ENZYMATIC CONTENT OF NURSERY STOCK ROOTS: ITS DETERMINATION BY A SIMPLE METHOD[1]

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The enzymatic content of feeder rootlets was directly related to the amount of solar radiation received, the soil supply of toxic chemicals, soil drainage, and the abundance of short roots.

Recent observations have indicated that the relative abundance of wartlike or verrucose root appendages, commonly called "short roots" or "mycorrhizal short roots," reflects several important conditions of nursery culture prevalent both above and below the ground. A drastic reduction in the development of short roots was observed due to diminished exposure of the foliage to direct sunlight in heavily fertilized or densely seeded nursery beds of 2- or 3-year-old seedlings (*1*). Complete elimination of short roots was inflicted by high concentration of fungicidal eradicants of either growth-stimulating or growth-depressing influence (*2,3*). Partial or complete suppression of short roots took place under the influence of periodically impeded drainage (*4*).

In recent years, attempts have been made to record the root morphology of nursery stock by counting stained, fungus-mantled short roots and expressing their abundance as percentages of feeder roots. This procedure is too laborious and costly to be applied in appraisals of planting stock produced in nurseries varying in size from 40 to over 200 acres. Moreover, percentages are totally unreliable values in this type of analysis.

No one can claim the critical importance of short roots in the growth of trees; in numerous cases the symbiosis of fungi and naturally reproduced seedlings is accomplished in a complete absence of short roots (*5,6*). *Nevertheless, under nursery conditions, short roots, regardless of their nature, do increase the adsorbing surface of roots and do reveal the influences of solar radiation, toxic chemicals, and impeded drainage.*

A simple and rapid method of quantitative appraisal of short

Table 1.—*Catalytic potentials of tree roots determined with 2-gram air-dry samples, derived from six average size trees. Average results of five determinations expressed in terms of mercury column (mm Hg) per gram of analyzed material*

Analyzed materials	Catalytic potential mm Hg/g
2-year-old *Pinus strobus* nursery seedlings	
Total roots	20.3 ± 2.6
Roots without mycorrhizal rootlets	11.0 ± 1.3
Mycorrhizal rootlets less than 2 mm in diameter	32.1 ± 2.4
Nonmycorrhizal rootlets less than 2 mm in diameter	13.5 ± 1.3
3-year-old *Picea glauca* nursery seedlings	
Total roots	21.2 ± 2.7
Roots without mycorrhizal rootlets	10.9 ± 1.4
Mycorrhizal rootlets less than 2 mm in diameter	27.3 ± 3.1
These trees exhibited no rootlets less than 2 mm in diameter free from short roots.	—
2- to 5-year-old *Pinus resinosa* of natural reproduction	
Total roots, lacking mycorrhizal short roots	12.2 ± 2.4
Nonmycorrhizal rootlets less than 2 mm in diameter	14.8 ± 2.0

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roots was offered by the determination of their enzymatic content or catalytic potential (*7*). This analysis can be applied to either the entire root system or, more accurately, to the feeder rootlets less than 2 millimeters in diameter. The determination of the catalytic potential discloses the volume of short roots because they are the principal producers of catalase and related enzymatic substances. Table 1 outlines the relation in question.

Method of Analysis

The determination of the catalytic potential is very simple, but the analysis of roots of nursery stock requires meticulously careful sampling. All samples of trees must be collected from sections exhibiting uniform nursery stock, preferably of full density, and from the very centers of nursery beds. The abundance of short roots varies greatly between centrally and border-located trees in accordance with the amount of radiation received by their foliage (fig. 1). The trees should be excavated by a sharp spade entering the soil in a vertical position and removal of an approximately 10- by 10-inch block. Trees with partly cut-off roots are excluded from the sample.

The sampled trees are washed in cold water and the roots are separated from the tops by cutting just above the top root. The rootsystems are dried between heavy blotters. All rootlets less than 2 millimeters in diameter are separated from the larger roots and cut into approximately 1/2-inch lengths. The cut rootlets

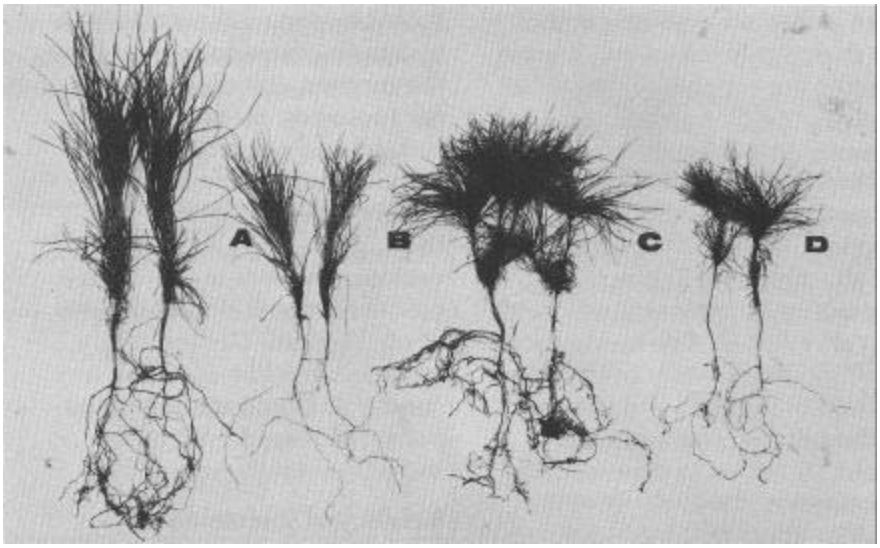

Figure 1.—*Incidence of mycorrhizal short roots in 2-year-old red pine (Pinus resinosa) and white pine (Pinus strobus), as influenced by the density of stock and exposure of the foliage to sunlight. A and C: Red and white pine seedlings sampled from the border of nursery beds with catalytic potential of 48 and 50 millimeters of mercury per gram, respectively; B and D: Similar seedlings sampled from the center of the same beds with catalytic potential of 21 and 24 millimeters of mercury per gram, respectively.*

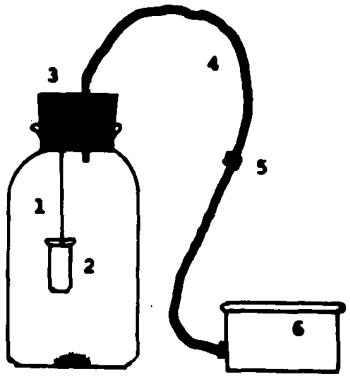

Figure 2.—*Equipment for determination of the enzymatic content of tree rootlets: (1) 250-milliliter reaction flask, (2) 20-milliliter reagent container, (3) No. 10 rubber stopper, (4) Tygon tubing, (5) leuer connector, (6) aneroid manometer.*

from at least six average plants are thoroughly mixed and 2-gram samples are weighed on an accurate balance. The sample is placed into a 250-milliliter wide-necked flask provided with a perforated No. 10 stopper. The stopper has a small tube inserted for attachment of Tygon tubing, and a 20-milliliter container, held by a wire (fig. 2). The rootlets are distributed uniformly over the bottom of the flask and pressed to the bottom with a plunger about 1½ inches in diameter. The container is filled with 18 milliliters of dilute hydrogen peroxide (one part 30-percent peroxide and four parts water). The flask is firmly stoppered and the Tygon tubing is attached by means of a leuer connector to an aneroid manometer (an inexpensive 200-millimeter pressure gauge). The flask is tipped to allow all of the hydrogen peroxide to pour onto the rootlets, vigorously shaken for 3 seconds, placed on a level surface, and permitted to stand without disturbance for exactly 1 minute when the manometer reading is taken. To prevent explosion or escape of free oxygen, the stopper should be held firmly by hand. The results are recorded in millimeters of mercury (Hg). Duplicate determinations, as a rule, do not differ more than 4 millimeters.

Results and Discussion

Tables 2 and 3 include the results obtained from analyses of seedlings produced in five nurseries of the Lake States Region. These analyses were performed on nursery stock of full density, using 2-gram samples of air-dry rootlets not exceeding 2 millimeters in diameter, 18 milliliters of 6-percent hydrogen peroxide, and 1 minute reaction time. The averages were derived from at least five determinations. The analyses were accomplished during the period from early July to the middle of September 1978.

These results strikingly reveal an extremely important, often overlooked, detail—the existence of a correlation between the abundance of mycorrhizal short roots and the amount of radiation received by the tree foliage. In most cases, the effect of sunlight is expressed in the relationship between the incidence of short roots and the density of nursery stock attained at different ages. Pooling together the results of all our analyses of more than 250 samples, the relationship between the age of pine and spruce seedlings and the catalytic potential of their rootlets is given by the following averages: 1-year-old stock: 68 ± 5.1, 2-year-old stock: 43 ± 6.5, and 3-year-old stock: 26 ± 5.1 millimeters of mercury per gram. These averages indicate a linear relationship with a band of normality of approximately 6 millimeters of mercury per gram.

Under present conditions, we must accept these averages as values indicating that nursery stock of *Pinus banksiana, P. resinosa*, *P. strobus*, and *Picea glauca* is not significantly affected by

Table 2.—*Incidence of verrucose short roots in planting stock of Wisconsin nurseries, determined on the basis of the enzymatic content of rootlets less than 2 millimeters in diameter.*

Nature of analyzed stock	Catalytic potential, mm Hg/g and sampled nurseries		
	Griffith	Hayward	Wilson
Pinus resinosa, 1 year	39 ± 1.6	71 ± 2.7	58 ± 1.7
2 years	29 ± 1.2	49 ± 3.2	58 ± 1.7
3 years	20 ± 2.8	32 ± 3.3	30 ± 3.0
Pinus strobus, 2 years	38 ± 3.9	53 ± 1.1	32 ± 2.4
3 years	19 ± 1.2	23 ± 2.1	21 ± 2.8
Picea glauca, 3 years	21 ± 2.0	29 ± 1.5	27 ± 3.1

Table 3.—*Incidence of verrucose short roots in planting stock produced in J. W. Tourney nursery of Michigan and Eveleth nursery of Minnesota, determined on the basis of enzymatic content of rootlets less than 2 millimeters in diameter.*

Nature of analyzed stock	Catalytic potential, mm Hg/g and sampled nurseries	
	Eveleth	J. W. Tourney
Pinus banksiana, 1 year	62 ± 1.6	—
2 years	41 ± 1.7	56 ± 3.2
Pinus resinosa, 2 years	38 ± 3.1	33 ± 2.4
3 years	38 ± 3.1	26 ± 4.9
Picea glauca, 2 years	66 ± 2.9	48 ± 3.7
3 years	—	30 ± 1.6

adverse climatic or soil conditions. It is obvious that, in attempts to relate the incidence of short roots to the effect of eradicants, fertilizers, inadequate drainage, or other soil conditions, it is imperative to consider the age of nursery stock and its corresponding density. Nursery stock of low density due to poor germination of seed or thinning by parasites may show a very high incidence of short roots even at the age of 3 years.

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