THESIS

THE EFFECTS OF ETHEPHON ON THE ROOTING OF CUTTINGS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KENNETH W. MUDGE ENTITLED THE EFFECTS OF ETHEPHON ON THE ROOTING OF CUTTINGS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

THE EFFECTS OF ETHEPHON ON THE ROOTING OF CUTTINGS

Ethylene has been shown to promote rooting in a number of species of herbaceous and woody plants. Because ethylene is a gas it is impractical for treating cuttings. Ethephon, a liquid which releases ethylene to plant tissue, offers a practical means of treating cuttings with ethylene. This research was designed to investigate the effect of ethephon solutions at various pH's on the rooting of cuttings and on ethylene levels within cuttings.

Woody cuttings of Salix caprea, Ribes alpinum, Salix alba, Potentilla fruticosa, Rosa hybrida, Rosa laxa, Forestiera neomexicana and Populus deltoides were treated with unbuffered solutions of ethephon and rooted under intermittant mist in a greenhouse. The latter five species were also treated with IBA and ethephon plus IBA. Response to the treatments was measured as percent rooted, number of roots per cutting, and root dry weight per cutting. Ethephon promoted rooting of S. caprea and P. fruticosa only. In no case was ethephon as effective as IBA.

Light grown mung bean cuttings were treated with solutions of ethephon, IBA and the combination of both. Treatment solutions were unbuffered, or buffered at pH 3.7, 5.7 and 7.4. Ethylene levels in treated cuttings were determined by gas chromatography and rooting was measured as number of roots per cutting. Ethephon treatment resulted

in increased tissue ethylene levels with increasing solution pH, but no effect on rooting occurred at any pH. IBA treatment had no effect on tissue ethylene levels, but it did strongly promote rooting.

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CHAPTER 1

INTRODUCTION

When the effects of the plant growth regulator ethylene are enumerated in modern plant physiology and plant propagation texts, the list often includes the promotion of rooting (42, 60, 41, 6, 27). This response, first reported in 1933 (76), was one of the earliest known plant responses to ethylene.

For commercial plant propagation, the promotion of rooting by ethylene gas was of no value because of the difficulty of treating cuttings with a gas. However, with the development of ethephon, a liquid which releases ethylene gas to plant tissues, a practical method became available for treating cuttings with ethylene.

Although the stimulation of rooting in response to ethylene reported by Zimmerman et al. (76) was quite dramatic, numerous studies since then with both ethylene gas and ethephon have been less conclusive. There are nearly as many reports that ethylene inhibits (59, 53, 64, 49, 44) or does not affect rooting (47, 64, 61, 65, 62, 54, 68, 8) as there are reports that it promotes rooting (55, 77, 76, 59, 15, 35, 47, 16, 38, 39, 40, 8, 7, 63, 12, 68). The response appears to be species dependent (59, 76, 47, 63, 68).

At about the same time that ethylene was found to promote rooting synthetic auxins were also reported to be effective in stimulating this response (32). Ethylene is not widely used commercially as a root promoting compound; however, auxins are used for this purpose

throughout the plant propagation industry. That auxins have been shown to stimulate ethylene production in many plant tissues (1) suggests that the ability of both to promote rooting may be more than coincidental.

The objective of this study was twofold. First, to evaluate the effectiveness of ethephon as a root promoting compound for cuttings from several sepcies of woody plants, and secondly, to investigate the correlation between the level of ethylene gas within cuttings resulting from ethephon and auxin treatments and the rooting response to these treatments.

CHAPTER 2

REVIEW OF THE LITERATURE ON ETHYLENE IN PROPAGATION

Nomenclature

Prior to discussing the literature concerning the effects of ethylene on rooting, a few definitions and clarifications are in order. The term "rooting" itself is somewhat imprecise. Rather than a single event, rooting refers to an ordered developmental sequence beginning with the first biochemical event prior to any anatomical changes and culminating in the emergence of a visible root from the tissue from which it developed. For clarification several authors (19, 22, 27, 26, 53) have found it helpful to subdivide rooting into a number of distinct stages. However, rooting is a continuum rather than a series of distinct stages. The term root formation is used in the same sense as rooting, and points out the developmental nature of the process.

When rooting occurs in response to an external stimulus such as a hormone treatment or wounding, the period between the stimulus and the first anatomical change is referred to as dedifferentiation (27, 22, 19). Root initiation refers to the initial cell divisions which determine the number and position of the developing roots (27, 22, 53). Root initiation results in an undifferentiated clump of meristematic cells called root initials (27). Root initials then undergo differentiation into root primordia (27, 19, 53). Root primordia undergo elongation (19) and finally emergence (27, 53) from the tissue from which they arose. Unless otherwise indicated, this reveiw will

deal with the formation of adventitious roots defined by Esau (21) as roots not arising in normal sequence from the root pole of the embryo or a branch thereof.

A special class of adventitious roots is preformed adventitious roots or preformed root primordia (PRP). These refer to root primordia laid down in the stems of some plant species during the normal course of stem development (27, 11, 19, 21, 24, 26, 69, 72). Preformed root primordia usually lie dormant within the stem unless stimulated to elongate and emerge by wounding or some other external stimulus. Adventitious roots which are not preformed and which initiate only in response to an external stimulus are referred to as induced roots (26).

The term cutting refers to a plant part excised from the whole plant, from which the missing organs may be induced to regenerate (27). Although the phrase "stem cutting" is in common usage, and will be used here, in most cases it would be more proper to use the phrase "shoot cutting" since most stem cuttings include both stem tissues and leaf or leaf bud tissues, making them shoots (21).

Ethephon as an Ethylene-Releasing Compound

In the introduction it was pointed out that the use of ethephon, an ethylene-generating liquid, was potentially a more practical means of treating cuttings with ethylene than the use of the gas itself. Ethephon is the common name for 2-chloroethylphosphonic acid (Figure 1). It is also referred to as CEPA (74), Amchem 66-329 (3) and Ethrel® (3). The compound was first synthesized in 1946 as cited by deWilde (18), but not until 1963 was the formation of ethylene from it

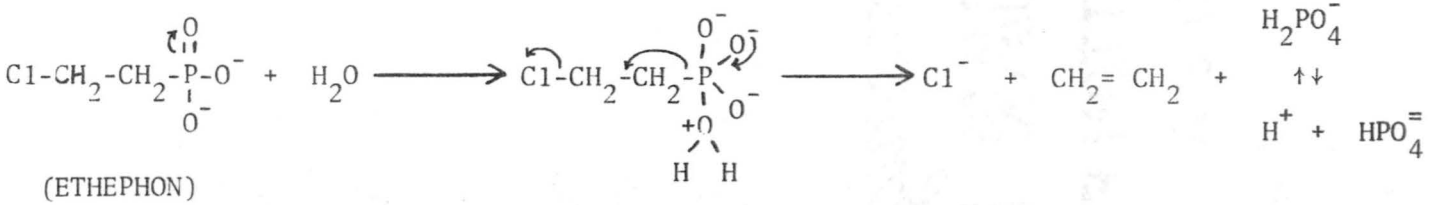

Figure 1. The proposed reaction mechanism for the release of ethylene from ethephon (47).

described by Maynard and Swan (46). Warner and Leopold (70) were the first to describe the use of ethephon as a plant growth regulator. Apparently unaware of Maynard and Swan's earlier demonstration that ethephon breaks down non-enzymatically to yield ethylene plus a chloride and a phosphate ion (46), Warner and Leopold suggested that ethephon stimulated the plant to produce endogenous ethylene in a manner analogous to auxin-stimulated ethylene production. This notion was convincingly put to rest by Lougheed and Franklin (45) who demonstrated that ethephon-treated tomato fruits had equivalent amounts of ethylene gas when treated in a nitrogen atmosphere or in air. Since tomato fruits are incapable of endogenous ethylene production under anaerobic (N_2) conditions, the ethephon molecule itself was implicated as the source of ethylene.

The true mechanism of ethylene generation from ethephon was again accurately described by Edgerton and Blanpied (20) (without acknowledging Maynard and Swan's (46) earlier work) and verified by Yang (74). The reaction mechanism (Figure 1) involves the base catalyzed nucleophilic attack of the phosphonate dianion (electrophile) by water or a hydroxyl ion (nucleophile). A series of electron shifts results in the net withdrawal of an electron by chlorine and the consequent fragmentation of the molecule into a chloride ion, a molecule of ethylene and a phosphate ion(46, 20, 74). Maynard and Swan (46) showed that the phosphonate group of the ethephon molecule must be in the dianion form ($R\text{-}PO_3^{=}$) for the reaction to take place. They suggested that the dianion is more strongly polarized ($P^{\sigma+}\!\!-O^-$) than the monoanion ($R\text{-}PO_3H^-$), and thus acts as a more effective electrophile. This requirement for the dianion form explains the observed pH dependency of

the reaction. The breakdown of ethephon occurs very slowly at pH's below 4.5 and increases as the pH is raised above that point (46, 20). At pH 4.5, the second -OH on the phosphonate group begins to ionize, resulting in the formation of the dianion.

The role of pH in the breakdown of ethephon is of importance because the pH of even a dilute ethephon solution is quite acidic. A 10 ppm solution of ethephon has a pH of 3.7, and a 1000 ppm solution has a pH of 2.7 (personal observation). At these acidic pH's, the breakdown of ethephon would be extremely slow (20). Only if the treatment solution was buffered in some manner by the plant tissue would the rate of ethylene production be faster. Some investigators (18, 13) have assumed that the release of ethylene from ethephon occurs within plant cells where the pH is buffered relatively high (>pH 4). This assumption is based on the results of a single study by Warner and Leopold (71). They measured ethylene evolved from ethephon-treated leaves of Bryophyllum cruentum grown under long day or short day conditions. Leaves grown under long day conditions have a higher tissue pH than short day grown leaves, as well as a significantly faster rate of ethylene release from ethephon. Warner and Leopold (71) assumed that the difference in tissue pH was due entirely to a difference in intracellular pH and therefore the release of ethylene from ethephon must occur intracellularly to account for the different rates of ethylene evolution. However, if the difference in tissue pH between long day and short day plants is extracellular as well as intracellular, as seems likely (though unproven), then no such conclusions can be made about the site of ethephon breakdown.

Since the ethephon molecule is an anion and "foreign" to the plant, it seems unlikely that its transport into plant cells is very rapid. Prior to absorption, ethephon would remain in the relatively unbuffered extracellular spaces (cell walls and xylem elements) where the original pH of the treatment solution would be expected to influence the rate of ethylene release from ethephon. It is surprising then, that none of the experiments discussed in this review mentions the pH of the ethephon treatment solutions, nor have buffered treatment solutions been used.

The Effect of Ethylene on Rooting

In the nomenclature section of this review the process of rooting was arbitrarily divided into five stages--dedifferentiation, initiation, differentiation, elongation and emergence. Ideally, in studying the effect(s) of a growth regulator on rooting, it would be desirable to be able to determine at what stage(s) of the rooting process the growth regulator has its effect. In practice, however, this is rarely done because of the difficulty involved in such a highly specific determination. Instead, results are usually reported simply as an effect on rooting in general rather than some specific stage of rooting. Some investigators (76, 53) have assumed that an ethylene treatment which promotes the rooting of a plant species which has preformed root primordia represents a stimulation of the emergence of the preformed root primordia. This assumption is not necessarily correct since neither investigator ruled out the possibility that ethylene could be promoting formation of new roots. The same assumption will not be made in this review. Table I summarizes the reports in the literature on

Table 1. Continued.

Species	I/C	PRP	Source of Ethylene	Concentration (ppm)	Parameter Measured	Effect	Reference
Phaseolus aureus (dark grown)	C	no	C_2H_4	10	root initials/cutting	-	54
Pisum sativum	C	no	C_2H_4	nr	roots/cutting	0	48
Zea mays	I	yes	ethephon	nr	subjective evaluation	+	8
Zea mays	I	yes	C_2H_4	10 to 2000	subjective evaluation	+	75
Dianthus caryophyllus	C	no	ethephon	200	nr	0	65
Euphorbia pulcherrima	C	no	ethephon	200	nr	0	65
Crysanthemum morifolium	C	no	ethephon	200	nr	0	65
Crysanthemum morifolium	C	no	ethephon	1	roots/cutting	0	62
Populus tremuloides	C	no	ethephon	25	roots/cutting	0	64
Populus tremuloides	C	no	ethephon	"...range of conc..."	nr	0	63
Populus nigra	C	no	ethephon	100	roots/cutting	+	64
Juglans nigra	C	no	ethephon	nr	nr	-	12
Rhododendron sp.	C	no	ethephon	1000	subjective evaluation	0	55
Forestiera neomexicana	C	no	ethephon	240	roots/cutting	+	68
Cotoneaster racemifolia	C	no	ethephon	1440	percent rooted	+	68
Rhamnus cathartica	C	no	ethephon	240	percent rooted	+	68
Prunus tomentosa	C	no	ethephon	480	percent rooted	+	68
Amorpha fragrans	C	no	ethephon	480	percent rooted	+	68
Juniperus scopulorum	C	no	ethephon	240-1920	percent rooted	0	68
Rosa sp.	C	no	ethephon	200	nr	-	65

Table I. Continued.

Species	I/C	PRP	Source of Ethylene	Concentration (ppm)	Parameter Measured	Effect	References
Ribes nigrum	C	yes	ethephon	10-250	nr	+	45
Ribes nigrum	C	yes	ethephon	>250	nr	-	45
Impatiens balsamina	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Begonia semperflorens	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Bryophyllum pinnatum	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Coleus blumei	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Cosmos bipinnatus	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Xoanoa sulphureus	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Fuschia hybrida	I	no	C_2H_4	10-2000	subjecitve evaluation	+	76
Galinsoga parviflora	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Heliotropium peruvianum	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Hydrangea macrophylla	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Nicotiana tabacum	I	no	C_2H_4	10-2000	subjective evaluation	+	76
Twelve unidentified species	nr	nr	C_2H_4	10-2000	subjective evaluation	0	76
"Ornamental cuttings"	C	nr	ethephon	nr	subjective evaluation	0	8
Sedum rubrotinctum (leaves)	C	no	ethephon	500	roots/cutting	+	7
Sedum rubrotinctum (leaves)	C	no	ethephon	1000	roots/cutting	+	7
Sedum rubrotinctum (leaves)	C	no	ethephon	2000	roots/cutting	+	7

Table 1. Continued.

Species	I/C	PRP	Source of Ethylene	Concentration (ppm)	Parameter Measured	Effect	Reference
Raphanus sativa (roots)	C	no	C_2H_4	>0.31	roots/cutting	-	57
Sinapis sp. (cotyledons in vitro)	C	no	C_2H_4	nr	nr	-	51
Solanum tuberosum (shoots in vitro)	C	no	C_2H_4	5	subjective evaluation	-	50

[a] I/C = intact plant or cutting
PRP = preformed root primordia
(+) = promotion of rooting
(-) = inhibition of rooting
(0) = no effect on rooting
nr = not reported

the effect of ethylene on rooting. The species investigated are divided into those with preformed root primordia and those without.

Of the studies included in Table I, several different parameters have been used as measures of rooting. This makes comparisons between studies less meaningful. The most commonly reported parameter is number of roots per cutting. The percentage of cuttings rooted has also been used as a quantitative measure of rooting. In a number of studies, the effect of ethylene has been reported qualitatively, simply by indicating the results in subjective terms such as "slight," "moderate" or "heavy" rooting. To facilitate comparisons between studies, Table I also indicates the concentration of growth regulator used and whether the source of ethylene was ethephon or ethylene gas.

The first and by far the most extensive study of the effects of ethylene on rooting was carried out by Zimmerman et al. during the 1930's (76, 77, 14, 34). Rooting was one of several plant responses to ethylene which they discovered. Of the 27 different species or varieties of plants which they exposed to ethylene gas, 15 responded by formation of adventitious roots (77). Fourteen of the 15 were herbaceous intact plants. The only woody plant, Salix babylonica, was made into cuttings. In all species the stimulation of rooting in response to ethylene fumigation consisted of the appearance of roots along the above-ground stems, and in several species roots formed on the underside of leaves and petioles as well. Then ethylene gas was dissolved in lanolin and applied locally to stems of intact tomato plants, root formation occurred only where the lanolin contacted the stem (14).

Since this initial work (77, 76, 14, 34), the promotion of rooting by ethylene has been verified by other investigators for several of the same or related kinds of plants (47, 35, 53, 59, 55, 8, 36). For example, the rooting of Salix cuttings was promoted by ethylene gas (35, 36, 47) and by ethephon (35, 36). Mullins (53) verified the findings of Zimmerman et al. (76) with intact marigold plants (Tagetes erecta). Both reported a promotion of rooting by ethylene. Using ethephon rather than ethylene, Roy et al. (59) showed that rooting of marigold stem cuttings was also stimulated. In contrast, the rooting of tomato cuttings was slightly inhibited by ethephon (59) whereas ethylene gas promoted rooting of intact plants of this same species (76). Rooting of intact tomato plants was also promoted by ethephon applied as a soil drench (55). The promotion of aerial prop root formation on intact corn plants reported by Zimmerman et al. (76) was verified by Briggs (8), except that he used ethephon instead of ethylene gas.

The mung bean (Phaseolus aureus) rooting bioassay developed by Hess (28, 29, 30) has been used by several authors to investigate the effect of ethylene on rooting. Using dark grown mung bean cuttings as stipulated in the original mung bean bioassay, Mullins (53) reported that ethephon inhibited rooting of dark grown mung bean seedlings but not to the extent reported by Mullins (53) using ethylene gas. Krishnamoorthy's (38. 39, 40) results with light grown mung bean cuttings differ strongly from those obtained using dark grown cuttings (53, 39). Rooting of light grown cuttings regardless of their age (5-, 10-, 15-days-old) was promoted by ethephon (39, 40). Roy et al. (59) also

reported that the rooting of light grown mung bean cuttings was promoted by ethephon.

Curtis and Fellenberg (16) reported a two-fold increase in the rooting of another species of bean, Phaseolus vulgaris, in response to treatment with ethephon. Linkins et al. (43), however, reported that the rooting of this species was unaffected by ethylene gas.

In two separate experiments (61, 64) ethephon was reported to have no effect on the rooting of chrysanthemum (Crysanthemum morifolium) cuttings. Likewise, two independent studies (62, 63) reported that rooting of aspen (Populus tremuloides) cuttings was unaffected by ethephon.

Overall, there does appear to be fairly good agreement between different investigators working with the same species of plant. What is most apparent from the research conducted to date (Table I) is that the rooting of plants in response to ethylene is species-dependent (59, 76, 68, 62, 53, 47). By dividing the experiments listed in Table I into various "either-or" categories for comparative purposes, it is possible to make some generalizations as to the characteristics of plants which do or do not respond to ethylene with improved rooting. Table II is arranged to show comparisons between several of these "either-or" categories. The few entries on Table I dealing with root formation on organs other than stems (7, 56, 49, 50) are not included in the comparisons made in Table II since they may represent physiologically dissimilar systems and will be discussed as a separate case.

The comparisons in Table II between herbaceous vs. woody plants, intact plants vs. cuttings, and plants with preformed roots vs. those without, do not include in the calculations the 12 types of plants

Table II. The effects of ethylene on the rooting of various categories of plants.

Comparisons	Promotion of Rooting	Inhibition of Rooting	No Effect on Rooting
Herbaceous	76%	6%	18%
Woody	68%	11%	21%
Intact	100%	0%	0%
Cuttings	59%	12%	30%
With preformed root primordia	86%	14%	0%
Without preformed root primordia	69%	5%	26%
Ethephon	65%	10%	26%
Ethylene gas	56%	3%	41%
Total	60%	6%	34%

which did not respond to ethylene in the study by Zimmerman et al. (76). They did not identify these 12 plants by name, nor did they indicate whether they were herbaceous or woody, intact or cuttings, or whether they did or did not have preformed root primordia. The same calculations also do not include the unspecified "ornamental cuttings" in the report by Briggs (8).

The percentages in Table II were calculated based on the relative occurrence of a (+), (-), or (0) in the "effects" column of Table I. For example, 39 of the 65 entries in Table I which deal with stems, report a promotion of rooting by ethylene. Thus, Table II indicates that 59% of these experiments indicate a promotion of rooting on stems by ethylene. Furthermore, Table II indicates that ethylene is not much more effective as a root promoting compound with herbaceous plants (76%) than it is with woody plants (68%).

While only 59% of the experiments with cuttings report a promotion of rooting by ethylene, a full 100% of the experiments with intact plants report a promotion of rooting. It should be pointed out that aerial root formation on stems of intact plants in response to ethylene is usually an "all or nothing" response in that intact plants not treated with ethylene usually do not form any aerial roots. Untreated cuttings, on the other hand, often root but to a lesser extent than treated cuttings. Thus, the literature indicates that ethylene is more effective in promoting rooting of intact plants than of cuttings.

Table II also compares species which have preformed root primordia to species which do not. Nearly 90% of the experiments on species which have preformed root primordia report a promotion of rooting by ethylene whereas less than 70% of the experiments on species without

preformed root primordia report a promotion of rooting by ethylene. This indicates that ethylene is a more effective root promoting compound with species which have preformed root primordia than species which do not.

Finally, Table II compares the effectiveness of ethephon as a source of ethylene to the application of ethylene gas directly. There are approximately as high a percentage of reports that ethephon promotes rooting (65%) as there are reports that ethylene gas promotes rooting (56%). This suggests that ethephon is an effective ethylene-releasing compound in this type of experiment regardless of the low pH of the treatment solution.

When a comparison is made between the three responses to ethylene included in Table II--promotion, inhibition, or no effect on rooting--it is apparent that reports of a promotion of rooting by ethylene (60% of total) are about ten-fold more frequent than reports of inhibition (6%). There are approximately half as many reports of "no effect" as there are reports of promotion of rooting. While ethylene is not an effective root promoting compound for all types of plants, it is effective more often than not.

Table I also includes reports of the effect of ethylene on rooting of plant tissue other than stems, i.e. leaves, roots, tissue grown *in vitro* (tissue culture). Of the 15 species in the study by Zimmerman *et al.* (76) which formed stem-born adventitious roots in response to ethylene, three also formed roots on their leaves. These were *Cosmos sulphureus*, *Heliotropium peruvianum* and *Tagetes erecta*. Boe *et al.* (7) reported that both ethylene gas and ethephon promoted rooting of sedum leaf cuttings. The only report on the effect of ethylene on lateral

root formation on roots was radish (56) wherein ethylene gas inhibited lateral root formation.

Finally, there are two reports of the effect of ethylene on root formation on plant tissue grown *in vitro*. They were with cultured *Sinapis* cotyledons (50) and cultured potato shoot tips (49). Rooting of both was inhibited by ethylene gas.

Ethylene and Auxin Interactions in Rooting

Much of the research concerned with the effects of ethylene on rooting has not only compared an ethylene treatment(s) with an untreated control, but has also compared it with an auxin treatment and/or a combined treatment of auxin plus ethylene. The rationale for including an auxin treatment in these experiments is obvious if one considers the importance of auxins in the commercial plant propagation "industry." There would be no point in encouraging the use of ethylene or ethephon as commercial root promoting compounds if they were not at least as effective or more effective than currently available auxin preparations. When ethylene has been compared directly to auxin in a single experiment, the auxin treatment is usually more effective in stimulating rooting (58, 37, 39, 40, 53, 43, 54). There are exceptions to this generalization. Krishnamoorthy (38, 39, 40) and Roy *et al*. (59) reported that ethephon significantly promoted rooting of mung bean cuttings, whereas the auxin, IAA, was nearly without effect. Two synthetic auxins, IBA and NAA, are more effective than ethephon in promoting rooting of mung beans (59, 40). The lack of response to the native auxin IAA is a characteristic of the mung bean rooting bioassay (28).

Ethephon has also been reported to be more effective than auxin in promoting rooting of aspen stem cuttings (63) and sedum leaf cuttings (7). In general, auxins are more effective promoters of rooting than ethylene. Audus (4) compiled the results of 1240 rooting experiments with auxin and found that 86% reported a promotion by auxin. In contrast, only 60% of rooting experiments with ethylene resulted in a promotion (Table II).

Whereas ethylene alone is generally not as effective in promoting rooting as auxins, the combination of ethylene plus auxin has been reported in a number of experiments to be more effective than either treatment alone (38, 39, 40, 59, 63, 47). Table III lists experiments in which auxin and ethylene have been used in combination. It indicates that the effect of the combined treatment is often synergistic. Synergism is indicated when the auxin plus ethylene treatment results in a greater stimulation of rooting than the sum of the two separate treatments. This is a positive interaction (66). Antagonism (negative interaction) is indicated when the ethylene plus auxin treatment results in less rooting than the sum of the two separate treatments. Synergism or antagonism can be determined by the following formula (25):

$S = C - (A+B)$, where

A is the difference between the auxin treatment and the untreated control,

B is the difference between the ethylene treatment and the control,

C is the difference between the combined ethylene plus auxin treatment and the control.

Table III. Summary of experiments on the effect of auxin plus ethylene on rooting.

Plant Type	Type of Auxin	Source of Ethylene	Concentration (in ppm) Auxin	Ethylene	Parameter Measured	Effect of Auxin plus Ethylene	Reference
Populus tremuloides	IBA	ethephon	10	25	roots/cutting	synergistic	64
Populus nigra	IBA	ethephon	10	100	roots/cutting	additive	64
Juglans nigra	IBA	ethephon	nr	nr	nr	"not effective"	12
Salix sp.	IAA	C_2H_4	1000	1000	roots/cutting	synergistic	48
Prunus tomentosa	IBA	ethephon	500	960	percent rooted	=control >ethephon treatment	68
Amorpha fragrans	IBA	ethephon	5000	960	percent rooted	>ethephon treatment >control	68
Rhamnus cathartica	IBA	ethephon	5000	960	percent rooted	=ethephon treatment >control	68
Forestiera neomexicana	IBA	ethephon	500	960	percent rooted	=ethephon treatment =control	68
Juniperus scopulorum	IBA	ethephon	5000	960	percent rooted	>ethephon treamtnet >control	68
Cotoneaster racemifolia	IBA	ethephon	5000	960	percent rooted	=ethephon treatment >control	68
Phaseolus aureus	IAA	ethephon	10	10	roots/cutting	synergistic	39
Phaseolus aureus	IAA	ethephon	10	10	roots/cutting	synergistic	41
Phaseolus aureus	IBA	ethephon	10	10	roots/cutting	synergistic	41
Phaseolus aureus	NAA	ethephon	10	10	roots/cutting	synergistic	41
Phaseolus aureus	IAA	ethephon	10	10	roots/cutting	synergistic	40
Phaseolus aureus	IAA	ethephon	1	1	roots/cutting	synergistic	40
Phaseolus aureus	IAA	ethephon	1	10	roots/cutting	synergistic	40

Table III. Continued.

Plant Type	Type of Auxin	Source of Ethylene	Concentration (in ppm) Auxin	Ethylene	Parameter Measured	Effect of Auxin plus Ethylene	Reference
Phaseolus aureus	IAA	ethephon	10	1	roots/cutting	synergistic	40
Phaseolus aureus (5-day-old)	IAA	ethephon	10	10	roots/cutting	antagonistic	40
Phaseolus aureus (10-day-old)	IAA	ethephon	10	10	roots/cutting	synergistic	40
Phaseolus aureus (15-day-old)	IAA	ethephon	10	10	roots/cutting	synergistic	40
Phaseolus aureus	IAA	ethephon	8.8	0.5	roots/cutting	antagonistic	60
Phaseolus aureus	IBA	ethephon	3.7	0.5	roots/cutting	antagonistic	60
Tagetes erecta	IAA	ethephon	8.8	0.5	roots/cutting	antagonistic	60
Tagetes erecta	IBA	ethephon	3.7	0.5	roots/cutting	antagonistic	60
Lycopersicon esculentum	IAA	ethephon	8.8	0.5	roots/cutting	synergistic	60
Lycopersicon esculentum	IBA	ethephon	3.7	0.5	roots/cutting	synergistic	60
Lycopersicon esculentum	IBA	C_2H_4	nr	saturated H_2O soln.	subjective evaluation	"induced rooting at a lower concentration than IBA alone"	34
Phaseolus vulgaris	IAA	C_2H_4	1.75	5	subjective evaluation	"no increase over auxin treatment"	44

A positive value for S indicates that the ethylene plus auxin treatment had a synergistic effect on rooting. A negative value for S indicates that the ethylene plus auxin treatment had an antagonistic effect on rooting. Obviously, four treatments must be included in an experiment to determine S: (1) an untreated control, (2) an ethylene treatment, (3) an auxin treatment, and (4) an ethylene plus auxin treatment.

A synergistic (or antagonistic) effect on rooting indicates that the two compounds do not act independently, but rather that they interact in some manner. An example of such an interaction is that auxin stimulates ethylene production in many plant tissues (1). This is only one of a number of possible interactions between auxin and ethylene that might be responsible for their synergistic effect on rooting. The underlying causes for synergism will be discussed in the section on mechansims of action.

Of the experiments listed in Table III which included the four treatments necessary for determining additivity, synergism, or antagonism, the majority showed a synergism between ethylene and auxin (63, 47, 38, 39, 40, 59). Two reported an antagonism (40, 59). Only one experiment (63) reported that the effect of ethylene plus auxin was strictly additive (S=0). This type of data indicates that ethylene and auxin do in fact interact with each other in some way to promote rooting. However, the nature of the interaction is not known.

For lack of a better explanation for the occurrence of both synergism and antagonism in Table III, one might say that they are due to differences between species; however, within the single species, mung bean, there are reports of both. Different results with a single

species might be due to differences in the experimental methods of different investigators, or due to differences in the concentrations of growth regulators, or as Krishnamoorthy (39) has suggested, differences in the age of the mung bean seedlings. Obviously, it is difficult to make interspecific or intraspecific comparisons when the experimental conditions differ from experiment to experiment. Roy et al. (59) compared the rooting response of three different species using identical experimental conditions and growth regulator concentrations for all three, thus making interspecific comparisons more meaningful. They found with tomato cuttings that ethylene plus auxin promoted rooting synergistically, whereas the same treatment antagonized rooting of mung bean and marigold cuttings. These results suggest that the response to auxin plus ethylene is species-dependent.

Auxin plus ethylene may result in a greater stimulation of rooting than either compound alone. Linkins et al. (43) have suggested that there may be an absolute requirement for ethylene in auxin-stimulated rooting. This conclusion was based on experiments with Red Kidney bean (Phaseolus vulgaris) in which they used a mercuric perchlorate trap to remove endogenous ethylene from auxin-treated cuttings. In the absence of ethylene the auxin treatment resulted in some initial cell division, but no rooting ensued. Conversely, when they treated bean cuttings with ethylene but no auxin, cortical swelling and cell separation occurred, but again no rooting took place. Only when auxin and the endogenous ethylene synthesized upon auxin treatment were present together did rooting occur. Additional exogenous application of ethylene gas to auxin-treated cuttings had no further effect on rooting.

Likewise, Schmid et al. (63) reported that Populus tremuloides cuttings treated with IBA alone did not root but cuttings treated with ethylene (or ethephon) plus IBA did root. These results support the conclusions of Linkins et al. (43) but they differ in that endogenously-generated ethylene was sufficient in the bean cuttings, whereas the P. tremuloides cuttings required exogenous ethylene.

To summarize the results of experiments performed to date, ethylene promotes rooting in some instances, but generally not as well as auxin. The rooting of intact plants and plants with preformed root primordia is more likely to be promoted by ethylene than the rooting of cuttings and plants without preformed root primordia. Often, the combined treatment of auxin plus ethylene is more effective in promoting rooting than either treatment alone.

Mechanism of Action

To discuss the effect(s) of ethylene on rooting, be it endogenously generated or exogenously applied, one must keep in mind the overall heterogenous sequence of events which is referred to as "rooting." A simple model was outlined in the nomenclature section of this review as follows:

Dedifferentiation → Initiation → Differentiation → Elongation → Emergence

These five arbitrary stages actually represent a continuum. Since dedifferentiation has not been mentioned in the literature with respect to the effect of ethylene or auxin on rooting, it will not be further discussed in this review. In the case of preformed root primordia, development is arrested for an indefinite period of time between differentiation and elongation (11).

It is reasonable to assume that various compounds which affect rooting act at different points along the sequence. It is also conceivable that a single growth regulator might have an effect at two or more points along the sequence. It has been suggested, for example, that auxin might have an effect on root initiation at the level of transcription or protein synthesis (27), while ethylene affects the sequence by promoting elongation of root primordia (53, 76). Auxin then might have a secondary effect on elongation via its stimulation of ethylene production.

Several hypotheses that have been put forth to account for the effect(s) of ethylene and auxin on rooting are summarized in Figure 2. This summary is not intended to be all-inclusive. It simply indicates the most likely hypotheses. In the remainder of this section, each hypothesis will be discussed individually. This review will not attempt to discuss primary mechanisms involving transcription, translation or allosteric mechanisms, as experimental evidence at this level is extremely limited.

Auxin Effects

A Direct Effect of Auxin on Root Initiation (A1 of Figure 2)

Since this mechanism of action for auxin does not pertain directly to the topic of this review, it will be discussed only in so far as it relates to the effect(s) of ethylene on rooting. Boulline's well-known and long-discussed Rhizocaline Theory as cited in Dore (19), and the Auxin/Cofactor Theory of Hess (31) specifically addresses the mechanism of a direct auxin effect on root initiation.

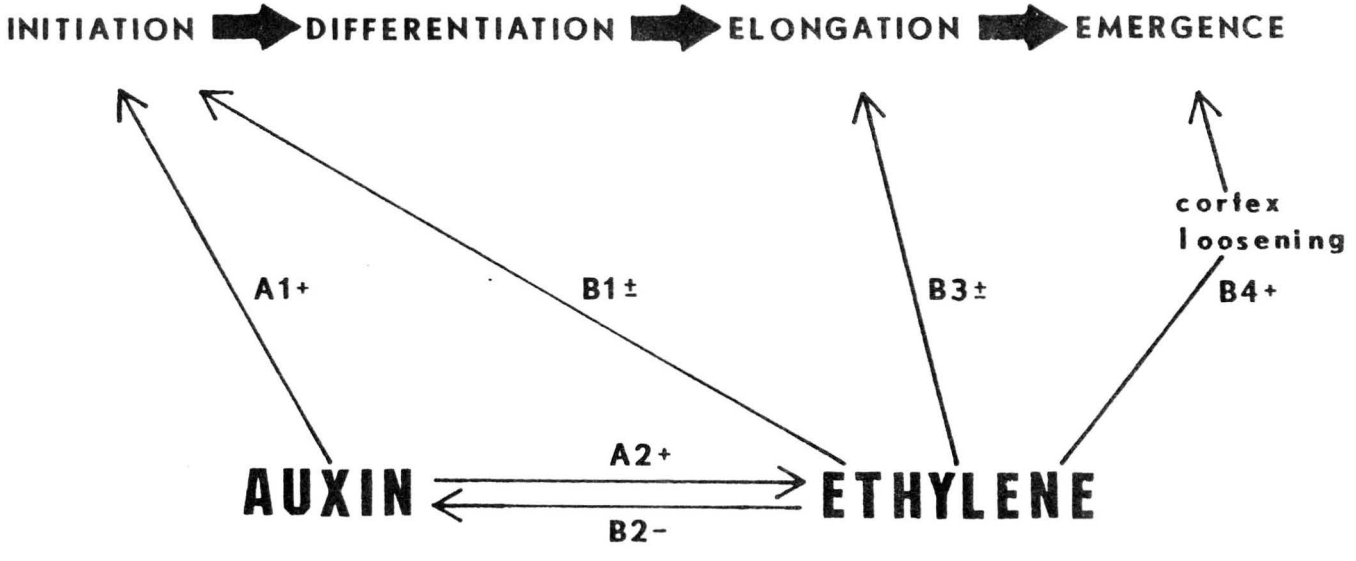

Figure 2. Hypotheses proposed to account for the effect(s) of auxin (A1, A2) and ethylene (B1 through B5) on rooting. A plus sign (+) indicates a promotive effect and a minus sign (-) indicates an inhibitory effect.

An Indirect Effect of Auxin on Rooting Via
Its Effect on Ethylene Synthesis
(A2 of Figure 2)

The concept of auxin affecting a physiological process via an ethylene intermediate is not restricted to rooting. This role of auxin has been experimentally verified for a number of physiological processes including abscission, leaf epinasty, initiation of flowering in Xanthium, inhibition of stem, root and leaf elongation, promotion of flowering in bromeliads, inhibition of bud growth, fading of orchid flowers, isocoumarin formation in carrots, latex flow in rubber trees, inhibition of epicotyl or hypocotyl hook opening in dicot seedlings, swelling of onion leaf bases, induction of phenylalanine ammonia lyase in parsnip root, and increasing the percentage of female flowers in dioecious plants (1). Abeles (1) has proposed five experimental criteria for establishing that an effect of auxin on a given physiological process is mediated by an ethylene intermediate. They are as follows:

1. demonstration of auxin-induced ethylene production;
2. demonstration that ethylene mimics the effects of auxin;
3. demonstration that auxin effects are lost or reduced in the presence of saturating amounts of ethylene;
4. demonstration that removal of ethylene by absorption, flushing with air, or hypobaric storage decreases the effectiveness of auxin;
5. demonstration of a decrease in the response to auxin in the presence of CO_2. This is based on the observation that CO_2 competitively inhibits ethylene action in many systems. In cases where CO_2 has secondary, non-competitive, or toxic effects, the interpretation would be more difficult.

Criteria 1, 2 and 4 are discussed below. Criteria 3 and 5 have not been investigated with respect to auxin-stimulated rooting and will not be discussed in this review.

Criterion 1. Demonstration of auxin-induced ethylene production. Auxin-induced ethylene production has been demonstrated in a wide variety of plant material. It was first demonstrated in 1935 by Zimmerman and Wilcoxon (77) prior to the development of gas chromatography. Marigold plants, known to be more sensitive to ethylene than tomato, were placed under a bell jar with auxin-treated tomato plants. The observation that untreated marigolds underwent epinasty was interpreted as a response to the ethylene produced by the auxin-treated tomatoes since marigolds enclosed with untreated tomato plants did not undergo epinasty.

With the development of gas chromatography, it has been possible to quantitatively measure ethylene production from a wide variety of auxin-treated tissues. In fact, auxin stimulates ethylene production in all cases investigated except in seeds, cell suspension cultures of horseradish and sweet clover, and in several mature fruits (1). Nevertheless, there are only two instances in which ethylene production has been measured in auxin-treated tissue undergoing a rooting response to that auxin (43, 53). Linkins et al. (43) reported that 10^{-5}M IAA, applied in lanolin paste to petiole stumps of debladed bean cuttings, resulted in a stimulation of ethylene production. This auxin treatment also resulted in root initiation as well as other anatomical and biochemical changes, including swelling and separation of cells in the cortex and pith, xylem formation, and changes in cellulase isozyme patterns.

Mullins (53) reported that auxin-stimulated ethylene production was negatively correlated with rooting. Dark grown mung bean cuttings treated with IAA produced significantly more ethylene than IBA-treated

cuttings, but rooting was unaffected by IAA and strongly stimulated by IBA. Greater stimulation of ethylene production by IAA than by IBA was also reported by Abeles and Rubenstein (2) with Phaseolus vulgaris, but rooting was not investigated. Not only did Mullins (53) report that the auxin which stimulated the most ethylene production resulted in the least rooting, but also he reported that exogenously applied ethylene gas (in the absence of auxin) inhibited rooting. Mullins' (53) results argue against the hypothesis that ethylene acts as an intermediate in auxin-stimulated rooting in this species. Rather, they suggest that auxin has some direct effect on root initiation (Figure 1, A1). However, it should be stressed that Mullins' (53) results are atypical in that others (38, 39, 40, 59) have reported that ethylene promotes rather than inhibits rooting of light grown mung bean cuttings. Also, the lack of response to IAA in the mung bean rooting bioassay is unique to this system (28). IAA promotes rooting of most other plants (27), but usually to a lesser extent than synthetic auxins (e.g. IBA). This has been attributed to the more rapid degradation of IAA than IBA (19) since degradative enzymes specific for the naturally-occurring auxin IAA (e.g. IAA oxidase) would be present in the plant, but enzymes specific for the "foreign" auxin IBA would not be present. Neither this explanation nor Mullins' (53) explanation that the greater effectiveness of IBA is due to the fact that it stimulates the least ethylene production can be ruled out based on the experimental evidence available.

Criterion 2. Demonstration that ethylene mimics the effect of auxin. The evidence pertaining to this criterion for an ethylene intermediate in an auxin response has been discussed in the previous section of this review on the effects of ethylene on rooting as well as, to some extent, in the paragraph immediately above. To summarize, ethylene, like auxin, does promote rooting in some situations (55, 77, 76, 59, 15, 35, 47, 16, 38, 39, 40, 8, 7, 63, 12, 68) but not in others (59, 53, 64, 49, 44, 47, 64, 61, 63, 62, 54, 68, 8). The rooting response to auxins is, however, more ubiquitous, occurring in a greater number of species than ethylene-stimulated rooting. Within a given species, auxin is usually the stronger stimulator of rooting.

Criterion 4. Demonstration that removal of ethylene by absorption, flushing with air, or hypobaric storage decreases the effectiveness of auxin. The satisfaction of this criterion has been demonstrated by Linkins et al. (43). They used a mercuric perchlorate trap to remove endogenous ethylene from auxin-treated bean cuttings and reported that rooting was no longer promoted by auxin.

Ethylene Effects

Direct Effect of Ethylene on Root Initiation (B1 of Figure 2)

Zimmerman et al. (76) concluded that ethylene promoted root initiation. This conclusion was based on the observation that ethylene caused aerial rooting on stems of intact plants which did not have preformed root primordia. The same conclusions cannot be drawn from studies involving cuttings without preformed root primordia because of the possibility that the very act of cutting might provide the

stimulus for root initiation. Ethylene then might be promoting the differentiation, elongation and/or emergence of the wound-stimulated root initials rather than the initiation of the root initials per se.

There is another interpretation for the experiments of Zimmerman et al. (76) with intact plants besides a direct ethylene effect on root initiation. Rooting could also be cuased by an ethylene induced alteration of auxin transport (9, 52, 51) as discussed in the next section.

Unambiguous proof of a direct effect of ethylene on root initiation would necessarily involve microscopic and/or histological staining techniques to determine the presence or absence of root initials prior to differentiation, elongation, or emergence. A promotive effect of ethylene on rooting could be interpreted as an effect on root initiation if and only if it could be demonstrated that plants not treated with ethylene had significantly fewer root initials than treated cuttings. Microhistological techniques have not been employed in any study in which ethylene promoted rooting; therefore, no direct evidence exists to support the hypothesis that ethylene has a direct effect on root initiation.

An Indirect Effect of Ethylene on Rooting Via a Modification of Auxin Distribution (B2 of Figure 2)

This hypothesis assumes that auxin has some direct effect on root initiation (Figure 1, A1). Further, it assumes that a disruption of "normal" auxin distribution could trigger auxin-stimulated rooting. None of the studies concerned with the effects of ethylene on rooting have addressed this hypothesis directly. However, there are several studies which show that ethylene does have an effect on auxin

distribution within the plant. Several of these studies have dealt with the ability of ethylene to interfere with polar auxin transport (9, 52, 51). It is conceivable that an inhibition of auxin transport might cause localized auxin accumulation and consequently root initiation in those areas.

An Effect of Ethylene on the Elongation of Root Primordia (B3 of Figure 2)

This hypothesis has been suggested by Zimmerman et al. (76) and by Mullins (53). There is no direct evidence to support it. Indirect evidence comes from the empirical observation that ethylene promotes the rooting of a number of species of plants which have preformed root primordia (see Table I). Table II shows that 86% of all experiments on species with preformed root primordia resulted in promotion of rooting by ethylene, while only 69% of the experiments on species without preformed root primordia resulted in a promotion of rooting by ethylene. This strongly suggests that ethylene has some specific promotive effect on root elongation. However, this must be considered indirect evidence, since none of these studies verified that the roots which emerged in response to ethylene were from the preformed root primordia rather than newly initiated roots.

Also, there is evidence which tends to discredit this hypothesis. Ethylene inhibits the elongation of excised, previously emerged radish roots (56) and previously emerged, intact pea roots (57). Also, Krishnamoorthy (38, 39, 40) and Curtis and Fellenberg (16) reported that even though ethylene promoted rooting of mung bean and pea cuttings, the roots were shorter on ethylene-treated cuttings than on untreated

cuttings. There is evidence, then, which indicates that ethylene inhibits rather than promotes root elongation.

An Indirect Effect of Ethylene on the Emergence of Root Primordia Via a Decrease in the Mechanical Resistance of the Cortex to the Elongating Root Primordia (B4 of Figure 2)

This hypothesis was proposed by Linkins et al. (43) to explain the heterogenous effects of auxin on the anatomy and cellulase isozymes of Phaseolus vulgaris cuttings. IAA at 10^{-5}M induced ethylene production, cortical swelling, cell separation, xylem initiation, cell division, an increase in the activity of acidic and basic pI[a] cellulase enzymes, and finally, root emergence. When the auxin-stimulated ethylene was removed with a mercuric perchlorate trap, a few initial cell divisions and the xylem differentiation occurred along with an increase in the acidic pI cellulase activity, but the cortical swelling, the increase in the basic pI cellulase, and the root differentiation and emergence did not occur. Furthermore, exogenous ethylene without auxin resulted in cortical swelling and an increase in the basic pI cellulase activity but no cell division or rooting occurred. Linkins et al. (43) suggested that the primary auxin effect was to increase the activity of an acidic pI cellulase which was responsible in part, or in full, for the xylem differentiation and initial cell divisions associated with root initiation. A secondary but essential auxin effect was to stimulate ethylene production. Ethylene then resulted in the increase in the activity of the basic pI cellulase which resulted in cortical swelling and a concomitant weakening of the mechanical resistance of

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[a]pI or isoelectric point is the pH at which there is no electric charge on a protein (67).

the cortex. According to this hypothesis, rooting requires both auxin and ethylene (endogenous or exogenous). To support their hypothesis, Linkins et al. cited work by Ferrari (23) which shows that ethylene treatment causes a mobilization of cellulase from the cytoplasm to the cell walls where it is presumably active in cell wall degradation. This might account for the cortical swelling and cell separation in response to ethylene which Linkins et al. (43) observed.

Although this hypothesis is internally consistent and well-documented by the data presented, it lacks experimental support by other researchers.

CHAPTER 3

THE EFFECTS OF ETHEPHON ON THE ROOTING OF SEVERAL SPECIES OF WOODY PLANTS

Previous studies indicate that ethephon may have potential as a commercial root promoting compound (7, 76, 60, 77, 35, 47, 38, 39, 40, 63, 12, 68, 44). However, ethephon is usually not as effective a stimulator of rooting as auxin (38, 39, 40, 59, 54). The response appears to be species-dependent (63, 68, 76, 59, 8). Before useful recommendations can be made to the commercial plant propagation industry, more information is necessary. The following research on woody cuttings was designed to evaluate the potential of ethephon as a root-promoting compound for several species of woody plants.

Experiment I: The Effect of Ethephon Concentration on the Rooting of Five Species of Woody Plants

This experiment was designed to determine the optimum ethephon concentration for root promotion for several species of woody plants and to determine whether the occurrence or absence of preformed root primordia (26) had an effect on the response.

Materials and Methods

Cuttings of five species of woody plants were treated with a broad range of ethephon concentrations: 0 ppm, 21 ppm, 213 ppm, and 1600 ppm. Salix alba and Ribes alpinum were chosen because they have preformed root primordia (26, 24). Rosa hybrida var. 'Forever Yours,'

Potentilla fruticosa and Salix caprea do not have preformed root primordia (10). The treatments were arranged as a complete factorial (Table IV) in a completely randomized design. Each treatment included ten replications (cuttings) for each species.

Leafy shoot cuttings of greenhouse-grown Rosa hybrida consisted of one node and were approximately 2.5 inches long. Leafy shoot cuttings from the remaining four species were approximately six inches long and included several nodes, all from the current season's growth. The terminal end of all branches was discarded to eliminate the influence of an apical meristem on rooting. Cuttings were taken on 9/25/75.

Ethephon solutions were prepared with Ethrel[a] and tap water immediately prior to treatment. The basal ends of the cuttings were soaked in one inch of treatment solution for three minutes and then planted about 1-1.5 inches deep in a greenhouse propagation bench containing perlite, with 27°C bottom heat, under intermittent mist. The rooting time for each species was as follows: R. hybrida--36 days; P. fruticosa--36 days; S. caprea--29 days; Salix alba--32 days; and R. alpinum--26 days.

Upon recording the percentage of cuttings rooted for each treatment, rooted cuttings were washed and the number of roots per cutting was determined. Detached roots were dried at 68°C for at least 24 hours and root dry weight per cutting was determined.

A separate two-way analysis of variance (AOV) was calculated for each of the three parameters measured. Since only a single percentage rooted value was calculated for each treatment, it was necessary to use

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[a]Ethrel is a commercially available preparation of ethephon produced by Amchem Products, Inc. (21.3% a.i.).

ETHEPHON CONCENTRATION, PPM	SALIX CAPREA	ROSA HYBRIDA	POTENTILLA FRUTICOSA	RIBES ALPINUM	SALIX ALBA
0	100%	60%	100%	100%	100%
21	100%	100%	100%	90%	100%
213	100%	80%	90%	100%	100%
1600	100%	60%	100%	100%	100%
SPECIES MEAN :	100%	75%	98%	98%	100%

the mean square term for the species x ethephon interaction to calculate the appropriate F ratio (66). Root number and root dry weight data were not available for cuttings which did not root. They were treated as missing values rather than zeros since, in most cases, they were dead (as opposed to alive but not rooted). Missing values resulted in unequal replication and necessitated performing an unbalanced AOV using the method of weighted squares (32). The method of least significant differences was used to determine which ethephon treatments differed significantly from the controls (66).

Results and Discussion

Percent Rooted

Except for R. hybrida, the controls for all species in this experiment rooted 100%, leaving no room for improvement of this parameter by ethephon (Table IV). In no case was there a significant inhibition of rooting percentage by ethephon. Although the controls for R. hybrida rooted only 60%, this was not significantly increased by any of the ethephon treatments (Appendix A).

Number of Root Per Cutting

Both a significant species effect and a significant species x ethephon interaction are present (Appendix B). The significant interaction means that ethephon did have a significant effect on the rooting of some but not all species. LSD comparisons (Figure 3) showed that the rooting of only one species was significantly promoted by ethephon--Salix caprea. Cuttings treated with 21 ppm ethephon had about twice as many roots as untreated controls. There was also a

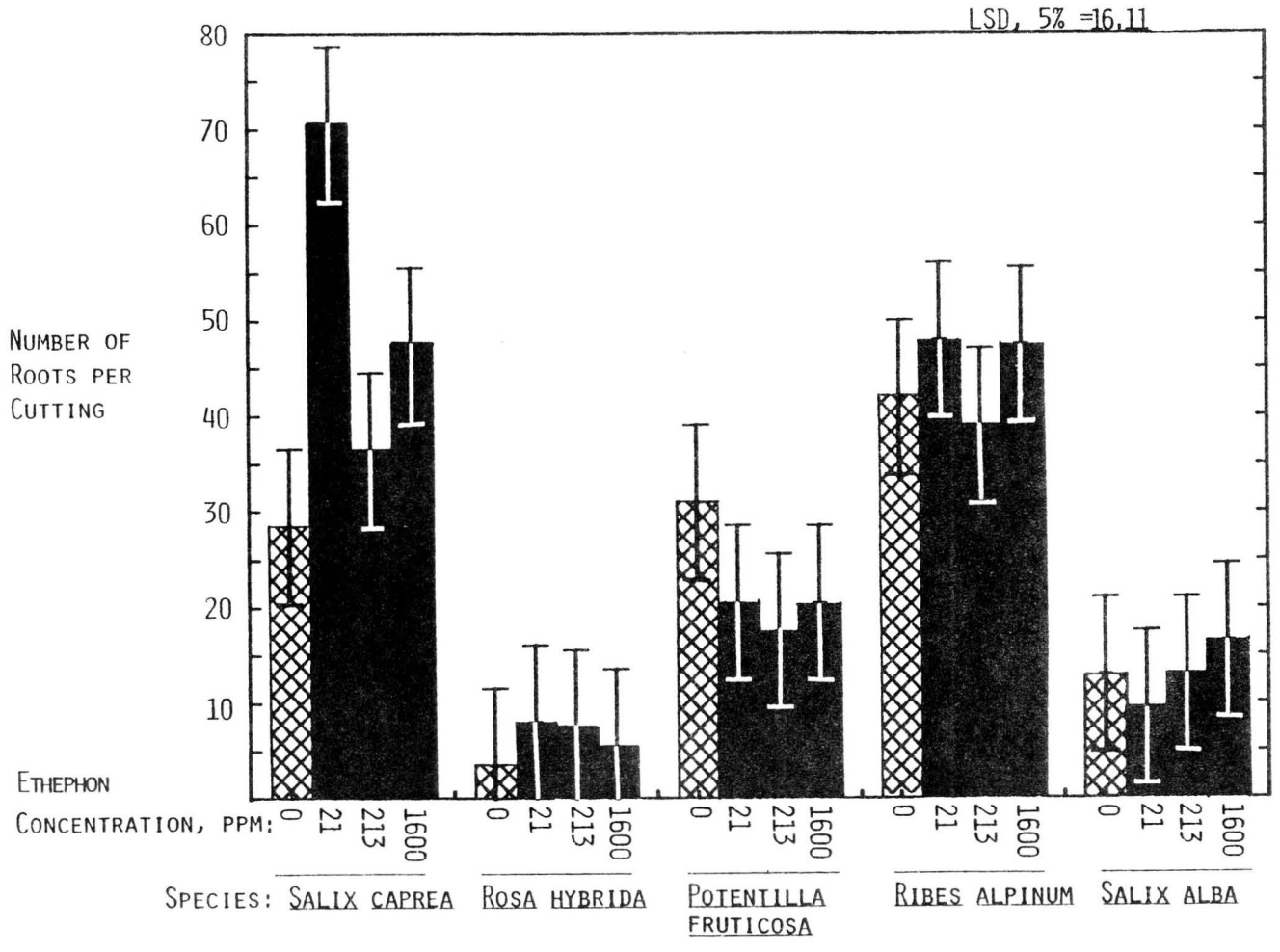

Figure 3. The effect of ethephon concentration on the number of roots per cutting for five species of woody plants (Experiment I). Treatments are significantly different when the LSD intervals at the top of the treatment mean bars do not overlap. LSD's should only be used to compare the ethephon treatment means with the control for a given species.

significant promotion of rooting with 1600 ppm ethephon, but not as much as with 20 ppm. Neither R. alpinum nor S. alba, both of which have preformed root primordia, showed a response to ethephon. This was not in agreement with similar research with other plants having preformed root primordia (54, 76, 77, 59, 53, 40, 47, 44). All such experiments showed a promotion of rooting by ethephon. Rooting of Ribes nigrum (45) and Salix fragilis (41), closely related to R. alpinum and S. alba, was promoted by ethephon concentrations similar to those used in this study. S. caprea was the only species in this experiment in which root number was increased by ethephon. It is, however, an atypical species of Salix in that it does not have preformed root primordia (10).

Root Dry Weight Per Cutting

None of the ethephon treatments had a significant effect on root dry weight for any of the five species in this experiment (Table V; Appendix C). No previous research reports the effect of ethephon (or ethylene gas) as root dry (or fresh) weight per cutting. Thus, comparative data are not available. The fact that ethephon, in this experiment, had an effect on root number per cutting but not on root dry weight per cutting for S. caprea suggests that regardless of the effect of ethephon on the number of roots per cutting, the total root "bulk" remains essentially constant.

In summary, ethephon promoted rooting of cuttings of only one of the five species of woody plants--S. caprea. This response was measureable only as an increase in the number of roots per cutting.

Ethephon Concentration, ppm	Salix caprea	Rosa hybrida	Potentilla fruticosa	Ribes alpinum	Salix alba
0	.0397	.0017	.0879	.0319	.0561
21	.0916	.0060	.0588	.0316	.0438
213	.0671	.0039	.0376	.0261	.0402
1600	.0405	.0051	.0700	.0190	.0427
Species mean :	.0597	.0044	.0642	.0273	.0457

Table V. The effect of ethephon concentration on the root dry weight (grams) per cutting for five species of woody plants (Experiment I).

Experiment II: The Effect of Treatment Duration and Ethephon Concentration on the Rooting of *Salix caprea* and *Potentilla fruticosa*

The objective of this experiment was to determine if soaking time is related to the effectiveness of ethephon at different concentrations.

Materials and Methods

Salix caprea and *Potentilla fruticosa* were treated with three ethephon concentrations (0, 21 and 1600 ppm) at five soaking times ranging from 0.5 minute to one hour. The treatments were arranged as an incomplete factorial with a completely randomized design (Table VI).

The incomplete factorial arrangement of the treatments is indicated in Tables VI and VII by the blocks which are crossed out. These treatments were not included in the experiment because 0.5 minute/21 ppm was considered insufficient, and 10 minutes/1600 ppm and 60 minutes/1600 ppm treatments were considered excessive.[a]

Each treatment included ten replications (cuttings). The treatment procedures, experimental conditions and the data collections were as described for Experiment I. Hardwood cuttings were treated on 11/30/75 and the experiment was terminated after 22 days for *S. caprea* and 31 days for *P. fruticosa*.

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[a]After the experiment had been completed, a reference was found in the literature to a successful promotion of rooting of *Salix fragilis* cuttings with a 24-hour/1000 ppm ethephon treatment (40). Hence, the 10 minutes/1600 ppm and 60 minutes/1600 ppm treatments might well have been included in this experiment without toxic effects.

	Ethephon Concentration, ppm	Treatment Duration, minutes 0.5	1	3	10	60
Potentilla fruticosa	0	100%	100%	90%	100%	100%
	21		100%	90%	100%	80%
	1600	100%	100%	100%		
Salix caprea	0	67%	89%	78%	78%	78%
	21		100%	67%	67%	78%
	1600	100%	100%	67%		

Table VI. The effect of treatment duration and ethephon concentration on the percentage of cuttings rooted for Salix caprea and Potentilla fruticosa (Experiment II).

A. Number of Roots Per Cutting

Ethephon Concentration, ppm	Treatment Duration, minutes 0.5	1	3	10	60
0	7.3	2.3	3.9	6.4	3.7
21		3.6	6.7	5.0	2.4
1600	4.7	7.1	4.8		
SPECIES MEAN :	5.1	4.4	5.1	5.8	3.1

B. Root Dry Weight Per Cutting

Treatment Duration, minutes 0.5	1	3	10	60
.0045	.0024	.0029	.0074	.0029
	.0033	.0051	.0045	.0016
.0043	.0068	.0030		
.0043	.0042	.0037	.0061	.0023

Table VII. The effect of treatment duration and ethephon concentration on (A) the number of roots per cutting and (B) the root dry weight (grams) per cutting for Salix caprea (Experiment II).

It was not possible to statistically analyze the percent rooted data for this experiment because the 10 cuttings provided only one observation per treatment and because of the incomplete factorial. For root number and root dry weight per cutting, separate AOV's were calculated for each species. Unrooted cuttings were treated as missing values. These and the incomplete factorial were accommodated in the AOV by the method of fitting constants (32). LSD intervals were computed for AOV's which had significant treatment effects.

Results and Discussion

Percent Rooted

Nearly 100% of the P. fruticosa cuttings rooted regardless of treatment (Table VI). S. caprea, on the other hand, had a lower overall percentage of cuttings rooted (≈80%), but there was no apparent effect of ethephon concentration or treatment duration on this parameter.

Number of Roots per Cutting

Neither ethephon concentration nor treatment duration had a significant effect on the number of roots per cutting for S. caprea (Appendix D and Table VII). Root number for P. fruticosa, however, was significantly affected by some of the treatments (Appendix E and Figure 4). Soaking time (treatment duration) did not have a significant effect on the number of roots per cutting (Appendix E), but ethephon at 3 minutes/1600 ppm produced significantly more roots than the untreated controls (Figure 4, LSD).

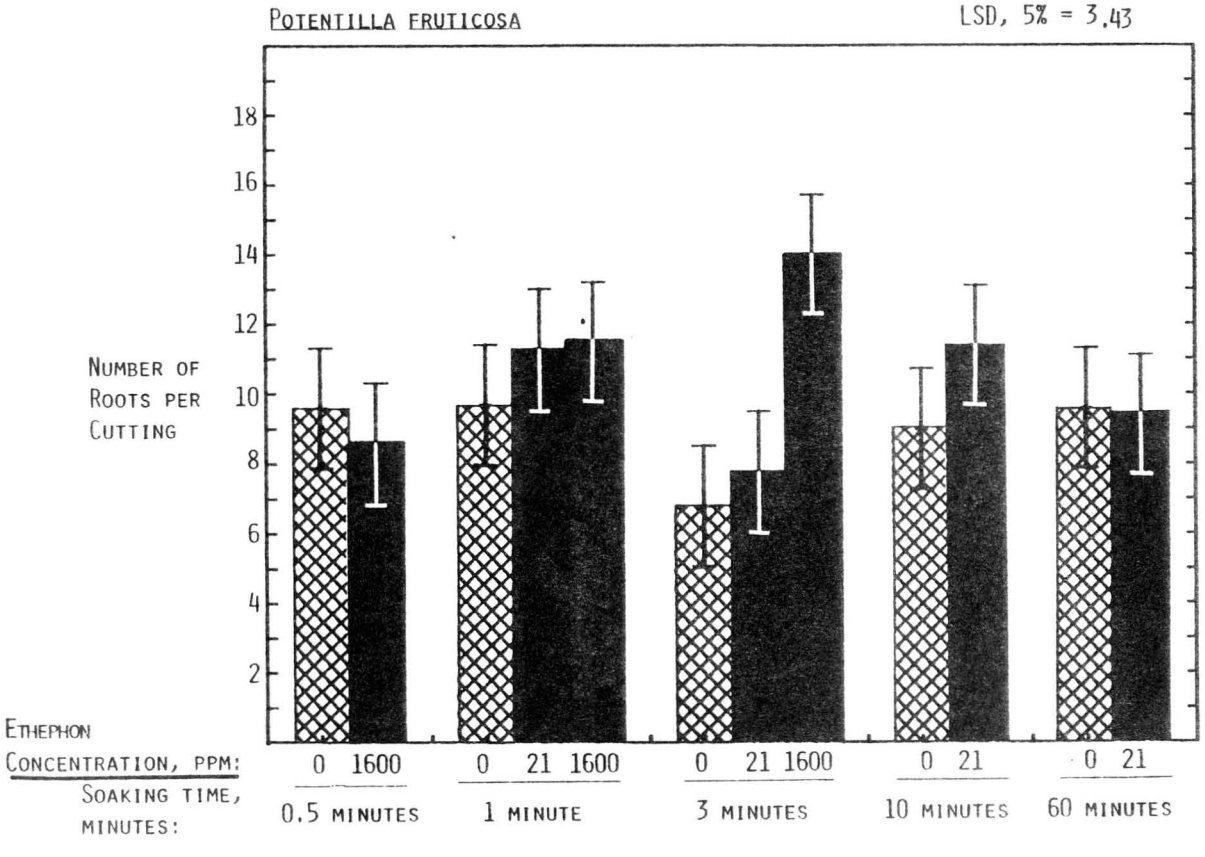


Figure 4. The effect of treatment duration and ethephon concentration on the number of roots per cutting for Potentilla fruticosa (Experiment II). LSD's are indicated by the intervals at the top of each treatment mean bar, and treatments are significantly different only if they do not overlap.

Root Dry Weight Per Cutting

For S. caprea, neither treatment duration nor ethephon concentration had a significant effect on root dry weight (Appendix F and Table VII). Thus, for all three rooting parameters, the treatments in this experiment did not have any significant effects on S. caprea.

For P. fruticosa root dry weight per cutting was significantly increased by ethephon concentration, but not by treatment duration (Appendix G). Root weight was increased by 1600 ppm ethephon at all soaking times (Figure 5). Twenty-one ppm ethephon significantly increased root weight at the 10 minute soaking time, but not as much as the 1600 ppm treatments at 0.5 and 3 minutes.

To summarize the results of Experiment II, the rooting of P. fruticosa measured as root number or root dry weight was significantly promoted by ethephon. There were significant differences between different concentrations of ethephon, but no differences between soaking times. The more effective concentration was 1600 ppm regardless of soaking time. Ethephon had a greater effect on root dry weight than it did on root number. Rooting of S. caprea was not affected by any treatment in this experiment.

Experiment III: A Comparison of the Effects of Ethephon, IBA and the Combination of Both on the Rooting of Five Speices of Woody Plants

This experiment was designed to further investigate the potential of ethephon as a commercial root promoting compound by comparing it separately and in combination with a commercially proven auxin preparation, Amchem 60-88, which contains the synthetic auxin, indole butyric acid (IBA).

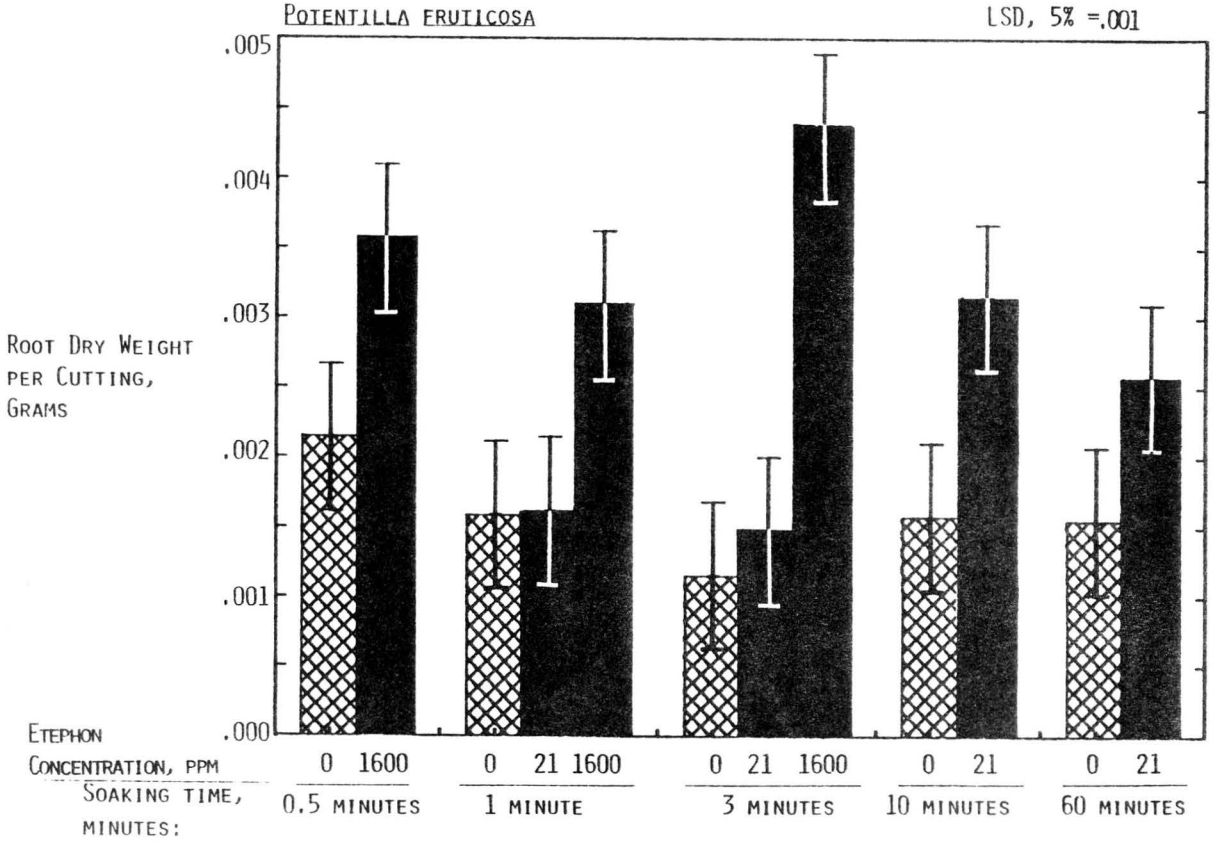


Figure 5. The effect of treatment duration and ethephon concentration on the root dry weight (grams) per cutting for Potentilla fruticosa (Experiment II). LSD's are indicated by the intervals at the top of each treatment mean bar, and treatments are significantly different only if they do not overlap.

Materials and Methods

Five species and two levels of both IBA and ethephon were arranged as a complete factorial in a completely randomized design with 15 replications (cuttings) per treatment (Table VIII). Cuttings were soaked in the treatment solutions for 18 hours. The choice of 18 hours/1000 ppm ethephon was based on the work of Kawase (40) with *Salix fragilis* in which a 24 hour/1000 ppm ethephon treatment strongly promoted rooting of that species. Experimental methods, conditions, and data collection were as indicated in Experiment I with the following exceptions: because of the length of the treatment period, a plastic bag was placed over the soaking cuttings to prevent wilting. Cuttings were treated on 6/11/76 and the rooting period for each species was as follows: *S. alba*--11 days; *R. laxa*--26 days; *F. neomexicana*--25 days; *P. fruticosa*--17 days; and *P. deltoides*--19 days. These rooting times are considerably shorter than those for the previous experiments because this experiment was performed in the early summer during the period of vigorous vegetative growth rather than during the end of the growing season as with the previous two experiments.

Data for the three rooting parameters were evaluated separately for each species. Unrooted cuttings were treated as missing values except for *P. deltoides*, for which many of the cuttings which did not root were alive and callused. For these, root number and root dry weight data was recorded as a zero rather than as a missing value.

Determination of significant differences between treatment means was based on significant F probabilities for each of the three factors (auxin, ethephon and the auxin x ethephon interaction).

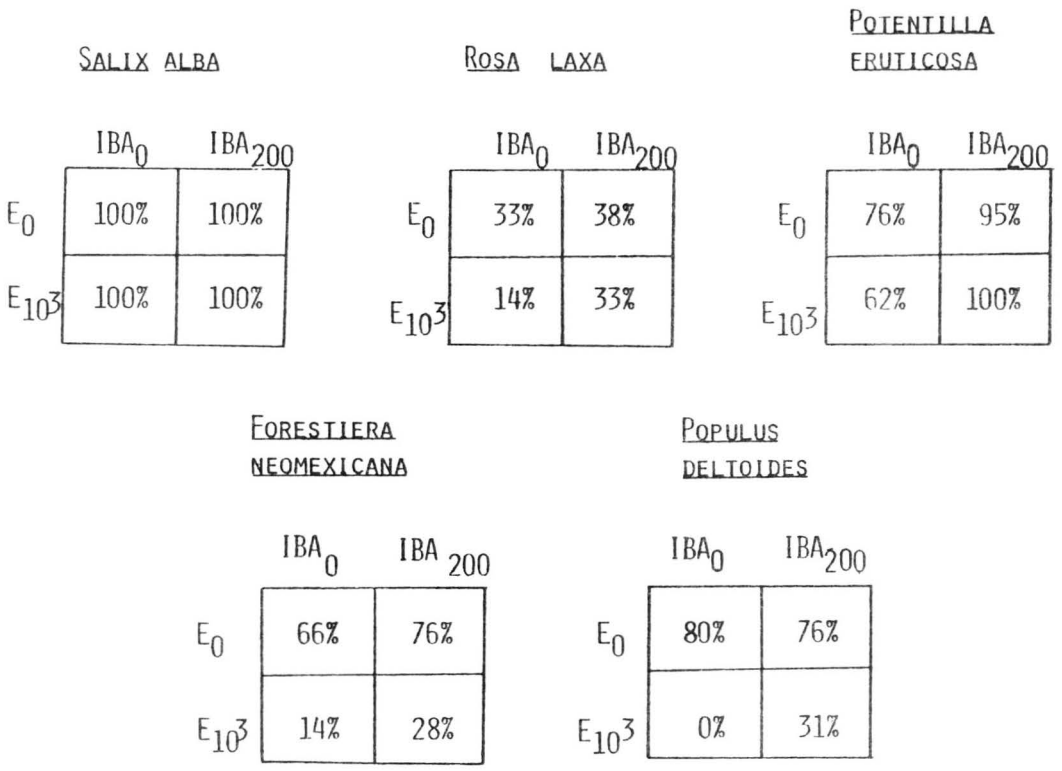


Table VIII. The effect of ethephon, IBA and the combination of both on the percentage of cuttings rooted for five species of woody plants (Experiment III). E = ethephon, subscripts indicate the concentration of growth regulator in ppm.

Results and Discussion

Percent Rooted

Salix alba was the only species for which there was 100% rooting (Table VIII). *Rosa laxa* had the poorest rooting (40%).

Neither ethephon nor IBA had a significant effect on the percentage of cuttings rooted for *S. alba*, *R. laxa* and *P. fruticosa* (Appendices I and J). In contrast, the rooting percentage for *F. neomexicana* and *P. deltoides* was significantly inhibited by ethephon either with or without IBA (Appendices K and L; Table VIII). Swanson (68) reported that the percentage of *F. neomexicana* cuttings which rooted was significantly promoted by ethephon at nearly the same concentration (960 ppm) but for only a 3-minute soaking time instead of 18 hours.

Number of Roots Per Cutting

For each significant treatment effect, the appropriate F probability appears in Figure 6 above the species to which it applies. For example, the "P(B) = .004" at the top of Figure 6 above *Salix alba* indicates that IBA ("B" in Figure 6) had a highly significant promotive effect on the number of roots per cutting for this species. P(E) and P(BxE) refer to the F probabilities for ethephon and the IBA x ethephon interaction, respectively. F probabilities not significant at the 5% level are not included.

These results indicate that for *Salix alba*, IBA increased the number of roots per cutting. Although there was no significant ethephon effect for this species, there was a significant IBA x ethephon interaction (Appendix M). This interaction (Figure 7) indicates that ethephon, in the absence of auxin (lower line), increased the

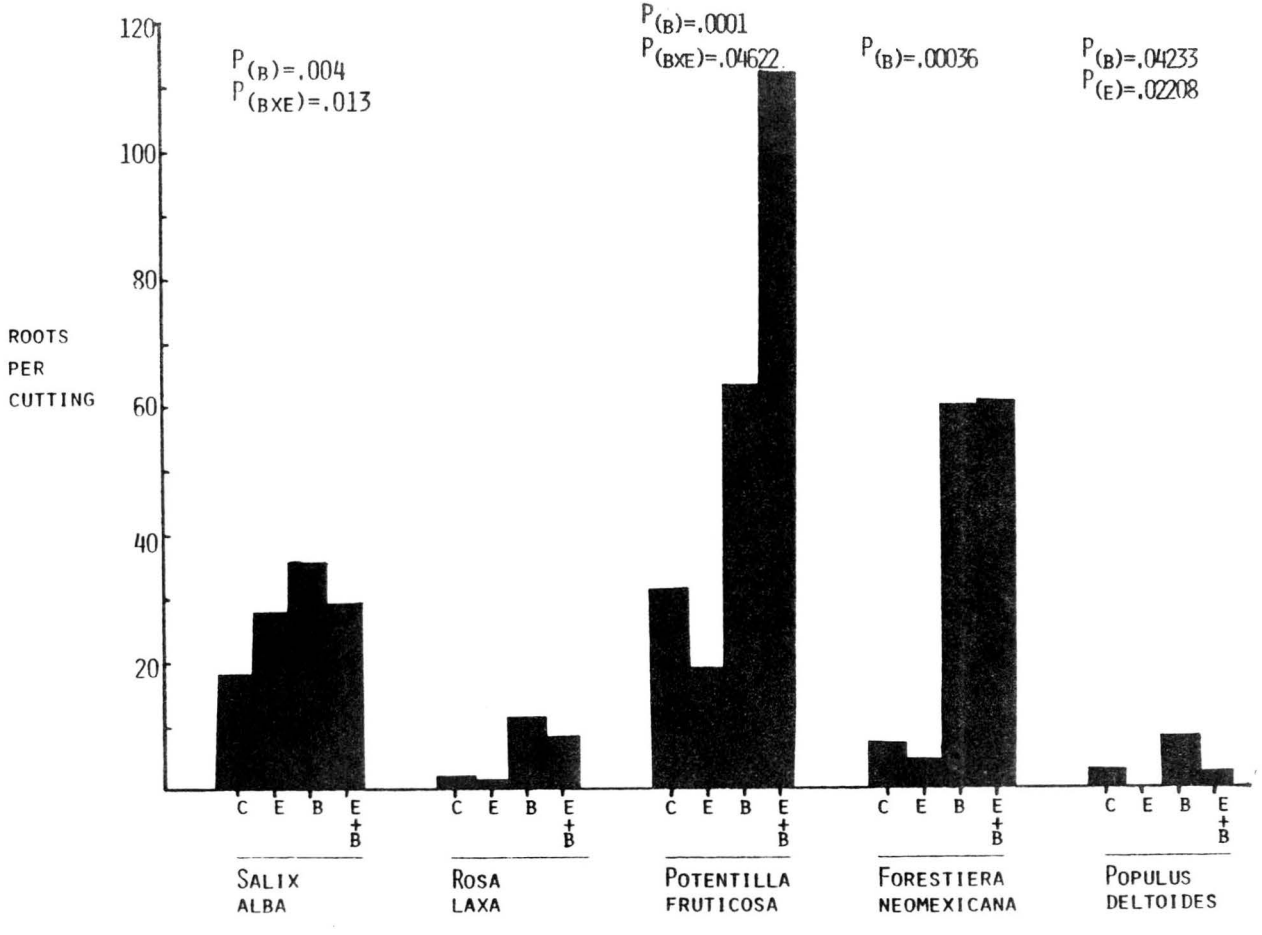


Figure 6. The effect of ethephon, IBA and the combination of both on the number of roots per cutting for five species of woody plants (Experiment III). Treatments were as follows: C=control; E=1000 ppm ethephon; B=200 ppm IBA; E+B=10 ppm ethephon plus 10 ppm IBA. The F probabilities ($P_{(\ )}$) associated with significant treatment effects are indicated above the species to which they apply.

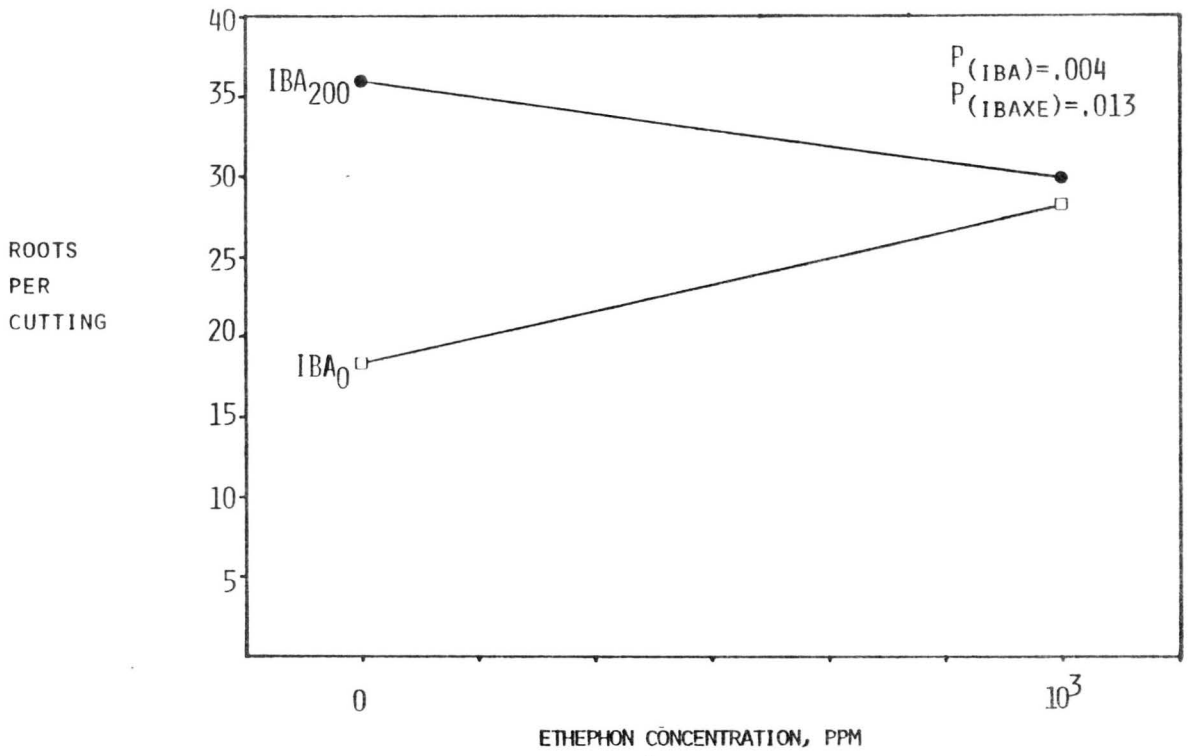


Figure 7. The interaction between ethephon and IBA for Salix alba with respect to the number of roots per cutting (Experiment III). The F probabilities associated with significant treatment effects are indicated in the top right corner.

number of roots per cutting over the control. However, in the presence of IBA (upper line), ethephon decreased root number (compared to IBA alone). This is a negative interaction or an antagonism of IBA-stimulated rooting by ethephon. In contrast, Michner (47) reported that ethylene plus IAA synergized (instead of antagonized) the number of roots per cutting for Salix sp.

The number of roots per cutting for R. laxa was not affected by any of the treatments (Appendix N). For P. fruticosa the number of roots per cutting was increased by IBA alone, and to a greater extent by IBA in combination with ethephon. This combined effect is reflected in the significant positive interaction or synergism as shown in Figure 8 (Appendix O). For F. neomexicana, IBA had a highly significant promotive effect on the number of roots per cutting (Appendix P). This response to IBA was not affected either way by the addition of ethephon, nor did ethephon alone have any effect on the number of roots per cutting. Swanson (68), however, reported that F. neomexicana was the only one of six species of woody plants for which ethephon increased root number per cutting both alone and when used in combination with IBA or with NAA.

For P. deltoides, IBA significantly increased root number per cutting, whereas ethephon had the opposite effect (Figure 6; Appendix Q).

Root Dry Weight Per Cutting

None of the four treatments had a significant effect on this parameter for S. alba or R. laxa (Figure 9; Appendices R and S). IBA significantly increased root dry weight per cutting for P. fruticosa and for F. neomexicana (Appendices T and U) but had no effect on root dry

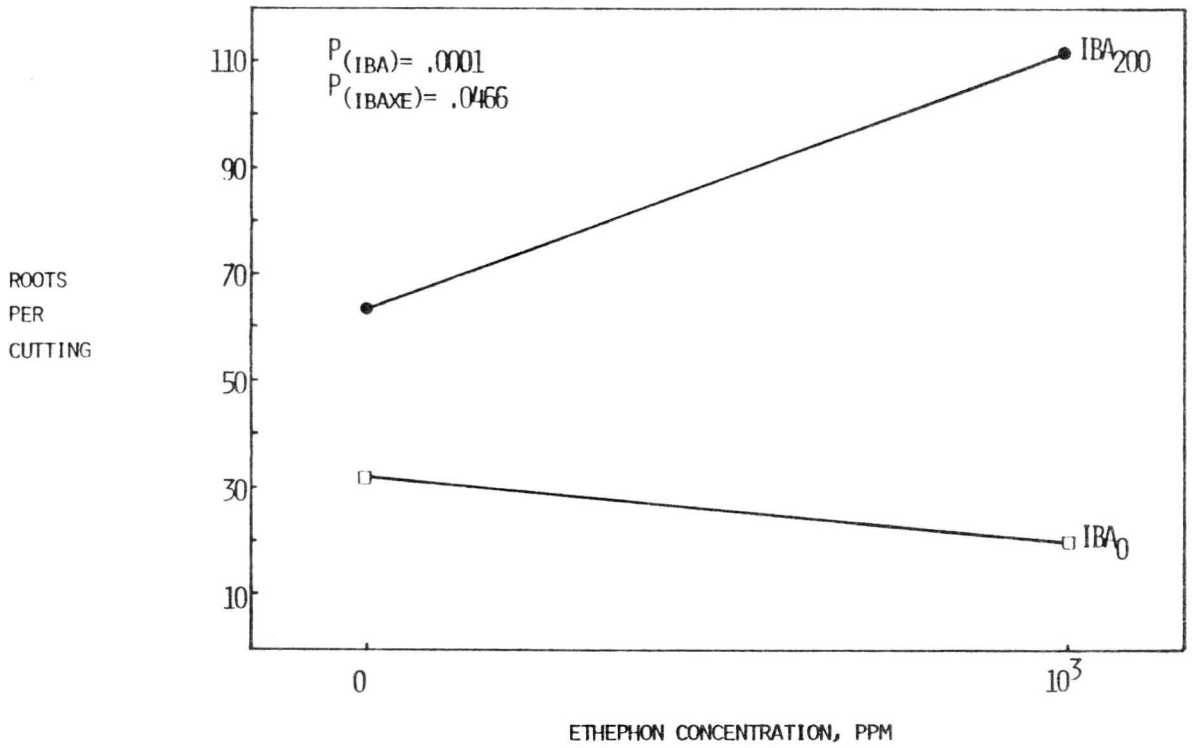


Figure 8. The interaction between ethephon and IBA for Potentilla fruticosa with respect to the number of roots per cutting (Experiment III). The F probabilities associated with significant treatment effects are indicated in the top right corner.

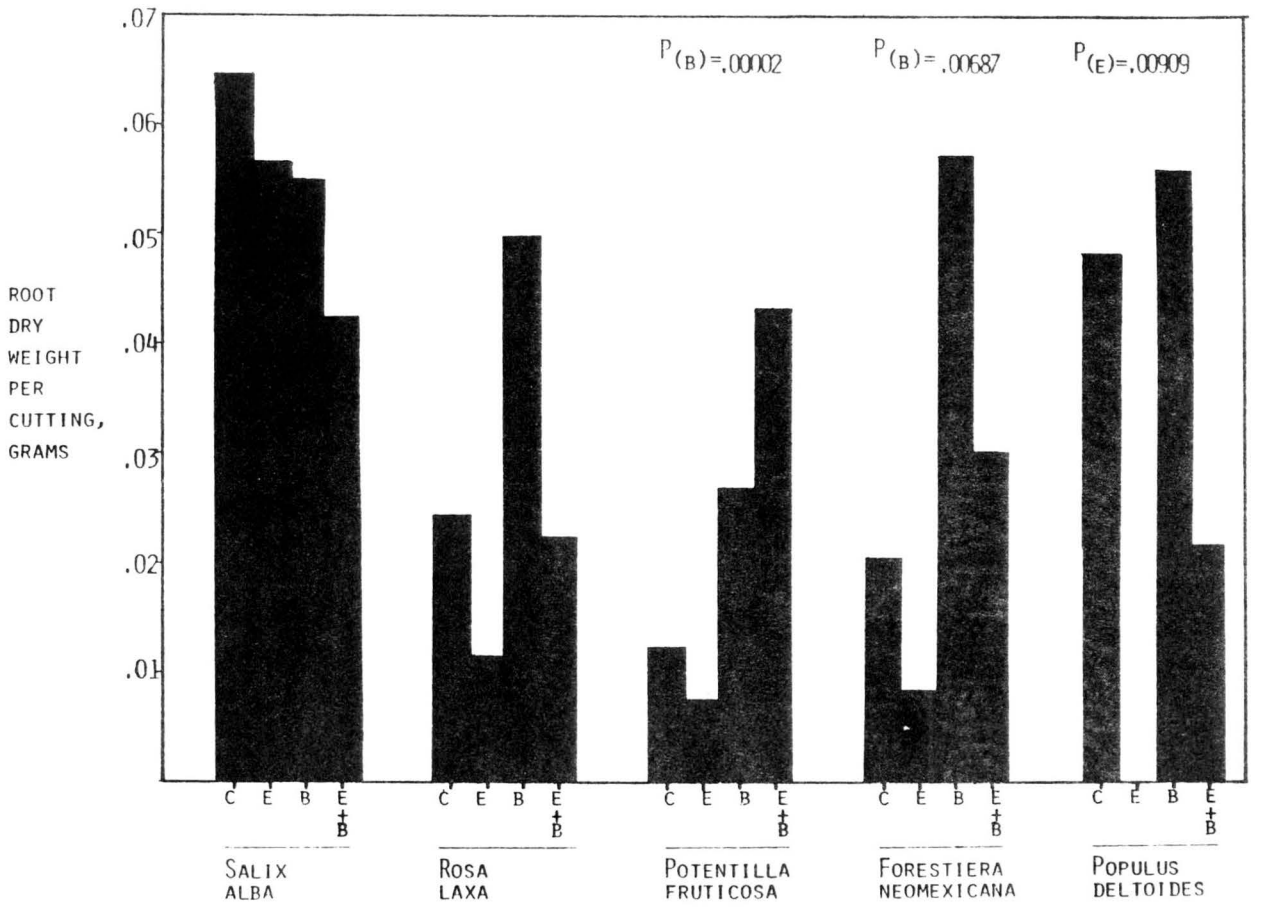


Figure 9. The effect of ethephon, IBA and the combination of both on the root dry weight (grams) per cutting for five species of woody plants (Experiment III). Treatments were as follows: C= control; 3=1000 ppm ethephon; B=200 ppm IBA; E+B=10 ppm ethephon plus 10 ppm IBA. The F probabilities ($P_{(\ )}$) associated with significant treatment effects are indicated above the species to which they apply.

weight for these species. The significant IBA x ethephon interactions present in the root number data for S. alba (antagonism, Figure 7) and for P. fruticosa (synergism, Figure 8) were not present in the root dry weight data. The only significant ethephon effect on root dry weight was with P. deltoides (Appendix V). As in the root number data, ethephon inhibited the root dry weight for this species (Figure 9).

In summary, IBA promoted rooting considerably more effectively than ethephon. The only promotion of rooting by ethephon in Experiment III was with P. fruticosa, where IBA plus ethephon synergistically promoted root number per cutting. Otherwise the only statistically significant effect of ethephon was to inhibit the percentage of cuttings rooted for F. neomexicana and P. deltoides and to antagonize IBA-stimulated rooting of S. alba as measured by root number per cutting.

It should be pointed out that these inhibitory effects might not necessarily have been due to ethylene gas released from ethephon. The data do not rule out the possibility that the inhibitions of rooting by ethephon might have been due to the very acidic pH (2.7) of the 1000 ppm ethephon solution.

Summary and Conclusions

Ethephon increased rooting of only two of eight species: S. caprea and P. fruticosa. These results are not in complete agreement with prior studies where ethephon or ethylene gas promoted rooting in 12 of the 16 species investigated (15, 40, 76, 47, 63, 12, 54, 68, 44, 64). Rooting of all five of the species with preformed root primordia was promoted by ethephon (or ethylene) (40, 76, 47, 15, 44). S. alba

and R. alpinum have preformed root primordia, but their rooting was not affected by ethephon.

The relative lack of root promotion in the experiments reported here as compared to other experiments in the literature is not readily apparent. The methods and conditions were standard for rooting of woody cuttings, and the ethephon concentrations were comparable to those used in other experiments (40, 63, 68, 44).

In Experiment I the only measurable effect of ethephon was on number of roots per cutting (S. caprea). In Experiment II ethephon had a slight effect on number of roots per cutting for P. fruticosa but a considerably stronger effect on root dry weight for this species. In Experiment III the only promotive effect of ethephon (ethephon plus IBA synergism with P. fruticosa) was measured as an increase in the number of roots per cutting. In none of these experiments did ethephon increase the percentage of cuttings which rooted.

The lack of consistency from experiment to experiment deserves comment. In Experiment I ethephon significantly increased root number per cutting for S. caprea and had no effect on P. fruticosa. In Experiment II almost the opposite results occurred. Rooting of S. caprea was not significantly affected by ethephon, whereas ethephon promoted rooting of P. fruticosa (root number and root weight). S. caprea was not included in Experiment III. The rooting of P. fruticosa in Experiment III was unaffected by ethephon alone but was promoted synergistically by IBA plus ethephon.

The ethephon concentrations and soaking times were comparable for Experiments I and II and yet, nearly opposite results were observed. Possibly the time of year was an important factor in this apparent

inconsistency. Experiment I was started in mid-September when woody plants are beginning to enter a dormant period. Experiment II was started at the beginning of December when most woody plants would be fully dormant. The rooting of woody plants is known to be seasonal (27, 19). Plant tissues are known to respond differently to ethylene with respect to abscission depending on the physiological age of the tissue (17). This might conceivably be true for rooting as well although there is no experimental evidence to this effect.

Based on the results of this study, ethephon could not be recommended to the plant propagation industry as a root-promoting compound. The extremely low pH of ethephon treatment solutions and the slow release of ethylene from ethephon at low pH suggests an experimental approach for further research in this area. The use of buffer solutions to increase the pH of ethephon treatment solutions might enhance its effectiveness. Chapter 4 of this thesis deals in part with the investigation of this hypothesis.

CHAPTER 4

THE EFFECT OF ETHEPHON, IBA AND TREATMENT SOLUTION pH ON ROOTING AND ON ETHYLENE LEVELS WITHIN MUNG BEAN CUTTINGS

Ethephon has been reported to promote (55, 77, 76, 59, 15, 35, 47, 16, 38, 39, 40, 8, 7, 63, 12, 68), inhibit (59, 53, 64, 49, 44), or have no effect (47, 64, 61, 63, 62, 54, 68, 8) on rooting of cuttings of various species of plants. When ethephon does not promote rooting, the possibility exists that the cause is the ineffective release of ethylene gas from ethephon, rather than a lack of response to ethylene gas per se. No reports were found which correlate tissue ethylene levels with rooting in ethephon-treated cuttings.

Likewise, auxin-stimulated rooting may result from auxin-stimulated ethylene production by the treated tissue (77). Correlations of ethylene production with auxin-stimulated rooting have yielded contradictory results. Linkins et al. (43) presented tissue ethylene data which, at least in part, support the hypothesis, whereas Mullins' data (53) contradict it.

The objective of the following two experiments was to correlate internal ethylene levels with the rooting response of mung beans treated with ethephon and auxin.

Experiment I: The Effect of Unbuffered Solutions of Ethephon, IBA and the Combinations of Both on Rooting and on Ethylene Levels in Mung Bean Cuttings

This experiment was designed to determine if unbuffered solutions of ethephon, IBA and the combination of both promote rooting of light grown mung bean cuttings, and to investigate the relationship between rooting and tissue ethylene levels in response to these growth regulators.

Materials and Methods

The mung bean rooting bioassay (28) was chosen because preliminary studies indicated that the rooting of mung beans is less variable and faster, and it is better characterized in the literature than rooting of woody cuttings. Seeds of mung bean (*Phaseolus aureus* var. 'Berken') were germinated and grown on moist sand in a non-humidified growth chamber under continuous illumination of approximately 400 foot candles from cool-white flourescent tubes. The temperature of the growth chamber was 27 $\pm$ 1°C. Cuttings from these seedlings were treated and rooted under the same conditions except that the relative humidity was raised to almost 100% since preliminary experiments indicated that mung bean cuttings grown under low humidity rooted slowly or not at all. Cuttings were prepared from 11-day-old seedlings and consisted of 3 cm of hypocotyl and the entire epicotyl including two primary leaves and the apical meristem. The overall length of the cuttings (depending on variation in the length of the epicotyl) was 11 to 14 cm.

Eight treatments were arranged as a complete factorial with two levels of IBA (0, 10 ppm) and four levels of ethephon (0, 5, 10, 15 ppm). Treatment solutions were prepared with distilled water. Cuttings were placed in 25 x 100 mm glass vials (5 cuttings/vial) containing 10 ml of treatment solution and were placed in the humidified growth chamber. After 24 hours the treatment solutions were poured off and replaced with 13 ml of distilled water. The rooting experiments were terminated after five days by killing and preserving the rooted cuttings in 95% ethanol. The number of roots on each cutting was then determined.

Cuttings used for ethylene determinations were treated in the same manner, except that the treatment period was shorter. Internal tissue gas was extracted and analyzed for ethylene 12 hours after the start of the treatments. The decision to sample tissue ethylene at 12 hours was based on the work of Mullins (53) and our own preliminary work which indicated no change in tissue ethylene levels of ethephon-treated cuttings between 6 and 18 hours after the start of treatment. Internal tissue gas for ethylene analysis was extracted from the cuttings by the method of Kawase (36) modified as follows (Figure 10):

A 400 ml beaker was placed inside a large vacuum desiccator filled with degassed water. Fifteen mung bean cuttings were placed in the beaker and covered by an inverted funnel. The funnel was entirely filled with degassed water and its neck was sealed with a rubber serum cap. The desiccator was then evacuated with a vacuum pump for five minutes. While under vacuum, the gas within the cuttings bubbled out, mostly from the cut ends, and collected in the neck of the funnel. Fifteen cuttings yielded 1-2 ml of gas. One-half ml of this gas was

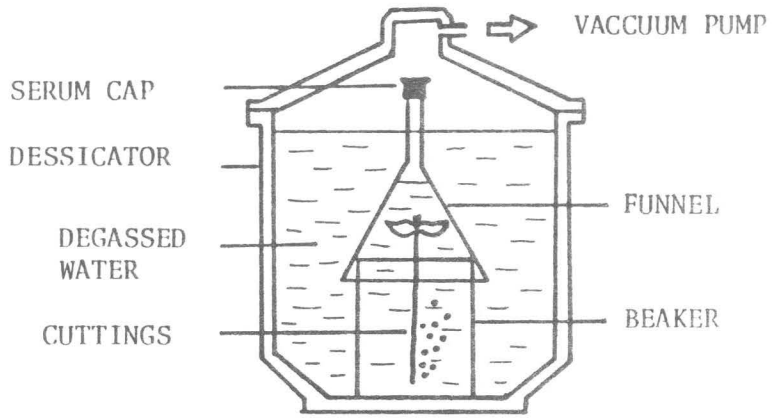


Figure 10. The apparatus used to extract gas samples for ethylene determinations from mung bean cuttings. See text for explanation.

removed with an air-tight gas syringe, through the serum cap, and immediately injected into a Hewlett Packard model 5750-B gas chromatograph with a flame ionization detector and a column 86 cm long packed with Porapak® type N (100/120 mesh). The amount of ethylene in a treatment sample was determined by comparing the ethylene peak height of the unknown sample to the peak height of a standard containing 4.53 ppm ethylene.

All treatments for the rooting portion of the experiment were replicated at least 20 times, except for the following four treatments which were replicated 30 times: control, 10 ppm ethephon, 10 ppm IBA, and 10 ppm ethephon plus 10 ppm IBA. Tissue ethylene determinations were replicated at least three times. Rooting data were analyzed as a two-way unbalanced analysis of variance (AOV) using the method of weighted squares (32).

Results and Discussion

Ethephon alone, regardless of concentration, had no significant effect on the number of roots per cutting (Figure 11B and Appendix W). IBA, however, strongly promoted rooting, either alone or with any concentration of ethephon. None of the ethephon levels synergized or antagonized IBA-stimulated rooting. Thus, ethephon had no effect on the rooting of these mung bean cuttings. Similarly, Mullins (53) reported that ethylene gas inhibited the rooting of dark grown mung bean cuttings. In contrast, Krishnamoorthy (38, 39, 40) and Roy *et al.* (59) reported that ethephon promoted rooting of light grown mung bean cuttings. Krishnamoorthy (39) reported that IBA plus ethephon promoted

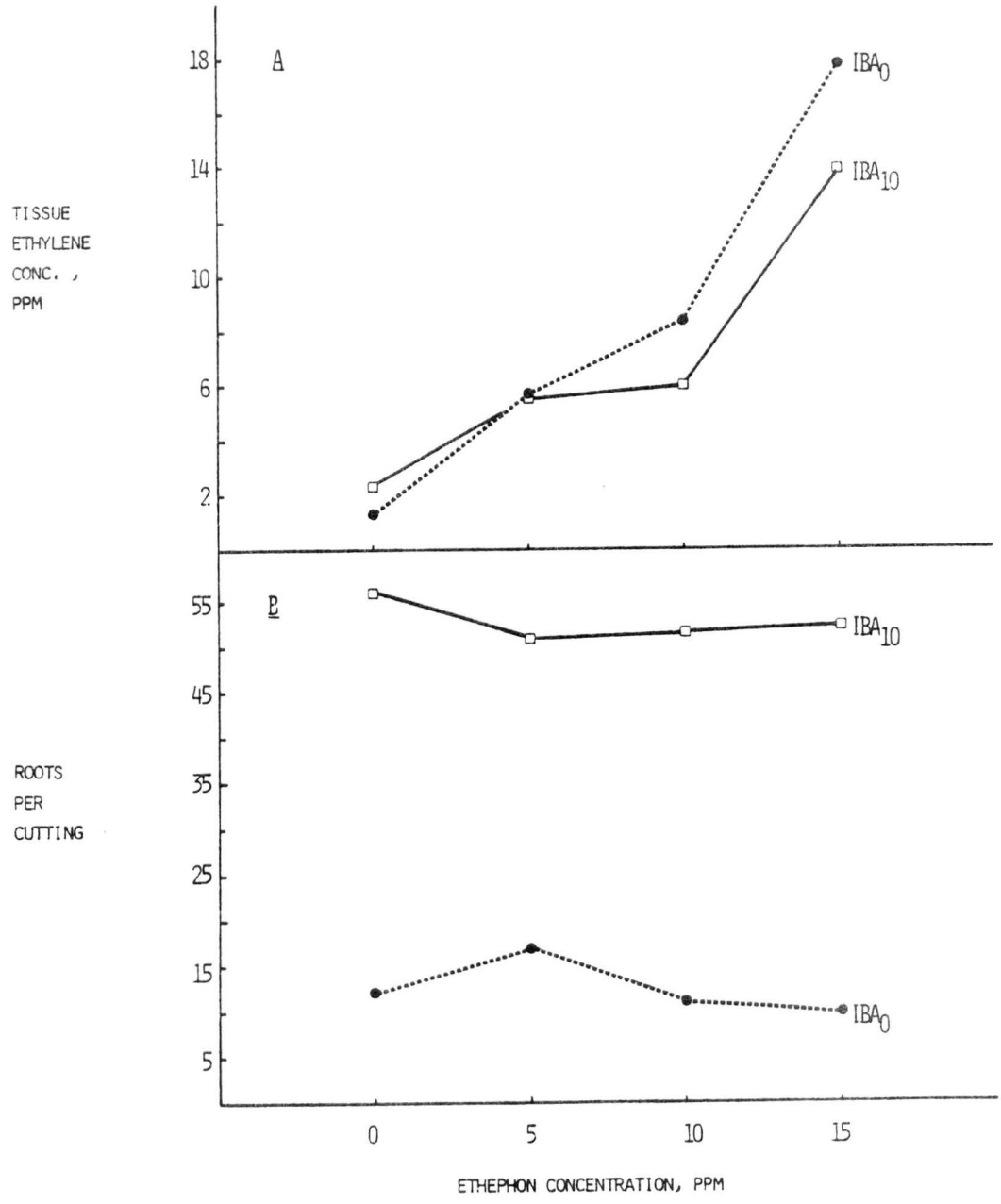


Figure 11. The effect of ethephon, IBA and combinations of both on (A) tissue ethylene levels, and (B) the number of roots per mung bean cutting (Experiment I). Subscripts indicate IBA concentration in ppm.

rooting synergistically, whereas Roy et al. (59) found the combination slightly antagonistic to rooting.

The tissue ethylene levels in response to the treatments in this experiment do not indicate a correlation between ethylene and rooting (Figure 11). Increasing concentration of ethephon in the treatment solutions resulted in increased levels of ethylene within the cuttings, but no concomitant increase in rooting. Likewise, the large stimulation of rooting in response to IBA was not accompanied by a significant increase of tissue ethylene levels. In fact, the data do not indicate any IBA stimulation of ethylene production whether the treatment solution contained IBA alone or in combination with ethephon. In contrast, Mullins (53) reported significant stimulation of ethylene production in dark grown mung bean cuttings by IBA and an even greater stimulation by IAA. Of the two auxins in Mullins' (53) experiment, the one which stimulated the lesser ethylene production, IBA, resulted in the greater stimulation of rooting. Although Mullins' (53) results differ from those reported here in that auxin-stimulated ethylene production was reported in his experiment but not in this one, the results of both experiments tend to discredit the hypothesis that auxin-stimulated rooting is due to an ethylene intermediate. However, with a related species of bean, Phaseolus vulgaris, Linkins et al. (43) not only measured IAA-stimulated ethylene production, but also presented evidence which suggested that this ethylene was necessary for auxin-stimulated rooting.

In summary, this experiment indicates that rooting of mung bean cuttings was not affected by ethephon in spite of increased tissue

ethylene levels, and that IBA promoted rooting without affecting tissue ethylene levels.

Experiment II: The Effect of Treatment Solution pH on Rooting and Ethylene Levels in Mung Bean Cuttings Treated with Ethephon, IBA and the Combination of Both

Several authors have assumed that the generation of ethylene from ethephon occurs primarily intracellularly (18, 13), where it would occur rapidly due to the relatively high pH of the cell (~pH 6) (20, 46). However, this assumption is based on only one study (71) involving Bryophyllum cruentum. Since the uptake by cells of a "foreign" anion, like ethephon, would not be expected to be rapid, and since the pH of an ethephon treatment solution is quite low (a 10 ppm solution has a pH of 3.7) it is a reasonable hypothesis that the use of buffers to increase the pH of ethephon treatment solutions would result in increased rooting due to increased ethylene evolution. This experiment was designed to test this hypothesis by investigating the effect of buffered ethephon solutions on rooting and ethylene levels in mung bean cuttings.

Materials and Methods

Mung bean seedlings were grown and cuttings prepared as described for Experiment I. Four growth regulator solutions were used, including the following: a control, 10 ppm ethephon, 10 ppm IBA, and 10 ppm IBA plus 10 ppm ethephon. Each of these solutions was buffered at four different pH's including pH 3.7, pH 5.7, pH 7.4 and an unbuffered solution prepared from distilled water (Table IX). The pH of each of

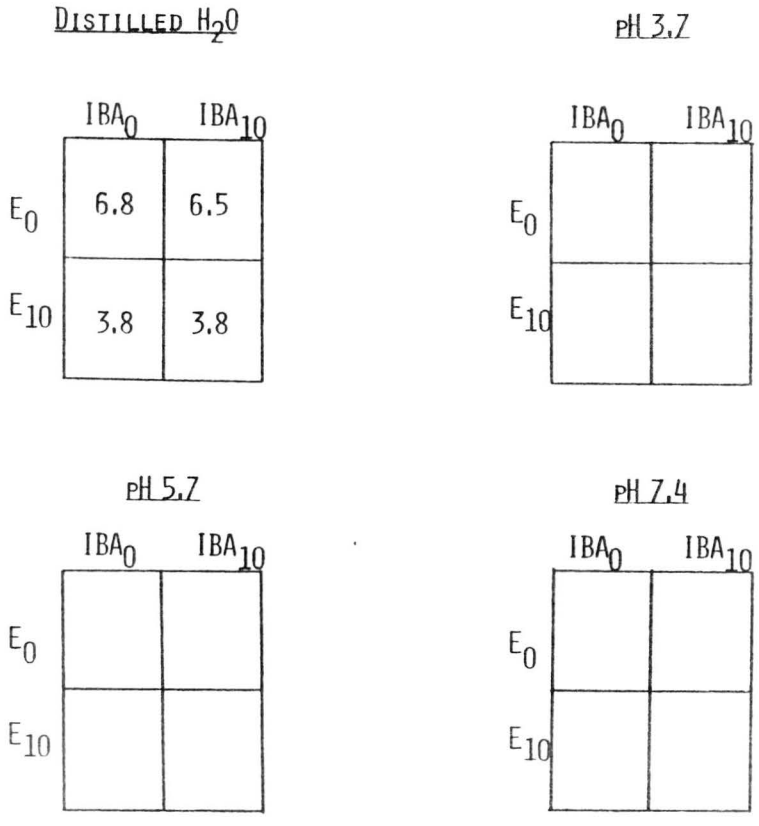


Table IX. The arrangement of treatments in Experiment II. E = ethephon. Subscripts indicate the concentration of growth regulators in ppm. The numbers in the blocks corresponding to the four distilled water treatments refer to the pH of each solution.

the unbuffered treatment solutions is indicated within the appropriate block in the distilled water set treatments in Table IX. This table also indicates the complete three-way factorial arrangement of the treatments. The pH 3.7 buffer was prepared from citric acid/sodium citrate and the pH 5.7 and 7.4 buffers were prepared from potassium phosphate/potassium hydroxide. All buffers were 5 mM since a preliminary experiment indicated that 50 mM buffers resulted in slat injury to the mung bean cuttings. Five mM was sufficiently strong to buffer the growth regulator solutions to within $\pm$0.2 pH units of the pH's indicated (Table IX).

For the rooting part of the experiment, each treatment was replicated 30 times (30 cuttings). Root number per cutting data was analyzed in a balanced three-way AOV. Tukey's Honestly Significant Difference (HSD) was used to determine which means differed significantly (66). Tissue ethylene determinations were replicated at least three times and standard deviations were determined.

Results and Discussion

Regardless of buffer solution pH, ethephon-treated cuttings did not have significantly more (or less) roots than untreated controls (Figure 12; Appendices X and Y). Likewise, cuttings treated with IBA plus ethephon did not have significantly more (or less) roots than cuttings treated with IBA alone. Thus, the data does not support the hypothesis that increasing the pH of an ethephon treatment solution will increase the effectiveness of ethephon as a root-promoting compound. The literature contains no references to other experiments in

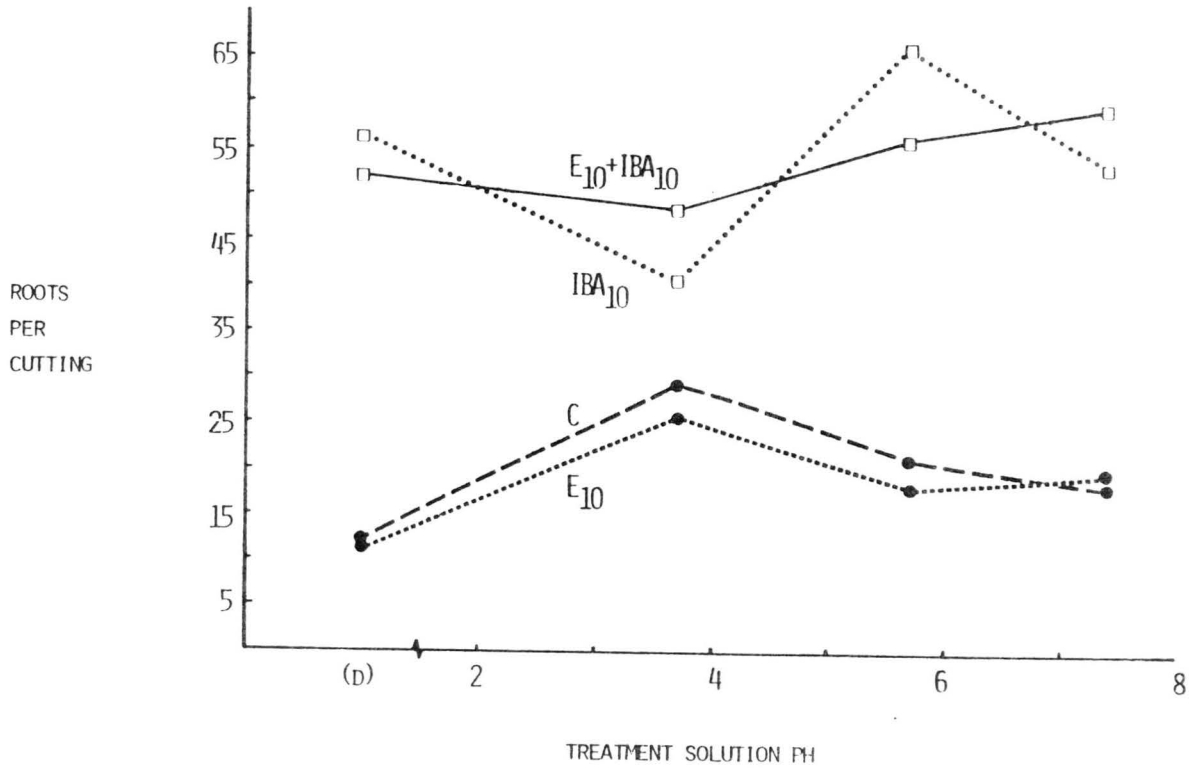


Figure 12. The effect of treatment solution pH on the number of roots per mung bean cutting treated with ethephon, IBA and the combination of both. (D) = distilled water (unbuffered); C = control; E = ethephon. Subscripts indicate growth regulator concentrations in ppm.

which treatment solution pH was examined for its effect on rooting in response to ethephon.

The amount of ethylene within ethephon-treated cuttings was dependent on the pH of the treatment solution (Figure 13). Cuttings treated with ethephon, or IBA plus ethephon, at pH 7.4 had a greater than five-fold increase in tissue ethylene levels over those treated at any of the lower pH's. The approximately exponential increase in tissue ethylene level with increasing treatment solution pH is not unexpected considering the logarithmic nature of the pH scale. As in Experiment I, the data do not indicate any significant stimulation of ethylene production by IBA alone or when applied in combination with ethephon (Figure 13).

Figure 14 shows the lack of correlation between rooting and ethylene levels in cuttings treated with ethephon or IBA plus ethephon. Increasing the pH of the treatment solutions did not increase the effectiveness of ethephon (alone or with IBA) as a root-promoting compound (Figure 14B), even though it did result in a large increase in tissue ethylene levels (Figure 14A).

Summary and Conclusions

Both experiments indicated clearly that ethephon did not promote the rooting of mung bean cuttings regardless of the treatment solution pH, even though measurable amounts of ethylene within the cuttings was detected. A large stimulation of rooting by IBA occurred without detectable increase in tissue ethylene levels.

This lack of a promotion of rooting by ethephon is in marked contrast to the results of Krishnamoorthy (38, 39, 40) who reported that

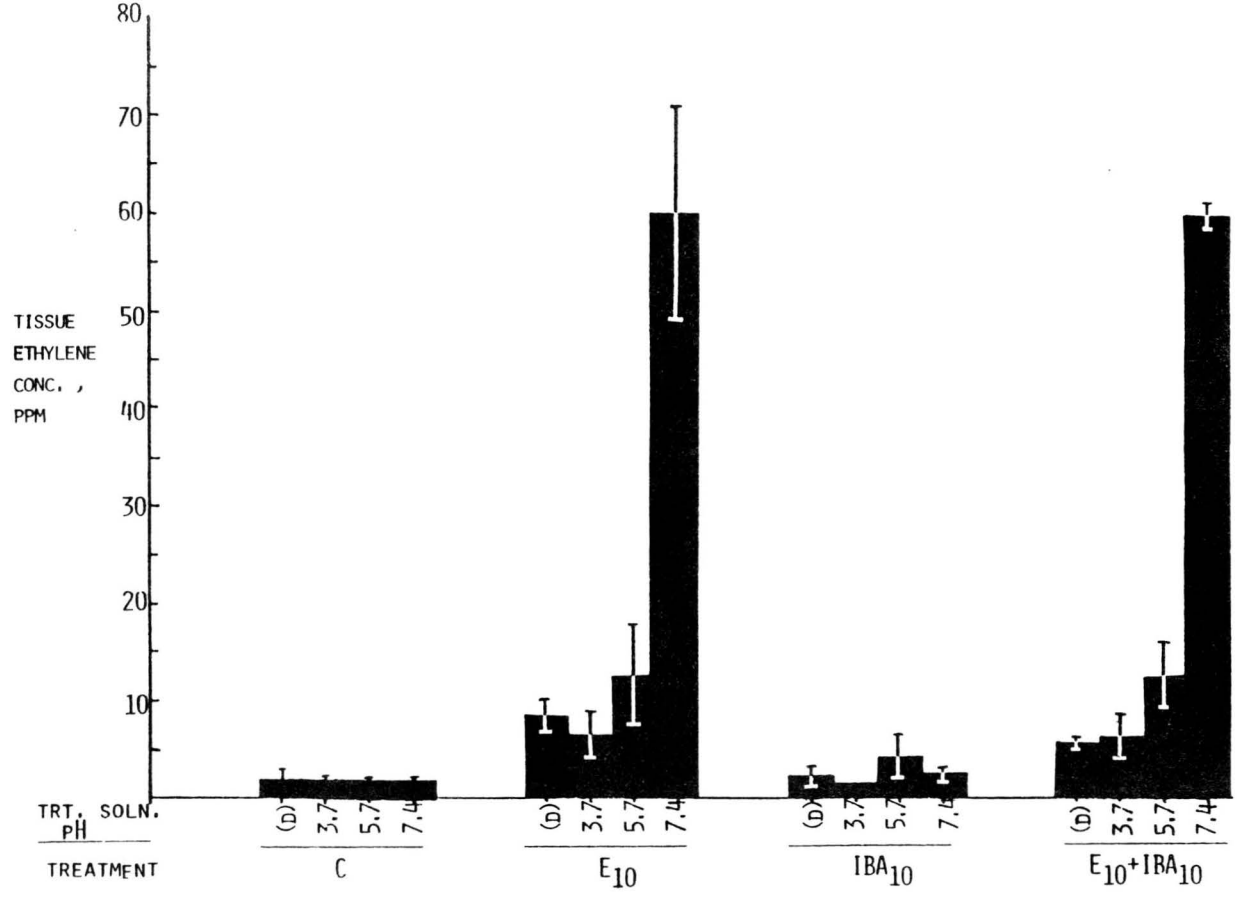
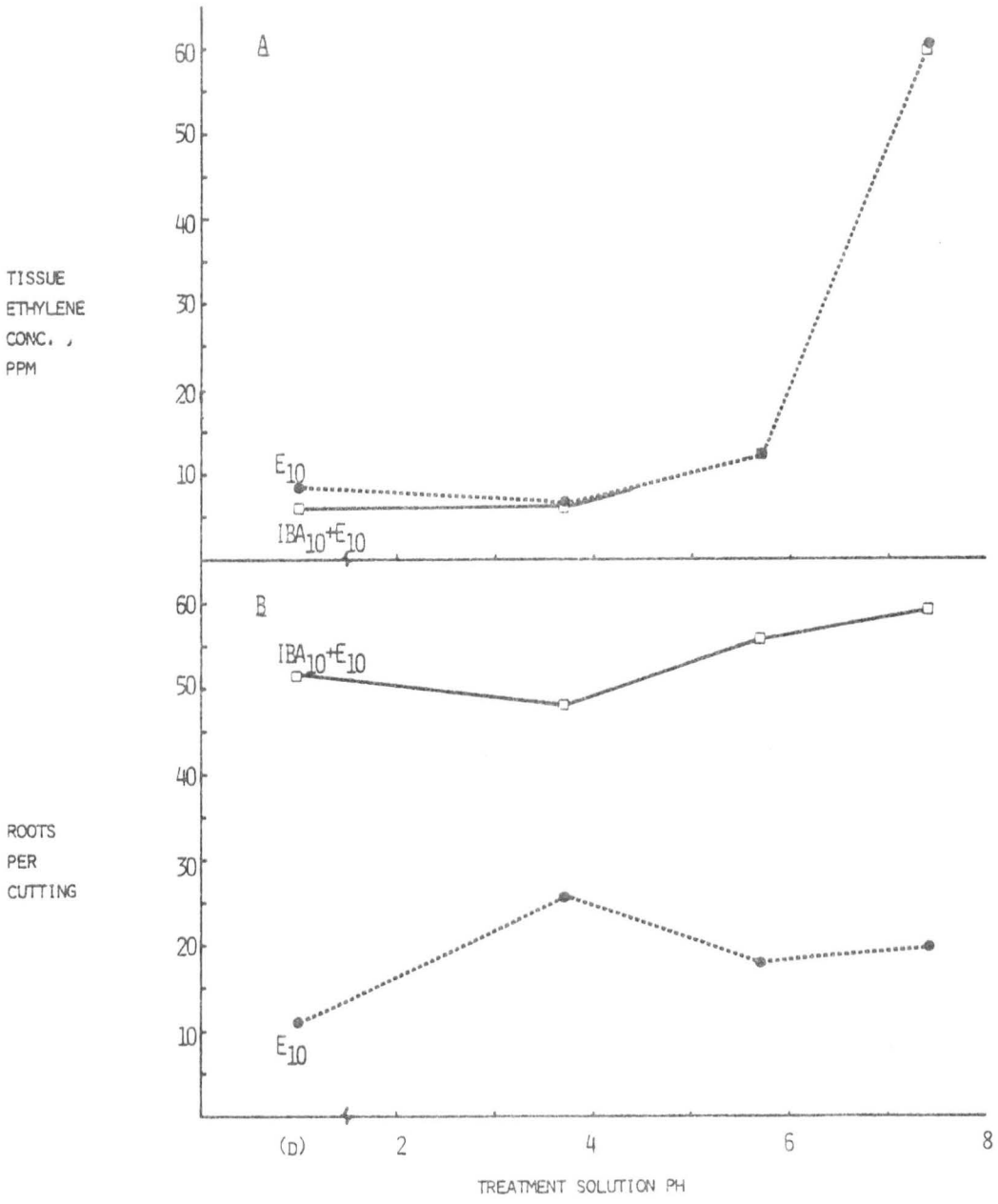


Figure 13. The effect of treatment solution pH on tissue ethylene levels in mung bean cuttings treated with ethephon, IBA and the combination of both. (D) = distilled water (unbuffered); C = control; E = ethephon. Subscripts indicate growth regulator concentrations in ppm. Standard deviations are indicated by the intervals at the top of each treatment bar.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TREATMENT SOLUTION PH

ethephon promoted the rooting of mung bean cuttings. The only difference between the two experiments were the variety of mung bean used, and the relative humidity under which the cuttings were rooted. Krishnamoorthy (38, 39, 40) used P. aureus var. 'T-54', whereas the variety 'Berken' was used in this study. The two varieties may respond differently since similar differences between varieties of other species have been reported (27).

There appears to have been a difference in the relative humidities under which the two studies were carried out. Krishnamoorthy (38, 39, 40) did not specify the relative humidity for his experiments. It is possible that they were conducted at less than 100% r.h. since the ambient relative humidity of Hisar, India, is approximately 50% and ranges from 75% to 20% (65, 73). In preliminary experiments in our laboratory, untreated mung bean cuttings produced about three to four roots per cutting in unhumidified air in contrast to an average of 12.3 roots per cutting for untreated cuttings rooted at approximately 100% r.h. In Krishnamoorthy's (38, 39, 40) experiments, the untreated cuttings had about five roots per cutting. This suggests that those cuttings were rooted in a comparatively low humidity situation. It appears that mung bean cuttings rooted at lower humidities have a reduced potential to root. This is probably due to reduced turgor at lower humidities and consequently reduced root growth.

Light appears to be another factor which reduces the rooting potential of mung bean cuttings. Balzich and Heuser (5) reported that lower light intensities greatly increased the number of roots per mung bean cutting. Mullins (53) reported that untreated controls of dark grown mung bean cuttings had 23.7 roots per cutting, which is more than

the number reported for controls of any experiment involving light grown cuttings. In this same study, Mullins (53) reported that ethylene gas inhibited rooting. It appears that ethylene (or ethephon) promotes rooting primarily in situations in which the full rooting potential of mung bean cuttings is reduced by some environmental factor such as low relative humidity, and/or high light intensity. This interpretation would explain the seemingly contradictory results among the studies by Krishnamoorthy (38, 39 40), Mullins (53) and the study reported here.

Tissue ethylene determinations in both Experiment I and II indicated that the lack of a promotion of rooting by ethephon was indeed a lack of response to ethylene gas and not just a matter of the ineffective conversion of ethephon to ethylene. In Experiment I, with unbuffered ethephon solutions, there were increasing levels of tissue ethylene with increasing ethephon concentrations (0, 5, 10, 15 ppm) but no difference in rooting. In Experiment II tissue ethylene levels increased with increasing pH, but again, no increase in rooting. Experimental systems in which rooting has been shown to be promoted by ethylene (55, 77, 76, 59, 15, 35, 47, 16, 38, 39, 40, 8. 7, 63, 12, 68) need to be investigated with respect to the use of buffered solutions to increase the effectiveness of ethephon.

The failure to demonstrate auxin-induced ethylene production in these experiments was somewhat surprising since it has been reported by others in a wide variety of plant tissues (1), including dark grown mung bean cuttings (53). Perhaps IBA-stimulated ethylene production occurred in our experiments as well, but went undetected because of the technique used to extract ethylene from the cuttings. This technique

(Figure 10) involved extracting gas from the tissue without diluting or concentrating it so that the ethylene (if any) in the sample would have been at approximately the same concentration as it was in vivo. Mullins' (53) reports of IBA-stimulated ethylene production from dark grown mung bean cuttings involved incubation of auxin-treated tissue in an enclosed flask for several hours. With this technique ethylene gas may have increased to levels greater than would have existed in vivo with non-enclosed cuttings. In a close system like this, auto-catalytic ethylene production may occur (1) in response to even a minute amount of auxin-stimulated ethylene, resulting in a substantial magnification of the auxin effect.

Regardless of whether IBA-stimulated ethylene production occurred at an undetectable level or not in these experiments, the fact remains that there were considerably greater levels of ethylene (measureable levels) in ethephon-treated cuttings (Figure 13), and yet ethephon had no effect on rooting whereas IBA promoted it strongly. This evidence strongly suggests that IBA promotes rooting by some mechanism other than stimulation of the plant to produce ethylene.

Although several hypotheses have been proposed to explain the effect of ethylene on root formation (76, 77, 43, 53), the one proposed by Linkins et al. (43) best accounts for the fact that ethylene promotes rooting in some experimental systems but not others (e.g. this study). Based on experiments with Phaseolus vulgaris var. 'Red Kidney', Linkins et al. (43) proposed that ethylene promotes the development, elongation, and ultimately the emergence of auxin-stimulated root initials by reducing the mechanical resistance of the cortex to root enlargement. They were able to show that ethylene

caused an increase in the activity of an extracellular cellulase enzyme which presumable acted on cortical cell walls resulting in cortical swelling and cell separation (i.e. mechanical weakening). Although the system with which they worked required exogenous application of auxin for root initiation, it is probable that in many species of plants the auxin is supplied endogenously. Extending their hypothesis, it is possible that plants which do not respond to exogenously applied ethylene with increased rooting either do not have any mechanical barrier to root emergence (e.g. cortex) or they have sufficient endogenous ethylene to loosen that barrier (such as wound ethylene). Such a hypothesis might explain the seemingly contradictory results between this study and those of Krishnamoorthy (38, 39, 40) and of Mullins (53). In the study reported here, the high humidity in which the cuttings were rooted may have had the effect of increasing turgor and thereby increasing root growth to the point where the cortex was not an effective barrier to root emergence. If this were the case, ethephon would have no effect on rooting. Mung bean cuttings rooted at lower humidity may have had roots with less turgor, resulting in reduced ability to overcome the mechanical resistance offered by the cortex. In this situation a "loosening" effect of ethylene on the cortex would promote rooting. Light intensity might have a similar effect on the ability of elongating roots to overcome cortical resistance.

The results of this study and of other studies on the effect of ethylene on rooting indicates that the response of cuttings to ethylene is determined at least in part by the environmental conditions under which rooting occurs.

CHAPTER 5

SUMMARY

Research was conducted on the effects of ethephon on the rooting of cuttings from the following species of woody plants: Salix caprea, Ribes alpinum, Salix alba, Potentilla fruticosa, Rosa hybrida, Rosa laxa, Forestiera neomexicana and Populus deltoides. Ethephon promoted the rooting of S. caprea and P. fruticosa only. A three-minute soak in 21 ppm and in 1600 ppm ethephon incerased the number of roots per cutting for S. caprea. Number of roots per cutting for P. fruticosa was promoted by a three-minute soak in 1600 ppm ethephon. Root dry weight per cutting for this species was promoted by 0.5, 1 and 3 minute soaks in 1600 ppm ethephon and a 10 minute soak in 21 ppm ethephon. An 18 hour soak in 200 ppm IBA was more effective than an 18 hour soak in 1000 ppm ethephon in terms of both root number and root dry weight for P. fruticosa. An 18 hour soak in 1000 ppm ethephon plus 200 ppm IBA promoted rooting synergistically for this species. Overall, rooting of woody cuttings was not sufficiently promoted by ethephon to warrant recommending its use to commerical plant propagators.

The mung bean rooting bioassay was used to investigate the influence of treatment solution pH on the effectiveness of ethephon, IBA, and ethephon plus IBA on rooting and ethylene levels in treated cuttings. Ethephon at 5, 10 and 15 ppm did not promote rooting regardless

of treatment solution pH (3.7, 5.7 and 7.4), even though it did result in a pH dependent increase in tissue ethylene levels. IBA strongly promoted rooting but had no effect on tissue ethylene levels. These studies indicate that ethylene applied as ethephon does not promote rooting of mung beans and that the promotion of rooting by IBA is not caused by stimulation of ethylene synthesis.

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APPENDICES

Appendix A. Analysis of variance of percent rooted, Experiment I.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	19	3080.0	162.105		
Species	4	1830.0	457.500	4.858	.015
Ethephon	3	120.0	40.000	0.425	.739
Interaction	12	1130.0	94.167		
Error	0	0.0			

Appendix B. Analysis of variance of number of roots per cutting, Experiment I.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	187	110712.829			
Species	4	46395.260	11598.815	37.457	.000
Ethephon	3	2333.871	777.957	2.512	.060
Interaction	12	9798.591	816.549	2.637	.003
Error	168	52022.778	309.659		

Appendix C. Analysis of variance of root dry weight per cutting, Experiment I.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	185	.3820			
Species	4	.0792	.0198	12.3162	.000
Ethephon	3	.0045	.0015	.9387	.423
Interaction	12	.0287	.0024	1.4852	.134
Error	166	.2669	.0016		

Appendix D. Analysis of variance of number of roots per cutting for *Salix caprea*, Experiment II.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	86	1347.931			
Soaking Time	4	60.066	15.017	1.022	.401
Ethephon	2	13.891	6.945	.473	.625
Interaction	5	159.555	31.911	2.172	.066
Error	75	1101.825	14.69		

Appendix E. Analysis of variance of number of roots per cutting for Potentilla fruticosa, Experiment II.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	118	2661.983			
Soaking Time	4	62.496	15.624	.740	.567
Ethephon	2	126.937	63.468	3.005	.054
Interaction	5	228.409	45.682	2.163	.064
Error	107	2259.856	21.120		

Appendix F. Analysis of variance of root dry weight per cutting for Salix caprea, Experiment II.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	85	.002			
Soaking Time	4	.000	.0000	1.411	.239
Ethephon	2	.000	.0000	.691	.504
Interaction	5	.000	.0000	1.272	.285
Error	74	.001	.0000		

Appendix G. Analysis of variance of root dry weight per cutting for Potentilla fruticosa, Experiment II.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	115	.000			
Soaking Time	4	.000	.000	1.234	.301
Ethephon	2	.000	.000	18.269	.000
Interaction	5	.000	.000	1.774	.125
Error	104	.000	.000		

Appendix I. Analysis of variance of percent rooted for Rosa laxa, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	12	15772.0			
Mean	1	10680.333			
IBA	1	432.0	432.0	.847	.384
Ethephon	1	432.0	432.0	.847	.384
Interaction	1	147.0	147.0	.288	.606
Error	8	4080.667	510.083		

Appendix J. Analysis of variance of percent rooted for Potentilla fruticosa, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	12	91878.0			
Mean	1	83333.333			
IBA	1	2465.333	2465.333	3.446	.101
Ethephon	1	75.0	75.0	.105	.754
Interaction	1	280.333	280.333	3.392	.103
Error	8	5724.000	715.500		

Appendix K. Analysis of variance of percent rooted for Forestiera neomexicana, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	12	41401.0			
Mean	1	25854.083			
IBA	1	420.083	420.083	.444	.524
Ethephon	1	7550.083	7550.083	7.987	.022
Interaction	1	14.083	14.083	.015	.906
Error	8	7562.667	945.333		

Appendix L. Analysis of variance of percent rooted for Populus deltoides, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	12	42920.000			
Mean	1	26320.000			
IBA	1	560.330	560.330	1.340	.280
Ethephon	1	11781.330	11781.330	28.080	.001
Interaction	1	901.330	901.330	2.150	.181
Error	8	3356.670	419.580		

Appendix M. Analysis of variance of number of roots per cutting for Salix alba, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	59	10602.183			
IBA	1	1334.817	1334.817	9.070	.004
Ethephon	1	58.017	58.017	3.980	.533
Interaction	1	968.017	968.017	6.578	.013
Error	56				

Appendix N. Analysis of variance of number of roots per cutting for *Rosa laxa*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	24	3334.960			
IBA	1	394.423	394.423	2.822	.108
Ethephon	1	21.890	21.890	.157	.696
Interaction	1	3.497	3.497	.025	.876
Error	21				

Appendix O. Analysis of variance of number of roots per cutting for *Potentilla fruticosa*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	53	214629.037			
IBA	1	51041.933	51041.933	17.420	.000
Ethephon	1	4316.890	4316.890	1.480	.230
Interaction	1	12144.159	12144.159	4.163	.047
Error	50	145865.571	2917.311		

Appendix P. Analysis ov variance of number of roots per cutting for *Foresteria neomexicana*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	33	71808.029			
IBA	1	24928.933	24928.933	16.164	.000
Ethephon	1	4.453	4.453	.003	.958
Interaction	1	21.153	21.153	.014	.908
Error	30	46268.776	1542.293		

Appendix Q. Analysis of variance of number of roots per cutting for *Populus deltoides*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	37	1189.263			
IBA	1	125.893	125.893	4.450	.042
Ethephon	1	162.7851	162.785	5.755	.022
Interaction	1	4.1885	4.189	.148	.703
Error	34	961.783	28.289		

Appendix R. Analysis of variance of root dry weight per cutting for *Salix alba*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	60	.208			
Mean	1	.180			
IBA	1	.002	.002	4.642	.036
Ethephon	1	.002	.002	3.538	.065
Interaction	1	.000	.000	.166	.685
Error	56	.024	.000		

Appendix S. Analysis of variance of root dry weight per cutting in *Rosa laxa*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	23	.054			
IBA	1	.002	.002	.861	.365
Ethephon	1	.003	.003	1.115	.304
Interaction	1	.000	.000	.107	.747
Error	20	.050	.003		

Appendix T. Analysis of variance of root dry weight per cutting for *Potentilla fruticosa*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	53	.030			
IBA	1	.008	.008	21.654	.000
Ethephon	1	.000	.000	1.094	.301
Interaction	1	.001	.001	3.657	.062
Error	50	.091	.000		

Appendix U. Analysis of variance of root dry weight per cutting for *Forestiera neomexicana*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	32	.039			
Ethephon	1	.003	.003	2.849	.102
IBA	1	.009	.009	8.471	.007
Interaction	1	.000	.000	.336	.567
Error	29	.029	.001		

Appendix V. Analysis of variance of root dry weight per cutting for *Populus deltoides*, Experiment III.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	37	.062			
Ethephon	1	.011	.011	7.655	.009
IBA	1	.001	.001	.760	.390
Interaction	1	.000	.000	.036	.852
Error	34	.051	.002		

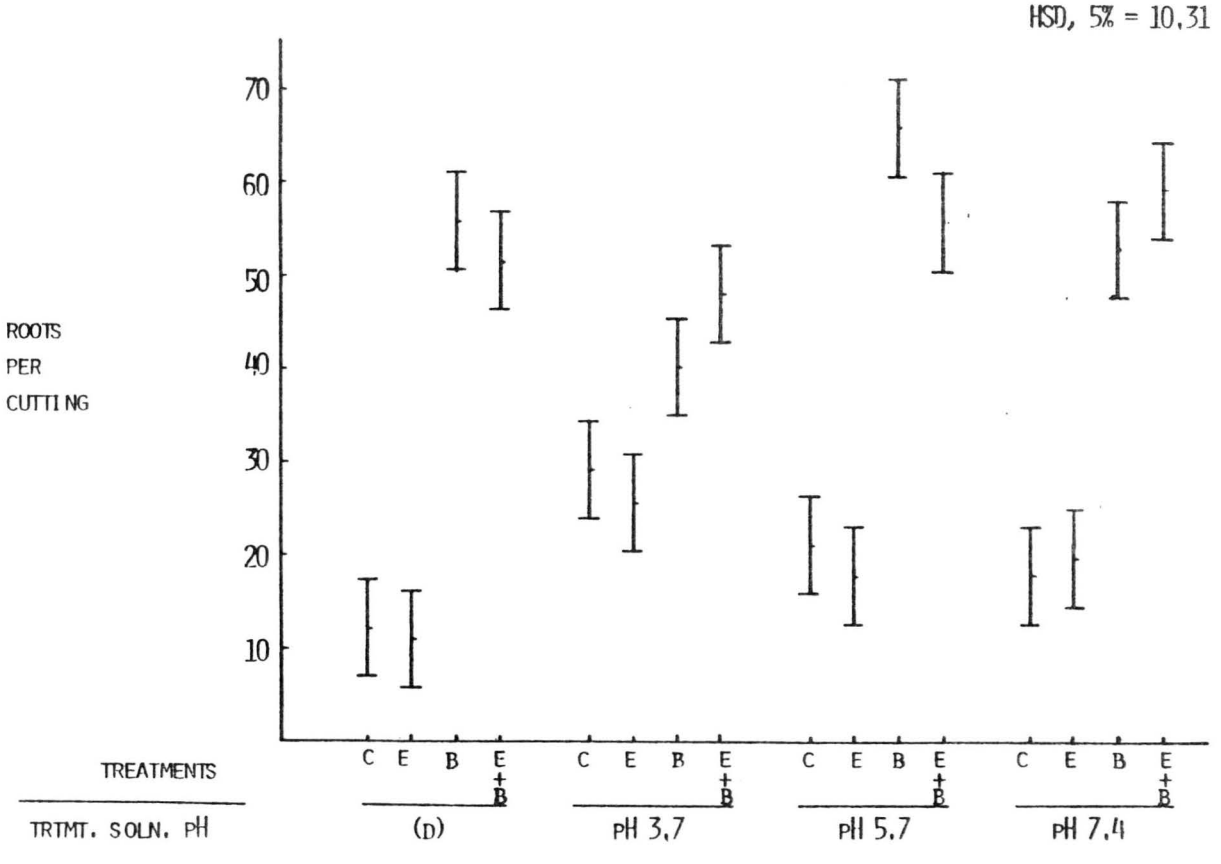
Appendix W. Analysis of variance of number of roots per cutting for mung beans, Experiment I.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total	198	100325.739			
Ethephon	3	628.387	209.462	2.036	.110
IBA	1	74137.985	74137.985	720.682	.000
Interaction	3	586.135	195.378	1.899	.131
Error	191	19648.540	102.872		

Appendix X. Analysis of variance of number of roots per cutting for mung beans, Experiment II.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F	F Probability
Total		224479.167			
pH	3	3496.833	1165.611	8.597	.000
Ethephon	1	78.408	78.408	.578	.448
IBA	1	141384.675	141384.675	1042.800	.000
pH x Ethephon	3	2134.9583	711.653	5.249	.001
pH x IBA	3	12932.225	4310.742	31.795	.000
IBA x Ethephon	1	73.633	73.633	.5431	.462
pHxIBAxEthephon	3	1470.500	490.167	3.615	.013
Error	464	62907.933	135.577		

Appendix Y


HSD, 5% = 10.31
ROOTS PER CUTTING
70
60
50
40
30
20
10
C E B E+B C E B E+B C E B E+B C E B E+B
TREATMENTS
TRTMT. SOLN. pH
(D)
pH 3.7
pH 5.7
pH 7.4