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# Effect of Tissue Removal and Hormone Application on Rooting of Hazelnut Layers

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***Abstract*. 'Barcelona' hazelnut (*C. avellana* L.) shoots were girdled and stool layered in a factorial design with three tissue removal (leaf, bud and meristem removal) and two hormone (with or without 750 ppm IBA) treatments. Percent rooting, rooting grade (0 to 5), shoot length, shoot diameter, and total number of buds were determined. Average percent rooting was >90% for all treatments. Girdling alone gave as high percent rooting as hormone application. The main effect of IBA was on root quality rather than percent rooting. About 75% of the hormone-treated rooted layers could be directly planted (grades 3 to 5), compared to 44% for the control, but shoot length, shoot diameter and total number of buds decreased with IBA application. Bud removal did not affect average percent rooting while meristem removal reduced it slightly. The percentage of layers having grades 3 to 5 was lower for the meristem and bud removal treatments than for leaf removal. Our results support the adoption of stool layerage with girdling and IBA application for the production of strong, well-rooted trees suitable for planting directly in the orchard.**

Hazelnut (*Corylus avellana* L.), an important nut crop, is clonally propagated for orchard establishment. Several propagation methods can be used including layering (Caliskan and Koc, 1998; Hartman et al., 1990; Pierce, 1991), hardwood or softwood cuttings (Bassil et al., 1991; Ercisli and Read, 2001; Kantarci and Ayfer, 1989; Lagerstedt, 1982; Proebsting and Reish, 1991; Soylu and Erturk, 1997), budding or grafting (Lagerstedt, 1981; Pierce, 1991) and micropropagation (Proebsting and Meneghelli, 2000; Yu and Reed, 1995).

Although other propagation methods are available, simple layering has been the usual method of commercial nurseries (Achim et al., 2001; Olsen 2002; Pierce, 1991) because of difficulties inherent in other methods (Bergougnoux et al., 1976). To make a simple layer, a shovel is stuck into the ground next to the mother plant about the time the tree begins leafing out. Then, a 1-year-old stool shoot is bent in an S-shaped fashion and inserted into the shovel slit. The terminal 20 to 30 cm of the shoot stick up out of the ground. The shoot remains attached to the mother plant and roots form on the underground portion.

The use of softwood or hardwood stem cuttings has been difficult either due to poor root initiation or abscission of the vegetative buds (Bergougnoux et al., 1976; Ercisli and Read, 2001; Falaschi and Loreti, 1969; Kantarci and Ayfer, 1989; Kilavuz and Cetiner, 1991; Kucuk and Yakut, 1983; Lagerstedt, 1970; Ozbek and Yilmaz, 1970; Rodriguez et al., 1988; Soylu and Erturk, 1997). Bud abscission is a serious problem even though the rooting percentage may be acceptable (Lagerstedt, 1982). Also the rooted cuttings are small and require a second year of growth in the nursery.

Grafting is another way to propagate hazelnut trees, and the nonsuckering rootstocks 'Newberg' and 'Dundee' were developed for use with this method. A high temperature is needed for callusing at the graft union. Thus, grafting has been successful when plants are grafted and grown under greenhouse conditions or when using hot callusing pipe to maintain the temperature at the graft union at 27 °C (Achim and Parnia, 1997; Achim et al., 2001; Lagerstedt, 1981; Lagerstedt and Byers, 1969; Olsen, 2002; Pierce, 1991; Wyzgolik and Piskornik, 2001).

In simple layering losses are high. Usually 10% of the shoots can not be used because they are crooked or originated too high to be bent to the ground. 15% to 20% of the shoots are lost to breakage when they are bent. Harvesting the trees without damage is difficult under wet conditions and 10% to 20% of the trees can be lost due to breakage. In addition, during the growing season, the shoots in the middle of the stool, which are to be layered the next year, compete with the current year's shoots for light and cause the shoots to have a bow in them from growing away from the center of the stool (Pierce, 1991).

Simple layerage has been replaced by stool layerage, also known as tie-off or mound layerage, by an increasing number of nurseries. Stooling is more efficient than simple layerage in that fewer shoots are lost to breakage and harvesting is easier under wet conditions. Per year production is about 60% higher and the trees produced are straighter and more profusely rooted than trees produced traditionally (Pierce, 1991). According to Caliskan and Koc (1998), percent rooting in stool layering (95%) was higher than simple layering (44.6%). About 2.2 times more layers per hectare could be obtained from stool layering than simple layering (Achim et al., 2001).

Stooling of hazelnuts involves girdling the current season's shoots with hog-ring type metal staples around the base of the shoot, applying rooting hormone to the lower part of the shoot and mounding moist sawdust, bark dust, or leaf mold over the suckers to a depth of about 20 cm (Olsen, 2002; Pierce, 1991). Stool layering is used to propagate currant, gooseberry, and clonal rootstocks of apple, pear and quince, but without the hog-ring staple (Hartmann et al., 1990).

Although girdling plus hormone application is now the standard practice in hazelnut nurseries in Oregon, it was adopted in the absence of hard data. Furthermore, it is not the standard in other hazelnut-producing countries. In Turkey and Spain, new orchards are established using rooted suckers from existing orchards rather than trees form commercial nurseries (M. Rovira, personal communication). In France and Italy, the tie-off method is used but without IBA application (Germain et al., 2004; Roversi and Mozzone, 1998). The objective of this study was to investigate the effect of six treatment combinations of tissue removal and hormone application on rooting and layer quality.

## Materials and Methods

*Plant material.* The experiment was conducted using 'Barcelona' stools spaced 1 × 1 m at the Lewis-Brown Research Farm of Oregon State University in Corvallis. The suckers were propagated by modified stooling similar to that described by Pathak et al. (1978) and Ahn et al. (1982). Ten to sixteen current-season sucker shoots about 60 to 90 cm in height and about 0.5 to 0.8 cm in diameter were kept and smaller shoots were removed using pruning shears.

*Treatments.* Four stools were randomly assigned to each of six treatment combinations including three tissue removal and two rooting hormone applications. Tissue removal treatments were applied to the lower 30 cm of each sucker. 1) Leaf removal = only leaves were removed with pruning shears leaving some of the pedicel on the stem. Leaf removal makes the girdling easier and is the usual practice and so serves as the control treatment. 2) Bud removal = leaves and buds were removed by rubbing the buds by hand. 3) Meristem removal = leaves, buds and primary meristem tissue at the nodes where buds emerge (bud shield) were excised by slicing off the bud with a razor blade utility knife leaving a wound 2 to 5 mm wide and 5 to 8 mm in length. The removed bud shield included a small amount of wood from the stem. Hormone treatments were sprayed on the lower 15 cm of each sucker: 1) 750

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Table 1. ANOVA of percent rooting of hazelnut layers after hormone application and tissue removal treatments.$^{z}$

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Hormone | 1 | 421.0 | 421.0 | 11.71 | 0.003$^{*}$ |
| Tissue removal | 2 | 276.5 | 138.3 | 3.85 | 0.041$^{*}$ |
| Interaction | 2 | 76.1 | 38.1 | 1.06 | 0.367$^{NS}$ |
| Error | 18 | 647.0 | 35.9 | | |
| Total | 23 | 1420.7 | | | |

$^{z}$Data were transformed by arcsin√% for statistical analysis.
$^{NS,*}$Nonsignificant or significant at $P = 0.05$.

Table 2. Effect of hormone and tissue removal treatments on percent rooting of hazelnut layers. Note that ethanol is the control for IBA, and leaf removal is the control for bud and tissue removal treatments. The percent rooting in leaf removal with ethanol application reflects the effect of girdling.

| Tissue | Hormone application | | |
|---|---|---|---|
| removal | IBA | Ethanol | Avg |
| Leaf removal | 100.0 | 98.2 | 99.1 a$^{z}$ |
| Bud removal | 100.0 | 95.6 | 97.8 **ab** |
| Meristem removal | 98.2 | 91.5 | 94.9 b |
| Average | 99.4 a | 95.1 b | |

$^{z}$Means not followed by a common letter are statistically different at $P = 0.05$ level. Mean separation by LSD test.

Table 3. Friedman test for the effect of hormone application on average rooting grade of layers across tissue removal treatments.

| Treatment mean | Rank |
|---|---|
| IBA | 1.70 a$^{z}$ |
| Ethanol | 1.30 b |

$^{z}$Means not followed by a common letter are statistically different at $P = 0.05$ level

ppm IBA (indole-3-butyric acid, Sigma, St. Louis, Mo.), dissolved in 50% ethanol, and 2) 50% ethanol which served as the control. A steel hog-ring style staple was applied around the base of each sucker after tissue removal applications but prior to hormone application with Stanley-Bostitch P-7 hog ring pliers. Moist sawdust, primarily from Douglas fir [*Pseudotsuga menziesii* (Mirbel) Franco] was placed around the suckers to a depth of about 20 cm, held in place by black asphalt-impregnated roofing paper (#30 roofing felt). The sawdust was periodically watered during the growing season. The layers were harvested the following January and residual sawdust washed from the shoots.

The number of rooted layers was counted and root mass rated on a scale of 0 to 5: 0 = no roots, 1 = very weak, 2 = weak, 3 = medium, 4 = good, 5 = very good. Layers with a root mass considered the minimum acceptable for planting directly in an orchard were given 100 points and assigned a grade of 3. Then layers initially assigned scores of 0, 25, 50, 200, and 400 points were given grades of 0, 1, 2, 4, and 5, respectively. Each layer was assigned a score based on the size of the root mass and then a grade. In addition, shoot length and shoot diameter were measured and number of buds was counted.

The experimental design was a completely randomized design with a full factorial set of six treatments and four replications (10 to 16 layers in each) per treatment. Percent rooting, shoot length, shoot diameter and bud number data were subjected to analysis of variance using the GLM procedure of SAS. The rooting grade data was analyzed by the nonparametric Friedman test.

## Results

The ANOVA for percent rooting (Table 1) shows no interaction between hormone and tissue removal treatments. More than 90% of shoots rooted under all six treatments. Application of IBA resulted in a slight but significant increase in the percentage of suckers producing roots, but did not significantly affect rooting percentage across the tissue removal treatments (Table 2). Bud removal did not affect average percent rooting while meristem removal significantly reduced the percent rooting although it was only 4.2% less than leaf removal (Table 2).

IBA application significantly increased the average rooting grade (Table 3) as a result of an increase in root quantity (Fig. 1). However, shoot diameter and length, and bud number were decreased (Table 4). Tissue removal treatments did not significantly affect the average rooting grade, but the percentage of layers having a grade of #3 or above was slightly lower in the meristem and bud removal treatments than for leaf removal (Fig. 2).

## Discussion

In our experiment, we observed >90% rooting, similar to the 92.9% reported by Caliskan and Koc (1998) for girdling plus IBA application on stool layers of 'Tombul' hazelnut. The average number of rooted layers was significantly higher for the IBA treatment than the control (ethanol), but the difference was only 4.3% (Table 2). The leaf removal treatment gave the highest percent rooting (98.2%) compared to bud and meristem removal in the absence of hormone (Table 2). Leaf removal is regularly used by nurseries since it makes the girdling easier. Thus, it is not a tissue removal treatment and it was used as a control for bud and meristem removal treatments. The very high percent rooting in leaf removal without application of rooting hormone reflects the effect of girdling by the hog-ring staple. Similar results were found in olive (Petridou and Voyiatzis, 1994; Porlingis et al., 1999) and pecan (Wood, 1989). In general, girdling the stem induces adventitious root formation. It causes an interruption in the downward translocation of organic materials such as carbohydrates, auxins, and other growth factors from the leaves and growing shoot tips. Rooting occurs just above the constriction point (Hartmann et al., 1990; Westwood, 1993). Our observations indicate that at least one mother shoot in each stool should be left ungirdled to nurse the roots. Otherwise, the number of shoots per stool declines because of the limited assimilates for root growth.

Wounded tissues are stimulated into cell division and production of root primordia on cuttings. This could be due to accumulation of carbohydrates and auxin and formation of a new sink area (Hartmann et al., 1990). We considered bud and meristem (bud plus bud shield) removal treatments as wounding in addition to girdling on the lower part of the layers. However, we found that bud removal did not affect average percent rooting while meristem removal significantly reduced the percent rooting although it was only 4.2% less than leaf removal (Table 2). Porlingis et

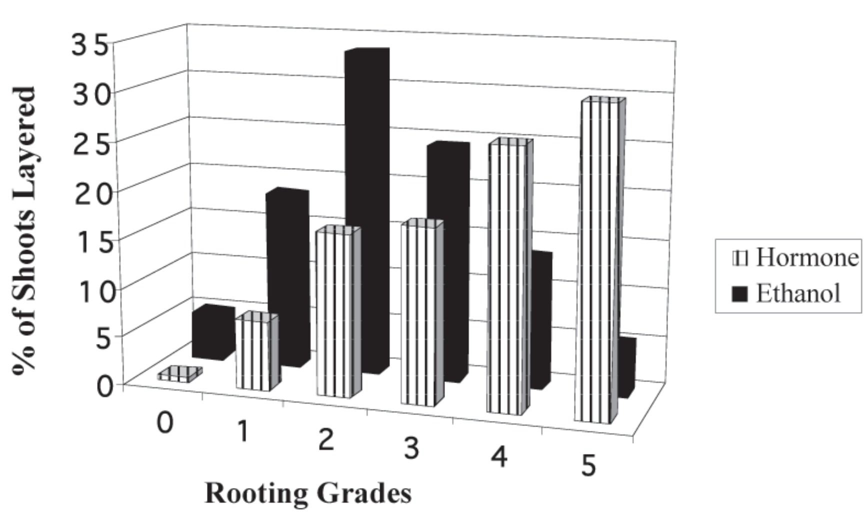


Fig. 1. Distribution of layers into rooting grades with and without hormone treatments.

Table 4. Effect of hormone applications on shoot length and diameter, and bud number on layers across tissue removal treatments.

| Parameter | Ethanol | IBA | $P$ value |
|---|---|---|---|
| Length (cm)[z] | 126.39 a[y] | 114.17 b | 0.000* |
| Diameter (cm)[x] | 0.97 a | 0.86 b | 0.000* |
| Bud number[w] | 24.71 a | 23.16 b | 0.005* |

[z]Mean length of layers.
[y]Means in the same row not followed by a common letter are statistically different at $P$ = 0.05. Mean separation by LSD test.
[x]Mean diameter of layers.
[w]Mean number of buds on layers.

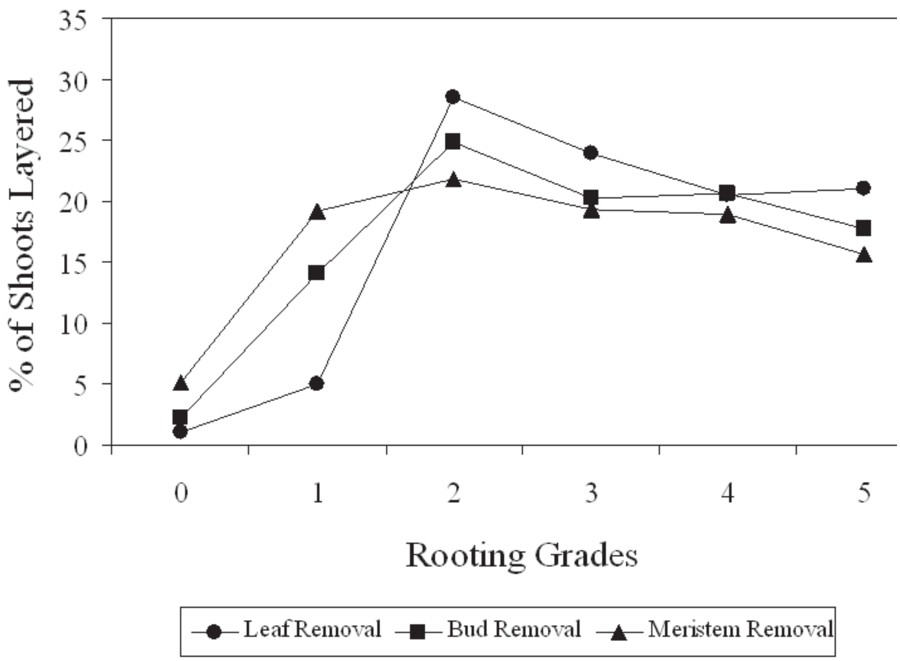


Fig. 2. Distribution of layers into rooting grades for three tissue removal treatments.

al. (1999) reported that girdling and wounding were necessary and their combined effect was more than additive for rooting of olive layers. The reason we did not see a beneficial effect of additional wounding is that we did not do wounding at the internodes. Instead we removed buds and meristems (buds and bud shields). Thus, the lower percent rooting could be due to reduced endogenous auxin levels since buds are powerful auxin producers (Hartmann et al., 1990).

The effect of IBA was more pronounced (Asymp. Sig. 0.000) on root quality than on average percent rooting (Table 3). In our grading, we considered grade #3 (medium rooting) as acceptable for planting in an orchard while grades of #4 (good) and #5 (very good) are preferred. The number of layers having grade #3 or above was much higher with IBA application (75.5%) than ethanol (43.6%) (Fig. 1). These results clearly indicate that auxin is needed in combination with girdling to obtain well-rooted layers suitable for orchard planting. Similar results were found in olive, where IBA accelerated the rooting of layers and increased the number and fresh weight of roots and thus increased root quality (Petridou and Voyiatzis, 1994; Porlingis et al., 1999). In pecan, IBA without girdling was incapable of inducing rooting, and girdling plus IBA application doubled the number of shoots producing roots and increased by 5-fold the number of primary roots per shoot as compared to girdling alone (Wood, 1989).

There was no significant difference among tissue removal treatments for distribution of layers into the rooting grades. However, the percentage of layers having a grade of #3 or above was slightly lower in the meristem and bud removal treatments than in the control (Fig. 2).

We found that average shoot length and diameter were 114.2 and 0.86 cm, respectively, for 'Barcelona' layers. Caliskan and Koc (1998) reported 119.8 and 1.18 cm, respectively, for 'Tombul' layers. The difference could be due to use of a different cultivar. There were no significant differences among tissue removal treatments for shoot length and diameter, and total number of buds of rooted layers. However, these differences were significant for hormone treatments. Shoot length and diameter, and total number of buds decreased with IBA application compared to the control (Table 4). Application of IBA promoted rooting but it seems that vegetative growth was limited. That is probably due to use of assimilates for rooting rather than for vegetative growth.

The Oregon hazelnut industry has been dependent on a single cultivar 'Barcelona' for the in-shell market for a long time. However, the industry began to change in the early 1980s seeking new cultivars for the kernel market. In recent years, susceptibility to eastern filbert blight caused by *Anisogramma anomala* (Peck) E. Müller has been a major consideration in the selection of cultivars and pollenizers (Lagerstedt, 1980, 1990; Mehlenbacher et al., 2000, 2001; Pierce, 1991; Smith and Mehlenbacher, 2002). New cultivar demands and the pressure of eastern filbert blight are forcing the industry to look for a more efficient and faster method of propagation. Stool layering is a suitable method for cultivars that will remain in use for long periods. The main drawback of layering is that it is relatively inefficient. Establishment of layer beds may take ≥5 years (Pierce, 1991; Proebsting and Meneghelli, 2000). In this case, the alternative method is micropropagation, which has the potential of very high rates of multiplication (Proebsting and Meneghelli, 2000; Yu and Reed, 1995).

Turkey is a leading producer, accounting for about 70% of the annual world hazelnut crop. Yet there is not a single hazelnut nursery in Turkey. The traditional way to establish an orchard has been the use of rooted suckers from neighbors' orchards. There is no available land for expansion of hazelnut plantings in the Eastern Black Sea region, and there is no strong pressure for the industry to change cultivars. However, the orchards are old and in need of renovation. Thus, the establishment of nurseries and determination of appropriate methods of propagation are needed. Stool layering could be an efficient propagation method in this regard.

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