# Vacuum Infiltration of Gibberellic Acid Stimulates Germination of Dormant Black Walnut Seeds[1]

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## Abstract

Black walnut (*Juglans nigra* L.) seed normally requires cold stratification to overcome dormancy. In 2 experiments, dormant, unstratified seeds were treated with gibberellic acid ($GA_3$) in attempts to overcome dormancy. To facilitate uptake of treatment solutions, seeds were scarified by making 2 small notches through the shell with a grinding wheel. Treatment solutions of 0, 50, 150, or 250 mg/l (ppm) $GA_3$ were applied either by soaking seeds for 24 hours or by vacuum infiltration (VI) for 30 minutes. VI of $GA_3$ stimulated germination in both experiments to a level equal to or greater than germination of seeds receiving cold-moist stratification for 45 days. A brief 15 day stratification period followed by $GA_3$ was more effective than VI alone. Soaking in $GA_3$ was effective in the first experiment but not the second. Germination rate and seedling height was increased by $GA_3$.

**Index words:** *Juglans nigra,* seed scarification, seed stratification

## Introduction

Seeds from temperate zone nut trees have a requirement for cold-moist stratification to overcome internal dormancy (6). The stratification period commonly recommended for black walnut is 60-120 days (14) at 1-5 °C (34-41 °F) which typically results in 50% to 60% germination (2,13). Frequently with this species, direct field planting in the fall or spring is practiced (2). With the former, stratification occurs naturally during the winter period while in the latter, refrigerated stratification of seed in a moist medium is necessary. However, field-planted seeds are subject to rodent and bird predation, decay, and, when fall-planted, they are susceptible to low temperature injury as well (5,11). Delayed germination in the spring is cited as another factor which limits seedling production of black walnut (2).

Gibberellins have been used to stimulate the germination of seeds of several species of temperate zone nut species including Chinese chestnut (*Castanea mollissima* Bl.) (8), Persian walnut (*Juglans regia* L.) (8), pecan (*Carya illinoensis* (Wang.) K. Koch) (8), filbert (*Corylus avellana* L.) (8), European hazel (*C. avellana* L.) (3,4) and beech (*Fagus sylvatica* L.) (4). Gibberellins have been shown to increase in parallel with the loss of dormancy in European hazel (1), *Fraxinus excelsior* L. (European ash) (7), *Prunus domestica* (plum) (9), and *Acer tartaricum* L. L. (Tartarian maple) (12).

Thus it seems likely that treatment of dormant black walnut seeds with gibberellic acid ($GA_3$) might overcome dormancy in lieu of cold-moist stratification. Frankland and Waring (4), however, found that $GA_3$ stimulated germination of European hazel seeds only if the pericarp (shell) was removed to facilitate uptake of the treatment solution. Similarly, for four other nut species for which this technique has been successful (filbert, Chinese chestnut, Persian walnut and pecan) it was necessary to crack the shell and extract the intact embryo (kernel) which was then soaked for 6 to 12 hours in 25-100 mg/l (ppm) (.001-.004 oz/qt) $GA_3$ (8). Embryo extraction was necessary since very little uptake of $GA_3$ solution would occur through the relatively impermeable pericarp of an intact nut. In the case of black walnut, the pericarp is extremely hard to crack and intermingles with and partially separates the cotyledons, making embryo extraction impossible without damage to the embryo. To overcome this problem, a technique was developed for vacuum infiltrating solutions of $GA_3$ into scarified, intact seeds of this species.

## Materials and Methods

The walnut seed germination experiments described below were conducted on 2 separate years using a different seed source each year. For the first experiment, seeds were obtained from Herbst Bros. Seedsmen, Inc. (Brewster, NY) on November 28, 1982, and stored dry in fishnet bags at 18.3 °C (65 °F). On January 6, 25 of these seeds were placed in a loosely closed plastic bag containing moist peat moss and stored at 4 °C (39.2 °F) for a 45 day cold-moist stratification. On February 20, the remaining unstratified seed were scarified, treated with $GA_3$ as described below, and sown along with the stratified seed. For the second experiment, seeds were collected from an open pollinated tree in the vicinity of Ithaca, NY on September 24, 1983, dehusked and stored dry as described above. Three days later, 25 of these seeds were placed in cold-moist stratification for 45 days prior to sowing, and on October 27, an additional 25 seeds were placed in cold-moist stratification for 15 days. On November 11, the remaining unstratified seeds as well as those which had been stratified for 15 days were scarified and treated with $GA_3$ and were sown along with the 45 day stratified seed.

Mechanical scarification rather than embryo extraction was used to facilitate uptake of treatment solutions. Scarification involved making 2 notches in the shell with

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an electric grinder. The notches were approximately 6 mm (0.25 in) up from the styler (root) end of the seed, on opposite sides from each other and just deep enough to penetrate the shell without damaging the underlying embryo. Mechanical scarification could be performed rapidly (approx. 185 seeds in 25 min).

$GA_3$ (Sigma Chemical Co., St. Louis, MO) was dissolved in 5 ml of 95% ethanol and then diluted to one liter with water to prepare treatment solution of 50, 150 and 250 mg/l (.002, .006, .01 oz/qt). The control solution (0 mg/l $GA_3$) consisted of 5 ml of ethanol per liter of water. Treatment solutions were applied either by soaking scarified seeds for 24 hours or by vacuum infiltration (VI) for 30 min. In either case, for each treatment, 25 scarified seeds were placed in a 1 liter (1.06 qt) glass beaker, weighted down with a steel ring, and sufficient treatment solution was added to cover the seeds (approx. 500 ml).

The apparatus used for VI is illustrated in Fig. 1. VI involed placing a beaker containing seeds and treatment solution into a 25 cm (10 in) i.d. glass desiccator jar covered with a lid having a hose connection sleeve. This, in turn, was connected in series via rubber vacuum tubing, to a vacuum gauge, a threaded glass needle valve (VWR Scientific, Rochester, NY), a backflow trap (1 liter filter flask, 6 mm (0.25 in) o.d. glass tubing and rubber stopper), and a faucet-type filter vacuum pump (Markson Scientific). Once the faucet was turned on, approximately 2 min were required to establish a vacuum of 55.9 mm (22 in) Hg. The needle valve was adjusted manually to maintain it at this level for an additional 15 min. During this period, internal gasses bubbled out of the seed through the notches in the pericarp. Once the vacuum was released by opening the needle valve, the seeds absorbed a quantity of treatment solution to replace the amount of gas initially withdrawn. The uptake period took an additional 15 minutes after vacuum release.

Immediately after treatment, seeds were sown in 35x50x10 cm deep (13.8x19.7x4 in) wooden flats containing 1:1:1 (v/v) mixture of peat:perlite:soil. Flats were placed in a greenhouse maintained at approx. 21 °C (70 °F) under intermittent mist set to come on for 5 seconds every 24 minutes during daylight hours. Counts of emerged seedlings were made approximately every other day throughout the 45 day germination period.

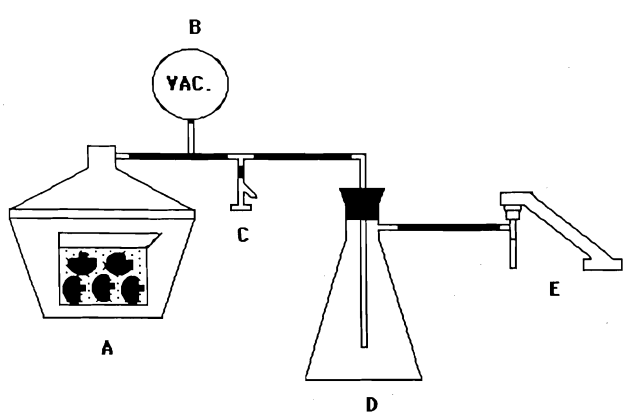


**Fig. 1. Apparatus used for vacuum infiltration (VI) of $GA_3$ solutions into scarified black walnut seeds. Scarified seeds in a beaker were covered with treatment solution and placed inside a glass desiccator (A) which was connected via rubber vacuum tubing to a vacuum gauge (B), needle valve (C), backflow trap (D), and a vacuum filter pump and water faucet (E). A vacuum was applied for 15 min. at 22 in. Hg, followed by a 15 min. uptake period after release of the vacuum.**

For statistical analysis, a Chi square test was performed on the germination data for the first experiment. Since the test involved a single degree of freedom, Yeats correction was used (10). The expected values used to calculate $X^2$ were the average of the number of seeds germinated or not at the zero (control) levels of $GA_3$ (VI and soak), i.e., the analysis tested the hypothesis that germination of $GA_3$ treated seeds differed from that of water controls. A Chi square test was not performed on data for the second experiment because the test is considered inappropriate when the smallest expected value is less than 5, as it was in this case.

## Results and Discussion

Overall, treatment of unstratified black walnut seeds with $GA_3$ stimulated germination when compared to either stratified or unstratified seeds which did not receive $GA_3$ (Fig. 2). In the first experiment, 45 days of cold stratification resulted in 48% germination, which approaches the 50 to 60% considered typical and acceptable for this species (2,3). Vacuum infiltration of

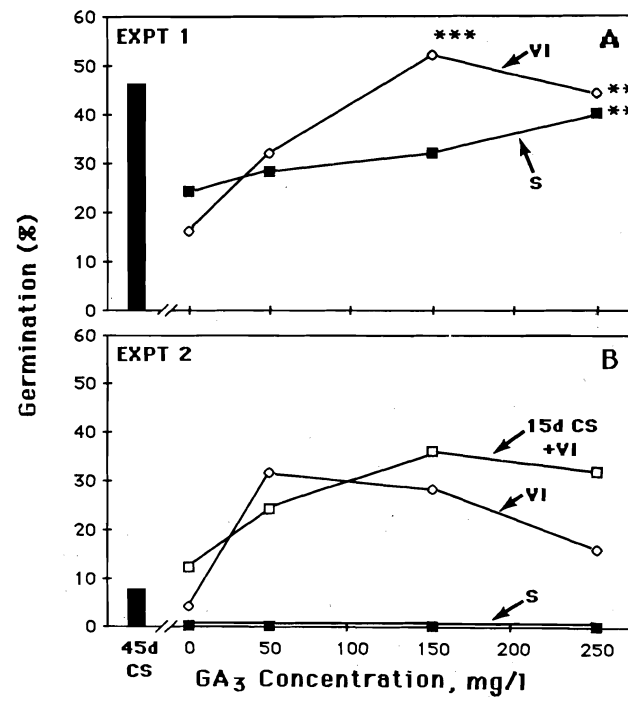


**Fig. 2. Effect of $GA_3$ concentration and/or cold stratification on the percentage of black walnut seed germinated after 45 days. In the first (A) and second (B) experiments one treatment consisted of seeds which were cold stratified (CS) for 45 days and, in the second experiment only (B) another treatment consisted of stratification for 15 days followed by scarification and vacuum infiltration (VI) of $GA_3$ solutions. Non-stratified seeds in both trials were scarified and treated with $GA_3$ solutions by soaking (S) or by VI. Asterisks indicate statistical significance at the .01 level (**) or .001 level (***) based on Chi square analysis of seed number data (first experiment only).**

Table 1. Effect of $GA_3$ concentration, method of application and/or cold stratification on black walnut seed germination rate[z] and seedling height (Experiment 1).

| $GA_3$ concentration, (mg/l) | Days to 50% of maximum germination | | Seedling height 45 days after sowing[y], (cm) | |
|---|---|---|---|---|
| | VI[x] | Soak | VI | Soak |
| 0 | 30 | 32 | 8.25 ± 1.03 | 6.0 ± 0.55 |
| 50 | 28 | 28 | 9.42 ± 1.17 | 9.29 ± 1.27 |
| 150 | 18 | 25 | 14.17 ± 0.63 | 10.6 ± 1.02 |
| 250 | 23 | 23 | 12.25 ± 1.10 | 15.4 ± 1.13 |
| 45 day stratification (no $GA_3$) | 18 | | nd[w] | |

[z]Expressed as the time required to achieve one-half maximum germination.

[y]Mean ± standard error.

[x]VI = vacuum infiltration.

[w]nd = no data.

150 mg/l (.006 oz/qt) $GA_3$ was more effective than cold-moist stratification, resulting in 52% germination. Either VI or soaking in 250 mg/l (.01 oz/qt) $GA_3$ were nearly as effective (44 and 40%, respectively). All three of these $GA_3$ treatments were significantly more effective than either VI or soaking in plain water which resulted in only 24 and 16% germination, respectively.

In the second experiment, germination of the 45-day cold stratified seed was considerably less (8%) than in the first experiment (48%). This experiment also differed from the first in the response of seeds to soaking as a means of applying $GA_3$. While in the first experiment soaking in 250 mg/l (.01 oz/qt) $GA_3$ significantly stimulated germination, in the second trial soaked seeds completely failed to germinate regardless of $GA_3$ concentration. In the second experiment, as in the first, VI of $GA_3$ stimulated germination; the most effective concentration being 50 mg/l (.002 oz/qt), which resulted in 32% germination. The combination of a brief (15 day) cold stratification followed by VI of 150 mg/l (.006 oz/qt) $GA_3$ resulted in 36% germination. This was superior to the maximum response to VI without prior stratification. Overall, the results of the 2 experiments were similar in that $GA_3$ stimulated germination of black walnut seeds, and VI was more effective than soaking. In both cases the most effective $GA_3$ treatment was more effective than 45 days of cold-moist stratification. Differences between the 2 experiments, including the germination of the 45 day stratified seeds, the effectiveness of soaking in $GA_3$, and the optimal $GA_3$ concentration for VI may have been related to differences in the date of seed harvest, duration of storage, or the date of initiation of the treatments. For example, if the seeds used in the first experiment were collected later in the fall than those used in the second experiment (their actual collection date is not known to us), they may have received more natural chilling, resulting in partial afterripening, and a possible change in their responsiveness to $GA_3$. Geographic or genetic differences in the seed lots may also have contributed to the observed differences in response to treatments. Despite the differences, however, the results of both trials confirm the original hypothesis that $GA_3$ might stimulate germination of nonstratified walnut seed. Based on the results of these experiments, the treatment which is likely to be most effective is a 15 day cold-moist stratification followed by scarification and VI with 150 mg/l (.006 oz/qt) $GA_3$.

The time to one-half maximum germination ans seedling height 45 days after sowing was determined for the first trial (Table 1). It is apparent that regardless of method of applications, seeds treated with the highest 2 concentrations of $GA_3$ resulted in taller seedlings than those treated with 0 mg/liter $GA_3$. This difference in height after 45 days apparently was due, at least in part, to the effect of $GA_3$ treatment on the rate of germination, i.e., seeds treated with 150 or 250 mg/liter $GA_3$ tended to germinate sooner than those treated with 0 or 50 mg/liter, as indicated by the time required for 50% maximum germination and thus had more time to elongate during the 45-day period. Also, $GA_3$-treated seedlings appeared to have longer internodes but this response was not quantified.

Use of a grinding wheel for scarification followed by VI constitutes a novel method by which $GA_3$ can be effectively applied to black walnut seed which would otherwise be damaged in the process of embryo extraction. An electric grinder, which allows rapid scarification, is available in most nurseries and the equipment necessary for VI of $GA_3$ solutions is relatively inexpensive and easily assembled and operated. The use of a faucet operated filter vacuum pump is much cheaper and requires far less maintenance than an electric vacuum pump. This report adds black walnut to the list of woody plant seed for which germination is stimulated by $GA_3$.

## Significance to the Nursery Industry

These results demonstrate that gibberellic acid ($GA_3$) treatment is an alternative to long-term stratification as a means of overcoming black walnut seed dormancy. Because the amount of time after harvest required to germinate seeds is substantially reduced by this method, several advantages may accrue. Propagation can be started in the winter (in a greenhouse) in order to allow a longer growth period during the first year. This benefit

could be particularly significant since delayed germination of field sown seeds can be a problem with this species (2). Greenhouse propagation would also eliminate loss of seeds due to freezing injury and rodent predation. Furthermore, once outplanted, $GA_3$-accelerated seedlings could put on more growth early in the season before environmental conditions became unfavorable for growth due to drought and high temperatures.

It should be pointed out that it is in relatively small-scale nursery operations that the somewhat labor-intensive techniques described here are most likely to be practical alternatives to conventional propagation methods. Application to larger operations will depend upon the development of a more rapid method of scarification, and a larger apparatus for vacuum infiltration of $GA_3$.

## Literature Cited


1. Bradbeer, J.W. and N.J. Pinfield. 1967. Studies in seed dormancy. III. The effects of gibberellin on dormant seeds of *Corylus avellana* L. New Phytol. 66:515-23.

2. DeHayes, D.H. and C.E. Waite. 1982. The influence of spring sowing on black walnut germination in northern Vermont. Tree Planters' Notes 33(4):16-18.

3. Frankland, B. and P.F. Waring. 1962. Changes in endogenous gibberellins in relation to chilling of dormant seed. Nature (London). 194:313.

4. Frankland, B. and P.F. Waring, 1966. Hormonal regulation of seed dormancy in Hazel (*Corylus avellana* L.) and beech (*Fagus sylvatica* L.). J. Exp. Bot. 17:596-611.

5. Funk, D.T. 1979. Black walnuts for nuts and timber, p. 51-74. *In:* R.A. Jaynes (ed.). Nut Tree Culture in North America. Northern Nut Growers Assn., Inc., Hamden, CT.

6. Hartman, H.T. and D.E. Kester. 1983. Plant Propagation, Principles and Practices. 4th ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.

7. Kentzer, T. 1966. Gibberellin-like substances and growth inhibitors in relation to the dormancy and afterripening of ash seed (*Fraxinus excelsior* L.). Acta Soc. Bot. Pol. 35:575.

8. Lagerstedt, H.B. 1979. Propagation—seed, grafting, budding, p. 240-271. *In:* R.A. Jaynes (ed.). Nut Tree Culture in North America. Northern Nut Growers Assn., Inc., Hamden, CT.

9. Lin, C.F. and A.A. Boe. 1972. Effects of some endogenous and exogenous growth regulators on plum seed dormancy. J. Amer. Soc. Hort. Sci. 97:41-44.

10. Little, T.M. and F.J. Hills. 1972. Statistical Methods in Agricultural Research, UCD Book Store, Davis, CA.

11. McMillan Browse P.D.A. 1979. Hardy woody plants from seed. Grower Books, London.

12. Nikolaeva, M.G. 1977. Factors controlling the seed dormancy pattern, p. 51-76. *In:* A.A. Kahn (ed.). The physiology and biochemistry of seed germination. North Holland Publ. Co., NY.

13. Schopmeyer, C.S., (ed.). 1974. Seeds of Woody Plants in the United States. USDA Handbook No. 450. U.S. Govt. Printing Office, Washington, DC.

14. Stefferud, A., (ed.). 1961. Seeds, The Yearbook of Agriculture. p. 550-551. U.S. Department of Agriculture. U.S. Gov't. Printing office, Washington, DC.