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Seed Germination of *Rhamnus caroliniana*: Implications for Ecology and Horticulture

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***Abstract*. Little is known about the reproductive biology of carolina buckthorn [*Rhamnus caroliniana* Walt. or *Frangula caroliniana* (Walt.) Gray], an attractive North American shrub or small tree that might merit increased use in managed landscapes. The fecundity and high germinability of seeds of the Eurasian common buckthorn (*Rhamnus cathartica* L.), however, have been characterized as factors contributing to its invasiveness. We compared seed germination of these species to ascertain how easily carolina buckthorn could be grown from seed in nurseries and to acquire data for predicting whether carolina buckthorn might be invasive if introduced into managed landscapes. Fruits of carolina buckthorn were collected from indigenous plants in central Missouri, southern Oklahoma, and southern Texas. Fruits of common buckthorn were collected from shrubs naturalized in central Iowa. Seeds of both species were stratified for up to 112 days in darkness at 4 ºC; germination at 24 ºC in the dark was then evaluated for 56 days. Quadratic functions best described how time of stratification influenced germination value and germination percentage of common buckthorn, whereas these measures of carolina buckthorn were best represented by exponential (value) or linear (percentage) functions. Stratification for 112 days maximized germination value and percentage for carolina buckthorn within the 56-day germination period, but shorter stratifications were sufficient to optimize germination of common buckthorn. While the overall mean germination of carolina buckthorn was 40%, results varied by provenance and ranged from 25% (Missouri) to 56% (Oklahoma). Mean germination of common buckthorn over times of stratification was 71%, and the overall mean daily germination of common buckthorn, 1.3, was 86% greater than that of carolina buckthorn, 0.7. We conclude that seeds of carolina buckthorn are more resistant to germination than seeds of common buckthorn. Our results suggest that plant propagators should cold-stratify seeds of carolina buckthorn for up to 112 days, and suggest that carolina buckthorn has a lower potential to be invasive than does common buckthorn.**

Carolina buckthorn, also known as indian cherry, is an attractive shrub indigenous to the south-central and southeastern United States. The species has glossy leaves that turn orange, yellow, and red in the autumn. Plants grow to about 12 m tall (Dirr, 1998) and form open crowns of slender branches. The distinctive fruits of carolina buckthorn progressively change from green to light yellow to red, and finally turn blueberry-blue to black in late autumn (Graves, 2002). Across its natural range, the species is found in riparian areas as well as on dry, upland, limestone ridges (Brown and Kirkman, 1990; Foote and Jones, 1989). Consistent with this pattern of occurrence, young plants of carolina buckthorn tolerate both drought and partially flooded soils (Stewart and Graves, 2004).

Its stress resistance (Stewart and Graves, 2004), ease of vegetative propagation (Graves, 2002), and ornamental appeal suggest carolina buckthorn could be promoted for use in horticultural landscapes. Several members of the *Rhamnus* L. genus, including common buckthorn (*Rhamnus cathartica* L.), however, are invasive in the north-central and northeastern United States. Although there are several reasons for the pervasiveness of common buckthorn in disturbed habitats (Gourley, 1985), the fecundity of common buckthorn is a dominant element contributing to its invasiveness (Archibold et al., 1997; Hubbard, 1974; Kollman and Grubb, 1999). Gourley (1985) cited the high viability and rapid germination of common buckthorn seeds as factors that contribute to the aggressive invasiveness of the species, and germination percentage of common buckthorn exceeded that of seeds from two cultivars of *Rhamnus frangula* L. (Wheeler and Starrett, 2001). The buried seed bank beneath mature plants of common buckthorn averaged 620 viable seeds/m^2 in natural areas near Saskatoon, Saskatchewan, and the mean germination rate of overwintered seeds of common buckthorn was 85% (Archibold et al., 1997). These findings are consistent with several reports indicating that introduced species that become invasive have higher rates of germination than those of closely related native species (Mihulka et al., 2003; Radford and Cousens, 2000; van Clef and Stiles, 2001).

Little information is available about the reproductive biology of carolina buckthorn. Fresh seeds of carolina buckthorn may require no pretreatment to germinate (Esquivel, 2001; Nokes, 2001). However, fruits of the species do not ripen until late in the growing season and often are retained on plants through at least early winter. Thus, in a temperate climate, seedlings from these fruits are not likely to establish due to the onset of winter. It has been suggested that stored seeds should be stratified for 30 d at 5 °C to evoke germination within five weeks (Nokes, 2001), but no data related to seed germination of carolina buckthorn have been published.

Our objective was to characterize effects of cold stratification on seed germination of carolina buckthorn. Our rationale for this research was 2-fold. Because carolina buckthorn is an appealing, native species (Dirr, 1998) that may merit use in managed landscapes (Graves, 2002), our data should benefit those who may wish to propagate plants for commerce. In addition, our results provide new insights about the degree of resistance to germination inherent among seeds of carolina buckthorn. While far from describing the invasive potential of the species fully, this information represents a first step toward that goal, particularly because germination of seeds of carolina buckthorn from multiple provenances was compared with germination of the invasive common buckthorn. Including multiple seed sources in our experiments was important because of interspecific and intraspecific variation in resistance to germination of seeds of members of the *Rhamnus* L. genus (Hubbard, 1974; Young and Young, 1992).

Materials and Methods

Fruits of carolina buckthorn were collected during October 2001. Collections were made from five plants in Brazito, Mo. (lat. 38°26'44"N; long. 92°18'9"W), from five plants along the Blue River in Johnston County, Okla. (lat. 34°19'40"N; long. 96°42'20"W), and from five plants in Kerrville, Texas (lat. 30°02'50"N; long. 99°08'24"W). Fruits of common buckthorn were collected from five naturalized plants in Ames, Iowa (lat. 42°2'5"N; long. 93°37'11"W). Seeds were separated from fruits 7 to 14 d after collection and stored in paper bags at 20 °C for 3 months until treatment initiation. Only seeds weighing ≥20 mg each were used. Seed moisture content on 5 Apr. 2002 was 4.2% and 5.5% for carolina buckthorn and common buckthorn, respectively (International Seed Testing Association, 1985).

Each experimental unit consisted of 34 half-sibling seeds from one maternal source that were held between two pieces of 90-mm-diameter filter paper within a 100 × 15-mm plastic petri dish. Due to seed availability, there were 23 half-sibling seeds per petri dish from one of the five maternal sources in Missouri. Each experimental unit to be stratified received 1.75 mL distilled H_2O and then was placed into a dark growth chamber at 4 °C. Dates in 2002 when stratification began differed based on the various ascribed durations so that the date treatments ended was the same (7 May) for all

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Table 1. Measures of germination of seeds of *Rhamnus cathartica* (common buckthorn) and *Rhamnus caroliniana* (carolina buckthorn) from Missouri, Oklahoma, and Texas. Seeds were stratified for 0, 14, 28, 42, 56, 84, and 112 d in the dark at 4 °C. Data are the means over the seven stratification periods. Values for species are means of 35 and 105 multiseed replications for *Rhamnus cathartica* and *Rhamnus caroliniana*, respectively. Values partitioned by provenance are means of 35 multiseed replications.

Species and population	df	Germination Value	Germination %	Germination Distribution (d)	Peak day	Peak value	Mean daily germination (%/d)
Rhamnus cathartica		3.5 A[z]	71.2 A	25.2 A	25.8 A	2.3 A	1.3 A
Rhamnus caroliniana		2.7 A	39.9 B	23.6 A	25.1 A	2.0 A	0.7 B
Missouri		1.3 b[y]	25.2 b	16.8 b	25.0 a	1.5 a	0.4 b
Oklahoma		4.0 a	56.3 a	28.9 a	26.3 a	2.6 a	1.0 a
Texas		2.6 ab	38.3 b	25.1 ab	24.0 a	2.1 a	0.7 b
Mean square							
Population (P)	2	63	8558***	1337**	45	12	3***
Time of stratification (T)	6	177***	5556***	487	1748***	76	2
P × T	12	23	193	281	95	6	0.1
Error	84	21	782	266	247	7	0.3

[z]Species means within each column followed by the same capital letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.
[y]Provenance means within each column followed by the same lower-case letter are not different at $P \leq 0.05$ according to Tukey's honestly significant difference test.
,*Significant at $P \leq 0.01$ or 0.001, respectively.

experimental units. Treatment durations of 0, 14, 28, 42, 56, 84, and 112 d were initiated on 7 May, 23 Apr., 9 Apr., 26 Mar., 12 Mar., 12 Feb., and 15 Jan., respectively. Experimental units were arranged in a completely randomized design with five replications (each with seeds from only one of the five maternal sources) per treatment (common buckthorn) or provenance within treatment (carolina buckthorn). On 7 May we applied 3.5 mL distilled H_2O to experimental units assigned to the 0-d treatment, applied an additional 1.75 mL to all other units, and then randomized them all in a dark growth chamber at 24 °C. Each petri dish was sealed in an individual plastic bag to minimize moisture loss during the stratification and germination periods. During the germination period, we added 1 to 1.75 mL distilled H_2O to experimental units in which the filter paper began to appear dry (every 5 to 10 d). Filter paper was replaced as needed to prevent fungal growth.

Germination was defined as the emergence of a radicle, which was confirmed by viewing seeds with a 15× dissecting microscope. Germinated seeds were counted every 3 to 4 d for 56 d, and germination value (Czabator, 1962), germination percentage, germination distribution, peak day, peak value, and mean daily germination were calculated (Schrader and Graves, 2000a). Germination value is the product of peak value and mean daily germination and is a composite expression of the speed and completeness of germination (Czabator, 1962), which is useful for measurement of seeds of woody plants that may germinate slowly. Germination percentage is the number of germinated seeds divided by the total number of seeds in a given seed lot. Mean daily germination is the total germination percentage divided by the number of days of the germination period. Germination distribution is the number of days over which germination occurred. The day during the germination period on which the highest number of seeds germinated is defined as the peak day. Peak value is the cumulative germination percentage for each experimental unit on its peak day, divided by the number of days that were required to reach that percentage. Tetrazolium tests (Peters, 2000) were performed after the final day of germination assessment to determine the viability of ungerminated seeds.

The effects of species and time of stratification on seed germination were analyzed by analysis of variance and Tukey's honestly significant difference option of SAS/STAT software, Version 8.2 (SAS Inst., Cary, N.C., 1999–2001) using the general linear models procedure. Data from carolina buckthorn also were analyzed separately from those of common buckthorn to assess for differences between populations. Analysis of variance showed no interaction between the main effects of species and time of stratification. There was also no interaction between the main effects of populations of carolina buckthorn and time of stratification (Table 1). We were able to analyze these effects separately due to their independence (Cochran and Cox, 1992). Regression analysis was performed to test for effects of cold stratification on seeds of both species.

Results

Averaged over all stratification periods, germination value did not differ between species as a whole, but germination percentage of common buckthorn was 78% greater than that of carolina buckthorn (Table 1). Tetrazolium tests revealed that 16% and 49% of the ungerminated seeds of common buckthorn and carolina buckthorn were viable, respectively. Germination distribution, peak day, and peak value of both species were similar (Table 1). Mean daily germination of common buckthorn was nearly twice that of carolina buckthorn (Table 1).

The germination value of seeds of carolina buckthorn from Oklahoma was three times that of seeds from Missouri (Table 1). Seeds from Oklahoma germinated at the highest percentage among the three provenances and showed a greater germination distribution than did seeds from Missouri (Table 1). There were no differences among provenances in peak day or peak value, but seeds from Oklahoma had the highest mean daily germination (Table 1).

Germination value of seeds of carolina buckthorn increased exponentially as time of stratification increased; values ranged from 0.2 at 0 d of stratification to 9.9 at 112 d of stratification (Fig. 1A). Germination value of seeds of common buckthorn changed quadratically over time of stratification and was as high as 4.7 at 56 d of stratification but decreased thereafter to 3.9 at 112 d (Fig. 1A). Germination percentage of seeds of carolina buckthorn increased linearly over time of stratification from 18% to 69% (Fig. 1B). Germination percentage of common buckthorn was 48% after 0 d of stratification, increased to 85% with 42 d of stratification, and declined slightly at 112 d (Fig. 1B). Time of stratification decreased peak day for seeds of carolina buckthorn linearly from 36 d at 0 d of stratification to 9 d at 112 d (Fig. 1C). Peak day for non-stratified seeds of common buckthorn was similar to that of non-stratified carolina buckthorn, but the decrease in peak day over time of stratification for common buckthorn ceased after 42 d (Fig. 1C). Peak value of seeds of carolina buckthorn increased exponentially over time of stratification from 0.3 at 0 d of stratification to 7 at 112 d (Fig. 1D). At 0 d of stratification, peak value for common buckthorn was 1 (Fig. 1D). It increased to 3 at 56 d of stratification and decreased to 2.6 at 112 d (Fig. 1D). Mean daily germination of carolina buckthorn increased linearly over time of stratification from 0.3 to 1.2 %/d (Fig. 1E). Mean daily germination of common buckthorn consistently exceeded that of carolina buckthorn, and the linear increase due to stratification was less pronounced for common buckthorn than for carolina buckthorn (Fig. 1E). Quadratic regression functions best described the relationship of time of stratification and germination distribution of both species (*Rhamnus caroliniana*: $y = 14.2 + 0.38[day] - 0.0025[day^2]$, $r^2 = 0.87$; *Rhamnus cathartica*: $y = 18.4 + 0.32[day] - 0.0023[day^2]$, $r^2 = 0.78$). Germination distribution for seeds of carolina buckthorn was 17 d at 0 d of stratification, reached a maximum of 30 d at 56 d of stratification, and declined to 26 at 112 d. Similarly, germination distribution for seeds of common buckthorn was 19 d at 0 d of stratification, 32 d after 84 d, and only 24 d at 112 d.

Discussion

Our results are significant to horticulture in three ways. First, we have shown that cold stratification enhances the germination of seeds

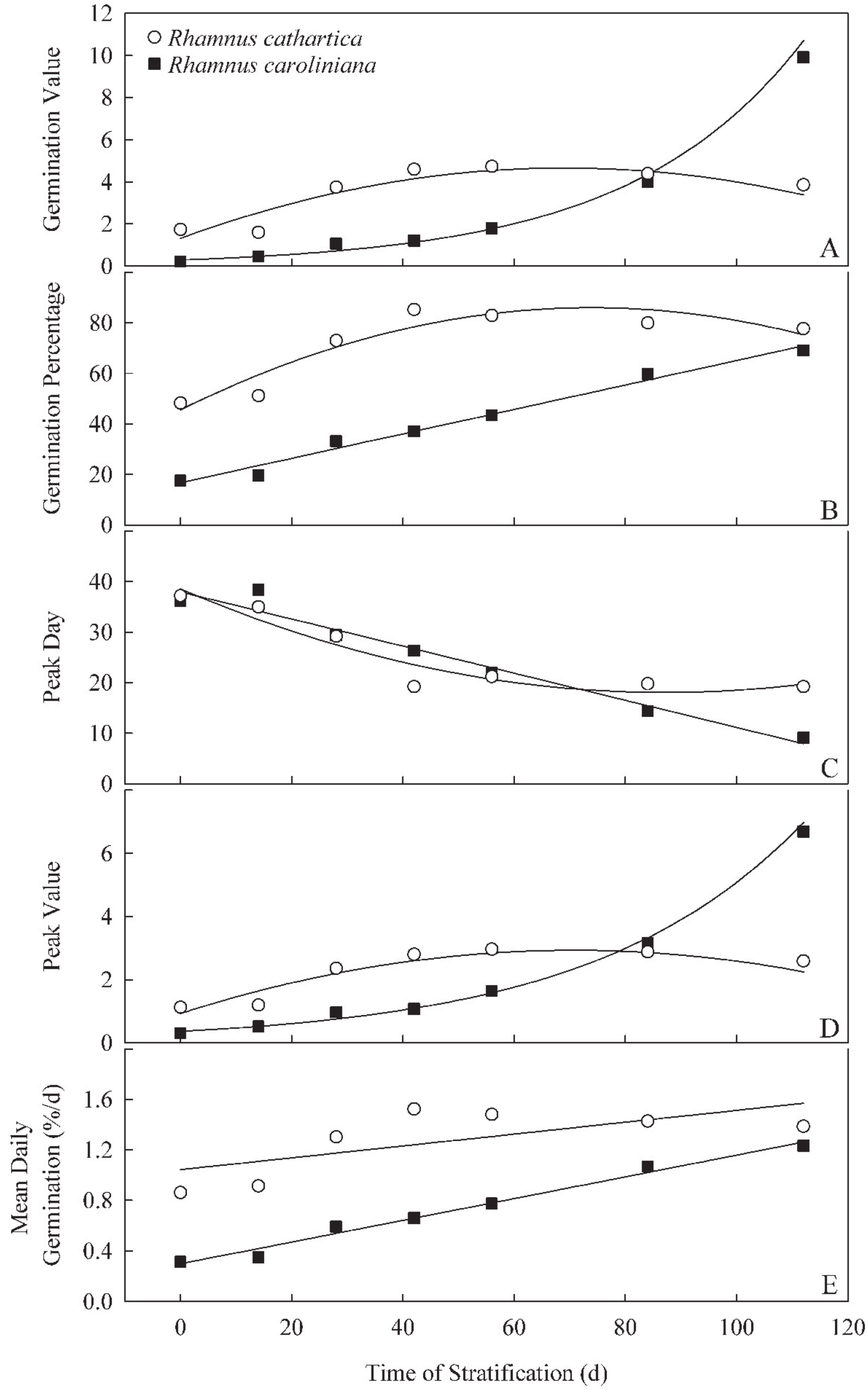

Fig. 1. Effects of stratification duration on seeds of *Rhamnus caroliniana* (carolina buckthorn) and *Rhamnus cathartica* (common buckthorn). (**A**) Germination value (*R. caroliniana*: $y = 0.29e^{0.032[\text{day}]}$, $r^2 = 0.97$; *R. cathartica*: $y = 1.31 + 0.097[\text{day}] - 0.0007[\text{day}^2]$, $r^2 = 0.86$). (**B**) Germination percentage (*R. caroliniana*: $y = 16.7 + 0.48[\text{day}]$, $r^2 = 0.99$); *R. cathartica*: $y = 45.5 + 1.09[\text{day}] - 0.0074[\text{day}^2]$, $r^2 = 0.88$). (**C**) Peak day (*R. caroliniana*: $y = 37.99 - 0.27[\text{day}]$, $r^2 = 0.96$; *R. cathartica*: $y = 38.57 - 0.47[\text{day}] + 0.0027[\text{day}^2]$, $r^2 = 0.91$). (**D**) Peak value (*R. caroliniana*: $y = 0.36e^{0.0265[\text{day}]}$, $r^2 = 0.98$; *R. cathartica*: $y = 0.93 + 0.057[\text{day}] - 0.0004[\text{day}^2]$, $r^2 = 0.92$). (**E**) Mean daily germination (*R. caroliniana*: $y = 0.3 + 0.009[\text{day}]$, $r^2 = 0.99$; *R. cathartica*: $y = 1.04 + 0.005[\text{day}]$, $r^2 = 0.48$). Values are means of five and 15 multiseed replications for *R. cathartica* and *R. caroliniana*, respectively.

of carolina buckthorn. Second, the inclusion of seeds from different provenances of carolina buckthorn in our work indicates that the germination characteristics of the species vary with seed source. Lastly, we conclude that, in general, seeds of carolina buckthorn are more resistant to germination than are seeds of common buckthorn. Although more research is needed to assess whether carolina buckthorn merits increased horticultural use, our findings provide potential growers with useful information on propagation and represent a first step toward resolving questions regarding the potential invasiveness of the species.

Both germination value and germination percentage increased with increasing duration of cold stratification for seeds of carolina buckthorn, but the pattern of trends over time differed. The exponential increase in germination value over time of stratification shows that relatively long stratification periods are particularly beneficial (Fig. 1A). In contrast, increases in germination percentage (Fig 1B) and mean daily germination (Fig. 1E) were linear, indicating that improvements in how quickly and uniformly seeds germinate, rather than gains in germination percentage, account for the more pronounced increases in germination value predicted by the exponential function after day 60 (Fig. 1A). This conclusion is consistent with other indicators of the speed of germination; as time of stratification increased, peak day decreased (Fig. 1C) and peak value (Fig. 1D), which is derived from peak day, increased. While these results illustrate the benefits of cold-stratifying seeds, the minimal duration of stratification considered acceptable will vary among growers of carolina buckthorn and will depend on the source of the seed (Table 1); on average, about 80 d should elicit 50% germination (Fig. 1B), and at least 112 d would optimize germination value (Fig. 1A). Future research should be designed to determine the dormancy mechanisms that account for the relatively high percentage of viable seeds of carolina buckthorn that do not germinate after cold stratification.

Research representing more provenances is needed to determine whether the degree of resistance to germination among seeds of carolina buckthorn is random or can be predicted based on geographic origin. The provenance differences we observed are consistent with previous work with other woody species from temperate climates. For example, seeds of *Betula papyrifera* Marsh. from the northern limits of its natural distribution germinate faster than seeds of southern origin (Bevington, 1986), and germination varies among seeds of *Alnus maritima* (Marsh.) Nutt. depending on the disjunct provenance from which seeds are obtained (Schrader and Graves, 2000a). No cultivars of carolina buckthorn have been selected. If the low germinability of seeds from Missouri is consistent among plants within that provenance and over multiple years of seed development, selections from that region may have an inherently low likelihood of becoming invasive when used in amenity landscaping. The environmental benefits of low seed germination, however, must be reconciled with the need for higher seed germination by growers.

We included common buckthorn in this research so that the influence of cold stratification on seed germination of carolina buckthorn could be compared to that of a closely related species that is known to be invasive. The comparatively high germination percentage and mean daily germination of common buckthorn over times of stratification (Table 1) indicate that seeds of carolina buckthorn are comparatively resistant to germination, particularly when times of stratification are brief (Fig. 1B, E). While these data provide some basis to claim that carolina buckthorns introduced into horticultural landscapes may prove less invasive than common buckthorns, it is important to recognize the limitations over what can be concluded. While fecundity and high seed viability and germination lead to the invasiveness of some *Rhamnus* spp. introduced to North America from other continents (Archibold et al., 1997; Gourley, 1985; Hubbard, 1974; Kollman and Grubb, 1999; Wheeler and Starrett, 2001), our results alone do not permit definitive conclusions about the potential for invasiveness of carolina buckthorn. Field studies to evaluate the reproductive biology of the species could be designed to determine seed viability, the fate of seeds consumed by animals, and environmental factors that influence the establishment of unintended seedlings. In particular, work needs to be done to determine annual fruit and seed production of carolina buckthorn to verify our postulation that plants from Missouri might be less invasive than other seed sources. Seeds of some woody plants are less tolerant of low temperatures than are the plants on which they arise (Schrader and Graves, 2000b). Although plants of carolina buckthorn may survive if introduced to areas with colder winters than those where the species is native, seeds that develop on plants installed in cold climates may lose viability during winter. Pooler et al. (2002) showed that seeds from an interspecific cross of an invasive species introduced to North America, *Celastrus orbiculatus* Thunb., and its North American relative, *Celastrus scandens* L., were held under weaker dormancy than were seeds from intraspecific crosses of *C. scandens*. Thus, whether carolina buckthorn can hybridize with invasive members of its genus also should be determined.

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