BIOCHEMICAL COMPARISONS OF EXTRACTS FROM PHYSIOLOGICALLY JUVENILE AND MATURE QUAKING ASPEN CUTTINGS[1]

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Vegetative propagation is an important tool in genetic improvement of quaking aspen (Populus tremuloides Michx). Cuttings from stump sprouts or root suckers are considered physiologically juvenile and root easily (Farmer, 1963), but physiologically mature cuttings from the crown of older trees are difficult to root (Stairs and Jeffers, 1967). The purpose of our study was to compare juvenile and mature aspen cuttings with respect to certain indole and phenolic compounds which are reputed to affect the rooting process (Farmer, 1963; Basu, et al. 1969).

An anatomical investigation of physiologically juvenile and mature aspen cuttings (Hicks and Gladstone, 1971) preceeded biochemical investigations. Microscopic examination revealed that reduced rooting of mature aspen cuttings was due to failure to form root primordia, a process which is apparently metabolically controlled.

METHODS

A single aspen clone was selected for study to limit genetic variability. A spectrum of age classes was available within the clone. Root suckers of the current year provided juvenile material and more mature cuttings were collected from current-year twigs of trees 1 through 10 years of age.

In preliminary rooting trials approximately 60 percent of current-year root suckers rooted compared to 8 and 4 percent, respectively for cuttings from 1- and 2-year old trees. Cuttings from trees older than 2 years failed to root, therefore a well defined difference in rooting ability existed within a single clone.

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Extract Preparation:

Extracts were prepared from root suckers and other age classes up to 10 years of age. The extraction process is outlined as follows:

1) A 50 g sample of freshly collected stem tissue from an age class was stripped of leaves and buds and macerated in a. Waring blender with 200 ml of methanol.

2) The macerated tissue was left standing in this solution for 12 hours at -10 °C.

3) The liquid was separated from the tissue by vacuum filtration and taken to dryness with a rotary evaporator.

4) The residue was redissolved in 50 ml of ethyl acetate and partitioned into 75 ml of 1% $NaHCO_3$.

5) The ethyl acetate fraction was discarded and the aqueous fraction was acidified to pH 3.0 with 0.5 N H_2SO_4 and re-extracted with 50 ml of ethyl acetate.

6) The aqueous fraction was discarded and the final ethyl acetate fraction was retained.

Thin-layer Chromatography:

Extracts were further fractionated by thin-layer chromatography (TLC). Two-dimensional chromatographs were prepared using the following solvent systems: benzene - 1, 4-dioxane (20:30) and methylene chloride-methanol-acetic acid (99:10:0.5). Two chromatographs per age class were developed; one was sprayed with Salkowski Reagent and the other with diazotized sulfanilic acid. These detection reagents are sensitive to indole and phenolic compounds, respectively. Spots resulting from colorometric reactions with these reagents are numbered serially and prefixed by i or p for the indole and phenolic indicators, respectively.

Bioassay:

One-dimensional thin-layer chromatographs were prepared using the benzene-dioxane solvent system. The developed plates were divided into horizontal strips which were eluted in methanol for bioassay. The sensitized wheat coleoptile straight growth test, in which quantitative determination of auxin is based on elongation of coleoptile segments (Nitsch and Nitsch, 1956), and the "aspen rooting test" (Hicks, 1972) were employed in testing the fractions. The aspen rooting test was developed for this study and was sensitive to indole-3-acetic acid (IAA) and several other plant growth regulators at physiological concentrations. In this test, the number of roots produced by aspen sucker cuttings was the criterion used to evaluate biological activity.

RESULTS

Chromatographic evidence indicated qualitative and quantitative differences in extracts from different age classes (Table 1). Spots i-la and p-2a appeared only in older age classes. Spots number i-3, i-5, p-4, p-5, and p-7 were larger in chromatographs from older age classes, indicating increasing concentrations. No indole spot appeared in the chromatograph of the extract for any age class at the position of an IAA standard.

A 200 ul sample of extract from each age class including root suckers was fractionated by TLC for wheat coleoptile bioassays. Chromatographs were divided into 10 horizontal strips which were eluted in methanol and the eluates tested separately. Response to root sucker extracts differed little from controls but extracts from older age classes inhibited coleoptile elongation (Fig. 1). Chromatograph fractions 1 and 7 showed a trend toward increasing inhibitor concentrations with older age classes. Endogenous IAA, which, if present, should have appeared in fraction 8, was not detectable by wheat coleoptile tests.

Extracts from root suckers and the four-year age class were fractionated by TLC and assayed with the aspen rooting test. Eluates from chromatographs were diluted to a concentration approximating the cellular solution of the material extracted. These tests revealed in the extracts no substances which significantly affected rooting of aspen sucker cuttings. No rooting stimulator was found in the chromatographic region of IAA.

SUMMARY AND CONCLUSIONS

Chromatographic evidence indicates that qualitative and quantitative differences in indole and phenolic compounds exist between juvenile and mature quaking aspen cuttings. Wheat coleoptile tests revealed the presence of growth inhibitors in extracts of cuttings from older trees and no indication of the presence of IAA or other growth stimulators. Aspen rooting tests of the same extracts, however, failed to reveal any substance which significantly promoted or inhibited rooting of aspen cuttings.

Endogenous IAA does not appear to be involved in rooting differences of juvenile and mature aspen cuttings. Growth inhibiting substances were present in cuttings from older trees; however, these substances apparently do not account for reduced root initiation.

Table 1. Relative spot size of putative indole (Salkowski positive) and phenolic diazotized sulfanilic acid positive) compounds in extracts from aspen cuttings.

Salkowski positive spots

spot number	age class			
	1	2	3	4
i-1	0.45	0.42	0.42	0.40
i-1a	-	-	-	0.36
i-2	0.12	0.52	0.40	0.45
*i-3	0.55	0.80	1.00**	1.50
i-4	0.45	0.45	0.60	0.55
*i-5	0.25	0.40	0.72	1.10

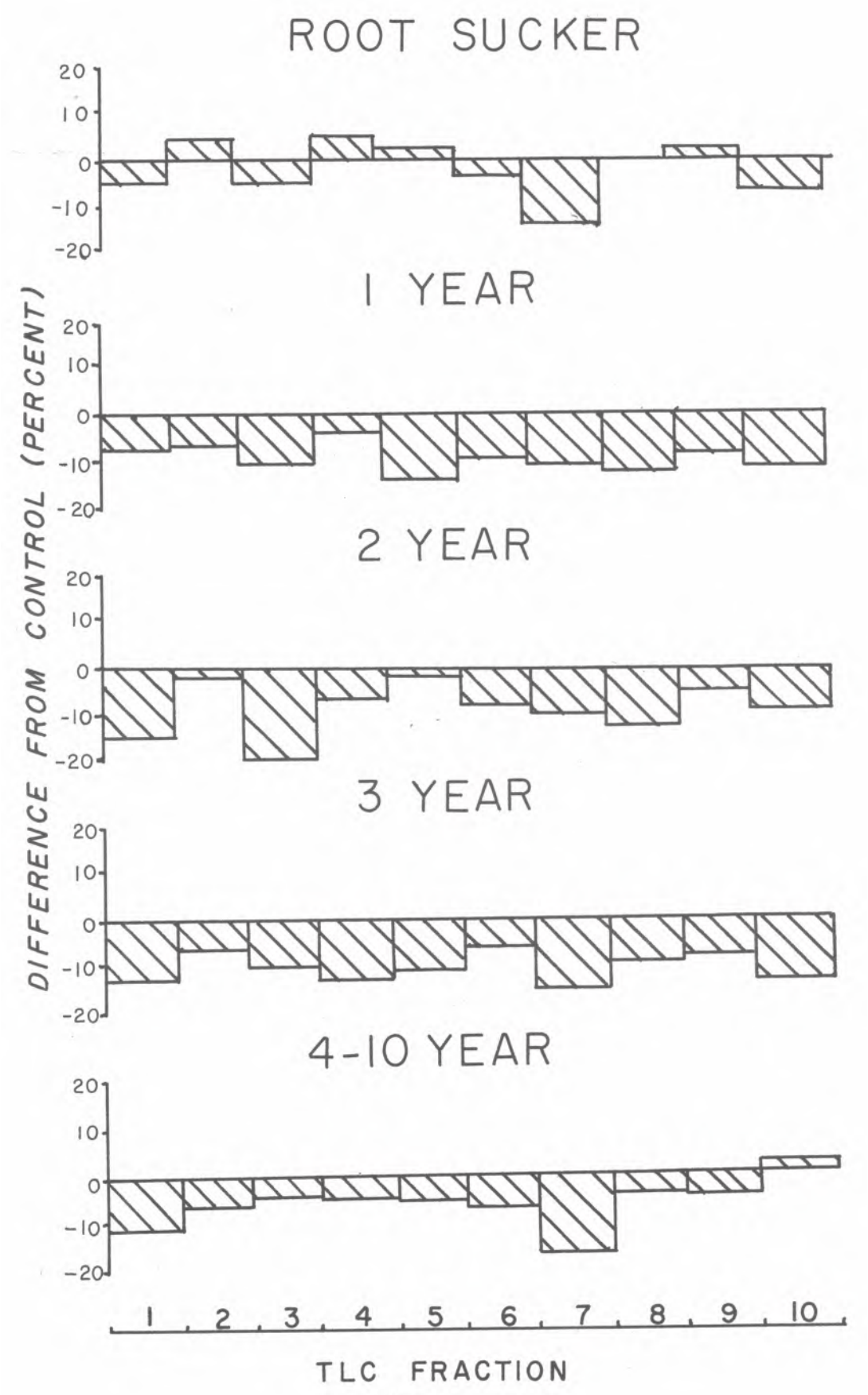
Diazotized sulfanilic acid positive spots

spot number	age class			
	1	2	3	4
p-1	0.15	0.35	0.35	0.40
p-2	0.20	0.35	0.25	0.35
*p-2a	-	-	0.25	0.35
p-3	0.50	0.55	0.45	0.70
*p-4	0.45	0.70	0.62	0.82
*p-5	0.55	0.45	0.80	0.75
p-6	0.45	0.70	0.45	0.70
*p-7	0.50	0.45	0.50	0.85

* - spots showing increasing size with increasing age

** - spot areas are expressed as a decimal fraction of this spot

Figure 1. Biohistograms showing elongation response of wheat coleoptile segments to extracts from greenwood aspen cuttings from various age classes.



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A paper on rooting Douglas-fir was presented at the Conference by Edward Palpant but was not received for publication. However, the discussion on this paper is included in the Proceedings.

DISCUSSION

Larsson -How did you apply your indoleacetic acid? Was it by basal treatment or soaking and if by soaking, for how long?

Hicks - I think he is referring to the aspen rooting test where in development of the test, I wanted to find out whether or not aspen root suckers could be used as a bioassay, and, therefore, whether they were sensitive to auxin and some other known growth-regulating compounds. Initially, we tried an aqueous, but we found that if the cuttings remain in a solution of indoleacetic acid during the whole process of rooting, it increases root numbers, but inhibits root elongation. So it makes it difficult to detect the roots that have been stimulated. From anatomical work, we found that it took about five days for root initiation in these cuttings. So what I finally did was to use an IAA soak of five days followed by a transfer to distilled water on the fifth day. The result was a very marked response to auxin in terms of numbers of roots plus elongated roots that could be detected easily.

Connola - Regarding the Douglas fir with the flat top, how did these characters behave later on? When you're beginning to develop toward a mature tree, whether it be Christmas tree or otherwise, do you have the same effect of multiple leaders.?

Palpant - I guess you are suggesting that maybe this is a multiple leader? I have no reason to suspect that they would maintain a multiple leader condition. To me this is a rather normal development for this age of tree. It is certainly not optimum, but it approaches what I would expect to be normal for this age, and I would expect that in two or three years that this would maintain a dominance greater than it shows here.

Connola - But you have no older tree that you could compare with?

Palpant - A few, but they tend to be the same conditions that I have shown you here; either showing a tendancy to grow laterally or grow upright, depending upon where we collected the cuttings. Again there is a clonal difference. Some clones, whether we collected them laterally or not, tend to turn upright. I don't have any strong data to back this up. This is a judgment on my part.

Rauter - A couple of questions. You showed a picture of a wild stand and you said you could go in and select trees. What kind of rooting success do you get with these older trees, and how old can these trees be to still produce cuttings which will root.

Palpant - The first chart that I showed gave a rooting index with very few cuttings that actually rooted.

Rauter - So your rooting index shows that your percent success is very low.

Palpant - Yes, this is a combined factor of top growth, callus formation, and rooting.

Rauter - But your actual percent of rooting is very low?

Palpant - Right.

Rauter - Have you done any work with any of the other coniferous species, namely spruce? You mentioned some others briefly.

Palpant - No. The only ones we used were the commercially propagated ones as kind of a back-up standard.

Rauter - And you said you were talking of establishing rooting beds and taking rooted cuttings from these beds. How far along are you into this process and what type of success are you getting?

Palpant - We're not into this, actually. In order to produce two hundred ramets of this clone, we need to plant ten to twenty thousand cuttings.

Rauter - We are considering going into a similar program with spruce and think we can have reasonably good success with it, and we were wondering if you had started any such program?

Palpant - No, we haven't.

Schreiner - Have you tried taking cuttings from these?

Palpant - No, we haven't, and this is just getting to the stage now where we can try this. We wouldn't take the lateral.

Schreiner - Poplar ramets derived from branches from the lower portion of the crown of an old tree will often be more or less procumbent. In the middle 1920's I used the current-year wood from all branches of a 30-year-old P. maxomowiczii tree; we had straight trees only from one- to 4-year-old branches and progressively more procumbent ramets from the older branches. When these ramets were cut back, they did produce upright growth. Sucker growth from pollarded old branches will also produce straight rooted trees. Have you tried cutting-back your ramets from lateral branches?

Palpant - No, we haven't. Again this is just getting to a stage now where we can try it.

Zufa - I have a question about your suggestion for these cutting orchards. At one point you mentioned about top pruning the trees to produce more cuttings on lateral branches, and then later on you mentioned that topophysis affected the growth of the rooted cuttings taken from lateral branches. How would these two things compare?

Palpant - What we are hoping for, and I saw this in some of our best Douglas fir plantations where people have topped the trees. We ended up with trees that have no shape, but many leaders that are growing perpendicular to the earth's surface and apparently this is what we are talking about. It's not whether they come from a lateral position or not, but whether they are growing perpendicular to the earth's surface. This seems to be the key to whether they are going to continue to grow that way.

Zufa - I did not notice topophysis on rooted white pine cuttings taken from lateral branches of trees of different ages.

Palpant - Some cuttings that we have grafted from Maple, Ontario, white pine have shown no topophysis.

Zufa - Ray, I wondered if the cuttings you've taken from root suckers and one-year-old aspens were of the same kind? How did this great decline in rooting come about?

Hicks - The root suckers were material that we got from the greenhouse. I went out in the woods, collected roots and put them in the flats. When the suckers were about three inches tall, I could root 100% of them. A one-year cutting was a root sucker that had developed in the field and was collected the following spring from the field. Therefore, a one-year cutting was the green wood material of the current year's growth from a stem that was one-year-old from sprout. You could collect root suckers from the field, but it was much easier to grow them in the greenhouse and have a ready source of them. A four-inch section of root can produce 20 or 30 cuttings.

Kiang - I have one comment related to Palpant's question about lateral branch cuttings. In coast redwood the cuttings taken from the lateral branches usually root better than those taken from the crown; but they also have the same growth habit as you mentioned in Douglas fir--usually prostrate. However, when the rooted cuttings are planted in the field, in thesecond or third year, sprouts, which grow from the base of the cuttings, will shoot up and take over. I wondered if this might happen with Douglas fir? Do you find any seasonal difference in rooting performance in Douglas fir?

Palpant - In our seasonal study, no, but incidentally to our work and Henry elluded to the fact that we have been collecting material off of Christmas trees. We have taken cuttings from three trees, two of President Oswald's Christmas trees, and one of my own, and all three of these trees have rooted reasonably well. Last year was tops, this year second to the best, and the tree I had for my own Christmas tree, which was the best one we have had in twenty-five years, rooted very well. So it indicates to me that collecting material, at least sometime in December, repeating what we have done with those trees, specifically in the past, keeping them outside in a sheltered condition, recutting the material, to the preferred size, and sticking them around the first of March has some hope of increasing rooting.

Schreiner - I presume these were sheared trees. Do you think the shearing over a period of six or eight years influenced your results?

Palpant - We believe this, Ernie, but I had no data to back it up. This is why I mentioned the matter of cutting diameter. As you shear, you tend to reduce the diameter of the cutting. And we did find that smaller diameter cuttings rooted better than the larger diameter cuttings. Maybe Miss Rauter noticed this.

Rauter - Yes, I'd like to make a comment on this last statement about lateral branches. In fact one of the reasons why I don't take terminal branches is because I feel that they are just too hefty--just by the look of the cutting. I get much better success with the laterals, but I do find for the first and sometimes the second year, after they have rooted, they tend to develop in a lateral direction. After that, they begin to grow in a vertical direction. We have beautiful trees in the nursery that people won't believe came from cuttings--they are nice-looking little trees. And they are all from lateral material. We are working with many different species of Picea.

Palpant - We do have two Douglas fir, between 25 and 30 years old (Childs) that people have topped at about three feet. They have about 200 or so cuttings that you could collect all growing perpendicular to the ground and we have rooted a few of these.

Farmer - I'm still a little uncertain about the variance that you are getting. Quantitatively, how much variance is associated with clones? How many ramets do you have involved? How are you testing this clonal effect? What percentage are you getting for genetic variance.

Palpant - These are the number of observations, perhaps you can't read it back there, that make up these various components. If you would like to take a look at this later, perhaps we can discuss it in detail.

Farmer - Those are variance components?

Palpant - These are the number of observations that make up these components of variance. Looking at all clones here, we probably have in the neighborhood of five percent that root across all trees, which is a small amount. Obviously a lot of the treatments have zero, a lot of the clones have zero rooting.

Farmer - How many ramets are involved in one of these tests, so that you can compare your clones in the breakdown that you have there?

Palpant - In this particular test, about a hundred ramets.

Farmer - Do all of your cuttings come from the ortet or how many ramets?

Palpant - We haven't reproduced these yet--we are still working with the mother tree.

Farmer - Then you really can't determine the genetic variance until you develop some ramets of your ortets?

Palpant - Right.

Farmer - Is it possible then that your clonal variance in this instance, is, perhaps, confounded with the physiological variance that is present from tree to tree?

Palpant - Yes.

Farmer.- Do you think this is a big factor in Douglas fir?

Palpant - Yes, I have to say this because I tried to summarize all the selections that we made to see the trends by years. Some were consistent and some were not. One tree that I mentioned had zero percent last year but had the highest rooting percent this year.

Farmer - So there is a big physiological effect, perhaps?

Palpant - We fertilized the tree and took off every primary cutting. Whether that affected the size of the cutting or just what caused the reversal of the rooting percent, I can't say.

Farmer - Now that you do have some clones developed, do you have plans for designing a genetic test from which you can get some estimate of genetic variance?

Palpant - We always have plans, but funding limits our activities.

Rauter - You said that you fertilized the trees. What type of fertilizer are you using? Are you trying to alter your C/N ratio?

Palpant - Yes, we did. I had some data that I'm not prepared to present at this stage. We were not able to detect any positive relationship.