PROGRESS REPORT OF ROOT PROMOTING ACTIVITY IN JUVENILE AND ADULT PHASES OF MALUS ROBUSTA 5 APPLE ROOTSTOCK

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Since World War II, juvenility in plants has gained a certain prominence in the research of a number of countries. Specifically with *Malus*, reports of juvenility began to appear in European literature in the late 1940's and a summary of this was published in 1956 by Blair. MacArthur and Nelson (3) to substantiate the finding of the juvenile and adult forms in *Malus* and *Pyrus* at Ottawa. Subsequent to that report, both physiological growth phases were clearly evident on the same tree of a German rootstock-stembuilder selection received from Professor Maurer of Berlin in the late 1950's.

Of prime interest to the propagator is the fact that the juvenile form of *Malus* roots readily from softwood cuttings while the adult roots poorly; not only in low percentages but also with few points of attachment (11). Although there have been reports of the successful rooting of apples, I feel that the situation is fairly well summed up in the East Malling Report for 1964 (1) and I quote, "Some 30 scion varieties of apples have been propagated on their own roots: none were found to root as readily as even the shyest-rooting commercial rootstocks."

The quest for information on juvenility is of prime commercial importance as the propagation of own-rooted fruit trees would eliminate rootstock problems in all their ramifications. Conversely, if it was found, by chance, that the adult growth phases contained the controlling growth substance and the juvenile could be so treated, tremendous saving of time to the plant breeder would greatly accelerate the long program now required. Although many environmental treatments have been tried in an attempt to shorten the juvenile period, it has been generally accepted that these have not been successful or could not be applied to any appreciable breeding program. Recently, (Andersen (2), a marked shortening of the juvenile period was achieved by grafting to the very dwarfing rootstock, 3431, although considerable extra work is involved. Although the rooting of material is of greater importance to this group, we cannot ignore the other possibility and the plant breeder who is closely associated with us.

Even though juvenility in *Malus* had been identified at Ottawa and work with other crops by Hess (5) strongly suggested a further area of investigation, it was not until 1962 at the University of Saskatchewan that this work could be started. The first phase of the work by Quamme and Nelson (13) terminated in 1963. Briefly, it was found that a typical cofactor response, adopting Kefford's (9) extraction methods, was found

in the acid-ether fraction of the juvenile phase. The alkaline-ether fraction failed to give a cofactor-type response although increased rooting occurred near the base line of the chromatogram in both the adult and juvenile phases. The aqueous extract showed severe inhibition of rooting and did not vary in the two growth phases. Straight-growth tests with Avena coleoptiles were also conducted (Nitsch (12)) and although some promotion was present, it was not significant between the two growth phases and the greatest promotion occurred on the chromatograms in areas other than that of the cofactor response.

Subsequent to this work, another student, Miss Hwang (7), concentrated on the alkaline-ether fraction using thinlayer chromatography and varying solvent systems. The solvent system of normal hexane, ethyl acetate, as suggested by Hess (6), proved to give us the best visual separation under daylight and ultraviolet light but, although responses in rooting have occurred, it was not demonstrated to the workers' satisfaction that a cofactor response existed in this fraction of *Malus*. In this work, the acid-ether fraction was obtained in each extraction. As suggested by Kefeli and Turelskaya (8), toluene was used to remove fats and pigments. The alcohol extract was washed three times with toluene immediately after the sample was macerated and filtered. The area that only gave a cofactor response in the juvenile form for Quamme and Nelson (13) was now giving a cofactor-type response in both phases. This may be similar to the findings of Zimmerman (14) with *Pinus*. Obviously, by chemical manipulation, using toluene, something was removed from or released in the adult extract that allowed it to behave in the same manner as the juvenile. To date, no clues have been obtained from further studies with the toluene fraction.

During the summer, a chemistry technician was obtained and seconded to the Department of Chemistry under the direction of Dr. J. M. Pepper. A completely chemical approach was taken and exhaustive soxhlet extractions, with varying solvents, were made by the Department of Chemistry. The water soluble portions of the crude and fractionated extracts were returned to the Department of Horticulture and bioassayed with the mung bean rooting test without chromatographic separation. A number of root-promoting solutions were obtained and are illustrated in the three tables. Values listed in the upper left are number of runs, number of roots on water check and number of roots on the IAA (10^{-4}M) check respectively. Values under the columns represent the mean percentage increase in rooting over the respective check treatments.

(a) As shown in Figure 1, root promotion was found in the ether and alkaline water soluble fraction. It will be noted that ether alone gave practically no response but when the ether was washed with NaOH, the rooting-promoting substance(s) went into the water portion. When the water portion was reacidified, the root-promoting substance(s) were again ether soluble and were taken up in water when the ether was evaporated. It

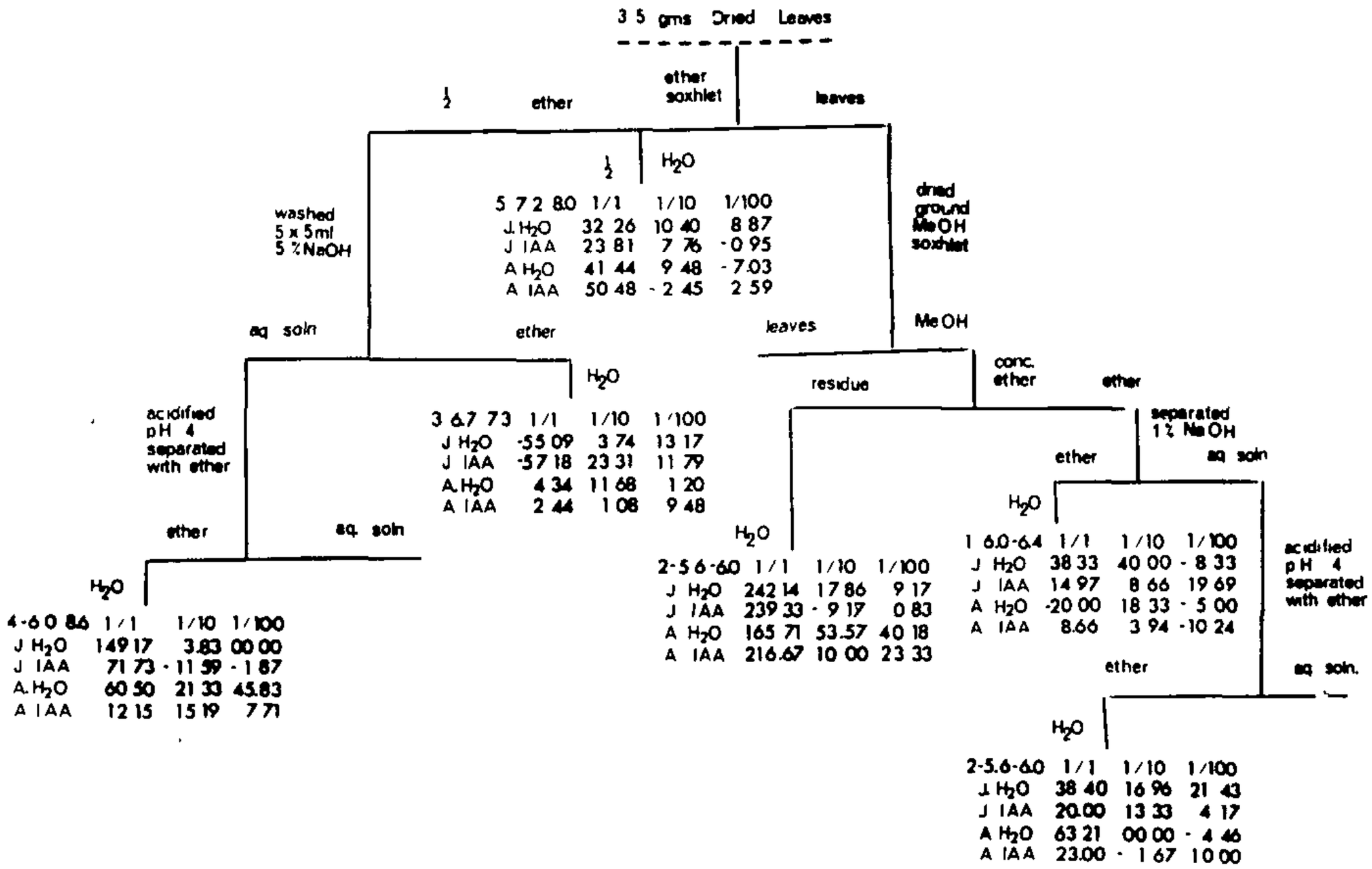

Figure 1. Average rooting response (over check treatment) of mung bean cuttings in solutions from various stages of ether, methanol and water extractions.

should be further noted that an inhibitor became evident in the ether after the alkaline wash. The level of root activity and inhibition was greatest in the juvenile.

(b) Also in Figure 1, another solution is exhibited that contained substance(s) which were ether insoluble, methanol soluble and water soluble. This root promotion was found in both of the growth phases.

(c) Rooting responses were obtained from both the juvenile and adult growth phases (Fig. 2) from substances that were benzene insoluble, methanol insoluble, ether soluble and water soluble.

(d) Also in this Figure is exhibited a solution that contained substance(s) that were benzene insoluble, methanol soluble, ether soluble and water soluble. The very high response is likely the result of the greater amount of original material.

(e) Still referring to Figure 2, another area of root promotion was from substance(s) that were benzene insoluble, methanol soluble, ether insoluble and water soluble. This residue showed considerable phytotoxicity but the 1/10 dilution yielded rooting levels far higher than experienced for this dilution in any of the other extractions.

(f) Finally in Figure 2, substance(s) that were benzene soluble, ether soluble, alkaline water soluble, but returned to the ether portion when reacidified and were finally water soluble when the ether was evaporated.

Because of fewer solvents in (a), it is possible that the substances discussed in one of (c), (d) or (f) could be the same as (a). Also (e) could be the same as (b).

Figure 3 illustrates extractions where the order of methanol and ether was reversed. It was noted that, where the root-promoting substance(s) were both methanol and ether soluble, it seemed preferable to use methanol first in the extraction.

As shown in Figure 2, one cofactor type of response was found in the juvenile phases but not in the adult. The substance(s) was benzene soluble, ether soluble and remained in the ether when it was washed with NaOH but was water soluble when the ether was evaporated.

It will be further noted that final aqueous separations were ignored in the extractions. This was because of the earlier findings of Quamme and Nelson (13). Obviously from work this fall however, there is considerable root-promotion in aqueous solutions but in most cases, a dilution of 100 times was needed.

As stated in the title, this is a preliminary report. The root-promoting substances discovered in these extractions will have to be further fractionated by chromatography so that they

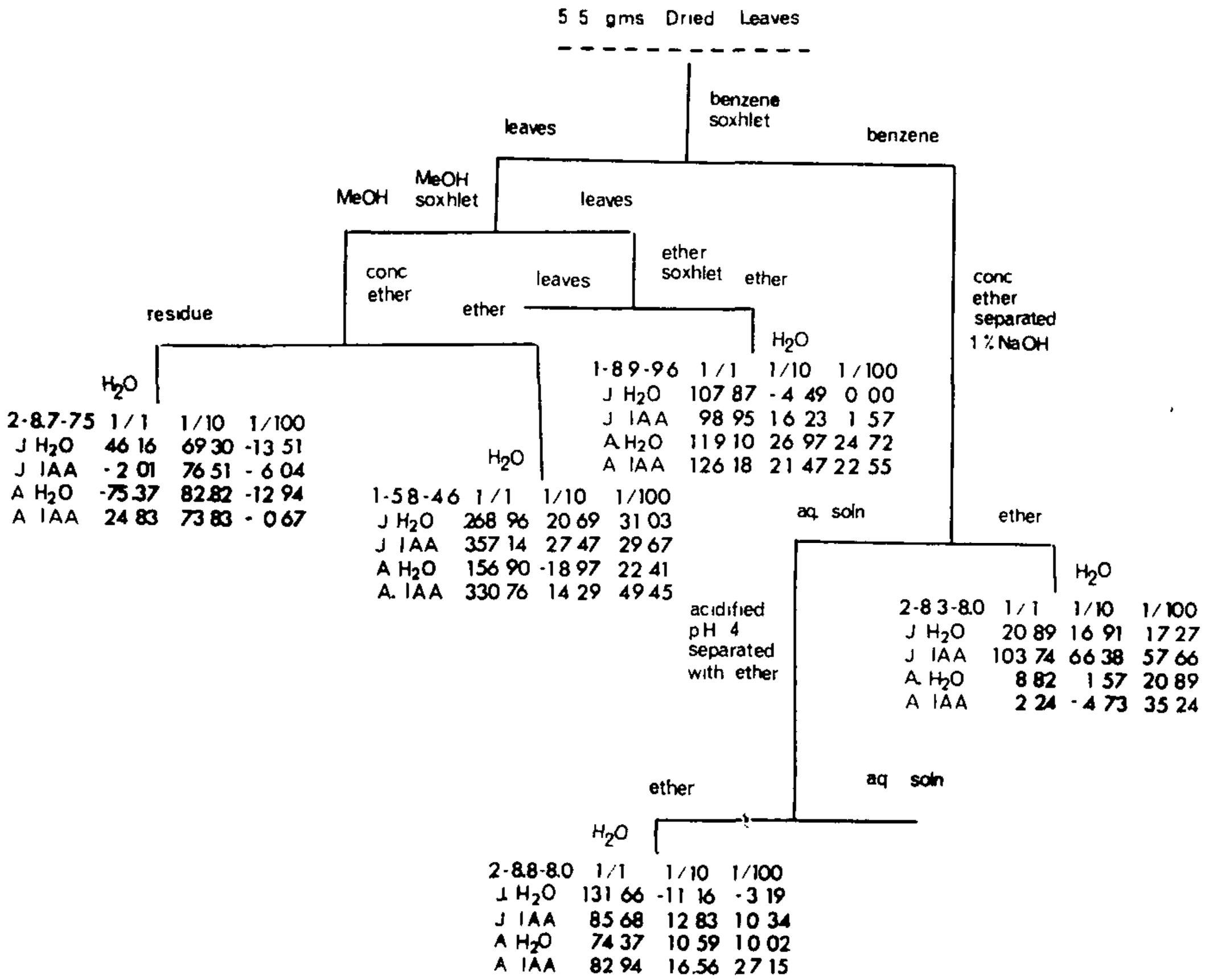

Figure 2 Average rooting response (over check treatment) of mung bean cuttings in solutions from various stages of benzene, ether, methanol and water extractions

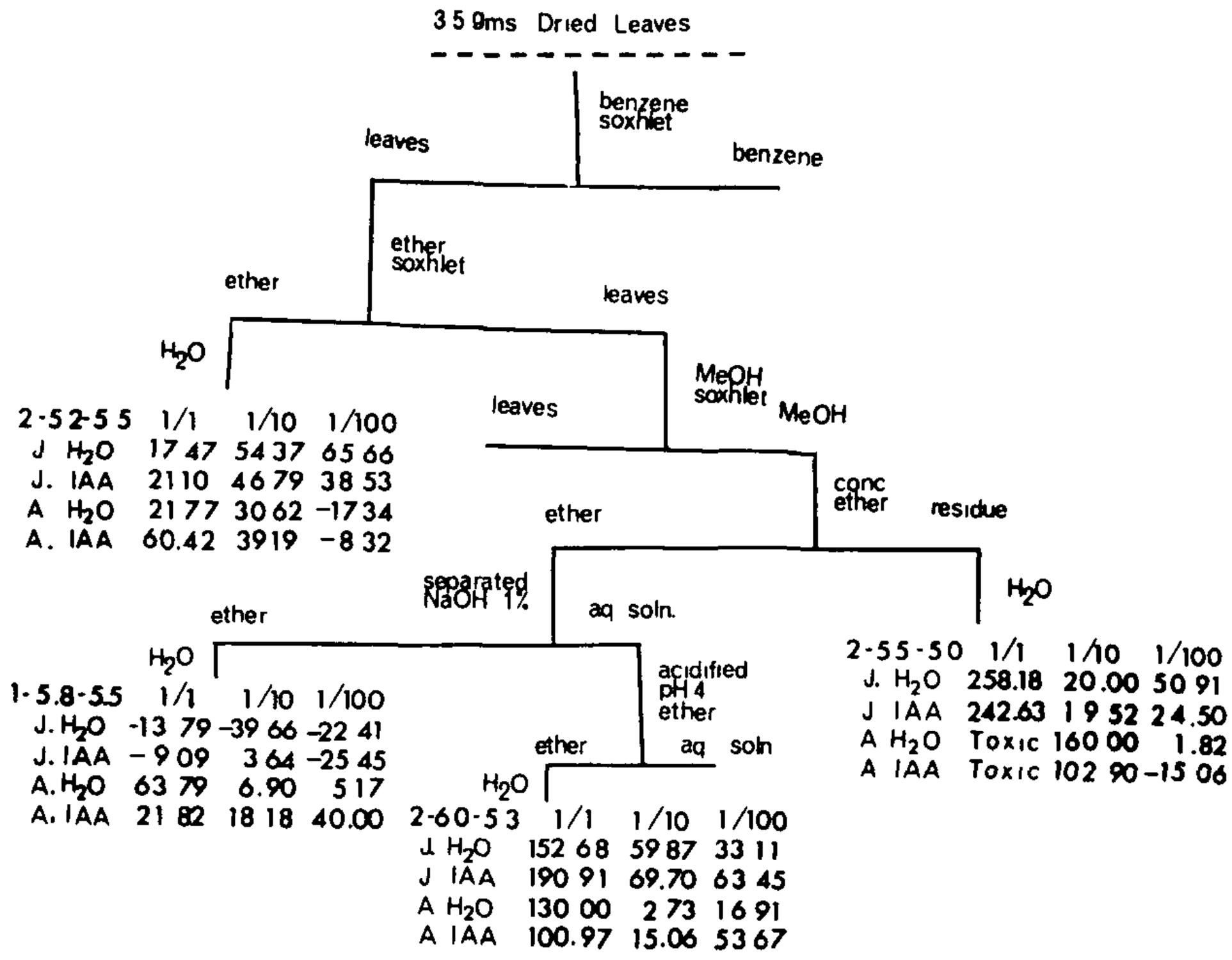

Figure 3. Average rooting response (over check treatment) of mung bean cuttings from various stages of benzene, methanol, ether and water extractions.

can be characterized. An attempt will be made to correlate them to the root-promoting substances reported by Luckwill (10) but of the "auxins" he reported in apple, only two were said to have root-promoting activities. Furthermore, the cofactor response found in this study will have to be characterized by chromatographic methods. This cofactor-type response is obviously not the same as that reported by Challenger, Lacey and Howard (4) as theirs was obtained in the acid fraction. The cofactor response, originally reported by Nelson and Quamme (13), however, may be the same as that reported by these workers in England but appearing at a different Rf. because of isopropanol:water (8:2) was used at Saskatoon and isopropanol:water:ammonia (8:1:1) was used in England.

Acknowledgements

The authors would like to acknowledge that they have referred freely to the thesis work by Miss Hwang and Mr. Quamme. They would also like to acknowledge the contributions of Mr. A. T. Ward, Technician, Department of Horticulture and Miss Quan who was seconded to the Department of Chemistry during the summer.

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HANS HESS: Our next speaker this morning is Ralph Shugert who will talk about *Phomopsis* blight.

CONTROL OF PHOMOPSIS BLIGHT IN JUNIPERUS VIRGINIANA SEEDLINGS

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One of the most serious plant diseases of *Juniperus virginiana* is Phomopsis blight (*Phomopsis juniperovora*). In our seedlng operation at Plumfield Nurseries, *Juniper virginiana* is a valuable crop, since we drill one hundred pounds of seed each year, and take off about one hundred and fifty thousand seedlings, 2-0 and 3-0, annually.

Anyone who has grown an extensive amount of *Juniperus virginiana* is well acquainted with cedar blight. I have never seen seed beds of this species that have not been infected with this insidious fungus to some extent. This particular fungus has the disconcerting characteristic of attacking the growing tip of the evergreen, thus necessitating a good spray program throughout the growing season.

Over the years the Plains nurseries, including Plumfield, have tried several fungicides but the control has not been satisfactory. For many years Bordeaux mixture was used, and this was followed by a material called Special Semesan, which is no longer manufactured. In the late 1950's Puratized Agricultural