MODERATOR STOUTEMYER: We will now hear from Dr. Charles E. Hess, Associate Professor of Horticulture, Purdue University, Lafayette, Indiana, who will discuss his work on the physiology of rooting. We will also be privileged to see the excellent movie film he has prepared, showing step-by-step the methods used and results obtained during his studies. Dr. Charles Hess.

A PHYSIOLOGICAL COMPARISON OF ROOTING IN EASY AND DIFFICULT-TO ROOT CUTTINGS

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In order to obtain an understanding of the substances involved in the processes of root initiation, a comparative study of rooting has been made in the juvenile and mature forms of *Hedera helix* L. *Hedera* was selected as the experimental material because as shown in Figure 1 both the juvenile and the mature form may be found on the same plant. However, the rooting ability of the two forms is very different. The juvenile form roots very easily; 100% rooting is not uncommon. The mature form of *Hedera*, on the other hand, is very difficult to root, 16% rooting being the maximum under our conditions. Therefore we have plants which should be genetically identical and are grown under the same environment, and yet are very different in their rooting ability.

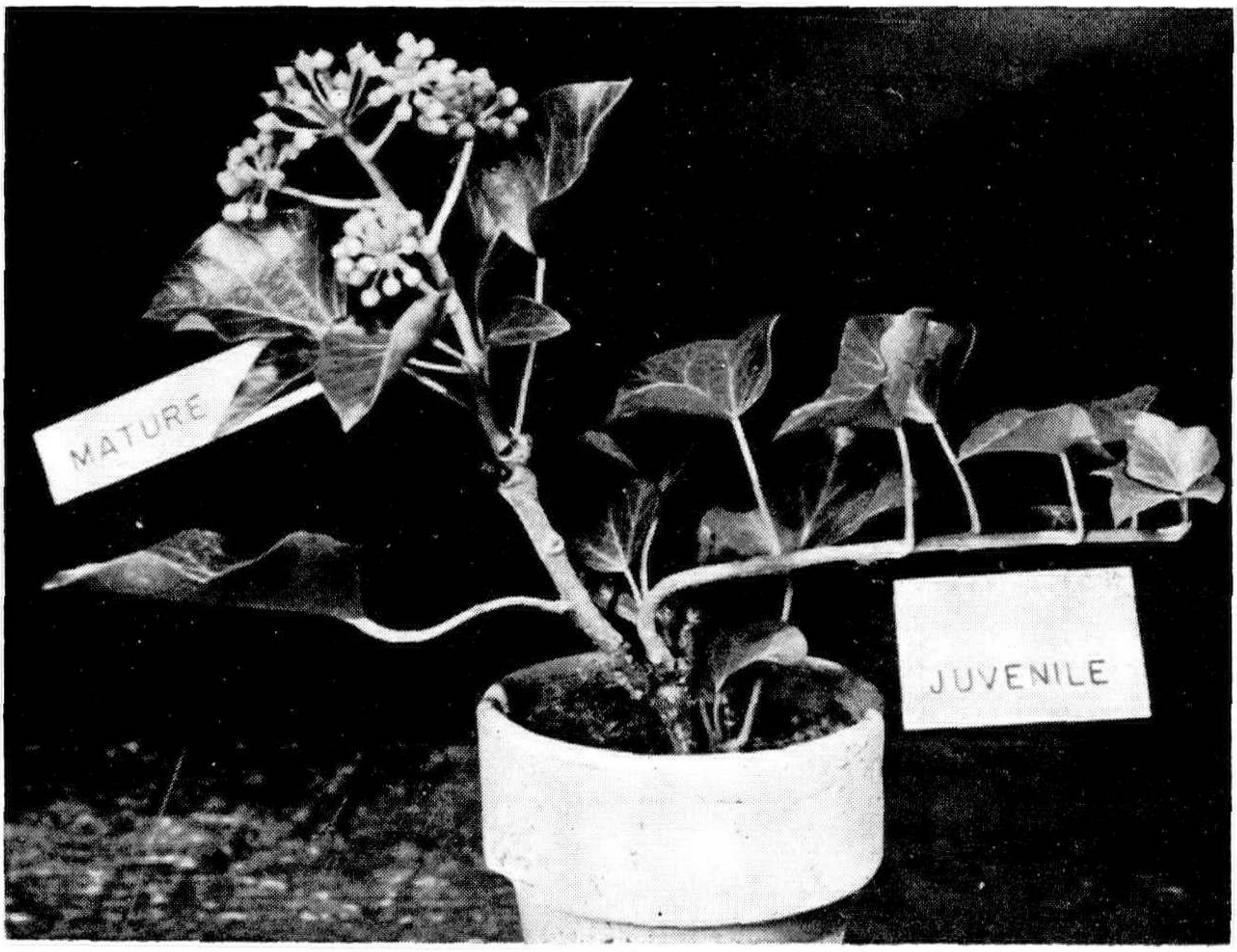

Figure 1. Juvenile and mature forms of *Hedera helix* appear on the same plant.

The first approach in the study was to determine the auxin and inhibitor content of the two growth forms. The purpose was to see if the easy-to-root juvenile form contained an auxin which was lacking in the mature form, or if the mature tissue contained an inhibitor which blocked root initiation. The former possibility did not seem likely since the application of known auxins did not alter substantially the rooting response of the mature cuttings. However the latter possibility, that is the presence of an inhibitor, has been described by Spiegel (3) in difficult-to-root grape cuttings.

Methyl alcohol extracts were made of dried tissue from juvenile and mature cuttings. The extracts were fractionated by paper chromatography. The presence of auxins and inhibitors was determined by use of the *Avena* coleoptile straight growth test as described by Nitsch and Nitsch (2).

The results of such an experiment is shown in figure 2. The bars above the horizontal line represent areas of growth promotion or auxins, and the bars below the line represent inhibitors. IAA, IAN, and IAE represent indoleacetic acid, indoleacetonitrile, and ethyl-3-indoleacetate. The three substances are known auxins which have been found in plant tissues and were co-chromatographed with the tissue extracts for the purpose of comparison.

The important things to see in Figure 2 are (1) that there are several auxins and inhibitors present in the juvenile and mature tissues, and (2) the auxins and inhibitors are present in approximately the same amounts in the juvenile and mature tissues. Therefore, it did not seem possible that the great difference in rooting abili-

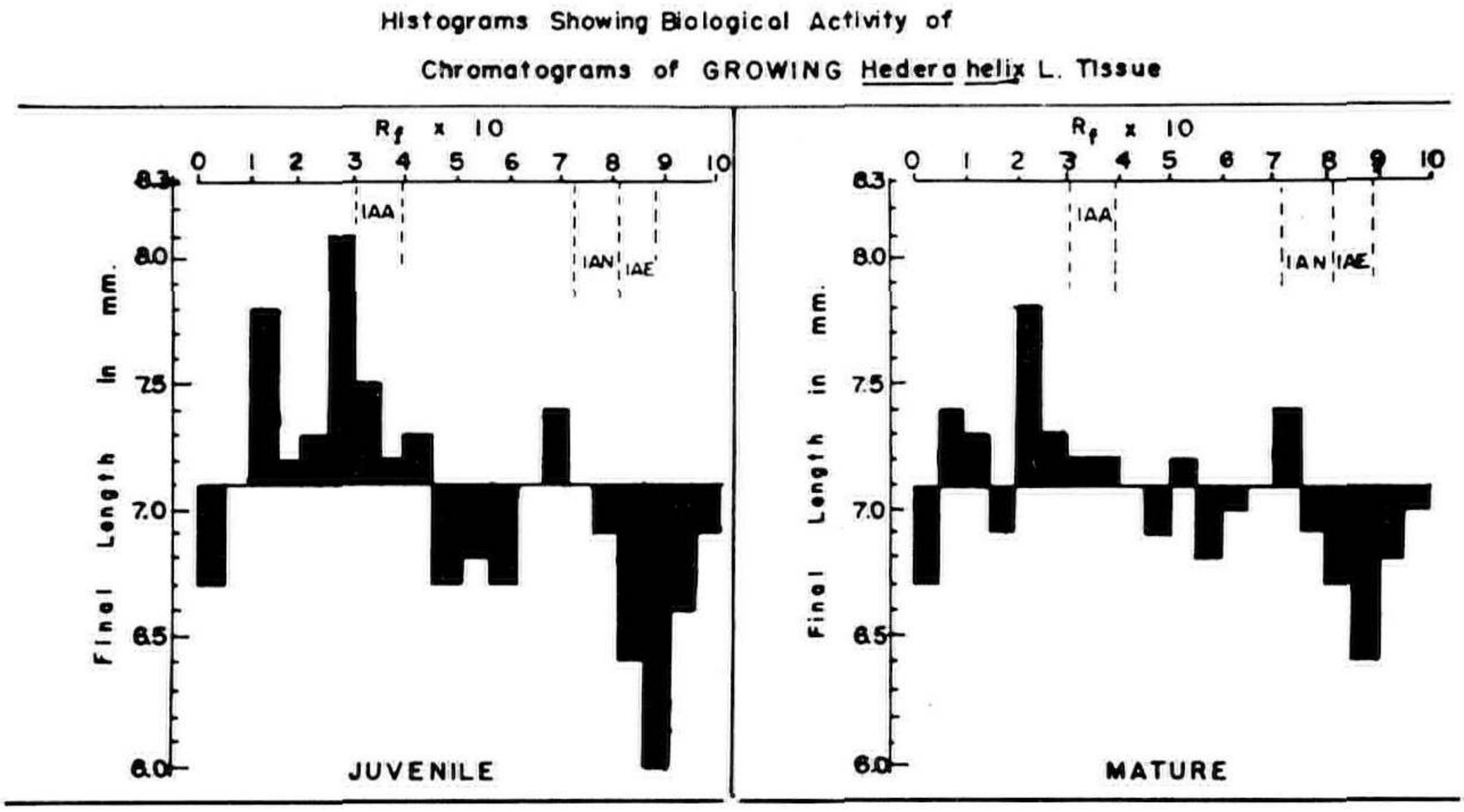

Figure 2.

50mg lyophilized tissue, extracted with methanol for 2 hours at 0°C. Extract chromatographed in isopropanol - ammonia - water (8,1,1). Bioassay - Avena coleoptiles 4mm. long, taken 3mm. below tip.

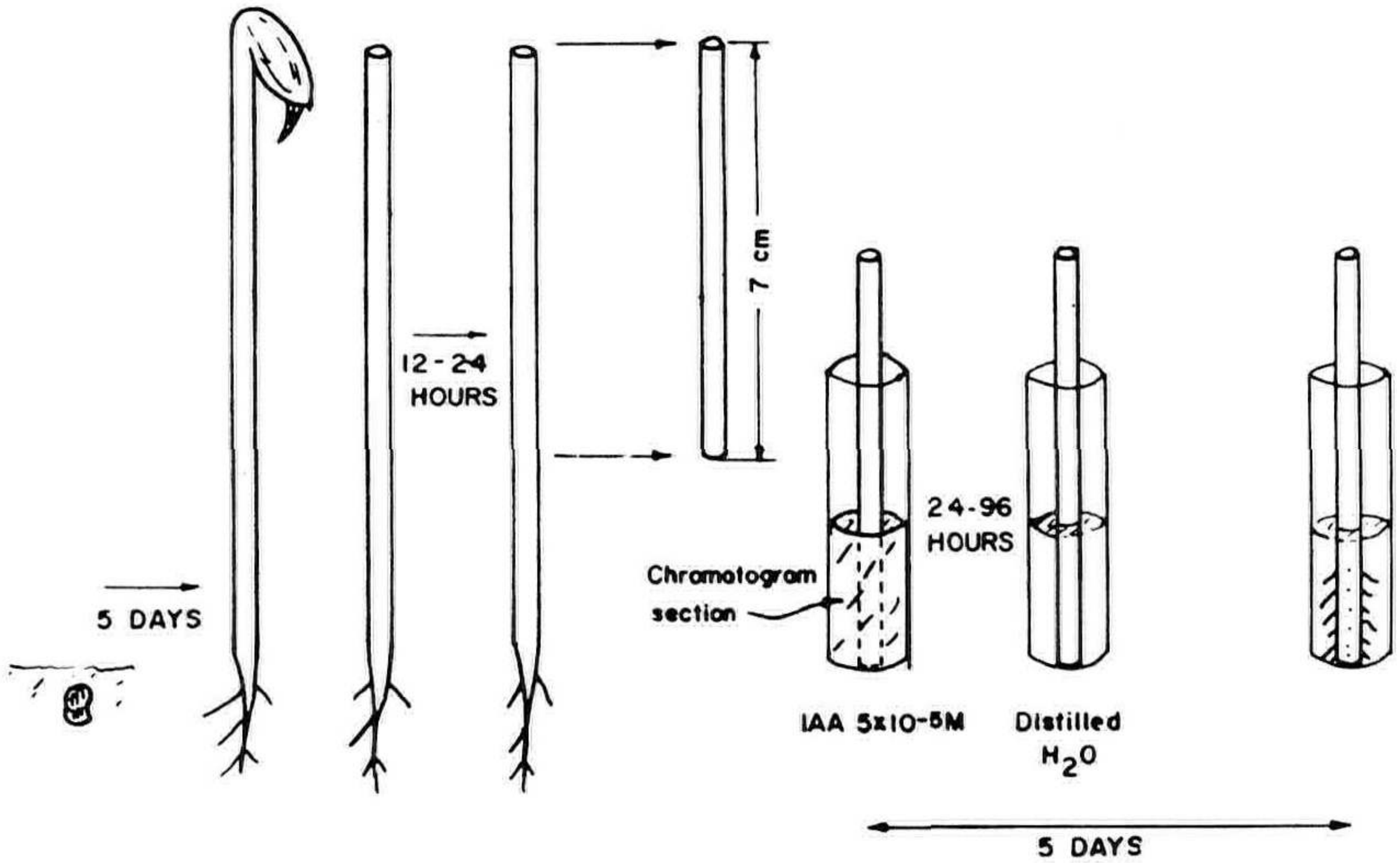

Figure 3.

ty between the juvenile and mature *Hedera* could be explained on the basis of a difference in auxin or inhibitor content.

The next approach was to analyze methyl alcohol extracts of the juvenile and mature *Hedera* with a test based on rooting. Figure 3 shows one of the rooting tests that was used.

Seeds of the mung bean (*Phaseolus aureus*, Roxb.) are planted in vermiculite and are placed in a dark room with a temperature of approximately 27°C and 85% relative humidity. In 5 days the etiolated seedlings are ready for use. The cotyledons are cut off and the remainder of the seedling is allowed to stand for 24 hours. During this time the internal root promoting substances dissipate, leaving the cuttings more sensitive. At the end of the 24 hour period, 7 cm cuttings are taken. Ten cuttings are placed in each vial which contains an extract or piece of chromatogram. After 24 to 96 hours the cuttings are transferred to distilled water. The roots on the cutting are ready to count in 5 days from the time the cuttings were first made. The average number of roots per cutting in each vial is used to determine the presence of a root promoting substance.

Figure 4 shows the results of an experiment using the mung bean test. The juvenile extract, when supplied to the cuttings in the presence of indoleacetic acid (IAA at 5×10^{-5}M), substantially increased the amount of root initiation. The mature extract did not stimulate rooting.

When the juvenile extract is fractionated by paper chromatography and the chromatogram is assayed with the mung-bean rooting test, it is found that the root-promoting activity is due to at least four

substances. Figure 5 shows such a chromatogram and each of the peaks above the horizontal line (average number of roots per cutting on the controls) represents an individual root promoting substance.

Although the third (R^F0.6) and fourth R^F0.8) peaks are quite close in this chromatogram, it has been possible to separate them using other solvents.

On the basis of this and similar experiments with *Hibiscus Rosa-sinensis* L., our present concept is that the differences in the rooting ability of cuttings is due to the presence of at least four substances in the easy-to-root cuttings. The difficult-to-root cuttings either lack these substances or contain smaller amounts of them.

The next step in this study is the purification and identification of the root-promoting substances found in the juvenile *Hedera*. With this information it will be possible to test our hypothesis and also find out how the substances actually work in stimuating root initiation. The techniques used for partial purification and some effects of chemical structure upon the activity of a root promoting substance are presented in a previous issue of the Proceedings (1) and will not be included here.

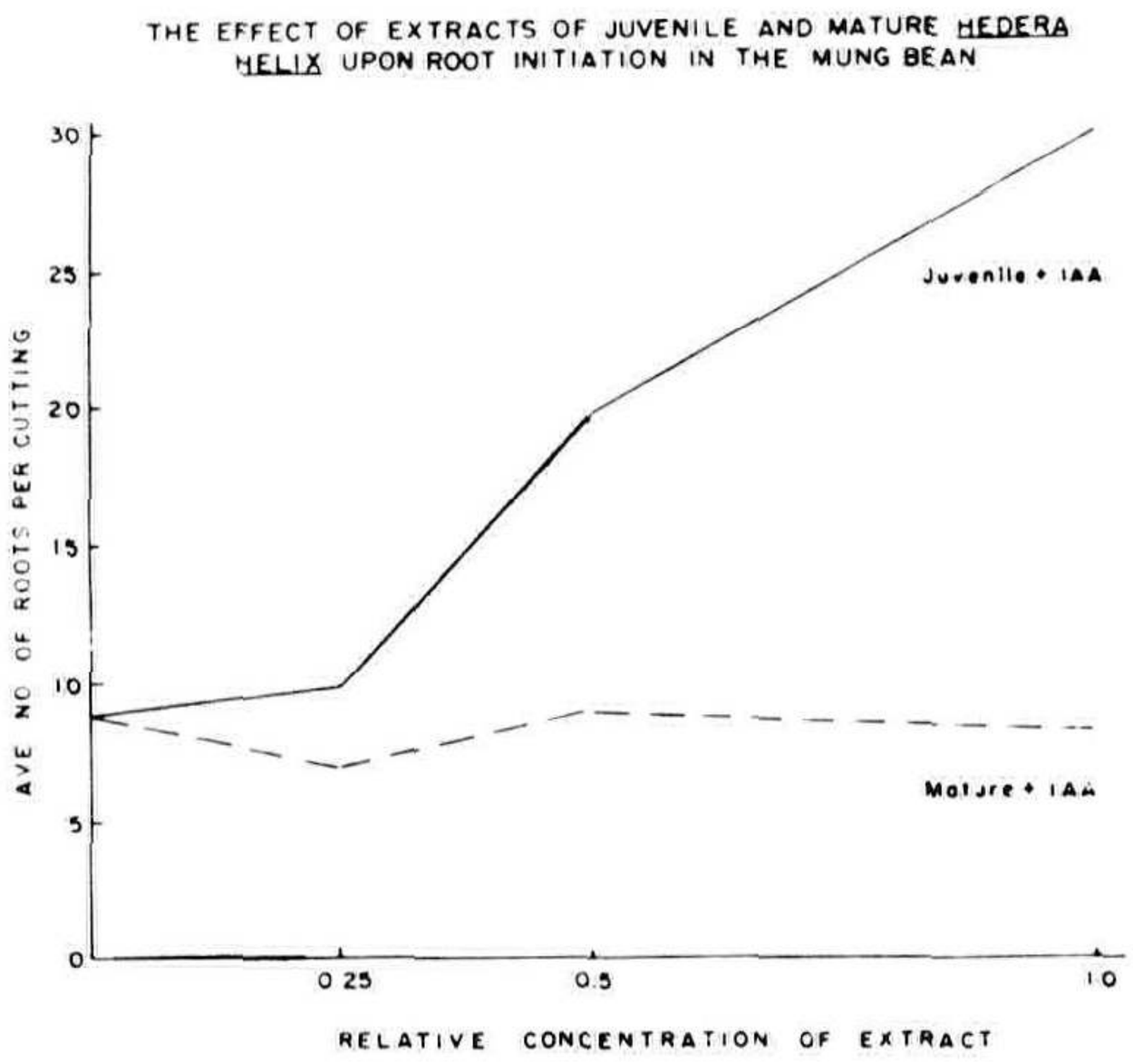

Figure 4.

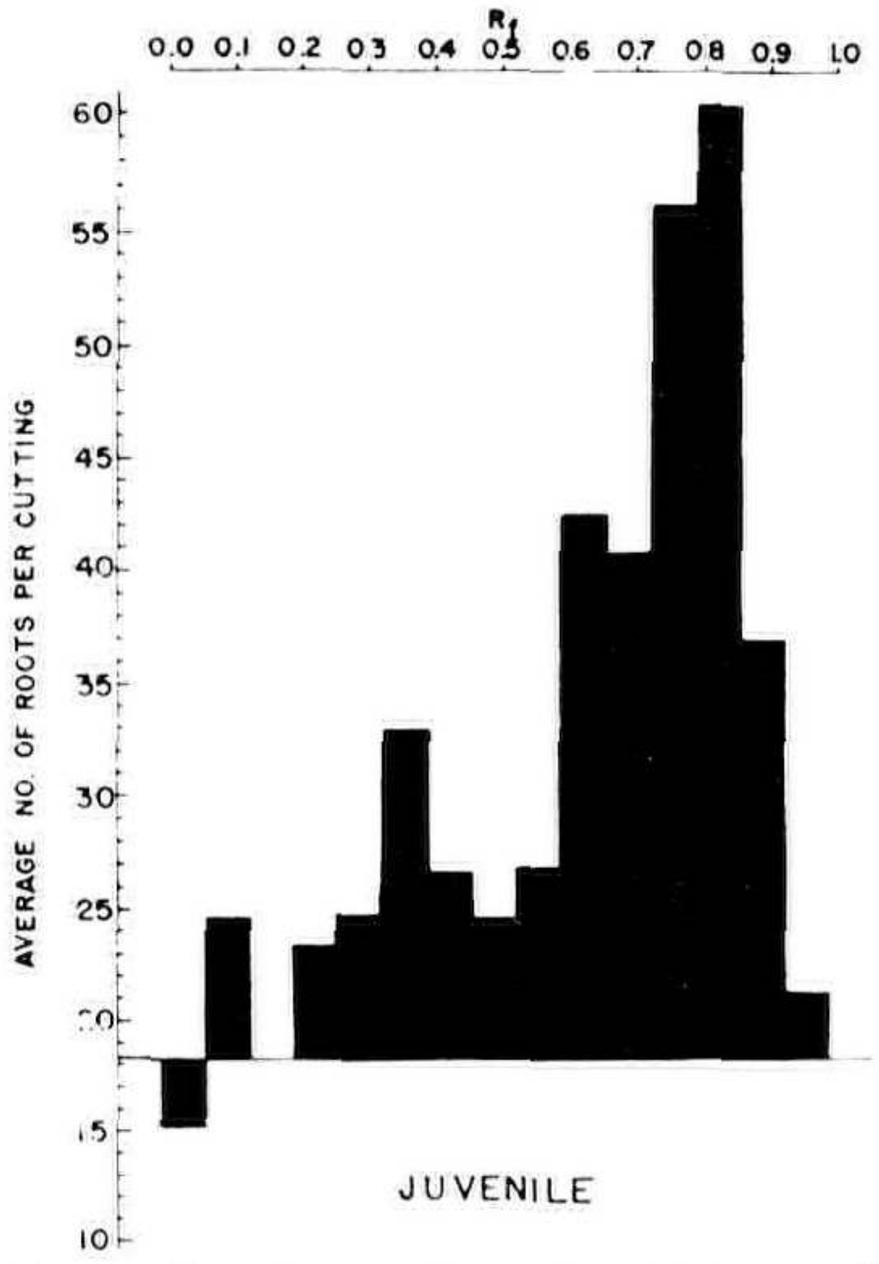

Figure 5. A methanol extract of juvenile *Hedera helix* tissue. water (8.2 v/v). Bioassay: Mung Chromatogram with isopropanol: Bean Cuttings.

LITERATURE CITED

1. Hess, Charles E. 1961. Characterization of the rooting cofactors extracted from *Hedera helix* L. and *Hibiscus Rosasinensis* L. Proceedings Plant Propagators Soc. 1961: 51-57.
2. Nitsch, J. P. and C. Nitsch. 1956. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight growth test for auxins. Plant Physiol. 31:94-111.
3. Spiegel, P. 1954. Auxin and inhibitors in canes of Vitis. Bull. Research Council of Israel 4:176-183.