Quicker Methods of Establishing Forest Genetic Test Plantations

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An active tree improvement program involves scores of permanent test plantations. Their rapid and efficient establishment becomes a matter of concern. Aside from the cost, time itself is important. Trees are living and the longer they remain out of the ground, the poorer their chances of survival. Hence speed is in itself desirable.

During the past 8 years the Michigan State University forest genetic project has established 121 test plantations within the state. Currently 10 to 20 new test plantations are established each year. These vary in size from a few hundred to several thousand trees and contain from 20 to 300 different seedlots, each replicated several times. Of necessity, all the preparation and much of the actual planting is done by the same three men during a 3- to 4-week period.

This experience prompted the development of new methods of experimental plantation establishment which are described in this paper.

Quality in Permanent Test Plantations

High survival, good growth, low error variance and ease of measurement are the principal characteristics of a good test plantation. The first three factors are strongly interrelated. Conditions which result in poor initial survival are often the same ones responsible for poor growth and low statistical precision. That has been evident in the NC-51 provenance- and progeny-testing program in which the Michigan Agricultural Experiment Station participates. Most experiments have included several different plantations. And in most experiments the vast majority (possibly 95 per cent) of the useful information has been derived from plantations in which initial mortality was 20 per cent or less.

There is no set standard for the maximum size of the error variance. In several recent experiments the standard error of estimate of a single seedlot mean has been 6 or 7 per cent of the plantation mean. That is so high as to make difficult the detection of small differences (i. e. differences less than 15 per cent of the mean). But such differences are important enough to be detected, and improvement is needed. Precision can be increased by smaller plots and more replications but a limit is soon reached. Greater gains can be made by refinement in nursery and planting techniques.

Plantation measurements is a costly item at best. Cost can be kept reasonably low if spacing is regular and plantations are free of brush. Costs may become prohibitive in irregular plantations.

These are the standards to be maintained when applying new methods to the establishment of plantations to test scores or hundreds of genetically different entities.

Nursery Handling of Stock

Lifting *and* bundling trees from unreplicated *nursery* experiments

The standard nursery experiment at Michigan State University consists of four randomized replications containing small plots and a fifth replication containing large plots of several hundred trees each. The four small-plot replications are measured to provide statistically adequate data on nursery performance. Most or all of the trees for field planting are derived from the fifth, unmeasured replicate.

Lifting and bundling procedures are the same for such an experiment as for an unreplicated one because all material of a seedlot destined for one plantation is taken from one portion of a seedbed. The following description applies specifically to seedlings lifted for a single plantation to contain 108 seedlots, 10 randomized complete blocks, and 4-tree plots. The steps are as follows.

(1) Using the nursery map, check all nursery labels and replace broken or illegible ones.

(2) Place masking-tape labels on 5 trees (if there are to be 5 similar plantations) per plot. Use masking tape because it is cheap and will not bind the trees as wired labels would; the tape lasts about one year. Write the seedlot numbers with a black China-marking pencil — other pens or pencils fade. Attach the masking tape labels right-side up near the tops of the seedlings.

(3) Run the tractor-drawn lifter under the seedbed to loosen the seedlings; observe the operation to insure that no seedlings are misplaced.

(4) Starting in the corner opposite the semi-permanent plot marker, pull 40 seedlings (including one with a masking-tape label) plus a few extras for culling. Tie them together and proceed to the other 107 seedlots. Transport all the 40-tree bundles to the packing shed. The opposite-corner starting point is important because it helps to maintain plot identity until all trees are lifted.

(5) Untie a 40-tree bundle, make a check mark against a master sheet, discard the smaller extra trees, and place masking-tape labels near the tops of nine more trees. Check these new labels. Make ten 4-tree bundles, each containing one labelled tree. Fasten each bundle with a rubber band; use 2 loops only because each bundle must be unfastened later in a second or two. Root prune if necessary, dip the roots in water and place one 4-tree bundle in Replicate 1, one in Replicate 2, etc. Proceed to the next 40-tree bundle.

(6) After completion of the 4-tree bundling, pack and label each replicate separately.

This schedule permits prompt handling of the trees without undue desiccation or confusion. The work generally proceeds at the rate of about 140 trees per man-hour when preparing 4-tree bundles. That includes all labelling, lifting and packing. The accuracy record has been good. That has been checked in several provenance tests in which the extreme between-provenance differences make mistakes easy to detect. One mistake occurred in 25,000 plot-bundles packed in 1961; none has been found in the several experiments packed since then.

One crew of 3 or 4 men does all the lifting and packing as well as much of the planting of several experiments per year in the 3- to 4-week planting season found in southern Michigan. Thus the work is hurried. The crew pays most attention to maintenance of proper identity and to proper treatment of the trees. But no double-checks can be made on some other details. Hence, of 1,000 plot-bundles a few may contain extra trees (thrown away during planting) or too few trees (an empty space is left during plant-

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ing). Occasionally a seedlot is omitted or duplicated in a particular replicate. This type of mistake has the same consequences as does 0.1 to 0.5 per cent first-year mortality.

Lifting from replicated experiments. — Several investigators have selected phenotypically excellent nursery trees and followed them for several years after planting. In many cases the size effects have persisted. In the NC-51 provenance testing work both H. B. Kriebel of Ohio and I have several experiments in which size differences due to nursery treatment have persisted for at least 6 to 8 years.

These persistent nursery effects have serious consequences if we follow the usual practice of lifting all trees of one seedlot from one portion of a seedbed. Statistically significant differences may have an environmental rather than a genetic basis. One can measure the probable magnitude of the nursery-size effects but cannot correct for them. This criticism applies to most published data on early mortality, to short-term greenhouse or growth-chamber experiments using nursery-grown seedlings lifted a short time previously, to several reports of plantation × genotype interaction, and to many small differences reported in ordinary provenance and progeny tests.

The only adequate safeguard is to carry replication through from nursery to plantation, as R. T. Bingham of Moscow, Idaho did for some of his western white pine progeny tests. This increases the cost of nursery handling but appears necessary.

When dealing with a 10-replicated field plantation, the most reasonable way to accomplish this is to establish a 5-replicated nursery experiment and to place masking-tape labels on one of every four trees per nursery-plot (if there are to be four trees per plantation-plot). Then, in the first nursery-replicate, eight trees would be pulled per plot and made into two 4-tree bundles (each to contain a labelled tree). One 4-tree bundle would be placed in a box for Plantation-replicate No. 1 and one in a box for Plantation-replicate No. 2. Then, when those two replicates were lifted and packed, work could proceed on the other eight replicates. Alternatively, there could be one nursery-replicate for each field-replicate.

When working with scores of seedlots and thousands of seedlings, it is impractical to attempt any scheme which involves transport of all seedlings to a packing shed with the purpose of combining trees from different nursery plots before packing. We have attempted this but have never found enough space or time to do the work properly.

I have written in terms of seedlings grown in one place for a few years and then planted directly in the field. The same procedures can be followed in the transfer of seedlings to transplant beds and the later transfer to permanent plantations.

Lifting and packing of potted seedlings. — In South America and many eucalypt-growing regions, seed is germinated in large pots and after a few weeks the seedlings are transferred to long narrow pots. There they remain for about 6 months before planting in the field.

Wilfredo H. G. Barrett of Argentina successfully established a series of many-replicated, small-plot experimental plantations in 1967 using potted seedlings. To do this he used the following schedule. (1) Sow the seed in pots in the winter of 1966. (2) Transfer the young seedlings to pots and make one long row (50 seedlings) for each seedlot in each nursery replicate, labelling the first pot in each row. (3) After 6 months lift 8 potted seedlings from each row and place in one line in a specially built wood flat just wide enough to contain 8 rows of pots. (4) Proceed to the next row until all flats needed to complete one plantation-replicate are filled. (5) Number the flats and load on a truck for transportation to the planting site several hundred miles distant. (6) Unload and make certain that each flat is oriented correctly and in the proper order. (7) Plant, preserving the same arrangement as in the flats.

Unequal numbers per seedlot. — It is common to have unequal numbers of seedlings per seedlot. The simplest and most satisfactory solution is to distribute one plot-bundle per seedlot to the first replicate, one plot-bundle of each remaining seedlot to the second replicate, etc. As done in a white spruce test, this resulted in complete representation of 24 seedlots in the first 5 replicates, of 22 seedlots in the first 7 replicates, and of 16 seedlots in all 10 replicates. Statistically, the plantation consisted of three separate experiments but only one analysis of variance (for 16 seedlots and 10 replicates) was computed because the sparsely represented seedlots grew slowly and were of little interest.

This procedure has been used in 60 plantations, without complications in planting or data analysis. Usually, data for incompletely represented seedlots are entered and crossed off, to be ignored in the analyses of variance. With hundreds of degrees of freedom, the loss of a few is of no consequence. If incompletely represented seedlots are among the most valuable, separate partial analyses are made.

Plantation Establishment by Hand

Hand planting methods are used to establish small experimental plantations or when the terrain is rough. Most Michigan soils are such that the trees can be planted in slits made with spades and closed by foot. The former practice of scalping has been superseded by chemical weed control or by plowed furrows.

Our plantation establishment method differs from those commonly used in two basic respects. First, the plots are randomized within blocks during planting rather than beforehand. This has a two-fold advantage. It permits planting to proceed at the same rate as in commercial operations. Also, it permits planters to devote full attention to the job of planting the trees well because they do not have to look for a particular plot-bundle or check identity before planting.

Second, we insist on accurate placement of the trees within ± 3 inches in either direction when there are no obstacles. This makes the job of measurement much easier. Good alinement is more a matter of method and care than of time. Either of two techniques have proven satisfactory. (1) Spray strips or otherwise mark the ground in both directions and plant at intersecting lines. (2) Plow furrows or mark lines in one direction only. Then mark rows at right angles to those lines by setting *two* stakes at the end of each row. It helps if a different color is used for each row. String lines have not been satisfactory. Nor have rows been straight when planters lined on single stakes or on themselves.

The general procedure used for a hand-planted plantation is as follows. (1) Poison or otherwise kill woody vegetation 2 years prior to planting. (2) Kill grass on strips by chemical spray (dalapon, amino-triazole or simazine at manufacturers' recommended dosages) 6 months prior to planting. Or, plow furrows for weed control. (3) On the day of planting, mark a plantation corner and place temporary replicate boundaries on the ground. Also, fix guide stakes as necessary to mark the planting rows. (4) Open the first replicate-bundle and apportion the plot-bundles in a random order to planting trays. Adequate randomization is obtained if each tray is loaded with plot-bundles taken from several different portions of the replicate-bundle. Protect the roots from desiccation. (5) Choose a plot-bundle at random and untie it. Plant the labelled tree in the first space in the plot and the other trees in the remaining spaces. If there is an extra tree, throw it away. If there is one tree too few, leave a blank space. (6) Proceed to the next plot and continue until all plot-bundles for that replicate are planted. (7) Do not leave blank plots but revise the replicate boundary to show its actual contents. (8) Proceed to the next replicate. Plant border rows. (9) Prepare the plantation map by walking across the end of each plot. Make sure that the map is oriented correctly at all times and that row and column designations are entered before actual mapping is started. Also, make cross checks between series of plots at frequent intervals.

Any number of planters can be used and each may work at his own speed. Supervision is limited to planting technique, conformity with plot boundaries and conformity with replicate boundaries. If the planting crew is conscientious enough to plant trees well, the supervisor can help plant or proceed with mapping after the first half-hour. The mapping can be done in almost any sequence and with frequent interruptions.

The major statistical requirement about replicate shape is that site conditions within replicates be as uniform as possible. Replicates should be nearly square on flat ground or elongated along contours on hilly ground. They need not be contiguous nor regular in outline.

One to two border rows of the same species surround most of our experimental plantations. These are given the same care and mapped with the same accuracy as the experimental trees and often find experimental use in later years. Preferably the border trees are extras of seedlots which have performed well in the nursery.

Fresh and high-quality nursery stock, proper site selection, good planting and adequate site preparation are requisites of a good plantation, experimental or otherwise. When these conditions have been met the above schedule has given uniformly satisfactory results at a cost only 10 to 15 per cent higher than for a non-experimental plantation of the same size.

The instructions given here are semi-detailed. In practice, it is desirable to prepare very detailed instructions and ask that they be followed rigidly. Issuance of such instructions in the past would have prevented the following actual occurrences — placement of the labels so close to the ground line that most were buried in planting; sorting of plot-bundles so that the tallest progenies were placed near the top of each replicate; cross alignment so poor that some 80-tree rows were 60 feet shorter than others; use of one experiment as border stock for another; simultaneous opening and randomization of the contents of all replicate bundles; and deliberate placement of site-demanding species on sites selected for their poor quality. Of course, such plantations yielded little useful information.

Plantation Establishment by Machine

Michigan is relatively level with large areas of light sandy soils. It is well adapted to the use of planting machines which now are used in nearly all the commercial planting. This prompted a trial of machine-planting of a much replicated small-plot experiment in 1961. That trial was successful and 32 more plantations have since been established by machine. Most have been on level to gently rolling ground but one small plantation includes 30° slopes. We now prefer machine establishment for large plantations whenever the topography is suitable.

The university's tree planting machine, which was used in most cases, is of medium-weight construction and is drawn by a wheeled tractor. The machine consists of a U-shaped "shoe" which opens a narrow trench 8 to 10 inches deep, a seat above and to the rear of the shoe, planting trays, and two rubber-tired wheels to support the rider and close the trench. A rider holds a tree by its top, places the roots in the open trench and holds the seedling until the roots are firmly grasped by the soil.

On rough brushy sites it was necessary to borrow sturdier equipment drawn by caterpillar-type tractors. Some had plow attachments to open a double-furrow before the planting shoe. One had a sturdily constructed cage over the rider's seat and could be used when planting areas containing trees up to 4 inches in diameter. As long as the machine was adequate for the site conditions, its type did not matter.

We maintain almost the same standards for machine as for hand planting. Trees must be in straight lines in both directions, plots of the same size and shape, and replicates of the proper shape for the site conditions. Also, quality of planting must be good. That is no problem because one good man can be chosen to do all planting by machine and can do better work than the average planting crew.

Good alinement proved much easier to achieve than was anticipated. At the beginning it seemed necessary to mark lines on the ground at right angles to the direction of tractor travel. This is no longer done and all alinement is accomplished by eye. We start with two parallel rows of guide stakes along one edge of a plantation and plant the first row of trees opposite those stakes. Then trees in the second row are placed opposite those in the first, etc.

Some planters can maintain alinement within 2 or 3 inches for 50 consecutive rows. Others place occasional trees 6 to 12 inches out of line. It is helpful to have an extra man following the planting machine to replant occasional trees. When this is done the finished product is often indistuinguishable from a hand-planted plantation.

A 4-man crew works well. There should be a tractor driver responsible for alinement in one direction; a planter responsible for planting and alinement in the other direction; a "walker" to untie plot bundles, hand trees to the planter, assist in alinement and watch for plot and replicate changes; a "trouble-shooter" to walk close behind with a spade and replant occasional trees, to load trees on the planting machine and to map. With an inexperienced crew or under difficult conditions it is best to have a fifth man who maps and supervises.

A constant tractor speed of 1½ miles per hour is used which permits a comfortable planting rate of one tree each 6 seconds. It has proven best to maintain this rate and to stop only when absolutely necessary. Some mistakes such as planting the labelled tree in the second rather than the first position in a plot are overlooked because their only consequence is a slight inconvenience when mapping. Most other mistakes, if not too frequent, can be corrected by the trouble-shooter who follows the planter. Complete stoppage of the entire planting operation does in itself cause confusion, and is limited to correction of persistent mis-planting practices or to mistakes which cause erroneous identifications. With a good crew such stoppages occur only once each 1,000 to 2,000 trees.

The detailed procedure is as follows. In this example I assume that the trees are spaced 8 feet apart in 4-tree plots and that the plantation is 768 feet (= 8 feet × 4 trees × 6 plots × 4 replicates) long in the direction of tractor travel. Four replicates are to be planted simultaneously. (1) Establish a base line parallel to the direction of tractor travel. Place single stakes at 8-foot intervals, double stakes at 32-foot intervals to mark plot boundaries and extralarge stakes at 192-foot intervals to mark replicate boundaries. (2) Unload four replicate-bundles between the second and third replicates, place in the same order as the replicates to be planted, and open them. (3) Place 6 plot-bundles from Replicate No. 1 in Planting Tray No. 1, and 6 from Replicate No. 2 in Planting Tray No. 2. Drive to edge of plantation to start planting. (4) Remove a plot-bundle at random from Tray No. 1, untie the bundle, and plant the labelled tree opposite the first double stake. Plant the remaining three trees opposite the next three stakes. (5) Proceed to the next five plots. (6) After 192 feet remove a plot-bundle at random from Tray No. 2, untie the bundle, plant the labelled tree opposite the replicate stake and the remaining three trees opposite the next three stakes. (7) Plant the next five plots with plot-bundles taken from Tray No. 2. (8) Load Tray No. 1 with 12-plot-bundles from Replicate No. 3 and load Tray No. 2 with 12 plot-bundles from Replicate No. 4. (9) Proceed with planting. Use plot-bundles from Tray No. 1 in Replicate No. 3 and from Tray No. 2 in Replicate No. 4. (10) At the end of the row turn around and reverse the direction of planting. On the return trip remove plot-bundles first from Tray No. 2 and then from Tray No. 1. Plant the labelled tree last in each plot. At the center line reload for Replicates 2 and 1. (11) Occasionally move the stakes marking plot and replicate boundaries so that they remain visible. (12) When a replicate-bundle is finished, start a new replicate immediately, leaving no plots vacant. Slight extra care in loading and mapping is necessary when completing irregularly shaped replicates. (13) Plant the border rows. (14) Map the plantation, including border rows. Mapping may be done at odd intervals.

In most machine-established plantations, the planting rate has varied between 500 and 700 trees per hour for a 4-man crew, including cleanup and mapping. Results have generally been as good as or better than obtained with hand planting on similar sites.

Replacement

First-year losses were replaced in most plantations made from 1960 through 1962, using specially reserved planting stock of the same seedlots. This was very laborious and required that each nursery experiment be maintained for an extra year or two. In some experiments with limited numbers of trees, the need for replacement stock prevented full use of experimental material in the original plantations.

At best replacement could not be handled as methodically as the original planting. Thus mortality was usually higher — sometimes very much higher — than among the original trees. The living replacements filled vacant spaces and thus fulfilled part of their purpose. However, they have rarely grown the same as the original trees and thus must be separated or ignored during measurement. Thus, on the whole the results have been disappointing.

Starting in 1963 we adopted another philosophy — mortality is avoidable and every effort should be made to insure success of the original trees. That is, the work must be done correctly the first time or an experiment will be wasted. Gradually the new philosophy has become more realistic and the quality of work has improved. It has become much easier to plan the nursery and field planting work when no replacements are to be made. Overall results have been better when the philosophy of good first-time work has been followed.

Accomodation to Other Designs

The quoted examples are in terms of a randomized complete block design, with each seedlot represented once in each block. That is the most generally applicable design and can be made to give as great precision as a lattice design if appropriate corrections are made for the location of seedlots within replicates. That is a laborious process but no more laborious than the statistics connected with a Lattice design. Also, the extra work may not be needed.

Latin squares, which can be used only with small numbers of seedlots, cannot be established with the methods outlined here because of the need to plant each seedlot in a pre-determined location.

There is no such need in incomplete block or lattice designs, which can be used with the present techniques. The major procedural change would be in the nursery packing operation. After the trees were lifted and tied in plot-bundles it would be necessary to refer to a master-sheet and determine the appropriate block-bundle in which to place each plot-bundle. Planting and mapping could proceed in the same manner as for a randomized complete block experiment.

Summary

Two new methods of establishing experimental forest genetics plantations are described. They have been tested under a variety of field conditions during the past 8 years. The methods offer a saving in time without a sacrifice in quality of work.

Careful preparation of the planting stock is necessary when establishing a plantation by hand. Trees are labelled, lifted from nursery seedbeds, and packed in plot-bundles which are grouped into replicate-bundles. Each replicate-bundle is packed separately. In the field one replicate-bundle is opened at a time and the plots are planted in random order, to be mapped later. To facilitate later study, all plots are made of uniform size and shape and the trees are planted in straight rows in both directions. The best alinement methods are discussed. Planting can proceed at the same rate as in ordinary commercial practice.

Machine establishment of much-replicated small-plot experiments has proven successful under all conditions suited to the use of machines for commercial planting. Planting stock is prepared in the same manner as when planting by hand. With little practice it is possible to plant at the rate of 500 to 700 trees per hour and obtain near-perfect alinement. The quality of planting is often better than by hand.