EVALUATING ROOT REGENERATION POTENTIAL OF BARE-ROOT NURSERY STOCK

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ABSTRACT

The terms Root Regenerating Potential (RRP) and Root Growth Capacity are reviewed to overcome current confusion in the literature. It is suggested that the term Root Regenerating Potential be used exclusively to describe the potential of transplanted or outplanted nursery stock root systems to initiate or elongate new white roots shortly after transplanting or outplanting.

The literature on RRP is reviewed with respect to the development of the methodology used to evaluate this important physiological attribute of nursery stock. The review of literature includes the work done at the Faculty of Forestry at the University of Toronto and at the School of Forestry at Lakehead University during the last decade. The review also includes the initial work done by Dr. E.C. Stone and his associates in California.

The physiological quality and RRP work now in progress at the School of Forestry at Lakehead University is described and the methods used to bioassay the nursery stock are listed: 1) the pot bioassay method; 2) bioassay in the root mist chamber; and 3) bioassay in the root growth box.

INTRODUCTION

The terms Root Regenerating Potential (RRP) and/or Root Growth Capacity (RGP) are not clearly or concisely defined in spite of their extensive use in describing the post-planting root behavior of nursery stock.

According to the Concise Oxford English Dictionary (Sykes 1976) the word *potential means 'capable of coming into being or action' and capacity means 'power of* containing or producing'. As both RRP and RGP are usually measured by the number, length, area, volume, or weight of the roots produced by nursery stock after transplanting *or outplanting, it seems that the two terms are almost identical in meaning. As Root* Regenerating Potential was the first term used by Stone and his co-workers (Stone and Schubert 1956, 1959a) and as new roots are not 'contained' within the old root system but 'come into being' from it, *the term Root* Regenerating Potential is *recommended.* The following definition of Root Regenerating Potential is proposed:

> The potential of transplanted or outplanted nursery stock root systems to initiate or elongate new white roots shortly after transplanting or outplanting.

1959a). Even by 1958, Stone and Schubert (1959) had developed their methodology so that they were

> digging seedlings from the nursery every 15 days, replanting them in the greenhouse after 0, 1, 2, and 3 months in cold storage and redigging them 30 days later and recording any new root growth.... each seedling shipment was subjected to the following treatment. First the white root tips were pinched off. Then the 20-seedling lot was planted--10 seedlings per galvanized container--in sand loam. These planted seedlings were then watered and drained, and the containers were placed in a 20˚C (68˚F) waterbath in the greenhouse .... The air temperature in the greenhouse did not fall below 20˚C (68˚F) at night but with few exceptions did not exceed 35˚C (95˚F) in the daytime .... After one month in this greenhouse environment, each galvanized container was lifted from the waterbath and the soil carefully washed from the seedlings .... The root systems of the seedlings were then examined and elongating laterals which had just started to suberize, but which could still be recongized as new roots were counted.

By use of these techniques Stone and Schubert were able to show that the RRP of ponderosa pine *(Pinus ponderosa* Laws.) varied greatly with the time of year in which the seedlings were lifted and outplanted. Later Stone *et al.* (1962) also showed that the RRP of Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) varied with lifting and outplanting date. In this work a more comprehensive analysis of the seedling root systems and the measurement of RRP was made:

> Douglas-fir seedlings in common with most conifers have an heterorhizic root system with a primary root, several elongated first and second order laterals and many short first, second, third, and fourth order laterals varying in size from short protuberances to laterals several inches or more in length. When these seedlings are removed from the seedbed or transplant bed, varying portions of the the primary roots as well as most of the elongated laterals and many of the short laterals, particularly if they are in a growing state, are broken off and left in the ground. Thus when seedlings are planted in the planting site, the root systems that are regenerated subsequently must come from one or more of the following: (1) short unbroken laterals that begin to elongate rapidly; (2) lateral roots that originate at points where older roots have been removed; (3) lateral roots that originate some distance back from wound tissues and apparently are not associated with them.... Seasonal variation (in RRP) is measured in both lateral root elongation potential and lateral initiation potential which together are hereafter referred to as "root regenerating potential"....

In 1963, Stone *et al.* showed the root regeneration potential of ponderosa pine seedlings produced by four California nurseries varied not only with lifting and outplanting date but also with the source nursery. In 1966, Krugman and Stone showed that cold nights enhanced the RRP of ponderosa pine. In this study they still used the methods described in Stone and Schubert (1959a). By 1967, Stone had begun to improve RRP methodology:

> According to the technique that we have used, seedlings whose RRP is to be evaluated are (root) pruned .... and all the white root

> tips longer than 0.25 cm are pinched off.... merely to simplify subsequent counting procedures. The seedings are planted in screened blended forest soil .... Following planting the trays are suspended in 20°C water baths for 28 days .... Roots that have grown 1.0 cm or more -all new growth is white and easily recognized-are measured and recorded with respect to origin .... Until recently, the controlled temperature baths were located in glass houses in which air temperatures fluctuated widely but were not allowed to fall below 18°C during the night. The water baths are now located in controlled environment chambers in which the air temperature is maintained at 25°C during the day and at 18°C during the night; the light intensity is maintained, with water cooled Zenon-arc lamps, at 3,500 foot candles (37,650 lux).

Stone (1967) and Stone and Jenkinson (1970) showed that ponderosa pine seedlings transplanted into soils initially moistened over a range of available soil moisture percentages varied in RRP. In most seasons, soil moisture tensions greater than 0.5 to 1.0 bar appeared to limit RRP. However, in January when RRP is highest in California, roots were regenerated in soils with tensions up to 7 bar.

RRP research in Canada began after a visit by Dr. E.C. Stone to the Faculty of Forestry at the University of Toronto in 1969. Initial studies at Toronto (Hambly 1973) were followed by more comprehensive studies of RRP at the School of Forestry at Lakehead University in Thunder Bay (Stupendick 1973; Day and Stupendick 1974; Day and Butler *1975;* Day and MacGillivray 1975; Mahon 1976; Kenny 1976; Fraser 1976; Day 1976). These studies were in most respects similar to those carried out by Stone and his associates with the exception of the use of the Rhizometer (Morrison and Armson 1968) and glass-fronted root boxes (Lavin 1961; Larson 1962; Musik et al. 1962). Day and Stupendick point out the change in approach to RRP study as follows:

> An important difference between this study and that of previous workers is the use of the Rhizometer (Morrison and Armson 1968) for the measurement of 'Root Area Index' or 'RAI' of seedlings under study. Previous workers' methods of counting and measuring the length of newly regenerated roots was not used because of limited time and funds .... Sixty seedlings were potted as before in waxed cardboard containers for study of RAI response and twenty seedlings were planted against the glass in 30.5 cm (12 in) by 15.75 cm (6 in) by 3.0 cm (1.2 in) glass fronted root boxes. The root *boxes were sloped at 45^0 during the root response period so that* newly elongating roots could be demarkated and measured against the glass.

In the United States, Stone and his co-workers continued their research on RRP in the 1970s. This work resulted in publications on the modification of nursery climate to improve RRP (Stone and Norberg 1971) and on physiological grading (Stone and Jenkinson 1971) and culminated in a summary of all the RRP work in 'Reforestation practices for conifers in California' by Schubert and Adams (1971). In Canada, Day and MacGillivray (1975), Day and Butler (1975), and Polhill (1975) were working on the RRP of nursery stock after transplanting in soil at field capacity, 0.1 bar, and at 0.5, and 1.5 bars soil moisture tension. Day and MacGillivray (1975) describe the work:

> Eight seedlings were planted in each of three sets of root boxes that contained sandy loam soil adjusted to 0.1, 0.5 and 1.5 bars

> SUIT (soil moisture tension) .... The root boxes were 30.5 x 15.25 x 2.54 cm (12 x 6 x I in) ... and there were 16 equally spaced 2 mm holes in the back (for irrigation to replace water used by pipette) ....Newly developed roots and the extension of old roots were traced on the glass at 4- and 6-day intervals. At the end of the 40-day period .... The RAI of each seedling was remeasured and compared with the initial RAI to obtain the increment or decrement that had occurred....

These studies showed that the RRP of white spruce (Picea glauca (Moench) Voss), black spruce (Picea rnariana (Mill.) B.S.P.) and jack pine (Pinus *banksiana* Lamb.) was generally high in the spring, low during the summer, and moderate in the fall. They also showed that RRP tended to be very low in soils at more than 0.5 bar tension, although jack pine was less severely affected than the spruces.

In 1976, RRP studies at Lakehead University were continued with: 1) a study of plant moisture stress (-water potential) - RRP relationships (Mahon 1976); 2) a study of fall chilling on the RRP of overwinter cold-stored red pine (Pinus resinosa Ait.) (Fraser 1976); and 3) a study of cool (+2°C) and frozen (-2°C) storage on the RRP of spring lifted white and black spruce stock (Kenny 1976). In 1976, Day and Day et al. summarized the results of their work on the effects of planting date and the moisture content of the outplanting soil on RRP. Finally, in 1977, Day and Breunig showed that the RRP of white spruce was significantly better for seedlings with large (>30.0 cm^2) RAIs than of those with either 10 or 20 cm .

In 1976, Lee and Hackett published their work on the RRP of Pistacia chinensis in a 'bottom mist chamber'. Although this work was not known to the Canadian workers, it is of considerable interest because it antedates the root misting work now in progress at Lakehead University:

> The root regenerating potential (RRP) of one year old Pistacia chinensis seedlings at different growth stages was determined by recording the number of newly initiated roots during the period of weeks after bare-root transplanting into a bottom mist chamber.... Root systems were mist irrigated in a chamber covered on the sides and ends with black polyethylene film to exclude light and aluminium foil to reduce radient heating. The top of the chamber was covered with pieces of 1.8 cm (3/4 inch) thick styrofoam between which the seedlings were inserted for support .... Misting frequency for roots was 5 sec. per 5 min. 24 hours a day....

In 1977 and 1979, Burdett wrote on assessing stock quality by means of physiological tests carried out in an inexpensive semicontrolled environment chamber, and on new methods of evaluating RRP. One of the methods was the well-known displacement technique (Iyer, not dated) that provided root volume measurements. The other was a coding system devised by the author. Burdett (1979) described his work in British Columbia as follows:

> The survival and growth of lodgepole pine (Pinus contorta Dougl. ex. Loud.) planted in the spring under a variety of conditions was found to be closely related to its root growth capacity as measured by two newly developed methods. One method employed a displacement technique to measure the root volume of the test seedlings, nondestructively, both at the beginning and end of the

> period of growth under standard conditions. The change in root volume that occurred during the test was taken as a measure of the root growth capacity. The other method of measuring was to record, by means of a semi-quantitative scale, the number of newly elongated roots possessed by the test seedlings after 1-week.

In fact, the semiquantitative scale proposed by Burdett (1979) (i.e., 0 = no new roots; 1 = some new roots, none over 1 cm of length; 2 = 1 to 3 new roots over 1 cm in length; 3 = 4 to 10 new roots over 1 cm in length; 4 = 11 to 30 new roots over 1 cm in length; and 5 = more than 30 new roots over 1 cm in length) was similar to the 'root phenology codes' described by Kenny (1976), Day and Breuning (1977), and Mears (1978).

In 1979, Herman and Lavender outlined methods for testing the vigor of Oregon species in pots filled with forest soil and placed either in a growth room or greenhouse. Although RRP was not evaluated and bud flush and survival alone were used to evaluate physiological quality, the tests are pertinent because both nonstressed and stressed seedlings are tested:

> The physiological quality of the seedlings will become apparent more quickly if the seedlings are stressed .... The mot common stress treatment is to expose the roots and shoots to 90 F (32.2°C) and a relative humidity of 30 percent for 15 minutes just before potting the seedlings .... After 4 weeks, bud flush and survival will indicate the vigour of most trees.

In 1979, MacDonnel (1980) began to develop an intermittent root mist chamber (RMC) at Lakehead University in order to attempt to find a rapid practical method for *evaluating RRP before stock is shipped to the planting site.*

> The (root mist) chamber was developed to provide a rapid method of estimating root regeneration potential of nursery stock by evaluating root growth capacity before stock is shipped to the planting site .... The results of initial tests show that the RMC gave values for root growth capacity (in terms of percent root activity) that were not significantly different from tests carried out in soil (peat-vermiculite mix in pots) .... This suggests that the RMC method will be satisfactory in providing an accurate and rapid measure of root growth capacity for an estimation of root regeneration potential.

The current methods of evaluating RRP now in use at Lakehead University will be the focus of the remainder of this paper.

CURRENT METHODS OF EVALUATING ROOT REGENERATING POTENTIAL

From 1980 to 1983, a new program of research on 'Morphological and physiological stock quality in relation to field outplanting performance' is being conducted at Lakehead University.

The objectives of the research are:

To prepare test lots of 1V2+1S4 white and black spruce and 2+0 jack pine nursery stock by varying lifting data and storage treatment, i.e., 1) spring lifted, 2) spring lifted and cold stored at +2°C, 3) fall lifted and frozen stored at -2°C, and 4) fall lifted and frozen stored at -2°C, but preconditioned for 3 and 6 weeks at +2°C before planting.

To plant the test lots of stock over a 63-day period in the spring, i.e., 1) May 5, 2) May 26, 3) June 6, and 4) July 7, both in pots in a standard growth chamber environment for bioassay of RRP and on two field sites.

To measure the physiological and morphological quality of each lot of stock before and after planting and to correlate these measurements with survival, growth, and other measures of performance.

In 1981 it was possible to bioassay sample lots of 15 to 25 seedlings from each of the treatments included in the research in 1) pots, 2) root mist chambers, and 3) root growth boxes. In each case the seedlings were measured, grown for 21 days, and remeasured to evaluate the change in top and root characteristics. In addition, identical lots of stock were planted on two field sites, a sandy outwash plain and a shallow till, 80 km north of Thunder Bay.

It is too early to comment on the results of this research, but it appears that the results of the three bioassay methods are very similar to field results.

In the laboratory bioassays and field trials all seedlings are measured, grown for 21 days, and remeasured. In the root mist chamber and root growth boxes the seedlings were also remeasured at 10 days to provide a rapid estimate of RRP. In the field trials, one-third of the seedlings planted were removed 21 days after planting. In all tests, 80-column data processing forms were used to record information on individual seedlings. Columns 8 to 16 were for Identification. As the study involved initial (x) and final (y) measurements, both were recorded as follows:

TOP CHARACTERISTICS--Columns 17 to 41

17 & 18	Total Height in cm (x)		
19 & 20	Root Collar Diameter in mm (x)		
21 to 24	Height Growth in cm (x & y)		
25 to 28	Shoot Volume in cm^3 (x & y)		
29 & 30	Physiological Condition Code (x & y)		
	0 =	Foliage Healthy (Green)	
	1 =	0-25	Brown and/or Defoliated
	2 =	26-50	
	3 =	51-75	
	4 =	76-100	
	5 =	Dead (Buds and Inner Bark Dry)	
31 & 32	Phenological Condition Code (x & y)		
	0 =	Buds Dormant	
	1 =	Buds Swelling	
	2 =	Buds Bursting	
	3 =	Short Shoot Elongation	
	4 =	Medium Shoot Elongation	
	5 =	Long Shoot Elongation	
33 to 38	Plant Moisture Stress (PMS in bars) (x & y)		

39 to 40 — PMS Calculator Code (Day 1980) (x & y)

1 = Normal
2 = Moderate
3 = High
4 = Excessive
5 = Lethal

ROOT CHARACTERISTICS--Columns 41 to 78

41 & 42 — Root Area Index in cm^2 (x & y)

45 to 52 — Number and Length of Small Root Tips (0.0-2.0 mm, mean 0.2 mm)

Code	Number	Equivalent Length in cm
0	0	0.0
1	1-10	0.5
2	11-20	1.5
3	21-30	2.5
4	31-40	3.5
5	41-50	4.5
6	51-60	5.5
7	61-70	6.5
8	71-80	7.5
9	>80	8.5

53 to 60 — Number and Length of Medium Root Tips (3.0 to 10.0 mm, mean 0.6 mm)

0	0	0.0
1	1-10	3.0
2	11-20	9.0
3	21-30	15.0
4	31-40	21.0
5	41-50	27.0
6	51-60	33.0
7	61-70	39.0
8	71-80	45.0
9	>80	51.0

61 to 66 — Number of Long Root Tips (>1.0 cm) (x & y)

67 to 72 — Length of Long Root Tips in cm (x & y)

73 to 78 — Length of All Root Tips in cm (x & y)

The above recording system provides most of the essential information needed to evaluate the post-transplanting or post-outplanting response of the seedlings under test. It is obviously designed for scientific rather than operational use of the nursery; however, the above coding system could be made more operational by providing a coding system for long root tips (>1.0 cm).

The pots, root mist chambers, and root growth boxes used for the bioassay are briefly described in the following conclusion.

I. Pot Bioassay Method

a. From three to five replications of five seedlings (the number planted per pot will depend on root system size) are planted in a standard 2:1 peat:vermiculite mix in 23-cm (9-in.) plastic bulb pans (the size of the bulb pan is increased if necessary).

b. The peat-vermiculite mix is irrigated to field capacity and the bulb pans of test seedlings are placed in a growth chamber programmed to provide:

Light- 16 to 20 hour photoperiod with 12 hours >50 000 lux.
Temperature- 25°C day and 17.5 °C night. Humidity- 50-60% RH day and 80-100% RH night.

2. Root Mist Chamber (Figure 1)

a. From three to five replications of five seedlings are vertically fixed in the RMC support frames so that their roots hang down in the enclosed chamber and their shoots extend upward outside it. The spray-jets in the chamber are programmed to provide 5 seconds of mist every 30 minutes, or sufficient mist so that the roots are always coated with a fine moisture film.

b. The root mist chambers are placed in growth chambers that are programmed to provide exactly the same environment as in l.b above.

3. Root Growth Boxes (Figure 2)

a. From three to five replications of five seedlings are set, three to a pad, sequentially in the root growth boxes. The seedlings are slipped between the jacket or sleeve of polyethylene and on top of the pad of polyurethane foam. The roots must be spread with care and the planted pads must be packed tightly between the polyfoam spacers so that the seedlings remain in a vertical position.

b. The polyurethane foam adjacent to the seedling roots is fully irrigated night and morning to ensure that the pads are moist at all times. The root growth boxes are placed in growth chambers programmed to provide exactly the same environment as in l.b above.

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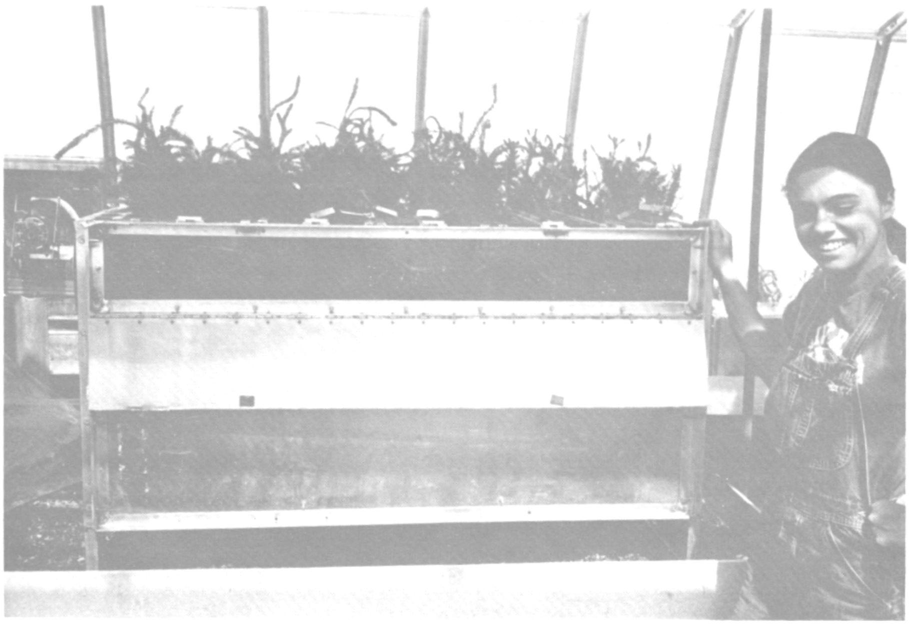
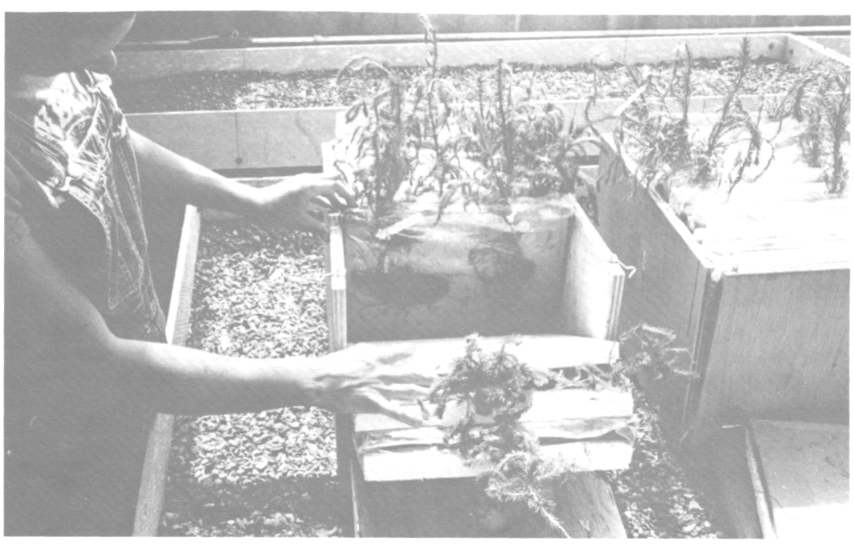


Figure 1. The root mist chamber with window exposed for viewing roots.

Figure 2. Root growth boxes opened to show roots.



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