J. Range Manage.
47:503-504 November 1994

Technical Note: Measuring Post-germination growth

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Abstract

Measurement of heterotrophic plant growth is important for evaluating seed or seedling vigor. Hand measurement of large numbers of germinants and seedlings is tedious, can injure young plants, and is often inexact when curved roots are involved. Using the Cobb-Jones germination method with a digitizing tablet, a personal computer, apparatus, and methods we developed, reduces the time, tedium, injury, and guesswork often associated with hand measurements. Evaluation of measurement precision using our methods indicates that repeated measurements of individual germinants/seedlings will have average differences that are less than 1.0 mm.

Key Words: seedling vigor, germination method, seedling measurement, slant-board, Cobb-Jones apparatus, digitizing tablet

Sequential measurements of the elongating axes of germinants and seedlings are important to studies of early growth; but, can be tedious, time consuming, and can injure the plants. Several methods have been developed, particularly for plant roots, to reduce measurement time and tedium. Newman (1966) placed roots on a flat surface and counted the number of intersections between roots and random straight lines. This line-intersect method was quicker than direct measurements. Shearer [1968 as cited by Reicosky et al. (1970)] measured root length by running an opisometer (an instrument used to determine the length of an irregular line) over a projected image of the root. Reicosky et al. (1970) compared the opisometer and line-intersect methods and found little difference in precision, but that the line-intersect was quickest. Marsh (1971) modified Newman's method by utilizing a grid instead of random straight lines. Rowse and Phillips (1974) mechanized Marsh's grid by developing an instrument for estimating the root length of a sample using a photoelectric method to count intersections. Tennant (1975) retested Marsh's technique utilizing 4 grid sizes and found that precision could be increased to around 1% coefficient of variation by increasing the number of intercept counts. Voorhees et al. (1980) scanned photographs of root samples using a computer-controlled video system with a digitizer. Length measurement was based on root intersection with video scan lines. Modern digitizing methods also use line-intersect with video scan lines or with Marsh's grid (digitizing tablet) as the basis for length measurement.

In 1984 we attempted to use a video scanning system to determine the axil length of post-germination plants. That system has been described, with improvements, by Harris and Campbell (1989). Although different light filters, backgrounds, and light conditions were tested, we found the system could not consistently, fully distinguish small, light colored radicles and roots from the background without time-consuming adjustments for each individual seedling. We subsequently tested, and currently use a digitizing tablet (Booth 1992, 1993). Here we describe our current procedure for measuring post-germination growth.

BASIC METHOD

Plants are germinated and grown using the Cobb-Jones or slant-board method (Jones and Cobb 1963). Seeds are placed on moist germination paper supported by a 20 x 15 x 3-mm rectangular plate of acrylic plastic inclined at 67° from horizontal (slant-board). We use regular weight (38 lb) germination paper, Anchor Paper Co, Saint Paul, MN.[1] The wet germination paper clings to the board. The seeds are held to the germination paper by a 11 x 22-cm sheet of moistened cellulose tissue. The slant is maintained by inserting the lower edge of the board into a slotted rack or base. The rack holds several boards and is placed in a water reservoir so that the lower edge of the boards and germination papers are immersed.

Axial measurements of germinants and seedlings are made by laying the slant-board on a digitizing tablet controlled by a personal computer. A glass "table" is placed over the board to afford a smooth, dry surface upon which to trace axial length (Fig. 1). The table is supported by 6-mm-thick rubber pads glued to the underside of the glass. Measurements are automatically recorded and tabulated by the software as each seedling is traced ("Sigma Scan," Jandel Scientific, San Rafael, CA.)[1]. Individual plants can be measured repeatedly with a minimum of disturbance to the elongating roots.

Two problems slow measurements. It is difficult to detect very light colored roots, and the roots do not grow straight - often they intersect, or grow contiguous to neighboring roots. These problems require that the table be lifted and the plants more closely examined by further wetting the cellulose tissue cover to improve transparency, or that the tissue cover be carefully pulled back to more clearly reveal the roots. Various approaches were tested to improve our technique. We found a dark background, such as a dark-blue acetate sheet under the reservoir, and/or an adjustable lamp with the beam directed from the side, increased visibility of fine roots. A shallow reservoir large enough to partially immerse a horizontally placed

Manuscript accepted 7 Mar '94

[1]Mention of products is for information only and is not an endorsement.

Fig. 1. Use of glass table with digitizing tablet for measuring axil length of germinants and seedlings.

slant-board was constructed by applying a bead of silicon caulk around the edge of a glass sheet. The reservoir was used by placing it on the digitizing tablet, placing a slant-board in the reservoir, adding water until the board was mostly immersed, then covering the slant-board with the glass table so that the tissue covering the seeds would adhere to the bottom of the table. This magnified fine roots making them easier to see and thus improved measuring accuracy.

The reservoir has become part of our standard equipment. We use it to protect the tablet from water, to prevent drying of germination paper, and to allow the operator the option of filling the reservoir versus lifting the table and peeling back the tissue to distinguish hard-to-see roots. Since root measurement with a filled reservoir is also time consuming, it is a matter of preference whether to "lift and peel" or to "fill." Peeling back the tissue is usually most practical when roots are relatively short. Filling the reservoir is best for longer roots that are more likely to be damaged by peeling back the tissue.

We tried, and rejected (1) adding fushin stain to the water reservoir, (2) using only a sheet of cellulose tissue with grooved slant-boards, and (3) using blue blotters with tissue covering. The grooved boards allowed us to measure axial length more quickly because roots were straighter; however, growth rates were reduced. Blue blotters plus tissue increased the number of non-straight roots, increasing measurement time.

Howarth and Stanwood (1993) are using blue blotters on slant-boards without a covering tissue and have little problem with curved roots (Stanwood, personal communication). This approach requires seeds be hydrated before being placed on the blotter.

Test of Precision

We evaluated our precision with this procedure by measuring axil length of 42 bitterbrush [*Purshia tridentata* (Pursh) DC.] seedlings at 3 separate times (replications) within one hour. All measurements were made by the same person. Statistical analysis consisted of computing the maximum difference (range) among the 3 measurements made for each seedling (42 maximum-difference values), and using linear regression analysis to test for a correlation between measurement error and axil length. Also, differences in seedling length by replication were tested by two-way analysis of variance (replication 1, 3; seedlings 1, 42).

The range in axil length among all observations was 15.7 to 142.8 mm. The greatest maximum difference among measurements of any one seedling was 2.7 mm, the least maximum difference was 0.2 mm, the average difference was 0.6 mm, and the standard deviation among maximum differences was 1.1 mm. One would expect that seedlings with short axil lengths would tend to have smaller maximum difference values than seedlings with large axil lengths. This was not the case. The correlation coefficient relating maximum difference with seedling length was 0.007 and the observed significance level of the slope of the regression line was 0.96. Means for seedling length among replications were 99.8, 99.6, and 99.7 mm. The observed significance level for a difference among replications was 0.453. These analyses suggest that measurement errors tended to be random, are therefore compensating, and are not related to axil length. With due care, a person can measure seedling axil lengths with an average precision, where n=42, of $\pm$ 0.2 mm (the range among replication means). Individual measurements will usually be $\pm$ 0.6 mm of the true value, assuming that the true values for the bitterbrush seedlings were within the range of values (maximum differences) obtained for each seedling.

SUMMARY AND CONCLUSIONS

Measurements of the axes of germinants and heterotrophic seedlings is used to evaluate seedling vigor. The Cobb-Jones (slant-board) germination method facilitates using a digitizing tablet to measure early growth. When used with a glass table and a shallow reservoir, the digitizing-tablet method causes less damage to seedlings than hand measurement. We note that new technology for measuring seedling growth is being developed (Howarth and Stanwood 1993). Until that technology is available and affordable, we recommend our methods for monitoring the growth of large numbers of plants needed to evaluate factors that influence seedling vigor.

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