

Title: Preemptive Harvest: Early Collection and Germination in Doubly Dormant and Recalcitrant Seeds

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Abstract:

Dormancy occurs in the seeds of many plant species. Dormant seeds will not germinate readily; instead the range of conditions under which they will germinate is plastic and regulated by characteristics within the seed itself. Doubly dormant species, such as many viburnums, require a period of both warm, moist stratification and cold, moist stratification before the epicotyl will emerge forming shoot tissue. Other species exhibit a reverse tendency, they will germinate readily but viability does not last under storage and/or desiccation – these seeds are called recalcitrant. We wished to discover if viability and/or dormancy, for doubly dormant and recalcitrant seeds, were different for seeds collected at different stages of maturity. We collected both ripe and unripe seeds from the species *Betula allegheniensis*, *Betula lenta*, *Corylus fargesii*, *Rosa palustris*, *Viburnum cassanoides*, *Viburnum lentago*, and *Viburnum prunifolium*. Seeds were sown immediately in seed propagation media and placed in the greenhouse. If, after 14 weeks, the seeds of doubly dormant species had not germinated they were placed in a refrigerator at 4° C for 12 weeks before being returned to the greenhouse.

Stage of collection did not affect dormancy or germination rate for any of the doubly dormant species (*R. palustris*, *V. cassanoides*, *V. lentago*, and *V. prunifolium*). *V. cassanoides* however was found not to exhibit double dormancy as previously thought and instead germinated after a period of warm stratification only. For both *B. allegheniensis* and *B. lenta* germination rate was higher for seeds collected prior to full maturity and desiccation.

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Introduction

Seed germination requires that certain conditions of light, water, temperature, and nitrates be met (Crocker 1916). In some species however, particularly in temperate regions, fresh seeds will not germinate even given these conditions. Instead, the range of conditions at which a seed may germinate is plastic and regulated by characteristics within the seed itself (Vleeshouwers et al. 1995). Such seeds are said to be dormant. Several types of seed dormancy occur, each requiring a period at particular temperatures under moist conditions before germination becomes possible.

Some species, including several viburnums, exhibit separate physiological dormancies for the radicle (forming roots) and epicotyle (forming shoots)(Browse 1997). In this case a period of warm temperatures (~21° C) is necessary for the radicle to emerge. The epicotyl, however, will not germinate until after a period of cold (~4° C) subsequent to radicle emergence. Such seeds are called 2 year seeds - in nature germination would not occur until the second spring after seed maturity.

Dormancy in seeds is regulated by several plant hormones (Baskin and Baskin 1989) and, while only partially understood, it is believed that dormancy does not develop until late in seed maturity (Hartmann et al. 1997). Several authors have noted that if viburnum seeds are harvested prior to full maturity they are “less dormant” and may even germinate without pre-treatments (Browse 1997, Dirr 2007). The best time for harvest and the extent to which dormancy is lessened, however, is unknown.

The goal of this project was to determine whether physiological seed dormancy may be overcome or lessened in select species through the collection and sowing of seed prior to full maturity. In addition, we wished to determine the optimal period of time at which to collect seed for this purpose.

Removal of fruit from the seeds of viburnums is both a tedious and lengthy process. As a result we decided to use some of the seeds collected to test for differences in germination between seeds that had been cleaned of fruit and those that had not.

Several species that do not exhibit double dormancy were also of interest to us. For these species we wished to discover how germination rate changed in the transition through maturity and desiccation of fruits and seeds prior to release from the parent plant. The seeds of some species are recalcitrant, being unable to survive a period of desiccation. The genus *Betula* as well as several genera of tree nuts such as *Corylus* are among this group (King and Roberts, 1980). Early collection may be important in these species in order to avoid desiccation as well as herbivory from seed predators.

Materials and Methods

Species selected included a number of ornamental species that produce fruit in the late summer and fall. They included *Betula allegheniensis*, *Betula lenta*, *Corylus fargesii*, *Rosa palustris*, *Viburnum cassanoides*, *Viburnum lentago*, and *Viburnum prunifolium*. Seed collection began in late August, 2008 and continued in 10-12 day intervals until late September, 2008. Collections were made from Hickory Run State Park, French Creek State Park, and from specimens already in the Morris Arboretum collection. Fruits and seeds were determined to be ripe if more than 50% of their surface had taken on the color of full maturity and unripe if they had not. In addition, the growing degree days (GDD) for 2008 were determined for each date of collection. Seeds were cleaned and sown the day of harvest in Fafard 2 propagation media and placed in the greenhouse with 21° C bottom heat. Collections were made from the same area, and same parent plants when possible, for each subsequent collection following the same protocol and continued until either September 31 or when 100% of the fruit were fully mature at collection, whichever came first. Individual parent plants were recorded and distributed evenly among treatments when possible.

Thirty six replicates of 3-25 seeds each (actual number for each species listed in Table 1) were used for each treatment with a maximum of 5 different collection dates with both ripe and unripe seeds being collected during collections. Each replicate was marked for collection date and maturity and placed in a grid pattern with other treatments. Germination percentages were recorded 5 and 15 days after initial germination. For *Viburnum*, *Carpinus*, and *Rosa* species, trays with no germination after 14 weeks were put into cold stratification for 12 weeks and subsequently re-sown. Again, germination percentages were determined at 5 and 15 days after initial germination. We used a 2-way Analysis of Variance (ANOVA) to test for differences in germination between collection periods and stage of ripeness when both factors were present. For species that only a single collection was made a t-test was used to test for differences in germination between the treatments.

For *V. cassanoides* and *V. prunifolium* twenty-eight replicates were used to compare germination between cleaned (fruit removed) and un-cleaned seeds. Each replicate contained the same number of seeds as in the previous experiments (Table 1). A t-test was used to test for differences in mean germination.

Results

Doubly dormant species

No germination occurred during the initial 14 week germination phase for *Viburnum lentago*, *V. prunifolium*, or *Rosa palustris*. For *Viburnum cassanoides* germination did occur for all treatments. Un-cleaned seeds were significantly less likely to germinate than un-cleaned seeds (ttest, $p = 0.021$, $n = 28$). Mean germination ratios were 0.575 (SD = 0.229) for un-cleaned seeds and 0.704 (SD = 0.173) for cleaned seeds. Because of this seeds without the fruit removed were not included in the ANOVA for used to test differences in germination due to time of collection or ripeness of fruit. ANOVA showed no difference in germination due to time of collection ($p = 0.414$), ripeness of fruit ($p = 0.885$), or their interaction ($p = 0.030$). Mean germination ratios were 0.707 (SD = 0.172) for unripe seeds and 0.670 (SD = 0.193) for ripe seeds (Table 2).

For *V. lentago*, *V. prunifolium*, and *R. palustris* little to no germination occurred during the second warm period following 12 weeks cold stratification. Cold stratification was interrupted early for these species due to an equipment failure that brought the ambient temperature to above 40° C. There was no germination for *V. lentago* for any treatment; *V. prunifolium* had a total of 3 seeds germinate among all treatments; for *R. palustris* 22 seeds germinated. There was no difference in the germination due to ripeness of fruit for *R. palustris* (ttest, $p = 0.265$, $n = 36$).

Recalcitrant species

Germination occurred for both treatments for both *B. allegheniensis* and *B. lenta*. Germination was significantly higher in unripe seeds for both *B. allegheniensis* (ttest, $p < 0.0001$, $n = 36$) and *B. lenta* (ttest, $p < 0.0001$, $n = 36$). Mean germination (out of 25) for *B. allegheniensis* was 7.61 (SD = .292) for unripe seeds and 0.556 (SD = 0.773) for ripe seeds (Table 2). Mean germination (out of 25) for *B. lenta* was 7.83 (SD = 3.27) for unripe seeds and 3.81 (SD = 2.32) for ripe seeds.

For *Corylus fagesii* a cut test showed that only 2 of 10 fruits contained an embryo. Too few were collected and germinated to have statistical relevant results. However, of 21 fruits collected early and 56 fruits collected following maturity and desiccation, germination only occurred for the early collection (a single seedling).

Discussion

Doubly dormant species

The early harvest of seeds did not prevent double dormancy in the species selected. *V. cassanoides* however did not exhibit double dormancy in our populations, a result that conflicts with current literature (Dirr and Heuser 1987). Unripe seeds showed no lack of viability in this species and no significant difference in germination suggesting that propagators may be indiscriminate in their collection of seeds. The cleaning of fruit from seeds did improve germination though the magnitude of this difference was small (70% vs. 58%) and so would not warrant the effort involved unless only a very small quantity of seed was available.

Due to the equipment failure during cold stratification the results from *V. lentago*, *V. prunifolium*, and *R. palustris* are difficult to interpret. While no germination occurred in the initial 14 weeks of warm stratification, without subsequent germination after cold stratification it is impossible to know whether this is due to double dormancy or to lack of viability. For *V. prunifolium*, however, 50 seeds from the fifth collection were placed in warm stratification in perlite, moved to cold stratification after 14 weeks, and 5 seeds germinated while in cold stratification. This suggests that seeds from this collection (an unripe seed collection) were both viable and doubly dormant. Fifty seeds from each treatment of *R. palustris* were also stratified in perlite. Of the unripe seeds 3 germinated during the initial warm stratification, whereas none of the ripe seeds germinated during this period. It is difficult to explain this as none of the unripe seeds placed in warm stratification in soil germinated during the initial 14 week period. The ratio of seeds that germinated in warm stratification in perlite (0.06) was at least similar to the ratio of unripe seeds that eventually germinated in soil. If we could assume that the warm conditions produced by the equipment failure during cold stratification did not effect the germination of *R. palustris* this would suggest that the conditions during warm stratification in perlite are somehow different than in soil and that unripe seeds may germinate without cold stratification under these conditions. However, due to the lack of replication and the warm conditions produced by the equipment failure it is impossible to state this conclusively.

Betula species

Germination was significantly higher for unripe nutlets in both *Betula* species. The *Betula* species studied are wind dispersed (Houle 1998) but such dispersal does not appear to occur until after a degree of desiccation similar to that of the collections we made which had inferior germination. At this point the strobili may be easily crumbled in the hand into individual nutlets. Thus dispersal would not occur until the viability of seed was already reduced. This

seems contrary to the logic of evolutionary strategy and it has been suggested previously that recalcitrance is an ancestral trait (Pammenter and Berjak, 2000).

Literature cited

Baskin, J. M., and Baskin C. C. 1989. "Seed Germination Ecophysiology of *Jeffersonia Diphylla*, a Perennial Herb of Mesic Deciduous Forests". American Journal of Botany. 76: 1073-1080.

Browse, McMillan. 1979. Hardy Woody Plants from Seed. Grower Books.

Crocker, W. M. 1916. "Mechanics of Dormancy in Seeds". American Journal of Botany. 3: 99-120.

Dirr, M. A. 2007. Viburnums: Flowering Shrubs for Every Season. Timber Press.

Dirr, M. A. and Heuser, C. W. 1987. The Reference Manual of Woody Plant Propagation: From seed to Tissue Culture. Varsity Press.

Hartmann, H T., Kester, D. E., Davies F. T. Jr., and Geneve, R. L. 1997. Plant Propagation: Principles and Practices (6th edition). Simon & Shuster.

Houle, G. 1998. Seed dispersal and seedling recruitment of *Betula alleghaniensis*: spatial inconsistency in time. *Ecology*, 79(3): 807-818.

King, M. W., and Roberts, E. H. 1980. "Maintenance of recalcitrant seeds in storage". In H. F. Chin and E. H Roberts (eds). Recalcitrant Crop Seeds. Tropical Press SDN. BHD. Kuala Lumpur, Malaysia. Pp. 53-89.

Pammenter, N. W., and Berjak, P. 2000. "Evolutionary and ecological aspects of recalcitrant seed biology". Seed Science Research. 10: 201-306.

Vleeshouwers, L. M., Bouwmeester, H. J., and Karssen, C. M. 1995. "Redefining seed dormancy: an attempt to integrate physiology and ecology". Journal of Ecology. 83: 1031-1037.

Tables and Figures

Table 1: Species studied and corresponding number of seeds per replicate.

Species	Number of seeds/replicate	Number of collections
Betula allegheniensis	25	1
B. lenta	25	1
Carpinus caroliniana	10	1
Rosa palustris	10	1
Viburnum cassaniodes	10	3
V. lentago	4	2
V. prunifolium	3	5

Table 2: Mean number of seeds germinated per replicate by species and fruit maturity.

Species	Date collected	Mean number of seeds germinated	
		Ripe	Unripe
Betula allegheniensis	09/02/08	0.56	7.61
Betula lenta	09/02/08	3.80	7.83
Rosa palustris	09/05/08	0.39	0.25
Viburnum cassanoides		6.70	7.07
Collection 1	09/03/08	6.97	5.78
Collection 2	09/12/08	7.44	7.08
Collection 3	09/24/08	6.81	7.25
V. lentago*		0.0	0.0
Collection 1	08/20/08	0.0	0.0
Collection 2	08/29/08	0.0	0.0
V prunifolium*		0.0	~0.0
Collection 1	08/18/08		0.0
Collection 2	08/29/08		0.0
Collection 3	09/09/09		0.0
Collection 4	10/03/09		0.03
Collection 5	10/14/09	0.0	0.06

* = After 14 weeks warm stratification and 12 weeks cold stratification