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A Preliminary Identification of Morphological Indicators of Field Performance in Bare-Root Nursery Stock

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ABSTRACT

A general method for identifying key morphological attributes of bare-root nursery stock as indicators of early field performance is presented. The method is exploratory with emphasis on relating attributes of individual seedlings to their early field performance. The approach is illustrated using data from a recent study of longleaf pine (*Pinus palustris* Mill.) seedling quality.

Keywords: Experimental design, nursery data analysis, field data analysis, seedling quality, *Pinus palustris*.

Historically, the approach to determining seedling quality has involved setting a definition of seedling quality followed by outplanting a group of seedlings thus defined to observe average field performance (Brissette 1984). However, various relationships of morphological characteristics of bare-root nursery stock of the southern pines to field performance have eluded researchers in their attempts to identify or separate seedlings according to their potential for survival and growth in the field. For example, Wakeley (1954) concluded that his grading rules for morphological characteristics were inadequate. In many tests, he had found that grade 1 seedlings usually made the best growth among three grades but that they generally did not survive as well as grade 2 and sometimes not as well as grade 3 seedlings, which ordinarily were culled. Our approach features broad flexibility to infer seedling quality based on individual seedling field performance. This method evolved during a longleaf pine seedling quality study based on Wakeley's (1954) grades 1 and 2 and reported by Hatchell and Muse (1990).

The objective of this Paper is to develop a general method for identifying key morphological attributes of bare-root nursery stock as indicators of field performance. Areas of consideration include nursery and outplanting study objectives, field data analysis, and seedling quality inference. The resulting method is illustrated using data from the above-mentioned longleaf pine seedling quality study.

Methods

Our method focuses on the morphological characteristics of individual seedlings at lifting time in relation to subsequent field performance. Since an attribute may vary considerably among seedlings, large sample sizes are needed; otherwise, the joint consideration of two attributes in relation to field performance would probably not be feasible. Also, large sample sizes are needed for powerful analysis of nominal scale field data such as survival. Since seedling survival is a necessary condition for further study of field performance, the first stage of our method addresses the identification of key morphological attributes related to survival. The resulting sample stratification could provide either a basis for delineating seedling quality or a basis for utilizing a second measure of field performance to infer seedling quality.

Our primary objective in the nursery phase is to produce a large set of plantable seedlings for each nursery treatment regime. A minimum of 1,000 seedlings is desirable with each seedling identified, tagged, and measured at lifting time. For study purposes, culling, if any, should be limited to seedlings on which damage or disease has substantially altered a morphological attribute of primary interest.

Our main objective in outplanting experimental design is to be able to draw inference about seedling quality for each nursery–field treatment combination of interest. Therefore, a large set of plantable seedlings is needed for each of these combinations.

Our approach utilizes the assumption that individual seedling field responses are independent. Consequently, the field design should feature the random assignment of seedlings produced under each nursery treatment regime to single-tree plots associated with each field treatment.

Our approach to seedling quality inference involves partitioning the data set into a sequence of nested sets associated with one or more attributes such that advancing nested sets possess higher sample seedling quality. More specifically, let G_1, G_2, . . . , G_k denote K nested sets where G_2 is contained in G_1, G_3 is nested in G_2, etc., and sample quality increases with the set subscript. For survival studies, G_1 is the set of all plantable seedlings. If the field response is not survival, then G_1 may be a subset of the set of plantable seedlings such that survival rate is acceptable. In any event, the object is to find a set G_j, $j > 1$, such that the sample quality for G_j is significantly higher than the sample quality for G_1. If such a set exists, there may also exist a set G_j^*, $j^* > j$, such that the sample quality for G_j^* is significantly higher than the sample quality for G_j. Consequently, levels of seedling quality may be inferred. For large samples, the null hypothesis of no change in seedling quality from G_i to G_j, $j > i$, versus the alternative hypothesis that seedling quality increases from G_i to G_j can be tested using the following procedures:

1. If the field response is a nominal scale measure involving exactly two levels of response, say acceptable and unacceptable, then under the null hypothesis, the test statistic Z is an approximate standard normal statistic

where

$$Z = (f_j - f_i)/ \sqrt{f_j(1 - f_j)/n_j + f_i(1 - f_i)/m_i},$$

and where

X_j = the number of acceptable seedlings in G_j,

n_j = the total number of seedlings in G_j,

$G_i - G_j$ = the set of seedlings contained in G_i but not in G_j,

W_i = the number of acceptable seedlings in $G_i - G_j$,

m_i = the total number of seedlings in $G_i - G_j$,

$f_j = X_j/n_j$, and $f_i = W_i/m_i$.

Although the above statistic Z compares the performance levels for G_j and $G_i - G_j$, it is appropriate for testing the above hypothesis involving G_i and G_j. This can be seen from the following derivation where Z_1 is appropriate for comparing the performance levels of G_i and G_j. Using the above notation, let

$$Z_1 = Y/ \sqrt{\text{estimated var}(Y)},$$

where

$$Y = X_j/n_j - (W_i + X_j)/(m_i + n_j)$$
$$= (m_i X_j/n_j - W_i)/(m_i + n_j)$$
$$= m_i(f_j - f_i)/(m_i + n_j),$$

$W_i + X_j$ = the number of acceptable seedlings in G_i,

$m_i + n_j$ = the total number of seedlings in G_i,

and

$$\text{estimated var}(Y) = m_i^2[f_j(1 - f_j)/n_j + f_i(1 - f_i)/m_i]/(m_i + n_j)^2.$$

Substituting for Y and estimated var(Y), Z_1 reduces to Z.

2. If the field response is a nominal scale measure involving exactly q levels of response, $q > 2$, then under the null hypothesis, the test statistic Z is an approximate chi-square statistic with $q - 1$ degrees of freedom

where

$$Z = \sum_{h-1}^{q} (f_{jh} - f_{ih})^2/[f_{jh}(1 - f_{jh})/n_j + f_{ih}(1 - f_{ih})/m_i],$$

n_j, $G_i - G_j$, and m_i are defined in procedure 1 above,

X_{jh} = the number of seedlings in G_j exhibiting a level h response,

W_{ih} = the number of seedlings in $G_i - G_j$ exhibiting a level h response,

$f_{jh} = X_{jh}/n_j$, and $f_{ih} = W_{ih}/m_i$.

Note that the statistic given in procedure 1 can also be used if the q levels of response can be condensed to two meaningful levels of response.

3. If the field response involves interval scale data, then under the null hypothesis, the test statistic Z is an approximate standard normal statistic where Z, Y, n_j, G_i - G_j, and m_i are defined in procedure 1,

X_j = the observed total for seedlings from G_j,

W_i = the observed total for seedlings from G_i - G_j,

and

$$\text{estimated var}(Y) = m_i^2(S_i^2/m_i + S_j^2/n_j)/\,(m_i + n_j)^2$$

where S_i^2 and S_j^2 denote the sample variances for sets G_i - G_j and G_j, respectively. Survival, vigor, and height are examples of early field performance measures for which the three approaches are applicable.

Since partitioning the data set into a sequence of nested sets of increasing quality is not a uniquely defined process, some guidelines would be useful. In our study, sample seedling quality tended to increase as the morphological attribute of interest increased. Therefore, each set in the nested sequence of sample quality sets could be defined by increasing the lower bound of an attribute used to define the previous set in the sequence. If the sequence is based on one morphological attribute, then the partitioning is straightforward. If the sequence is based on two or more attributes, then several approaches are possible. In our approach, one attribute at a time was allowed to increase, thereby obtaining a trial set G_i - G_{i+1} for each attribute involved in defining the sequence. Since G_i - G_{i+1} denotes the set of seedlings deleted at the (i + 1) stage of the nesting process, the trial set having lowest sample quality is an attractive set to delete. G_{i+1} was defined on this basis. The above approach is easily generalized to the case where sample quality peaks within the interval of observed values for one or more attributes.

An Illustration

The above method was developed in conjunction with a longleaf pine study reported by Hatchell (1987) and Hatchell and Muse (1990). The nursery phase of this study involved a 2 x 2 x 2 factorial experiment laid out in four replications in a completely randomized design. The factors were: vertical root pruning versus nonpruned control; seedbed density, 65 or 129 seedlings per square meter; and seed drill spacing, 15 or 30 centimeters. At lifting time, 50 plantable seedlings, based on Wakeley's (1954) criteria for grades 1 and 2 stock, were randomly selected from each of the 32 plots in the nursery. Individual seedling attributes measured included root-collar diameter (RCD) in millimeters; number of strong, first-order lateral roots (NSFOLR), which included only those with diameters larger than 1 millimeter (Kormanik 1986); and fibrosity, an ocular rating of the abundance of fibrous roots. Assigned fibrosity ratings were low (L), medium (M), and high (H). These seedlings were subsequently outplanted on a site at the Savannah River Forest Station, Aiken, SC, using one field treatment and a completely randomized design with single-tree plots. Seedlings were measured after the second growing season.

As the study progressed and our approach to seedling quality inference evolved, two shortcomings of the study became evident. First, using Wakeley's specification for grades 1 and 2 stock may have been too restrictive. In any event, our inferences about seedling quality were limited to the subpopulation of grades 1 and 2 seedlings. Second, each nursery treatment regime resulted in only 200 seedlings; thus, sample sizes were small. Subsequent analysis of variance indicated that pooling across levels of drill spacing and density in the nursery were justified, thereby resulting in pooled sets of size 800 for pruned and nonpruned seedlings. Since the survival rate was significantly higher ($P = 0.01$) for pruned seedlings (69%) than for nonpruned seedlings (48%), the pruned seedling data set was chosen for our illustration. Active height growth results are included for completeness.

Results reported in table 1 indicate that fibrosity classes could be used to delineate levels of relative seedling quality. Medium- or high-fibrosity seedlings had a significantly higher survival rate (72%) than all plantable seedlings (69%). High-fibrosity seedlings performed significantly better (82%) than medium- plus high-fibrosity seedlings (72%). The rate of active height growth (height $\geq$ 10 cm) was also significantly higher for high-fibrosity seedlings (78%) than for medium- plus high-fibrosity seedlings (71%).

Values in table 2 indicate that NSFOLR offers several alternatives for defining relative seedling quality. For example, if fibrosity is M or H, then the sample survival rate for G_5 (NSFOLR > 3) is 79 percent, and 80 percent of survivors have active height growth by age 2. The corresponding data for the complementary set G_1 - G_5, the set of seedlings contained in G_1 but not G_5, can be easily derived. Using the counts and survival percentages for G_1 and G_5, survival counts for G_1 and G_5 are 472 and 355 seedlings, respectively. Therefore, G_1 - G_5 contains 652 - 448 = 204 seedlings with 472 - 355 = 117 surviving, and the survival rate for G_1 - G_5 is 57 percent. Using survival counts and active height growth percentages for G_1 and G_5, respective height growth counts for G_1 and G_5 are 337 and 284. Therefore, G_1 - G_5 contains 337 - 284 = 53 seedlings in active height growth, and the percentage in active height growth is 45. Consequently, for medium- or high-fibrosity seedlings, G_5 represents a statistically significant improvement in quality over G_1 - G_5. Furthermore, G_5 constitutes a large proportion of the sample seedling population with 69 percent of the medium- or high-fibrosity seedlings and 56 percent of all plantable seedlings represented. As a second example, consider G_6 with fibrosity unrestricted (fibrosity = L, M, or H). The sample survival rate for G_6 is 75 percent with 80 percent in active height growth; corresponding data for G_1 - G_6 include a 59-percent survival rate with 48 percent in active height growth. Again, the delineation in relative seedling quality is striking. Continuing the example, G_1 - G_6 could be further partitioned into two sets of significantly different quality. Survival and active height growth rates for G_1 - G_4 (NSFOLR < 3) are 55 and 39 percent, respectively, while the corresponding rates for G_4 - G_6 (NSFOLR = 3 or 4) are 63 and 59 percent. Hence, three levels of significantly different seedling quality have been defined.

Results pertaining to RCD are given in table 3. Again several alternatives for delineating sample quality are evident. In particular, G_4 (RCD > 8) contains seedlings of significantly higher quality than G_1 - G_4. If fibrosity = M or H, the survival and active height growth rates for G_4 are 76 and 75 percent, respectively; the corresponding rates for G_1 - G_4 are 53 and 45 percent. If fibrosity is not restricted, the survival rate and active height growth rates for G_4 are 72 and 73 percent, respectively; the corresponding rates for G_1 - G_4 are 53 and 47 percent.

Table 4 contains results for RCD and NSFOLR as joint indicators of seedling quality. Each set G_{i+1}, i = 1, . . . , 10, in the nested sequence was generated from G_i by: (1) allowing the lower bound for RCD to increase while holding the lower bound for NSFOLR constant to produce a trial set G_i - G_{i+1}, (2) repeating the process with the roles of RCD and NSFOLR reversed to produce a second trial set, and (3) setting G_{i+1}, to be the portion of G_i complementary to the trial set of lowest seedling quality based on survival. Sets G_2, G_3, and G_4 are based on increases in RCD, while the remaining sets involve increases in NSFOLR. Results based on table 4 are comparable to table 2 results except that sample quality levels for sets G_4 through G_{11} of table 4 are higher than the corresponding sample quality levels of table 2. The largest difference in sample quality occurs for G_4 of table 4 versus G_1 of table 2; however, as the nested level increases, the corresponding differences in sample quality tend to decrease. Consequently, the advantages of joint consideration of RCD and NSFOLR in table 4 versus NSFOLR or RCD alone for assessing seedling quality are probably negligible.

Conclusions

From the above discussion, the exploratory nature of our methodology is evident. Nevertheless, the approach offers considerable flexibility for relating nursery morphological attributes to early field performance. Based on our experience with the longleaf pine quality study, the preliminary method presented in this Paper provides a strong basis for identifying key morphological attributes of bare-root nursery stock as potential indicators of field performance.

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Table 1—Fibrosity as an indicator of second-year survival and active height growth given vertical root-pruned seedings

Nested sets	Fibrosity rating[1]	Initial number	Survival		Active height growth	
			Count	Percent	Count	Percent
G_1	L, M, or H	800	552	69.0[a]	389	70.5[a]
G_2	M or H	652	472	72.4[b]	337	71.4[a]
G_3	H	170	140	82.4[c]	109	77.9[b]

Within columns, survival and active height growth percentages followed by the same letter do not differ significantly ($P = 0.05$). The percentage in active height growth is based on surviving seedlings.

[1]L = low; M = medium; H = high.

Table 2—Number of strong, first-order lateral roots (NSFOLR) as an indicator of second-year survival and active height growth in vertically root-pruned seedlings with specified fibrosities

Nested sets	NSFOLR	Initial number by fibrosity class[1]		Survival by fibrosity class[1]		Active height growth fibrosity class[1]	
		L, M, or H	M or H	L, M, or H	M or H	L, M, or H	M or H
				- - - *Percent* - - -		- - - *Percent* - - -	
G_1	≥0	800	652	69.0^{a}	72.4^{a}	70.5^{a}	71.4^{a}
G_2	≥1	747	602	70.0^{b}	73.6^{b}	72.3^{b}	73.6^{b}
G_3	≥2	692	555	71.2^{c}	76.0^{c}	74.6^{c}	75.8^{c}
G_4	≥3	633	501	72.7^{d}	77.2^{d}	76.7^{d}	78.0^{d}
G_5	≥4	572	448	74.1^{e}	79.2^{e}	78.5^{e}	80.0^{e}
G_6	≥5	510	392	74.9^{e}	$80.1^{e,f}$	80.4^{f}	82.5^{f}
G_7	≥6	452	347	76.8^{f}	81.3^{f}	81.8^{g}	84.0^{f}
G_8	≥7	386	298	77.2^{f}	81.5^{f}	83.6^{h}	84.4^{f}

Within columns, survival and active height growth percentages followed by the same letter do not differ significantly ($P = 0.05$). The percentage in active height growth is based on surviving seedlings.

[1]L = low; M = medium; H = high.

Table 3—Root-collar diameter (RCD) as an indicator of second-year survival and active height growth of vertically root-pruned seedlings with specified fibrosities

Nested sets	RCD	Initial number by fibrosity class[1]: L, M, or H	Initial number by fibrosity class[1]: M or H	Survival by fibrosity class[1]: L, M, or H	Survival by fibrosity class[1]: M or H	Active height growth fibrosity class[1]: L, M, or H	Active height growth fibrosity class[1]: M or H
				Percent	*Percent*	*Percent*	*Percent*
G_1	≥6	799	651	69.1^a	72.5^a	70.5^a	71.4^a
G_2	≥7	780	633	69.5^a	73.0^a	71.2^b	72.3^b
G_3	≥8	744	599	70.3^b	74.1^b	72.1^c	73.4^c
G_4	≥9	681	542	72.0^c	76.4^c	73.5^d	75.1^d
G_5	≥10	592	471	72.3^c	76.4^c	78.3^e	80.0^e
G_6	≥11	455	359	73.2^c	76.9^c	82.0^f	83.3^f

Within columns, survival and active height growth percentages followed by the same letter do not differ significantly ($P = 0.05$). The percentage in active height growth is based on surviving seedlings.

[1]L = low; M = medium; H = high.

Table 4—Number of strong, first-order lateral roots (NSFOLR) and root-collar diameter (RCD) as joint indicators of second-year survival and active height growth of vertically root-pruned seedlings with specified fibrosities

Nested sets	NSFOLR	RCD	Initial number by fibrosity class[1]		Survival by fibrosity class[1]		Active height growth fibrosity class[1]	
			L, M, or H	M or H	L, M, or H	M or H	L, M, or H	M or H
					- - - *Percent* - - -		- - - *Percent* - - -	
G_1	≥0	≥6	799	651	69.1^a	72.5^a	70.5^a	71.4^a
G_2	≥0	≥7	780	633	69.5^a	73.0^a	71.2^b	72.3^b
G_3	≥0	≥8	744	599	70.3^b	74.1^c	72.1^c	73.4^c
G_4	≥0	≥9	681	542	72.0^c	76.4^c	73.5^d	75.1^d
G_5	≥1	≥9	670	533	72.2^c	$76.5^{c,d}$	73.8^d	75.5^d
G_6	≥2	≥9	646	513	$72.4^{c,d}$	$77.1^{d,e}$	75.6^e	77.2^e
G_7	≥3	≥9	610	482	73.1^d	77.8^e	76.9^f	78.4^f
G_8	≥4	≥9	559	438	74.6^e	79.7^f	78.4^g	79.9^g
G_9	≥5	≥9	503	387	75.3^e	80.6^g	80.5^h	82.7^h
G_{10}	≥6	≥9	450	346	76.7^f	81.2^g	81.7^i	84.0^h
G_{11}	≥7	≥9	384	297	77.1^f	81.5^g	83.4^j	84.3^h

Within columns, survival and active height growth percentages followed by the same letter do not differ significantly ($P = 0.05$). The percentage in active height growth is based on surviving seedlings.

[1]L = low; M = medium; H = high.

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