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Comparison of Auxin Formulations and Concentrations on Rooting Woody Softwood Cuttings

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Summary

This study evaluated a range of auxin formulations and concentrations for their effects on root initiation on difficult-to-root, softwood cuttings including *Acer palmatum*, *Cornus* [(*Cornus florida* x *kousa*) x *florida*], *Metasequoia glyptostroboides* 'Ogon', and *Zelkova serrata* 'Goshiki'. Auxin formulations included gels, powders, and liquid quick-dips. Auxin concentrations ranged from 0 to 16,000 ppm as either IBA or a mixture of KIBA and KNAA. Results varied by taxa. The highest rooting percentages for *Cornus* (75.3 – 92.9%) were achieved with rooting gels containing 3,100-5,500 ppm IBA, a powder containing 16,000 ppm IBA, and liquid quick-dips containing 5,000 – 10,000 ppm auxin (mixture of 2/3 KIBA and 1/3 KNAA). For *Metasequoia glyptostroboides* 'Ogon', the treatments with the highest rooting percentages (19.1 – 37.2%) included two gels, powders ranging from 1,000 to 16,000 ppm IBA, and liquid quick-dip containing 5,000-10,000 ppm auxin (mixture of 2/3 KIBA and 1/3 KNAA), plus the control. Rooting percentages for *Acer palmatum* and *Zelkova serrata* 'Goshiki' ranged from 2.4 to 23.5% and 0 to 9.5%, respectively, with no significant treatment effects. These results identify numerous treatments that were effective for rooting selected cultivars of *Cornus* and *Metasequoia*; the effectiveness of rooting treatments varied considerably among taxa and environment.

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INTRODUCTION

The age-old practice of propagating plants by stem cuttings has progressed by leaps and bounds with the widespread adoption of the nursery industry in applying synthetic auxins to cuttings (Mendel, 1992). Of the many methods of applying auxin to cuttings, liquid quick-dip and powder applications remain the industry standard (Blythe et al., 2007). Even so, some taxa of plants remain difficult to root by liquid and powder means (Dirr and Heuser, Jr. 2006).

Since the 1980s, several commercial rooting gels have been introduced that promise another method of delivering auxin to cuttings. The gels are marketed as more effective and safer to use than other auxin delivery mechanisms because of their ability to adhere to where they are applied, thus delivering auxin for a longer period while reducing the risk of inhalation (Hydrodynamics International, 2022; Technaflora Plant Products, 2022). Producers of other types of rooting products have countered by claiming gels produce a hypoxic environment at the cutting base which leads to root rot (Hormex, 2020). Despite these claims, commercial rooting gels have seen widespread adoption by hydroponic growers and home propagators but are less common in the industry (Amazon, 2022). However, if a commercial rooting gel were to significantly outperform other auxin delivery methods in harder to root cuttings, it could be an economically desirable alternative for commercial propagators.

Previous research into rooting gels has been paltry and mixed. Ismaili (2016) reported that Clonex® Rooting Gel outperformed a 3,000 ppm liquid treatment of IBA (acid form) on softwood cuttings of seven varieties of olive (*Olea europaea*). Hepburn and Matthews (1985) did not observe a significant difference in roots per cutting between an IBA-based powder and an NAA-based gel in the potato (*Solanum tuberosum*) cultivar 'Désirée'. Caplan et. al, (2018) found that 2,000 ppm IBA gel outperformed a 2,000 ppm *Salix alba* extract gel for *Cannabis sativa*, but did not evaluate against more conventional powder or liquid formulations of IBA.

For this experiment, gels were selected based on Google and Amazon rankings, which approximate sales numbers (Amazon, 2022; Google, 2022). Therefore, these gels represent what a typical consumer might find when searching for a rooting gel in the USA. The selection of the powder and liquid products was based on the standard operating procedure of the Mountain Crop Improvement (MCI) Lab and common nursery practice (Dirr and Heuser, Jr., 2006).

Taxa were selected for propagation difficulty, diversity, prevalence in the trade, and availability. Taxa that were moderately difficult-to-root from softwood cuttings were chosen, based on industry consensus (Pers. Communication) and comments by Dirr and Heuser (2006). Botanical diversity was also emphasized, with taxa from 4 families and both gymnosperms and angiosperms selected. The 4 taxa selected were: 1) *Acer palmatum* (unnamed selection), 2) *Cornus* H2016-019-001 [(*Cornus florida* x *kousa*) x *florida*], 3) *Metasequoia glyptostroboides* 'Ogon', and 4) *Zelkova serrata* 'Goshiki'.

The objective of this study was to evaluate the effectiveness of a diverse collection of rooting gels, powders, and liquids on softwood cuttings of four moderately-difficult-to-root taxa of woody plants.

MATERIALS AND METHODS

The studies were conducted as a randomized complete block design (RCBD). There were 12 treatments plus one control. Four proprietary cloning gels were selected including Clonex® Rooting Gel (3,100 ppm IBA; Hydrodynamics International, Lansing, MI), Midas Hydro Rooting Gel (3,500 ppm IBA, Midas Hydro, Southfield MI), Rootech Cloning Gel™ (5,500 ppm IBA, Technaflora Plant Products, Mission, B.C.), and FOOP Clone Gel (natural ingredients including fish excrement, mineralized aquaculture water, mycorrhizal fungi, willow water, aloe vera, hydrolyzed fish, sea kelp, volcanic silica, peppermint oil, and xanthan gum; FOOP Organic Biosciences, Silver Spring, Maryland), along with 4 concentrations of Hormex Rooting Hormone Powder (1000, 3000, 8000, and 16000 ppm IBA; Maia Products, Westlake Village, CA), and 4 concentrations of a liquid 5-sec. quick-dip of ⅔ K-IBA and ⅓ K-NAA (2500, 5000, 7500, and 10000 ppm total auxin concentration, Sigma-Aldrich, Saint Louis, Mo.). The control consisted of a quick dip in distilled water. A single rep consisted of 7 cuttings (subsamples), with 6 reps (complete blocks) for a total of n = 6 per taxa. Taxa were treated as separate experiments.

Softwood cuttings were taken between 8:30 and 14:00 pm on 26-27 May 26, 2022, from stock plants grown in full sun between 6 and 30 years old, depending on the taxa. All stock plants were established in the landscape of the Mountain Horticultural Crops Research and Extension Center in Mills River, NC (USA zone 7a). Cuttings were taken from the portions of the stock plants which were in full sun. Once cut, cuttings were transported and stored in a moistened cooler and placed in a 4 °C (40 °F) refrigerator until sticking time.

At sticking time, cuttings were retrimmed and leaves from the bottom half of the cuttings were removed. The bottom 2.5 cm (1 in) of the cutting was moistened before being dipped into either the liquid quick-dip, gel, or powder treatments. Cuttings for the control and liquid quick-dip sections were kept in the auxin solution for 5 seconds. Excess powder and gel were shaken off. All cuttings were then stuck in a 48 x 51 x 11 cm (18.9 x 20 x 4.3 in) flat to a depth of 8 - 10 cm (3.2 - 3.9 in), with each flat treated as a complete block. All cuttings in a given flat/block were as similar in length and node number as practical.

The rooting media consisted of a mixture of 66% horticultural perlite (Krum, Carolina Perlite Company, Gold Hill, NC) with 33% ground Canadian sphagnum peat moss (Sunshine®, Sun Gro® Horticulture, Agawam, MA; ground with a W.W. grinder, Wichita, KS). Stuck flats were placed under intermittent mist at a rate of 10 seconds every 10 minutes, with flats containing the same taxa randomly grouped together on a bench. No bottom heat was provided. Cuttings were harvested 52 to 91 days after sticking, when most cuttings of the taxa showed some sign of rooting or callusing.

Data collected included the percentage of cuttings in a rep that had rooted, the average number of primary roots per rooted cutting per rep and the average length of the primary roots per rooted cutting per rep. Data were analyzed using a one-way ANOVA (Proc GLM, SAS Institute, NC) with significant difference between means determined by Fisher's protected least significant difference. In addition, a two-way ANCOVA was performed for all 3 dependent variables (Proc GLM, SAS). Additional regression analyses (Proc GLM, SAS) were performed for auxin concentration for liquid and powder treatments based on the ANCOVA (Proc GLM, SAS). Taxa specific differences in method are recounted below:

***Cornus* H2016-019-001.** Terminal cuttings were taken from six-year-old stock plants between 8:30 and 9:30 am on May 27 in partially cloudy conditions. These 3 node cuttings were stuck at 9:40 am on May 27 after being recut to between 8 cm (3.5 in) to 16 cm (6.3 in). They were harvested 52 days after sticking on July 18, 2022.

***Metasequoia glyptostroboides* 'Ogon'.** Terminal cuttings were taken from a 20-year-old stock plant between 9:35 and 11:00 am on May 26 after a rainstorm to ensure proper hydration. These cuttings were stuck at 2:30 pm on May 26 after being recut to between 10 cm (3.0 in) to 14 cm (5.5 in). They were harvested 78 days after sticking on August 12, 2022.

Acer palmatum. Terminal cuttings were taken from a 30-year-old stock plant between 8:30 and 9:30 am on May 26 during a rainstorm to ensure proper hydration. These 5 node cuttings were stuck at 11:10 am on May 26 after being recut to between 8 cm (3.5 in) to 16 cm (6.3 in). They were harvested 78 days after sticking on August 12, 2022.

Zelkova serrata 'Goshiki'. Terminal cuttings were taken from a 20-year-old stock plant between 1:00 and 2:00 pm on May 27 in sunny conditions. These 6-8 node cuttings were stuck at 2:05 pm on May 27 after being recut to between 10 cm (3.9 in) and 16 cm (6.3 in). They were harvested 91 days after sticking on August 26, 2022.

RESULTS

Significant differences and ranking of rooting responses as a function of treatments varied substantially among the different taxa.

***Cornus* H2016-019-001**. The ANOVA showed that treatments were significant for all three dependent variables (percent rooting, root number and root length). The treatments with the highest rooting percentages included Midas Hydro Rooting Gel, Clonex® Rooting Gel, Rootech Cloning Gel™, Hormex powder with 16,000 ppm auxin, and the 5,000, 7,500, or 10,000 ppm auxin quick-dip liquid treatments (**Table 1**). For average root number, Rootech Cloning Gel™ significantly outperformed all other treatments with an average of 18.8 roots per cutting compared to the control with 4.2 roots. Average root length varied from 0.8 cm for Foop Clone Gel to 2.5 cm for Rootech Cloning Gel™ (that was not significantly different from Clonex® Rooting Gel or the 7,500 or 10,000 ppm auxin quick-dip treatments). The ANCOVA showed that there were significant interactions between auxin formulation and auxin concentration for all three dependent variables (α=0.05). There was a significant quadradic regression response for percent rooting as a function of auxin concentration for liquid quick-dip treatments with percent rooting = 43.16 + 0.011 auxin concentration – 0.00000073 (auxin concentration)^2, but not for powders.

Table 1. Effect of different auxin formulations and concentrations on rooting of *Cornus* H2016-019-001.

Treatment	Auxin Concentration (ppm)	Rooting Percentage (n=78)	Average Root Number (n=77)	Average Root Length (cm) (n=77)
Control	0	40.5 f	4.2 g	1.2 fg
Clonex® Rooting Gel	3,100 IBA	88.1ab	12.7 bcd	2.5 a
Midas Hydro Rooting Gel	3,500 IBA	92.9 a	13.3 bc	2.3 ab
Rootech Cloning Gel™	5,500 IBA	85.7 ab	18.8 a	2.5 a
Foop Clone Gel	0	44.4 ef	3.1g	0.80 g
Hormex (Powder)	1,000 IBA	67.9 bcd	5.9 fg	1.7 bcdef
Hormex (Powder)	3,000 IBA	59.5 def	6.0 fg	1.2 efg
Hormex (Powder)	8,000 IBA	64.3 cde	8.5 ef	1.9 bcd
Hormex (Powder)	16,000 IBA	75.3 abcd	10.6 cde	1.6 cdef
Quick-Dip	2,500 ⅔ KIBA + ⅓ KNAA	69.9 bcd	6.1fg	1.4 def
Quick-Dip	5,000 ⅔ KIBA + ⅓ KNAA	76.2 abcd	11.9 bcde	1.8 bcde
Quick-Dip	7,500 ⅔ KIBA + ⅓ KNAA	84.5 abc	9.8 de	2.3 ab
Quick-Dip	10,000 ⅔ KIBA + ⅓ KNAA	81.0 abc	14.3 b	2.1 abc

Means in the same column with the same letter are not significantly different at $\alpha=0.05$.

Visually, some cuttings treated with higher powder and liquid concentrations showed signs of auxin toxicity, defined as browning and necrosis at the dipped portion with roots forming above the dipped portion. Cuttings treated with the gels did not display this negative effect (**Fig. 1**).

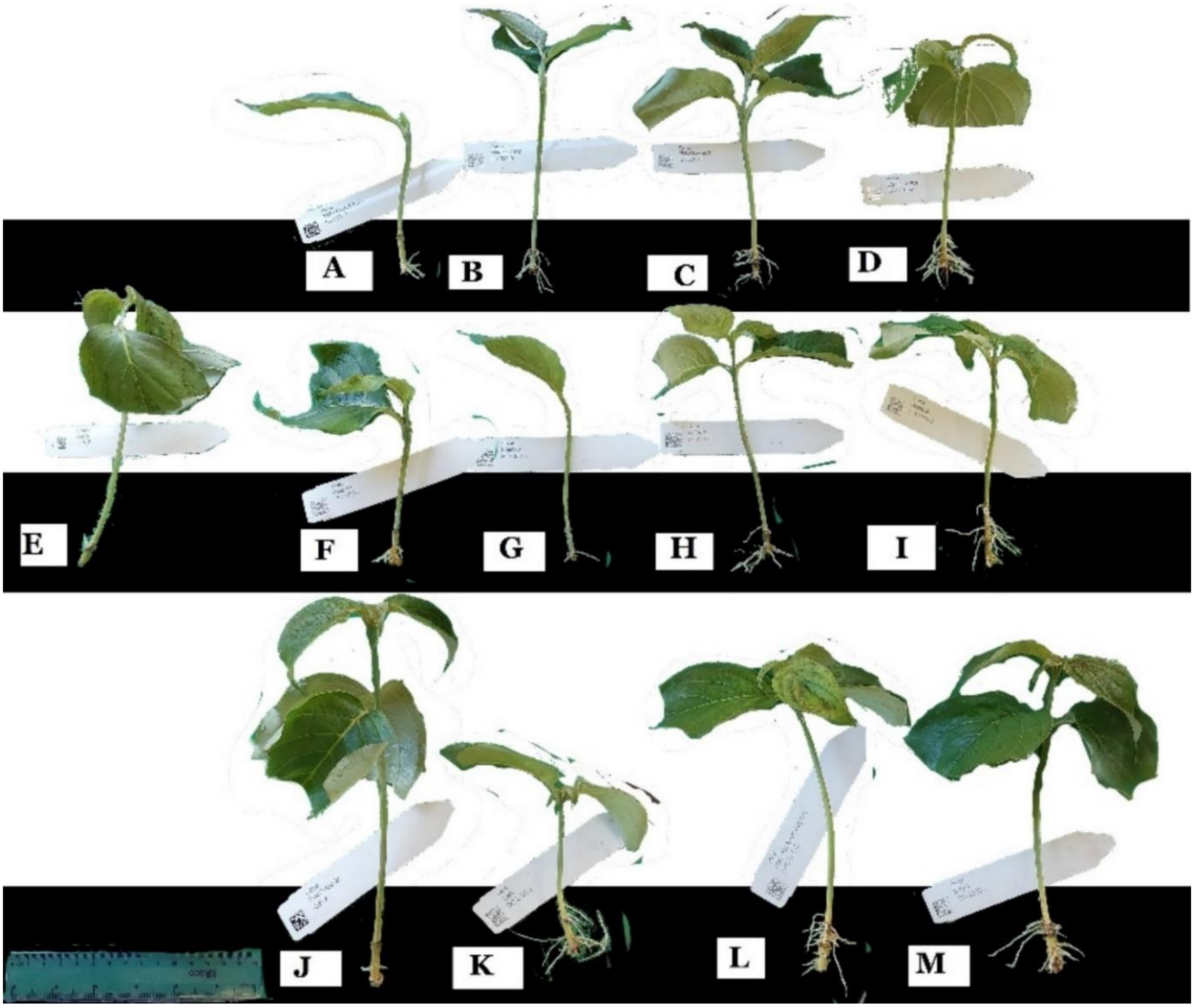

Figure 1. Representative examples of rooting of *Cornus* H2016-019-001 in response to: A-D: Liquid Quick-Dip 2,500, 5,000, 7,500, 10,000 Auxin. E: Control F-I: Hormex Rooting Powder 1,000, 3,000, 8,000, 16,000 J: FOOP Clone Gel, K: Clonex® Rooting Gel, L: Midas Hydro Rooting Gel M:Rootech Cloning Gel.

***Metasequoia glyptostroboides* 'Ogon'.** The ANOVA showed that treatments were significant for percent rooting, but not for root number and root length. The treatments with the highest rooting percentage included Clonex® Rooting Gel, Midas Hydro Rooting Gel, powders ranging from 1,000 to 16,000 ppm auxin, and liquid quick-dips between 5,000 and 10,000 ppm auxin, plus the control (**Table 2**). The ANCOVAs for rooting percentage and average root length were not significant while the ANCOVA for root number showed that there were significant interactions between auxin formulation and auxin concentration (α=0.05).

Table 2. Effect of different auxin formulations and concentrations on rooting of *Metasequoia glyptostroboides* 'Ogon'.

Treatment	Auxin Concentration (ppm)	Rooting Percentage (n=78)	Average Root Number (n=77)^{NS}	Average Root Length (cm) (n=77)^{NS}
Control	0	19.1 abc	1.1	10.8.
Clonex® Rooting Gel	3,100 IBA	35.7 a	1.4	9.1
Midas Hydro Rooting Gel	3,500 IBA	21.4 abc	1.1	11.7
Rootech Cloning Gel™	5,500 IBA	4.8 bc	4.0	1.2
Foop Clone Gel	0	2.4c	1.0	3.6
Hormex (Powder)	1,000 IBA	22.9 ab	1.6	12.2.
Hormex (Powder)	3,000 IBA	36.5 a	1.4	16.1
Hormex (Powder)	8,000 IBA	32.1 a	1.6	11.7
Hormex (Powder)	16,000 IBA	37.2 a	1.7	10.5
Quick-Dip	2,500 ⅔ KIBA + ⅓ KNAA	9.5 bc	1.5	12.8
Quick-Dip	5,000 ⅔ KIBA + ⅓ KNAA	19.1 abc	1.1	14.7
Quick-Dip	7,500 ⅔ KIBA + ⅓ KNAA	23.8 ab	1.3 .	11.3
Quick-Dip	10,000 ⅔ KIBA + ⅓ KNAA	29.5 a	1.2	6.9

Means in the same column with the same letter are nor significantly different at α=0.05. **NS** indicates that an LSD was not performed because the ANOVA was not significant to α = 0.05.

There were no significant linear or quadradic regression responses for any of the dependent variables as a function of auxin concentration for liquid quick-dips or powders. Visually, cuttings treated with high concentrations of the liquid quick-dip treatment showed signs of auxin toxicity, while cuttings treated with any kind of gel showed signs of necrosis around the dipped area (**Fig. 2**).

***Acer palmatum*.** Although percent rooting varied from 2.4 to 23.5%, there were no significant treatment effects for the ANOVA, ANCOVA, or regression analyses.

***Zelkova serrata* 'Goshiki'**. Although percent rooting varied from 0 to 9.5%, there were no significant treatment effects for the ANOVA, ANCOVA, or regression analyses.

DISCUSSION

Consistent with many propagation studies, the efficacy of different auxin formulations and concentrations on root initiation and growth varied considerably among taxa and environment (Dirr and Heuser, 2006). For *Cornus* H2016-019-001, the highest rooting percentages were achieved with rooting gels containing 3,500-5,500 ppm auxin (IBA), powder containing 16,000 ppm auxin, and liquid quick-dips containing 5,000 – 10,000 ppm auxin (mixture of 2/3 KIBA and 1/3 KNAA). It is noteworthy that cuttings treated with these gel formulations often had high rooting percentages, yet with lower auxin concentrations compared with liquid quick-dips or powders. It may be that the gels deliver a greater volume of material that adheres to the cuttings, than do liquids or powders, and thereby proved a similar dose of total auxin at a lower auxin concentration. Some of the gel formulation also include carriers/solvents like propylene glycol (e.g., Rootech Cloning Gel™) that may also enhance auxin uptake. For *Metasequoia glyptostroboides* 'Ogon', the treatments with the highest rooting percentage included two gels, powders ranging from 1,000 to 16,000 ppm auxin, and liquid quick-dips ranging from 5,000 to 10,000 ppm auxin, plus the control. That necrotic symptoms at the base of cuttings treated with gels for this conifer was a negative effect. The *Acer palmatum* and *Zelkova serrata* 'Goshiki' had low rooting percentages with no treatment effects. Consistent with previous research, these two taxa can be exceedingly difficult to root (Dirr and Heuser, 2006) and none of the treatments evaluated here proved exceptional.

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