Literature Cited

1. Dickson, A.G. and E.W. Samuels. 1956. The mechanism of controlled growth of dwarf apple trees. J. Arnold Arb. 37:307–313.

2. Garner, R.J. 1979. The grafter's handbook. Ed. 4, Oxford Univ. Press, 319 p.

3. Mosse, B. 1960. Further observations on the effects of ring-grafting peaches with an incompatible rootstock variety. J. Hort Sci. 35:275–281.

4. Santamour, F.S., Jr. 1980. How to build a better tree. New Horizons from the Horticultural Research Institute—1980, p. 1–3.

5. Santamour, F.S., Jr. 1988. Graft compatibility in woody plants: An expanded perspective. J. Environ. Hort. 6:27–32.

6. Santamour, F.S., Jr., A.J. McArdle, and R.A. Jaynes. 1986. Cambial isoperoxidase patterns in *Castanea*. J. Environ. Hort. 4:14–16.

7. Sax, K. 1954. The control of tree growth by phloem blocks. J. Arnold Arb. 35:251–257.

8. Sax, K. 1957. Dwarf ornamental and fruit trees. Proc. Intern. Plant Prop. Soc. 7:146–153.

9. Sax, K. and A.Q. Dickson. 1956. Phloem polarity in bark regeneration. J. Arnold Arb. 37:173–179.

10. Thair, B.W. and T.A. Steeves. 1976. Response of the vascular cambium to reorientation in patch grafts. Can. J. Bot. 54:361–373.

Chemical and Biological Stability of Indole-3-Butyric Acid (IBA) After Long-term Storage at Selected Temperatures and Light Regimes[1]

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Abstract

Concentrated [4.9 mM (1,000 ppm) and 24.6 mM (5,000 ppm)] IBA solutions in 50% isopropyl alcohol were stored in amber and clear glass bottles at 3 temperatures [22–25°, 6°, 0°C (72–77°, 43°, 32°F)]. No significant change in biological activity of the solutions or breakdown of IBA was observed for solutions stored for 4 and 6 months. Solution color changed during storage. Color development was dependant on storage temperature, but not on exposure to light. *Chemical names used:* IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; NAA = 1-naphthaleneacetic acid

Index words: propagation, growth regulators, rooting, auxins, cuttings

Introduction

Auxins are the class of plant growth substances most often associated with the promotion of adventitious root formation. Zimmerman and Wilcoxon (11) demonstrated that two synthetic auxins, IBA and α-NAA, induce greater rooting than the naturally occurring auxin, IAA. Auxins, when used as rooting compounds are often applied as concentrated solutions (2, 7, 8, 10). Although the use of synthetic auxins (IBA, NAA) is of central importance in plant propagation (1), little is known about the shelf-life of concentrated solutions.

Hartmann and Kester (3) indicate that an uncontaminated solution of NAA maintains its activity for as long as a year and is entirely light-stable although no data are presented to support this claim. For IBA, they state that a 20hr exposure to strong sunlight causes only a slight change in concentration. Pinney (8) suggests that a concentrated solution of IBA (in 95% ethyl alcohol) can be stored for up to 3 months in the dark at 4°C (39°F) with neglible change in concentration. Again, no data are presented to substantiate this claim. To date, no quantitative information has been found in the literature on the shelf-life of IBA solutions.

[1]Received for publication June 8, 1987; in revised form January 8, 1988.

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The purpose of this study was to provide practical information about the effect of storage conditions on the long-term shelf-life of concentrated IBA solutions. The concentration, solvent, and storage environment were chosen to represent conditions that a plant propagator would encounter.

Materials and Methods

IBA was purchased from two suppliers, United States Biochemical (USB) (Cleveland, OH 44122) and Sigma (St. Lous, MO 63178). A bulk quantity of IBA was prepared for each concentration [24.6 mM (5,000 ppm), 4.9 mM (1,000 ppm), 0 mM] and chemical supplier. Fifty percent isopropyl alcohol, as opposed to ethyl alcohol, was used as the solvent since isopropyl alcohol is more readily available to the public. The two alcohols yield equivalent rooting results (9). The color of freshly prepared solutions depended on the chemical supplier. A 24.6 mM (5,000 ppm) solution of IBA prepared using the USB product was a light-yellow color. The intensity of this color is proportional to concentration. The solution [24.6 mM (5,000 ppm)] prepared from the Sigma product was clear.

The bulk supply for each solution was dispensed (60 ml/bottle) into clear or amber glass bottles. Caps for the bottles were vinyl-lined. Two bottles were prepared for each treatment (temperature, bottle color, concentration, and chemical supplier). During the storage period, bottles were located in one of three locations (laboratory shelf, refrigerator, freezer). One series of bottles was stored on an open shelf

in a laboratory [22–25°C (72–77°F), 6 μmoles m^{-2} sec^{-1}]. The remaining bottles were stored in the dark in either a refrigerator [6°C (43°F)] or freezer [0°C (32°F)]. To repeat the experiment, an identical set of solutions was prepared 2 months after the first set. Results from the second experiment are not shown since they were similar to those for the first. Solutions were analyzed by HPLC and the rooting bioassay at the beginning and end of the storage period. HPLC was used as the method for chemical analysis. The rooting bioassay (4) was used to monitor the biological activity of IBA and to detect the presence of inhibitory or promotive compounds that might be produced during storage.

HPLC. The liquid chromatography system consisted of a Hewlett-Packard (Palo Alto, CA 94304) 1084B chromatograph equipped with a variable wavelength spectrophotometric detector and a 5 micron Econosphere column 25 cm × 4.6 mm (9.8 × 0.2in) (Alltech, Deerfield, IL 60015).

Solutions were analyzed by first diluting them to a concentration of 50 μM with glass distilled water. Fifty μl of the diluted sample was then injected onto the column and eluted with a gradient of 5→ 50% acetonitrile in water: 4.0→ 2.0 mM potassium phosphate buffer (pH = 6.1) over 15 min. The flow rate was 1.5 ml/min. The potassium phosphate was analytical grade; all solvents HPLC grade. The column temperature was 40°C (104°F). IBA was detected by monitoring absorbance at 280 nm (A_{280}). The composition of the sample was determined by comparison to the retention time and peak area of a freshly prepared standard of IBA.

Rooting Bioassay. Mung bean (*Vigna radiata* (L.) R. Wilcz.) seedlings were grown in coarse vermiculite in a growth chamber at a constant temperature [27°C (81°F)] and relative humidity (65%) under a 16hr photoperiod with a photosynthetic photon flux density (PPFD) of 300 μmoles m^{-2} sec^{-1} (Cool-White fluorescent tubes and incandescent bulbs). Plants were watered when necessary with half-strength Hoagland's solution (6).

Cuttings 10 to 12cm (3.9 to 4.7in) long were made from uniform 7-day-old seedlings. Each cutting consisted of a terminal bud, 2 primary leaves, the epicotyl, and 3cm (1.2 in) of hypocotyl. Cuttings were treated by placing the entire hypocotyl section in shell vials [21 × 70mm (0.8 × 2.8in)] containing 10ml of treatment solution for 15 sec. The cuttings were then rinsed in deionized water and placed 5 per vial. Six vials were used per treatment. Rooting solution for both systems consisted of deionized water containing 1 mM $CaCl_2$ and 9 μM H_3BO_3. Solution volume in the shell vials was maintained at 10ml by refilling 2 or 3 times daily with the same solution during the rooting period. Rooting experiments were performed under the environmental conditions used for growing the seedlings. Number of visible roots was counted after 5 days.

Results and Discussion

Results indicated that concentrated [24.6 mM (5,000 ppm)] IBA solutions (USB) can be stored at room temperature [22–25°C (72–77°F)] in a clear glass bottle for at least 4 months without a significant loss in biological activity of the solutions (Table 1) or breakdown of the compound (Table 2). This result was rather surprising because solutions stored at room temperature had changed from the initial light-yellow, to a bronze color. A similar change in color was observed for solutions prepared from the Sigma product. Exposure to low light does not appear to influence color production as solutions stored in a clear or amber bottle were identical in color. Presumably the color could be a result of a highly colored breakdown product(s) of IBA. The product(s) must be produced in very small quantities since no significant amount of breakdown could be detected (Table 2). The color could also be produced by a contaminant that has no influence on the biological activity of a concentrated IBA solution (Table 1).

The development of the colored product(s) was influenced by temperature. A significant change in color occurred in solutions stored at room temperature, while only a slight change in color occurred in refrigerated solutions. Solutions stored in a freezer maintained their original color. No difference in biological activity or amount of breakdown was detected between the Sigma and USB products stored at room temperature (data not shown) even though the color of the original solutions was different.

Analysis of solutions from the first experiment after an additional 2 months of storage at room temperature (6 months total storage) indicated no significant loss in biological activity of the solutions (data not shown) or breakdown of the compound (Table 2). Solutions prepared for a preliminary experiment that had been stored at room temperature for 19 months contained only 26% of the original amount of IBA.

To determine if concentration influenced the shelf-life of IBA, a series of lower concentration [4.9 mM (1,000 ppm)]

Table 1. Effect of 4 months of storage on the biological activity of a 24.6 mM (5,000 ppm) IBA solution.

Storage Conditions		
Bottle color	Temp °C (°F)	Mean number of roots per cutting[z]
clear	22–25 (72–77)	84
amber	22–25 (72–77)	83
clear	6 (43)	90
clear	0 (32)	83
—control, fresh—		79

[z]Means of 30 cuttings, all of which rooted. Means are not significantly different. LSD (p = 0.05) = 11.3

Table 2. Percent of a 24.6 mM (5,000 ppm) IBA solution remaining after storage.

Storage Conditions		Percent IBA Remaining after storage, months	
Bottle color	Temp. °C (°F)	4	6
clear	22–25 (72–77)	102[z]	102
amber	22–25 (72–77)	106	106
clear	6 (43)	102	109
clear	0 (32)	110	110

[z]Accuracy of the HPLC method (dilution and analysis) is ±6%.

solutions were stored along with the 24.6 mM (5,000 ppm) solutions. The lower concentration solutions also showed no loss in activity or breakdown after 4 months of storage (data not shown).

In addition to the pure acid, IBA is also available as the potassium salt. Although the salt is slightly more expensive than the acid, it has the advantage of being freely soluble in water. The potassium salt of IBA is generally more effective at stimulating rooting than is the acid (5). Because the solvent for the salt would normally be water, bacterial growth might be a problem in stored aqueous solutions. It is reasonable to assume that results on the shelf-life of the salt would be similar if the salt were dissolved in alcohol.

Significance to the Nursery Industry

The use of concentrated IBA solutions is widespread in the nursery industry, however, no data are presently available on the shelf-life of these solutions under conditions that a plant propagator would encounter.

Results from our study suggest that concentrated IBA solutions prepared in 50% isopropyl alcohol are quite stable. IBA concentrations up to 5,000 ppm (24.6 mM) can be stored in a clear glass bottle for up to 6 months at room temperature with no significant loss in activity even though there is a significant change in the solution color.

Literature Cited

1. Dirr, M.A. 1982. Rooting compounds and their use in plant propagation. Proc. Intern. Plant Prop. Soc. 31:472–479.

2. Gray, H. 1959. The quick dip, alcoholic solution as an aid to rooting cuttings. Proc. Intern. Plant Prop. Soc. 9:47–48.

3. Hartmann, H.T. and D.E. Kester. 1983. Plant propagation: Principles and Practices. Prentice-Hall, Englewood Cliffs, New Jersey.

4. Hess, C.E. 1957. A physiological analysis of rooting in cuttings of juvenile and mature *Hedera helix* L. Ph.D Thesis, Cornell Univ., Ithaca, New York.

5. Hitchcock, A.E. and P.W. Zimmerman. 1939. Comparative activity of root-inducing substances and methods for treating cuttings. Contrib. Boyce Thompson Inst. 10:461–480.

6. Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Cir. 347.

7. Meahl, R.P. and F.O. Lanphear. 1967. Evaluation of the quick-dip method of treating stem cuttings with rooting hormones. The Plant Propagator. 13(2):13–15.

8. Pinney, T.S. 1959. The method of quick dip hormone treatment of cutting wood at the Evergreen Nursery Co. Proc. Intern. Plant Prop. Soc. 9:48–50.

9. Roller, J.B. 1959. Preparation and use of quick dip solutions on cuttings. Proc. Intern. Plant Prop. Soc. 9:50–51.

10. Stroombeek, E. 1959. Hormone application by the quick dip method. Proc. Intern. Plant Prop. Soc. 9:51–54.

11. Zimmerman, P.W. and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. 7:209–229.