25,000 mg·liter^{-1} IBA + 12,500 mg·liter^{-1} NAA. Cuttings were placed inside a clear polyvinyl mist tent constructed over the bench and received a maximum PPF of 272 μmol·m^{-2}·s^{-1} with maximum/minimum air temperatures of 38/25C. Mist settings averaged 2 sec duration with 6 min frequency between 0800 HR and 1800 HR.

July auxins 1993. Semi-hardwood cuttings were collected on 15 July. Auxin application rates were 1) 20,000 mg·liter^{-1} IBA, 2) 30,000 mg·liter^{-1} IBA, 3) 7,500 mg·liter^{-1} NAA, 4) 15,000 mg·liter^{-1} NAA, 5) 20,000 mg·liter^{-1} IBA + 7,500 mg·liter^{-1} NAA, 6) 30,000 mg·liter^{-1} IBA + 15,000 mg·liter^{-1} NAA. Cuttings were

placed into a tent with the same mist settings as in June, and exposed to a maximum PPF 239 μmol·m^{-2}·s^{-1} with maximum/minimum air temperatures of 37/24C.

The statistical design for experiments during May, June, and July was a randomized complete block with 3 replications containing 10 subsamples per treatment. Cuttings were evaluated 12 weeks after planting using a rating scale as follows: 0=dead, 1=alive, no callus or roots, 2=callus on stem or cut surface, 3=root tip visible, 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8= ≥3 primary roots with secondary roots. Analysis of variance procedures and paired *t* tests were performed on all data.

Auxins 1994 Terminal cuttings from lateral shoots were taken on 9 May and 18 May from 20-year-old trees. The 9 May cutting corresponded to about 404 degree days, and 18 May corresponded to 518 degree days from orange budbreak. Degree days were obtained using a threshold temperature of 7.2C. (Chapter II). Cuttings were re-cut under a bud to about 9 cm long, and then given a 5 sec dip of the following treatments: 1) 5,000 mg·liter^{-1} IBA; 2) 10,000 mg·liter^{-1} IBA; 3) 15,000 mg·liter^{-1} IBA; 4) 5,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA; 5) 5,000 mg·liter^{-1} IBA + 10,000 mg·liter^{-1} NAA; 6) 10,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA; 7) 10,000 mg·liter^{-1} IBA + 10,000 mg·liter^{-1} NAA; 8) 15,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA; 9) 15,000 mg·liter^{-1} IBA + 10,000 mg·liter^{-1} NAA; 10) 5,000 mg·liter^{-1} NAA; 11) 10,000 mg·liter^{-1} NAA; 12) alcohol; 13) no auxin. Cuttings were placed in 25 cm wide x 16 cm long x 8 cm deep plastic rooting flats containing 1 peat: 3 perlite (by volume). Cuttings were kept under natural photoperiod in a

polyethylene greenhouse with a maximum PPF of 845 μmol$\cdot$m^{-2}s^{-1} and maximum/minimum air temperatures of 36/22C. Benches were equipped with DGT mist nozzles with an output of 2 LPM (Hummerts, St. Louis, Mo.) placed 50 cm above the flats and spaced at 121 cm intervals. Mist settings were adjusted to changing environmental conditions as needed, averaging 2 sec duration with 2 min frequency from 0800 HR to 1700 HR daily.

A randomized complete block design was used with 10 replications of 5 subsamples for each cutting date. Cuttings were evaluated 12 weeks after collection for primary root number, mean primary root length of the three longest roots, mean number of secondary roots on the three longest roots, and amount of callus produced.

Bud position and retention, auxin, and gender effects on rooting response. Cuttings were collected from four male and four female 34-year-old trees at the Oklahoma State University Nursery Research Station, Stillwater, Okla. on 16 May and 23 May 1994. The 16 May cutting corresponded to about 520 degree days, and 23 May corresponded to 635 degree days from orange budbreak (Fig. 2.1B). Degree days were obtained using a threshold temperature of 7.2C. (Chapter II). Terminal cuttings were taken from lateral shoots in the upper canopy of the trees. Cuttings were brought into the greenhouse and recut for the following treatments: 1) cut below the sixth bud, buds left intact; 2) cut below the sixth bud and the fourth, fifth and sixth buds removed; 3) cut above the sixth bud, buds left intact; 4) cut above the sixth bud and the fourth and fifth buds removed (Fig. 3.1). The basal 1 cm of

cuttings was dipped 5 sec in one of the following IBA treatments 1) no IBA, 2) 7,500 mg·liter^{-1}, 3) 15,000 mg·liter^{-1}, and 4) 22,500 mg·liter^{-1}. Cuttings were placed in 25 x 16 x 8 cm plastic containers containing 1 peat: 3 perlite (by volume), and kept under natural photoperiod in a polyethylene greenhouse with a maximum PPF of 835 μmol·m^{-2}·s^{-1}, and maximum/minimum air temperatures of 36/22C. Benches were equipped with 2 LPM DGT mist nozzles set at 2 sec duration with 2 min frequency between 0800 HR and 1800 HR daily.

A split-split block statistical design was used with 10 replications of 5 subsamples. Bud position and retention was the main treatment, IBA concentration was the sub-treatment, and gender was the sub-sub treatment. Cuttings were evaluated 14 weeks after planting for the same criteria as the 1994 auxin experiment.

Bottom Heat. Juvenile and adult terminal cuttings from lateral shoots about 12 cm long were removed from trees on 1 Oct. 1993. Cuttings were re-cut to 10 cm long immediately below a bud. Adult cuttings were collected from the upper canopy of a 34-year-old male tree, and juvenile cuttings were collected from several 22-month-old Chinese pistache trees. Two temperature treatments were used, 30C basal heat provided by 56 cm x 152 cm Progrow (Hummert, St. Louis, Mo.) propagation mats or no bottom heat on a polyethylene covered bench. Cuttings were kept under natural photoperiod in a polyethylene greenhouse with a maximum PPF of 835 μmol·m^{-2}·s^{-1} and maximum/minimum air temperatures of 33/13C. Benches were equipped with Flora-Mist nozzles (Hummert, St. Louis, Mo.) with an output of 32 LPH, placed 50 cm above the flats and spaced at 121 cm intervals set at 4 sec

duration with 32 min frequency. Medium temperatures were monitored twice weekly and averaged 30C with bottom heat, and 19C without bottom heat. All cuttings were placed into 12 cm wide x 36 cm long x 6 cm deep plastic flats containing 1 peat:3 perlite (by volume). The peat moss was previously soaked to saturation in a 1 molar $Ca(OH)_2$ solution. Cuttings were randomized, then divided into the two temperature treatments.

For the non-chilled treatment, half of the cuttings were dipped 5 sec in 20,000 $mg \cdot liter^{-1}$ IBA applied to the basal 1 cm, then in a 50% benomyl talc (Chevron, San Ramon, Calif.), and directly planted.

For the chilled treatment, basal ends of the remaining cuttings were dipped in benomyl talc only, then they were completely covered with sphagnum peat previously saturated with 1 molar $Ca(OH)_2$ and placed in Ziplock plastic sandwich bags (Dow, Indianapolis, Ind.). Sixty bags with twenty cuttings per bag were prepared, then placed into a cardboard box which was closed and stored in a cooler at 5C for 75 days. On 15 Dec. cuttings were removed from the cooler, peat and benomyl were washed off with tap water, and then the chilled cuttings received the same IBA and benomyl dip as the directly planted non-chilled cuttings.

On 5 Nov. 1993 cuttings were taken from the same trees and treated as previously described for October. Chilled cuttings were removed from the cooler on 21 Jan.

On 1 Jan. 1994, juvenile cuttings were taken from 25-month-old seedlings that were kept outside in 3.8 liter containers, and adult cuttings were taken from the

same trees used in October and November. Cuttings were treated with 20,000 mg·liter^{-1} IBA, followed by benomyl, and then directly planted. Flats were randomized and then placed into each of the two temperature treatments. January cuttings received natural environmental cooling conditions prior to cutting so artificial chilling was not provided.

A split-plot design with bottom heat as the main plot was used with 3 replications and 20 sub-samples. Rooting response was evaluated after 12 weeks. The rating scale was as follows: 0=dead, 1=alive, no callus or roots, 2=callus on stem or cut surface area, 3=root tip showing, 4=1-2 primary roots, and 5=≥3 primary roots. General linear model analysis was performed with SAS statistical software (SAS Institute, Cary, N.C.).

Results

May auxin 1993 Cuttings receiving no acid and 10,000 mg·liter^{-1} IBA + 2,500 mg·liter^{-1} NAA had significantly higher ratings than the no acid/ no auxin control (Table 3.1). The following auxin treatments, all preceded by acid applications, had significantly lower ratings than the no acid/ no auxin control: 20,000 mg·liter^{-1} IBA, 2,500 mg·liter^{-1} NAA; and 20,000 mg·liter^{-1} IBA + 10,000 mg·liter^{-1} NAA.

June auxin 1993 Treatment with H_2SO_4 and 15,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA or 25,000 mg·liter^{-1} IBA + 12,500 mg·liter^{-1} NAA reduced the visual root rating compared to the control. Several other treatment combinations improved the visual root rating compared to the control; however, none resulted in acceptable rooting.

July auxin 1993 Cuttings treated with no acid and 7,500 mg·liter^{-1} NAA had a higher visual rating than the no acid/ no auxin control. (Table 3.3).

Auxins 1994. The only cuttings with roots received a rating of 4, indicating that root formation only occurred in the presence of callus (Table 3.4). The number of dead cuttings after 12 weeks was greater in the 18 May cuttings than the 9 May cuttings. The IBA by NAA by cutting time interaction was significant at $P \leq 0.01$ for primary root number (Table 3.4). The largest number of primary roots per cutting (0.56) was produced on cuttings collected on 9 May and treated with 5,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA. Roots tended to be longer on cuttings which had a smaller number of roots. Cuttings with longer roots tended to produce more secondary roots (Table 3.4) than cuttings with shorter roots. The 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA and cut on 18 May produced the largest number of rooted cuttings (38%) (Table 3.4). Regardless of cutting date 5,000 mg·liter^{-1} IBA with 5,000 mg·liter^{-1} NAA produced the largest number of rooted cuttings. The IBA by NAA interaction was significant for callus production (Table 3.5) with 5,000 mg·liter^{-1} IBA + 5,000 mg·liter^{-1} NAA producing the largest amount of callus.

Bud position and retention, auxin, and gender. With the exception of four cuttings rated as 3, all rooted cuttings received a rating of four, indicating the importance of callus formation on root production (Table 3.6). The number of dead cuttings was greater for cuttings taken on 23 May compared to 16 May.

The IBA concentration by cutting time by bud treatment interaction was significant for root number, root length, and secondary roots (Table 3.7). Male and female cuttings taken on 16 May and given 15,000 mg·liter^{-1} IBA, with buds retained and stems cut below the bud, produced the largest number of primary and secondary roots, and longest primary roots. The same IBA concentration and bud treatment also produced the largest number of primary and secondary roots and longest roots on 23 May.

Cutting time by IBA concentration, IBA by bud treatment, tree gender by bud treatment, cutting time by tree gender, and cutting time by bud treatment were all significant interactions for callus production (Tables 3.8). Cutting time by IBA curvilinear was significant for callus production with cuttings collected on 16 May and treated with 22,500 mg·liter^{-1} IBA producing the largest amount of callus (Tables 3.8, 3.9). IBA by bud treatment was significant at $P \leq 0.01$ (Table 3.8). Cuttings treated with 15,000 mg·liter^{-1} IBA with buds retained and cut below the bud produced the largest amount of callus, 2.77 mm per cutting (Table 3.10). The interaction of tree gender and bud treatment was significant (Table 3.8) with female cuttings that had buds retained and cut below a bud producing the largest amount of callus (Table 3.11). Tree gender by cutting time was significant at $P \leq 0.05$ (Table 3.8, 3.12). The interaction of cutting date and bud position was significant at $P \leq 0.001$ (Table 3.8) with cuttings taken on 16 May with buds retained and cut below a bud producing a mean of 2.6 mm of callus per cutting (Table 3.13).

The largest number of rooted cuttings (22%) was from female trees cut on 16 May, given 7,500 mg·liter^{-1} IBA with buds retained and stems cut below the sixth bud.

Bottom Heat. Twelve hundred cuttings were evaluated in this experiment with with only two producing roots. Cuttings exposed to 30C bottom heat had significantly less callus and lower survival rates than those not receiving supplemental heat (Table 3.14). Age was also significant with cuttings from the adult tree receiving higher ratings than juvenile cuttings. The time cuttings were exposed to chilling, which is a combination of the month cut and the chilling treatment, was highly significant with highest root ratings on October cuttings that were given the 75 day chilling treatment. The chilling time by heat interaction was significant at $P \leq 0.001$ with October chilled cuttings, which received no bottom heat, producing the highest root ratings. Chilling by age interaction was significant at $P \leq 0.001$ with adult cuttings collected in November producing more callus and the highest survival rates.

Discussion

Cuttings in 1993 were given a H_2SO_4 treatment prior to an auxin treatment, based on research by Lee et al. (1976). In this study, cuttings were severely injured and root formation did not occur. These results differ with those of Lee et al. (1976) who found that H_2SO_4 promoted rooting of Chinese pistache cuttings. This difference may be due to different morphological stages or degrees of lignification in the cuttings used. Hitchcock and Zimmerman (1939) found that higher concentrations of hormones were required on cuttings harvested later in the season

than on those harvested earlier to promote equal rooting in *Rhododendron* species. The results for the May, June, and July 1993 auxin treatments did not agree with this premise. In May, cuttings receiving no acid treatment and 10,000 mg·liter^{-1} IBA + 2,500 mg·liter^{-1} NAA had significantly higher root ratings than the no acid/no auxin control (Table 3.1). In July, only the no acid 7,500 mg·liter^{-1} NAA treated cuttings significantly differed from the no acid/no auxin control (Table 3.2) Increased rooting with an IBA + NAA combination was established in the 1994 auxin experiments. The 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA produced the largest number of rooted cuttings (38%) regardless of cutting harvest date (Table 3.4). This agrees with Hitchcock and Zimmerman (1940) who found that equal parts of IBA and NAA, when used on a wide diversity of species, induced a higher percentage of cuttings to root and more roots per cutting than either chemical alone.

In both the 1994 auxin study and the time-IBA-bud-gender study, callus formation was associated with root formation. In the auxin study there was a significant interaction between IBA and NAA, and in the time-IBA-bud-gender study there were several significant interactions involved in callus formation. Although formation of callus and formation of roots are independent of each other, in some species callus formation is a precursor of adventitious root formation. Adventitious roots from callus tissue have been associated with difficult-to-root species such as *Abies, Cedrus, Ginkgo, Taxodium*, and *Pinus* (Hartmann, et al. 1990). In *Pinus radiata* D. Don (Cameron and Thomson, 1969), *Sedum* L. (Wells, 1963), and adult

phase *Hedera helix* L. (Girouard, 1967) adventitious roots originate in callus tissue that has formed at the base of the cutting.

The IBA concentration by cutting time by bud treatment interaction was significant for root number, root length, and secondary root number (Table 3.7). Male and female cuttings taken on 16 May, given 15,000 mg·liter^{-1} IBA with buds retained, and the stem cut below the bud, produced the largest number of primary and secondary roots and produced the longest primary roots.

Regardless of auxin or bud treatment, cuttings taken on 16 May, which was equivalent to 520 degree days from budbreak (7.2C threshold), produced significantly more primary and secondary roots, and longer roots than those taken on 23 May, which was 635 degree days from budbreak (Table 3.7). This confirms the conclusion in Chapter II that rooting potential is diminished with increasing calendar and degree day accumulation. In comparsion, cuttings taken at 380 degree days in a previous study (Chapter II) produced the largest percentage of rooted cuttings with the window of rooting almost closed at an accumulation of 573 degree days. This could explain the low rooting percentages in this study.

Gender of the source tree was not significant in treatment interactions, except in callus production. This does not support results using the same trees in Chapter II, which showed male trees consistently produced more rooted cuttings than female trees, or Snow (1942) who found cuttings from male *Acer rubrum* L. trees rooted at higher percentages than those taken from female trees. This could be due to heavier seed production by the females in 1993 affecting carbohydrate reserves.

Regardless of other treatments, retaining the reproductive buds on the cuttings and the position of the stem cutting in relation to the bud was important for primary and secondary root production, root length, and callus production (Tables 3.7, 3.8, 3.10, 3.11, 3.13). Cuttings with buds retained and cut below the bud, promoted rooting and callus production more than the other three bud treatments. With all IBA concentrations, cuttings with the buds retained produced more primary and secondary roots, longer primary roots, more callus, and a larger number of rooted cuttings compared to cuttings that had their buds removed. This was first established by Went and Thimann (1937) when they found that exogenous auxin would not substitute for buds as a stimulus to root formation.

Van der Lek (1925) was one of the first to report that position of the basal cut was important. He established that preformed root initials or the formation of root initials were most abundant in the first one-half inch below the node. Spiegel (1954) found that the presence of the lowest bud on a *Vitis* L. cutting promoted rooting. Harada and Nakayama (1957) found that rooting was highest in *Camellia sinensis* L. cuttings with 2 or 3 buds and lowest in those without buds. In cuttings with only one bud, roots appeared directly below that bud. Root formation directly under the bud was also observed in most rooted Chinese pistache cuttings in these studies. Increased root formation associated with the presence of flower buds, could be due to endogenous levels of auxins in the buds. Biran and Halevy, (1973) found that flower buds form a stronger "sink" for photosynthates than vegetative buds.

In the time-IBA-bud-gender experiment, the IBA concentration of 15,000 mg·liter^{-1} combined with bud retention and cutting below the bud produced the most roots. This IBA concentration was determined less effective than 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA in the 1994 auxin experiment, which also had buds retained and were cut below the bud.

Loreti and Morini (1977) reported a detrimental effect of bottom heat on the rooting of hardwood cuttings of *Pyrus betulaefolia*. Alternatively, bottom heat ranging from 25C to 30C has been used in rooting of *Pistacia vera* L. (Al Barazi and Schwabe, 1982, 1985). Bottom heat of 30C used on Chinese pistache produced a lower number of callused cuttings, lower survival rates, and did not promote rooting. These results agree with the morphology study in Chapter II which showed that hardwood cuttings do not normally produce roots.

Based on these studies we recommend an auxin treatment of 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA used on green softwood cuttings. A H_2SO_4 treatment should not be used. All buds should be retained on cuttings, and the cut should be made directly under a bud. Bottom heat on hardwood cuttings would not be recommended.

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Table 3.1. Visual root rating of cuttings taken in May 1993 from Chinese pistache and treated with sulfuric acid, IBA and NAA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Acid	Auxin (mg·liter^{-1})	Visual rating[z]
No	None	1.6
No	IBA 10,000	1.9
No	IBA 20,000	1.7
No	NAA 2,500	1.8
No	NAA 10,000	2.0
No	IBA 10,000+NAA 2,500	2.4*
No	IBA 20,000+NAA 10,000	2.0
Yes	IBA 10,000	1.5
Yes	IBA 20,000	0.6**
Yes	NAA 2,500	0.6**
Yes	NAA 10,000	1.2
Yes	IBA 10,000+NAA 2,500	1.0
Yes	IBA 20,000+NAA 10,000	0.6**
No	Alcohol	1.9
Yes	None	0.4**
Yes	Alcohol	1.0*

[z]Mean of 30 cuttings.

*,** Significantly different from cutting receiving no acid or auxin at $P \leq 0.05$, or 0.01, respectively.

Table 3.2. Visual root rating of cuttings taken in June 1993 from Chinese pistache and treated with sulfuric acid, IBA and NAA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots,7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Acid	Auxin (mg·liter^{-1})	Visual rating[z]
No	None	1.2
No	IBA 15,000	1.5
No	IBA 25,000	1.3
No	NAA 5,000	1.5
No	NAA 12,500	1.6*
No	IBA 15,000 + NAA 5,000	2.2***
No	IBA 25,000 + NAA 12,500	2.0***
Yes	IBA 15,000	1.8**
Yes	IBA 25,000	2.2***
Yes	NAA 5,000	1.8**
Yes	NAA 12,500	1.7*
Yes	IBA 15,000 + NAA 5,000	0.7*
Yes	IBA 25,000 + NAA 12,500	0.3***
No	Alcohol	1.0
Yes	None	0.5**
Yes	Alcohol	0.8

[z]Mean of 30 cuttings.
*,**,*** Significantly different from cuttings receiving no acid or auxin at $P \leq 0.05$, 0.01, 0.001, respectively.

Table 3.3. Visual root rating of cuttings taken from Chinese pistache and treated with sulfuric acid, IBA and NAA. Rating scale: 0=dead, 1=alive-no callus or roots, 2=callus, 3=root tip(s), 4=1-2 primary roots, 5=3-4 primary roots, 6=≥5 primary roots, 7=1-2 primary roots with secondary roots, 8=≥3 primary roots with secondary roots.

Acid	Auxin (mg·liter^{-1})	Visual rating[z]
No	None	0.2
No	IBA 20,000	0.1
No	IBA 30,000	0.2
No	NAA 7,500	1.6***
No	NAA 15,000	0.2
No	IBA 20,000 + NAA 7,500	0.8
No	IBA 30,000 + NAA 15,000	0.1
Yes	IBA 20,000	0.2
Yes	IBA 30,000	0.7
Yes	NAA 7,500	0.0
Yes	NAA 15,000	0.0
Yes	IBA 20,000 + NAA 7,500	0.0
Yes	IBA 30,000 + NAA 15,000	0.0
No	Alcohol	0.0
Yes	None	0.2
Yes	Alcohol	0.1

[z]Mean of 30 cuttings.
*** Significantly different from cuttings receiving no acid or auxin at $P \leq 0.001$.

Table 3.4. Number of Chinese pistache cuttings receiving 0; 5; 10; or 15 g·liter^{-1} IBA and 0; 5 or 10 g·liter^{-1} NAA rated on a scale of 0 to 5 with 0= dead, 1= no callus/no roots, 2= callus present/ no roots, 3= roots present/no callus formed, 4= roots and callus, roots originating from callus, 5= roots and callus present but in different locations. Primary and secondary roots were counted, primary root length was measured, and percentage of rooted cuttings per treatment was calculated.

Cutting time	IBA (g·liter^{-1})	NAA (g·liter^{-1})	Number of cuttings Rating 0	1	2	3	4	5	Primary root number[z]	Root length[y] (mm)	Secondary root number[x]	Rooted cuttings (%)
9 May	0	0[w]	0	13	37	0	0	0	0.00	----	----	0
	0	5	0	9	39	0	2	0	0.46	123.75	19.30	4
	0	10	0	5	41	0	4	0	0.22	27.40	1.30	8
	5	0	2	2	43	0	3	0	0.12	32.33	0.00	6
	5	5	0	2	37	0	11	0	0.56	33.45	5.00	22
	5	10	1	4	44	0	1	0	0.06	136.00	8.00	2
	10	0	2	0	48	0	0	0	0.00	----	----	0
	10	5	2	3	44	0	1	0	0.02	8.00	0.00	2
	10	10	1	10	39	0	0	0	0.00	----	----	0
	15	0	5	4	40	0	1	0	0.08	11.33	1.00	2
	15	5	3	12	35	0	0	0	0.00	----	----	0
	15	10	3	12	35	0	0	0	0.00	----	----	0
18 May	0	0	5	15	30	0	0	0	0.00	----	----	0
	0	5	5	4	37	0	4	0	0.12	19.50	3.00	8
	0	10	5	6	33	0	6	0	0.52	73.17	11.80	12
	5	0	7	2	37	0	4	0	0.18	32.75	4.00	8
	5	5	5	0	26	0	19	0	3.48	38.70	11.60	38
	5	10	5	10	35	0	0	0	0.00	----	----	0

Table 3.4 continued

10	0	5	1	43	0	1	0	0.18	29.00	1.00	2
10	5	7	3	38	0	2	0	0.26	7.00	0.00	4
10	10	6	7	36	0	1	0	0.02	8.00	0.00	2
15	0	6	1	43	0	0	0	0.00	----	----	0
15	5	8	12	30	0	0	0	0.00	----	----	0
15	10	8	4	33	0	0	0	0.00	----	----	0

Significance:	
I (L)	NS
I (Q)	NS
I (C)	*
A (L)	NS
A (Q)	**
I x A	NS
I (Q) x A (L)	NS
I (C) x A (L)	NS
I (L) x A (Q)	NS
I (Q) x A (Q)	**
I (C) x A (Q)	**
Time	NS
I (L) x Time	NS
I (Q) x Time	NS
I (C) x Time	NS
A (L) x Time	NS
A (Q) x Time	NS
I x A x Time	NS
I (Q) x A x Time	NS

Table 3.4 continued

I (C) x A x Time	NS
I (L) x A (Q) x Time	NS
I (Q) x A (Q) x Time	*
I (C) x A (Q) x Time	**

I = IBA, A = NAA, L = Linear, Q = Quadratic, C = Cubic.
[NS], *,**, Non-significant, and significant at $P \leq 0.05$ and 0.01, respectively.
[z] Mean number of roots per cutting.
[y] Mean root length of the 3 longest roots per cutting.
[x] Mean number of secondary roots per cutting produced on the 3 longest roots per cutting.
[w] 50 observations per treatment, except on 18 May at 15 g·liter^{-1} IBA and 10 g·liter^{-1} NAA had 45 observations.
[v] Due to low numbers of observation, statistical analysis was not appropriate.

Table 3.5. The effect of 0; 5; 10; or 15 g·liter^{-1} IBA and 0; 5; or 10 g·liter^{-1} NAA on Chinese pistache cuttings callus production.

IBA (g·liter^{-1})	NAA (g·liter^{-1})	Callus production (mm)[z]
0	0	1.40
0	5	2.29
0	10	2.20
5	0	2.48
5	5	3.32
5	10	2.07
10	0	3.10
10	5	2.71
10	10	2.38
15	0	2.76
15	5	1.81
15	10	2.01
Significance:		
I (L)		NS
I (Q)		***
I (C)		NS
A (L)		NS
A (Q)		NS
I x A		**
I (Q) x A (L)		NS
I (C) x A (L)		NS
I (L) x A (Q)		**
I (Q) x A (Q)		NS
I (C) x A (Q)		NS
Time		***
I (L) x Time		NS
I (Q) x Time		NS
I (C) x Time		NS
A (L) x Time		NS
A (Q) x Time		NS
I x A x Time		NS
I (Q) x A x Time		NS
I (C) x A x Time		NS
I (L) x A (Q) x Time		NS

Table 3.5 continued

I (Q) x A (Q) x Time	NS
I (C) x A (Q) x Time	NS

I=IBA, A=NAA, L=Linear, Q=Quadratic, C=Cubic

NS, **, ***, Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

[z]Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminal.

Table 3.6. Visual rating 12 weeks after planting male and female Chinese pistache cuttings treated with 0; 7,500; 15,000; or 22,500 mg·liter^{-1} IBA. Cuts were made above or below the sixth bud below the terminus and the fourth, fifth and sixth buds were removed or left intact. Rating scale: 0= dead, 1= no callus/no roots, 2= callus present/ no roots, 3= roots present/ no callus formed, 4= roots and callus, roots originating from callus, 5= roots and callus present but in different locations.

Gender	IBA (mg·liter^{-1})	Buds retained	Position of cut	Number of cuttings rating 0	1	2	3	4	5
			16 May 1994						
Male	0	Yes	Above	2	28	20	0	0	0
			Below	0	14	35	0	1	0
		No	Above	6	34	10	0	0	0
			Below	4	34	12	0	0	0
Male	7,500	Yes	Above	0	5	40	0	5	0
			Below	0	5	39	0	6	0
		No	Above	2	23	25	0	0	0
			Below	0	22	28	0	0	0
Male	15,000	Yes	Above	0	6	41	0	3	0
			Below	0	0	41	0	9	0
		No	Above	0	14	34	2	0	0
			Below	6	15	29	0	0	0
Male	22,500	Yes	Above	0	4	45	0	1	0
			Below	0	0	47	0	3	0
		No	Above	6	14	29	0	1	0
			Below	12	12	26	0	0	0
Female	0	Yes	Above	2	19	27	0	2	0
			Below	0	2	44	0	4	0
		No	Above	8	20	22	0	0	0
			Below	2	24	23	0	1	0
Female	7,500	Yes	Above	1	3	45	0	1	0
			Below	0	3	36	0	11	0
		No	Above	3	15	32	0	0	0
			Below	0	5	44	0	1	0
Female	15,000	Yes	Above	1	5	42	0	2	0
			Below	0	0	40	0	10	0
		No	Above	2	11	36	0	1	0
			Below	2	14	34	0	0	0
Female	22,500	Yes	Above	0	4	44	0	2	0
			Below	1	2	42	0	5	0

Table 3.6 continued

		No	Above	4	10	35	0	1	0
			Below	3	6	41	0	0	0
			23 May, 1994						
Male	0	Yes	Above	2	5	43	0	0	0
			Below	2	9	39	0	1	0
		No	Above	7	29	14	0	0	0
			Below	4	22	24	0	0	0
Male	7,500	Yes	Above	1	2	46	0	1	0
			Below	2	5	42	0	1	0
		No	Above	8	11	31	0	0	0
			Below	5	12	33	0	0	0
Male	15,000	Yes	Above	3	3	40	0	4	0
			Below	3	5	42	0	0	0
		No	Above	5	11	34	2	0	0
			Below	7	11	32	0	0	0
Male	22,500	Yes	Above	21	1	28	0	0	0
			Below	7	3	40	0	0	0
		No	Above	13	8	29	0	0	0
			Below	12	13	25	0	0	0
Female	0	Yes	Above	1	9	40	0	0	0
			Below	2	5	43	0	0	0
		No	Above	7	23	20	0	0	0
			Below	0	18	32	0	0	0
Female	7,500	Yes	Above	2	3	45	0	0	0
			Below	0	3	45	0	2	0
		No	Above	13	20	17	0	0	0
			Below	5	14	31	0	0	0
Female	15,000	Yes	Above	3	3	44	0	0	0
			Below	3	6	36	0	5	0
		No	Above	5	5	40	0	0	0
			Below	6	8	36	0	0	0
Female	22,500	Yes	Above	15	5	30	0	0	0
			Below	4	5	40	0	1	0
		No	Above	10	6	34	0	0	0
			Below	15	9	26	0	0	0

[z] 50 cuttings per treatment

Table 3.7. Effect of cutting date, gender, 0, 7,500, 15,000, 22,500 mg·liter^{-1} IBA, bud retention, and position of cut in relation to the sixth bud, on root length, secondary roots, and percentage of rooted cuttings.

IBA (mg·liter^{-1})	Buds retained	Position of cut	Primary root number[z]	Root length (cm)[y]	Secondary root number[x]	Rooted cuttings (%)[w]
			16 May 1994			
0	Yes	Above	0.09	0.57	0.00	1
		Below	0.08	1.33	0.01	5
	No	Above	0.00	----	----	0
		Below	0.04	0.12	0.02	2
7,500	Yes	Above	0.11	1.48	0.04	17
		Below	0.29	6.07	0.24	0
	No	Above	0.00	----	----	0
		Below	0.02	0.37	0.01	2
15,000	Yes	Above	0.07	1.15	0.03	2
		Below	0.44	7.33	0.28	19
	No	Above	0.05	1.03	0.01	3
		Below	0.00	----	----	0
22,500	Yes	Above	0.07	0.96	0.00	3
		Below	0.13	3.05	0.14	8
	No	Above	0.03	1.25	0.03	2
		Below	0.00	----	----	0
			23 May 1994			
0	Yes	Above	0.00	----	----	0
		Below	0.04	0.98	0.00	1
	No	Above	0.00	----	----	0
		Below	0.00	----	----	0
7,500	Yes	Above	0.01	0.37	0.00	1
		Below	0.04	0.72	0.01	3
	No	Above	0.00	----	----	0
		Below	0.00	----	----	0
15,000	Yes	Above	0.04	1.76	0.12	4
		Below	0.11	1.06	0.03	5
	No	Above	0.07	0.79	0.01	2
		Below	0.00	----	----	0
22,500	Yes	Above	0.00	----	----	0

Table 3.7 continued

		Below	0.01	0.03	0.00	1
	No	Above	0.00	----	----	0
		Below	0.00	----	----	0
ANOVA						
Time			***	***	***	
Rep			NS	NS	NS	
Gender			NS	NS	NS	
Bud[v]			***	***	***	
I(L)			NS	NS	NS	
I(Q)			**	**	**	
I(C)			NS	NS	NS	
I(L) x Time			NS	NS	NS	
I(Q) x Time			**	NS	NS	
I(C) x Time			NS	NS	NS	
I(L) x Gender			NS	NS	NS	
I(Q) x Gender			NS	NS	NS	
I(C) x Gender			NS	NS	NS	
I(L) x Bud			NS	NS	NS	
I(Q) x Bud			***	***	**	
I(C) x Bud			NS	NS	NS	
Gender x Bud			NS	NS	NS	
Time x Gender			NS	NS	NS	
Time x Bud			***	***	***	
I(L) x Time x Gender			NS	NS	NS	
I(Q) x Time x Gender			NS	NS	NS	
I(C) x Time x Gender			NS	NS	NS	
I(L) x Time x Bud			NS	NS	NS	
I(Q) x Time x Bud			**	**	**	
I(C) x Time x Bud			NS	NS	NS	
I(L) x Gender x Bud			NS	NS	NS	
I(Q) x Gender x Bud			NS	NS	NS	
I(C) x Gender x Bud			NS	NS	NS	
I(L) x Time x Gender x Bud			NS	NS	NS	
I(Q) x Time x Gender x Bud			NS	NS	NS	
I(C) x Time x Gender x Bud			NS	NS	NS	
Significance ($LSD_{0.05}$)						
Cutting time means for the same IBA and bud treatment			0.29	4.86	0.28	
Bud treatment for the same time and the same or different IBA			0.29	50.21	0.27	

Table 3.7 continued

IBA means for the same or different bud treatment and cutting time	0.28	20.35	0.03

I = IBA, L = Linear, Q = Quadratic, C = Cubic

[NS],**,*** Nonsignificant or significant at $P \leq 0.01$, 0.001, respectively.

[z] Mean number of roots per cutting.

[y] Mean root length of the 3 longest roots per cutting.

[x] Mean number of secondary roots per cutting produced on the 3 longest roots per cutting.

[w] 100 cuttings per treatment

[v] The bud treatment consists of a factorial combination of bud retention and position of cut (Fig. 3.1)

Table 3.8. ANOVA table for callus production data presented in tables 3.9, 3.10, 3.12, and 3.13.

ANOVA	
Time	NS
Rep	**
Gender	**
Bud[z]	***
I(L)	**
I(Q)	**
I(C)	NS
I(L) x Time	***
I(Q) x Time	NS
I(C) x Time	*
I(L) x Gender	NS
I(Q) x Gender	NS
I(C) x Gender	NS
I(L) x Bud	NS
I(Q) x Bud	**
I(C) x Bud	NS
Gender x Bud	*
Time x Gender	*
Time x Bud	***
I(L) x Time x Gender	NS
I(Q) x Time x Gender	NS
I(C) x Time x Gender	NS
I(L) x Time x Bud	NS
I(Q) x Time x Bud	NS
I(C) x Time x Bud	NS
I(L) x Gender x Bud	NS
I(Q) x Gender x Bud	NS
I(C) x Gender x Bud	NS
I(L) x Time x Gender x Bud	NS
I(Q) x Time x Gender x Bud	NS
I(C) x Time x Gender x Bud	NS

L=Linear, Q=Quadratic, C=Cubic, I=IBA concentration

[NS], *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, respectively.

[z] The bud treatment consists of a factorial combination of bud retention and position of cut (Fig. 3.1).

Table 3.9. Effect of cutting date and 0; 7,500; 15,000; and 22,500 mg·liter^{-1} IBA on Chinese pistache callus production 12 weeks after planting.

Cutting date	IBA concn. (mg·liter^{-1})	Callus (mm)[z]
16 May	0	1.04[y]
	7,500	1.81
	15,000	1.83
	22,500	1.84
23 May	0	1.37
	7,500	1.58
	15,000	1.75
	22,500	1.51

[z] Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminus.
[y] Mean of 400 cuttings.

Table 3.10. Effect of 0, 7,500, 15,000, 22,500 mg·liter^{-1} IBA, bud retention and cutting position in relation to the sixth bud, on Chinese pistache callus production.

IBA concn. (mg·liter^{-1})	Buds retained	Postion of cut	Callus (mm)[z]
0	Yes	Above	1.42[y]
		Below	1.90
	No	Above	0.56
		Below	0.93
7,500	Yes	Above	2.02
		Below	2.56
	No	Above	0.99
		Below	1.20
15,000	Yes	Above	2.12
		Below	2.77
	No	Above	1.20
		Below	1.06
22,500	Yes	Above	1.80
		Below	2.27
	No	Above	1.26
		Below	1.37

[z] Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminus.

[y] Mean of 400 cuttings.

Table 3.11. Effect of tree gender, bud retention, and cutting position in relation to the sixth bud on Chinese pistache callus production 12 weeks after planting.

Tree gender	Buds retained	Position of cut	Callus (mm)[z]
Male	Yes	Above	1.75[y]
		Below	2.10
	No	Above	0.95
		Below	0.89
Female	Yes	Above	1.94
		Below	2.65
	No	Above	1.06
		Below	1.39

[z] Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminus.

[y] Mean of 400 cuttings.

Table 3.12. Effect of tree gender and two cutting times on Chinese pistache callus production 12 weeks after planting.

Cutting date	Tree gender	Callus (mm)[z]
16 May	Male	1.39[y]
	Female	1.87
23 May	Male	1.46
	Female	1.65

[z] Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminus.

[y] Mean of 800 cuttings.

Table 3.13. Effect of cutting time, bud retention, and cutting position in relation to the sixth bud on Chinese pistache callus production 12 weeks after planting.

Cutting date	Buds retained	Position of cut	Callus (mm)[z]
16 May	Yes	Above	1.82[y]
		Below	2.60
	No	Above	0.99
		Below	1.10
23 May	Yes	Above	1.86
		Below	2.15
	No	Above	1.02
		Below	1.18

[z] Diameter of the callus at the widest point on the cutting minus stem diameter measured under the third bud basipetal from the terminus.

[y] Mean of 400 cuttings.

Table 3.14. Visual rating of rooting response of juvenile and adult Chinese pistache cuttings taken during October, November, and January and placed directly in media or exposed to 75 days chilling at 5C, then kept at greenhouse or 30C bottom heat temperature. Rating scale was: 0=dead, 1=alive-no activity, 2=callus, 3=root initials 4=1-2 primary roots, 5=≥3 primary roots.

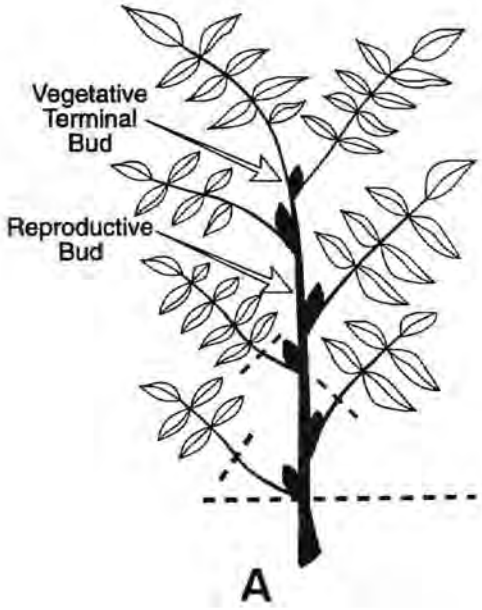
Month cut	Artificial chilling	Bottom heat	Visual rating at 12 weeks[z]
	Juvenile		
October	Yes	Yes	0
October	Yes	No	1
October	No	Yes	1
October	No	No	1
November	Yes	Yes	0
November	Yes	No	1
November	No	Yes	1
November	No	No	1
January	No	Yes	0
January	No	No	1
	Adult		
October	Yes	Yes	0
October	Yes	No	1
October	No	Yes	1
October	No	No	2
November	Yes	Yes	0
November	Yes	No	1
November	No	Yes	1
November	No	No	1
January	No	Yes	2
January	No	No	1
Significance:			
Treatment (T)			***
Age (A)			***
Coldtime[y](C)			***
Coldtime*Treatment			***
Treatment*Age			NS
Coldtime*Age			***
Coldtime*Treatment*Age			NS

[NS], ***, Nonsignificant or significant at $P \leq 0.001$, respectively.

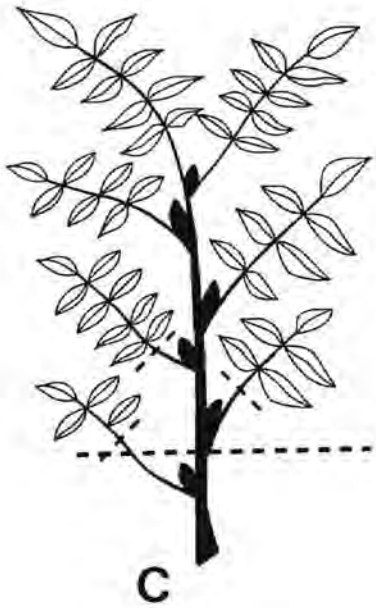
[z]Mean of 60 cuttings, evaluated after 12 weeks.

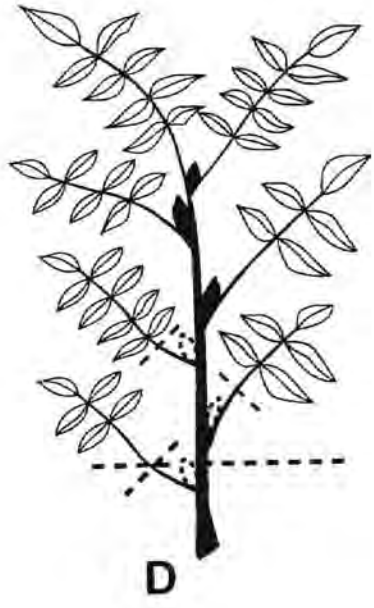
[y]Coldtime consists of months and chilling treatments combined into a total of 5 coldtime treatments.

Figure 3.1 Chinese pistache cuttings given four cutting treatments. (A) cut below the sixth bud, buds left intact; (B) cut below the sixth bud with fourth, fifth, and sixth bud removed; (C) cut above the sixth bud with buds left intact; (D) cut above the sixth bud with fourth and fifth buds removed. The basal 3 leaf petioles were cut near the buds on all treatments.


Vegetative
Terminal
Bud
Reproductive
Bud
A


B


C


D

Chapter IV

Propagation of *Pistacia chinensis* by Mound Layering

Diane E. Dunn[1] and Janet C. Cole[2]
Department of Horticulture and Landscape Architecture
Oklahoma State University
Stillwater, OK 74078

Additional index words: stooling, Chinese pistache

Abstract. **Chinese pistache (*Pistacia chinensis*, Bunge) is a commonly recommended ornamental shade tree in the nursery and landscape industry. Currently, Chinese pistache trees are propagated commercially from seed, resulting in highly variable branch habit and fall color. Mature Chinese pistache, like other members of the *Pistacia* sp., have proven difficult to root, graft, or bud successfully. This study was initiated to investigate the potential of mound layering as an alternative vegetative propagation method for producing genetically identical clones of superior mature Chinese pistache trees. Results from a greenhouse pre-trial in which trees were cut at two different heights and at three different morphological stages showed that significantly more shoots were produced when stock plants were cut at 5 cm compared to 1 cm, and when trees completely broke dormancy before cutting. Field trials during two consecutive years evaluated four treatments consisting of 1) wound; 2) 17,500 mg·liter^{-1} IBA; 3) wound and 17,500 mg·liter^{-1} IBA 4) no wound, no IBA. In 1993, 77% and in 1994, 75% of wounded shoots treated with IBA at 17,500 mg·liter^{-1} produced roots. Chemical name used: indolebutyric acid (IBA).**

Brought to America from China in the late 1800s, Chinese pistache is hardy in USDA hardiness zones 6 through 9 (Dirr, 1985). It flourishes in full sun, and reaches a mature height of 30 to 40 feet with a 20 to 30 foot spread (Whitcomb, 1985). It develops an oval, umbrella-like crown providing generally light-textured shade throughout the growing season. Chinese pistache is native to well drained alkaline soils, but is tolerant to most soil conditions (Lee et al., 1976a). In California, it is recommended for its xerophilous qualities and salt tolerance (Crockett, 1972). It is also drought tolerant and endures extreme heat and drying winds (Dewers, 1981; Behboudian et al., 1986; Spiegel-Roy et al., 1977). It survives winter temperatures to -26C (Koller, 1978), but is not adapted to zones where late spring frost occurs after budbreak. As a street tree, it continues healthy growth even when planted on narrow spacings (Long, 1960). Fall color is variable, ranging from dark red to yellowish-green. Most trees display some shade of brilliant orange-red, yet extreme diversity can exist even within the same tree (MacMillian Browse, 1988). Foliage is lush throughout the season since it doesn't suffer any insect or disease problems (Crockett, 1972; Whitcomb, 1985; Dirr, 1985).

Vegetative propagation of Chinese pistache would offer many benefits. Asexual reproduction in general, preserves desirable genetic traits by producing genetically identical clones. In Chinese pistache, benefits would include establishment of cultivars for marketing in the nursery industry. These cultivars would be propagated from superior, mature trees that have been evaluated for vigor, consistent fall color, disease and insect resistance, and branch habit. Establishment

of cultivars based on fruit bearing would also be beneficial where the presence of fruit would be a problem. Besides eliminating seedling variability, the selection of superior cultivars could eliminate problems such as freeze damage, weak crotch angles (Whitehouse, 1957), crooked trunks and multiple leaders (Dirr, 1985), root defects (Harris, et al., 1971), and problems with transplanting (Lee, et al., 1976a).

Rooting ability of cuttings from many woody plant species declines with increasing age of seedling-derived mother plants (Davis et.al, 1988). This inverse relationship between ontogenetic age and rooting was reported by Gardner (1929). The loss of rooting potential with increasing maturity is particularly severe in many long lived tree species and limits the success or efficiency in clonally propagating desirable mature individuals (Davis et al., 1988). Mature cuttings of Chinese pistache have proven difficult to root (Joley, 1960), however some rooting success has been experienced with cuttings from seedlings (Pair and Khatamian, 1982; Lee et al. 1976b). This is typical of the difficult-to-root *Pistacia* genus. Attempts to root *Pistacia vera* cuttings from mature trees have been unsuccessful (Joley and Opitz, 1971) or limited, with very high concentrations of IBA (Al Barazi and Schwabe, 1982; 1984; 1985). Maturity in Chinese pistache seems to be reached after two years (Pair and Khatamian, 1982; Joley, 1960; Lee, et al., 1976b), making asexual propagation more difficult.

Mound layering offers a method of manipulating stock plants to regain the high rooting potential of juvenile trees. Mound layering, or stooling involves the establishment of a parent stock plant which is then cut back to a very short stub--the

stool (MacMillian Browse, 1980). The process of stooling involves the initiation and development of roots on a stem before that stem is removed from its parent plant. Howard, et al. (1988) stated that severe pruning and the induction of adventitious shoots, produce plants which are juvenile-like in appearance and vigorous in growth. In an attempt to return *Ulmus americana* to a juvenile state, Schreiber and Kawase (1975) cut back 12-year-old elms from 23 to 200 cm in height. Cuttings were collected from shoots and then rooted, with most successful rooting (83%) resulting from the 23 cm stools. Maintenance of the juvenile state in radiata pine *(Pinus radiata* L.) is successful by growing stock plants in hedge rows kept continually pruned (Libby and Hood, 1976; Menzies, 1985). A stool bed or hedge can be used for 15 to 20 years providing it is maintained in a vigorous condition (Hartmann, et al., 1990). Propagation of cashew (*Anacardium occidentale* L.) is accomplished by pruning the whole plant and mound layering vigorous juvenile shoots (Ohler, 1979). Mound layering today is an efficient, mechanized, economical propagation system (MacDonald, 1986). The purpose of this study was to determine whether mound layering is an effective propagation method for Chinese pistache.

Materials and Methods

Greenhouse cutting time and height pre-trial. This experiment utilized 457 ten-month-old seedlings planted in 7 by 7 by 14 cm deep bottomless waxed cardboard boxes (705 ml) containing 1 peat:1 perlite (by volume) amended with 2.3 $kg \cdot m^{-3}$ 17N-3P-10K slow release fertilizer (Osmocote, Grace-Sierra, Milpitas, Calif.) and 0.6 $kg \cdot m^{-3}$ micronutrients (Micromax, Grace Sierra). Trees were kept in a shade house

at a maximum temperature of 32C under a maximum photosynthetic photon flux (PPF) of 945 $\mu mol \cdot m^{-2} \cdot s^{-1}$ until 26 October 1992. Trees were then placed in a cooler at 5C until 4 January 1993. Upon removal, trees were placed in a polyethylene covered greenhouse at the Oklahoma State University Nursery Research Station in Stillwater, Okla. They were maintained at a maximum/minimum air temperature of 37/18C and PPF of 815 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under natural photoperiod for the remainder of the experiment. Trees were fertilized with 20N-4.3P-16.6K water soluble fertilizer (Peter's Peat Light Formula, Grace-Sierra) at 22.5 mg N $liter^{-1}$ and micronutrients (STEM, Grace Sierra) at 0.06 $g \cdot liter^{-1}$ and at two week intervals until the experiments conclusion.

Seedlings were cut at 1 or 5 cm above the soil line on 4 Jan., 25 Jan., and 17 Feb. 1993. These dates corresponded to dormancy (buds not open), majority of buds broken, and leaves unfurled, respectively. Plants cut 4 Jan. were evaluated after 59 days, while those cut 25 Jan. and 17 Feb. were evaluated after 38 days. Evaluation occurred when most shoots were at least 10 cm long. All shoots were counted regardless of length and treatments were evaluated for number of shoots produced. Trees were randomized in a split plot design with cutting height as the main plot and cutting time as the subplot. Forty replications were used with a total of 457 subsamples. Analysis of variance procedures were performed on the data, and the differences between the treatment means were further determined with the least significant difference (LSD) test using SAS statistical software (SAS Institute, 1989).

Field study 1993. On 1 Oct. 1992, 56 ten-month-old trees were removed from 3.7 liter pots and field planted with 2.5 m between rows and 2.5 m between trees within rows at the Oklahoma State University Nursery Research Station. Trees were cut to one trunk per stool at that time. The soil type was a Norge loam (fine silty, mixed, thermic Udic Paleustolls) with a pH ranging from 4.9 to 5.2 throughout the field. Calcium carbonate was applied at 428 Kg/ha on 1 April. Maximum PPF was 1,780 $\mu mol \cdot m^{-2} \cdot s^{-1}$. On 14 May 1993, all trees were actively growing (leaves unfurled) and were cut to a height of 18 cm above the soil line and new shoots were allowed to develop. Shoots grew horizontally for about 10 mm, then parallel to the stool. Wounding consisted of slicing into the phloem and cambial tissue about 1 mm in depth and 8 mm in length along the top of the horizontal section of the shoots. The 17,500 $mg \cdot liter^{-1}$ IBA was applied by lightly rubbing the IBA onto this horizontal area. The morphological condition of the shoots was green softwood with the basal 2 cm beginning to lignify. Treatments were applied when shoots were 12 cm long, and all treated shoots were tagged to distinguish them from shoots that arose later. Sawdust was mounded around treated shoots to a depth of 8 cm. As new shoots developed above treated shoots they received the same treatment and the sawdust depth was increased. Trees received drip irrigation as necessary, sawdust was watered overhead every two days.

On 1 April 1994, sawdust was removed and each treated shoot was evaluated for primary root number, length of three longest primary roots, circumference of three largest primary roots, and number of secondary roots on the three longest

primary roots. Shoot height was measured from its point of emergence from the stool to the tip of the terminal.

Field Study 1994. Plants were treated as described in the 1993 field, study except that the trees were cut to 10 cm in height on 15 Apr. 1994. Thirteen blocks were used for a total of 52 trees. Trees were evaluated after 12 weeks for rooting response.

A randomized complete block design was used with seven and thirteen replications for 1993 and 1994, respectively. Each block consisted of four treatments 1) wound; 2) 17,500 mg·liter^{-1} IBA liquid application; 3) wound and 17,500 mg·liter^{-1} liquid IBA; 4) no wound and no IBA. IBA concentration was based on data from a greenhouse auxin trial (Appendix A). Data were subjected to analysis of variance (GLM), and means were separated by least significant difference (LSD) at $P \leq 0.05$ using SAS (SAS Institute, 1989).

Results

Greenhouse cutting time and height pre-trial. The interaction between cutting height and morphological condition of the seedlings was significant at $P \leq 0.01$ (Table 4.1). Cutting the stock plants at 5 cm after emergence of shoots and leaves produced the largest number of shoots. Regardless of whether the trees were dormant, had broken bud, or had unfurled leaves, the 5 cm height produced significantly more shoots than the 1 cm cutting height.

Field study 1993. Wounded shoots treated with 17,500 mg·liter^{-1} IBA produced significantly more primary roots than the other three treatments (Table

4.2). Mean root length, root circumference, and secondary root number for the wound/IBA combination treatment were also significantly greater than for the other three treatments. There was no significant difference in shoot height, regardless of treatment.

Field study 1994. Wounded shoots treated with 17,500 mg·liter^{-1} IBA produced significantly more primary roots than the other three treatments (Table 4.3). Since no primary roots formed on non-wounded plants, no root lengths, root circumferences, or secondary root numbers were available. There was no signficant difference in mean root length, root circumference, or secondary root number, regardless of IBA treatment in wounded stools. Shoots receiving the no wound/no IBA, or no wound/IBA treatment, were taller than wounded IBA treated shoots.

Discussion

Nurseries in Europe during the 18th, 19th, and early 20th centuries relied heavily on layering for vegetative propagation of plants (MacDonald, 1986). It was also often used to increase populations of plants collected by plant explorers after the parent plants had established themselves. Despite the advances in cutting propagation with the advent of mist benches and auxins, mound layering is the main technique used in both Europe and North America to clonally propagate *Malus* rootstocks such as Malling 9, Malling 26, and Malling Merton 106 (MacDonald, 1986). It is also a standard propagation practice for ornamentals, such as *Tilia* species, *Prunus cerasifera* J.F. Ehrh 'Nigra' (Black Myrobalan Plum), *Cotinus coggygria* Scop., *Chaenomeles* species, and *Castanea sativa* Mill. (Hartmann et al.

1990; MacMillian Browse, 1969; MacDonald, 1986). Mound layering is used with *Corylus maxima* 'Purpurea' Mill. (Purple Giant Filbert) and *Prunus tenella* Batsch 'Firehill' (Dwarf Russian Almond cv.) to produce larger plants over a shorter time as compared to cutting propagation (MacMillian Browse, 1980). The greenhouse pretrial established the most advantageous morphological cutting time and stock plant height for Chinese pistache field layering. These results were used in the field trials, except that height was increased to provide more potential shoots. Stock plant height the second year was decreased to eliminate any influence due to treatments during the previous year and to encourage shoots to be produced at the same time on the base. The 10 cm height accomplished these goals and would be recommended for future field studies.

The first year stock plants produced an average of 2 to 3 treatable shoots. The second year, many stock plants produced 8 to 12 shoots, so the number of shoots per tree were reduced during 1994 to the six largest to maintain uniformity across treatments. Commercially, the use of multiple leaders, multiple shoots, and close tree spacings would result in large numbers of marketable size Chinese pistache clones. Rooting percentage of shoots treated with a wound followed by IBA was 77% in 1993 and 75% in 1994. Comparably, M.9 apple rootstocks, with an average rooting of 70% when planted at standard 0.3 m x 1.8 m spacing yield 30,000 clones per acre (MacDonald, 1986). This suggests that a similar number of clones might be expected from mound layered Chinese pistache.

A high correlation between treatment and primary root number, length, circumference, secondary roots, and shoot height occurred both years. Wounding the shoots is usually necessary for root production in Chinese pistache but must be combined with the auxin treatment for high rooting percentages. Wounding removes the hardened periderm that may restrict root emergence. Wounding also allows the IBA to be placed directly on the cambium. Use of auxin is not a traditional procedure in mound layering but has been shown to be important in air layering of many species such as pecan (*Carya illinoinensis*)(Sparks and Chapman, 1970), chestnut (Vieitez, 1974) and cashew (*Anacardium occidentale*)(Ohler, 1979). Application of IBA was necessary for rooting of Chinese pistache, but only when combined with wounding.

In conclusion, wounding combined with 17,500 mg·liter^{-1} IBA produced roots on 75-77% of the treated shoots. Due to low rooting percentages of adult Chinese pistache cuttings, mound layering may offer a feasible alternative to producing well rooted cuttings of a new cultivar. A Chinese pistache tree with exemplary characteristics could be chosen, multiple cuttings taken, rooted in a greenhouse, and then planted in a field and mound layered indefinitely. Mound layering could also be used in conjunction with budding and grafting. Trees could be budded or grafted at ground level, then layered as described.

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Table 4.1. Influence of stock plant cutting heights of 1 and 5 cm, and three morphological times of cutting on Chinese pistache shoot production.

Cutting date	Stock plant morphology	Stock plant height (cm)	Number of shoots
4 Jan	Dormant	1	0.88[z]
4 Jan	Dormant	5	2.76
25 Jan	Budbreak	1	0.76
25 Jan	Budbreak	5	3.33
17 Feb	Shoots and leaves out	1	0.74
17 Feb	Shoots and leaves out	5	4.76
Significance $LSD_{0.05}$			
Date treatment for same height			.88
Date treatment for different height			.68
Height			***
Date			*
Height*Date			**

[z]Mean of 40 replications.
*,**,*** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 4.2. Number, length and circumference of primary roots, number of secondary roots and shoot height 40 weeks after wounding and application of 17,500 mg·liter^{-1} IBA to 18 month-old Chinese pistache seedlings in 1993.

Treatments						
Wound	IBA	Primary root number[z]	Root length[y] (cm)	Root circumference[x] (mm)	Secondary root number[w]	Shoot height (cm)
Yes	Yes	7.39a[v]	26.49a	9.10a	18.62a	48.92a
Yes	No	0.56b	2.82b	0.50b	1.33b	51.75a
No	Yes	0.67b	5.63b	0.90b	4.31b	45.53a
No	No	0.13b	0.60b	0.20b	2.53b	59.00a

[z]Mean number of primary roots per treated shoot.
[y]Mean length of three longest primary roots.
[x]Mean circumference of three largest primary roots.
[w]Mean number of secondary roots on 3 longest primary roots.
[v]Mean separation within columns by LSD at $P \leq 0.05$.

Table 4.3. Number, length and circumference of primary roots, number of secondary roots and shoot height 12 weeks after wounding and application of 17,500 mg·liter^{-1} IBA to 18 month-old Chinese pistache seedlings in 1994.

Treatments						
Wound	IBA	Primary root number[z]	Root length[y] (cm)	Root circumference[x] (mm)	Secondary root number[w]	Shoot height (cm)
Yes	Yes	10.97a[v]	75.12a	1.65a	21.92a	63.03c
Yes	No	3.26b	15.20a	1.22a	3.20a	80.84bc
No	Yes	0.00b	-----	-----	------	91.82ab
No	No	0.00b	-----	-----	------	106.85a

[z]Mean number of primary roots per treated shoot.
[y]Mean length of three longest primary roots.
[x]Mean circumference of three largest primary roots.
[w]Mean number of secondary roots on 3 longest primary roots.

Chapter V

Preliminary Studies to Determine the Micropropagation Potential of *Pistacia chinensis*

Diane E. Dunn and Janet C. Cole
Department of Horticulture and Landscape Architecture
Oklahoma State University
Stillwater, OK 74078

Additional index words. tissue culture, in vitro, Chinese pistache

Abstract. **Chinese pistache (*Pistacia chinensis* Bunge.) is a desirable ornamental shade tree in the nursery and landscape industries. A cultivar with reliable characteristics such as fall color and branch habit would be a welcome addition to these industries. Shoot tip culture was investigated as a method of cloning Chinese pistache. Shoot tips were grown in Driver-Kuniyuki Walnut (DKW) media amended with four concentrations of IBA, 0.01, 1.5, 2.5, and 3.5 mg·liter^{-1}, and BA concentrations of 1.0, 2.5, 3.5, and 4.5 mg·liter^{-1} in a completely randomized factorial design. The 16 treatments were evaluated at eight and ten weeks for callus, shoots, and root formation. Root formation did not occur, but callus formation was documented on explants. Upon expansion, terminal and axillary buds were excised from shoots during the first two weeks that the shoots were in culture. Excised buds displayed more callus formation and higher survival rates than shoot tips. There was**

no significant difference in shoot or bud survival with the various media combinations. Shoots developed from the callused base of two explants.

Chemical names used: indole-3-butyric acid (IBA), 6-benzylaminopurine (BA).

Chinese pistache is an ornamental shade tree which displays bright autumn color, heat and drought tolerance, and resistance to insects and diseases. Vegetative propagation of Chinese pistache would offer many benefits. Asexual reproduction preserves desirable genetic traits by producing genetically identical clones. The ability to asexually propagate Chinese pistache, would allow the nursery industry to market cultivars with desirable characteristics such as cold hardiness, brilliant fall color, strong branching habit, interesting bark texture and color, dense canopy, and disease and insect resistance. Male Chinese pistache cultivars could be marketed for use in locations reserved for trees guaranteed to be fruitless.

Marketing of a Chinese pistache cultivar would require development of a technique for mass propagation; however, mature Chinese pistache, like other *Pistacia*, have proven difficult to root, graft, or bud successfully (Joley, 1960; Long, 1960; Hall, 1975). Currently there is no report of Chinese pistache propagation by tissue culture; however, there has been some success with micropropagation of other *Pistacia* species. Shoot tips and nodal bud segments from aseptically germinated seedlings of *Pistacia vera* L. were successfully cultured by Barghchi and Alderson (1983). Nodal bud segments from mature *Pistacia terebinthus* L. were propagated by

Pontikis (1984). Other *Pistacia* micropropagation has been accomplished by Martinelli (1987), Parfitt and Almehdi (1991, 1992), Parfitt et al. (1990), Picchioni and Davies (1990), and Barghchi (1985).

The objectives of these studies were 1) evaluate the effect of light intensity and PVP on contamination and phenolic build-up; 2) determine the effect of combinations of IBA and BA on explant survival and callus and shoot formation; and 3) evaluate the effect of antibiotics on explants and contaminants.

Materials and Methods

Effect of Light Intensity and PVP on Restricting Contamination. A 30 g·liter^{-1} sucrose solution was adjusted to a pH of 5.5 with HCl. Agar was added at 10 g·liter^{-1} and the medium was boiled in a microwave oven until the agar was in solution. Polyvinylpyrrolidone (PVP) was added at 7 g·liter^{-1} to half of the medium. No additional amendment was added to the remaining medium. Both media (with or without PVP) were heated until the PVP completely dissolved in the medium containing it. Pyrex culture tubes (15 x 150 mm) were filled with 10 ml of medium and then heated at 121C and 0.1 MPa for 20 min in an autoclave. Tubes were then placed in slant racks and allowed to cool at a 45° angle.

On 27 Aug. 1993 shoot tips were cut from 19 month-old Chinese pistache maintained in a shade house with a maximum photosynthetic photon flux (PPF) of 945 μmol·m^{-2}·s^{-1} at the Oklahoma State University Nursery Research Station in Stillwater, Okla. Trees had been cut to 10 cm in height 10 months earlier to promote budbreak and shoot growth. Shoot tips 7 cm long were cut and leaves were

removed as close to the stem as possible. Shoots were then placed in a plastic container with tap water and transported to the lab and re-cut to a length of 5 cm. They were placed in a colander under 24C fast running tap water for 50 min and then placed in a beaker containing 70% ethyl alcohol (ETOH) for 1 min. Shoots were then placed in a disinfestant solution on a stirring plate at medium speed for 30 min. The disinfestant solution contained 0.05% NaOCl and 1.0% Liqui-Nox (Alconox, New York, N.Y.), adjusted to a pH of 5.0 with HCl. Shoots were placed in distilled, deionized water (DDH_2O) for 10 min on a stirring plate. Shoots then received two 5 min rinses in DDH_2O and were placed in a laminar air-flow hood and rinsed for 10 min in sterile DDH_2O. Shoots remained in sterile DDH_2O until they were re-cut with a scalpel to 3 cm in length to remove any tissue damaged by disinfesting treatments and then placed into media filled tubes. Five shoots from each medium type were placed in the following light treatments: high (36.3 μmol·m^{-2}·s^{-1}), medium (13.9 μmol·m^{-2}·s^{-1}), low (8.4 μmol·m^{-2}·s^{-1}), dark (6.3 μmol·m^{-2}·s^{-1}), and medium light with aluminum foil wrapped around the outside of the bottom of the tube to exclude light from the medium. Lamps used were cool white fluorescent Bright Stiks (General Electric, Cleveland, Ohio) placed 34 cm above each shelf.

After 18 days, number of shoots without contamination was evaluated for each medium and light treatment. The X^2 statistic was used to test the independence between light treatments.

Fall semi-hardwood shoot tip culture. Shoot tips were collected from 21 month-old Chinese pistache trees at the Oklahoma State University Nursery Research Station in Stillwater, Okla. on 28 Sep. 1993. Terminal shoot tip cuttings about 7 cm long were collected and leaves were removed from the cuttings as close to the stem as possible. Shoots with expanded terminal or axillary buds were avoided. Shoot tips were placed in a plastic storage container filled with tap water during collection and transported to the lab. In the lab, cuttings were placed in a strainer and kept under 24C running water for 1 h. Cuttings were cut with a scalpel to 5 cm and all remaining petioles were removed, without damaging remaining buds. Surface disinfestation was accomplished by placing cuttings in a 0.16% NaOCl, 2% tween 20 (Sigma, St. Louis, Mo), 95% DDH_2O solution neutralized to pH 7 with HCl, and agitating for 30 min. Shoots were immersed in 70% ETOH for 1 min, moved to a laminar air-flow hood, exposed to three sterile DDH_2O rinses, and then retained in DDH_2O. Shoots were individually removed from the water, cut to a final length of 3 cm, and placed in 25 x 150 mm Pyrex culture tubes. Each tube contained 10 ml of medium consisting of a modified Driver-Kuniyuki Walnut (DKW) medium (Parfitt and Almehdi, 1991 and 1992, Parfitt, et al., 1990). This media consisted of pre-packaged DKW basal salt mix (Sigma, St. Louis, Mo.) supplemented with 0.25 $mg \cdot liter^{-1}$ B, 8.5 $mg \cdot liter^{-1}$ Zn, and 1 $g \cdot liter^{-1}$ KNO_3. (Appendix B, Table B.1). To this, 30 $g \cdot liter^{-1}$ sucrose, and a vitamin solution containing 100 $mg \cdot liter^{-1}$ myo-inositol, 1.0 $mg \cdot liter^{-1}$ nicotinic acid, 2.0 $mg \cdot liter^{-1}$ glycine, and 2.0 $mg \cdot liter^{-1}$ thiamine was added. Initially, 2.2 $g \cdot liter^{-1}$ phytogel (Sigma, St. Louis, Mo.) was

added as a gelling agent, but all subsequent transfers were placed in DKW containing 2.5 g·liter^{-1} phytogel to prevent liquification. Media pH was adjusted to 5.5 with NaOH prior to adding phytogel.

BA was incorporated into the medium at 1.5, 2.5, 3.5, and 4.5 mg·liter^{-1} and IBA was incorporated at 0.01, 1.0, 2.5, 3.5 mg·liter^{-1}. The media were then boiled in a microwave until all phytogel was dissolved, dispensed into 25 x 150 mm Pyrex culture tubes, autoclaved for 20 minutes at 121C, and then allowed to cool upright. All tubes were sealed with Kaput-caps (Sigma, St. Louis, Mo.). Culture tubes containing explants were randomized and placed into slant racks and maintained under a 16-hour photoperiod. A factorial completely randomized design was used with ten replications per hormone combination. Shoots were subjected to low light (8.4 μmol·m^{-2}·s^{-1}) for seven days, then to an average PPF of 35.0 μmol·m^{-2}·s^{-1} at media height for the remainder of the experiment. Explants were maintained the first five weeks with average day/night air temperatures of 33/25C. Daytime temperature averaged 35C inside the tubes. Average air temperatures after the first five weeks were 28/23C and 27C within the tubes.

As terminal and axillary buds opened during the first two weeks, they were removed and placed in the amended DKW medium with 2.5 mg·liter^{-1} IBA to encourage rooting. After two weeks in culture, excised buds were transferred to basal DKW (0.01 mg·liter^{-1} IBA combined with 1.00 mg·liter^{-1} BA) for the remainder of the experiment. Sucrose was also decreased from 3 g·liter^{-1} to 2 g·liter^{-1} to reduce bacterial growth. On 16 Oct., about 3 weeks from initial culture,

about 3 mm was removed from the basal end of each explant to decrease bacterial growth.

Original terminal shoots were transferred to new media about every seven days. Excised buds were transferred about every three days. Criteria for determining transfer consisted of 1) darkening of the media, a sign of phenolic build up; 2) yellow or milky substance around the base of the explant; 3) presence of a clear bacterial halo formed in the media. Any sign of fungi constituted termination. At four, six, and eight weeks all explants were scrutinized for callus formation, stem tissue or bud necrosis, and signs of bacteria. Those with necrosis or bacteria were terminated. Explants remaining in culture were evaluated at 10 weeks for callus and shoot formation before final termination.

Number of excised shoots per treatment was subjected to analysis of variance (ANOVA) and separated by least significant difference (LSD) at $P \leq 0.05$ using SAS statistical software (SAS Institute, Cary, N.C.). Goodness of fit between observed and expected shoot tip and excised bud survival and tests of independence between IBA\BA treatments were performed using the X^2 statistic.

Spring softwood shoot tip culture. All leaves were removed prior to placing the trees used in the previous experiment in a cooler at 5C. Trees remained in the cooler from 1 Oct. 1993 until 14 Dec. 1993. Upon removal from the cooler they were placed in a greenhouse and drip irrigated. Cuttings were taken from the soft, succulent new growth on 20 Feb. These cuttings were exposed to: a) 45 min under 26C fast running tap water; b) immersion in pH neutralized disinfestant solution

consisting of 2% Liquinox, 0.16% NaOCl, and 95% sterile DDH_2O for 45 min; c) two sterile DDH_2O rinses in the lab for 10 and 5 min, respectively; d) two sterile DDH_2O rinses in the laminar air flow hood for 5 min each. Shoot tips were re-cut to 3 cm in length and placed in modified DKW medium amended with 6 $mg \cdot liter^{-1}$ polymyxin B sulfate, 6 $mg \cdot liter^{-1}$ rifampicin, and 25 $mg \cdot liter^{-1}$ tetracycline which were previously filter sterilized using disposable filters (Nalgene, Rochester, NY). All antibiotics were added to the medium after it had been autoclaved.

Due to a high shoot tip death rate from the antibiotic medium, a second disinfestation procedure utilizing the same antibiotics was initiated on 11 March. The second disinfestation procedure consisted of a) sterile DDH_2O rinse in beakers placed on an orbital shaker at high speed for 45 min.; b) placement in a pH neutralized 2% Liquinox, 0.16% NaOCl, and 95% sterile DDH_2O disinfestant solution on an orbital shaker for 45 min.; c) sterile DDH_2O rinse on shaker for 3 min; d) antibiotic rinse consisting of sterile DDH_2O with 6 $mg \cdot liter^{-1}$ polymyxin B sulfate, 6 $mg \cdot liter^{-1}$ rifampicin, and 25 $mg \cdot liter^{-1}$ tetracycline for 15 min.; e) sterile DDH_2O rinse on shaker for 5 min; f) sterile DDH_2O rinse in laminar hood for 10 min. Shoots were re-cut to 3 cm and placed into antibiotic-free modified DKW media amended with the same auxin/cytokinin concentrations used in the fall experiment.

Results

Effect of light intensity and PVP on restricting contamination. After 18 days, regardless of light treatments, agar medium without PVP remained clear, while agar

medium with PVP had turned brown. There was no significant difference in survival of shoot tips placed in PVP amended medium, regardless of light treatment (X^2 = 5.00, nonsignificant at $P \leq 0.05$) (Fig. 5.1). Survival did differ among the light treatments when shoot tips were placed in medium without PVP (X^2 = 10.22, significant at $P \leq 0.05$) (Fig. 5.2). High light produced the highest rate of budbreak and shoot and leaf production. Fungal and bacterial contamination was most apparent in the dark treatment, and nonexistent under low light after 18 days. The surface disinfestation procedure was inadaquate since 44% of shoots were contaminated after 18 days.

Fall semi-hardwood shoot tip cultures. High temperatures initially caused the medium to partially liquify, resulting in shoot tips submerging into the medium. Subsequently, fungal and bacterial contamination occurred. The shoots in 0.01 mg·liter^{-1} IBA with 3.5 mg·liter^{-1} BA medium were most affected. At ten weeks shoot loss was attributed to the following causes: 37.5% to bacteria, 32.5% to fungi, 8.75% to phenolics, and 3.75% to unknown causes.

There was no significant difference in shoot tip survival among media treatments after four, six, or eight weeks (X^2 = 18.1, 18.3 and 23.0, respectively). There was a tendency toward more live shoot tips in the medium consisting of 0.01 mg·liter^{-1} IBA combined with 4.5 mg·liter^{-1} BA (Fig. 5.3), however, the 0.01 mg·liter^{-1} IBA combined with 4.5 mg·liter^{-1} BA treatment had 90% of shoot tips alive with next best treatments having only 50% live shoot tips. At eight weeks 40%

of shoot tips in the 1.5 mg·liter^{-1} IBA combined with 2.5 mg·liter^{-1} BA medium were alive while there was no surviving explant in any of the five other media (Fig. 5.3).

Upon budbreak, 107 buds were excised from shoot tips during the first two weeks in culture. The 0.01 mg·liter^{-1} IBA/1.0 mg·liter^{-1} BA and 1.5 mg·liter^{-1} IBA/1.0 mg·liter^{-1} BA yielded the most excised buds (Fig. 5.4). The 2.5 mg·liter^{-1} IBA/1.0 mg·liter^{-1} BA medium treatment produced less buds for sub-culturing than the other treatments. There was no significant difference in bud survival among medium treatments after four, six, or eight weeks (X^2 = 16.14, 13.72, 8.74, respectively) (Fig. 5.5). After eight weeks, six excised buds and one shoot tip showed swelling and callus. After 10 weeks, 5 additional terminal buds and one more shoot tip had formed callus. At termination, there were 11 callused terminal buds and 2 callused shoots. Two callused terminals had small shoots at the union of the base and callus. One bud had 3 new shoots and a callus base 8 mm in diameter (Fig. 5.6). The other bud had 2 new shoots with a callus base 6 mm in diameter. It was not possible to determine whether shoots originated from callus or from latent buds preexisting at the base of the excised bud.

Spring softwood shoot tips. The combination of three antibiotics was toxic to the shoots, and caused immediate death of approximately 80% of the shoots. Necrotic tissue originated at the basal end and progressed upward. The experiment was terminated after 3 days. In the second spring shoot tip trial, approximately 50% of shoots died after 3 days in culture. The other 50% were not effected by the antibiotics and no contamination appeared by the time the experiment was

terminated fifteen days later. Necrotic shoots were more succulent at the time of induction than unaffected shoots.

Discussion

DKW was developed as a walnut specific medium (Driver and Kuniyuki, 1984). McGranahan et. al. (1987) used a corrected version of DKW medium. DKW was designed to be used exclusively with the gelling agent phytogel, which does not contain the contaminants that agar does. An amended DKW medium was chosen for this study based on the work of Parfitt and Almehdi (1991, 1992, 1994) and Parfitt et al.(1990). A comparison of 3 media, MS, Anderson, and DKW showed DKW to be superior for micropropagation of *Pistacia* UCB. Stefan and Millikan (1984) found PVP reduced oxidation of phenolic compounds and lethal browning in black walnut. Cotton (1983) found that PVP, a one-week dark treatment, and activated charcoal (3.0 $g \cdot liter^{-1}$) reduced the amount of phenolic compounds released in the media by pecan explants. Somers et al. (1982), found that PVP did not absorb exudates and may have promoted explant necrosis. In this study, PVP inclusion did not control phenolics and shoot survival was lower in medium containing PVP. Harmful phenolic oxidation products are formed in the presence of light, so reduction of light intensity at the initial stage was explored. Less than 4% browning was reported when garlic meristems were placed at 150 lux compared to 18% browning at high light (Wang and Huang 1975). Initial dark incubation of eucalyptus shoots prevented the production and oxidation of some inhibitory phenolic compounds (Durand-Cresswell and Nitsch, 1977). The low light level in the first

experiment resulted in the highest rate of shoot survival regardless of treatment.

Two of the media containing BA at 1.00 mg·liter^{-1} produced the most budbreak on shoots, resulting in the most excised terminal and axillary buds (Fig. 5.4). This agrees with the conclusion by Parfitt and Almehdi (1992) which stated that BA concentrations of 2.5 or 5 mg·liter^{-1} produced better bud break and better growth in vitro with *Pistacia atlantica* and *Pistacia integerima*. However, the treatment producing significantly less buds, also contained 1.00 mg·liter^{-1} BA (Fig. 5.4).

There was no significant correlation between callus formation and original IBA/BA media treatment. Callus was formed on 11 buds and 2 shoots, with massive callus (6 mm and 8 mm) on 2 of the excised buds. Callus formation on buds may be linked to endogenous auxin associated with terminal buds, or it may be associated with buds having been on 2.5 mg·liter^{-1} IBA medium for two weeks. Excessively applied auxin has been implicated in the stimulation of profuse callus growth and suppression of shoot proliferation in cultures of *Pistacia* and other woody species (Barghchi and Alderson, 1983; Tisserat, 1984).

Lack of regeneration may be accounted to the impending dormancy of the fall-harvested shoot tips. Shoots taken at this time had more woody, lignified tissue than those taken in the spring, and their growth rate had slowed. Dodds (1983) stated that the concentration of growth regulators in trees, particularly hardwoods, shows seasonal variation and specifically that only spring cambial explants of trees

were suitable for tissue culture purposes due to their ability to undergo a rapid increase in the rate of cell division.

Most buds, excised from shoots which eventually were lost to bacterial contamination, also became contaminated, indicating that the bacteria were present internally in terminal and basal portions of the shoots before excision of the buds. Buds survived longer when removed from the parent shoot tip than when they were left intact on the shoot. The majority of transfers were performed to prevent bacterial and phenolic build-up. Re-cutting basal portions at 3 weeks caused additional phenolic compounds to be released, thus requiring more frequent in vitro transfers. If bacteria and phenolic release could be controlled in future experiments with Chinese pistache, transfer rates in subsequent experiments could be decreased. McGranahan et al. (1987) concluded with black walnut shoot tips that daily transfers were required for the first week, then weekly to reduce phenolic build-up. In our experiments, reducing sucrose to 2% slowed bacterial spread, thereby extending the time between transfers. Zimmerman et al. (1991) found 1% sucrose to be more effective than 2% sucrose at suppressing apparent production of phenolics. Parfitt and Almehdi (1991, 1992) found that eliminating sucrose from the medium and exposing shoots to gaseous CO_2 induced shoots and roots on *Pistacia vera*.

Since internal within tube temperature averaged 35C, the explants may have been stressed and thus exhibited the external tissue darkening observed after the second week. High temperatures may have increased bacterial activity and phenolic compound release since both seemed to decrease once the temperature was reduced.

Pierik (1987), concluded that addition of antibiotics to media in concentrations high enough to control contaminants may inhibit growth and differentiation of higher plants. However, Young et al. (1984) found that when contaminated shoot cultures of apple and rhododendron were treated with cefotaxime, tetracycline, rifampicin, and polymyxin B sulfate in combination at 25, 25, 6, and 6 $mg \cdot liter^{-1}$, respectively, bacteria were completely eliminated. No deleterious effects to shoots occurred at these concentrations. Cotton (1983), tested a number of antibiotics (gentamicin sulfate, penicillin-G, streptomycin, and nystatin) for control of bacterial and fungal contamination in pecan micropropagation. In these experiments, however, 100% of pecan explants became contaminated, probably because the antibiotics were added to the medium before autoclaving. Autoclaving antibiotics degrades their formulation and eliminates their effectiveness (Pierik, 1987).

Based on these studies we can conclude that inclusion of PVP in the medium does not reduce phenolic build-up in the medium. Low light levels resulted in less observable contamination than higher light treatments or darkness. We observed less contamination after the temperature was lowered and after the sucrose was reduced from 3% to 2%. The combinations of IBA and BA in the DKW medium had no significant effect on callus formation, shoot formation, or shoot survivability. Excised buds placed in culture tended to live longer and produce more callus. The antibiotic combination used in the medium was toxic and would not be recommended. Antibiotics as a rinse were toxic to approximately half of the shoots. The surviving

shoots were free of contamination even after 18 days without being transferred, so the rinse did show some effectiveness preventing contamination.

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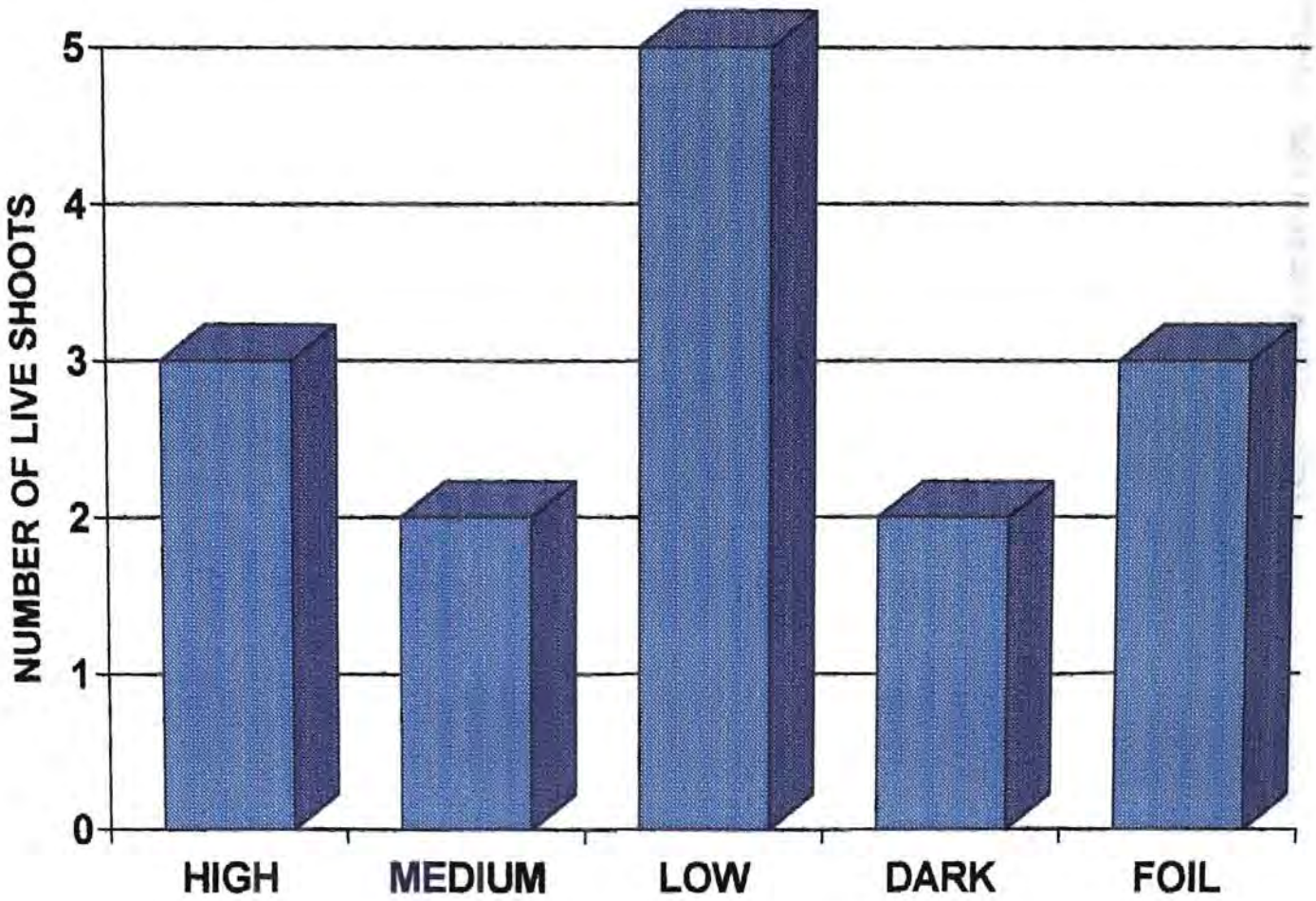
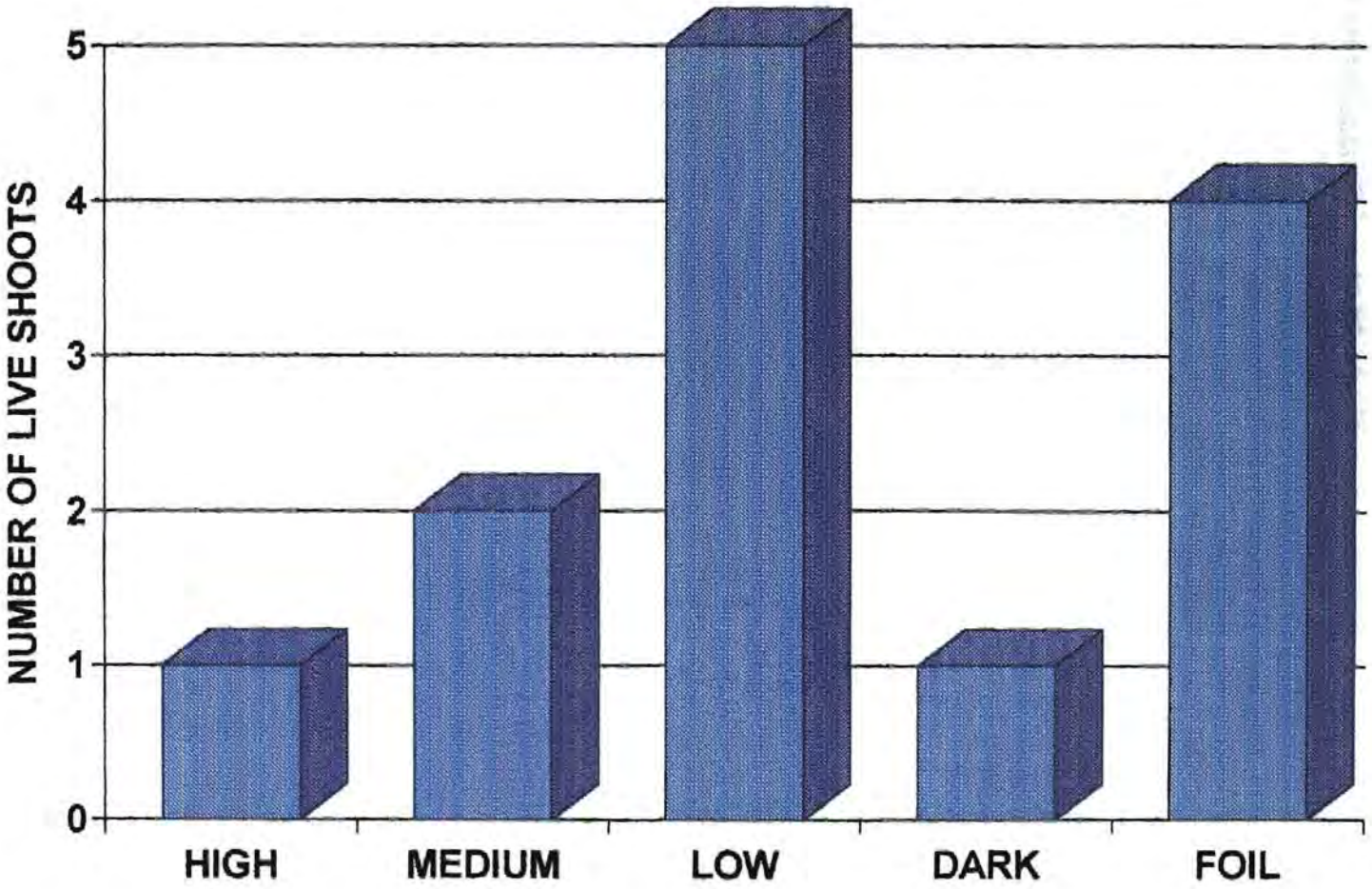

NUMBER OF LIVE SHOOTS
5
4
3
2
1
0
HIGH
MEDIUM
LOW
DARK
FOIL

Figure 5.1. Chinese pistache shoot survival 18 days after shoot induction in medium containing PVP, and exposed to five light treatments. There was no significant difference between light treatments ($X^2 = 5.00$, nonsignificant at $P \leq 0.05$).


NUMBER OF LIVE SHOOTS
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3
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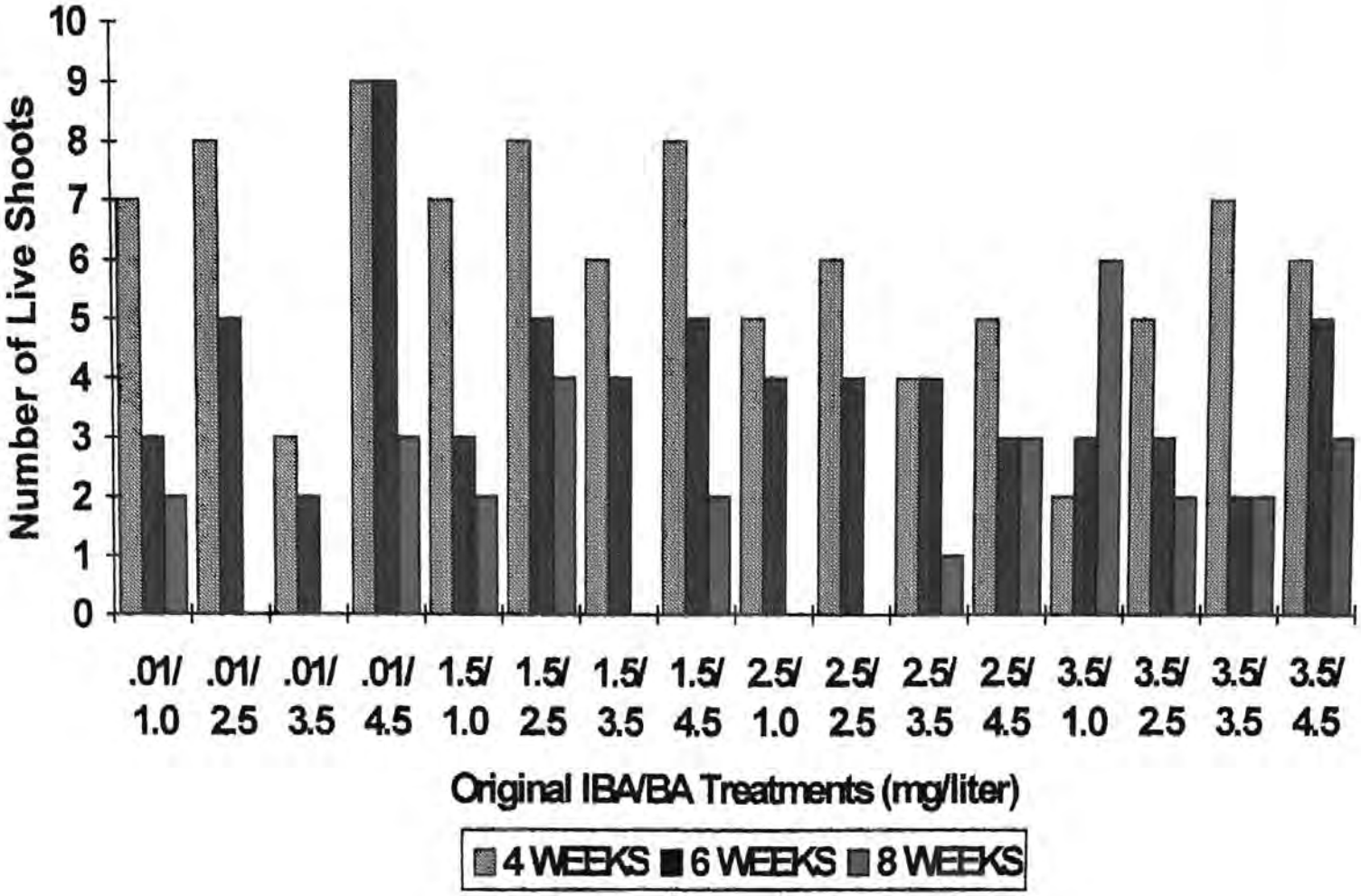
LOW
DARK
FOIL


Number of Live Shoots
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4
3
2
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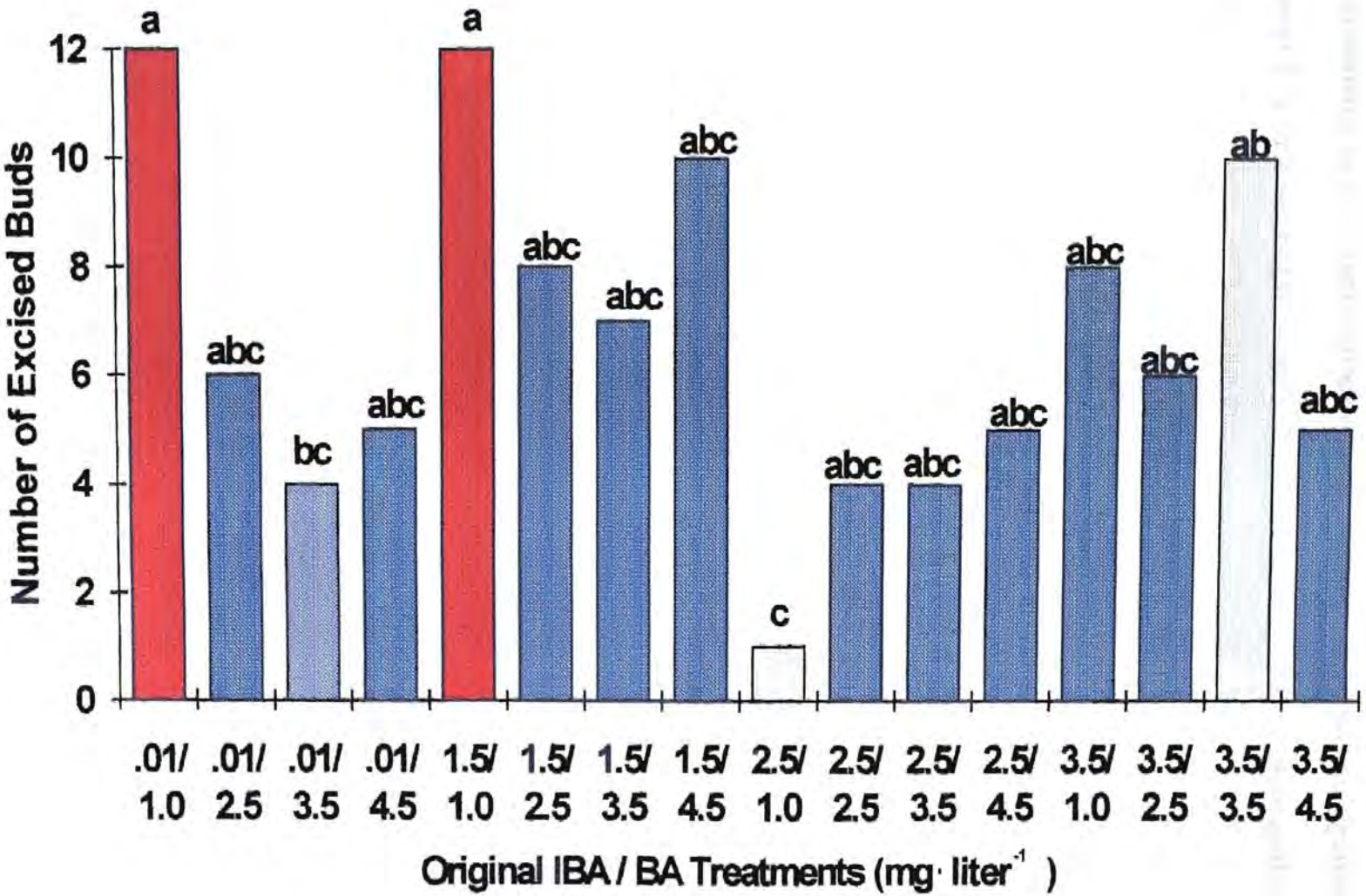
.01/ 2.5
.01/ 3.5
.01/ 4.5
1.5/ 1.0
1.5/ 2.5
1.5/ 3.5
1.5/ 4.5
2.5/ 1.0
2.5/ 2.5
2.5/ 3.5
2.5/ 4.5
3.5/ 1.0
3.5/ 2.5
3.5/ 3.5
3.5/ 4.5
Original IBA/BA Treatments (mg/liter)
4 WEEKS
6 WEEKS
8 WEEKS

Figure 5.3. Number of live shoot tips 4, 6, and 8 weeks after induction in medium containing various combinations of IBA and BA. There was no significant difference in survival among medium treatments at 4, 6, or 8 weeks (X^2 = 18.1, 18.3, and 23.0, respectively, non-significant at $P \leq 0.05$).

Figure 5.4. Number of Chinese pistache buds subcultured from shoots established on medium containing various combinations of IBA and BA. Treatments with the same letter were not significantly different ($LSD_{0.05}$).


Number of Excised Buds
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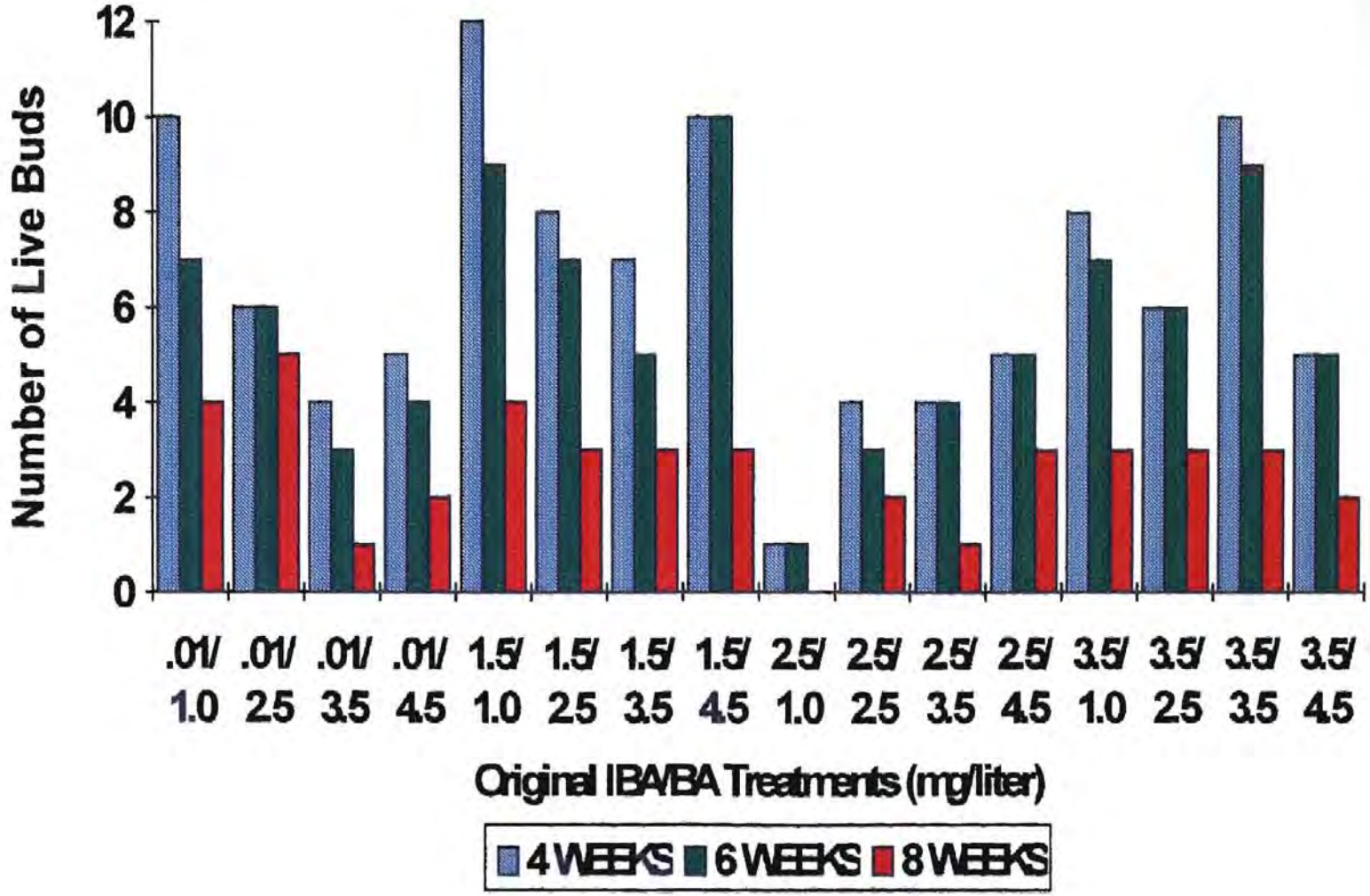
4
6
8
10
12
a
abc
bc
abc
a
abc
abc
abc
c
abc
abc
abc
abc
abc
ab
abc
.01/ 1.0
.01/ 2.5
.01/ 3.5
.01/ 4.5
1.5/ 1.0
1.5/ 2.5
1.5/ 3.5
1.5/ 4.5
2.5/ 1.0
2.5/ 2.5
2.5/ 3.5
2.5/ 4.5
3.5/ 1.0
3.5/ 2.5
3.5/ 3.5
3.5/ 4.5
Original IBA / BA Treatments (mg· liter-1)

Figure 5.5. Chinese pistache bud survival 4, 6, and 8 weeks after shoot induction on medium containing various combinations of IBA and BA. There was no significant difference at 4, 6, or 8 weeks (X^2 = 16.1, 13.8, and 8.5, respectively, nonsignificant at $P \leq 0.05$).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8
6
4
2
0
.01/ 1.0
.01/ 2.5
.01/ 3.5
.01/ 4.5
1.5/ 1.0
1.5/ 2.5
1.5/ 3.5
1.5/ 4.5
2.5/ 1.0
2.5/ 2.5
2.5/ 3.5
2.5/ 4.5
3.5/ 1.0
3.5/ 2.5
3.5/ 3.5
3.5/ 4.5
Original IBA/BA Treatments (mg/liter)
4 WEEKS
6 WEEKS
8 WEEKS

Figure 5.6. An excised Chinese pistache terminal bud ten weeks after induction, with a base of brown woody callus. Multiple shoots arise from the base.

roots per cutting were observed on cuttings which had been taken on 9 May and which had received 8,750 mg·liter^{-1} IBA. Male Chinese pistache cuttings should be taken from green softwood stems before 799 degree days have accumulated after orange budbreak.

The effect of auxins, postion of cut, bud retention, gender and bottom heat were studied from May 1993 through Sept. 1994. In May 1993, auxin treatments consisted of 1) 10,000 mg·liter^{-1} IBA, 2) 20,000 mg·liter^{-1} IBA, 3) 2,500 mg·liter^{-1} NAA, 4) 10,000 mg·liter^{-1} NAA, 5) 10,000 mg·liter^{-1} IBA+2,500 mg·liter^{-1} NAA, and 6) 20,000 mg·liter^{-1} IBA+10,000 mg·liter^{-1} NAA. One-half of the cuttings from each of the above treatments received a H_2SO_4 pre-treatment. Cuttings without H_2SO_4 but with 10,000 mg·liter^{-1} IBA+2,500 mg·liter^{-1} NAA had significantly higher root ratings than cuttings receiving no acid or auxin. On 9 May and 18 May 1994, auxin treatments consisted of 0; 5,000; 10,000; or 15,000 mg·liter^{-1} IBA, or 0; 5,000; or 10,000 mg·liter^{-1} NAA alone or in IBA/NAA factorial combination. Cuttings receiving 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA produced more rooted cuttings (38%) than other treatments. The IBA by NAA by time interaction for root number was significant at $P \leq 0.01$.

Cuttings collected on 16 May and 23 May were dipped in 0; 7,500; 15,000; or 22,500 mg·liter^{-1} IBA and cut either above or below the bud, with remaining buds either retained or removed. Cuttings taken on 16 May and given 15,000 mg·liter^{-1} IBA, with buds retained and stems cut below a bud, produced more primary and secondary roots with longer primary roots than those of other treatments.

A total of 1200 juvenile and adult hardwood cuttings exposed to greenhouse temperature or 30C bottom heat and cut in October, November, and January produced only two rooted cuttings. Bottom heat did not promote rooting.

Due to the low rooting percentages obtained from adult Chinese pistache cuttings, mound layering was a feasible alternative method of propagation. Results from a greenhouse pre-trial showed that significantly more shoots were produced when stock plants were cut at 5 cm compared to 1 cm, and when trees completely broke dormancy before cutting. Field trials performed during two consecutive years which evaluated the effect of wounding and IBA application, resulted in 77% rooted shoots in 1993, and 75% rooted shoots in 1994 when shoots were wounded, and then treated with 17,500 mg·liter^{-1} IBA.

Preliminary studies to determine the micropropagation potential of Chinese pistache were performed. A study to determine the effect of light intensity and use of PVP in the medium to restrict contamination showed no significant difference in survival of shoot tips placed in PVP amended medium regardless of light treatment. Survival did differ among the light treatments when shoot tips were placed in medium without PVP. High light produced the highest rate of budbreak and shoot and leaf production. In the fall semi-hardwood shoot tip trial, there was no significant difference in shoot tip survival among medium treatments after four, six, or eight weeks. There was no significant difference in bud survival among medium treatments after four, six, or eight weeks. At termination, there were 11 callused

terminal buds and 2 callused shoots. Two callused terminals had small shoots at the union of the base and callus.

In conclusion, vegetative propagation of Chinese pistache with cuttings is possible as long as the cuttings are taken as green softwood cuttings. Cuttings should be collected before 799 degree days have accumulated, based on a threshold temperature of 7.2C. Auxin treatments of 8,750 mg·liter^{-1} IBA or 5,000 mg·liter^{-1} IBA combined with 5,000 mg·liter^{-1} NAA were most successful in initiating roots on cuttings. All buds should be retained on cuttings, and the cut position should be directly beneath a bud. Mound layering offers an alternative to cutting propagation, producing enough rooted shoots to be commercially feasible. Because of endogenous bacteria and other contamination problems, shoot tip culture of Chinese pistache is not recommended.

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Appendix A

Propagation of *Pistacia chinensis* By Mound Layering

Two greenhouse experiments were performed to determine the rooting potential of shoots produced from Chinese pistache trees that had been cut back to heights of 1 cm or 5 cm. The first trial was performed to determine the auxin concentration, formulation, and application method that would result in the most root production on non-excised shoots in future mound layering (stooling) experiments. Also, the necessity of wounding was evaluated. The second trial determined the rooting potential of shoots severed from the trees and placed under mist in a greenhouse.

Methods and Materials

Greenhouse auxin and wounding trial. On 31 March 1993, shoots from 15 month-old trees that had been maintained in a greenhouse with maximum/minimum air temperature of 37/18C and maximum PPF of 815 μmol·m^{-2}·s^{-1} under a natural photoperiod and cut to a height of 5 cm were given one of the following treatments: 1) wounding + Hormodin 3 IBA talc (MSDAGVET, Rahway, N.J.), 2) wound + 8,750 mg·liter^{-1} IBA, 3) 0.05 ml injection of 8,750 mg·liter^{-1} IBA, 4) wound + 17,500 mg·liter^{-1} IBA, 5) 0.05 ml injection of 17,500 mg·liter^{-1} IBA, 6) wound only, 7) no wound + no auxin. The knife wound consisted of an angled cut through the epidermis and phloem to the cambium. This was performed on the upper surface

of the horizontal portion of the shoot. The injection treatment consisted of a puncture wound with a 0.01 mm hypodermic needle followed by a 0.05 ml injection of IBA. Sawdust was mounded around the shoots after treatment and kept moist. There were twenty-four shoots per treatment. After 5 weeks the sawdust was removed and the number of shoots that produced roots was counted.

Excised shoot trial. On 10 April 1993, shoots were excised from trees that had been cut back to 1 cm or 5 cm. Ten shoots were excised from each height treatment and given a 5 sec quick dip of one of the following treatments 1) 8,750 mg·liter^{-1} IBA, 2) 17,500 mg·liter^{-1} IBA, 3) 35,000 mg·liter^{-1} IBA, 4) H_2SO_4 (sulfuric acid) followed by 17,500 mg·liter^{-1} IBA. Shoots were placed in 12 cm wide x 36 cm long x 6 cm deep plastic rooting flats containing 1 peat:3 perlite (by volume) and kept under a mist cycle of 2 sec duration with 2 min frequency. After 5 weeks the number of rooted shoots was counted.

Results and Discussion

Greenhouse auxin and wounding trial. Auxin treatments were significantly different at $P \leq 0.001$ ($X^2 = 94.4$) with wounding followed by 17,500 mg·liter^{-1} IBA and injected 8,750 mg·liter^{-1} IBA producing the most rooted shoots (Table A.1). Since the auxin was applied directly to the cambium, the injected 17,500 mg·liter^{-1} IBA treatment burned the surrounding tissue causing tissue necrosis. Based on these results, wounding with a knife followed by an application of 17,500 mg·liter^{-1}

IBA had the potential to produce the most roots on non-excised shoots, and was used in the 1993 field experiment.

Excised shoot trial. The auxin treatments used on the shoots were significantly different at $P \leq 0.001$ ($X^2 = 65.0$) with 17,500 mg·liter^{-1} IBA applied to shoots from trees cut to 1 cm producing 100% rooted shoots (Table A.2). The 35,000 mg·liter^{-1} IBA treatment and application of H_2SO_4 followed by 17,500 mg·liter^{-1} IBA treatments burned the shoots, resulting in tissue necrosis and failure to root. Shoots from the 1 cm treatment were less lignified and therefore rooted slightly better than the shoots from the 5 cm tree height.

Table A.1. Number of Chinese pistache shoots that produced roots after receiving an IBA talc dip (Hormodin 3), an IBA quick dip at 8,750 mg·liter^{-1} or 17,500 mg·liter^{-1}, or no IBA. The shoot was wounded by an angled cut (W) or a puncture with a hypodermic needle (I) through the epidermis and phloem to the cambium, or received no wound (N). They were then mound layered in a box in a greenhouse. Treatments were significantly different at $P \leq 0.001$ ($X^2 = 94.4$).

Auxin treatments (mg·liter^{-1})	Wound treatment	Number of rooted shoots
Hormodin 3[z]	W	0[y]
IBA 8,750	W	10
IBA 8,750	I	20
IBA 17,500	W	21
IBA 17,500	I	2
None	W	4
None	None	0

[z] Hormodin 3 concentration is 8,000 mg·kg^{-1} IBA.
[y] 24 shoots were treated per treatment.

Table A.2 Rooting response of Chinese pistache shoots excised from stock plants cut to a height of 1 cm or 5 cm then treated with 8,750, 17,500, or 35,000 mg·liter^{-1} IBA or dipped in H_2SO_4 plus the 17,500 mg liter IBA, and placed under mist in rooting media. Treatments were significantly different at $P \leq 0.001$ ($X^2 = 65.0$).

Auxin treatment (mg·liter^{-1})	Original cutting height (cm)	Number of rooted shoots
IBA 8,750	1	8[z]
IBA 8,750	5	7
IBA 17,500	1	10
IBA 17,500	5	8
IBA 35,000	1	0
IBA 35,000	5	0
H_2SO_4 + IBA 17,500	1	0
H_2SO_4 + IBA 17,500	5	0

[z] Ten shoots were used per treatment.

Appendix B.

Table B.1 Final concentration of *Pistacia chinensis* media.

Component	Mm	mg·liter^{-1}
NH_4NO_3	17.7	1416.0
$Ca(NO_3)_2 * H_2O$	8.3	1968.0
K_2SO_4	8.9	1559.0
$MgSO_4 * 7H_2O$	3.0	740.0
$CaCl_2 * 2H_2O$	1.0	149.0
KH_2PO_4	1.9	265.0
m-Inositol	0.55	100.0
Sucrose[z]	87.6	30,000.0
	Um	**mg·liter^{-1}**
$Zn(NO_3)_2 * 6H_2O$	85.8	25.5
$MnSO_4 * H_2O$	198.2	33.5
$CuSO_4 * 5H_2O$	2.0	0.5
H_3BO_3	77.6	4.8
$Na_2MoO_4 * 2H_2O$	1.6	0.39
$FeSO_4 * 7H_2O$	121.5	33.8
Na_2EDTA	135.0	45.4
$NiSO_4 * 6H_2O$	0.02	0.005
Thiamin * HCl	5.9	2.0
Nicotinic acid	8.1	1.0
Glycine	26.6	2.0
KNO_3		1,000.0

[z] Adjusted to 20,000.0 mg·liter^{-1} at 3 weeks into Fall Shoot Tip Experiment, and for Spring Softwood Experiments.

VITA

Diane Dunn

Candidate for the Degree of

Master of Science

Thesis: VEGETATIVE PROPAGATION OF CHINESE PISTACHE

Major Field: Horticulture

Biographical:

Personal Data: Born in Kansas City, Missouri on May 29, 1957.

Education: Received Associate of Applied Science degree in Horticulture Technology from Oklahoma State University, Oklahoma City in 1991, and Bachelor of Science degree in Horticulture from Oklahoma State University, Stillwater in 1993. Requirements for the Master of Science degree with a major in Horticulture at Oklahoma State University were completed in May 1995.

Experience: Employed by Oklahoma State University Department of Horticulture as a graduate research assistant 1993 to present.