

dilution (500 ppm IBA), as a quick dip, is applied to the basal portion of the bundled cuttings. Although rooting would occur without the use of hormone, its use provides more consistent rooting.

Following sticking the cutting, the flat moves through a watering tunnel that thoroughly waters the medium and prevents desiccation before being placed under mist or in a fog environment. The flats containing *W. florida* WINE & ROSES™ weigela are placed on the concrete floor of Stuppy gutter connected greenhouses. Two systems for rooting are used. One method is under a traveling irrigator from Growing Systems with misting frequencies dependant on the time of day and light intensity controlled by a PRIVA environmental computer system. The other method is in a high humidity environment (90% to 95%) provided by a high pressure Mee Fog system. Traveling irrigators are used once or twice per hour on hot, sunny days to prevent overheating of the unrooted cutting.

Root initials appear within 7 to 10 days in both environments and after 4 weeks are rooted sufficiently to undergo hardening off by reducing mist frequency to 0 to 10 times per day or being moved out of the fog environment. Rooting percentages have been 90% to 95% in either environment with few disease problems noted.

***Chionanthus virginicus*: Embryo Culture vs. Traditional Germination**

Charlotte R. Chan and Robert D. Marquard

The Holden Arboretum, 9500 Sperry Road, Kirtland, Ohio 44094-5172

INTRODUCTION

Chionanthus virginicus is traditionally propagated by seed sown outdoors, with germination taking 2 years to break double dormancy. Cuttings have not been as successful (Dirr, 1987; Nicholson, 1990), and grafting to *Fraxinus excelsior* rootstock (Dirr, 1994) or *F. ornus* (Fagan, 1980; Young, 1992) has met with limited success. Work with embryos cultured on a gibberellic-acid-enhanced medium (Redcay and Frett, 1990) and with removal of the epidermis, pericarp, and endocarp to accelerate germination (Carpenter et al., 1991) suggested a possible method to overcome the dormancy and to compress the time to obtain marketable plants. The objective of this investigation was to compare traditionally propagated and embryo-cultured *C. virginicus* for percent germination, plant size, and vigor over a duration of 2 years.

MATERIALS AND METHODS

Embryo Culture. Fruit (still green) of *C. virginicus* were collected 9 Aug., 16 Aug., and 23 Aug. 1995. The fruits were surface sterilized with 95% ethanol for 30 sec, then in 20% household bleach and 0.1% Tween with agitation for 20 min, rinsed 3 times for 4 min each in sterile distilled water, then left in the final rinse until excision. Ninety embryos were excised for each collection date and placed three per baby food jar for a total of 90 jars with 270 embryos.

Andersons rhododendron medium (Anderson, 1978) was prepared a day in advance and included: 3.0% sucrose, 0.7% Difco-bacto agar, M.S. vitamins, and brought to pH

5.8 with 1M KOH. Medium was melted in a microwave (15 min for 2 liters of medium) to evenly distribute the agar. Thirty milliliters of medium was distributed to each jar, with the jars loosely capped, and autoclaved for 15 min at 121 C.

The embryos were excised under sterile conditions in a laminar flow hood. The radicle end of the embryo is at the distal end of the fruit. To excise, the fruit was held laterally with forceps, while the stem end was cut off just enough to remove part of the endocarp. The fruit was placed cut surface down, held in place with forceps, and cut again along the side only enough to expose the endosperm. By carefully running the scalpel between the endocarp and endosperm, the embryo dislodged and generally lay along the side of the scalpel blade. The embryo was then placed with radicle in the sterile medium and cotyledons above the medium surface. After capping the jars tightly, the lids were sealed with parafilm. On average, 30 embryos could be excised per hour.

Cultures were placed in growth chambers at 25C with 16-h illumination (40 watt cool white fluorescent bulbs). After 4 weeks incubation, the germinated embryos were transferred to flats that contained a sterile perlite and peat mix, watered-in, covered with plastic, and placed in a greenhouse (high humidity at 18 to 24C). Plastic was removed after 1 week. Flats were moved to cold storage (4C) on 12 Jan. 1996 for 2 months. On 8 March 96 the flats were moved out of cold storage to a greenhouse at 18 to 24C. The seedlings were transplanted to pots 12 June 96 into a standard potting mix and returned to the greenhouse. The pots were moved into cold storage (0 to 4C) 4 Nov. 96, then out again 7 May 1997, and transplanted to 1-gal bags 27 June 1997.

Traditional Germination. Fruit (158) from the same seed source were collected on 20 Sept. 1995, pulped and cleaned, then stratified 79 seeds per seed pan in sterile germination medium (peat and perlite). The pots were placed in a greenhouse at 21 to 27C with mist for 5 months.

The pots were moved to 4C on 20 Feb. 96 for one month, then returned to the greenhouse. Carpenter et.al. (1992) reported on the ineffectiveness of additional cold treatments as a means of shortening the germination period. Therefore, pots remained in the greenhouse until the appearance of cotyledons. On 21 April 1997 the seedlings were transplanted from the seed pans to individual pots.

Data including germination, seedling heights, numbers of leaves, and stem calipers were collected at regular intervals for all seedlings. In Oct. 1997, 10% of each of the two groups of seedlings were randomly selected, cleaned of all soil, thoroughly dried in a 95C oven for 24 h and weighed (roots, stems, and leaves) for mass comparison.

RESULTS AND DISCUSSION

Excised embryos started to expand and grow within 3 days. One month after initiation on sterile medium, most of the embryos had true leaves and radicles over 4 cm in length and were transplanted to sterile soil. Mortality occurred due to contamination in culture, as well as during the seedling stage. By Oct. 1997, the survivorship was 6% (for excision on 9 Aug.), 18% (16 Aug.), 21% (23 Aug.), and 44% for traditionally grown seedlings.

On 15 May 1997, measurements were made of seedling heights and numbers of leaves for both groups and averages calculated: average height for embryo cultured

was 12.7 cm and 2.7 cm for traditional, and average number of leaves was 8.8 for embryo cultured and 2.8 for traditional. Stem caliper measurements made at root collar level compared as follows: embryo cultured seedlings averaged 7.9 mm and traditional averaged 2.6 mm.

Dry weight data, collected Oct. 1997 demonstrated an overall increase for the embryo-cultured group of 13.4-fold over that of the traditionally germinated group of seedlings.

In conclusion, additional time and labor were required for the embryo-cultured seedlings and the rate of germination success was lower than for the traditionally-germinated seedlings. However, a comparison of culture methods showed a remarkable difference in both size and vigor, suggesting embryo-culture to be a viable alternative to traditional propagation.

LITERATURE CITED

- Anderson, W.C.** 1978. Tissue culture of rhododendrons. *In Vitro* 14:434.
- Carpenter, W.J., E.R. Ostmark, and T.J. Sheehan.** 1991. Recommendations for germinating fringetree *Chionanthus virginicus* L. seed. *Proc. Fla. State Hort. Soc.* 104:337-340.
- Dirr, M.A.** 1994. The fringe tree, spectacular for its variability in growth habit, leaf color, and size. *Nursery Manager*. June, pp. 24-26.
- Dirr, M.A. and C.W. Heuser, Jr.** 1987. The reference manual of woody plant propagation from seed to tissue culture. Varsity Press, Athens, Georgia.
- Fagan, A.E. and M.A. Dirr.** 1980. Fringetrees — ready to be propagated. *Amer. Nurseryman*. 152(7):14-15,114-117.
- Nicholson, R.** 1990. The fringe tree and its far-flung cousins. *Arnoldia* 50(4):24-31.
- Redcay, S. and J.J. Frett.** 1990. Germination of doubly dormant *Chionanthus virginicus* seeds. *HortScience* 25:627 (Abstr.).
- Young, J.A. and C.G. Young.** 1992. Seeds of woody plants in North America. Dioscorides Press, Portland, Oregon.