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MR. ART LANCASTER: Thank you very much, Charley. The next paper will be given by Dr. Richard Zimmerman, Texas Forest Service, College Station, Texas.

ROOTING COFACTORS IN SOME SOUTHERN PINES

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INTRODUCTION

The Tree Improvement Program of the Texas Forest Service is concerned with the selection and breeding of superior strains of southern pines for paper pulp and lumber. One of the problems encountered in this program has been the vegetative propagation of selected trees. Since only mature trees are selected, rooting capacity is low (6, 7) and the selections have been propagated by grafting in the past. For our purposes, this has the disadvantages of (1) a different genetic constitution of the stock and scion, (2) possible incompatability between the stock and scion, and (3) higher cost.

Attempts at working out a satisfactory technique for propagating older pines from cuttings met with little success. Accordingly the decision was made to begin a basic investigation of rooting in pines. The purposes were, first, to study root initiation in pines and, second, to determine the relationshp between juvenility and root initiation. The first phase of the research has been to determine if the rooting cofactors that Hess (1) reported are present in pines.

MATERIALS AND METHODS

Pine needles are collected and dried by lyophilization or "freeze-drying." The dried tissue is ground up and a 100 milligram portion weighed out. This is extracted with absolute methanol for two hours at 0° C. The extracts are filtered, dried, and dissolved in a small amount of 95% ethanol. The concentrated extract is applied as a streak on a two inch wide strip of Whatman 3MM chromatographic paper. The chromatograms are developed by ascending chromatography with isopropanol-water (4:1 v/v) following a 15 hour equilibration period. Development of the chromatograms is stopped when the solvent

has ascended 22.5 cm above the line where the extract was applied. The chromatogram is dried and cut into fifteen 1.5 cm sections, each corresponding to 0.067 R-f unit.

To determine root initiating activity of the substances in the pine needle extract, we use the mung bean rooting bioassay developed by Hess (3) with some slight modifications. The mung bean seeds are sowed in water-saturated vermiculite and placed in a controlled environment chamber. The growing conditions in this chamber consist of a 16-hour photoperiod with a light intensity of 2000 foot-candles at plant level, a temperature of 77° F in the light and 73° F in the dark, and a relative humidity of approximately 40%. At the end of ten days, cuttings are made from the seedlings by making a cut 3 cm below the cotyledonary node. At this time the primary leaves are fully developed and the first trifoliate leaf is in the bud stage. Five of these cuttings are placed in a vial containing four milliliters of 5 x 10^{-6} M indoleacetic acid plus a section from the chromatogram. After 15 hours, the cuttings have taken up nearly all the solution and glass-distilled water is added to the vials. Glass-distilled water is added twice daily until the roots are counted. The roots are long enough to count after four or five days.

RESULTS AND DISCUSSION

The research was started with seedlings of loblolly pine and has been expanded to include older trees of this species and trees of other species as well. In needles of seven month old seedlings of loblolly pine, several substances are present which stimulate root initiation in the mung bean cuttings. The most active substances were found at R-f values of 0.45, 0.55 and 0.85. These correspond approximately to the values Hess has published for cofactors 2, 3, and 4 respectively (2, 3). In addition, a slightly active substance was found at R-f 0.1, corresponding to cofactor 1 (2, 3).

Preliminary results with slightly older loblolly pines indicated a significant reduction in the rooting cofactor content of the needles. Upon repeating this work and extending it to even older trees, we found no apparent reduction in rooting cofactor content occurred even in trees 22 years old. There were definite indications of wide variation from tree to tree however.

In one eight year old tree, active substances were found only at R-f values of 0.5-0.6 and 0.8-1.0, corresponding to cofactors 3 and 4. Another tree of the same age, from the same seed source, and growing under the same conditions gave very different results. Active substances at R-f values of 0.1, 0.65, and 0.9-1.0 correspond to cofactors 1, 3, and 4 respectively. In addition active substances occurred at R-f values of 0.3 and 0.45. The latter probably corresponds to cofactor 2. It appears that there may be more than four co-factors in some trees. As yet we have not compared the rooting ability of cuttings from these two trees.

We have also sampled needles from twelve year old loblolly

pines from the same seed source and growing on the same site as the eight year old trees. These contain active substances at R-f values of 0.5, 0.65, and 0.9-1.0, which correspond to cofactors 2, 3, and 4. A slightly active substances has been found occasionally at an R-f of 0.75, between the normal positions of cofactors 3 and 4 on the chromatogram.

While the eight and twelve year old trees were old enough to flower, we could find no evidence that these individual trees had flowered. Samples were collected from branches with and without cones on 22 year old trees. The results were similar in both cases. Active substances were present at R-f values of 0.45, 0.55-0.75, and 0.9-1.0 and a substance with a slight amount of activity at R-f 0.1.

Extracts were made from needles of eleven month old seedlings and eight year old trees of slash pine. The seedlings contained active substances at R-f values of 0.4-0.6, 0.7, and 0.9-1.0 with a slightly active substance at 0.1-0.2. The older trees contained active substances at 0.5-0.65 and 0.9-1.0.

After we had determined that rooting cofactors were present in pine needles, the next problem became that of identifying the active substances. On this we have just begun.

Hess has reported that cofactor 4 can be separated from the others by partitioning the extract between water and chloroform (4). Cofactor 4 and the chlorophylls will be found in the chloroform layer while the other cofactors will be found in the water layer. Cofactor 4 can then be separated from the chlorophylls by running the chloroform fraction through a column of activated charcoal and celite (4). Hess has also reported that cofactor 4 consists of four separate compounds (5).

Working with extracts from loblolly pine seedlings, we have been able to separate cofactor 4 from the other active substances by the same technique. We have also been able to separate cofactor 4 from the chlorophylls in the same manner. By using different solvent systems for developing the paper chromatograms, we have found that there are several components to cofactor 4 extracted from loblolly pines also. Our only attempt to separate the cofactors in the water layer so far have been unsuccessful.

We have observed an interesting phenomenon with the substances which have R-f values of approximately 0.45-0.5 and 0.6-0.65, that is cofactors 2 and 3. Often on the mung bean cuttings used to test the chromatogram sections carrying these substances, the roots which are initiated develop very little. In some cases the epidermis of the stem will not be broken; in others, the roots will be no more than 2-3 mm long. The base of the stem is usually dead. At times, the number of roots initiated will be greatly reduced, even to the point where none are initiated. In the same amount of time, roots will be at least 8-15 mm long on cuttings used to test other sections of the same chromatogram and on the control cuttings. At first, the cause of this inhibition of root development appears to be too high a

concentration of the active substance. Yet, when the crude extract is diluted so that inhibition of root development no longer occurs, stimulation of root initiation is greatly reduced or disappears. The same phenomenon has been found when mung bean cuttings are treated with such compounds as thiamin, ascorbic acid and arginine.

SUMMARY

From the results just presented, it is clear that needles of loblolly and slash pines contain substances which are very active in the mung bean rooting bioassay. Most of these substances appear to be very similar to, if not the same as, the rooting cofactors Hess has discovered in *Hedera* and other plants. Whether these substances will stimulate rooting in pine cuttings as well as they do in mung bean cuttings is not yet known. The quantities of these rooting cofactors appear to be as great in 22 year old flowering trees as in seedlings less than a year old.

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MR. ART LANCASTER: Thank you very much, Dr. Zimmerman. The next paper will be given by Peter Vermeulen, John Vermeulen and Son Nursery, Neshanic Station, New Jersey.

MIST PROPAGATION OF CUTTINGS INSERTED DIRECTLY INTO THE ROOTING-GROWING MEDIUM

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The subject of Mist Propagation of Cuttings Inserted Directly into the Rooting-Growing Medium is not a new one. I recall saying to this society last year that none of us have completely original thoughts. I am sure that there are many propagators who have at some time or other rooted cuttings inserted