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Growth Regulator Effects on Adventitious Root Formation in Leaf Bud Cuttings of Juvenile and Mature *Ficus pumila*[1]

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Abstract. **Adventitious root formation was stimulated with foliar application of indolebutyric acid (IBA) from 1000 to 1500 mg/liter for juvenile and 2000 to 3000 mg/liter for mature leaf bud cuttings of *Ficus pumila* L. IBA increased cambial activity, root initial formation, and primordia differentiation and elongation. IBA stimulated rooting when applied to juvenile cuttings at 3, 5, or 7 days after experiment initiation, but had no effect on mature cuttings when applied at day 15, the final treatment period. The interaction of IBA/gibberellic acid (GA_3) did not affect early root development stages, but reduced root elongation and quality once primorida had differentiated. IBA/6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) inhibited rooting at early initiation stages.**

Recent researchers have generally agreed that adventitious root formation (ARF) involve sequences of histological steps with each step having different requirements for growth substances (5, 8, 9, 10, 11). Eriksen (5) and Mohammed and Eriksen (8) found that auxins and cytokinins had different affects on ARF depending on developmental stage. Sircar (11) reported 5 different histological stages in which GA_3 and IAA alternately promoted or inhibited ARF. Hypocotyl cuttings of herbaceous annuals have been used in previous sequencing

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experiments, but herbaceous material may not give a true index of changes occurring in mature woody materials.

The woody ornamental creeping fig *(Ficus pumila)* exhibits strong dimorphism (2) and differences in rooting between the juvenile and mature forms. Objectives of this study were to determine the effect of IBA, PBA, and GA_3 applied at different rooting developmental stages to juvenile and mature leaf-bud cuttings (LBC) of *F. pumila*.

Materials and Methods

F. pumila cultivated on the University of Florida campus at Gainesville were used as stock plants. Leaf bud cuttings (LBC-lamina, petiole and 2.5 cm piece of stem with attached axillary bud) were rooted under an intermittent mist system in a medium of sterilized mason sand maintained at 24°C with a 2 hr night light interruption previously described (4). Juvenile LBC were harvested after 21 days and mature cuttings 42 days after experiments were initiated. All growth regulators were applied as aqueous sprays with 0.25 ml/liter of surfactant, emulsifiable A-C polyethylene and octyl phenoxy polyethoxy ethanol (Plyac).

In an experiment to establish optimum IBA concentration required for rooting, cuttings were taken in November and IBA applied at 500, 1000, 1500, 2000, 3000, and 10,000 mg/liter to juvenile and 2000, 2500, 3000, 4000, 5000, and 10,000 mg/liter to mature LBC at time of insertion. The design was a randomized complete block with 4 replications and 40 cuttings per treatment.

To characterize growth regulator effects at different root development stages a factorial experiment was initiated in May with 2 forms (juvenile, mature LBC) × 2 IBA pretreatments (control, treated) × 3 growth regulators (IBA, PBA, GA_3) × 3 application dates. An IBA spray of 1000 mg/liter was applied to half the juvenile cuttings and 3000 mg/liter to half the mature material at the time of insertion. Growth regulators were then applied after 3, 5, or 7 days for juvenile and 3, 9 or 15 days for mature cuttings: IBA at 1000 mg/liter for juvenile and 3000 mg/liter for mature cuttings, 1000 mg/liter PBA and 3000 mg/liter GA_3 for both types. The design was a randomized complete block with 4 replications and 32 cuttings per treatment. To determine stage of ARF 10 cuttings of each treatment combination were selected at each of the 3 time intervals and fixed in formalin-acetic acid-ethanol (FAA) *in vacuo*, dehydrated in ethanol-tertiary butyl alcohol series and embedded in Paraplast-plus. Blocks containing stem pieces with one surface exposed were soaked in distilled water *in vacuo* for 5 days to soften tissues prior to sectioning. Serial cross and longitudinal sections were cut at 8 and 11 um and strained with safranin and fast green.

Cuttings were measured for percent rooting, root number, and root length (average of 3 longest roots) and rated on a quality scale of 1 to 4 with 1 = no rooting, 2 = small root system, 3 = intermediate root system and 4 = extensive root system.

Results

Optimum IBA concentration. IBA treatments stimulated ARF in both juvenile and mature LBC (Fig. 1, 2, 3, 4). At high IBA levels root length was reduced in both forms (Fig. 3) and root quality in juvenile cuttings was poor (Fig. 4). Best horticultural responses were obtained in juvenile material treated with 1000-1500 mg/liter and mature cuttings treated with 2000-3000 mg/liter IBA considering root number, length and quality (Fig. 2, 3, 4). The performance of IBA-treated juvenile LBC was better than IBA-treated mature cuttings.

Hormonal effects during rooting stages. Percent rooting in IBA pretreated cuttings was unaffected by additional IBA at any of the 3 time intervals after insertion, however, root length was reduced in all treatments (Table 1, 2). In juvenile LBC receiving no IBA pretreatment, later IBA application increased rooting in all dates (Table 1), but in mature cuttings only the first or second application period was stimulatory (Table 2).

GA_3 reduced root length and quality in IBA-pretreated cuttings (Table 1, 2 and Fig. 5, 6). In juvenile cuttings without IBA pretreatment, GA_3 reduced root length (Table 1), but had no effect on mature LBC without IBA pretreatment (Table 2).

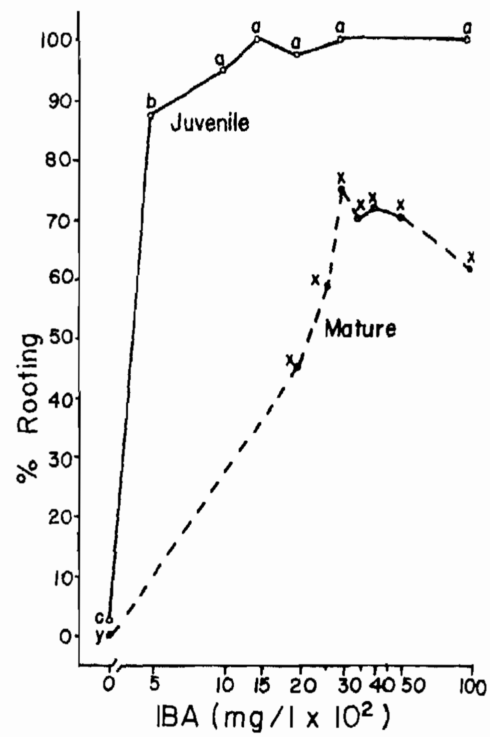

Fig. 1. Effect of IBA on rooting in juvenile and mature leaf bud cuttings of *Ficus pumila*. Points with same lower case letters are not significantly different.

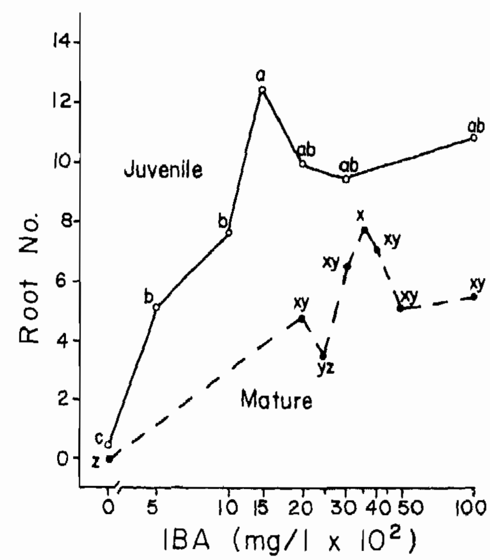

Fig. 2. Effect of IBA on root number in juvenile and mature leaf bud cuttings of *Ficus pumila*. Points with same lower case letters are not significantly different.

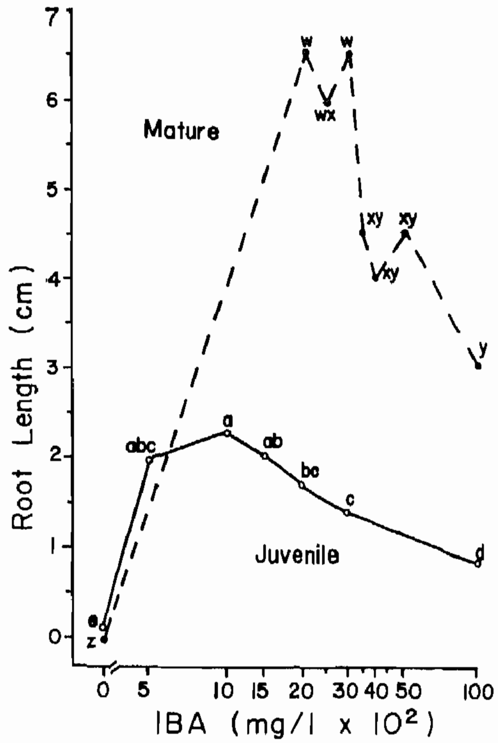

Fig. 3. Effect of IBA on root length in juvenile and mature leaf bud cuttings on *Ficus pumila*. Points with same lower case letters are not significantly different.

PBA effectively limited ARF in IBA-pretreated cuttings when applied during the first or second time intervals (Tables 1, 2). In juvenile LBC the greatest inhibition occurred during the first time interval which coincided with increased cambial activity associated with the dedifferentiation phase of ARF (Table 3). PBA caused less inhibition of ARF the second appli-

Table 1. Adventitious root formation in juvenile leaf bud cuttings of *Ficus pumila* treated with 3 growth regulators at 3, 5, or 7 days after experiment initiation. Half the cuttings were pretreated with 1000 mg/liter IBA.

IBA pre-treatment (mg/liter)	Growth regulator post treatment	Rooting (%)	No. roots	Root length (cm)	Root quality scale[z]
0	IBA				
	(1000 mg/liter)				
	day 3	100a[z]	9.5e	1.1bcde	2.6de
	day 5	100a	11.0bcde	1.1bcde	2.8cd
	day 7	100a	10.3cde	1.0bcde	2.5de
	GA_3				
	(3000 mg/liter)				
	day 3	31c	0.7h	0.8cde	1.3gh
	day 5	28c	0.8h	0.7de	1.3gh
	day 7	34c	1.0h	1.5bcd	1.5g
	PBA				
	(1000 mg/liter)				
	day 3	0d	0h	0h	1.0h
	day 5	25c	0.9h	1.2bcde	1.3gh
	day 7	25c	0.9h	1.4bcd	1.3gh
	Control	31c	0.8h	1.7b	1.3gh
1000	IBA				
	(1000 mg/liter)				
	day 3	100a	12.7b	1.5bc	3.0abc
	day 5	100a	15.2a	1.3bcd	3.2ab
	day 7	100a	12.4bc	1.0bcde	2.7cd
	GA_3				
	(3000 mg/liter)				
	day 3	100a	10.8bcd	1.3bc	2.7cde
	day 5	100a	9.0ef	1.5bc	2.8cd
	day 7	100a	10.2de	1.7b	2.8bcd
	PBA				
	(1000 mg/liter)				
	day 3	38c	1.3h	0.5ef	1.4gh
	day 5	66b	5.3g	1.3cde	2.0f
	day 7	88a	7.2fg	1.2bcde	2.3ef
	Control	100a	11.9bcd	2.5a	3.4a

[z]Root quality scale range from 1 to 4 with 1 = no root system, 2 = small root system, 3 = intermediate root system and 4 = extensive root system.
[y]Mean separation in columns by Duncan's multiple range test, 5% level.

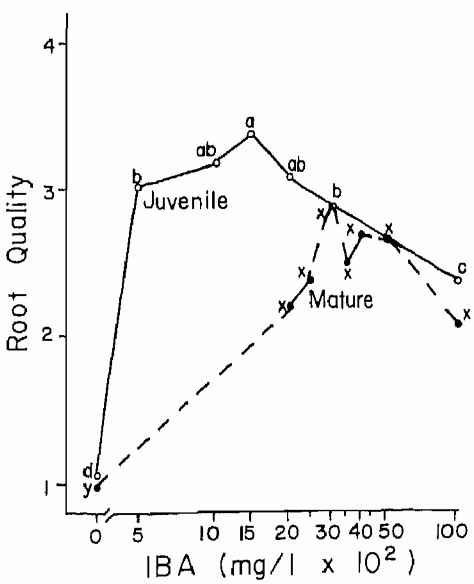

Fig. 4. Effect of IBA on root quality in juvenile and mature leaf bud cuttings of *Ficus pumila*. Points with same lower case numbers are not significantly different.

cation period when root initials and primordia were first observed. Half the LBC rooted by the third interval (Table 3); thus PBA application at this time did not affect % rooting but did reduce root number, length and quality. In mature cuttings PBA treatment at first application period completely inhibited ARF (Table 2) when no cambial activity was observed. PBA was less effective in inhibiting ARF during second application when cambial activity was first observed (Table 2, 4). Root length and quality were reduced with PBA application at any period, but had no effect on % rooting or number during the third treatment period.

PBA reduced rooting in juvenile cuttings not pretreated with IBA when applied during the first treatment period when neither root initials nor primordia were observed (Table 1, 3). In mature cuttings PBA had no statistical effect on rooting; however, none of the treated cuttings formed roots, nor were root initials or primordia observed (Table 2, 4).

Discussion

Mature *F. pumila* cuttings did not root as efficiently as juvenile material. Thus, IBA-treated mature cuttings required higher exogenous auxin levels and longer time to obtain

Table 2. Adventitious root formation in mature leaf bud cuttings of *Ficus pumila* treated with 3 growth regulators at 3, 9, or 15 days after experiment initiation. Half the cuttings were pretreated with 3000 mg/liter IBA.

IBA pre-treatment (mg/liter)	Growth regulator post-treatment	Rooting (%)	No. roots	Root length (cm)	Root quality scale[z]
0	IBA (3000 mg/liter)				
	day 3	84abc[z]	13.1abc	3.4ab	3.0ab
	day 9	94ab	8.6cde	3.0ab	2.7abc
	day 15	53cdefg	2.7fg	1.0cde	1.7efg
	GA_3 (3000 mg/liter)				
	day 3	44efg	2.0fg	0.7de	1.5fgh
	day 9	41fg	1.9fg	0.8cde	1.5fgh
	day 15	38fg	1.1fg	0.8cde	1.4gh
	PBA (1000 mg/liter)				
	day 3	0h	0g	0e	1.0h
	day 9	0h	0g	0e	1.0h
	day 15	0h	0g	0e	1.0h
	Control	22gh	1.5fg	1.1cde	1.3gh
3000	IBA (3000 mg/liter)				
	day 3	81abcd[z]	11.1bcd	2.1bcd	2.6bcd
	day 9	100a	16.1a	3.1ab	3.2ab
	day 15	91ab	13.7ab	2.1bcd	2.7abc
	GA_3 (3000 mg/liter)				
	day 3	66bcdef	8.4cde	1.6cd	2.0def
	day 9	50defg	6.0ef	1.7cd	1.8efg
	day 15	66bcdef	7.3de	2.2bc	2.1cde
	PBA (1000 mg/liter)				
	day 3	0h	0e	0e	1.0h
	day 9	28gh	1.6fg	1.0cde	1.3h
	day 15	75abcde	9.2bcde	1.3cde	2.2cde
	Control	94ab	13.3abc	3.8a	3.2a

[z]Root quality scale ranged from 1 to 4 with 1 = no root system, 2 = small root system, 3 = intermediate root system and 4 = extensive root system.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

maximum rooting (3) than juvenile LBC. Mature cuttings may have lower endogenous auxin levels and/or other endogenous chemicals needed to stimulate root initiation. When ARF was measured on a daily basis (3), IBA-treated mature cuttings rooted slower than juvenile LBC, but equalled juvenile controls by day 20, giving strong evidence that endogenous auxin levels were acting as a possible limiting factor in root initiation.

IBA increased ARF in both juvenile and mature cuttings by stimulating initiation of cambial activity, root initials and primordia, which agrees with reports that auxins trigger early formation of root primordia (6). However in *F. pumila*, application of auxin above the optimum level reduced root length and quality indicating that primordia elongation was decreased.

In both juvenile and mature cuttings the combination of IBA/GA_3 retarded rooting after primorida were differentiated, since % rooting was not influenced but root length and quality were reduced. The conflicting reports on exogenous gibberellin influence on rooting (1, 7, 12) may be related to species differences. Our results agree with Hassig (7) who reported that initiating primordia were least affected by GA_3 but that cell number was reduced in older established primordia which was deleterious to root formation.

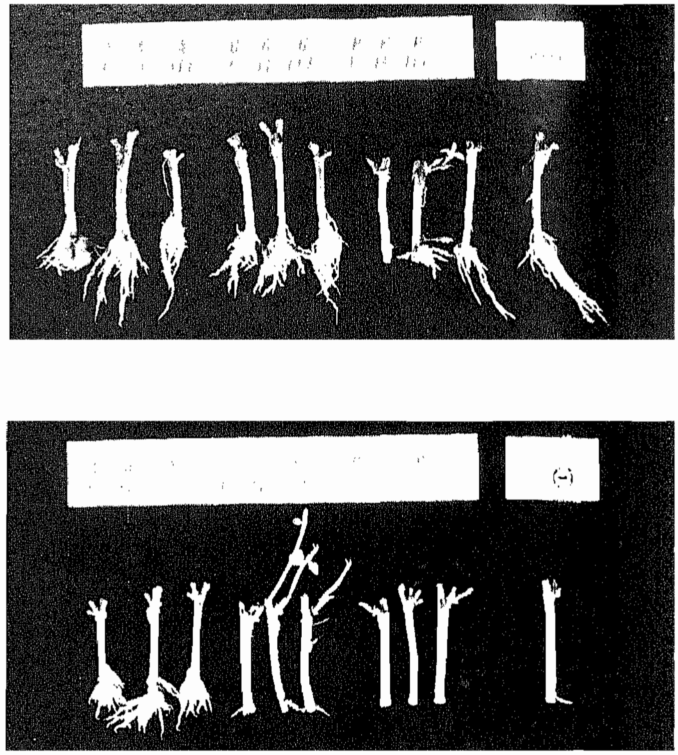

Fig. 5. Effect of IBA, GA_3 and PBA on adventitious root formation when applied at 3 time intervals to juvenile leaf bud cuttings. (top) Pretreated with (IBA). (bottom) No pretreatment with IBA.

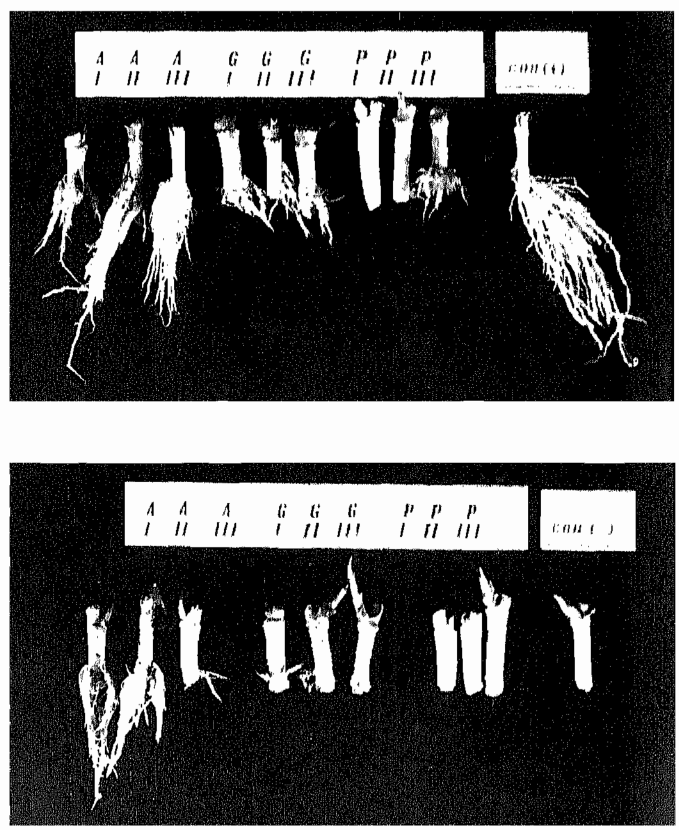

Fig. 6. Effect of IBA, GA_3 and PBA on adventitious root formation when applied at 3 time intervals to mature leaf bud cuttings. (top) Pretreated with IBA. (bottom) No pretreatment with IBA.

Table 3. Stage of adventitious root formation of juvenile leaf bud cuttings of *Ficus pumila* at 3 time intervals.

Treatment	Increased cambial activity	Root initials	Root primordia	Rooting (%)	No. roots	Root length (cm)	Root quality scale[z]
IBA pretreatment at (1000 mg/liter)							
day 3	yes	none	none	0	0	0	1.0
day 5	yes	yes	yes	0	0	0	1.0
day 7	yes	yes	yes	50	6.2	0.7	1.6
No IBA pretreatment							
day 3	none	none	none	0	0	0	1.0
day 5	yes	none	none	0	0	0	1.0
day 7	yes	yes	yes	20	0.4	0.5	1.2

[z]Root quality scale ranged from 1 to 4 with 1 = no root system, 2 = poor root system, 3 = intermediate root system and 4 = extensive root system.

Table 4. Stage of adventitious root formation of mature leaf bud cuttings of *Ficus pumila* at 3 time intervals.

Treatment	Increased cambial activity	Root initials	Root primordia	Rooting (%)	No. roots	Root length (cm)	Root quality scale[z]
IBA pretreatment at (3000 mg/liter)							
day 3	none	none	none	0	0	0	1.0
day 9	yes	none	none	0	0	0	1.0
day 15	yes	yes	yes	20	1.7	0.5	1.2
No IBA pretreatment							
day 3	none	none	none	0	0	0	1.0
day 5	none	none	none	0	0	0	1.0
day 15	yes	none	none	0	0	0	1.0

[z]Root quality scale ranged from 1 to 4 with 1 = no root system, 2 = poor root system, 3 = intermediate root system and 4 = extensive root system.

The rooting inhibition of PBA on juvenile and mature *F. pumila* concur with reports that cytokinins inhibit preinduction phases of rooting (12) with a loss of inhibitory effect at later stages (6).

Differences in adventitious rooting between juvenile and mature cuttings may be partially attributed to endogenous auxin levels, since lower IBA levels were required for optimal rooting in juvenile compared to mature LBC. However, other factors such as auxin/cytokinin and auxin/GA_3 ratios, cofactors and inhibitors may be involved, since exogenous IBA applications did not overcome root formation differences between IBA-pretreated juvenile vs. mature tissue.

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