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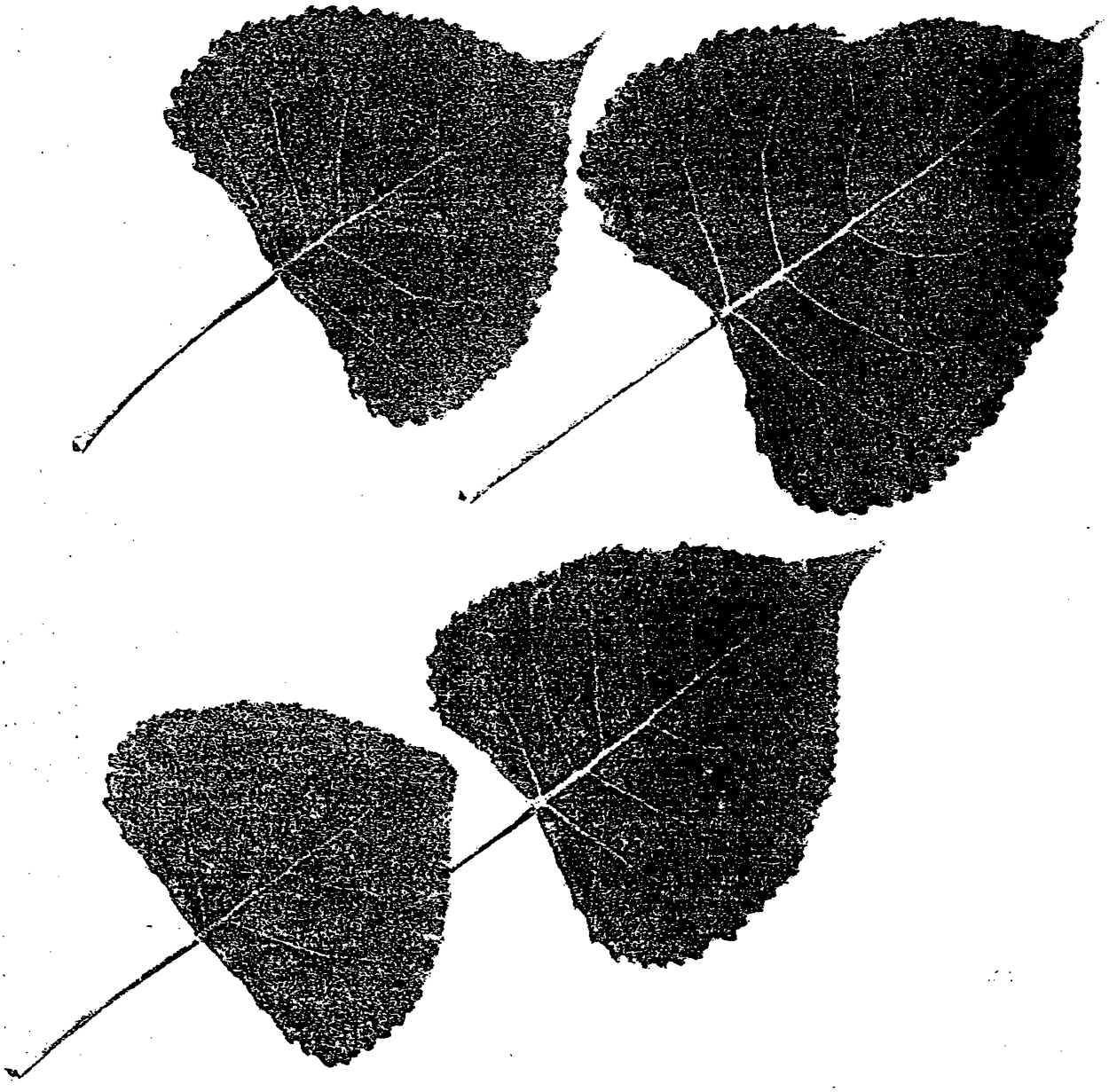
Report NC-81

Methods of Rapid, Early Selection of Poplar Clones For Maximum Yield Potential:

A Manual of Procedures



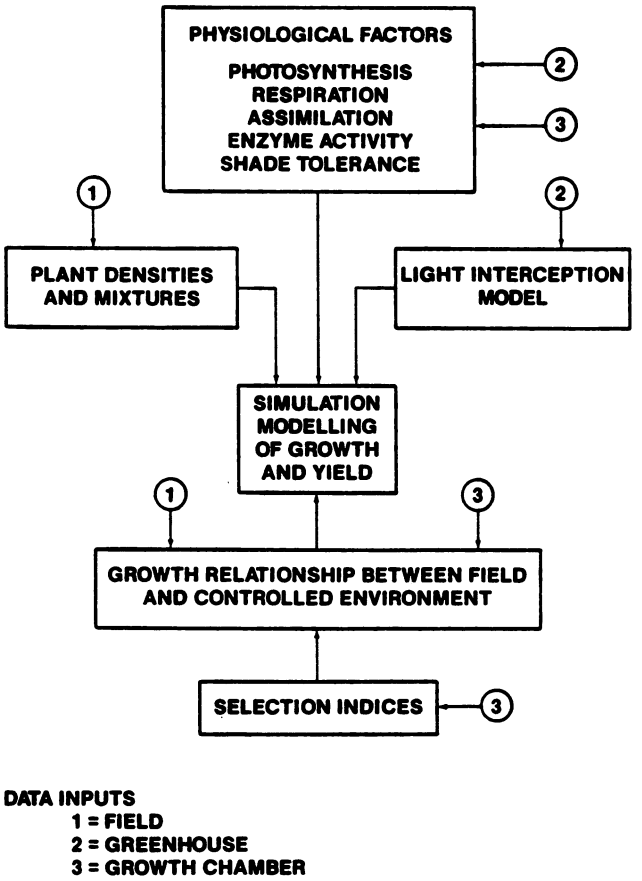
FOREWORD

Since 1920, large increases in productivity have been achieved with agricultural plants such as corn, but no comparable increases have been attained with woody plants. Thus, in the last 10 years, increased attention has been focused on the need for more intensive silvicultural practices to increase fiber production per unit area of land.

Populus clones are currently being examined for use in intensive silvicultural systems because of their rapid growth, ease of propagation, and high utility for a variety of wood-fiber products (Schreiner 1959, Cram 1960, Larson and Gordon 1969, Dawson and Hutchinson 1973). Because more *Populus* species and variants are available than can be reasonably field tested, a rapid technique for selecting superior clones must be devised. A desirable technique must be simple and fast, in contrast to field-growth studies that might take from 3 to 20 years and would occupy large areas. If controlled-environment growth studies and physiological indicators can be used to select genotypes capable of rapid growth, field trials can be smaller, with attendant savings in time, effort, and money.

The chances of successful early selection of poplar clones are enhanced by the genetic constancy of clonal material, by the great amount of knowledge about poplars and their culture already accumulated, and by the well-defined cultural conditions and relatively short rotations used in the intensive silviculture systems now emerging (e.g., Larson and Gordon 1969). Intensive culture and short rotations, particularly, improve the chances of successful selection because environmental variation and time are both small.

For maximum efficiency, early selection systems must be integrated with overall yield improvement efforts. Selection systems should also be capable of continuous improvement while in use. The early selection methods described here are to be used in the yield improvement model illustrated below.



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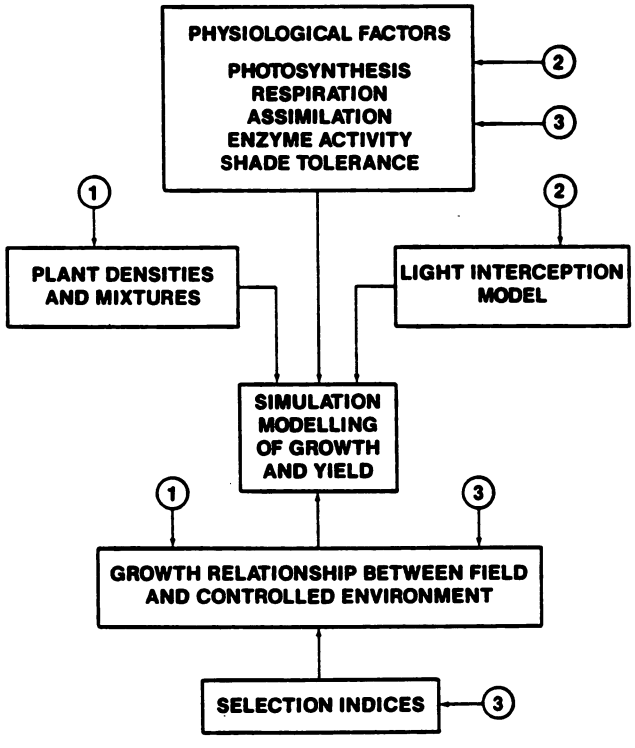

DATA INPUTS
1 = FIELD
2 = GREENHOUSE
3 = GROWTH CHAMBER

Described herein are methods of propagation, culture in controlled environments, and construction and testing of selection indices for poplar clones. The next obvious improvement is the use of physiological, as well as growth, measurements to improve the predictive capability of selection indices. Such possibilities for poplars have been examined (Gordon and Promnitz 1976) but will not be discussed here.

The primary objective of this manual is to describe, for researchers in intensive poplar culture, ways of using controlled environments to select clones that have a high probability of rapid growth in the field. Our system has some limitations: We have no guidelines yet for early selection for insect and disease resistance, nor for resistance to extreme environmental stress (late frost, drought). We can identify clones with high growth potential relative to the tested group of clones and we can make probabalistic statements about the stability of performance across environments for tested clones. **J. Gordon and L. Promnitz**

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PROPAGATION OF POPLAR CLONES FOR CONTROLLED-ENVIRONMENT STUDIES

R. Faltonson, *Research Facilities Supervisor, Forestry, Iowa State University, Ames, Iowa,*
D. Thompson, *Research Assistant, Department of Forest Science, Oregon State University, Corvallis, Oregon,*
and **J. C. Gordon,** *Head, Department of Forest Science, Oregon State University, Corvallis, Oregon*

Producing *Populus* clone materials for research necessitates an efficient, reliable, uniform system of vegetative reproduction: efficient, in the sense that there be little time lag between demand and material availability; reliable, in that materials should be available with reasonable expectation of success; and uniform, in that growth studies should not have to cope with a great deal of variability in plant size, form, and vigor.

We have developed a system using greenhouse culture of stock plants, and an intermittent-mist system for vegetative propagation of softwood tip cuttings. The system described here may prove practical not only for research, but for nursery production and forest industry intensive-culture systems. We have also begun development of a tissue culture, nutrient-film technique method of poplar propagation, which provides savings in propagating "easy" poplars, and hope for mass propagation of "difficult" ones.

Intermittent-Mist Facilities

Propagation of leafy cuttings requires that a high-humidity environment be maintained. Without such an environment leafy cuttings quickly become stressed, and fail within minutes.

The intermittent-mist system maintains a film of water on the leaf surface from the time of sticking (placing new cuttings in the mist bench) until roots are well established. An intermittent mist is desirable to reduce excessive leaching of nutrients from the leaves that would result from a continuous mist. The system is composed of a bench, water source, line strainer, solenoid valve, mist line, nozzles, and a control unit.

1. We use elevated **benches** made with asbestos bottoms and sides, supported by a galvanized pipe structure. This construction is preferred over wood because of the continual wetting of the surface from the mist, and because algae accumulations can be more easily cleaned from the metal and asbestos surfaces. The pipe frame also allows better air circulation under the benches.

Bench dimensions can be adjusted to meet the needs of the operation, size of the propagation area, available water pressure, and spread of the mist nozzles selected. In a 20- x 20-foot greenhouse bay, three benches (18 x 2.5 x 0.5 feet) will fit nicely. This provides ample working space between benches and adequate access to the perimeter of the benches. A bench height of 45 inches provides a comfortable work area.

2. The **water source** is important; water high in calcium and magnesium salts will result in unsightly and perhaps detrimental accumulations on leaf surfaces. Although often not economically practical, steam distillate as a by-product of the greenhouse heating system makes an excellent choice. Where standard water sources are unacceptable, another alternative is to construct a rainwater impoundment. Water cycled through a water softener should not be used.

3. **Line strainers** with 100- or 200-mesh screens should be placed in between the water source and any booster pump, solenoid valve, or mist nozzle. The strainer filters out damaging particles and thereby protects mist system components.

4. The **solenoid valve** is an electrically activated valve in the water line. Its function is to provide an intermittent flow of water, and it is interconnected with the control unit. Two types of solenoid valves are available: normally open and normally closed. The normally open type is preferred if power interruptions are expected. Because it opens when the current is interrupted, a power failure will not desiccate the cuttings—the mist will run continuously

as long as the power is off. Waterproof solenoid valves are also desirable. This can be accomplished by covering the valve coil with a silicone sealer. The solenoid valve should be located below the level of the mist line to avoid dripping of the mist nozzles during the off cycle.

5. The **mist lines** may be made of iron pipe, PVC (plastic), or copper. The lines should be of sufficient diameter to serve the pressure needs of the system. Several commercially available mist systems may be selected, depending on preferences dictated by use, durability, and initial cost.

PVC has become increasingly popular because of its ease of assembly and low cost. Iron pipe is difficult to fit. Copper tubing is initially more expensive, but is durable, fairly easy to work with, and is essentially trouble-free. Also, copper fittings are readily available through local plumbing supply outlets.

6. Two general types of **mist nozzles** are available: deflection and oil burner. Deflection nozzles operate by water striking a flat surface, which may be simply a wire located above the orifice, or a solid metal or plastic deflection plate. Several types of deflection nozzles are available. Some are specially constructed for PVC systems, and all are relatively trouble-free. Oil burner nozzles work on a principle of whirling the water through the orifice. This type, though often more expensive, is very durable, particularly when equipped with a stainless steel tip. The oil burner nozzle is also better suited for situations requiring water conservation, since the deflection nozzle uses considerably more water. Oil burner nozzles work well at 30 psi, while deflection nozzles may require 50 to 60 psi to obtain the fine mist desired.

The nozzles may be placed on the mist line in a number of ways. Some are positioned on risers sufficiently above the line to clear the plant materials, while others may be positioned on overhead lines directed downward. Another method is to elevate the mist line, with the nozzles teed from the line upward, to provide a uniform umbrella of spray over the bench surface. The line can be located approximately 10 inches above the bench, with the tee and nozzle extending an additional 5 inches. This provides excellent access to the bench without interference from lines and nozzles. It allows for maximum distribution of the mist spray, and the line is easily reached for maintenance without disturbing plant materials on the bench. Overhead lines with nozzles directed downward tend to drip more freely, and the mist line must be recharged with water with each "on" cycle because of water lost through dripping. This causes problems with uniformity, because the nozzles are activated beginning with the nozzle closest to the water source and progressing outward.

Depending on the type of mist nozzle selected, nozzles should be spaced from 2.5 to 3 feet apart. The primary consideration is to provide uniform coverage over the surface to be misted. Natural drafts and drafts associated with the heating and air circulation systems may make closer spacing more desirable.

7. The **control unit** must regulate the mist cycle so that leaf surfaces of the cuttings are wet at all times. Five general types are available: (a) electronic leaf, (b) thermostat, (c) counter- balance, (d) photoelectric, and (e) clock.

The electronic leaf is a sensor made up of two electrodes. As the water film evaporates from its surface, simulating a leaf surface, the electrical current between the electrodes is broken, activating the mist cycle until the surface is wet again.

A **thermostat** control monitors the temperature of the leaf surface. As the water evaporates, the temperature rises to a critical level, activating the mist cycle.

The **counter-balance** control is regulated by a simulated leaf surface, normally a wire screen surface. This is counter-balanced with a weight at the opposite end; the assembly is then hung on a fulcrum with a mercury switch to activate the solenoid. As water evaporates from the screen, the imbalance causes the weight to shift, allowing the mercury switch to turn on the mist system. Algal and metallic salt accumulations cause this system to lose its precision over time and periodic cleaning of the screen is necessary.

Photoelectric cells work on the relation of light intensity and evapotranspiration—the higher the light intensity, the more frequently the mist cycle is activated. They are used, but not commonly.

Control units utilizing **clocks**, which may be hooked together to provide just about any uniform mist cycle over a 24-hour period, have been successfully employed by many propagators. Normally two clocks are hooked together to provide on and off periods for day and night, and mist cycles within the on period of about any duration desired. This system has been employed by Iowa State University researchers and has proved most reliable. One time

clock is used for the 24-hour day ight sequence with the second clock providing 30-second mist bursts.

Different mist cycles will be dictated by environmental factors such as relative humidity, light intensity, and temperature. Closer frequencies, even continuous mist, may be required during summer months. Fresh, succulent cuttings may require a continuous mist for the first 2 or 3 days during daylight hours. High temperatures and light intensities in late spring and summer cause stresses on the fresh cutting that must be neutralized. *Populus* cuttings do not appear to be severely affected by nutrient leaching that would otherwise make a continuous mist undesirable. For most circumstances, a cycle of 30 seconds on and 30 seconds off is adequate.

The interval between mist bursts may be lengthened during winter. Thirty seconds on and 2.5 minutes off has worked successfully, but propagators must vary the cycle according to weather and condition of cuttings.

Misting during the dark should be avoided when possible to prevent disease. In practice, however, dark-period misting may be necessary, particularly during the winter when the heating system can lower relative humidity considerably. One or two mists during the dark period is ordinarily sufficient.

The primary purpose of the intermittent-mist system is to allow as much light as possible without stressing the cutting. However, shading may prove desirable during high light intensity periods of early summer. Shade cloth can be hung above the bench, but it should be high enough to ensure good air movement within the cuttings. Incandescent lights to extend the photoperiod to 18 hours are also used.

Special circumstances, or individual preferences, may require mist durations of less than 30 seconds. For example, cuttings propagated with flower buds intact, although ordinarily not acceptable, may be needed for hybridization studies. A 30-second mist provides too much moisture and will damage the catkin. An additional timer allowing 1- or 2-second bursts can be added to the system to accommodate such special situations.

Bottom heat provided by thermostatically controlled soil cables is helpful, especially during winter. Air temperatures of 21 to 24°C and bottom heat temperatures of 3°C higher than air work well. The bench temperature does not appear significant during late spring and summer propagation periods. This is probably due to the propagation medium absorbing heat and naturally retaining somewhat more warmth than the surrounding environment.

Stock Plants

A reserve of stock plants must be maintained in the greenhouse to supply cuttings. A schedule should be established to provide suitable cuttings whenever they are needed.

A good cutting comes from a vigorous stock plant showing no signs of disease or serious insect infestation. Although succulence leads to susceptibility to pathogen attack, it is the nature of *Populus* that vigor and succulence are virtually inseparable. Because some succulence will have to be accepted, lateral cuttings are preferred over apical. The carbohydrate-to-nitrogen ratio has been suggested as the primary influence related to succulence and rooting ability. High nitrogen and low carbohydrate concentrations provide soft, succulent tissues that often develop stem rot. This may be offset somewhat by using lateral shoots as previously suggested, by reducing fertilization somewhat, and by reducing the amount of water provided the stock plant.

To provide a continuous supply of cuttings through the year and to maintain stock plant vigor, a rotation schedule should be followed. Stock plants are most productive if changed every 4 or 5 months. The 4-month schedule offers the most cuttings over time, with the 5-month schedule extending the use of the stock plant for one more cutting collection.

A 4-month rotation can be set up to provide cuttings every 3 weeks throughout the year. This will accomplish the objective of having plant material available for propagation without undue delay. The 4-month rotation involves eight actual rotations within a 12-month period. The sequence of 4 weeks, 6 weeks, 3 weeks, and 3 weeks, illustrated below, is standard. The number of rotations can be adjusted to fit the needs of the operation.

- Week 1—Start cuttings to be used for rotation I.
- Week 4—Pot rooted cuttings as stock plants for rotation I. Grow for 6 weeks, pruning lateral branches as needed to provide a single stem. Height at 6 weeks should be 60 to 80 cm.
- Week 10—Decapitate approximately 20 cm from stem to force lateral growth on rotation I.

- Week 13—First cuttings should be available from rotation I plants. An average of seven cuttings per stock plant can be expected from this first cutting. (See section on clonal differences.) At this point the stock plant should be pruned to two to four strong laterals. The selected laterals must be headed back to approximately 15 cm, with four to five available buds per lateral for forcing a second cutting.
- Week 16—Second cutting of rotation I. An average of 10 cuttings per stock plant can be expected. At this time the stock plant can be cut back to approximately 25 cm, with any lateral shoots below this height pruned off, provided there are buds remaining for additional growth. This will allow a few more cuttings in another month. The stock plant is preferably discarded at this point, in favor of the better cuttings that will be available in 3 weeks from rotation II plants.

Annual Rotation Schedule

Rotation I	Rotation II
Jan. 1—Start cuttings	Feb. 15—Start cuttings
Feb. 1—Pot rooted cuttings	Mar. 15—Pot rooted cuttings
Mar. 15—Decapitate	May 1—Decapitate
Apr. 7—First cuttings	May 21—First cuttings
May 1—Second cuttings	Jun. 15—Second cuttings
Rotation III	**Rotation IV**
Apr. 1—Start cuttings	May 15—Start cuttings
May 1—Pot rooted cuttings	Jun. 15—Pot rooted cuttings
Jun. 15—Decapitate	Aug. 1—Decapitate
Jul. 7—First cuttings	Aug. 21—First cuttings
Aug. 1—Second cuttings	Sep. 15—Second cuttings
Rotation V	**Rotation VI**
Jul. 1—Start cuttings	Aug. 15—Start cuttings
Aug. 1—Pot rooted cuttings	Sep. 15—Pot rooted cuttings
Sep. 15—Decapitate	Nov. 1—Decapitate
Oct. 7—First cuttings	Nov. 21—First cuttings
Nov. 1—Second cuttings	Dec. 15—Second cuttings
Rotation VII	**Rotation VIII**
Oct. 1—Start cuttings	Nov. 15—Start cuttings
Nov. 1—Pot rooted cuttings	Dec. 15—Pot rooted cuttings
Dec. 15—Decapitate	Feb. 1—Decapitate
Jan. 7—First cuttings	Feb. 21—First cuttings
Feb. 1—Second cuttings	Mar. 15—Second cuttings

This system conserves space, since there are never more than two sets of stock plants on hand at any time. As the second cutting is made, stock plants can be discarded and replaced with materials scheduled from the mist bench. It should be noted that there is a 1-week discrepancy between the propagation date for stock plants and first cuttings available from two rotations behind. For example, cuttings for January 1 (Rotation I) must be taken from cuttings scheduled for January 7 (Rotation VII). Ordinarily, there are enough good cuttings after 2 weeks' growth to provide for the few that will be needed for replacement stock plants.

Rotations such as this have been kept active for several years with no apparent degeneration of the clone. However, it is a good practice to annually renew each of the stock clones using cuttings obtained from field-grown clonal orchards. Care should be taken not to introduce pathogen infections from field-grown materials.

The rotation described has some inherent unpredictability. The average number of cuttings taken from either the first or second propagation is usually as indicated. But there may be times when more stock plants will be necessary to ensure an adequate supply of good material because the number of good shoots for cuttings is less than expected. Generally, it is a good idea to have as many stock plants on hand as space permits. This is especially true when preparing for field studies where scheduling may be critical.

Most of the principles of rotation scheduling can also be applied to field-study scheduling. The field study is especially critical, because planting dates are often unyielding. The first rule in field studies is not to rely on hardwood cuttings as a shortcut for stock plant material. Hardwood cuttings may be rooted and in cutting production 6 weeks before the standard stock plant is ready, but growth is often much more procumbent, requiring two or three stakes per pot. The lateral regeneration, after cutting back to force growth, is markedly slower due to a much poorer root system. Finally, the cuttings themselves are often too succulent from trying to push things too fast culturally.

A production schedule for field planting may look something like the following:

March 1—Start cuttings to be used as stock plants
April 1—Pot rooted and weaned cuttings as stock plants
May 6—Cut to force first shoots
May 19—Cut to force second shoots
June 6—Take cuttings
June 20—Cuttings rooted, begin weaning
June 26—Cuttings ready for planting

One final note on stock plants: As may be evident from the above schedule, growth is often somewhat faster during spring than during the rest of the year.

Rooting Medium

A rooting medium should be porous enough to allow good aeration, uniformly retentive of moisture, and physically able to support the cutting. A great variety of plantable containers satisfy these criteria. The Jiffy-7[1] peat pellet is one of these, and has provided excellent results for *Populus* cuttings.

The Jiffy-7 (No. 700) is composed of peat with a small amount of added nutrients, bound in a plastic mesh net. It stores as a wafer 1-3/4 inches in diameter by inch thick. This saves storage space in the greenhouse. When water is added it expands to 1-3/4 inches in diameter by 2 inches thick. The pellet has a pH of 5.5 to 6.0.

The real advantage in rooting cuttings in the Jiffy-7 is that the complete unit is plantable upon root initiation. The rooted cutting can be moved from the bench to the pot or field location with very little root disturbance, thus avoiding much of the transplant shock associated with bare-root methods.

The propagation bench should be filled with horticultural, coarse-grade perlite. This may also be used as a rooting medium for special use situations where a plantable container is not desirable. In addition, the perlite provides a sterile bed for the cuttings in Jiffy-7's to rest on. The emerging roots can continue into the perlite without danger of desiccation.

Making and Rooting Cuttings

Three types of cuttings are associated with propagation of woody plant materials: (a) softwood, (b) semi-hardwood, and (c) hardwood. Each requires a different technique, although much of the operation is the same.

Softwood cuttings, sometimes called "greenwood" or "softwood tip" cuttings, are used in the majority of research applications. Studies involving top growth comparisons where form and initial uniformity are important require softwood cuttings.

[1]*Mention of trade names does not constitute endorsement of the products by the USDA Forest Service.*

Attention must be given to propagating in a sanitary environment. The bench and perlite should be sterilized with a dilute household bleach solution prior to setting out the Jiffy-7's. The peat pellets are placed on the bench after running the mist system for an hour or two to leach out any remaining bleach. The peat pellets should be spaced to allow plenty of room for air circulation between the cuttings and to minimize leaf overlap after the cuttings have been stuck. As an example for spacing, a bench 2.5 x 18 feet will accommodate 700 peat pellets. This provides 9.25 square inches per peat pellet, or one pellet in the center of a 3- x 3-inch area. Adequate space is extremely important, since closer spacing almost invariably results in pathogen problems, particularly stem rot.

After the peat pellets have been placed, the mist system is again turned on to a constant mist and allowed to run for another hour or two until the Jiffy-7 is thoroughly expanded and uniformly wet. The peat pellets are then dibbled, being careful not to insert the dibble much more than three-fourths of the way through the pellet. Otherwise, the cutting may be inserted through the pellet, producing an undesirable rooting environment. Dibbles made of nylon are available through horticulture suppliers. The nylon keeps the peat from sticking to the dibble, thus avoiding tearing up the pellet. The dibble should be inserted and given a slight twist before extracting.

After the pellets have been dibbled, a drench of Captan wettable powder fungicide should be applied. Use 8 teaspoons per gallon of water per 100 square feet, and apply over the surface of the pellets and entire bench surface using a sprinkling can. The propagation medium is now ready to accept the vegetative cuttings.

A few easily obtained items for taking (or making) cuttings should be assembled. These include single-edge razor blades, a rubber stopper (size 11 or 12), two plastic beakers (1,000 ml), and a lab cart for a working surface.

Razor blades are suggested rather than pruning shears, because they are sterile and provide a much cleaner cut. The cleaner cut allows less vascular damage, better water and nutrient uptake, and less general tissue damage that might provide pathogen entry. The razor blades should be changed at least every 50 cuttings, and even from plant to plant if there is any suggestion of disease in the stock plants. (Sometimes it may be necessary to propagate suspect

plants to find out if their disorder is pathogenic or physiological by growing new ramets.)

A rubber stopper is used as a backing to ensure a clean cut. The stopper tends to keep the plant, and not fingers, properly positioned for severing.

Two plastic beakers are used when there are two or multiples of two people making cuttings. One person prepares the cuttings and keeps them fresh by inserting them in a beaker containing water. The second individual takes this beaker (with 30 or 40 cuttings) to the mist bench and sticks the cuttings. The person making cuttings can then continue with the second beaker. The person sticking cuttings returns with the empty beaker, rinses it out and refills it with fresh tap water. The use of water in a beaker is contradictory to some sanitation principles. But, due to the extreme succulence of most *Populus* cuttings, methods such as wet toweling do not work well. The fresh cuttings will droop from wilt in a very short time if not kept in water. Working with clean hands and not smoking while taking cuttings are other important sanitation axioms that should be observed.

The lab cart provides a handy place to keep the razor blades and beakers. The cart can also be used as a receptacle for leaves trimmed from the cutting. Trimming the cuttings over the cart is more convenient than cleaning the floor after the job is finished.

The cuttings should be taken when possible from lateral growth on the stock plant. Terminal cuttings may root, but generally do not make good propagation material. If the rotation schedule for stock plants is followed, the first and second cutting dates will only have lateral growth available anyway. A 4-inch cutting is normally selected, although sometimes during early summer a 3-inch cutting may be preferred. The shorter cutting has less tendency to stress with the accompanying droop. The smaller cuttings will survive this stress better, have less overlap in the bed from drooping, and stand up under the rigors of field planting more satisfactorily. In either case, leaves should be removed from approximately two-thirds of the stem, retaining the leaves at the apex. The pruning of leaves should in most cases be done with a razor blade. The removal of leaves reduces transpiration and limits leaf overlap in the bench. Overlap should be avoided as much as possible because the mist may not reach some leaves and leaves sticking together often decay.

Cuttings should be taken, whenever possible, in the morning when the stock plants are fully turgid. This usually means sometime prior to 10:00 a.m. An alternative is early evening or at night, providing the stock plants have been watered and are not under any transpiration stress. Cool, cloudy days are also good for making cuttings.

Usually, however, the mist system should be operating during sticking. On cloudy, cool, humid days one may stick the cuttings without the mist system being on, except while the "sticker" is out of the propagation bay collecting another batch of cuttings.

In sticking the cuttings, the peat should be firmly tamped around the stem of the cutting. It is important that there be good contact on as much of the stem within the pellet as possible.

Our experience has shown that with softwood cuttings, with few exceptions, no hormone treatment is necessary. In fact, treating with hormones often results in stem burn and accompanying rot. One exception may be the Crandon clone (NCFES 5339). Crandon can be propagated without hormones, but seems to respond favorably to a 500-ppm IBA solution applied as a dip.

There is evidence that Captan, used as a fungicide, may have an additional benefit to the cutting. With some species, fungicides and particularly Captan, seem to have a hormonal effect in stimulation of rooting.

Rooting for most clones will take place in about 2 weeks. It is preferable to allow 3 weeks before transferring to the container. Some clones, such as NCFES 5323, 5377, and 5339, may need 3 weeks to properly root. This may vary with rooting conditions. Generally, one should allow an additional week to wean the cuttings from the mist. Weaning involves reducing the mist frequency gradually down to once every 15 minutes. For example, change from 30 seconds on and 30 seconds off, to 30 seconds on and 5 minutes off for the first 2 days. The second 2 days increase to 10 minutes off. Increase to 15 minutes off for the last 2 or 3 days.

If the cuttings are going to the field, this hardening-off process should be given special attention. Be sure the cuttings will be able to survive before subjecting them to a field environment. Any shading that has been provided should also be removed for at least a week prior to field planting. Hardened materials, however, can be placed in the shade for a few days, such as under a greenhouse bench if such accommodations are available. A watchful eye and a fog nozzle can prevent damage to newly potted

trees retained in the greenhouse. This care is seldom practical in field plantings, which again emphasizes the need for special care in hardening materials for such use.

Semi-hardwood cuttings are actually stem-section cuttings that include at least one node and internode segment. These cuttings, if taken from actively growing greenhouse stock plants, will root easily. Their use is generally limited to studies where form is not a factor. Similar cuttings can be taken from field-grown trees during late summer or early fall. In this case, it is advantageous to use a rooting hormone. Such nongrowing cuttings will usually root, but there may be some difficulty in forcing new bud growth.

Hardwood cuttings can also be rooted under the mist. Late-season cuttings—taken when buds are swelling—may even require mist propagation. The advanced stage of bud development will usually result in the cutting leafing out before the roots are sufficient to support transpiration.

For hardwood cuttings it is desirable to either stick the cutting directly into the perlite, or partially bury the peat pellet containing the cutting. This allows better response from the soil cables. In some cases, when treated this way roots may emerge in 3 or 4 days. Ordinarily, if cuttings are taken during the winter months, there is no need for the assistance of a mist system. Cuttings may be stuck directly into the container or field-planted when conditions are favorable.

By far the most cuttings for research are established as softwood cuttings, unless a large cutting orchard has been established to provide hardwood cuttings for some field applications. Nearly 100 percent success can be expected from softwood cuttings propagated as described. As a cushion, an additional 20 percent can be started to allow for some selection at time of planting.

Outplanting

Assuming that the rooted cuttings have been adequately weaned from the mist environment, the next step may be planting them to establish a field study. Some protection should be provided in transportation to the planting site. If transported in an open truck bed, winds will severely desiccate and bruise leaves if the materials are not covered.

When planting the cutting it is important to be sure the root system is well distributed and not twisted. Otherwise, the young tree will develop roots that will encircle and eventually strangle the remaining roots. Poor root arrangement will also favor windthrow.

Where irrigation is available the trees should be "watered in". This helps to reduce stresses associated with transplanting and serves to settle the soil around the root system uniformly.

Clonal Variation

Clones used in vegetative propagation have shown variation in: (a) the number of available cuttings from the stock plants and (b) time required to root.

Clone	Cuttings Available Per Plant—First Cutting Mean number/stock plant	Range
5377	7.7	5-12
5321	6.9	5-10
5323	7.4	5-10
5328	6.1	5-10
5339	7.6	5-10
5260	9.4	6-13
5351	7.8	6-11

Clone	Cuttings Available Per Plant—Second Cutting Mean number/stock plant	Range
5377	11.1	7-16
5321	11.3	10-15
5323	10.4	8-13
5328	9.0	8-11
5339	11.9	8-15
5260	7.1	5-9
5351	9.0	6-13

Differences in time required to root are closely associated with rooting ability and general rooting success. Clone 5260 can be expected to root in 10 days and rarely has difficulty in getting established. Most clones will root in approximately 14 days. Clones 5323 and 5377 may lag behind by 5 to 7 days; they generally are more difficult to root, and have occasional stem rot difficulties. Clone 5377 is somewhat slower than 5323. Clone 5339 (Crandon) is the most difficult to root, although it seldom takes longer than 4 weeks. The difficulty with the Crandon clone is probably associated with its aspen and European white poplar parentage. Clone 4877, a *P. alba* clone, is very similar to 5339. Neither 5339 nor 4877 will root with much success as hardwood cuttings.

The intermittent-mist system provides the production manager a relatively simple way of producing a large number of genetically and physically uniform plants in a limited space. The simplicity of the system also allows minimally trained personnel to do the work with high probability of success.

Propagation of Poplars by Shoot Apex Culture and Nutrient Film Technique

Plant tissue culture offers a form of vegetative propagation that may be able to overcome some of the problems encountered in the standard methods of woody plant propagation. Shoots have been initiated on callus cultures of a number of woody plants. Several reviews are available (Durzan and Campbell 1974, Pierik 1975, Winton and Huhtinen 1976). The production of shoots from callus cultures, however, has several limitations. Shoots may appear irregularly and in limited numbers on the callus. For this reason, the production of large numbers of woody plants is not usually possible.

A second problem with shoots produced on callus cultures is that callus tissue in culture tends toward endopolyploidy (Murashige and Nakano 1965, Partanen 1963). This increase in the ploidy level in culture may result in the loss of totipotency, the ability of every individual cell in a plant to regenerate a complete plant. Perhaps more importantly, polyploidy may result in production of a plant genetically different from the original plant, and hence the loss of the originally desired characteristics.

The culturing of shoot apices has been used in the large-scale, clonal propagation of a wide variety of herbaceous, horticultural plants (Murashige 1974). By using shoot apices of Gerbera daisy, the production of 1 million plants from one original shoot apex in 1 year is possible (Murashige 1974). The shoot apex is perhaps the most totipotent part of the growing plant. The cells of the shoot apex are less differentiated and more uniformly diploid than those of most other parts of the plant (D'Amato 1952, Partanen *et al.* 1955). Thus, the production of only a few polyploid plants would be expected in plants regenerated from shoot apices, and this has been demonstrated (Hasegawa *et al.* 1973, Murashige *et al.* 1974).

Because shoot apex culture offers a system for the large-scale clonal propagation of herbaceous plants, we attempted to determine if the same basic techniques could be applied to the propagation of woody plants.

Culture medium

The complete medium consisted of an inorganic and organic portion. The inorganic portion was the basic Murashige and Skoog (1962) inorganic salt mixture. Phosphate was added to this (170 mg/1) in the form $Na\ H_2\ PO_4 \times H_2O$. The organic portion consisted of 0.4 mg/1 thiamine HC1, 80 mg/1 adenine sulfate, 100 mg/1 myo-inositol, 20 g/1 sucrose and 10 g/1 Difco Bacto agar. To determine the most effective hormone combination for the multiplication of shoots, 30 combinations of five levels of indoleacetic acid (IAA) (0.0 M, 5.7×10^{-8}M, 5.7×10^{-7}M, 5.7×10^{-6}M and 5.7×10^{-5}M) and six levels of benzylaminopurine (BAP) (0.0 M, 1.3×10^{-7}M, 1.3×10^{-6}M, 1.3×10^{-5}M, 6.6×10^{-5}M and 1.3×10^{-4}M) were tested.

The IAA and BAP were dissolved in water by heating in an autoclave for a few minutes and then added in the appropriate amounts to the medium to produce the 30 hormone combinations. The medium pH was adjusted to 5.7 with 1 N KOH or HC1. The sucrose and agar were then added and dissolved by heating in an autoclave, and 25 ml of the medium was poured into each 25- x 150-mm culture tube. The tubes were capped with Bellco Kaputs and all components were autoclaved together at 121°C for 15 minutes.

Plant material

Shoot tips of *Populus tristis* x *P. balsamifera* cv. Tristis #1 (NCFES 5260) were used in this study (Cram 1960). They were collected in mid-August from a plantation near Ames, Iowa. All leaves were removed and the shoot tips were washed in a detergent solution. Surface sterilization was found to be unnecessary. The bud scales and all but the last pair of leaf primordia were removed under a dissecting microscope. A shoot apex 1- to 2-mm tall was planted in each tube of culture medium.

Multiplication of shoots

Tubes containing the excised shoot apices were placed in a growth chamber with a day temperature of 24°C and a night temperature of 18°C. The irradiance was 90.0 microeinsteins/m^2 sec^{-1} and was supplied by fluorescent lamps for 14 hours of every 24.

After the first 2 to 3 weeks in culture, some of the apices had senesced and died, while others had grown to five to six times their original size. Some of the treatments stimulated the formation of small amounts of callus where the base of the original apex came in contact with the medium. After 3 to 4 weeks in culture, some of the apices initiated small green areas of organized growth on the callus. These areas continued to grow and became the sites for the formation of adventitious buds that developed into adventitious shoots.

These adventitious shoots occurred where treatment consisted of 1.3×10^{-7}M, 1.3×10^{-6}M, and 1.3×10^{-5}M BAP with 0.0 M, 5.7×10^{-8}M, 5.7×10^{-7}M, and 5.7×10^{-6}M IAA. The largest number of shoots occurred when the medium contained 1.3×10^{-6}M BAP and 5.7×10^{-7}M IAA.

This treatment produced an average of 8 to 12 adventitious shoots from each original shoot apex, and it was selected as the medium for all further shoot multiplication experiments. The adventitious shoots developed on the callus at the base of the original apex, and as they grew, they produced a mass of leaves that surrounded the original apex. These shoots typically grew as a whorl of leaves without well-developed stems. The stem internodes failed to elongate on the shoot multiplication medium, perhaps because of the high level of cytokinin.

These adventitious shoots could be divided and placed on fresh multiplication medium, which resulted in the initiation of new adventitious shoots without the formation of an intermediate callus. Again, the shoots consisted only of a whorl of leaves without well-developed stems.

Rooting of shoots

To induce root formation on the multiplied shoots, the basal medium was modified by the omission of both the adenine sulfate and the additional phosphate. Also the rooting medium did not contain any BAP. Six levels of IAA (5.7×10^{-7}M, 5.7×10^{-6}M, 2.8×10^{-5}M, 5.7×10^{-5}M, 1.1×10^{-4}M, and 2.8×10^{-4}M) were tested for their ability to initiate roots. Later, six levels of indolebutyric acid (IBA) (4.9×10^{-7}M, 4.9×10^{-5}M, 2.5×10^{-5}M, 4.9×10^{-5}M, 9.8×10^{-5}M, and 2.5×10^{-4}M) were also tested for their ability to initiate roots. Shoots to be rooted were placed in 117.6-cm^3 french square bottles containing 40 ml of rooting medium. Cultures were placed in a growth chamber with a day temperature of 24°C and a night temperature of 18°C. The irradiance was 125.0 microeinsteins/m^2 sec^{-1} supplied by a mixture of fluorescent and incandescent lamps for 14 hours of every 24.

When adventitious shoots were placed on a rooting medium containing either 5.7×10^{-7}M or 5.7×10^{-6}M IAA, without any BAP in the medium, the internodes of the stems began to elongate, producing normal-looking stems. None of these shoots, however, formed roots even after 5 weeks on this medium. IBA was tested because of its ability to stimulate roots on cuttings of many plants. In the presence of 4.9×10^{-7}M, 4.9×10^{-6}M, 2.5×10^{-5}M, and 9.8×10^{-5}M IBA, shoot internodes elongated and roots were formed. The 4.9×10^{-7}M IBA treatment resulted in formation of the most normal-looking root system, complete with lateral roots. A second rooting experiment was conducted to determine the optimal IBA concentration for root formation. IBA was applied at 0.0 M, 2.5×10^{-7}M, 4.9×10^{-7}M, 2.5×10^{-6}M, 4.9×10^{-6}M, 1.2×10^{-5}M, and 2.5×10^{-5}M. Again, the 4.9×10^{-7} IBA treatment resulted in the formation of the most normal-looking root system.

Once the roots begin to appear on the shoots on the rooting medium, they should be transplanted to minimize the damage done to the root system during transfer. Rooted shoots were removed from the bottle of rooting medium and, after the medium was washed from the roots, planted in a peat pellet placed under a shaded, intermittent-mist system in the greenhouse. In this way, plants could be "hardened off" gradually to the environment outside the culture bottle. Once plants were hardened off they were moved to a Nutrient Film Technique (NFT) (Cooper 1975) system for further growth.

Applications

Tristis #1 is routinely propagated by rooting shoot tip cuttings. In this way, one shoot tip cutting can produce one complete plant in 2 weeks. By using excised shoot apices, however, each Tristis #1 apex can produce between eight and 12 adventitious shoots in 4 to 6 weeks, each of which can be rooted to produce a complete plant. If the adventitious shoots are divided and placed on fresh multiplication medium instead of rooting medium, each shoot can produce another eight to 12 adventitious shoots. Thus, the number of shoots increases geometrically each time the shoots are subcultured.

In shoot apex propagation of Gerbera daisy, each subculture results in a fivefold increase in the number of shoots, and if the subcultures are made every 4 weeks, 1 million plants can be produced in 1 year (Murashige *et al.* 1974). Similar results have been obtained by using the runner apex of Boston fern (Burr 1976). One runner apex, subcultured three times during a 5½-month period, can produce 5,000 plants. Adding a fourth subculture and extending the time period to 6½ months allows production of between 10,000 and 20,000 plants, all from one original runner apex. Unfortunately, Boston fern has not been found to be very stable in culture for long periods. The longer it is kept in culture, the greater the chance that "sports" or mutant plants will appear. When

subcultured a fourth time, between 10 and 20 percent of the plants are mutants. This can be avoided simply by subculturing only three times and then starting with new cultures freshly initiated from excised runner apices.

The stability of each plant to be propagated in culture should be established before large-scale propagation is begun. Not all plants may exhibit the genetic instability of Boston fern; for example, there has been little evidence of the development of sports in the apex propagation of asparagus and Gerbera daisy (Hasegawa *et al.* 1973, Murashige *et al.* 1974). Even if sports do develop, it is a simple matter of subculturing less than the number of times when the sports become a problem. Apex propagation of Boston fern is currently done on a commercial scale in California; the cultures are subcultured only three times, and then new cultures are started. In this way, one nursery has been able to produce about 80,000 plants per month.

Because shoots are grown and multiplied in culture tubes 25 x 150 mm, a large number of shoots can be grown in a relatively small space. Cultures can be grown on shelves illuminated by fluorescent lamps mounted on the bottom of the shelf above. With this system, it has been estimated that a room 13 x 13 x 9 feet could contain about 10,000 tubes. In the propagation of Tristis #1, each tube would contain between 8 and 12 adventitious shoots, each capable of producing a complete plant. By using conventional shoot tip propagation techniques, a 13- x 13-foot space in a greenhouse could support between 150 and 200 stock plants, each of which could produce about 20 cuttings. By using shoot tip propagation, between 3,000 and 4,000 plants could be produced in this area. Shoot apex propagation using Tristis #1 has the potential of producing between 80,000 and 120,000 shoots after 4 to 6 weeks in culture without any subculturing.

Another advantage of shoot apex propagation is that because there is a multiplication of shoots in culture, fewer stock plants need to be maintained in the greenhouse. This lowers the cost of the greenhouse operation by reducing the amount of space taken up by stock plants.

The facilities for a shoot apex propagation operation consist of laboratory and greenhouse space. Ideally, the laboratory consists of separate areas for washing glassware, preparing media, transferring cultures, and growing cultures (fig. 1). The use of separate rooms for these operations greatly reduces the risk of contaminating the cultures. The glassware washing area should contain an autoclave, sink, dishwashing machine, and storage area for glassware. It should also be used in preparing plant material to be used to initiate cultures. The media preparation area should contain balances, refrigerators, a water deionizer, a pH meter, storage area for glassware and reagents, and bench space. The transfer room should have storage space for freshly prepared media and also a sterile area with either a filtered air or laminar air flow hood for initiating or subculturing cultures. The culture room should contain several sets of shelves with fluorescent lamps. Control of photoperiod and light intensity is useful. Each room should have separate controls for temperature, especially the culture room, because of the sensitivity of cultures to extremes in temperature. The greenhouse should have space for the maintenance of a few stock plants and also a shaded, intermittent-mist system for the plants after they have been removed from culture.

About 25 commercial nurseries in California presently use shoot apex propagation of herbaceous, horticultural plants. The smaller nurseries produce 10,000 to 20,000 plants per month, while the larger ones are capable of producing up to 100,000 plants per month. One of the larger nuseries propagating Boston ferns employs about 11 people and can produce about 80,000 plants per month. The initial cost

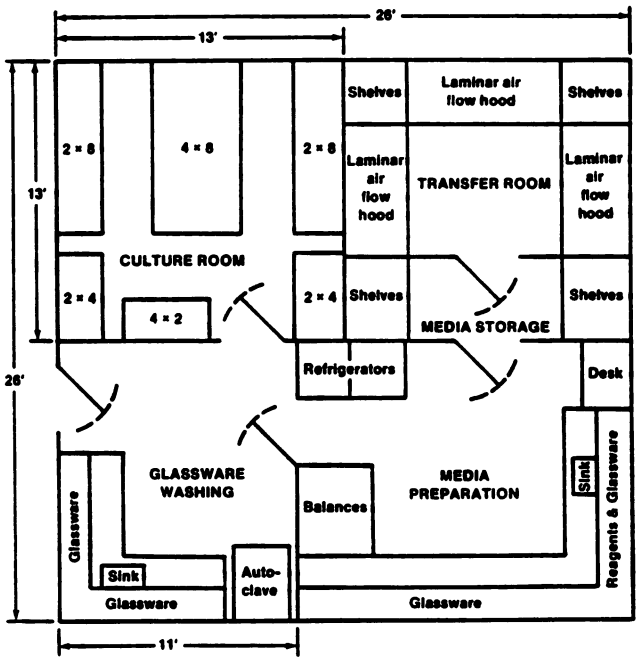

Figure 1.—*Floor plan of a hypothetical laboratory facility for the propagation of plants through shoot apex culture.*

for starting a small operation has been estimated at between $20,000 and $30,000, while a larger one would require between $60,000 and $100,000.

The low-capital-cost hydroponic technique known as Nutrient Film Technique (NFT) may have several advantages over other systems for further growth of the propagules derived from apex culture. This system is commercially used for the greenhouse culture of vegetable crops and has been advocated for production of ornamental nursery stock (Cooper 1975). Propagules in which root primordia have been induced are transferred to troughs containing trickling nutrient solution and grown until they reach a desirable size for field planting. The system is flexible in that propagules can be grown-on in containers such as Jiffy-7's or fiber blocks, or as bare-rooted stock. The latter option greatly reduces root malformation and binding that occur in container-grown stock. Bare-root plants grown in NFT gutters produce a uniform, continuous root mass which, when separated into individual plants, shows rapid root regeneration.[2] Plants could conceivably be shipped and machine-planted as a continuous strip, separated only before insertion into the soil. The advantages of NFT culture over other growing-on methods are:

1. Precise control of plant nutrition and control of diseases and insects are possible through the use of nutrient solutions containing systemic pesticides.

2. Continuous recirculation allows complete use of fertilizer and minimum evaporation loss of water.

3. Root temperatures can be precisely specified and maintained.

4. Optimal nutrition and root temperature result in better growth rates.

Gutter and other equipment for NFT system are now commercially available.

Another possible advantage of shoot apex propagation is the production of pathogen-free plants. In standard propagation of plants, the shoot tip is dissected down to an apex 1- to 2-mm tall, which consists of the apical dome and several pairs of leaf primordia. If the explant is made smaller (between 0.05- and 1.0-mm tall), a pathogen-free plant may be produced (ten Houten *et al.* 1968). The culturing of the apical dome alone makes possible the production of plants free from fungi, bacteria, viruses, viroids, mycoplasmas, spiroplasmas, and rickettsias. These organisms cause plant disease and thus restrict the growth and productivity of the host plant. The apical dome is one of the few parts of the growing plant that may be free of these organisms. Pathogen-free plants will grow rapidly after they are field-planted until they become naturally reinfested. The increased growth rate after planting, however, may be critical to the survival and subsequent growth of the plant. Plants so produced could also meet the pathogen-free requirements encountered in the international exchange of plant material, an important part of many tree improvement programs.

Apex propagation is usually easiest with both herbaceous and woody plants whose cuttings can be rooted without difficulty (Murashige 1974). This, however, does not mean that only "easy" plants can be propagated by these techniques. The shoot apex is probably the most totipotent part of the growing plant, second only to the embryo of the seed or a young seedling. Indeed, recent success in producing shoots from callus cultures of white spruce and Douglas-fir has depended on the use of excised embryos or hypocotyls of young seedlings (Campbell and Durzan 1975). The advantage of using excised shoot apices is that they can be collected from mature trees selected for their observable desirable characteristics. The use of excised embryos or seedling parts depends on the assumption that they will retain the desired characteristics of the parent tree.

Because of the totipotency of the shoot apex, propagation should be possible in a wide range of woody plants. With further experimentation, these techniques can possibly be applied to other hardwood species and perhaps, in time, to the conifers, for which standard methods of vegetative propagation have not been very successful.

Shoot apex culture offers the possibility of rapid, large-scale, clonal propagation of plants. This can be done in a relatively small amount of space, thus reducing propagation costs. However, the cost of propagating a particular plant by apex culture should first be compared with the cost of propagating the plant by existing vegetative means. Where the time required for a tree to reach seed-producing age is long and where standard methods of vegetative propagation may not be successful or practical on a large scale, tissue culture (in particular, apex culture) may offer a solution.

[2]*Personal communication with A. J. Cooper.*

CONTROLLED-ENVIRONMENT CULTURE OF *POPULUS* CLONES

R. Faltonson, *Research Facilities Supervisor, Forestry, Iowa State University, Ames, Iowa*

Growing *Populus* in the greenhouse presents some challenges not ordinarily faced in greenhouse production of horticultural plants. The forestry researcher is often confronted with problems more common to nursery container production than to typical greenhouse applications. The following sections describe equipment and procedures that have been used to successfully culture *Populus* clones in the greenhouse.

Facilities

Forest researchers at Iowa State University have use of 9,000 square feet of greenhouse space under glass and an additional 2,800 square feet of headhouse space. The portion under glass is divided into 14 bays of varying dimensions, with 7,600 square feet of usable space. The headhouse provides office, laboratory, storage, and utility space.

Each of the bays is equipped with individual coolers for ventilation and cooling, ridge and sidewall ventilation, unit heaters, perimeter radiation heat, overhead electrical unistruts, a concrete floor and gutter system, and movable benches. This equipment is described in detail below:

1. Evaporative coolers of varying sizes are provided individually for smaller bays, or in pairs for larger bays. Effective cooling from April through September is essential for maintaining temperatures suitable for plant growth. Poplars respond best to day temperatures of 75°F (24°C) and night temperatures of 65°F (18°C). The capacity of the coolers is frequently insufficient for maintaining these greenhouse temperatures when outdoor temperatures reach or exceed 85°F (29°C). Shading the glass with a spray-on compound is used to compensate, but temperatures during late June through August are ordinarily too high for studies involving growth responses.

2. Ridge and sidewall, manually operated, sash ventilation systems are essential for effective air circulation and cooling. Although the greenhouse was designed to provide adequate air exchange using shutter-like exhaust vents in the curtain wall, this has proved ineffective without supplementary shading. During summer the ridge vents are opened sufficiently to allow warm air to rise and escape, when the cool air from the evaporative coolers displaces it. The sidewall ventilators are rarely used, except when there is a cooler failure, requiring natural cooling of the bay.

3. Pressurized steam unit heaters are used during the cold months for a thermostatically controlled source of heat. The unit heaters are thermostatically interconnected with the cooling system to provide a reasonably constant temperature. Equally important is the function of the heater fan in providing air movement. The unit heaters are hung overhead (9 feet), with a horizontal air draft that provides critical air circulation during both summer and winter, although during summer the steam lines remain closed. During sub-zero weather this mixing property of the unit heater is especially evident. On days when outdoor temperatures drop to zero or below, the unit heaters are unable to provide sufficient air movement below the benches. Air stratification may result in temperature differences of 30 to 40°F (-1.1 to +4.4°C) from the bay floor to the bench level of 40 inches (100 cm). Adequate temperatures normally can be maintained at the plant level, but irrigation hoses will freeze at the floor level. This points out the importance of raised benches for plant growth under acceptable temperatures. The complication that arises from trying to compensate for stratification is that trees grown on the raised benches have limited space for height growth. They may either grow beyond the artificial light source, or if directly in the path of the unit heater, suffer desiccation from the warm air flow. Additionally, mite populations may prosper on tall trees in line with the warm air movement. This problem can be alleviated by growing trees during more favorable growing periods, or by keeping taller trees out of areas in the bay subject to this warm air blast.

4. Perimeter hot (finned radiation) heat is used as a constant heat source. The amount is regulated by an outside thermostat that calls for additional heat to supplement the unit heater when temperatures fall. Heat from this source alone is not sufficient to keep bay temperatures at a desired level. It does aid in supplying a more uniform heat for the bays and is the sole source of heat in the headhouse.

5. Overhead electrical unistruts provide structural support for artificial lighting, and electrical outlets for lights and instruments.

6. Floors are concrete with a gutter and sump system at the lower end of a slight slope to provide drainage of irrigation water. Although concrete makes maintaining humidity somewhat more difficult, this liability is more than offset by its ease of cleaning, an important factor in successful sanitation. Also it is easier to move benches on rollers on concrete than on gravel or packed earth.

7. Movable benches are used in most studies to facilitate changing bench patterns within the bays, cleaning bay floors, gaining access to overhead structures and equipment, and transporting plant materials from one location to another. Each bench is constructed of galvanized pipe with 5-inch casters for wheels. The bench top, a 4-inch-high box, is constructed of redwood with inside dimensions of 2.5 by 6 feet. The bench also has a redwood pallet at 12 inches above the floor, which is useful for hardening succulent materials in partial shade before placing them on top of the bench.

Sanitation

Proper sanitation is the key to any greenhouse operation. The following rules should be observed:

1. Keep floors clean and free from algae. Periodically scrub with soap and water, then hose with a high-pressure nozzle. When weather permits venting of sidewall and ridge, sanitize by scrubbing with soap and water; then apply a dilute bleach mixture (1 pint bleach to 8 quarts water) with a watering can.

2. Periodically scrub benches with soap and water. Follow up with a rinse of dilute bleach, allow to stand for a few minutes, then rinse off.

3. Keep leaves that have fallen into pots or bench tops picked up. Remove weeds that become established in pots or benches.

4. Avoid splashing soil particles around when cleaning or watering. (This is another advantage of elevated benches.)

5. Hang up hose ends. If the breaker comes in contact with the floor, be sure to clean it prior to using for irrigation.

6. Use sterile tools when mixing soils. A dilute formaldehyde solution (1:18) works well. Keep unsanitary tools and unwashed hands out of containers full of soil.

7. Use a sterile soil medium.

8. Keep pots and flats sanitary. Wash them thoroughly after use. Do not store cleaned containers near used and contaminated containers.

9. Provide good air circulation. This seems to be particularly important. For example, it is standard practice to have only 12 2-gallon (8-inch) containers on a 2.5- x 6-foot bench. Considerably closer spacing is acceptable when trees are getting started, but space them out amply when they begin touching each other. This not only provides a less desirable environment for pathogens, but aids in miticide application by allowing more thorough coverage.

Pest Control

The most sensible approach to greenhouse pest management is to effectively control pest populations while minimizing human health hazard. With this in mind, many of the traditional pesticides have been withdrawn from use with no apparent backslide in control effectiveness.

In *Populus* culture, spider mites are the major pest. Effective control can be accomplished with Pentac miticide (Pentachloro-2, 4-Cyclopentadien-1-y1). Dosage rates of 2 level teaspoons per gallon of water, plus an added surfactant such as Triton B-1956 spreader-sticker at teaspoon per gallon, have proved effective with no apparent phytotoxicity. Application with a 3-gallon, hand-operated pressure sprayer is adequate. However, a gasoline-powered KWH Knapsack Mistblower/Duster will improve coverage and reduce application time. Label regulations should be rigorously followed, of course.

Sanitation, pest control, and adequate air circulation are interrelated. During summer, whenever possible, remove all plant materials from a bay. Clean thoroughly as previously described, spray with an

ovicide, and allow the bay to "dry out" for 2 or more weeks.

Soiless Mix

Soiless mixes get around the problem of obtaining uniform soil from year to year, plus the associated complications of residual herbicides or high salt content in field soils. Originally, we selected a soiless mix using two parts Jiffy-Mix and one part coarse-grade horticultural perlite. We later tested this ratio against several other combinations of Jiffy-Mix to perlite (1:0, 1:1, 2:1, 3:1, and 0:1), with and without Mag-Amp slow-release fertilizer. The results showed clearly that a ratio of 2:1 with Mag-Amp is superior.

Soiless mixes provide the researcher with several advantages:

1. Precise repeatability of growth medium for research.

2. A sterile medium with no potential for herbicide, weed seed, insect, or pathogen contamination.

3. Expensive steam or electric sterilization equipment is unnecessary, because the mix is sterile when purchased.

4. Toxicity problems that often accompany conventional sterilization processes are avoided.

5. Ease in preparation, which again economizes labor and time.

6. Light in weight, allowing fairly large trees and containers to be moved without back-breaking effort.

7. Problems of salt accumulation are minimal due to the looseness of the mixture and free drainage.

8. Excellent, uniform air-water-particle relation.

9. Ease of storage. Roughly 50 8-inch containers can be filled with a single batch of soiless mix. This involves two bags of Jiffy-Mix and one bag of perlite. An adequate supply can be stored in a comparatively small space, without special holding bins as would be necessary for soil.

10. Season-to-season mix uniformity allows a standardized fertilization program. Trace elements, plus N-P-K in small amounts, are included in the mix.

The soiless mix is prepared by sterilizing a portion of a bay floor. Two bags of Jiffy-Mix and one bag of perlite are then dumped onto the floor. (Jiffy-Mix and perlite are obtained in 4-cubic-foot bags.) Terra-Lite brand perlite is used, because the coarse grade has larger particle size than some other brands. Perl-Gro is another brand that has been used. Some brands have a smaller particle size, in fact rather fine, and do not provide the proper knit. They tend to drain too fast as a mix, thus they do not retain nutrients well and must be fertilized more frequently. Medium granual Mag-Amp (7-40-6) at a rate of 2.95 Kg (6.5 pounds) per 12 cubic feet is then added to the pile. The components are then mixed thoroughly with a sterile shovel. Both the Jiffy-Mix and perlite have a lot of fine dust-like particles that can get into eyes or impair breathing. It is a good idea to wear a dust mask or respirator and goggles when pouring out and mixing the components. Also, any fans that could pick up the dust should be turned off.

Pots and Potting

After thoroughly blending the soiless mix components, pots and potting is the next consideration. A 1-quart aluminum scoop is ordinarily used, although a bleach bottle cut to a scoop shape also works nicely. Bronze screening material is cut into 1.25-inch squares and placed over the drainage holes of the containers. The copper in the bronze alloy helps keep the roots from escaping the pot when plants are grown for long periods. Aluminum screening can be used for short-duration studies, but should not be used for plants grown more than 2 months. Enough potting medium is put in the container to fill it to within 1 inch of the top. The peat fraction of the soil mix is difficult to wet, so at this stage, the container should be watered two or three times to settle the mix and provide uniform wetting. Settling should be to about 2 inches from the top of the pot, so that an adequate amount of water can be applied during irrigation to permit drain-through without floating out the perlite or washing out other medium components. Some of the soil should be scooped out (with clean hands) to provide a cavity for the young tree; after planting, water again lightly to settle the soil and clean up the container.

For most studies, and particularly for stock plants that may be grown 3 to 4 months, an 8-inch, 2-gallon plastic pot is suggested. Polyethylene field containers are light, durable, and reusable. Individual preferences vary concerning the use of plastic pots rather than clay. Although clay pots are porous, allowing

better aeration, they also dry out faster and tend to encourage root growth along the sides of the pot rather than uniformly throughout. With soiless mixes aeration is provided by the loose properties of the medium. Plants in clay pots require watering 1.5 to 2 times more often than plants of the same size grown in plastic containers (Ball 1975). All-purpose, all-weather, heavy-duty polyethylene containers are available in sizes ranging from 1 quart to 7 gallons. Having several sizes on hand will meet the changing needs of a research greenhouse.

Irrigation and Fertilization

Because of the loose properties of soiless growth media, watering is not as critical as it would otherwise be. But do not allow this to permit laxity in one of the most important phases of plant growth. Timely irrigation according to plant size, light, temperature, and air circulation is still extremely important. Ironically, one of the most difficult things for the greenhouse manager to get across to his or her help is the importance of proper irrigation. Here is where the free-draining property of the soiless growth medium is perhaps most valuable.

Too little water applied too frequently will not allow the soil to become uniformly moist. It also will reduce oxygen penetration to the root system. Enough water should be applied to allow a small amount to drain from the pot. This flushing will leach out any salt accumulation and ensure uniform moistening of the soil. Plant size and "drying down" characteristics provide the best information on how often to water. Many of the larger stock plants may require irrigation twice daily. A good practice is to water thoroughly in the morning, then follow up in the afternoon where needed. About a 6-hour interval should be allowed between irrigations.

How the water is applied is also important. The nature of the soiless mix will indicate whether it is being done correctly. The perlite of the upper inch or so of the mix will float, and when it floats uniformly from side to side, enough water has been applied. If it floats out or is washed out by a sideways direction of the hose and water breaker, the plant is being improperly watered. The rate of water flow from the breaker should be adjusted to avoid washing the mix from the pot and the breaker should be held above the pot and aimed downward. Watering with too much force from the side will wash out the mix, distribute it unevenly within the pot, and create a sanitation problem on the bench. Irrigating until the perlite "floats" may be a little difficult to judge at first. Stating this more precisely, 1 liter of water should be applied to a 2-gallon container.

Although frequency of irrigation could be varied for individual plants, a happy medium is normally sought to avoid complicated and time-consuming adjustments. Fertilization includes the Mag-Amp slow-release fertilizer mentioned in the soiless mix section for a constant supply of nutrients. To complement this a Peters 20-20-20 water-soluble fertilizer is applied while irrigating with a 1:24 proportioner, a device that meters liquid fertilizer into a hose or irrigation system. One pound of this fertilizer is diluted in 5 gallons of water to provide, after the 1:24 proportioning, a 200 ppm N, 88 ppm P, and 166 ppm K nutrient boost. This is applied twice a week during favorable growing conditions, and once a week during cloudy winter months when watering is less frequent and salt accumulation becomes a threat.

Chelated iron is applied once a week and micronutrients are provided at about the 12-week stage of growth, or when deficiencies are noted. To avoid a precipitate, use distilled water for mixing the chelated iron and N-P-K fertilizers in the 5-gallon stock solution. Chelated iron is added at 2 ml per liter applied to the plant, or about 900 ml per 5 gallons stock solution. Micronutrients are added at 1 ml per liter applied. Half concentrations are provided newly planted rooted cuttings for the first 2 weeks. This is accomplished by saving these applications until most of the stock solution has been used, then simply adding enough water to double the existing volume. Any conifers in the greenhouse will also do better with this 100 ppm N concentration.

FeEDTA (Chelate of Iron Stock Solution)		1 ml/l = 5 ppm
Compound	(g/l)	(g/4 l)
Iron (II Sulfate $FeSO_4 \times 7H_2$)	24.9	99.6
Ethylenediaminetetraacetic acid EDTA	26.1	104.4
Sodium hydroxide NaOH	10.5	42.0

Mixing Apparatus

1. 4,000 ml beaker.
2. Magneticstirrer (use long rod magnets).
3. Aeration tube and tubing.

One-liter Preparation

1. Set up beaker on magnetic stirrer.
2. Fill beaker with approximately 900 ml distilled water.
3. Insert stirring rod.
4. Add 24.9 g $FeSO_4$ and 26.1 g EDTA.
5. Attach air tubing to air supply; couple tube with glass tubing and insert into beaker.
6. Gently aerate while mixing with magnetic stirrer for 1 hour before adding NaOH. Aerate at rate that minimizes spattering.
7. Add 10.5 g NaOH.
8. Add water to bring to 1,000 ml.
9. Continue aeration for 3 hours.
10. After the 3 hours, adjust pH to 5.0 for better storage characteristics. Normally involves adding additional NaOH.
11. Continue aeration and stirring overnight, or for several hours.
12. A **clear**, coffee-colored solution should be the final result. Store under refrigeration.

Micronutrient Stock Solution

Compound		Grams dissolved in 1 liter H_2O
H_3BO_3	Boric Acid	2.86
$MnCl_2 \times 4H_2O$	Manganese Chloride	1.81
$ZnSO_4 \times 7H_2O$	Zinc Sulfate	0.22
$CuSO_4 \times 5H_2O$	Copper Sulfate	.08
$H_2MoO_4 \times H_2O$	Molybdic Acid (assaying 85 percent MoO_3)	.02
$MgSO_4 \times 7H_2O$	Magnesium Sulfate	.20

Add 1.0 ml of this stock solution for each liter of water applied.

Element	Ppm for 1 liter nutrient solution applied
B	0.50
Mn	.50
Zn	.05
Cu	.02
Mo	.01

Lighting

Lighting consists of a very basic system of alternating incandescent and fluorescent fixtures. Spacing is approximately 3 feet apart, with the lamp 7 feet from the floor and 4 feet from the bench surface. Illuminance is only sufficient to extend the photoperiod and prohibit dark respiration, it is insufficient for active photosynthesis. The system provides for an 18-hour photoperiod from 6:00 a.m. until 12:00 midnight.

Miscellaneous Cultural Suggestions

Staking is necessary after about 2 weeks' growth. Without staking, and in the absence of winds to provide an environmental stimulus for initiating supporting fibers, the tree will grow over to one side. Increased branching will accompany this procumbent form. Four-foot bamboo cane stakes are used, with "twist-em" ties located as necessary to provide support. Tie loosely and check frequently to avoid constricting the expanding stem.

A time-saver in applying miticides is to spray only the bottom two-thirds of the tree when it gets about 3 feet high. This can be done because normally the tree will grow faster than the mites can migrate upward. It also provides an advantage in that the new, more succulent growth is not subjected to potentially phytotoxic chemicals.

The soiless mix cultural system described in this section allows essentially all clones to be treated alike, thus vastly reducing management complexities. The system not only works well with all *Populus* clones, but with most other woody plants as well, except conifers. They just do not do well with the soiless mix suggested. About the only exception to the blanket applicability of this system to *Populus* is with Tristis #1 (NCFES 5260). It does not require as much water for some reason, which in turn means it does not need to be fertilized as frequently. When fertilized like the rest, however, it does not seem to suffer.

Temperatures fluctuate diurnally between 75°F day and 65°F night as a normal reaction to light and dark periods. It has never been necessary to daily set and reset the thermostats of each individual bay.

Watering, potting, and potting mixes described for greenhouse use are equally suitable for growth-room culture of poplar clones. Nutriculture techniques for the production of uniform poplars have been described by Dykstra (1972), but are not routinely used for rapid-selection purposes.

USE OF CONTROLLED-ENVIRONMENT AND STATISTICAL TECHNIQUES TO MAXIMIZE DISCRIMINATION AMONG CLONES

H. Zuuring, *Assistant Professor, School of Forestry, University of Montana, Missoula, Montana,*
P. Wray, *Assistant Professor, Department of Forestry, Iowa State University, Ames, Iowa,*
L. Promnitz, *Head, Forest Biometry Research, Crown Zellerbach Corporation, Wilsonville, Oregon,*
and **J. C. Gordon,** *Head, Department of Forest Science, Oregon State University, Corvallis, Oregon*

On the basis of several studies, we have established principles for deriving best estimates of growth potential differences for poplar clones from controlled environment studies. These are:

1. Environments that are most favorable for growth expose greatest differences among clonal growth potentials. Thus, long photoperiods, higher light intensities, and rich nutrient and water regimes are preferred.
2. For estimating environmental stability (the ability of a given clone to grow similarly under a wide range of environments), the use of a few, widely divergent environments is preferred to the use of a larger number of similar environments.
3. With limited controlled-environment capacity, the number of clones should be increased at the expense of a larger number of environments in selection trials. At least three environments must be included if estimates of environmental stability are to be calculated, however. Three to five replications per clone and environment are needed for materials as variable as the poplar clones used in these studies.
4. Selection efficiency, both in resource and statistical terms, increases with the number of clones included in a given trial.
5. In a time series of selection trials, a minimum of one common clone should be included in all trials as an internal standard. Internal standard clones should be good growers that are environmentally stable.
6. Elaborate environmental control systems are not necessary for early selection trials of the kind described here, in that no attempt to simulate "field" environments is called for. Repeatability and reliability are more important than varying environmental parameters over wide ranges or establishing elaborate temporal programs.
7. Photoperiod and temperature, manipulated for plants optimally supplied with nutrients and water, can provide sufficient environmental difference to expose the relative environmental stabilities of several clones. For stability across nutrient and water potential gradients, clones should, of course, be evaluated in environments differing in these parameters. Stability to specific pathogen levels could probably be evaluated in the same way.
8. At least 6 weeks are required to conduct selection trials in controlled environments, assuming clones are rooted and established in pots at the beginning.
9. Height is not well-correlated with other growth variables (e.g., leaf area, dry weight) as a rule. Thus, if ability to accumulate dry weight is of interest, dry weight must be measured—i.e., it probably cannot be predicted accurately from height measurements.
10. Total dry weight accumulation over the trial period appears to be the single best predictor of field growth potential.

Two iterations of controlled-environment selection trials for field growth potential are described below. The data from both are used to calculate selection indices. The outcome of the trial is a ranking according to estimated growth potential. Any subset of the ranked test group can be chosen for field trial. The smaller the subset, the greater will be the chance of excluding good clones and including bad ones.

Steps in a generalized trial are:

1. Decide upon specific objectives, and choose environments and measurements.
2. Assemble and propagate plant material.
3. Grow and measure clones in controlled environment.
4. Analyze variances to see if clones differ.
5. Rank clones by mean performance.
6. Do "distance" multivariate analyses.
7. Do "environmental stability" analyses.
8. Choose clone subset for field trials.
9. Do field trials.
10. Calculate field/controlled-environment correlations for use in future selection trials.

Controlled-Environment Trial I

Twenty-five *Populus* clones were chosen from those gathered by the Maximum Yield Project of the North Central Forest Experiment Station for possible use in field trials (table 1). No particular mix of parentage or origin was chosen; for some clones (e.g., 5258), no reliable information on lineage was available. (Clones 2 and 19 probably are the same; this was discovered after all the data were analyzed.)

In all three environments (Greenhouse I, Greenhouse II, Growth Chamber), apical cuttings rooted under mist were grown in 2-gallon plastic pots containing a 3:1 Jiffy-Mix:perlite artificial substrate. The growth chamber environment had the following characteristics: growth period, 6 weeks; photoperiod, 18 hours (in Percival, Model PT-80 growth chambers); and temperature, 25°C day and 15°C night. The growth-chamber environment had the least variation in photoperiod, light intensity, and temperature. Greenhouse II had a much higher temperature and a longer natural light photoperiod than did Greenhouse I. Light intensities were highest in Greenhouse II because of seasonal changes in solar position. In both greenhouse experiments natural photoperiod was supplemented with additional artificial light to extend daylength to 18 hours.

The plants were placed on benches in the greenhouse and in growth chambers at random.

Growth rates were determined from weekly total leaf counts and total height measurements (cm), beginning with initial measurements when the rooted cuttings were removed from the propagation bench. Leaf ovendry weight (g) and stem ovendry weight (g) were determined at the end of the growth period.

Table 1.—Populus *clones included in trials*

Iowa State University clone number	North Central Forest Experiment Station number	Name and parentage
1	4877	*Populus alba* L.
2	4878 (5327)	*Populus* x *euramericana* Guinier *(deltoides* x *nigra)*
3	4879	*Populus* x *euramericana* Guinier
4	5258	*Populus* sp.
5	5262	*Populus candicans* Ait. x *Populus berolinensis* Dipp.
6	5263	*Populus candicans* Ait. x *Populus berolinensis* Dipp.
7	5264	*Populus deltoides* Marsh. x *Populus plantierensis* Schneid.
8	5265	*Populus deltoides* Marsh. x *Populus trichocarpa* Torr. et Gray
9	5266	*Populus deltoides* Marsh. x *Populus trichocarpa* Torr. et Gray
10	5267	*Populus deltoides* Marsh. x *Populus caudina*
11	5271	*Populus charkoviensis* Schroed. x *Populus deltoides* Marsh.
12	5272	*Populus nigra* L. x *Populus laurifolia* Ledeb.
13	5321	*Populus* x *euramericana* Guinier
14	5322	*Populus* x *euramericana* Guinier
15	5323	*Populus* x *euramericana* Guinier
16	5324	*Populus* x *euramericana* Guinier
17	5325	*Populus* x *euramericana* Guinier
18	5326	*Populus* x *euramericana* Guinier
19	5327 (4878)	*Populus* x *euramericana* Guinier
20	5328	*Populus* x *euramericana* Guinier
21	5331	*Populus betulafolia* Dipp. x *Populus trichocarpa* Torr. et Gray
22	5332	*Populus betulafolia* Dipp. x *Populus trichocarpa* Torr. et Gray
23	5334	*Populus deltoides* Marsh. x *Populus trichocarpa* Torr. et Gray
24	5260	*Populus tristis* Fish. x *Populus balsamifera* L.
25	5377	*Populus* x *euramericana* Guinier, "Wisconsin Number 5"

Results

Clones varied greatly in all growth characteristics measured, but showed some consistency when compared across the three test environments. Total height and dry weight of leaves and stems varied widely among clones and environments (tables 2, 3, and 4).

When all clones were pooled, mean leaf and stem dry weight and total height were all greatest in Greenhouse II (table 5). The greater total solar radiation due to longer natural daylight, greater average light intensity during the longer days, and a greater proportion of clear days during the 8-week growth period all contributed to greater growth in this environment. The growth chamber means were smaller than the greenhouse means, primarily because of the shorter growth period (6 rather than 8 weeks), but also because of lower growth-chamber light intensities. Because total photoperiod was the same in all three environments, photoperiodic reactions should not have caused differences in growth among environments.

Table 2.—*Duncan's new multiple range test for significant differences in stem dry weight for the three environments (any two means not next to a common line are significantly different)*

Growth chambers		Greenhouse I		Greenhouse II	
Clone	Mean	Clone	Mean	Clone	Mean
	Grams		*Grams*		*Grams*
24	7.96	3	18.80	4	32.49
4	6.71	4	18.01	23	31.89
18	6.68	5	17.58	18	31.28
5	6.65	9	17.32	25	30.88
25	6.62	23	15.40	9	27.97
3	5.95	8	14.73	3	27.25
9	5.91	15	14.57	17	24.81
1	5.83	18	14.42	15	23.82
6	5.78	21	14.31	7	23.54
17	5.55	16	14.19	5	23.16
21	5.46	17	14.14	20	22.78
23	5.33	12	14.13	8	22.19
15	5.32	22	13.52	6	20.62
7	4.95	7	13.46	1	20.23
12	4.94	25	12.64	12	19.51
22	4.91	6	12.10	2	18.88
8	4.77	24	12.01	16	18.11
13	4.66	19	11.54	14	18.08
2	4.47	14	11.35	19	17.34
16	4.30	2	10.35	22	17.26
19	3.98	13	10.15	21	16.43
20	3.38	20	8.39	24	15.51
14	3.27	10	6.91	10	10.47
11	3.20	11	6.87	13	10.25
10	2.57	1	6.12	11	7.38

Table 3.—*Duncan's new multiple range test for significant differences in leaf dry weight for the three environments (any two means not next to a common line are significantly different)*

Growth chambers		Greenhouse I		Greenhouse II	
Clone	Mean	Clone	Mean	Clone	Mean
	Grams		*Grams*		*Grams*
9	22.37	9	32.49	23	54.88
23	19.84	8	29.71	9	49.27
8	19.52	23	29.59	8	45.46
4	19.02	4	27.38	7	43.76
25	18.63	3	27.28	4	42.99
6	18.37	7	25.36	15	39.22
18	17.75	15	24.36	18	39.01
17	17.40	5	24.33	17	37.90
5	16.96	17	23.86	25	37.27
1	16.86	18	21.75	3	36.34
7	16.77	16	21.50	14	34.63
15	16.76	14	21.32	5	32.82
12	16.06	21	21.22	20	31.44
21	15.88	12	20.44	1	31.23
24	15.51	20	20.41	2	30.69
3	15.18	19	18.42	19	29.56
20	14.85	22	18.31	12	28.73
16	14.83	2	18.04	6	28.59
13	14.28	13	17.95	16	28.26
2	13.74	25	17.93	21	26.14
22	13.60	24	17.40	24	22.07
19	12.44	6	16.94	22	21.80
14	12.09	10	13.00	10	18.59
10	10.33	1	12.85	13	17.74
11	7.90	11	9.91	11	11.45

Within each environment a high rank for one variable did not necessarily indicate a high rank for other variables. Moreover, clonal ranking based on individual variables or sums varied from environment to environment. Further analysis beyond simple ranking and summing was necessary to indicate clearly which clones had the greatest juvenile growth potential and stability across environments. Therefore, growth variables for clones within environments were subjected to analysis of variance, and differences among clones (for each growth variable in each environment) were examined by use of Duncan's new multiple range test (tables 2, 3, and 4).

Greenhouse II produced the greatest number of significant differences among clones in stem weight as well as the greatest stem weights (table 2). Seven clones produced significantly greater mean stem weight than others regardless of environment. These seven clones (3, 4, 9, 17, 18, 23, and 25) may be regarded as consistent producers of heavy stems across all environments.

Table 4.—*Duncan's new multiple range test for significant differences in total height for the three environments (any two means not next to a common line are significantly different)*

Growth chambers		Greenhouse I		Greenhouse II	
Clone	Mean	Clone	Mean	Clone	Mean
	cm		*cm*		*cm*
5	94.6	5	181.2	23	192.8
6	86.9	3	163.4	5	188.8
24	85.5	9	158.2	9	180.0
3	82.1	22	157.6	3	172.1
1	81.9	6	156.4	18	171.6
12	80.7	23	155.9	7	170.6
2	79.7	12	151.0	25	170.4
9	79.0	19	144.0	6	170.2
18	78.9	15	141.8	17	169.5
23	78.6	8	141.4	22	167.7
22	78.1	16	140.8	4	166.6
7	77.0	4	140.4	12	164.9
15	76.5	17	137.9	15	160.6
25	75.6	2	136.8	2	160.0
17	75.2	7	135.4	8	156.4
4	75.1	25	134.2	21	154.2
21	74.9	18	130.5	1	153.8
19	72.8	24	124.4	16	147.9
8	71.9	13	121.8	19	142.6
16	65.5	1	120.9	24	134.7
13	65.4	14	117.5	14	133.4
11	64.3	21	115.2	20	124.6
10	58.6	10	110.3	10	118.7
14	55.6	11	104.8	11	112.6
20	49.9	20	97.9	13	108.8

Clones producing the greatest total leaf weight were also consistent for all three environments (table 3). As with stem weight, Greenhouse II produced the greatest number of significant differences in leaf weight. Three clones, 8, 9, and 23, constituted the top group. Two of these (9 and 23) also were in the top group for all environments in stem-weight production.

Total height analysis presented a somewhat different picture. Greenhouse I produced the greatest number of significant differences in total height (table 4). But of the six tallest clones in Greenhouse I, only three (3, 9, and 23) appeared in the group showing consistently greatest stem weight, and only two (9 and 23) appeared in the group showing consistently greatest leaf weight. Thus, high potential for weight production is not necessarily related to high potential for height growth.

Leaf number increased roughly with time; therefore, regressions of leaf number on time were examined to see if rate of leaf production, as indicated by the slope of this regression line, was an indicator of final weight or height or both. If it were, it might be possible to reduce or eliminate destructive measurement. Again, Greenhouse II produced the greatest slopes, reiterating the generally better growing conditions in this environment. There was little consistency, however, between leaf production and final weight and (or) height. For example, clone 23, one of the best performers in terms of stem and leaf weight and height growth, had one of the lowest rates of leaf production.

Table 5.—*Averages and ranges for leaf and stem weight and total height for all clones pooled in each environment*

	Growth chambers	Greenhouse I	Greenhouse II
Leaf weight		*Grams*	
Mean	15.79	20.68	32.79
High	22.37	32.49	54.88
Low	7.90	5.82	11.45
Variance	15.46	39.92	55.12
Stem weight		*Grams*	
Mean	5.18	12.54	21.29
High	7.96	18.80	32.49
Low	2.57	3.04	7.38
Variance	2.72	15.10	23.69
Total height		*cm*	
Mean	74.3	134.7	155.7
High	94.8	181.2	192.8
Low	49.9	82.5	98.8
Variance	68.24	25.79	219.28

Controlled-Environment Trail II

Growth chamber procedures

The experiment was conducted in Percival, Model PT-80 growth chambers. The average light intensity at the top of the plant crowns was maintained at approximately 3,000 foot-candles. The relative humidity was not controlled. Plants were watered to the saturation point (water dripping out the pot bottom) once every 2 days. Each week every pot was flushed with demineralized water followed by 200 mls of the prepared nutrient-micronutrient solution. The temperature was controlled to within ±2°C and the photoperiod to within ±15 minutes. Row temperature treatments with day temperatures (D) and night temperatures (N) corresponding to light and dark (D) portions of low light treatments, making a total of 16 treatment combinations, were randomly assigned to growth chambers. Temperature treatment levels were: 17D-5N, 23D-11N, 29D-17N, 35D-23N; light treatment levels were: 12D-12N, 14D-10N, 16D-8N, 18D-6N.

During the 6 weeks that the plants remained in the chambers, the following measurements were recorded: total number of leaves, length and width of every leaf to the nearest 0.1 cm, total plant height to the nearest 0.1 cm (HT), and basal diameter to the nearest 0.1 mm (DIA).

When the plants were harvested at the end of 6 weeks the following measurements were also recorded: leaf dry weight (LFWT), stem dry weight (STMWT), and root dry weight (RTWT). Several additional variables were calculated from these latter three: total plant weight (TOTWT), stem-to-root ratio (SRR), top-to-root ratio (TRR), and leaf, stem, and root weight ratios (LWR, SWR, RWR).

Leaf surface area relations

Many direct measurement methods to obtain leaf surface area are available (Sesták *et al.* 1971). All of them involve destroying the leaves and are tedious and time-consuming. Therefore, we used an indirect method that involved developing regression equations using leaf length (L) and width (W) to predict leaf area (A).

Plants were selected at random to yield a sample of at least 30 leaves for each of the eight clones. All the leaves, both juvenile and expanding, were taken from these plants; the leaf circumferences were then traced on paper. The areas were obtained by using a planimeter that measured to the nearest 0.1 cm². It was assumed for all clones that leaf shape was independent of the environment in which the leaf was grown.

Various models were analyzed using ordinary least squares; the final model chosen was

$$A = a + b(LW) + c(W^2).$$

Parameter estimates and associated statistics were obtained for each clone (table 6). To test the reliability of prediction of the above model, additional data were collected for clones 5321, 5323, 5326, and 5377.

Four statistics were calculated for these four clones using the observed and predicted leaf surface areas (table 7). The predicted areas were obtained by substituting the corresponding values of the independent variables associated with a particular observed area into the correct clonal prediction equation. The statistics were:

$$T_1 = \frac{(\hat{\beta}-\beta_o)' X'X (\hat{\beta}-\beta_o)}{ks^2} \sim F_{k,n-p}, \text{ where}$$

k = number of parameters in joint null hypothesis under test, and
n = number of observations (Kempthorne 1972)

$$\text{and } T_2 = \frac{1}{4n^2}\left\{ \sum_{i=1}^{n} [R(X^{(i)}) - 2i]^2 + \sum_{j=1}^{m} R(Y^{(j)}) - 2j^2 \right\};$$

$$T_3 = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2,$$

the sum of squares between observed and predicted leaf areas and

$$T_4 = \sum_{i=1}^{n} |y_i - \hat{y}_i|,$$

the sum of absolute deviations.

Table 6.—*Regression coefficients and associated statistics for eight clones included in Trial II*

Clone	Coefficient	Southeast of coefficient	n	S^{2}	R^2
5321	0.50868	0.52286	37	3.184	0.9972
	.54004	.03332			
	.09037	.03587			
5323	.43073	1.05231	40	10.389	.9977
	.52406	.05146			
	.14628	.04666			
5326	− .07917	1.32275	39	14.590	.9953
	.27336	.04602			
	.38605	.04257			
5328	−3.48530	1.15573	36	12.001	.9982
	.64992	.04883			
	.12364	.04511			
5377	1.26054	.64691	50	7.383	.9975
	.29245	.02778			
	.36252	.02795			
5260	− .58338	.73483	50	4.856	.9964
	.63068	.05760			
	.06288	.07197			
5339	.81738	.85277	41	8.087	.9967
	.51805	.04154			
	.21589	.05168			
Balsam	− .06247	.82671	39	6.448	.9945
	.69543	.04729			
	.01553	.08489			

Table 7.—*Several statistics to show the prediction reliability of the leaf area model Y = a + b(LW) + c(W²) for four clones (Y = leaf area, L = leaf length, W = leaf width)*

Clone	n	T_1	T_2	T_3	T_4	Range of $Y_i - Y_i$
						cm²
5321	2	[1]26.12	[2]0.025	548.63	97.25	− 5.21 to 7.50
5323	25	[1]7.58	[2].020	248.56	63.89	− 6.10 to 3.55
5326	28	[1]10.82	[2].054	1,111.81	125.70	−15.94 to 2.11
5377	26	[1]49.35	[2].067	707.03	112.90	− 8.99 to 1.61

[1]Significant at $P < 0.01$.
[2]Not significant at $P < 0.05$.

The Cramer-von Mises Two-Sample test was used to check

H_o: $F(x) = G(x)$ for all x

vs. H_1: $F(x) \neq G(x)$ for at least one value of x

where n = m = number of observations in each sample,

$F(X^{(i)})$ = the rank of the i-th smallest of the X's in the combined ordered sample, and

$R(Y^{(j)})$ = the rank of the j-th smallest of the Y's in the combined order sample (Conover 1971).

Test statistic T_1 is significant at $\alpha = 0.01$ for all four clones, implying that the joint null hypothesis $\beta = {}^{0}_{1}$ is rejected and that the equations do not fit the new data very well. The test statistic T_2 is not significant at $\alpha = 0.10$ for all four clones, implying that the null hypothesis $F(x) = G(x)$ is accepted, and that the equations do fit the new data.

These two statistics give conflicting results, but based on the fact that the leaves of these clones vary somewhat in shape, the estimate, s^2, in the denominator of statistic T_1 may be difficult to estimate. With the exception of clone 5326, the estimated leaf areas were within ±10.0 cm² of the actual leaf areas (table 7). For clone 5326 only five out of the 28 areas calculated exceeded this tolerance. Therefore, the model $Y = a + b(LW) + c(W^2)$ appears satisfactory for estimating leaf surface areas for the four clones (table 7).

In addition to the estimated leaf surface area (LFAREA), we calculated specific leaf area (SLA), leaf weight ratio (LWR), and leaf area ratio (LAR). The relations are:

$$LAR = \frac{LFAREA}{TOTWT} = \frac{LFAREA}{LFWT} \times \frac{LFWT}{TOTWT}$$

$$= (SLA) \times (LWR) \text{ (Sesták } et\ al. \text{ 1971),}$$

where

LFWT = leaf dry weight, and

TOTWT = total plant dry weight.

Vegetative growth

Because the three-factor interaction (photoperiod x temperature x clone) was not significant to the 5 percent level for each of the three vegetative growth variables (DIA, HT, and LFAREA), we considered the mean response of each of the eight clones over levels of photoperiod and temperature for each of these variables (table 8). With a few exceptions, growth in basal stem diameter, height, and leaf area increased rapidly as photoperiod increased from 12 to 16 hours for all eight clones, although not at the same rate for each clone. The increase in growth was less rapid as photoperiod was increased further to 18 hours. Clone 5260 had the greatest height growth rate as photoperiod increased from 16 to 18 hours, and attained the greatest mean height of all eight clones at 18-hour photoperiod.

Balsam poplar exhibited the poorest growth for all three variables (DIA, HT, and LFAREA), while clone 5328 displayed the best growth in diameter and leaf area over all photoperiod levels. Clone 5323 showed the greatest growth in height over all photoperiods.

The effect of temperature on growth in diameter and leaf area was quadratic, in that DIA and LFAREA increased rapidly as the day-night temperature increased from 17-5°C to 29-17°C, then decreased as

Table 8.—*F-values for vegetative growth variables of eight* Populus *clones associated with eight major sources of variation (P = photoperiod, T = temperature, C = clone)*

Source	Dependent variables		
	DIA	HT	LFAREA
Replicate	[1]12.55	[1]9.62	[1]11.56
P	[1]11.65	[1]31.34	[1]17.66
T	[1]29.55	[1]51.08	[1]37.78
P x T	[2]32.35	[3]2.59	[2]2.41
C	[1]24.16	[1]24.51	[1]32.23
P x C	[2]31.06	[1]2.09	[2]1.25
T x C	[2]31.55	[1]2.31	[1]3.30
P x T x C	[2]31.21	[2]1.22	[2]1.00

[1]Significant at $P < 0.01$.
[2]Not significant at $P < 0.05$.
[3]Significant at $P < 0.05$.

day-night temperature was further increased to 35°C. This trend was not so obvious for height growth. Generally, height growth levelled off as the day-night temperature increased from 29-17°C to 35-23°C for all eight clones, although there were some exceptions. The rate of growth over temperature level was not the same for each clone.

All main effects—experiment, photoperiod, temperature, and clone—were significant at the 1 percent level for all three growth variables; the temperature x clone interaction was significant for the variables HT and LFAREA, and the photoperiod x clone interaction was significant for variable HT (table 6). This meant that the clones responded significantly differently over temperature levels for the variables HT and LFAREA and for HT over photoperiod levels.

Dry weight

Again, the three-factor interaction, photoperiod x temperature x clone, was not significant at the 5 percent level for each of the four dry weight variables LFWT, STMWT, RTWT, and TOTWT (table 9). With the exception of clone 5339 and balsam poplar, dry weights of leaves, stem, and total plant increased rapidly with increase in photoperiod from 12 to 16 hours and less rapidly from 16 to 18 hours, although not at the same rate for each clone.

Balsam poplar yielded the lowest dry weight accumulation with respect to leaves, stem, roots, and total plant, while clones 5328 and 5323 yielded the highest dry weight production for all the dry weight variables except STMWT over all photoperiod levels. Clones 5377 and 5326 produced the highest STMWT in all photoperiods except for 18 hours.

Table 9.—*F-values for dry weight variables of eight* Populus *clones associated with eight major sources of variation*

Source	Dependent variables			
	LFWT	STMWT	RTWT	TOTWT
Replicate	[1]5.96	[2]9.39	[1]6.01	[1]7.91
P	[2]9.91	[2]13.30	[3]0.97	[2]6.61
T	[2]26.98	[2]22.16	[2]15.78	[2]25.96
PxT	[3]1.69	[3]1.60	[3]0.89	[3]1.37
C	[2]25.82	[2]13.80	[2]22.04	[2]20.73
PxC	[3]1.00	[3]1.36	[3]0.62	[3]0.94
TxC	[2]2.60	[1]1.66	[1]1.67	[2]2.06
PxTxC	[3]0.91	[3]1.08	[3]1.01	[3]0.96

[1]Significant at $P < 0.05$.
[2]Significant at $P < 0.01$.
[3]Not significant at $P < 0.05$.

The dry weight of leaves, stem, and roots increased sharply as the day-night temperature increased from 17-5°C to 29-17°C, and decreased as the temperature increased from 29-17°C to 35-23°C. The classic quadratic response to temperature was exhibited by all eight clones.

The photoperiod, temperature, and clone sources of variation were all significant at the 1 percent level for all four dry weight variables, with the exception of the photoperiod source of variation for RTWT. The effect of photoperiod on RTWT was not even significant at the 10 percent level (table 9).

The temperature x clone interaction was significant at the 1 percent level for LFWT and TOTWT and at the 5 percent level for STMWT and RTWT. This indicated that the response of each clone over temperature was significantly different, one from another. In fact, the differential response of clones over temperature with respect to LFWT was much more pronounced than the responses with respect to either STMWT or RTWT.

The effect of the experiment was significant at the 1 percent level for STMWT only and significant at the 5 percent level for the other dry weight variables. This may indicate that stem dry weight production was altered to a greater degree than either leaf or root dry weight production by the change in "soil" composition from Experiment 1 to Experiment 2.

Distribution of assimilate

The distribution of assimilate was expressed in dry stem weight-to-root weight and top-to-root ratios as well as leaf, stem, and root dry weights as proportions of total dry weight. With the exception of SWR, the photoperiod x temperature x clone interaction again was not significant at the 5 percent level for all the other assimilate distribution variables (table 10).

The SRR and TRR increased rapidly as photoperiod increased from 12 to 16 hours for all clones with the exception of balsam poplar. As photoperiod continued to increase from 16 to 18 hours, clones 5260, 5339, and balsam poplar continued to increase in SRR and TRR at a high rate. Clones 5377, 5323, and 5328 continued to increase at a low rate and clones 5321 and 5326 decreased in SRR and TRR (fig. 2).

As the day-night temperature increased from 17-5°C to 35-23°C, most of the clones increased rapidly in SRR and TRR.

Table 10.—*F-values for assimilate distribution variables of eight* Populus *clones associated with eight major sources of variation (P = photoperiod, T = temperature, C = clone)*

Source	Dependent variables				
	SRR	TRR	LWR[1]	SWR[1]	RWR[1]
Replicate	[2]2.81	[2]0.13	[2]0.80	[3]9.98	[2]0.78
P	[3]33.27	[3]20.53	[4]3.66	[3]29.15	[3]22.65
T	[3]30.73	[3]16.11	[2]0.91	[3]31.24	[3]13.19
PxT	[2]1.67	[2]0.72	[2]1.89	[2]2.44	[2]1.18
C	[3]22.43	[3]29.49	[3]59.18	[3]38.73	[3]26.72
PxC	[3]2.13	[2]1.46	[3]2.26	[3]2.39	[3]3.74
TxC	[4]1.74	[3]2.62	[2]1.62	[2]0.96	[3]2.49
PxTxC	[2]1.31	[2]0.97	[2]1.03	[4]1.61	[2]1.06

[1]These proportions were transformed by the function arcs in $\sqrt{P}$. The analyses of variance were performed on these transformed variables.
[2]Not significant at $P < 0.05$.
[3]Significant at $P < 0.01$.
[4]Significant at $P < 0.05$.

The main effects—photoperiod, temperature, and clone—were significant at the 1 percent level except for the variable LWR. Here the photoperiod effect was only significant at the 5 percent level and the temperature effect was not significant at all. Possibly photoperiod affects LWR more than temperature.

The effect of the experiment was not significant at the 5 percent level for any of the assimilate distribution variables except SWR, for which it was highly significant at the 1 percent level.

The photoperiod x clone interaction was significant at the 1 percent level for all the variables except TRR. For this variable the above interaction was not significant at the 5 percent level. This implied that there was no significant difference in response with respect to TRR from one clone to another over levels of photoperiod. Similarly, the temperature x clone interaction was significant at the 1 percent level for

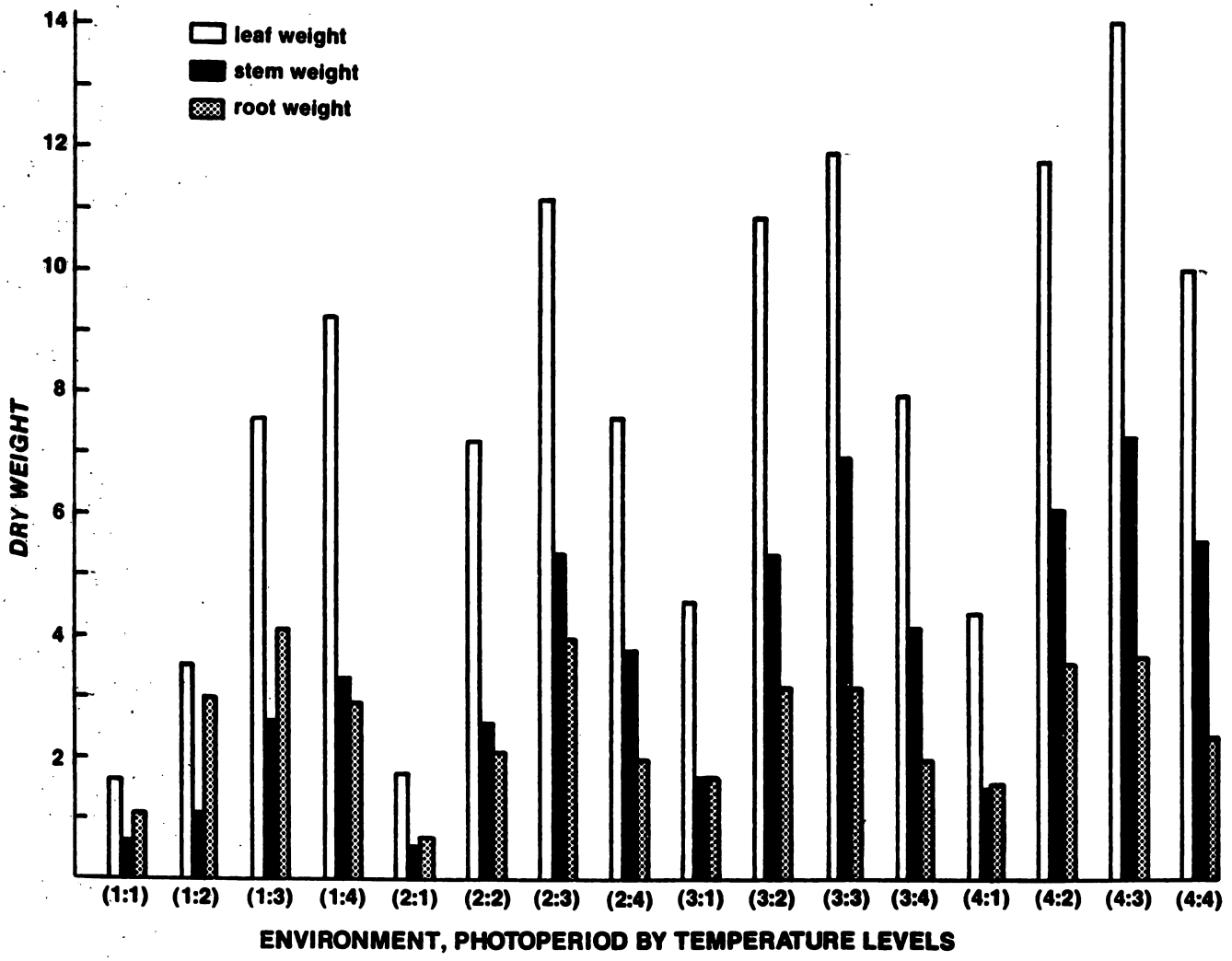

Figure 2.—*Average dry weight distribution over all clones in each of 16 environments (numbers in parentheses along the x-axis represent photoperiod and temperature levels, respectively).*

TRR and RWR, at the 5 percent level for SRR, and not significant at the 5 percent level for LWR and SWR. The response of RWR from one clone to another, therefore, was significantly different over temperature, but the responses of LWR and SWR were not.

The combined action of photoperiod and temperature produced noticeable shifts in the average dry weight distribution of all eight clones combined (fig. 2), even though the photoperiod x clone interaction for LWR, SWR, and RWR was not significant at the 5 percent level (table 10).

Relative size of assimilatory apparatus

The photoperiod x temperature x clone interaction was not significant at the 5 percent level, so we considered leaf area ratio (LAR) and specific leaf area (SLA) versus levels of photoperiod and temperature separately for all eight clones (table 11).

Generally, the relative size of the leaves on a square decimeter per gram basis yields information about leaf density. The magnitude of the variables SLA (=LFAREA/LFWT) and LAR (=LFAREA/TOTWT) indicates the degree to which assimilation rate and efficiency are affected by changes in photoperiod and temperature.

SLA was definitely more sensitive to changes in environment than LAR. The former varied from 1.6 to 2.6 and the latter from 0.8 to 1.5. Clone 5339 had the densest leaves (large SLA) and balsam poplar had the least dense leaves (small SLA) over all temperature levels.

Table 11.—*F-values for relative leaf size variables of eight* Populus *clones associated with eight major sources of variation (P = photoperiod, T = temperature, C = clone)*

Source	Dependent variable	
	LAR	SLA
Replicate	[1]0.88	[1]3.90
P	[2]10.69	[2]7.74
T	[2]14.12	[2]17.16
PxT	[1].57	[1]1.40
C	[2]8.46	[2]20.24
PxC	[1]1.23	[1]1.40
TxC	[1]1.39	[1]1.23
PxTxC	[1].82	[1]1.02

[1]Not significant at $P < 0.05$.
[2]Significant at $P < 0.01$.

Only the main effects—photoperiod, temperature, and clone source—were significant at the 1 percent level. The remaining sources were not significant at the 5 percent level (table 10).

Except for clone 5260, which decreased sharply, all clones increased in SLA and LAR as photoperiod increased from 16 to 18 hours. As temperature increased, LAR and SLA generally increased for all clones; no quadratic response was observed. With the exception of clones 5323 and 5377, all clones increased rapidly in SLA at first, then moderately, and finally very sharply as the day-night temperature increased from 17-5°C to 35-23°C.

Selection Indices

Selection indices have been used for selection in animal and plant breeding programs when several quantitative characters are considered (Elston 1963, Hazel 1943, Hazel and Lush 1942, Kempthorne and Nordskog 1959, Panse 1946, Smith 1936, and Tallis 1962). Hanson and Johnson (1957) have discussed methods for calculating a general selection index based on pooled information from two or more experiments. Their index was similar to our index I_1, which is discussed below. Two data sets were combined to minimize sampling errors and to improve the estimation of the genotype by environment interaction. The two combined populations were grown under identical environments. In this manner a selection index determined from one data source could be used successfully as a general index. The expected genetic advance was used as a means of index reliability.

Okuna *et al.* (1971) evaluated the performance of 29 rice varieties grown in several environments using seven different methods, and one of the methods they used involved principal component analysis.

A selection index often considered appropriate is a function of the form:

$$I = w_1x_1 + w_2x_2 + \ldots + w_px_p;\ i = 1, \ldots p,$$

where:

w_i = i-th known or unknown economic weight, and

x_i = i-th measured trait (Hazel 1943, Smith 1936). However, such a linear function may not be appropriate, or, if the economic weights are unknown, the experimenter may not want to go through the in-

volved estimation procedure necessary. The researcher may not even want to consider any weights.

An alternative is to consider a nonlinear function of the form

$I = (w_1x_1)\ (w_2x_2)\ \ldots\ (w_px_p) = w'x_1x_2\ \ldots\ x_p$. For simplicity, suppose $p = 2$ so that only two traits are being considered. Then figure 3 illustrates selection on the basis of a linear index, $x_1 + wx_2$, and figure 4 illustrates selection on the basis of a nonlinear index, wx_1x_2. In both cases, the shaded area represents the specified fraction, λ, of the individuals to be selected from the known population. This fraction is obtained by assigning ranks within the group of clonal values for each index and selecting the clones with highest rank from each group. This way the selection ability of each index can be compared with that of another.

In addition to linear or nonlinear indices of the forms mentioned above, the literature suggests a variety of approaches to the problem of discriminating among individuals on the basis of several traits. These are discussed below, together with our own further development of some of them.

H-S index.—Consider the index (in matrix notation) of the form

$$H = a'X,$$

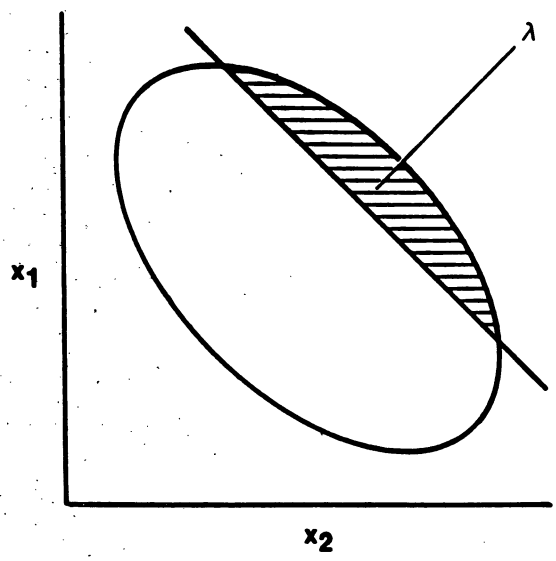

Figure 3.—*Selection based on the linear index $x_1 + wx_2$ for the selection portion (λ) of the population.*

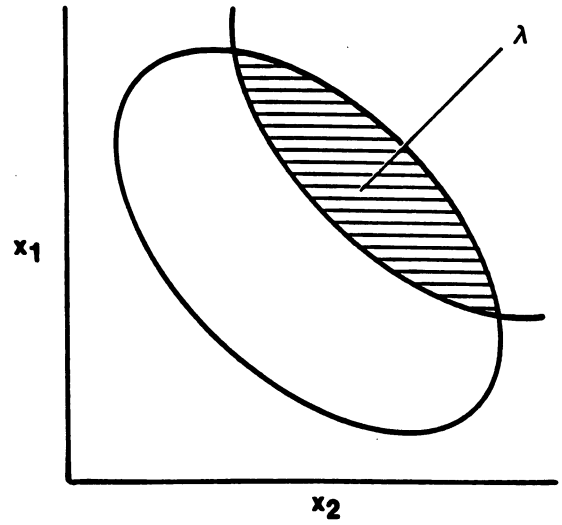

Figure 4.—*Selection based on the nonlinear index wx_1x_2 for the selection portion (λ) of the population.*

where

a = vector of known economic weights, and

X = vector (mxl) of unknown genotypic values of the individual clone for the m attributes of interest on which selection is to be based.

Because index H cannot be easily estimated, let selection be based on a linear function, I, which correlates best with the index H of the form

$$I_1 = b'Y,$$

where

Y = vector (mxl) of phenotypic values of the individual clone for the m attributes of interest on which selection is to be based, and

b = vector of coefficients to be determined from the system $Pb = Ga$ (Hazel 1943, Smith 1936), and where

P = matrix of phenotypic variances and covariances,

G = matrix of genotypic variances and covariances, and

a = vector of previously defined constants.

This implies $b = P^{-1}Ga$ and $I_1 = (P^{-1}Ga)'Y$.

Provided the following conditions hold, the selection index, I_1, will be a correct predictor of superior growth potential:

(1) The phenotypic value, P_i, for the i-th trait of an individual clone will be made up of the sum of two parts, the genotypic value, G_i, defined as the average of the phenotypic values possible over a range of environments and the environmental contribution, E_i—i.e., $P_i = G_i + E_i$. The $Cov(G_iE_i) = 0$, but genotypic by environment interactions, $(GE)_i$, can be presented provided genotypes and environments are associated with each other at random and $(GE)_i$ is incorporated with E_i.

(2) The genetic value, G_i, is composed entirely of additive gene effects.

(3) The quantities Y_i and H are such that the regression of H on any linear function of the Y_i is linear (Kempthorne and Nordskog 1959).

(4) The matrices of variances and covariances P and G are known.

Weight-free index.—Elston (1963) suggested the following nonlinear index for selection with respect to p traits at a time:

$$I_2 = \prod_{i=1}^{p} (x_i - k_i)$$

where

x_i = i-th trait measured on a particular individual clone, and
k_i = greatest lower bound of the x_i for all the individuals under consideration for selection.

However, this index is not independent of the scales used to measure the x_i's, thus:

$$x_i' = \log_{10}(x_i - k_i),$$

and the index becomes

$$\sum_{i=1}^{p} x_i'$$

If the index is to be based on weighted measurements, w_1x_1, w_2x_2, ..., w_px_p, and the w_i's are unknown, then an index that is invariant under the choice of the w_i's should be used. The index becomes:

$$I_2' = \prod_{i=1}^{p} (x_i' - k_i'),$$

where

$x_i' = \log_{10}(x_i - k_i)$, and
K_i' = lower bound of x_i'.

The index I_2 was evaluated for each clone within each environment and, since environments were assumed independent of each other, a simple average value for each clone over all environments was calculated.

Adaptation index.—Finlay and Wilkinson (1963) proposed a method of analyzing the adaptation of a randomly chosen group of 277 varieties of barley from a world collection, grown in replicated trials for several seasons at three sites in South Australia. For each variety, they computed a linear regression of individual grain yield on mean grain yield over all varieties for each environment (site and season). A slope of 1.00 meant that the variety was well adapted to all environments. This regression coefficient was then a measure of variety adaptation. The authors transformed their data logarithmically to index independence between means and their variances.

The study was assisted by the use of a scatter diagram that plotted variety regression coefficients (slopes) against variety means. However, no attempts were made by the authors to select a fraction, λ, of the varieties showing superior growth potential with respect to yield over all environments. We propose to go a step further than Finlay and Wilkinson and create the following index:

where $I_3 = (\hat{\mu}_{i.} - k_1)(b_i - 1.0)^2 - k_2$,

$\hat{\mu}_{i.}$ = mean of i-th variety over all environments for a particular variate of interest on which selection is to be based, and

b_i = regression coefficient (slope) of Y_{ij} on $\hat{\mu}_{.j}$,

where

Y_{ij} = mean of i-th variety at the j-th environment,
$\hat{\mu}_{.j}$ = mean of j-th environment over all varieties,
k_1 = greatest lower bound of $\hat{\mu}_{i.}$ and
k_2 = greatest lower bound of $(b_i - 1.0)^2$.

The index I_3 was evaluated for each clone for each of several variates of interest.

An alternative approach was to consider a canonical variable (Morrison 1967)—which is a linear combination of all the variates of interest in the selection process—as a variate on which selection is to be based. New environment and variety of environment means were found and the coefficients $b_i^{(\vartheta)}$ were obtained by linear regression techniques.

Then the index:

$$I_3^{(\vartheta)} = (\hat{\mu}_i^{(\vartheta)} - k_1)(b_i^{(\vartheta)} - 1.0)^2 - k_2 \qquad (6)$$

was calculated for each clone, where the superscript ϑ stands for the ϑ-th canonical variable used to evaluate the above previously defined parameters.

Curvature index.—Wu (1973) proposed that if the response of a varietyto various environments is quadratic, that is:

$$Y_i = a + bx_i + cx_i^2,\ i = 1,m,$$

where

m = number of environments,
Y_i = observed response of a clone at the i-th environment, and
x_i = independent i-th environmental measure,

then a measure of plant stability is the reciprocal of the radius of curvature as expressed below:

$$\rho = \left|\frac{1 + (y')2^{3/2}}{y''}\right|, \text{ and}$$

$$y' = b + 2cx = r, \text{ and}$$

$$y'' = 2c. \text{ Hence,}$$

$$\rho^{-1} = \left|\frac{1 + (b + 2cx)^{2^{3/2}}}{2c}\right|^{-1},$$

where x is day temperature and is evaluated at $\bar{x}$, and b anc c are coefficients. (A curvature of nearly zero implied the curve was nearly linear and clonal response to various environments was stable.) Next consider the index:

$$I_4 = (\bar{y}_i - k_1)(k_2 - |\rho^{-1} - (\bar{\rho})^{-1}|),$$

where

$\bar{y}_i$ = i-th clonal mean over all environments for a particular trait,
ρ^{-1} = curvature of the i-th clone,
$(\bar{\rho})^{-1}$ = average curvature over all clones,
k_1 = greatest lower bound of y_i, and
k_2 = greatest lower bound of $|\rho^{-1} - (\bar{\rho})^{-1}|$

However, this index deals with only one trait at a time, so consider the index:

$$I_4^{(\vartheta)} = (\bar{y}_i^{(\vartheta)} - k_1)\, k_2 - |\rho_\vartheta^{-1} - (\bar{\rho})_\vartheta^{-1}|,$$

where

$\bar{y}_i^{(\vartheta)}$ = i-th clonal mean of the ϑ-th canonical variable associated with the clone source of variation in the MANOVA,
ρ_ϑ^{-1} = curvature of the i-th clone based on ϑ-th canonical variable,
$(\bar{\rho})_\vartheta^{-1}$ = average curvature over all clones based on ϑ-th canonical variable,
k_1 = greatest lower bound of $\bar{y}_i^{(\vartheta)}$, and
k_2 = greatest lower bound of $|\rho_\vartheta^{-1} - (\bar{\rho})_\vartheta^{-1}|$.

With this index, clones with curvature near the average will be preferred over those with curvature far from the average.

Hamiltonian index.—Wu (1973) also considered the function $H = f(y,r)$, where $y = g(x)$ and $r = h(x)$. Here y and r are total growth and growth rate of the clones or varieties over various environments. The function, H, is the Hamiltonian for the system represented by the equations:

$$y' = b + 2cx = r,$$
$$y'' = 2c$$

(Brauer and Nohel 1969). If the above system is written in the form:

$$\frac{dy}{dx} = y' = \frac{\partial H}{\partial r},$$

$$\frac{dr}{dx} = y'' = -\frac{\partial H}{\partial y}.$$

With H defined as the total energy of the system and, assuming the principle of conservation of energy, the function H is a constant for different values of x. Therefore:

$$\frac{dH}{dx} = 0, \text{ and}$$

using one of the chain rules of calculus

$$\frac{dH}{dx} = \frac{\partial H}{\partial y}\frac{dy}{dx} + \frac{\partial H}{\partial r}\frac{dr}{dx}$$

which leads to the system derived previously:

$$\frac{dy}{dx} = y' = \frac{\partial H}{\partial r}$$

$$\frac{dr}{dx} = y'' = \frac{\partial H}{\partial y}$$

Substituting values for y' and y" the system (Equation 10) becomes:

$$\frac{\partial H}{\partial r} = r$$

$$\frac{\partial H}{\partial y} = -2c.$$

The solutions of this system of equations are $H = r^2/2 + c_1$ and $H = -2cy + c_2$, where c_1 and c_2 are constants. Since a linear combination of these two solutions is also a solution to the system,

$$H = \frac{r^2}{2} - 2cy$$

is also a solution; substituting for y and r produces

$$H = \frac{1}{2}b^2 - 2ac,$$

where a, b, and c are coefficients. Similar to the curvature index, consider

$$I_i^{(\vartheta)} = (\bar{y}_i^{(\vartheta)} - k_1)\,(k_2 - |H_\vartheta - \bar{H}_\vartheta|), \qquad (11)$$

where $y_i^{(\vartheta)}$ and k_1 are defined as the index $I_i^{(\vartheta)}$ and

H_ϑ = Hamiltonian of i-th clone based on ϑ-th canonical variable,

$\bar{H}_\vartheta$ = average Hamiltonian over all clones based on ϑ-th canonical variable, and

k_2 = greatest lower bound of $|H_\vartheta - \bar{H}_\vartheta|$.

Again, clones with a Hamiltonian, H close to $\bar{H}$, will be preferred.

Distance index.—The first step in this procedure is to calculate all the possible pairwise squared generalized distances between the clones of interest using a pooled variance-covariance matrix whose rank equals the number of variates used as discriminators. Then

$$D^2(i|j) = (\bar{x}_i - \bar{x}_j)'\,\mathrm{Cov}^{-1}(\bar{x}_i - \bar{x}_j),$$

where

$\bar{x}_i - \bar{x}_j$ = a vector (s x 1) of clonal mean differences,

Cov = pooled variance-covariance matrix (s x s), and

s = number of variates used as discriminators.

Next, these distances are used to cluster the clones into subgroups which are more homogeneous than those determined by the unweighted pair group method; the results are then displayed as a dendrograph (McCammon and Wenninger 1970). The dendrograph is looked at subjectively to determine which clones demonstrate superior growth.

Canonical index.—A canonical analysis is performed on the combined data over all environments for the variates (dependent variables) on which selection is to be based. Canonical variables of the form

$$Y^{\vartheta(\vartheta)} = R'_\vartheta X$$

are formed

where

$\vartheta = 1, \ldots, s$,

s = number of variates considered in the analysis,

X = a vector of correlated dependent variables on which selection is to be based, and

R_ϑ = ϑ-th normalized characteristic vector of $E^{-1}H$

where

$R'_\vartheta E R_\vartheta = 1$ (a scalar),

E = pooled error matrix, and

H = partial sums of squares and cross products matrix due to clone by environment source of variation in the Multivariate Analysis of Variance (MANOVA) table.

Means of the canonical variables are obtained by replacing the vector X by $\bar{X}$ such that

$$\bar{Y}_{ij}^{(\vartheta)} = R'_\vartheta\,\bar{X}_{ij}$$

where

$i = 1, \ldots, p$,

$j = 1, \ldots, m$,

p = number of clones,

m = number of environments, and

x_{ij} = a vector of means (s x 1) for the i-th clone at the j-th environment.

Then $\bar{Y}^{(2)}$ is plotted against $\bar{Y}^{(3)}$ and the resultant plot viewed subjectively. One looks for the clones in each environment that consistently are farthest from the origin. These are the superior clones.

Difficulties

As stated earlier, the H-S index is a correct predictor of superior growth potential only if all the mentioned assumptions hold. Immediately one observes that the variance-covariance matrices, P and

G, are now known and that only estimates of these are available. Data are often insufficient to estimate G. So the index is subject to sampling and estimation errors. Also the assumption that $P_i = G_i + E_i$ is usually unrealistic and Cov $(G_iE_i \neq 0)$. This would change the estimation procedure for obtaining the vector of coefficients, b. There also exists the problem of assigning the proper weight to each trait in the index.

It should be noted that even if some of the weights in the original index, H, are zero, all of the coefficients estimated for I_1 will be **nonzero.**

The weight-free index, contrary to its name, is really an index which assigns equal weights to all variates or traits. This may not be desirable, since one may want to select a small fraction of individuals on the basis of several traits that are to be unequally emphasized. This index also selects individuals with larger measurements on each trait, so one must arrange to measure each trait on a scale that will meet this criterion. Thus, if there is a trait for which a smaller measurement is desired, one would change the direction of the scale on which the trait is measured.

The distributions of the measurements on each of the traits should be as similar as possible for the weight-free index to be reliable. Frequently, when histograms are drawn up for the various traits contained in the index, the distributions of the measurements on some of the traits are of an entirely different type—e.g., bimodal instead of unimodal—from those of others. Those measurements should be transformed to lessen the differences.

The adaptation index seems the most promising, although index I_3 selects individuals on the basis of only one trait at a time. One must either look at each group of clonal values for index I_3 for each trait of interest in the selection process separately, remembering that these traits are highly correlated with each other, or use index $I_3^{(\phi)}$. With this index, however, it is difficult to know which canonical variable to use.

The curvature index, like the adaptation index, selects individuals based on only one trait at a time. It also requires an independent variable with at least three levels to fit the assumed quadratic. The radius of curvature of this function varies with different values of the independent variable (some environmental measure). For comparison, index I_4 is evaluated at the mean of the independent variable, but this may not be appropriate.

The Hamiltonian index comes from a principle of motion in physics which states that the total energy of a system is equal to the sum of the potential and kinetic energy. Applying this idea to biology involves substituting growth and growth rate for potential and kinetic energy. The index I_5 represents the biological total energy of the system. The principle of conservation of energy is assumed so that along any solution of a Hamiltonian system the total energy is constant. This may not be true when one considers growth and growth rate of a plant as position and speed of a particle.

The problem with the distance index is that it involves a subjective instead of objective procedure. Certain clones will be segregated from the main group but it will not be clear from the dendrograph whether these segregated clones are superior or inferior. Additional prior information is needed to interpret the results correctly.

The canonical index again is a subjective procedure, but seems to hold great promise, especially if $Y^{(2)}$ and $Y^{(3)}$ are plotted against each other instead of $Y^{(1)}$ and $Y^{(2)}$. This procedure appears to work best when the number of clones is greater than the number of environments.

Reliability of indices

To make reliability statements (such as confidence intervals) about the various indices, their probability density functions (p.d.f.'s) or distributions must be known exactly or approximated by some known tabulated distributions. An alternative is to assume some limiting distributions.

For the H-S index, I_1, one could easily find the expected value of I_1, $E(I_1)$, and the variance of I_1, $(V(I_1)$, if the P and G matrices were known. Then, if one assumed that the phenotypic values, Y, were distributed normally with some finite mean and variance, the distribution of index I_1 could be found. But matrices P and G must be estimated because they are usually not known. This makes it extremely difficult to find the variance of I_1.

On the other hand, the p.d.f. for the weight-free index, I_2, and adaptation index, I_3, can be found, but only under very restricted conditions. Without these restrictions some limiting distributions must be assumed.

For example, let selection be based on two traits only $(p = 2)$ and assume x_1 and x_2, the two traits,

are distributed lognormal with means, μ_1 and μ_2 and variances, σ_1^2 and σ_2^2, respectively. Then if one imposes the restrictions $k_1 = k_2 = 0$ (lower bound for x_1 and x_2 is zero) and x_1 and x_2 independent, then the index $I_2 = t = x_1x_2$ has the following p.d.f.:

$$g(t) = \frac{1}{\sqrt{2\pi}\, t \sqrt{\sigma_1^2+\sigma_2^2}} e^{-\frac{1}{2}(\log t-(\mu_1+\mu_2))^2/(\sigma_1^2+\sigma_2^2)}, \; 0<t<\infty.$$

If $_1 = {}_2 = 0$ and $_1^2 = {}_2^2 = 1$ then the above p.d.f. becomes

$$g(t) = \frac{1}{2t\sqrt{\pi}} e^{-\frac{1}{4}(\log t)^2}, \; 0<t<\infty.$$

If $k_1 / k_2 / 0$ then the p.d.f. cannot be found analytically. Note that the restriction x_1 and x_2 independent has not been relaxed, which is the case with this study.

The next step would be to find the first and second moments of indices I_2 and I_3, assuming the traits on which selection was to be based are normally distributed with finite mean and variance. Then Monte Carlo techniques could be used to artificially construct indices I_2 and I_3 based on sampling procedures, and frequency curves could be drawn. Next, known distributions such as t or F would be used to approximate the p.d.f.'s of index I_2 and I_3 based on the previously calculated moments, and frequency curves would again be drawn. Goodness-of-fit tests could then be used to determine how well the known distributions approximate the sample-based p.d.f.'s of indices I_2 and I_3.

The simplest method is as follows: Deming (1943) stated that the variance of a function g of a number of correlated random variables $x_1, \ldots, x_n$ could be approximated by the expression

$$V(g) \approx \sum_{i=1}^{n} \sum_{j=1}^{n} \left|\frac{\partial g}{\partial x_i}\right| \left|\frac{\partial g}{\partial x_j}\right| \mathrm{Cov}(x_i x_j).$$

Now recall the Central Limit Theorem (Hogg and Craig 1969), which states that if $x_1, \ldots, x_n$ denote the items of a random sample of size n from any distribution having finite variance σ^2, then the random variable $\sqrt{n}\,(\bar{x} - \mu)/\sigma$ has a limiting normal distribution with zero mean and unit variance. Utilizing this fact, assume the expression

$$\frac{\hat{I}_\vartheta - I_\vartheta}{(I_\vartheta)} \sim N(0,1) \text{ as } n \to \infty$$

$$\vartheta = 2,3;$$

then confidence intervals can be constructed of the form

$$\text{Prob } (L < I_\vartheta < u) \geq 1 - \alpha \quad \vartheta = 2,3,$$

where

L = some lower limit,

u = some upper limit,

$1-\alpha$ = confidence coefficient, and

α = significance level.

Likewise, the variances of indices I_4 and I_5 can also be found. It should be noted that a given index is more reliable if it fails to select a superior clone than if it includes a bad clone.

Evaluation of indices

Data collected by Wray (1974) (Trial I) were used to evaluate the seven indices described on the preceding pages. One growth chamber and two greenhouse environments were utilized to assess the juvenile growth potential of 25 *Populus* clones. There were four replications in each environment, yielding 300 observations. The variables measured were total plant height (HT), and stem and leaf dry weights (STMWT and LFWT).

Data from Trial II were also used to evaluate seven of the selection indices. Eight clones were grown twice in 16 environments, yielding 256 observations. Six variables were measured: basal diameter (DIA), total plant height (HT), leaf surface area (LFAREA), and leaf, stem, and root dry weights (LFWT, STMWT, RTWT).

H-S index.—Before estimating the coefficients of this index, the P and G matrices had to be obtained. The elements of these variance-covariance matrices were procured by performing all possible analyses of variance (ANOVA) and cross-product analyses for the traits of interest (three traits for Trial I and six traits for Trial II). Assuming all factors as random, except replicates within environments for Trial I, the expected mean squares were determined.

Assuming the model

$$P = G + E + EG$$

and $\quad \sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{EG}^2$

where

P = phenotypic value,
G = genotypic value,
E = environment component,
EG = genotype by environment interaction component,
σ^2_P = phenotypic variance,
σ^2_G = genotypic variance,
σ^2_E = environmental variance, and
σ^2_{EG} = genotype by environment variance,

the component of variance estimates, $\hat{G}_{ij}$ and $\hat{P}_{i,j}$, were obtained (table 12).

Elements of the vector, α, of economic weights were set equal to 1. Coefficients for index I_1 associated with each trait were obtained for the original data and the data transformed by means of logarithms to the base ten.

From the signs and magnitudes of the coefficients, index I_1 appeared to be a contrast between LFWT and STMWT in Trial I (table 13). For Trial II, however, index I_1 appeared to be a comparison of LFWT and STMWT versus RTWT and LFAREA, and DIA, HT and RTWT versus STMWT and LFAREA for the original and transformed data, respectively (table 13).

Index I_1 was evaluated by environment for Trial I for both the original and transformed data. Ranks were assigned to the index values within each group. The ranking of the clones within each environment varied substantially from one environment to another for both the original and transformed data (table 14). However, within each environment the ranks were approximately the same for both the original and transformed data. Since the three environments were independent of each other, these index values were summed for each clone and ranks were assigned to these values for both the original and transformed data.

Index I_1 was not evaluated for each environment in Trial II because the main objective was to select

Table 12.—*Formulas to obtain component of variance estimates for each trial*

Component[1]	Trial I	Trial II
$\hat{G}_{ij} = \sigma_{C_{ij}}$	$\frac{\text{M.S. (Clone)}_{ij} - \text{M.S. (E x C)}_{ij}}{12}$	$\frac{\text{M.S. (Clone)}_{ij} - \text{M.S. (E x C)}_{ij}}{32}$
$\hat{\sigma}^2_{EG_{ij}} = \hat{\sigma}^2_{EC_{ij}}$	$\frac{\text{M.S. (E x C)}_{ij} - \text{M.S. (Error)}_{ij}}{4}$	$\frac{\text{M.S. (E x C)}_{ij} - \text{M.S. (Error (b))}_{ij}}{2}$
$\sigma^2_{E_{ij}}$	$\frac{\text{M.S. (Envir)}_{ij} - \text{M.S. (E x C)}_{ij}}{100}$	$\{\text{M.S. (Envir)}_{ij} - \text{M.S. (Error (a))}_{ij} - \text{M.S. (E x C)}_{ij} + \text{M.S. (Error (b))}_{ij}\}/16$
	for i,j = 1,2,3 (number of variables)	for i,j = 1, . . . , 6 (number of variables)

[1]The three components listed were used to obtain the quantity:

$$\hat{P}_{ij} = \hat{\sigma}^2_{C_{ij}} + \hat{\sigma}^2_{EC_{ij}} + \hat{\sigma}^2_{E_{ij}}$$

Table 13.—*Estimated coefficients for index I_1 associated with various traits by data base and trial*

Trait	Trial I Data base Original	Trial I Data base Transformed	Trial II Data base Original	Trial II Data base Transformed
DIA	—	—	6.8974	27.4666
HT	0.3137	0.6631	1.0832	9.8186
LFWT	2.1883	1.9500	118.9613	1.9062
STMWT	−3.3192	−1.1332	66.0984	−4.4460
RTWT	—	—	−35.5430	8.0979
LFAREA	—	—	−61.9364	−15.7343

Table 14.—*Ranks associated with index I_1 values by environment for both the original and transformed data in Trial I*

Clone	Environment 1 O[1]	Environment 1 T[2]	Environment 2 O	Environment 2 T	Environment 3 O	Environment 3 T
4877	11	12	20	22	14	15
4878	16	19	10	14	8	11
4879	19	22	8	7	18	12
5258	5	5	18	8	22	9
5262	13	20	6	10	9	10
5263	10	14	13	20	16	20
5264	4	3	5	5	4	4
5265	2	2	3	2	1	2
5266	1	1	1	1	3	3
5267	22	17	22	24	17	21
5271	25	25	25	25	23	25
5272	9	8	15	16	13	17
5321	20	18	17	18	21	24
5322	24	24	14	11	5	8
5323	7	7	7	6	6	5
5324	17	15	19	13	15	18
5325	6	6	9	9	10	7
5326	8	11	21	15	24	13
5327	14	13	11	17	11	14
5328	21	9	4	4	7	6
5331	15	16	12	12	12	19
5332	18	21	16	19	19	22
5334	3	4	2	3	2	1
5260	23	23	24	23	20	23
5377	12	10	23	21	25	16

[1]Original data.
[2]Transformed data.

clones exhibiting rapid juvenile growth over all 16 environments. Marked variation in the ranking of the clones was found from one environment to the next.

For Trial I, clones 5266, 5334, 5265, 5264, and 5323 were selected when the original data were considered, and clones 5266, 5265, 5334, 5264, and 5328 when the transformed data were used (table 15). This selection was based on the assumption that the top 20 percent of the clones under consideration was to be chosen. Essentially the same clones were chosen, regardless of the data base (original or transformed). The data, therefore, need not have been transformed from a selection viewpoint.

In Trial II, clone 5323, balsam poplar, and clones 5339 and 5321 were selected based on the original and transformed data, respectively (table 16). The results of this index are unexpected, since clone 5339 and balsam poplar are definitely not superior.

Table 15.—*Ranks associated with the values of five different indices in Trial I*

Clone	Index 1		2	3				6	7
	O[1]	T[2]	O	TLF[4]	TST[5]	THT[6]	OC[7]	O	O
4877	14	19	17	18	19	18	14		
4878	9	13	18	16	17	13	6		
4879	18	12	5	8	4	3	13	7	
5258	17	7	3	2	1	8	23	9	
5262	6	9	11	11	6	1	2		4
5263	13	20	15	15	10	2	7		5
5264	4	4	8	9	16	14	5	4	
5265	3	2	6	3	9	16	11	3	3
5266	1	1	2	1	3	4	1	1	1
5267	22	22	24	21	23	21	17		
5271	25	25	25	24	24	22	25		
5272	11	11	14	12	13	6	8		
5321	20	21	23	23	21	20	18		
5322	15	15	13	25	22	23	21		
5323	5	6	10	6	8	9	3	10	
5324	16	16	16	13	14	19	15		
5325	7	8	9	5	7	7	4		
5326	21	10	4	4	2	10	22	5	
5327	10	14	20	14	18	17	9		
5328	8	5	12	10	20	24	16		
5331	12	17	19	17	11	12	10		
5332	19	23	22	20	12	5	12		
5334	2	3	1	19	15	15	19	2	2
5260	24	24	21	22	25	25	20	8	
5377	23	18	7	7	5	11	24	6	

[1]Original data based on three traits.
[2]Transformed data based on three traits.
[3]Original data based on two traits.
[4]Transformed data based on LFWT.
[5]Transformed data based on STMWT.
[6]Transformed data based on HT.
[7]Original data utilizing a canonical variable.

Table 16.—*Ranks associated with the values of seven different indices in Trial II*

Clone	Index 1		2	3	4	5	6	7	
	O[1]	T[2]	O	OC[3]	OC	OC	O	OC1[4]	OC2[5]
5321	3	2	5	5	4	4	3	3	
5323	1	4	1	4	2	5	2	2	
5326	4	7	2	1	1	3			3
5328	7	5	4	7	6	6	1	1	2
5377	6	6	3	6	3	1			
5260	5	3	6	3	5	2		8	1
5339	8	1	7	2	7	7			
Balsam	2	8	8	8	8	8	8		8

[1]Original data based on six traits.
[2]Transformed data based on six traits.
[3]Original data utilizing a canonical variable.
[4]Canonical variables associated with Photo/Clone interaction.
[5]Canonical variables associated with Temp/Clone interaction.

One critical assumption associated with this index states that index I_1 is effective as a discriminator only when the genotypic correlations between the traits included in the index are high. The following statistics were calculated from the relation:

$$r_g = \hat{\sigma}G_{ij} \quad \sqrt{\hat{\sigma}^2_{G_{ii}} \cdot \hat{\sigma}^2_{G_{jj}}}$$

$$i,j = 1, \ldots, 3 \text{ (Falconer 1960)}$$

for both the original and transformed data.

All the correlations between the three traits of interest in Trial I were high, with the exception of the genotypic correlation between LFWT and HT for both the original and transformed data. Correlations based on the transformed data were slightly higher than those based on the original data (table 17). Index I_1, therefore, is an effective discriminator for Trial I. In Trial II, however, there are low genotypic correlations between the traits DIA and HT, and LFWT and HT for both the original and transformed data (table 18). This may indicate that index I_1 is not an effective discriminator in Trial II, possibly because of the large number of traits being considered.

Weight-free index.—Before evaluating this index, the assumptions that the distributions of the traits are similar (at least unimodal) and that selection of individuals with large measurements on each trait is desired were verified.

For Trial I, the trait HT was found not to be as important as the traits LFWT and STMWT, thus HT was omitted. Lower bounds for the remaining two

Table 17.—*Genotypic correlations between three traits based on original transformed data (n = 300) for Trial I*

Trait	Trait		
	LFWT	STMWT	HT
LFWT	1.0000[1]	0.8490	0.5139
	1.0000	.8464	.5574
STMWT		1.0000	.7513
		1.0000	.7974
HT			1.0000
			1.0000

[1]Upper figure is based on original data and lower figure on transformed data.

traits were obtained for each environment and then ranks were assigned to the index values within each environment. The lower bounds for both traits increased from environment 1 to environment 3, indicating improved growing conditions from the growth chambers to the greenhouse environments (table 19).

Ranks associated with index I_2 showed marked variation from one environment to another. For example, clones 5260, 5326, 5258, 5266, and 5377 were selected in environment 1, while clones 5266, 4879, 5258, 5334, and 5265 and clones 5334, 5258, 5266, 5326, and 5377 were selected in environments 2 and 3, respectively. The only clones selected in all three environments were 5266 and 5258 (table 20). This index appeared to be less consistent than index I_1 from one environment to another.

Index values were also summed over environments for each clone and ranks were assigned. Clones

Table 18.—*Genotypic correlations (over all clones) for six traits based on the original and transformed data (n = 256) for Trial II*

Trait	Trait					
	DIA	HT	LFWT	STMWT	RTWT	LFAREA
DIA	1.0000[1]	0.3267	0.9256	0.7143	0.8512	0.9619
	1.0000	.2764	.9031	.6923	.7879	.9222
HT		1.0000	.4451	.8644	.6731	.6060
		1.0000	.4730	.8251	.6724	.6056
LFWT			1.0000	.7774	.9192	.9707
			1.0000	.8248	.8921	.9620
STMWT				1.0000	.8588	.8676
				1.0000	.9132	.9048
RTWT					1.0000	.9455
					1.0000	.9569
LFAREA						1.0000
						1.0000

[1]Upper figure based on original data and lower figure based on transformed data.

Table 19.—*Lower bounds for two traits LFWT and STMWT by environment*

Trait	Environment		
	1	2	3
LFWT	7.223	9.458	11.451
STMWT	2.580	5.987	7.379

5334, 5266, 5258, 5326, and 4879 were chosen (table 15). For both index I_1 and I_2 only clones 5334 and 5266 were chosen by each index.

For Trial II, index I_2 was not evaluated for each of the 16 environments. Based on the ranks associated with index I_2 values over all environments, clones 5323 and 5326 were selected (table 16). Only clone 5323 was chosen by both I_1 and I_2.

Adaptation index.—Regression analyses of the clone by environment means on the environment means (over all clones) were performed for each trait. Only the transformed data were used, since the logarithmic transformation helped to linearize the data as well as decrease the dependence between means and variances.

Table 20.—*Ranks associated with index I_2 values based on two traits by environment for Trial I*

Clone	Environment		
	1	2	3
4877	9	24	13
4878	21	22	16
4879	11	2	8
5258	3	3	2
5262	14	6	11
5263	8	20	15
5264	12	8	6
5265	13	5	7
5266	4	1	3
5267	23	23	23
5271	24	25	25
5272	19	13	17
5321	20	21	24
5322	25	16	14
5323	10	7	10
5324	17	11	19
5325	6	9	9
5326	2	10	4
5327	18	18	18
5328	22	14	12
5331	15	12	20
5332	16	15	21
5334	7	4	1
5260	1	19	22
5377	5	17	5

By definition, a clone is stable across all environments for a particular trait if the slope of the straight line fitted through the above-mentioned means is near 1.0 (Finlay and Wilkinson 1963). Via the method of simple least squares, these lines were fitted and regression coefficients were obtained. Considering Trial I and the trait LFWT, clones 5258, 5325, 5326, 5327, and 5377 are stable, while clones 5321, 5322, 5334, and 5260 are highly unstable. For the trait STMWT, clones 4878, 5324, 5325, 5326, 5327, and 5377 are stable, while clones 5271, 5322, 5334, and 5260 are unstable (table 21). For the trait HT, clones 5258, 5262, 5264, 5265, 5272, 5323, 5325, and 5377 are stable, while clones 5271, 5334, and 5260 are unstable. All three traits considered jointly revealed clones 5325 and 5377 as stable, and clones 5334 and 5260 as unstable.

To assist data interpretation, we made scatter diagrams by plotting clonal regression coefficients (slopes) against clone means (over all environments) for each of the three traits. Using figure 5, we determined the general significance of the location of the points

Table 21.—*Estimated regression coefficients associated with the simple linear regression of clone by environment means on environment means for three traits, based on the transformed data*

	LFWT		STMWT		HT	
Clone	a[1]	b[2]	a	b	a	b
4877	0.044	0.915	0.128	0.798	0.471	0.769
4878	-.206	1.126	-.034	.989	.182	.920
4879	-.322	1.287	.029	1.108	-.073	1.063
5258	.166	.981	.096	1.083	-.018	1.019
5262	-.252	1.206	.000	1.088	.027	1.036
5263	.148	.862	.123	.907	.176	.942
5264	-.371	1.340	-.162	1.157	-.049	1.030
5265	-.106	1.190	-.092	1.127	-.066	1.035
5266	.063	1.099	.037	1.097	-.197	1.122
5267	.239	.675	-.207	.934	.056	.925
5271	.184	.583	.078	.598	.314	.794
5272	.077	.929	-.117	1.112	.002	1.016
5321	.797	.308	.163	.695	.246	.834
5322	-1.118	1.804	-.658	1.510	-.901	1.386
5323	-.172	1.189	-.023	1.089	.076	.973
5324	.187	.846	-.029	1.014	-.418	1.191
5325	.049	1.022	.062	1.018	-.002	1.012
5326	.091	.993	.202	.956	.160	.934
5327	-.049	1.009	-.007	.960	.234	.887
5328	-.304	1.258	-.365	1.261	-.546	1.206
5331	.349	.722	.163	.848	-.094	1.055
5332	.379	.649	.092	.905	-.067	1.054
5334	-.623	1.590	-.245	1.341	-.442	1.241
5260	.785	.363	.705	.353	.943	.540
5377	-.036	1.054	.060	1.051	-.012	1.015

[1]Intercept.
[2]Slope.

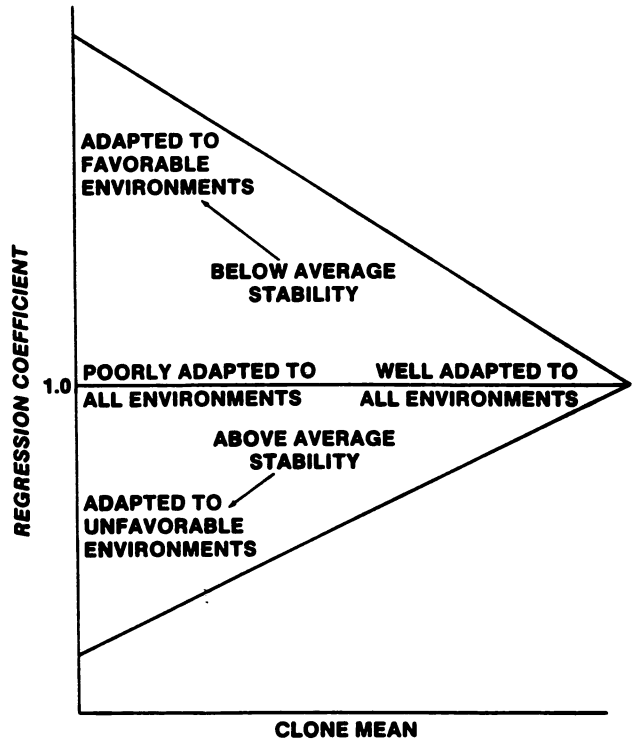

Figure 5.—*General interpretation of the scatter diagram when clonal regression coefficients are plotted against clone means.*

on these scatter diagrams. For the trait LFWT, clones 5258(4), 5265(8), and 5266(9) appear well-adapted to all environments; for the trait STMWT, clones 4879(3), 5258(4), 5266(9), and 5326(18); and for the trait HT, clones 4879(3), 5262(5), 5263(6), and 5266(9) (figs. 6, 7, and 8).

In Trial II the slopes of the fitted lines indicated that balsam poplar was unstable for all traits while clone 5326 was stable for all traits except DIA (table 22).

Index I_3 (adaptation index) has a form similar to index I_2 because individuals are selected on the basis of large values of clone means (over all environments). In addition, individuals are selected on the basis of small values of the squared deviations of the slope about the mean slope, 1.0 (i.e., min $(b_i-1.0)^2$). These requirements led to the form of index I_3:

$$I_3 = (\hat{\mu}_{i.} - k_1)\{k_2 - (b_i - 1.0)^2\}$$

The lower bounds for the expressions μ_i and $(b_i-1.0)^2$ were obtained for the traits used in Trials I and II (table 23), and index I_3 values and associated ranks were calculated. For Trial I, clones 5266, 5258, 5265, 5326, and 5325 were chosen for the trait LFWT; clones

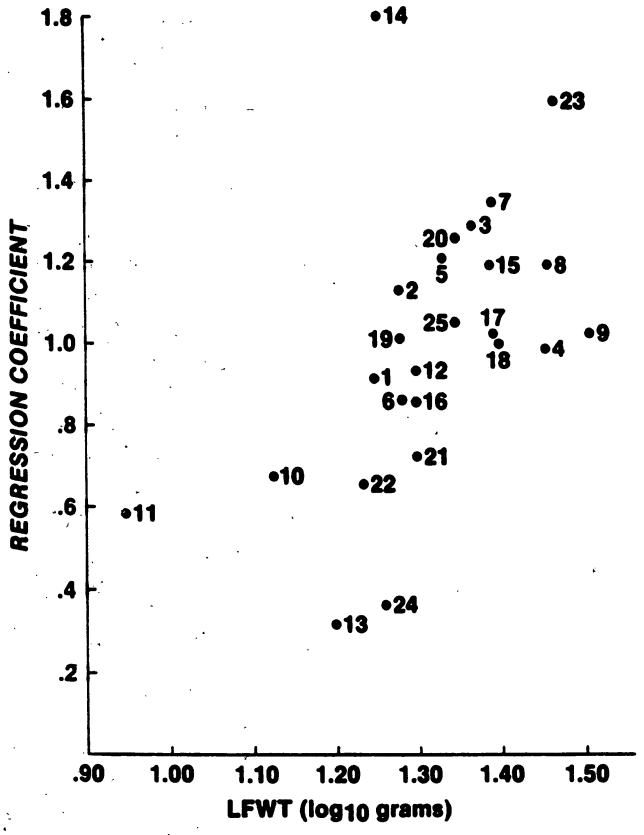

Figure 6.—*Clonal regression coefficients (slopes) plotted against clone means (over all environments) for the trait LFWT, based on the transformed data.*

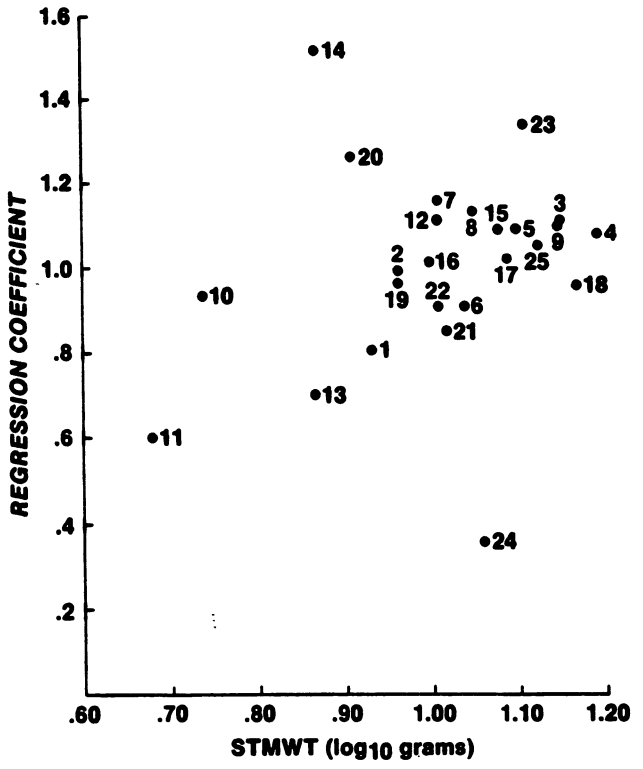

Figure 7.—*Clonal regression coefficients (slopes) plotted against clone means (over all environments) for the trait STMWT, based on the transformed data.*

5258, 5326, 5266, 4879, and 5377 for the trait STMWT; and clones 5262, 5263, 4879, 5266, and 5332 for the trait HT (table 15). When all three traits were considered jointly, only clone 5266 was selected. Due to the dependence between traits, the index values could not be summed over all traits for each clone.

In Trial II, clones 5323 and 5326 were chosen when each of the six traits were considered separately, even though clone 5326 ranked low with respect to the trait DIA. Balsam poplar ranked last for all six traits (table 24), which is understandable since this clone is a naturally occurring *Populus* species and not a purposely chosen hybrid like the other seven clones.

An alternative procedure was to construct index $I_3^{(\theta)}$ utilizing the th canonical variable associated with the clone source of variation in the MANOVA table (see Canonical index). The canonical variable:

$Y^{(2)} = 0.02016(LFWT) - 0.03383(STMWT) + 0.00455(HT)$ was chosen for Trial I and the canonical variable

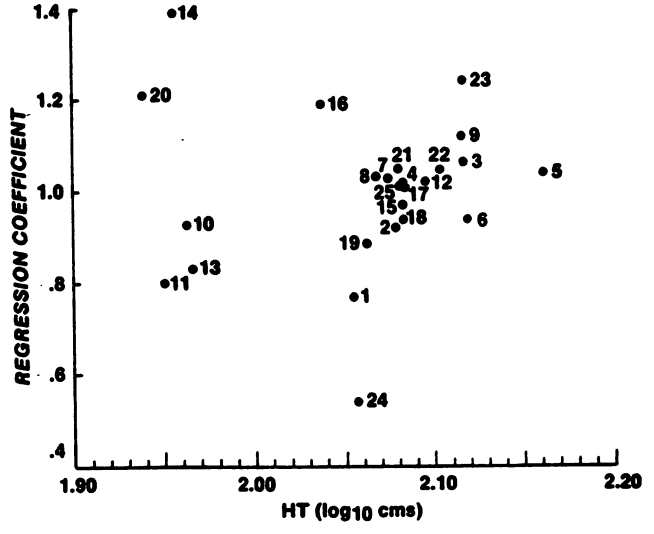

Figure 8.—*Clonal regression coefficients (slopes) plotted against clone means (over all environments) for the trait HT, based on the transformed data.*

Table 22.—*Estimated slopes of the straight lines fitted by regressing the clone by environment means on the environment means for six traits, based on transformed data (Trial II)*

Clone	Trait					
	DIA	HT	LFWT	STMWT	RTWT	LFAREA
5321	0.942	0.926	0.881	0.942	1.019	0.872
5323	.927	1.008	1.111	1.093	1.132	1.034
5326	.490	.970	.992	.990	.983	1.020
5328	1.028	1.097	1.241	1.171	1.155	1.238
5377	1.243	1.087	1.243	1.159	1.164	1.142
5260	1.039	1.225	.959	1.016	.823	1.018
5339	1.189	.983	1.047	1.071	1.037	1.061
Balsam	.546	.703	.525	.556	.687	.614

$$Y^{(2)} = -0.03020(DIA) + 0.00437(HT) - 0.07773(LFWT) - 0.05162(STMWT) + 0.11780(RTWT) + 0.03984(LFAREA)$$

was chosen for Trial II.

Through this canonical variable, the clone, environment, and clone by environment means were transformed to produce new canonical means. Slopes were obtained by fitting lines through these new means—i.e., regressing the canonical clone by environment means on the canonical environment means.

Index $I_3^{(2)}$ was of the form

$$I_3^{(2)} = (\overline{Y}_i^{(2)} - 0.4258)\{0.8604 - (b_i^{(2)} - 1.0^2\}$$

for Trial I and

$$I_3^{(2)} = (\overline{Y}_i^{(2)} - 0.00272)\{0.4009 - (b_i^{(2)} - 1.0^2\}$$

for Trial II, where
$\overline{Y}_i^{(2)}$ = clone mean of canonical variable 2, and
$b_i^{(2)}$ = slope of line fitted through canonical variable 2 means.

Table 23.—*Lower bounds for two expressions which form index I_3 for the appropriate traits by trial, based on transformed data*

Trait	Trial I expression		Trial II expression	
	$\mu_{i.}$	$(b_i-1.0)^2$	$\mu_{i.}$	$(b_i-1.0)^2$
DIA	—	—	0.7623	0.2601
HT	1.9385	0.2116	1.5492	.0882
LFWT	.9522	.6464	.5170	.2256
STMWT	.6838	.4186	.1258	.1971
RTWT	—	—	-.0954	.0980
LFAREA	—	—	.7687	.1490

Table 24.—*Ranks associated with values of index I_3 for six traits, based on transformed data in Trial II*

Clone	Trait					
	DIA	HT	LFWT	STMWT	RTWT	LFAREA
5321	4	5	4	5	2	4
5323	2	1	1	2	3	1
5326	7	2	2	1	1	2
5328	1	7	3	6	4	5
5377	3	3	5	4	7	3
5260	6	6	6	3	6	6
5339	5	4	7	7	5	7
Balsam	8	8	8	8	8	8

From the ranks associated with the values of index $I_3^{(2)}$, clones 5266, 5262, 5323, 5325, and 5264 were selected for Trial I and clones 5326 and 5339 for Trial II (tables 15 and 16).

For Trial I there seems to be no consistency between this index and the previous two indices. Perhaps another canonical variable should have been chosen, although the signs and magnitudes of the coefficients of $Y^{(2)}$ are acceptable from a selection viewpoint. In Trial II, the lack of consistency between the indices may be due to the large number of traits on which selection is based.

Curvature index.—Like the adaptation index, this index selects clones on the basis of one trait at a time over the range of some independent environmental measure. Because none of the clonal responses over environments in Trial I was quadratic, this index was not evaluated. In Trial II, this index was evaluated but an interpretation problem existed when the ranks associated with the index values for each of the six traits were considered simultaneously.

Consequently, the canonical variable 2 given in the previous subsection was used to obtain a linear combination of all six traits. For each clone the quadratic

$$Y_{ij}^{(2)} = a + bT_i + cT_i^2$$

where

T_i = day temperature (17, 23, 29, 35°C) and
$i = 1, \ldots, 4; j = 1, \ldots, 8$

was fitted, as well as the quadratic over all clones. The curvature parameter, ρ, for each clone and over all clones was evaluated by setting $T = \overline{T} = 26$. Clones with curvatures near the average were considered stable and those with curvatures much above

or below the average were considered to have below- and above-average stability, respectively. Clone 5326 exhibited average stability, while clone 5339 and balsam poplar showed below- and above-average stability, respectively (table 25).

Index I_4 was of the form

$$I_4 = (\overline{Y}_i^{(2)} - 0.00272)(0.00205 - [\rho_i - 0.00304])$$

where

$\overline{Y}_i^{(2)}$ has previously been defined,

ρ_i = i-th clonal curvature,

and the constant inside the absolute value signs is ρ.

From the ranks associated with the values of index I_4, clones 5326 and 5323 were selected (table 16). These results are consistent with those obtained via index I_2 and are inconsistent with the results obtained via indices I_1 and I_3.

Table 25.—*Regression coefficients for the model* $Y_{ij} = a + bT_i + cT^2_i$, *the parameters* ρ *and H for each clone, and the overall average*

Clone	Regression coefficients	ρ	H
5321	-1.26370 .10552 -.00170	0.00340	0.00127
5323	-1.26032 .10678 -.00173	.00346	.00134
5326	-1.04387 .09194 -.00147	.00294	.00115
5328	-1.57985 .12965 -.00217	.00434	.00154
5377	-1.08102 .08832 -.00132	.00264	.00104
5260	-.87136 .07313 -.00102	.00203	.00090
5339	-1.66792 .14504 -.00254	.00509	.00203
Balsam	-.29345 .01662 -.00019	.00038	.00003
Average	-1.13267 .09462 -.00152	.00304	.00104

Hamiltonian index.—Using only the data from Trial II and the estimated coefficients of the quadratic equations developed for the curvature index, the parameter H was calculated for each clone and over all clones. Clone 5377 exhibited average stability, while clone 5339 and balsam poplar showed below- and above-average stability, respectively (table 25). The interpretation of H is the same as that for parameter ρ.

From the ranks associated with the values of index I_5, which has the form $I_5 = (\overline{Y}_i^{(2)} - 0.00272)\{0.00101 - [H_i - 0.00104]\}$ with the constant inside the absolute value signs denoting H, clones 5377 and 5260 were selected (table 16). The results obtained from this index were completely different from those obtained from any of the other indices. Possibly the principle of conservation of energy does not hold and the function $H = f(g(x), h(x))$ is not constant for different values of x.

Distance index.—A discriminant analysis was performed on the original data and the generalized squared distance between each possible pair of clones was calculated considering the traits LFWT, STMWT, and HT. A pooled covariance matrix was used to ensure consistency with the standard analysis of variance assumption of equality of variance. Although a test for the equality of a group of variance-covariance matrices was made and resulted in the rejection of the null hypothesis H_o: $\Sigma_1 = \Sigma_2 = \ldots = \Sigma_p = \Sigma$ where p equals the number of clones, this test is not a good one. Even if one element of one of these matrices is different from the corresponding elements in the other matrices, H_o will be rejected.

A better plan is to look at the correlation matrices, one for each clone, and see if the signs and magnitudes of the coefficients are similar from one clone to the next. When this was done the correlation matrices were found to be quite similar.

Next a cluster analysis was performed on these clonal distances and a dendrograph was constructed to display the results. From this graph clones 5266, 5334, 5265, 5264, and 5326 were chosen for Trial I. In Trial II, clone 5328 and balsam poplar were selected (fig. 9). Recall that this technique, however, separates very poor clones also. Based on the clonal means over all environments for each of the six traits, balsam poplar was the poorest overall performer of the eight clones. Balsam poplar, therefore, was rejected and clone 5323 was selected instead (fig. 10). With the exception of index I_1 based on the original data, balsam poplar was ranked the lowest of all eight clones by all the other indices considered so

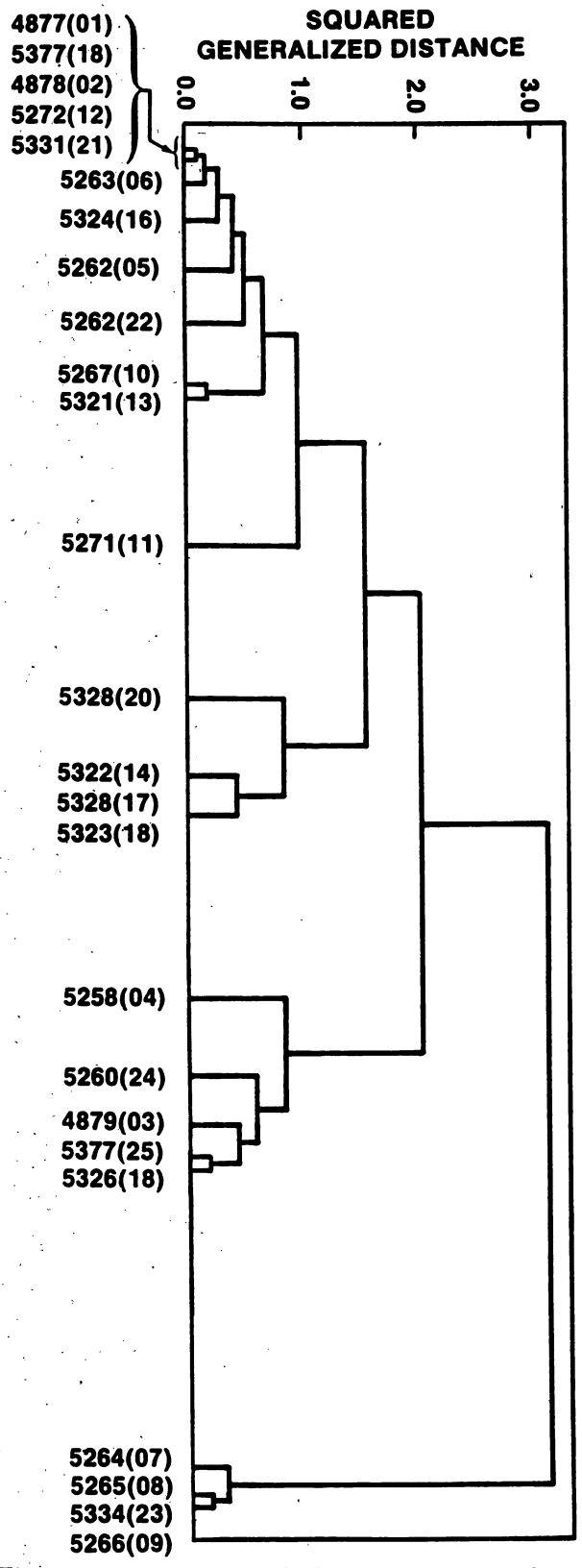

Figure 9.—*Dendrograph displaying the results of a cluster analysis performed on the generalized squared distances between 25 clones based on three traits in Trial I.*

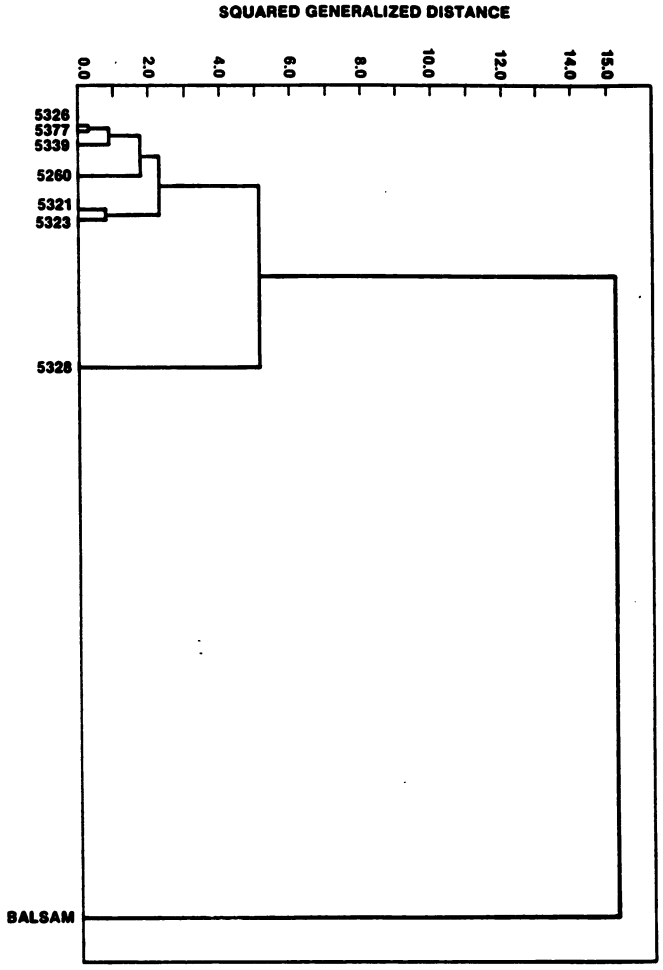

Figure 10.—*Dendrograph displaying the results of a cluster analysis performed on the generalized squared distances between eight clones based on six traits.*

far. To interpret the dendrograph correctly when widely different clones are present, the mean clonal response over all environments for the traits of interest must be considered.

The results obtained via index I_6 agree closely with those obtained by index I_1 for Trial I, but are inconsistent with all the other indices for Trial II.

Canonical index.—A multivariate analysis of variance was performed on the combined data over all environments with all traits considered jointly for both Trials I and II. The form of the univariate analysis of variance associated with each trait prior to the multivariate analysis was identical to that assumed for the H-S index in Trials I and II (table 11).

Canonical variables were derived from the partial sums of squares and cross-products matrix due to the

clone by environment interaction for Trial I. For Trial II, canonical variables associated with both the partial sums of squares and cross-products matrices due to the photoperiod by clone and temperature by clone interactions, respectively, were calculated.

Generally the first two canonical variables account for 80 percent or more of the total variation in the data and thus are good for data condensation and description purposes (table 26). These first two canonical variables, however, are poor discriminators. The means of canonical variable 1 (one for each clone) within each environment tend to cluster around the overall mean. This is not true for the means of canonical variables 2 and 3. As a result, the plotting of canonical variable 1 means versus canonical variable 2 means yielded no good clonal separation, but the plotting of the means of canonical variables 2 against 3 did.

For trial I, three distinct clusters of points appeared, one for each environment, and the superior clones stood out clearly. Clones 5266(9), 5334(23), 5265(8), 5262(5), and 5263(6) were selected (fig. 11). The results of this index also compare favorably with those obtained by the H-S index, I_1, and the distance index, I_6.

For Trial II, four distinct groups of points were exhibited by both plots, one for each photoperiod or temperature level.

Considering the means of canonical variables 2 and 3 associated with the photoperiod x clone interaction, clones 5323(2) and 5328(4) were chosen (fig. 12). When the means of $Y^{(2)}$ and $Y^{(3)}$ associated with the temperature x clone interaction were considered, the selection of clones was not so obvious. Balsam poplar (8) was definitely the poorest performer, while clones 5260(6) and 5339(7) were "best" at two out of the four temperature levels (fig. 13). One reason for this dilemma might be the magnitude and signs associated with the coefficients of the canonical variables. For example, the signs of the coefficients for the traits LFWT and RTWT for the temperature x clone interaction are reversed as compared to coefficients for the same traits and corresponding canonical variable related to the photoperiod x clone interaction.

Table 26.—*Coefficients of each canonical variable (CANVAR) and the percentage of the total variation explained by each CANVAR for Trial I*

Canonical variable	Trait			Percentage
	LFWT	STMWT	HT	
1	-0.01614	0.04589	-0.00257	59.12
2	.01842	-.02041	.00257	24.57
3	-.00804	-.00979	.00709	16.31

Another reason was that the elements of the partial sums of squares and cross-products matrix associated with the temperature x clone interaction were much larger than the elements of the corresponding matrix related to the photoperiod x clone interaction. Thus, the temperature x clone means were more variable than the photoperiod x clone means.

The results of I_7 based on the photoperiod x clone interaction agreed with those obtained from index I_6, but not with any of the other index results.

Appraisal of index reliability

Confidence intervals about the values of indices I_2 through I_5 were determined to appraise the reliability of the indices. However, due to the large variances and positive covariances between traits, the approximated variances associated with some index values were so large that confidence intervals were meaningless. A more useful approach was to apply an index with coefficients estimated from existing data to new data and vice versa. This was done for indices I_1 through I_3.

Index I_1 with coefficients derived from Trial I was applied to both the original and transformed data from Trial II, and ranks were assigned to the resulting index values. Clones 5323 and 5328 were chosen. These results agreed with those obtained by index I_6 and I_7 based on the data from Trial II only.

To apply index I_1 with coefficients, b, derived from Trial II to Trial I, the coefficients had to be recalculated because not all traits were present in both data sets. From the ranks associated with the values of this latter index, clones 5326, 5377, 5334, 5266, and 5325 were selected. With the original index I_1 clones 5334 and 5266 were chosen. Apparently the index derived from an experiment involving few environments and many clones gives more reliable results than one obtained from an experiment involving many environments and few clones.

Similarly, index I_2 with coefficients derived from Trial II was applied to Trial I, and clones 5334, 5266, 5258, 5326, and 4879 were chosen. These results compared favorably with those obtained by index I_2 when it was derived from and used in Trial I. When

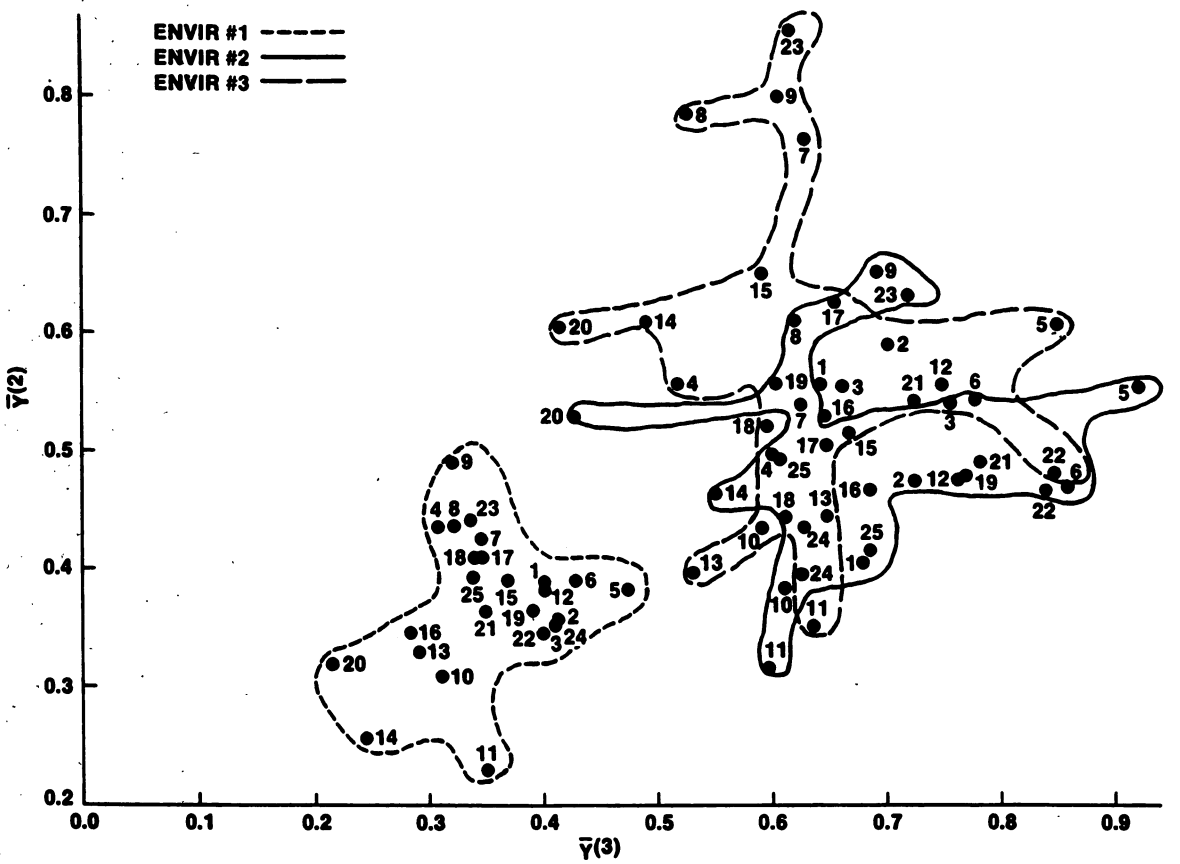

Figure 11.—*Plot of the means of canonical variable 2 versus the means of canonical variable 3 for each environment based on three traits for Trial I ($Y^{(2)}$ and $Y^{(3)}$ are associated with H_{CE}).*

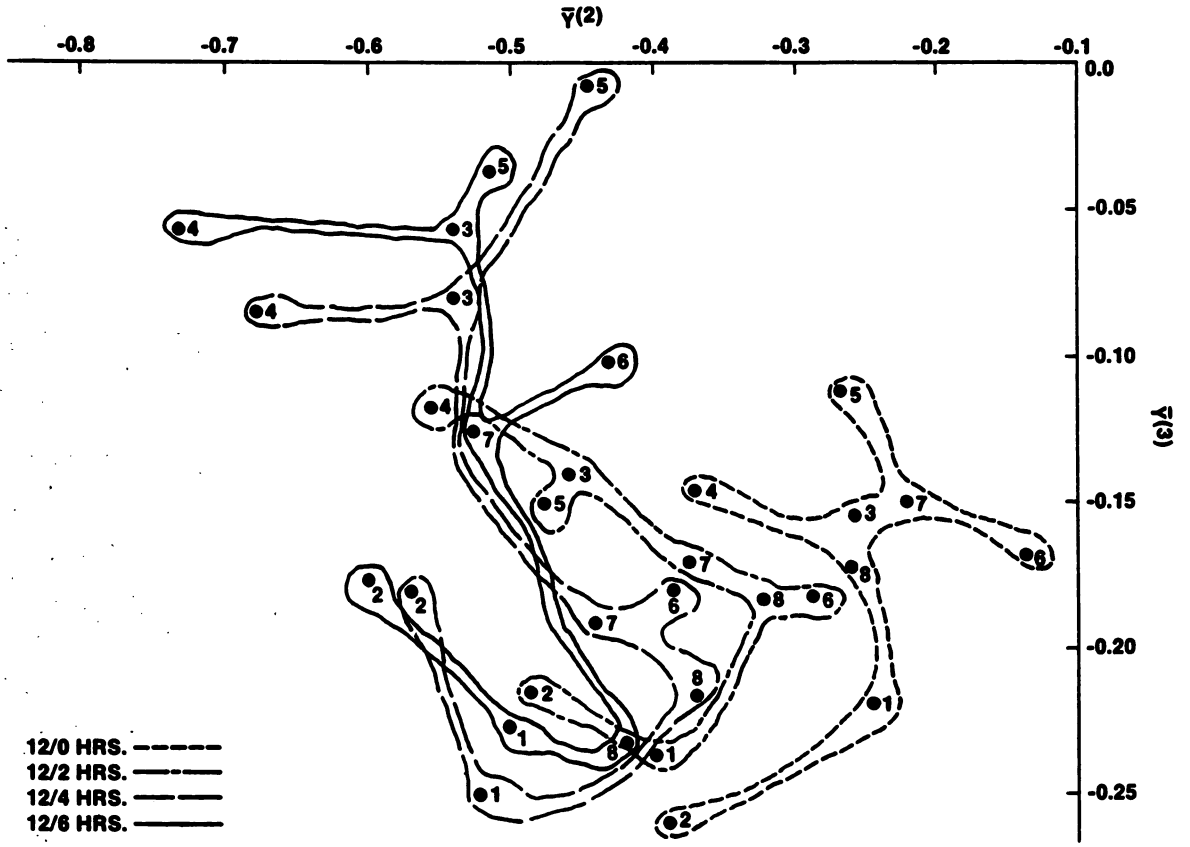

Figure 12.—*Plot of the means of canonical variable 2 versus the means of canonical variable 3 for each photoperiod level based on six traits in Trial II ($Y^{(2)}$ and $Y^{(3)}$ are associated with H_{PC}).*

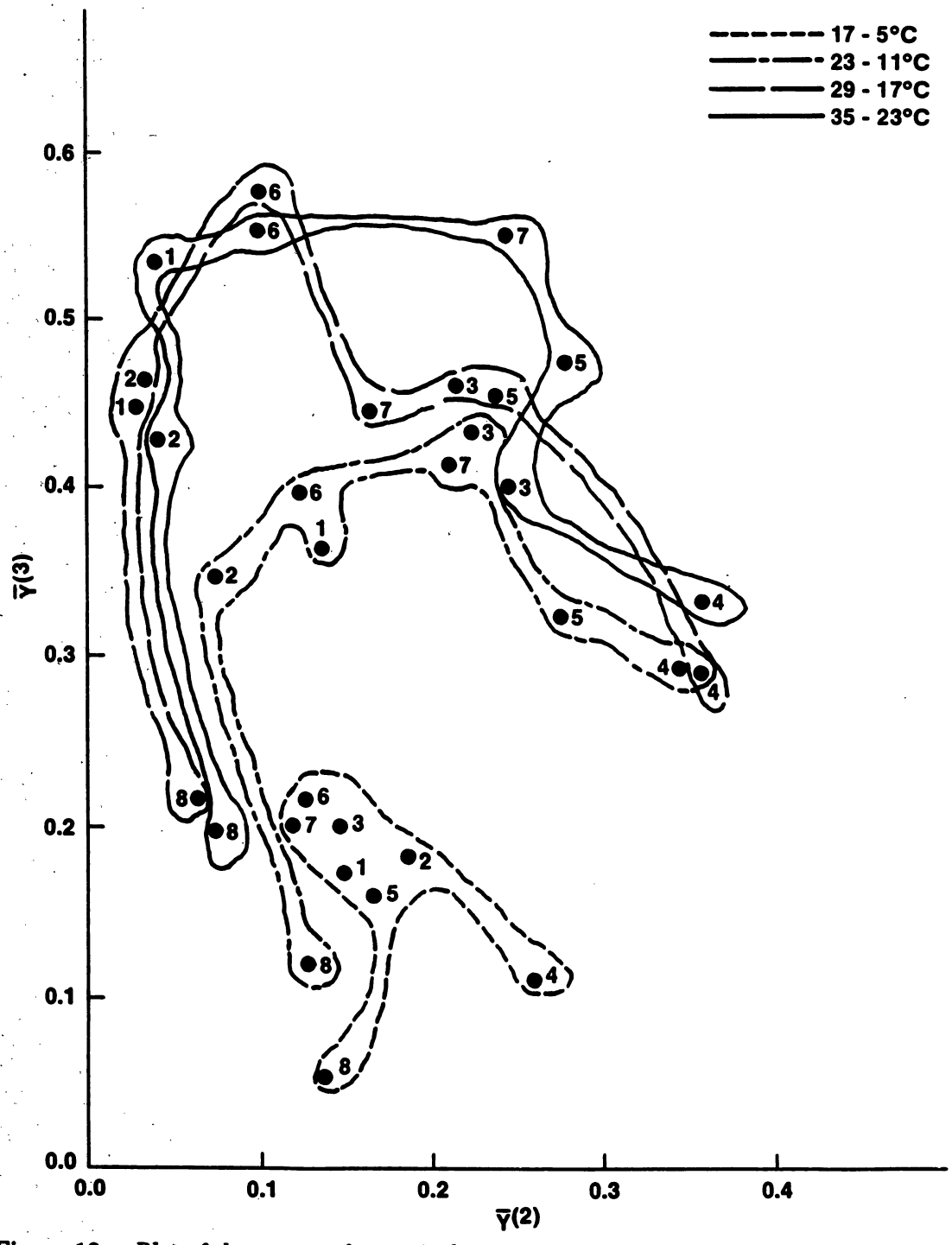

Figure 13.—*Plot of the means of canonical variable 2 versus the means of canonical variable 3 for each temperature level based on six traits in Trial II ($Y^{(2)}$ and $Y^{(3)}$ are associated with H_{TC}).*

index I^2 with coefficients derived from Trial I was applied to Trial II, clones 5323 and 5326 were selected. Lower bounds derived from Trial I, however, could not be used in Trial II since these bounds were too high. These bounds, therefore, were set equal to zero because two of the three environments in Trial I were greenhouse environments with means for all traits significantly higher than those for the growth chamber environments of Trial II. Consequently, no valid comparisons could be made for index I^2 other than those already mentioned.

Similar problems with lower bounds arose when index I_3, based on Trial I, was applied to Trial II. Also, index I_3, with coefficients derived from Trial II, could not be evaluated for all clones when applied in Trial I. Only clones common to both experiments could be evaluated.

These results demonstrate the data-dependence of all the proposed indices and their coefficients. In other words, two experiments could not be combined if the clones associated with Trial II were grown under conditions different from those of Trial I.

The number of traits on which selection is based appears to influence the reliability of an index. For example, when the number of traits associated with index I_2 in Trial II was reduced from six to two—STMWT and SLA—and individuals were selected on the basis of large values of STMWT and small values of SLA, clones 5323 and 5328 were selected. When all six original traits were considered, clones 5323 and 5326 were chosen.

As a further extension, index I_1 with coefficients derived from Trial I was applied to first-year field data composed of clones 5321, 5326, 5323, and 5377. Clone 5323 was chosen after assigning ranks to the index values. This compares favorably with results obtained by indices I_6 and I_7 applied to Trial II.

The computations associated with indices I_6 and I_7 must be executed again whenever a new group of clones is tested.

Indices and their reliability

Five of the seven proposed selection indices were evaluated using data obtained from Trial I. The curvature and Hamiltonian indices were not evaluated because none of the clonal responses over environments was quadratic and no independent environmental measure could be associated with each environment.

All seven selection indices were evaluated using data obtained from Trial II.

In Trial I, reasonable consistency was exhibited by indices I_1, I_2, I_6, and I_7, which selected clones 5265, 5266, and 5334 in the top group of five superior clones. These results compare favorably with those obtained by Wray (1974).

In Trial II, the indices produced less consistent results than they did in Trial I. For example, out of nine cases (seven indices of which indices I_1 and I_2 were based on both original and transformed data), clone 5323 was in the top 20 percent five times and clones 5326 and 5328 were each included three times. The reliability of an index apparently decreases as the number of traits on which selection is based increases.

In Trials I and II the coefficients of index I_1 were estimated from both original and transformed data. Since the variances associated with the traits of interest tended to increase with increasing mean, and since the coefficients of index I_1 were based on estimated phenotypic and genotypic variances, the transformed data were thought to improve the reliability of index I_1. A comparison of index I_1 in Trial I based on both the original and transformed data revealed identical results. In Trial II the results of index I_1 based on both types of data were dissimilar. The data transformation (logarithms to the base ten), therefore, did not improve the reliability of index I_1.

Unlike the other indices, index I_1 has the built-in facility of assigning weights, either economic or biological, to the traits on which selection is to be based. The sign and magnitude of these weights depend on the goals of clonal selection. In Trial I equal weights were assumed for lack of other information. As information about the relative importance of these traits becomes available it can be incorporated into the index and a new set of coefficients will be formed.

Kempthorne and Nordskog (1959) point out that index I_1 is reliable only when the genotypic correlations between the traits forming the index are high. These correlations were high for the traits in Trial I, and most, but not all, traits in Trial II.

Bridgwater (1972) used different weights and various combinations of six traits (height, diameter, total dry weight, specific gravity, volume, and number of limbs per foot) to construct many indices. The form of these indices was the same as index I_1 in our study. The expected gain (Falconer 1960) was employed as a means of deciding which index was best according to the selection goals. The expected gain was found to be high only when the traits height, diameter, and total dry weight were incorporated into the index. Bridgwater (1972) found that a reliable index was one containing traits with high genotypic correlations, such as the three listed above. Our results tend to support these findings.

Index I_1 with coefficients estimated from Trial I was applied to Trial II, and clones 5323 and 5328 were selected. This procedure produced good results compared with other indices. After some additional

calculations, indices I_1 and I_2 based on data from Trial II were applied to Trial I, and clones 5334 and 5266 were among the top five superior clones. These results are acceptable when compared with the results of other indices.

Index I_1, when derived from an experiment involving few environments and many clones, gave more reliable results than when obtained from an experiment involving many environments and few clones.

Index I_2 is easy to calculate and, unlike index I_3, which bases selection on one trait at a time over several environments, index I_2 bases selection on several traits over one environment. The latter index is preferred because an average value over all environments can be calculated (environments are assumed independent). This cannot be done with index I_3 because the traits measured on the same plant are correlated with each other. Index I_3 must, therefore, be evaluated for each trait separately, which leads to interpretation problems if there are many traits. Even if a canonical variable is used to evaluate index I_3, its reliability is totally dependent on the canonical variable. A canonical variable which "explains" a large percentage of the total variation in the data—say 50 to 80 percent—is not desirable. Canonical variable 2 was usually a better discriminator, even though it "explained" only 10 to 30 percent of the total variation.

Wu (1973) originally proposed using the curvature of a quadratic equation that was a function of some independent environmental variable as a measure of adaptation, in contrast to the procedure outlined by Finlay and Wilkinson (1963). Wu (1973), however, stated that a variety is stable when its curvature approaches zero. We feel that a clone is stable across all temperatures if the curvature of a particular clonal response was near the overall average curvature. This idea was incorporated into index I_4.

Index I_5 was also evaluated based on data from Trial II, but did not perform as well as index I_4. Possibly no function, formed by the product of a growth and growth rate function, which remains constant over increasing temperature exists. Indices I_4 and I_5 were not evaluated in Trial I because some necessary information was absent. Index I_4 performed as well as index I_2 when both were based on data from Trial II. Clones 5323 and 5326 were selected in both cases.

Indices I_6 and I_7 are constructed by methods that involve complex computations, but existing computer programs partially solve this problem. Since final selection is a visual process, no mathematical functions are needed. Only the plotting of the computer results is required to produce a visual display.

The computations associated with index I_6 can easily be performed on any computer accepting FORTRAN. The computations associated with index I_7 are performed by an IBM-dependent package called SAS, which is not available for use on a non-IBM computer. A procedure called REGR, a subprogram of the SAS package, was used to perform MANOVAs and produce the canonical variables associated with various sources of variation of the assumed model. There is the limitation that the degrees of freedom associated with the source of variation due to regression must be 80 or less. Otherwise the procedure REGR breaks down and the plot of canonical means cannot be constructed.

In summary, an index based on existing data can be used reliably to select clones from a new data set or a combined data set of new and previously tested clones—provided the new clones are grown under environmental conditions similar to those under which the previously tested clones were grown. Given similar environments, all indices present useful information in condensed form. Because indices I_4 and I_5 can be computed only when some independent measured environmental factor produces a quadratic growth response, the other indices should be considered more general. Index I_2 should be chosen if computational base is important. However, index I_1 is best if selection is to be based on unequally weighted traits. Indices I_6 and I_7 are most useful if a graphical display is desirable.

PREDICTION OF FIELD GROWTH POTENTIAL

T. Hennessey, *Assistant Professor, Department of Forestry, Oklahoma State University, Stillwater, Oklahoma, and* **J. C. Gordon,** *Head, Department of Forest Science, Oregon State University, Corvallis, Oregon*

How well do controlled-environment measurements predict growth in the field? We present here guidelines for conducting field trials, methods for relating controlled-environment to field results, and actual results of controlled-environment field-correlation studies.

Guidelines for Field Trials

The most important consideration is to conduct replicated field trials under the environmental and cultural conditions that will prevail in actual practice. Weed control, planting density, water management, and location should all be similar to intended practice. Plots should be large enough to exclude edge effects, and genotypic composition should be what might be used in practice if specific clones were accepted. In short, proven field procedures with proper statistical design should be used.

For example, a study could run from 1 year to 3 years, or to rotation age. Sixteen clones could be grown at four locations in a two-replicate, randomized-block layout. The experimental plots would each contain four clones spaced 6 feet apart. To eliminate border effects, each block would be bordered by two rows of whatever clone the experimenter desires. Basal diameter, total height, and top dry weight should be the variables on which selection is based. Root variables would be of interest to many silviculturists; sampling methods for poplar are available. Plot averages would be analyzed so that the analysis would be balanced even if mortality occurred in some of the plots.

The ANOVA table analyses could be carried out for each year separately, then combined for all years.

Source	d.f.
Blocks/Locations	4
Locations (L)	3
Clones (C)	15
L x C	45
Error	60
Total	127

Several authors have examined the analysis of repeated-measurements experiments. Generally, analyses are kept simple if equal subclass numbers can be maintained. By utilizing plot averages, the experimenter has greater assurance of a balanced experiment.

The problem of plot size has also been investigated. They state that if most of the total cost is assignable on a per-plot basis, a small number of trees should be assigned to each plot. However, if the plot is too small, the problem of missing plots arises. Finally, the authors suggest that the biological adequacy of data collected from small plots depends on the magnitude of the correlation between the performance of varieties grown in mixture and that of the same varieties grown in pure stands. Four clones per experimental plot, therefore, appear minimally adequate for a field study.

Field study

In this study, three *Populus* clones, adapted to southern Canada (5260), southern Wisconsin (5377), and central Iowa (5339) were used. Tip cuttings taken from stock plants growing in the greenhouse were individually planted in commercial Jiffy-7 pellets, then placed under an alternating mist system on greenhouse benches. When the roots emerged from the pellets, 45 of the plants were planted in five Latin

square designs at each of two locations—the State Nursery in Ames, Iowa (latitude 42°N) and the Hugo Sauer Nursery in Rhinelander, Wisconsin (latitude 45°N). Three degrees difference in latitude was enough to give markedly different environments, and hence different growth patterns were expected. High levels of nutrients and moisture were maintained in both locations. Stem height and number of leaves were recorded every 2 weeks. In addition, at approximately 30-day intervals (July, August, and September), a destructive harvest was made of one Latin square (three plants per clone, three clones), and stem height, stem diameter, stem dry weight, leaf number, leaf area, leaf dry weight, and total top dry weight were measured. This procedure was repeated in 1971, 1972, and 1973 at both locations.

Growth room

The three *Populus* clones were placed in Latin square designs in Percival growth chambers (three plants per clone, three clones, three photoperiods) to examine the productivity of individuals as affected by genotype and photoperiod. Cuttings were taken from stock plants and rooted under mist, then transferred into photoperiods of 13, 14, and 15 hours, with day temperature of 25°C and night temperature of 15°C. High nutrient and moisture levels were maintained throughout the experiment. Stem height and number of leaves were recorded approximately every 4 days until the end of the experiment, when all plants were harvested and measured as in the field study. This portion of the study was replicated four times, with the total growing period being either 6 weeks (one time) or 7½ weeks (three times). Results were analyzed with correlation analysis as well as simple ranking according to size.

Field, first-year growth

Clones were ranked first, second, or third on the basis of their size at the end of each growing period for all 3 years. This was done for all seven variables measured at both locations.

Wisconsin #5 (W-5) ranked first or tied for first with Crandon (Cr) for all variables measured for all three growing seasons at both locations. In Ames, the ranking was W-5, Cr, Tristis #1 (Tr) for each variable measured for both 1971 and 1973; for 1972, the ranking was always W-5, Tr, Cr. Similarly, in Rhinelander, W-5 ranked first or tied for first with Crandon for all variables measured for years 1971 and 1973; for 1972, the ranking was always W-5, Tr, Cr. In general, growth trends were the same at both locations for the years 1971 and 1973; 1972 growth differed from those 2 years, but differed in the same way at both locations.

To supplement the harvest data, the seasonal growth patterns of the clones were examined. Tr consistently set bud by mid-July in Ames, whereas in Rhinelander it grew longer but more slowly than during the first half of the growing season. In Ames, Tr showed approximately the same growth trends for all 3 years. At Rhinelander, however, Tr grew differently in different years. At the end of the 1972 growing season, for example, the total top dry weight of Tr in Rhinelander was almost twice that in Ames, although in 1973 the total top dry weights were almost identical.

Cr grew throughout the growing season at both locations for all 3 years, although it did not grow well at either location in 1972. In 1972, Cr grew best in Ames with respect to stem height, stem diameter, and leaf number, while in Rhinelander it grew best with respect to leaf weight, total top dry weight, and leaf area; stem weights were nearly identical. In 1973, Cr grew much better in Rhinelander with respect to stem height, stem diameter, and leaf area; other variables were similar in both locations, with the exception of stem weight and total top weight, which were slightly larger in Ames.

By the end of each of the three growing seasons in Ames, W-5 far surpassed Cr and Tr for all variables measured. In Rhinelander, W-5 usually ranked ahead of the other two clones. The difference between the first- and second-ranking clones, however, was not as consistently large as in Ames. In 1972, W-5 grew better with respect to six variables at the Rhinelander site, with only tree diameter being slightly larger at Ames. In 1973, W-5 grew better with respect to six variables at the Ames site, with only leaf area being slightly larger at Rhinelander.

Field, 2- and 3-year growth

Trees that were not harvested by the end of the 1971 and 1972 growing seasons were left to grow until the end of the 1973 season. Rankings for 2-year-old material in Ames were W-5, Cr, Tr for the variables stem height and stem diameter, and W-5, Tr, Cr for stem weight. Rankings for 2-year-old material left at Rhinelander were W-5, Tr, Cr for all variables measured. All three clones, however, were larger in Rhinelander than in Ames after 2 years. For example, Tristis stem weight at Rhinelander was approximately six times that in Ames, and W-5 stem weight was approximately twice that in Ames. This is most probably a result of more frequent irrigation

and fertilization in Rhinelander. Rankings for material left 3 years (planted spring 1971, harvested fall 1973) at the Ames location were Cr, W-5, Tr for all variables measured. At Rhinelander, the rankings were W-5, Cr, Tr for all variables measured.

In general, after 3 years' growth, Cr grew much better at Ames, W-5 grew somewhat better at Rhinelander, and Tr grew only slightly better at Ames.

Growth chamber

Clones were ranked first, second, or third for each variable at the end of the growing period. The one growth period of 6 weeks was combined with the three growth periods of 7½ weeks, and the pooled means were used as a basis of comparison. Thus, each mean value represented 12 trees (three trees per clone, four replications).

In the 13-hour photoperiod the ranking was W-5, Cr, Tr for the variables stem height, stem diameter, leaf weight, number of leaves, and total top weight; for the variable leaf area, ranking was Cr, W-5, Tr. In the 14-hour photoperiod the ranking was again W-5, Cr, Tr for the variables number of leaves, leaf weight, leaf area, stem weight, and total top weight; but the stem diameter ranking was W-5, Tr, Cr. Stem height exhibited a third order: Cr, W-5, Tr. In the 15-hour photoperiod treatment, the ranking was W-5, Cr, Tr for leaf weight, number of leaves, and leaf area. Ranking for stem height, stem weight, and stem diameter was W-5, Tr, Cr. Thus, W-5 ranked first in all variables except 13-hour leaf area and 14-hour stem height. Tr ranked last in 16 of the 21 measurements. Differences among clones were least in the 13-hour photoperiod, greater in the 14-hour photoperiod, and usually greatest in the 15-hour photoperiod.

To quantify the relations for the measured variables between growth room and field growth, correlation matrices were calculated for three combinations of variables: (1) all variables in one location with all variables in the same location; (2) each variable in one photoperiod with each variable in the same photoperiod; and (3) each variable in each location with each variable in the different photoperiods. Thus, it was possible to get "r" values, for example, between stem height in Ames and stem height in a certain photoperiod (table 27).

Discussion

By examining the values in table 27, it can be seen that the 13-hour photoperiod yielded the poorest growth-chamber and field correlations. This would indicate there is less discrimination in ranking of clones in this photoperiodic treatment than in the longer photoperiods. The results, indeed, showed that the magnitude of the difference in performance between the three clones was least under the 13-hour treatment. Higher "r" values were obtained between field and 14-hour growth-chamber performance, with the highest values being obtained between field growth and 15-hour growth. Greatest differences in performance between clones were observed in the growth room at the longer photoperiods.

An average correlation value was calculated for each location and photoperiod; this value increased progressively by photoperiod for both locations. Thus, ranking of clones between the growth room and the field appears consistent for each variable measured, and variability in the field when averaged over several trials is evidently not large enough to disrupt this ranking.

Table 27.—*Correlation coefficients ("r") between growth chamber and field growth, by growth chamber photoperiod and field location, with clones and years pooled*

Field location	Growth chamber photoperiod	SH	SD	LW	TTW	LA	LN	SW	XR
Ames	13HR	0.82	0.44	0.44	0.57	0.31	0.60	0.90	0.58
	14HR	.33	.91	.79	.83	.79	.81	.82	.75
	15HR	.80	.85	.86	.88	.78	.89	.90	.85
Rhinelander	13HR	.65	.34	.26	.34	.32	.47	.65	.43
	14HR	.30	.65	.70	.59	.69	.66	.53	.59
	15HR	.61	.61	.56	.59	.68	.73	.65	.63

Values for the correlations between Ames and the three photoperiods are larger than those between Rhinelander and the three photoperiods. This may be because there was less difference between the first- and second-ranked clones at Rhinelander for many variables.

Ranking was consistent in the field at both locations for 1971 and 1973. Although W-5 did rank first in 1972 also, the fact that the rankings were inconsistent with the other 2 years with respect to Tristis and Crandon was due to the poor growth of Crandon in 1972. It is possible that differences in climate caused this difference in growth pattern. First, the monthly averages in temperature for June, July, August, and September were all below the 10-year average at the Ames location (National Oceanic and Atmospheric Administration 1972). Second, the monthly solar radiation totals for June, July, and August were lower in 1972 than in 1971 at Ames. Third, the percentage of possible sunshine days (100 percent = full sun) was significantly below average for July, August, and September in 1972, and below average for July and August in 1971 and 1973 (Waite and Shaw 1961). Temperature readings also were below normal for June, July, August, and September at the Rhinelander location in 1972.

By the end of the third year at Ames, Crandon was firmly in first place. Tristis continued to set bud early in the season at Ames, resulting in its being considerably behind the other two clones after 3 years.

This study showed that there was consistency in ranking of clones between the growth chamber and the field for many variables for 1- and 2-year growth. Although growth differences did occur between years, the variability, when averaged over several years, was not enough to disrupt these rankings.

Therefore, it seems that it may be possible to estimate initial field growth potential of clonal material by means of a preliminary analysis of selected variables when the material is grown in controlled environments.

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