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Seed Germination of *Dirca* (Leatherwood): Pretreatments and Interspecific Comparisons

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Abstract. **The genus *Dirca* L. (Thymelaeaceae) consists of three species of understory shrubs. *Dirca palustris* L. is sparsely distributed across eastern North America, *D. occidentalis* Gray is endemic near the San Francisco Bay, and *D. mexicana* Nesom & Mayfield is known only in one population in northeastern Mexico. Despite interest in the horticultural use of *Dirca*, plants seldom are marketed. Difficult propagation impedes production of *Dirca*. We sought to define protocols that promote uniform seed germination of all three *Dirca* spp. Endodormancy and paradormancy cause sporadic germination over several years under natural conditions, but endocarp removal, cold stratification, and treatment with GA_3 increased germination percentage, speed, and uniformity. *Dirca occidentalis* was most responsive; up to 94% of seeds germinated after endocarp removal, 24 hours in GA_3 at 50 mg·L^{-1}, and stratification at 4 °C for 30 days. Treatments also were effective for *D. palustris* (up to 68% germination), but seeds of *D. mexicana* were unresponsive and germinated at 25% or less. Seed treatments should facilitate production of *D. occidentalis* and *D. palustris*, but further research is needed to define methods to propagate *D. mexicana* for horticultural use and for conserving this rare species in the wild.**

Information on the biology of *Dirca* (leatherwood) is becoming increasingly important. Wild populations of *D. palustris* are not considered threatened, but the other two species are more vulnerable. *Dirca occidentalis* is imperiled to vulnerable at the state, national, and worldwide levels due to its endemic distribution and to human-induced and natural threats (Johnson, 1994; NatureServe, 2004; Walter and Gillett, 1998). The status of *D. mexicana* has not been published, but the existence of only one population indicates that it should be considered critically imperiled or endangered (Graves, 2004; Nesom and Mayfield, 1995). Cultivation can be one component of preservation, and *Dirca* spp. are valued for their extreme shade tolerance, aesthetic form, yellow leaves in autumn, and delicate yellow flowers that emerge before foliage in spring (Del Tredici, 1991; Dirr, 1998). There has been interest for many years in using *D. palustris* in managed landscapes, and native-plant enthusiasts have created a demand at specialty nurseries in California for *D. occidentalis*. Even amid concerns regarding conservation and interest in cultivation, regeneration in the wild is poorly understood, and reliable horticultural methods of propagation have remained elusive. Attempts to root stem cuttings of *D. palustris* have been unsuccessful (Dirr and Heuser, 1987). Seeds of *D. palustris* develop tenacious dormancy at maturity, and it is believed that no commonly used treatment will improve seed germination (Del Tredici, 1991).

A water-impermeable seed or fruit coat can impart seed dormancy, but in many woody species, disruption of the impermeable tissue allows seeds to imbibe water and germinate (Baskin et al., 2000; Hartmann et al., 2002). We questioned whether such paradormancy exists in the mature fruits of *Dirca* spp. Early botanists referred to the fruit of *Dirca* as a berry (Bigelow, 1818; Darlington, 1837; Marshall, 1785), but more recent descriptions classify it as a drupe (Bailey and Bailey, 1976; Bradford et al., 1968; Cooperrider, 1995; McVaugh, 1941; Munz, 1959; Pammel, 1911). Although the fruit of *Dirca* deviates slightly from the typical morphology of a drupe [e.g., a thinner and glossier pit wall or stony layer (Esau, 1977) than most drupes and a relatively thin mesocarp that shrinks to a meager remnant when dry] and the specific origin of each layer has not been documented for *Dirca*, we have described the fruit based on the modern definition of a drupe (Fig. 1). The fruit of the three *Dirca* spp. consists of a dicotyledonous embryo surrounded by four anatomical layers. The outermost layer, the exocarp, is tough and surrounds a relatively thin, fleshy layer, the mesocarp. The innermost layer of the pericarp, the endocarp, is hard and impermeable. Beneath the hard endocarp is a soft, semi-permeable seed coat (testa) that surrounds the embryo (Fig. 1).

Endodormancy, regulated by physiological factors inside the seed, can also inhibit germination, and some species exhibit both endodormancy and paradormancy (Baskin et al., 2000; Hartmann et al., 2002). Several commonly used treatments can overcome endodormancy, but results vary with species and seed or fruit type. Cold stratification alone may overcome endodormancy (Hartmann et al., 2002; Schrader and Graves, 2000; Shopmeyer, 1974). Gibberellic acid (GA) used with or without stratification increases the germination of many species, including those with drupes (Bradbeer and Pinfield, 1967; Geneve, 1991; Gianfagna and Rachmiel, 1986). Cytokinins overcome the effects of germination inhibitors in some species (Hartmann et al., 2002; Khan, 1971). Gibberellic acid and kinetin (a cytokinin) both improved germination of *Myrica pensylvanica* Loisel., a drupaceous species, when seeds were scarified before chemical treatment and then stratified for 30 d (Hamilton and Carpenter, 1977). Treatment with hydrogen peroxide (H_2O_2) has improved germination of some seeds (Chien and Lin, 1994; Duval and NeSmith, 2000; Ibanez and Passera, 1997).

Our goal was to establish protocols for all three *Dirca* spp. that promote uniform seed germination. We performed five experiments to examine the effects of stratification, endocarp modification, chemical treatments, and species on germination. Our results indicate that consistent propagation of *Dirca* spp. from seed is attainable, but that success varies among the three species.

Materials and Methods

Collection and storage of seeds. For all experiments that included seeds of *D. palustris*, ripened drupes were collected from plants growing at Ledges State Park and the Iowa Arboretum in Boone Co., Iowa, and Retz Memorial Forest in Clayton Co., Iowa. Ripened drupes of *D. occidentalis* were collected from plants growing in Contra Costa and Alameda Counties of western California, and ripened drupes of *D. mexicana* were collected from plants of the single population in Tamaulipas, Mexico. To ensure that fruits were mature and ripened, they were collected only after natural abscission or when gentle agitation caused abscission. Collection times each year were from late May through early June for *D. palustris*, mid-June for *D. occidentalis*, and mid-May for *D. mexicana*. For all experiments, drupes were stored in paper envelopes until treatments commenced. This allowed the fruit and seed to dry and be maintained close to ambient relative humidity (RH) and discouraged damage by microbes. For experiment 1, drupes were held at 4 ± 1 °C and

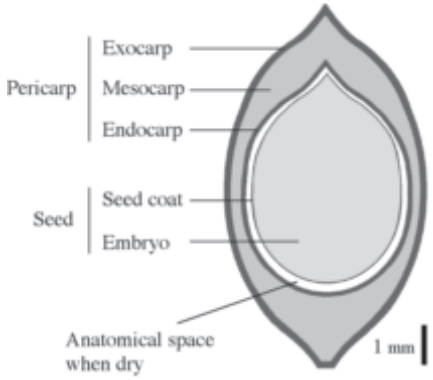

Fig. 1. Diagram of the drupe of *Dirca* spp. in longitudinal section parallel with the plane of the cotyledons. The three outer layers, the leathery exocarp, fleshy mesocarp, and hard endocarp, constitute the fruit or pericarp of the drupe. The embryo and semipermeable seed coat constitute the seed. The diagram also illustrates the anatomical space that develops between the endocarp and the seed as the drupe dries at maturity.

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30% ± 5% RH until the start of stratification. For the other four experiments, drupes were stored at 22 ± 2 °C and 40% ± 10% RH.

Experiment 1: Responses of D. occidentalis and D. palustris to stratification. In 2001, we collected ripe drupes of *D. occidentalis* and *D. palustris* from five plants of each species to evaluate their response to initial stratification treatments of 0, 5, 10, 15, and 20 weeks followed by germination conditions, then two more 5-week episodes of stratification. Seven intact drupes from each of 10 source plants (five of each species) were placed individually in clay pots (top diameter = 7.5 cm, height = 10 cm) filled with peat-based, soilless medium on each of five dates separated by 5 weeks. Initial stratification began on 17 Oct. 2001 for units assigned to the 20-week treatment. Each pot with one seed was an experimental unit in a completely randomized design (Cochran and Cox, 1992). Drupes were placed 3 mm below the surface of the root medium, which was kept moist with tap water. Stratification was done in the dark at 4 ± 1 °C. Staggering the initiation of stratification allowed us to move all stratified units to germination conditions on the same date that the 0-week experimental units were prepared and placed under germination conditions. All units were arranged randomly on a bench in a glass-glazed greenhouse [temperature: maximum = 26.3 °C, mean = 18.5 °C, minimum = 16.1 °C; mean RH= 63.4%; and mean photosynthetically active radiation (PAR) between 1000 and 1400 $_{HR}$ = 330 μmol·m^{-2}·s^{-1}]. Visible emergence of the shoot from the root medium was considered proof that a unit had germinated. Units not emerged after the initial stratification and 6-week germination episode (including controls) were subjected to a second episode of stratification for 5 weeks, followed by 4 weeks of germination conditions. Units still not emerged received a third 5-week stratification and then were held under germination conditions for 7 weeks. After the third germination period, all non-emerged seeds were removed from the root medium, examined visually for germination (radicle emergence to a length of 2 mm), and tested for viability by using the tetrazolium test (International Seed Testing Association, 1985).

Experiment 2: Responses of D. occidentalis to endocarp and chemical pretreatments. Ripened drupes of *D. occidentalis* were collected from five plants in 2002 and were combined. On 10 Oct. 2002, the exocarp and mesocarp layers were removed from all fruits by using tweezers, and fruits were randomly assigned to three endocarp treatments (intact, cracked, and removed) in factorial with four chemical treatments [GA_3 at 1000 mg·L^{-1}, kinetin at 100 mg·L^{-1}, 3% hydrogen peroxide, and distilled-deionized (dd) H_2O (control)]. Chemical-treatment concentrations were derived from the methods of Duval and NeSmith (2000), Geneve (1991), and Hamilton and Carpenter (1977). Experimental units contained 20 seeds each, and the experimental design was a completely randomized, 3 × 5 factorial (Cochran and Cox, 1992). Replication was n = 25 for endocarp treatments, n = 15 for chemical treatments, and n = 5 for endocarp × chemical cross classification. The Cracked treatment was accomplished by applying pressure with a scalpel across the end opposite the radicle until the scalpel broke through the hard endocarp. For the Removed treatment, endocarp removal was accomplished by using a dental pick. This was done with minimal damage to the embryo and seed coat, because in mature fruits of *Dirca* spp. these tissues are not in contact with the hard endocarp (Fig. 1). After applying the endocarp treatments, seeds were chemically pretreated by soaking them in their assigned treatment solution for 24 h. Controls were soaked for 24 h in dd H_2O. Directly after chemical treatment, seeds from each unit were drained, placed between two pieces of 90-mm-diameter filter paper within a 100 × 15-mm plastic petri plate, and given 2 mL dd H_2O. Units were placed in individual sealed, plastic bags to minimize evaporation and were placed into stratification (4 ± 1 °C in the dark) for 30 d. After stratification, experimental units were provided with an additional 0.75 mL dd H_2O and placed in germination conditions of 20 °C in a dark growth chamber for 40 d.

Emergence of a radicle to a length of 2 mm constituted germination, and seeds germinated in each unit were counted every 2 d. Germination percentage and peak day were calculated from the results. Germination percentage was the total percentage of seeds in each unit that germinated over the 40-d period. Peak day was the day of the trial on which the greatest number of seeds germinated and reflects germination rate. Units with no germinated seeds after 40 d had no true peak day and were ascribed a 40, the poorest score possible.

Experiment 3: Responses of excised seeds of all three species to GA_3 pretreatment. In 2002, ripened drupes were collected from nine plants of *D. mexicana*, 10 plants of *D. occidentalis*, and seven plants of *D. palustris*. Based on the results of experiment 2, we removed the entire pericarp, including the hard endocarp, from all seeds used in the final three experiments. Experimental units contained 10 seeds each, and the experimental design was completely randomized. Seeds were randomly assigned to one of three chemical treatments [GA_3 at 1000 mg·L^{-1}, GA_3 at 500 mg·L^{-1}, or dd H_2O (control)]. Replication was n = 25 for species, n = 15 for chemical treatments, and n = 5 for species × chemical cross classification. Seeds from each unit were soaked for 24 h in their assigned treatment solution, were stratified for 30 d, and were placed under germination conditions as in experiment 2. We limited the trial to 21 d under germination conditions because all excised seeds that germinated during experiment 2 had done so by day 20. After the 30-d stratification period, seeds were tested for viability by using a nondestructive test of seed firmness. All seeds were assessed to be either firm (viable) or flaccid (nonviable) by applying a consistent, gentle pressure. Experimental units were evaluated for germination percentage, percentage of viable seeds, germination of viable seeds, and peak day. The poorest possible score for peak day was 21 d.

To assess the effects of GA_3 treatments on the initial growth of seedlings, germinated seeds were removed from petri plates, placed in peat-based, soilless root medium in separate plastic pots, and arranged randomly on a greenhouse bench (mean temperature = 20.5 °C, mean RH = 70.5%, and mean PAR between 1000 and 1400 $_{HR}$ = 373 μmol·m^{-2}·s^{-1}). Shoot length and number of leaves per unit length of shoot were measured 8 weeks later. Treatment means that deviated ≥25% of the control mean were considered abnormal.

Experiment 4: Responses of excised seeds of D. occidentalis to GA_3 concentrations. Because the results of experiment 3 indicated that treatments with GA_3 at 500 and 1000 mg·L^{-1} caused seedlings to elongate abnormally, we examined the effects of several concentrations of GA_3 on the seed germination and initial seedling growth of *Dirca* spp. Ripened drupes of *D. occidentalis* were collected from 12 plants in 2003. On 16 Jan. 2004, the pericarp was removed from all seeds. Experimental units contained 10 excised seeds each, and the experimental design was completely randomized. Experimental units were randomly assigned to one of 10 GA_3 treatments (GA_3 at 0, 25, 50, 75, 100, 200, 300, 400, 500, or 1000 mg·L^{-1}). Replication was n = 10. Treatments, stratification, and germination conditions were administered as in experiment 3, and germination percentage and peak day were measured. The effects of GA_3 treatments on the initial growth of seedlings were assessed by measuring survival and shoot length after 8 weeks as was done during experiment 3.

Experiment 5: Responses of all three species to the most favorable pretreatment. Ripened drupes of *D. mexicana*, *D. occidentalis*, and *D. palustris* were collected in 2003 from eight, nine, and six plants, respectively. Experimental units contained 10 excised seeds each, and the experimental design was completely randomized. Because of limited seed availability, this experiment had unbalanced replication with n = 30 for *D. mexicana* and *D. occidentalis*, n = 22 for *D. palustris*, n = 41 for chemical treatments, and n = 15 or 11 for species × chemical cross classification. On 3 June 2004, experimental units were randomly assigned to one of two chemical treatments [GA_3 at 50 mg·L^{-1} or dd H_2O (control)]. Treatments, stratification, and germination conditions were administered as in experiment 3, and seed viability and the same three measures of germination were assessed. Because of the low viability of seeds of *D. mexicana* after the 30-d stratification period, we performed an additional trial with this species during which we applied GA_3 to excised seeds but did not stratify them.

Statistical analysis. Data for all experiments were analyzed for main effects, interactions, and mean-separation statistics by using the general linear models (GLM) procedure and the least significant difference (LSD) option of SAS/STAT, Version 6.12 (SAS Institute, Cary, N.C.). Data sets were tested for homogeneity of variance by using Levene's test (SAS Institute, Cary, N.C.), and nonhomogeneous data were transformed by a log or square-root function. Means were calculated from raw data, while the mean-separation statistics were calculated from raw or transformed data as necessary. Regression procedures of JMP, Version 3.2.6 (SAS Institute) were used to assess effects of stratification duration on seed germination and viability (experiment 1) and to evaluate effects

Table 1. Emergence percentage and viability of ungerminated[z] seeds of *Dirca occidentalis* and *Dirca palustris* in response to five durations of initial stratification followed by germination conditions and two more episodes of stratification for 5 weeks each. Intact drupes were held individually in clay pots filled with moist root medium, received stratification treatments, and were held under germination conditions in a greenhouse for 6, 4, and 7 weeks after the initial, second, and third stratifications, respectively. Emergence percentages for the second and third stratification episodes represent the cumulative percentages for all units that received those respective total treatment durations.

	Emergence (%)			Viability of
	Initial stratification	Second stratification	Third stratification	ungerminated[z] seeds (%)
Total weeks of stratification				
0	0 b[y]			
5	0 b	2.9 b		
10	1.4 a	11.3 a	10.4 a	50.7 a
15	5.7 a	9.6 a	10.1 a	56.5 a
20	2.9 a	8.8 a	8.3 a	47.0 a
25		7.4 a	9.0 a	42.6 b
30			0 b	15.9 c
Main effect of species				
Dirca occidentalis			13.1 a	41.1 b
Dirca palustris			2.9 b	53.1 a

[z]Percentage of ungerminated seeds (seeds still ungerminated at the end of the trial) that were determined to be viable according to the tetrazolium viability test (International Seed Testing Association, 1985).
[y]Means within columns in each category followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test; n = 175 for species, n = 70 for initial stratification treatments.

Table 2. Germination percentage and peak day[z] for seeds of *Dirca occidentalis* in response to three endocarp treatments in factorial with four chemical treatments. There were interactions ($P \leq 0.0001$) between the two treatment types for both dependent variables. Experimental units consisted of 20 seeds held between two sheets of moist filter paper in a plastic petri plate and were held under germination conditions (20 °C in a dark growth chamber) for 40 d.

Treatment combinations	Germination (%)	Peak day
Removed × GA_3	84 a	5 a
Removed × Kinetin	30 c	12 a
Removed × H_2O_2	2 d	8 a
Removed × control	11 d	10 a
Cracked × GA_3	65 b	4 a
Cracked × Kinetin	37 c	4 a
Cracked × H_2O_2	1 d	15 ab
Cracked × control	1 d	29 bc
Intact × GA_3	1 d	27 bc
Intact × Kinetin	2 d	26 b
Intact × H_2O_2	0 d	40 c
Intact × control	0 d	40 c

[z]Peak day was the day of the trial on which the greatest number of seeds germinated; it is a measure that reflects the rate of germination. Units with no germinated seeds after 40 d had no true peak day and were ascribed a 40, the poorest score possible.
[y]Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test; n = 25 for endocarp treatments, n = 15 for chemical treatments; n = 5 for endocarp × chemical cross classification.

of GA_3 concentration on germination, survival, and seedling height (experiment 4).

Results

Experiment 1: Responses of D. occidentalis and D. palustris to stratification. There was no interaction between the effects of species and treatment, indicating that stratification affected *D. occidentalis* and *D. palustris* similarly. Across the two species, initial stratification for 10, 15, and 20 weeks increased emergence compared to stratification for 0 or 5 weeks (Table 1). Additional units emerged after the second stratification episode, but only one additional unit emerged after the third stratification. Units that received three stratifications for a total of 30 weeks did not emerge (Table 1). Visual examination of seeds not emerged after the third stratification and germination period confirmed that only germinated units had emerged from the root medium and no nonemerged units had germinated. Total stratification durations of 25 and 30 weeks reduced the percentage of ungerminated seeds that were viable, and regression analysis revealed linear and quadratic effects in which viability decreased with total stratification duration >20 weeks [viability = $-0.002(\text{weeks})^2 + 0.045(\text{weeks}) + 0.192$, $r^2 = 0.08$, $P < 0.0001$]. Averaged over stratification durations, seeds of *D. occidentalis* germinated at a greater percentage than did those of *D. palustris* (Table 1). Both species had a high percentage of viable, ungerminated seeds at the end of the third germination period, but the percentage was greater for *D. palustris* than for *D. occidentalis* (Table 1).

Experiment 2: Responses of D. occidentalis to endocarp and chemical pretreatment. Both endocarp and chemical treatments affected the percentage and speed (peak day) of germination, and there were interactions ($P \leq 0.0001$) between the two treatment types for both dependent variables. GA_3 and kinetin increased germination of seeds with endocarps removed or cracked but had no effect on intact seeds (Table 2). Chemical treatments also had a greater effect on germination speed (peak day) when endocarps were removed or cracked than when seeds were intact (Table 2). Germination percentage was optimized at 84% by removing the endocarp and using GA_3 at 1000 mg·L^{-1}. All the seeds that germinated did so within the first 20 d in germination conditions. Seedlings from seeds treated with kinetin at 100 mg·L^{-1} or 3% H_2O_2 developed abnormally and eventually died, while seedlings from seeds treated with GA_3 at 1000 mg·L^{-1} developed elongated stems and unusually narrow leaves.

Experiment 3: Responses of excised seeds of all three species to GA_3 pretreatment. Across treatments, seeds of *D. occidentalis* showed the greatest germination percentage, viability, and germination of viable seeds of the three species, but peak day for all species was similar (Table 3). *Dirca mexicana* had a lower percentage of viable seeds after the 30-d stratification than did *D. occidentalis* and *D. palustris* (Table 3). Across species, GA_3 at both 500 and 1000 mg·L^{-1} increased germination percentage, seed viability, germination of viable seeds, and speed of germination (decreased peak day) (Table 3). There were no differences between the effects of the two concentrations of GA_3.

There were species-by-treatment interactions for germination percentage ($P \leq 0.0001$), seed viability ($P = 0.05$), and germination of viable seeds ($P = 0.042$), but not for peak day ($P = 0.21$). Interaction means showed a greater increase in germination percentage for seeds of *D. occidentalis* that received GA_3 than for the other two species, and seeds of *D. mexicana* showed no increase in germination percentage in response to GA_3 (Table 3). The interaction for seed viability was due to the lower viability of seeds of only *D. palustris* that received no GA_3 compared to those that did receive GA_3. The interaction for germination of viable seeds was due to the greater germination of viable seeds of only *D. occidentalis* and *D. palustris* in response to GA_3 (Table 3). Seeds of *D. occidentalis* that received a GA_3 treatment showed the greatest germination percentage (up to 95.5%) and germination of viable seeds (up to 98.2%).

Shoots of *D. occidentalis* were taller after 8 weeks than shoots of *D. mexicana* (Table 4). *Dirca mexicana* had the greatest number of leaves per unit stem length and *D. palustris* had the least, reflecting a greater degree of stem elongation for *D. palustris*. There was no interaction between species and treatment for growth parameters. Across the three species, both GA_3 concentrations increased shoot height and decreased leaves per unit stem length, confirming that treating seeds with GA_3 at these two concentrations leads to abnormally long shoots (Table 4).

Experiment 4: Responses of excised seeds of D. occidentalis to GA_3 concentration. GA_3 at 25 to 1000 mg·L^{-1} increased germination percentage and decreased peak day compared to the control treatment (Table 5). There were no differences in peak day among seeds treated with any concentration of GA_3, and the only difference for germination percentage among concentrations was that GA_3 at 50 and 500 mg·L^{-1} induced greater germination (94%) than did GA_3 at 1000 mg·L^{-1} (86%). Treating seeds with GA_3 at 50 and 75 mg·L^{-1} increased survival of the resulting seedlings after 8 weeks compared to the control treatment (Table 5). Most other GA_3 concentrations had no effect on seedling survival compared to the control, but GA_3 at 500 and 1000 mg·L^{-1} decreased survival to 61% and 33%, respectively. Regression analysis also indicated that survival decreased with increasing GA_3 concentration greater than 100 mg·L^{-1} [survival = $-0.00004(GA_3)^2 - 0.005(GA_3) + 94.1$, $r^2 = 0.16$, $P < 0.0001$]. All of the GA_3 concentrations applied to seeds increased shoot

Table 3. Seed viability and germination of *Dirca mexicana*, *D. occidentalis*, and *D. palustris* in response to GA_3 at 500 and 1000 mg·L^{-1}. Seed viability was assessed by manually testing seed firmness after treatments and 30 d stratification and before being held under germination conditions for a maximum of 21 d. All seeds had the three pericarp layers removed.

Effect	Germination (%)	Seed viability (%)	Germination of viable seeds (%)[z]	Peak day
Main effect of species				
D. mexicana	10.0 b[y]	21.7 c	40.0 b	11 a
D. occidentalis	69.7 a	94.8 a	72.8 a	9 a
D. palustris	18.6 b	30.9 b	52.4 b	10 a
Main effect of treatment				
GA_3 1000 mg·L^{-1}	45.7 a	52.7 a	76.2 a	6 a
GA_3 500 mg·L^{-1}	49.3 a	58.6 a	70.7 a	8 a
Control, distilled H_2O	11.9 b	44.7 b	25.6 b	15 b
Interaction (species × treatment)				
D. mexicana × GA_3 1000	8.8 cd	18.8 d	50.0 bc	8 ab
D. mexicana × GA_3 500	13.8 cd	28.8 cd	35.8 cd	13 bc
D.. mexicana × control	7.1 d	17.1 d	33.3 cd	14 c
D. occidentalis × GA_3 1000	95.5 a	97.3 a	98.2 a	7 a
D. occidentalis × GA_3 500	87.3 a	93.6 a	91.3 a	6 a
D. occidentalis × control	26.4 bc	93.6 a	29.1 cd	13 bc
D. palustris × GA_3 1000	22.7 bc	32.7 bc	73.4 ab	5 a
D. palustris × GA_3 500	36.0 b	44.0 b	75.9 ab	7 a
D. palustris × control	2.9 d	20.0 d	19.0 d	17 c

[z]Germination of viable seeds is the percentage of viable seeds in each unit that germinated (number of germinated seeds ÷ number of viable seeds × 100).
[y]Means within columns in each category followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test; n = 25 for species treatments, n = 15 for chemical treatments, n = 5 for species × chemical cross classification.

Table 4. Effects of GA_3 treatments on the initial growth of *Dirca* spp. Shoot height and the number of leaves per unit length of stem were evaluated 8 weeks after germination.

Effect	Shoot ht (cm)	Leaves/ cm of stem[z]
Main effect of species		
D. mexicana	3.3 b[y]	1.21 a
D. occidentalis	6.0 a	0.64 b
D. palustris	4.5 ab	0.41 c
Main effect of treatment		
GA_3 1000 mg·L^{-1}	6.2 a	0.54 b
GA_3 500 mg·L^{-1}	6.0 a	0.61 b
Control, distilled H_2O	2.1 b	1.15 a

[z]Leaves per cm of stem is the shoot height divided by the number of leaves per plant and is an indicator of stem elongation.
[y]Means within columns in each category followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test; n = 11 for *D. mexicana*, n = 128 for *D. occidentalis*, and n = 24 for *D. palustris*; n = 85 for GA_3 1000 mg·L^{-1}, n = 62 for GA_3 500 mg·L^{-1}, and n = 16 for control treatments.

height of the resulting seedlings (Table 5), but this effect was most pronounced for seedlings from seeds treated with ≥300 mg·L^{-1}. Regression analysis confirmed that shoot height increased with increasing GA_3 [height = $-0.00001(GA_3)^2 + 0.015(GA_3) + 6.8$, $r^2 = 0.28$, $P < 0.0001$]. Seedlings from seeds pretreated with GA_3 at 300 to 1000 mg·L^{-1} appeared to have narrower leaves and fewer leaves per unit stem length, whereas seedlings from seeds treated with GA_3 at <200 mg·L^{-1} showed few or no leaf abnormalities. Pretreating seeds with GA_3 at 25, 50, and 75 mg·L^{-1} seemed to result in particularly healthy and vigorous seedlings. GA_3 at 50 mg·L^{-1} was chosen for use in experiment 5 because it evoked favorable responses across all measured parameters.

Experiment 5: Responses of all three species to the most favorable pretreatment. As in experiment 3, excised seeds of *D. occidentalis* achieved the greatest germination percentage (60%), percentage of viable seeds after the 30 d of stratification (95.3%), and germination of viable seeds (63.5%) across the two treatments (Table 6). *Dirca mexicana* again had the lowest percentage of viable seeds, but unlike during experiment 3, it also had the lowest germination percentage, the lowest germination of viable seeds, and the greatest peak day of the three species (Table 6). GA_3 at 50 mg·L^{-1} increased germination percentage and germination of viable seeds and decreased peak day across the three species, but there were species-by-treatment interactions for these parameters ($P \leq 0.0001$ for each). GA_3 improved germination percentage, germination of viable seeds, and peak day for *D. occidentalis* and *D. palustris* but not *D. mexicana* (Table 6). Additional, nonstratified seeds of *D. mexicana* showed greater viability and germination than had seeds after stratification, but GA_3 again did not effect germination (Table 6). Optimum germination for nonstratified seeds of *D. mexicana* (25.3%) was lower than the germination of GA_3-treated and stratified *D. occidentalis* (90%) and *D. palustris* (68.2%).

Discussion

The most thorough prior report on the propagation of *Dirca* spp. included only *D. palustris* and concluded that seed germination is complicated by dormancy, and that the best technique is to sow seeds outdoors and let nature run its course (Del Tredici, 1984 and 1991). Our results with the three *Dirca* spp. agree in some ways with those conclusions. Seeds of *D. palustris* do possess dormancy that causes them to germinate slowly and sporadically over several years under simulated natural conditions (Schrader and Graves, 2003). *Dirca palustris* and *D. occidentalis* had a high percentage of viable, ungerminated seeds at the end of the germination period of experiment 1 (Table 1), demonstrating that both species possess dormancy not overcome by stratification alone. Removal of the hard endocarp increased germination percentage and speed over that of seeds with the endocarp intact (Table 2), indicating that ripe seeds of all three species of *Dirca* are paradormant due to an impermeable endocarp (Hartmann et al. 2002). Even after endocarp removal, treatment with GA_3 along with stratification increased germination of *D. occidentalis* and *D. palustris* further (Tables 2 and 3), evidence that seeds of *Dirca* also are endodormant (Hartmann et al., 2002). Future experiments on after-ripening, imbibition, stratification, GA requirements, and inhibitor presence should clarify the underlying mechanisms of seed dormancy in *Dirca*.

Unlike the results of Del Tredici (1984 and 1991), our findings reveal that endocarp and chemical treatments improve germination. Treatments were effective for seeds of *D. occidentalis* and *D. palustris*, and we propose that growers propagate these species from seeds by removing the pericarp (Fig. 1), soaking excised seeds in GA_3 at 50 mg·L^{-1} for 24 h, stratifying treated seeds at 4 °C for 30 d, and allowing germination to occur at about 20 °C. This technique is not recommended for *D. mexicana*. Based on our two experiments that included *D. mexicana*, we conclude that pericarp removal can improve its germination, but treatment with GA_3 has no effect, and stratification can be detrimental after pericarp removal (Tables 3 and 6). Although other concentrations of GA_3 also improved germination of *D. occidentalis* and *D. palustris*, treatment with GA_3 at 50 mg·L^{-1} led to particularly vigorous seedlings with no apparent detrimental side effects (Table 5). GA_3 at ≥300 mg·L^{-1} should be avoided due to morphological abnormalities and reduced survival of seedlings.

Although the ornamental value of *Dirca* is recognized (Dirr and Heuser, 1987; Del Tredici, 1991), the genus is poorly understood from a horticultural perspective. Our data provide important new information. *Dirca occidentalis* is the easiest of the species to propagate from seed. In the two experiments that compared the three species, *D. occidentalis* had the greatest seed viability, germination, and germination of viable seeds (Tables 3 and 6). In our preliminary assessments of seedlings cultured in greenhouses, *D. occidentalis* has shown the greatest survival and the fastest growth. Two potential limits to the widespread use of *D. occidentalis* as a nursery crop are related to its adaptation to the Mediterranean climate of western California. First, the cold hardiness of *D. occidentalis* may restrict its use because annual minima in its native habitat rarely are <0 °C. Second, at least some populations of *D. occidentalis* are summer-deciduous during the dry season in the San Francisco Bay area (Ackerly, 2004; Schrader and Graves, 2004). The tendency of *D. occidentalis* to lose foliage by mid-summer under dry conditions might limit the commercial

Table 5. Germination percentage, peak day,[z] and survival and shoot height 8 weeks after germination for seeds of *Dirca occidentalis* in response to 10 levels of GA_3 treatments. All seeds had the three pericarp layers removed, were placed in petri plates between two sheets of moist filter paper, and were held under germination conditions for a maximum of 21 d.

GA_3 treatment ($mg \cdot L^{-1}$)	Germination (%)	Peak day	Survival[y] (%)	Shoot ht[x] (cm)
0 (control)	49 c[w]	8 b	78 bc	3.7 a
25	93 ab	6 a	84 ab	5.8 b
50	94 a	6 a	93 a	6.7 bc
75	89 ab	6 a	92 a	7.0 c
100	92 ab	6 a	88 ab	7.5 c
200	91 ab	5 a	70 cd	6.7 bc
300	88 ab	6 a	81 abc	9.1 d
400	87 ab	6 a	79 bc	9.3 d
500	94 a	5 a	61 d	9.9 d
1000	86 b	6 a	33 e	8.8 d

[z]Peak day was the day of the trial on which the greatest number of seeds germinated; it is a measure that reflects the rate of germination. Units with no germinated seeds after 21 d had no true peak day and were ascribed a 21, the poorest score possible.
[y]Percentage of germinated seeds from each treatment that survived for 8 weeks after the start of germination conditions; n = 50 for control, n = 75 for all other treatments.
[x]Shoot height of live seedlings measured 8 weeks after the start of germination conditions.
[w]Means within each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test. n = 10 for all treatments for results of germination percentage and peak day.

Table 6. Seed viability and germination of *Dirca mexicana*, *Dirca occidentalis*, and *Dirca palustris* in response to pericarp removal and GA_3 at 50 $mg \cdot L^{-1}$ (best treatment from experiment 4). Seed viability was assessed by manually testing seed firmness after treatments and 30 d stratification and before being held under germination conditions for a maximum of 21 d.

Effect	Germination (%)	Seed viability (%)	Germination of viable seeds[z] (%)	Peak day
Main effect of species				
D. mexicana	4.6 c[y]	30.0 c	10.8 c	17 b
D. occidentalis	60.0 a	95.3 a	63.5 a	9 a
D. palustris	39.5 b	85.0 b	46.5 b	11 a
Main effect of treatment				
GA_3 50 $mg \cdot L^{-1}$	52.7 a	65.1 a	60.7 a	10 a
Control, distilled H_2O	15.9 b	72.2 a	18.5 b	15 b
Interaction (species × treatment)				
D. mexicana × GA_3 50	4.0 d	24.0 d	10.5 d	18 c
D. mexicana × control	5.3 d	36.0 c	11.1 d	17 c
D. occidentalis × GA_3 50	90.0 a	94.0 ab	95.8 a	5 a
D. occidentalis × control	30.0 c	96.6 a	31.2 c	12 b
D. palustris × GA_3 50	68.2 b	81.8 b	81.5 b	6 a
D. palustris × control	10.9 d	88.2 ab	11.4 d	16 c
Dirca mexicana nonstratified[x]				
D. mexicana × GA_3 50	24.0 a	84.0 a	27.2 a	11.3 a
D. mexicana × control	25.3 a	82.6 a	30.9 a	12.7 a

[z]Germination of viable seeds is the percentage of viable seeds in each unit that germinated (number of germinated seeds ÷ number of viable seeds × 100).
[y]Means within columns in each category followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test; n = 30 for *D. mexicana* and *D. occidentalis*, n = 22 for *D. palustris*, n = 41 for chemical treatments, n = 15 or 11 for species × chemical cross classification.
[x]Supplemental trial to assess viability of seeds of *Dirca mexicana* with pericarp removed and given GA_3 treatment but no stratification. Mean separation statistics for this trial are separate from those of the full trial; n = 15.

appeal of the species. Nonetheless, although growers regard *D. occidentalis* difficult to produce, they believe a strong market for the species exists if cultural protocols are defined (M. Teel, Yerba Buena Nursery, Woodside, Calif., personal communication).

In contrast to *D. occidentalis*, the broad, temperate distribution of *D. palustris* suggests ample cold hardiness, which may make it suitable for more widespread use in the landscape. Seeds of *D. palustris* responded well to our treatments (Table 6), and there is no evidence that plants are summer-deciduous. The rarity of *D. mexicana* justifies continued research on propagation strategies to foster conservation efforts, which could include cultivation of plants in public gardens and landscapes.

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