

Variation in Seed Viability and Dormancy of 17 Weed Species after 24.7 Years of Burial: The Concept of Buried Seed Safe Sites

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A 50-yr study at Fairbanks, AK, was started in 1984 to determine soil seed longevity of 17 weed species. Seeds were buried in mesh bags 2- and 15-cm deep and were recovered 0.7, 1.7, 2.7, 3.7, 4.7, 6.7, 9.7, 19.7, and 24.7 yr later. Viability was determined using germination and tetrazolium tests. By 24.7 yr after burial (YAB), no viable seeds were found for common hempnettle, flixweed, foxtail barley, quackgrass, and wild oat. Bluejoint reedgrass, which had no live seed 19.7 YAB, again had viability (1% at 15 cm) 24.7 YAB. Seeds of 11 other species were still viable: American dragonhead (52%), marsh yellowcress (11 and 3.0% at 2 and 15 cm respectively), common lambsquarters (2.8%), prostrate knotweed (2.8% at 15 cm), shepherd's-purse (2.8%), pineapple-weed (2.6%), rough cinquefoil (2.3%), Pennsylvania smartweed (1.1%), common chickweed (0.4%), wild buckwheat (0.3%), and corn spurry (0.1%). Seed dormancy 24.7 YAB was very low (< 10%) for all species except American dragonhead (99%), shepherd's-purse (40%), marsh yellowcress (23% at 2 cm), Pennsylvania smartweed (18%), and rough cinquefoil (14%). At the beginning of the study, declines in seed longevity were uniform between replicates, but variability between replicates increased over time for all species except American dragonhead, suggesting that some soil microsites are more favorable for seed survival and may be seedbank "safe sites." Results of this study demonstrate that nonrandom seed mortality contributes to the spatial heterogeneity of seed populations in the soil seedbank.

Nomenclature: American dragonhead, *Dracocephalum parviflorum* Nutt. DRAPA; bluejoint reedgrass, *Calamagrostis canadensis* (Michx.) Beauv. CLMCD; common chickweed, *Stellaria media* (L.) Vill. STEME; common hempnettle, *Galeopsis tetrahit* L. GAETE; common lambsquarters, *Chenopodium album* L. CHEAL; corn spurry, *Spergula arvensis* L. SPRAR; flixweed, *Descurainia sophia* (L.) Webb. ex Prantl DESSO; foxtail barley, *Hordeum jubatum* L. HORJU; marsh yellowcress, *Rorippa palustris* (L.) Bess. RORIS (in previous manuscripts called *Rorippa islandica* (Oeder) Borbas); Pennsylvania smartweed, *Polygonum pensylvanicum* L. POLPY; pineapple-weed, *Matricaria discoidea* DC. MATMT (in previous manuscripts called *Matricaria matricariodes* (Less.) C.L. Porter); prostrate knotweed, *Polygonum aviculare* L. POLAV; quackgrass, *Elymus repens* (L.) Gould AGRRE (in previous manuscripts called *Elytrigia repens* (L.) Nevski); rough cinquefoil, *Potentilla norvegica* L. PTLNO; shepherd's-purse, *Capsella bursa-pastoris* (L.) Medik. CAPBP; wild buckwheat, *Polygonum convolvulus* L. POLCO; wild oat, *Avena fatua* L. AVEFA.

Key words: Buried seed, seed longevity, soil seedbank, weed seed decline, buried seed safe site.

The understanding of plant population biology and weed management is aided by knowledge of seed longevity in soil. Seeds in the soil seedbank continue to germinate and form competitive populations as long as a viable seedbank remains. Reducing the persistence of seeds in the soil seedbank is central to weed management (Davis et al. 2008) and knowledge of seedbank persistence is necessary to determine the duration of control measures and whether eradication is feasible (Panetta and Timmins 2004). Seed longevity data also have been used as criteria to assess the invasive potential of weeds (Daehler et al. 2004; Hiebert 1997).

Despite the need for seed longevity data, only this study and two others have examined seed longevity over a period lasting more than 20 yr. Beal's ongoing study of soil seed longevity of 21 species at Michigan State University has spanned over 120 yr (Darlington 1951; Telewski and Zeevaart 2002), and Duvel's study of 107 crop and weed species in Virginia continued for 39 yr (Toole and Brown 1946). Two additional studies examined seed longevity for a 20-yr period: Burnside et al. (1996) studied seed longevity of 41 species at two locations in Nebraska, and Lewis (1973) studied 39 crop and weed species in Wales, U.K.

This 50-yr buried seed longevity study was started at Fairbanks, AK in 1984 to determine the seed longevity and dormancy patterns of 17 plant species under subarctic conditions. We hypothesized that cold soil temperatures would increase seed longevity in soil, since examples of extreme seed longevity have occurred in frozen soil (McGraw et al. 1991; Shafer and Chilcote 1970).

Earlier results of this study showed no significant relationship between initial dormancy and longevity (Conn and Farris 1987). Dormancy was initially high but rapidly decreased for most species. By 4.7 YAB, only two species, common hempnettle and quackgrass, had lost all seed viability (Conn 1990). Foxtail barley and wild oat lost all viability by 9.7 YAB (Conn and Deck 1995). By 9.7 YAB, \leq 5% seed viability remained for bluejoint reedgrass, common chickweed, common lambsquarters, corn spurry, flixweed, marsh yellowcress, Pennsylvania smartweed, pineapple-weed, prostrate knotweed, rough cinquefoil, shepherd's-purse, and wild buckwheat, whereas 62% of American dragonhead seeds were still viable (Conn and Deck 1995). Despite the low viability for most species, only bluejoint reedgrass lost all viability between 9.7 and 19.7 YAB (Conn et al. 2006).

The 19.7-YAB seed data were described with an exponential model using nonlinear regression (Conn et al. 2006), and burial depth for each species was tested using a likelihood ratio test (Haddon 2001) to predict viability. Depth of burial was a significant factor explaining decline of seed viability for only 4 of the 17 species: bluejoint reedgrass, marsh yellowcress, prostrate knotweed, and wild oat.

Variability between replicates in weed seed longevity has been noted (Gardarin and Colbach 2009). In long-term seed viability studies it is common that species will appear to lose

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Figure 1. Changes in Morisita's index of dispersion and the percentage of replicates containing viable seed for 17 weed species over 24.7 yr. Data from the 2- and 15-cm soil depths were combined for species where depth was not a significant factor influencing seed viability decline at 19.7 yr after burial. A Morishita's index of 0 indicates uniform dispersal, 1 indicates random dispersal, and a number equal to the number of samples (*n*) indicates the most clumped dispersal, where all viable seed occurred in one seed packet.

viability and then later in the study replicates will be exhumed that contain viable seed. Such "reversals" in viability were found for species in Beal's (Darlington 1951; Telewski and Zeevaart 2002) and Duvel's (Toole and Brown 1946) studies. The possibility exists that some soil microsites are more conducive for seed longevity than are others.

This paper reports on the viability and dormancy of the buried seeds 24.7 YAB and examines the variability in seed



Figure 1. Continued.

viability over the course of the study. We also examined the variability in seed longevity between replicates of seed packets over time to determine whether some seed burial sites are safer than others.

Materials and Methods

Burial and Viability Methods. A complete description of seed burial and viability testing procedures was detailed in



Figure 1. Continued.

previous papers (Conn 1990; Conn and Farris 1987). On October 2, 1984, 100 seeds of each weed species were buried in mesh bags at 2- and 15-cm soil depth. Enough bags were buried to allow for four replicates of each species and burial depth to be recovered on 15 dates spanning 50 yr. Seeds were previously recovered in mid-May 0.7, 1.7, 2.7, 3.7, 4.7, 6.7, 9.7, and 19.7 YAB.

Seeds were recovered by replicate on different dates from May 26 to June 3, 2009, 24.7 YAB. Immediately after seeds were exhumed, intact seeds were removed from the bags and



Figure 1. Continued.

were placed on moist filter papers and incubated for 14 d at temperatures favorable for germination (Conn and Farris 1987). Seed coats of seeds that did not germinate were then scarified and incubated for an additional 7 d. Nongerminated seeds were then placed in a 3,000 parts per million (ppm) 2,3,5-triphenyltetrazolium chloride (TTC) solution (pH 7.0) for 7 to 14 d. Seeds were then dissected, and seed with pink to red embryos were recorded as viable.

Statistical Analysis. Total seed viability (TOTAL) was calculated as the sum of seeds that germinated before scarification plus seeds that germinated after scarification (GAS) plus nongerminated seeds with TTC-stained embryos (STAINED). The percentage of seeds that were dormant was calculated as ([{GAS + STAINED}/TOTAL]100). Means and standard errors were calculated for all species; however, for mean percentage dormancy, only replicates with viable seed were included. We combined data from 2- and 15-cm depths for all species where our analysis of the 19.7-YAB data (Conn et al. 2006) showed that burial depth was not a significant variable explaining weed seed viability decline. We made separate analyses of the two burial depths for bluejoint reedgrass, marsh yellowcress, prostrate knotweed, and wild oat.

To examine the variability in seed viability decline, we calculated Morisita's index of dispersion (Morisita 1962) and the proportion of seed packets containing viable seed for each species in each year. Morisita's index is a more appropriate index of dispersion than indices based on variance-to-mean ratios, since it is independent of sample mean and size (Elliott 1977). Morisita's index of dispersion (I_d) was calculated for each species and each year seeds were exhumed:

$$I_{\rm d} = n \left(\left[\Sigma x^2 - N \right] / \left[N \{ N - 1 \} \right] \right)$$

where *n* is the number of seed packet replicates, *N* is the total number of viable seed in all *n* seed packets, and Σx^2 is the square of the numbers of individuals per packet, summed over all packets.

Results and Discussion

Flixweed and prostrate knotweed at the 2-cm depth were the only species to lose viability during the 5-yr period since seeds were last exhumed (Table 1). At 24.7 YAB, less than 5% seed viability was observed for all species except American dragonhead (52% seed viability) and marsh yellowcress (11% at 2-cm depth). American dragonhead continued its slow seed decline (Conn et al. 2006), and appears to have extreme seed longevity. It is a successional species following forest fires and is found in the seedbank of subarctic forests and agricultural fields (Conn et al. 1984). American dragonhead seed is a preferred food of Alaskan birds in winter because of its high fat content, but it is highly dormant, with germination only effectively enhanced in the laboratory by the extreme treatments of soaking in hot water and 500 ppm gibberellic acid (Van Veldhuizen and Knight 2006).

At 19.7 YAB, we recognized a suite of 11 species that were characterized by rapid initial viability loss followed by very slow viability loss: bluejoint reedgrass, common chickweed, common lambsquarters, corn spurry, flixweed, marsh yellowcress, Pennsylvania smartweed, pineapple-weed, prostrate knotweed, rough cinquefoil, and shepherd's-purse. Species exhibiting such a pattern have been categorized as "long-term persistent" (Thompson et al. 1997). Viability of these species did not decline appreciably in the 5 yr between 19.7 and 24.7 YAB, except for flixweed and the 2-cm depth of prostrate knotweed, which had no viable seeds at 24.7 YAB. The weed species with remaining viability may have seeds located in

Table 1. Mean and standard errors of seed viability and dormancy 19.7 and 24.7 yr after burial (YAB) at Fairbanks, AK. Dormancy was calculated only with replicates (*n*) with viable seed. Data from 2- and 15-cm depths were combined for species where depth was not a significant factor influencing seed viability decline at 19.7 YAB.

Common name	Depth	Viability		Dormancy	
		19.7	24.7	19.7	24.7
	cm				
American dragonhead	2 + 15	62.3 ± 4.5	51.9 ± 8.9	$99.6 \pm 0.2 \ (8)^{a}$	$99.3 \pm 0.5 (8)$
Bluejoint reedgrass	2	$0.0~\pm~0.0$	0.0 ± 0.0	_b	_
	15	$0.0~\pm~0.0$	1.0 ± 1.0	_	0.0 (1)
Common chickweed	2 + 15	1.6 ± 1.6	0.4 ± 0.3	0.0 (1)	0.0 ± 0.0 (2)
Common hempnettle	2 + 15	$0.0~\pm~0.0$	0.0 ± 0.0	_	_
Common lambsquarters	2 + 15	3.0 ± 1.8	2.8 ± 1.7	26.0 ± 13.7 (5)	9.8 ± 6.1 (4)
Corn spurry	2 + 15	0.1 ± 0.1	0.1 ± 0.1	0.0 (1)	0.0 (1)
Flixweed	2 + 15	0.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0 (2)	_
Foxtail barley	2 + 15	$0.0~\pm~0.0$	$0.0~\pm~0.0$	_	-
Marsh yellowcress	2	8.5 ± 7.2	10.8 ± 5.1	0.0 ± 0.0 (3)	23.0 ± 23.0 (4)
	15	0.3 ± 0.3	3.0 ± 1.5	0.0 (1)	0.0 ± 0.0 (3)
Pennsylvania smartweed	2 + 15	2.4 ± 1.2	1.1 ± 0.7	50.0 ± 28.9 (4)	17.8 ± 9.7 (3)
Pineapple-weed	2 + 15	1.6 ± 0.9	2.6 ± 1.3	7.1 ± 7.1 (4)	0.0 ± 0.0 (4)
Prostrate knotweed	2	0.3 ± 0.3	0.0 ± 0.0	0.0 (1)	_
	15	$0.8~\pm~0.8$	2.8 ± 2.1	0.0 (1)	0.0 ± 0.0 (3)
Quackgrass	2 + 15	0.0 ± 0.0	0.0 ± 0.0	_	_
Rough cinquefoil	2 + 15	1.6 ± 1.2	2.3 ± 0.8	0.0 ± 0.0 (3)	$14.0 \pm 5.8 (5)$
Shepherd's-purse	2 + 15	1.3 ± 0.6	2.8 ± 2.3	75.0 ± 17.7 (4)	39.5 ± 24.4 (4)
Wild buckwheat	2 + 15	1.5 ± 0.6	0.3 ± 0.3	0.0 ± 0.0 (5)	0.0 (1)
Wild oat	2	0.0 ± 0.0	0.0 ± 0.0	_	<u> </u>
	15	$0.0~\pm~0.0$	0.0 ± 0.0	_	_

^a Number in parentheses is the number of replicates (n) with viable seed.

^b Unable to calculate with zero in denominator.

advantageous soil microsites or they may be genotypes within the seed population that intrinsically have a greater ability to survive for long periods. For example, foxtail (*Setaria* spp.) plants are known to produce seeds with varying dormancy and germination characteristics, resulting in seedlings that emerge at different times during the cropping cycle and in different years, thus allowing the species to survive detrimental changes in farming practices and weather (Dekker and Hargrove 2002).

Most species had very low dormancy (< 10%) and germinated without scarification. Exceptions to this were American dragonhead, shepherd's-purse, marsh yellowcress at 2-cm depth, Pennsylvania smartweed, and rough cinquefoil, which had seed dormancies of 99, 40, 23, 18, and 14%, respectively.

Bluejoint reedgrass, which did not have viable seed at 19.7 YAB, again exhibited viability at 24.7 YAB. There were four viable seeds in one of the eight seed packets recovered. A similar "reversal" in loss of viability was found early in this study for common hempnettle, which had no viable seed 2.7, 3.7, and 4.7 YAB, but had two viable seeds in a single replicate at 6.7 YAB. Similar "reversals" in loss of viability were found for species in Beal's (Darlington 1951; Telewski and Zeevaart 2002) and Duvel's (Toole and Brown 1946) studies.

The variability in seed viability decline was studied using Morisita's index of dispersion and the percentage of replicates with viable seed (Figure 1). A Morishita's index of 0 indicates uniform dispersal, 1 indicates random dispersal, and an index value equal to the number of seed packet replicates indicates the most clumped arrangement when all viable seeds occurred in one seed packet (Morisita 1962). At the beginning of the study, there was nearly uniform seed viability in all replicates for each species. As time progressed, the variability in seed viability between replicates increased dramatically for all species except American dragonhead. Morisita's index increased from values indicating random dispersal of viable seed (approximately 1) in the early years of the study to values indicating a clumped dispersal of viable seed (4 for 2- or 15cm depths analyzed separately or 8 for combined depths). This trend is also reflected by the decline of percentage of replicates with viable seed over time. Morisita's index increased somewhat for most species as the number of viable seeds remaining declined (Figure 1). Gardarin and Colbach (2009) also noted variability in seed survival among replicates in a buried seed study at Dijon, France.

The nonrandom, clumped distribution of seed viability between replicates suggests that some seed bag replicates were in soil microsites that favored the maintenance of seed viability, whereas others were not. There were numerous instances where all viability had been lost in some replicates, whereas in other replicates of the same species, viability still remained near 100%. Results of this study demonstrate that rates of seed mortality are variable within the soil seedbank. The soil environment contains a wide array of microhabitats with different physical, chemical, and biological properties that may interact differently with buried seeds (Chee-Sanford et al. 2006). Similar to safe sites for seed germination (Harper et al. 1965), certain soil microsites may be safe sites for seed survival.

Although the mesh bags used in this study protected seed from predation by large insects, the seeds were still susceptible to attack by fungi and microorganisms. However, the high density of seeds in burial containers may enhance seed mortality from pathogenic fungi because of enhanced seed-toseed contact (Van Mourik et al. 2005).

Soil seedbanks are known to be spatially variable, but this is generally thought to be caused by the nonrandom spatial patterns of plant populations, associated seed rain, and cropping practices (Dessaint et al. 1991). Results of this study demonstrate that nonrandom seed mortality patterns contribute to the spatial heterogeneity of seed populations in the soil seedbank. Our results suggest that the number of replicates be increased for the later years of seedbank viability studies because of the increased variability caused by nonrandom seed mortality. The continued viability of 12 of 17 weed species after nearly 25 yr of burial is a strong argument for preventing any seed rain by new weed species.

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