KEN SINK: We have that in mind, however, at this point we have no source of seed.

CHARLES HEUSER: Have you tried to lay the shoots down in a horizontal fashion to possibly disrupt apical dominance and stimulate bud break?

KEN SINK: No.

BRENT McCOWN: We have found with birch that the important consideration is just time in culture. We can get birch seedlings into culture in 6 months but mature birch requires 2 to 3 years. Some physiological change is occurring within the mature tissue.

INITIAL TRIALS WITH COMMERCIAL MICROPROPAGATION OF BIRCH SELECTIONS[1]

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Abstract. The rapid multiplication of *Betula platyphylla* var. *azechuanica* by micropropagation using shoot-tip cultures has been demonstrated on a commercially-feasible basis. Shoot-tips and nodal explants placed on Gresshof-Doy nutrient agar medium supplemented with 4μm benzyladenine produced actively growing shoot cultures within 6 months. Stocks could be maintained or increased by monthly subculturing after removal of the elongating shoots and division of the resultant shoot-mass. Twenty to 30 utilizable shoots could be harvested from each culture in 6 to 8 weeks after subculturing. Harvested shoots rooted with 100% success within 2 weeks when placed in peat/perlite in a rooting chamber. After a period of acclimation, these plants could be treated like young seedlings in commercial production. A comparison of the field growth of seedling and micropropagated birch showed that both had identical growth rates in the spring and summer; however the micropropagated plants stopped growth one month earlier than the average seedling. This resulted in the micropropagated plants having a smaller size at grading than the seedlings. Whether this difference was genetic or a result of the propagation technique is unknown. The micropropagated plants were highly uniform in growth and grade as compared to the seedling propagated plants.

Birch has long been a prized ornamental tree. However, its use has been limited by important pest problems, particularly bronze birch borer, *Certaocystis fagacearum*. A number of selection programs are now finding birch genotypes that appear resistant to this pest. In addition, resistance to birch leaf miner and early coloration of the bark in young propagules are also desired traits. Once final selections are made, they will proba-

[1] The assistance and advice of Evergreen Nursery, Sturgeon Bay, Wisconsin, and Dr. Edward Hasselkus was essential to the successful completion of this work.

bly have to be maintained as clones; this may present problems since most birch do not readily root from cuttings. Budding and grafting are feasible but add considerable expense in production. Micropropagation may be a practical solution for the multiplication of unique and desirable birch selections.

With the support, advice, and cooperative assistance of Evergreen Nursery, Sturgeon Bay, Wisconsin, we evaluated the use of micropropagation in birch production with the hope that this technology would complement their active selection program. Since this nursery produces at least ¼ million birch liners each year and is developing an accelerated growth program, they would most likely be able to capitalize on the tremendous multiplication potential that micropropagation offers.

The use of microculture in plant culture is now very well established. Several excellent and recent reviews and symposia are available and amply cover the extent to which microculture, particularly micropropagation, is being used in the horticulture industry, e.g. Barz, Reinhard and Zenk (1); Hughes, Henke, and Constantin (3); Reinert and Bajaj (4); and the Fourth (1978) International Congress of Plant Tissue and Cell Culture. Indeed, the papers presented at this meeting represent the current state-of-the-art. Although the incorporation of micropropagation into woody plant culture has been slower than the use in herbaceous plant production, the progress with such plants as fruits and rhododendrons show that success is imminent. To our knowledge, our work with birch is the first demonstrated use of micropropagation in the culture of an ornamental tree on a commercially-feasible scale.

MATERIALS AND METHODS

Seedlings of the Asiatic white birch, *Betula platyphylla* var *azechuanica*, were obtained from Evergreen Nursery. Stem tips and node sections were removed from 12 actively-growing plants and after removal of most of the leaves, were sterilized in 10% household bleach (sodium hypochlorite) with a wetting agent added (0.05% Tween-20). After rinsing in two washes of sterile distilled water and removing any injured tissue, the explants were placed on agar solidified medium. If any exudation from the cut surfaces occurred, the culture was shifted to fresh medium.

All cultures were grown on modified Gresshof-Doy (2) nutrient medium supplement with 0.6 to 0.8% agar, 10% sucrose, thiamine (1 mg/l), nicotinic acid (0.1 mg/l), myo-inositol (10 mg/l), pyridoxine (0.1 mg/l) and benzyladenine (4μM). Cultures were grown in rooms with 24 hour fluorescent lighting (300 ft-c) and temperatures that averaged 28° to 32°C (82° to

90°F). Culture vessels were either 1 oz or 4 oz heavy glass bottles capped with Parafilm-M. Cultures were subcultured to fresh medium at least every month and at this time, any malformed tissues were discarded.

The growth characteristics of the micropropagated birch were tested in field plantings. Shoots from the cultures of one of the seedlings were rooted and grown along with a set of seed propagated plants originating from the same seed source. These two sets of plants, 600 originating from micropropagation and 600 originating from seed, were interplanted in guarded blocks of 150 plants in the field. Growth rates were recorded on 20 randomly selected plants in each block. In the fall, the plants were dug by machine and graded.

RESULTS

Explants from all the seedlings responded similarly to the microculture conditions. Lateral buds and often the apical meristem of the initial explants showed new leaf expansion and stem elongation within the first month in culture. As this new growth was subcultured on a monthly basis, growth became more rapid and lateral bud break (and thus shoot multiplication) was progressively easier to stimulate. After 6 months in culture, explants from all the genotypes that had initially survived the first subculture showed active and uniform shoot growth and multiplication (Figure 1).

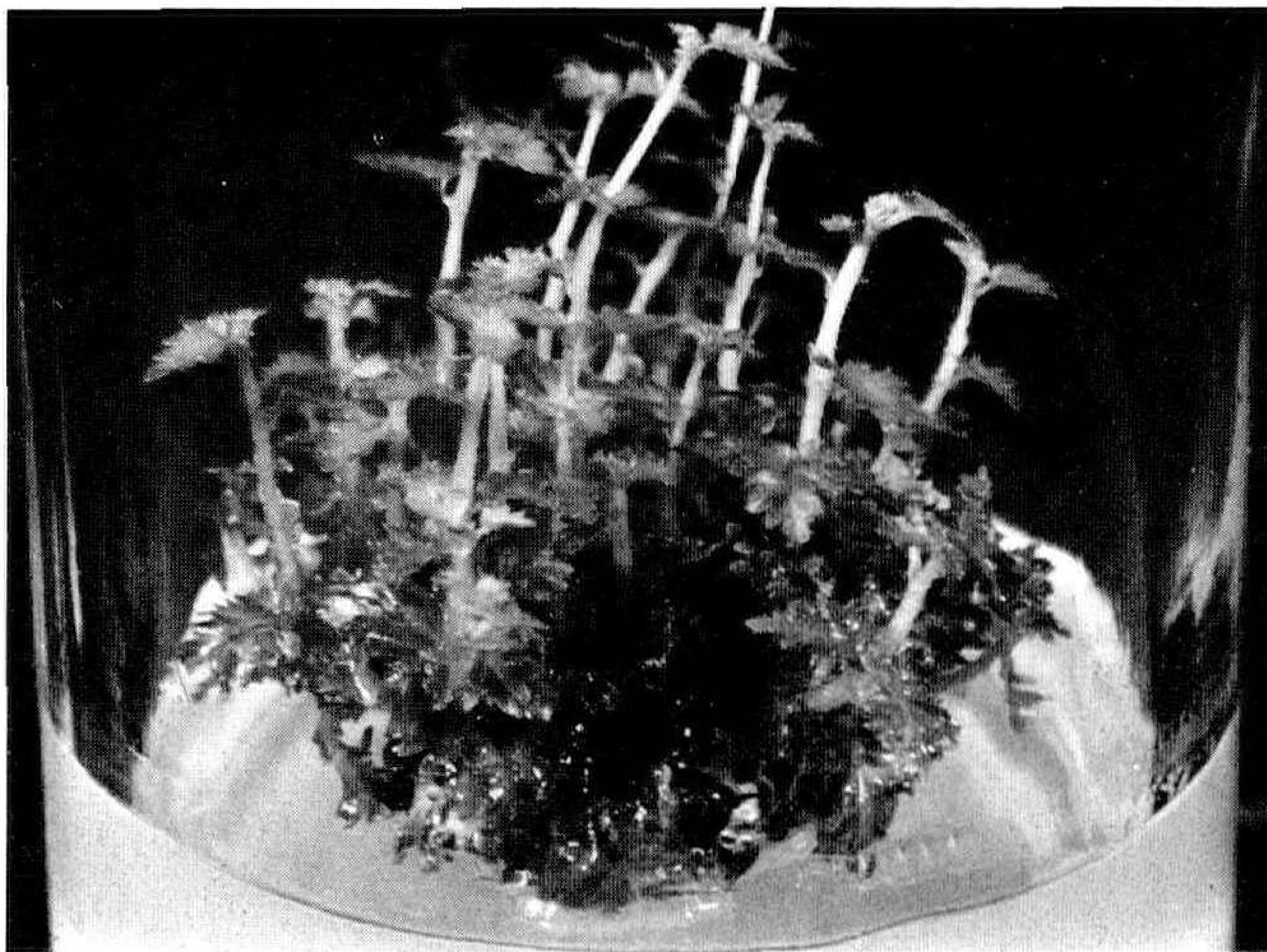

Figure 1. A shoot-tip culture of birch, *Betula platyphylla* var. *azechuanica*, showing multiple shoot development. At least 20 uniform shoots can be harvested from such cultures. After rooting, shoots can then be treated as seedlings in production programs.

The shoot-tip cultures could be multiplied by removing the

elongating shoots, dividing the basal shoot mass into 3 to 5 sections, and placing each section onto new medium. Such stock cultures were readily maintained as long as the subculturing was repeated every 3 to 4 weeks.

Shoots could be harvested from actively-growing cultures after 4 to 6 weeks of growth after subculturing. At this time an average of 20 to 30 shoots of 3 to 6 nodes in length could be obtained from each culture. It was possible to allow the same cultures to sprout a new crop of shoots and although these were numerous (up to 60 shoots per culture), the shoots were small, difficult to handle, and variable. Harvested shoots were very subject to desiccation and thus were cut into water and remained floating until placed in the rooting environment.

Shoots were rooted in 1:1 peat/perlite in a warm 30°-35°C (86°-95°F) high humidity (greater than 80%) chamber. Rooting under mist was also feasible but desiccation was more difficult to prevent. Rooting occurred within 2 weeks and was essentially 100% successful. Hormone treatments were not necessary. Once rooting occurred, the plants were shifted to a greenhouse and gradually given full sunlight and lower humidity over a 2 week period. From this time on, the plants could be treated as seedlings.

Even though the initial size of the seedlings was larger than the micropropagated plants at the time of the field planting, the growth of the two sets of plants was identical through the summer (Figure 2). However, after early September, the mi-

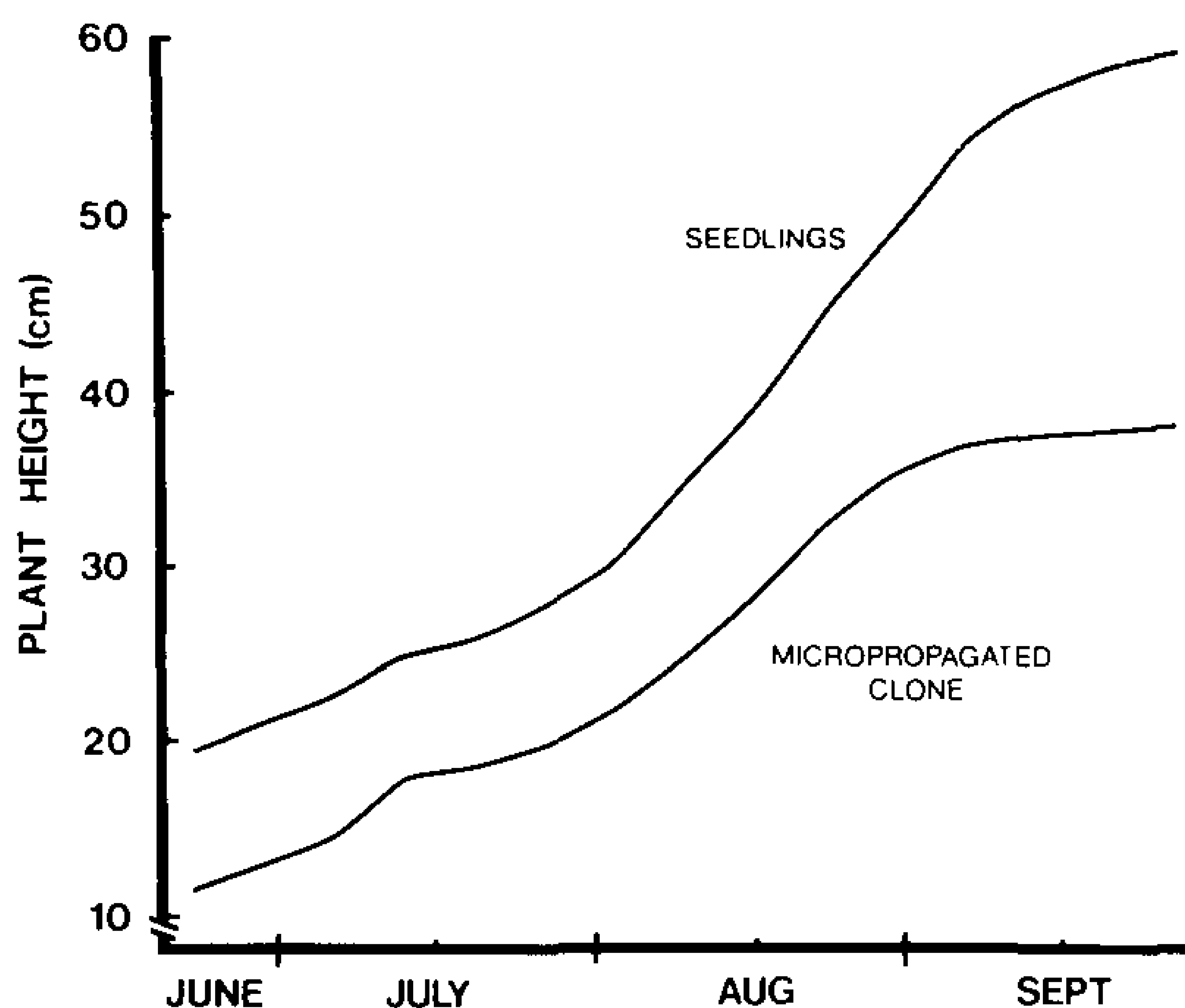

Figure 2. The growth in height of two sources of birch liners. *Betula platyphylla* var. *azechuanica*, in the field for one season. The micropropagated plants came from cultures of a seedling from the same seed source as the seedling plants. The date are from 40 plants of each source randomly selected from experimental blocks grown in the same field.

cropropagated birch slowed and stopped growth while the seedlings continued growth until the end of September. This differences in cessation of growth was also evident in the early fall coloration of the micropropagated birch.

The grading data show the result of the earlier growth cessation of the micropropagated birch (Figure 3). The seedlings averaged larger in size (seedling population peaked at 21 to 30 in. in height) as compared to the micropropagated plants (peak at 12 to 15 in. size class). However, the seedlings also showed a in. much larger range of sizes (6 to 36 in.) than did the micropropagated plants (6 to 21 in).

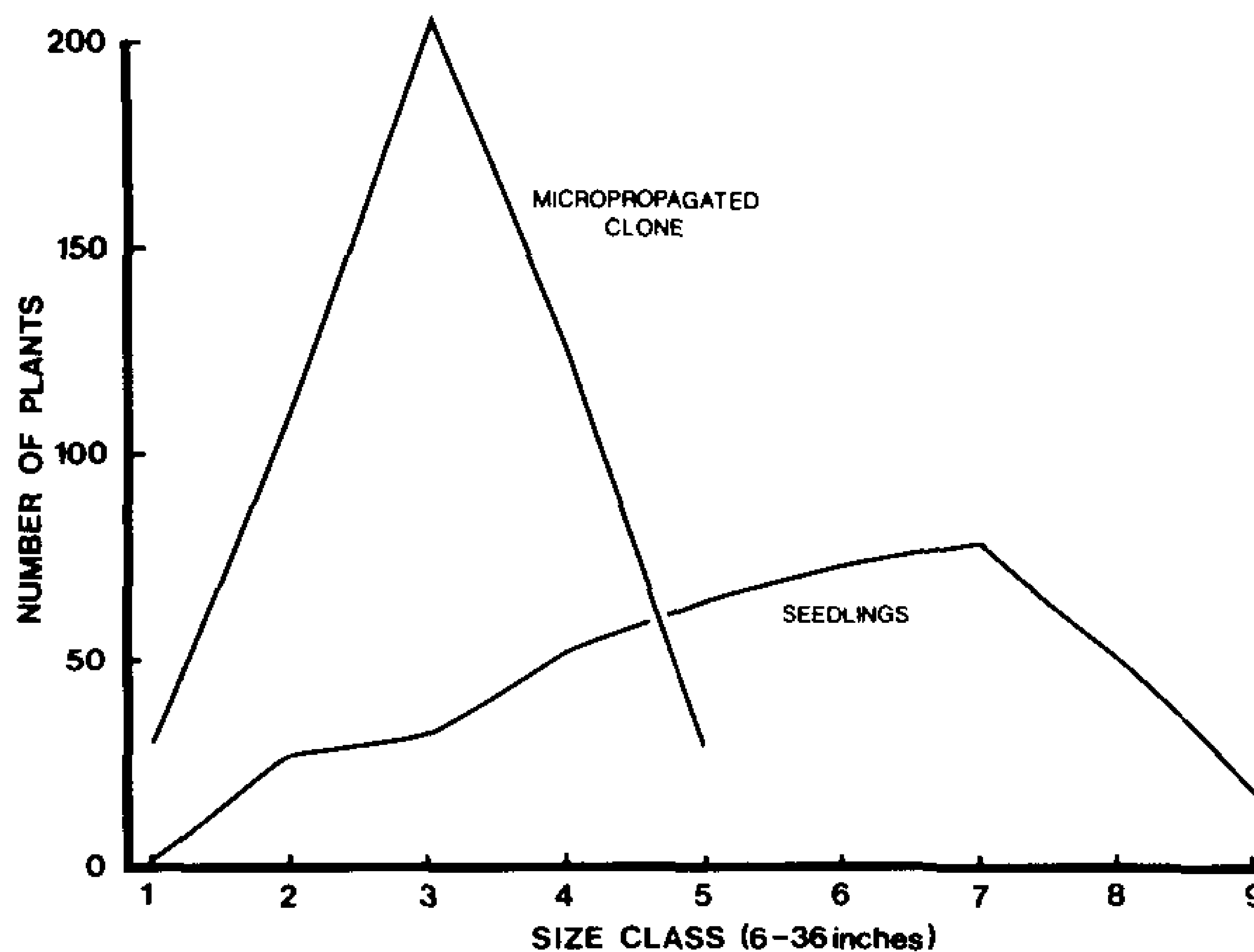

Figure 3. The grading by size (height) of birch liners, *Betula platyphylla* var. *azechuanica*, propagated by two techniques and grown in the same field for one season. The size classes were 3 inch intervals except the last class which included all plants greater than 30 inches in height. The data are from 600 plants from each source.

DISCUSSION

To our knowledge, this is the first well-documented use of micropropagation in the production of a tree species on a commercially-feasible basis. Besides rapid multiplication, additional benefits of the use of micropropagation are a minimal space allocated for stock plant maintenance, potentially disease-free propagules, and dependable, easily controlled uniformity of propagules.

Because the micropropagation techniques used here employed shoot-tip cultures and not adventitious shoot regeneration from callus, shoots evolved from preformed meristems on the original explant. Thus, given a relatively stable genotype initially, the genetic stability of the culture should remain high. Indeed, even after the hundreds-of-thousands of shoots that we

have generated, only one visually abnormal shoot has been observed. This was an albino/normal chimera, an aberrance that is readily selected against.

The observation that the microcultured shoots root rapidly and dependably contrasts with the general rooting potential of most birch species. This may indicate that micropropagated shoots differ in their physiological state (juvenility?) from cuttings taken from the usual stock plants.

Although these experiments used explants originally taken from seedlings, success has also been achieved with tissues from mature trees. In the latter case, the time to "acclimate" the explant to culture so that rapid and reproducible shoot-tip cultures are obtained may take up to several years. Again, this may indicate a gradual change in the physiological state of the cultured tissues.

The rate of multiplication appears adequate for commercial purposes. Given a conservative average of 20 shoots being produced per culture in 6 to 8 weeks, some 4000 shoots can be generated per square foot of culture shelf space per year. To produce ½ million propagules, only 125 square feet of culture shelf space would be necessary.

The performance of microcultured birch in the field is not fully understood. The observation that the growth rate of micropropagated plants was identical to that of seedlings in the spring and summer indicates that the two sets of propagules had the same growth capability. However, the early cessation of the growth of the micropropagated plants may have two explanations. Since the growth characteristics of the original seedlings that generated the cultures used to produce the microcultured plants is not known, the difference may be genetic — by chance, a genotype was cultured that characteristically goes dormant early in fall. A second explanation is that the differences in growth are a result of a physiological difference between microcultured plants and seedlings. Such differences may be traced to differences in juvenility, rooting behavior, collar physiology, or some other as yet unexplained factor. Future research will focus on this phenomenon.

One advantage of clonal propagation, in this case by micropropagation, is clearly demonstrated in the grading results. The micropropagated plants showed high uniformity as compared to the seedlings. Such uniformity in growth is particularly advantageous in accelerated growth programs where a predictable response to cultural conditions is paramount.

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JOHN HART: How do we control genetic vulnerability of the plants we produce by tissue culture?

BRENT McGOWN: This technique can be misused like any other technique in propagation. It is a point of professionalism. We can use it just as readily for good benefit. We can produce multiple genotypes for forest planting in culture easier than by standard means. It is, therefore, possible to protect against the introduction of single genotypes that would be vulnerable to plant pest problems.

DISEASE-FREE PLANTS THROUGH MICROPROPAGATION[1]

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Asexual or vegetative propagation of plants is practiced when the qualities of elite clones are not maintained in seedling progenies. Asexual propagation also perpetuates any systemic infection caused by viruses or vascular wilt organisms. Micropropagation can eliminate such infections and, in some cases, is the only available method for obtaining healthy plants. Four methods of micropropagation currently are being practiced. Three of these have limited usefulness due to the possibility of propagating somatic mutations. In as much as current and future investigations could change this, they will be briefly discussed. The fourth, tip meristem culture, has been used for a quarter of a century to eliminate viruses from infected plants and is the basis for the commercial production of cultured car-

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