ROOTING COFACTORS — IDENTIFICATION AND FUNCTIONS

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The presence of root promoting substances in cuttings has been postulated since at least 1880 (4). The hypothesis is that when a cutting is taken, substances synthesized in the leaves and buds move down the stem, accumulate at the base of the cutting, and stimulate root initiation. The basis for this hypothesis can be demonstrated experimently by removing leaves, and/or buds, reducing leaf area, or by girdling the stem of a cutting. In each case the rooting response of the cutting will be reduced. Selim (5) has been shown in *Perilla* cuttings, the leaves contribute up to 78 precent of the rooting response, the buds contribute 15 percent and the stems, 7 percent. The fact that girdling the stem of a cutting below the leaves, but above the rooting medium, blocks rooting indicates not only the source of root promoting substances, but also that the substances are translocated in the phloem.

The presence of naturally occurring substances can also be demonstrated in grafting experiments (3). If a scion from an easy-to-root plant is grafted on a difficult-to-root cutting, the rooting of the latter can be improved. If the leaves of the scion are removed, or if the stem is girdled below the graft union, the promotive effect of the scion is lost.

Since girdling blocks the downward movement of root promoting substances, the technique can be used to enhance root initiation and also to provide information as to the kind of substances which are moving down the phloem. Root initiation can be enhanced by girdling a stem prior to taking a cutting. The longer the girdled stem remains on the plant, up to 34 days, before the cutting is taken, the better will be the improvement of the rooting response. This is because root promoting substances are accumulating in the tissue above the girdle.

The accumulation of substances in the tissue above the girdle can be shown by extracting the substances at several intervals after girdling (6, 7). The substance which accumulates in the largest amounts is sugar. Amino acids accumulate in the first five or ten days after girdling and then decline in concentration. As shown in Figure 1, rooting cofactor 4 accumulates in the tissue above the girdle of an easy-to-root, red variety of *Hibiscus rosa-sinensis* but not in the difficult-to-root variety, Wilson's White, or in the tissues below the girdle of either variety (7). As will be mentioned later, rooting cofactor 4 is a mixture of oxygenated terpenoid compounds (2).

Still another example of the accumulation of root promoting substances is shown in experiments by Cooper (1). Cooper worked with lemon cuttings which were treated with Indoleacetic acid (IAA). In some cases the base of the cutting was cut off after the IAA treatment, and in others, the cuttings were

retreated after the base had been removed. The results are shown in Table I.

The control cuttings, treated with tap water, produced an average of 1.7 roots per cutting. When the cuttings were treated with IAA, they produced 12.6 roots per cutting. When the treated area at the base of the cutting was cut off, the average number of roots dropped to 4.9. It could be said that the de-

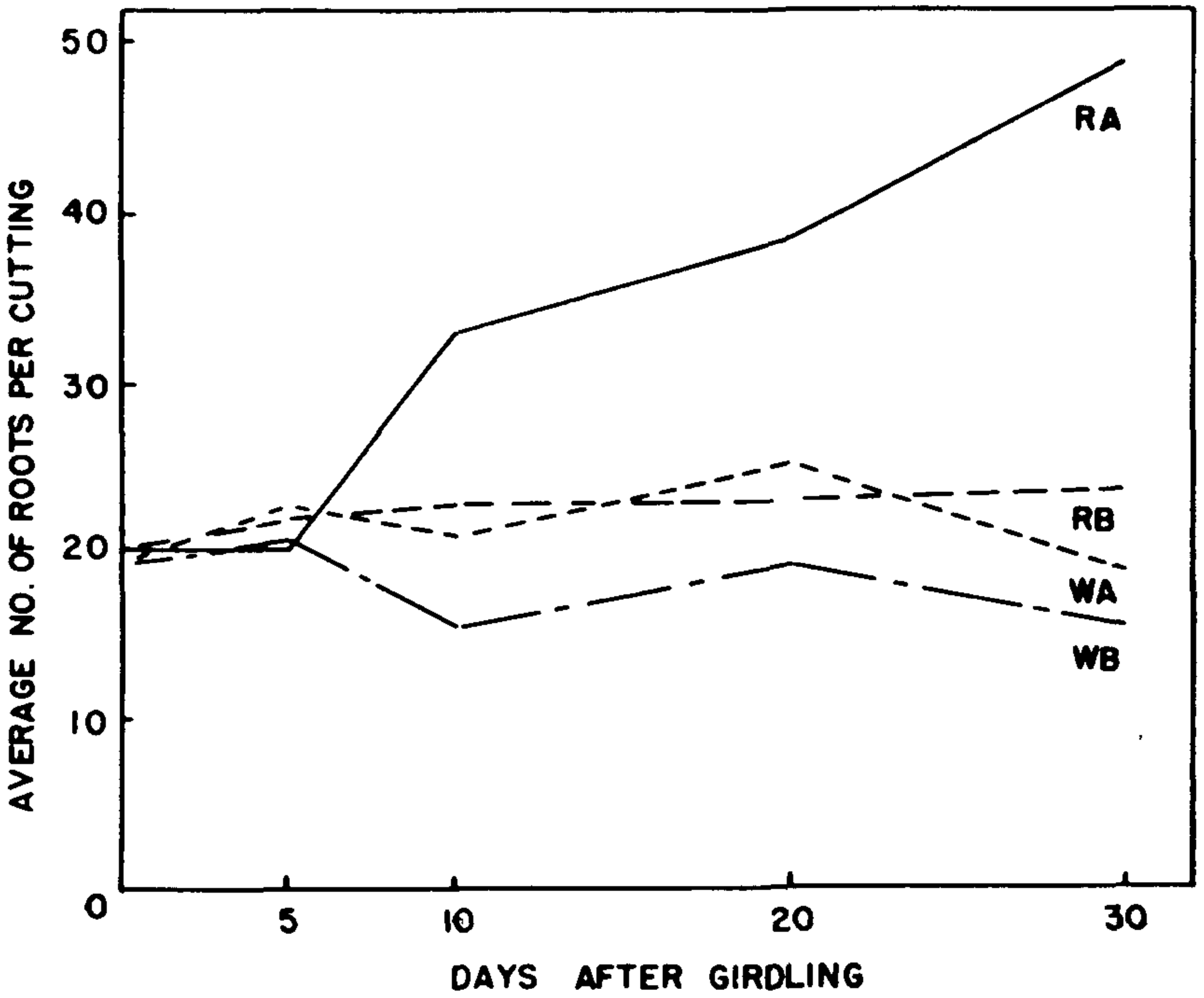


Figure 1. The accumulation of rooting cofactor 4 in girdled tissues RA — red Hibiscus easy to root), tissue *above* girdle RB — red Hibiscus, tissue *below* girdle. WA — Wilson's White Hibiscus (difficult to root), tissue *above* girdle. WB — Wilson's White Hibiscus, tissue *below* girdle.

Table 1 Interaction between indoleacetic acid and other naturallly occurring root promoting substances.

	Average Number of Roots per Cutting
Tap Water	1.7
Base treated*	12.6
Base treated and cut off	4.9
Base treated, cut off, cutting retreated*	4.8

*Treated — Fifteen hour soak in 0.17 mg/cc IAA.

crease was mainly due to the removal of the IAA which had been absorbed during the 15 hour soak. However, as shown in the last column, cuttings which had been treated, then had the treated area removed, and finally retreated only produced 4.8 roots. Even though the IAA was replenished, the cuttings were not able to initiate any more than 4.8 roots. The interpretation of the results is that a naturally occurring root promoting substance accumulated at the base of the cutting during the 15 hour soak in the IAA solution. When the base was removed, not only was the IAA removed but also other root promoting substances which had accumulated. Apparently these sub-

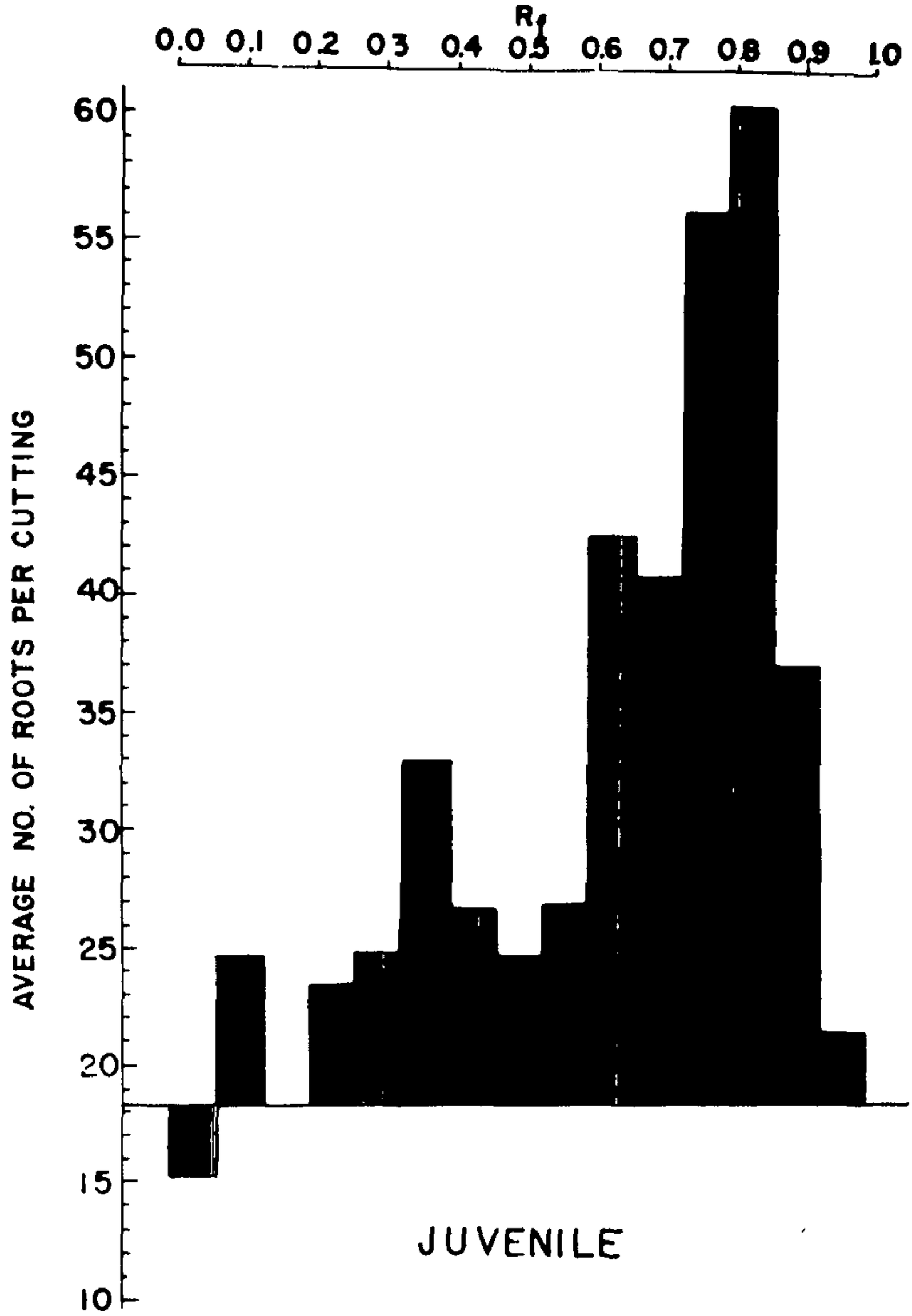


Figure 2 Histogram showing the biological activity in an extract of Juvenile *Hedera helix* Seventy-five mg of lyophilized tissue extracted with methanol and chromatographed with isopropanol and water (8 2, v/v) Bioassay. Mung bean rooting test

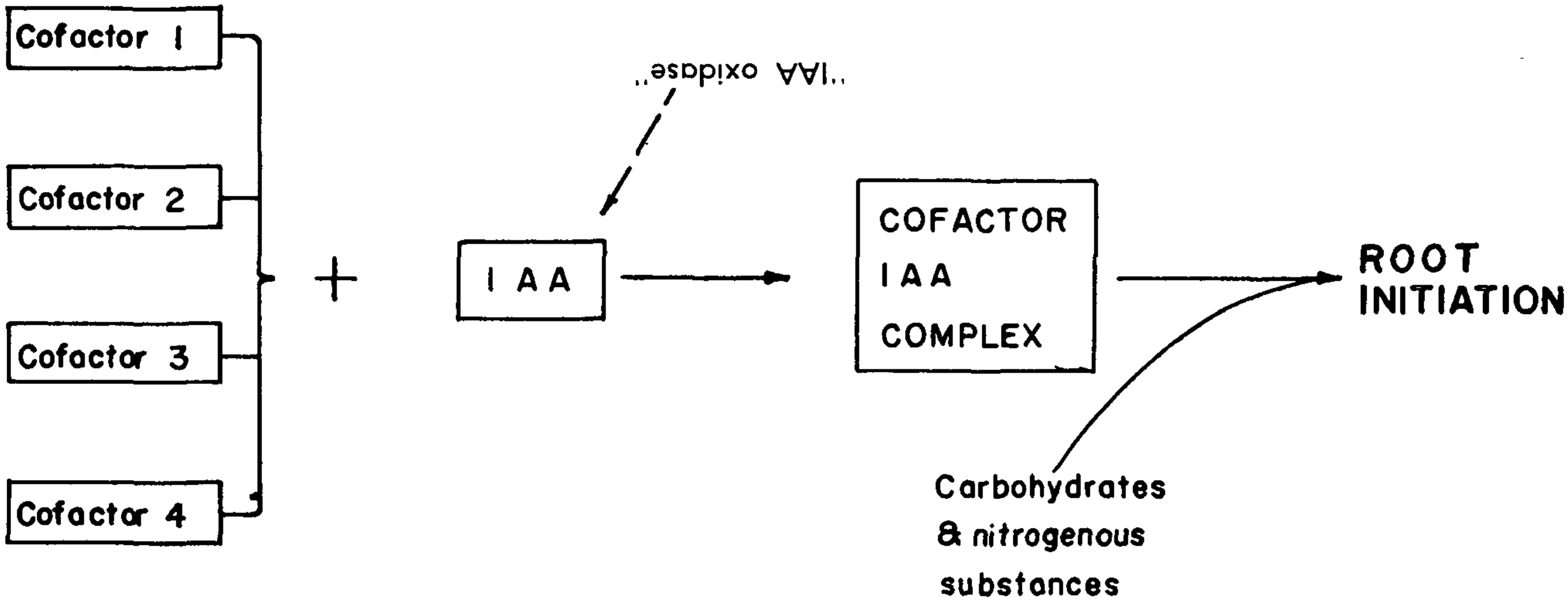
stances are synthesized slowly in lemon cuttings and the supply was depleted because a retreatment of the cuttings, after the base had been removed, did not increase the rooting response.

As has been previously described it is possible, to demonstrate the presence of root promoting substances in extracts of easy-to-root plants such as the juvenile form of English ivy, *Hedera helix* (2). As shown in Figure 2 the extract is partially purified by paper chromatography and the active, root promoting substances are located with the mung bean bioassay. There are four areas of activity and they are referred to as rooting cofactors 1, 2, 3, and 4, starting with rooting cofactor 1 closest to the origin.

By physical and chemical tests such as ultraviolet spectral analysis and functional group analysis, it has been determined that at least part of the biological activity in the area of rooting cofactor 3 can be attributed to the compound isochlorogenic acid. The subsance is a phenolic compound which has at least three isomers. By feeding cuttings a combination of isochlorogenic acid and IAA C^{-14} (radioactive indoleacetic acid), it was possible to find that a large part of the root promoting activity of isochlorogenic acid and other phenolic compounds with similar orthodihydroxy structures is due to their ability to prevent the inactivaction of IAA by the IAA oxidizing system. Zenk (8) and others have found a similar relationship between IAA and phenolic compounds in *Avena* coleoptiles.

Rooting cofactor 4 is a group of oxygenated terpenoid compounds (2). These compounds appear to be the most active of all the naturally occurring root promoting substances. They react synergistically with indoleacetic acid, but it is not known as yet how they function in the cutting.

A hypothetical scheme of where the rooting cofactors fit into the process of root initiation is shown in Figure 3. From the standpoint of an easy-to-root cutting, all four rooting cofactors would be present, IAA would be present, and a sufficient supply of carbohydrates and nitrogenous materials would be available for energy and synthesis. If the cutting is difficult-to-root, it may lack IAA because of an active oxidizing system which destroys it before enough IAA can accumulate to stimulate root initiation. In this case a synthetic auxin such as Naphthalene acetic acid (NAA) could be used because the plant can not readily destroy this auxin. However, as is the case with many difficult-to-root cuttings, the synthetic auxin may have little or no effect. In this case it is believed that one or more of the rooting cofactors are missing. The degree of difficulty, which varies from variety to variety, is an expression of how many and/or how much of the rooting cofactors are missing. Theoretically, if all the cofactors could be supplied to a cutting and there were cells available to divide, the cuttings should become easy to root.



A HYPOTHETICAL SCHEME OF ADVENTITIOUS ROOT INITIATION

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MODERATOR PINNEY: Our next speaker is Dr. James Kelley from the University of Kentucky.

ROOTING OF CUTTINGS AS INFLUENCED BY THE PHOTOPERIOD OF THE STOCK PLANT

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Photoperiodism is the phenomena in which the relative length of light and darkness influences the development of plants and animals. The influence of photoperiod on the development of plants was first recognized in 1923 (1). Since then the majority of plant physiologists have focused their attention on the flowering phenomena, however they were aware that photoperiod influenced the vegetative growth of many herbaceous and woody plants.

In more recent years the effect of photoperiod on woody plants has studied by Waxman (5) and others. It has been shown that if one divides a group of actively growing dogwoods (*Cornus florida* L.) into two groups and places one of them under long days of 15 hours or more and the other one under short days of 12 hours or less, one will observe that the plants under long days will continue to grow but those under short days will stop growth within 2 weeks. In other words, these plants become dormant.

Waxman (5) showed that when cuttings are taken from *Cornus florida* or *Weigela* plants growing under long days and rooted under various photoperiodic treatments, the number of roots produced per cutting was lower under short-day than under long-day treatments. Piringer (3) has reported that the rooting of holly and boxwood was favorably influenced when the