

## Shoot RNA, cambial activity and indolebutyric acid effectivity in seasonal rooting of juvenile and mature *Ficus pumila* cuttings

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The seasonal influence on adventitious root formation was studied in woody leaf bud cuttings of *Ficus pumila* L., creeping fig. Juvenile cuttings rooted easily, whereas only mature cuttings treated with indolebutyric acid (IBA) exceeded 30% rooting. Greater rooting occurred in IBA-treated juvenile and mature cuttings than controls, regardless of the month each experiment was initiated. Seasonal changes influenced rooting in all treatments except IBA treated juvenile cuttings where percentage rooting was not affected. Higher vascular cambial activity and shoot RNA levels occurred in juvenile and mature forms during peak rooting periods. Highest RNA was recorded with juvenile materials during maximum rooting periods, while lowest RNA was observed in mature shoots during low rooting intervals.

*Additional key words* - Adventitious root formation, leaf bud cuttings.

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### Introduction

Adventitious root formation is essential for the successful propagation of many woody plants. Ease of adventitious root formation generally declines with increasing age of parent stock, which is an important problem because desirable characteristics are frequently not expressed until plants have reached maturity. The season in which cuttings are taken is another important factor in cutting propagation which may influence adventitious root formation as reported by Roberts (1969). Mixed reports exist in the literature on relationships among auxin (Anand and Heberlein 1975, Greenwood et al. 1976), cambial activity (Anand and Heberlein 1975), RNA (Riding 1976, Trippi and Brulfert 1973) and DNA (Alliata and Polito 1982) in seasonal rooting pattern and tissue culture of mature and juvenile material.

*Ficus pumila* L. is a woody ornamental vine that exhibits dimorphism and differences in adventitious root formation between juvenile and mature forms (Davies et al. 1982). Research with *F. pumila* has shown that the different histological phases have different re-

quirements for growth substances and that differences in rooting ability can only be partially attributed to auxin responses (Davies and Joiner 1980). Objectives of this study were to determine relationships among shoot RNA, cambial activity and exogenous auxin application on seasonal rooting response of easy-to-root juvenile and mature, difficult-to-root *F. pumila* cuttings.

*Abbreviations* - CZ, vascular cambial zones; IBA, indolebutyric acid.

### Materials and methods

*F. pumila*, cultivated outdoors on the University of Florida campus (Gainesville, FL, USA) were used as stock plants. Climatic data for the Gainesville campus was provided by the IFAS Agronomy Dept and the UF Dept of Physics and Astronomy (Tab. 1). Leaf bud cuttings with a 2.5 cm stem segment were rooted under an intermittent mist system in sterilized mason sand and

Tab. 1. Climate conditions of *Ficus pumila* stock plants grown on the University of Florida Campus, Gainesville, FL, USA.

Climatic conditions	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov
Photoperiod (h)	10/28	11/10	12/01	12/53	13/39	14/03	13/51	13/14	12/21	11/29	10/40
Thermoperiod (°C)	17/4	17/4	23/9	29/13	31/18	33/22	33/22	33/22	33/21	27/13	22/7
Solar radiation-PAR ( $\mu\text{mol m}^{-2}$ )	664	651	1030	1347	1349	1259	1180	1227	1041	-	-
Precipitation (mm)	157	127	114	15	89	99	264	244	142	94	48
Minimum percentage relative humidity	38	41	35	31	32	42	54	48	43	45	43

maintained at 24°C. Long days were maintained in the propagation house with supplemental incandescent light from 2300 to 0100 h ( $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), as previously described (Davies and Joiner 1978). Juvenile and mature leaf bud cuttings were evaluated for percentage rooting and root number after 21 and 42 days, respectively; the rooting termination period was based on peak rooting cycles of IBA pretreated juvenile and mature *F. pumila* (Davies et al. 1982).

The effect of IBA on seasonal rooting of juvenile and mature *F. pumila* was characterized in a study established utilizing a  $2 \times 2 \times 11$  factorial, complete block design with 4 replications and 40 cuttings per treatment. Juvenile cuttings were treated at insertion with aqueous sprays of 0 and 1 g IBA l<sup>-1</sup>, while mature leaf bud cuttings were treated with 0 and 3 g IBA l<sup>-1</sup> with experiments initiated monthly for 11 months. All auxin treatments were with an IBA potassium salt formulation and a surfactant (Plyac) (E. C. Geiger, Harleysville, PA, USA). Previous research (Davies and Joiner 1978) had shown optimal rooting of juvenile and mature leaf bud cuttings with aqueous sprays of 1 and 3 g IBA l<sup>-1</sup>, respectively, when applied with 0.25 ml of a surfactant (emulsifiable A-C polyethylene and octyl phenoxy polyethoxy ethanol (Plyac) l<sup>-1</sup>).

Stem sections from stock plants were harvested monthly concurrent with rooting experiments to determine the association between vascular cambial activity and seasonal rooting. Stem sections were fixed in formalin-acetic acid-ethanol *in vacuo* and stored for later analysis. Fixed material was dehydrated in an 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 (Davies et al. 1982). Serial cross and longitudinal sections were cut at 8 and 11  $\mu\text{m}$  and stained with safranin and fast green.

The relationship between shoot apical RNA and seasonal rooting was studied by harvesting stem sections from stock plants monthly and concurrent with rooting experiments. Serial longitudinal sections were cut at 9  $\mu\text{m}$  thickness and Azure B was used in the differential staining for RNA following the procedures of Jensen (1981). RNA was quantified with a Zeiss scanning microspectrophotometer at 688–655 nm with 160 $\times$ . Depending on meristem size, scans in the shoot apices encompassed 45–90 cells in the tunica and part of the corpus region.

## Results

Higher percentage rooting and number of roots per cutting occurred in IBA-treated juvenile and mature cuttings compared to controls, regardless of the month the experiment was initiated (Figs 1–4). Adventitious root formation was influenced by season in all treatments except IBA-treated juvenile cuttings where percentage rooting was not affected (Fig. 1). Poorest rooting occurred in November and January in juvenile controls, and January and February in IBA-treated mature cuttings (Figs 1–4). Adventitious root formation occurred in mature control cuttings only in April and May. IBA-treated mature cuttings showed less rooting in April compared to those made in March or May (Figs 3 and 4).

During peak rooting periods in both juvenile and mature stems, the cambium was in an active state of division and had a larger number of undifferentiated layers compared to low rooting periods when the vascular cambial zone narrowed and differentiating cell layers decreased (Tab. 2, Figs 5a–d). The cambial zone was closely packed in November and January and remained so until March, April and May when increased cambial activity occurred. Likewise, easy-to-root juvenile and difficult-to-root mature *F. pumila* had higher RNA levels in shoot apices during maximum rooting periods

Tab. 2. Cambial activity of juvenile and mature *Ficus pumila* shoot sections during low and high rooting activity periods. The cambial zone was measured by the number of undifferentiated cell layers (O'Brien and McCully 1981). A minimum of eight shoot sections per plant age per month was sampled. Cambial activity was evaluated 2.5 cm below the terminal bud.

Plant age	Month	Rooting level	Average number of undifferentiated cell layers in the cambial zone
Juvenile	January	Low	3.3
	November	Low	3.2
	March	High	7.9
	April	High	7.8
	May	High	7.5
Mature	January	Low	3.7
	November	Low	4.0
	March	High	8.0
	April	High	7.3
	May	High	6.6

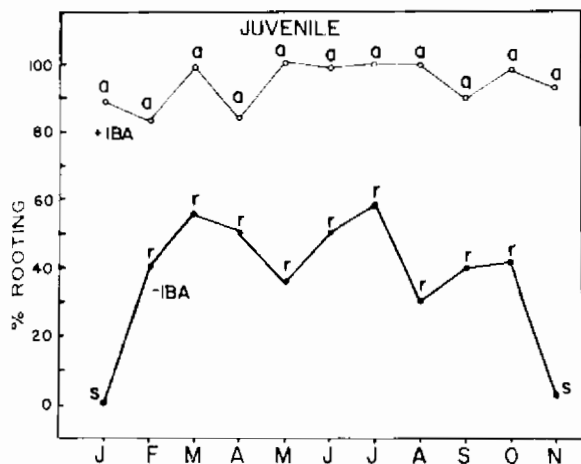


Fig. 1. The influence of season and IBA treatment on percentage of juvenile *F. pumila* cuttings forming adventitious roots. Cuttings were pretreated with auxin (+IBA) or left as controls (-IBA). Points with same lower case letters are not significantly different.

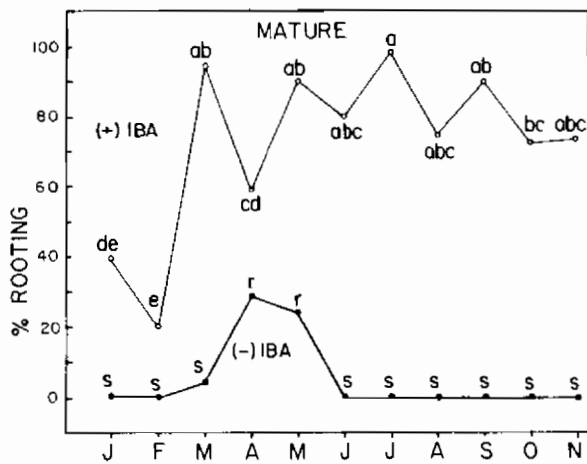


Fig. 3. The influence of season and IBA treatment on percentage of mature *F. pumila* cuttings forming adventitious roots. Cuttings were pretreated with auxin (+IBA) or left as controls (-IBA). Points with the same lower case letters were not significantly different.

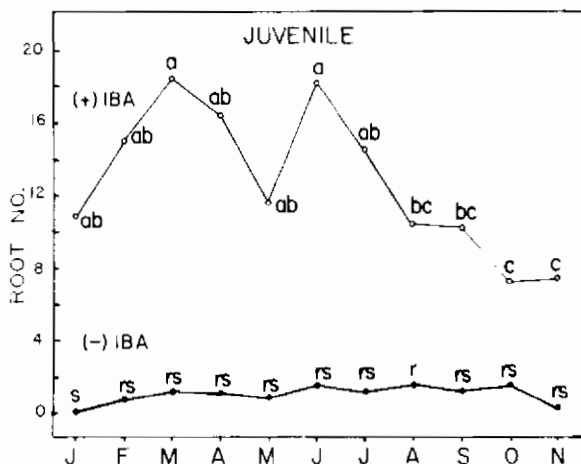


Fig. 2. The influence of season and IBA treatment on average number of adventitious roots formed per juvenile *F. pumila* cutting. Cuttings were pretreated with auxin (+IBA) or left as controls (-IBA). Points with same lower case letters are not significantly different.

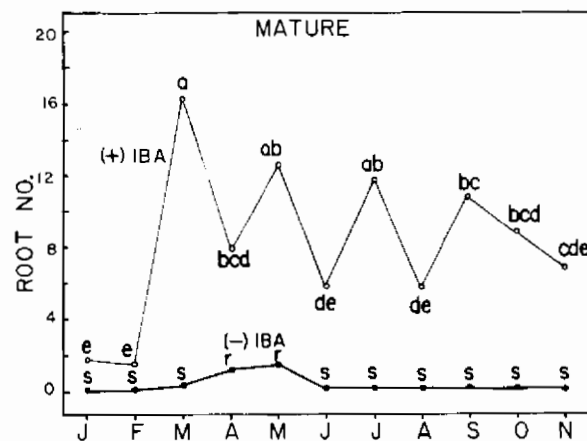


Fig. 4. The influence of season and IBA treatment on average number of adventitious roots formed per mature *F. pumila* cutting. Cuttings were pretreated with auxin (+IBA) or left as controls (-IBA). Points with the same lower case letters are not significantly different.

(Tab. 3). Highest RNA was recorded with juvenile material during peak rooting periods, while lowest RNA was found in mature shoots during low rooting intervals (Tab. 3).

### Discussion

In *Ficus infectoria* Roxb., a deciduous tree with a true dormancy period, high rooting phases coincided with resumption of growth (Anand and Heberlein 1975). However, the high rooting phase was auxin sensitive while the low rooting phase was auxin insensitive. In

contrast, the evergreen *F. pumila* which undergoes no apparent dormancy other than a quiescent period in response to cooler temperature, was auxin sensitive throughout its seasonal rooting cycle. In the present study, IBA-treated juvenile cuttings had sufficient yearly auxin sensitivity to eliminate seasonal fluctuations in percentage rooting, but not in root number (Figs 2 and 4). A more rapid increase in root number occurred in IBA-pretreated juvenile (February) compared to mature material (March), suggesting inherent abilities of juvenile tissue to dedifferentiate more efficiently into roots and/or seasonal differences between both forms in endogenous auxin, auxin synergists, etc.

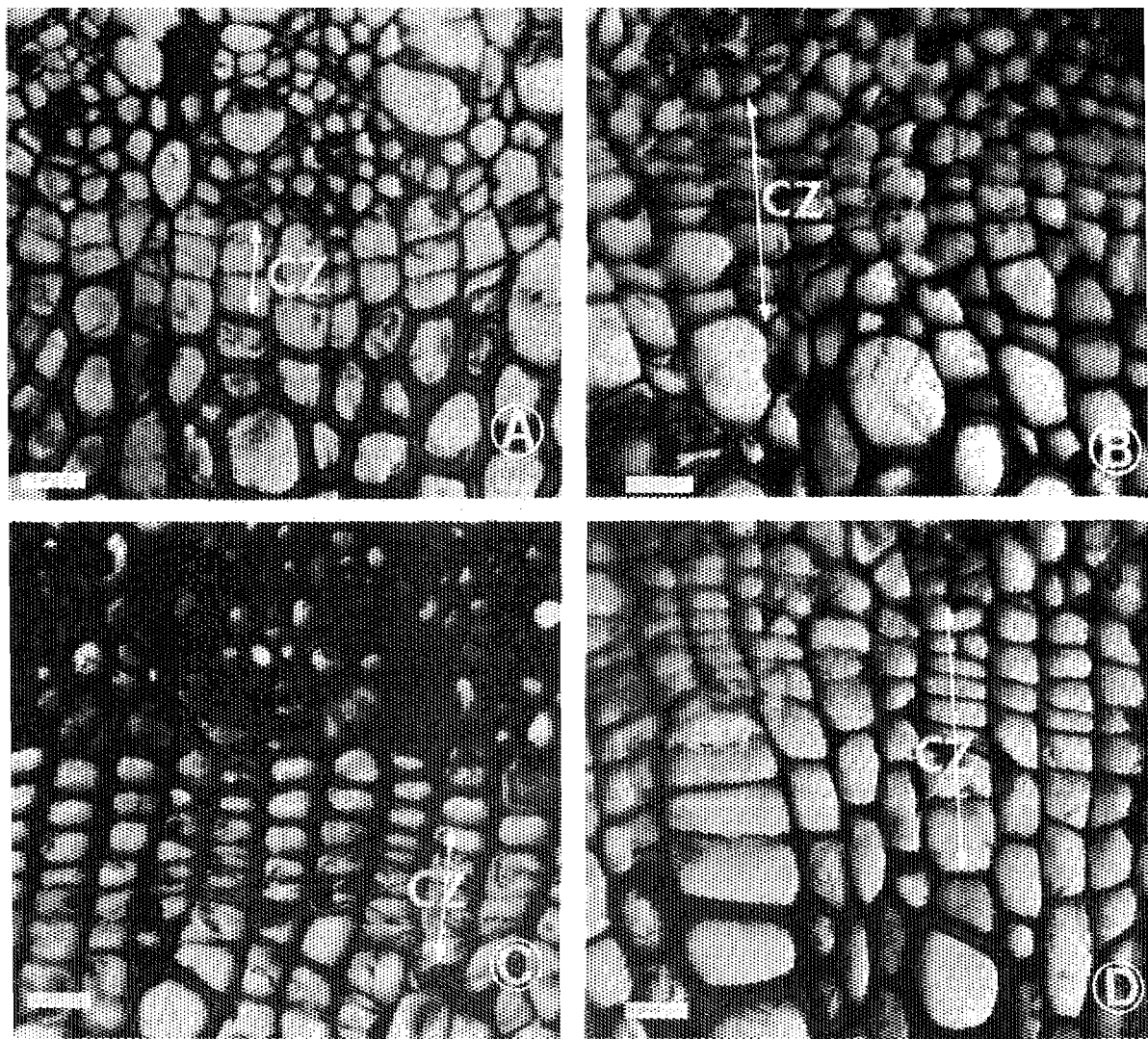


Fig. 5a-d. Vascular cambial zones (CZ) of *F. pumila* stems. Photomicrographs are from stem cross sections harvested during peak rooting months of March, April and May and low rooting months of November and January. A, juvenile stem during low rooting intervals; B, juvenile stem during high rooting intervals; C, mature stem during low rooting intervals; D, mature stem during peak rooting intervals. Bar represents 25  $\mu$ m.

Tab. 3. The relationship between shoot RNA and adventitious root formation in *Vitis pumila* cuttings. Shoot apices were harvested from stock plants during high rooting months of March, April and May and during low rooting months of November and January. Optical density measurement of RNA was by azure blue absorption, 688-655 nm. Results presented are the mean separation by Duncan's multiple range test, 5% level. Means followed by the same lower case letters are not significantly different.

Plant age	Rooting level	RNA
Juvenile	High	0.801a
Mature	High	0.647b
Juvenile	Low	0.631b
Mature	Low	0.478c

It was shown in difficult vs. easy-to-root *Acer saccharum* (sugar maple) ortets that bases of easily rooted ortets accumulated larger amounts of IAA (Greenwood et al. 1976). As with *F. infectoria*, there was an auxin-insensitive phase in *Acer saccharum* where exogenous auxin application did not stimulate adventitious root formation. Rooting in *F. pumila* may be more sensitive to auxin than in species with an auxin insensitive phase where auxin synergists, rooting cofactors and/or inhibitors may be of greater significance.

In the present study, IBA treated mature *F. pumila* cuttings had a lower percentage rooting in April compared to those made in March or May, suggesting that in April seasonally higher endogenous auxin levels may

have produced a supra optimal response. In a previous experiment when high levels of exogenous IBA were applied to either juvenile or mature *F. pumila* cuttings, there was a subsequent decline in adventitious root formation compared to optimal IBA levels (Davies and Joiner 1980).

Peak rooting periods in *F. pumila* corresponded with increased cambial activity. Mature controls formed roots only during periods of higher cambial activity, which suggests the importance of endogenous auxin from shoot apices and other rooting factors in stimulating vascular cambial development and associated rooting (Anand and Heberlein 1975, Wodzicki 1978). It was observed that juvenile and mature IBA pretreated *F. pumila* cuttings had increased vascular cambial activity which preceded histological events leading to root initial and root primordia formation (Davies et al. 1982).

Shoot RNA in *F. pumila* was an index of bud activity and subsequent seasonal rooting differences. It was reported that seasonal variation in auxin and subsequent rooting was attributable to changes in bud activity (Roberts and Fuchigami 1973). As an index of rooting, shoot apex RNA in *F. pumila* was influenced both by season and physiological age of plants. Riding (1976) reported a positive correlation between rooting potential and RNA in the cells of the shoot apex of the gymnosperm *Picea mariana*. Trippi and Brulfert (1973) reported the progressive loss of rooting capacity with decreased shoot RNA in the herbaceous plant *Anagallis arvensis*.

Shoot apex RNA levels paralleled increases in cambial activity and adventitious root formation in *F. pumila*. Working with tissue-cultured meristems of pea root, Webster and Van't Hof (1970) concluded that RNA and protein synthesis were prerequisites for cell division and DNA synthesis. In the rooting of *Hydrangea macrophylla* cuttings, Molnar and La Croix (1972) reported that both DNA and some protein synthesis must occur before cell division of the root initials can take place. It was suggested that the organization of a root meristem from the cambial region requires a complex sequence of nuclear events regulated by a developmental time clock of DNA/or RNA (Greenwood et al. 1976). Seasonal and growth phase differences in rooting of *F. pumila* may well be attributed to gene regulation of protein synthesis, which is manifested by

increased shoot RNA activity, production of auxin and other metabolites, and subsequent changes in cambial activity.

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