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Effects of Phenolic Compounds and Indoleacetic Acid on Adventitious Root Initiation in Cuttings of *Phaseolus aureus*, *Acer saccharinum*, and *Acer griseum*

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Abstract. **Twenty-one phenolic compounds in combination with indoleacetic acid (IAA) were applied to mung bean (*Phaseolus aureus* Roxb.) cuttings. Catechol, pyrogallol, salicylic acid, and tannic acid stimulated adventitious root initiation in mung bean and were further tested on *Acer saccharinum* L. Catechol stimulated root initiation in softwood cuttings of *Acer saccharinum*. Softwood cuttings of *Acer griseum* Pax. rooted 100% when treated with a combination of catechol (4.5×10^{-3} M) and IAA (1.1×10^{-3} M) for 24 hours and several combinations of catechol and IAA also stimulated the number of roots per cutting of this species.**

The stimulation of adventitious roots by phenolic compounds has been reported for a number of plant species. Catechol, *p*-hydroxybenzoic acid, pyrogallol, and salicylic acid stimulated root initiation in mung bean cuttings (9, 12, 13). Other phenolic compounds, anthranilic, caffeic, chlorogenic, coumaric, gentisic, quinic, shikimic, and vanillic acids, phenol and phloroglucinol, had little effect on adventitious root initiation in mung bean cuttings (9, 13). Rutin and tannic acid were reported to inhibit root initiation in mung bean cuttings (13).

Hess (9) found that catechol acted synergistically with IAA to stimulate rooting of mung bean cuttings. He attributed the activity of catechol in this combination to adjacent hydroxyl groups and a free *para* position on the aromatic ring. Phenolic compounds without adjacent hydroxyl groups and a free *para* position would not stimulate rooting in combination with IAA.

Gorter (7) found that 1-naphthol, 2-naphthol, and phenol interacted with IAA to stimulate root initiation in cuttings of *Phaseolus vulgaris*. Bose et al. (2) found that *p*-coumaric, *p*-hydroxybenzoic, salicylic, and tannic acids acted synergistically with IAA to stimulate rooting in cuttings of 10 subtropical shrubs and vines.

The following experiments tested the effects of 21 phenolic compounds on root initiation in mung bean cuttings. Compounds which stimulated root initiation in mung been cuttings were tested on cuttings of *Acer saccharinum*, an easy-to-root woody species (5). A phenolic compound which stimulated rooting in *A. saccharinum* was further tested on cuttings of *A. griseum*, a difficult-to-root species (3, 4, 6).

Phaseolus aureus. Mung bean seeds were treated and germinated as described by Hess (8) and Mitchell and Livingston (11). Seedlings were grown in a growth room maintained at 25 ± 3°C at the plant canopy. Light was provided 16 hr a day by a 1000W metal halide (Sylvania Metal Arc) lamp. Light intensity was reduced by Saran shade cloth to 65 to 70 μE $m^{-2}sec^{-1}$ (400–700 nm) or 25 W m^{-2} measured at the plant canopy by a Li-Cor Lambda meter with Li-Cor quantum and pyranometer sensors. Relative humidity was maintained at 50 to 60% as measured with an aspirated psychrometer.

Phenolic compounds tested for stimulation of adventitious rooting included: anthranilic, caffeic, chlorogenic, *p*-coumaric, ferulic, gentisic, p-hydroxybenzoic, D(-) quinic, salicylic, (-) shikimic, tannic and vanillic acids, catechol, hydroquinone, 1-naphthol, 2-naphthol, phenol, phloroglucinol, pyrogallol, resorcinol, and rutin. Factorial combinations of phenolic compounds (0, 10^{-6}, and 10^{-4} M) and IAA (0, 5×10^{-6}, and 5×10^{-5} M) were used as the test solutions. All solutions were made with distilled, deionized, autoclaved water (1) and contained 2 ml of 95% ethanol per liter. Cuttings were made from uniform 7- and 8-day-old mung bean seedlings (11) and placed in 17 × 60-mm shell vials containing 4 ml of the test solutions. The vials containing the cuttings were placed in the growth room and refilled to 4 ml as required. The roots visible on each cutting were counted after 6 days. Each experiment was a completely randomized design with 3 replicates of 3 samples.

Catechol, pyrogallol, salicylic acid, and tannic acid stimulated adventitious root initiation in mung bean cuttings (Table 1). Catechol also interacted (F test, 5% level) with IAA to stimulate rooting. Cuttings treated with catechol at 10^{-4} M in combination with 5×10^{-5} M IAA produced 39.8 more roots per cutting than the check (Table 2). Pyrogallol was only slightly stimulatory to rooting. The stimulation of root initiation by catechol and pyrogallol in these studies agrees with the findings of Hess (9). The slight stimulation of root initiation by salicylic acid in these experiments agrees with the findings of Still et al. (13). Tannic acid (10^{-4} M), which was slightly stimulatory to root initiation in these experiments, was reported to inhibit rooting at a concentration of 2×10^{-4} M (13). The complex structure (14) of tannic acid or differences in experimental conditions (1) may account for these differences. Blazich and Heuser (1) found that differences in rooting response in the mung bean bioassay could be attributed to light intensity, water quality, and age of seedlings.

Structural examination of these compounds reveals that catechol and pyrogallol have multiple hydroxyl groups, including 2 adjacent hydroxyl groups on the aromatic ring with a free *para* position. Salicylic acid is

Table 1. Effects of 21 phenolic compounds and their interactions with IAA on adventitious root initiation in mung bean cuttings.[z]

Phenolic compound	Effect on root initiation[y]	Interaction with IAA[y]
Anthranilic acid	0	0
Caffeic acid	0	0
Catechol	+	0
Chlorogenic acid	0	0
p-Coumaric acid	0	0
Ferulic acid	0	0
Gentisic acid	0	0
p-Hydroxybenzoic acid	0	–
Hydroquinone	0	0
1-Naphthol	0	0
2-Naphthol	0	0
Phenol	0	0
Phloroglucinol	0	0
Pyrogallol	+	0
Quinic acid	0	0
Resorcinol	0	0
Rutin	0	0
Salicylic acid	+	+
Shikimic acid	0	0
Tannic acid	+	0
Vanillic acid	0	0

[z]Phenolic compounds applied at 0, 10^{-6}, and 10^{-4} M; IAA applied at 0, 5×10^{-6}, and 5×10^{-5} M.
[y]Significant F test at 5% level; + = stimulatory, – = inhibitory and 0 = no effect.

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Table 2. Effects of 5 phenols at 3 concentrations in combination with 3 concentrations of IAA on adventitious root initiation in *Phaseolus aureus* cuttings.[z]

Phenol	Phenol concn (M)	No. roots initiated per cutting[y] IAA concn 0	5×10^{-6} M	5×10^{-5} M
Catechol	0	6.9 c[x]	7.1 c	12.9 c
	10^{-6}	7.1 c	8.1 c	13.0 c
	10^{-4}	19.7 bc	32.1 b	46.7 a
p-Hydroxybenzoic acid	0	6.2 bc	6.2 bc	11.2 a
	10^{-6}	6.8 b	7.7 b	7.2 b
	10^{-4}	7.0 b	4.6 c	8.0 b
Pyrogallol	0	5.8 b	7.0 ab	10.9 ab
	10^{-6}	6.1 b	6.2 b	7.0 ab
	10^{-4}	10.4 ab	10.4 ab	14.3 a
Salicylic acid	0	6.3 de	6.7 de	7.4 cd
	10^{-6}	6.8 b	7.7 b	7.2 b
	10^{-4}	5.0 e	6.6 de	10.1 ab
Tannic acid	0	5.0 c	5.4 c	14.1 ab
	10^{-6}	6.1 c	6.9 c	7.6 bc
	10^{-4}	5.4 c	9.1 bc	19.7 a

[z]Phenols which did not significantly affect rooting of mung bean cuttings (Table 1) were omitted from Table 2.
[y]A mean of 3 replicates of 3 samples each.
[x]Mean separation by Duncan's multiple range test, 5% level. Means are for comparison within each phenolic compound only.

not a dihydroxyphenol; however, it does have a hydroxyl group adjacent to a carboxyl group, which is also a weak acid. Since the *para* position on this molecule is free, salicylic acid could be functioning through the same mechanism.

Anthranilic, caffeic, chlorogenic, *p*-coumaric, ferulic, gentisic, quinic, shikimic and vanillic acids, and hydroquinone, 1-naphthol, 2-naphthol, phenol, phloroglucinol, resorcinol, and rutin did not stimulate adventitious root initiation in the mung bean bioassay (Table 1). These findings agree with the predictions made by Hess (9), since these compounds are not *o*-dihydroxyphenols with free *para* positions. Lee and Tukey (10) found that a combination of rutin and IBA stimulated more rooting of *Euonymous alatus* Sieb. 'Compactus' than IBA alone. These workers did not apply rutin without IBA.

The compound, *p*-hydroxybenzoic acid, did not inhibit or stimulate root initiation alone; however, it did interact (Table 1) with IAA in these experiments to inhibit root initiation (Table 2). Salicylic acid interacted with IAA to cause a slight increase in the number of roots which initiated (Table 2).

The phenolic compounds, p-coumaric acid, 1-naphthol, 2-naphthol, and phenol, did not interact (F test, 5% level) with IAA to stimulate root initiation in these experiments with mung bean. Interactions or synergism between IAA and these compounds have been reported to stimulate rooting in cuttings of *P. vulgaris* (7) and several woody plants (2).

Acer saccharinum. Phenolic compounds that increased rooting in mung bean cuttings were tested on cuttings of *A. saccharinum*. Stock plants of *A. saccharinum* were grown from seed in a greenhouse maintained at 25 ± 3°C. Natural daylength was extended to a 16-hr photoperiod with 100W incandescent lamps placed 40 cm above the plant canopy at 1-m intervals. Plants were fertilized biweekly with a solution of 200, 18, and 133 mg/liter of N, P, and K, respectively. Silver maple cuttings were made from 30- and 40-day-old shoots of seedling stock plants which had been cut back. Cuttings contained 2 nodes with leaves and 2 to 3 lower nodes with leaves removed. Cuttings were disinfected by soaking in 0.25% sodium hypochlorite for 10 minutes. Cuttings were rinsed with sterile water and inserted individually into vials of the test solutions.

Factorial combinations of phenolic compounds (0 and 10^{-4} M) and IAA (0 and 5×10^{-5} M) were used as the test solutions. The vials were supported by Styrofoam blocks inside of plastic storage boxes (28 × 41 × 17 cm) which maintained a high level of humidity around the cuttings. Boxes were sealed with a single layer of clear plastic film wrap. Cuttings were quite succulent at this stage and had to be sprayed twice daily for the first 3 days and once per day thereafter to maintain turgidity. The boxes containing the cuttings were placed on the floor of the growth room where the light intensity was 15 to 19 μE $m^{-2}sec^{-1}$. Temperatures inside the boxes were 1 to 2°C higher than the surrounding air of the growth room. The roots visible on each cutting were counted after 21 days. The experimental design was completely randomized with 5 replicates per treatment.

Catechol was the only phenolic compound which stimulated adventitious root initiation in *A. saccharinum* (Table 3). Since catechol was the most stimulatory phenolic compound in *P. aureus* and *A. saccharinum*, the mechanism of action could be the same. Pyrogallol and salicylic acid, which were mildly stimulatory to root initiation in mung bean cuttings (Table 2), did not stimulate rooting in *A. saccharinum* (Table 3). Tannic acid, which stimulated root initiation in mung bean cuttings (Table 2); inhibited root initiation in cuttings of *A. saccharinum* (Table 3). None of the 4 phenolic compounds used in the maple bioassay interacted with IAA. The apparent interaction between catechol and IAA (Table 3) was not significant at the 5% (F test) level.

Acer griseum. Catechol was tested on softwood stem cuttings of *A. griseum*. Tip cuttings were taken from 2-year-old seedling stock plants produced under the cultural conditions described for *A. saccharinum*. The cuttings consisted of 4 nodes with 1 pair of mature leaves and a 2nd immature pair which had reached the stage of development in which they had just lost their reddish color. Leaves on the lower nodes were removed. Cuttings were wounded on one side by slicing off a thin layer of bark at the basal 1 cm of the stem.

Treatments consisted of factorial combinations of IAA (2.9×10^{-4}, 5.7×10^{-4} and 1.1×10^{-3} M) and cathechol (1.8×10^{-4}, 9.1×10^{-4}, and 4.5×10^{-3} M). All

Table 3. Effects of 4 phenolic compounds applied in combination with IAA on adventitious root initiation in softwood tip cuttings of *Acer saccharinum*.

Compound	Concn (M)	No. roots initiated per cutting[z] IAA concn 0	5×10^{-5} M
Catechol	0	8.4 b[y]	19.0 b
	10^{-4}	14.0 b	37.0 a
Pyrogallol	0	17.1 a	26.6 a
	10^{-4}	27.0 a	27.2 a
Salicylic acid	0	16.2 a	27.3 a
	10^{-4}	17.6 a	24.4 a
Tannic acid	0	16.8 ab	27.6 a
	10^{-4}	9.4 b	15.4 ab

[z]A mean of 5 cuttings.
[y]Mean separation by Duncan's multiple range test, 5% level. Means are for comparison within each phenolic compound only.

Table 4. Effects of combinations of catechol and IAA on rooting percentages and number of adventitious roots in *Acer griseum* softwood tip cuttings.

Catechol concn (M)	Rooting percentage (no. roots per cutting)[z] IAA concn 2.9×10^{-4} M	5.7×10^{-4} M	1.1×10^{-3} M
1.8×10^{-4}	40 (1.2 b[y])	40 (5.6 ab)	40 (2.8 b)
9.1×10^{-4}	60 (8.2 ab)	60 (2.0 b)	80 (13.0 a)
4.5×10^{-3}	80 (3.3 b)	80 (5.0 ab)	100 (5.8 ab)

[z]A mean of 5 cuttings per treatment.
[y]Mean separation by Duncan's multiple range test, 5% level.

solutions contained 20 ml of 95% ethanol per liter. The *A. griseum* cuttings were soaked in a 3-cm depth of the treatment solutions in beakers on a mist bench for 24 hr. Cuttings were then inserted into wooden flats containing a steam-pasteurized medium of peat moss and perlite (1:1, v:v). The daylength was extended to 16 hr by 100W incandescent light bulbs placed 60 cm above the plant canopy at 50-cm intervals from 6 to 11 pm. The experiment was a completely randomized design with 5 replicates per treatment. The number of roots and root quality were determined after 63 days.

A 24-hr treatment of stem tip cuttings of *A. griseum* with 1.1×10^{-3} M IAA in combination with 4.5×10^{-3} M catechol resulted in 100% rooting (Table 4). All other treatments gave lower rooting percentages. Treatment of *A. griseum* cuttings with a combination of 1.1×10^{-3} M IAA and 9.1×10^{-4} M catechol resulted in production of a mean of 13 roots per cutting with 80% rooting. Treatment with all other combinations of IAA and catechol resulted in the formation of fewer roots per cutting. Roots which did form were brittle and difficult to transplant.

The 100% rooting of *A. griseum* cuttings treated with a combination of catechol and IAA represents an improvement over rooting percentages of 30% (3) and less than 1% (4) reported by other workers. Fordham (6) reported 100% rooting of *A. griseum* cuttings taken from a 6-year-old-plant; however, cuttings taken from younger plants the same day showed only 46% rooting. Fordham offered no explanation for these differences.

These experiments demonstrate that treatment of cuttings with catechol in combination with IAA can be of value in the propagation of woody plants. Additional experiments should be carried out to assess the use of catechol and auxins to stimulate root initiation in cuttings of other difficult-to-root species.

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Distance Measuring and Signaling Device for a Mechanical Tree Planter

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Abstract. **Depending on soil-surface conditions, an electronic pick-up and counting device to signal within-row tree distances using a mechanical tree planter produced planting distances as accurate or more accurate than those obtained with traditional planting techniques.**

Traditionally, orchards have been laid out by measuring and staking 2 outside rows and 2 or more rows perpendicular to the outside rows. The remainder of the planting sites were established by "sighting-in" on the previously staked rows. A 2nd method commonly used was to measure and stake the outside rows of the orchard and then, by sighting on these outside stakes and with the aid of a sub-soiler, from a grid pattern to locate tree position. Trees were then planted in a hole dug by a tractor-mounted auger at the intersection of the layout lines. Both methods (2, 3) of staking out and planting an orchard required considerable time and labor. To reduce planting costs and to increase planting efficiency in high-density orchards, a continuous mechanical tree planter was developed by the U.S. Department of Agriculture (4). Recent studies have shown that shoot growth and anchorage of mechanically planted trees are superior to trees planted with a conventional auger (1).

In addition to traditional orchard layout techniques described, other options are available for use with the mechanical planter. All methods required staking the ends of the row to establish the spacing between the rows. To establish the spacing within the row, the first row was usually staked using a measuring tape to mark the planting distance between individual trees. Succeeding within-row spacing was determined by a person walking beside the planter and sighting-in adjacent rows and signaling the person planting trees when to plant. The person on the planter also could sight-in the planting location. Staking every 3rd or 4th row often increased accuracy. Another way to establish within-row distance was to have a cable trailing the planter that was the distance of the spacing required; a tree was planted when the tip of the cable passed the previously planted tree. Both methods often resulted in less-than-desired accuracy for within-row spacing. At the 1981 West Virginia State Horticultural Convention, held in Martinsburg in January, K.C. Elliott et al. described a proposed ground-driven measuring wheel attached to a tree planter and an electronic counting device for determining within-row planting distance. Field testing and results were not reported.

The objective of the present study was to develop tractor- and planter-mounted equipment which would determine within-row tree spacing accurately and provide a signal as to when to plant. Flexibility to allow rapid adjustment for different tree-spacing distances was considered an essential design feature.

An inductive proximity switch sensing on a 50-tooth, 10-pitch gear mounted to a hub on one of the front tires of the tractor (Fig. 1) was used to gauge distance traveled. Each passing of a spur-gear tooth by the proximity switch corresponded to 1/50 of the rolling

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