Effect of pH and 1*H*-indole-3-butyric acid (IBA) on Rooting of Apple Microcuttings

James F. Harbage[1] and Dennis P. Stimart[2]
Department of Horticulture, University of Wisconsin, Madison, WI 53706

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Abstract. Involvement of pH and IBA on adventitious root initiation was investigated with *Malus domestica* Borkh. microcuttings. The pH of unbuffered root initiation medium (RIM) increased from 5.6 to 7 within 2 days. Buffering with 2[*N*-morpholino] ethanesulfonic acid (MES) adjusted to specific pHs with potassium hydroxide prevented pH changes and resulted in a 2-fold higher root count at pH 5.5 compared to pH 7 or unbuffered medium. As pH decreased, lower concentrations of IBA were required to increase root counts. Colorimetric measurement of IBA in buffered RIM showed greater IBA loss and higher root count were associated with lower pH levels in all cultivars. This suggests that IBA loss from RIM depends on medium pH, which affects root count. Root count differences between easy-to-root through difficult-to-root cultivars were not consistent with amount of IBA loss from RIM. Cultivar differences in root count could not be explained solely by IBA loss from RIM.

Adventitious root formation on microcuttings of *Malus pumila* Mill. involves an auxin-sensitive phase during root initiation followed by an auxin-insensitive phase during root development (James, 1983). Similarly, anatomical work with *Malus domestica* microcuttings corroborates identification of the two-phase rooting protocol of root initiation and root development (Harbage et al., 1993). Four days after transfer to root initiation medium containing 1.5 µM IBA, microcuttings of *M. domestica* 'Gala' initiated synchronous root primordia at 10 to 12 sites. By 14 d, roots emerged from these primordia with no further emergence after an additional 7 d.

The role of pH on adventitious root formation is not well understood. Improved adventitious root formation has been associated with acid pH (Lee et al., 1976; Stone, 1963: Williams et al., 1985), alkaline pH (Lee et al., 1976), and near-neutral pH (Mellor and Stace-Smith, 1969). The inconsistencies may exist from failure to distinguish between the phases of root initiation and root development (Lovel and White, 1986). Since movement of auxin into isolated cells and tissues depends on pH (Rubery and Sheldrake, 1973), it would be during root initiation that pH might be involved in auxin-dependent adventitious rooting.

Microculture and microcuttings present a unique opportunity to investigate adventitious rooting. The purpose of this study was to investigate pH buffering and IBA on adventitious root initiation of microcuttings in vitro of several apple genotypes.

Materials and Methods

Microcutting production. Shoot cultures of *Malus domestica* 'Gala', 'Golden Delicious', 'Jonathan', 'McIntosh', 'Vermont Spur Delicious', and 'Triple Red Delicious' were obtained from Richard Zimmerman, U.S. Dept. of Agriculture, Beltsville, Md. These cultivars represent a broad range of rooting in response to methods developed by Zimmerman and Fordham (1985). Shoots were subcultured every 28 d onto 100 mL of Murashige-Skoog (MS) medium (Murashige-Skoog, 1962) modified to contain 0.56

mM myo-inositol, 1.2 µM thiamine hydrochloride, 1.3 µM gibberellic acid (GA$_3$), 0.49 µM IBA, 4.44 µM benzyladenine, 88 mM sucrose, and 7 g·L^{-1} Difco Bacto-agar. pH was adjusted to 5.2 before addition of agar. All ingredients were added before autoclaving at 1.3 kg·cm^{-2} at 121 °C for 15 min. Culture vessels were 475-mL (9.0 cm diameter × 9.5 cm height) clear glass jars covered with glass petri plate bottoms (10 cm in diameter) and a layer of plastic Saran film over the entire jar. Ten to twelve shoots were placed in each jar. Cultures were maintained under continuous cool-white fluorescent lamps (40 µmol·m^{-2}·s^{-1}) at 21 °C unless specified. Shoots were subcultured every 28 d by basally detaching shoots with scissors, excising shoot tips, and lightly pressing each shoot tip horizontally into fresh culture medium.

Shoots were harvested 21 d after subculturing for rooting. Shoot clusters were covered with sterile water in culture jars to prevent drying. Shoots were detached from the original explant at their bases with scissors and cut to shoot lengths from the apex down, having five nodes for 'Gala', 'Golden Delicious', and 'Jonathan', which had uniform internode lengths, and 2 to 3 cm for 'McIntosh', 'Triple Red Delicious', and 'Vermont Spur Delicious', which had variable internode lengths. Cuts were made just above a node. The resulting cuttings, hereafter, are referred to as microcuttings or by cultivar names. Shoots were kept in sterile water for up to 5 min until placed on treatment medium.

Microcutting rooting

Root initiation. Root initiation medium (RIM) contained 43.8 mM sucrose and 1.5 µM IBA (Zimmerman and Fordham, 1985) unless otherwise specified. The pH of unbuffered RIM was adjusted initially to 5.5 to 5.6 before autoclaving. This pH was verified after autoclaving and found to be unchanged (Harbage, 1991). Treatment was in darkness at 30 °C for 4 d (Zimmerman and Fordham, 1985). Histological observations showed initiation of adventitious root primordia occurred during this period (Harbage et al., 1993).

Root development. Microcuttings were transferred from RIM to a root development medium (RDM) consisting of half-strength MS salts without Fe-EDTA and 21.9 mM sucrose and maintained at 23 °C under a 16-h photoperiod provided by cool-white fluorescent lamps (30 µmol·m^{-2}·s^{-1}). This protocol was shown to facilitate uniform development of adventitious root primordia initiated during root initiation treatment (Harbage, 1991).

Root initiation and root development occurred in 25-mm-diameter × 90-mm-tall glass shell vials covered with plastic caps. One microcutting was placed in a plastic support in each shell vial with the basal 4 mm of the microcutting submerged in 3 mL of liquid RIM or RDM.

Root initiation experiments on microcuttings

pH change and buffering. A first study was undertaken to determine change in pH of unbuffered RIM. The pH of RIM was set initially at pH 5.6 and measured 0, 1, 2, 3, and 4 d after transfer of microcuttings of 'Gala' from RIM and 4 d after transfer of 'Golden Delicious', 'Jonathan', and 'McIntosh' from RIM. Controls without microcuttings were run for 4 d to check on pH change of medium. Completely random designs were used with 10 replications/d for 'Gala' and four replications resulting from pooling 10 samples for each of 'Golden Delicious', 'Jonathan', and 'McIntosh'. A Beckman ϕ 40 pH meter and calomel semimicro combination electrode (Beckman Instruments, Fullerton, Calif.) were used for all pH measurements after two-point calibration with pH 4.0 and 7.0 calibration buffers.

A second study was undertaken to determine the effect of pH on rooting in buffered RIM. 2[*N*-morpholino] ethanesulfonic acid (MES) at 0.1, 1.0, and 10.0 mM was added to RIM and pH was adjusted to 5.5 with KOH, NaOH, or Ca(OH)$_2$. An unbuffered control at pH 5.5 was included for comparison. A randomized complete block design was used with 13 blocks and one sample per block using 'Gala'. The pH of RIM was measured 4 d after transfer of microcuttings to RIM and root counts were taken 14 d after transfer to RDM. Medium pH in subsequent experiments was maintained with 10 mM MES plus KOH unless otherwise specified.

A third study was undertaken to determine the effect of pH level on rooting. Unbuffered and buffered RIM at pH 5.5, 6.0, 6.5, and 7.0 were evaluated with 'Gala'. A completely random design was used with 10 replications per treatment. Root counts were determined after 7 and 14 d on RDM.

pH and IBA concentration. This study was undertaken to determine the effect of IBA concentration and pH on rooting.
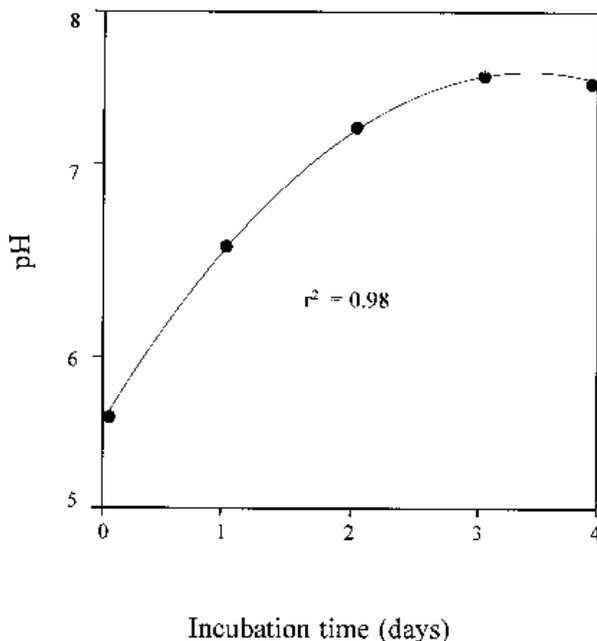
Microcuttings of 'Gala' were placed on RIM containing IBA at 0, 0.15, 1.5, 15.0, and 150.0 μM and adjusted to pHs of 5.5, 6.25, and 7. Twenty replications were used per treatment in a completely random design. Roots were counted 14 d after transfer to RDM.

IBA measurement. A colorimetric assay (Ehmann, 1977) was used to estimate loss (reduction) of IBA in RIM. After shoot removal, 1.5 mL of RIM was placed in a 13×100-mm test tube. One half ml of Van Urk reagent (4.0 g p-dimethylamino-benzaldehyde dissolved in 200 mL concentrated HCl then added to 200 mL 100% ethyl alcohol) and 0.5 mL Salkowski reagent (0.62 g anhydrous FeCl$_3$ dissolved in 250 mL H$_2$O followed by addition of 150 mL concentrated H$_2$SO$_4$, performed on ice) were added to the RIM sample. Absorbance was measured at 615 nm using a spectrophotometer (model 390; Sequoia-Turner Corporation, Mountainview, Calif.). Reduction of IBA was expressed as a percent difference between absorbance of RIM incubated with a microcutting and absorbance of RIM incubated without a microcutting. Preliminary experiments showed a linear relation between absorbance and IBA concentration (data not presented). Medium lacking IBA, incubated with microcuttings, had zero absorbance.

The relationship between cultivar and pH on IBA loss from RIM by microcuttings was evaluated with 'Gala', 'Jonathan',

Table 1. Changes in pH of unbuffered root initiation medium after 4 d incubation with *Malus domestica* microcuttings.

Cultivar	Initial pH[z]	Final pH	pH change
Golden Delicious	5.60	6.98 (0.04)[y]	1.38
Jonathan	5.60	6.98 (0.06)	1.38
McIntosh	5.60	7.10 (0.02)	1.50

[z]Initial pH adjusted with HCl. Controls without microcuttings remained at pH 5.60.
[y]Standard error of the mean.

Table 2. Final pH of root initiation medium and root count of *Malus domestica* 'Gala' microcuttings treated with MES and counterions during root initiation.

MES (mM)	Counterion	Final pH[z]	Root count[y]
0.1	KOH	6.38	10.3 a[x]
	NaOH	6.25	8.8 a
	Ca(OH)$_2$	6.62	10.2 a
1.0	KOH	5.74	10.7 a
	NaOH	5.78	10.1 a
	Ca(OH)$_2$	5.75	11.1 a
10.0	KOH	5.52	14.3 a
	NaOH	5.52	14.5 a
	Ca(OH)$_2$	5.50	6.3 b
Unbuffered[w]		6.97	7.8

ANOVA

Source of variation	df	Mean squares	
		Final pH	Root count
Block	12	0.065[NS]	35.855[NS]
MES	2	4.915[***]	38.179[NS]
Counterion	2	1.328[NS]	70.949[NS]
M × C	4	1.653[NS]	113.628[**]
Error	96	0.612	25.27

[z]Initial pH 5.5 and final pH 96 h later.
[y]Fourteen days after transfer to root development medium.
[x]Mean separation by Duncan's multiple range test (*P* = 0.05).
[w]Unbuffered control not included in analysis of variance.
[NS,**,***]Nonsignificant and significant at *P* = 0.01 and 0.001, respectively.

Fig. 1. Change in pH of unbuffered root initiation medium during incubation of *Malus domestica* 'Gala' microcuttings.

Table 3. Effect of pH of MES buffered root initiation medium on root count in *Malus domestica* 'Gala' microcuttings 7 and 14 d after transfer to root initiation medium.

		Root count[z]	
pH		7 d	14 d
5.5		11.9 a	14.2 a
6.0		7.7 b	10.9 ab
6.5		7.0 b	8.7 bc
7.0		5.2 b	7.1 c
Unbuffered		6.6 b	7.6 bc
ANOVA			
		Mean squares	
Source of variation	df	7 d	14 d
pH	4	63.970[**]	84.650[***]
Error	45	16.467	15.598

[z]Mean separation within columns by Duncan's multiple range test ($P = 0.05$).
[**],[***]Significant at $P = 0.01$ or 0.001, respectively.

Table 4. Analysis of variance for the influence of pH and IBA concentration during root initiation on root count of *Malus domestica* 'Gala' microcuttings.

Source of variation	df	Mean squares Root count[z]
pH	2	809.6[***]
IBA	4	1655.4[***]
pH × IBA	8	1552.9[***]
Error	255	47.4

[z]Fourteen days after transfer to root development medium.
[***]Significant at $P = 0.001$.

'Vermont Spur Delicious', and 'Triple Red Delicious'. pH was adjusted to 5.5 or 7. The RIM in this experiment contained 30 μM IBA and 43.8 mM sucrose. After 1 d incubation in RIM, microcuttings were transferred to RDM to prevent prolonged exposure to IBA. No changes to root initiation or root development environments were made. Treatments were arranged in a randomized complete block design with two blocks and five samples per block. IBA loss from RIM was determined 1 d after transfer of microcuttings and root counts were made 14 d after transfer of microcuttings to RDM.

The pH response curve for IBA loss from RIM by microcuttings was evaluated further using pH 5, 5.5, 6, 6.5, and 7 with 'Triple Red Delicious'. Microcuttings were incubated in RIM containing 30 μM IBA and 43.8 mM sucrose for 1 d and then transferred to RDM as in the previous experiment. A completely random design was used with 13 replications per treatment. Loss of IBA from RIM after 1 d and root count 14 d after transfer of microcuttings to RDM was measured.

The pH response curve for IBA reduction in RIM was expanded to include pH 3.1, 3.5, 4, 4.5, 5, 5.5, 6, and 7. 'Gala' microcuttings were incubated in RIM containing 30 μM IBA, 10 mM MES, H_3PO_4 to pH 3.1, and final pH adjustments by addition of KOH. Microcuttings were incubated in RIM for 11 h followed by transfer to RDM as with the two previous experiments. A completely random design was used with nine replications per treatment. The IBA loss from RIM and root count 14 d after transfer to RDM were determined as described previously.

Growth regulators and MES were from Sigma Chemical Co., St. Louis. Other chemicals were ACS reagent grade. Data for all experiments were analyzed using the General Linear Models Procedure (SAS Institute, Cary, N.C.).

Results

pH change and buffering

First study. The pH of unbuffered RIM incubated with 'Gala' microcuttings rose from pH 5.6 to 7.5 over a 4-d period (Fig. 1) following a second-order regression ($r^2 = 0.98$). A major increase in pH occurred from 0 to 2 d with smaller subsequent increases. The pH rose from 5.6 to 7.0 for 'Golden Delicious' and 'Jonathan' and to pH 7.1 for 'McIntosh' after 4 d (Table 1). Controls without microcuttings remained at pH 5.6 after 4 d incubation.

Second study. Final pH was affected significantly by MES concentration (Table 2). The difference between the initial pH (5.5) and the final pH decreased as MES concentration increased from 0.1 to 10 mM. Unbuffered medium (pH 5.5) changed most to a final pH of 7.0. The final pH was not affected significantly by counterion.

Root count was affected significantly by an MES × counterion interaction (Table 2). The interaction resulted from reduced root count when $Ca(OH)_2$ was combined with 10 mM MES. Medium with 10 mM MES and KOH or NaOH remained at pH 5.5 with root counts 1.8-fold greater than unbuffered medium.

Third study. Root count of 'Gala' was influenced significantly by pH ($P = 0.001$, Table 3). At 7 and 14 d, root count at pH 5.5 was higher than root count at pH 6, 6.5, and 7. Root count 14 d after transfer of microcuttings to RDM decreased as pH increased in RIM. Microcuttings from unbuffered RIM had lowest root counts.

pH and IBA concentration

Root count of 'Gala' was affected significantly by a pH × IBA interaction (Table 4). In the presence of 0, 0.15, and 1.5 μM IBA, root count was highest at pH 5.5, intermediate at pH 6.25, and lowest at pH 7 (Fig. 2). In the presence of 15 μM IBA, root count was highest at pH 6.25, intermediate at pH 5.5 and lowest at pH 7. In the presence of 150 μM IBA, root counts were similar at all pHs. Root counts at pH 5.5 and 6.25 were related positively between 0 and 15 μM IBA, but at 150 μM IBA root counts decreased. Root count at pH 7 was related positively to IBA between 0 and 150 μM.
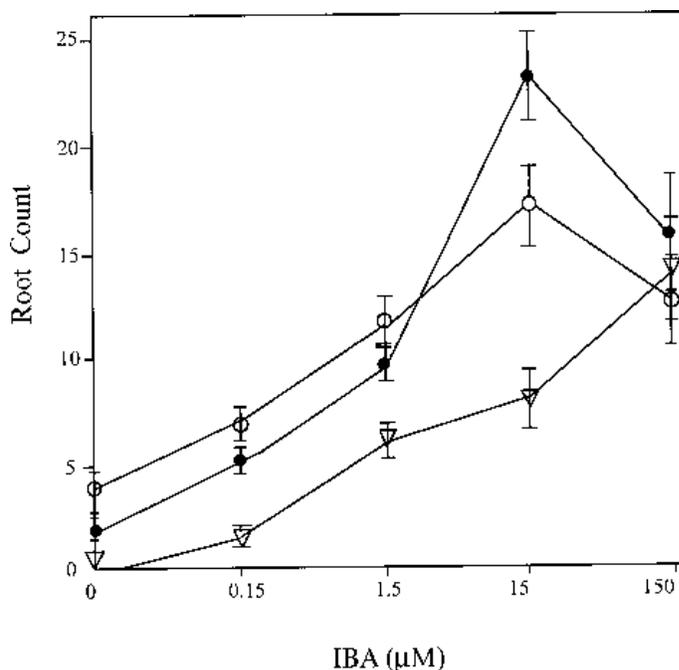

Fig. 2. Influence of IBA and pH at 5.5 (○), 6.25 (●), and 7.0 (▽) of root initiation medium on root count of *Malus domestica* 'Gala' microcuttings 14 d after transfer to root development medium. Vertical bars represent ±SE.

Table 5. Analysis of variance for the influence of pH and cultivar on IBA loss from root initiation medium during root initiation and root count on *Malus domestica* microcuttings.

Source of variation	df	Mean squares	
		IBA loss[z]	Root counts[y]
Block	1	16.04[NS]	64.71[NS]
pH	1	1078.14[***]	540.55[***]
Cultivar	3	51.51[***]	284.69[***]
pH × cultivar	3	12.02[NS]	46.19[NS]
Error	5	12.17	

[z]Loss of 1*H*-indole-3-butyric acid from root initiation medium measured colorimetrically.
[y]Fourteen days after transfer to root development medium.
[NS,***]Nonsignificant and significant at $P = 0.001$, respectively.

IBA measurement

The IBA loss from RIM was affected significantly ($P = 0.001$) by pH and cultivar (Table 5); therefore, main effect means are presented (Fig. 3). Greater IBA loss from RIM occurred at pH 5.5 than at pH 7 (Fig. 3A). IBA loss from RIM was least with 'Jonathan' microcuttings compared to 'Gala', 'Triple Red Delicious', or 'Vermont Spur Delicious' microcuttings, which had similar responses (Fig. 3B).

Root count was affected significantly by pH and cultivar (Table 5). Therefore, main effect means are presented (Fig. 3). Root count at pH 5.5 was higher than at pH 7 (Fig. 3C). Root count was highest on microcuttings of 'Gala', intermediate on 'Jonathan', and lowest on 'Triple Red Delicious' and 'Vermont Spur Delicious', which responded similarly (Fig. 3D).

IBA loss from RIM with 'Triple Red Delicious' was greatest at pH 5, decreased at pH 6.5 (Fig. 4A), and did not change further at pH 7. Root count decreased as pH increased.

In 'Gala', IBA loss was highest at pH 3.5, with loss decreasing between pH 3.5 and 7 (Fig. 4B). Root count was highest between pH 4 and 5 and decreased sharply at pH <4 and at pH >5. A brown discoloration was observed on the basal 1 to 2 mm of microcuttings at pH 3.1.
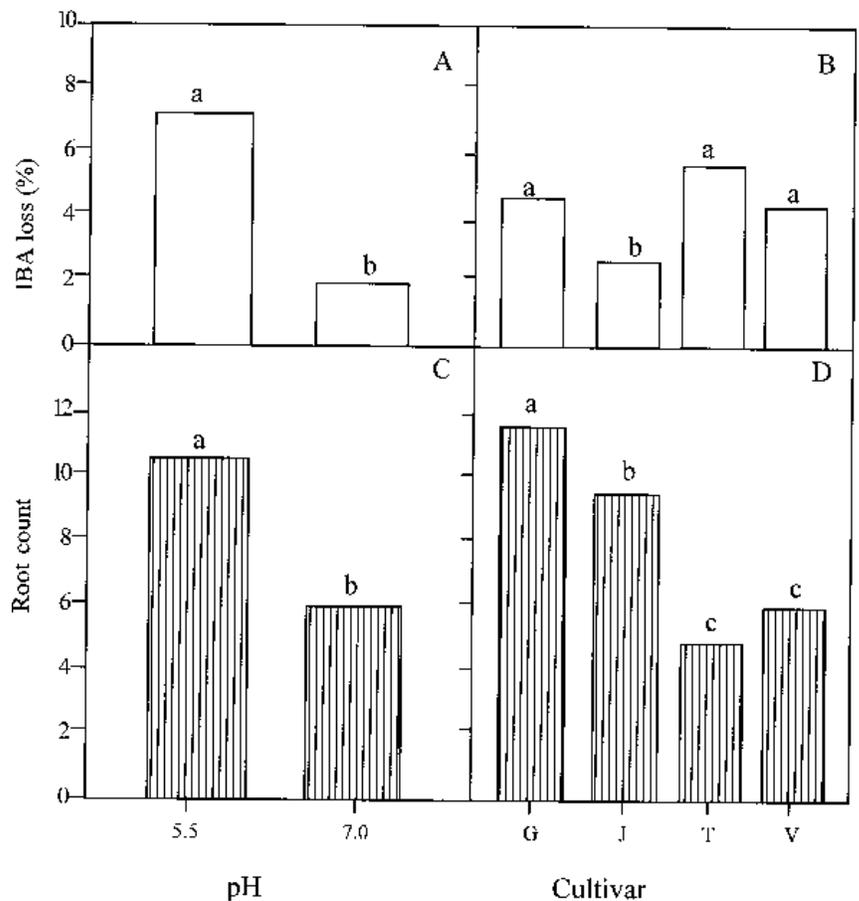
Discussion

This study found that adventitious root count on microcuttings of apple was affected by buffering RIM with MES, pH, IBA concentration, and genotype. The pH of unbuffered medium rose approximately two units within 4 d (Fig. 1) and was associated with nearly a 50% decrease in root count (Table 2) compared with medium buffered at pH 5.5. Medium buffered at acidic pHs remained at initial pHs during root initiation (Table 2). These treatments had increased root count when compared to neutral pH. The relationship of pH on adventitious root formation showed a trend toward increasing root count at pH 5.5 compared to pH 6.25 and 7.0 (Fig. 2). Colorimetric analysis of IBA loss from RIM confirmed greatest loss of IBA at lowest pHs (Figs. 3 and 4). Anatomical analysis of 'Gala' microcuttings 4 d after treatment on medium with 0 or 1.5 μM IBA revealed synchronous root primordia initiation at 0 to 2 and 10 to 12 initiation sites, respectively (Harbage et al., 1993).

Considering the association between presence of IBA and increased root primordia initiation and the results from the present study, the importance of a buffered medium, at the optimal pH and optimal IBA level clearly are important to maximize root count.

Previous reports differ on the influence of pH on adventitious root formation. Acid pretreatment of stem cuttings increased auxin-induced rooting in some species while alkaline treatment increased it in others (Lee et al., 1976). Rooting of in vitro cultured *Dianthus caryophyllus* L. shoot apices was greater at pH 5.5 (59%) than at pH 6.0 (4%) (Stone, 1963). Rooting of cultured *Solanum tuberosum* L. buds was highest at pH 5.7 compared to pH 4.8 or 6.2 (Mellor and Stace-Smith, 1969). Williams et al. (1985) found that microcuttings of *Prostanthera striatiflora* F. Muell. and *Correa decumbens* F. Muell. formed no roots at pH 5.5, but up to 95% rooted at pH 4.0. In the present study, buffered RIM was used across pH and IBA concentrations to increase root counts. The lack of consistency among previous studies may have resulted from use of unbuffered culture media.

Combining 10 mM MES with KOH, NaOH, or Ca(OH)$_2$ prevented pH change during root initiation; however, Ca(OH)$_2$ appeared to inhibit root formation (Table 2). Calcium has been shown to synergistically enhance boron stimulation of root development (Jarvis, 1986). This study showed an inhibitory affect of calcium on the auxin-requiring root initiation phase. When associating

Fig. 3. Influence of root initiation medium (RIM) pH (**A** and **C**) and *Malus domestica* (**B** and **D**) 'Gala', 'Jonathan', 'Triple Red Delicious', and 'Vermont Spur Delicious' microcuttings on IBA loss from RIM (**A** and **B**) and root count (**C** and **D**) 14 d after transfer to root development medium. IBA loss and root count values for pH and cultivar represent main effect means. Mean separation by Duncan's multiple-range test ($P = 0.05$).
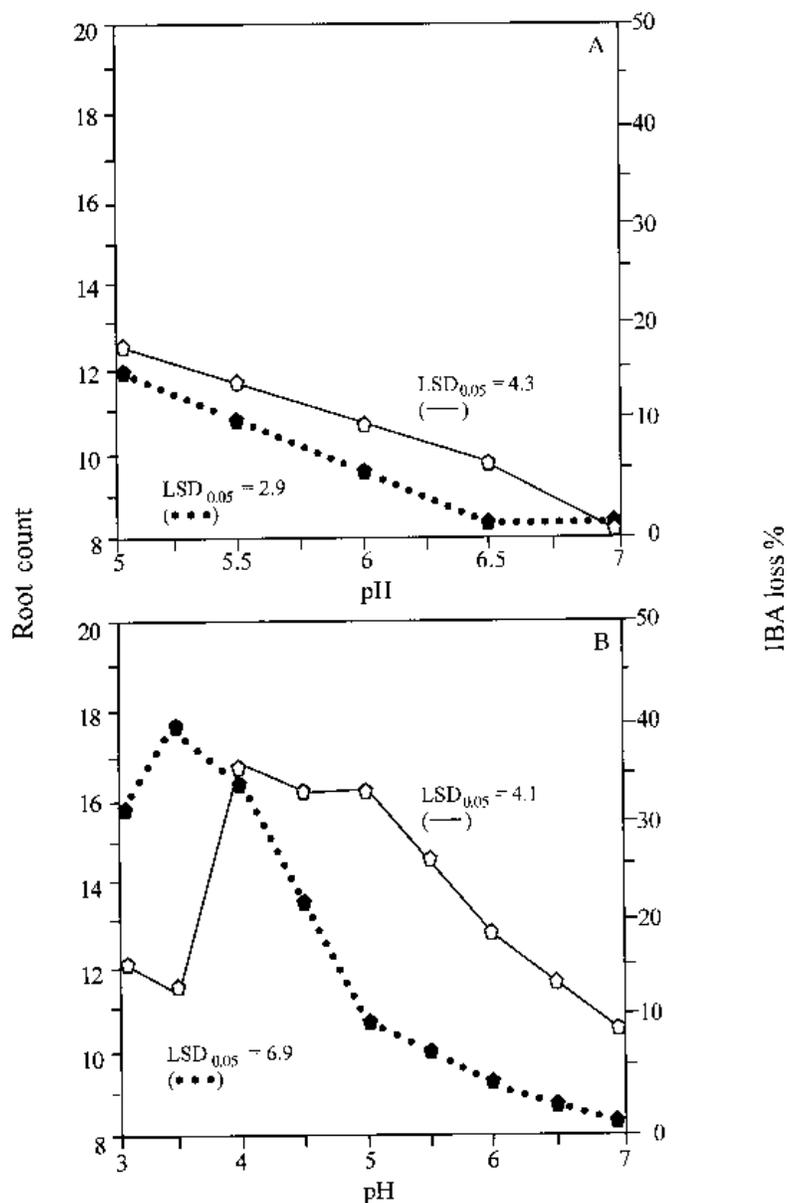
Fig. 4. Influence of root initiation medium pH on IBA loss (•-•-•) and root count (——) 14 d after transfer of microcuttings of *Malus domestica* 'Triple Red Delicious' (**A**) and 'Gala' (**B**) to root development medium.

from RIM and root counts were greater at pH 5.5 than pH 7.0. (Fig. 3 A and C). The sigmoidal pattern of IBA reduction by 'Gala' microcuttings at pH between 3.5 and 7.0 (Fig. 4B) was consistent for a weak acid like IBA (pK 4.7) crossing a lipophilic barrier (Rubery and Sheldrake, 1973). Our data and those of Blakely et al. (1986) suggest that auxin-dependent root formation is influenced by pH. In our study, pH may have affected uptake of auxin into apple microcuttings.

Independent of pH, the loss of IBA from RIM was similar for 'Gala', 'Triple Red Delicious', and 'Vermont Spur Delicious', which suggests adequate amounts of IBA were available for root initiation (Fig 3B). However, root count was highest on easy-to-root 'Gala', intermediate on moderately easy-to-root 'Jonathan', and lowest on most difficult-to-root 'Triple Red Delicious' and 'Vermont Spur Delicious'. Even though rooting response was enhanced by a pH and IBA treatment for all cultivars, an additional barrier to root induction of difficult-to-root cultivars persisted (Fig. 3D), suggesting different capacities to respond to IBA.

Future investigations should focus on the quantification of endogenous IBA in relation to root initiation phase pH, IBA treatment, and plant genotype.

Literature Cited

Blakely, L.M., R.M. Blakely, and C.M. Galloway. 1986. Effects of dimethyl sulfoxide and pH on indoleacetic acid-induced lateral root formation in the radish seedling root. Plant Physiol. 80:790–791.

Ehmann, A. 1977. The Van Urk-Salkowski reagent—A sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J. Chromatogr. 132:267–276.

Harbage, J.F. 1991. Anatomy and physiology of adventitious root formation in *Malus domestica* microcuttings. PhD diss., Univ. of Wisconsin, Madison (Abstr. 91–28915).

Harbage, J.F., D.P. Stimart, and R.F. Evert. 1993. Anatomy of adventitious root formation in microcuttings of *Malus domestica* 'Gala'. J. Amer. Soc. Hort. Sci. 118:680–688.

James, D.J. 1983. Adventitious root formation 'in vitro' in apple rootstocks (*Malus pumila*) II. Uptake and distribution of indol-3yl-acetic acid during the auxin-sensitive phase in M.9 and M.26. Physiol. Plant. 57:154–158.

Jarvis, B.C. 1986. Endogenous control of adventitious rooting in nonwoody cuttings, p. 191–221. In: M.B. Jackson (ed.). New root formation in plants and cuttings. Martinus Nijhoff, Dordrecht, Netherlands.

Lee, C.I., J.L. Paul, and W.P. Hackett. 1976. Root promotion on stem cuttings of several ornamental plant species by acid or base pretreatment. Comb. Proc. Inter. Plant Prop. Soc. 26:95–99.

Lovel, H.L. and J. White. 1986. Anatomical changes during adventitious root formation, p. 111–140. In: M.B. Jackson (ed.). New root formation in plants and cuttings. Martinus Nijhoff, Dodrecht, Netherlands.

Mellor, F.C. and R. Stace-Smith. 1969. Development of excised potato buds in nutrient culture. Can. J. Bot. 47:1615–1621.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Rubery, P.H. and A.R. Sheldrake. 1973. Effect of pH and surface charge on cell uptake of auxin. Nature New Biol. 244:285–288.

Stone, O.M. 1963. Factors affecting the growth of carnation plants from shoot apices. Ann. Appl. Biol. 52:199–209.

Williams, R.R., A.M. Taji, and J.A. Bolton. 1985. Specificity and interaction among auxins, light, and pH in rooting of Australian woody species in vitro. HortScience 20:1052–1053.

Zimmerman, R.H. and I. Fordham. 1985. Simplified method for rooting apple cultivars in vitro. J. Amer. Soc. Hort. Sci. 110:34–38.

auxin sensitivity to root primordia formation during root initiation phase (Harbage and Stimart, 1993) and low root counts observed in this study with $Ca(OH)_2$ it appears calcium acted antagonistically with the auxin-mediated process during root initiation.

The interaction between pH and IBA concentration on final root count (Table 5) suggests that pH affected microcutting auxin perception. Altered perception could result from modification of auxin uptake. The rate of auxin uptake into cells is dependent on the equilibrium between the free acid and the anionic form which is determined by pH (Rubery and Sheldrake, 1973). In *Raphanus sativus*, pH dependence of auxin-induced lateral root formation was overcome by addition of dimethylsulfoxide to increase plasma membrane permeability (Blakely et al., 1986). This suggested a permeability barrier to auxin uptake influenced lateral root formation and was dependent on pH, although auxin uptake was not measured. In the present study, root counts were correlated negatively with pH at 0.15 and 1.5 μM IBA (Fig. 2). At 150 μM IBA, similar root count occurred at all pH values. Additionally, IBA loss