RESEARCH IN ROOT INITIATION — A Progress Report

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As many of you will remember from last year's talk (2), we are attempting to establish chemical differences between easy-to-root and difficult-to-root cuttings. .One of the plants we are working with is *Hibiscus Rosa-sinensis.* The red flowering form of this plant is easy to root and the white form is difficult to root. In addition we have a third variety which is intermediate in its ability to root. By extracting the tissue of the three forms of *Hibiscus* we find four separate root promoting substances in the red *Hibiscus,* three root promoting substances in the *Hibiscus* which is intermediate in its rooting ability, and two in the difficult-to-root *Hibiscus.* In other words, a correlation exists between the number and the amount of root promoting substances we can extract and the rooting ability of the cutting — the easier a cutting is to root, the more root promoting substances can be extracted. The root promoting substances are not related to indoleacetic acid (IAA), the natural plant auxin, but interestingly enough, they require the presence of IAA for maximum activity.

The major effort in the present *Hibiscus* research is to isolate the root promoting substances in pure form and identify them. This is a very formidable problem because plant tissues contain many thousands of substances and at times I feel our extracts must contain all of them. However, progress is being made and we now know some of the physical characteristics of the root promoting substances. They are soluble in water and in methyl and ethyl alcohol. They appear to be insoluble, as a group, in chloroform and ether. The root promoting substances are thermostable; that is, they can be exposed to high temperatures (in this case 250° F. at 15 p.s.i. for 20 min.) and still remain active. The thermostability information provides an indication that the substances are quite stable, at least in a semi-purified condition. This is encouraging information because it means that we can work with the rooting substances at room temperature without the fear that they will break down into other substances which probably would not be active. Finally, the root promoting substances are nondiolytic. This property is determined by placing the substances in a cellophane bag which is sealed. The bag is next surrounded with a solvent such as water. Substances of small molecular size pass through cellophane membrane into the surrounding water. Substances of large molecular size stay within the cellophane bag. It turns out that the root promoting substances all stayed within the bag. Although this indicates the substances are of large molecular size, the possibility exists that the root promoting substances are attached to a large molecule and this prevented their escape from the bag or that the substances absorbed to the inside surface of the bag. You can see from this example that in research you very seldom get clear cut answers. Instead you find indications which lead to other experiments which in turn provide more indications and other experiments and so it goes until you finally have enough evidence accumulated to make a valid conclusion.

Now I would like to describe some of the work we have been doing with *Hedera helix,* the English Ivy. *Hedera* is an ideal species for studying root initiation. Both the easy-to-root juvenile form and the difficut-to-root mature form can be found on the same plant. The juvenile condition is characterized by a horizontal growth, and as you know, is used as a ground cover. The leaves are lobed, the stems are often reddish, and grow rapidly. The mature form, in contrast, grows upright, has entire leaves, the stems are usually green, and growth is slow. The best indication of maturity is that when plants reach this stage of growth, they are capable of flowering. However, the particular difference in which we are interested is rooting ability. The juvenile form, which will initiate aerial roots, can be rooted at almost any time of the year with nearly 100% success. The best we have been able to do with the mature cuttings, up to this year, has been 16% rooting with an average of 2 roots per cutting. So you can see there is a tremendous difference in the rooting ability of the 2 forms, and yet we are sure that the tissues are genetically similar, if not identical.

Many other, if not all plants have a juvenile phase of growth. The juvenile form of *Euonymus coloratus,* for example, has leaves which color to a dark purple-red in the winter. The leaves on the mature form, which will develop if the plant is allowed to grow up a support, do not "color" nearly as well as the juvenile form. The best way to detect the male form, as with the *Hedera,* is to check for evidences of flowering.

The beech expresses its juvenility in a little different way. Young seedlings in the juvenile stage of growth retain their leaves well into winter. Mature plants lose most of their leaves early in the season. However, in many cases, the base of a mature tree will retain its leaves. The reason is that this portion of the tree remains physiologically juvenile. If scions are taken from this area, the plants will be juvenile. If scions are taken from the upper portions of the tree they will be mature and will lose their leaves early in the season.

Mature apple trees also are physiologically juvenile at the base. If an apple tree is girdled or heavily pruned, shoots will arise from or near the base of the plant. These shoots are physiologically juvenile and because of their rapid growth are called water sprouts. Conifers also go through a juvenile phase of growth. In the juniper the young seedlings have needles, and as the plants become mature, the needles are reduced to scale-like leaves. Conifers provide a particularly good example of the relationship between juvenility and rooting ability. If cuttings are taken from young seedlings of pine or spruce, they are relatively easy to root. As the plants mature, propagation by cuttings becomes increasingly more difficult, until finally, they are classified as being "impossible to root."

You can see, therefore, from the few examples that I have given, the phenomenon of juvenility can be found in a wide range of plant material, and that when plants are in the juvenile stage of growth they are easier to root. As I reported last year, we found that the juvenile *Hedera helix* contains more root promoting substances than does the mature *Hedera*. Since then we have found that the juvenile *Hedera* extracts contain four individual root promoting substances which are similar to, but not exactly the same as those extracted from the *Hibiscus*.

With this background, the widespread phenomenon of juvenility, the association of juvenility with rooting ability, and the fact that the juvenile tissue contains more root promoting substances than does the mature, we became interested in the possibility of rejuvenating a mature plant. If this were possible we could obtain a better understanding of the mechanisms which control the expression of juvenility and maturity. With this knowledge it might be possible to prolong the period of juvenility for the benefit of the propagator or hasten maturity for the benefit of the plant breeder. The experiment was conducted by grafting scions of mature *Hedera* on established juvenile understock. Scions bearing fruit were selected so as to be sure the scions were physiologically mature. The fruit was cut off prior to grafting. Grafting was done in March, 1960. The sequence of growth following union formation is shown diagramatically in figure 1. After the union had formed, all juvenile growth was removed. The first new growth from the scion was morphologically mature — i.e. the leaves were entire and the shoots were upright. This first spurt of growth was probably already differentiated in the bud at the time the scion was taken. Shortly after the first spurt of growth was completed, new growth developed which was morphologically juvenile. The leaves were lobed and the shoot was horizontal. The mature tissue therefore had been rejuvenated. Doorenbos (1) and Stoutemyer (4) have reported that cuttings taken from the rejuvenated growth was easy-to-root. However, our grafts did not stop at the rejuvenated stage. After a period of approxi-

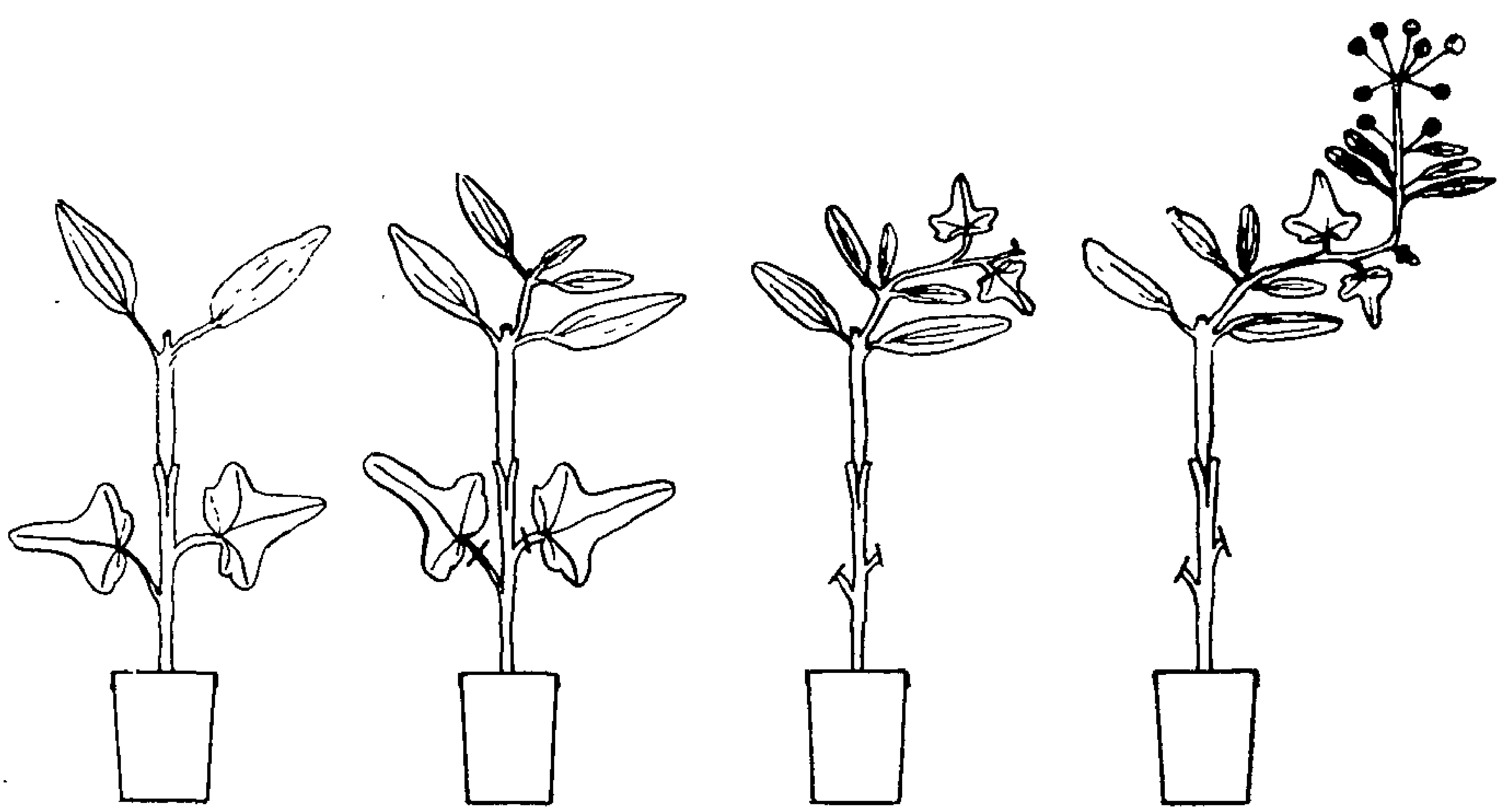

Figure 1.—Induction Of Juvenility And Reversal To The Mature State

mately 4 weeks of juvenile growth the shoots reverted back to a morphologically mature condition. Not only did the leaves become entire and the shoots grow upright, but they also flowered and produced fruit. There was no question about the maturity of the new growth.

Now that our scions had produced growth that went the full cycle from mature to juvenile, back to mature, we wanted to see how the final mature growth would root. We took cuttings and also tissue samples for extraction. The original mature growth, if used as cuttings rather than scions, rooted at 16% with an average of 2 roots per cutting. When cuttings were taken from the "reverted" mature shoots we obtained 96% rooting with an average of 17 roots per cutting. So, although the tissue was morphologically mature, the cuttings rooted nearly as well as juvenile cuttings (juvenile cuttings taken at the same time rooted 100% with an average of 30 roots per cuttings). When we analyzed the extracts from the "easy-to-root" manure shoots, we found four cofactors, identical with those present in juvenile tissue. However, the amount of each cofactor present in the "reverted" mature tissue was less

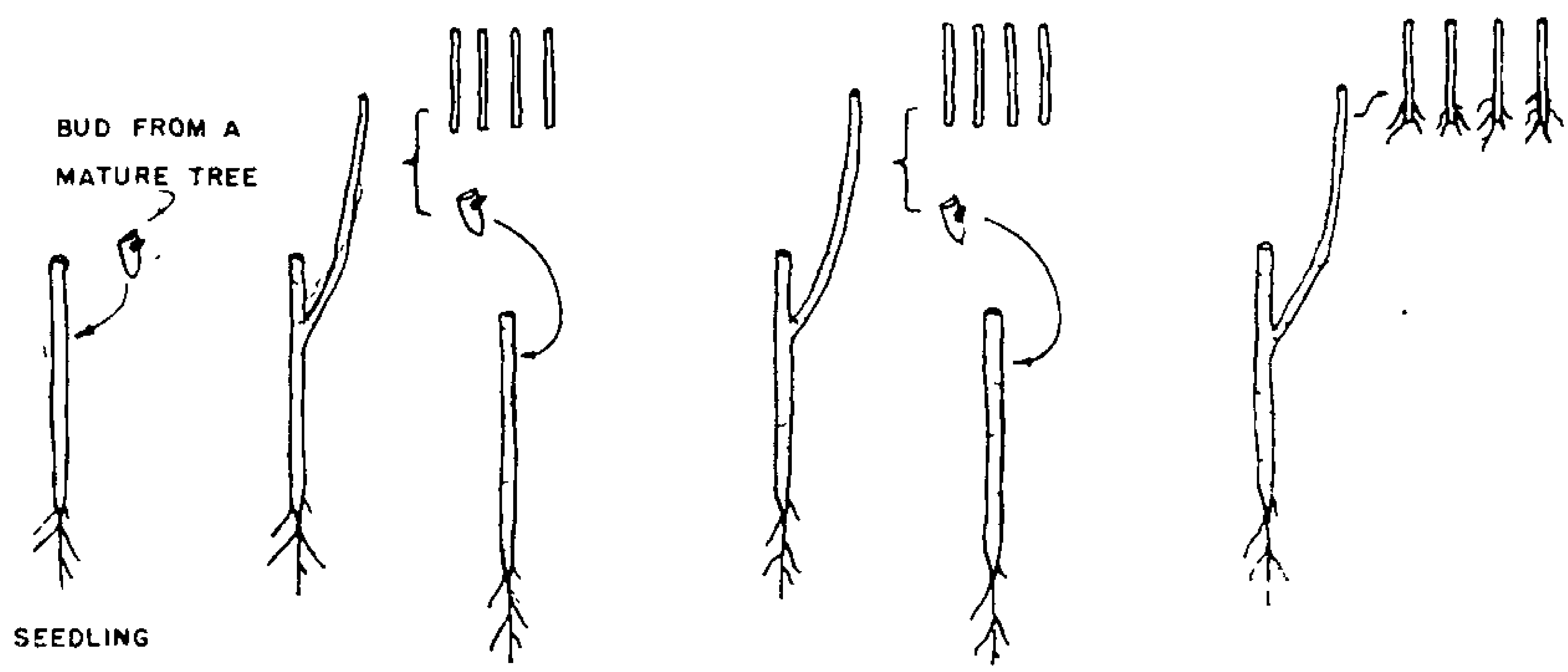

Figure 2.—Accumulation Of Rooting Abiilty By Budding On Seedlings

than that in the juvenile tissue. So, once again, we have a correlation between rooting ability and the presence of root promoting substances. The mature cuttings before grafting were difficult-to-root and contained little or no root promoting substance, after grafting the "reverted" mature tissue was relatively easy-to-root and contained all 4 root promoting substances although in a reduced amount when compared to juvenile tissue. Another conclusion can be drawn from these experiments — although juvenility and rooting are very closely associated, they are not necessarily controlled by the same mechanisms, since the morphologically and physiologically mature, flowering shoots were easy-to-root. If the same substances are involved in the rooting and juvenility, then the assumption may be made that a higher level of these substances is required for the expression of juvenility as compared to rooting ability.

Figure 2 shows that the transfer of rooting ability from a juvenile stock to a mature scion or bud does not necessarily occur in one operation as it did with *Hedera*. In this case Muzik (3) budded mature form of *Hevea*, rubber tree, on *Hevea* seedlings. When new growth was produced by the mature bud, cuttings were taken and also a bud was taken from the new growth and was budded on another seedling. The cuttings taken from the first budding operation failed to root. When the second budding produced growth, cuttings were taken and again a bud was budded onto a third seedling. The cuttings from the second budding operation also failed to root. When the third budding produced growth, cuttings and buds were taken again. The cuttings still did not root. But when cuttings were taken from the fourth budding operation 30% rooting was obtained. The results of this experiment indicate a gradual accumulation of root promoting substances and that it was not until the fourth budding that the level was high enough to stimulate rooting. In this case there was no apparent morphological change to the juvenile condition. The *Hevea* experiment is similar, in a sense, to the example I gave previously about conifers. Of course, with conifers the rooting ability gradually decreases as the plant matures. I bring this point up again for consideration when deciding to establish a stock block. Careful records should be maintained so that any trends in rooting ability would be immediately apparent. If there is an apparent "maturation effect," the stock block may be rejuvenated by severe pruning which forces shoots from or near the base of the plant, which as I have pointed out, often remains physiologically juvenile.

In summary, we are continuing to work on the identification of the four root promoting substances extracted from *Hibiscus* and *Hedera*. Realizing that as many plants grow from the juvenile, seedling stage to maturity, there is a gradual decrease in rooting ability, we attempted to rejuvenate mature scions of *Hedera* by grafting on juvenile understock. Rejuvenation was obtained, but the shoots reverted back to the mature form. However, the "reverted" mature growth was now almost as easy to root as juvenile cuttings. Analysis of the extracts from the juvenile and mature tissues showed that the "reverted" mature tissue contained almost the same amount of root promoting substances as did the juvenile tissue.

I would like to mention one other area of research that was stimulated by last year's question box, relative to the identity of Chloromone. After a comprehensive study using paper chromatography, *Avena* coleoptile straight growth and mung bean rooting assays, and ultraviolet spectral analysis, we concluded that the principal auxin in Chloromone was naphthaleneacetic acid. It was present in our sample at a concentration of at least 3 mg/cc (3,000 p.p.m) in an aqueous solution at a pH of 8.2. Thank you very much.

References

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3. Muzik, T. J., and H. J. Cruzada. 1958. Transmission of Juvenile Rooting Ability from Seedlings to Adults of *Hevea brasiliensis,* Nature 181:1288,
4. Stoutemyer, V. T., O. K. Britt, and J. R. Goodin. 1960. The Propagation of Tree Ivies by Cuttings. Abstracts of Papers Presented Before the Amer. Soc. for Hort. Sci., Oklahoma State University.

MODERATOR HILL: That was certainly outstanding, Charlie. I am sure everybody in the audience was impressed with the depth and detail of this type of truly scientific investigation.

We must also recognize that investigations of this kind are best left in the hands of those capable of undertaking them, analyzing them, and we look forward to the day, Charlie, that you will give us a small black box that is readily portable, that we can take with us and take a small piece of juniper cutting, close the door and it will light up either green or red ,saying now or wait another week. Thank you very much.

The session recessed at 12:00 o'clock.

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