Plant Source and Seed Parasitism Influence Seed Viability in Redbud (*Cercis* spp.)[1]

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Abstract Eastern redbud, *Cercis canadensis* L., trees are difficult to vegetatively propagate. Named cultivars of Eastern redbud do not grow "true" from seeds forcing growers to invest resources to graft buds or scions onto seed-grown liners. Whereas the unnamed North American native Eastern redbud trees produce large numbers of easily-collected seedpods that contain abundant numbers of seeds, these seeds are frequently infested with seed-parasitic beetles and associated wasp parasitoids. Of the redbuds investigated, we found that unnamed Eastern redbuds were preferred hosts for redbud seed beetles, *Gibbobruchus mimus* (Say). However, named and unnamed redbud types as well as *C. chinensis* 'Avondale' were suitable hosts for *G. mimus*. In addition, several new wasp parasitoids were recovered from seeds although it was not apparent whether these were attacking seed endosperm, *G. mimus*, or both. A float test successfully discriminated dead or infested seeds from seeds that were mostly viable. The majority of seeds that floated were empty, dead or infested. Growers can optimize seed collection efficiency by screening native Eastern redbud seeds for size and subjecting seeds to a float test thereby minimizing time, labor and land resources that would otherwise be expended for preparation and maintenance of fields planted with potentially nonviable seeds.

Key Words *Cercis* sp., *Dinarmus* sp., Eulophidae, Eurytomidae, *Gibbobruchus mimus*, *Horismenus* sp., parasitism, pest resistance, Pteromalidae

Eastern redbud, *Cercis canadensis* L., trees are difficult to propagate by stem or root cuttings (Dirr and Heuser 1989, Bir 1992). Both unnamed, seed-grown redbud trees and named cultivars produce abundant seedpods. When abundant fruit are set, the winter appearance of trees becomes unsightly (Dirr 1998). Named cultivars of Eastern redbud do not grow "true" from seeds, yet trees of named cultivars can provide growers with seed sources for field-grown rootstocks. To meet consumer demand for horticulturally-selected cultivars, growers must graft buds or scions onto seedling liners, which is a time-consuming and costly process (Geneve 1991).

Initiating a crop with high-quality seed is advantageous for commercial growers who face rising production costs and less available labor. Couvillon (2002) demonstrated that redbud seed could be segregated by mass to confer advantages in subsequent germination rate and growth of seedlings. Heavier redbud seeds germinated more rapidly, in greater numbers, and yielded larger seedlings across several growth parameters than lighter seeds.

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Seeds are easily collected in late summer through November (Dirr and Heuser 1989, Young and Young 1992). Young and Young (1992), however, reported that redbud seeds in pods in Oklahoma are infested with insects by November, suggesting that seeds be collected in late August or early September once seedpods have ripened.

Several species of insect pests have been identified from redbud seeds. In the western U.S., two bruchid beetles, the groundnut seed beetle, *Caryedon serratus* (Olivier) (Prevett 1967) and the redbud seed beetle, *Gibbobruchus mimus* (Say), infest *C. canadensis* and *C. occidentalis* Torrey redbud seeds. *Gibbobruchus mimus* occurs throughout the range of native *Cercis* sp. (Whitehead and Kingsolver 1975, Center and Johnson 1976, Pfaffenberger 1986). The redbud seed beetle is readily observed on redbuds during flowering, after which it can infest large numbers of seeds (Whitehead and Kingsolver 1975, Pfaffenberger 1986). Early work has identified several hymenopterans thought to be parasitoids of redbud seed beetles, including a braconid (*Glyptolocastes bruchivorus* Crawford), a eupelmid (*Cerambycobius cyaniceps* Ashmead), and a eulophid (*Horismenus* sp.) that emerged from bruchid beetle-infested redbud seeds collected in Texas and Arizona (Cushman 1911, Center and Johnson 1976).

The ability to collect and segregate redbud seeds into high quality lots with similar vigor is confounded by the possibility that seeds may be infested with seed beetles and phytophagous or parasitic wasps. Redbud susceptibility to *G. mimus* and, subsequently wasp parasitism, is unknown. Commercial producers would benefit from nondestructive seed assessment strategies that discriminate infested from noninfested seeds, thus maximizing seasonal investments in labor and land use. Therefore, the objective of this research was to identify arthropod pests and asso ciated parasitoids infesting seeds from mature landscape groupings of six redbud types and cultivars that are available in commercial trade (Dirr 1998, Pooler and Dix 2001).

Materials and Methods

During November 1999 and 2000, fully-developed redbud seedpods from 6 individual trees were collected from each landscape group of either ungrafted, seed-grown North American native Eastern redbuds (unnamed *C. canadensis*), or grafted 'Alba', 'Forest Pansy', 'Oklahoma', and 'Rubye Atkinson' *C. canadensis* redbud cultivars and 'Avondale' (*C. chinensis* 'Avondale') Chinese redbuds. Trees of horticulturally named Eastern redbud cultivars had been grafted or budded onto seed-grown *C. canadensis* rootstocks. All 36 trees were part of 10-yr-old, massed, roadside plantings near the University of Tennessee (Knoxville, TN) (35°58′ N x 83°55′ W).

Seedpods were maintained in a walk-in cooler (Hobart Medalist, Troy, OH) at 4 ± 1°C and 70 ± 5% RH for 4 wk. Individual seeds from seedpods were graded into size classes using U.S. Standard Test Sieves such that Size 7 = ≥2.80 mm; Size 8 = 2.36-2.79 mm; Size 10 = 2.00-2.35 mm; and Size 12 = ≤1.99 mm diam. The remaining, unexamined seedpods were held in the laboratory at room temperature (23 ± 3°C). Once sized, seeds were subjected to a simple float test (e.g., Young and Young 1992, Gribko and Jones 1995) to quantify the benefit of using seed immersion to determine seed viability. Seeds in each size class were directly immersed in tap water, and the surface tension of the water was broken using 5-sec gentle agitation

Table 1. Determination of redbud seed status by dissection (100 seedpods per tree)

Redbud	Seed size*	Number of seeds	Dead** (%)	Infested† (%)	Number of seeds	Dead (%)	Infested (%)
			1999			2000	
Unnamed *Cercis canadensis*	7	216.7	2.3	7.8	239.0	1.5	17.1
Large seed rank: 1	8	73.0 ab††	5.1 bc	10.2 ab	97.2 a	9.3 a	32.5 a
Seed viability rank: 2	10	12.8	93.8	1.5	13.7	38.7	33.3
	12	10.0	95.0	0.0	9.0	70.0	17.4
C. canadensis 'Alba'	7	214.0	1.7	8.4	185.7	1.2	21.9
Large seed rank: 2	8	17.8 abc	35.4 c	4.2 bc	12.0 b	2.8 a	20.0 bc
Seed viability rank: 3	10	3.8	97.4	0.0	4.0	95.0	0.0
	12	0.0	0.0	0.0	1.3	100.0	0.0
C. canadensis 'Forest Pansy'	7	34.2	2.3	0.0			
Large seed rank: *NA*	8	172.3 a	5.9 b	2.9 cd	—produced no seedpods in 2000—		
Seed viability rank: *NA*	10	105.0	1.2	1.4			
	12	8.5	82.3	0.0			
C. canadensis 'Oklahoma'	7	213.2	1.9	5.2	126.7	5.9	31.1
Large seed rank: 4	8	16.8 bc	59.5 c	5.1 bc	9.7 b	44.3 a	18.3 bc
Seed viability rank: 4	10	0.8	87.5	3.3	6.5	100.0	0.0
	12	0.0	0.0	0.0	3.8	100.0	0.0

Table 1. Continued.

Redbud	Seed size*	Number of seeds	Dead** (%)	Infested† (%)	Number of seeds	Dead (%)	Infested (%)
		1999			2000		
C. canadensis 'Rubye Atkinson'	7	178.3	4.3	17.0	157.8	0.2	28.7
Large seed rank: 5	8	16.7 c	83.8 bc	3.6 a	20.7 b	20.8 a	16.3 b
Seed viability rank: 5	10	4.5	95.6	2.4	9.5	61.1	7.0
	12	3.0	100.0	0.0	8.5	90.6	4.7
C. chinensis 'Avondale'	7	167.2	10.0	0.1	143.5	8.4	1.2
Large seed rank: 3	8	78.7 abc	85.5 a	0.5 d	40.3 b	11.2 a	7.5 b
Seed viability rank: 1	10	24.3	100.0	0.0	16.8	48.8	4.0
	12	3.2	100.0	0.0	5.0	60.0	10.4
Significance†††							
Cultivar		*	***	***	***	NS	***
Seed size		***	***	***	***	*	***
Cultivar × seed size		***	***	***	**	NS	***

* Seeds classes established with U.S. Standard Testing Sieves such that 7 = ≥2.80 mm; 8 = 2.36 to 2.79 mm; 10 = 2.0 to 2.35 mm; and 12 = ≤1.99 mm diameter.

** Dead seed percentages are calculated within each size class. Dead seed were either aborted, contained no endosperm or were decomposing. Seeds with an intact, cream-colored endosperm were considered viable. Parasitized seeds are not viable.

† Ratio calculated as the proportion of seeds within each size class parasitized by bruchid beetles, phytophagous or parasitic wasps.

†† For each year, LSMEAN values followed by the same letter, within columns, are not significantly different at the 0.05% level by Duncan's multiple range test.

††† NS and ***, **, or * indicate that F ratios were not significant or significant at $P \leq 0.001$, 0.01, or 0.05, respectively.

with a glass rod. Due to constraints of time and the objective to quantify insect infestation, germination tests on seeds that sank were not conducted. Instead, all floating seeds and up to 200 sinking seeds in each size class were dissected and classified as viable, dead or infested. Seeds were considered viable if they contained complete, yellow cotyledons and an intact, cream-colored endosperm. Seeds were considered dead if they were empty or had darkened cotyledons with signs of decomposition. Infested seeds had an emergence hole or contained a larva, pupa, or cadaver. Several wasps emerged from these seeds and were submitted to the Plant Industry Division of the North Carolina Department of Agriculture for identification.

Data were subjected to analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute, 1999). Linear contrasts described the ability of the float test to discern redbud seeds as viable, dead or infested. LSMEAN values among statistically significant variables were separated using Duncan's multiple range test value ($P \leq 0.05$). Large seed rankings reflect only Size 7 and 8 classes whereas seed viability rankings consider Size 8 class seeds (see Table 1). Rankings were averaged across years to provide recommendations about optimal redbud seed resources for growers. *Cercis canadensis* 'Forest Pansy' seeds are not ranked because trees did not produce seedpods in 2000.

Results and Discussion

Small seeds of a redbud species or cultivar are expected to grow less quickly and with less vigor than larger seeds (Couvillon 2002). Because smaller seeds also yield fewer food resources for phytophagous seed beetles like *G. mimus*, they are expected to be used less by pests. Seed counts and seed status LSMEAN values from redbud seedpod lots differed in 1999 compared with 2000 ($F = 10.22$-66.69; df = 1, 249; $P = 0.002$ to < 0.0001), thus data were analyzed separately by year. In both years, redbud seeds collected from seedpods were mostly large seeds (≥ 2.8 mm diam) that can be readily separated using a No. 7 U.S. Standard Testing Sieve (Table 1). In 1999, 'Forest Pansy' redbuds yielded proportionally smaller seeds (2.0-2.49 mm diam) than other *Cercis* types. Unlike the other redbud trees, the smaller seeds of 'Forest Pansy' redbuds were also dark brown to black and had a wrinkled seed coat. Because 'Forest Pansy' trees in the landscape plantings produced no seedpods in 2000, and seedpods were also unavailable in 2003, we could not ascertain if this cultivar produces consistently smaller seeds.

In 1999, seed numbers per 100 seedpods were highest in *C. chinensis* 'Avondale' and unnamed *C. canadensis* trees, and 'Alba' and 'Forest Pansy' Eastern redbud cultivars. In 2000, unnamed Eastern redbud trees yielded the most seeds per 100 seedpods (Table 1). Seed viability in 1999, determined by seed dissections, was highest for unnamed *C. canadensis* and 'Forest Pansy' redbuds followed by 'Alba' and 'Oklahoma' cultivars. In 2000, unnamed Eastern redbud trees produced the largest number of viable seeds. In 1999, seedpods from *C. chinensis* 'Avondale' redbuds yielded the greatest numbers of dead (empty or decaying) seeds. Differences in number of dead seeds were not apparent among redbuds in 2000.

Contrary to erroneous reports by Cushman (1911) and Young and Young (1992), redbud seed beetles oviposit on young seedpods, and their parasites are active early in the season (Pfaffenbarger 1976). Cushman (1911) reported that *G. mimus* is a "continuous breeder" capable of reproducing on old (darkened) pods. Yet, subsequent studies have demonstrated that beetle oviposition is restricted to green pods.

Eggs may be deposited individually or in clusters of about three eggs and are most frequently found along the rib of developing pods in early summer. Once eggs are deposited, the surface of the pod begins to turn brown underneath the egg (Pfaffenberger 1986). Pfaffenberger (1986) hypothesized that parasitoids of bruchid beetles use rapid pod discoloration, which follows beetle egg deposition, as a cue to locate their host organisms.

Seed parasitism, whether by *G. mimus* or its associated hymenopteran parasitoids, was greatest in 1999 among unnamed *C. canadensis* and 'Rubye Atkinson' trees and for unnamed Eastern redbuds in 2000 (Table 1). Considering the proportion of all seeds collected per redbud type, infestation was most prevalent in 1999 for 'Rubye Atkinson' and unnamed *C. canadensis* followed by 'Alba' and 'Oklahoma' cultivars and for unnamed redbuds in 2000. Whereas *C. chinensis* 'Avondale' can support *G. mimus* development, 1999 data suggest that 'Avondale' trees are a less preferred host.

Seeds that overwintered at room temperature (20 ± 3C) yielded *G. mimus* and several parasitic wasps that emerged from fall-collected seedpods. Other seed parasitoids were excised from seeds during dissections. In 1999 and 2000, the most abundant parasitic wasp that emerged from seeds was identified as the pteromalid wasp, *Dinarmus basalis* (Rondoni), which is a common parasitoid of stored-legume pests (Sanon et al. 2002). Another wasp parasite that emerged from seeds was in the genus *Horismenus* (Hymenoptera: Eulophidae). Whereas specimens could not be more precisely identified, many species within this genus are beetle parasites. Two different species of eurytomid wasp also were recovered but could not be further classified (K. Ahlstrom, pers. comm.). Voucher specimens are deposited in the Department of Entomology and Plant Pathology Museum Collection of the University of Tennessee's Institute of Agriculture in Knoxville, TN.

Whereas *Horismenus* sp. wasps have been associated with redbuds (Cushman 1911, Center and Johnson 1976), neither *Dinarmus* sp. nor wasps in the Family Eurytomidae have been reported to emerge from redbud seeds. These eurytomid wasp species could either be parasites of *G. mimus* or directly feed on *C. canadensis* seeds, or both. At present, the developmental resources required by these wasps are unclear. Regardless, parasitism of beetle larvae and subsequent development of wasps within the protective seed coat yields infested redbud seeds unviable.

Float testing redbud seeds was an effective tool for segregating infested and noninfested seeds. Averaged among years without regard to seed size or cultivars, 93% of the seeds that floated were dead or infested (Table 2). Only 7% (2 of 28) of floating seeds contained cotyledons and an intact endosperm when dissected. The

Table 2. Seed status (averaged among years without regard to seed size or cultivars) determined after exposure to a simple float test

Response	Total seeds n (%)	Viable seeds n (%)	Dead seeds n (%)	Parasitized seeds n (%)
Seeds sank	216 (100) a*	185 (85) a	24 (11) a	8 (4) b
Seeds floated	28 (100) b	2 (7) b	7 (25) b	19 (68) a

* Means within column followed by different letters are significantly different by Duncan's multiple range test ($P < 0.05$).

float test was a less accurate predictor of seed status when seeds sank, however. About 11% of seeds that sank showed signs of decay within the endosperm and were actually dead. Further, about 4% of the seeds that sank contained a larva, pupa or cadaver of a phytophagous beetle or parasitic wasp when dissected. Still, the float test correctly diagnosed about 12% of all redbud seeds in each lot to be dead or infested, allowing growers to eliminate them and preserve production resources (Table 2). Because redbud seeds are seasonally abundant, no economic advantage would be gained from more thorough screening methods.

The higher seed:seedpod ratio and larger seed size of unnamed, seed-grown Eastern redbuds (Table 1) underscores their value as a good seed collection resource. On average, seed from unnamed Eastern redbuds and *C. canadensis* 'Alba' yield the greatest likelihood for large seed with low rates of infestation. Seedling tree propagators should examine a subsample of each collected seedlot. Average seed size and seed parasitism rate will vary both regionally and seasonally, but growers are not expected to gain advantages by selectively collecting and propagating seed of *C. canadensis* 'Rubye Atkinson', 'Forest Pansy' or 'Oklahoma' cultivars. *Cercis canadensis* 'Rubye Atkinson' and *C. chinensis* 'Avondale' redbuds also produced smaller seedpod profiles and had seedpods that yield fewer seeds with low levels of arthropod infestation. These redbud selections fill a niche for small landscape trees that require less maintenance or offer aesthetically tidier winter landscapes and can reduce host plant resources for *G. mimus* and its associated parasitoids.

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