BILL CUNNINGHAM: I might point out that a Bunsen burner or alcohol lamp is not a satisfactory way to sterilize tools. An incinerator is better.

LEN STOLTZ: You are right; however, it will burn up your tools which is costly. We use 95% ethyl alcohol because we had contamination problems with 70%.

DICK ZIMMERMAN: Alton Jones offers two courses on tissue culture. One course is 3 days long and the other is 2 weeks.

PROGRESS ON *IN VITRO* PROPAGATION OF RED MAPLE[1]

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Abstract. Tissue culture experiments were conducted using shoot-tips (5 to 10 mm) or single node sections to devise an *in vitro* propagation scheme for red maple (*Acer rubrum*). Inconsistent proliferation of axillary buds on shoot-tip explants occurred when they were aseptically cultured on modified Linsmaier and Skoog (LS) medium containing kinetin (K), 6-benzylaminopurine (6·BAP) or 6(*ν*-*ν*-dimethylallylamino)-purine (2ip) at 0.1, 1.0, 5 or 10 mg/l.

Actively growing shoots were a prerequisite for a high percent rooting of proliferated shoots. Eighty-five percent of shoots cultured on ½ strenght LS + 0.5 mg/l indolebutyric acid (IBA) developed roots within 10 days. Phenolic secretion that inhibited growth was controlled by preconditioning explants on potato dextrose agar or LS basal medium by a series of 3-day subcultures.

In the past few years, research efforts on *in vitro* propagation of woody plant species have experienced increased activity. Research reports have been issued on apple (1,5,6,7,12), almond (11), blackberry (3), bougainvillea (4), pear (5), and plum (5). In addition, Winton (13) in a recent review stated that at least 37 angiosperm and 19 gymnosperm trees have been regenerated as individual rooted shoots. This listing may, upon initial examination, appear impressive but for many speices regeneration was low in frequency, from embryonic tissues, or from callus. Winton's list (13) includes *Betula*, *Citrus* spp., *Hamamelis*, *Populus* and *Ulmus americana* that are of interest to nurserymen. An earlier review by Abbott (2) presents a summary of present *in vitro* successes with evergreen and conifer species.

Commercial cultivars of red maple (*Acer rubrum*) are generally propagated by budding or grafting onto red maple seedlings. In many horticultural species, this propagation technique

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is acceptable. However, red maple often exhibits graft failure sometime after planting in the landscape setting or even as young 2-2½" trees in the commercial nursery. For this reason, as well as to hasten the evaluation process and release of superior red maple cultivars into commerce, we initiated a tissue culture propagation study. The information presented herein is the result of our first two years of effort.

MATERIALS AND METHODS

Plant material for *in vitro* culture was obtained from two-year-old red maple seedlings or from mature trees of the cultivar 'Red Sunset'. Experiments were conducted with terminal and sub-terminal shoot-tips and with stem node sections. These explants, as shown by preliminary trials, were routinely disinfected in a 3% sodium hypochlorite solution for 30 min followed by 4 to 5 sterile water rinses. The shoot-tip explants were subsequently prepared for culture by aseptically removing all expanded young leaves.

Three basal media at full and one-half strength were tested: Abbott and Whiteley (1), Linsmaier and Skoog (8) salts plus Nitsch and Nitsch vitamins (10), and Cheng (5) medium. These three basal media were supplemented with auxins (IAA, IBA, NAA) or cytokinins (6·BAP, 2ip, kinetin) singly or in combination from 0.01 to 10 mg/l.

All media were adjusted to pH 5.7 after adding the agar and 25 ml was dispensed into each 125 × 25 mm culture tube. The culture tubes were sealed with Kaputs, autoclaved at 121°C (249°F) for 20 min and cooled on a slant. Cultures were placed at 1500 lux (cool-white) on a 16 h photoperiod. The temperature was 26°C±2 (79°F).

Early experiments indicated that substantial phenolic secretion was present soon after placing shoot tips in culture, particularly on those shoot tips taken from mature trees as growth declined in late July to August. Three chemicals were screened to determine their effectiveness in controlling this secretion: ascorbic acid (150 mg/l), L-cysteine (50 mg/l) and polyvinylpyrrolidone (PVP) 40,000 mol. wt. (5,10,20 mg/l). The ascorbic acid was used as a pre-soak solution on disinfected explants; whereas the latter two compounds were incorporated in the basal medium. A preconditioning treatment (5) was also evaluated for control of phenolic secretion.

RESULTS

Phenols. Phenolic secretion and subsequent injury to buds or shoot tips could not be controlled nor prevented by any of the chemicals tested in this study. There was no difference, by

visual observation, in amount of phenols produced when these materials were employed when compared to the control.

The preconditioning treatment did provide an effective method of controlling phenolic damage to plant material *in vitro*. Plant material that was badly damaged could be excluded or the slightly damaged tissues could be removed; the explant was recultured on preconditioning medium or placed into defined medium culture.

Effect of Cytokinins on Shoot Proliferation. Multiple shoot development was observed when shoot tips were cultured on Abbott and Whiteley's basal medium (1) containing kinetin (K), 6·BAP or 2ip at 0.1, 1.0, 5.0 and 10.0 mg/l. From one to 8 axillary shoots arose per shoot tip. However, this response was inconsistent with respect to number of shoots and number of explants responding with multiple shoots at each concentration of the different cytokinins. Considerable variation in multiple shoot formation was observed between identical experiments conducted with shoot tips from mature trees at different growth stages.

Experiments were also conducted using uniform, actively growing shoot tips from the preconditioning treatment. These shoots were cultured on Cheng's medium (5) plus 6·BAP at 0.1, 1.0, 5.0 and 10.0 mg/l. Initial results were promising at 1.0 mg/l 6·BAP with the induction of 2 to 5 axillary shoots per shoot tip. These results, however, could not be consistently repeated in subsequent experiments.

Jones (7) suggested that phloroglucinol (126 mg/l) in combination with cytokinins promoted shoot proliferation in apple shoot tips. This finding was not observed when applied to red maple in our studies. Node sections were cultured on Abbott and Whiteley's medium (1) plus 2ip at 0.1, 1.0, 5.0 and 10.0 mg/l either with or without phloroglucinol. All node sections placed on these media containing phloroglucinol died within one week while explants cultured without it remained alive.

Effect of Cytokinin/Auxin Combinations on Shoot Proliferation and Rooting. Abbott and Whiteley's medium (1) supplemented with combinations of K and NAA were tested for induction of shoot proliferation and rooting. These two growth regulators were used at 0.01, 0.1, 1.0, and 5.0 mg/l. No multiple shoot development was observed under these *in vitro* culture conditions. Root initiation was observed, however at 0.1 mg/l K plus 0.1 or 1.0 mg/l NAA. Varying amounts of callus were also observed at the base of these shoot tips depending on the concentration of K and NAA. The largest amount of callus occurred at the highest level (5.0 mg/l) of K and NAA.

Shoot tips were also cultured on Cheng's medium (5) con-

taining 6·BAP at 0.1, 1.0, 5.0 and 10.0 mg/l combined with 0.1 to 1.0 mg/l IBA. As with the K+NAA combinations, no shoot proliferation was observed. Rooting did occur at 0.01 mg/l 6·BAP plus 0.1 mg/l IBA. Two of four shoots on this medium combination developed roots. Those which developed roots also unfolded new leaves while those which had not developed roots did not.

Effect of Auxins on Rooting. Consistent, repeatable root initiation and elongation was observed on shoot tips cultured on Cheng's medium (15) plus 0.5 mg/l IBA. Consistently, 85 to 90% of the shoot tips developed roots within 10 days after being placed in culture. These shoot tips had been preconditioned and they were growing actively in a single stem manner with new leaf development occurring. Three lower IBA concentrations: 0.01, 0.05 and 0.1 mg/l were also tested but were not as effective in promoting root initiation and development. Higher levels of IBA were also tested but they induced undesirable large amounts of callus.

DISCUSSION

There are several possible reasons for the observed inconsistent shoot proliferation with red maple. In initial experiments, it was observed that there was a great deal of variation in the growth rate of the plant material being placed into culture. Because of this difficulty, it became necessary to establish a system to select uniform plant material for explants. This was accomplished by placing disinfected shoot-tips onto a preconditioning medium (5). However, the assumption that uniform plant material would lead to consistent shoot proliferation proved false at least in preliminary experiments since multiple shoot initiation was still inconsistent with the use of preconditioned plant material.

These findings suggest that the inconsistent shoot development may be due to limitations in the experimental or environmental parameters. Nitsch, et al. (9) reported that adenine together with a cytokinin was essential for the initiation of adventitious buds in *Plumbago indica*. Experiments with adenine and several other chemical addenda are currently underway to test their influence on red maple shoot proliferation.

We found that root development on shoot tips from cultures and from seedlings and mature trees of red maple is closely linked to their physiological stage of development. This conclusion was derived from the observation that roots developed only on shoots which were actively growing and did not when terminal growth ceased and new leaves were not being produced. This response was also confirmed by the high per-

centage of shoots which rooted when placed on IBA at 0.5 mg/l following the preconditioning treatment. These findings suggest that active shoot growth is necessary for a high percentage of rooting. Since shoots which develop in culture are growing actively, this method of rooting could be readily accomplished on a commercial scale.

The preconditioning treatment as suggested by Cheng (5) provided an excellent way to prevent phenolic damage or to remove contaminated plant material from culture prior to placing it in culture. One disadvantage with the system is the amount of labor involved since the plant material must be handled several times before it can be used *in vitro*.

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BRUCE BRIGGS: Have you tried to sterilize seeds, germinate them under sterile conditions and use the resulting plant material as your source of explant material?

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

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.