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EFFECT OF ALCOHOL SOLVENTS AND IBA ON ROOTING OF SELECT WOODY ORNAMENTAL AND HERBACEOUS PLANTS

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

Alcohol toxicity based on solvent has been reported to be a problem in the propagation of certain woody ornamental plants. The purposes of this study were: (1) to evaluate the phytotoxicity of several alcohol solvents; (2) to evaluate the effect of IBA levels on several varieties of woody and herbaceous plants; (3) to determine whether or not the alcohol solvents could reduce the rate of IBA oxidation in solution.

Ethanol, polyethylene glycol 400, propylene glycol and isopropanol were selected as solvents for IBA. Terminal cuttings of several varieties of herbaceous and woody plants suspected to be alcohol sensitive were treated with a five-second quick-dip of the alcohol IBA solutions. After the cuttings were rooted and evaluated, no meaningful significant difference on rooting was found between the four alcohols tested, and no phytotoxicity was observed on any of the plant varieties tested. The primary influence on rooting was the concentration of IBA in the quick-dips.

A mung bean hypocotyl rooting bioassay was developed to study the effect of the alcohol solvents in the reduction rate of IBA oxidation in solution. Analysis of variance showed a significant influence of IBA, solvent, and age on root number of rooted mung bean hypocotyl cuttings. All interactions of these factors other than the IBA and age interaction were significant as well.

Duncan's New Multiple Range Test showed that one year old solutions of 1 and 10 ppm IBA in polyethylene glycol 400 produced significantly fewer roots per experimental unit than did freshly prepared solutions of 1 and 10 ppm IBA in PEG at the 1 percent level of significance. The results indicate that ETOH, PG and IPA reduce the oxidation rate of IBA in solution compared to PEG, and that no auxin activity is lost after one year in solution. Visual observation showed differences in color among freshly prepared and aged 1000 ppm IBA solutions in ETOH, PEG, PG and IPA. Spectrophotometric analysis was used to analyze the differences among these IBA solutions. Spectrophotometric analysis revealed a large peak at approximately 225 nm in all of the freshly prepared IBA solutions. Aged solutions of IBA in ETOH, PG and IPA produced lower peaks than the comparable freshly prepared solutions at 225 nm, whereas the aged IBA solution in PEG produced no peak whatsoever at 225 nm. The absence of a peak at 225 nm in the aged IBA solution in PEG and the lower peak for PG corresponded with the reduced rooting in the mung bean bioassay.

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I. INTRODUCTION

Indole-3-acetic acid (IAA), a naturally occuring compound, was isolated and subsequently shown to have considerable auxin activity in 1934 (20). IAA was found to promote the formation of adventitious roots in the following year (5), and the synthetic auxins indolebutyric acid (IBA) and napthaleneacetic acid (NAA) were shown to have even greater auxin activity than IAA (24). It has been shown that auxin is essential for initiation of adventitious roots (7).

The use of auxins in the asexual propagation of plants is a common practice in the nursery industry. IBA and NAA have been selected as the most reliable synthetic auxins in stimulating adventitious root production in cuttings (9). These auxins can be purchased as talc or liquid preparations or as pure chemicals that propagators can use in formulating their own preparations.

There are several ways of applying an auxin to a cutting: talc preparations, dilute solution soaking method and concentrated solution quick-dip method. Talc preparations are readily available and easy to use, but do not always give consistent results due to variation in the quantity of material adhering to the cutting (9). The dilute solution soaking method consists of soaking cuttings in a dilute solution of an auxin for about 24 hours before being inserted into the rooting medium. The main disadvantage of the dilute solution soaking method is the long

period of time required to treat cuttings. In contrast, the concentrated solution quick-dip method consists of dipping the basal end of cuttings in an auxin solution typically in the range of 1000 or greater parts per million (ppm) for 1 to 10 seconds. The concentrated solution quick-dip method is the commonly used method in the nursery industry today, since uniform results are much more likely than with either the talc preparations or the dilute solution soaking method (9). The concentrated solution quick dip method allows for easy preparation of solutions spanning a wide range of auxin concentrations, as well as easy and rapid treatment of large numbers of cuttings.

Alcohol quick-dips usually become discolored within a few days after preparation. Most propagators believe that this discoloration is associated with the loss of auxin activity in the quick-dip. Therefore, the unused portions of quick-dips are often discarded less than one week after being prepared.

Liquid quick-dips are either purchased as commercial formulations or are prepared by the propagator. An alcohol is generally used as a solvent for the auxin. Unfortunately, cuttings of certain varieties of plants appear to be alcohol sensitive. In these instances the propagator must rely on using talc preparations or water-soluble potassium salt IBA (K-IBA) in water to root these plants.

The purposes of this study were: (1) to evaluate the phytotoxicity of several alcohol solvents, (2) to evaluate the

effect of IBA levels on several varieties of woody and herbaceous plants, and (3) to determine whether or not the alcohol solvents could reduce the rate of IBA oxidation in solution.

II. LITERATURE REVIEW

A. Alcohol Toxicity

Ethanol and isopropanol are commonly used as solvents for IBA. To date, few studies referring to alcohol sensitive species have been performed. Water-soluble potassium salt IBA is often used with alcohol sensitive Indica azaleas such as 'Salmon Beauty' (3). Kalmia latifolia and Pieris floribunda rooted poorly using an IBA quick-dip with 95% ethanol, however, several chestnut clones rooted well with 95% ethanol as a solvent (12). Berberis thunbergii atropurpurea 'Crimson Pygmy' and other Berberis thunbergii cultivars are reported to be alcohol sensitive (8)(24). Ilex vomitoria 'Schilling's Dwarf' and other Ilex vomitoria cultivars often undergo rapid defoliation and have basal necrosis caused by the alcohol solvent for IBA (15)(22). Good results have been reported in propagating Pieris japonica and Pieris formosa using 2500 ppm IBA and NAA as a 5-second quick-dip (14). The alcohol used in the quick-dip was not specified.

B. Solvents Used in Propagation

Ethanol and isopropanol are the most commonly used alcohols in propagation. Polyethylene glycol is occasionally used in

preparing liquid quick-dips (6). Solvents such as mannitol, sorbitol, glycerine and propylene glycol have been tested as solvents and carriers of IBA because of evidence of tissue penetration and tissue tolerance (23).

C. Auxin Transportation

Wounding of cuttings does not appear to be necessary for optimal rooting unless the wound is the only available port of entry for IAA. It has been found that IAA is able to enter cuttings through leaf scars (16). Cuttings with and without terminal buds removed that were treated at the terminal end with labeled IAA transported the same amount of auxin to basal areas of cuttings.

D. Tomato Leaf Rooting Bioassay

A tomato leaf rooting bioassay consisting of leaf cuttings that are treated with growth promoting substances has been useful in testing for auxin activity. These cuttings root in five to seven days and the control cuttings produce few or no roots within this time (11). This test is simple, economical, quick and is useful in determining auxin activity. The tomato leaf bioassay is

also highly sensitive to the solvents used with root promoting chemicals (9).

E. Mung Bean Rooting Bioassay

The mungbean bioassay has been used for determining auxin activity of a wide range of compounds (10). Considerable variation has been observed in the mungbean bioassay. It has been found that seed source (2), seed size and cotyledon removal (1) produce variability in root number per cutting during rooting studies of mungbean. Removal of cotyledons reduced the endogenous auxin level and allowed treatments to produce greater effects on root number. Root regeneration is pH influenced and has a maximum response with pH 6.5 (25). Robbins et al. (9) designed a system to maintain a constant pH throughout the rooting bioassay (18). It was found that a constant pH was not significant in influencing root number per cutting compared to a system that allowed pH to fluctuate during the bioassay. Microbial contamination has been found to cause inconsistent results in the mungbean rooting bioassay (4). Boiling and filter sterilization of rooting medium did not completely eliminate the inconsistent results, but autoclaving the rooting medium eliminated the variability in the bioassay.

Mungbean cuttings rooted in distilled water produced 4 to 5 roots per cutting while 2 ppm IBA produced 7 to 11 roots per

cuttings (2). Four hundred ppm IAA is inhibitory to rooting and can greatly decrease the total root length by 61.6% per cutting relative to the control, whereas 50 ppm IAA increased root length per cutting by 51.9%.

F. Auxin Oxidation

Indole-3-acetic acid has been shown to be catabolyzed by peroxidative decarboxylation to 3-methyleneoxindole, 3-methyl-oxindole or indole-3-carboxylic acid in the presence of 'IAA oxidases' found in higher plants (19). Catabolism of IBA can also occur by oxidation to form oxindole-3-acetic acid (17). The presence of light can inactivate IAA *in vitro* (21). A 10 ppm concentration of IAA is destroyed by strong sunlight in about 15 minutes (9). Twenty hours of strong sunlight will only produce a slight change in a 10 ppm concentration of IBA.

III. MATERIALS AND METHODS

A. Survey of Southeastern Nurseries

To determine the magnitude of the alcohol toxicity problem, a survey (Appendix 1) was sent to 88 nurseries throughout the southeast. The sampling area covered Alabama, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas and Virginia. Only nurseries that were known to propagate large numbers of plants were included in the survey. The survey was designed to provide information on current problems associated with alcohol quick-dips.

B. Selection of Non-phytotoxic Alcohols by Means of the Tomato Leaf Rooting Bioassay

Tomato leaf cuttings were used to select alcohols that exhibited very low phytotoxicity. Alcohols that are not phytotoxic to tender tissue generally are not phytotoxic to woody plants. Elimination of some alcohols may not have been necessary if a woody plant species had been used in the screening since herbaceous plants exhibit alcohol phytotoxicity that might not occur in woody plants. However, the long period of time needed to perform alcohol screening with a woody plant species was not practical.

A series of 25%, 50%, 75% and 100% concentrations of seven alcohols (ethanol, polyethylene glycol 400, propylene glycol, isopropanol, methanol, ethylene glycol and glycol) plus a distilled water control was prepared for the screening process. Solvent codes and descriptions are listed in Appendix 2.

Tomato 'Royal Ace' seeds were planted in a commercial seed starting medium. Seedlings were transplanted into 48 cell plastic cell packs when the first pair of true leaves had developed. Plants were placed in a greenhouse and fertilized every watering with Peter's General Purpose Fertilizer 20-20-20- (W. R. Grace and Company, Fogelsville, PA) at 150 ppm nitrogen.

Leaf cuttings were taken when plants were approximately one foot tall. Cuttings, consisting of a leaf with petiole, were taken from approximately the same node of each plant. Five cuttings were used per experimental unit.

Cuttings were treated with a 5-second quick-dip and stuck under intermittent mist in a medium of 1 part peat moss : 1 part horticultural grade perlite. Mist frequency was set for one 6-second burst per 6 minutes during daylight hours. Cuttings were arranged in a randomized complete block experimental design consisting of 3 replications with 5 cuttings per treatment for a total of 435 cuttings. Block locations were chosen that minimized variation caused by differences in propagation bench location.

Cuttings were removed from the propagation bench after seven days, at which time the degree of rooting of the cuttings was

recorded, as was any extent of necrosis or chlorosis of the leaflets.

C. Evaluation of Several Alcohols as Non-toxic Carriers of IBA

A factorial design consisting of four solvents (ETOH, PEG, PG and IPA) at 50% by volume and four concentrations of the solute IBA (0, 1000, 3000 and 5000 ppm, unless otherwise specified) in each of the solvents was used in the experiments.

Cuttings were treated with a 5-second quick-dip and stuck under intermittent mist. Mist frequency was set for one 6-second burst per 6 minutes during daylight hours. Cuttings were arranged in a randomized complete block experimental design. Block locations were chosen that minimized variation caused by differences in propagation bench location.

Data were collected and then subjected to analysis of variance (ANOVA) using Statistical Analysis System software (SAS Institute, Inc., Cary, NC) on IBM 4381 computer facilities through the University of Tennessee Computing Center. Mean separations were performed using Duncan's New Multiple Range Test.

1. 'Royal Ace' Tomato Leaf Propagation Study

Treatments consisted of 2000 and 4000 ppm IBA in addition to the rates listed above. Tomato 'Royal Ace' plants were grown and cuttings prepared as described in Chapter III-B.

Cuttings were treated on April 5, 1986, and stuck in a medium of 1 part peat moss : 2 parts horticultural grade perlite. Experimental design consisted of 10 replications with 1 cutting per treatment for a total of 240 cuttings.

On April 17, 1986, cuttings were removed from the propagation bench and visually evaluated for root number and root growth using an arbitrary numerical scale of 1-5 (Figure 1). Any basal necrosis or chlorosis of the leaflets was also recorded. The 5% significance level was selected as an acceptable error for all analysis conducted in this experiment.

2. 'Aurora' Geranium Propagation Study

Treatments were the same as in the 'Royal Ace' tomato propagation study. Terminal cuttings 4" long were taken from disbudded stock plants of 'Aurora' geranium on March 15, 1986. The lower 1" of each cutting was stripped of leaves.

Cuttings were treated on March 15, 1986, and stuck in a medium of 1 part peat moss : 3 parts horticultural grade perlite.

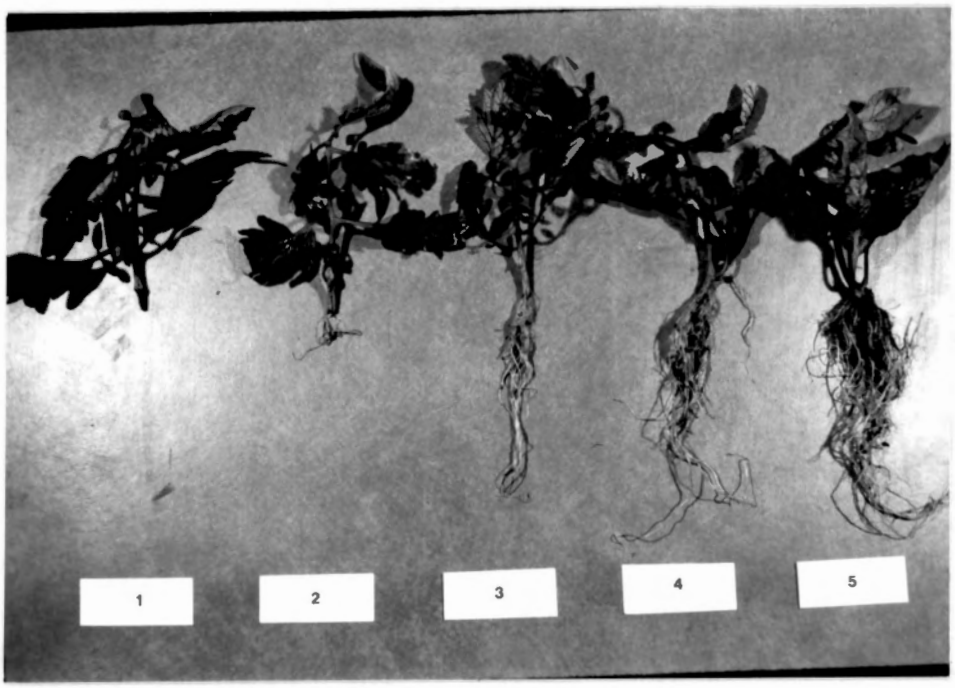


Figure 1. Visual Root Rating Scale for 'Royal Ace' Tomato Cuttings.

Experimental design consisted of 10 replications with 1 cutting per treatment for a total of 240 cuttings.

On April 18, 1986, cuttings were removed from the propagation bench and evaluated for root growth by measuring root ball diameter in millimeters. Basal necrosis or foliar chlorosis was also recorded. The 1% significance level was selected as an acceptable error for all analysis conducted in this experiment.

3. 'Crimson Pygmy' Barberry Propagation Study

A group of semi-hardwood cuttings of 'Crimson Pygmy' Barberry that had terminated growth and having decreased physiological activity, and almost completely lignified (hardened off) was obtained via two day United Parcel Service delivery from Wight Nursery (Cairo, GA) on July 24, 1986. A second group of semi-hardwood cuttings that were softer and still actively growing (cuttings that still maintain a high level of physiological ativity, yet have not become completely lignified) was collected at Little Green Garden (Knoxville, TN) on July 28, 1986. Terminals of cuttings from Little Green Garden were removed to make all cuttings 4" long. Cuttings from Wight Nursery were harder and more mature than cuttings from Little Green Garden. Basal ends were recut since the lower ¼" of the cuttings had become

dessicated during shipping. After recutting, all cuttings from Wight Nursery were 3" long. The lower ¾" of each cutting in each group was stripped of leaves and thorns, to provide adequate wounding.

Cuttings from Wight Nursery were treated on July 24, 1986, and those from Little Green Garden were treated four days later. Following treatment, cuttings were stuck in a medium of 1 part peat moss : 3 parts horticultural grade perlite. Experimental design consisted of 4 replications with 4 cuttings per treatment for a total of 256 cuttings from Little Green Garden and 6 replications with 4 cuttings per treatment for a total of 384 cuttings from Wight Nursery.

Cuttings from Wight Nursery and Little Green Garden were removed from the propagation bench on September 11 and 15, 1986 respectively and evaluated for root number and root growth using a subjective visual rating scale of 1-10 (Figure 2). The presence of defoliation or basal necrosis was also recorded. The 5% significance level was selected as an acceptable error for all analysis conducted in this experiment.

4. 'Schilling's Dwarf' Yaupon Holly Propagation Study

Semi-hardwood cuttings of 'Schilling's Dwarf' yaupon holly were obtained via two day United Parcel Service delivery from Wight Nursery (Cairo, GA) on July 24, 1986. The basal ends of



Figure 2. Visual Root Rating Scale for 'Crimson Pygmy' Barberry Cuttings.

cuttings were recut since they were dessicated upon arrival. The terminal end of cuttings was removed to make all cuttings 3" long, and the lower ¾" of each cutting was stripped of leaves and a single ¼" long wound was made at the base. Cuttings were separated into two groups; those with three or less branches (lightly branched) and those with more than three branches (heavily branched).

Cuttings were treated on July 24, 1986, and stuck in a medium of 1 part peat moss : 2 parts horticultural grade perlite. Experimental design consisted of 6 replications (three replications each of lightly and heavily branched cuttings) with 4 cuttings per treatment for a total of 384 cuttings.

On September 9, 1986 cuttings were removed from the propagation bench and evaluated for root number and root growth using a subjective visual rating scale of 1-5 (Figure 3). Percent defoliation was rated on a scale of 1-5 (100% defoliation - 0% defoliation). Any basal necrosis was also recorded. The 5% significance level was selected as an acceptable error for all analysis conducted in this experiment.

5. Variegated Privet Propagation Study

Semi-hardwood cuttings of variegated privet were collected from a stock block at the University of Tennessee (Knoxville, TN) on July 28, 1986. The terminal ends of cuttings were removed to

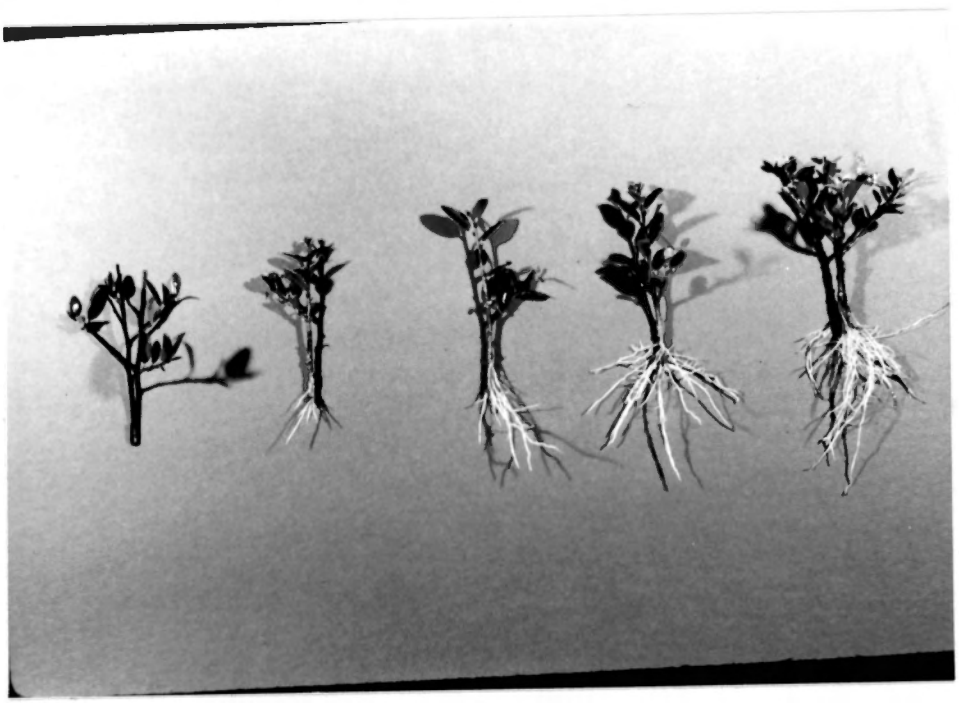


Figure 3. Visual Root Rating Scale for 'Schilling's Dwarf' Yaupon Holly Cuttings.

make all cuttings 4" long, and the lower 1" of each cutting was stripped of leaves. A single ½" long wound was made at the base of each cutting.

Cuttings were treated on July 28, 1986, and stuck in a medium of 1 part peat moss : 2 parts horticultural grade perlite. Experimental design consisted of 4 replications with 4 cuttings per treatment for a total of 256 cuttings.

On September 9, 1986, cuttings were removed from the propagation bench and evaluated for root number. Basal necrosis or defoliation was also recorded. The 5% significance level was selected as an acceptable error for all analysis conducted in this experiment.

D. Determination of Alcohol Potential to Reduce the Oxidation Rate of IBA

1. Mung Bean Rooting Bioassay

An incomplete factorial design was used in this experiment. Treatments consisted of four solvents (ETOH, PEG, PG and IPA) at 0.5% by volume and IBA at 0.01, 0.1, 1.0 and 10.0 ppm in each of the solvents. The final IBA concentrations were prepared from 1000 ppm IBA stock solutions that were prepared either immediately prior to treatment (new) or one year in advance of treatment

(old). The aged alcohols without IBA were not used since aged and fresh alcohol would elicit the same results.

Mung bean seeds were obtained from Natural and Organic Foods (Knoxville, TN), a local health food store. Seeds were surface sterilized with 25% Clorox and 0.04% Triton X-100 for 10 minutes and then rinsed three times with sterile double distilled water. Sterilized mung bean seeds were germinated on 0.7% water agar in 4" deep GA7 culture boxes (25 seeds and 50 ml agar per culture box), which were placed in a growth chamber with 1324 lux and a 16 hour photoperiod. The temperature was maintained at 24° C plus or minus 0.1° C. Planting of seeds and processing of cuttings were done under sterile conditions under a laminar flow hood. By four days after planting, seedlings had developed sufficiently to take cuttings. Seeds germinated on 0.8% water agar required 10 days to develop enough to take cuttings. Cuttings were taken from seedlings that had the first pair of true leaves still folded and epicotyl beginning to straighten out. Each cutting consisted of 3 cm of hypocotyl, epicotyl (1 cm or less in length) and the first pair of true leaves. Cotyledons were removed to reduce the endogenous auxin level in the seedlings. After cuttings were prepared, they were placed in sterile double distilled water to prevent drying out until treatment (less than 30 minutes).

Hormone treatments were prepared by serial dilution of fresh and aged 1000 ppm IBA in ETOH, PEG, PG and IPA at 50% by volume. Final IBA stock solution concentrations were 1, 10, 100 and 1000

ppm. Media, which were mixed in GA7 culture boxes, consisted of 148.5 ml of 0.3% agar adjusted to pH 6.5 and 1.5 ml of the IBA stock solutions. Each medium was poured into a GA7 culture box and then autoclaved for 25 minutes at 230° C and 20 psi, then vented for 45 minutes. Final IBA concentrations in culture box media were 0.01, 0.1, 1.0 and 10.0 ppm and 0.5% alcohol by volume. Solvent controls at 0.0 ppm IBA were also included.

Each cutting was inserted so that the cotyledonary node was at the surface of the medium, with four cuttings per culture box (experimental unit). Culture boxes were placed 8" below 40 watt fluorescent grow lights with a 16 hour photoperiod. The boxes were arranged in a randomized complete block experimental design consisting of 3 replications with one experimental unit per treatment for a total of 108 culture boxes or 432 mung bean hypocotyl cuttings. The entire experiment was repeated two weeks later using a different seed lot from the same supplier and a similar experimental design to determine if consistent results could be obtained. Three rather than 4 cuttings were used as an experimental design because of the poor germination rate found in the second seed lot. Thus, data from the second repetition were transformed to a 4 cutting per culture box basis in order to allow statistical analysis of both repetitions together.

Seven days after treatment, cuttings were removed from culture boxes and evaluated for root number per cutting. Roots 1mm or longer were counted. Any basal necrosis and differences in

root length among treatments were recorded. Root number was totaled for each experimental unit for statistical analysis.

Data were subjected to analysis of variance using the general linear model using Statistical Analysis System software (SAS Institute, Inc., Cary, NC) on IBM 4381 computer facilities through the University of Tennessee Computing Center. Mean separations were performed using Duncan's New Multiple Range Test. The 1% significance level was selected as an acceptable error for all analysis conducted in this experiment because of the precision of the data collected.

2. Spectrophotometric Analysis of IBA Breakdown Products

Immediately prior to spectrophotometric analysis, 1 ppm concentrations of IBA were prepared by serial dilutions from freshly prepared (new) and year-old (aged) 1000 ppm IBA stock solutions using ETOH, PEG, PG and IPA at 50% by volume.

IBA-alcohol samples were analyzed against the respective alcohol solvent without IBA by a Beckman Spectrophotometer Model 35. Spectral analysis was from 200-750 nm in wavelength.

IV. RESULTS AND DISCUSSION

A. Survey of Southeastern Nurseries

A response was received from 57 of 88 (64.8%) nurseries that were asked to participate in the survey. A liquid quick-dip was used by 49 (84%) of the 57 nurseries that responded. Of the 49 nurseries using quick-dips, 28 (58%) prepared their own formulations using a range from 0.5 to 110 gallons of alcohol per nursery per year, with a mean of 17.8 gallons. Of the 20 nurseries that purchased commercial preparations, 11 (55%) used Dip-N-Grow, 3 (15%) used Chloromone, 6 (30%) used Wood's Rooting Compound, and 2 (10%) used C-Mone. Two nurseries used more than one proprietary preparation.

The questions pertaining to alcohol toxicity were answered by 38 (67%) of the 57 nurseries that responded to the survey. Phytotoxicity symptoms thought to be due to alcohol were reported by 23 (61%) of these 38 nurseries during cutting propagation of certain plants (Table 1). In order to avoid perceived alcohol toxicity, 19 (83%) of the 23 nurseries reporting phytotoxicity symptoms used K-IBA in water or IBA in talc on the cuttings of plants thought to be alcohol sensitive. Also, 9 (35%) of the 26 nurseries using quick-dips but not actually reporting alcohol toxicity were wary enough of the perceived problem to use IBA in

Table 1. Plants Reported to Show Alcohol Toxicity Symptoms.

	ALCOHOL						
Plant Name	DA	DG	E	I	M	R	NS
Acer rubrum					X		
Berberis thunbergii atr. 'Crimson Pygmy'			X	X		X	
Berberis thunbergii 'Aurea'				X		X	
Betula nigra 'Heritage'	X						
Bignonia capreolata				X			
Cornus florida				X			
Cotinus species (unspecified)					X		
Cotoneaster species				X			
Elaeagnus species							X
Hibiscus syriacus				X			
Ilex crenata cultivars (unspecified)				X			
Ilex opaca				X			
Ilex vomitoria 'Folsom's Weeping'			X	X			
Ilex vomitoria 'Schillings'			X	X			
Ilex vomitoria 'Stoke's Dwarf'			X	X			
Juniperus species (unspecified)				X			
Juniperus squamata 'Blue Star'		X					
Lagerstroemia indica				X			
Leucophyllum species (unspecified)							X
Magnolia species (unspecified)					X	X	
Metasequoia glyptostroboides					X		
Philadelphus coronarius					X		
Pieris japonica							X
Pittosporum tobira		X		X			
Pittosporum tobira 'Variegata'		X		X			
Prunus species (unspecified)					X		
Raphiolepis indica				X			
Rhododendron species				X			
Viburnum species (unspecified)	X			X			
Vinca minor				X			

DA=Denatured Alcohol. DG=Dip-N-Grow. E=Ethanol. I=Isopropanol. M=Methanol. R=Reagent Alcohol. NS=Not Specified.

talc or K-IBA in water as a rooting hormone for plants that seem alcohol sensitive. The survey was useful because it demonstrated that the alcohol toxicity associated with IBA/alcohol quick-dips on certain plants is perceived as a problem by the nursery industry and that research into the area of alcohol toxicity is needed. The survey also pinpointed the kinds of plants thought to be alcohol sensitive in the Southeast.

B. Selection of Non-phytotoxic Alcohols by Means of the Tomato Leaf Rooting Bioassay

Three days after treatment, tomato leaves treated with METH, EG and G were exhibiting chlorosis. After seven days all leaves were showing chlorosis regardless of the treatment. This may have been caused by nutrients being leached from leaf tissue by the mist. Cuttings treated with METH and G, at all concentrations, were completely necrotic and had no living tissue remaining at the basal end. Cuttings treated with EG, at all concentrations, exhibited extensive phytotoxicity symptoms and had completely rotted within seven days. Cuttings treated with ETOH, PEG, PG and IPA at 75% and 100% had some necrosis at the basal end, but there was no damage at 25% or 50% from these four solvents.

Rooting may have been promoted slightly by ETOH, PEG, PG and IPA over the control. This root promotion was slight and did not appear to be of statistical significance.

This screening process eliminated METH, EG, and G from further propagation research due to their relative phytotoxicity. ETOH, PEG, PG and IPA all appeared to be non-toxic to 'Royal Ace' tomato at 25% and 50%. Due to the solubility of IBA in some of these alcohol solvents, 50% alcohol was selected for subsequent propagation research.

C. Evaluation of Several Alcohols as Non-toxic Carriers of IBA

1. 'Royal Ace' Tomato Leaf Propagation Study

No basal necrosis or chlorosis of the leaves was observed in any of the treatments. Ninety nine percent of the cuttings rooted.

The ANOVA showed a significant influence of IBA on root number and root growth (root rating) of 'Royal Ace' tomato leaf cuttings. Treatments containing IBA were not significantly different from each other (Table 2).

The results indicated that 0 ppm IBA in PG would be as beneficial as 1000 ppm IBA in any of the solvents tested for

Table 2. Effect of IBA Levels and Solvents on Root Rating* of 'Royal Ace' Tomato Leaf Cuttings.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	2.8c**	2.8c	3.5b	2.4c
1000	4.0ab	4.0ab	4.1ab	4.0ab
2000	4.2a	4.3a	4.2a	4.2a
3000	4.2a	4.5a	4.6a	4.2a
4000	4.3a	4.4a	4.3a	4.4a
5000	4.3a	4.4a	4.1ab	4.5a

*Visual rating of 1-5. 1 = no roots - 5 = best rooting.

**Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

rooting tomato leaf cuttings. The results also suggest that IBA influences rooting of tomato leaf cuttings more than the solvent used.

2. 'Aurora' Geranium Propagation Study

No basal necrosis or defoliation was observed in any of the treatments. The geranium cuttings rooted one hundred percent.

The ANOVA showed significant influence of IBA and solvent on root number and root growth (rootball diameter) of 'Aurora' geranium.

Largest rootball diameter of geranium was found at 5000 ppm IBA in PEG (Table 3). Smallest rootball diameters were found at: 0, 1000 and 2000 ppm IBA in ETOH and PEG, all except 4000 ppm in PG and in all IBA concentrations except 5000 ppm in IPA. It appears that higher levels of IBA in any solvent would be beneficial in rooting cuttings of 'Aurora' geranium when compared to untreated cuttings.

3. 'Crimson Pygmy' Barberry Propagation Study

Cuttings from Little Green Garden rooted 100% and no basal necrosis or defoliation was observed. The ANOVA showed a significant influence of IBA and solvent on root rating (root

Table 3. Effect of IBA Levels and Solvents on Rootball Diameter* of 'Aurora' Geranium cuttings.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	32.8d**	35.7cd	35.5cd	32.0d
1000	45.9bcd	49.3bcd	51.4bcd	40.7bcd
2000	59.6bcd	62.9bcd	44.4bcd	55.4bcd
3000	66.3bc	70.0b	63.3bcd	64.9bcd
4000	72.5b	70.9b	69.6b	58.4bcd
5000	67.5bc	100.7a	56.1bcd	69.4b

*Average rootball diameter in millimeters.

**Means followed by the same letter are not significantly different at the 1% level according to Duncan's New Multiple Range Test.

number and root growth) of 'Crimson Pygmy' barberry cuttings from Little Green Garden.

Significantly better root ratings were produced by 0 ppm IBA in ETOH and IPA than 0 ppm IBA in PG (Table 4). Five thousand ppm IBA in ETOH produced a better root rating than 0 and 1000 ppm IBA in ETOH, 0, 1000 and 5000 ppm IBA in PEG, 0 and 3000 ppm IBA in PG and 0 ppm IBA in IPA.

Cuttings from Wight Nursery rooted 67% (Table 5) and no basal necrosis or defoliation was observed. Overall root rating means were depressed greatly by the large number of unrooted cuttings.

The ANOVA showed a significant influence of IBA on root rating of 'Crimson Pygmy' barberry cuttings from Wight Nursery. IBA did not significantly influence root number and root growth (root rating) when PEG, PG and IPA were the solvents (Table 6). Only in ETOH was a higher level of IBA effective in increasing root rating over the control. IBA was probably not a significant influence on root rating due to the variability found between replications. There were no significant differences between solvents at any given IBA level (Table 6).

Three thousand ppm IBA in PEG produced a significantly better root rating than 0 and 1000 ppm IBA in ETOH and 0 and 5000 ppm IBA in IPA (Table 6). Three thousand ppm IBA in ETOH was significantly better than 0 and 1000 ppm IBA in ETOH and 0 ppm IBA

Table 4. Effect of IBA Levels and Solvents on Root Rating* of 'Crimson Pygmy' Barberry Cuttings From Little Green Garden.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	6.3de**	6.0ef	5.1f	6.5cde
1000	7.1bcde	7.1bcde	7.7abc	7.7abc
3000	8.0ab	7.6abc	6.8bcde	7.8ab
5000	8.7a	7.3bcd	7.7abc	7.7abc

*Visual rating of 1-10. 1 = no roots, 10 = best rooting.

**Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 5. Effect of IBA Levels and Solvents on Rooting Percentage of 'Crimson Pygmy' Barberry Cuttings From Wight Nursery.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	50% *	75%	79%	50%
1000	54%	58%	71%	67%
3000	75%	79%	75%	71%
5000	71%	71%	76%	50%

*Percent rooting based on 24 cuttings per treatment.

Table 6. Effect of IBA Levels and Solvents on Root Rating* of 'Crimson Pygmy' Barberry Cuttings From Wight Nursery.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	3.7d**	5.3abcd	5.2abcd	3.8cd
1000	3.6d	4.3abcd	5.0abcd	4.7abcd
3000	6.2ab	6.3a	6.0abc	4.3abcd
5000	5.6abcd	4.8abcd	5.3abcd	3.9bcd

*Visual rating of 1-10. 1 = no roots - 10 = best rooting.

**Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

in IPA. However, there did not appear to be a meaningful pattern of response in this experiment.

The experiments showed that 4" semi-hardwood cuttings (from Little Green Garden) rooted much better than the 3" semi-hardwood cuttings that had ceased growth (from Wight Nursery). These findings are consistent with the fact that most deciduous plants are more easily rooted using softer cuttings, since softer cuttings are more physiologically responsive to rooting. The greater number of leaves on the longer cuttings suggest increased carbohydrate synthesis and thus greater root growth. The slight dessication of the Wight Nursery cuttings during shipping may account for the lower rooting percentage.

4. 'Schilling's Dwarf' Yaupon Holly Propagation Study

No basal necrosis was observed in any of the treatments. Only 24.7% of the yaupon holly cuttings rooted. This may have been caused by keeping the cuttings too wet during propagation.

The ANOVA showed a significant influence of IBA on root number and root growth (root rating), but there was no difference between solvents at any level of IBA (Table 7). Three thousand ppm IBA in PEG produced a higher root rating than 0 ppm IBA in ETOH, 0 ppm IBA in PEG and 0 and 1000 ppm IBA in IPA (Table 7).

Table 7. Effect of IBA Levels and Solvents on Root Rating* of 'Schilling's Dwarf' Yaupon Holly.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	1.0d**	1.2bcd	1.5abcd	1.1cd
1000	1.5abcd	1.6abcd	1.5abcd	1.2bcd
3000	1.4abcd	1.9a	1.5abcd	1.8ab
5000	1.8ab	1.7abc	1.4abcd	1.5abcd

*Visual rating 1-5. 1 = no roots - 5 = best rooting.

**Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

IBA did not significantly influence root rating when PG was the solvent (Table 7).

The ANOVA showed no significant influence of IBA or solvent on percent defoliation of 'Schilling's Dwarf' yaupon holly cuttings (Table 8). Replications containing the heavily branched cuttings had significantly less defoliation than those containing the lightly branched cuttings (Table 9).

Fifty five percent of the cuttings showed no defoliation, 13.0% of the cuttings were 25% defoliated, 8.6% of the cuttings were 50% defoliated, 7.0% of the cuttings were 75% defoliated and 16.4% of the cuttings were 100% defoliated and dead.

There was only a slight effect of solvents and IBA levels on root rating and no effect on percent defoliation of 'Schilling's Dwarf' yaupon holly cuttings. The mist frequency and water holding capacity of the rooting medium may be more important (22), since yaupon holly cuttings are sensitive to too much water.

5. Variegated Privet Propagation Study

The ANOVA showed no significant influence of IBA or solvent on root number of variegated privet. Solvents were not significantly different from each other at any given IBA level, nor were there any significant differences between IBA levels for any given solvent (Table 10), with the exception that 3000 ppm IBA

Table 8. Effect of IBA Levels and Solvents on Defoliation* of 'Schilling's Dwarf' Yaupon Holly Cuttings.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	3.8ab**	3.8b	4.6a	4.2ab
1000	4.2ab	3.6b	3.8ab	3.5b
3000	3.7ab	3.8ab	3.6b	3.8ab
5000	3.9ab	3.7ab	3.6b	3.3b

*Percent defoliation rated 1-5. 1 = 100% defoliated, 2 = 75% defoliated, 3 = 50% defoliated, 4 = 25% defoliated and 5 = 0% defoliated.

**Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 9. Effect of Replication on Defoliation* of 'Schilling's Dwarf' Yaupon Holly Cuttings.

Replication**	
1	4.1bc ***
2	4.5ab
3	4.8a
4	3.8c
5	3.0d
6	2.8d

*Percent defoliation rated 1-5. 1 = 100% defoliated, 2 = 75% defoliated, 3 = 50% defoliated, 4 = 25% defoliated and 5 = 0% defoliated.

**Replications 1-3 = heavily branched cuttings. Replications 4-6 = lightly branched cuttings.

***Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 10. Effect of IBA Levels and Solvents on Root Number per Cutting of Variegated Privet.

	Solvent			
ppm IBA	ETOH	PEG	PG	IPA
0	1.9ab*	1.5b	1.9ab	1.4b
1000	1.9ab	2.0ab	1.9ab	1.7ab
3000	2.4a	1.5b	2.2ab	1.6ab
5000	2.0ab	1.8ab	1.9ab	1.9ab

*Means followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

in ETOH produced a greater number of roots per cutting than did 0 or 3000 ppm IBA in PEG or 0 ppm IBA in IPA (Table 10). No basal necrosis or defoliation was observed in any of the treatments. One hundred percent of the variegated privet cuttings rooted.

The results indicate that treating variegated privet with an IBA quick-dip is not needed. IBA concentrations greater than the 5000 ppm IBA tested may be beneficial in rooting variegated privet, but levels of 5000 ppm IBA or lower in ETOH, PEG, PG and IPA were almost equal to each other in this experiment.

D. Determination of Potential of the Alcohols to Reduce the Oxidation Rate of IBA

1. Mung Bean Rooting Bioassay

The ANOVA showed significant influence of IBA, solvent, age and all interactions of these factors other than the IBA and age interaction on root initiation and root number of mung bean hypocotyl cuttings (from second repetition of experiment).

Figure 4 shows there was no effect from the two lowest IBA levels (0.01 and 0.10 ppm) in any solvent in either fresh or aged solutions. Nor were IBA levels in the experiment high enough to depress rooting as one would expect in a classical hormone response curve. For future work with this system, researchers

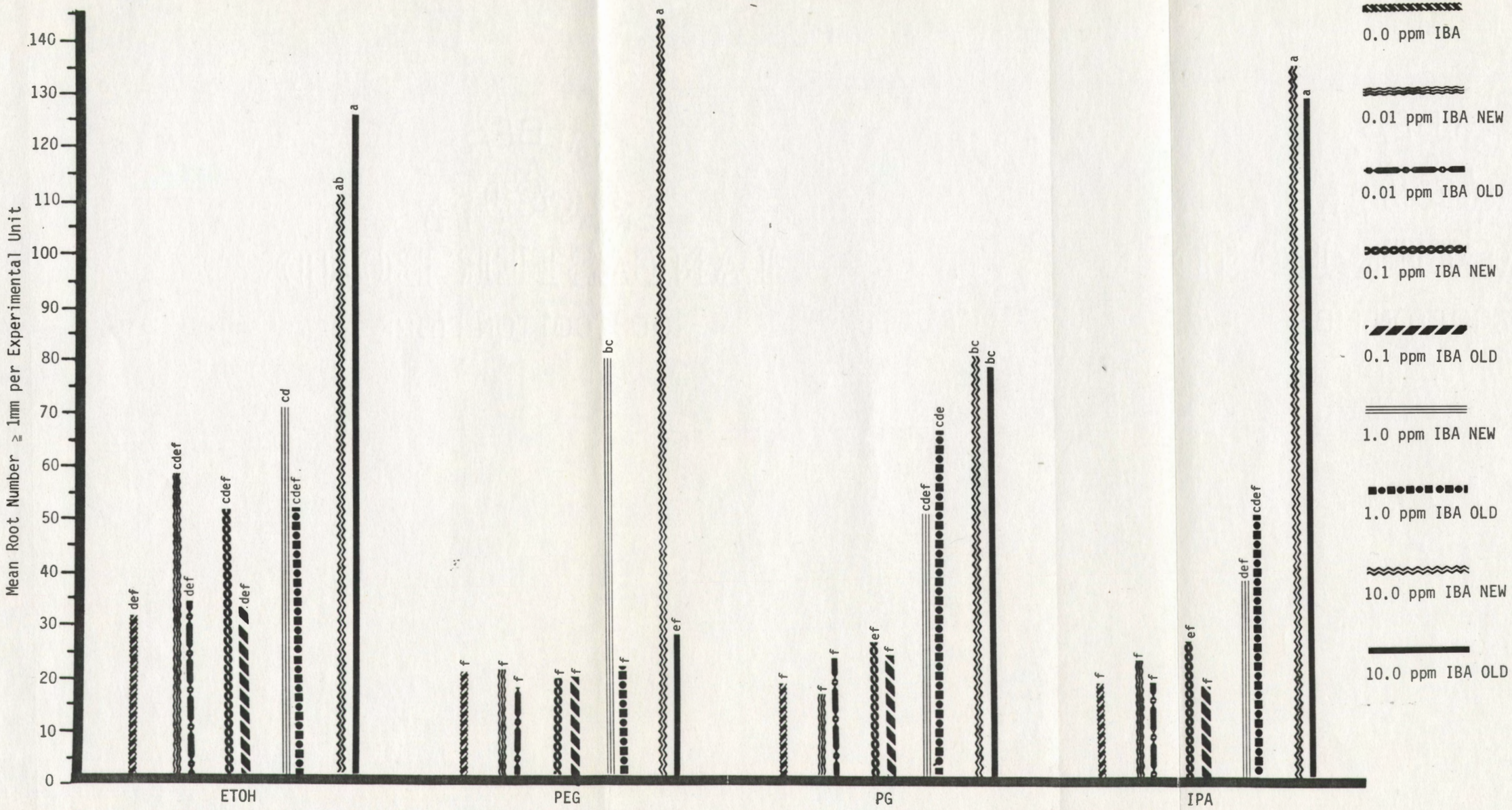


Figure 4. Effect of IBA Levels, Solvents and Solution Age on Root Initiation of Phaseolus aureus.

*Means followed by the same letter are not significantly different at the 1% level according to Duncan's New Multiple Range Test.

would be well advised to eliminate IBA levels below 1 ppm and to add IBA levels above 10 ppm.

As expected, higher levels of IBA generally produced more roots per experimental unit, despite certain interactions which will be addressed later. The most roots were produced at 10 ppm IBA in both fresh and aged solutions of ETOH and IPA, and in fresh PEG. The solvent PG appeared to depress rooting in both fresh and aged 10 ppm IBA solutions compared to the treatments mentioned above (except for the fresh 10 ppm IBA in ETOH). Lower IBA levels in PG were not depressed, compared to equivalent levels in the other solvents.

The most interesting result was the loss of root promoting activity in aged solutions of 1 and 10 ppm IBA in PEG, which were no different from controls. This was unexpected as the aged IBA in PEG solutions had discolored the least compared to the other solvents.

The initial supposition was that discoloration would be related to chemical breakdown, hence IBA activity would be expected to be least in the strongly discolored solutions. This was not the case. Figure 5 shows that for IBA in alcohol solutions aged one year in stoppered glass flasks on a laboratory shelf, PEG discolored the least, PG was intermediate, and IPA and ETOH were most strongly discolored. Figure 4 shows that for aged 10 ppm in IBA/alcohol solutions, the highest root number occurred

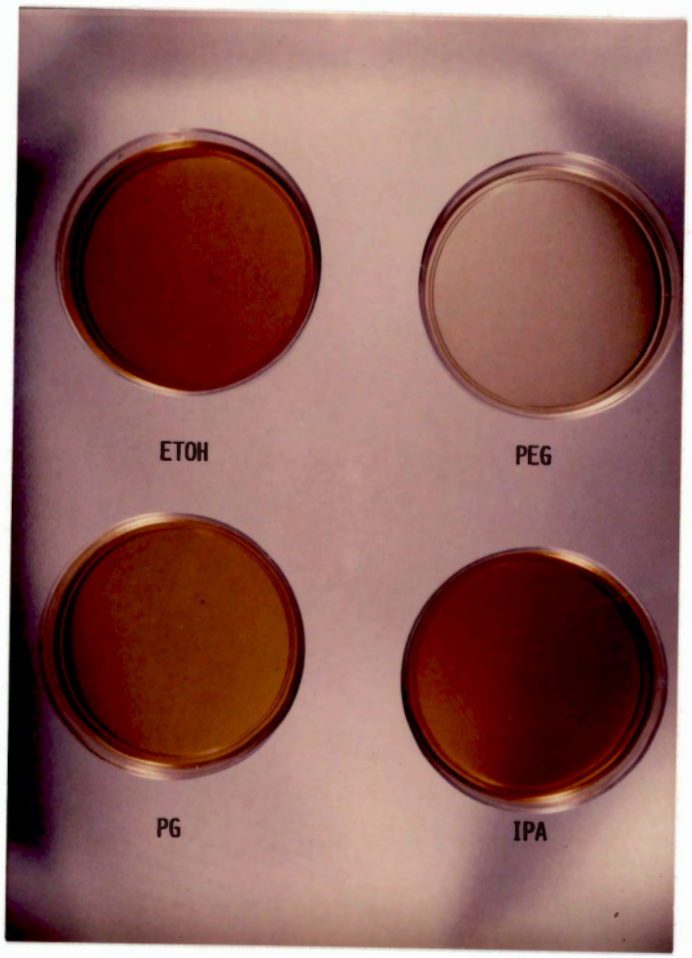


Figure 5. Comparison of Discoloration observed in Year Old IBA-Alcohol Solutions.

in IPA and ETOH, PG was intermediate, and PEG was least. Thus, for aged 10 ppm IBA in alcohol solutions in this experiment, visual discoloration and root promoting activity were diametrically opposed. Previous work has shown that solvents can affect the oxidation rate of auxins (13). No basal necrosis was observed in any of the treatments. Root length was greatest (up to 3 cm in length) in 0.0 and 0.01 ppm IBA (freshly prepared and aged) in ETOH, PEG, PG and IPA. Root length was shortest (approximately 1 to 5 mm) in 10.0 ppm IBA (freshly prepared and aged) in ETOH, PEG, PG and IPA.

The first and second repetitions of this experiment showed similar trends. However, the first repetition had significantly fewer roots per cutting, which could be caused by the genetic variability found between the seed lots as described by Bassuk (2). The second repetition of the experiment was selected for presentation due to the greater response found in root number per cutting when treated with IBA.

It is possible that the IBA oxidation rate was reduced much more in PEG than in ETOH, PG or IPA. As IBA oxidizes, the initial oxidation products are powerful anti-auxins. As these products continue to oxidize, they may lose their anti-auxin effects and may possibly produce auxin-like effects. If PEG reduces the oxidation rate, there could still be a relatively high concentration of the initial oxidation products (anti-auxins)

present after one year of oxidation. If ETOH, PG and IPA allowed IBA to oxidize at a faster rate than PEG, then it is possible that the initial anti-auxins produced could be oxidized to the point that they no longer produce anti-auxin effects on root number per cutting of mung bean. In order to determine if this is actually happening, it would be necessary to test the IBA solutions (using high pressure liquid chromatography or a rooting bioassay) at several intervals during the first year of aging. This would allow for determination of the relative oxidation rate of IBA in each of the solvents.

2. Spectrophotometric Analysis of Fresh and Aged IBA Solutions

Spectrophotometric analysis revealed a difference between freshly prepared and aged IBA solutions in all four alcohol solvents in the ultraviolet absorption bands (Figures 6, 7, 8 and 9). All the freshly prepared solutions produced a weak absorption band centered at approximately 285 nm and a large peak at approximately 225 nm with a shoulder at approximately 205 nm. Aged solutions of IBA in ETOH, PG and IPA resulted in reduced peaks at 225 nm (Figures 6,8 and 9), whereas the aged solution in PEG lost the 225 nm peak entirely (Figure 7). Only the aged solution of IBA in PG exhibited a higher peak at 285 nm than the fresh solution (Figure 8). The absence of a peak at 225 nm in the

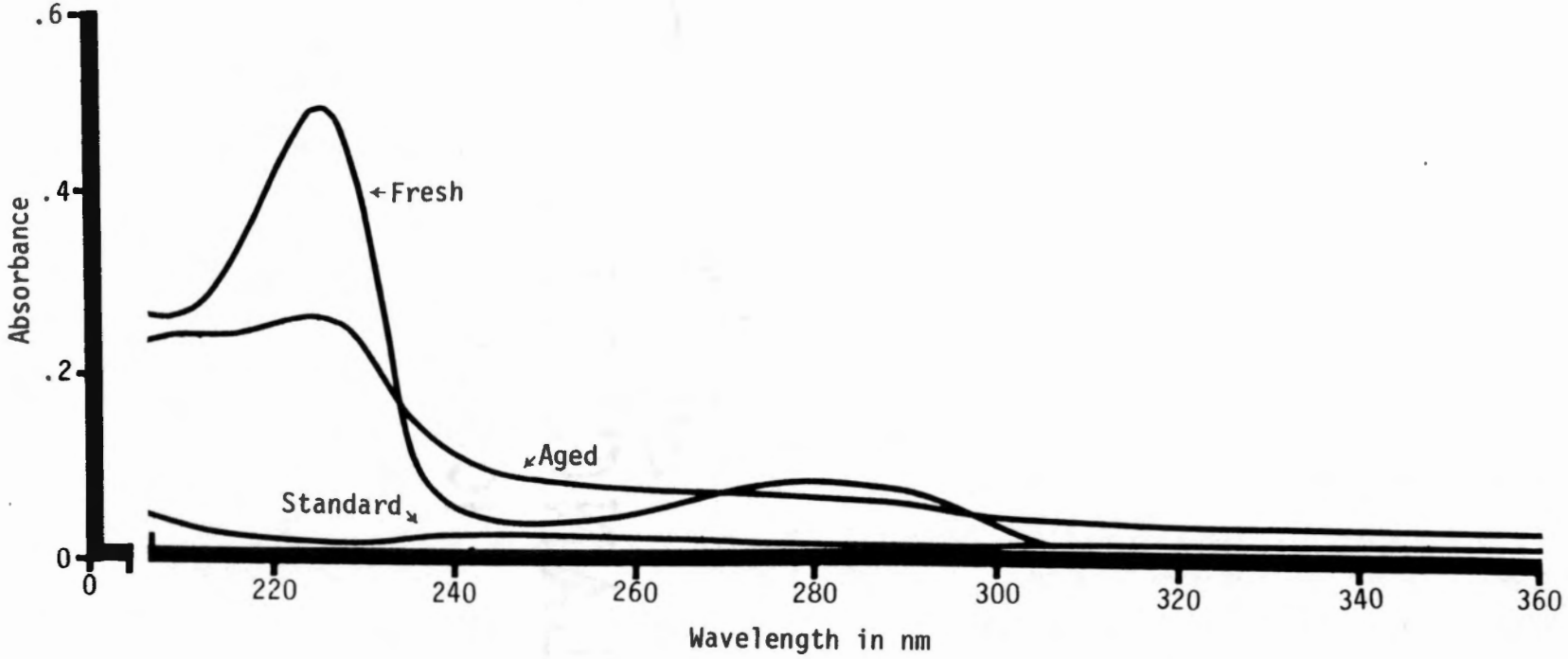


Figure 6. Spectrophotometric Analysis of Freshly Prepared and Aged Solutions of IBA in ETOH.

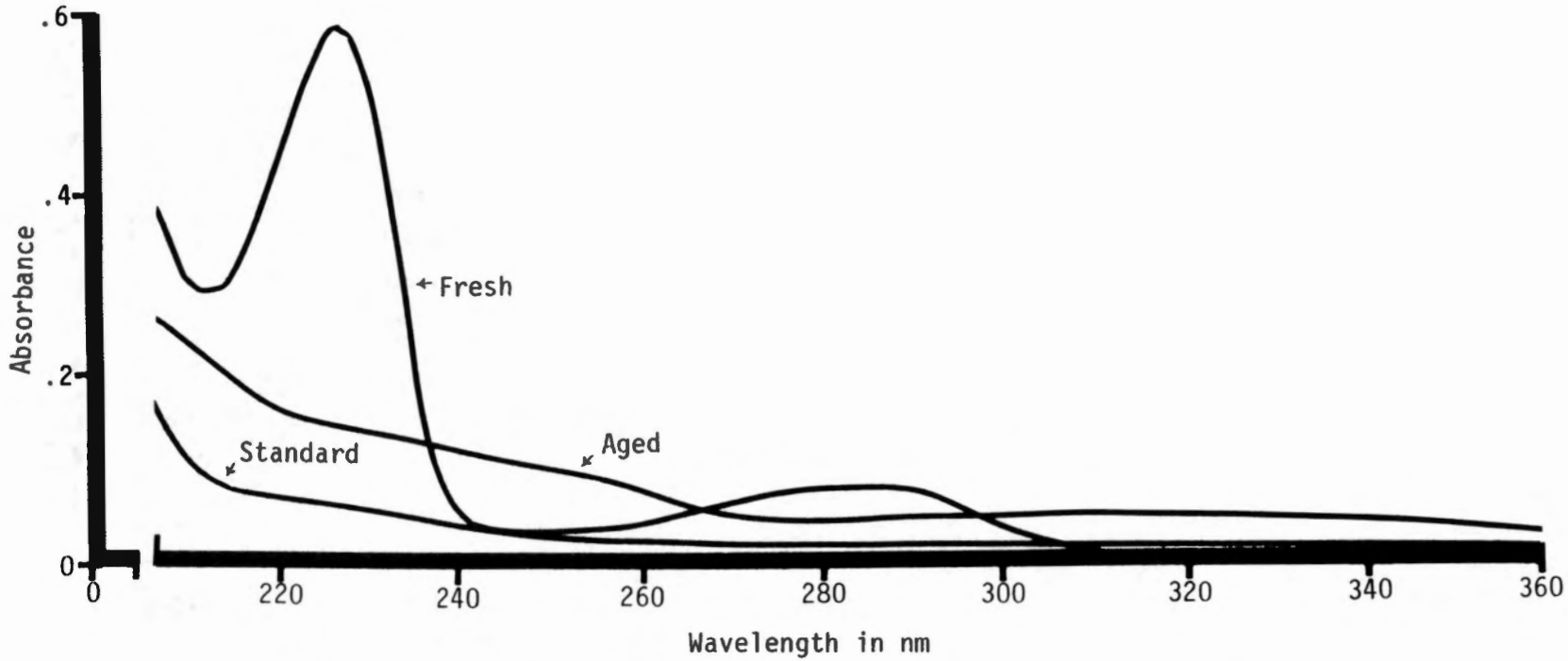


Figure 7. Spectrophotometric Analysis of Freshly Prepared and Aged Solutions of IBA in PEG.

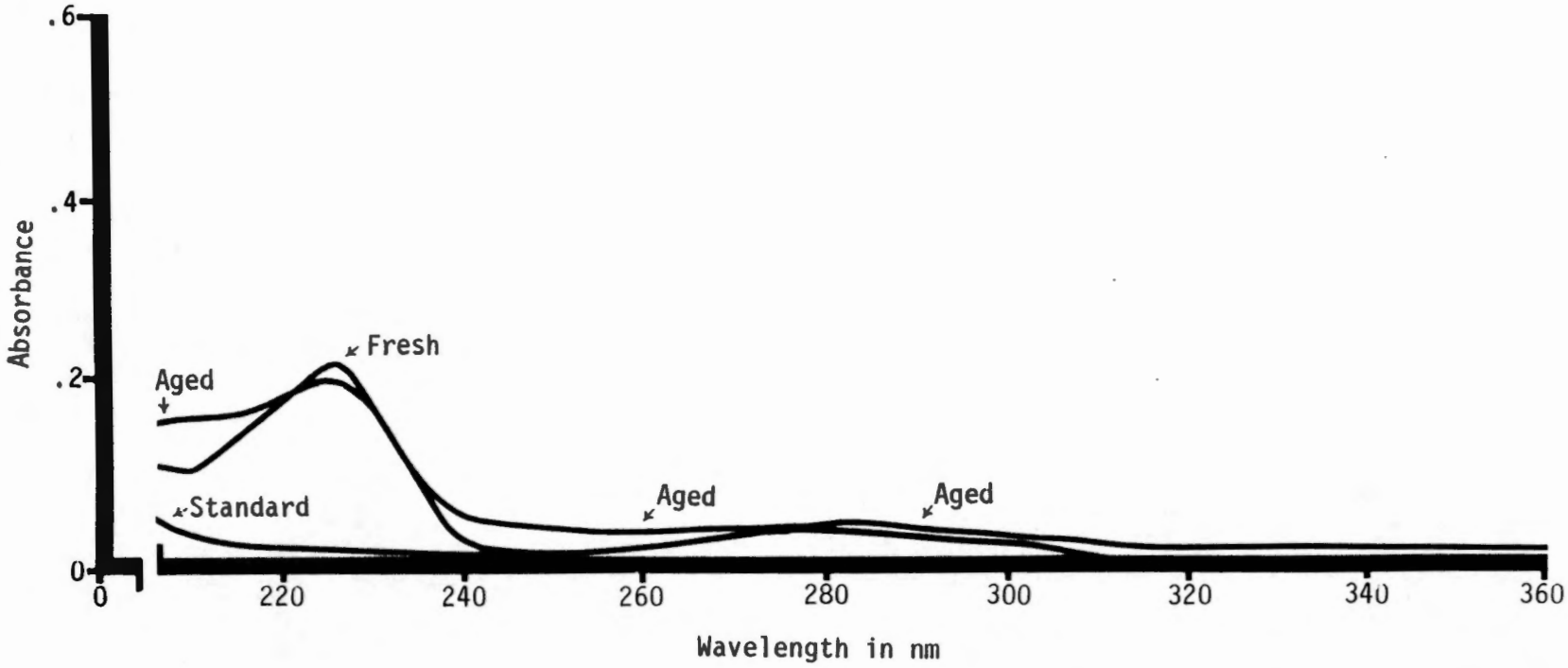


Figure 8. Spectrophotometric Analysis of Freshly Prepared and Aged Solutions of IBA in PG.

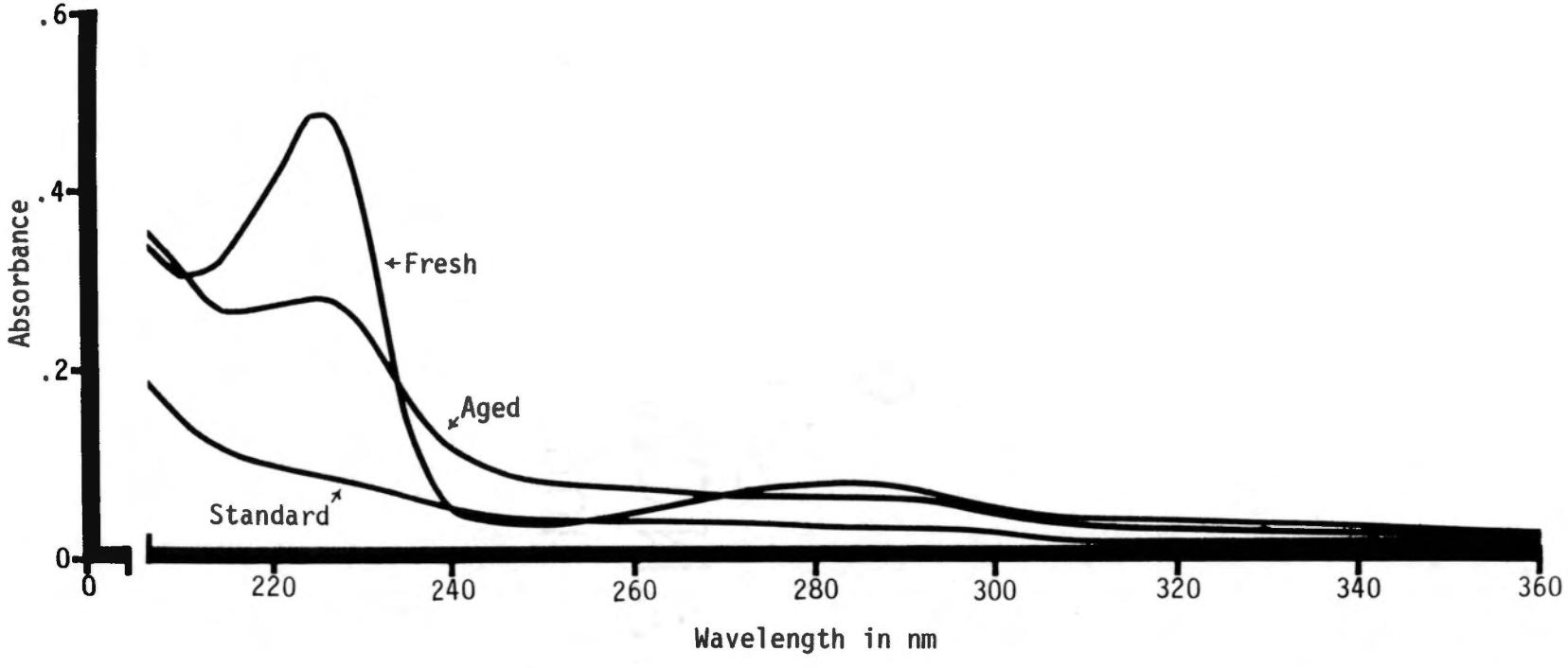


Figure 9. Spectrophotometric Analysis of Freshly Prepared and Aged Solutions of IBA in IPA.

aged IBA solution in PEG corresponded with the reduced rooting in the mung bean bioassay (Figure 4) as did the lower peaks for PG. However, the lower 225 nm peak for aged ETOH and IPA did not. Even though the compound(s) found in the aged IBA solutions have not been identified, it appears that compounds with a high absorbance at 225 nm may have an auxin-like effect on rooting mung bean hypocotyl cuttings.

High pressure liquid chromatography could possibly be utilized to determine the number and identity of breakdown products found in the aged solutions of IBA in the alcohol solvents. Identification of the breakdown products is needed in order to determine the specific reason for conservation or loss of root inducing potential of the solutions. If the breakdown of IBA during aging in PEG and PG was extensive, only a small amount of the original compound would remain, which could explain the loss of effectiveness of the aged solutions to induce rooting. Alternately, rooting may be directly inhibited by anti-auxins produced as oxidation products of the original IBA.

When comparing the findings from the mung bean rooting bioassay and spectrophotometric analysis (Chapter IV-D) with the results of the five propagation studies (Chapter IV-C), we observe only a tenuous correlation. While the rooting studies were designed primarily to test for phytotoxicity, they should also have served to demonstrate IBA effectiveness, or the lack of it,

and in retrospect we should have seen less rooting in PG treatments at the higher IBA levels. There was a slight indication of this in the rootball diameter of geranium at the 5000 ppm IBA level (Table 3) but not in the other studies (Tables 2, 4, 5, 6) at any IBA level. It may be that the kinds of plants chosen for the propagation studies were not as responsive to IBA as the mung beans. Or additional samples and replications may have been necessary to reduce error variance and allow for more precision in separating treatment levels in the propagation experiments. More refinement in experimental approaches in future work may resolve this dilemma.

CHAPTER V

SUMMARY AND CONCLUSIONS

The tomato leaf rooting bioassay eliminated METH, EG and G for consideration as solvents for IBA due to the extreme phytotoxicity exhibited by these alcohols. This screening procedure showed that ETOH, PEG, PG and IPA produced no phytotoxicity symptoms on the tender tomato tissue, and would therefore not be phytotoxic to the less tender woody species used in these experiments.

There appears to be little meaningful difference between ETOH, PEG, PG and IPA as solvents for IBA quick-dips on rooting woody ornamental and herbaceous plants in either efficacy or phytotoxicity. Therefore, selection of the alcohol solvent for IBA does not need to be dictated by relative phytotoxicities of ETOH, PEG, PG or IPA. Selection can be based on physical properties such as viscosity or evaporation rate, by low human toxicity, or by cost.

PEG and PG are both more viscous than ETOH or IPA thereby having slower evaporation rates. Viscosity of alcohol solvents may play a role in propagation. Alcohols such as PEG and PG adhere to cuttings much better than ETOH or IPA. Since more viscous alcohols have greater adherence, there is a greater volume of IBA remaining on each cutting. Results have shown that a

larger amount of IBA is necessary for root initiation in some plants.

The evaporation of PEG is negligible over a period of several days, whereas ETOH and IPA can lose over 50% of their volume in this time (unpublished results). Since PEG and PG evaporate much more slowly than ETOH or IPA, they tend to maintain the desired IBA concentrations for a much longer time even in a hot propagation house. Therefore, PEG or PG may be preferred over ETOH or IPA when very precise IBA concentrations are needed and evaporation is a problem.

PEG and PG have much lower acute oral mammalian toxicities than ETOH or IPA (Appendix). If workers must be exposed to the alcohol quick-dips for long periods of time it may be preferable to select PEG or PG for the IBA solvent to reduce the risk to the workers.

The results from the mung bean bioassay showed no meaningful loss of root promoting activity after one year in solutions of IBA when ETOH, PG or IPA were used as solvents. Thus, the discoloration seen in old IBA-alcohol solutions should not necessarily be taken as an indication of loss of IBA activity. Propagators can probably use IBA solutions in ETOH, PG or IPA for much longer periods of time than what seems to be the current practice of disposing of them in less than one week. This could result in substantial savings for the nurseries.

The most significant factor on rooting in these experiments was the IBA concentration. The higher IBA concentrations tended to be beneficial for rooting the plants tested in these experiments. Therefore, it would be better to determine the optimal IBA concentration for a given plant variety than to worry about what solvent to use.

Since there was no phytotoxicity observed on any of the plants tested in these experiments the reported alcohol toxicity problems would seem to be unfounded. However, reagent grade chemicals used in these experiments may not have produced the same responses as commercial grade chemicals used in the nursery industry. ETOH used by commercial propagators has been usually been denatured and IPA purchased off the drugstore shelf usually has a chemical additive. The denaturants used for ETOH include such phytotoxic chemicals as methanol and kerosene. Additionally there are other chemicals that are commonly used as denaturants that may be phytotoxic. It may be that some reports of alcohol toxicity are due to the denaturants found in the alcohols and not the alcohols themselves. Research is needed to determine which denaturants, if any, are responsible for the reported alcohol toxicity and what amount induces phytotoxicity. It would then be necessary to determine if it is feasible to denature ETOH with non-phytotoxic chemicals. IPA could be used as the sole denaturant to ETOH since it was not toxic to the reported alcohol sensitive plants in our experiments and can legally be used to

denature ETOH. If IPA or other non-phytotoxic chemicals can be successfully used as ETOH denaturants on alcohol sensitive plants, then the nursery industry needs to request that the alcohol manufacturers produce a "nursery grade" ETOH that is safe to use on sensitive varieties of plants.

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APPENDIXES

APPENDIX 1

SURVEY SENT TO SOUTHEASTERN NURSERIES

Head Propagator
Name of Nursery
Address

Date

Dear Propagator:

We have a research study going on with different alcohol solvents for IBA. We would like to get a handle on use of alcohol quick-dips by commercial propagators in the southeast. Please take five minutes to fill out our survey form. Thanks in advance for your prompt reply.

Sincerely,

Willard T. Witte
Assoc. Prof. - Nursery Crops Research

1. Are you currently using a liquid quick-dip hormone solution in propagation? Yes___, No___.

2. If yes, do you make your own liquid quick-dip___ or do you buy a commercially prepared quick-dip___? (If so, what brand?)

_____

3. If you make your own quick-dip, what alcohol or solvent are you using? Ethanol___, Denatured Alcohol___, Isopropyl Alcohol (Rubbing Alcohol)___, Other (please specify) _____

4. If you use alcohol for making your own quick-dips, please write the name and complete address and phone number of your supplier. _____

5. Approximately how many gallons of alcohol do you use per year for propagation? _____

6. Have you ever observed any injury you thought was alcohol toxicity on any of your cuttings? Yes___, No___.

7. If yes, what species or cultivars have given you problems?

_____

8. Do you use talc or K-IBA in water on the plants that seem to be alcohol sensitive? Yes___, No___.

Your name (please print)_____

APPENDIX 2

SOLVENT CODES AND DESCRIPTIONS

Solvent code	Description
EG	Ethylene glycol (1,2-ethanediol) Molecular weight : 62.07 Molecular formula : CH_2OHCH_2OH Oral LD_{50} in rats : 8.54 g/kg
ETOH	Ethanol Molecular weight : 46.07 Molecular formula : CH_3CH_2OH Oral LD_{50} in rats : 13.7 g/kg
G	Glycerol (1,2,3-propanetriol) Molecular weight : 92.09 Molecular formula : $CH_2OHCHOHCH_2OH$ Oral LD_{50} in rats : 31.5 g/kg
IPA	Isopropanol (2-propanol) Molecular weight : 60.09 Molecular formula : $CH_3CHOHCH_3$ Oral LD_{50} in rats : 5.8 g/kg
METH	Methanol Molecular weight : 32.04 Molecular formula : CH_3OH Oral LD_{50} in rats : not available
PEG	Polyethylene glycol Molecular weight : 380-420 Molecular formula : $H(OCH_2CH_2)_nOH$ n is between 8.2 and 9.1 Oral LD_{50} in rats : 43.6 g/kg

Solvent code	Description
PG	Propylene glycol (1,2-propanediol) Molecular weight : 76.09 Molecular formula : $CH_3CHOHCH_2OH$ Oral LD_{50} in rats : 30 g/kg

VITA

Thomas P. McCracken was born on September 30, 1962 in New Orleans, Louisiana. He was graduated from Lenoir City High School in Lenoir City, Tennessee in 1981. In 1985, he received the Bachelor of Science degree in Ornamental Horticulture and Landscape Design from The University of Tennessee, Knoxville. In September 1985, he entered the Graduate School of the University of Tennessee, Knoxville. While working toward the Master of Science degree in Ornamental Horticulture and Landscape Design, he was employed as a Graduate Research Assistant for the Department by The University of Tennessee Agricultural Experiment Station. He was granted the degree in December 1987. The author is a member of Alpha Zeta and Pi Alpha Xi.