Methods for Stimulating Green Ash Seed Germination[1]

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Seeds of green ash were subjected to several pregermination treatments including various combinations of stratification, partial endosperm removal, complete embryo dissection, and water-soaking. All treatments enhanced germination percentage, but were either time consuming or inhibited subsequent seedling growth.

Mature seeds of ash (*Fraxinus* sp.) are frequently deeply dormant. Causes of this dormancy vary among ash species (*1, 8, 10*). Seeds of white ash (*F. americana* L.) may be kept dormant by high concentrations of abscisic acid in the endosperm and embryo (*8*). This causes embryo dormancy. Seeds of European ash (*F. excelsior* L.) often have pericarps impermeable to oxygen (seed coat dormancy), immature embryos, metabolic inhibitors in the endosperm and embryos, and deficiencies of growth-promoting substances in embryos *(10*). As a result of variable dormancy causes, recommendations for treating ash seeds to break dormancy or stimulate germination are quite variable and include stratification (*1, 9*), after-ripening followed by stratification (*1, 9, 10*), water-soaking (*9*), treatment with a variety of growth regulatory chemicals (*2, 4, 5, 8*), embryo dissection (*4*), steam-heating (*7*), and others. Unless one pays close attention to species, such recommendations can be quite confusing.

Seeds of green ash (*F. pennsylvanica* Marsh.) have been reported to have an embryo dormancy that is commonly broken by a combination of warm and cold stratification (*1*) or by cold stratification alone (*1*). Applications of growth regulators have been used (*3*), but information on methods to stimulate germination for this species is not extensive. In an effort to find a suitable method to germinate a high percentage of green ash seeds and to produce seedlings of uniform size (short germination period) for use in other studies, I tested a variety of methods previously used for various ash species.

Methods

Green ash seeds were collected in southeastern Michigan in October 1978. Damaged or unusually small seeds were discarded, and remaining seeds were air-dried for at least a week. Dried seeds were stored in plastic bags at 4° C. In May 1979, all seeds were surface sterilized in 1-percent sodium hypochlorite, rinsed, then randomly divided into lots with 200 seeds per lot. Single lots were subjected to the treatments described in table 1.

Table 1.—*Pregermination treatments of green ash seeds using 200 seeds per treatment*

Treatment designation	Treatment description
A.	Seeds placed in plastic bags and stratified for 88 days at 4° C as described by Bonner (*1*). Pericarps left intact after stratification.
B.	Same as in "A," above, but pericarps removed after stratification and before sowing.
C.	Same as in "B," above, but endosperm removed from around radicle tips of embryo (a 22-gauge hypodermic needle used).
D.	Same as in "B," above, but embryos were completely dissected from endosperm.
E.	Unatratified seeds were soaked in aerated, demineralized water for 20 days. The water was changed daily for 3 days, then changed at 5-day intervals. Pericarps and endosperm were left intact after soaking.
F.	Same as in "E," above, except pericarps removed and endosperm removed from around radicle tips of embryos.
G.	Seeds not stratified or soaked. Pericarps were left intact when seeds sown.
H.	Same as in "G," above, but pericarps were removed when seeds sown.

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After the treatments, intact seeds, depericarped seeds, seeds with partially exposed embryos, or naked embryos were sown in plastic trays (23 by 30 by 6 cm) lined with paper toweling saturated with demineralized water. Trays were covered with clear plastic film and left on a laboratory bench at ambient room temperatures. Trays were examined weekly for 3 weeks. Seeds were considered to have germinated when radicles protruded through seed coats (treatments A, B, E, G, H) or when exposed radicles responded geotropically and had measurably elongated (treatments C, D, F). Data were analyzed with a one-way analysis of variance by using the MIDAS statistical package available through the University of Michigan's NITS Computer system (*3*).

Results and Discussion

The number of seedlings obtained by using only stratification (treatment A) was quite small and germination rate was rather slow, but germination was markedly improved by depericarping and partial and complete embryo dissection (fig. 1). Water-soaking of seeds (treatment E) improved germination percentage when compared to unstratified and unsoaked seeds (treatment G), but germination rates were still quite slow (fig. 1). Partial embryo dissection after soaking significantly ($p \leq 0.05$) stimulated germination percentage when compared to germination after soaking only (fig. 1).

The pericarp appears to inhibit germination of stratified seed, but its removal had no effect on nonstratified seed. Depericarping is a relatively simple procedure for seeds stratified in plastic bags, and it also serves as a final screening step for eliminating damaged or unfilled seeds in situations where high germination percentages are desired. Villiers and Wareing (*10*) found no metabolic inhibitory chemicals in pericarps of European ash, but stated that pericarps were impermeable to oxygen diffusion.

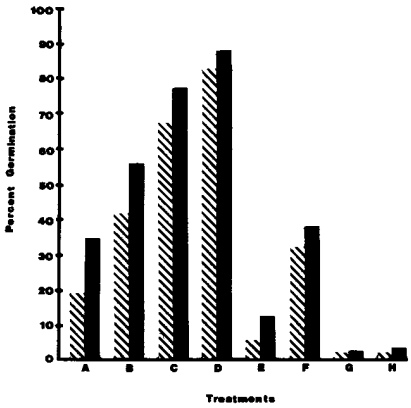

Figure 1.—*Response of green ash seeds to germination pretreatments. See table 1 for treatment descriptions. Cross-hatched bars represent data 2 weeks after sowing. Solid bars represent data 3 weeks after sowing.*

Partial dissection of embryos from stratified green ash seeds stimulated germination percentages significantly ($p \leq 0.05$) more than did pericarp removal, but this is considerably more time consuming. Endosperm removal from embryonic radicle tips probably eliminates some growth inhibitory substances (*8*) and may increase embryonic water absorption. Increased embryonic hydration may sufficiently dilute growth inhibitors in the embryo to permit embryo growth to begin (*10*).

Partial dissection of embryos led to subsequent problems with seedling growth. When germinated seeds were sown in soil-filled flats after this study and when hypocotyls carried cotyledons above the soil surface (epigeous germination), cotyledons were frequently trapped within the dried, adhering seed coat. In many cases, cotyledons could not expand and subsequent seedling growth was inhibited.

Best germination percentages and rates were obtained when embryos were completely dissected from stratified seeds. Gendel and other (4) found this technique worked well with white ash, and they concluded that embryo dissection was a valuable tool for circumventing problems with dormant seeds and for improving the yield of seedlings. Although this tech-

nique does markedly improve germination, it frequently results in subsequent seedling growth problems. Marshall and Kozlowski (*6*) grew seedlings from naked and partially dissected green ash embryos and found that, while endosperm was not essential for germination, early seedling growth was markedly stimulated when cotyledons were in contact with endosperm at least 4 days after the onset of germination. Ten-day-old seedlings grown from naked embryos were considerably shorter and had smaller dry weights than seedlings that were grown with their cotyledons in contact with endosperm for more than 4 days (*6*).

Water-soaking of green ash seeds followed by partial endosperm removal (treatment F) may be a suitable alternative to long stratification when green ash seedlings are needed in a hurry. Seedlings produced by this technique may have problems shedding seed coats from cotyledons as previously, described but adhering seed coats can be removed manually.

Conclusions

Several techniques can be used to improve germination rates and percentages of green ash seeds, but all have some drawbacks in that they are marginally effective, time consuming, or lead to subsequent seedling growth problems. If seedlings are needed rapidly, water-soaking followed by depericarping and partial endosperm removal may suffice. If time permits stratification, seed germination can be quite easily stimulated by depericarping stratified seeds before sowing.

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