Optimizing Temperature and Humidity for Rooting Hybrid Hazelnuts from Hardwood Stem Cuttings

Lois Braun and Donald Wyse

Abstract

Hybrid hazelnuts are being developed as a new crop for the Upper Midwest for their ecological and economic value, but lack of economically viable propagation methods is a significant bottleneck to their wide scale adoption. In previous trials we found that hardwood stem cuttings could be propagated in low cost humidity tents constructed of molded plastic tubs covered with white 70% shade plastic. When the plastic was sealed tightly at the sides, these tubs maintained relative humidity near saturation, but also tended to overheat. This trial experimented with the use of ordinary household humidifiers as an alternative way of maintaining humidity while avoiding overheating. We found that it is not necessary to maintain RH near 100% as we had been doing when we kept the humidity tents tightly sealed. Stem survival and, as a consequence, rooting were improved in vented tents in which humidity was maintained with humidifiers, though these required much more management than the sealed tents.

Index words: Propagation, Corylus americana (Walter), Corylus avellana (L.), rooting, indole-3-butyric acid.

Species used in this study: hybrid hazelnuts [Corylus americana (Walter) x Corylus avellana (L.)].

Significance to the Horticulture Industry

Hybrid hazelnuts (C. americana x avellana) are one of several new perennial and winter annual crops being developed as part of the University of Minnesota’s Forever Green Initiative, to provide continuous living cover on a landscape that would otherwise be bare through the non-growing season. The annual row crops that currently dominate the Upper Midwest keep the landscape green for only four to five months of the year, leaving it brown the majority of the time. Unvegetated soil is vulnerable to soil erosion, leaching of nutrients and loss of organic matter, which lead to contamination of surface and ground waters, and loss of productivity and ecological resilience. Forever Green crops provide farmers and other landowners with economically profitable alternatives to summer annuals. Hazelnuts also provide a healthful and flavorful human food. The primary obstacle to adoption of hybrid hazelnuts thus far has been lack of improved germplasm. Hazelnut breeders at the University of Minnesota are working to develop improved varieties, but these need to be propagated for deployment to growers. Micropropagation is likely the only method capable of producing large numbers, but thus far, success with micropropagation has been variable. Mound layering is an option, but only produces small numbers of clones. Propagation from stem cuttings is an alternative that can augment mound layering to produce modest numbers of new plants needed for research trials or for small-scale commercial plantings. This paper is the second in a series describing trials to optimize propagation of hybrid hazelnuts from hardwood stem cuttings.

Introduction

Lack of economically viable methods of propagation is a major bottleneck in the development of hybrid hazelnuts [Corylus americana (Walter) x C. avellana (L.)] as a new environmentally sustainable crop for the Upper Midwest of the US. A breeding program has been initiated to improve the quality of locally adapted hazelnut germplasm. Vegetative propagation of the best selections is needed to produce clonal material for evaluation in replicated trials as part of this program. Although we expect that micropropagation will ultimately be the method used for disseminating plant releases to the public, micropropagation must be optimized for specific genotypes, and thus may not be cost effective for the small numbers needed in a breeding program. We have been using mound layering, but it is not capable of producing the numbers of plants needed. Grafting is not viable for these multi-stemmed shrubs. Therefore, although propagation by stem cuttings has not been found to be as reliable a method as layerage for hazelnuts (Solar et al. 1994), it may be the best short term alternative.

This paper reports on one of a series of hardwood stem cutting trials we conducted over a period of years, with the goal of optimizing our protocol for producing clonal plant material for our own germplasm evaluation trials. We did not repeat any trial with the exact array of treatments, but used the best treatment from each trial as the control for the next.

In a preliminary trial, we found that rooting was better in sealed humidity tents than under a mist system. We assumed that was because sealed humidity tents are better than fog or mist systems at maintaining relative humidity (RH) close to saturation, as is needed for many species.
were covered with white 70% shade plastic and the other and 3-mm (0.1 in) drip irrigation tubes were used to keep plastic of each tent. The humidifier tanks were removed ordinary household evaporative humidifier underneath the shut on the sides. Humidity was maintained by placing an wide paper clamps, to maintain RH close to 100%, as we had done with earlier trials. For the vented tents, plastic might not be needed in unsealed tents, and that rooting might improve with increased light transmission, as has been observed for many species (Harrison-Murray and Howard 1998). This experiment also tested whether cuttings could survive and form roots with lower levels of RH than the nearly 100% RH at which sealed humidity tents maintained it. The results of this trial were the impetus for a follow-up trial to determine optimal humidity conditions (Rusnak and Braun 2017).

Materials and Methods

All humidity tents consisted of 90 by 57 by 22 cm (35 by 22 by 9 in) molded plastic utility tubs, into which we drilled drainage holes in the bottom. We fitted them with 1.8 m by 1 cm (6 ft by 0.5 in) PVC pipes bent into arches, one at each end of the tubs, plus a third in the middle, to hold up plastic sheeting. Materials cost about $30 per humidity tent. These humidity tents can accommodate 180 cuttings each, spaced 4 by 6 cm (1.5 by 2 in) apart. They can accommodate cuttings up to 75 cm (30 in) tall at the centers of the tents, but only about 45 cm (18 in) tall at the perimeters. Tubs were placed in a greenhouse with temperatures set at 21 C (70 F) day and 18 C (64 F) night, under halogen lamps set for a 16-hour day length. We filled the tubs to a depth of 20 cm (8 in) with a 1:4 mixture of peat and perlite, which was then soaked and allowed to drain through holes drilled in the bottom.

In this experiment, we compared three types of humidity tents, replicated three times each: 1) white sealed, 2) white vented and 3) clear vented. The white plastic was 70% shade polycarbonate. The three sealed tents were covered with white plastic that was folded tightly at the corners and clamped tightly to the sides of the tubs with 5 cm (2 in) wide paper clamps, to maintain RH close to 100%, as we had done with earlier trials. For the vented tents, plastic was merely draped over the PVC hoops, but was not sealed shut on the sides. Humidity was maintained by placing an ordinary household evaporative humidifier underneath the plastic of each tent. The humidifier tanks were removed and 3-mm (0.1 in) drip irrigation tubes were used to keep the water wells full at all times. Three of the vented tents were covered with white 70% shade plastic and the other three with clear plastic. As much as possible, placement of the tents within the greenhouse was blocked to control for variability in microclimate within the greenhouse.

We collected dormant crown suckers from nine hybrid hazelnut and five American hazelnut bushes soon after leaf drop in late October 2012, and stored them in a cooler at 2 C (36 F) and high humidity. See Braun and Wyse (2019) for more detail on the collection and preparation of the crown suckers. On Feb. 6, 2013, working with one genotype at a time, we cut the stems into segments short enough to fit inside the tents, and then sorted them into nine groups that contained a roughly equal array of segment sizes, one group for each of the nine tents. Having initially collected between 9 and 257 stems from each mother plant, due to the highly variable sizes of mother plants, we ended up with between 3 and 29 cut segments of each genotype in each of the nine tents. We then dipped the basal 2 cm (1 in) of segments into 2 g L\(^{-1}\) (2,000 ppm) indole-3-butyric acid (IBA) in a 1:1 solution of ethanol and deionized water. Finally we randomly assigned each group of stems to a humidity tent, where we stuck the bases of the stems into the rooting medium just far enough that they would stand up. In this way, we placed approximately 176 cuttings in each tent.

We placed two Hobo (Onset Computer Corporation, Bourne, MA) temperature and light data loggers within each tent, one on the surface of the rooting medium and one about 3 cm (1.5 in) below the surface. We recorded RH manually every day from mechanical non-recording hygrometers suspended from the PVC hoops within the plastic. We recorded RH in mid-afternoon, as close as possible to the peak temperature of the day, which coincided with the lowest humidity of the day. We watered any tents found to have less than 60% RH, using a hose until water ran out the holes in the bottoms of the tubs.

We evaluated rooting on April 10 (62 days after the start), May 1 (83 d), May 21 (103 d), June 24 (134 d) and July 20 (163 d). See Braun and Wyse (2019) for more detail on how we conducted evaluations, and how we potted rooted cuttings and cared for them after potting.

Statistical analysis. We used JMP software (v. 12, Copyright 2015, SAS Institute, Inc., Cary, NC) for ANOVA and means comparisons. We used XLISP-STAT software (version 3.52.17, Copyright 1989-1999, by Luke Tierney) for regression analysis. We used binomial regression for rooting and survival data (rooted = 1, unrooted = 0), and linear regression for root and leaf quality ratings. We considered single cuttings as the experimental units and controlled for other factors, such as genotype, by including them as covariates in the statistical models.

Results and Discussion

Air temperatures in the white vented tents were lower than temperatures in the sealed tents or the tents with clear plastic for the duration of the experiment (Fig. 1). Whereas the sealed tents had the highest maximum air temperatures in the first part the season, starting in late April, the clear vented tents started to match them, and by late June, the
clear tents were consistently the hottest. Temperatures in the rooting medium were generally about 6°C (43°F) cooler than air temperatures, with which they fluctuated. The rooting medium in the sealed tents was significantly warmer than in the white vented tents \((p < 0.05)\), with the temperature of the medium in the clear vented tents intermediate between the two.

As expected, the sealed tents maintained high humidity through the entire experiment much better than either type of vented tent (Fig. 2). The white vented tents maintained humidity slightly higher than the clear vented tents. Although both kinds of vented tents generally kept humidity above ambient greenhouse levels, for a period in April when the greenhouse was filled with many other transpiring plants, ambient humidity in the greenhouse was on average higher than in the vented tents. Maximum humidity did not vary much between types of tent because humidity was maintained by watering whenever it dipped below 60% (Table 1). By contrast, minimum humidity varied radically. Low humidity in the vented tents necessitated watering every 5 to 6 days, whereas the sealed tents needed to be watered only about once a month.

**Fig. 1.** Maximum temperatures within three types of humidity tents, averaged weekly over the duration of the rooting trial. Solid line = sealed with white plastic; dashed line = vented clear plastic; dash-dot-dot line = vented white plastic.

**Fig. 2.** Relative humidity within three types of humidity tents measured daily over the duration of the rooting trial. Solid line = sealed with white plastic; dashed line = vented clear plastic; dash-dot-dot line = vented white plastic; dotted line = ambient RH in the greenhouse outside the tents.
Mortality of cuttings that had leafed out but not rooted was high in all tents on the first evaluation date, April 10, at 62 d (Table 2). Most cuttings which died had broken bud, but died before full leaf expansion. Leaves had a wilted appearance, but did not appear diseased. Mortality was dramatically higher in the sealed tents than in the two types of vented tents: 78% of cuttings in the sealed tents died within the first two months versus 53% in the vented tents. Production of rooted cuttings at the end of the experiment as a percentage of the number of cuttings initially prepared was significantly lower in the sealed tents than in the vented ones at p < 0.001 (Fig. 3). However, this was mostly a function of the higher mortality in the sealed tents: the percentage of surviving cuttings that formed roots by the end of the experiment did not differ significantly between treatments. Type of plastic, clear versus white, did not make a difference until the very end of the trial, when rooting in the clear vented tents was slightly lower than in the white vented tents at p = 0.06. Whereas root quality did not differ between the three tent types, leaf quality was significantly better in the tents with white plastic than in the ones with clear plastic (p < 0.03).

It was not possible to separate the effects of heat and humidity because the tents with the highest mean temperatures were also the ones with the highest humidity (p < 0.0001). It appears that rooting was not directly affected by either factor, but both temperature and humidity were strongly and negatively correlated with stem cutting survival, and thereby had an indirect negative effect on rooting. At the first evaluation date, 62 d, mortality was about 50% in tents which had average temperatures of 21 C (70 F) or less, but mortality rose sharply in tents with average temperatures above 22 C (72 F) (Fig. 4). We observed similar patterns at subsequent evaluation dates High mortality was also correlated with high humidity. Mortality increased linearly with increasing average humidity (p < 0.02 at the first evaluation date, and p < 0.05 at the end of the trial), but it also increased linearly with increasing minimum measured humidity (p < 0.01 at the first evaluation date, and p <0.05 at the end of the trial).

The first challenge in getting difficult-to-root species to root from cuttings is keeping them alive long enough to form roots. This experiment made that clear because cuttings that survived the first two months subsequently rooted equally well in all treatments. Our data suggest that both high humidity and high temperatures were to blame for the high mortality in the sealed tents during the first two months of the experiment, when the greatest mortality was observed.

Excessive heat was likely the greater problem in later months, as suggested by the slight reduction in rooting observed in the clear plastic tents at the end of the experiment. The surviving cuttings in the clear tents were also more visibly stressed at the end of the experiment than the cuttings in other tents. For this reason, we do not recommend clear plastic. Even though it transmitted more light than the white 70% shade plastic, this did not improve rooting.

Prior to this experiment, we had assumed that higher humidity would allow for more root formation. Many authors have reported a positive correlation between rooting success and moisture content of the rooting medium (Loach 1988a). Rein et al. (1991) found that rooting of juniper (Juniperus spp.), azalea (Rhododendron spp.) and holly (Ilex spp.) cuttings improved even up to 500% moisture by weight in the rooting substrate, which had a 1:1 ratio of peat to perlite. Harrison-Murray and Howard (1998) observed that whereas maintaining RH close to saturation enhanced rooting for some species, it inhibited it for others, such as Cryptomeria japonica (L.f.) D. Don. They speculated that some transpiration is needed in this species to prevent damaging over-hydration of intercellular air spaces in tissues close to the basal wound. Alternatively, they proposed that some degree of water stress is required to trigger root initiation in C. japonica.

LeBude et al. (2004) found that moderate water stress on cuttings of loblolly pine (Pinus taeda L.) actually improved rooting. They proposed that cuttings exposed repeatedly to sub-lethal water stress may undergo osmotic adjustment, which might induce hormonal changes that stimulate

Table 1. Minimum, maximum and average relative humidity (RH) values observed mid-afternoon before watering, in three kinds of humidity tents: white sealed plastic, white vented plastic, and clear vented plastic; number of times RH dropped below thresholds of 50% and 60% over the 164 day duration of the experiment; and percentage of stems that died by day 62 and rooted by day 164. Tents were watered when RH dropped below 60%. Note that because RH was measured at the time of day when it was expected to be lowest, and before watering, average and maximum RH shown in the table are underestimate. Humidity in all tent types rose to nearly 98% soon after watering; the maximums shown in this table are humidity measured a day later.

<table>
<thead>
<tr>
<th>Measured Relative Humidity</th>
<th>Number of times RH &lt; 50%</th>
<th>Number of times RH &lt; 60%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>White Sealed</td>
<td>41%</td>
<td>90%</td>
</tr>
<tr>
<td>White Vented</td>
<td>28%</td>
<td>90%</td>
</tr>
<tr>
<td>Clear Vented</td>
<td>19%</td>
<td>85%</td>
</tr>
</tbody>
</table>

Table 2. Percentage mortality after 62 days and 164 days, and percentage rooting after 164 days, averaged across genotypes.

<table>
<thead>
<tr>
<th>Survival after 62 days</th>
<th>Survival after 164 days (%) of initial cuttings</th>
<th>Rooting after 164 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Sealed</td>
<td>22%</td>
<td>16%</td>
</tr>
<tr>
<td>White Vented</td>
<td>47%</td>
<td>35%</td>
</tr>
<tr>
<td>Clear Vented</td>
<td>46%</td>
<td>32%</td>
</tr>
</tbody>
</table>
rooting. This may be analogous to how root growth is stimulated in nutrient-poor soils. Since plants function like an economy, and allocate resources towards those structures needed to acquire more of whatever resource is most limiting to growth, as described by Bloom et al. (1985), moisture stress might induce growth of roots, the organ required for acquiring moisture.

We did not measure medium water content for this experiment, but in a subsequent experiment we found that the 1:4 peat:perlite mix stayed moist for months in sealed tents with no additional inputs of water: two months after the medium had last been soaked and drained, substrate moisture was still 2.5 g g⁻¹. This was approximately 50% of saturation, so although it was still moist to touch, the rooting zone was well aerated. There was little cutting mortality in that subsequent experiment, and no basal necrosis of those cuttings that were dead. However, for the experiment reported in this paper it is likely that substrate moisture content in the sealed tents was higher than 2.5 g g⁻¹ during the first two months when most of the mortality occurred, because they were watered five times in that period. Thus waterlogging and O₂ starvation of the cutting bases may in fact have been the cause of the mortality.

Conversely, the low humidity in the vented tents, which got as low as 20%, was not as big a problem as we anticipated, possibly because, due to daily monitoring, the low humidity was only of low duration. Because the stem cuttings were dormant at the start of the experiment, the leaves that emerged when they broke dormancy were never subjected to conditions radically different from the ones in which they developed, and thus they were likely to have been somewhat adapted to those conditions. We cannot say whether or not short periods of water deficit were beneficial, as they were for loblolly pine in the experiment of LeBude et al. (2004).

Although the results of this experiment suggest that sealed tents should not be used, the rooting percentages observed in the vented tents were no higher than we had observed with sealed tents in previous trials. For subsequent trials, we used sealed tents and did not water them during the first few months, when dry winter air made it difficult to maintain high humidity, then opened the tents and added humidifiers when the potential for overheating became a concern in the spring, which was about the same time as higher ambient humidity made it easier to maintain

Fig 3. Cumulative rooting of hybrid and American hazelnut hardwood stem cuttings in three types of humidity tents, averaged across genotypes. Solid line = sealed with white plastic; dashed line = vented clear plastic; dash-dot-dot line = vented white plastic.

Fig 4. Mortality of stems on April 10 (62 days after the start of the trial) related to average temperature within three types of humidity tents. Squares = sealed with white plastic; triangles = vented clear plastic; circles = vented white plastic.

y = 0.0159x² - 0.6236x + 6.5773
R² = 0.9607
humidity with humidifiers. In those trials, we obtained rooting percentages comparable to the vented tents in this experiment. This supports the theory that the mortality observed in the sealed tents in this experiment may have been due to overwatering and not due to lack of ventilation.

Although the vented tents reduced cutting mortality, and consequently produced more rooted plants, they required daily attention. By contrast, the sealed tents could be ignored for long periods. For low-resource growers, or growers who only need to generate a small number of plants from a large number of available stems, sealed tents might still be a viable option. Based on the observation that short-term low humidity is not as great a problem as we initially thought, it may be possible to capture the advantages of the vented tents without the inconvenience of maintaining humidifiers simply by watering them when humidity falls below a critical threshold. We used this approach in a subsequent trial (Rusnak and Braun 2017), in which we determined the optimal RH to be between 50 and 70%. In actuality, RH is likely not so critical as maintaining an optimum water content in the rooting medium.

This trial and our previous trial on size of stems and stem segments (Braun and Wyse 2019) collectively demonstrate a basic principle of propagation from stem cuttings: keeping cuttings alive long enough for them to form roots is critical, especially for slow-to-root species such as hazelnuts. Not only must stress from obvious factors such as heat load, desiccation, and waterlogging be avoided, but the carbohydrate balance in the stems must be maintained by keeping respiration low and by enhancing photosynthetic potential.

**Literature Cited**


