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BRUCE BRIGGS: What hormone and what concentrations do you use?

DAN STUDEBAKER: Wood's Rooting Compound at a 1:3 or 1:5 v/v, dilution or 1:2, v/v, for the most difficult types.

BRUCE BRIGGS: Just a comment. Your results show how a hormone does not work without bottom heat.

MICROPROPAGATION OF *OXYDENDRUM ARBOREUM*

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Abstract. Micropropagation and acclimatization of *Oxydendrum arboreum* (L.) DC. (sourwood) is described. Explants were axillary shoots forced from dormant stems of a mature tree, or nodal stem sections obtained from spring growth of the same tree. Optimum shoot growth was obtained with WPM supplemented with 0.4 to 0.8 mg/l zeatin. Shoots could be divided and subcultured after 6 weeks. Microcuttings were rooted on WPM supplemented with 0.5 to 2 mg/l IBA, or in peat/vermiculite after treatment with 0.8% IBA in talc. Mist, plastic bags, and a wet fabric tent were compared for acclimatization and promotion of normal stomatal functioning. The wet tent appeared to promote the most rapid acclimatization.

REVIEW OF LITERATURE

Oxydendrum arboreum (sourwood, sorrel, or lily-of-the-valley tree) is an attractive, slow-growing tree native to the eastern and southeastern United States, that has considerable value for use in the landscape. While *Oxydendrum* can be propagated easily from seed, there are significant variations in growth habit, flowering characteristics, and fall color. Cuttings are difficult to root (3). *Oxydendrum* is a member of the Ericaceae. It was considered to be a

good candidate for micropropagation, since several other ericaceous plants have been propagated *in vitro,* including rhododendron, blueberry, deciduous azalea, kalmia, and lingonberry (1,4,5,6,7).

MATERIALS AND METHODS

Collection and Preparation of Explants. Explants have been successfully collected for culture in two different ways:

1) Dormant stems of previous season's growth were collected from a mature tree (24 years old) in late February, 1987, and placed in a floral preservative solution (Floralife, Inc., Burr Ridge, IL, 9 g/l) in a greenhouse to force new shoot growth. The forced axillary shoots (1 to 2 cm in length) were used as explants.

2) Actively growing stems were collected after growth started in the spring (late April). Stem sections consisting of 2 nodes each were used as explants.

The explants were washed in distilled water containing 0.1% Tween 20 for 10 min. followed by stirring in 0.5% sodium hypochlorite solution for 5 to 10 min.

The explants were initially placed in culture tubes with 20 ml Woody Plant Medium (WPM) (6) supplemented with 0.8% Difco-Bacto agar, 1 mg/l zeatin, and 2 mg/l indole-3-acetic acid (IAA). Preliminary experiments showed zeatin to be superior to 6-(y y-dimethylallyamino) purine (2iP) and N_6 benzyladenine (BA) for the initiation of shoot growth. The pH of the medium was adjusted to 5.2 prior to the addition of agar and before autoclaving at 121°C for 15 min. The cultures were maintained at 24 to 26°C under a 16-hour photoperiod provided by cool-white fluorescent lamps.

Multiplication. Uniform shoots (10 mm in length) were excised from previously-established cultures and placed individually into 25 × 150 mm culture tubes containing 20 ml WPM with 0.8% Difco-Bacto agar supplemented with 0, 0.9, or 1.8 mg/l IAA, and 0, 0.4, 0.8, 1.6, 3.2, or 6.4 mg/l zeatin. Culture conditions were maintained as previously described.

Rooting. Experiment 1. Shoots longer than 10 mm were excised from an established culture and placed in culture tubes containing WPM supplemented with 0.8% Difco-Bacto agar, and 0, 0.5, 1.0, 1.5, or 2 mg/l indole-3-butyric acid (IBA). Root development was evaluated after 6 weeks.

Experiment 2. Shoots longer than 10 mm were excised and soaked in a 400 ppm IBA solution for 0 (untreated), 5, 10, 20, or 30 min, or the basal ends were dipped in Hormodin No. 1, 2, or 3 (0.1, 0.3, or 0.8% IBA in talc). These treated shoots were then placed in baby food jars containing a sterile medium of peat moss and fine vermiculite (1:1 by volume), moistened with sterile distilled water. The jars were capped with magenta B-Caps and placed under the conditions described for shoot initiation.

Acclimatization. Rooted microcuttings were removed from the culture tubes and potted into ProGro 300S in 2 in. plastic containers. These plants were then placed under 3 different conditions for acclimatization:

1) A wet fabric tent similar to that described by Whitcomb (8). The tent was constructed on a greenhouse bench with a fabric of 85% polyester/15% linen used to wick water down the sides.
2) Intermittent mist, 5 sec. every 5 min, in the same section of the greenhouse as the fabric test.
3) Enclosure within clear plastic bags.

The plants in mist and plastic bags were shaded with the same fabric covering the humid tent, so that light levels would be the same for all three treatments. Each day for 10 days following the start of acclimatization, 8 plants were removed from each of the above treatments and placed under 55% shade cloth in the greenhouse. At that time one leaf from each of three plants per treatment was excised and placed on moist filter paper in a glass petri dish. Comparable leaves from already acclimated plants were also collected in the same way. The excised leaves were then taken to the laboratory where the petri plates were opened and the leaves were allowed to lie abaxial side up for 15 min. prior to obtaining epidermal peels for microscopic determination of stomatal closure. One week after all plants had been removed from their respective acclimatization treatments, they were evaluated for leaf burn and growth.

RESULTS AND DISCUSSION

Collection and Establishment of Explants. Explants obtained either from actively growing stems, or by forcing dormant stems, were successfully established *in vitro*. Although the explants were initially established on WPM containing 1 mg/l zeatin and 2 mg/l IAA, it has since been determined that an auxin is not needed at this stage, and that 0.4 to 0.8 mg/l zeatin is sufficient to promote shoot proliferation.

We have recently established and multiplied cultures from five additional mature trees, including a tree estimated to be 75 to 100 years old, with actively growing stems collected in August.

Multiplication. As the concentration of zeatin was increased, the total number of shoots produced also increased; however, the addition of IAA to the medium reduced shoot numbers. Although the greatest total number of shoots was produced with 1.6 to 3.2 mg/l zeatin, most of these shoots were very short, suggesting excessive amounts of cytokinin. The largest number of shoots longer than 10 mm was produced with 0.4 to 0.8 mg/l zeatin (2).

Rooting. Experiment 1. A preliminary experiment showed IAA and NAA to be ineffective in promoting root development of sourwood *in vitro*. However, in this experiment, the addition of IBA to the medium (0.5 to 2 mg/l) promoted roots on 88 to 95% of the cuttings. Only 5% of the untreated cuttings rooted.

Experiment 2. Rooting in the peat/vermiculite medium was less successful. The most effective treatment was the 0.3% IBA in talc, which resulted in about 50% rooting. Almost all of the microcuttings that were treated with the 400 ppm IBA soak died.

Acclimatization. The three acclimatization treatments provided a range of humidity conditions. Over the 10 day period, the relative humidity (RH) in the plastic bags averaged 96%, while the mean RH of the wet tent was 76%. The air in the vicinity of the intermittent mist had a mean RH of 58%; however, the surfaces of the leaves were wet at all times. The RH for the rest of the greenhouse averaged 40% during this period.

The percentage open stomata data determined for each of the 10 days of acclimatization shows that the stomata of the plants from the wet tent started to close after 3 days (indicating that the stomata were functional and that the plants were successfully acclimating) and, after 8 days, had the same percentage of closed stomata as the fully acclimatized plants (Figure 1). However, the stomata from the plants kept in the plastic bags were only slightly more functional after 10 days than they were at the start of the experiment. The stomata from the mist treatment started to close at 5 days and were at about the same level as the tent treatment at 8 days.

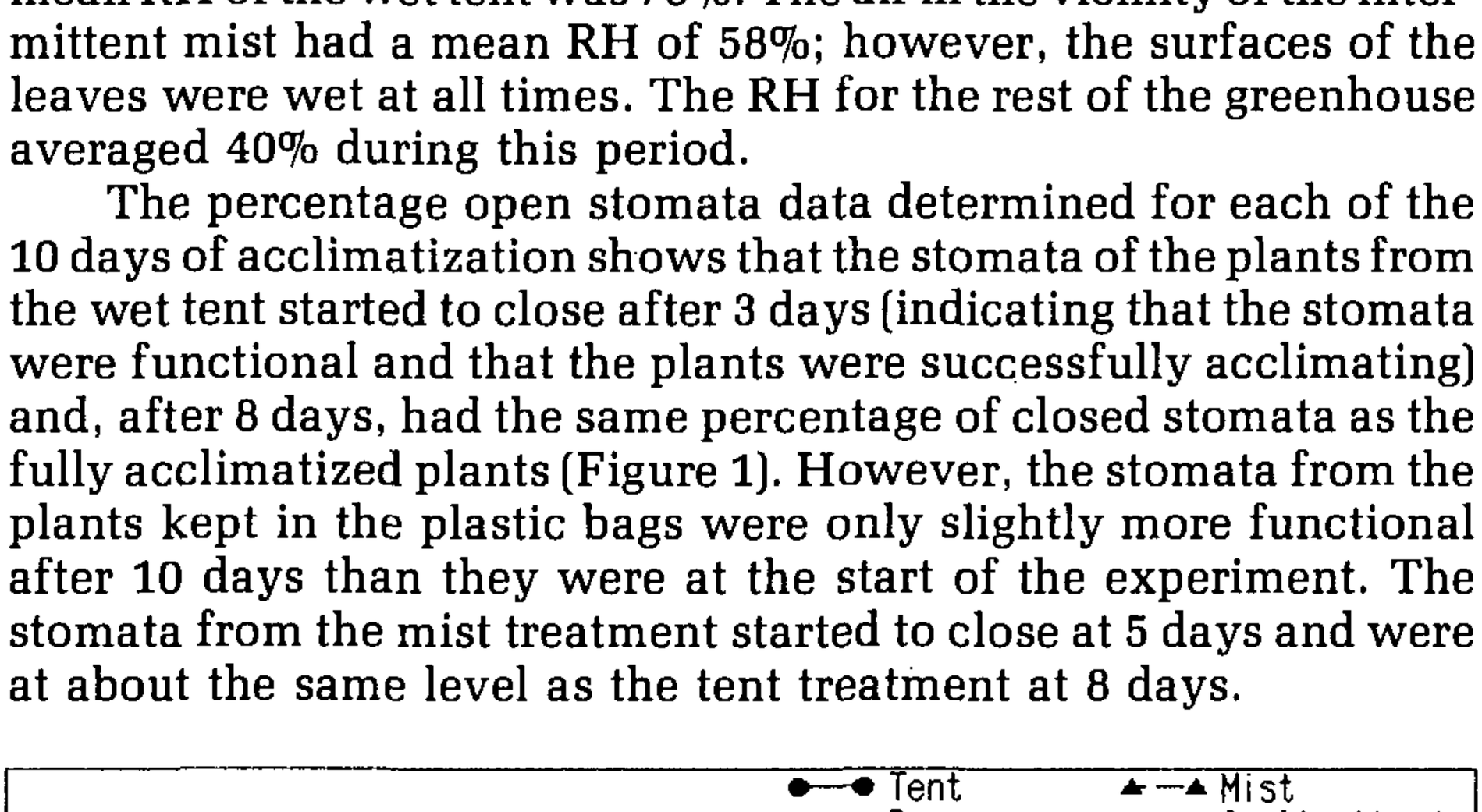

Figure 1. Percent open stomata for excised leaves of tissue-cultured plants after increasing periods of acclimatization in poly bags, intermittent mist, or wet tent.

The leaf-burn ratings showed that the plants from the plastic bags had consistently more leaf burn than the plants from the other two treatments (Figure 2). The growth ratings show that after 7 days, the plants from the tent had the greatest amount of growth and survival, while those from the bags had the lowest ratings (Figure 3). This is consistent with the data on stomate function for the plants from the three different treatments.

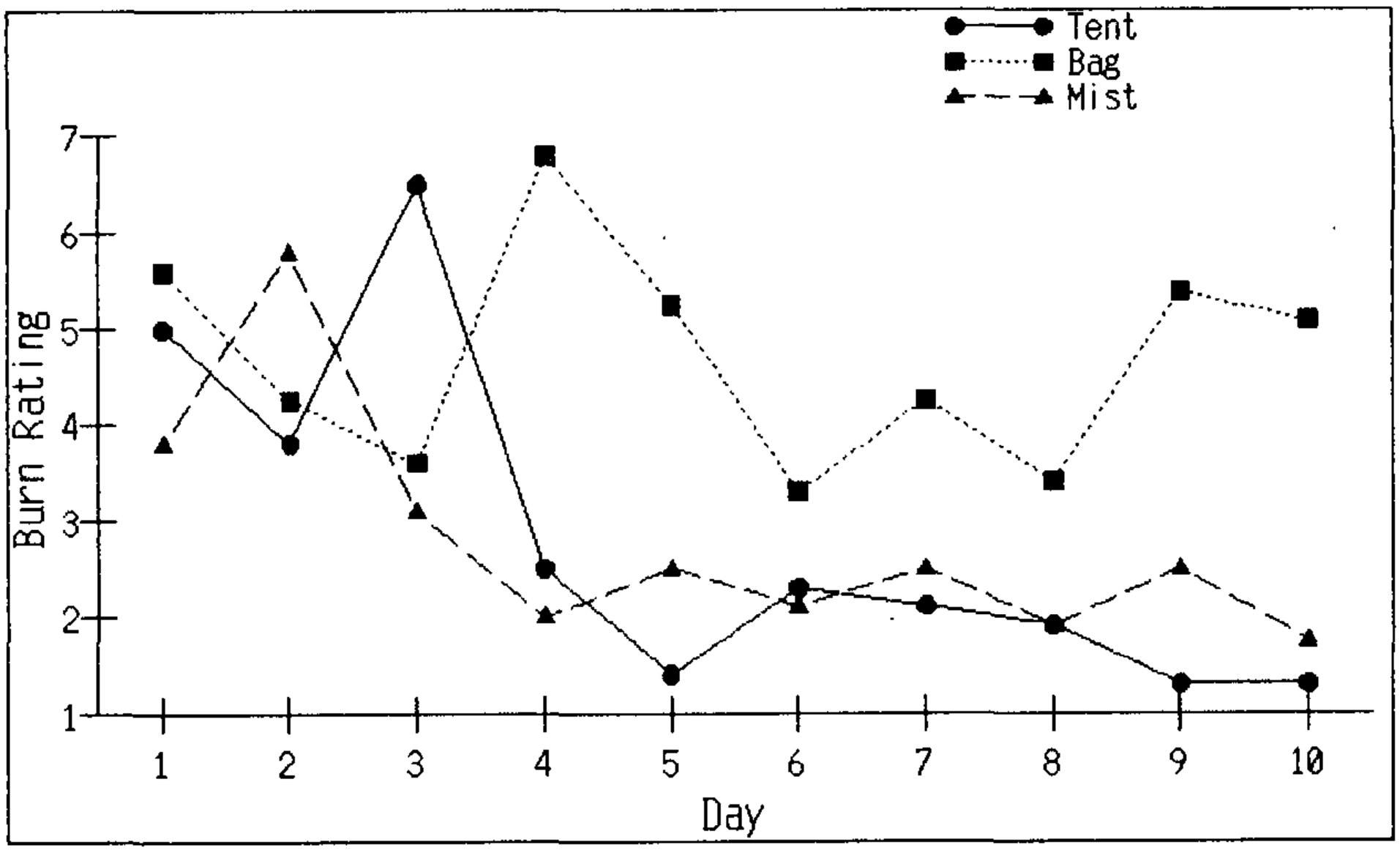

Figure 2. Leaf burn rating for plants removed from three different acclimatization treatments after 1 to 10 days of acclimatization. 1 = no leaf burn; 10 = all leaves burned, plant dead.

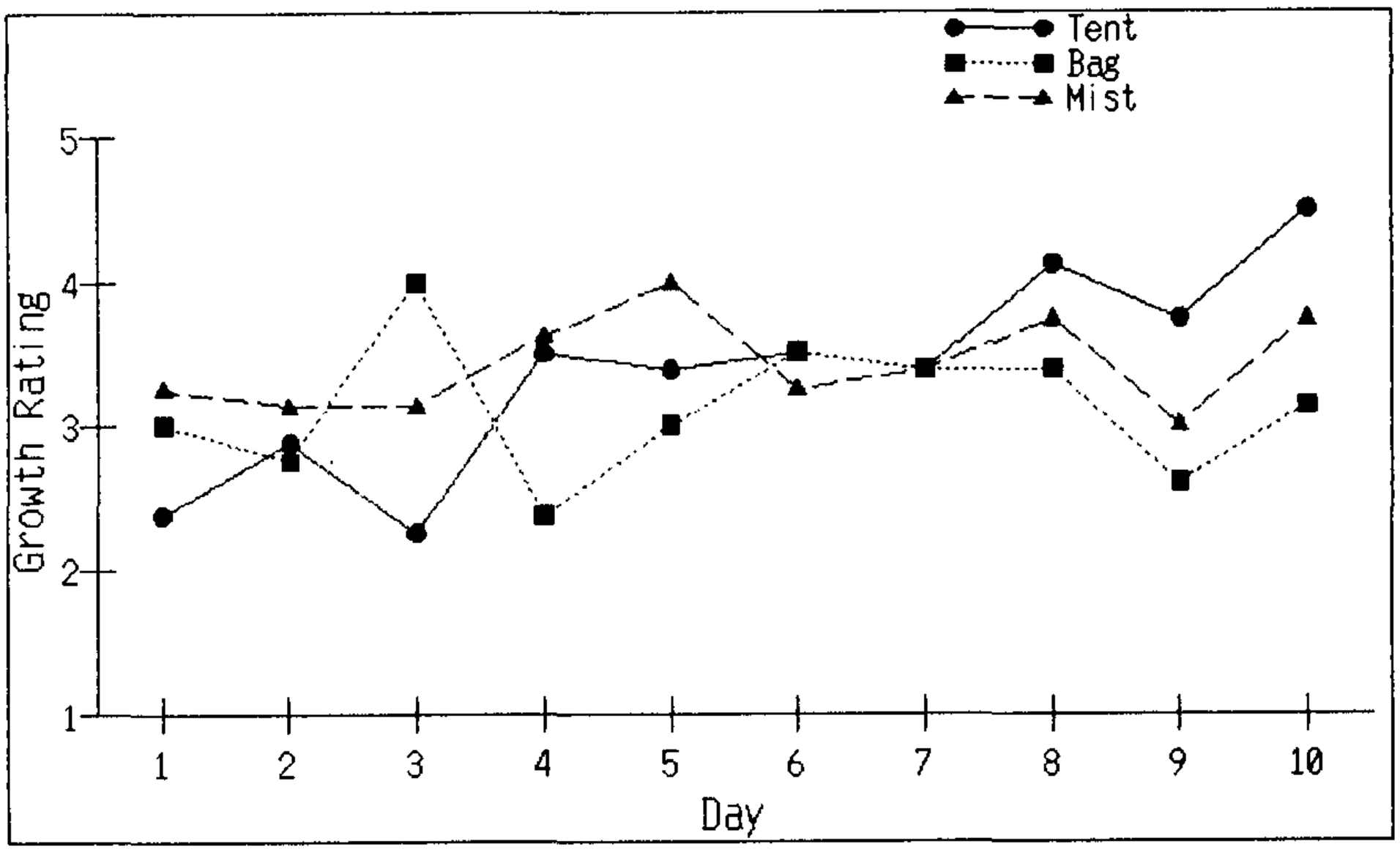

Figure 3. Growth ratings for plants removed from three different acclimatization treatments after 1 to 10 days of acclimatization. 1 = no growth; 5 = vigorous new growth.

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VOICE: What was the growth rate of the shoots after rooting, and did you get multiple shoots?

TOM BANKO: Yes, they do throw multiple shoots and that needs to be worked on. The growth rate can be quite fast. With heavy fertilization, we have obtained 3 ft the first year.

BOXWOOD PRODUCTION IN THE U.S. MIDWEST

KEN ROE AND PHILIP SOMMER

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Gardeners have known the value of *Buxus* species (boxwood) for thousands of years as: specimens, foundation plants, hedges, edging for knot gardens, accent plants, topiary, bonsai, and many other applications limited only by the imagination.

Boxwoods are native to Europe and parts of Asia. *Buxus sempervirens* has been in cultivation since the time of ancient Rome. During the middle ages it was cultivated in castle gardens and monasteries. During the seventeenth and eighteenth centuries it was in general use in Europe. It was during that time that colonists brought boxwood to America.

Along the coast in the states of Virginia, Maryland, and the Carolinas *B. sempervirens* cultivars did beautifully, but as the people moved westward, midwestern nurserymen learned of the limits of its range. Midwestern nurserymen have suffered many