PRESIDENT KRAUSE: Would those new members whose names have been read and who are present please stand? We would like to recognize you. Let's all give them a hand.

I wish to make a plug for the 1970 meetings, which will be a joint venture of the Western and Eastern Regions of the International Plant Propagators' Society. This meeting will be held in St. Paul, Minnesota, September 9 to 12. Both regions will be participating in setting up the program and I am sure this will be a very fascinating one. You will find envelopes describing this meeting on the table in the foyer; please take several of these and hand some to your friends or fellow members who are not here. We would like to see a real good representation from the Western Region in St. Paul next year.

It is time to get on with our program, but first let me make some announcements. Those who are participating in the program, would you take seats in the front prior to the session in which you will be participating. We are very time-conscious at our Plant Propagators' meetings; in fact, we are so bold as to have a warning light that will flick on and off when your time is up, and we will also even be so bold as to cut you off. We want to hear what all the speakers have to say and we do not want to deprive anyone of the time for his presentation. All the proceedings of our meetings are printed. I am sorry that we do not yet have our last year's Proceedings at hand now to show you; they are still in the process of being printed.

The first talk on our program today will be on root initiation in easy and difficult-to-root plants, by Dr. Wesley Hackett.

THE INFLUENCE OF AUXIN, CATECHOL AND METHANOLIC TISSUE EXTRACTS ON ROOT INITIATION IN ASEPTICALLY CULTURED SHOOT APICES OF THE JUVENILE AND ADULT FORMS OF HEDERA HELIX

WESLEY P. HACKETT
Department of Environmental Horticulture
University of California, Davis

INTRODUCTION

One of the characteristics of the juvenile, non-flowering phase of *Hedera helix* is its striking ability to form adventitious roots on the stem of intact plants. In contrast, the adult flowering phase of this species does not form aerial adventitious roots and is difficult to root when leafy stem cuttings are placed under favorable environmental conditions (7). Many observations indicate that cuttings of most plants in the seedling (juvenile) state initiate roots more readily than in any other stage of development.

It has been known for many years that auxins stimulate adventitious root formation (13, 17). However, cuttings of many difficult-to-root plants, including the adult phase of

Hedera helix, respond very little to auxin treatment (2, 9). There is evidence that endogenous factors, other than auxin, are important in the control of adventitious root initiation (4, 7, 8, 9). More specifically there is evidence that phenolic compounds such as catechol, pyrogallol, caffeic acid and chlorogenic acid interact with auxin to induce root initiation (8, 14).

Using root formation on mung bean cuttings as a bioassay, Hess has shown that fractionated extracts of easy-to-root, juvenile *Hedera helix* shoots contain several root-promoting substances while extracts of the difficult-to-root adult form have less activity (7, 8, 9). He postulates that the presence of greater amounts of the root-promoting substances in the juvenile form than in the adult form may account for the higher rooting capacity of the juvenile form as compared to the adult form. Fadl and Hartmann (4), also using mung beans as a bioassay for rooting, found high levels of root-promoting activity in fractionated extracts from easy-to-root 'Old Home' pear cuttings. Extracts from difficult-to-root 'Bartlett' pear cuttings showed considerably less root-promoting activity but did show high levels of inhibitory activity.

The general objective of this investigation was to determine if aseptically cultured shoot apices of *Hedera helix* could be used to study factors influencing root initiation in easy and difficult-to-root plants. A more specific objective was to determine the root initiation activity of fractionated extracts from shoots of juvenile and adult *Hedera helix* plants when shoot apices of these plants are used as a test for root initiation.

MATERIALS AND METHODS

Tissue for extracts and shoot apices for rooting tests were obtained from vegetatively propagated juvenile and adult *Hedera helix* plants which originated from the same plant. Tissue for extracts was from newly matured leaves plus the node and internode directly associated with these leaves. The tissue was lyophilized, ground to pass through a 40-mesh screen and stored at -20°C until extracted. Tissue samples of 0.5 gm were extracted 3 times in 50 ml portions of methanol. The extracts were combined, concentrated under reduced pressure and an aliquot equivalent to 225 mg or 112 mg of tissue was streaked on 4-inch wide strips of Whatman No. 3MM chromatographic filter paper. The chromatograms were developed in isopropanol and water (4:1 v/v) after an overnight equilibration period. Development was stopped when the front had moved 10 inches from the origin.

Rooting tests were performed aseptically in 6-dram flint glass vials using the basal culture medium shown in Table 1. (Modified from a formulation described by J. A. Romberger of U.S.D.A., Beltsville, Maryland, unpublished). Chemicals to be tested for their effect on root initiation (auxins and catechol) were added to the basal medium as supplements. A 0.5 x

4-inch strip of Whatman No. 3MM filter paper folded and placed in vials as shown in Fig. 1 served as a wick and a platform for the shoot apices. The basal medium with supplements was sterilized by passage through a bacterial Millipore filter and vials, filter paper wicks and strips of paper chromatograms (where used) were sterilized by autoclaving at 15-lbs/in^2 for 20 minutes. Ten ml of medium was dispensed into each vial.

For experiments involving fractionated methanolic extracts, chromatograms were cut into 20 strips each equal to 0.5 R_f unit. Each strip was put into 4 pieces and placed in a separate vial prior to autoclaving and subsequently filled with sterile medium.

Shoot apices 2-3 mm in height were excised aseptically and placed on the filter paper wicks in the vials. One apex was used per vial and 10 vials were used per treatment. Rooting tests were run at 21°C and, except as noted, a light intensity of 500 ft. c. from daylight fluorescent lamps was maintained for 16 hours per day. Roots were counted 28 days after the apices were implanted.

RESULTS

Because of well established differences in the effects of various auxins on promotion of adventitious root initiation in cuttings, an experiment was performed to test the effects of

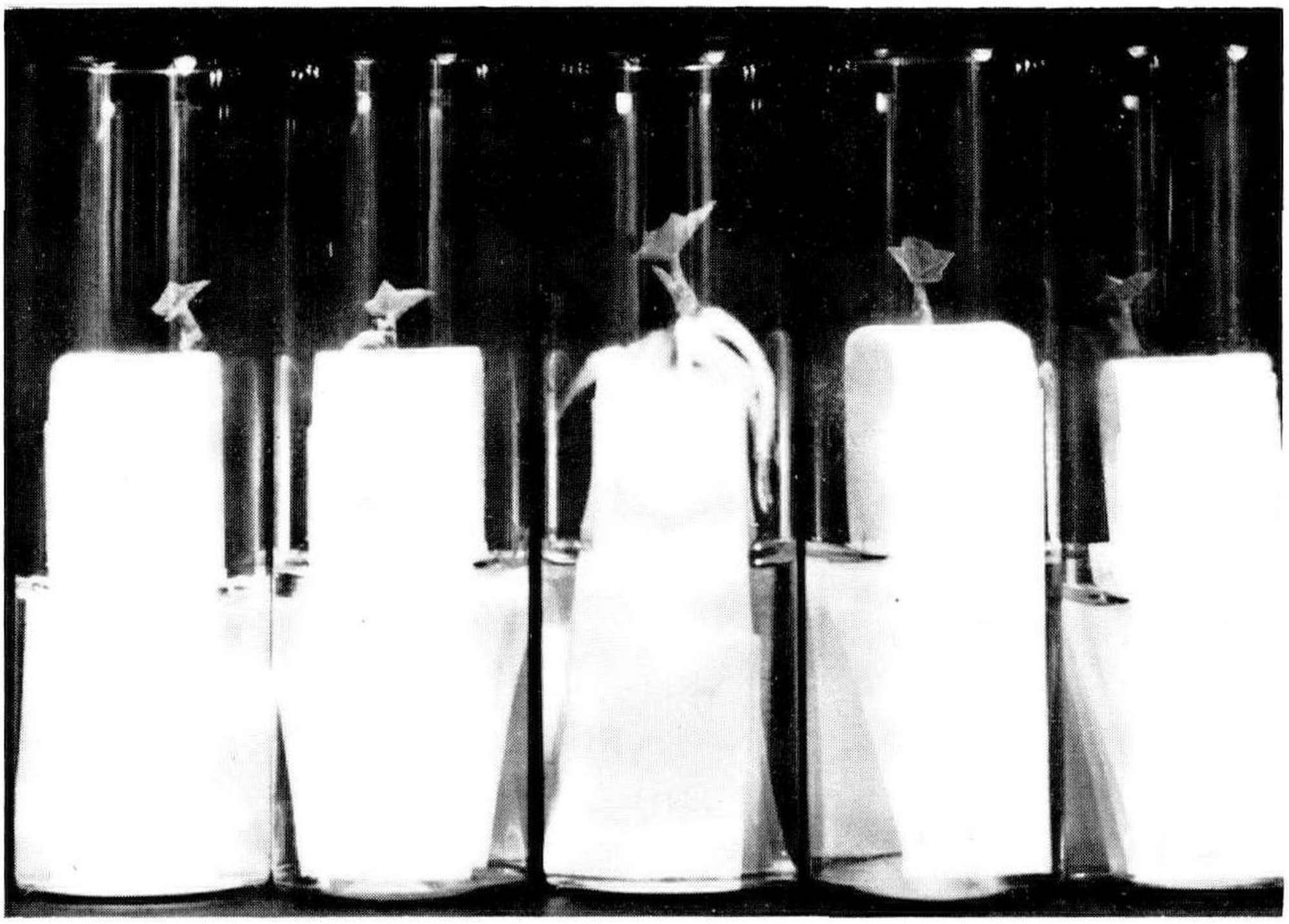

Fig. 1. Juvenile shoot apices in place on filter paper wicks in flint glass vials at the termination of a rooting test.

indoleacetic acid (IAA) indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) on root initiation in shoot apices of adult and juvenile ivy. Although they elongated slightly and unfolded new leaves, none of the 230 adult apices implanted formed any roots with concentrations of IAA, IBA or NAA ranging from 0 to 50 mg/l. In contrast, juvenile shoot apices (Fig. 2) displayed marked response to kind and concentration

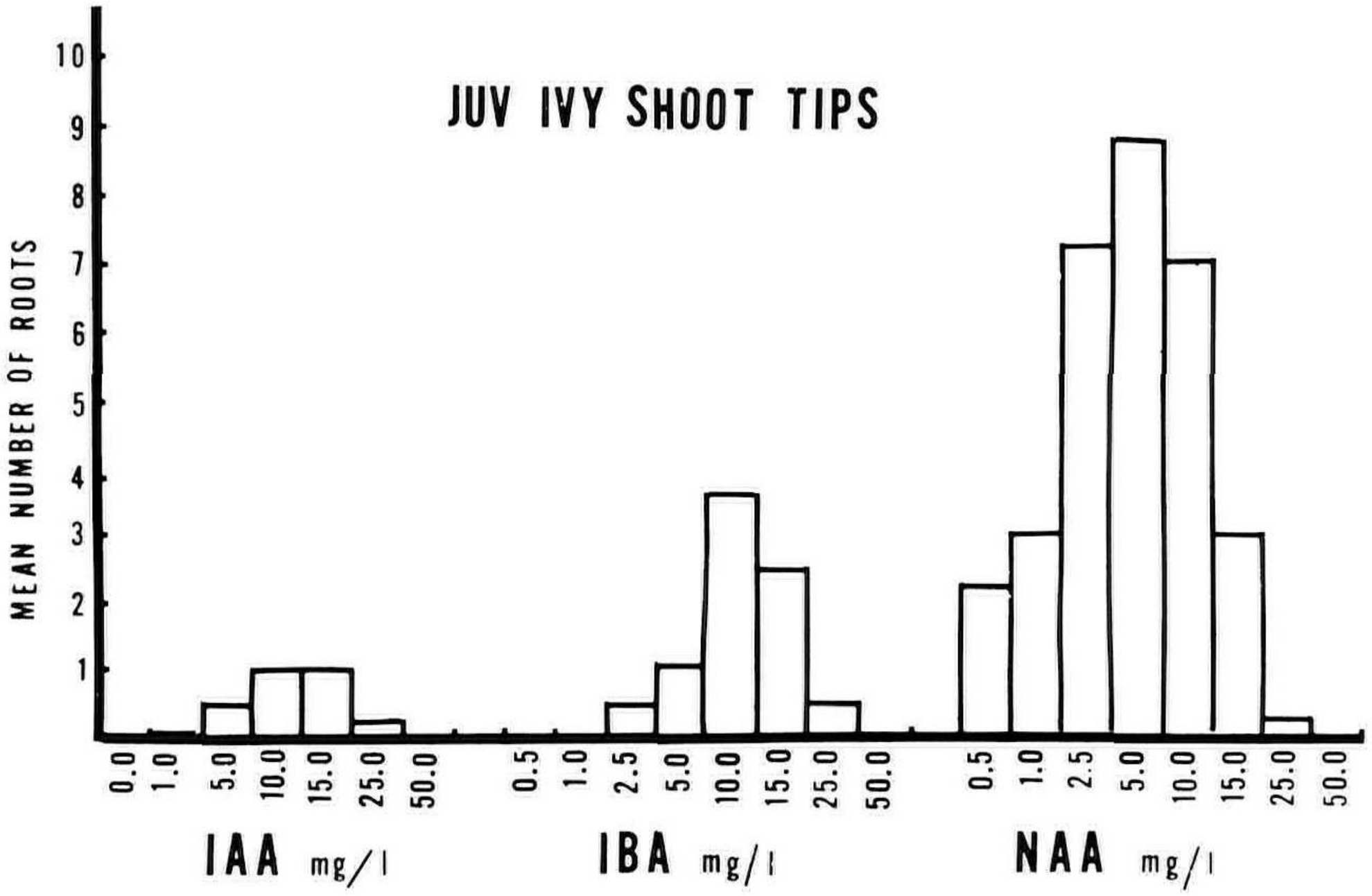

Fig. 2. The rooting response of juvenile shoot apices to kind and concentration of auxin.

Table 1. Composition of basal culture medium. pH adjusted to 5.8.

Component	mg/liters
KH_2PO_4	170.2
KC1	149.2
NaC1	2.3
$MgSO_4 \cdot 7H_2O$	123.3
Na_4 Fe EDTA[1]	25.3
$Ca(NO_3)_2 \cdot 4H_2O$	472.4
$MnSO_4 \cdot H_2O$	1.7
KI	0.17
$ZnSO_4 \cdot 7H_2O$	0.29
H_3BO_3	0.12
$CuSO_4 \cdot 5H_2O$	0.25
$NaMO\ O_4 \cdot 2H_2O$	0.24
myo-Inositol	90.1
thiamin - HC1	0.17
urea	300.5
sucrose	20,000.0

[1]Tetra-sodium-ferric-ethylenediaminetetracetic acid

of auxin. Naphthaleneacetic acid was by far the most active, with IBA intermediate and IAA least active. The optimum concentrations were not greatly different for the three auxins, being 5 mg/1 (2.7 x 10^{-5}M) for NAA, 10 mg/1 (4.9 x 10^{-5}M) for IBA and 10 mg/1 (5.7x 10^{-5}M) for IAA. This is in marked contrast to the great difference in rooting response to the three auxins at their optimum concentrations.

Figure 3 shows the synergism of IAA and catechol in promoting root initiation in juvenile shoot apices. Notice that catechol has no effect on rooting in the absence of IAA. With IAA at 10 mg/1 (5.7 x 10^{-5}M) the optimum concentration of catechol is 6 x 10^{-5}M; with IAA at 5 mg/1 (2.85 x 10^{-5}M) the response to catechol levels off at 3 x 10^{-5}M. So it appears that the maximum response to catechol occurs at a concentration equimolar to the IAA concentration. As was true in the previously described experiment, none of the apices from adult plants rooted.

The data presented in Figure 4 show that there is no response of juvenile shoot apices to catechol when NAA is used as an auxin. A combination of IAA at 5 or 10 mg/1 and catechol at 5 x 10^{-5}M gave a rooting response equal to or better than that obtained with NAA at its optimal concentration of 5 mg/1. Combinations of NAA and catechol were not effective in stimulating initiation of roots on apices from adult plants.

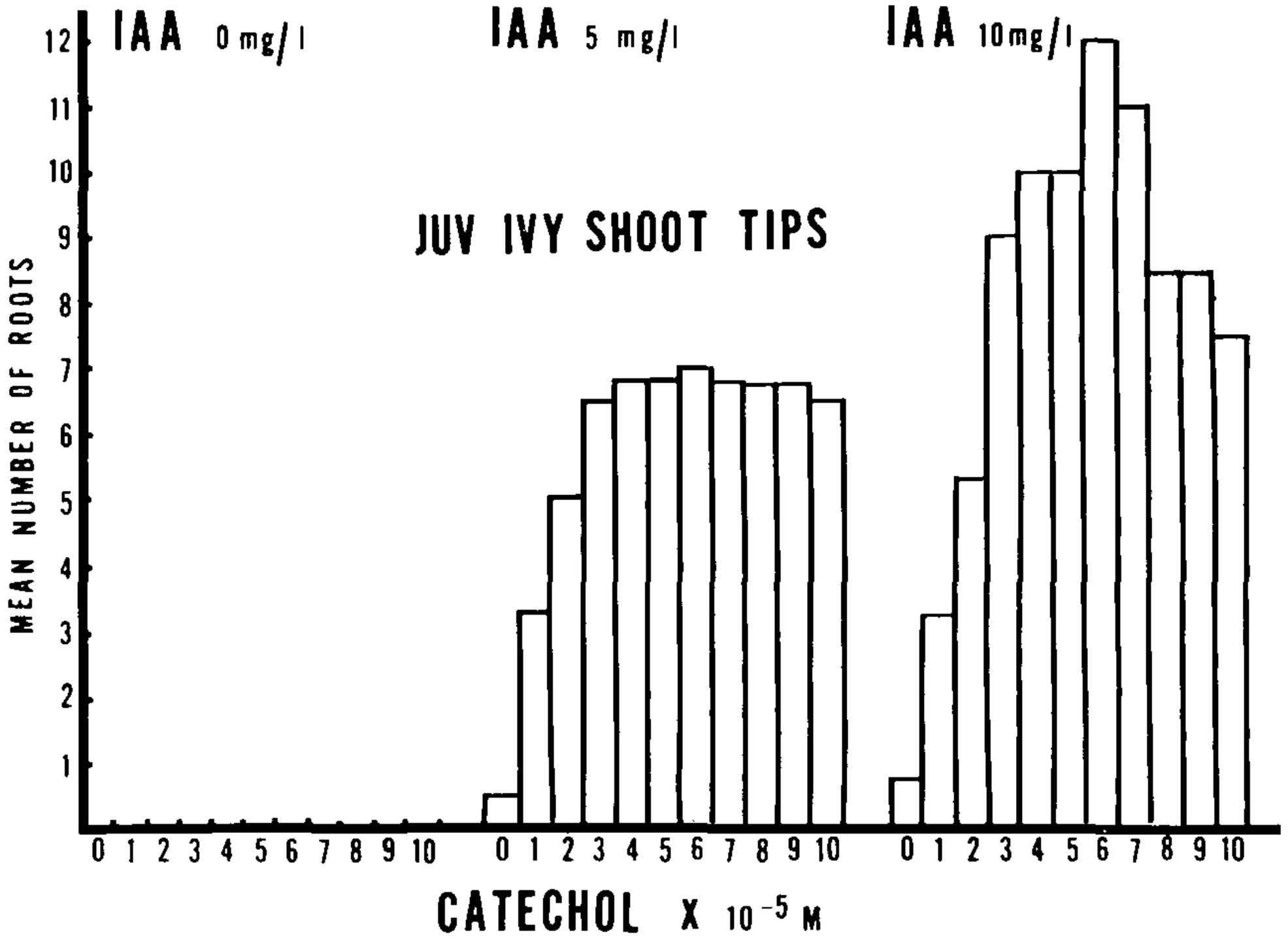

Fig. 3. The synergism of indole-acetic acid and catechol in promoting rooting of juvenile shoot apices.

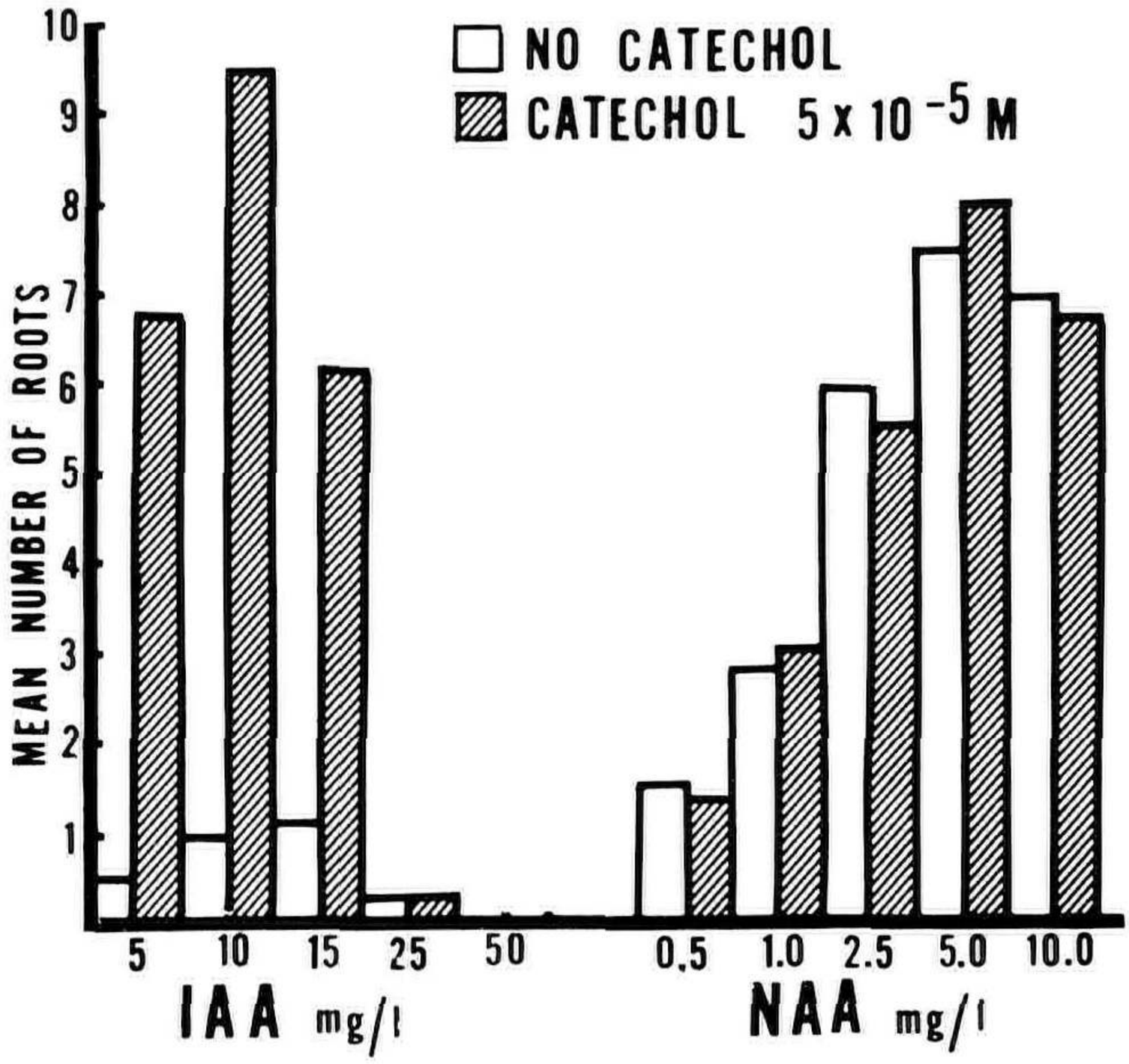

Fig. 4. A comparison of rooting response of juvenile shoot apices to IAA-catechol and NAA-catechol combinations.

When fractionated extracts of adult and juvenile ivy shoot tissue were assayed for root promoting activity using juvenile shoot apices, results as shown in Fig. 5 were obtained. The histograms show peaks of activity at R_f's 0.3-0.4, and 0.5-0.65 and possibly a weak peak at R_f 0.1 (See Fig. 8 also). The area from R_f 0.8-1.0 is somewhat inhibitory to rooting. The root-promoting peak at R_f 0.3-0.4 was not greatly affected by decreasing the amount of extract streaked from 225 mg equivalent of dry tissue to 112 mg but the peak at R_f 0.5-0.65 was substantially decreased. Notice that extracts from adult and juvenile shoot tissue give similar results when assayed using juvenile shoot tips. Fractionated extracts of neither juvenile nor adult shoot tissue were effective in stimulating initiation of roots on apices from adult plants.

In an attempt to stimulate rooting of shoot tips from adult plants, the quality and intensity of light under which the rooting tests were conducted was varied. The following four regimes were used: 1) daylight fluorescent light at 500 ft. c.; 2) incandescent light at 500 ft. c.; 3) incandescent light at 50 ft. c.; and 4) darkness. Figs. 6 and 7 show the response of adult and juvenile shoot tips to light and catechol. Notice that under low intensity incandescent light, and in darkness, adult shoot

tips formed about two roots per tip when IAA was provided at 10 mg/1. These treatments gave the first observed instance of root initiation on adult shoot tips. Catechol had no effect on rooting of adult tips in high intensity fluorescent or incandescent light but promoted rooting by 100 to 300% in low intensity incandescent light and darkness. Rooting of juvenile shoot tips was also promoted by reduction or exclusion of light.

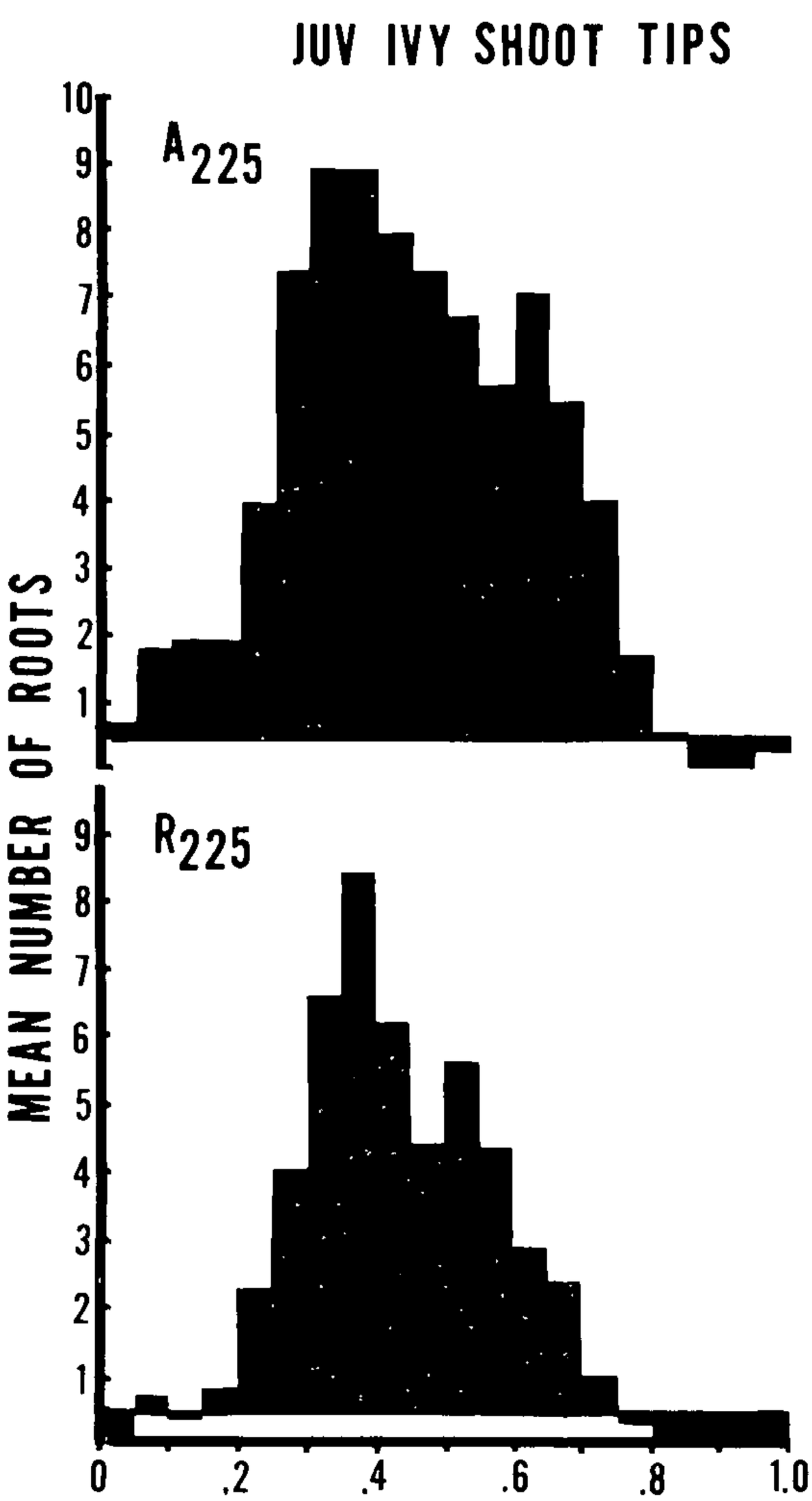

Fig. 5. Histograms showing the rooting response of juvenile shoot apices to chromatographically fractionated methanolic extracts of juvenile (bottom) and adult (top) *Hedera helix* shoot tissue. Extracts from 225 mg. of lyophilized tissue chromatographed on paper with isopropanol and water (4:1 v/v). Basal culture medium supplemented with IAA at 10 mg/1.

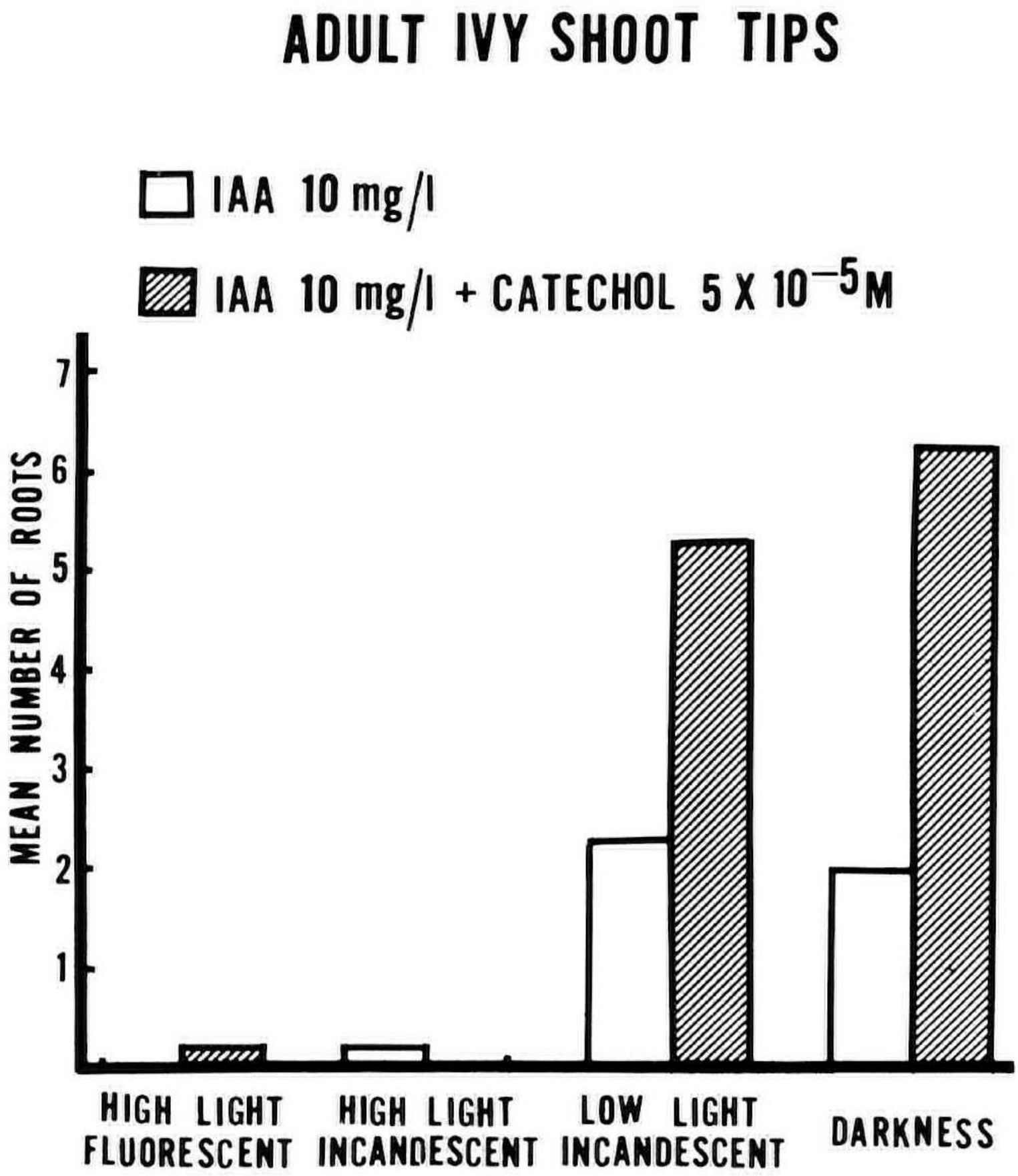

Fig. 6. The influence of light and catechol on the rooting of adult shoot apices.

However, with this tissue catechol was less promotive at low light intensity than it was at high intensity. In darkness catechol had little effect on rooting.

The results of the experiment with light indicate that high intensity light is strongly inhibitory to rooting. A subsequent experiment showed that etiolation of stems on intact adult plants caused the stems to form aerial roots quite profusely. Because of the evidence that low light intensity or darkness is promotive to rooting, fractionated methanolic extracts of etiolated and light-grown shoots were prepared and assayed for root promoting activity under high intensity light (500 ft. c.). Fig. 8 shows the results of this experiment using juvenile shoot tips as the test for rooting. Fractionated extracts from etiolated tissue gave only one peak of root promoting activity at R_f 0.5-0.65 while the light-grown tissue once again showed two large peaks and one small peak of activity. The magnitude of activity at R_f 0.5-0.6 was very similar for extracts from etiolated and light-grown tissue and there was no difference between extracts from adult and juvenile tissue. When these same extracts were assayed for rooting using adult shoot tips there was no stimulation of root initiation.

DISCUSSION

The experimental evidence reported here indicates that auxin is an important factor in the control of rooting in juvenile ivy shoot tips. There is, however, a strong synergism between IAA (but not NAA) and catechol. This strong synergism can possibly be explained on the basis of decreased destruction of IAA in the presence of catechol. It is known that polyphenols such as catechol inhibit the peroxidase type indoleacetic acid oxidase system in peas and wheat (15) and also the photo-oxidation and chemical oxidation of IAA (1, 12).

The fact that NAA is much more active than IAA in promoting rooting could also be explained on the basis of IAA destruction, as NAA is not destroyed by IAA oxidase (3) and is much more resistant to photo-oxidation and chemical oxidation. Indolebutyric acid which has intermediate root promoting activity is intermediate in its susceptibility to various kinds of oxidative destruction (10). However, the fact that high concentrations of IAA do not promote rooting of juvenile

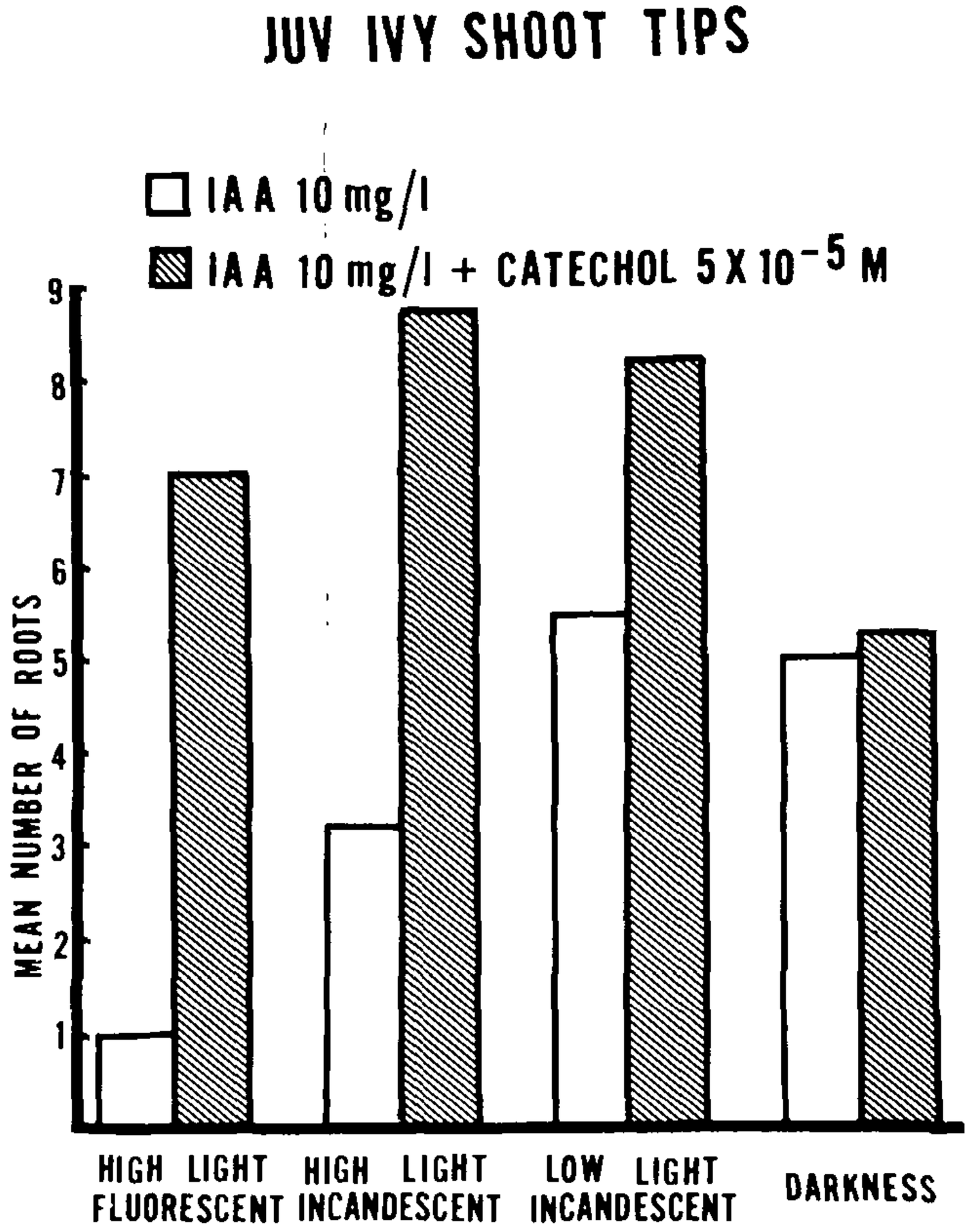

Fig. 7. The influence of light and catechol on the rooting of juvenile shoot apices.

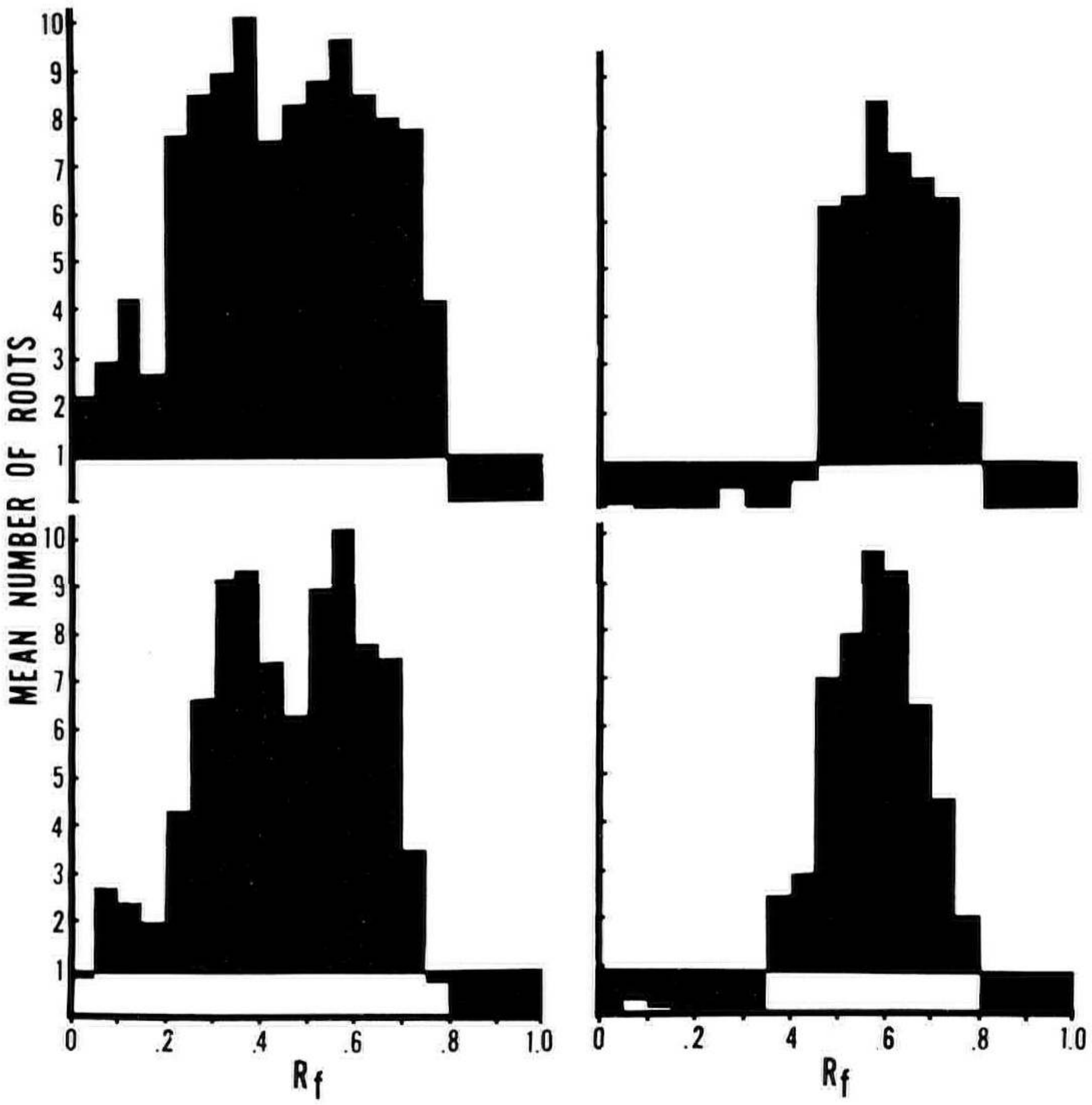

Fig. 8. Histograms comparing the rooting response of juvenile shoot apices to chromatographically fractionated methanol extracts of light grown (*lower left*) and dark grown (*lower right*) juvenile and light grown (*upper left*) and dark grown (*upper right*) adult *Hedera helix* shoot tissue, chromatographed on paper with isopropanol and water (4:1 v/v). Basic culture medium supplemented with IAA at 10 mg/l.

shoot tips (Fig. 2) is difficult to explain unless it is assumed that the products of IAA breakdown are inhibitory to rooting.

Destruction or inactivation of IAA is also indicated by the fact that rooting is much higher in juvenile shoot tips at low light intensities and in darkness than at high light intensities. Fletcher and Zalik (5) have shown that white light reduces elongation of bean seedlings in comparison to those grown in darkness and that there is a direct relationship between IAA content and plant height. They also found a marked influence of light on the metabolism of exogenously applied IAA and speculate that red light stimulates the oxidation of IAA (6). Light promotes oxidation of IAA in some crude enzyme extracts (16) and of course is essential for photo-oxidation.

The synergism between IAA and catechol in promoting root initiation in juvenile shoot tips is very great in high intensity light but much reduced or absent in low intensity light or darkness. It may be that catechol and reduced light are

promoting root initiation of juvenile shoot tips through a similar mechanism.

Another possible explanation for the synergism between IAA and catechol in root initiation is the formation of an IAA-catechol complex which is more effective in promoting rooting than either component. Polyphenol oxidase enzymes are known to oxidize catechol to a quinone and quinones are known to condense with IAA giving a colored pigment (11). Fadl and Hartmann (4) have isolated a root-promoting factor which has tentatively been identified as an auxin-phenol complex. The results reported here would indicate that NAA is active without formation of a catechol complex.

The three peaks of root-promoting activity found in fractionated methanolic extracts of adult and juvenile ivy stem tissue have R_f values which correspond with three of the cofactors reported by Hess (7, 9) using a mung bean bioassay. Juvenile shoot tips showed no response to the R_f 0.8-1.0 area of chromatograms which was the area of highest activity reported by Hess. While Hess (9) found greater activity in extracts from juvenile than adult shoots, the results presented here using juvenile shoot tips to assay rooting show no difference between adult and juvenile extracts.

Whereas juvenile shoot tips grown in light respond to auxins and combinations of IAA and catechol by forming more roots, adult shoot tips do not respond to these factors. Likewise, adult shoot tips do not respond to fractionated extracts of adult and juvenile stem tissue whereas juvenile shoot tips do respond to these extracts. This indicates that these factors do not limit root initiation in this difficult-to-root adult shoot tissue and points out a danger in using easy-to-root tissue as a rooting assay in studies of root initiation in difficult-to-root plants.

Reduction in light intensity brings about a qualitative change in the rooting response of adult shoot tips to auxin and catechol (Fig. 6). There is essentially no response to IAA and catechol when adult tips are grown in high intensity light, but when grown in low intensity light adult tips respond markedly to these factors, and in much the same manner that juvenile shoot tips respond to these factors in high intensity light.

There is ample evidence for methanol extractable factors which promote rooting in easy-to-root juvenile ivy shoot tips but no evidence for similar factors which promote rooting of difficult-to-root adult tips. Even the light controlled factor which stimulated rooting of adult shoot tips was not methanol extractable. Further work with different extraction solvents is needed to determine the factors that control rooting in adult ivy shoot tips. It is possible that these factors reside in a fraction of the cell which is not readily extractable or transmissible.

LITERATURE CITED

1. Brauner, L. and M. Brauner. 1954. Untersuchungen uber die Photolyse des Hetero-auxins II. *Z. Bot.* 42:83-124.
2. Cooper, W. C. 1935. Hormones in relation to root formation on stem cuttings. *Plant Physiol.* 10:789-794.
3. Donoho, C. W., A. E. Mitchell and H. M. Sell. 1962. Enzymatic destruction of C^{14} labelled indoleacetic acid and naphthaleneacetic acid by developing apple and peach seeds. *Proc. Amer. Soc. Hort. Sci.* 80:43-49.
4. Fadl, M. S. and H. T. Hartmann. 1967. Isolation, purification and charac-. terization of an endogenous root-promoting factor obtained from the basal sections of pear hardwood cuttings. *Plant Physiol.* 42:541-549.
5. Fletcher, R. A. and S. Zalik. 1964. Effect of light quality on growth and free indoleacetic acid content in *Phaseolus vulgaris* L. *Plant Physiol.* 39:328-331.
6. Fletcher, R. A. and S. Zalik. 1965. Effect of light of several spectral bands on the metabolism of radioactive IAA in bean seedlings. *Plant Physiol.* 40: 549-552.
7. Hess, C. E. 1962. A physiological analysis of root initiation in easy-and difficult-to-root cuttings. *Proc. 16th Int. Hort. Cong.* pp. 375-381.
8. Hess, C. E. 1962. Characterization of rooting cofactors extracted from *Hedera helix* L. and *Hibiscus rosa-sinensis* L. *Proc. 16th Int. Hort. Cong.* pp. 328-388.
9. Hess, C. E. 1963. Naturally-occurring substances which stimulate root initiation. In: J. P. Nitsch, ed., Regulateurs Naturels de la Croissance Vegetale, C.N.R.S., Gifs/Yvette. pp. 517-527.
10. Kenton, R. H. 1955. The oxidation of B (3-indolyl) propionic acid and (3-indolyl) n-butyric acid by peroxidose and Mn^{2+}. *Biochem. Journ.* 61: 353-359.
11. Leopold, A. C. and T. H Plummer. 1961. Auxin-phenol complexes. *Plant Physiol.* 36:589-592.
12. Platt, R. S., Jr., and K. V. Thimann. 1956. Interference in Salkowski assay of indoleacetic acid. *Science* 123:105-106.
13. Thimann. K. V. and J. B. Koepfli 1935. Identity of the growth—promoting and root-forming substances of plants. *Nature* 135:101-102.
14. Tomazewski, M. 1963. The mechanism of synergistic effects between auxin and some natural phenolic substances. In: J. P. Nitsch, ed. Regulateurs Naturels de la Croissance Vegetale, C.N.R.S., Gifs/Yvette pp. 335-351.
15. Waygood, E. R., A. Oaks and G. A. MacLachlin. 1956. The enzymatically catalyzed oxidation of indoleacetic acid. *Canad. Journ. Bot.* 34:905-926.
16. Waygood, E. R. and G. A. MacLachlan. 1956. The effect of catalase, riboflavin and light on the oxidation of indoleacetic acid. *Physiol. Plant.* 9:607-617.
17. Zimmerman, P. W. and T. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thomp. Inst.* 7:209-229.

PRESIDENT KRAUSE: Thank you very much, Wes. After our next two speakers we will have a Question and Answer period, so reserve your questions until then. We will have adequate time set aside for questions and answers.

Our next speaker will talk on etiolation as an aid in propagation. Dr. George Ryan: