

The Effects of Relative Humidity and Substrate Moisture on Rooting of Hybrid Hazelnuts from Hardwood Stem Cuttings¹

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Abstract

Hybrid hazelnuts (*Corylus americana* Walter x *C. avellana* L.) are currently being bred for use as a cold-hardy perennial crop that could produce profits for Midwestern farms while supporting agroecosystem sustainability. However, asexual propagation techniques for producing germplasm for breeding and dissemination purposes have proven difficult. This study was one of a series attempting to develop a protocol for hardwood stem cutting propagation. This study assessed the impact that relative humidity (RH) and substrate moisture has on rooting of hardwood stem cuttings propagated in low-cost humidity tents. Hardwood stem cuttings retrieved from 14 hybrid hazelnut genotypes were planted into enclosed humidity tents, each housing 64 cuttings. Treatments were RH thresholds of 30%, 50%, 70%, and 90%, which were maintained by daily monitoring and watering to saturate the substrate when a tent's RH fell below its specified RH threshold. Cuttings propagated in 50% and 70% RH tents showed the highest rates of rooting at 16% and 12% respectively, whereas only 7% of cuttings rooted at 90% RH and 3% at 30% RH. By showing that intermediate RH levels and watering regimes are optimal for rooting, these results suggest daily monitoring is not necessary for hardwood stem cutting propagation of hybrid hazelnuts.

Index words: propagation, relative humidity, substrate moisture, hardwood stem cuttings, hazelnuts, adventitious rooting.

Chemicals used in this study: IBA (indole-3-butyric acid).

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

Significance to the Horticulture Industry

Farmers, scientists, and policy-makers have become increasingly aware of the negative effects that cultivation can have on a landscape. In particular, regions of the Midwest dominated by corn/soybean monocultures often see increased soil erosion and runoff of agricultural chemicals into water. Of particular concern are nitrates from fertilizers, which are implicated in the contamination of ground water and hypoxia in the Gulf of Mexico. One solution to mitigate these problems involves planting perennial crops such as hybrid hazelnuts in riparian buffer zones to reduce erosion and agricultural runoff year round. American hazelnuts (*Corylus americana* Walter) are cold hardy, eastern filbert blight- (EFB) resistant native woody suckering shrubs whose native habitat extends as far south as Louisiana and as far north as Quebec and Manitoba (USDA 2017). Current commercial varieties of European hazelnuts (*C. avellana* L.), on the other hand, are neither resistant to EFB nor cold hardy for the Midwest. High performing hybrid genotypes retain the desirable EFB resistance and cold hardiness but are difficult to propagate asexually, which is typically done through mound layering or hardwood stem cutting, with micropropagation methods currently under development. This study sought to identify optimal relative humidity (RH) ranges in which to successfully propagate hardwood stem cuttings in low-cost humidity tents. Results indicated that moderate RH levels

(50% to 70%), which were maintained by watering, on average, 16% to 48% of the days, yielded the highest rooting percentages, indicating daily monitoring is not needed.

Introduction

Hybrid hazelnuts (*Corylus americana* Walter x *C. avellana* L.) are currently being bred for agricultural production in the Midwest. These hybrids cross native cold-hardy and eastern filbert blight- (EFB) tolerant American hazelnuts with high yielding, commercially grown European hazelnut varieties. The hybrids retain the bush-like habit of the American hazelnut, while improving nut size and quality to rival the tree-like European hazelnuts. The goal of these breeding efforts is to develop hybrid hazelnut germplasm adapted for production in the Midwest, where they could be grown for both economic and environmental benefits by providing a perennial cover that will diversify Midwestern agriculture. Such a crop would be particularly well suited as a buffer around riparian habitats in order to decrease erosion and runoff of agrochemicals into waterways, which have been implicated in hypoxic zones in the Gulf of Mexico and in contaminated ground water that locals depend upon for drinking.

In order to successfully introduce hybrid hazelnut crops onto Midwestern farms, disseminating improved genotypes will be key. To keep these desirable genotypes true to type, asexual propagation is necessary and may be achieved in hazelnuts through layering, tissue culture, or stem cuttings (Hartmann et al. 1990). Mound layering is a commonly used method for cloning small amounts of hybrid hazelnut germplasm (Olsen and Smith 2013), but is also labor intensive (Braun 2015a). Grafting is not feasible due to the

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heavy suckering habit typical of hybrid hazelnuts. Micro-propagation is another method being developed to rapidly create large numbers of hazelnut clones (Kreiser et al. 2016), but thus far it has failed to produce the plants needed for our breeding and research trials. Propagation by stem cuttings is a relatively low-cost alternative (Hartmann et al. 1990), though hazelnuts with *C. americana* parentage in particular have been notoriously difficult to root (Ercisli and Read 2001; Kreiser et al. 2016). Therefore propagation through stem cuttings is being improved as an alternative for producing more clones per plant with lower costs and less effort than other methods (Braun 2015b).

Although we found in previous work that softwood stem cuttings can be rooted fairly readily, their survival after rooting was too low for the method to be viable. By contrast, we found that hardwood stem cuttings survived at rates close to 100% after rooting⁴. Thus, although still difficult to root, hardwood stem cuttings proved to be a viable alternative to mound layering for clonal propagation of hybrid hazelnut germplasm.

The goal of this study was to seek the optimum relative humidity levels for propagating hybrid hazelnut germplasm through hardwood stem cuttings. Traditionally, hardwood cuttings have been propagated with near 100% RH in controlled environment rooms or sealed tents with a constant mist to maximize rooting success (Okoro and Grace 1976; Elgimabi 2009). We believed nearly saturated air to be necessary for successful propagation since the hardwood cuttings leaf out before rooting. As is typical for these hardwood cuttings, bud break began only about 3 weeks after planting, while rooting took well over 2 months to occur in the majority of cuttings, which is consistent with other work with hardwood hazelnut cuttings (Kantarci and Ayfer 1994). In addition to the risk after leaves have formed, hardwood cuttings are sensitive to water loss at low RH values even while leafless (Howard and Harrison-Murray 1988). As such, in our previous research we had used sealed tents that kept RH near 100% RH, but later found that rooting could be improved by venting these tents, which caused RH to occasionally decrease significantly below 100%⁴. This was mitigated with daily watering. By identifying the optimal RH levels for propagation, this study aimed to improve rooting success by reducing the heat stress that occurs in closed humidity tents while eliminating the need for the daily maintenance required to keep RH at 100%.

Materials and Methods

The humidity tents used in this experiment were intended as a low cost alternative to growth chambers for maintaining the high humidity levels believed to be needed for rooting hardwood stem cuttings. They were constructed from 90 by 57 by 22 cm (36 by 22.5 by 8.5 in) Menards® molded plastic utility tubs (Menard, Inc., Eau Claire, WI). Each tub was fitted with three 1.8 m by 1.3 cm (7 ft by 0.5

in.) PVC pipes bent into arches and secured with U brackets on the sides to support 70% white shade plastic, which was secured to the sides and ends of the tub with metal clips. Small drainage holes were drilled into the bottom of the tubs, which were filled with 20 cm (7.9 in) of a peat moss:perlite (1:4 v/v) mixture. Twelve tents total were prepared to accommodate three replications each of four RH levels (30%, 50%, 70%, and 90%).

Hardwood stem cuttings were collected from one year old suckers from 14 different genotypes after leaf drop on Oct. 21, 2015. They were kept in cold storage at 2 C (35 F) and 98% RH until Dec. 12, 2015, when, working with one genotype at a time, stems were prepared by trimming about 1 cm (0.4 in) off the proximal ends and dividing stems that were too long to fit under the plastic into two or three sections ranging from 30 to 50 cm (11.8 to 19.7 in.) in length so they would fit under the plastic. These sections were sorted by size and by whether the sections were proximal, medial, or distal. To control for variability in rooting potential of these different kinds of sections (Ughini and Roversi 2005, Tombesi et al. 2015), the proximal, medial, and distal sections were distributed evenly between the 12 humidity tents. Finally, the cuttings were dipped for ten seconds in 2,000 mg·L⁻¹ indole-3-butyric acid (IBA) in a 50% ethanol solution right before being planted 5 cm deep in their humidity tent, leaving 3 to 10 nodes exposed to atmospheric conditions. Each tent held 64 cuttings total. The number of cuttings of each genotype varied between 1 and 10 cuttings per tent depending on the number of cuttings available, but each individual genotype was equally represented in each tent. The tents were housed in a greenhouse lit with halogen lighting set to 16 hours on and 8 hours off each day. Irradiance under the tents was 32% of ambient solar irradiance, which is consistent with the 70% shade rating of the plastic. The average irradiance in St. Paul, MN is 1.37 kWh·m⁻²·day⁻¹ in December, and it gradually increases to 3.44 kWh·m⁻²·day⁻¹ in March (Boxwell 2017), which puts average irradiance in the tents between 0.44 kWh·m⁻²·day⁻¹ and 1.10 kWh·m⁻²·day⁻¹ during the course of the experiment. Temperatures were set to 21 C (70 F) during the day and 18 C (64.4 F) at night.

Each tent was assigned a minimum RH threshold value of either 30%, 50%, 70%, or 90% RH in randomized blocks with three replications (tents) of each threshold. From Dec. 12, 2015 to Mar. 23, 2016 (102 days total), each tent was monitored once daily at approximately noon. Current temperature and RH were recorded from a dual Inkbird® thermometer/hygrometer (Inkbird Tech. Co., Ltd., Shenzhen, PRC.) kept in each tent on a stake at about 10 cm (4 in) above the level of the medium. Weekly maximum and minimum temperature and RH were recorded once a week. Two Hobo® data loggers (Onset Computer Corp., Bourne, MA) per tent (24 total) were used to track hourly temperature variation. To keep tents above their assigned threshold RH values, tents found to have a RH value below their threshold value at the time of checking were immediately watered until the rooting medium was fully saturated and excess water began to drain from the holes in the tubs, which corresponds to an

⁴Braun, L. unpublished. Optimizing temperature and humidity for propagating hybrid hazelnuts from hardwood stem cuttings in low-cost humidity tents. Unpublished manuscript, University of Minnesota, St. Paul.

Table 1. Average relative humidity, average temperature, percent rooting, survival, average root and shoot lengths, and average root and shoot ratings of hardwood hybrid hazelnut (*Corylus americana* (Walter) x *C. avellana* (L.)) stem cuttings propagated in humidity tents held above relative humidity thresholds of 30%, 50%, 70%, and 90% relative humidity over a 102 day period in from Dec. 2015 to Mar. 2016. Means within a row that share a letter do not differ significantly at Tukey HSD $\alpha = 0.05$.

	Relative Humidity Threshold (%)				ANOVA P-Value
	30	50	70	90	
Average Relative Humidity (%)					
Relative Humidity (RH)	47.1c	63.2b	62.8b	70.8a	0.0003
Weekly Min RH	27.8b	33.4a	34.9a	38.9a	0.0006
Weekly Max RH	79.5b	96.3a	96.2a	96.2a	<0.0001
Average Temperatures ^z (C)					
Temperature	20.8d	21.7a	20.8cd	21.4bc	<0.0001
Daily Min Temperature	16.6b	17.2a	16.9ab	17.2a	<0.0001
Daily Max Temperature	25.4b	26.7a	25.4b	26.4a	0.0001
Weekly Min Temperature	16.1	16.5	16.0	16.4	0.706
Weekly Max Temperature	32.5	33.0	31.6	32.0	0.276
Average Rooting and Survival ^y (%)					
Survival	14.7c	51.9a	49.2a	33.0b	<0.0001
Rooted after 102 days	3.1c	15.9a	12.0ab	6.8bc	<0.0001
Rooting (alive only) ^x	11.3	30.9	24.5	21.0	0.492
Length ^w (cm)					
Longest Root	3.3	6.5	7.8	6.5	0.412
Longest Shoot	0.7	4.1	4.2	4.7	0.206
Cutting Quality ^v (subjective)					
Root Rating	1.7	2.2	2.3	2.1	0.821
Shoot Rating	1.5b	3.6a	3.8a	3.5a	0.0001

^zReadings taken from pairs of HOBO loggers in the same tent were averaged together.

^yN = 191, 189, 191, 191 cuttings for 30%, 50%, 70%, and 90% RH treatments, respectively, for rooting and mortality.

^xRooting (alive only) indicates the cuttings that rooted as a percentage of the number (n) of cuttings still alive by day 102, discounting any that died before then. n = 28, 99, 94, and 63 cuttings still alive on day 102 for 30%, 50%, 70%, and 90% RH treatments, respectively

^wN = 6, 30, 23, and 13 rooted cuttings for 30%, 50%, 70%, and 90% RH treatments, respectively, for which longest root and longest shoot measurements were recorded.

^vSubjective quality rating on a scale of 1 to 5, with 5 being the highest. N = 5, 14, 16, and 9 rooted cuttings for 30%, 50%, 70%, and 90% RH treatments respectively for which root and shoot ratings were recorded.

average gravimetric water content of 3.3 g g⁻¹ for our 1:4 peat moss:perlite substrate when excess water has finished draining.

On week 12 (February 28) and week 15 (March 23) cuttings were evaluated for survival and rooting. Cuttings were carefully removed one by one from the rooting medium and their condition recorded as dead, alive but unrooted, or rooted. Cuttings that were obviously dead, with shriveled foliage or buds, were recorded as such and discarded from the tents. Live cuttings with no roots were marked as alive and returned to their respective tent. For rooted cuttings, root and shoot quality were rated on a subjective scale from 0 to 5 based on estimated length, number, color, and thickness/size of roots and shoots, with 5 signifying extensive, healthy roots and shoots respectively, and 0 signifying absent, insignificant, or sickly roots and shoots. Results were analyzed using analysis of variance (ANOVA) and Tukey HSD calculated in RStudio.

Results and Discussion

Rooting percentage was significantly higher for the 50% RH thresholds than for either the 30% or 90% RH

thresholds, but did not differ significantly from the 70% RH threshold ($P < 0.001$) (Table 1). Survival of cuttings showed a similar pattern: survival was significantly higher with the 50% and 70% RH thresholds than with 90% RH, which in turn was significantly higher than 30% RH ($P < 0.001$). However, when rooting was taken as a percentage of surviving cuttings as opposed to total cuttings prepared (that is, disregarding any that died prior to the second and evaluation), there were no differences in rooting success. This indicates that any affect RH or substrate moisture may have had on propagation success occurred by altering mortality levels. Substrate moisture may have been implicated in rooting success: tents with the 90% RH thresholds were watered almost every day, and thus had a saturated substrate a good portion of the time, whereas tents with the 30% RH thresholds went an average of 28 days between waterings (Table 2) and thus often had a nearly dry substrate.

Few significant differences in quality of rooted cuttings were noted between treatments (Table 1). Length of longest root, length of longest shoot, and subjective root ratings did not differ. However, there was a difference in subjective shoot ratings, with cuttings in the 30% RH threshold

Table 2. Number of days out of 102 that humidity tents were watered averaged between the three replicates of each RH threshold treatment. Tents were watered until the substrate was saturated. Means within a row that share a letter do not differ significantly at Tukey HSD $\alpha = 0.05$.

	Relative Humidity Threshold (%)				ANOVA P-value
	30	50	70	90	
Average Number of Days Watered	3.7d	16.3c	49.0b	76.7a	<0.0001

treatment receiving lower ratings than those in the three other threshold treatments due to smaller leaves ($P < 0.001$). In general, more shoot development was observed at higher RH thresholds with few differences noted between the 50%, 70%, and 90% RH thresholds. An exception was that shoots in the 90% RH threshold tents, while highly developed, showed spotty yellowing due to chlorosis, which may partly explain the higher mortality in those tents relative to the tents with intermediate humidity (50 and 70% RH). Chlorosis was likely caused by leaching of nutrients from leaves due to almost daily watering.

Heat stress within this kind of humidity tent was a concern due to a clear variation in temperature within the greenhouse due to proximity to heaters and ventilation fans, which we attempted to control for in laying out the treatment blocks. Statistically significant differences in temperature between treatments, however, did not correlate with either humidity, mortality or rooting, so it is unlikely that heat stress was an important factor in the observed rooting differences.

Rooting was highly dependent on genotype, ranging from 0% to 26.0% (Table 3), which is consistent with previous findings and is the subject of on-going research in our program (Kreiser et al. 2016). For the best rooting genotype, rooting was 17%, 46%, 29% and 13% for the 30, 50, 70, and 90% RH thresholds, respectively (Table 4).

The results of this study indicate that, contrary to prior belief, maintaining close to 100% RH may not in fact be necessary for optimizing rooting of hardwood hazelnut stem cuttings. Generally, high RH levels decrease transpiration and water loss, which supports the high turgor pressure necessary for cell expansion and growth in adventitious roots and for preventing desiccation (Loach 1988). This explains why cuttings in the 30% RH threshold treatment had the lowest rooting and survival percentages. However, our results show that 50% RH was adequate to prevent desiccation, whereas other factors reduced rooting success at higher levels of RH.

This study supports our previous observation that intermediate RH levels may actually promote adventitious root formation⁴. Some studies using hardwood stem cuttings reported that a moderate level of water stress, as likely would be caused by the periodic drying out of the substrate in our experiment, may actually be necessary to initiate root formation and optimize rooting success (Harrison-Murray and Howard 1998, Lebude et al. 2004). For instance, in one study of hazelnut propagation, hardwood cutting treatments that experienced lower RH (at times below 70%), a higher air vapor pressure deficit,

Table 3. Overall percentage rooting and survival of hybrid hazelnut (*Corylus americana* Walter x *C. avellana* L.) stem cuttings by genotype.

Genotype	Cuttings Per Tent	Survival (%)	Rooting (%)
Shep 2-3	5	33	2
Shep 1-17	8	55	26
Shep 1-6	7	50	23
Shep 2-7	4	37	4
Shep 3-1	4	45	15
Rose 14-7	4	50	4
Rose 18-10	3	11	3
Rose 11-8	10	17	4
Rose 14-10	4	52	10
Rose 2-8	4	29	2
Gibs 5-8	7	61	2
Swamp 1-6	2	29	6
Eric NOGA	1	31	8
Edge 5f	1	8	0

and lower water potential actually had the highest rooting and lowest mortality (Tombesi et al. 2015). An ecological explanation is that plants put resources into growing those structures that enable them to obtain more of the resources that limit them which, in the case of stem cuttings under moisture stress, would be roots. Loach (1988) suggested that water stress causes an increase in production of abscisic acid (ABA) which, in turn, might help root formation. However, more recent research with *Vitis sp.* (Kelen and Ozkan 2003) and *Picea sitchensis* (Selby et al. 1992) suggests that ABA is more likely to inhibit rooting, so we do not have a physiological explanation.

Conversely, Loach (1988) claims that in mist systems most of the water uptake in cuttings occurs through the cut basal ends absorbing water from the rooting media, as opposed to through the leaves. Thus substrate water potential affects cutting water potential, which can in turn affect rooting success of hardwood cuttings (Lebude et al. 2004). Therefore, maintaining high ambient RH may not be as important as maintaining adequate levels of moisture in the growing medium for supplying water to the hardwood hazelnut cuttings. Furthermore, one study found that rooting success was optimized at 20% moisture content in the substrate, with more moisture leading to decreasing returns (Tsiouridis and Thomidis 2004). We found that rooting was highest when the substrate was saturated for a brief period about once every six days on average in the 50% RH treatment and every two days on average in the 70% RH treatments (Table 4) and that after draining,

Table 4. Rooting success of the three hybrid hazelnut (*Corylus americana* Walter x *C. avellana* L.) genotypes with the best overall percentage rooted, broken down by relative humidity threshold.

Genotype	Rooting (%)			
	Relative Humidity Threshold (%)			
	30	50	70	90
Shep 1-17	17	46	29	13
Shep 1-6	10	33	24	24
Shep 3-1	0	25	25	8

substrate moisture content equilibrated at about 3 g g^{-1} . It appears that our periodic watering schedule provided the cuttings with sufficient plant-available moisture to replace the transpired water, while the daily oversaturation caused by almost daily watering of the 90% RH tents may have waterlogged cuttings and thereby reduced root formation. The fact that no differences in rooting were observed between the 50% and 70% RH treatments suggests that the optimum watering regime covers a range of possible watering frequencies.

Our conclusions from this trial have already been validated by a subsequent trial in which we obtained 54% rooting (unpublished data), with excellent quality roots and shoots, in a growth chamber managed at 60% RH, which is intermediate between the two levels this trial identified. The success of the growth chamber trial also supports the conclusion that heat stress, which was avoided in the growth chamber, likely was a major contributor to the mortality observed in the humidity tents. It appears that adequate control of temperature is key to success with stem cuttings and may be impossible with humidity tents.

This trial has important implications for the success of our hybrid hazelnut breeding program by enabling us to produce the clonal material needed for advanced performance and agronomic trials. Whereas in the past we were challenged by equipment failures and laborious daily monitoring and watering meant to maintain RH levels near 100%, while still only achieving rooting rates near 30%, recognizing the fact that similar or better results can be achieved at lower RH levels will save time and money. Although we hope that micropropagation protocols will eventually be developed for mass propagation of our germplasm, propagation by this hardwood stem cutting technique is allowing us to proceed until then. Future hardwood stem cutting research will address the effects that dark pretreatments have on rooting success.

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