Sexual and Asexual Propagation of Japanese Stewartia©

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The ornamental qualities of Japanese stewartia [*Stewartia pseudocamellia* (Maxim.)] are well documented (Spongberg and Fordham, 1976 and Dirr, 1998). However, Japanese stewartia availability is limited. Major barriers to wider availability are the lack of sexual and asexual propagation protocols.

This article summarizes recent work on sexual and asexual propagation of Japanese stewartia. For more detailed information, consult the following articles: Oleksak and Struve, 1999; Struve, et al., 1999; and Struve and Lagrimini, 1999.

ASEXUAL PROPAGATION

Stewartia taxa can be rooted from softwood and semihardwood cuttings using relatively high auxin (typically IBA) concentrations ranging from 3000 to 8000 ppm (Curtis, et al, 1996; Ekstrom and Ekstrom, 1988; Flemer, 1982; Fordham, 1982; Gouveia, 1995; Halward, 1966; Perkins and Bassuk, 1995; and Spongberg and Fordham, 1975). However, the propagation window is narrow and first winter survival of rooted cuttings is low.

We thought that poor overwinter survival of rooted cuttings was linked to the high auxin levels used to root cuttings. If cuttings could be rooted using lower auxin concentrations, then overwintering survival might be improved. We questioned why such relatively high auxin levels are used to root *Stewartia*. *Stewartia* is a member of the tea family, a family characterized by high levels of phenolic compounds and high peroxidase activity. Phenolic compounds are progenitors of defensive, taste, and odor compounds. Peroxidases and phenolics can inactivate auxin. Thus, high exogenous auxin levels are used because high peroxidase and phenolic levels inactivate the applied auxin, leaving low levels of metabolically active auxin. To effect rooting at lower auxin levels, we applied a peroxidase inhibitor, 0.1 mM ascorbic acid, and a competitor, 0.1 mM caffeic acid, before treating the cuttings with 100 ppm IBA.

On 24 June, cuttings were collected from 3- to 4-ft tall, 2-year-old seedling stock plants grown in #3 nursery containers. Four-inch-long co-dominant leaders were used as cuttings. Immediately after severing the cuttings from the stock plant the basal ends were dipped for 5 sec in either 0.1 M ascorbic acid or caffeic acid, or tap water and placed on a tray out of direct sunlight. When all the cuttings were collected, they were given a 5-sec quick dip in 100 ppm IBA and placed under intermittent mist to root. Natural photoperiods were used throughout the experiments.

Rooting response was evaluated 1 and 3 months after sticking. At the end of 3 months, cuttings treated with ascorbic acid, caffeic acid, and water were 90%, 96%, and 90% rooted, respectively. Rooted cuttings were potted into quart containers and placed in a heated greenhouse under natural photoperiods until December when they were moved to a minimum heat polyhouse (33°F) for the winter. In February, the cuttings were returned to a heated greenhouse. Cuttings overwintered with 100% survival. A second experiment was conducted in August. The treatments used were: ascorbic and caffeic acid pre-treatments followed by 100 ppm IBA as done in

June, a water pre-dip followed by 100 ppm IBA basal dip, or 100 ppm IBA dip. As a control treatment, cuttings were severed from the stock plant and placed directly in the propagation medium without a basal dip in water or IBA. Cuttings were evaluated on 15 October. Rooting percentage was lower in the second experiment. Cuttings pre-treated with ascorbic or caffeic acid and then IBA rooted at 64% and 67%, respectively. Cuttings treated with water and IBA, water alone, or no pre-treatment, rooted at 28%, 31%, and 38%, respectively. The cuttings propagated in August were handled similarly to the June-propagated cuttings, but overwintered in a 45°F walk-in cooler. Overwintering success was 89% for all cuttings. There was no difference in overwintering success among the treatments.

Semihardwood stem cuttings of Japanese stewartia can be rooted in high percentages using low rates of IBA if first treated with ascorbic or caffeic acid. These cuttings can be transplanted and overwintered successfully in a minimum heat polyhouse. Propagation success is attributed to pre-treatment with either a peroxidase inhibitor or competitor, which results in higher effective auxin levels with relatively low exogenous auxin application.

SEXUAL PROPAGATION

Current seed propagation protocols are based on the recommendations of Fordham and Spongberg (1976) and Fordham (1982). Fordham's procedure was to extract seeds from dried capsules in October, mix the seeds with moist peat and sand, place the

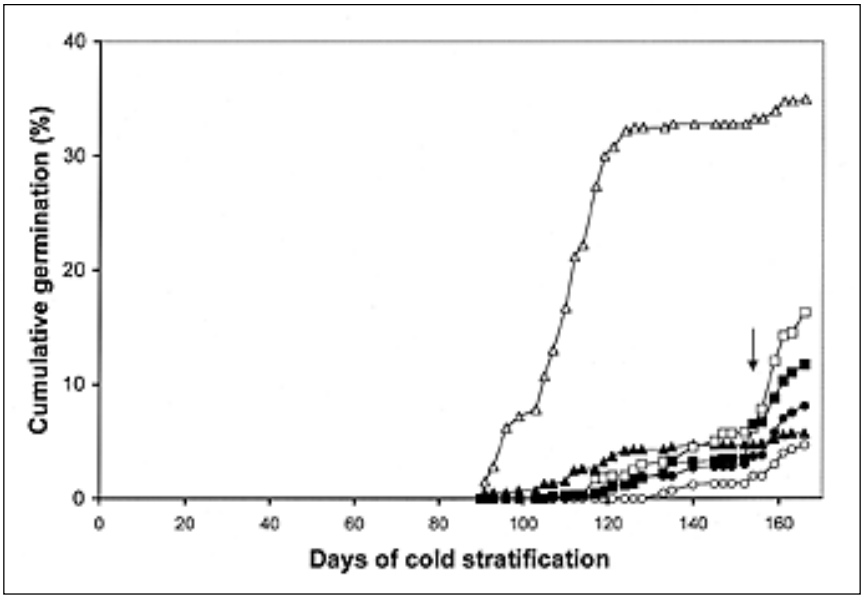

Figure 1. Cumulative percent germination of Japanese stewartia seeds following 3 months warm moist stratification at various temperatures and cold (7°C, 45°F) moist stratification. The arrow indicates when seeds were removed from cold stratification to 25°C for a 7-day germination test. Each value is the mean of 6100 seed replications. ○ = constant 25°C (77°F), ● = constant 20°C (68°F), △ = constant 15°C (59°F), ▲ = constant 10C (50°F), □ = 12-h cycles of 23/18°C (73/65°F), and ■ = 12-h cycles of 18/12°C (65/55°F).

mixture in plastic bags, seal the bags, and expose the seeds to 4 months of "natural" warm stratification on a greenhouse bench out of direct sunlight. Warm stratification temperatures ranged from 40 to 100°F. Afterwarm stratification, seeds were given a 3-month cold (40°F) stratification treatment. Seeds germinated when sown in flats in a greenhouse. However, no germination percentage was reported.

The warm/cold stratification conditions are required to mature an immature embryo (Oleksak and Struve, 1999) and to overcome embryo dormancy. The optimum warm and cold stratification temperatures and durations were unknown.

In a series of experiments, Japanese stewartia seeds were identified as recalcitrant, that the optimum warm stratification temperature was 50°F for at least 3 months and long (at least 150 days) cold stratification at 45°F (Figs. 1 and 2). Although seeds could be germinated, final germination percentage tended to be low (35%) and asynchronous; germination began as early as 90 days and after as much as 310 days cold stratification. A contributing factor to low germination may be the genetics of the mother trees. We used seeds collected from three trees at the Dawes Arboretum in Newark, OH. Seeds sent from one tree in Japan germinated over 95% (Fig. 3).

Currently, we are researching methods to increase germination percentage and synchrony. Using these techniques, Japanese stewartia propagation should be more successful, resulting in increased availability of one of our most ornamental species.

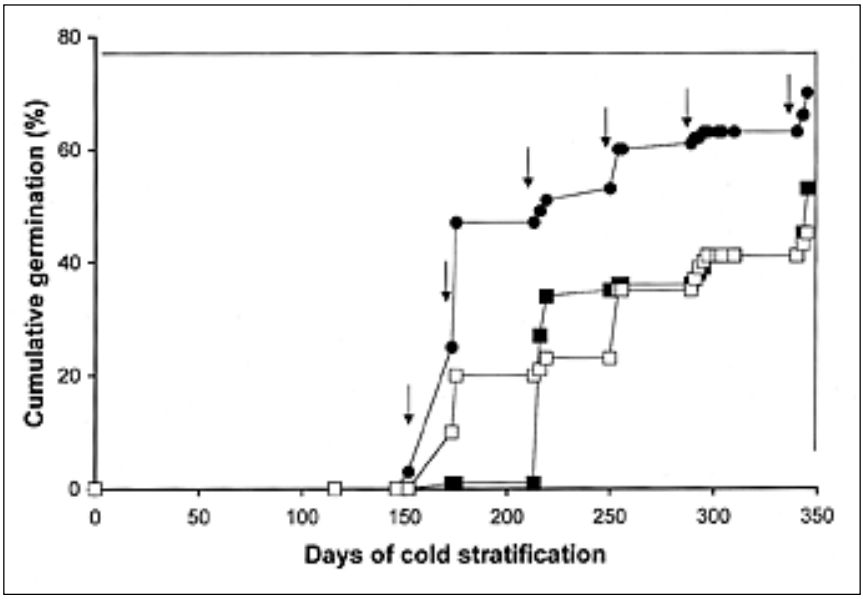

Figure 2. Cumulative percent germination of Japanese stewartia seeds following a 3-day aerated water soak in 1 mM GA_3, 3 months warm moist stratification at a constant 25°C (77°F) and 4, 5, or 6 months cold (7°C, 45°F) moist stratification. Arrows indicate when seeds given 4 months cold moist stratification were removed from cold stratification to 25°C for a 7-day germination test after which ungerminated seeds were returned to cold stratification. For seeds given 5 and 6 months cold stratification, this process was begun 173 and 213 days, respectively, after initiation of cold stratification. ● = 4 months, □ = 5 months, and ■ = 6 months cold stratification. Each value is the mean of three 50-seed replications.

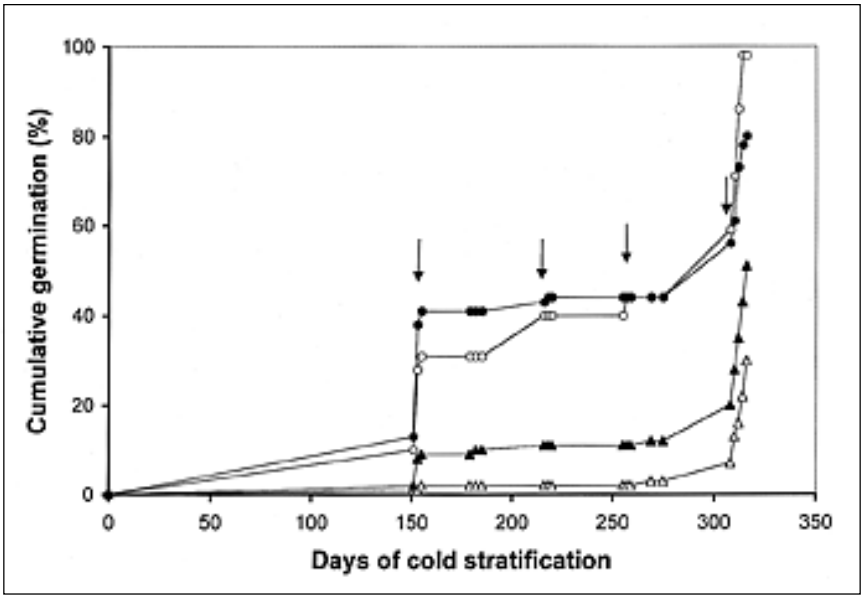

Figure 3. Cumulative percent germination of Japanese stewartia seeds from 4 mother trees following a 3-day aerated water soak in 1 mM GA_3, 3 months warm moist stratification, 12-h cycle of 20/12°C (68/54°F) alternating, and cold (7°C, 45°F) moist stratification. Arrows indicate when seeds were removed from cold stratification to 25°C for a 7-day germination test after which ungerminated seeds were returned to cold stratification. ● = Tsukuba 1, ○ = Tsukuba 3, ▲ = Tsukuba 4, △ = Tsukuba 5. Each value is the mean of three 50-seed replications.

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Improved Adventitious Rooting in *Quercus* Through the Use of a Modified Stoolbed Technique©

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INTRODUCTION

Vegetative propagation of the oaks (*Quercus*) is a difficult undertaking. Previous attempts at propagation using the methods of grafting, cutting propagation, and tissue culture have produced only very limited success (Drew and Dirr, 1989; Zaczek et. al., 1997). Recently, however, the propagation of oaks has been improved by using the practice of etiolation in conjunction with a modified stoolbed technique. A newer approach to improving propagation is through practices that are thought to reduce the plants' endogenous cytokinins, a class of hormones that is generally thought to inhibit adventitious rooting. The idea behind this approach is that a reduction in the plants' endogenous cytokinin levels may improve the potential for adventitious root formation. These two approaches to propagation, the use of etiolation in conjunction with the modified stoolbed technique and the use of practices aimed at reducing endogenous cytokinin levels, will be discussed here.

ETIOLATION

Etiolation, or the practice of excluding light from the plant environment, has previously been shown to improve adventitious rooting (Bassuk and Maynard, 1987). The benefit of etiolation on adventitious rooting comes, in part, by way of its positive influence on the shoot's anatomical development. Past research with *Carpinus betulus* indicated that by etiolating shoots during their initial development, the shoots would exhibit reduced lignification of the secondary xylem and reduced sclereid development. The net effect of these anatomical changes in the shoots seems to have been an increase in the potential sites for adventitious root development (Maynard and Bassuk, 1996).

The use of etiolation in conjunction with a modified stoolbed technique has also been used to improve adventitious rooting in *Quercus* (Griffin and Bassuk, 1996). This approach requires that the hormone auxin, mixed into an aqueous carrier (DMSO), be applied directly onto the plant's newly emerging shoots.

THE AUXIN/CYTOKININ RELATIONSHIP

When approaching work in vegetative propagation, one of the primary considerations is that of the relationship, and interaction, between the plant hormones auxin