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Evaluation of Honey as a Rooting Adjuvant for Cutting Propagation of Three Common Nursery Crops †

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Abstract: Plant propagation is a labor-intensive process in the nursery and greenhouse industry, with labor accounting for 41.4% of expenditures in 2019—$4.8 billion of the $11.6 billion total. Labor availability remains a critical issue, and current methods of applying root-promoting compounds to cuttings often yield inconsistent rooting responses. This research investigated honey as a rooting adjuvant and its effects on rooting in Red Cascade™ miniature rose (Rosa 'MOORcap'), common camellia (*Camellia japonica*), and southern magnolia (*Magnolia grandiflora* 'Little Gem'). For Red Cascade™ rose, adding honey to water-soluble indole-3-butyric acid (IBA) solutions did not improve root counts compared to IBA alone. However, 1000 μL·L^{-1} IBA produced more roots than 250 μL·L^{-1} IBA. Camellia and magnolia cuttings were treated with multiflora, Manuka, or commercial honey, alongside IBA rates of 0 to 4500 μL·L^{-1}. In camellia, honey type or auxin rate did not significantly affect rooting, but local and multiflora honey combined with higher IBA rates increased root counts. For magnolia, multiflora honey improved both root number and quality, outperforming other treatments. While honey showed limited benefits for camellia and rose, multiflora honey demonstrated potential economic advantages for magnolia propagation, enhancing root quality and quantity for producers.

Keywords: adventitious rooting; camellia; indole-3-butyric acid (IBA); magnolia

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1. Introduction

Nursery owners worldwide consistently trial novel methods for applying root-promoting compounds during the propagation process [1,2]. Implementing new propagation methods to enhance rooting and subsequent transplant success is a major priority for plant production [3]. In addition to decreasing rooting times, nursery owners are also exploring new methods to reduce the labor required during the propagation process. Within the nursery industry, propagation places the greatest demand on labor in the United States [4]. A survey of nursery growers in the Willamette Valley in Oregon reported that propagation accounted for >50% of their annual labor budget [5]. Further, it was estimated that to produce a crop of red tip photinia (*Photinia* × *fraseri*) and tam juniper (*Juniperus*

sabina 'Tamariscifolia'), propagation accounted for 22% and 27% of the total cost of production, respectively [5]. In the U.S. Census of Horticulture Specialties, nursery operations reported $11.6 billion in expenditures, with labor representing 41.4% ($4.8 billion) of that amount [4]. With record sales being reported by nurseries as people look for alternative activities due to government-imposed lockdowns as a result of the COVID-19 pandemic, decreasing the amount of time a plant spends in the production process before reaching the consumer is an even greater priority for nursery crop producers around the globe [6,7].

In the nursery industry, sugars (as carbohydrates) positively impact the rooting of cuttings. They are frequently used in Stage III of tissue culture as an energy source for micro-cuttings [8]. Numerous clinical studies have confirmed the broad-spectrum antimicrobial properties of honey, which are theorized to be due to naturally low pH, osmotic effect, high sugar concentration, and presence of bacteriostatic and bactericidal factors [9–16]. Historically, honey has been frequently used as a human wound treatment, dating back to the writings of the Egyptians over 5000 years ago. In these early writings, honey was reportedly made into an ointment to treat skin and eye diseases and applied as a dressing for burns and wounds [11,17]. While it was popular as an ancient wound-healing compound, the mechanism of its antimicrobial properties was unknown until the middle 20th century [18–20]. Research in the early 1960s revealed that hydrogen peroxide is the principal antimicrobial agent acting within honey [18,20]. Further research into hydrogen peroxide concentration in honey revealed that dilution of honey with water to levels between 15% and 67% led to significant hydrogen peroxide accumulation [19].

There is one honey produced in the world today that is colloquially referred to as a "super honey" for its antimicrobial activity is Manuka honey, which is solely produced in New Zealand from the nectar of the Manuka shrub (*Leptospermum scoparium*) [2]. The Manuka shrub grows natively along the eastern coast of Australia and the entirely of islands of New Zealand [11]. In its native habitat, it serves as a primary successional species in areas of disturbed forest and serves as the primary understory shrub in its native range [11].

Unlike other honey, the antimicrobial properties of Manuka honey are derived not from hydrogen peroxide, but rather from a compound called methylglyoxal (MGO), produced from the conversion of dihydroxyacetone (DHA), which is abundant in fresh Manuka honey [21,22]. The antimicrobial properties of Manuka honey are determined by testing, and the results are used to calculate the unique Manuka factor (UMF), which ranges in potency from 1–25 [2]. To be classified as Manuka honey, it must be produced in New Zealand. It must pass independent laboratory tests to ensure that it meets the minimum standard for concentrations of methylglyoxal (MGO), dihydroxyacetone (DHA), and leptosperin [19,21,22].

To date, there has been limited nursery trialing evaluating the effectiveness of honey as a rooting hormone. Whalley (2009) [2] conducted an on-nursery trial using honey as a stand-alone rooting hormone when their primary rooting hormone powder was discontinued. Whalley trialed Manuka honey (UMF 15+), commercially-available multiflora honey, a commercially-available root-promoting compound, and a non-treated control. These treatments were applied to cuttings of the following six New Zealand natives: brachyglottis (*Brachyglottis compacta* 'Sunshine'), sand coprosma (*Coprosma acerosa*), creeping mirror plant (*Coprosma* × *kirkii* 'Kirkii'), kapuka (*Griselinia littoralis* 'Broadway Mint'), mousehole tree (*Myoporum laetum*), and small-leaved tree daisy (*Olearia virgata* var. *lineata*) [2]. Both varieties of honey were used to prepare solutions containing honey and hot water (1:2 v/v), and solutions were refrigerated for 24 h; afterward, cuttings were placed into solutions 30 min before sticking and placed onto a mist bed with bottom heat [2]. Cuttings treated with solutions containing multiflora honey had the fewest unrooted cuttings across all four treatments and a high number of cuttings with a good (4+) and average (3 or less) root

rating. In comparison, Manuka solutions had the lowest number of roots with a good (4+) rating and a higher number of unrooted cuttings among all four tested treatments [2]. While Whalley (2009) demonstrated that multiflora honey resulted in fewer unrooted cuttings than cuttings treated with Manuka honey, we chose to continue examining Manuka honey as other research has shown that darker-color honey (e.g., Manuka honey) has greater antimicrobial activity than lighter-color honey [10,14,15].

Since sugars are commonly used as an energy source in tissue culture production, we hypothesized that treating cuttings with honey solutions would provide cuttings with a "starter" charge of energy for use in adventitious root formation. Therefore, this research aimed to evaluate whether adding honey to water-soluble auxin solutions increased root growth and uniformity of stem cuttings of 'Red Cascade' miniature climbing rose (*Rosa* 'MOORcap), common camellia (*Camellia* japonica) and 'Little Gem' southern magnolia (*Magnolia grandiflora* 'Little Gem') compared to auxin solutions without honey.

2. Materials and Methods

The research was conducted at the South Mississippi Branch Experiment Station in Poplarville, Mississippi, USA (latitude 30°50′38.328″ N, longitude 89°32′13.704″ W). Three plant species were selected for these studies due to their popularity in the nursery trade or relevance to propagation research: Red Cascade™ miniature climbing rose (*Rosa* 'MOORcap'), 'Little Gem' southern magnolia (*Magnolia grandiflora* L. 'Little Gem'), and common camellia (*Camellia japonica* L.).

2.1. Plant Material and Preparation

For the 'Red Cascade™' rose, medial, 2.4-inch (6-cm) softwood cuttings were harvested on 5 June 2020 from actively growing, well-fertilized greenhouse-maintained stock plants. For 'Little Gem' southern magnolia, 7-inch (18-cm) semi-hardwood terminal cuttings were collected on 20 August 2020 from an actively growing, established cutting block with all but the top 3 to 4 leaves removed, and basal wounds made on opposite sides to simulate commercial propagation techniques. Common camellia cuttings were collected on 18 August 2020 from an actively growing, established landscape planting, with 5-inch (13-cm) terminal, semi-hardwood cuttings retaining the top two leaves and a single 1.5-inch (4-cm) basal wound applied to one side.

2.2. Treatments

Three honey sources were evaluated: UMF 15+-rated Manuka honey (New Zealand Honey Co., Wanaka, New Zealand), commercially available multiflora honey (Sam's Choice; Bentonville, AR, USA), and locally sourced multiflora honey from USDA-ARS beehives in Poplarville, MI, USA. Solutions were created with indole-3-butyric acid (IBA) (Hortus Water Soluble Salts; Phytotronics Inc., Earth City, MO, USA) dissolved in deionized (DI) water. For Red Cascade™ roses, IBA concentrations were 0, 250, 500, 750, and 1000 $\mu L \cdot L^{-1}$. For camellia, concentrations were 0, 3000, 3500, 4000, and 4500 $\mu L \cdot L^{-1}$, while magnolia concentrations ranged from 0, 2500, 3000, 3500, to 4000 $\mu L \cdot L^{-1}$. Honey was added to the IBA solutions at ratios of 2 parts water to one part honey for all tested species. Prepared solutions were refrigerated for 24 h at 37.4 °F (3 °C) before use. The brix readings determined at 24 hr after dilution were 36.8, 34.7, and 37.6 for the Local, Multi, and Manuka solutions, respectively whereas the pH values of the solutions were 5.0, 5.1, and 5.1.

2.3. Planting and Environmental Conditions

Cuttings were stuck into nursery pots filled with 100% pine bark (3.5-in [8.9 cm] pots for camellia and roses, 4.5-in [11.4 cm] pots for magnolia). Pine bark was a mix of 50% aged and 50% fresh bark passed through a 3/8-inch (0.95-cm) screen, sourced from Eakes'

Nursery Materials (Seminary, MS, USA). Cuttings were placed under intermittent mist systems at the following misting intervals:

Rose: 6 s every 10 min.
Camellia: 6 s every 10 min.
Magnolia: 4 s every 4 min.

Misting intervals were periodically adjusted as needed. Environmental conditions were controlled by Phytotronics NOVA 1626ET six-zone analog controllers (Phytotronics, Inc.; Earth City, MO, USA).

2.4. Measurements

2.4.1. Rose

Evaluations after 42 days included rooting percentage, root number, length of three longest roots, shoot length, and root quality (rated 1–5, with 1 indicating callus-only cuttings and 5 indicating 10^+ roots). Photosynthesis and stomatal conductance were measured on terminal leaflets using a LiCOR® 6800 Portable Photosynthesis Platform with a Multiphase Flash™ fluorometer (LiCOR Inc., Lincoln, NE, USA). Measurements were taken between 0730 and 1130 HR under greenhouse conditions (70.4 °F [21.6 °C], 450 μmol m^{-2}·s^{-1} light, 600 μmol s^{-1} airflow, 65.3% relative humidity, 400 ppm CO_2).

2.4.2. Camellia

Evaluations after 60 days included rooting percentage, root number, average root length, shoot length, and root quality (1–5 scale). Photosynthesis and stomatal conductance were measured under conditions similar to those for roses (82.3 °F [27.9 °C]).

2.4.3. Magnolia

Evaluations after 120 days included rooting percentage, root number, average root length, shoot length, and root quality (1–5 scale). Photosynthesis and stomatal conductance data were not collected due to leaf senescence.

2.5. Experimental Design and Analysis

A 4 × 5 (honey × IBA) factorial treatment structure was used within a completely randomized design with 15 cuttings per treatment. Data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA), with mean separation performed using the Holm-Simulated Method for multiple comparisons ($\alpha = 0.05$).

3. Results

3.1. Rose

Rooting ranged from 87% to 100%, although rooting percentages were not impacted by the honey type or auxin rate (Table 1). Net photosynthesis and stomatal conductance of cuttings were similarly not influenced by honey type or auxin rate (Table 1). Although honey type did not impact root number or root quality ratings, root number and root quality ratings were greater for cuttings receiving 1000 μL·L^{-1} IBA than those treated with 250 μL·L^{-1} IBA (Table 1).

Table 1. Results of honey source and auxin rate on rooting percentage, root number, average root length, root quality, and growth of medial stem cuttings of Red Cascade™ miniature climbing rose (*Rosa* 'MOORcap').

	Rooting (%)		Roots (no.)	Avg. Length of Three Longest Roots (cm)	Shoot Height (cm)	Root Quality Rating [z]	Net Photosynthesis (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal Conductance (gsw) ($mol \cdot m^{-2} \cdot s^{-1}$)
				Significance of treatment f	actors			
Honey Type [y]	NS		NS	NS	NS	NS	NS	NS
Auxin Rate [x]	NS		0.017	NS	NS	0.0368	NS	NS
Honey Type × Auxin Rate	NS		NS	0.001	0.0074	NS	NS	NS
			L	east Squares means for mai	n effects			
Honey Type / None	Auxin Rate	87%	1.9 a [w]	10.0 a	4.7 a	3.4 a	7.1 a [z]	0.19 a
Local		100%	2.0 a	10.1 a	4.8 a	3.4 a	8.7 a	0.19 a
Manuka (15+ UMF)		97%	1.9 a	10.3 a	5.1 a	3.3 a	7.4 a	0.16 a
Multiflora		100%	2.0 a	9.3 a	4.6 a	3.4 a	8.6 a	0.18 a
	0 $\mu L \cdot L^{-1}$ IBA	87%	2.0 AB	9.8 A	4.9 A	3.4 AB	8.4 A	0.16 A
	250 $\mu L \cdot L^{-1}$ IBA	90%	1.8 B	8.8 A	4.4 A	3.2 B	9.0 A	0.18 A
	500 $\mu L \cdot L^{-1}$ IBA	90%	2.0 AB	10.4 A	5.3 A	3.4 AB	8.4 A	0.19 A
	750 $\mu L \cdot L^{-1}$ IBA	93%	2.0 AB	10.3 A	4.8 A	3.4 AB	6.8 A	0.15 A
	1000 $\mu L \cdot L^{-1}$ IBA	100%	2.1 A	10.2 a	4.8 A	3.4 A	7.2 A	0.2 A
			Treatm	ent least squares means gro	uped by honey			
Honey Type / None	Auxin Rate 0 $\mu L \cdot L^{-1}$ IBA	-	1.9	8.4 b [z]	5 ab [z]	3.1	3.2	0.07
	250 $\mu L \cdot L^{-1}$ IBA	-	1.8	9.8 ab	4.4 ab	3.2	3.6	0.08
	500 $\mu L \cdot L^{-1}$ IBA	-	1.8	8 b	3.9 ab	3.1	3.2	0.08
	750 $\mu L \cdot L^{-1}$ IBA	-	2	12.2 ab	4.9 ab	3.3	3.9	0.14
	1000 $\mu L \cdot L^{-1}$ IBA	-	2.2	11.5 ab	5.4 ab	3.6	2.8	0.09
	0 $\mu L \cdot L^{-1}$ IBA	-	2.1	9.8 ab	5.1 ab	3.6	3.6	0.02
	250 $\mu L \cdot L^{-1}$ IBA	-	1.8	10.2 ab	4.6 ab	3.1	3.4	0.01
Local	500 $\mu L \cdot L^{-1}$ IBA	-	2	10.3 ab	5.1 ab	3.4	2.9	-
	750 $\mu L \cdot L^{-1}$ IBA	-	2	10.6 ab	5.3 b	3.4	4.2	0.05
	1000 $\mu L \cdot L^{-1}$ IBA	-	2	9.6 ab	3.7 b	3.4	2.9	-
	0 $\mu L \cdot L^{-1}$ IBA	-	1.8	10.3 ab	4.8 ab	3.1	3.8	0.004
	250 $\mu L \cdot L^{-1}$ IBA	-	1.7	7.3 b	3.4 b	3.1	3.8	0.06
Manuka (15+ UMF)	500 $\mu L \cdot L^{-1}$ IBA	-	2.1	13.4 a	7.1 a	3.5	4.8	0.07
	750 $\mu L \cdot L^{-1}$ IBA	-	1.9	9.4 ab	4.2 ab	3.3	1.8	-
	1000 $\mu L \cdot L^{-1}$ IBA	-	2	11 ab	6.3 ab	3.3	3.6	-
	0 $\mu L \cdot L^{-1}$ IBA	-	2.2	10.8 ab	4.6 ab	3.6	3.7	0.16
	250 $\mu L \cdot L^{-1}$ IBA	-	1.9	7.8 b	5 ab	3.2	4.2	-
Multiflora	500 $\mu L \cdot L^{-1}$ IBA	-	2	9.8 ab	5 ab	3.4	3	0.06
	750 $\mu L \cdot L^{-1}$ IBA	-	2	9.2 ab	4.9 ab	3.3	3.2	0.09
	1000 $\mu L \cdot L^{-1}$ IBA	-	2.1	9 ab	3.8 b	3.4	3.1	0.09

[z] Root Quality (1–5, with 1 = callused cutting without roots and 5 = ≥10 roots); [y] Honey: None, Locally sourced, Manuka (15+ UMF), and multiflora; [x] Auxin rate: 0, 250, 500, 750, or 1000 $\mu L \cdot L^{-1}$ IBA applied as a 3-s basal quick-dip; [w] When the interaction term (Honey Type × Auxin Rate) in the model is not significant ($p > 0.10$), main effects means for levels within each treatment factor followed by the same lower-case or upper-case letter are not significantly different using the Holm-Simulated method for multiple comparisons ($\alpha = 0.05$). When the interaction term in the model is significant ($p \leq 0.10$), simple effects means. (treatment means for honey type grouped within auxin rate) followed by the same lower-case or upper case letter are not significantly different using the Holm-simulated method for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes. NS = not significant.

There was an interaction between auxin rate and honey type for the average length of the three longest roots and shoot height (Table 1). Cuttings dipped in a Manuka solution with 500 $\mu L \cdot L^{-1}$ IBA had a greater average root length of the three longest roots than cuttings that received no honey or auxin, no honey and 500 $\mu L \cdot L^{-1}$ IBA, or multiflora

or Manuka solutions and 250 $\mu L \cdot L^{-1}$ IBA. The average lengths of the three longest roots were similar for all other treatment combinations (Table 1). Shoot heights were greatest for cuttings dipped in a Manuka solution with 500 $\mu L \cdot L^{-1}$ IBA compared to cuttings treated with multiflora or local honey solutions with 1000 $\mu L \cdot L^{-1}$ IBA, cuttings treated with a Manuka honey solution with 250 $\mu L \cdot L^{-1}$ IBA, and cuttings treated with a local honey solution with 750 $\mu L \cdot L^{-1}$ IBA (Table 1). All other treatment combinations had similar shoot growth (Table 1).

3.2. Camellia

The rooting percentage of camellia ranged from 26.7% to 60%, but neither the honey type nor the auxin rate impacted the rooting percentage (Table 2). Also, neither the honey type nor the auxin rate affected the average length of the three longest roots, shoot height, or root quality ratings (Table 2). There was an interaction between the main effects of auxin rate and honey type for root number (Table 2). Cuttings treated with solutions of local or multiflora honey combined with 4000 $\mu L \cdot L^{-1}$ IBA and a locally sourced multiflora honey solution with 4500 $\mu L \cdot L^{-1}$ IBA resulted in greater root numbers than cuttings dipped in locally sourced multiflora honey or a commercially available multiflora honey solution without auxin or 3000 $\mu L \cdot L^{-1}$ IBA treatments without honey (Table 2). Honey type significantly impacted the net photosynthesis rate (A) (Table 2). Treating cuttings with a commercial multiflora honey resulted in a higher photosynthetic rate than cuttings treated with local multiflora honey. Auxin rate and honey type significantly impacted the stomatal conductance values (Table 2). Cuttings treated with any type of honey (commercial multiflora, Manuka, or local multiflora) had greater stomatal conductance values than cuttings in the control treatment group. For auxin rate, cuttings receiving no auxin and cuttings receiving 4500 $\mu L \cdot L^{-1}$ IBA resulted in greater stomatal gas exchange values than cuttings treated with 4000 $\mu L \cdot L^{-1}$ IBA.

Table 2. Impact of four different honey sources and five varying auxin rates on rooting percentage, root number, average root length, growth, root quality, net photosynthesis, and stomatal conductance values of terminal stem cuttings of camellia (*Camellia japonica*).

	Rooting (%)	Roots (no.)	Avg. Length of Three Longest Roots (cm)	Shoot Height (cm)	Root Quality Rating [z]	Net Photosynthesis (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal Conductance (gsw) ($mol \cdot m^{-2} \cdot s^{-1}$)
Significance of treatment factors							
Honey Type [y]	NS	NS	NS	NS	NS	**	**
Auxin Rate [x]	NS	0.003	NS	NS	NS	NS	**
Honey Type × Auxin Rate	NS	<0.0001	0.0764	NS	NS	NS	NS
Least Squares means for main effects							
Honey Type / Auxin Rate							
None	60	2.6 a	2.4 a	0.1 a	3.4 a	0.3 ab [w]	0.002 b
Local Multiflora	50	2.6 a	2.6 a	0.1 a	3.5 a	0.3 b	0.01 a
Manuka (15+ UMF)	60	2.5 a	2.9 a	0.3 a	3.3 a	0.7 ab	0.02 a
Commercial Multiflora	26	2.6 a	2.4 a	0.2 a	3.5 a	0.8 a	0.02 a
0 $\mu L \cdot L^{-1}$ IBA	60	2.3 B	2.5 A	0.2 A	3.3 A	0.5 A	0.02 A
3000 $\mu L \cdot L^{-1}$ IBA	46	2.6 A	2.6 A	0.2 A	3.5 A	0.7 A	0.01 AB
3500 $\mu L \cdot L^{-1}$ IBA	60	2.6 A	3.0 A	0.2 A	3.5 A	0.5 A	0.01 AB
4000 $\mu L \cdot L^{-1}$ IBA	60	2.7 A	2.5 A	0.1 A	3.5 A	0.4 A	0.0003 B
4500 $\mu L \cdot L^{-1}$ IBA	46	2.6 A	2.3 A	0.1 A	3.4 A	0.4 A	0.01 A

Table 2. *Cont.*

	Rooting (%)		Roots (no.)	Avg. Length of Three Longest Roots (cm)	Shoot Height (cm)	Root Quality Rating [z]	Net Photosynthesis (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal Conductance (gsw) ($mol \cdot m^{-2} \cdot s^{-1}$)
Treatment least squares means grouped by honey								
Honey Type	Auxin Rate							
None	0 $\mu L \cdot L^{-1}$ IBA	-	2.5 abc [z]	2.4 ab	0.1	3.2	1.3	0.02
	3000 $\mu L \cdot L^{-1}$ IBA	-	2.1 bc	-	-	-	-	-
	3500 $\mu L \cdot L^{-1}$ IBA	-	2.4 abc	-	-	-	-	-
	4000 $\mu L \cdot L^{-1}$ IBA	-	2.5 abc	-	-	-	-	-
	4500 $\mu L \cdot L^{-1}$ IBA	-	2.3 abc	-	-	-	-	-
Local Multiflora	0 $\mu L \cdot L^{-1}$ IBA	-	2.2 bc	2.4 ab	0.1	3.1	1.3	0.03
	3000 $\mu L \cdot L^{-1}$ IBA	-	2.6 ab	2.7 ab	-	3.5	1.2	0.01
	3500 $\mu L \cdot L^{-1}$ IBA	-	2.3 abc	3.1 ab	0.1	3.6	1.4	0.02
	4000 $\mu L \cdot L^{-1}$ IBA	-	2.8 a	2.5 ab	0.3	3.8	1.2	0.01
	4500 $\mu L \cdot L^{-1}$ IBA	-	2.8 a	2.5 ab	0.1	3.6	1.4	0.04
Manuka (15+ UMF)	0 $\mu L \cdot L^{-1}$ IBA	-	2.6 abc	3.5 ab	0.4	3.5	1.5	0.04
	3000 $\mu L \cdot L^{-1}$ IBA	-	2.3 abc	2.4 ab	0.6	-	2.3	0.04
	3500 $\mu L \cdot L^{-1}$ IBA	-	2.6 ab	3.8 a	0.2	3.4	1.8	0.02
	4000 $\mu L \cdot L^{-1}$ IBA	-	2.3 abc	2.5 ab	0.2	3.4	1.5	0.01
	4500 $\mu L \cdot L^{-1}$ IBA	-	2.4 abc	2.4 ab	0.1	3.2	1.3	0.03
Commercial Multiflora	0 $\mu L \cdot L^{-1}$ IBA	-	1.8 c	1.8 b	0.2	3.3	2.1	0.03
	3000 $\mu L \cdot L^{-1}$ IBA	-	2.7 ab	2.9 ab	0.2	3.7	1.7	0.03
	3500 $\mu L \cdot L^{-1}$ IBA	-	2.8 ab	2.5 ab	0.3	3.5	1.5	0.02
	4000 $\mu L \cdot L^{-1}$ IBA	-	2.8 a	2.6 ab	-	3.5	1.8	0.02
	4500 $\mu L \cdot L^{-1}$ IBA	-	2.4 abc	2.2 ab	-	3.4	1.6	0.02

[z] Root Quality (1–5, with 1 = callused cutting without roots and 5 = ≥10 roots); [y] Honey: None, local multiflora honey, Manuka (15+ UMF) honey, and a commercial multiflora honey; [x] Auxin rate: 0, 3000, 3500, 4000, or 4500 $\mu L \cdot L^{-1}$ IBA applied as a 3-s basal quick-dip; [w] When the interaction term (Honey Type × Auxin Rate) in the model is not significant ($p > 0.10$), main effects means for levels within each treatment factor followed by the same lower-case (honey type) or upper-case letter (auxin rate) are not significantly different using the Holm-Simulated method for multiple comparisons ($\alpha = 0.05$). When the interaction term in the model is significant ($p \leq 0.10$), simple effects means (treatment means for honey type grouped within auxin rate) followed by the same lower-case or upper-case letter are not significantly different using the Holm-simulated method for multiple comparisons ($\alpha = 0.05$); otherwise, the treatment means are presented without letter groupings for informational purposes. Significant at the 0.05 (**). NS = not significant.

3.3. Magnolia

The rooting percentage of magnolia ranged from 6.7% to 20%; but the percentages were statistically similar (Table 3). Similarly, the auxin rate also had no impact on root number, the average length of the three longest roots, or root quality ratings (Table 3). However, honey type did influence both root number and root quality ratings. Cuttings treated with a commercial multiflora honey had more roots than cuttings treated with either Manuka or no honey (Table 3). Cuttings treated with multiflora honey had higher root quality ratings compared to cuttings treated with no honey (Table 3). At study conclusion, no visible shoot growth was observed for the magnolia cuttings. Gross photosynthesis and stomatal conductance data were not collected for magnolia cuttings due to leaf senescence.

Table 3. Results of three different honey sources and five varying auxin rates on rooting percentage, root number, average root length, root quality, and growth of terminal stem cuttings of southern magnolia (*Magnolia grandiflora* 'Little Gem').

	Rooting (%)	Roots (no.)	Avg. Length of Three Longest Roots (cm)	Root Quality Rating [z]
Significance of treatment factors				
Honey Type [y]	NS	0.0002	NS	0.0377
Auxin Rate [x]	NS	NS	NS	NS
Honey Type × Auxin Rate	NS	NS	NS	NS

Table 3. *Cont.*

		Rooting (%)	Roots (no.)	Avg. Length of Three Longest Roots (cm)	Root Quality Rating [z]
Least squares means for main effects					
Honey Type	Auxin Rate				
None		13.3	2.1 c [w]	9.1 a	3.4 b
Local Multiflora		13.3	2.7 ab	9.1 a	3.8 ab
Manuka (15+ UMF)		6.7	2.4 bc	9.7 a	3.6 ab
Commercial Multiflora		3.3	2.9 a	9.4 a	4.1 a
	0 $\mu L \cdot L^{-1}$ IBA	13.3	2.1 A	8.7 A	3.5 A
	2500 $\mu L \cdot L^{-1}$ IBA	13.3	2.7 A	10.0 A	3.9 A
	3000 $\mu L \cdot L^{-1}$ IBA	20.0	2.7 A	9.2 A	3.8 A
	3500 $\mu L \cdot L^{-1}$ IBA	13.3	2.6 A	10.0 A	3.8 A
	4000 $\mu L \cdot L^{-1}$ IBA	10.0	2.4 A	8.7 A	3.6 A

[z] Root Quality (1–5, with 1 = callused cutting without roots and 5 = ≥10 roots); [y] Honey: None, local multiflora honey, Manuka (15+ UMF) honey, and a commercial multiflora honey; [x] Auxin rate: 0, 250, 500, 750, or 1000 $\mu L \cdot L^{-1}$ IBA applied as a 3-s basal quick-dip; [w] When the interaction term (Honey Type × Auxin Rate) in the model is not significant ($p > 0.10$), main effects means for levels within each treatment factor followed by the same lower-case (honey type) or upper-case letter (auxin rate) are not significantly different using the Holm-Simulated method for multiple comparisons ($\alpha = 0.05$). NS = not significant.

4. Discussion

4.1. Rose

Our results differ from Whalley (2009), who reported using multiflora honey enhanced rooting, including a healthier or higher quality root system, compared to a commercial root-promoting compound. In contrast, in the present study, honey type did not impact rooting percentage, root number, or root quality ratings (Table 1). Whalley (2009) inserted cuttings into a mist bed with bottom heat. In propagation, the use of bottom heat has been well-documented to enhance the rooting of difficult-to-root species [8]. While their results indicate using multiflora honey enhanced rooting compared to a root-promoting compound, the use of bottom heat may have enhanced root growth.

Additionally, cuttings were stuck into either pit sand or a cutting mix [2]. Our study selected 100% pine bark to replicate the most common propagation media used in the Southeastern United States. Whalley using pit sand provided an inert medium that could limit any potential interaction between the rooting environment and the treatments. Inert media are preferred in agricultural research as they do not decompose or provide nutrition to experimental plants and are void of pathogens that could cause harm. While pit sand falls under the 'inert media' category, it is not mentioned in the article if the pit sand was sterilized before use. If it was not sterilized, it could have artificially impacted the study by making the use of honey that could have a greater impact on controlling pathogens that may have been present on the sand. In their results, cuttings treated with the commercial root-promoting compound had the greatest number of cuttings with 4 or more roots and a higher rooting percentage (80%) than those treated with a multiflora honey (65%). However, using a commercial cutting mix resulted in a rooting percentage of nearly 55% when using either multiflora honey or the commercial root-promoting compound. In the nursery and greenhouse industry, many pre-packaged soilless media contain a starter 'nutrient package' to provide nutrition for the plant until further nutrition is applied. The bagged cutting mix may have influenced the rooting of the cuttings by providing supplemental nutrition to those cuttings grown in the cutting mix compared to cuttings grown in the inert pit sand media.

For the average length of the three longest roots, combining Manuka honey with 500 ppm auxin resulted in greater average lengths compared to cuttings treated with 500 ppm auxin and no honey (Table 1). Shoot heights were greatest for cuttings dipped in a Manuka honey solution with 500 ppm IBA compared to the highest rates of auxin

in solution with commercially available multiflora or locally sourced multiflora honey, cuttings dipped in 250 ppm IBA in a solution with Manuka honey, or cuttings dipped in a local honey solution combined with 750 ppm auxin (Table 1).

Manuka honey, commercially-sourced multiflora honey, or locally-sourced multiflora honey alone did not lead to enhanced rooting without auxin. While the exact mechanism of how adding Manuka honey to 500 ppm IBA led to increased root length and shoot height over 500 ppm IBA alone is unknown, the ratio of sugars present in Manuka honey solutions may provide a nutritional source for the cuttings. Further research into the carbohydrate breakdown of Manuka honey has shown that Manuka honey possesses many oligosaccharides [23]. Oligosaccharides are more complex sugars formed during the ripening stage of honey in which enzymatic activity and acids are more active. These complex sugars work synergistically with methylglyoxal (MGO) to provide antimicrobial properties against biological pathogens. Further, the main bioactive compound in Manuka honey, methylglyoxal, could have impacted the increased shoot length and root length observed on cuttings treated with 500 ppm plus Manuka honey [24].

Additionally, our results differ from previous research regarding the effects of the IBA rate on the rooting of Red Cascade™ miniature rose [1]. Previous work reported that the rooting response of Red Cascade™ rose increased with increasing auxin concentration. Our results indicated that the increased auxin rate did not impact tested rooting parameters. Cuttings treated with 1000 ppm IBA rooted similarly to those cuttings not receiving IBA at the time of treatment initiation. For both experiments, cuttings were taken from well-fertilized greenhouse-maintained stock plants. However, previous work used single-node cuttings, whereas multi-node cuttings were used for the experiment reported here. The use of multi-node cuttings is recommended in the literature for the propagation of Red Cascade™ miniature rose [25,26].

Further, multi-node cuttings of ornamental plants theoretically contain more endogenous auxin used for adventitious root formation; therefore, multi-node cuttings may have no impact on our tested rooting responses compared to the increase seen in previous work. This research focused on the long-term impact of rooting hormone and honey rates on rooting, previous research was focused on speed of rooting. Previous work had a rooting period of 30 days compared to 42 days for this study. Speed of rooting is important for maintaining the profitability in nurseries and greenhouses. When producers can root liner materials faster, greenhouses have increased availability for additional production.

4.2. Camellia

Our results indicate that using honey as a rooting aid was not beneficial for the root generation of common camellia if auxin rates were above 3000 $\mu L \cdot L^{-1}$. This result is like previously reported guidelines for camellia [25,26]. Previous research on camellia's propagation has shown that auxin rates between 3000 and 5000 $\mu L \cdot L^{-1}$ stimulate the adventitious rooting of camellia [25,26]. The slightly higher auxin level preferred by camellia cuttings in this study may result from differences in the nutritional status of the stock plants or variations in the growing environment. According to Dirr and Heuser (2006) [26], the time of year and age of stock plant material is critical for adventitious rooting of camellia. This is further supported by the work of Salami and Hesami [27] on their work with *Ficus religiosa*. Their work found that the physiological status of the mother plant is a critical prerequisite in achieving the adventitious rooting of stem cuttings. The cutting material used in this study was taken from well-established landscape plants estimated to exceed 30 years of age.

For camellia, using any honey type led to increased stomatal conductance values compared to the non-treated control. In contrast, using a commercially-available multiflora

honey increased net photosynthetic rates compared to a local multiflora honey. While this result is different from Whalley (2009), his work did not include common camellia, and it is not uncommon for difficult-to-root species to have vastly different propagation protocols.

4.3. Magnolia

Using a local multiflora or a commercial multiflora honey resulted in greater root numbers compared to cuttings that did not receive honey while using a commercial multiflora honey alone resulted in a higher quality root system compared to cuttings receiving no honey. However, we saw similar rooting percentages when cuttings received no auxin or were treated with the higher auxin rates recommended by Dirr and Heuser [26]. Previous work on the propagation of *Magnolia* species has shown that exogenously applied auxin was necessary to induce adventitious rooting [28]. Our results with 'Little Gem' differed, showing that adventitious rooting can occur without exogenously applied IBA.

Further, our study with 'Little Gem' varies from other studies on *Magnolia* species, which reported that treating cuttings with exogenous IBA significantly increased rooting ability [29]. Previous research suggested a range of 5000 $\mu L \cdot L^{-1}$ to 20,000 $\mu L \cdot L^{-1}$ IBA was required to stimulate the rooting of *Magnolia* species; however, variation has been reported [25,26,28,30]. Research by Castro et al. [31] found that oligosaccharides derived from red seaweed induced the partial suppression of viral, fungal, and bacterial infections due to the accumulations of phenylpropanoid compounds (PPCs) with antimicrobial activities. Their results suggest that the presence of oligosaccharides stimulate growth in plant materials and provide protection against pathogens, suggesting some synergy occurs between oligosaccharide concentration and perceived pathogenic control [32]. While we did not observe any presence or suppression of pathogens in this study with magnolia or camellia, further work is warranted on the control honey solutions may have on the presence of pathogens within the propagation environment.

5. Conclusions

While using honey alone did not enhance rooting across most measured parameters, a positive interaction was observed when Manuka honey was combined with 500 $\mu L \cdot L^{-1}$ IBA for the average length of the three longest roots and shoot growth in Red Cascade™ miniature climbing rose cuttings. This suggests a synergistic relationship between honey and auxin that warrants further investigation, particularly concerning the speed of root initiation. Honey's antimicrobial effects may also positively impact the rooting of species susceptible to pathogens during propagation, although no clear advantage was identified for the propagation of large numbers of easy-to-root species like Red Cascade™.

For camellia, while the honey source did not significantly impact most measured responses, it influenced net photosynthesis and stomatal conductance, suggesting that higher gas exchange could have affected other data had the study duration been extended. Additionally, a synergy between honey source and auxin rate was noted for root numbers in camellia, while multiflora honey positively impacted root numbers and root quality for 'Little Gem' southern magnolia. This indicates that honey could improve propagation outcomes for difficult-to-root or disease-susceptible species, making its use potentially advantageous in these contexts.

Despite these findings, the added labor costs associated with incorporating honey into water-soluble auxin solutions may render this approach impractical for large-scale nursery operations. Smaller operations, however, may benefit from the extra step, particularly when propagating species like 'Little Gem' southern magnolia. These operations could market plants grown with honey as environmentally friendly alternatives, free from synthetic chemicals. Additionally, the use of locally sourced multiflora honey provides opportunities

for value-added marketing, as nurseries could produce their own honey for propagation while selling any surplus as a supplemental revenue stream.

Further research is needed to explore the economic implications of honey in plant propagation, its long-term benefits on subsequent plant growth, and its effectiveness across a broader range of plant species. Such studies would help determine whether the potential benefits of honey justify its use in commercial nursery operations.

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