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Influence of Seed Depth and Pathogens on Fatal Germination of Velvetleaf (*Abutilon theophrasti*) and Giant Foxtail (*Setaria faberi*)

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Fatal germination of weed seeds occurs when a weed seed germinates, but the seedling dies before reaching the soil surface. Controlled-environment bioassays of velvetleaf and giant foxtail seed fate in Michigan field soil (Kalamazoo silt loam, 1.1% soil organic matter) were used to determine the role of pathogenic fungi and seed burial depth in fatal germination of these species. Fatal germination at 2 cm seed depth was nonexistent for giant foxtail, and rare (< 10% of seeds studied) for velvetleaf. At greater depths, fatal germination remained close to zero for giant foxtail, whereas it increased to as high as 40% for velvetleaf at a 10-cm burial depth. Cultures taken from fatally germinated velvetleaf seedlings were found to contain *Pythium ultimum*, a soilborne pathogen known as the causal agent for pea root rot. When samples of infected media taken from these cultures were used to inoculate field soil in pots, fatal germination of velvetleaf from depths of 4 to 6 cm increased, compared with field soil inoculated with sterile media. At seed burial depths of 8 and 10 cm, fatal germination of velvetleaf increased to 20 and 40%, respectively, and was the same for unsterilized soil and *P. ultimum*–inoculated soil. Given that maximal fatal germination of velvetleaf occurred in the unsterilized soil treatment at the 10 cm depth, burial of newly shed velvetleaf seeds to a 10 cm, or possibly greater, depth with tillage equipment may be a practical way of reducing velvetleaf seed banks through fatal germination.

Nomenclature: Giant foxtail, *Setaria faberi* Herrm. SETFA; velvetleaf, *Abutilon theophrasti* Medik. ABUTH.
Key words: Fatal germination, seed burial depth, soil fungal pathogens, *Pythium ultimum*, root rot, tillage.

Seed fate plays a central role in the population dynamics of annual weeds because all individuals in a population must pass through the seed life-stage (Cousens and Mortimer 1995). Fluctuations in weed seed-banks over time are governed by changes in persistent and nonpersistent seed (Schafer and Chilcote 1969). Persistent seeds remain in the seed bank but cycle through dormancy states over time in response to environmental variation (Baskin and Baskin 2001; Benech-Arnold et al. 2000). Nonpersistent seeds are lost from the seed bank through mortality because of aging (Telewski and Zeevaart 2002), attacks by seed predators (Westerman et al. 2003), seed decay (Schafer and Kotanen 2003), and germination (Schafer and Chilcote 1969). Germination of weed seeds often results in successful seedling emergence from the soil surface (Benvenuti et al. 2001), and much of the work done in weed science over the past two decades has been targeted at either predicting the timing and magnitude of weed seedling emergence or optimizing methods for controlling weed seedlings. Another possible, but poorly characterized, pathway for seeds in the weed seed bank is mortality of the seedling before emergence from the soil or ''fatal germination'' (Fenner and Thompson 2005). One of the major obstacles to quantifying fatal germination in weed populations is the rapid degradation of necrotic seedling tissue within the soil (Schafer and Chilcote 1969), causing seed losses through this pathway to be pooled with seed losses from decay (Davis et al. 2005).

Fatal germination of weeds has been studied primarily in relation to soil physical parameters, such as depth (Benvenuti et al. 2001), temperature and moisture (Schafer and Chilcote 1970). Emergence from the weed seed-bank declines as a function of increasing seed burial depth primarily because of depth inhibition by dormancy, although fatal germination also accounts for part of the decline in seedling emergence (Benvenuti et al. 2001). Successful weed seedling emergence also declines in response to soil moisture and temperature extremes (Schafer and Chilcote 1970). Seed germination and seedling survival of annual weeds can be reduced by exposure to pathogenic fungi, which have been found to reside, in some cases, on weed seed coats (Kirkpatrick and Bazzaz 1979). Our objective in this study was to determine whether there is a pathogenic component to fatal germination of weed seeds, and if so, how such a mechanism would interact with depth effects on seed germination. This information is important in understanding whether seed burial may hasten seed-bank decline over time for certain species by increasing fatal germination. Furthermore, if there is a pathogenic component to fatal germination, the mechanism could be exploited in the development of new biocontrol tactics that use pathogenic fungi (Hallett 2005).

We set out to determine the prevalence of, and mechanisms contributing to, fatal germination of velvetleaf and giant foxtail seeds. These weed species were chosen because of their economic importance in the Corn Belt of the north-central United States (Lindquist et al. 1996, 1999) and also because of their difference in seed mass (velvetleaf seed mass = 8.6 g 10^3 seeds^{-1}; giant foxtail seed mass = 2.1 g 10^3 seeds^{-1}), shown by Benvenuti et al. (2001) to correlate with depth inhibition of seedling germination. We hypothesized that fatal germination is caused not only by limitation of seedling emergence because of excessive seed burial depth (i.e., insufficient seedling ''push power'' to reach the soil surface) but by pathogenic attack of newly germinated seeds as well. Our investigation was divided into several research questions modeled after Koch's postulates (Agrios 1997): (1) does fatal germination occur for giant foxtail and velvetleaf seeds buried in soil at depths that do not limit seedling emergence? (2) does soil containing fatally germinated seedlings increase fatal germination in viable seeds? (3) can a pathogenic agent be isolated from fatally germinated seedlings? and (4) does inoculation of field soil with the putative pathogen alter seedling recruitment from depth compared with sterilized or untreated soil?

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Materials and Methods

Bioassays of Fatal Germination. Field soil for bioassays was collected in late March 2004, to a depth of 10 cm from an area of the Michigan State University, W. K. Kellogg Biological Station, in Hickory Corners, MI. This field was managed in an early successional state through burning and mowing. The soil collected from this site was a Kalamazoo silt loam (Typic Hapludalf; 43% sand, 40% silt, 17% clay, 1.1% organic matter, and pH 6.7). Soil samples were bulked to form composite samples and stored in sealed plastic bags at 4 C for up to 1 mo before use. Subsamples of the bulk soil were elutriated and analyzed for ambient densities of viable giant foxtail and velvetleaf seeds using tetrazolium assays (Peters 2000). No viable seeds of either species were found in the soil; therefore, ambient seed-bank was not included as a parameter in estimation of seed-fate fractions. Seed lots of giant foxtail and velvetleaf for use in bioassays were collected in October 2003 from populations in crop field margins at the Michigan State University Crop Sciences Farm, in East Lansing, MI. Seeds were collected by gently shaking mature seed heads over a bucket, and chaff or light seed was removed with a seed cleaner.[1] Cleaned seed lots were allowed to air dry in the open at 25 C for several days before being stored in the dark in air tight plastic containers at 4 C before use in April 2004. Several days before bioassays were initiated, initial viability of both seed lots was determined with tetrazolium assays.

Several different bioassay methods were used to address research questions 1 through 4. *Rag-doll Bioassays*. The method for studying question 1 employed ''rag doll'' bioassays assembled as described by Dabney et al. (1996). Each experimental unit in the study consisted of either 40 velvetleaf or giant foxtail seeds spaced in a line 10 cm from the top edge of a double layer of 25 cm by 38 cm germination paper[2] and moistened with 20 ml of distilled, deionized H_2O. The field-moist equivalent of 100-g dry weight soil was spread in a 1-cm-deep band, starting 2 cm above, and extending 12 cm below, the line of seeds. A third premoistened sheet of germination paper was placed on top of the soil layer, and the entire assembly was rolled along the short axis, wrapped in plastic film, and secured with rubber bands. Units were incubated vertically in a Conviron 125-L incubator[3] for 96 hr at 25 C in the light (16 hr) and 20 C in the dark (8 hr). Two consecutive runs of the bioassay with 10 replications per species were performed in a completely randomized design within runs. At the end of the incubation period, bioassay units were unrolled, and seed fates were recorded as belonging to one of four classes: fatal germination, emerged seedling, dormant seed, or decayed seed.

There are no published protocols for classifying weed seedlings as fatal germinants; therefore, we applied the following rule: fatal germinants were those seeds that obviously had no chance of reaching the surface, no matter how long the incubation period. Seeds had to match two or more of the following criteria to be classified as fatal germinants: (1) seed imbibed but no longer viable as determined by visual inspection and gentle pressure with a forceps followed by tetrazolium testing, (2) radicle lengths less than 25% of other seedlings within the experimental unit, (3) cotyledons depleted and withered but shoot tip still less than half the distance between original seed position and soil surface, and (4) necrotic lesions on the radicle. Seedlings with cotyledons that were fully emergent from the soil surface or that were still beneath the soil surface but did not meet the criteria for fatal germination were counted as emerged seedlings. Ungerminated seeds were classified as dormant or nonviable through tetrazolium testing.

Inoculation of Sterile Medium. To address research question 2, we used seedlings from each of the different seed-fate classes in the rag-doll bioassays to inoculate a sterile plant propagation medium,[4] containing 4.3 g Murashige and Skoog (MS) salts, 30.0 g sucrose, 0.17 g $NaPO_4$, 1.0 ml thiamine, 0.1 g myo-inositol, and 8.0 g Bacto-Agar[5] dissolved in 1 L of distilled, deionized H_2O, with no addition of antibiotics (D. S. Douches, personal communication). Each experimental unit in this bioassay consisted of one emerged seedling, fatal germinant, or dormant seed placed in the center of a 110-mm petri dish containing sterile growth medium and 40 viable seeds of either velvetleaf or giant foxtail, spaced at least 2 cm away from the inoculum. All seed transfers to plates were performed within a laminar flow hood to prevent accidental fungal or bacterial infection from nonexperimental sources. Experimental units were sealed with Parafilm[6] and then incubated within the same clean hood for 96 h at 25 C in the light. At the end of the incubation, percentage of germination and radicle length were recorded. Ungerminated seeds were subjected to tetrazolium testing to determine whether they were dormant or nonviable. Two consecutive runs of the experiment were performed in a completely randomized design with four replications per run.

Pathogen Culture and Identification. To address research question 3, at the end of the above bioassay, sterile inoculation loops were used to streak four new MS propagation medium plates per seed-fate class with culture samples from plates from the corresponding seed-fate class. One culture from each seed-fate class was submitted to the Michigan State University Extension Diagnostic Services Laboratory, and another set of cultures was submitted to the North Carolina State University Plant Pathogen Identification Laboratory, for identification of possible plant pathogens. Technicians at these locations then transferred the cultures to several media, including corn meal agar (CMA), potato dextrose agar, alkaline water agar, CMA plus antibiotics, and H_2O grass-leaf culture. Identification was made after a 2-wk incubation period based on hyphal structures and oospores. The remaining cultures from research question 2 were used as inoculum in the following greenhouse bioassay.

Greenhouse Bioassay. To address research question 4, we created a factorial treatment design of weed species, seed planting depth, and soil treatment. We planted 40 seeds of either giant foxtail or velvetleaf at 2, 4, 6, 8, or 10 cm depths in field soil within 15-cm-tall by 10-cm-diam pots in the greenhouse. Soil in each experimental unit was assigned to one of three soil treatments: unsterilized control, sterile, and inoculated. In preparation for the bioassays, field moist soil was sifted through a 2-mm standard soil sieve and homogenized. A test of four subsamples of the soil indicated that the gravimetric water content was consistent throughout the sample, with a mean content of 19 $\pm$ 0.3%, slightly drier than field capacity for this soil (A.J.M. Smucker, personal communication). Soil for the sterile treatment was autoclaved

within a polyethylene biohazard bag for 2 h at 128 C. Unsterilized soil was added to 4, 6, 8, 10, or 12 cm depths in the control and inoculated experimental units, and sterilized soil was added to the same depths in the sterile experimental units. A 1 by 1 cm square of agar was then added to the soil surface in each pot, with sterile agar added to the unsterilized control and sterile treatments and agar from the fatal germination treatment from research question 2 added to the inoculated treatment. The seeds were then arranged on the soil surface at 2 cm equidistant from the agar cube, and soil of the appropriate treatment was added to the pots to bring the soil level to 1 cm from the rim of the pot. The initial weight of each experimental unit was recorded and all units were subsequently weighed on a daily basis, with additional water added to make up the mass deficit. Bioassays were performed in a randomized complete-block design with four replications, blocked by location on the greenhouse bench, and were repeated in a second run that started 2 wk after the first. Day/night temperatures in the glasshouses were 25/15 C. Natural light was supplemented with high-intensity discharge lamps supplying 120-μmol photosynthetically active radiation. After 10 d, seed fates were analyzed for each experimental unit. First, emerged seedlings were counted; then, soil from each experimental unit was carefully removed, using the criteria listed for the rag-doll bioassays to identify fatally germinated, dormant, or decayed seed.

Data Analysis. Seed-fate data for velvetleaf and giant foxtail were subjected, within species, to ANOVA with the GLM subroutine of SYSTAT 11.0.[7] Models contained terms for experimental run, replication, seed fate, and for the greenhouse bioassay, soil management. Tests for normality and constant error variance (Neter et al. 1996) indicated that the data for research questions 1 and 2 met the requirements for ANOVA; therefore, data were not transformed before analysis. Data for research question 4, the greenhouse bioassay, were subjected to a $\sin^{-1}(x^{0.5})$–transformation before analysis to satisfy ANOVA requirements. There was no significant effect of experimental run in any of the bioassays; therefore, data for experimental runs were combined. Protected, Bonferroni-corrected multiple comparisons (Neter et al. 1996) were used in mean separations of seed fates.

Results and Discussion

Nondepth-Mediated Fatal Germination. The number of velvetleaf fatal germinants was numerically, but not statistically, different from zero in the rag-doll assays (Table 1). There were no giant foxtail fatal germinants in the rag-doll bioassays. Given that 800 seeds were screened per species, these results indicate that, at least for the bioassay conditions used here, nondepth-mediated fatal germination is a very rare event for velvetleaf populations and likely nonexistent for giant foxtail populations. For the purposes of this experiment, however, the presence of several fatally germinated velvetleaf seedlings provided the opportunity to obtain tissue from fatal germinants for further study.

Fatal germination was less prevalent, with the exception of seed decay, than other seed fates. The majority of velvetleaf (83%) and giant foxtail seeds (54%) in the rag-doll bioassays germinated and emerged from the soil (Table 1). The second most common seed fate was dormancy, with 16% of velvetleaf and 46% of giant foxtail seeds classified into this category. Germination of this latter class of velvetleaf seeds, when sliced open for subsequent tetrazolium tests, suggested that the dormancy observed in this experiment was primarily due to hard-seededness (Stoller and Wax 1974). The high germination rate of velvetleaf seeds in this experiment is somewhat unusual for this species, which more commonly has only moderate germination in a given year, forming highly persistent seed banks (Buhler and Hartzler 2001). Variations in maternal environment during seed development have large impacts upon the quality and dormancy state of seeds entering the soil seed-bank (Kegode and Pearce 1998; Nurse and DiTommaso 2005).

Table 1. Fates of velvetleaf and giant foxtail seeds in rag doll bioassays.

Seed fate[a]	Weed species[b]	
	Velvetleaf	Giant foxtail
	%	
Emerged seedling	82 c	54 c
Fatal germination	2 a	0 a
Dormant seed	16 b	46 b
Decayed seed	0 a	0 a

[a] Seed fate was calculated as a percentage of viable seeds, summing to 100% over the four seed fate classes; $n = 800$ seeds species^{-1}.

[b] Values represent the mean of two runs of 10 replications. Within species, means followed by different lowercase letters were determined to be significantly different at $P < 0.05$ by a protected Bonferroni-adjusted multiple comparison test.

There were no decayed seeds for either giant foxtail or velvetleaf in the rag-doll bioassays (Table 1), as would be expected for experiments lasting only 3 d. Field experiments with buried seeds of giant foxtail and velvetleaf have shown seed decay rates as high as 27 and 23%, respectively, for these species for the period from late October through late March (Davis et al. 2005). There are currently no data to quantify the short-term temporal profile of seed decay in soil seed banks. Shorter-duration burial experiments need to be performed to determine the onset of seed decay for different species.

Inoculum Effects on Seed Fate. Tissue from each of the seed-fate classes in the rag-doll bioassays were used in an inoculation bioassay addressing research question 2: Can fatally germinated seedlings induce fatal germination in viable seeds? Because there were no giant foxtail fatal germinants in the rag-doll bioassay, this seed-fate class was not included in the inoculation bioassay. Percentage of seed germination was unaffected by inoculum source for either species (Table 2). Velvetleaf radicle length was affected by inoculum source ($P < 0.01$) and was shortest for seeds exposed to fatal germinants, intermediate for seeds exposed to emerged seedlings, and greatest for seeds exposed to dormant seed. Giant foxtail radicle length was unaffected by inoculum source. The velvetleaf seedlings exposed to fatal germinant inoculum had radicles that were less than 25% of the length of seedlings exposed to other inoculum sources. When these seedlings were inspected under ×10 magnification, there were extensive lesions and sloughing of root hairs in seedlings exposed to fatal germinants compared with those exposed to other inoculum sources (data not shown). In addition, the seedlings clearly had stopped growing and were becoming necrotic. By the criteria described in the Material and Methods section that was used to identify fatal germinants

Table 2. Fates of velvetleaf and giant foxtail seeds on sterile agar inoculated with emerged seedlings, fatal germinants or dormant seeds from screening bioassay.[a,b]

Inoculation material	Velvetleaf		Giant foxtail	
	Germination	Radicle length	Germination	Radicle length
	%	mm	%	mm
Emerged seedling	83 a	59.2 b	44 a	47.0 a
Fatal germinant	87 a	13.8 a	—[c]	—
Dormant seed	83 a	72.3 c	47 a	46.5 a

[a] Germination percentages were taken within each type of inoculation material, and do not total 100% within columns.

[b] Values represent the mean of two runs of four replications. Within columns, means followed by different lowercase letters were determined to be significantly different at $P < 0.05$ by a protected Bonferroni-adjusted multiple comparison test.

[c] No fatal germination of giant foxtail seeds was found in rag-doll bioassays; therefore, there were no fatal germinants to use as inoculation material.

in the rag-doll bioassays, the seedlings grown in propagation media exposed to fatally germinated seedlings were classified as fatal germinants themselves.

Identification of Putative Pathogenic Agent. Cultures made from media exposed to seedlings from different seed-fate classes were used to address external research question 3: Can a pathogenic agent be isolated from fatally germinated seedlings? External experts from the Michigan State University Extension Diagnostic Services Laboratory and the North Carolina State University Plant Pathogen Identification Laboratory found no pathogenic agents in media exposed to dormant seeds or emerged seedlings. However, both labs identified media exposed to fatal germinants as being infected with *Pythium ultimum*, a pathogenic fungus that is a common cause of root rot of pea (*Pisum sativum* L.) (Malvick and Babadoost, 2002). Although other pathogenic fungi have been found on the surface of velvetleaf seeds (Kirkpatrick and Bazzaz 1979; Kremer 1986), *P. ultimum* is not one of them, being soilborne. It is important to note here that there are at least two possible relationships between *P. ultimum* and fatal germination of velvetleaf seeds in bioassays. First, *P. ultimum* may have attacked newly germinated velvetleaf seeds, causing damping off of the seedlings, as it does in peas, especially under poorly drained soil conditions. It is also possible that a different pathogen that could not be cultured, or some other unexplained factor, caused fatal germination in velvetleaf seedlings and that *P. ultimum* grew as a saprophyte on dying velvetleaf seedlings. Whatever the true relationship may be, it does appear that inoculation of sterile media with velvetleaf seedlings infected primarily by *P. ultimum* can induce fatal germination in viable velvetleaf seeds.

Soil Inoculation and Seed Depth Placement Effects on Fatal Germination. Greenhouse bioassays were used to address research question 4: Does inoculation of field soil with the putative pathogen alter seedling recruitment from depth compared with sterilized or untreated soil? Seedling emergence decreased with depth ($P < 0.001$) for both velvetleaf and giant foxtail (Figures 1a and 2a). For velvetleaf, however, the decline in seedling emergence with depth interacted with soil management treatment ($P < 0.05$). For the nonsterile control and sterile soil treatments, velvetleaf seedling emergence remained constant at about 60% from 2 to 6 cm burial depth, after which, it declined (Figure 1a). For the inoculated soil treatment, however, velvetleaf seedling

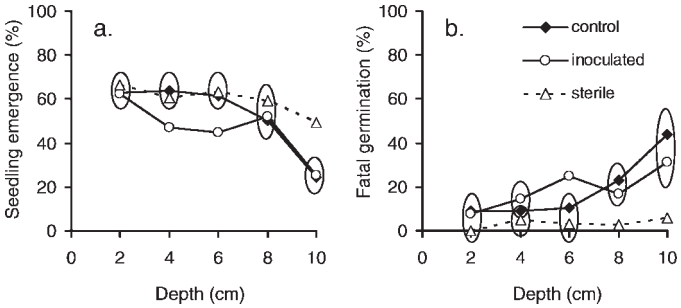

Figure 1. Velvetleaf seedling (a) emergence and (b) fatal germination with seed burial depth in sterile soil and unsterilized soil inoculated with sterile media, and soil inoculated with agar exposed to fatally germinated seedlings. Each point represents the untransformed mean of two runs of four replications. Points joined by ellipses were not significantly different at $P < 0.05$, by a Bonferroni-corrected multiple-comparison test. Analyses were performed on $\sin^{-1}(x^{0.5})$–transformed data.

emergence declined from 63% at the 2 cm depth to 47 and 44% at the 4 and 6 cm depths, respectively. This was significantly lower seedling emergence than the control or sterile treatments. At 8 cm seed burial depth, seedling emergence for the three soil treatments was not significantly different, but at 10 cm burial depth, velvetleaf seedling emergence in both the control and inoculated treatments declined sharply to 25%, whereas seedling emergence in the sterile treatment declined only slightly, to 49%. The effect of seed burial depth on giant foxtail seedling emergence was not influenced by soil management treatment (Figure 2b), with rapid declines in both the control and sterile treatments from 2 to 4 cm, a leveling off between 4 and 8 cm, and another large decline in seedling emergence between the 8 and 10 cm depth.

Velvetleaf fatal germination showed a strong interaction ($P < 0.01$) between soil treatment and seed burial depth (Figure 1b). For the sterile soil treatment, fatal germination remained relatively low across all depths, ranging between 0 and 6%. The control treatment had low levels of fatal germination (9 to 10%) between 2 and 6 cm, at which depths velvetleaf fatal germination was not significantly different among the control and sterile soil treatments. At 8 and 10 cm, fatal germination increased rapidly to 23 and 44%, respectively, for the nonsterile control treatment. Fatal germination of velvetleaf in the inoculated treatment was not significantly different from the control or the sterile treatments at the 2-cm

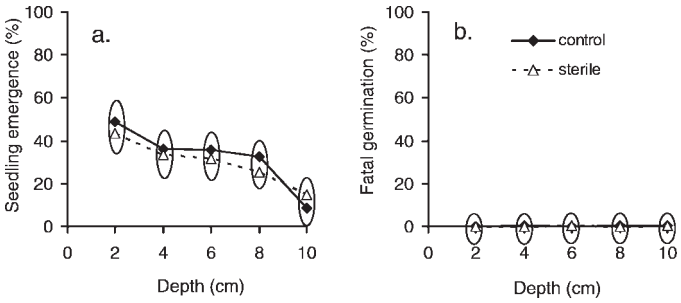

Figure 2. Giant foxtail seedling (a) emergence and (b) fatal germinations with seed burial depth in sterile soil and unsterilized soil inoculated with sterile media. Each point represents the untransformed mean of two runs of four replications. Points joined by ellipses were not significantly different at $P < 0.05$, by a Bonferroni-corrected multiple-comparison test. Analyses were performed on $\sin^{-1}(x^{0.5})$–transformed data.

seed burial depth, but fatal germination was greater in the sterile treatment at 4 cm and greater than both the sterile and control treatments at 6 cm. At the 8 and 10-cm soil burial depths, fatal germination levels in the inoculated treatment increased to 17 and 31%, respectively, in parallel to the control treatment, from which it was not significantly different.

Fatal germination remained close to zero at all depths for giant foxtail (Figure 2b). Therefore, it appears that reduction in seedling emergence for giant foxtail with increased seed burial depth is due mainly to decreased stimulation of germination rather than increased fatal germination. Benvenuti et al. (2001) found the same decrease in stimulation of seed germination with seed burial depth for 20 weed species, not including giant foxtail. In contrast, velvetleaf reductions in germination with increasing seed burial depth were at least partly due, in this study, to increased fatal germination. Of the 20 species assayed by Benvenuti et al. (2001), velvetleaf exhibited the least depth inhibition of germination. These results are consistent with ecological theory on the relationship between seed size and reliance upon germination cues (Bond et al. 1999; Fenner and Thompson 2005; Milberg et al. 2000): smaller-seeded species, such as giant foxtail, should be more dependent on depth cues for germination than larger-seeded species, such as velvetleaf, because there is a danger of smaller-seeded species running out of reserves before emergence when seeds are buried deeper than the top few centimeters.

Fatal germination does not appear to play an important role in giant foxtail germination biology or seed fate. In contrast, fatal germination in velvetleaf appears to be increasingly important with increased seed burial depth and appears to be driven by different processes, depending upon depth of seed burial. An important caveat for making inferences from these results is that our bioassays held temperature and soil moisture levels within a small subset of the range of conditions experienced by weed seeds in the field. These studies can be repeated under field conditions, but this will potentially confound soil moisture, temperature, depth, and other environmental effects on fatal germination. It is important, however, to determine the extent of fatal germination of weed seeds under field conditions.

Results from the rag-doll bioassays and the sterile soil treatment indicate that, at shallow seed burial depths that do not limit velvetleaf germination or in soils with low pathogen loads (autoclaving reduces, but cannot completely eliminate, pathogens from soil), fatal germination of velvetleaf seedlings occurs only rarely, most likely as the result of low-frequency pathogenic events. At intermediate depths that do not directly limit velvetleaf seedling emergence, an increased pathogen loads (as represented by the *Pythium* inoculation treatment) result in greater levels of fatal germination.

One possible explanation for the observation that inoculation with the putative pathogen increased fatal germination, compared with the sterile soil treatment, at 4 to 6 cm, but not at 2 cm, may be related to the decrease in germination velocity with increasing seed burial depth for many weed species, including velvetleaf (Benvenuti et al. 2001). Decrease in germination velocity with increasing burial depth is associated with increases in physical impedance, lower soil temperatures (at least early in the growing season), and reduced gas exchange at greater depths (Harper et al. 1955). Variation in germination velocity is inversely related to seedling susceptibility to attack by *P. ultimum* and other soilborne pathogens for spinach (*Spinacia oleracea* L.), wheat (*Triticum aestivum* L.), garden pea (*Pisum sativum* L.), sugarbeet (*Beta vulgaris* L.), and watermelon [*Citrullus lanatuss* (Thunb.) Matsum. & Nakai] (Leach 1947). This relationship arises because of the greater amount of seedling residence time with potential pathogens (Harper et al. 1955). Because germination velocity was not measured in this experiment, this line of reasoning is only speculative.

At intermediate burial depths, fatal germination was greatest in the inoculated soil treatment, however at 8 and 10 cm burial depths, fatal germination in the control treatment increased to match the levels in the inoculated treatment. Regardless of the mechanism, at all but the shallowest depths, seed burial depth appeared to synergize pathogenic contributions to fatal germination.

Future Directions and Implications for Weed Management. The data presented here indicate that the question of whether fatal germination can be harnessed to serve weed management ends must be answered on a species-specific basis. For giant foxtail, fatal germination does not appear to be an important seed-bank process or, at least, not one that can be easily manipulated. For velvetleaf, fatal germination appears to be both important and subject to manipulation. The inoculation of soil with *P. ultimum* was successful in increasing fatal germination of velvetleaf, relative to treatments inoculated with sterile media at intermediate soil depths, but not at greater depths, where ambient pathogen concentrations were sufficient to cause substantial levels of fatal germination. These results suggest two possible avenues for the development of biocontrol tactics. First, for increasing fatal germination of the soil seed-bank within the zone of optimal germination, 0 to 6 cm (Benvenuti et al. 2001), soil inoculation could potentially be useful. We do not advocate the use of *P. ultimum* as a biocontrol agent, given the wide range of crops it attacks; however, the principles outlined here may be useful for targeting the application of more selective agents. A second, more promising approach would be to select tillage tools for precise depth placement of the weed seed rain (Mohler 2001). The greatest levels of velvetleaf fatal germination in this experiment occurred at a depth of 10 cm, with no difference between the ambient and pathogen-inoculated treatments. In situations where high velvetleaf seed return is unlikely, sending newly produced seeds to a 10 cm depth, followed by one or more no-till cycles, might serve to reduce velvetleaf seed-bank expression through fatal germination. Such an approach, using moldboard tillage, was found to reduce velvetleaf seed bank densities more rapidly by an order of magnitude over a 17-yr period than by allowing velvetleaf seeds to remain near the soil surface (Lueschen et al. 1993).

Identification of other species to target with this tactic should begin by screening relatively large-seeded species that exhibit low depth inhibition of germination. An important parallel line of research should be to determine whether the 10-cm seed depth placement induces maximum fatal germination in velvetleaf or just represents a threshold for depth-synergized fatal germination that continues at similar rates at greater depths in the soil profile. The central importance of the weed seed-bank in annual weed population dynamics suggests that additional research to manage all seed fates, not only germination, will make positive contributions to integrated weed management systems.

Sources of Materials

[1] 757 South Dakota seed blower, Seedburo Equipment Company, 1022 W. Jackson Blvd., Chicago, IL 60607.

[2] 38-lb. germination paper, Anchor Paper, 480 Broadway Street, St. Paul, MN 55101.

[3] Controlled Environments Limited, 590 Berry St., Winnipeg, Manitoba, R3H 0R9, Canada.

[4] Reagents for Murashige and Skoog medium, Fisher Scientific, 4500 Turnberry Dr., Chicago, IL 60103.

[5] Bacto-Agar, Difco Laboratories, 920 Henry St, Detroit, MI 48201.

[6] American National Can, 1104 West 43rd St., Chicago, IL 60609.

[7] SYSTAT Software, Systat Software, Inc., 501 Canal Blvd, Suite E, Point Richmond, CA 94804.

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