#### CONCLUSIONS

Based on the results of this study, the commercial root treatments did not improve either plant survival or overall plant quality. The water dip control treatment had ratings as good or better, than any of the three products tested. Using a planting bar yielded higher survival rates across all species, followed by augering and mechanical planting. Intentionally deep planting adversely affected survival of concolor fir but not Douglas-fir, Colorado spruce, or Fraser fir. Overall, Douglas-fir exhibited the best performance in this study.

# Medium Development, Micropropagation, and Acclimatization of Difficult-to-Propagate Hazelnut (*Corylus*|sp.) Hybrids<sup>®</sup>

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A series of hybrid hazelnuts have gained interest as potential alternative crops for farmers in mid-western U.S.A. These hybrids possess tolerance to the harsh winter temperatures of the region and are relatively disease resistant, but they are difficult to propagate by conventional methods. Successful micropropagation has been achieved in our laboratory for selected genotypes by employing a medium based in part on the composition of the hazelnut kernel (Nas and Read, 2004). Acclimatization and subsequent establishment in the field proved to initially be an obstacle, but direct rooting of the microcuttings in rehydrated compressed peat pellets under conditions of high humidity, moderate light, and temperature led to successful production of potted plants of 0.5 to 1 m in height. Use of direct rooting in special plastic containers, together with a regimen of dilute nutrient sprays has facilitated more efficient and rapid multiplication, resulting in improved potential for scaled-up acclimatization and field establishment of the micropropagated hazelnut hybrids. Field plantings have been made in several locations and are being evaluated for trueness to type, winter survival, disease tolerance, growth characteristics, and productivity.

### INTRODUCTION

Micropropagation success is generally considered to be significantly influenced by the chemical composition of the culture medium (Nas and Read, 2000; Preece, 1995). Development of tissue culture media has often been time-consuming and laborious, e.g., the more than 5 years spent in finalizing the classic MS medium (Murashige and Skoog, 1962). Using hazelnut as the test species, we set out to create a systematic approach to the development of a tissue culture medium that may be suitable for other difficult-to-culture plants.

	NRM	MS	DKW	WPM	NN
Macrominerals (mg·L <sup>·1</sup> )					
NH <sub>4</sub> NO <sub>3</sub>	530	1650	1416	400	720
$Ca(NO_3)_2 \cdot 4H_20$	700		1960	556	
$CaCl_2 \cdot 2H_2 0$	90	440	147	96	202
$MgSO_4 \cdot 7H_20$	1600	370	740	370	185
KNO <sub>3</sub>	550	1900			950
KH <sub>2</sub> PO <sub>4</sub>	1300	170	259	170	68
$K_2SO_4$			1560	990	
Microminerals (mg·L <sup>-1</sup> )					
H <sub>3</sub> BO <sub>3</sub>	6.2	6.2	4.8	6.2	10
$CuSO_4 \cdot 5H_20$	2.5	0.025	0.25	0.25	0.025
$MnSO_4 \cdot H_2O)$	20	16.9	33.5	22.3	18.9
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O <sup>w</sup>	2.5	0.25	0.39	0.25	0.25
$ZnSO_4 \cdot 7H_20$	8.8	8.6		8.6	10
$Zn(NO_3)_2 \cdot 6H_20$			17		
Sequestrene 138 Fe	100				
$\mathrm{FeSO}_4{\cdot}7\mathrm{H}_2\mathrm{0}$		27.8	33.4	27.8	2.78
Na <sub>2</sub> EDTA		37.3	44.7	37.3	37.3
KI		0.83			
$\text{CoCl}_2 \cdot 6\text{H}_20$		0.025			
Vitamins (mg·L <sup>-1</sup> )					
Thiamine $(B_1)$	0.60	0.1	2.0	1.0	0.5
Riboflavin (B <sub>2</sub> )	0.21	-	-	-	-
Nicotinic acid (B <sub>3</sub> )	1.15	0.5	2.0	0.5	5.0
Pyrodoxine $(B_6)$	0.60	0.5		0.5	0.5
α-Tocopherol (E)	20.0				
Vitamin C (ascorbic acid)	1.0				
Glycine	0.85	2.0	2.0	2.0	2.0
Folic acid					0.5
Biotin					0.05
Myo-inositol	200	100	1000	100	100
Sucrose (g·L <sup>-1</sup> )	30	30	30	20	20
Agar (g·L <sup>-1</sup> )	5-6	10	-	6	8
Gelrite (g·L·1)	-	-	2		

Table 1. The composition of NRM in comparison with MS, DKW, WPM, and NN media<sup>z</sup>.

<sup>z</sup> NRM hazel nut medium; MS, Murashige and Skoog; DKW; WPM, Woody Plant Medium; and NN, Nitsch and Nitsch.

 $^{\rm w}$  The amount used during this study was 0.25 mg  ${\rm Na_2MoO_4\cdot 2H_2O}$  per Liter.



Figure 1. Proliferating culture of G-029N.



**Figure 2.** G-029N hybrid hazelnut in Jiffy 9 in a sundae cup.



**Figure 3.** Three plantlets of S-182 hybrid hazelnut in Jiffy 9.

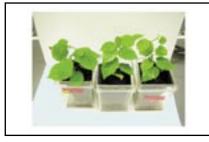


Figure 4. S-182 Hybrid hazelnut in soil mix.

# MATERIALS AND METHODS

We employed reported analyses of hazelnut kernel constituents (Ackurt, et al. 1999: Dzamic, et al., 1972) as a basis for the proposed medium. Initial experiments demonstrated that the resulting nitrogen content was excessive and caused death of the tissue (data not shown). We therefore chose a nitrogen level that was lower than MS, but similar to Woody Plant Medium (WPM) (Lloyd and McCown, 1980) and Nitsch's medium (NN) (Nitsch and Nitsch, 1969). Subsequent experiments led to the development of a medium suitable for hazelnut micropropagation (NRM medium Table 1).

## **RESULTS AND DISCUSSION**

Growth of hazelnut explants cultured on NRM medium was superior to that of explants cultured on MS medium (Table 2) Figs. 1, 2, 3, and 4 illustrate the system employed for direct rooting of hazelnut microcuttings and simultaneous acclimation. Plant quality was excellent and potted plants of  $\frac{1}{2}$  to 1 m in height have been established in several field locations for further evaluation.

## CONCLUSIONS

- A medium based in part on the composition of hazelnut kernels proved to be a key to successful micropropagation of hybrid hazelnuts.
- Direct rooting in rehydrated Jiffy 9 pellets under high humidity and moderate light facilitated acclimatization.
- Dilute nutrient sprays and rooting in special plastic containers accelerated acclimatization.

 Several hundred hybrid hazelnuts have been established in multiple field locations for further evaluation.

	Shoot le	Shoot lengths (mm)		buds /shoot
Genotype	NRM	MS	NRM	MS
E-295-S	15	11	4.0	1.3
G-029-N	7	4	2.3	1.4
S-182	6	4	2.8	1.2

Table 2. Mean shoot length and number of axillary buds of hybrid hazelnuts cultured on NRM medium and MS medium<sup>z</sup>.

<sup>z</sup>NRM, hazel nut medium; MS, Murashige and Skoog.

#### LITERATURE CITED

- Ackurt, F., M. Ozdemir, G. Bringen, and M. Loker. 1999. Effect of geographical origin and variety on vitamin and mineral composition of hazelnut (*Corylus avelland* L.) varieties cultivated in Turkey. Food Chem. 65:309-313.
- Anderson, W.C. 1983. Micropropagation of filberts, Corylus aveiland L. Comb. Proc. Intl. Plant Prop. Soc. 33:132-137.
- Dzamic, M., D. Jakovljev, B. Durdevic, B. Jelenkovic, and R. Dzamic. 1972. Chemical composition and technological values of some hazelnut varieties. University of Belgrade-Yugoslavia, Review of Research Work at the Faculty of Agriculture 20:1-10.
- Driver, J.A., and A.H. Kuniyuki. 1984. In vitro propagation of paradox walnut rootstock. HortScience 19:507-509.
- Lloyd, G., and B. McCown. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant Prop. Soc. 30: 421-427.
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-479.
- Nas, M.N., and P.E. Read. 2000. Micropropagation of hybrid hazelnut: medium composition, physical state and iron source affect shoot morphogenesis, multiplication and explant vitality. Acta Hort. 556:251-258.
- Nas, M.N., and P.E. Read. 2004. A hypothesis for the development of a defined tissue culture medium of higher plants and micropropagation of hazelnuts. Scientia Hort. 101:189-200.

Nitsch, J.P., and C. Nitsch. 1969. Haploid plants from pollen grains. Science 163, 85-87.

- Preece, J.E. 1995. Can nutrient salts partially substitute for growth regulators? Plant Tissue Cult. Biotech. 1:26-37.
- Yu, X.L., and B.M. Reed. 1995. A micropropagation system for hazelnut (Corylus species). HortScience 30:120-123.