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Germination of Japanese Stewartia Seeds: The Effects of Warm and Cold Stratification[1]

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Abstract

Japanese Stewartia (*Stewartia pseudocamellia* (Maxim.)) is a species with outstanding landscape qualities, but is not widely available because reliable propagation protocols have not been developed, including procedures for overcoming seed dormancy. Three experiments were conducted to determine the warm and cold stratification requirements of Japanese Stewartia seeds. In the first experiment, seeds given a 3-day aerated water soak in 1 mM GA_3, followed by 3 months warm moist stratification at 25C (77F) had greater germination 173 to 297 days after initiation of cold (7C, 45F) stratification than seeds given a 3-day aerated water soak and similar warm and cold stratification treatments. Final germination was 70%, but germination was not synchronous, it occurred over 172 days. In the second experiment, germination was low (less than 1%) for seeds given either 9 months cold moist stratification or 3 months warm moist stratification at 20C (68F) before 6 months cold moist stratification. Seeds given a 3-month warm moist stratification at an alternating 12 hr 20/12C (68/54F) cycles and 10 months cold moist stratification germinated from 30 to 93%, depending on mother tree. Germination was asynchronous, beginning after 150 days cold stratification and continuing for the next 165 days. In the third experiment, germination was not enhanced by a 3-day aerated water soak in either 1 mM GA_3 or water, compared with seeds given no aerated water soak prior to cold moist stratification. Germination was greatest, 35%, for seeds given a 3-month warm moist stratification at 15C (59F) followed by at least 4 months cold moist stratification. However, germination was asynchronous. The results begin to identify the warm and cold stratification treatments that result in high germination.

Index words: *Stewartia pseudocamellia*, seed germination, sexual propagation, gibberellic acid, seed dormancy, double dormancy.

Significance to the Nursery Industry

Japanese Stewartia seeds are difficult to germinate. The seeds are recalcitrant and dessicate rapidly. Japanese Stewartia seeds can be germinated in high percentages by preventing dessication and satisfying warm and cold stratification requirements. The best combination of warm and cold stratification durations and temperatures identified in this study are warm moist stratification at 15C (59F) for 3 months followed by an extended period, up to 300 days, of cold (7C, 45F) moist stratification. A 3-day aerated water soak, with or without 1 mM GA_3 did not increase germination. Under these conditions, germination was asynchronous, occurring over 165 days. Germination can occur over a 2-year period after harvest and under a wide temperature range.

Introduction

Japanese Stewartia has many desirable landscape characteristics, however it is difficult to propagate by seeds (2). Germination protocols (2, 3, 9) are based on work by Fordham (4, 8) who recommended that Stewartia seeds be extracted from capsules in October, mixed with moist peat moss and sand and then placed in a plastic bag, sealed and exposed to 4 months 'natural' warm stratification on a greenhouse bench out of direct sunlight. Warm stratification temperatures ranged from 4C to 38C (40 to 100F). After warm stratification, seeds were given a 3-month cold stratification treatment at 4C (40F) after which the seeds were sown in flats in a greenhouse. However, no data on germination rate or percentage were given. Three to 5 months warm stratification followed by 3 months cold stratification were recommended for *Stewartia monadelpha* (Sieb. & Zucc.) seeds, but stratification temperatures were not given (2). Others (4) have recommended that *Stewartia pseudocamellia* seeds be sown in flats immediately after harvest and checked for germination after 1 month warm stratification at 20C (70F). If no germination occurs, the flats should remain in a greenhouse an additional 18 months.

A 24-hour soak in GA_3 increased germination of *Stewartia koreana* (Rehd.) (8) and *S. pseudocamellia* (6) seeds. Both studies used a 3-month, 25C (77F) warm stratification period following the soak. For *S. koreana*, 42% germination occurred after 3 months cold stratification. For *S. pseudocamellia*, no germination occurred until the seeds received at least 5 months cold stratification (6). The greatest germination, 51%, occurred after 7 months cold stratification. One observation common to all Stewartia seed germination studies is non-uniform germination.

This study examined effects of pre-stratification GA_3 treatment, warm stratification temperature and the length of the cold stratification period on germination of Japanese Stewartia seeds. The objective was to develop pre-germination treatments that result in rapid and high germination so that direct seeding in a plug production system is economically feasible.

Materials and Methods

Experiment 1. GA_3 aerated water soak and length of cold stratification. Capsules were collected in mid-September 1997 from 3 trees at The Dawes Arboretum, Newark, OH, and seeds extracted as described previously (6). Seeds from the trees were mixed; the study was conducted with seeds randomly selected from the bulk sample. Except for 100

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seeds, all seeds received a 3-day aerated water soak in 1mM (1000 ppm) GA_3 (Sigma Chemical Co., St. Louis, MO). The GA_3 solution was changed daily due to discoloration. The 100 untreated seeds were placed between single layers of moist germination paper (Anchor Paper Co., Eau Claire, WI) inside an unsealed plastic bag while the other seeds received the aerated GA_3 soak. Groups of 50 seeds were counted on each of 11 sheets of moist germination paper, covered with another layer of moist germination paper, rolled and placed in individual, unsealed plastic bags. The seeds were placed in a dark incubator set at 25C ± 1C (77 F) for a 3-month warm stratification period. The seeds were then placed in a dark, walk-in cooler set at 7C ± 1C (45F) for 4, 5 or 6 months cold stratification. The untreated seeds received a similar warm stratification period and a 4-month cold stratification period. Thus, for the 4-month cold stratification period, there were 3 replications of 50 seeds each that received a 3-day aerated water soak in 1 mM GA_3 and 2 replications of 50 seeds each that did not. For the 5- and 6-month stratification periods, all seeds received a 3 day aerated water soak in 1 mM GA_3. There were 3 replications of 50 seeds each for each cold stratification period. A full factorial design was not possible due to a limited number of seeds.

On April 26, 1998, after the 4-month cold stratification period was completed, the seeds were removed from cold stratification and placed at room temperature 25C ± 2C (77F) for a 7-day germination test. Previous results showed that all germination occurred within 7 days after transferring the seeds to the warm temperature (6). Germinated seeds (radicles 0.2 cm (0.25 in) long) were counted at 2-day intervals and removed from the test. Ungerminated seeds were returned to cold stratification for another 3 weeks. This procedure was repeated for 6 months (until October 26, 1998) adding the 5- and 6-month cold stratification treatments on May 26 and June 26, respectively. From October 26 to November 16, seeds remained at 25C and then were returned to 7C for 1 month. On December 16, 1998, the final germination test was conducted.

In all experiments, germination counts were converted to percentages and analyzed as square root arcsin transformations by ANOVA using the repeated measures procedure within SPSS/PC+ for the personal computer. One degree of freedom was subtracted from the total and error sums of squares to account for estimating the third control replication mean. Transformed germination means were separated using the Student-Newman-Kuels test ($\alpha = 0.05$ level of significance) only when ANOVA indicated significant treatment differences. For the 3-month warm stratification treatment, an orthogonal contrast was used to determine the effect of a pre-warm stratification soak in GA_3.

Experiment 2. Constant or alternating warm stratification temperatures. Capsules collected from 7 trees in two locations (5 trees in the Arboretum of Forestry and Forest Products Research Institute (FFPRI) at Tsukub, Ibaraki, Japan, and 2 trees at FFPRI in Toyohira, Sapporo Hokkaido, Japan) in late October were packed on moist sphagnum peat moss in plastic bags, held at 7C until mailed to Columbus on October 31, 1997, where they were received on November 3, 1997.

The seeds were extracted from the capsules as before, while retaining mother tree identity, and given a 3-day aerated water soak in 1 mM GA_3. The seeds were then counted into groups of 50 seeds onto moist germination paper. Another layer of moist germination paper was placed over the seeds, and the germination paper rolled and placed in unsealed plastic bags. The seeds received one of three warm stratification treatments: placed directly into cold (7C, 45F) stratification on November 11, 1997; placed in a constant 20C ± 1C (68F) for 3 months; or placed in an alternating 20/12C ± 1C (68/54F) on a 12-hour cycle for 3 months. The number of seeds was limited, so only 4 trees (Tsukuba 1, 3, 4 and 5) were included in the alternating warm stratification treatment. On February 11, 1998, seeds from the 2 warm stratification treatments were placed in a dark, 7C walk-in cooler for 4 months cold stratification. After approximately 8 months cold stratification, November 11, 1997, to June 29, 1998, seeds of the cold stratification treatment were placed at 25C ± 2C (77F) for a 1-week germination test. As before, germinated seeds with radicles greater than 0.2 cm (0.25 in) were counted and removed. On July 6, 1998, the cold stratification treatment seeds were returned to 7C. At the same time, warm stratified seeds (which had received 3 months warm stratification and 5 months cold stratification) were placed at 25C for a 1-week germination test, germinated seeds removed and ungerminated seeds returned to 7C. After 3 additional weeks of cold stratification, all the seeds were removed from 7C for another 1-week germination test at 25C. This procedure was repeated on September 6, 1998, for the cold and constant warm stratification treatments. For the alternating warm stratified seeds, the 3 weeks cold, 1 week warm process was repeated until December, 1998.

Because of low germination in the no-warm and 20C-warm stratification treatments, the data was not subject to ANOVA. For the four Tsukub trees where there were enough seeds to include in the alternating warm stratification treatment, the transformed germination counts were subject to ANOVA using mother tree as the independent variable.

Experiment 3. Defining the optimum warm stratification temperature and the effect of GA_3 aerated water soaks. Capsules were collected from the same trees at Dawes Arboretum in early September 1998 as in 1997 and seeds extracted as before. A group of 1500 seeds was given a 3-day aerated water soak in 1 mM GA_3 as described earlier. Another group of 1500 seeds was given a 3-day aerated water soak in water only. A third group of 1500 seeds was placed between moist sheets of germination paper inside unsealed plastic bags for 3 days. From each group of 1500 seeds, 12 lots of 100 seeds were randomly selected and counted onto wire screens suspended above tap water in plastic accelerated ageing boxes (5) and covered. On September 14, 1998, all the seeds were placed in dark incubators at various warm stratification temperatures: a constant 25C, 20C, 15C, or 10C ± 1C, (77F, 68F, 59F, and 50F, respectively) or 12-hour cycles of fluctuating 23/18C ± 1C or 18/13C ± 1C (73/65F and 65/55F, respectively) for 3 months. On December 14, 1998, all the seeds were placed in a walk-in cooler at 7C ± 1C. Between March 10, 1999, and May 19, 1999 (96 and 159 days cold stratification), the boxes were inspected for germinated seeds using the same germination criteria as before. Germinated seeds were removed from the plastic boxes. On May 19, 1999, the seeds were placed in a lighted 25C ± 2C room for one week; the experiment was terminated on May 26, 1999.

The transformed data were subject ANOVA as described before. Transformed germination means were separated us-

ing the Student-Newman-Kuels test ($\alpha = 0.05$ level of significance) only when ANOVA indicated significant treatment differences.

Results and Discussion

Experiment 1. The first seeds germinated after 150 days cold stratification. Seeds treated with a 3-day aerated 1 mM GA_3 soak tended to have higher germination percentage; however the increase was not statistically significant (Fig. 1). The greatest difference, 15%, occurred between 219 and 222 days after cold stratification began.

For the seeds treated with GA_3, those given 4 months of cold stratification had significantly higher germination percentage between 173 and 297 days cold stratification than seeds given 5 or 6 months cold stratification (Fig. 2). The germination pattern was similar regardless of the length of cold stratification. Germination occurred during cold stratification followed by a two-fold germination increase when the seeds were exposed to warm germination test temperatures. Small increases in germination occurred following subsequent cycles of 3 weeks cold stratification and a 1 week warm germination test. For instance, germination increased from 2 to 51%, between 173 to 219 days cold stratification in the 4-month cold stratification treatment (Fig. 2). Half of the increase occurred during cold stratification, the remainder during the first 2 days of the germination test. Germination increased only 16% during the next 165 days. Similar increases in germination occurred during this same period (173 to 219 days) for seeds in the 5- and 6-month cold stratification treatments; 1 to 34 and 10 to 23%, respectively. The effect of exposing the seeds to periods of warm temperatures during the germination test periods on seed germination is unknown.

Experiment 2. Germination was low when seeds received either no warm stratification (11 months cold stratification) or warm stratification at a constant 20C (68F) followed by 7 months cold stratification, 1% and 0.4%, respectively. For seeds given alternating 20/12C (68/54F) warm stratification temperatures followed by cold stratification, some germination (10, 13, 1 and 2% for Tsukuba trees 1, 2, 3 and 4, respectively) occurred during the cold stratification period preceding the first germination test (Fig. 3). Germination at least doubled to 31, 41, 2 and 9% for trees 1, 2, 3, and 4, respectively, during the first 5 days of the germination test. However, another 3 cycles of 3 weeks cold and 1 week warm stratification (308 days since cold stratification began) were needed for an additional increase in germination (Fig. 3). As with the Dawes Arboretum seed source, germination occurred over an extended period, 165 days. There were significant ($P = 0.001$) differences among the mother trees in germination percentage during the experiment. Final percent germination ranged from 30 to 98%.

Experiment 3. No significant interaction was observed between the aerated water soak pre-stratification treatments and warm stratification temperature (data not presented). Treating seeds with a 3-day aerated water soak in 1 mM GA_3

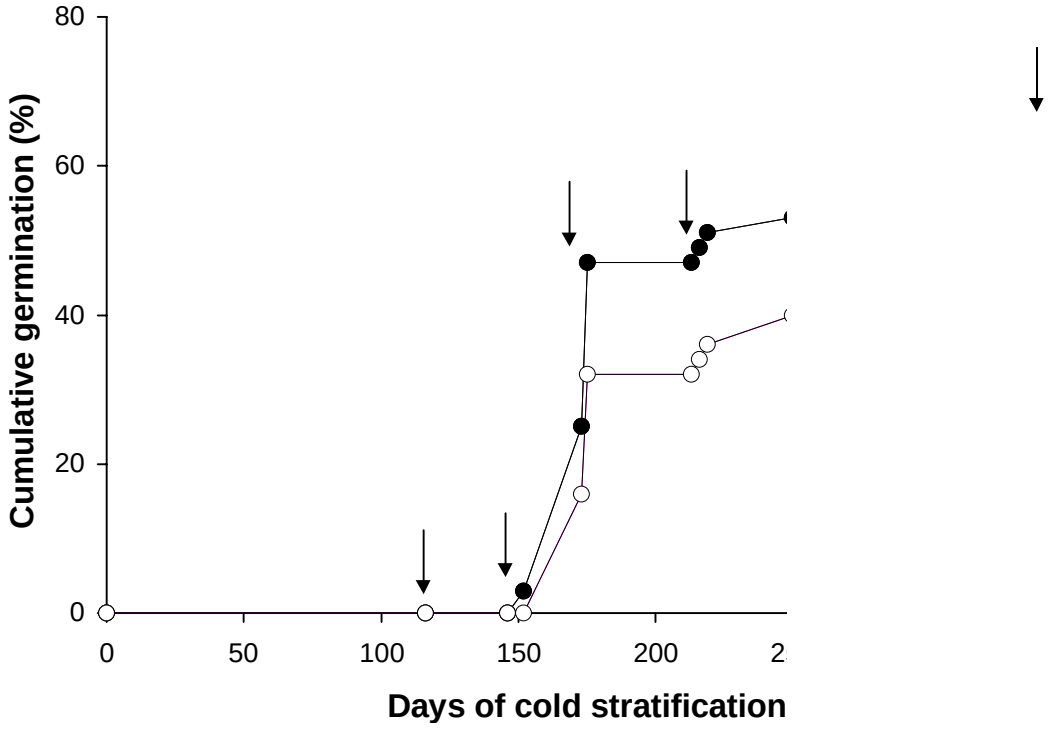

Fig. 1. Cumulative percent germination of Japanese Stewartia seeds following a 3-day aerated water soak in either 1 mM GA_3 or water followed by a 3-month warm moist stratification at a constant 25C (77F) and 4 months cold (7C, 45F) moist stratification. Arrows indicate when seeds were removed from cold stratification to 25C for a 7-day germination test after which ungerminated seeds were returned to cold stratification. ● = 3 day aerated water soak in 1 mM GA_3, ○ = 3 day aerated water soak. For the aerated water soak treatment in 1 mM GA_3, each value is the mean of 3, 50-seed replications. For the aerated water soak treatment without GA_3, each value is the mean of 2, 50-seed replications.

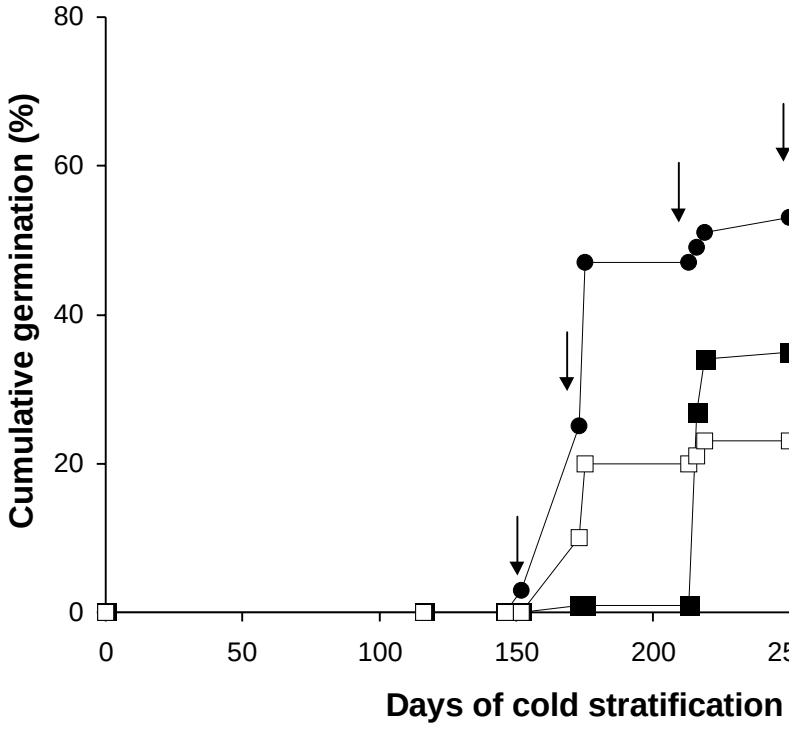

Fig. 2. **Cumulative percent germination of Japanese Stewartia seeds following a 3-day aerated water soak in 1 mM GA_3, 3 months warm moist stratification at a constant 25C (77F) and 4, 5 or 6 months cold (7C, 45F) moist stratification. Arrows indicate when seeds given 4 months cold moist stratification were removed from cold stratification to 25C 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 3, 50-seed replications.**

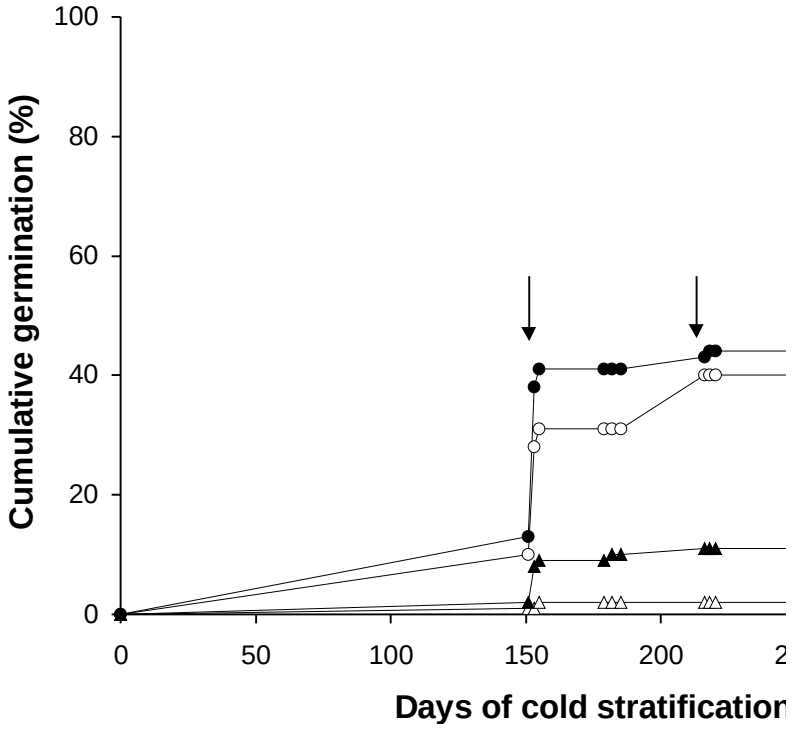

Fig. 3. **Cumulative percent germination of Japanese Stewartia seeds from 4 mother trees following a 3-day aerated water soak in 1 mM GA_3, 3 months warm moist stratification, a 12-hour cycle of 20/12C (68/54F) alternating, and cold (7C, 45F) moist stratification. Arrows indicate when seeds were removed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

significantly increased germination between 71 and 74 days of cold stratification compared with seeds given either no aerated water soak or an aerated water soak without GA_3. However, maximum seed germination during this period was less than 2%. During the aerated water soak, the water was changed daily due to discoloration, but apparently the compounds released during the aerated water soak were not germination inhibitors as germination was low. In contrast, a similar GA_3 soak significantly increased germination after 7 months cold moist stratification, 6 vs. 51% for untreated and GA-treated seeds, respectively (6) and a GA_3 soak was also effective in promoting *Stewartia koreana* seeds (7). Because there were no other aerated water soak treatment differences, the 3 treatments were combined within each warm stratification temperature.

Throughout the experiment, the warm stratification temperature significantly affected germination (P = 0.05). Seeds germinated most rapidly and in greatest numbers when exposed to a constant 15C (59F) before cold stratification (Fig. 4). Germination occurred rapidly from 0 to 30% between 96 and 127 days cold stratification. An additional 5% germination occurred during the remaining 47 days cold stratification. Germination percentage was significantly higher for seeds given 15C warm stratification than for the other warm stratification treatment between 96 and 174 days. Germination was less than 15% for all other warm stratification treatments until the seeds were removed from cold stratification and placed in a lighted 25C (77F) room, after 159 days cold stratification (Fig. 4). Germination percentage doubled in all warm stratification treatments, except the 15C treatment. Seeds given 15C warm stratification had significantly higher final germination percentage than all the other treatments. Seeds given an alternating 23/18C warm stratification treatment had significantly higher final germination percentage than seeds given 15, 20 or 25C warm stratification. There was no difference in final germination percentage between seeds given an alternating 23/28C and 18/12C warm stratification treatments.

The results of experiment 3 showed that a constant 15C warm stratification treatment promotes germination better than alternating temperatures. Final germination was 16 and 12% for the 23/18 and 18/13C warm stratification treatments, respectively, while it was 35% for seeds treated with a constant 15C. In contrast, final germination in experiment 2 was greater following an alternating warm stratification treatment than a constant 15C as in experiment 3. However, comparisons between experiments 2 and 3 must be made with caution. First, experiment 2 ran for almost twice as long as Experiment 3. When experiment 3 was concluded, after 174 days, germination was 35% for seeds given a constant 15C warm stratification treatment; after 174 days cold stratification, germination in experiment 2 ranged from 2 to 41%. Second, experiment 2 and 3 used seeds from different mother trees. The results of experiment 2 demonstrated significant differences among mother trees. Large differences in germination characteristics due to mother trees are common in unselected woody plants (1). For the Dawes source, there seems to be a narrow warm stratification temperature range that promotes germination. Warm stratification temperatures 5C (9F) lower or higher (the constant 10 and 20C warm strati-

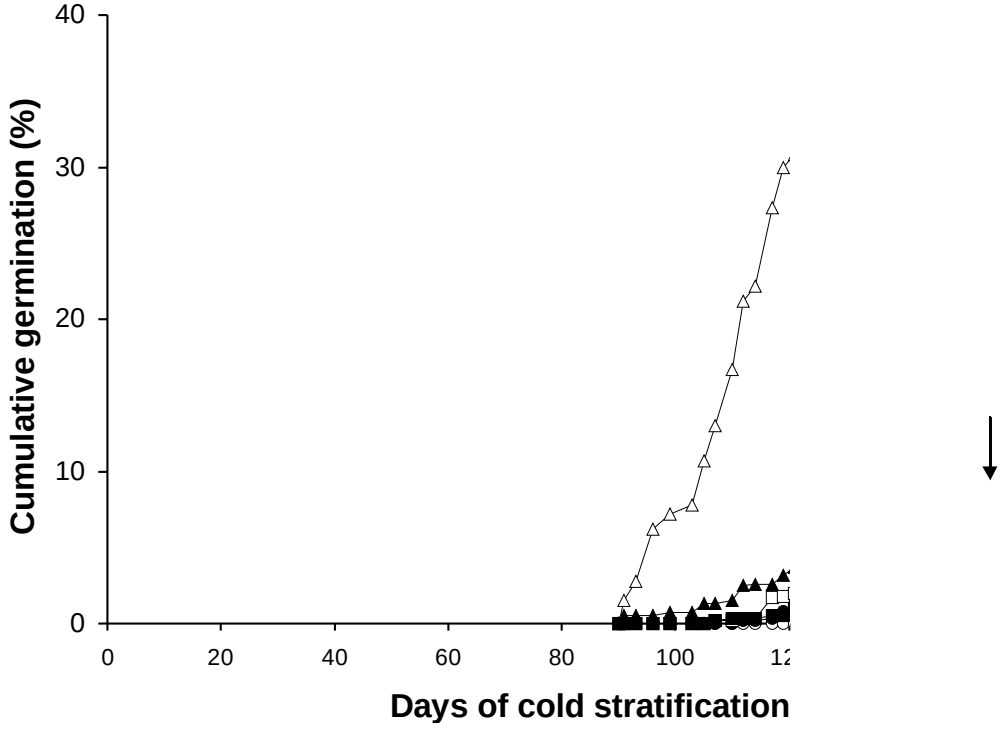

Fig. 4. Cumulative percent germination of Japanese Stewartia seeds following 3 months warm moist stratification at various temperatures and cold (7C, 45F) moist stratification. The arrow indicate when seeds were removed from cold stratification to 25C for a 7-day germination test. Each value is the mean of 6, 100-seed replications. ○ = constant 25C (77F), ● = constant 20C (68F), △ = constant 15C (59F), ▲ = constant 10C (50F), □ = 12 hour cycles of 23/18C (73/65F) and ■ = 12 hours cycles of 18/12C (65/55F).

fication treatments), or alternating temperatures 2.5C around a mean of 15C (18/13C warm stratification treatment) significantly reduced germination.

The warm stratification time used in this study is 1 month less than suggested (2, 4), but similar to that used by Shim, et al. (7). The optimum temperature found in this study is also lower than that found in other studies (4, 6, 7, 8).

There are still unknown or non-optimized factors affecting Japanese Stewartia seed germination. For instance, the optimum length of the warm stratification period is unknown; there may be a warm stratification temperature by time interaction; or a light by stratification temperature or time interaction; and a method for synchronizing germination has not been identified.

The results of this study begin to provide a scientific basis for the practices of successful Stewartia seed propagators. Heritage Seedling, Salem, OR, receives Stewartia fruit in fall and packs them in moist sand for a year of stratification (Mark Krautmann, personal communication). The seeds receive warm stratification in summer. In late winter, seeds are inspected. Germinated seeds are transplanted to containers, ungerminated seeds are left in the flat and transplanted as they germinate over the next 6 to 7 weeks. This process results in about a 50% seedling stand. Herman Losely and Son's Nursery (Painesville, OH) uses a similar method but artificially creates a 3-month warm stratification period (October through January) by placing Stewartia seeds in a warm (21C, 70F) room before a 3-month cold stratification period (Bentley Karran, personal communication). The seeds are then sown in flats and placed in a heated greenhouse. This results in 5% germination. These seedlings are transplanted. The ungerminated seeds remain in the flats exposed to natural temperature variations during spring and summer. In fall, the greenhouse temperature is kept above 2C (35F). Between February and April of the second year, most of the seeds germinate.

A 2-year dormancy breaking cycle is required under natural conditions because the first cold period is not perceived by the embryos. Presumably, some embryos are mature enough at harvest to germinate immediately (4) or mature enough to perceive the first cold stratification period (the 5% germination reported by Karran). For most seeds, the warm stratification period makes the embryos receptive to the cold stratification occurring the second winter. During the second winter, the embryo develops (6). The broad range of temperatures and times at which germination occurs represents a major scheduling challenge to propagators. We are currently studying methods to synchronize and increase Stewartia germination.

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