

Cutting Propagation with Auxin Applied Via a Stabilized Organic Rooting Substrate¹©

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Single-node cuttings of *Hedera helix* L. and *Rosa* 'Red Cascade' were inserted to a depth of 0.65 cm (0.25 inches) into 4-cm³ plugs of a stabilized organic rooting substrate that had been soaked in water or aqueous solutions of K-IBA + K-NAA at concentrations ranging, respectively, from 50 ppm + 25 ppm to 2000 ppm + 1000 ppm. With increasing auxin concentration in the plugs, treatments first provided a root-promoting response of the lower stem tissue of the cuttings, then a phytotoxic response of the lower stem tissue and some root-promoting response of the upper stem tissue, and finally a phytotoxic response by all stem tissue. Results suggested that cuttings inserted in plugs treated with a low concentration of auxin (below 100 ppm) could potentially provide results similar to cuttings receiving a conventional basal quick-dip. In a second experiment conducted in a similar manner, cuttings were inserted into plugs that had been soaked in water aqueous solutions of K-IBA at concentrations ranging from 15 ppm to 75 ppm, or aqueous solutions of K-IBA + K-NAA at concentrations ranging, respectively, from 15 ppm + 7.5 ppm to 60 ppm + 30 ppm, with results compared to cuttings receiving a conventional basal quick-dip in 1000 ppm K-IBA or 1000 ppm K-IBA + 500 ppm K-NAA. Although auxin was not essential for rooting, the number of roots and total root length on cuttings of *H. helix* were greater in plugs treated with 45 ppm or 60 ppm K-IBA and similar with other treatments compared to the basal quick-dip. Shoot length was similar on cuttings in untreated plugs and all K-IBA treatments compared to the basal quick-dip, and lower with all K-IBA + K-NAA treatments. Auxin was not essential for rooting cuttings of *R. 'Red Cascade'*; however, cutting response trends indicated that a low level of auxin (30 ppm or 45 ppm K-IBA) in the plugs could be beneficial.

INTRODUCTION

The use of auxins as root-promoting chemicals in cutting propagation has traditionally focused on their application to stem cuttings as a basal quick-dip using liquid or talc formulations or an extended basal soak using liquid formulations (Hartmann et al., 2002). Current worker protection standards in the United States mandate that employees using chemicals receive safety training and wear personal protective safety equipment (gloves, eyewear, and appropriate clothing). With simultaneous focus on improving employee safety and reducing production costs through improved labor processes and automation, propagators could benefit from alternative auxin delivery methods that permit reduced employee exposure to chemicals

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and use of lower chemical concentrations. Incorporation of auxins directly into the rooting substrate could be one means of accomplishing these objectives.

The literature contains no mention of auxin application to conventional stem cuttings by way of the rooting substrate; however, auxin-containing substrates for rooting are utilized in other methods of propagation. Microcuttings produced in tissue culture are often rooted in a Stage III substrate containing auxin (Kyte, 1987). Auxin can also be incorporated into the rooting substrate used for air layering (Wells, 1986). Various retail products used as a post-transplant soil drench contain low concentrations of auxin. While basally applied auxin is capable of encouraging adventitious rooting, at elevated levels it can also inhibit subsequent budbreak (DeVries and Dubois, 1988; Sun and Bassuk, 1993).

The objective of our first experiment was to screen the effectiveness of a broad range of concentrations of the potassium (K) salt formulations of indole-3-butyric acid (K-IBA) and 1-naphthalenacetic acid (K-NAA) applied in combination to single-node cuttings of *Hedera helix* and *R. 'Red Cascade'* via a stabilized organic substrate by examining cutting response in comparison to a conventional basal quick-dip application. Based upon these initial results, our second experiment was conducted using a narrower range of K-IBA + K-NAA and K-IBA alone to identify treatments that could be at least as effective as the basal quick-dip for rooting cuttings.

MATERIALS AND METHODS

Q Plug™ rooting plugs (International Horticultural Technologies, Hollister, California), stabilized organic substrate units containing peat moss and a polymer binder, were used to provide uniform, easily handled rooting substrate units. Oval plugs 2.5 cm × 1.6 cm (1-inch × 0.625-inch) (4 cm³ vol.) were dried for 24 h at 46 °C (115 °F) and then soaked by submergence for 24 h in water or in aqueous solutions of auxin. In Experiment 1, auxin solutions consisted of K-IBA + K-NAA (Sigma Chemical Company, St. Louis, Missouri) at 10 concentrations ranging, respectively, from 50 ppm + 25 ppm to 2000 ppm + 1000 ppm. In Experiment 2, auxin solutions consisted of K-IBA at 15, 30, 45, 60, and 75 ppm; and K-IBA + K-NAA at 15 + 7.5, 30 + 15, 45 + 22.5, and 60 + 30 ppm. Plugs were placed into alternate cells of a 384-cell polyethylene tray in a completely randomized design.

Single-node, 3.8-cm (1.5-inch) cuttings of *H. helix* and single-node, 1.9-cm (0.75-inch) cuttings of *R. 'Red Cascade'* were prepared from greenhouse-grown stock plants. In Experiment 1, cuttings in Treatment 1 received no auxin treatment, while cuttings in Treatment 2 received a basal quick-dip for 1 sec to a depth of 0.65 cm (0.25 inches) in 1000 ppm K-IBA + 500 ppm K-NAA. Cuttings were inserted to a depth of 0.65 cm (0.25 inches) into plugs that had been soaked in water. In Experiment 2, cuttings in Treatment 1 received no auxin treatment, while cuttings in Treatments 2 and 3 received a basal quick-dip for 1 sec in 1000 ppm K-IBA or 1000 ppm K-IBA + 500 ppm K-NAA, respectively. Cuttings were inserted to a depth of 0.65 cm (0.25-inches) into plugs that had been soaked in water. In both experiments, cuttings in the remaining treatments were inserted to the same depth into plugs that had been soaked in auxin solutions. Cuttings of the two species were inserted in separate plug trays with 15 replicates of each species per treatment and placed under a greenhouse mist system providing overhead mist for 6 sec every 16 min during daylight hours for a rooting period of 30 days in both experiments. All percentages were evaluated with logistic regression and compared with the basal

quick-dip treatment using single degree-of-freedom orthogonal contrasts. Mean number of roots, total root length, and shoot length (initial shoot growth during the rooting period) were evaluated with regression analysis and compared with the basal quick-dip treatments using Dunnett's Test.

RESULTS AND DISCUSSION

Experiment 1. Cuttings of *H. helix* rooted at 100% with the basal quick-dip treatment and in plugs treated with no auxin, 50 ppm K-IBA + 25 ppm K-NAA, and 100 ppm K-IBA + 50 ppm K-NAA (Table 1). All other treatments produced progressively lower rooting percentages with increasing auxin concentration, with no rooting occurring at 1000 ppm K-IBA + 500 ppm K-NAA or higher. At auxin concentrations of 250 ppm K-IBA + 125 ppm K-NAA and higher, some of the unrooted cuttings exhibited dead stem tissue within the auxin-treated plugs. At auxin concentrations of 750 ppm K-IBA + 375 ppm K-NAA and higher, there was a linear increase in the percent of cuttings with totally dead stem tissue. Short aerial roots formed from the stem tissue above the plug in most treatments with auxin-treated substrate. No cuttings produced callus growth only. When rooting occurred in auxin-treated plugs, number of roots on rooted cuttings was similar to cuttings that received the basal quick-dip, while total root length was lower. Shoot growth on rooted cuttings in untreated plugs was similar to cuttings receiving the basal quick-dip, while little or no shoot growth occurred on rooted cuttings in any auxin-treated substrate, indicating that shoot inhibition was occurring in response to auxin levels used for the substrate treatments.

Cuttings of *R. 'Red Cascade'* in substrate treated with 250 ppm K-IBA + 125 ppm K-NAA or less rooted similarly to cuttings receiving the basal quick-dip (100%); rooting percentage exhibited a linear decrease with increasing auxin concentration, with no cuttings producing roots at 1250 ppm K-IBA + 625 ppm K-NAA or higher (Table 1). Response in partial and total tissue death with increasing auxin concentration was generally similar to *Hedera*. Formation of aerial roots occurred in some cases, but to a lesser extent than with *Hedera*. Some of the unrooted rose cuttings produced callus; this was especially notable in plugs treated with 500 ppm K-IBA + 250 ppm K-NAA. Number of roots on cuttings in plugs treated with 50 ppm K-IBA + 25 ppm K-NAA and 100 ppm K-IBA + 50 ppm K-NAA was higher than for cuttings treated with the basal quick-dip, and similar with most other treatments in which rooting occurred. Total root length was less in auxin-treated plugs than with basally dipped cuttings. Limited or no shoot growth occurred on rooted cuttings in any auxin-treated substrate. Shoot inhibition associated with elevated levels of auxin has previously been noted (DeVries and Dubois, 1988) and tends to occur due to auxin-stimulated ethylene synthesis (Sun and Bassuk, 1993).

Overall results generally showed that, with increasing auxin concentration in the plugs, treatments first provided a root-promoting response of the lower stem tissue of the cuttings and suppression of shoot development, then a phytotoxic response of the lower stem tissue and some root-promoting response of the upper stem tissue, and then a phytotoxic response by all stem tissue. The auxin NAA has previously been shown to be phytotoxic when applied at elevated rates to *Oxalis* plants as a foliar spray (Holt and Chism, 1988). Results suggested that cuttings inserted in plugs treated with a low concentration of auxin (below 100 ppm) could potentially provide results similar to cuttings receiving a conventional basal quick-dip.

Table 1. Response of *Hedera helix* and *Rosa* 'Red Cascade' cuttings to K-IBA + K-NAA applied via a treated plug or a basal quick-dip (Experiment 1).

K-IBA + K-NAA Plug treatment (ppm)	Cuttings rooted in substrate (%)	Cuttings partially dead (%) ^z	Cuttings totally dead (%) ^y	Cuttings with aerial roots (%) ^x	Cuttings with callus (%) ^w	Roots (no.) ^v	Total root length (mm) ^y	Shoot length (mm) ^y
<i>Hedera helix</i>								
0 + 0	100	0	0	0	0	9.7	29	25
50 + 25	100	0	0	60 ‡	0	11.8	24 *	6 *
100 + 50	100	0	0	93 ‡	0	11.6	20 *	0 *
250 + 125	60 * ^u	40 ††	0	93 ‡	0	5.2	14 *	0 *
500 + 250	20 *	80 ‡	0	100 ‡	0	7.3	14 *	0 *
750 + 375	7 *	60 ‡	33 ‡	67 ‡	0	3.0	15 *	0 *
1000 + 500	0 *	53 ‡	47 ‡	40 ‡	0	-	-	-
1250 + 625	0 *	40 ‡	60 ‡	27 ‡	0	-	-	-
1500 + 750	0 *	20 ‡	80 ‡	7	0	-	-	-
1750 + 875	0 *	20 ‡	80 ‡	13	0	-	-	-
2000 + 1000	0 *	7	93 ‡	0	0	-	-	-
Response ^s	L***Q*	L*Q***	L***	Q***	NS	NS	L***Q*	Q*
Basal quick-dip ^r	100	0	0	0	0	9.0	31	29
<i>Rosa</i> 'Red Cascade'								
0 + 0	100	0	0	0	0	5.1	34	14
50 + 25	100	0	0	0	0	10.8 ‡	27 *	6 *
100 + 50	100	0	0	0	0	9.0 ‡	22 *	2 *
250 + 125	93	7	0	0	0	6.5	15 *	0 *
500 + 250	67 *	20 ‡	13	0	20 ‡	2.8 *	9 *	0 *

750 + 375	33 *	40 ‡	27 ‡	7	13	5.2	10 *	0 *
1000 + 500	20 *	53 ‡	27 ‡	20 ‡	7	3.7	9 *	0 *
1250 + 625	0 *	20 ‡	80 ‡	7	7	-	-	-
1500 + 750	0 *	33 ‡	67 ‡	7	7	-	-	-
1750 + 875	0 *	0	100 ‡	0	0	-	-	-
2000 + 1000	0 *	20 ‡	80 ‡	7	0	-	-	-
Response	L***	Q***	L***	NS	NS	L***	L***Q***	L*Q*
Basal quick-dip	100	0	0	0	0	5.8	3.8	18

^zCuttings with dead (brown) tissue within the substrate and live (green) tissue above the substrate. (No unrooted cuttings of *Hedera helix* showed live tissue within the substrate. A single cutting of *Rosa 'Red Cascade'* in the 250 ppm K-IBA + 125 ppm K-NAA treatment showed living tissue within the substrate, and is included in this column.)

^yCuttings with dead (brown) tissue both within and above the substrate.

^xCuttings with small aerial roots (up to ~5 mm) formed on the portion of the stem above the substrate.

^wUnrooted cuttings with callus formation on the stem just above the surface of the substrate.

^vLeast squares means calculated using rooted cuttings only.

^uPercentages and means followed by * within a species and column are significantly less than that of the basal quick-dip treatment according to single degree-of-freedom orthogonal contrasts for percentages and Dunnett's Test for all other measures ($\alpha=0.05$).

^tPercentages and means followed by † within a species and column are significantly greater than that of the basal quick-dip treatment according to single degree-of-freedom orthogonal contrasts for percentages and Dunnett's Test for all other measures ($\alpha=0.05$).

^sNon-significant (NS), linear (L), or quadratic (Q) regression response at $P = 0.05$ (*), 0.01 (**), or 0.001 (***) for treatments other than the basal quick-dip.

^rCuttings received a basal quick-dip for 1 sec in 1000 ppm K-IBA + 500 ppm K-NAA and were inserted in untreated plugs.

Table 2. Rooting and initial shoot growth response of *Hedera helix* and *Rosa* 'Red Cascade' cuttings to K-IBA or K-IBA + K-NAA applied via a treated plug or a basal quick-dip (Experiment 2).

Auxin treatment (ppm)	Rooting (%)	Roots (no.) ^z	Total root length (mm) ^z	Shoot length (mm) ^z
<i>Hedera helix</i>				
Untreated	100	10.5	260	9.3
15 K-IBA treated plug	100	11.5	236	8.7
30 K-IBA treated plug	100	11.9	262	7.1
45 K-IBA treated plug	100	13.4 † ^y	301 †	8.5
60 K-IBA treated plug	100	14.0 †	314 †	8.3
75 K-IBA treated plug	100	13.4 †	260	6.3
Response	NS	L***	L*	NS
1000 K-IBA basal quick dip	100	9.9	227	10.0
15 K-IBA + 7.5 K-NAA treated plug	100	11.6	261	6.3* ^w
30 K-IBA + 15 K-NAA treated plug	93.3	9.4	183	4.6*
45 K-IBA + 22.5 K-NAA treated plug	100	9.6	195	1.6*
60 K-IBA + 30 K-NAA treated plug	100	11.7	210	0.7*
Response	NS	NS	NS	L***
1000 K-IBA + 500 K-NAA basal quick dip	100	9.7	246	11.1
<i>Rosa</i> 'Red Cascade'				
Untreated	100	4.6	174	4.7
15 K-IBA treated plug	100	5.3	202	4.8
30 K-IBA treated plug	86.7	6.6	204	4.0
45 K-IBA treated plug	93.3	6.6	173	3.8

60 K-IBA treated plug	100				86 *	2.3 *
75 K-IBA treated plug	60.0 *				33 *	1.9 *
Response NS	L**Q***			L***Q***	L***	
1000 K-IBA basal quick dip	100			5.5	209	4.1
15 K-IBA + 7.5 K-NAA treated plug	100			4.7	155	3.6
30 K-IBA + 15 K-NAA treated plug	93.3			3.4	72 *	2.8
45 K-IBA + 22.5 K-NAA treated plug	80.0 *			2.8 *	59 *	2.8
60 K-IBA + 30 K-NAA treated plug	66.7 *			2.5 *	52 *	2.3
Response	L***			L***	L***	L***
1000 K-IBA + 500 K-NAA basal quick dip	100			4.9	182	3.4

^zLeast squares means calculated using rooted cuttings only.

^yMeans followed by ‡ within a species, column, and auxin formulation are significantly greater than the mean for the corresponding basal quick-dip treatment according to Dunnett's Test ($\alpha=0.05$).

^xNonsignificant (NS), linear (L), or quadratic (Q) regression response by species and auxin formulation at $P = 0.05$ (*), 0.01 (**), or 0.001 (***)). Untreated cuttings were included in each regression analysis.

^wPercentages and means followed by * within a species, column, and auxin formulation are significantly less than the percentage or mean for the corresponding basal quick-dip treatment according to single degree-of-freedom orthogonal contrasts for rooting percentage and Dunnett's Test for all other measures ($\alpha=0.05$).

Experiment 2. Cuttings of *H. helix* rooted at or near 100% in all treatments (Table 2). Cuttings in plugs treated with K-IBA alone exhibited a linear increase in number of roots with increasing auxin concentration. Cuttings in plugs treated with 45, 60, and 75 ppm K-IBA produced more roots than cuttings receiving a basal quick-dip in 1000 ppm K-IBA. Total root length also increased with increasing K-IBA concentration up to 60 ppm, and was greater with cuttings in plugs treated with 45 and 60 ppm K-IBA compared to the basal quick-dip. Shoot length on cuttings in plugs treated with K-IBA alone was similar to cuttings receiving the basal quick-dip. Number of roots and total root length on cuttings in plugs treated with K-IBA + K-NAA were similar to those in the basal quick-dip treatment. Shoot length on cuttings in plugs treated with K-IBA + K-NAA was lower compared to the basal quick-dip, and decreased linearly with increasing auxin concentration.

Cuttings of *R. 'Red Cascade'* in plugs treated with 0 to 60 ppm K-IBA produced rooting percentages similar to cuttings in the K-IBA basal quick-dip treatment; the highest concentration (75 ppm K-IBA) produced a lower rooting percentage than the basal quick-dip (Table 2). Cuttings in plugs treated with K-IBA exhibited a linearly decreasing and quadratic response in number of roots and total root length with increasing auxin concentration, while shoot length exhibited a linear decrease. Number of roots, total root length, and shoot length on cuttings in plugs treated with up to 45 ppm K-IBA were similar to the K-IBA basal quick-dip treatment, and lower on cuttings in plugs treated with 60 and 75 ppm K-IBA. All root and shoot development measurements on cuttings in plugs treated with K-IBA + K-NAA decreased linearly with increasing auxin concentration. Rooting percentage and number of roots were lower on cuttings in plugs treated with the two highest concentrations of K-IBA + K-NAA, while total root length was lower with the three highest concentrations, compared with the basal quick-dip in 1000 ppm K-IBA + 500 ppm K-NAA. Shoot length on cuttings in plugs treated with K-IBA + K-NAA was similar to cuttings receiving the basal quick-dip.

In general, K-IBA alone was preferable to K-IBA + K-NAA for rooting cuttings of these two species. In several cases, equal or greater root and shoot development was obtained on cuttings in auxin-treated plugs compared to the basal quick-dip treatments, and occurred using auxin concentrations substantially below those employed with the basal quick-dip treatments. Overall root development on cuttings of *H. helix* was best in plugs treated with 45 ppm or 60 ppm K-IBA. Auxin was not essential for rooting cuttings of *R. 'Red Cascade'*; however, response trends indicate that a low level of auxin (30 ppm or 45 ppm K-IBA) in the plugs could be helpful.

In summary, results indicate that cuttings of *H. helix* and *R. 'Red Cascade'* can be rooted successfully in a stabilized organic substrate that was pretreated with auxin. With the selection of an optimal auxin formulation and concentration, root development can equal, and sometimes exceed, that of cuttings receiving a conventional basal quick-dip, without an adverse effect on subsequent shoot development. Although auxin was not essential for rooting cuttings of either of the two species used in this study, the technique of applying auxin via the substrate could hold potential for harder-to-root species. This technique could also help to enhance employee safety and nursery productivity and is compatible with mechanized production systems.

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