HortScience 39(7):1695–1699. 2004.

Germination of *Leitneria floridana* Seeds from Disjunct Populations

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Additional index words. florida corkwood, gibberellic acid, leaching, scarification, viability, drupe phenology, seed dormancy, seed germination

***Abstract*. Attributes of *Leitneria floridana* Chapman have been recognized, but this North American shrub remains rare in commerce, and little information on propagation is available. We studied germination of seeds collected from several disjunct populations of *L. floridana* in 2002 and 2003. In 2002, ≤5% germination occurred when ripe drupes from Missouri and Florida were sown soon after collection. Effects of GA_3 (750 mg·L^{-1} for 24 hours) were assessed on stored drupes leached with water and on seeds excised from stored drupes. Germination percentages were 21 and 32 for leached drupes and excised seeds from Florida, respectively, but ≤5% germination occurred among germplasm from Missouri and among untreated drupes from both provenances. Viability of ungerminated seeds among treatments ranged from 0% to 7%. In 2003, fleshy, apparently unripe drupes from Texas, which were scarified with H_2SO_4 and then treated with 1000 mg·L^{-1} GA_3 showed 48% germination (germination value = 3.9). Up to 29% germination (germination value = 2.7) occurred when seeds were excised from unripe drupes from Arkansas and Missouri and then were treated for 24 hours with 750 or 1000 mg·L^{-1} GA_3. We conclude that provenance, developmental stage of drupes when collected, storage, and pregermination treatments influence viability and germination of seeds of *L. floridana*. Barriers to germination may be avoided by collecting drupes when they are green and fleshy.**

Native plants are in great demand in the United States, but, unfortunately, many species with horticultural merit have become rare in the wild, in part due to destruction of their natural habitat for anthropogenic use. Such losses prevent horticultural assessment of native plants and increase our reliance on nonnative species that do not reflect a region's natural heritage, may become invasive, or may introduce pests and pathogens. Use of native plants in natural and managed landscapes can help contribute to preserving the overall biodiversity and within-species genetic diversity of indigenous taxa.

Leitneria floridana is a rare shrub. The species has been classified as the sole member of the family Leitneriaceae but also has been placed in the Simaroubaceae. Plants of *L. floridana* occur in standing water, nontidal or brackish marshes and swamps, wet prairies, and swamp woodlands in disjunct populations in Florida, Georgia, Texas, Arkansas, and Missouri. Plants have attractive bright green foliage and brown, slender stems that remain unbranched up to 1 to 2 m. Maturing to about 5 m in its native habitat, the species tends to form thickets by producing suckers. *Leitneria floridana* has potential in horticultural commerce because of its affinity for poorly drained soils (Koller, 1997). Other attributes such as ecotypic variability in growth habit and tolerance of cold and drought could also be useful for selecting forms suited for managed landscapes. Reliable methods to germinate seeds will allow horticulturists to select individuals with desirable traits from diverse seedling populations. Dirr and Heuser (1987) indicated that "respectable germination" could be achieved after 90 d of cold stratification. However, to our knowledge, germination of *L. floridana* has not been quantified, nor have protocols been described for harvesting and handling the drupes and seeds of this species.

Many biochemical, physical, and morphological obstacles to germination are present in seeds of temperate origin (Baskin and Baskin, 1998). About two-thirds of North American tree species exhibit some form of seed dormancy (Schopmeyer, 1974), and considering that the natural distribution of *L. floridana* extends to 36°33'40" N latitude in southeastern Missouri (USDA hardiness zone 6b), it is likely that seeds of *L. floridana* possess one or more dormancy mechanisms. Most seeds lose water as they mature, and endogenous dormancy mechanisms often develop during this time (Baskin and Baskin, 1998; Hartmann et al., 2002; Jensen and Eriksen, 2001; Raven et al., 1999). Consequently, the developmental stage of seeds at the time of collection can affect germination. Harvesting seeds from immature fruits may result in comparatively rapid and complete germination (Bradbeer, 1988; Hartmann et al., 2002; Schopmeyer, 1974). Seemingly ripe drupes of *L. floridana*, which are up to 2.5 cm long and up to 1 cm wide, appear dry and brown upon natural abscission from the maternal parent in late June and July. If they do not germinate that same season, the seeds in Missouri are exposed during winter to average minima of –5 to 3 °C. Therefore, *L. floridana* may have developed physiological dormancy that is overcome by exposure to low temperatures. Additionally, the stony tissues surrounding the seeds of *L. floridana* may ensure exogenous dormancy by creating a physical barrier to germination; these tissues might also contain chemical inhibitors.

The objective of our research was to identify protocols for promoting germination of *L. floridana*. A preliminary trial was conducted in 2002 with untreated, whole drupes sown within a week after they were collected from native habitats in Florida and Missouri. We considered these drupes ripe because of their brownish-black exocarps. Although few seeds germinated (≤5%), those that did developed into healthy seedlings at frequencies as high as 87% depending on maternal parent. Based on these findings, we designed several experi-

Fig. 1. Map of the eastern United States showing the provenances where fruit of *Leitneria floridana* were collected to evaluate germination potential of seeds. Brown to black drupes were collected in 2002 from natural populations in southeastern Missouri on 28 June and along the northern Gulf Coast of Florida on 29 June. In 2003, green, fleshy drupes were collected near the Gulf Coast of Texas on 24 May, in central Arkansas on 1 July, and in southeastern Missouri on 3 July.

Received for publication 13 Jan. 2004. Accepted for publication 3 Mar. 2004. Journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, and supported by the Hatch Act and State of Iowa funds. We thank Jeff DiMaggio (Florida Department of Environmental Protection), Thomas Adam (U.S. Fish and Wildlife Service), Theo Witsell (Arkansas Natural Heritage Commission), and Mark Pelton (Missouri Department of Conservation) for their assistance in obtaining seeds of *Leitneria floridana* from Florida, Texas, Arkansas, and Missouri, respectively.
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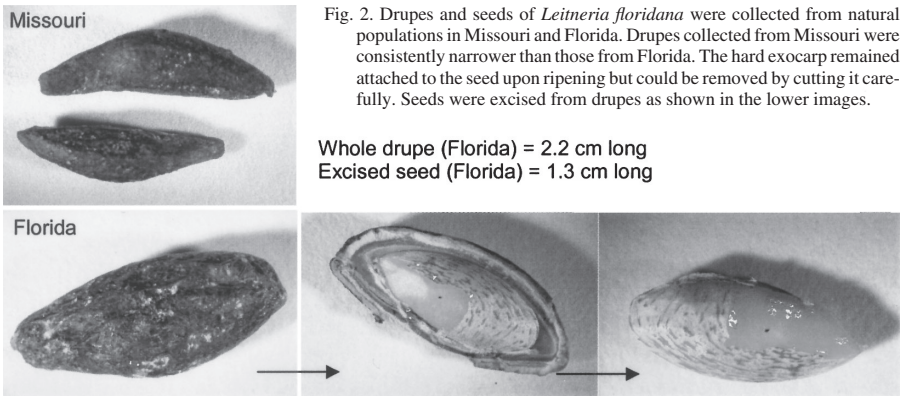

Fig. 2. Drupes and seeds of *Leitneria floridana* were collected from natural populations in Missouri and Florida. Drupes collected from Missouri were consistently narrower than those from Florida. The hard exocarp remained attached to the seed upon ripening but could be removed by cutting it carefully. Seeds were excised from drupes as shown in the lower images.

ments with both freshly harvested green, fleshy drupes and ripe, stored drupes to assess effects on germination of exocarp removal, stratification, leaching, gibberellic acid (GA_3), sulfuric acid (H_2SO_4), and sowing depth. Viability of ungerminated seeds also was determined, and development of young seedlings was compared to test for potential effects of pregermination treatments.

Materials and Methods

Germplasm collection. Ripe, abscising drupes were collected in 2002 when the outer tissues appeared dry, brownish-black, and wrinkled. Drupes were harvested on 28 June from plants in the Corkwood Natural Area in Butler County, Mo. (Fig. 1). Additional drupes were collected in Florida on 29 June from plants in two populations, one within the St. Marks National Wildlife Refuge in Wakulla County, the other within the Waccasassa Bay State Preserve in Levy County (Fig. 1). The Missouri and Florida populations represented the northern and southern extremes of the natural distribution of the species. Drupes were stored in paper bags at 25 °C for 1 week, and then they were held at 5 °C until they were treated in October. In 2003 we harvested unripe drupes that were green and fleshy from plants within San Bernard Wildlife Refuge in Brazoria County, Texas, Bayou Meto Wildlife Management Area in Arkansas County, Ark., and Corkwood Natural Area in Butler County, Mo. on 24 May, 1 July, and 3 July, respectively (Fig. 1). Drupes from Texas were stored in paper bags until 4 June at 23 °C. Germplasm from Arkansas and Missouri was held in paper bags at 23 °C until 15 July.

Moisture content. In both years, moisture content of excised seeds was determined (International Seed Testing Association, 1999). Two sets of 10 excised seeds were selected randomly from each provenance and were air-dried for 48 h on a laboratory bench. Seeds were placed in predried aluminium trays and weighed before we held them in an oven at 103 °C for 17 h. Upon removal from the oven, seeds and trays were cooled briefly on desiccant before dry weight was obtained. The difference between fresh and dry weights was expressed as a percentage of fresh weight.

Experiment 1: Ripe drupes from Florida and Missouri in 2002. Drupes from the two locations in Florida were combined. A factorial arrangement of two provenances (Florida and Missouri), three pregermination treatments, and two methods of sowing was established in a randomized complete block experimental design. The three pregermination treatments were 1) 100 drupes from each provenance were leached for 72 h under warm (35 to 40 °C), running tap water and subsequently were submersed for 24 h in 750 mg·L^{-1} GA_3; 2) surrounding tissues were removed (Fig. 2) from 100 seeds from each provenance, and the excised seeds were placed for 24 h in the GA_3 solution; and 3) 100 drupes from each provenance were left untreated. Each group of 100 drupes or excised seeds then was sown in a 2:1 (by volume) mixture of LC-1 medium (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta., Canada) and silica sand (Unimin Corp., Le Sueur, Minn.) as follows: 1) 50 excised seeds or drupes were placed on the surface and pressed lightly to ensure contact with the medium, and 2) 50 excised seeds or drupes were placed 2 cm deep and were covered with the medium. Each of the five blocks contained 10 seeds per treatment combination. Each drupe or seed was sown in an individual cell (length = 6.7 cm, width = 5.9 cm, height = 5.9 cm) of #3601 Com-Packs liners (T.O. Plastics Inc., Clearwater, Minn.). The temperature in the greenhouse averaged 25 °C. A 16-h photoperiod was maintained by providing supplemental irradiance with 400-W high-pressure sodium lamps. Developing seedlings received an average of 218 μmol·s^{-1}·m^{-2} photosynthetically active radiation during the illuminated period. The germination medium was kept moist with tap water throughout the experiment. Because seeds of *L. floridana* exhibit epigeous germination, a seed was considered to have germinated when the hypocotyl hook appeared (Hartmann et al., 2002). The number of germinants was counted 5 d after sowing and every 4 d thereafter until the test was concluded at day 33. When the first true leaves had expanded, the seedlings were transplanted singly into 15-cm-diameter standard pots (Kord Products, Brampton, Ont., Canada) in the same medium used for germination and were grown under the same environmental conditions. Plants were irrigated with tap water to container capacity and were fertilized once every 2 weeks with 4.6 mM N applied via 16.5N–2.2P–13.5K (3:1 mix of 15–5–15 Cal-Mag and 21–5–20 All Purpose, Miracle-Gro Excel, The Scotts Co., Marysville, Ohio). Percentage of surviving plants, height of the plants, number of leaves, length and width of the largest leaf, and diameter of stem at the root collar were determined 105 d after the germination experiment was begun.

Ungerminated seeds and drupes were cold-treated at 5 °C for 30 d and then held in the greenhouse at 25 °C for an additional 45 d. We then assessed germination and the viability of seeds within the ungerminated whole drupes. Excised seeds decomposed in the medium and could not be recovered for the viability test. Seeds were extracted from whole drupes by carefully cutting the surrounding tissues and then were submersed in deionized water for up to 5 h to soften the thin, papery coat. Decoated seeds were subjected to a 2, 3, 5-triphenyltetrazolium (TTC) staining test by submersing them in a solution of 2 mg·L^{-1} TTC in 0.05 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4) and incubating for up to 12 h in the dark at 23 °C.

Experiment 2: Ripe drupes from Missouri in 2002. Six pretreatments were applied to whole drupes collected from Missouri: 1) submersion for 20 min in 96% H_2SO_4 followed by a 6-week stratification at 5 °C; 2) leaching for 72 h under warm (35 to 40 °C), running tap water followed by a 6-week stratification at 5 °C; 3) submersion for 24 h in 1000 mg·L^{-1} GA_3 followed by a 6-week stratification at 5 °C; 4) submersion for 20 min in 96% H_2SO_4, submersion for 24 h in 1000 mg·L^{-1} GA_3, and subsequent six-week stratification at 5 °C; 5) leaching for 72 h under warm (35 to 40 °C), running tap water, submersion for 24 h in 1000 mg·L^{-1} GA_3, and subsequent 6-week stratification at 5 °C; and 6) untreated whole drupes. Each treatment was applied to 100 whole drupes. Containers, cultural, and environmental conditions were as described for Expt. 1, and germination and viability were assessed similarly.

Experiment 3: Green, fleshy drupes from Texas in 2003. After collection, drupes were held at 23 °C. Twenty-five drupes each were randomly assigned to four treatments on 4 June: 1) drupes were left intact; 2) seeds were excised from the drupes by removing the exocarp; 3) seeds were excised from drupes and then were submersed for 24 h in 1000 mg·L^{-1} GA_3; and 4) whole drupes were submersed for 25 min in 96% H_2SO_4 and subsequently treated for 24 h with 1000 mg·L^{-1} GA_3. The number of germinants was counted every 7 d after sowing until the test was concluded at day 28. The environment in the greenhouse and the cultural practices were similar to those of the previous experiments.

Experiment 4: Green, fleshy drupes from Arkansas and Missouri in 2003. Seeds were excised from drupes, and a factorial arrangement of two provenances (Arkansas and Missouri) and three GA_3 concentrations (24-h incubation in 0, 750, and 1000 mg·L^{-1}) was used in a completely randomized design. One-hundred seeds were assigned randomly to each treatment combination. Cultural practices were similar to those of other experiments, and germination was recorded similarly.

Statistical analyses. Analysis of variance (ANOVA) models were tested using the GLM procedure in SAS/STAT (Version 8.02, SAS

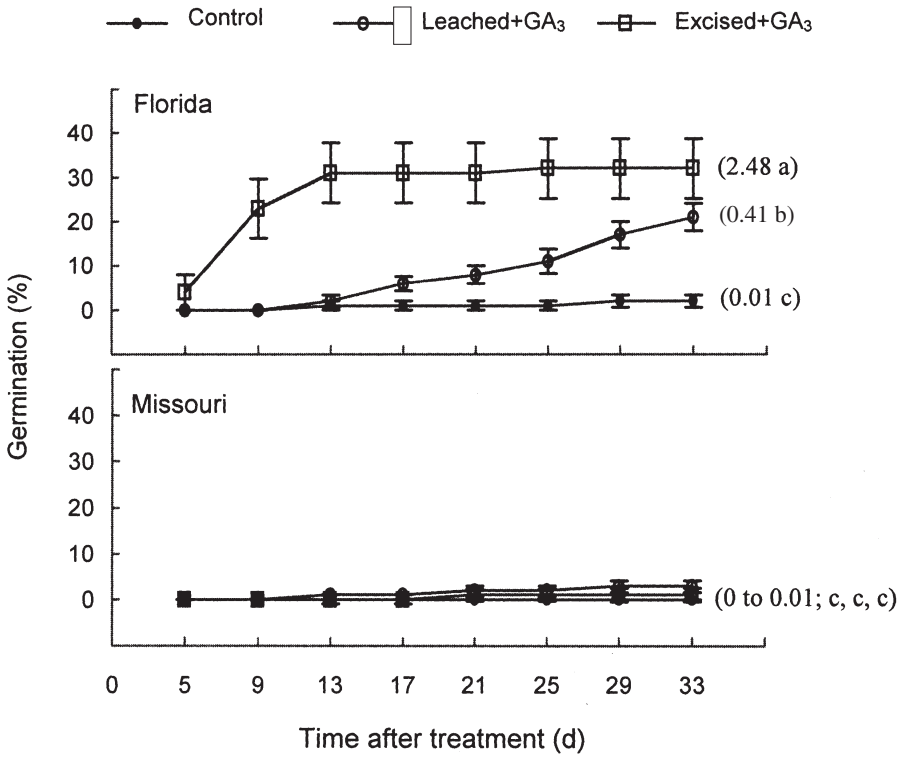

Fig. 3. Drupes of *Leitneria floridana* collected from Florida and Missouri were subjected to a 33-d germination test (Expt. 1) after treatment of whole drupes or seeds. Pregermination treatments included 1) untreated whole drupes, 2) leaching of whole drupes for 72 h under warm, running tap water and subsequent soaking for 24 h in 750 mg·L^{-1} gibberellic acid (GA_3), and 3) removal of fruit covering to excise the seed and subsequent soaking of seeds for 24 h in 750 mg·L^{-1} GA_3. Vertical bars about the data symbols represent ± the standard error of the mean germination percentages. Germination value, a composite measure of the speed and completeness, was determined, and values are presented in parentheses. Germination values followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

Table 1. Physical condition and viability of seeds of *Leitneria floridana* were assessed after they were treated in five ways following collection in late June 2002. The five treatments were 1) stored for 120 d at 5 °C; 2) stored for 120 d at 5 °C; placed for 45 d in germination medium at 25 °C; stratified for 30 d at 5 °C while in germination medium; then kept for 45 d at 25 °C (ungerminated seeds from Expt. 1); 3) stored for 135 d at 5 °C; stratified for 45 d at 5 °C; incubated in germination medium for 45 d at 25 °C (ungerminated seeds from Expt. 2); 4) placed for 135 d in germination medium at 22 °C; incubated for 90 d at 5 to 10 °C in a greenhouse, then kept for 45 d at 25 °C (ungerminated seeds); and 5) stored for 270 d at 5 °C. Intact seeds that appeared healthy (see Fig. 4) were counted as intact. Of the intact seeds, those staining pink to red (see Fig. 4) when treated with 2, 3, 5-triphenyltetrazolium chloride were counted as viable. Mean separation ($\alpha = 0.05$) was based on arcsin-transformed proportions and applied to nontransformed means. For each treatment, means followed by the same letter within a column are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

Provenance	Treatment	n	Intact (%)	Viability (%)	Evaluation date
Florida	1	93	75 a	Not assessed	25 Oct. 2002
Missouri	1	100	82 a	Not assessed	25 Oct. 2002
Florida	2	166	21 a	7 a	17 Feb. 2003
Missouri	2	188	2 b	2 b	17 Feb. 2003
Missouri	3	560	0	Not applicable	17 Feb. 2003
Wakulla County, Fla.	4	139	96 a	0 a	26 Mar. 2003
Levy County, Fla.	4	144	6 b	0 a	26 Mar. 2003
Missouri	4	288	93 a	0 a	26 Mar. 2003
Missouri	5	100	0	Not applicable	26 Mar. 2003

Institute, Inc., Cary, N.C.) with the proportion of germinants, proportion of intact seeds, and proportion of viable seeds as dependent variables. Proportion of surviving seedlings and the measures of seedling growth were analyzed by using separate ANOVA models. All proportions were arcsin-transformed before statistical analyses were conducted, however, nontransformed data are presented. When appropriate, means were separated by using Fisher's LSD ($\alpha = 0.05$). Germination value as described by Czabator (1962) was calculated to allow a comparison of the speed and completeness of germination.

Results

Mean moisture content of seeds in 2002 from Florida and Missouri was 29% (SE = 2) and 38% (SE = 3), respectively. In 2003, the moisture content was 39% (SE = 4), 40% (SE = 4), and 38% (SE = 3) among seeds extracted from unripe drupes from Texas, Arkansas, and Missouri, respectively.

Experiment 1: Ripe drupes from Florida and Missouri in 2002. Germination percentages were similar for seeds placed on the surface and for those inserted into the medium, and consequently, we pooled the data over the two sowing methods. Excised seeds from Florida began to germinate within 5 d of sowing, but the radicle of many germinants from seeds placed on the surface of the medium grew upward. In contrast, germinants from seeds sown in the medium were oriented normally and had true leaves within 10 d of sowing. Removal of the surrounding fruit tissues and a subsequent treatment with GA_3 improved germination of seeds from Florida (Fig. 3). Seeds in this treatment germinated at the highest frequency (32%; germination value = 2.48) compared to seeds in all other treatments. Leaching of whole drupes from Florida and a subsequent treatment with GA_3 also enhanced germination (21%; germination value = 0.41) over the control treatment, which resulted in 2% germination and a germination value of 0.01. Neither seed excision nor treating whole drupes with leaching and GA_3 influenced germination of seeds from Missouri, which did not exceed 3%. Germination values among treatments applied to seeds and drupes from Missouri ranged from 0 to 0.01.

Germinants originating from leached drupes survived more frequently (75%) than those originating from excised seeds (47%). Many of the excised seeds germinated when they were placed on the surface of the medium, but they often appeared disoriented with the radicle pointed upward and failing to establish. This phenomenon likely was responsible for the decreased survival of germinants originating from excised seeds. Although very few in number, all seedlings originating from seeds collected in Missouri survived. Plants of both provenances obtained from untreated drupes (control treatment) were shorter (26 cm; SE = 14), had fewer (10; SE = 5), smaller (8 cm; SE = 3) leaves, and had thinner stems (4 mm; SE = 1.5) than those obtained under other treatments, which grew to a height of 60 cm (SE = 4), had 20 (SE = 1) leaves that averaged 14 cm (SE = 1) long, and had a mean stem diameter of 8 mm (SE = 0.4).

Provenance differences existed in the percentage of ungerminated seeds that remained intact and viable after incubation in the germination medium, and TTC staining showed that many seeds that appeared intact and healthy often were not viable (Table 1, Fig. 4).

Experiment 2: Ripe drupes from Missouri in 2002. Germination did not occur among untreated drupes from Missouri or among drupes treated with acid, leaching, and/or GA_3. Viability estimates could not be made for the ungerminated seeds because all recovered drupes contained decomposed seeds (Table 1). We also examined seeds extracted from intact drupes that were stored at 5 °C for 270 d and found that 100% of these seeds (n = 100) were decomposed (Table 1). Fungal

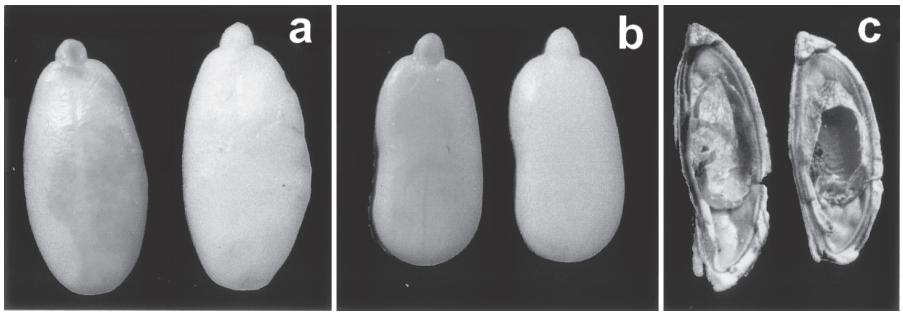

Fig. 4. Ungerminated seeds of *Leitneria floridana* from Florida (**a**) and Missouri (**b**) were recovered from germination medium and subjected to a 2, 3, 5-triphenyltetrazolium chloride (TTC) staining test to estimate viability (Expt. 1). Intact seeds staining pink to red were considered viable (seeds on the left in images **a** and **b**), whereas lack of these colors indicated nonviability. Many drupes contained decomposed seeds, which could not be recovered to estimate viability (**c**).

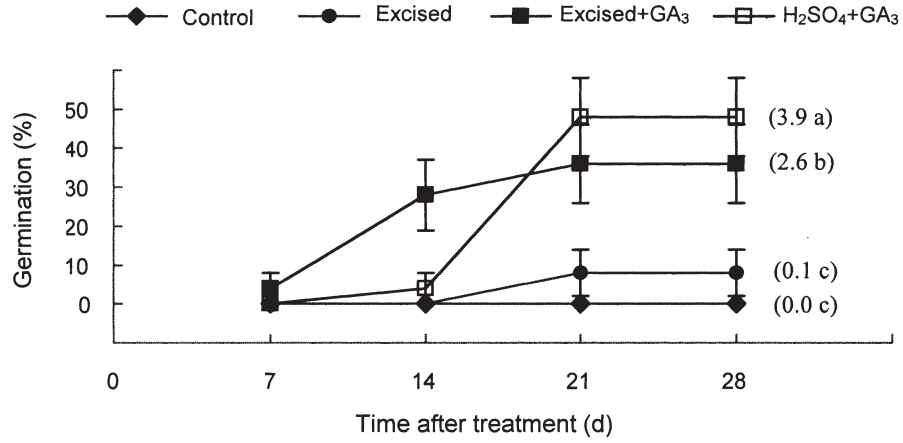

Fig. 5. Unripe drupes of *Leitneria floridana* were collected from Texas during the growing season of 2003 and subjected to a 28-d germination test (Expt. 3) after the following treatments were applied (n = 25): 1) drupes were left intact [Control]; 2) seeds were excised [Excised]; 3) excised seeds were submerged for 24 h in 1000 mg·L^{-1} GA_3 [Excised+GA_3]; and (4) whole drupes were submersed for 25 min in 96% H_2SO_4 and subsequently treated for 24 h with 1000 mg·L^{-1} GA_3 [H_2SO_4+GA_3]. Vertical bars about the data symbols represent ± the standard error of the mean germination percentages. Germination value, a composite measure of the speed and completeness of germination, was determined, and values are presented in parentheses. Germination values followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

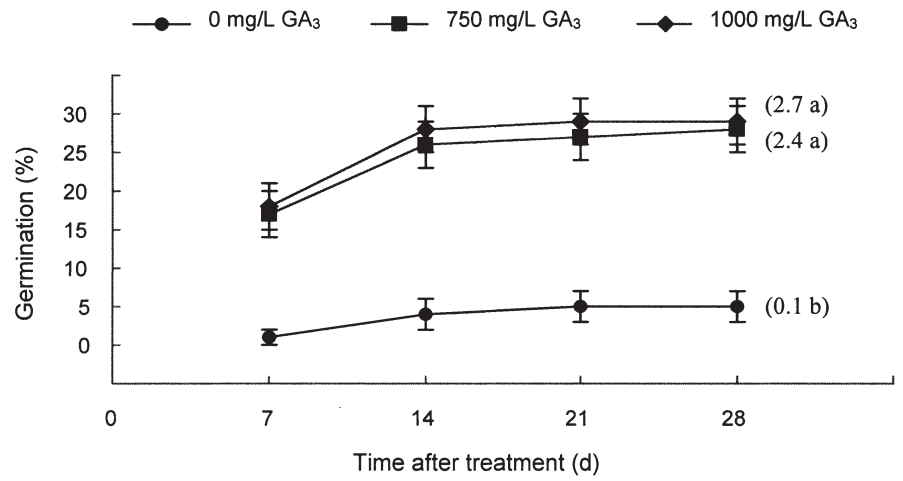

Fig. 6. Unripe drupes of *Leitneria floridana* were collected in Arkansas and Missouri during the growing season of 2003, and excised seeds were immediately subjected to a 28-d germination test (Expt. 4). A factorial arrangement of two provenances (Arkansas and Missouri) and three GA_3 concentrations (0, 750, or 1000 mg·L^{-1} applied as a 24 h soak) was used in a completely randomized design. Data from the two provenances were pooled (n = 200) because the germination response was similar. Vertical bars about the data symbols represent ± the standard error of the mean germination percentages. Germination value, a composite measure of the speed and completeness of germination, was determined, and values are presented in parentheses. Germination values followed by the same letter are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

growth was observed on these drupes and may have contributed to the fate of the seeds. While this experiment did not include seeds from Florida, we observed in other trials that a GA_3 pretreatment combined with cold stratification failed to induce germination in *L. floridana* seeds from Florida, whereas leached drupes or drupes scarified with 96% H_2SO_4 and subsequently treated for 24 h with 1000 mg·L^{-1} GA_3 and cold temperature resulted in 30% germination.

Experiment 3: Green, fleshy drupes from Texas in 2003. We obtained 48% germination (germination value = 3.9; Fig. 5) when unripe drupes collected in Texas were first scarified with H_2SO_4 and subsequently submerged for 24 h in 1000 mg·L^{-1} GA_3. Excising seeds and subsequently treating them with GA_3 increased germination to 36% (germination value = 2.6) in comparison to seed excision alone and the control treatment, which resulted in 8% and 0% germination, respectively, and the associated germination values ranged from 0 to 0.1.

Experiment 4: Green, fleshy drupes from Arkansas and Missouri in 2003. Up to 29% of the seeds from Arkansas and Missouri germinated when seeds were excised from unripe drupes and subsequently treated for 24 h with 750 or 1000 mg·L^{-1} GA_3 (germination values = 2.7 and 2.4, respectively; Fig. 6). In contrast, only 5% of untreated, excised seeds germinated, and the germination value was 0.1.

Discussion

Our data provide several important, new insights regarding the phenology of drupes of *L. floridana* and propagation of the species from seeds. Provenance differences in the time of fruit maturation were observed. Drupes became brown to black about 30 d earlier in the southern part of the natural range of *L. floridana* and appeared drier compared to drupes in Missouri. Considering that the drupes were collected only a day apart in the two provenances in 2002, perhaps drupes in Missouri were only partially dried because of climatic effects related to the more northern latitude. While the drupes in Florida and Missouri were abscising from plants when they were collected almost simultaneously in 2002, a 9% difference was found in moisture content of seeds. In 2003, we observed similar moisture content in seeds collected at similar developmental stages from three provenances. These green, fleshy drupes germinated to higher percentages when sown within a week after collection in comparison to germination during the previous year of freshly harvested or stored, ripe (brown to black) drupes. Physiological dormancy was not expressed or was overcome more easily among drupes that were green, fleshy, and presumably not fully ripe. While the moisture content of seeds collected in Missouri was similar in both 2002 and 2003, up to 29% germination occurred only when unripe drupes were used in 2003 (Figs. 3 and 6). Because the developmental stage of drupes appears to influence germination, germplasm should be collected on different dates in each provenance. Additionally, year-to-year variation in the

viability of seeds could lead to differences in germination, or seed viability could be affected by storage conditions. Loss of viability during storage and incubation is common among seeds of woody plants (Schopmeyer, 1974). It is possible that seeds of *L. floridana* may lose viability in storage. This loss, however, may not be evident in the physical appearance of the seeds, which can appear intact and healthy and yet be physiologically unviable (Table 1, Fig. 4). Data herein suggest the need for additional research to determine how seed moisture content and storage temperature can be regulated to sustain viability of *L. floridana*.

Provenance differences were observed in germination. While the lower germination among seeds from Missouri in 2002 in comparison to germination among seeds from Florida might reflect consistent ecotypic variation, temporal variation over years may also affect viability and germinability of seeds (Baskin and Baskin, 1998). Repeated samplings from the same populations and provenances could show whether inherent variation exists in drupe size and in the germination potential of seeds. Another insight revealed through our work is that the hard, outer exocarp creates a physical barrier to germination of *L. floridana*. Tissues surrounding a seed can present physical restriction by mechanically inhibiting embryo growth, can prevent leaching of chemical inhibitors from the embryos, or may contain inhibitors (Baskin and Baskin, 1998). The thick layer of tissues surrounding the seeds of *L. floridana* (Fig. 2) is not easily removable even with the aid of a scalpel. Considering that this species is native to wetlands where seeds may be submerged in saturated soils during the period after drupes abscise, perhaps some natural weathering of the exocarp occurs. The moist conditions may also favor leaching of inhibiting chemicals that might be present in the outer tissues.

Embryos of *L. floridana* also were inhibited physiologically from germinating. Freshly harvested seeds and seeds that were stored at 5 °C for several months showed improved germination after GA_3 was applied (Figs. 3, 5, and 6). Gibberellins are thought to activate α-amylase, which hydrolyses starch to produce sugars required for the development of germinating embryos, and the hormone induces cell elongation, which may enable the radicle to emerge (Raven et al., 1999). Gibberellins also may stimulate germination by overcoming the requirement for light or for cold stratification (Baskin and Baskin, 1971). GA_3 treatments have been effective in improving germination of seeds of several species including those that are rare (Bradbeer, 1988; Cochrane et al., 1999; Karam and Al-Salem, 2001; Shaltout and el-Shourbagy, 1989), and seeds of *L. floridana* are now known to benefit from a treatment with GA_3. While treatment with GA_3 of *L. floridana* seeds enhanced germination in this study, the possibility remains that before GA_3 was applied, the seeds inadvertently received cold stratification during storage at 5 °C. However, if this occurred, we would have expected improved germination among the seeds that did not receive pretreatments during Expt. 1. On the other hand, it is possible that a separate, GA_3-specific mechanism might be critical to release the physiological dormancy in seeds of *L. floridana*. This study has demonstrated that germination of freshly harvested and stored seeds of *L. floridana* can be improved considerably by using combinations of mechanical and chemical treatments. More experiments are warranted to improve our understanding of the reproductive biology of this rare species and to support its propagation for horticultural use.

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