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Effect of Ca Saturation and Air Filled Porosity of Sphagnum Peat on Root Regeneration of *Pistacia chinensis* Bunge and *Liquidambar styraciflua* L.[1]

C. I. Lee, J. L. Paul, and W. P. Hackett
Department of Environmental Horticulture, University of California, Davis, CA 95616

Additional index words. Root growth, soil pH, soil aeration

Abstract. **Root regeneration from root cuttings of both difficult-to-transplant *Pistacia chinensis* and moderately easy-to-transplant *Liquidambar styraciflua* was studied in a sphagnum peat medium varying from 0-100% Ca saturation and from 0-50% air filled porosity. Maximum root regeneration of *Pistacia* root cuttings was obtained at 75% Ca saturation and 30% and 40% air filled porosity, whereas *Liquidambar* root cuttings regenerated roots best at 25% Ca saturation and at 20% to 40% air filled porosity. Indolebutyric acid applied to the root cuttings greatly increased root-regenerating potential of *Pistacia* root cuttings but did not affect the optimum Ca and aeration requirement(s). Similarly, indolebutyric acid treatment greatly promoted the root-regeneration potential of *Liquidambar* root cuttings. Satisfactory root-regenerating conditions of both Ca saturation and air filled porosity for *Liquidambar* root cuttings were a little broadened by indolebutyric acid (IBA) application.**

***Pistacia* bare root seedlings also required high levels of Ca saturation and aeration for optimum root regeneration. Considerably greater numbers of roots were regenerated in peat having 75% Ca saturation and 20% air filled porosity than in peat having 0% Ca saturation and 5% air filled porosity. Root regeneration was not improved by increasing only the air filled porosity when Ca was low.**

Pistacia chinensis is an excellent shade tree but is difficult to transplant. *Pistacia* naturally grows in a well drained soil with high lime content (14) and thus root regenerating ability of *Pistacia* may be expected to increase if transplanted in a soil similar to its natural habitat. Adventitious root initiation of stem cuttings is known to be influenced by exchangeable Ca level (10, 11) and aeration in the rooting medium (5, 15) but rooting response to Ca in the rooting medium varies with species (9). Similar influences on the root regeneration of transplanted woody plants have not been discussed in the literature, but root regeneration may be similarly affected by these variables.

This study was performed to determine whether root regeneration of transplanted *Pistacia* is influenced by Ca saturation and aeration levels in sphagnum peat and to compare root regeneration with that of easy-to-transplant *Liquidambar*. *Liquidambar* naturally grows in poorly drained acid soils.

Materials and Methods

Preparation of peats for root cutting experiment. Canadian sphagnum peat was sieved through a 2 mm screen and air dry moisture content was determined. Six lots of air dry peat, equivalent to 75 g oven dry peat was placed in polyethylene bags. Appropriate amounts of $CaCO_3$ were mixed thoroughly with the air dry peat samples so that 0, 10, 25, 50, 75, and 100% of the titratable carboxylic groups were Ca saturated. Distilled water added in small increments to each bag with repeated vacuum applications to wet the peats to a final air filled porosity of 30% for *Pistacia* and 25% for *Liquidambar*. The pH's of the peats were 3.7, 3.9, 4.3, 5.0, 6.0 and 6.6. To vary the air filled porosity a moistened Ca-peat was placed in air-tight containers overnight wetted with appropriate volumes of distilled water to make a 0, 10, 20, 25, 30, 40 and 50% air filled porosity. The following relationship was used to estimate volume additions of water. Where E_a is the air filled porosity (percentage of the bulk volume occupied by air), P_V is the volumetric moisture content, and ρ_b and ρ_r are bulk density and particle density respectively. The bulk density was adjusted to 0.1 g/cc and the particle density of peat is 1.60 g/cc which was previously determined by standared picnometer method.

$$P_V = (1-\frac{\rho_b}{\rho_r})\ 100-E_a$$

Wetted peat equivalent to 75 g oven dry wt was placed into plastic containers (16 × 12 × 6 cm) and firmed to a bulk density of 0.1 g/cc. Root cuttings were taken from 2-year-old *Pistacia* and *Liquidambar* in April when the plants resumed spring

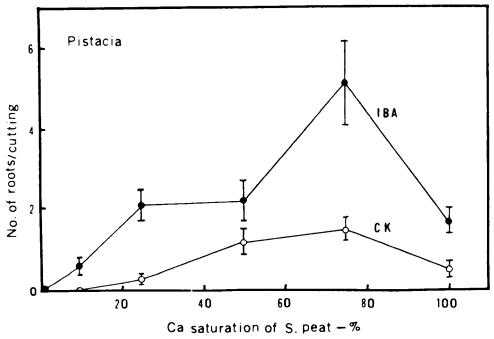

Fig. 1. Influence of Ca saturation of sphagnum peat on the root regeneration of *Pistacia chinensis* root cuttings. The peat has 30% air filled porosity.

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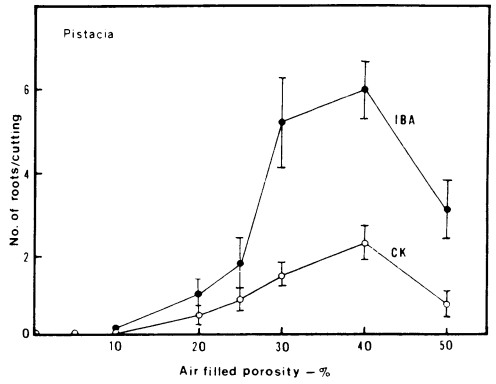

Fig. 2. Influence of air filled porosity of sphagnum peat on the root regeneration of *Pistacia chinensis* root cuttings. The peat has 75% Ca saturation.

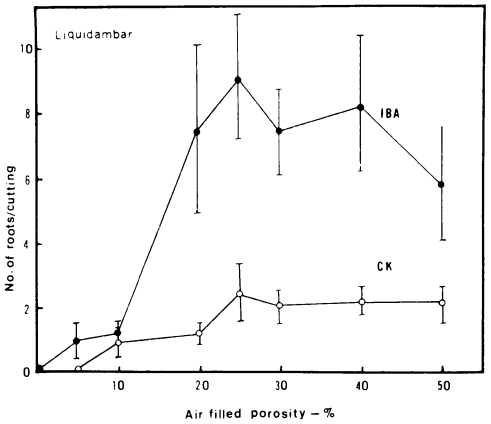

Fig. 4. Influence of air filled porosity of sphagnum peat on the root regeneration of *Liquidambar styraciflua* root cuttings. The peat has 75% Ca saturation.

growth. The root cuttings were washed thoroughly with distilled water. Each root cutting was 10 cm long and 2-3 mm in diam. Forty root cuttings were used in each level of Ca saturation and aeration. Twenty root cuttings were dipped in 3000 ppm solution of K-salt of IBA for 30 sec and 20 served as controls. Ten root cuttings per container were buried 3 cm deep in the peat. To insure saturation in the treatment where E_a equaled zero, a film of free water was maintained on the peat surface during the experiment. The containers were placed in unsealed polyethylene bags to reduce evaporation and placed in an incubator maintained at 20°C with no light. Moisture content in the peats was maintained by weighing at weekly intervals and adding distilled water to bring the containers to their initial weights. After 1 month, the cuttings were removed from the container and the no. of new roots was counted. Data are presented as means with standard errors.

Preparation of peats for Pistacia seedlings experiment. Peat equivalent to 200 g oven dry wt was prepared as it was for the root cutting experiment. The treatments were: 0% Ca saturation + 5% air filled porosity, 0% Ca saturation + 20% air filled porosity, 75% Ca saturation + 5% air filled porosity, and 75% Ca saturation + 20% air filled porosity. Wetted peats were then packed into 3.8 liter cans without drainage. Container grown 1-year-old *Pistacia* seedlings in an active state of growth were used. Growing medium was gently removed from the roots and the root system was washed with distilled water and pruned to 10 cm. All fibrous roots were removed leaving only 5 roots 2-3 mm in diam per seedling including the tap root. Shoot systems were defoliated and pruned to 60 cm in height. The pruned seedling contained about 30 lateral buds. The root system of the seedling was buried 10 cm deep in the peat which was 11 cm deep in the container. One seedling was planted per container and 9 seedlings were planted in each treatment. After transplanting, a 3 cm layer of perlite was placed on the surface of the peat to reduce evaporation. For further reduction of evaporation, each container was placed in a loosely sealed polyethylene bag. A near constant moisture content was maintained by adding water as needed every week. The transplanted seedlings were grown on a greenhouse bench with natural daylength but partially shaded with a single layer of cheese cloth. Average temp was 24°C (day) and 18° (night). Light intensity was 17.2 klx. After 1 month, seedlings were carefully removed from the cans, and no. of lateral buds broken and new roots regenerated were determined.

Results

Air filled porosity and Ca saturation in the peats greatly influenced root-regenerating potential (RRP) of root cuttings of both *Pistacia* and *Liquidambar*. The RRP of *Pistacia* root cuttings was completely inhibited in the peats having 0-10% Ca saturation with 30% air filled porosity (Fig. 1). Rooting gradually increased with Ca saturation. Maximum RRP was obtained at 75% Ca saturation, and a drop of RRP occurred at 100% Ca saturation. *Pistacia* root cuttings responded also to air filled porosity (Fig. 2). No roots were regenerated at 0-10% air filled porosity with 75% Ca saturation and a maximum RRP was

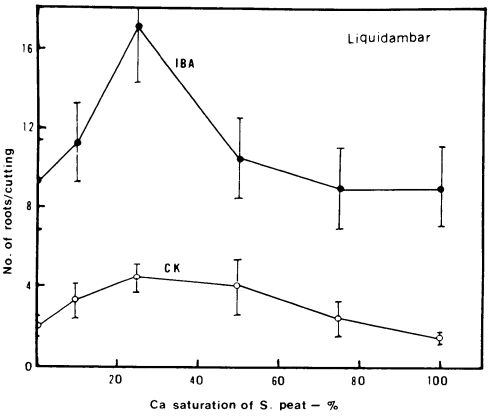

Fig. 3. Influence of Ca saturation of sphagnum peat on the root regeneration of *Liquidambar styraciflua* root cuttings. The peat has 25% air filled porosity.

Table 1. Root regeneration and lateral bud activity of defoliated, pruned *Pistacia chinensis* seedlings transplanted to sphagnum peat of different Ca saturation and air filled porosity.

Treatment	No. roots regenerated/root	No. lateral buds growing/seedling
0% Ca saturation + 5% air filled porosity	0.1 ± 0.1[z]	6.3 ± 1.4
0% Ca saturation + 20% air filled porosity	0.3 ± 0.2	6.2 ± 2.4
75% Ca saturation + 5% air filled porosity	3.2 ± 1.0	11.6 ± 2.3
75% Ca saturation + 20% air filled porosity	9.3 ± 1.0	12.6 ± 2.6

[z]Mean and SE.

found at 30% and 40% air filled porosity. The RRP was decreased again at 50% air filled porosity probably due to moisture stress.

Unlike *Pistacia, Liquidambar* root cuttings regenerated roots at 0% Ca saturation with 25% air filled porosity (Fig. 3). RRP reached a maximum at 25% Ca saturation and gradually decreased when the peats were saturated with more than 25% Ca. RRP of *Liquidambar* was also influenced by air filled porosity (Fig. 4). Low air filled porosity decreased RRP but minimum air filled porosity for the development of maximum RRP was 20% and the high RRP remained fairly constant even when air filled porosity was increased up to 50%.

IBA treatment greatly enhanced the RRP of *Pistacia* root cuttings, but it did not affect the optimum requirement for Ca and air in the peats (Fig. 1, 2). The promotive effect of IBA on root regeneration was similar in *Liquidambar* root cuttings, but IBA treatment enhanced the difference of RRP in various Ca saturation levels, although maximum RRP was still recorded at 25% Ca saturation (Fig. 3).

As shown in Table 1, *Pistacia* seedlings transplanted into peats responded distinctly in both lateral bud activity and RRP to the different levels of Ca saturation and air filled porosity. At 0% Ca saturation, root regeneration of *Pistacia* was almost completely inhibited and this inhibition was not overcome by increasing the air filled porosity. Lateral bud break was also restricted at 0% Ca saturation. The highest no. of roots was regenerated at 75% Ca saturation and 20% air filled porosity, and this RRP was comparable with that of *Pistacia* seedlings transplanted in a bottom misting chamber. Reduction of air filled porosity from 20% to 5% in peat with 75% Ca saturation did not affect no. of lateral buds broken but considerably decreased RRP.

Discussion

The foregoing results show that root regeneration of root cuttings of *Pistacia* and *Liquidambar* differ in response to variation in exchangeable Ca and aeration. The results may be broadly interpreted on the basis of native soil habitat. *Pistacia* is classified a gypso-calciphilous plant that grows in well drained soil (14), whereas *Liquidambar* grows naturally in alluvial swamps (3). That growth of roots of *Pistacia* requires higher Ca and good aeration than does *Liquidambar* may be related to the widely different natural adaptations of each species.

Root regeneration of *Pistacia* seedlings and in root cuttings of both species was markedly affected by aeration. The aeration requirement to produce a maximum number of roots was higher for *Pistacia* than *Liquidambar* due to different physical or metabolic characteristics of the root cuttings. Greenwood (4) suggests that differences in susceptibility of species to poor aeration may be related to ability to transport oxygen from aerial parts to the roots, but for root cuttings this argument is not valid. Roots do have gas spaces and if *Liquidambar* contained more air filled space when cuttings were taken, then additional stored oxygen might account for better rooting. This appears less convincing, however, in view of the amount of oxygen consumed by roots. According to Lemon and Wiegand (7), roots consume about 9 times their volume of oxygen gas each day. Luxmore et al. (8) have cited those variables which control oxygen absorption by roots and in the absence of transport from leaves, oxygen supply to roots is by radial diffusion from the medium. Root cuttings diam of both species were 2-3 mm and were of identical length; therefore, oxygen diffusion rates to the root surface should have been similar for both species if oxygen consumption was similar. Oxygen concn at the root surface is limiting when moisture films are thick at low air filled porosities but increases as aeration increases and as moisture films become thinner. Assuming that oxygen concn at the root surface are similar for both species at the same air filled porosity, then the difference in aeration requirement is due to root wall permeability or metabolic requirement for oxygen. Root cross-sections revealed that the periderm of *Pistacia* is thicker (4-5 cells) than that of *Liquidambar* (1-2 cells). A lower root wall permeability in *Pistacia* could account for a greater aeration requirement.

Cytochrome oxidase is usually considered the principal terminal oxidase in roots (4), but the absence of cytochrome oxidase in roots has been reported (12), and it is possible that terminal oxidases other than cytochrome may be present. The recent review by Meeuse (9) lists tissues where cyanide-resistant-respiration was found. This list includes roots. Thus, a terminal oxidase having a lower affinity for oxygen than cytochrome oxidase could account for the higher oxygen requirement in *Pistacia.* Conceivably, different oxygen requirements may be of metabolic origin and are not solely a reflection of differences in oxygen transport to roots from aerial parts.

In addition to aeration, exchangeable Ca level in the peat medium had a dramatic influence on root regeneration in *Pistacia.* By contrast *Liquidambar* was much less responsive and even grew new roots at "zero" Ca. Different Ca levels necessary for maximum root growth has been noted for rooting of stem cutting in other species (10). Paul and Leiser (10) found that rooting of *Rhododendron* and *Osmanthus* was independent of the exchangeable Ca level in peat while *Hebe, Euonymous,* and *Pyrancantha* rooted poorly at low Ca levels. For many plant species Ca is required at macro-nutrient concn to counteract the adverse effects of heavy metals and/or H^+ (1, 2, 13). In this study we assume that increased root regeneration of *Pistacia* is due to the ameliorating influence of Ca upon H^+. *Liquidambar* did respond to Ca at low Ca levels but since this species produced roots at "zero" Ca it may be required only at micro-nutrient concn under very acid conditions. Unlimed sphagnum peat does contain a trace of Ca (10). The physiological basis for different tolerances to acidity or different Ca requirements between species remains to be elucidated.

Results obtained with root cuttings generally correlate with the rooting behavior of bare root seedlings. *Liquidambar* seedlings regenerated roots in poorly aerated acid media (data not shown) while the results obtained with *Pistacia* (Table 1) show a response to Ca and aeration. In a preliminary experiment (data not shown) root regeneration in *Pistacia* seedlings was inhibited at $\geq$30% air filled porosity apparently due to moisture stress whereas root cuttings were not inhibited until 50% air filled porosity. It should be pointed out that 20% air filled porosity in 3.8 liter containers may not represent the actual aeration since some downward movement of water occurred causing greater aeration around at least part of the root system. The depressing effect of "zero" Ca saturation on lateral bud emergence may reflect a loss in permeability of roots to water transport. Buds which did emerge were smaller and appeared less turgid than those in high Ca.

Besides soil conditions there are other factors which affect root regeneration in *Pistacia* (6), but this study does emphasize the importance of site conditions. Successful establishment of *Pistacia* will depend on an adequate Ca level and a well drained soil.

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Root Regeneration of Transplanted *Pistacia chinensis* Bunge Seedlings at Different Growth Stages[1]

C. I. Lee and W. P. Hackett
Department of Environmental Horticulture, University of California, Davis, CA 95616

Additional index words. Lateral root initiation, auxin, sucrose

Abstract. **The root-regenerating potential (RRP) of one-year-old *Pistacia chinensis* seedlings at different growth stages was determined by recording the number of newly initiated roots during the period of 4 weeks after bare-root transplanting into a bottom misting chamber. RRP of intact pistacias was greatest when leaves were fully expanded and the terminal bud was forming (stage III) and lowest when seedlings were in a dormant condition (stages V and VI). However, seedlings disbudded before transplanting and also root cuttings showed two peaks in RRP; one at spring bud break (stage I) and the other at stage III. Removal of buds resulted in decreased RRP at stage I, but had little effect when plants were dormant. Treatments such as thiourea sprays of growing seedlings and chilling of dormant seedlings enhanced bud break and RRP. Potassium indolebutyrate applied to the root system promoted RRP of pistacia seedlings but did not eliminate the seasonal variation of RRP. Potassium indolebutyrate could replace the influence of buds only when seedlings were not in a dormant condition. Sucrose feeding via the stem substantially increased RRP at spring bud break. The results indicate that the dormant condition of buds and the availability of carbohydrates are the factors controlling the RRP of bare root transplanted pistacias.**

Pistacia chinensis Bunge, a widely used shade tree for fall color, is difficult to transplant bare root because of poor root-regenerating ability (4, 22). Root-regenerating potential (RRP) defined as the capacity of roots of transplanted seedlings to elongate existing lateral roots or initiate and elongate new lateral roots (18, 19) has been studied in several species. Seasonal variations in RRP of *Pinus ponderosa* Laws. (18), *Pseudotsuga menziesi* Mirb. (18) and *Taxus* spp. (9) have been reported and the optimum RRP occurred in early spring prior to terminal bud break. The RRP of *Quercus palustris* Muenchh. and *Q. coccinea* Muenchh. (11), however, reached a peak in April after bud break and leaf expansion. Physiologically non-dormant buds significantly increased the RRP of *Taxus* spp. (9), Pin oak (11) and Scarlet oak (11). Ponderosa pine seedlings required exposure to 150 cold nights to stimulate a significant increase of lateral root initiation and growth (7). Lathrop and Mecklenburg (9) reported that root dormancy as well as shoot dormancy appeared to be inversely related to RRP in *Taxus* spp.

The present experiments were performed to develop methods for studying root regeneration in transplanted woody plants, to determine the RRP of *Pistacia chinensis* in different growth stages, and to determine physiological factors controlling lateral root initiation on roots.

Materials and Methods

All plants used in this study were 1-year-old single stemmed *Pistacia chinensis* seedlings grown in 3.8 liter "crimpled" cans using a soil mix composed of sand: peat: redwood sawdust (v/v). Seeds collected on the campus of U.C. Davis were soaked in conc H_2SO_4 for 1 hr with frequent stirring, immersed in running water for 24 hrs and germinated in the above soil mix. The seedlings were left in the seed flats for a month, then transplanted to the 3.8 liter cans. Transplanted pistacias were grown in the greenhouse for 3 months before moving to an outdoor nursery area.

Expt. 1. Root-regenerating potential (RRP) of pistacia seedlings at different growth stages: The RRP of trees at 6 stages of growth (Table 1) was evaluated. Trees were prepared

Table 1. Morphological characteristics and bud activity of 1-year-old *Pistacia chinensis* seedlings at the time they were lifted for transplanting.

Growth stage	Shoot system
I	Terminal bud and some lateral buds broken and first leaf expansion.
II	Some leaves full size with rapid shoot elongation.
III	Completion of shoot elongation and inception of terminal bud scale formation.
IV	Development of fall coloration of leaves. Formation of brownish terminal bud scales. Onset of shoot dormancy.
V	Defoliation of colored leaves and shoot in deep dormancy.
VI	Terminal buds start to swell.

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