Physiological Comparisons between a Fast-growing and a Slow-growing Red Pine

by

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The present study stems from an interest in the various reasons for the inherent physiological differences that result in growth rate differences among individuals of the same species.

Plant growth can be thought of as the end product of an exceedingly complex series of physiological processes. Thus the over-all rate of such growth could be limited by an unfavorable condition at any of a large number of stages along various converging chains of events. A deficiency or partial block at any point might tend not only to raise the level of the immediate precursor, but also to change the levels of activity of all subsequent steps.

LITERATURE REVIEW

There are relatively few reports in the literature that relate the level or concentration of some substance to growth rate. Demonstrations of level of auxin production reflecting growth rate were made by Lehmann et al. (1936) using Epilobium and possibly by Abrams (1953) using Pisum. Overbeek (1935) uncovered a situation of greater enzymic destruction of auxins in nana forms of Zea; Kamerbeek (1956) demonstrated peroxidase activity to be higher than normal in nana varieties and lower than normal in gigas varieties of Zea, Tropaeolum, Nicotiana, and Phaseolus. Galston (1959) described a difference in growth rate in Pisum attributable to a difference in the amount of an inhibitor of auxin oxidase.

In the case of conifers, Mirov (1941) compared two similar groups of 6-year-old seedlings of western yellow pine (Pinus ponderosa Laws.), the one averaging about 1.8 meters in height and the other about half that size. He found that unpurified diffusates drained from the several tips of expanding shoots (candles) of the taller group exhibited almost twice the auxin activity of the others, but made no attempt to determine whether this difference was one of auxin level per se or the result of enzymic or inhibitor differences.

Tonzig and Marre (1961) working with Brassica have suggested the possibility that dehydroascorbic acid is a cell elongation inhibitor and that auxin can prevent the oxidation of ascorbic acid to this active form.

Brian and Hemming (1955) using Pisum and Phinney (1956) using Zea have demonstrated a correlation between the gibberellins and growth rate. McCune and Galston (1959) have found that the increased growth brought about by treatment with gibberellic

acid in Pisum was accompanied by a decrease in peroxidase activity; Kuraishi and Muir (1962) thus treating Pisum and Helianthus reported an accompanying increase in diffusible growth substances.

In the case of conifers, however, the gibberellins (at least applied gibberellins) seem to have generally slight effects on growth rate. An attempt on my part (1959) failed to obtain gibberellin from actively growing shoots of Monterey pine (Pinus radiata D. Don.).

In summation, some differences in growth rate seem correlated with the production of auxin, some with the destruction of auxin, and some with gibberellin activity. Certainly still other growth rate differences are explicable in terms of additional factors.

BASIS OF COMPARISON OF PHYSIOLOGICAL ACTIVITIES

In the present study it was decided to compare three factors which might reflect the differences in growth rate between a fast-growing and a slow-growing red pine (Pinus resinosa Ait.): growth substance activity, peroxidase activity, and ascorbic acid activity. The two saplings, both 13 years old, were growing side by side in an old field under virtually identical conditions. One was 262 cm tall, the other 408 (or 1.56 times taller). (The smaller tree had a diameter at breast height, i.e., 4.5 ft., of 32 mm, the larger 63.) The terminal portions (leaders) of the two trees were collected simultaneously in mid July while still actively growing; the length of the leader from the smaller tree was 56 cm, and from the other 82 (or 1.46 times longer). The leaders were frozen immediately upon collection and stored at -18 ^{o} C until analyzed.

In order to compare such physiological factors as growth substance activity, peroxidase activity, and ascorbic acid activity it was necessary to select a valid basis for these comparisons. Gross weight determinations were unsuitable because of the large amount of physiologically non-functional tissue found in woody plants. A comparison on the basis of protein content was also unfeasible because of the precipitation of proteins in pine extracts (presumably caused by the tannins present). As a result of these difficulties it was decided to make the comparison on the basis of deoxyribonucleic acid (DNA) content.

A DNA determination appears to be a particularly desirable means of arriving at a comparative measure of living, functioning cells in conifers. The amount of DNA per chromosomal set, and thus of cells of equal ploidy, is found to be remarkably constant in most cases (Swift, 1953). Although the occurrence of somatic aneuploidy (B chromosomes) (Darlington, 1956) and particularly of somatic polyploidy (polysomaty) (Geitler, 1953; D'Amato, 1952) appear to be of widespread, normal occurrence amongst the angiosperms, these phenomena seem to occur but rarely if at all during the normal development of the soma of gymnosperms (lilies, 1958; Tschermak-Woess and Doleal, 1953; Torok and White, 1960). Even in those species where polysomaty (often disomaty) does occur its regularity of distribution would possibly still make DNA the choice of the basis of comparison between like organs at similar stages of differentiation. However, the unpredictability of the distribution of B chromosomes probably would preclude the DNA basis in species where they occur. Fortunately they possibly do not occur normally in the long-lived woody plants (Darlington, 1956). Neoplastic and other abnormal growths have unpredictable

ploidies in both gymnosperms and angiosperms.

Periodicities correlated with the diurnal solar cycles have been reported for mitotic activity (Friesner, 1920; Brown, 1951), and with annual cycles for nucleoprotein levels (Celjniker, 1950), in some plants. Karsten (1918), investigating the apical meristems of Austrian pine (Pinus nigra Arn.) seedlings, found their mitotic activity to follow a daily cycle with maximum activity occurring between 2 and 4 a.m. Thus, collections for comparison must be made at a particular time of day and year.

METHODS OF ANALYSIS

Growth substance content was estimated in a procedure modified from that of Nitsch et ux. (1956). The growth substances were extracted with cold methanol; the methanol was then evaporated off and the growth substances extracted from the residue with water. This extract was then taken to dryness and the residue again dissolved in methanol for streaking onto chromatography paper. The material was then fractionated in an ascending direction using 80% v/v isopropanol as the solvent system. The paper column was divided into ten parts and each assayed by an Avenacoleoptile section straight growth test. The significance of the results was determined by an analysis of variance followed by J. W. Tukey's procedure for multiple comparisons (Steel and Torrie, 1960). Promotive activity (significant at the 1% level) was expressed in terms of the amount of indoleacetic acid (IAA) required to elicit the same response in the assay.

Peroxidase content was estimated by the Willstater reaction in a procedure modified from that of Sharpensteen et al.(Westing, 1960). The peroxidases were extracted with 0.1 M monobasic potassium phosphate (pH 4.7). Peroxidase activity was determined by the ability of this extract to oxidize the colorless compound pyrogallol in the presence of hydrogen peroxide to a yellow compound with maximum absorbance at 430 *mμ,* presumably purpurogallin. Activity was related to absolute units by a comparison with commercially obtained horseradish (Armoracia lapathifolia Gilib.) peroxidase (Nutritional Biochemicals Corp., Cleveland), a molecular weight of 40,000 being assumed.

Ascorbic acid content was estimated by the Roe reaction in a procedure modified from that of Schaffert and Kingsley (1955). The ascorbic and dehydroascorbic acids were extracted with 4×10^{-2} M oxalic acid in ca. 2 M acetic acid. This extract was partially purified by shaking with amorphous carbon, the amorphous carbon simultaneously serving to catalyze the oxidation of the ascorbic acid to the oxidized form, dehydroascorbic acid. Dehydroascorbic acid activity was determined by the capacity of this extract to reduce the colorless compound 2,4-diphenylhydrazine in the presence of sulfuric acid (and thiourea) to a red compound with maximum absorbance at 515 ma, presumably bis-2,4-diphenylhydrazone. The assay was standardized with ascorbic acid oxidizd as above.

DNA content was estimated by the Dische reaction in a procedure modified from that of Kupila et al. (1961). The insoluble pulp left by the first step of any of the above procedures was used as the source of DNA. This pulp was defatted by first washing with 0.05 M formic acid in methanol, then with a mixture of ethanol and ether (2:1, by volume), and finally with pure ether. The pulp was then dried and the DNA extracted with hot 1.7 M sodium chloride buffered at pH 7 using McIlvaine's buffer system (citric acid/K_2HPO4). DNA activity was determined by the capacity of this extract to convert acidified diphenylamine, light-yellow in color, (in the pres-

ence of acetaldehyde) to a blue compound with maximum absorbance at ca. 595 mg, possibly the condensation product of diphenylamine with 5- hydroxylaevulinic aldehyde. Owing to the immense size of the DNA molecule (the molecular weight being perhaps several million), its concentration actually was expressed in moles of its main nucleotide repeating units. Since the diphenylamine reacts with the purine and not the pyrimidine deoxyribosides, the activity of deoxyadenosine could be used to standardize the assay by considering each molecule to be the equivalent of two nucleotide repeating units.

RESULTS AND DISCUSSION

It was found that the extractible growth substance activity of the slow-growing pine was 9.79×10^{-3} while that of the fast-growing one was 17.80×10^{-3} mole equivalents of IAA activity per mole of nucleotide(or 1.82 times as much in the fast tree). Growth inhibitor activity brought to light by this procedure did not differ between the two trees. In the case of total ascorbic acid, the activity of the slow-growing pine was 9.96×10^{-2} while that of the fast-growing one was 8.61×10^{-2} mole equivalents of dehydroascorbic acid activity per mole of nucleotide (or 1.16 times as much in the slow tree). Peroxidase activity of the slow-growing pine was 8.29×10^{-4} while that of the fast-growing pine was 6.48×10^{-4} mole equivalents of horseradish peroxidase activity per mole of nucleotide (or 1.28 times as much in the slow tree).

It may be recalled that of the two trees being compared, the one was growing about 1.5 times as fast as the other. The higher activities in the slower growing tree of ascorbic acid (ca. 1.2 x) and peroxidase (ca. 1.3 x) are in the expected direction. In the case of the extracted growth promoting substances also, the activity in the faster growing tree was found to be greater (ca. 1.8 x) than in the slower growing one. Of course, whether the differences observed helped to bring about the differing growth rates or merely reflect them is not known. Finally, it would be of interest to learn to what extent the various physiological differences observed would be altered by environmentally imposed growth rate differences or even by plant age. With the procedures described above, these and other factors can now be investigated in detail.

SUMMARY

Procedures are presented that are suitable for determining growth-substance, perxidase, ascorbic acid, and deoxyribonucleic acid contests of coniferous stem samples. Justification is presented for the determination of deoxyribonucleic acid as an indication of the number of functional cells, and thus as a basis for comparing physiological activities of such samples.

As a preliminary investigation, extracts of the terminal shoots of two adjacent 13-year-old Pinus resinosa Ait. saplings - one growing 1.5 times as fast as the other - were compared on a functional cell basis in the above manner. The faster growing tree yielded 1.8 times as much growth substance activity, while the slower growing tree yielded 1.2 times as much ascorbic acid activity as well as 1.3 times as much peroxidase activity, all per nucleotide repeating unit of deoxyribonucleic acid.

Extracts appeared to contain, on the average, approximately 10^{-2} moles of growth

promoting substances, 10^{-1} moles of ascorbic acid, and 10^{-3} moles of peroxidase per mole of nucleotide repeating unit.

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