A HISTOLOGICAL AND PHYSIOLOGICAL ANALYSIS OF ADVENTITIOUS ROOT FORMATION IN JUVENILE AND MATURE CUTTINGS OF *Ficus pumila* L.

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1978

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TO MY MOTHER AND FATHER

ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. Jasper N. Joiner, Professor of Ornamental Horticulture, University of Florida, for his suggestions, enthusiasm, support and criticism during the course of this research and manuscript preparation.

Thanks are also extended to other members of the committee from the University of Florida, Dr. Robert H. Biggs, Professor of Fruit Crops, Dr. Indra K. Vasil, Professor of Botany, Dr. Charles R. Johnson, Associate Professor of Ornamental Horticulture and Dr. Dennis B. McConnell, Associate Professor of Ornamental Horticulture for their suggestions and criticism of this research and manuscript preparation.

I thank Mr. Walter Offen, Graduate Assistant, IFAS Statistics Unit, for his aid in programming statistical analysis. The assistance of the crew at the Ornamental Horticulture Greenhouses is greatly appreciated.

I am deeply indebted to my colleague, Dr. Jaime E. Lazarte, Vegetable Crops Department, University of Florida, for his advise and help during the course of this research and manuscript preparation.

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Doctor of Philosophy

A HISTOLOGICAL AND PHYSIOLOGICAL ANALYSIS OF ADVENTITIOUS ROOT FORMATION IN JUVENILE AND MATURE CUTTINGS OF *Ficus pumila* L.

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December 1978

Chairman: Jasper N. Joiner
Major Department: Ornamental Horticulture

Adventitious root formation (ARF) was studied in three cutting types of juvenile and mature *Ficus pumila* L. (Creeping Fig). Leaf bud cuttings (LBC) were established as the ideal type for developmental sequencing research since difficult to root mature LBC responded positively to auxin and rapid *de novo* ARF was obtained to minimize environmental and physiological variables.

Microscopic examination of ARF in juvenile and mature LBC indicated that both forms of cuttings exhibited remeristemation, early cell divisions (root initials), differentiation of primordia and root elongation. Primordia in juvenile LBC treated with indole-3-butyric acid (IBA) occurred at day 6 and in mature ones at day 10, roots emerged at days 7 and 20, respectively, with maximum rooting occurring at days 14

and 28. ARF originated primarily from phloem ray parenchyma. Root primordia differentiated from some basal callus of juvenile and mature LBC, but these nor the few primordia in mature controls elongated into well developed roots. IBA stimulated root initiation but did not effect primordia origin and perivascular fibers, sclerids and laticifers did not influence ARF. Time separation of root initiation phases and development allowed the study of concurrent physiological events in ARF of juvenile and mature LBC of this species.

Foliar application of IBA applied at day 1 was more effective than indole-3-acetic acid (IAA) in stimulating adventitious root formation, and 1000 to 1500 mg/l IBA was optimal for juvenile and 2000 to 3000 mg/l IBA for mature LBC. Foliar application of 2,3,5-triiodobenzoic acid (TIBA) and (2-chloroethyl) phosphonic acid (Ethrel) applied at day 1 did not effect ARF.

In a 2x3x3 factorial experiment half the cuttings were pretreated with IBA and three growth regulators - IBA, 6-benzylamino)-9 (2-tetrahydropyranyl)-9H-purine (PBA) and gibberellic acid (GA_3) were applied at 3 different time periods to both juvenile and mature forms. IBA applied at days 3,5 and 7 to pretreated IBA juvenile cuttings reduced root length and GA_3 and PBA inhibited ARF. IBA application at days 3,5 and 7 stimulated ARF in juvenile cuttings not pretreated with IBA. ARF in pretreated IBA mature cuttings was inhibited by GA_3 and PBA applications at days 3,9 and 15. IBA applied at days 3 and 9 stimulated ARF in mature cuttings

not pretreated with IBA.

IBA stimulated adventitious rooting by increased cambial activity, root initials and primordia differentiation and development in juvenile and mature cuttings. IBA/GA_3 did not effect early initiation stages, but reduced rooting once primordia had differentiated, while IBA/PBA inhibited rooting at early initiation stages.

Reasons for differences of growth regulator interactions between juvenile and mature LBC are discussed.

INTRODUCTION

Many plant species lose their ability to form adventitious roots (ARF) as their tissues pass from juvenility to maturity. Lignification, increased IAA oxidase production and other anatomical and physiological changes have been proposed to explain these differences. But only limited investigations have been conducted to determine whether ARF failure is due to inability of a recognizable root primordia to differentiate or inability of roots to develop from primordia.

Only limited information exists in the literature to explain ARF as an organized developmental process in economically important woody materials. Torrey stated that concepts of morphogenesis as they relate to regulation of organized development needed strengthening, (127). Inability of mature cuttings to form adventitious roots (which is an organized developmental process) may be attributed to inability of parenchymatous cells to undergo transformation into primordia for some physiological reasons. Several researchers (34,81,117) employing herbaceous materials have influenced ARF by applying growth regulators at different developmental stages, but no work has been reported with woody plants.

Ficus pumila L. is a woody ornamental vine and was used as the test material since it exhibits pronounced dimorphism and differences in ARF between juvenile and mature phases.

Objectives of this research were to: 1) separate time phases of root initiation and subsequent differentiation of root primordia via microtechniques, 2) study concurrent physiological events via growth regulator interactions and 3) compare above criteria between juvenile and mature states.

REVIEW OF LITERATURE

Vegetative Propagation

Vegetative or asexual propagation is an important commercial method for regenerating large quantities of genetically uniform plant materials. Asexual propagation entails reproduction from vegetative parts of plants such as stems, modified stems, roots, leaves, grafts or single cells. Stem cuttings are the most important propagation unit and are classified by the maturity of wood used into hardwood, semi-hard wood, softwood and herbaceous (55). Propagation using leaf cuttings is largely confined to herbaceous species and consist of an entire leaf (lamina and petiole) or leaf part. Leaf bud cuttings consist of a leaf blade, petiole and a short piece of stem with attached axillary bud and, in root cuttings shoot and root primordia are generated from root pieces (16). Adventitious roots must be generated for successful propagation, regardless of cutting type employed.

Adventitious roots (ARF) are those arising on aerial plant parts, underground stems and old root parts (35).

Morphological Factors

The origin of ARF is dependent on potentially meristematic tissue; hence they frequently arise from intercalary meristems at bases of internodes, meristematic tissue occurring at axils of leaves, lateral meristems (cambium and pericycle) and ray parenchyma (57).

Tissue from which roots originate in stem cuttings is determined partly by age of stem from which ARF arise (96). ARF in young stems often originate from pericyclic tissue arising from groups of cells and less frequently from phloem and ray parenchyma cells. Detection of a single cell around which a meristemoid developed was reported, but appeared to be without precedent in the literature (116). Origin of ARF in some crucifers is exogenous instead of endogenous (52) and proximity of new roots to main axis of stems permits rapid vascular connection between plant parts. Point of origin of root initials can vary between species and even within the same plant (116). Origin of wound induced roots in stems of woody plants is listed in Table 1. Problems in ARF are associated with retention of meristematic capacity in certain cells of the plant body.

ARF is an organized developmental process entailing synchronized histological and physiological changes within plant bodies (127) and has been considered a four-stage process: 1) dedifferentiation or remeristemation, 2) initition of slightly organized cell groups (root initials), 3) differentiation to form root primordia and 4) elongation or extension (8,38,43,44). Sircar and Chatterjee (111,112, 113) observed five histologically distinct stages in Vigna hypocotyl cuttings. Primordia have also been categorized for developmental stages (56,81), and Smith and Thorpe (117) reported that differentiation of primordia in hypocotyls of Pinus radiata consisted of three histological phases:

Table 1. Origin of wound induced roots in stems of woody plants[z]

Species	Origin
Abelia grandiflora	Callus.
Abies spp.	Callus.
Acanthopanax spinosa	Callus.
Acanthus montanus	Near vascular combium.
Camellia sinensis	Near vascular cambium.
Caragana arborescens	Secondary phloem, callus.
Carya illinoensis	Phloem/cortex callus, basal callus, cambium callus near leaf traces.
Chamaecyparis spp.	Vascular rays.
Clematis spp.	Vascular cambium.
Cryptomeria spp.	Vascular rays.
Cupressus spp.	Vascular rays.
Forsythia suspensa	Leaf and bud traces.
Hedera helix (juvenile)	Phloem ray parenchyma.
Hedera helix (mature)	Phloem ray parenchyma and callus.
Ilex opaca	Vascular cambium extended into callus, young phloem after auxin treatment.
Larix spp.	Bud traces.
Ligustrum vulgare	Outgrowths of lenticels.
Picea spp.	Leaf traces, callus.
Pinus spp.	Leaf traces, callus.
Pseudotsuga menziesii	Callus.
Ribes alpinum	Secondary xylem, cambium.
Rosa 'Dorthy Perkins'	Secondary phloem.
Rosa dilecta 'Better Times'	Phloem ray parenchyma.
Rubus idaeus	Primary rays by leaf traces.
Rubus occidentalis	Leaf and bud traces.
Sambucus nigra	Outgrowths of lenticels.
Sciadopitys spp.	Branch trees.
Taxus cuspidata	Phloem ray cells, 2^{o} Phloem.
Thuja spp.	Vascular rays.
Thujopsis spp.	Rays, leaf traces.
Thujopsis dolobrata	Callus.
Ulmus campestris	Outgrowths of lenticels.
Vaccinium corymbosum	Cambium, phloem, callus.

[z]from Girouard (45).

1) preinitiation, 2) initiation and 3) post initiation with continued divisions of derivatives to form meristemoids.

Strangler (121) noted that in ARF of woody materials few authors have adequately traced details of initiation and early cell division accompanied by convincing photomicrographs which was attributed to difficulty in sectioning, staining and making detailed cellular studies with highly lignified tissues (43).

Formation of callus and ARF are generally independent of each other, but often occur simultaneously due to dependence on similar internal and environmental conditions (55). ARF has been found to originate in callus tissue, particularly in cuttings from difficult to root species (13,44,66,108). Ali and Westwood (2) noted that adult Pyrus cuttings developed more callus, but juvenile ones rooted better.

Poor rooting of woody plants has been attributed to extensive sclerification (13,44,66,108). Beakbane (10,11) proposed that thick lignified walls of sclerenchyma tissues were mechanical or physiological barriers to root initiation and elongation and that secretory canals and secretions have hindered ARF. Several researchers were unable to find simple relationships between density and continuity of sclerenchyma and rooting potential (44). Sach et al. (107) considered differences in ease of rooting were related to ease at which root initials were formed, not to restriction of developing root primordia by sclerenchyma.

Environmental Factors

Environmental factors such as water, light, temperature and seasonal variation influence biochemical reactions and thus ARF of cuttings.

Water relations of plants in propagation beds are influenced by humidity and temperature. Intermittent mist techniques maintain water balance of cuttings under high light intensities enabling carbohydrate formation, but also leach out nutrients as well as substances stimulatory and inhibitory to ARF.

Effects of light perception by leaves on ARF is generally positive, but there are conflicting reports (129). Basically there are plants with enhanced ARF after irradiation with visible light and those exhibiting ARF inhibition. High light intensity and longday conditions usually promote ARF (39,58,59,105), since quantitative changes occur in endogenous growth regulators such as increased auxin and gibberellic acid levels. Under longday conditions ARF was stimulated by auxins (60), and *in vitro* and *in vivo* systems high auxin to cytokinin ratios stimulated ARF and reduced bud formation (115). Stem etiolation and internal carbohydrate reserves have been associated with decreased lignin formation and increased ARF in the absence of light (63).

ARF responded best to high temperatures (25-30^{o}C) (21, 28,58,88,105); this has been attributed to change in endogenous growth regulators, possible rooting cofactors and decreased carbohydrate translocation (21,28).

Heide (59) suggested that seasonal change in ARF was a complex interaction of temperature, day-length and daily light energy on levels of endogenous auxins, cytokinins and other growth regulators. ARF in Ficus infectoria was optimal in summer and poor in winter which coincided with winter dormancy and low cambial activity (5). Seasonal variation was reported in a number of species (27,95) and correlated to slower ARF (13), increased abscisic acid levels (ABA)(126), decreased auxin levels (118) or changes in endogenous promoters and inhibitors (36).

Relative bud activity is thought to be a primary determining factor in seasonal fluctuation of ARF (135). Early bud removal in Rhododendron enhanced ARF apparently by eliminating antagonistic floral initiation stimulus, while later bud removal eliminated competition for materials necessary in rooting (72). Dahlia cuttings with actively growing buds were difficult to root and reproductive buds suppressed ARF more than vegetative ones (15). Smith and Wareing (119) reported bud removal in poplar reduced preformed root initial formation. Hassig (56) proposed that actively growing buds adversely effected ARF by competing for metabolites, yet at other stages enhanced ARF by stimulating cambial activity which was related to primordia initiation. Seasonal changes influence buds which are a source of promoters and inhibitor effecting ARF (102). Auxins can not alter seasonal variations of buds, but do promote earlier ARF (103). Increased RNA in shoot apical

cells has also been correlated to increased rooting potential (100).

Mycorrhizal fungi is another factor which has enhanced ARF in woody plant cuttings (80).

Selection of Plant Material and Juvenility

Type of cutting utilized (55), timing relative to season (102), position on shoot, stock plant condition and environment (133) effect ARF. Herman and Hess (63) found that lack of lignin formation under conditions of etiolation or cuttings in an earlier physiological stage of development enhanced ARF.

Physiological age has been correlated to ease of rooting with juvenile material exhibiting the most ARF (41, 65,109). Elongation of primordia into roots decreases with age of Salix stems (20). Embryonization is a process where cells revert from functional specialization to reenter the cycle of embryonic functions. This occurs in the dedifferentiation phase of ARF and older regenerating cells revert to embryonization more slowly (120). Kester (73) attributed control of juvenility to stem apices where changes in meristem were attributed to endogenous and exogenous factors, aging of meristems or competition between growing points as plant size increased (101).

Many theories such as nutritional factors, carbohydrate levels (2,29), nucleic acids (1), magnesium and protein content (2,75) have been advanced to explain juvenility. Zimmerman (136) reported that conditions producing most vegetative growth of seedlings had largest effect on shortening juvenile periods. The "embryo-sac experience" was proposed

by Swingle (124) where a juvenility factor was introduced into the embryo during its development and subsequently diluted" by growth to the point where it was ineffective. The paramutation theory of cytoplasmic factors interacting with chromosomes and repressing genes controlling adult characteristics has been supported by increased RNA/DNA ratios within cells with age (109,122). Romberger (104) proposed that regulatory mechanisms or conditions determine when certain segments of genetic information and not others are operative in apical cells.

Hormonal theories have been advanced to explain juvenility. Hess (65) found rooting cofactors present in higher concentration in juvenile Hedera, which prevented distruction of indole-3-acetic acid (IAA) by IAA-oxidase and permitted rooting. Conversely, Hacket (50) found that methanolic extracts of juvenile shoot tissue did not effect ARF of adult Hedera apices. Higher Gibberellin (GA) like substances were found in juvenile shoot apices of certain conifers and Marsilea than adult counterparts (40); yet exogenous GA is known to hasten maturation (3). ABA has been proposed as an inhibitor of ARF; yet Hillman et al. (67) detected higher levels in juvenile Hedera leaves.

Biochemical Factors

Sachs (106) related regeneration ability to root forming substances originating in leaves in 1890 and postulated that such substances were transported basipetally and accumulate at base of cuttings. He further suggested that their distribution was influenced by light and gravity.

Diffusates from leaves have been reported to induce ARF in cuttings (132). In the early 1930's the rhizocaline hypothesis (132) was proposed for ARF as a complex entailing three factors: 1) auxin, a nonspecific and mobile factor present in physiological concentrations, 2) an oxygen requiring enzyme located in specific cells and tissues, and 3) highly specific and mobile factor with orthodiphenolic groups which is synthesized in leaves exposed to light.

Nutritional substances can effect ARF. Kraus and Kraybill (77) reported that root number increased with high carbohydrate to nitrogen ratio, while the reverse promoted root growth and Lovell, et al. (81) observed that low nitrogen to high carbohydrate levels stimulated optimal ARF. Gautheret (42) reported that ARF involved sequences of morphogenetic phenomena with differing requirements for nutrients. Van Overbeek, et al. (130) observed that leaves and auxins were necessary for ARF, but leaves could be replaced by a mixture of auxin, sucrose and nitrogen. Tissue culture of Sinapsis alba cotyledons in sucrose solution in the dark promoted ARF, while in light grown cultures sucrose modified biochemical and structural changes inhibiting extension phase of root primordia (82). Sucrose also had osmotic as well as nutritional effects on ARF (49). More difficult to root materials required high sugar concentration (94) while easy to root materials had higher endogenous total carbohydrate content (98). Nanda and Jain (91) observed that auxin

effects on rooting were influenced by nutritional status of stem cuttings and a proper balance of the two is needed for optimal ARF. Molnar and LaCroix (87) found a positive correlation between starch content and root number of cuttings.

Auxin increases percentage and speed of ARF, but does not cause rooting in plants incapable of rooting otherwise (48,85) and rooting has been related to amount of free auxins at base of cuttings (38,48,85,92). Altman and Wareing (4) suggested that IAA had a direct effect on transport and increased "root-sink". Differences between easy and hard to root Populus was reported due to downward transport of assimilates, suggesting an auxin mediated effect.

A number of indole compounds exhibit auxin activity and their responses are traced to conversion to IAA (92) via B-oxidation (37). Varying biological activity of auxins and other hormones are best understood as differences in ability to reach sites of action (penetration and mobility), stability in tissue (resistance to distruction and alteration such as by IAA oxidase) and relative affinities for reaction cites (128). A relatively immobile auxin is 2,4-dichlorophenoxyacetic acid. Indolebutyric acid (IBA) is less active than naphthaleneacetic acid (NAA), but has greater plant tissue tolerance and is more widely used. IBA was superior to NAA and IAA (37) in stimulating ARF in leaf bud cuttings of Ficus elastica (88) and other plant species (128).

Cofactors are naturally occurring substances that act synergistically with IAA in promoting ARF and levels of cofactors have been attributed to ease of rooting in a number

of species (64,79). Cofactors have been identified as oxygenated terpenoids, and chlorogenic, isochlorogenic, caffeic and ferulic acids (46). Hess (65) contended that oxidation of an ortho-dihydroxy phenol is a first step leading to root initiation. Biran and Halevy (14) found no differences in rooting cofactors, but rather higher inhibitor levels in difficult to root Dahlia. Other workers have reported correlations between activity level of inhibitors and difficulty of rooting in cuttings (79,125).

Cytokinins were reported to inhibit ARF (26,68), but Skoog and Miller (115) observed low ratios of cytokinin to auxin levels led to optimal ARF. Reports on inhibition of ARF were valid if cytokinins were present in high concentrations during initial stages of root induction. Inhibiting effects of cytokinins disappeared late in the induction period and subsequent development of root primordia depended on cytokinins (34). Ericksen (33) reported that IAA triggered early formation of root primordia, but subsequent differentiation of vascular tissues depended additionally on cytokinins.

Brian, et al. (17) were the first to record inhibitory effects of GA on ARF and this was followed by other negative reports (22,61,83). In these cases GA was applied at initiation of experiments. Recent reports have shown promotive and inhibitory effects of GA on ARF depending on light irradiance (53,54) and developmental stage of ARF (23,24). Coleman and Greyson (23) proposed that GA acted indirectly on ARF by increasing endogenous auxin through inhibition of IAA oxidase sparing effect and/or stimulation of auxin synthesis

(23) and that GA_3 could be replaced by $GA_{4/7}$. Roles of GA_3 inhibiting ARF have been attributed to inhibition of starch accumulation in early ARF stages (24), and GA_3 mediated distruction of late forming primordia by reducing intraprimordium cell divisions (56). Nanda et al. (90) reported a simultaneous promotion of rooting and sprouting with GA_3 on Ipomoea.

Ethylene and ethylene generating compounds have been reported to enhance ARF (71,78), inhibit primordia formation and promote emergence of roots in stems with preformed primordia (89); Mullins (89) proposed that promotive effects of auxins which resulted in induction of root primordia were inhibited by auxin induced ethylene feedback and that ARF was promoted when rates of ethylene production were low relative to auxin concentration.

Inhibitors of basipetal auxin transport, such as 2,3,5-triodobenzoic acid have inhibited (18) and stimulated (9) ARF, and high levels of morphactins inhibited ARF while low levels promoted ARF (74). Morphactin inhibited ARF in Salix despite continued cambial activity, suggesting its role was to prevent differentiation of cambial derivatives into vascular elements(76). Punjabi et al. (97) reported that in presence of auxins, cuttings from morphactin pretreated stock plants rooted better than cuttings from control plants. ABA has been reported to inhibit and stimulate ARF (55), and succinic acid-2,2-dimethyl hydrazide (SADH) improved ARF (98) but did not promote ARF via modification of root promoters (62).

ARF depends on auxin-induced RNA and protein synthesis (6). Jain and Nanda (69) reported that RNA synthesis is needed for development of root primordia and also for polarized initiation of root primordia at basal ends of cuttings, and that inhibitors of RNA and protein synthesis delayed polarity of cuttings (69). They also proposed that auxins acted as a triggering agent for synthesis of specific enzyme protein required for root primordia intitiation at the transcriptional level. Molnar and LaCroix (87) reported that changes of enzymatic activity could be correlated with various stages of root primordia development and felt that peroxidase was responsible for distruction of certain inhibitors which normally blocked metabolic processes in ARF.

Developmental Sequences

Researchers in the 1970's have approached ARF as involving sequences of histological phenomena with differing requirements for growth substances (89,34) which rendered physiological events more meaningful. Ericksen (32,33) and Mohammed and Ericksen (86) found that influences of auxins and cytokinins on ARF changed with developmental stage. Sircar and Chatterjee (113,114) reported five histologically different stages where GA_3 (113) and IAA had varying promotive and inhibitive actions on ARF, while Shibaoka (110) reported at least three phases in ARF of Azukia cuttings. IAA and auxin antalogs 2,4,6-trichlorophenoxyacetic (2,4,6-T) and p-chlorophenoxyisobutyric (PCIB) acids stimulated initial root induction, while IAA and PCIB stimulated root primordia development. In ARF of Phaseolus Anzai (7) found a phase

sensitive to inhibitors of RNA or protein synthesis and one sensitive to an inhibitor of DNA synthesis. Smith and Thorpe (117) reported three phases of rooting where IBA was required for primordia formation, while kinetin inhibited preinitiative phase. GA_3 inhibited preinitiative phase, but enhanced primordia development if applied during first stage of root initiation, but if applied following establishment of meristemoids inhibited ARF.

Materials used in developmental sequencing experiments have been herbaceous annuals or hypocotyl cuttings, but with maturity there are biochemical and histological changes associated with decreased ARF. Herbaceous materials may not give a true index on changes occurring in mature woody materials.

Ficus pumila

Ficus pumila L. (Creeping Fig), is a woody ornamental vine in the Moraceae family used for covering walls and sold in hanging baskets. Ficus pumila was chosen as the test material since it exhibits strong dimorphism (25) rendering the juvenile form easily identifiable from the mature and shows differences in ARF with juvenile cuttings rooting easily and mature ones with difficulty. Table 2 lists differences between juvenile and mature forms. There have been no reports in the literature on ARF of Ficus pumila.

Table 2. Dimorphic characteristics of *Ficus pumila* L.[z]

	Juvenile	Mature
Growth habit	Plagiotropic	Orthotropic
Flowers & fruits	Absent	Present
Leaves	Bathyphylls	Acrophylls
Leaf shape	Cordate-ovate	Elliptic to oblong-elliptic
Hydathodes in leaves	Present	Absent
Petioles	Sessile	Up to 2.5 cm
Shoot growth	Vigorous	Slight
Anthocyanin	Common	Uncommon
Aerial roots	Present	Absent
Rooting ability	Good	Poor

[z]from Davies (26)

CHAPTER ONE

ADVENTITIOUS ROOT FORMATION IN THREE CUTTINGS TYPES OF *Ficus pumila* L.

Introduction

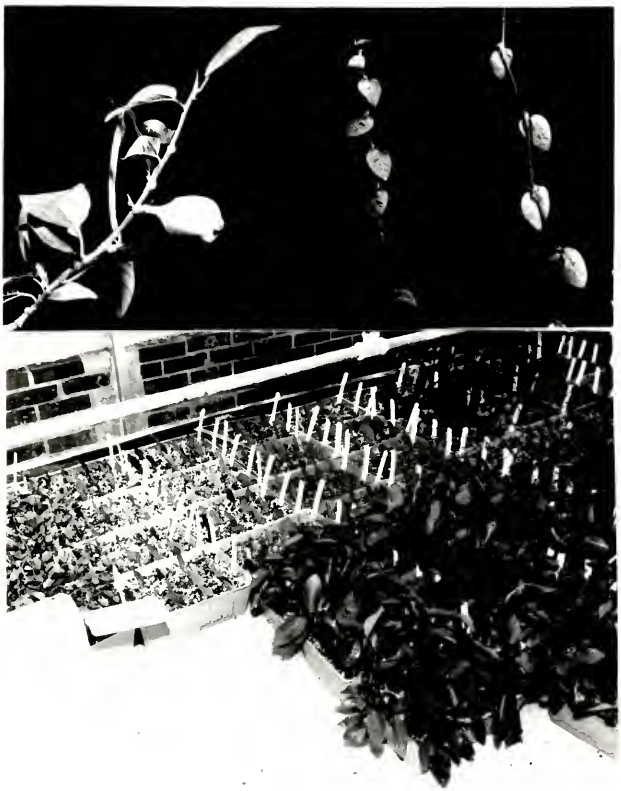
Objectives of these experiments were to establish optimal cuttings type in *Ficus pumila* among stem, leaf bud (LBC) and leaf cuttings. Criteria used to judge cuttings were difficult to root mature forms positive response to auxin treatment, ARF occurring via *de novo* root formation and rapid ARF occurrence minimizing environmental-physiological variables. With establishment of optimal cutting type future research could center on histological and physiological comparative studies on developmental sequencing of ARF in juvenile and mature form of this species.

Materials and Methods

Cuttings were taken from *Ficus pumila* stock plants cultivated on the University of Florida campus at Gainesville. Stem cuttings, leaf bud cuttings (LBC - lamina, petiole and 2.5 cm piece of stem with attached axillary bud) and leaf cuttings (lamina and petiole) were obtained from juvenile and mature terminal shoots (Fig. 1) and propagated under an intermittant mist system (Fig. 2) in a rooting medium of sterilized mason sand maintained at 24^{o}C and with a 2 hour night light interruption.

Fig. 1. Juvenile (J) and mature (M) forms of Ficus pumila.

Fig. 2. Standard propagation techniques for Ficus pumila during experiments.



Cuttings were treated with 3-indolebutyric acid and 2-naphthalenacetic acid in combination (IBA/NAA) and indoleacetic acid (IAA) applied in a 15 sec basal soak at experiment initiation. Juvenile stem, and mature stem and leaf cuttings were treated with 10000, 3000, 1000, 300, 100, 30, 10 mg/l IBA/NAA, while mature stem, LBC and leaf cuttings were treated with the same concentrations of IAA. Juvenile stem cuttings were treated with IAA at 10000, 1000, 10 mg/l. Mature LBC were treated with 10000, 1000, 100, 10 mg/l IBA/NAA.

There were 5 cuttings per experimental unit with 4 replications per treatment. The experiment was terminated after 90 days when cuttings were measured for percent rooting, root number and quality. Quality scale ranged from 1-4 with 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting. Data were analyzed by analysis of variance procedure and means of 20 measurements were compared at the 5% level of significance using Duncan's multiple range test.

Results

Juvenile stem cuttings treated with 1000 mg/l IBA/NAA and 10000 mg/l IBA/NAA and IAA had more roots than controls, and no auxin treatment had higher percent rooting and quality (Table 3). Macroscopic examination revealed few ARF occurred at base of cuttings and the majority occurring in nodal areas from preformed root initials (Fig. 3). Rooting was observed in all treatments by day 18.

Table 3. Adventitious root formation in Ficus pumila juvenile stem cuttings 90 days after auxin treatment.

Treatment (mg/l)	%Rooting[X]	No. Roots[X]	Quality[XY]
IBA-NAA			
10000	90ab	11.6ab	2.8bc
3000	90ab	10.3abc	3.1ab
1000	95ab	12.5a	3.3ab
300	100a	9.5bc	3.0abc
100	100a	9.0c	3.3ab
30	100a	8.5 c	3.4ab
10	100a	9.8bc	3.2ab
IAA			
10000	100a	11.5ab	3.2ab
1000	80b	8.3c	2.5c
10	100a	10.0bc	3.0abc
Control	95a	8.8c	2.9abc

[X]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[Y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Fig. 3-7. Origin of adventitious roots in juvenile and mature cuttings of Ficus pumila. Fig. 3-4, stem cuttings; Fig. 3, after 40 days: unrooted mature (M) and rooted juvenile (J); Fig. 4, 90 days experiment: rooted J. unrooted and rooted M. Fig. 5-7, leaf bud cuttings; Fig. 5-6, after 40 days: Fig. 5, juvenile: (A) de novo rooting, (B) nodal area rooting; Fig. 6, mature: roots from nodal areas of etiolated shoots; Fig. 7, 90 days experiment: unrooted M. and J. leaf cuttings vs. rooted leaf bud cuttings.

M
J
3
M
J
4
M
J
7
5
6

Mature stem cuttings treated with 10000, 3000, 1000 mg/l IBA/NAA had larger root number than controls (Table 4). ARF originated at base of cuttings (Fig. 4) by day 90, but at day 48 no cuttings sampled had visible roots.

ARF occurred readily in control juvenile LBC and origin was at nodal area and base of cuttings (Fig. 5).

At day 48 mature LBC sampled from 10000 and 1000 mg/l IBA/NAA and 3000 mg/l IAA had roots whereas control did not, but by day 90 no auxin treatment was better than control (Table 5). Roots were sometimes observed to form from nodal areas of etiolated shoots originating from axillary buds (Fig. 6).

No ARF occurred in juvenile leaf cuttings and only 10% rooting was recorded in mature leaf cuttings treated with 1000 mg/l IBA/NAA, but this was not significantly different from controls (Table 6). Juvenile leaves are sessile and mature petioles were considerably smaller than species normally propagated by leaves such as Peperomia (Fig. 7).

Discussion

LBC was the most satisfactory cuttings type since juvenile stem cuttings rooted predominantely from nodal areas and not de novo as desired and mature stem cuttings rooted too slowly, even with auxin treatment. Ficus pumila could not be propagated from leaf cuttings. Juvenile leaves were sessile, while petioles of mature leaves would only callus at the base with poor rooting. Leaf propagation has largely been confined to herbaceous species thus Moraceae genera are not commonly propagated by leaves (51).

Table 4. Adventitious root formation in Ficus pumila mature stem cuttings 90 days after auxin treatment.

Treatment (mg/l)	%Rooting[X]	No. Roots[X]	Quality[XY]
IBA-NAA			
10000	65a	8.3ab	2.7a
3000	80a	8.3ab	2.8a
1000	80a	9.5a	3.0a
300	80a	7.4abc	2.6a
100	70a	6.5abc	2.4a
30	80a	7.0abc	3.1a
10	60a	4.5abc	2.2a
IAA			
10000	55a	4.8abc	2.3a
3000	85a	5.5abc	2.3a
1000	85a	6.7abc	2.6a
300	55a	3.7bc	2.1a
100	80a	5.7abc	2.2a
30	60a	4.8abc	2.2a
10	55a	4.0abc	2.0a
Control	40a	2.0c	1.8a

[X]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[Y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 5. Adventitious root formation in Ficus pumila mature leaf bud cuttings 90 days after auxin treatment.

Treatment (mg/l)	%Rooting[x]	No. Roots[x]	Quality[xy]
IBA-NAA			
10000	55a	4.5ab	2.1a
1000	65a	5.3a	2.2a
100	45a	0.6b	1.3a
10	45a	2.8a	1.6a
IAA			
10000	20a	1.3ab	1.4a
3000	25a	1.5ab	1.9a
1000	25a	1.3ab	1.4a
300	25a	1.1ab	1.3a
100	20a	1.0ab	1.3a
30	40a	2.5ab	1.7a
10	40a	2.6ab	1.7a
Control	60a	2.9ab	2.3a

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 6. Adventitious root formation in Ficus pumila mature leaf cuttings 90 days after auxin treatment.

Treatment (mg/l)	%Rooting[x]	No. Roots[x]	Quality[xy]
IBA-NAA			
10000	0a	0a	1a
3000	0a	0a	1a
1000	10a	0.1a	1.1a
300	0a	0a	1a
100	0a	0a	1a
30	0a	0a	1a
10	0a	0a	1a
IAA			
10000	0a	0a	1a
3000	0a	0a	1a
1000	0a	0a	1a
300	0a	0a	1a
100	0a	0a	1a
30	0a	0a	1a
10	0a	0a	1a
Control	0a	0a	1a

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Control juvenile LBC rooted with ease while auxin treated mature LBC rooted faster than controls. Roots originated from nodes in juvenile cuttings and from etiolated shoots formed from axillary buds in mature LBC. Nodal areas had to be above rooting media in later experiments so that ARF would occur de novo at base of cutting.

Reasons for faster ARF in auxin treated LBC vs stem cuttings were not clear. LBC has only 1 leaf vs 4 to 5 leaves of stem cuttings which probably caused differences in quantity of such physiological materials as endogenous growth regulators, inhibitors and carbohydrates. LBC may have been less subject to stress since there was only 1 leaf exposed. There also may have been a tendency to insert base of stem cuttings further into media for better support where differences in carbon dioxide, oxygen and water saturation in pore spaces occurred.

LBC was established as the best system for ARF studies on developmental sequences in juvenile vs. mature Ficus pumila.

CHAPTER TWO

INITIATION AND DEVELOPMENT OF ADVENTITIOUS ROOTS IN JUVENILE AND MATURE CUTTINGS OF *Ficus pumila* L.

Introduction

A detailed ontogenetic study contrasting histological aspects of ARF between juvenile and mature *Ficus pumila* leaf bud cuttings (LBC) was established to characterize developmental sequencing and time of events so that future research could center on growth regulator interactions on ARF at discrete time intervals.

Materials and Methods

Ficus pumila L. stock plants cultivated on the University of Florida campus at Gainesville, provided cuttage material for this study. Terminal mature and juvenile shoots were employed to make leaf bud cuttings (LBC - lamina, petiole and 2.5cm piece of stem with attached axillary bud) which were propagated under an intermittant mist system in a rooting medium of sterilized mason sand which was maintained at 24^{o} C and with a 2 hour night light interruption.

A 2x2x21 factorial experiment was used with juvenile LBC pretreated with 0 and 1000 mg/l and mature LBC with 0 and 3000 mg/l IBA applied at experiment initiation as a foliar spray at 40 ml per flat containing 20 cuttings. Juvenile cuttings were harvested daily for 21 days and

mature ones every two days over a 42 day period to give 21 sampling times. Eight cuttings randomly selected constituted the experimental unit each sampling time to establish successive developmental stage and time of adventitious root formation (ARF). Cuttings were measured for rooting percentage, number and length, and data were analyzed with regression analysis at the 5% level of significance.

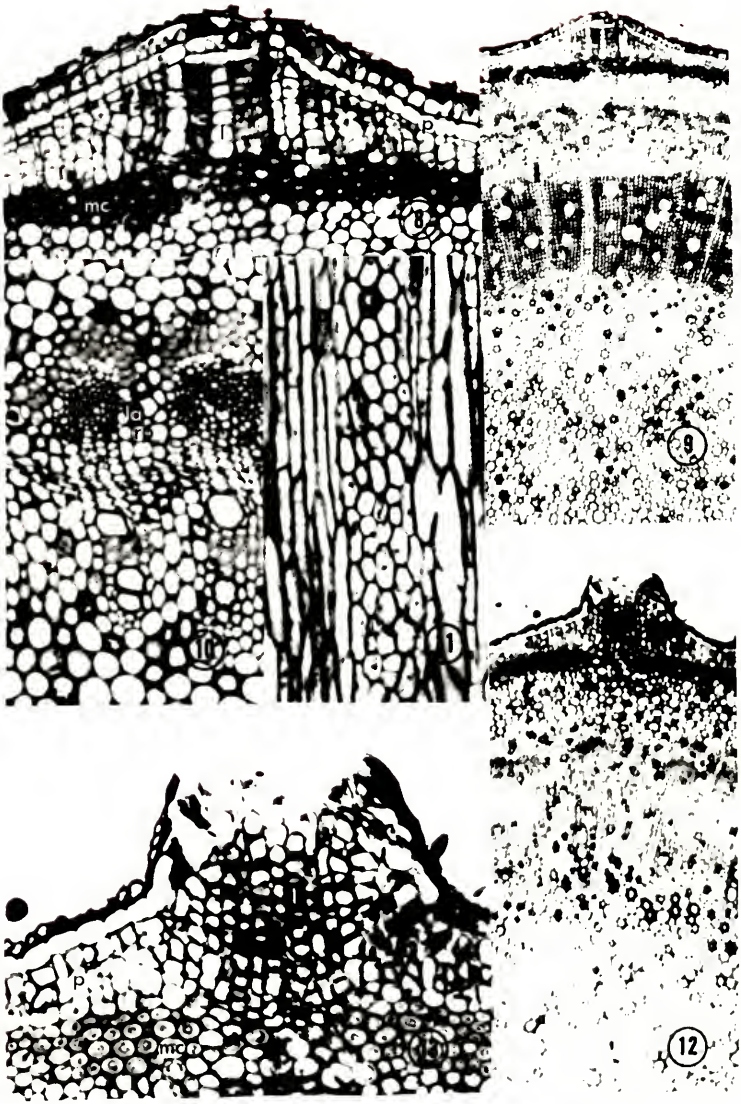
Basal 1 cm portion of each cutting was fixed in FAA (Formalin-acetic acid-ethanol) *in vacuo*, dehydrated in an ethanol-tertiary butyl alcohol series and embedded in Paraplast-plus. Ribbons satisfactory for developmental studies were difficult to make because of the lignified woody nature of *Ficus* unless blocks containing stem pieces with one surface exposed were soaked in water *in vacuo* for a minimum of five days to soften the material. Serial cross and longitudinal sections were cut at 8 and 11 um and stained with safranin and fast green.

Results

Juvenile Stem Anatomy

The periderm in juvenile stems was composed of a suberized phellem, phellogen and a phelloderm of 1 to 2 cell layers. Remains of the epidermal layer exterior to the phellem was sometimes present. Lenticels (Fig. 8) of the structural type represented by *Malus* and *Persea* (35) having a complementary or filling tissue composed of suberized cells were present. The cortex consisted of predominantly parenchyma cells with a continuous 1 to 3 cell layer thick

Fig. 8-13. Juvenile and mature stem anatomy. Photomicrographs are of stem cross, tangential or longitudinal sections. Fig. 8-11, details of juvenile stem; fig. 8, periderm (p), lenticel (l), 2-3 macrosclereids (mc) layers, x500; fig. 9, cross section of stem, x500; fig. 10, ray parenchyma (rp) blocked by laticifers (la) and phloem fibers (f), x500; fig. 11, tangential section: multiseriate ray parenchyma, x500. Fig. 12-13, details of mature stem; fig. 12, cross section of stem, x500; fig 13, periderm (p), lenticel (l), 4-5 macrosclereid layers, x500.


p
mc
8
Ja
Ji
10
1
9
12
p
men

ring of perivascular sclerenchyma (macrosclereids) to the exterior and phloem fibers to the interior (Figs. 8,9).

Vascular bundles were of the collateral type (Fig. 10) and vascular rays were multiseriate (Fig. 11). Phloem rays were sometimes blocked or partially separated from inner cortical cells by fibers and laticifers (Fig. 10). Phloem consisted of sieve tubes, companion cells, parenchyma and fibers, and thick walled xylem vessel elements were surrounded by fibers and rows of thick walled parenchyma. Pith was composed of parenchyma often containing crystals and laticifers. Laticifers were present in the stem as reported in other Ficus (131) and prismatic calcium oxalate crystals were found in all tissues, which rendered sectioning difficult.

Mature Stem Anatomy

The periderm, cortex, phloem, xylem and pith were similar in cell types but differed in cell number compared with juvenile stems (Fig. 12). A continuous ring of perivascular sclerenchyma (macrosclereids) exterior to the cortex had more cell layers than juveniles (Fig. 13) and phloem rays were sometimes blocked from inner cortical cells by fibers and laticifers.

Vascular bundles were collateral and vascular rays were multi-seriate as found in juveniles.

Juvenile Adventitious Root Formation

Three to four days after experiment initiation nuclei and cytoplasm became more prominent in distal phloem ray parenchyma and cambial activity increased which was associated

with dedifferentiation phase (Fig. 4). First anticlinal division of phloem ray parenchyma were observed four days after treatment application (Fig. 15). Root initials or slightly organized cells undergoing divisions formed in older phloem ray parenchyma and differentiation of cell groups into a recognizable root primordia were first observed at day 6 (Fig. 16). Periclinal divisions of cells at the outer surface of organized cell groups formed a tissue layer which subsequently gave rise to root caps in the cortex. Primordia began to elongate between vascular bundles and out into the cortical area (Fig. 17). Protoxylem differentiated acropetally to form tracheary elements while primordia continued to push through the cortex, ruptured the macrosclereid layer and emerged through the periderm (Fig. 18), and concurrently secondary xylem and phloem developed into vascular connections between young roots and stem vascular system. Primordia were observed to elongate through lenticels, but origin was from cambial phloem ray areas (Fig. 19).

Macroscopic examination at day 7 revealed the first emergence of roots at right angles to the main axis of stems, and by day 14 maximum rooting was recorded (Table 7 and Fig. 20).

There was no difference in origin or developmental stages in juvenile control; however, ARF was slower and no roots emerged till day 14.

Primordia were found in proliferating callus tissue at base of cuttings in control and IBA treatments, however,

Fig. 14-19. Developmental stage of rooting in juvenile leaf bud cuttings. Photomicrographs are of cross and longitudinal sections. Fig. 14, increase in cambial activity, (c)=cambium, (rp)=ray parenchyma, x780; fig. 15, first anticlinal division, x780; fig. 16, young primordia, x780; fig. 17, primordia elongation stage and rupture of macrosclereids and cortex, x265; fig. 18, adventitious root emerging from stem, x160; fig. 19, primordia developing through lenticel, x570.


14
15
16
17
18

Table 7. Time of adventitious root formation in juvenile and mature leaf bud cuttings of Ficus pumila treated with IBA.

	Juvenile	Mature
Cell Division	day 4	day 6
Primordia	day 6	day 10
1st Rooting[y]	day 7	day 20
Maximum Rooting[z]	day 14	day 28

[y]Linear regression with slope SD from 0 at 5% level.
[z]Based on 100% rooting, maximum root number and length analyzed by linear regression with slope SD from 0 at 5% level. See Figs. 20,26.

Fig. 20. Adventitious root formation in juvenile Ficus pumila leaf bud cuttings. Each point represents average of 8 LBC. Regression equations are as follows:

A) Control $Y = 0.018X - 0.005$, $r^2 = 0.45$

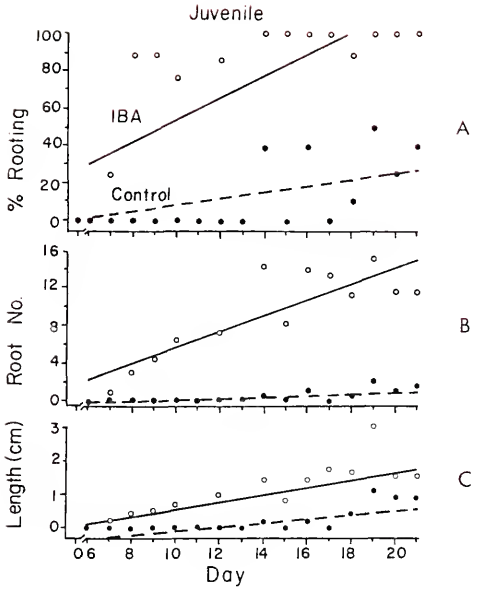
IBA $Y = 0.062 - 0.065$, $r^2 = 0.78$

B) Control $Y = 0.058X - 0.360$, $r^2 = 0.40$

IBA $Y = 0.841X - 2.684$, $r^2 = 0.84$

C) Control $Y = 0.039X - 0.247$, $r^2 = 0.49$

IBA $Y = 0.117X - 0.472$, $r^2 = 0.59$


Juvenile
% Rooting
IBA
Control
A
Root No.
B
Length (cm)
C
Day
100
80
60
40
20
0
16
12
8
4
3
2
1
06
8
10
12
14
16
18
20

these primordia were not observed to elongate into well developed roots.

Mature Adventitious Root Formation

Five to six days after experiment initiation, phloem ray parenchyma underwent similar dedifferentiation changes and increased cambial activity as reported in juvenile stems (Fig. 21). First anticlinal division occurred at day 6 (Fig. 22) followed by division of slightly organized cell groups into initials, primordia differentiation (Fig. 23) and elongation (Fig. 24,25). This followed the same developmental patterns as ARF in juvenile wood but occurred at later days (Table 7 and Fig. 26).

Primordia were found in proliferating callus tissue at base of control and IBA treated cuttings, although, identification of initiation stages of ARF in callus was extremely difficult. Root primordia originated in vicinity of differentiating tracheary elements with secondary wall thickenings (Fig. 27), which have been described as "callus xylem" or "tracheary nests" (19). In Ficus pumila callus primordia distinguished by these "tracheary nests" were connected with the main vascular system (Fig. 28), but were not observed to elongate into fully developed roots.

There was no difference in origin of primordia in controls, but they occurred at later periods than IBA treatments and no primordia elongated.

Discussion

Anatomical dissimilarities between juvenile and mature stems of Ficus did not account for differences in ARF.

Fig. 21-25. Developmental stage of rooting in mature leaf bud cuttings. Photomicrographs are of cross and longitudinal sections. Fig. 21, increase in cambial activity, (c)=cambium, (rp)=ray parenchyma, x1000; fig. 22, first anticlinal division, x1000; fig. 23, young primordia developing through the cortex, x700; fig. 24, later stage of primordia rupturing cortex and macrosclereids, x160.

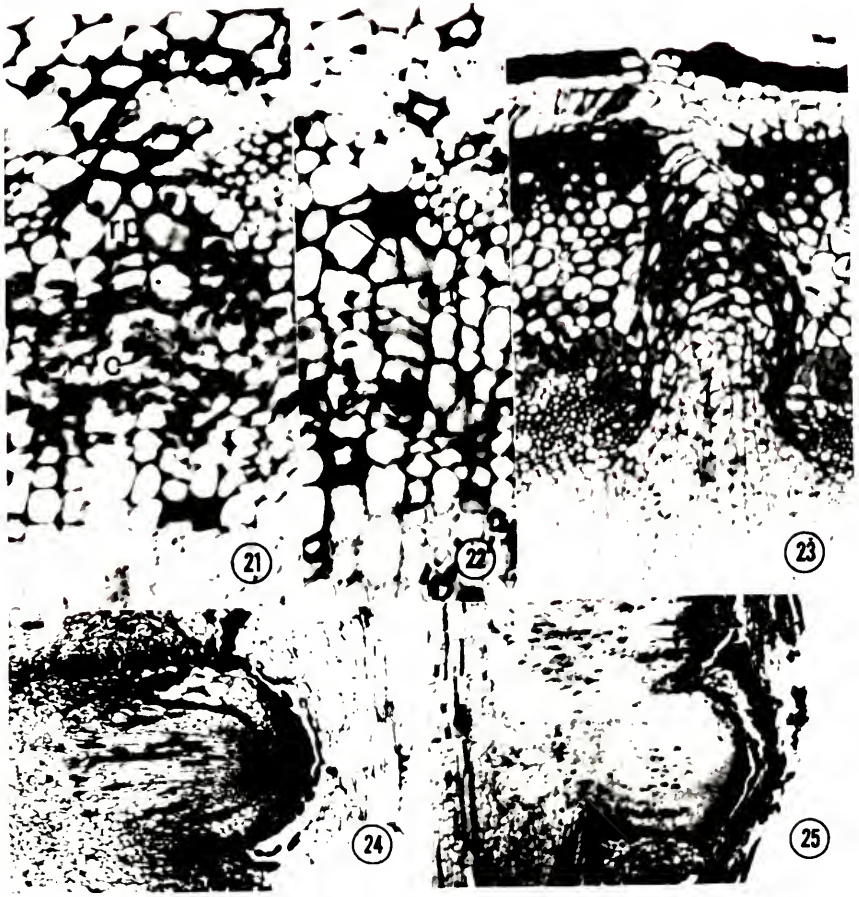

21
22
23
24
25

Fig. 26. Adventitious root formation in mature Ficus pumila leaf bud cuttings. Each point represents the average of 8 LBC. Regression equations are as follows:

A) Control $Y = 0$, $r^2 = 0$

IBA $Y = 0.053X - 0.171$, $r^2 = 0.79$

B) Control $Y = 0$, $r^2 = 0$

IBA $Y = 0.808X - 3.152$, $r^2 = 0.75$

C) Control $Y = 0$, $r^2 = 0$

IBA $Y = 0.091X - 0.319$, $r^2 = 0.76$

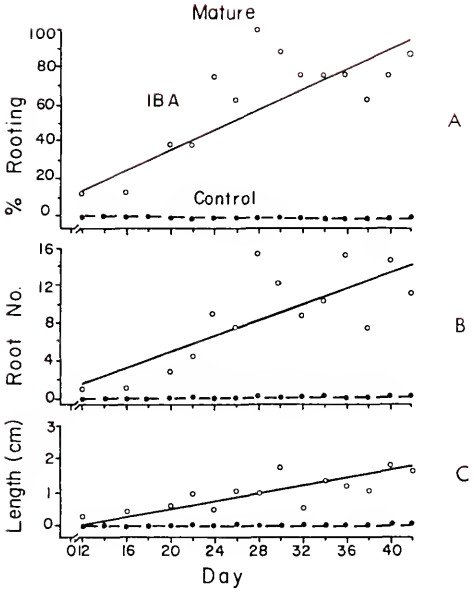

Mature
IBA
Control
% Rooting
100
80
60
40
20
0
A
Root No.
16
12
8
4
0
B
Length (cm)
3
2
1
0
C
012
16
20
24
28
32
36
40
Day

Fig. 27-28. Callus primordia formation in mature leaf bud cuttings. Photomicrograph are from longitudinal section of the stem. Fig. 27, "Tracheary nest", x1000; fig. 28, vascular connection developing between stem vascular system and "Tracheary nest", x750.


27
28

Perivascular sclereids (macrosclereids) were considerably thicker in mature stems, but primordia penetrated with relative ease through the cortex, pushing out 4 to 5 cell thick macrosclereids and emerging at right angles to plant stems. Bell and McCully (12) reported that in Zea lateral root development there was enzymatic and mechanical tearing of parent tissue, which may be the same process occurring in ARF in Ficus.

Whether differences in perivascular fibers and sclereids were associated with ARF in a cause and effect manner was difficult to determine. Doud and Carlson (31) reported that percent sclerification was negatively correlated with degree of rooting in Malus, which supported Beakbane's (11) observation that easy rooting species often had fewer schlerenchyma groups. Strong support for physiological rather than anatomical differences is shown in this research since at day 20, rooting responses of IBA treated LBC (Fig. 26) were equal to if not superior to juvenile controls (Fig. 20), and specialized cell groups were not an impediment for adventitious root initiation and development.

Laticifers common in the Moraceae (131) were found throughout plant stems of juvenile and mature Ficus pumila and often abutted the phloem ray, separating inner cortical cells. Contrary to reports that secretory canals and their secretions hinder ARF (11), this did not occur in either form of Ficus.

ARF initiated from phloem ray parenchyma in juvenile and mature Ficus as has been reported in other genera

(8,30,43,48,108,12). Closely associated with initiation was increased cambial activity, characteristic of an auxin response.

Callus primordia distinguished by differentiating tracheary elements (tracheary nests) with secondary wall thickenings were reported in Pseudotsuga (13) and Carya (19) to eventually connect with the vascular system. Similar observations were made in primordia developed in basal callus of IBA treated and untreated juvenile and mature LBC.

ARF has been reported to originate from outgrowth of lenticels (70,123). In Ficus pumila primordia were observed to elongate through lenticels but originated from the phloem ray and cambial area.

The process of ARF occurred more rapidly in juvenile than mature LBC and in IBA treated juvenile cuttings there was a 1 day lapse between primordia differentiation and first recorded rooting campared to 10 days in IBA treated mature ones. The 7 day time span between first rooting and maximum rooting in juvenile stems was comparable to the 8 day lapse in mature stems, taking into consideration that maximum rooting occurred at day 14 and 28 respectively in each form.

Wilson and Wilson (134) found few observations in the literature on rates of root production (i.e. number of roots developing per day) since it varies with time after cuttings are made. They reported that number of roots emerging daily from Sambus nigra cuttings maximized after 2 weeks and declined to near zero. They postulated that initiation rate was

inversely related to number of existing roots. Root emergence of IBA treated Ficus pumila juvenile and mature cuttings peaked at days 14 and 28 respectively and in later sampling periods percent rooting, root number and length did not increase. Primordia number were not evaluated, but there may have been similar numbers in day 14 compared to termination date of IBA treated Ficus juvenile cuttings and day 28 compared to termination date in mature ones.

IBA stimulated root initiation and may well effect elongation of primordia directly or indirectly since few primordia were observed in mature controls and none elongated into roots after 42 days. Origin of primordia was not affected by IBA.

Time separation of root initiation phases and development has made possible the study of concurrent physiological events in ARF of juvenile and mature Ficus pumila.

CHAPTER THREE

GROWTH REGULATOR EFFECT ON ADVENTITIOUS ROOT FORMATION APPLIED AT DISCRETE TIME INTERVALS TO JUVENILE AND MATURE *Ficus pumila* L. LEAF BUD CUTTINGS

Introduction

Ficus pumila L. (Creeping Fig) is a woody ornamental in the *Moraceae* family in which a detailed ontogenetic study constrasting histological aspects of ARF was reported (26). This study of concurrent physiological events due to growth regulator interactions at discrete time intervals has made possible correlations of morphological and physiological factors involved in adventitious rooting between juvenile and mature *Ficus pumila* LBC.

Materials and Methods

Ficus pumila L. stock plants cultivated on the University of Florida campus at Gainesville were used as cuttage material for this study. Terminal mature and juvenile shoots were employed to make leaf bud cuttings (LBC-lamina, petiole and 2.5 cm piece of stem with attached axillary bud) which were propagated under an intermittant mist system in a rooting medium of sterilized mason sand maintained at 24°C with a 4 hour light interruption previously described (26).

Growth regulators used in the 2x3x3 factorial experiment indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), gibberellic acid (GA_3), 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-

purine (PBA), (2-chloroethyl) phosphonic acid (Ethrel) and 2,3,5-tri-iodobenzoic acid (TIBA) applied as aqueous sprays with 0.25 ml per liter of the surfactant, emulsifiable A-C polyethylene and octyl phenoxy polyethoxy ethanol (Plyac).

IBA, IAA and GA_3 at 10, 100, 1000 mg/l each were screened on juvenile and mature LBC in experiment 1 to determine optimal auxin and GA_3 effects when applied prior to sticking. IBA was applied at 500, 1000, 1500, 2000, 3000, and 10000 mg/l to juvenile and 2000, 2500, 3000, 3500, 4000 and 10000 mg/l to mature LBC prior to sticking in experiment 2 to determine optimal concentration for ARF in juvenile and mature cuttings. Effects of PBA and TIBA at 10, 100, 1000 mg/l and Ethrel at 100, 1000, 3000 mg/l on ARF were compared on juvenile and mature LBC in experiment 3 to 100 mg/l IBA in juvenile and 3000 mg/l IBA in mature cuttings. A 2x3x3 factorial experiment was employed in experiment 4 in which pretreatment of 1000 mg/l was applied to half the juvenile cuttings and 3000 mg/l to half the mature ones at time of sticking. The other variables included three growth regulators -- IBA at 1000 mg/l for juvenile and 3000 mg/l for mature cuttings, 1000 mg/l PBA and 3000 mg/l GA_3 for both types -- were applied 3,5 and 7 days after sticking for juvenile and 3,9 and 15 days for mature cuttings. Ten cuttings of each treatment combination were selected at each of the three time intervals and fixed, dehydrated, embedded and sectioned under procedures previously reported (26) to determine stage of ARF.

Treatments in the four experiments were placed in a randomized complete block design with 10 LBC as the experimental unit and replicated 4 times, except in experiment 4 where 8 cuttings constituted the experimental unit. Juvenile cuttings were harvested 21 days after initiation and mature ones after 42 days. Cuttings were measured for percent rooting, numbers of roots, root length and rated on quality scale of 1 to 4 with 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting. Data were analyzed by analysis of variance and compared at the 5% level of significance using Duncan's multiple range test.

Results

Experiment 1

There were no differences between juvenile LBC treated with 1000 mg/l IBA or IAA in percent rooting or root length, but those receiving 1000 mg/l IBA averaged 10.4 root per cutting compared with those receiving IAA which averaged only 4.5 (Table 8). The only other treatment that produced a higher rooting percentage than controls was 100 mg/l IBA. No chemical treatment effected root length and GA_3 did not influence rooting parameters measured. IBA at 1000 mg/l was the only treatment that resulted in increased percent rooting, root number and length in mature LBC (Table 9), and IAA and GA_3 did not effect parameters measured.

IBA was selected as the most effective auxin to be used for future rooting experiments with *Ficus pumila*.

Table 8. Effects of IBA, IAA and GA_3 on adventitious root formation in *Ficus pumila* juvenile leaf bud cuttings after 21 days.

Treatment (mg/l)	%Roots[x]	No. Roots[x]	Root length[x]
IBA			
10	48cde	1.7cd	1.2abc
100	73abc	3.7bc	1.2abc
1000	100a	10.4a	1.6a
IAA			
10	70abcd	2.5bcd	0.9abc
100	53bcde	2.0bcd	1.2abc
1000	85ab	4.5b	1.6a
GA_3			
10	20e	0.5d	0.5c
100	30e	0.8d	0.7bc
1000	25e	1.1cd	0.3c
Control	30e	1.6cd	0.8abc
Control + Surfactant	35de	1.2cd	1.4ab

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.

Table 9. Effects of IBA, IAA and GA_3 on adventitious root formation in Ficus pumila mature leaf bud cuttings after 42 days.

Treatment (mg/l)	%Rooting[x]	No. Root[x]	Root length[x]
IBA			
10	0b	0b	0b
100	10b	0.2b	0.3b
1000	75a	5.9a	2.9a
IAA			
10	0b	0b	0b
100	0b	0b	0b
1000	8b	0.1b	0.2b
GA_3			
10			
100	0b	0b	0b
1000	0b	0b	0b
Control	0b	0b	0b
Control + Surfactant	0b	0b	0b

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.

Experiment 2

There were no treatments more effective in juvenile LBC than 1000-1500 mg/l IBA range in stimulating percent rooting and root number, length and quality (Fig. 29,30,31,32). The higher levels reduced root length and 3000 and 10000 mg/l reduced root quality, indicating supraoptimal levels, but these levels were still better than controls (Fig. 33).

There were no treatments more effective than the 2000-3000 mg/l IBA range in stimulating ARF in mature LBC (Fig. 29,30,31,32), but the 10000 mg/l level reduced root length indicating a supraoptimal effect (Fig. 33).

Experiment 3

TIBA and Ethrel did not effect ARF in juvenile or mature LBC (Tables 10,11), while 1000 mg/l IBA on juvenile and 3000 mg/l IBA in mature ones stimulated rooting parameters measured.

Experiment 4

Percent rooting in juvenile LBC pretreated with 1000 mg/l IBA were not effected by applying IBA 3,5 or 7 days after sticking (Table 12), however, root number was increased with IBA application at day 5. There was reduced quality when IBA was applied at day 7 and reduced root length at all day treatments.

GA_3 reduced root length and quality regardless of application time (Table 12 and Fig. 34) and root number was reduced when applied at day 5.

PBA treatment reduced root number, length and quality regardless of the period applied with the greatest inhibition

Fig. 29. Effect of IBA on percent rooting in juvenile and mature leaf bud cuttings of Ficus pumila. Points with same lower case letters are not significantly different.

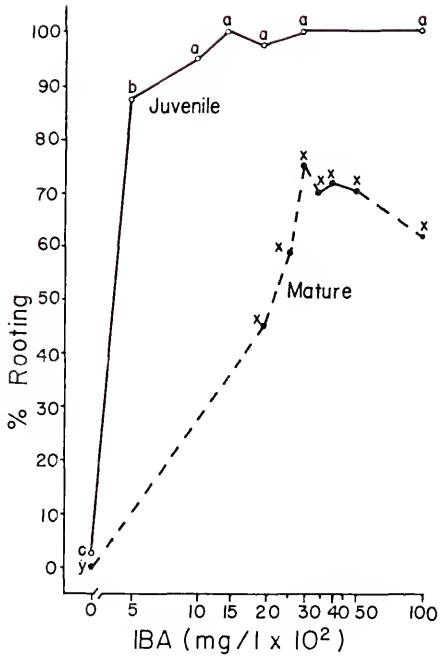

% Rooting
100
90
80
70
60
50
40
30
20
10
0
a
a
a
a
a
b
Juvenile
c
y
x
x
x
x
x
x
x
Mature
0
5
10
15
20
30
40
50
100
IBA (mg/l x 10²)

Fig. 30. 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.

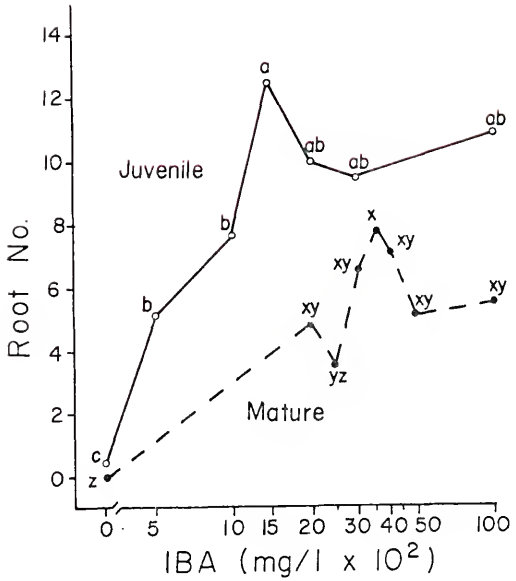

Root No.
14
12
10
8
6
4
2
0
Juvenile
Mature
a
ab
ab
ab
b
b
x
xy
xy
xy
xy
xy
c
z
yz
0
5
10
15
20
30
40
50
100
IBA (mg/l x 10²)

Fig. 31. Effect of IBA on root length in juvenile and mature leaf bud cuttings of Ficus pumila. Points with same lower case letters are not significantly different.

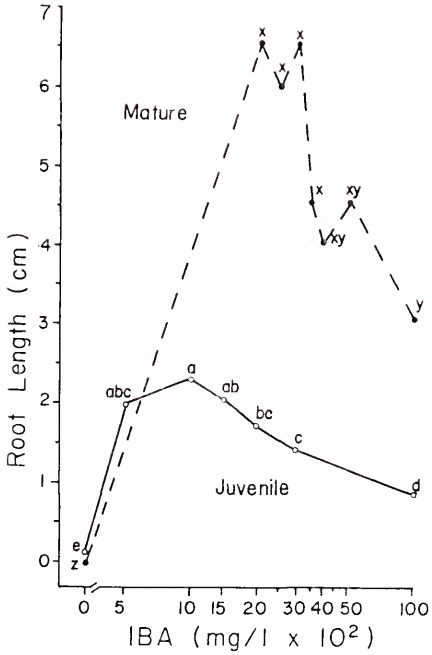

Root Length (cm)
7
6
5
4
3
2
1
0
Mature
Juvenile
x
x
x
x
xy
xy
y
a
ab
abc
bc
c
d
e
z
0
5
10
15
20
30
40
50
100
IBA (mg/l x 10²)

Fig. 32. 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.

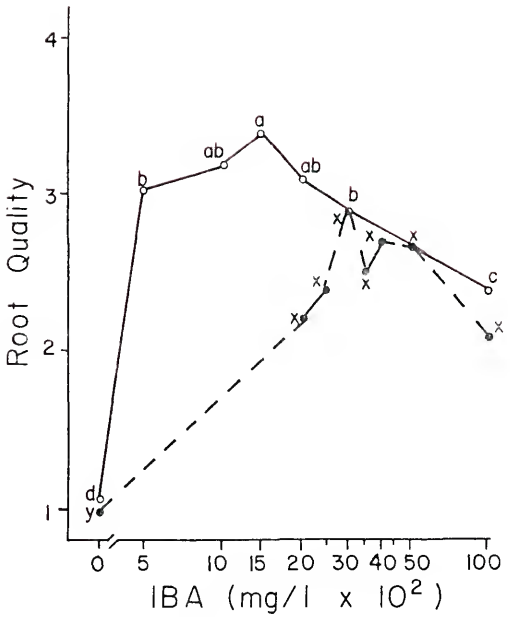
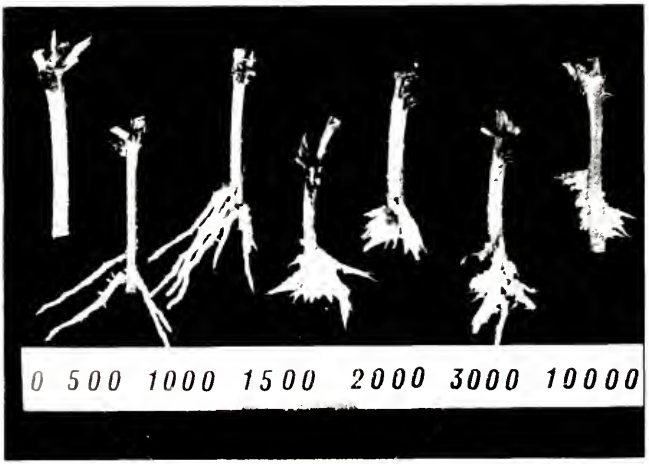
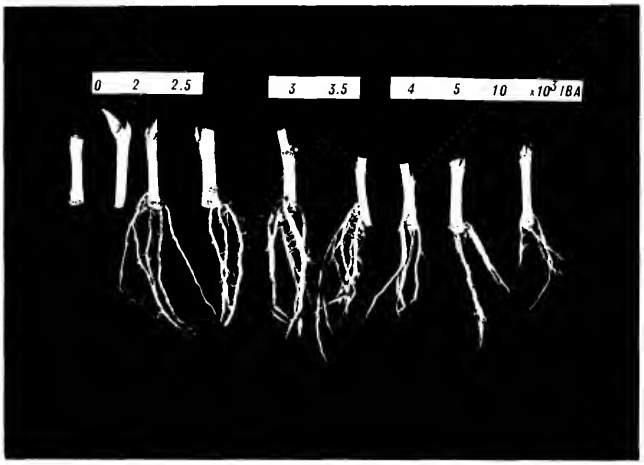

Root Quality
4
3
2
1
a
ab
ab
b
b
c
d
y
x
0
5
10
15
20
30
40
50
100
IBA (mg/l x 10²)

Fig. 33. Effect of IBA on adventitious root formation on A) juvenile and B) mature leaf bud cuttings of *Ficus pumila*.



A



B

Table 10. Effects of four growth regulators on adventitious root formation in Ficus pumila juvenile leaf bud cuttings after 21 days.

Treatment (mg/l)	%Rooting[X]	No. Root[X]	Root length[X]	Quality[XY]
IBA				
1000	95a	7.5a	3.8a	3.7a
PBA				
10	43b	1.1b	2.9ab	1.7b
100	28b	0.7b	2.3abc	1.4b
1000	15b	0.3b	0.9c	1.2b
TIBA				
10	35b	0.5b	2.1abc	1.4b
100	48b	1.6b	3.0ab	1.8b
1000	23b	0.4b	1.7bc	1.3b
Ethrel				
100	30b	0.6b	1.9bc	1.5b
1000	25b	0.5b	1.2bc	1.4b
3000	25b	0.7b	0.7b	1.3b
Control	43b	1.5b	1.4bc	1.7b
Control + Surfactant	53b	1.4b	1.9bc	1.8b

[X]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[Y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 11. Effects of four growth regulators on adventitious root formation in Ficus pumila mature leaf bud cuttings after 42 days.

Treatment (mg/l)	%Rooting[x]	No. Root[x]	Root length[x]	Qualitry[xy]
IBA				
3000	73b	8.9a	2.6a	2.7a
PBA				
10	0b	0b	0b	1b
100	0b	0b	0b	1b
1000	0b	0b	0b	1b
TIBA				
10	0b	0b	0b	1b
100	0b	0b	0b	1b
1000	0b	0b	0b	1b
Ethrel				
100	0b	0b	0b	1b
1000	0b	0b	0b	1b
3000	0b	0b	0b	1b
Control	0b	0b	0b	1b
Control + Surfactant	0b	0b	0b	1b

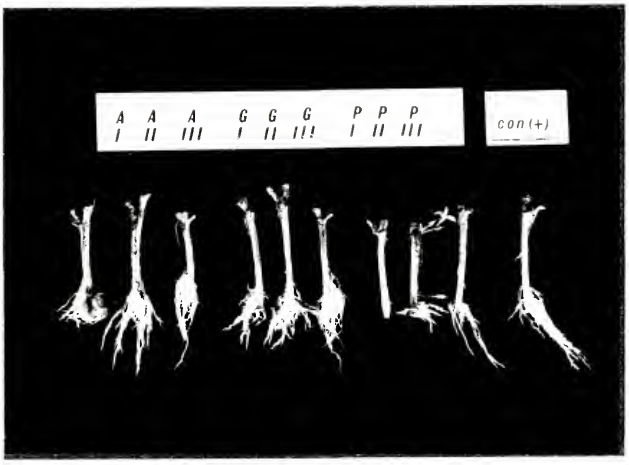
[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 12. Adventitious root formation in Ficus pumila juvenile leaf bud cuttings pretreated with 1000 mg/l IBA at time of sticking and three growth chemicals applied at three different time intervals in a 21 day experiment.

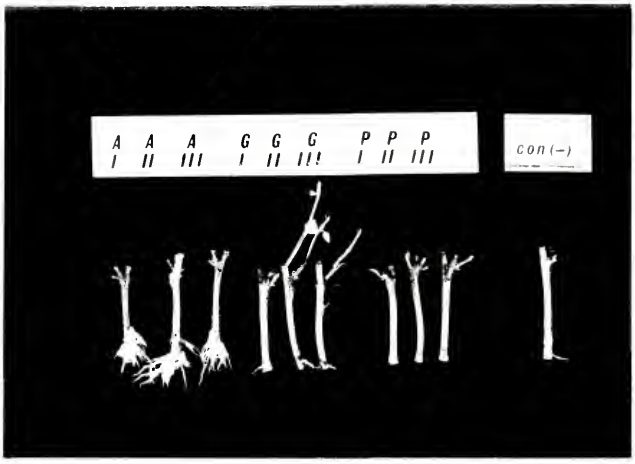
Treatment	%Rooting[x]	No. Roots[x]	Root length[x]	Quality[xy]
IBA (1000 mg/l)				
day 3	100a	12.7b	1.5bc	3.0babc
day 5	100a	15.2a	1.3bcd	3.16ab
day 7	100a	12.4bc	1.0bcde	2.72cd
GA_3 (3000 mg/l)				
day 3	100a	10.8bcd	1.3bc	2.69cde
day 5	100a	9.0ef	1.5bc	2.75cd
day 7	100a	10.2de	1.7b	2.84bcd
PBA (1000 mg/l)				
day 3	38c	1.3h	0.5ef	1.38gh
day 5	66b	5.3g	1.3cde	2.00f
day 7	88a	7.2fg	1.2bcde	2.31ef
Control	100	11.9bcd	2.5a	3.40a

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[Y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Fig. 34. Effect of IBA, GA_3 and PBA on adventitious root formation when applied at three time intervals to juvenile leaf bud cuttings A) treated with IBA B) untreated at day 1.


A A A G G G P P P
I II III I II III I II III
con(+)

A


A A A G G G P P P
I II III I II III I II III
con(−)

B

occurring when applied 3 days after cuttings were stuck (Table 12). Microscopic examination indicated that increased cambial activity associated with early ARF was occurring at this time (Table 13). PBA caused less inhibition of ARF when sprayed at day 5 than 3 when root initials and primordia were first observed. Half the LBC rooted by day 7 (Table 13), thus PBA application at this time did not effect percentage rooting, but did reduce root number, length and quality.

IBA treatment applied at days 3,5 and 7 increased ARF in juvenile LBC not pretreated with IBA on day 1 (Table 14). GA_3 had no effect on rooting (Fig. 34), which contrasted to reduced root length and quality of juvenile cuttings treated with IBA at day 1.

ARF in mature LBC pretreated with 3000 mg/l IBA was uneffected by later IBA treatment at 5,9 or 15 days after sticking (Table 15 and Fig. 35). GA_3 treatments reduced length and quality as was true with juvenile LBC pretreated with IBA, but reduced root numbers when applied at days 9 and 15.

In mature IBA pretreated cuttings PBA treatment at day 3 completely inhibited ARF and no cambial activity was observed (Table 16). PBA was less effective in inhibiting ARF at day 9 when cambial activity was first observed than in day 4 (Table 15). Root length and quality were reduced with PBA application at any period, but had no effect on percent rooting or number at day 15.

Table 13. Stage of adventitious root formation of juvenile leaf bud cuttings at three time intervals.

Treatment	Increased Cambial activity	Root Initials	Root primordia	%Rooting	No. Rooting	Root length	Quality[y]
IBA pretreatment at (1000 mg/l).							
day 3	yes	none	none	0	0	0	1
day 5	yes	yes	yes	0	0	0	1
day 7	yes	yes	yes	50	6.2	0.7	1.6
No IBA pretreatment							
day 3	none	none	none	0	0	0	1
day 5	yes	none	none	0	0	0	1
day 7	yes	yes	yes	20	0.4	0.5	1.2

[y]Quality scale ranged from 1=rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 14. Adventitious root formation in Ficus pumila juvenile leaf bud cuttings not pretreated with IBA at time of sticking and three growth chemicals applied at three different time intervals in a 21 day experiment.

Treatment	%Rooting[x]	No. Root[x]	Root length[x]	Quality[xy]
IBA (1000 mg/l)				
day 3	100a	9.5e	1.1bcde	2.59de
day 5	100a	11.0bcde	1.1bcde	2.75cd
day 7	100a	10.3cde	1.0bcde	2.53de
GA_3 (3000 mg/l)				
day 3	31c	0.7h	0.8cde	1.31gh
day 5	28c	0.8h	0.7de	1.28gh
day 7	34c	1.0h	1.5bcd	1.47g
PBA (1000 mg/l)				
day 3	0d	0h	0f	1.00h
day 5	25c	0.9h	1.2bcde	1.34gh
day 7	25c	0.9h	1.4bcd	1.31gh
Control	31c	0.8h	1.7b	1.34gh

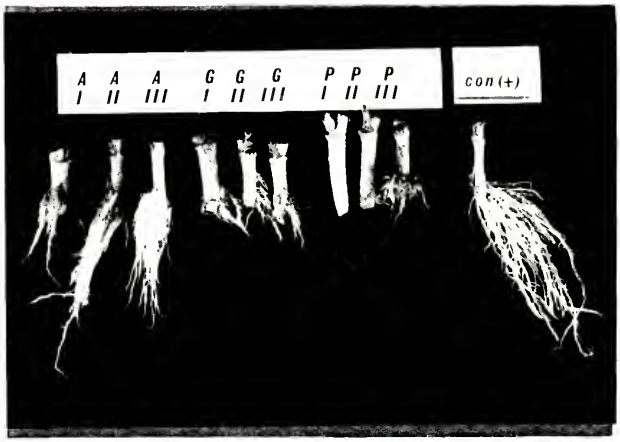
[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[Y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Table 15. Adventitious root formation in Ficus pumila mature leaf bud cuttings pretreated with 3000 mg/l IBA at time of sticking and three growth chemicals applied at three different time intervals in a 42 day experiment.

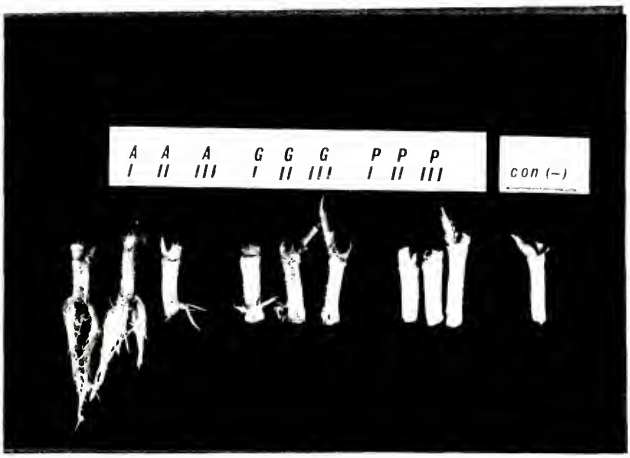
Treatment	%Rooting[x]	No. Root[x]	Root length[x]	Quality[xy]
IBA (3000 mg/l)				
day 3	81abcd	11.1bcd	2.1bcd	2.56bcd
day 9	100a	16.1a	3.1ab	3.16ab
day 15	91ab	13.7ab	2.1bcd	2.69abc
GA_3 (3000 mg/l)				
day 3	66bcdef	8.4cde	1.6cd	2.03def
day 9	50defg	6.0ef	1.7cd	1.81efg
day 15	66bcdef	7.3de	2.2bc	2.09cde
PBA (1000 mg/l)				
day 3	0h	0g	0e	1.00h
day 9	28gh	1.6fg	1.0cde	1.34h
day 15	75abcde	9.2bcde	1.3cde	2.21cde
Control	94ab	13.3abc	3.8a	3.25a

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

Fig. 35. Effect of IBA, GA_3 and PBA on adventitious root formation when applied at three time intervals to mature leaf bud cuttings A) treated with IBA and B) untreated at day 1.


A A A G G G P P P
I II III I II III I II III
con(+)

A


A A A G G G P P P
I II III I II III I II III
con(-)

B

Table 16. Stage of adventitious root formation of mature leaf bud cuttings at three time intervals.

Treatment	Increased cambial activity	Root initials	Root primordia	%Rooting	No. Rooting	Root length	Quality[y]
IBA pretreatment at (3000 mg/l)							
day 3	none	none	none	0	0	0	1
day 9	yes	none	none	0	0	0	1
day 15	yes	yes	yes	20	1.7	0.5	1.2
No IBA pretreatment							
day 3	none	none	none	0	0	0	1
day 9	none	none	none	0	0	0	1
day 15	yes	none	none	0	0	0	1

[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

IBA treatment at days 3 and 9 increased rooting parameters in mature LBC not pretreated with IBA (Table 17 and Fig. 35), but there were no differences between IBA treatment at day 15 and controls.

GA_3 and PBA did not effect rooting in mature LBC unless they were pretreated with IBA.

Discussion

IBA was more effective than IAA in stimulating ARF in Ficus pumila LBC which agrees with reports on other plant species (33,128). GA_3 at 10, 100, 1000 mg/l applied at day 1 did not influence ARF in juvenile or mature cuttings, which contrasted with reports of gibberellin inhibition (47,61, 83) and stimulation (22,53) of rooting in other species. GA_3 concentrations may have been too low to effect ARF in Ficus pumila, since 3000 mg/l reduced rooting.

Previous studies (26) indicated that maximum rooting occurred at day 14 in IBA treated juvenile LBC compared with day 28 for mature ones. Juvenile cuttings rooted best when treated with 1000 to 1500 mg/l IBA, whereas 2000 to 3000 mg/l gave best results with mature cuttings. IBA treated mature cuttings required higher IBA levels and longer time to obtain maximum rooting, which suggest they have lower endogenous auxin levels than juvenile LBC, or that they require higher auxin levels and/or other endogenous chemicals to stimulate cells to become remeristematic. ARF in IBA treated mature cuttings was slower than in juvenile LBC but equalled juvenile controls by day 20 giving strong evidence that endogenous auxin levels were acting as the rate limiting

Table 17. Adventitious root formation in Ficus pumila mature leaf bud cuttings not pretreated with IBA at time of sticking and three growth chemicals applied at three different time intervals in a 42 day experiment.

Treatment	%Rooting[x]	No. Root[x]	Root length[x]	Quality[xy]
IBA (3000 mg/l)				
day 3	84abc	13.1abc	3.4ab	3.03ab
day 9	94ab	8.6cde	3.0ab	2.66abc
day 15	53cdefg	2.7fg	1.0cde	1.66efg
GA_3 (3000 mg/l)				
day 3	44efg	2.0fg	0.7de	1.47fgh
day 9	41 fg	1.9fg	0.8cde	1.47fgh
day 15	38fg	1.1fg	0.8cde	1.34gh
PBA (1000 mg/l)				
day 3	0h	0g	0e	1.00h
day 9	0h	0g	0e	1.00h
day 15	0h	0g	0e	1.00h
Control	22gh	1.5fg	1.1cde	1.34gh

[x]Means followed by different letters are significantly different at 5% level, Duncan's multiple range test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting, 3=medium rooting and 4=heavy rooting.

factor in rooting this material.

Increasing IBA levels caused percent rooting and root number to peak and level off suggesting that high levels of auxin stimulate and do not hinder primordia differentiation which agrees with Miller and Skoog's (115) observation that high auxin to cytokinin levels encouraged root formation. Heide (59) found that too high an auxin to cytokinin level decreased root number in Begonia, which was contrary to these results with Ficus where only primordia elongation and root quality were reduced.

Root initials and root primordia were observed at day 5 in juvenile cuttings pretreated with IBA. At day 5 application of IBA increased and GA_3 decreased root number which may be related to the number of primordia formed, indicating that IBA has a stimulatory effect on root initiation and root primordia differentiation, while GA_3 has an inhibitory effect. PBA had the greatest inhibitory effect on early developmental stages of ARF when increased cambial activity was observed.

When juvenile LBC were not IBA pretreated, IBA treatment at days 3,5 and 7 probably increased cambial activity, root initials and primordia differentiation since percent rooting, root number and quality were increased.

ARF was generally higher in all IBA pretreated cuttings compared with untreated ones, except when IBA was applied at day 7 where rooting was equal. This correlated with observed root initials and primordia in pretreated and untreated cuttings.

An interaction occurred between GA_3 and IBA whereby root number (indicative of root initials and primordia differentiation and elongation) decreased at days 5 and 7 which correlated with observation that root initials and primordia were already present at this time.

Root initials and root primordia were observed at day 15 in IBA pretreated mature cuttings indicating that IBA stimulated cambial activity, root initials and primordia as in juveniles. Further treatments of IBA did not effect rooting.

The interaction of IBA and GA_3 at days 9 and 15 reduced root number in IBA pretreated mature LBC which can be correlated with increased cambial activity at day 9 and root initials and primordia at day 15. This indicates that in juvenile cuttings IBA/GA_3 effected existing cambial activity and root initials and primordia; whereas before cambial activity was observed IBA/GA_3 had no apparent effect.

PBA in IBA pretreated cuttings reduced percent rooting and root number at days 3 and 9 indicating an interaction, which was correlated with observations that no initials or primordia had differentiated during this time.

In non pretreated mature cuttings IBA application at days 3 and 9 increased rooting, which indicated that IBA may increase cambial activity since at these days, neither cambial activity, initials or primordia were observed. GA_3 and PBA alone had no effect on rooting.

Smith and Thorpe (117) did not take into account interactions between GA_3 and IBA even though all their chemical treatments were done in the presence of IBA. They reported three phases of primordia formation in which GA_3 inhibited the preinitiative phase, but enhanced primordia development if applied during the first stage of root initiation, and if applied following establishment of meristemoids inhibited ARF. This did not agree with results of this experiment, since in Ficus GA_3 had no effect on percent rooting or root number before (mature) and when (juvenile) cambial activity was first observed, and it had an inhibitory effect on root number when increased cambial activity, root initials and primordia were observed in mature and juvenile LBC. Results in this experiment agree with Hassig's work (56) who attributed GA_3 effect on reducing intraprimordia cell division.

Effects of PBA on Ficus coincide with reports that cytokinins inhibited preinduction phases of rooting (117) with a loss of inhibitory effect at later stages (34).

Results indicate that auxins effected initiation of cambial activity, and root initials and primordia which is in agreement with reports that IBA triggered early formation of root primordia (33).

Differences in adventitious rooting between juvenile and mature cuttings may be partially attributed to endogenous auxin levels. Support for this postulation is shown by lower IBA requirements for optimal rooting and ARF stimulation in IBA treated juvenile LBC compared to mature ones.

CONCLUSIONS

Primary objectives of this research were to describe and separate in time phases root initiation and subsequent differentiation and elongation of root primordia by microtechnique study concurrent physiological events of growth regulator interactions and compare these criteria between juvenile and mature forms.

Adventitious root formation (ARF) was studied in juvenile and mature leaf bud cuttings (LBC) of Ficus pumila L. (Creeping Fig) in experiments conducted under standard cultural conditions.

A detailed ontogenetic study contrasting histological aspects of ARF between juvenile and mature Ficus pumila LBC was reported to establish developmental sequencing so future research could center on growth regulator interactions on ARF at discrete time intervals.

Microscopic examination of ARF in juvenile and mature LBC indicated that both forms of cuttings exhibited remeristemation, early cell divisions (root initials), differentiation of primordia and root elongation. Primordia in juvenile LBC treated with indole-3-butyric acid (IBC) occurred at day 6 and in mature ones at day 10, roots emerged at days 7 and 20, respectively, with maximum rooting occurring at days 14 and 28. ARF originated primarily from phloem ray parenchyma.

Root primordia differentiated from some basal callus of juvenile and mature LBC, but these nor the few primordia in mature controls elongated into well developed roots. IBA stimulated root initiation but did not effect primordia origin and perivascular fibers, sclereids and laticifers did not influence ARF. Time separation of root initiation phases and development allowed the study of concurrent physiological events in ARF of juvenile and mature LBC of this species.

Foliar applications of IBA applied at day 1 was more effective than indole-3-acetic acid (IAA) in stimulating adventitious root formation and 1000 to 1500 mg/l IBA was optimal for juvenile and 2000 to 3000 mg/l IBA for mature LBC. Foliar application of 2,3,5-triiodobenzoic acid (TIBA) and (2-chloroethyl) phosphonic acid (Ethrel) applied at day 1 did not effect ARF.

In a 2x3x3 factorial experiment half the cuttings were pretreated with IBA and three growth regulators -IBA, (6-benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) and Gibberellic acid (GA_3) were applied at 3 different time periods to both juvenile and mature forms. IBA applied at days 3,5 and 7 to pretreated IBA juvenile cuttings reduced root length and GA_3 and PBA inhibited ARF. IBA application at days 3,5 and 7 stimulated ARF in juvenile cuttings not pretreated with IBA. ARF in pretreated IBA mature cuttings was inhibited by GA_3 and PBA applications at days 3,9 and 15. IBA applied at days 3 and 9 stimulated ARF in mature cuttings not pretreated with IBA.

IBA stimulated adventitious rooting by increased cambial activity, root initials and primordia differentiation and development in juvenile and mature cuttings. IBA/GA_3 did not effect early initiation stages, but reduced rooting once primordia had differentiated, while IBA/PBA inhibited rooting at early initiation stages.

Reasons for differences of growth regulator interactions between juvenile and mature LBC are discussed.

APPENDIX

ADVENTITIOUS ROOT FORMATION IN *Ficus pumila* MATURE LEAF BUD CUTTINGS TREATED WITH IBA IN A 42 DAY EXPERIMENT.

IBA (mg/l)	Rooting[x]	Root number[y]	Root length[y]	Quality[xy]
833	48b	3.9cd	2.3a	2.0b
2500	80a	11.1a	1.8a	2.8a
4167	65ab	6.1bc	1.1b	2.0b
8333	80a	8.7ab	1.9a	2.6ab
Control	0c	0d	0c	1.0c
Control + Surfactant	0c	0d	0c	1.0c

[x]Means followed by different letters are significantly different at 5% level, Duncan's Multiple Range Test.
[y]Quality scale ranged from 1=no rooting, 2=light rooting. 3=medium rooting and 4=heavy rooting.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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