Alternative Methods to Evaluate Root Growth Potential and Measure Root Growth[1]

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Rietveld, W.J.; Tinus, Richard W. 1987. Alternative Methods to Evaluate Root Growth Potential and Measure Root Growth. In: Landis, T.D., technical coordinator. Proceedings, Intermountain Forest Nursery Association; 1987 August 10-14; Oklahoma City, OK. General Technical Report RM-151. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 70-76. Available at: http://www.fcnanet.org/proceedings/1987/rietveld2.pdf

Abstract.--This paper reports experiments that compared root growth potential (RGP) testing methods, methods of quantifying root growth, and diagnostic ability of test methods. Factors that affect root growth in RGP tests are discussed. New root growth and plant water potential patterns of jack pine seedlings in pot, hydroponic, and aeroponic culture were similar, but new roots appeared first in hydroponic and aeroponic culture. The simplest method of quantifying root growth is to measure the number of roots longer than a minimum length. Electronic measurement of root area index is fast and well correlated with root number and length, but the equipment cost makes it most suitable for large operations. Test method and test length may affect results. Fourteen-day pot and aeroponic culture tests of jack pine seedlings subjected to root exposure treatments accurately diagnosed the weakened seedlings, but the seedlings recovered in 28-day tests, especially in aeroponic culture. For new applications, it is recommended that preliminary screening tests be run to determine the most suitable testing conditions.

INTRODUCTION

Root growth potential (RGP) is the most important measurable attribute of physiological quality because it quantifies the ability of seedlings to initiate and elongate new roots promptly and abundantly after transplanting. RGP is unique because it integrates an array of physiological factors into a single biologically meaningful estimate of performance potential -- the ability to grow new roots. Much information has been published on RGP in the past few years. Available evidence to date indicates a strong relation between RGP and field survival and growth (Ritchie 1985). Factors that affect the development and expression of RGP were extensively reviewed by Ritchie and Dunlap (1980), the relation of new root growth to several seedling and environmental factors was discussed by Carlson (1986), and the role of new root growth in the mechanism of transplanting stress was discussed by Sands (1984).

In contrast to most morphological quality measurements, which can be measured almost instantaneously, physiological quality attributes take time to measure (except for plant moisture stress). Consequently, it is not yet feasible to test stock and grade it physiologically before shipping. Until a faster method is available to estimate RGP, e.g. via a connection with cold hardiness (Ritchie 1985; Tinus, et al. 1986), we must be content to rely on present root growth tests to document RGP, and obtain the results in 2-4 weeks, usually after the seedlings have left the nursery.

Many people have hesitated to become involved in RGP testing because of: (1) equipment costs, (2) long test length, and (3) labor requirements and tedium of taking data. For the most part, these drawbacks are more imagined than real. The many variations on the original 28-day RGP test are summarized by Ritchie (1985). RGP tests may be shortened to as little as 7 days for certain species (Burdett 1979), and root growth may be quantified by new root number, length, volume, area index, or dry weight. In this paper we will focus on: (1) selection of methods to test the seedlings; (2) alternative methods to measure new root growth; and (3) the effects of testing

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[1]Paper presented at the Intermountain Forest Nursery Association Meeting, Oklahoma City, Oklahoma, August 10-14, 1987.

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method, test conditions, and test length on results.

COMPARISON OF TESTING METHODS

Although many different growing systems and media have been tried, the three main methods currently used to test seedlings are pot culture, hydroponic culture using an aquarium, and aeroponic culture using a root misting chamber. Pot culture is the traditional method (Stone 1955). It appears to be straight forward and inexpensive, but two important test conditions must be satisfied: (1) root temperature must be kept uniform, and (2) the growing medium must be well aerated. To provide a uniform root temperature, a growth room or water bath system is usually required, which raises the cost to a level comparable with other methods. Well-designed and relatively inexpensive hydroponic methods have recently been reported (DeWald et al. 1985, Palmer and Holen 1986). Hydroponic culture keeps the seedlings clean of growing medium, allows periodic observation of the progress of root growth, minimizes damage to new roots, and allows the test seedlings to be grown in fewer containers, while maintaining uniform root temperature and aeration within containers. It is important that aeration be gentle and uniform among containers, otherwise the agitation may inhibit root growth and increase variation. Aeroponic culture in a root misting chamber is another new technique. It was originally reported by Lee and Hackett (1976), refined by Harvey and Day (1983), and more recently refined by Rietveld and Tinus (1987). The root misting chamber has the same advantages as hydroponic culture, plus it is portable and provides a uniform temperature, humidity, and aeration environment for the roots in one container.

While developing the new root misting chamber, we needed documentation to show how the new device compares with existing methods for growing the test seedlings. To provide that documentation, we grew overwinter-stored 2+0 jack pine (Pinus banksiana Lamb) seedlings in pot culture, hydroponic culture, and aeroponic culture in the new root misting chamber, and compared new root production, among-seedling variation , and root size distribution. Potted seedlings were grown in a mixture of 1:1:1 sand /perlite/vermiculite with no fertilizer added. The hydroponic system consisted of tree holders laid across a large 20-cm-deep galvanized tank of water gently aerated through aquarium stones. The three growing systems were located in a growth room set at a constant 27°C temperature, 18 hour photoperiod, and light intensity of 165 uE/m^2/sec. The root misting chamber was also set at 27°C. Seedling root growth of 10 seedling samples was measured after 9, 11, 14, 16, 18, 21, 23, 25, and 28 days using a new root area index method (Rietveld and Tinus 1987). Number and length of new roots longer than 0.5 cm were also measured on day 28. Additionally, plant water potential of each test seedling was measured, using a pressure chamber, at the same time root growth was measured. Data were subjected to analysis of variance and Bartlett's test of homogeneity of variances.

New root growth was observed first in the root misting chamber and in hydroponic culture on day 9, then in pot culture on day 11 (fig. 1). Although seedlings grown in the root misting chamber had consistently higher levels of new root growth, the data were statistically indistinguishable from the hydroponic and pot methods on all measurement days, due to high among-seedling variation. The variances of the three methods, compared for the overall test and for days 14, 21, and 28, were likewise indistinguishable.

Root size distributions on day 28 for seedlings tested by the three methods are shown in figure 2. Although the patterns are similar for roots less than 15 cm long, seedlings grown in the root misting chamber and hydroponic culture had more long roots, reflecting the earlier and faster rooting apparent in figure 1. The response may also reflect the lack of soil resistance to root elongation.

The pattern of plant water potential in test seedlings is shown in figure 3. Average potential of seedlings taken from the cooler on day zero was -0.5 bar. Within 1 day in the growth room, potential dropped (became more negative) to approximately -6 bars, bottomed at approximately -6.5 bars on day two, then gradually increased during the course of the test to the range of -3 to -4 bars. The increase in plant water potential was weakly correlated with the initiation of new roots (r= -0.34), and may be better explained by osmotic adjustment. There were no significant differences in plant water potential among the cultural methods on any of the measurement days.

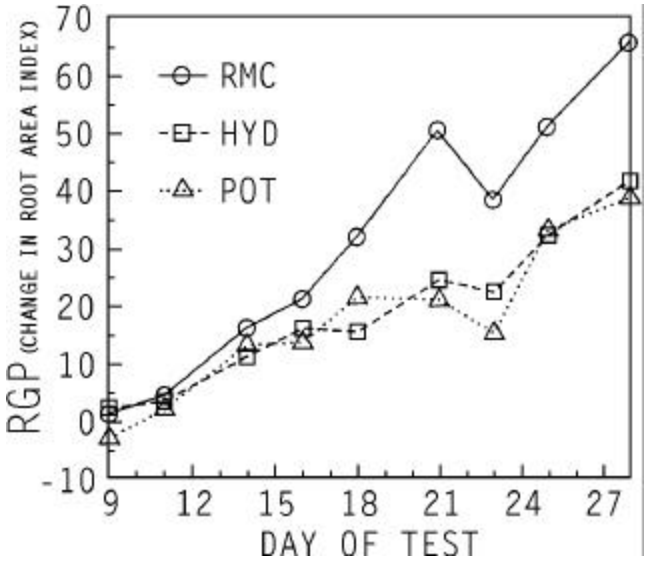

Figure 1. Root growth potential of 2+0 jack pine seedlings grown in aeroponic, hydroponic, and pot culture, quantified as change in root area index for nine test periods.

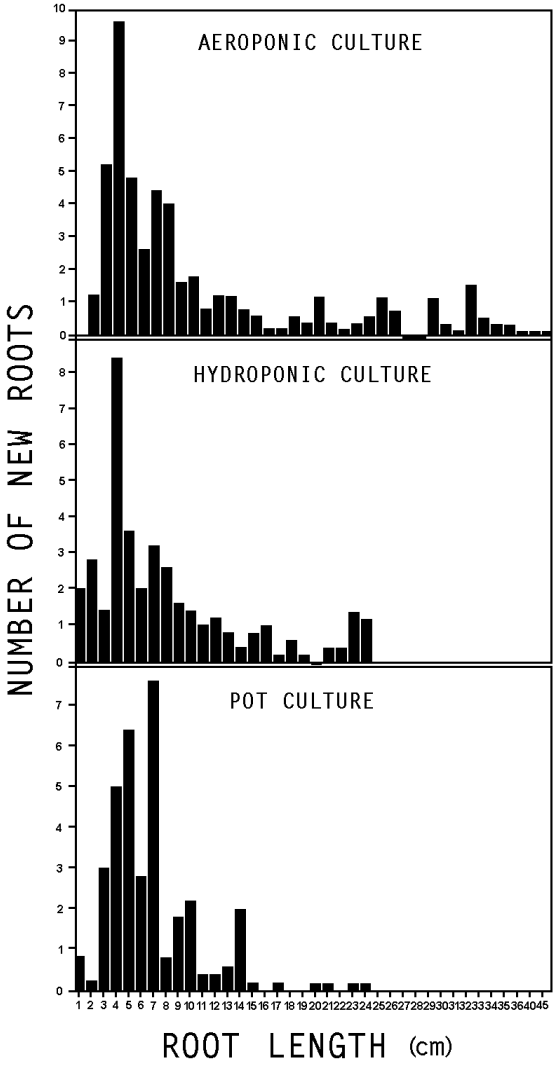

Figure 2. Size distribution of new roots of jack pine seedlings, on a per seedling basis, after 28 days of growth in aeroponic, hydroponic, and pot culture.

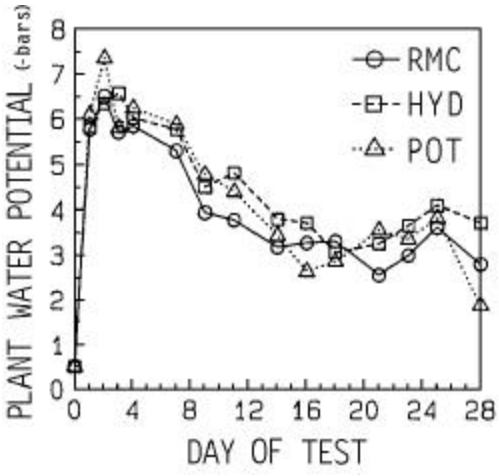

Figure 3. Plant moisture stress of 2+0 jack pine seedlings grown in aeroponic, hydroponic, and pot culture. Each point is the mean of 10 seedlings.

These data show that the three growing methods produce similar growth patterns for normal planting stock. Root growth was somewhat faster in the root misting chamber than in hydroponic or pot culture. For jack pine, 14 days appears to be the minimum test length to obtain an acceptable root growth response for evaluation.

COMPARISON OF METHODS TO MEASURE ROOT GROWTH

The task of quantifying new root growth may seem initially formidable when you look at a seedling that has up to 400 new roots on it, but the job is not as big as it looks. Researchers have devised many methods to lessen the task while still obtaining meaningful data. Originally both number of new roots and total length of new roots were measured. Eventually it was found that root number and root length are strongly correlated (Stone and Schubert 1959), so only number of roots longer than a minimum length was measured. Note, however that the correlation would be expected to decrease as test length increases because some of the new roots grow quite long (see fig. 2). Harvey and Day (1983) were the first to quantify new root growth in RGP tests by change in root area index using a Rhizometer (Morrison and Armson 1968), a photoelectric device developed for seedling morphology measurements in Ontario. Racey (1985) compared root measurement by root area index (using the Rhizometer), volume, and dry weight. He found strong correlations between the three quantification methods and the calculated area of new root tips, and recommended root volume because it was the easiest to measure. However, the Rhizometer has problems detecting new white roots at high light intensities (Racey 1985), and root volume determined by the Archimedes principle (measuring weight increase when the roots are dipped into a large beaker of water on a balance) has problems due to lack of repeatability of individual measurements (Ritchie 1985). A new root area index method for quantifying root growth in RGP tests was-developed by the authors (Rietveld and Tinus 1987). The method is based on a microprocessor area meter (Delta-T Devices, Cambridge, England3), and involves placing an intact root system on a light box in view of a black and white TV camera. The image is scanned by the area meter, and a microprocessor totals all the line segments in the viewing area that are covered by roots. The method is very fast (up to 500 seedlings/day), but the equipment cost much more ($3670) than that needed to count the new roots Manually.

To provide documentation for the microprocessor root area index method, we conducted a test to determine the relation among new root growth measured by change in root area index,

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[3]The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

counted number of new roots, and measured length of new roots. To compare the methods over a range of RGP, we gave 50 jack pine seedlings root exposures of 0, 10, 20, 30, and 40 min by placing them in a large forced-air oven at 40° C. The seedlings were grown in a root misting chamber located in a greenhouse with maximum air temperatures ranging between 18 and 28° C, minimum air temperature of 15.5° C, photoperiod extended to 18 hours with high pressure sodium lamps, and light intensity ranging from 300 to 800 μE/m^2/sec. The root misting chamber temperature was set at 27° C, which is favorable for jack pine. After 17 days, new roots > 0.5 cm on each seedling were measured manually, and all new roots were measured by the root area index method. Root growth measurements were compared by linear regressions using individual seedlings as observations (n=50). The coefficients of determination (r^2) for change in root area index on total number of new roots and total length of new roots were 0.88 and 0.90, respectively (fig. 4). Total number of new roots was closely related to total length of new roots (r^2=0.93). These strong relations indicate that measuring new root growth as change in root area index is a valid quantification method ,that provides a close estimate of actual root number and length.

Change in root area index may be a better estimate of rooting response than either root number or root length because (1) it measures all new roots, (2) it takes both root diameter and length into account, and (3) it detects root decrement as well as increment. However, the root area index method does not distinguish the origin of new roots and does not give any information on individual root size classes, i.e. the relative abundance of coarse and fine roots.

RGP TEST ENVIRONMENT AND SAMPLING

Although it is widely accepted that a uniform and favorable root environment is most important for conducting RGP tests, the shoot environment

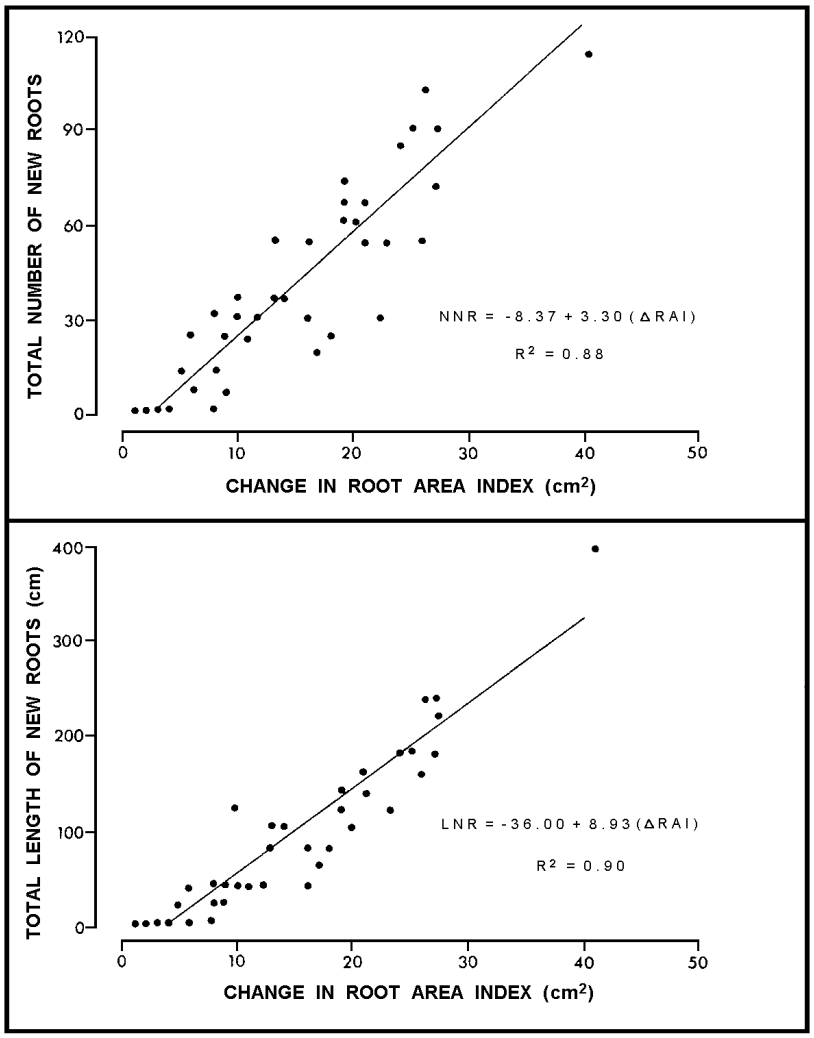

Figure 4. Regressions of root growth quantified by number of new roots (NNR) and length of new roots (LNR) on change in root area index (<>RAI), measured on the same seedlings. n=50. Several points represent multiple seedlings, especially those with zero NNR or LNR.

should also be favorable and repeatable when a series of RGP tests are run and the results compared. Abod et al. (1979) found that RGP of Pinus caribaea Mov. and P. kesiya Royle ex Gordon seedlings was optimized at air and soil temperatures between 24 and 30° C, and light intensity of approximately 50% of full sunlight (500-750 μE/m^2/sec). The optimum temperature for seedling root growth of many North American species is near 20° C (Ritchie 1985). Root growth potential tests are commonly run at elevated root and shoot temperatures and extended photoperiods. These conditions are well beyond the normal environment when seedlings are transplanted, but test results are obtained in a shorter time. Significant seed source and family differences in optimum temperature for root regeneration have been documented within a species (Carlson 1986, DeWald and Feret 1985, Jenkinson 1980, Nambiar et al. 1982). Therefore, it is advisable to experiment with root and shoot temperatures, and test length to determine the most suitable conditions for the species being evaluated, as well as seedlot or family variation in response to temperature. If seedlot or family variation is significant, it may be useful to adjust RGP to a base temperature (e.g. 20° C) for comparison.

Another factor to consider is seedling size. Seedlings with higher root volume have higher RGP (Carlson 1986), so it is important that the sample tested represents the range of seedling sizes in the stock lot. Note that selecting seedlings of uniform size for testing RGP would give a biased estimate of RGP if the average size of the sampled seedlings was not the same as the mean size for the stock lot. To obtain a true random sample that represents the range of seedling size and condition inn the seedlot, the seedlings to be tested must be sampled from many locations in the population.

For normal bed-run stock, we consider a sample size of 25 seedlings to be minimum because variation is often high in RGP tests (Ritchie 1985, Sutton 1983). Depending on the uniformity of the test plants and the precision desired, 50 seedlings or more may be necessary. Very uniform plant material, such as stock grown by family (e.g. from seed collected from a clone in a seed orchard), may require fewer test seedlings.

DIAGNOSTIC ABILITY OF THE TEST METHODS

An additional question that needs to be addressed is how do the methods compare in diagnosing stock that differs in vigor -- will the same conclusions be reached using different testing methods? To answer this question, we generated several levels of seedling vigor by subjecting jack pine seedlings from a common seedlot to root exposures of 0, 10, 20, 30, 40, and 50 min at 40° C in a large forced-air oven. We then assigned 15-seedling random samples to 14-day and 28-day RGP tests in the root misting chamber and pot culture. The experiment was conducted in a large root misting chamber (0.9 m wide x 3.7 m long) located in a greenhouse under the same environment as the previous experiment. Potted seedlings were suspended in the root misting chamber so that the root temperature in the pots was maintained at the same temperature as the misting chamber. New root growth was quantified by the root area index method described above.

The results were quite surprising. At 14 days the root misting chamber and pot culture methods gave the same diagnosis (fig. 5): i.e. RGP of all root exposure treatments was significantly lower than the control (0 min root exposure). The root growth difference between control and root exposed seedlings was substantially higher when seedlings were tested in the root misting chamber (fig. 5). In the 28-day test, however, seedlings from many of the root exposure treatments recovered, especially in the root misting chamber. The testing methods did not give the same diagnosis in the 28-day test: in pot culture, only seedlings root exposed for 10 min recovered (n.s. from control), while in the root misting chamber seedlings in all root exposure treatments recovered (all n.s. from control).

It appears that under some conditions the root misting chamber environment may be too favorable for root growth, so that weakened seedlings may recover in longer tests (28 days) and show acceptable RGP. This was true to some extent for the potting method as well. In a 14-day test, however, the two methods were equally capable of diagnosing the weakened seedlings. These results suggest that tests should be no longer than necessary to detect differences in quality; longer tests may result in greater variation among seedlings, recovery of weakened seedlings, and more roots to measure. Additional research is needed to determine all the implications of test method and test length.

This experiment also demonstrated clearly the difference in root growth rates between the root misting chamber and pot culture. For the 0 min root exposure treatment, root area index increment at 14 days was 16.2 for the root misting chamber and 12.1 in pot culture (significant at $\alpha = 0.05$); at 28 days it was 60.6 for the root misting chamber and 26.8 in pot culture (significant at $\alpha = 0.005$).

SUMMARY AND CONCLUSIONS

1. RGP is the most important measure of seedling physiological quality because it integrates an array of attributes into a single biologically meaningful measure - the ability to grow new roots. However, physiological grading is still not practical because RGP testing is not immediate like morphological measurements.

2. RGP testing in pot culture, hydroponic culture, and aeroponic culture (root misting chamber)

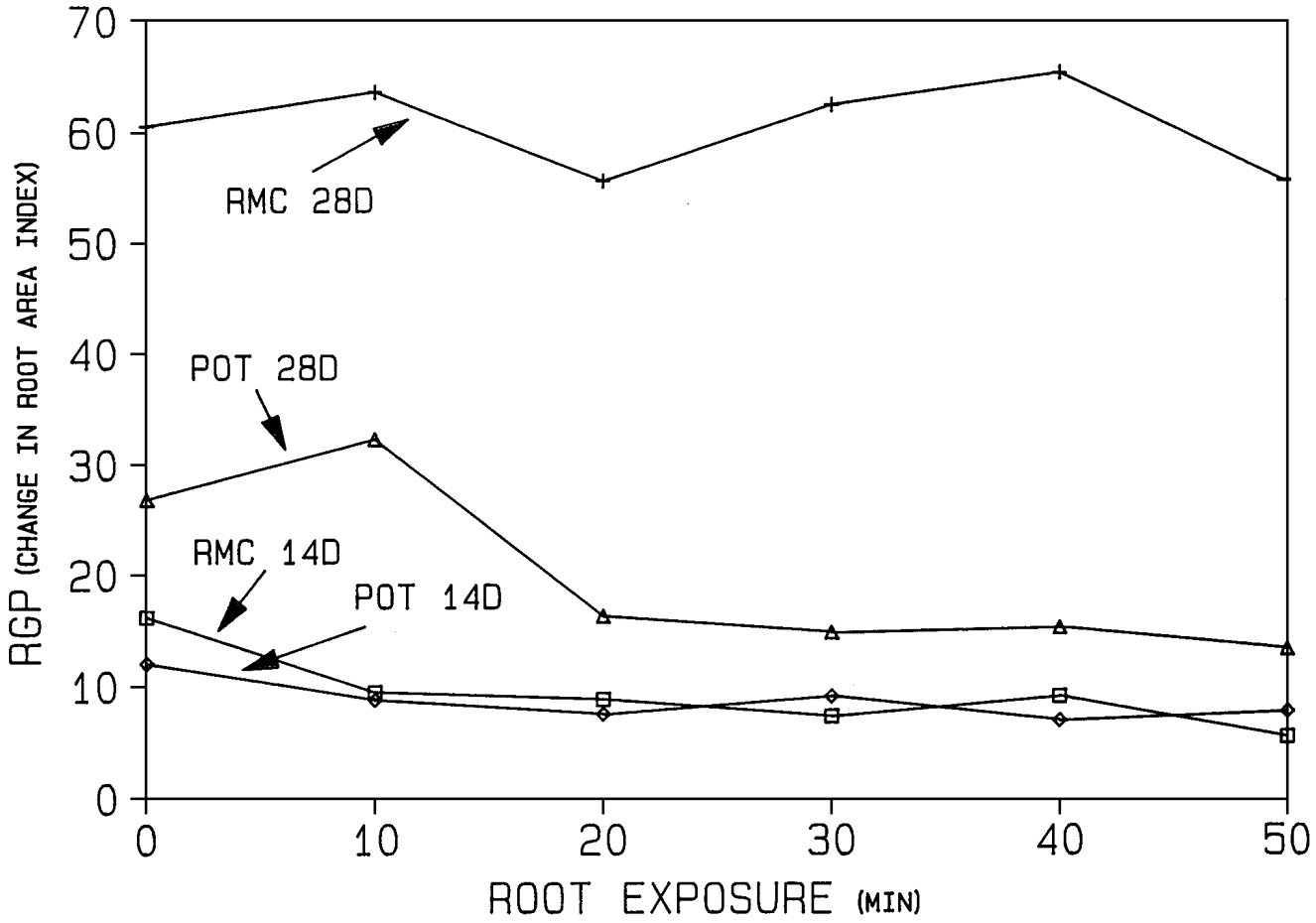

Figure 5. Root growth potential, measured as change in root area index, of 2+0 jack pine seedlings grown in aeroponic and pot culture for 14 and 28 days.

gives similar root growth patterns. New root growth was observed first in hydroponic and aeroponic culture. The three methods require approximately the same investment in equipment when maintenance of uniform root temperature is taken into account.

3. For smaller numbers of seedlings, it appears that the simplest and least expensive method of quantifying root growth is to count the number of new roots longer than a minimum length. This approach is based on a strong relation between root number and root length. The relation would be expected to weaken with longer test periods (some roots grow very long), but should still be satisfactory. Measurement of root area index increment is the easiest and fastest method of quantifying new root growth, and is well correlated with root number and length, but the equipment cost makes it more suitable for large operations.

4. Test method and test length may affect test results. Seedlings weakened from root exposure treatments were found to recover in 28-day aeroponic tests, and to some extent in pot culture. However, both methods accurately diagnosed differences in seedling vigor in 14-day tests.

5. Root temperature, light intensity, seedling size, test method, test length, species, and seed source/family within species have all been reported to affect RGP. If a series of RGP tests will be run and the results compared, it is advisable to run preliminary screening tests before a set of testing conditions is established. The "best" testing method and conditions are those that meet specific needs and objectives, and can distinguish differences in physiological quality in the least amount of time.

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