

Influence of Irrigation Timing on Disturbance-Induced Reductions in Soil Seedbank Density

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Studies suggest that disturbance-induced reductions in soil seedbank density are diminished by periods of water scarcity after soil disturbance; however, this hypothesis has yet to be tested. The objectives of this study were (1) to determine the effects of increasing time between soil disturbance and flood irrigation on disturbance-induced reductions in soil seedbank density, and (2) to identify specific soil moisture levels that cause seedbank reductions under flood irrigation. Weed species in this study were junglerice, Palmer amaranth, and yellow foxtail. For Objective 1, artificial seedbanks with known numbers of seeds were disturbed 10, 3, or 0 d prior to flood irrigations under field conditions. For Objective 2, seeds were buried in soil mesocosms that were hydrated to specific soil water potentials (flooded, 0 kPa, –30 kPa, –60 kPa, and –180 kPa) and placed in laboratory conditions favorable for germination. For both objectives, seeds were recovered to determine the percentages of buried seeds that survived the disturbance or moisture treatments. Results for the field study indicated that soil disturbances reduced seedbank persistence of Palmer amaranth but did not affect seedbank persistence of junglerice and yellow foxtail. Disturbance-induced reductions in seedbank density were greatest when soil was disturbed 0 and 3 d prior to flood irrigations. For the laboratory study, results showed that waterlogged soil was not required for seedbank losses because rates of seedbank persistence were greater in saturated soils (0 kPa and flooded) compared to the lower moisture levels. These studies indicate that delays in irrigation can reduce the seedbank reduction potentials of soil disturbance events. Further, irrigation timing effects on disturbed soil seedbanks are likely to occur in all irrigation systems, including those that reduce the amount of water applied compared to flood irrigation.

Nomenclature: Junglerice, *Echinochloa colona* (L.) Link ECHCO; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; yellow foxtail, *Setaria pumila* (Poir.) Roemer & J. A. Schultes SETLU.

Key words: Annual weeds, hypoxic stress, integrated weed management, seed mortality, seedbank persistence, seedling emergence, soil moisture.

Reducing soil seedbank densities is important for inhibiting population growth of weed species with annual life histories (Davis 2006; Jordan et al. 1995) and improving outcomes of specific weed control interventions (Schutte and Cunningham 2015; Sparks et al. 2004; Taylor and Hartzler 2000). Tactics that reduce soil seedbank densities include cultivation and other shallow soil disturbances (within top 10 cm of soil; hereafter “soil disturbances”) during periods when seedling emergence would normally take place (Mirsky et al. 2010; Mulugeta and Stoltenberg 1997; Taylor et al. 2005). These soil disturbances provide environmental cues for termination of physiological seed

dormancy, which is a type of dormancy caused by conditions of the embryo rather than embryo-covering structures that inhibit imbibition (Baskin and Baskin 2014; Finch-Savage and Leubner-Metzger 2006). Disturbance-associated environmental cues for dormancy termination modify buried seeds to subsequently respond to the thermal and moisture factors that stimulate the completion of germination (Benech-Arnold et al. 2000). Disturbance-associated environmental cues for dormancy termination include light, fluctuating temperatures, and gaseous stimulants (Benech-Arnold et al. 2000). Among these environmental cues, light is perhaps best understood (Pons 2000).

Previous studies conducted in laboratories showed that light-induced termination of seed dormancy is prevented by low water potentials in seed incubation mediums before or after light exposure (Berrie et al. 1974; de Miguel and Sanchez 1992; Vertucci et al. 1987). Laboratory studies that determined water-scarcity effects on positively photoblastic seeds (i.e., seeds with light require-

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ments for dormancy termination) were later corroborated by a field study that found that increasing the number of days between soil disturbance and irrigation reduced the population densities of seedling flushes occurring after irrigation (Botto et al. 2000). The gradual dissipation of disturbance effects on seedling emergence was attributed to instability in the physiologically active form of phytochrome, which is the chromoprotein that mediates light-induced dormancy termination (Botto et al. 2000). Considering that germination is a primary way through which seeds exit soil seedbanks (Forcella 2003), results from previous studies on water-deficit effects on light-induced seed dormancy termination (Berrie et al. 1974; de Miguel and Sanchez 1992; Vertucci et al. 1987) and postdisturbance emergence dynamics (Botto et al. 2000) suggest that water shortages following soil disturbances reduce their capacity for depleting weed seedbanks; however, this idea has yet to be tested.

Increased knowledge of moisture controls on seedbank persistence following soil disturbance will guide progress towards improved weed management strategies in irrigated agricultural systems. However, the diversity in irrigation water delivery systems (e.g., sprinkler, flood, drip) potentially complicates the development of a conceptual framework for understanding irrigation timing effects on disturbed soil seedbanks. This is because irrigation water-delivery systems that saturate upper soil layers for extended periods might confound disturbance-induced seedbank losses with hypoxic mortality, reflecting the fact that the low oxygen conditions that occur in waterlogged soils are damaging to seeds of some terrestrial plant species (Bekker et al. 1998; Morinaga 1926; Sarlistyaningsih et al. 1995; Thompson 2000). Thus, for studies that use flood irrigation to clarify seedbank responses to soil disturbance, the potential for seed mortality in saturated soil compels complementary experiments that determine the effects of waterlogging on seedbank persistence.

This study was designed to answer two research questions: (1) do delays in flood irrigation following soil disturbance promote seedbank persistence of weed species characterized by positively photoblastic seeds? (2) Do waterlogged soils intensify seedbank losses by creating low oxygen conditions that reduce seed viability? These questions were addressed with two experiments that determined the effects of soil disturbance and flood irrigation on seedbank persistence for three weed species with annual life

histories. Weed species in this study included Palmer amaranth, junglerice and yellow foxtail. Germination of Palmer amaranth and junglerice is considered light sensitive (Chauhan and Johnson 2009b; Jha et al. 2010), whereas germination of yellow foxtail is thought to occur independent of light (Steel et al. 1983). Accordingly, the hypotheses for this study were (1) disturbance-induced reductions in seedbank density for Palmer amaranth and junglerice are inversely related with the amount of time between disturbance and flood irrigation, but for yellow foxtail, disturbance-induced reductions in seedbank density are not related to the amount of time between disturbance and flood irrigation, and (2) for all species in this study, rates of seedbank persistence in an hypoxic, water-logged soil are less than rates of seedbank persistence in hydrated, nonsaturated soil.

Materials and Methods

Plant Materials. Seeds of Palmer amaranth, junglerice, and yellow foxtail were collected from the New Mexico State University, Leyendecker Plant Science Center (LPSC; 32.19°N, 106.74°W), which is a 82-hectare research farm that produces forage, fiber, nut, and vegetable crops using management practices typical for the region. For each species, seeds were collected from multiple crop fields over an 8-wk period beginning August 24, 2012. Separate seed collections were pooled to produce Palmer amaranth, junglerice, and yellow foxtail seed populations comprised of individuals with varied maturation conditions. Palmer amaranth seeds were obtained by first clipping seed-bearing inflorescences, which were then dried in an unheated greenhouse and in the absence of direct sunlight for 14 to 20 d. Dried inflorescences were hand-thrashed and sequential combinations of sieving and forced-air separation were used to separate seeds from chaff. Junglerice and yellow foxtail seeds were obtained by shaking seed-bearing inflorescences over receptacles that were held beneath plants in the field. Harvested plant materials were dried under conditions described above, and seeds were separated from chaff with the use of sieves and forced-air separation. After forced-air separation, mean 100-seed weights were $0.03 \pm \text{SE } 0.001$ g for Palmer amaranth, $0.09 \pm \text{SE } 0.008$ g for junglerice, and $0.28 \pm \text{SE } 0.006$ g for yellow foxtail. Cleaned seed populations were stored in airtight containers at 4 C. Prior to use in laboratory and field experiments, seeds were assayed for

viability with the use of a 0.6% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (Peters 2000). For each experiment, seed viability prior to burial was determined to be high (> 96%).

Field Experiment. To determine the effects of irrigation timing on disturbance-induced reductions in seedbank persistence, a field study was conducted at LPSC. The field study ran for 10 mo, consisted of two runs, and was initiated on December 17, 2012 and January 7, 2014. Annual runs were each arranged in a randomized complete-block design with eight replications.

Experimental units were artificial seedbanks within 10-cm diameter, 12-cm depth polyvinyl chloride pipes. To prevent downward seed losses, pipe bottoms were closed with nylon mesh (0.04-mm² openings). Pipes were incorporated into the soil at LPSC so that the pipe tops formed a lip 2 cm above the soil surface. Pipes were then filled with 800 ml of soil that was first autoclaved and subsequently augmented with 150 seeds of junglerice or Palmer amaranth, or 100 seeds of yellow foxtail. Preliminary experiments indicated that the autoclave procedure eliminated the indigenous soil seedbanks of the study species. Soil for artificial seedbanks was collected from the study site and was a Belen clay loam (clayey over loamy, smectitic over mixed, superactive, calcareous, thermic Vertic Torrifluvents, pH 7.5, 0.7% organic matter). Artificial seedbanks were covered with wire netting (0.04-cm² mesh) to block access by vertebrate seed predators. To prevent damage from larger animal pests, the study area was fenced with wire hex netting (2.5-cm openings). To determine the potential for unintended seed movement into pipes, the study included pipes (1 pipe replicate⁻¹) that were filled with only autoclaved soil.

For each annual run, soil moisture measurements were taken from four artificial seedbanks without seeds. Volumetric moisture measurements were also collected in soil outside of artificial seedbanks, 50 cm away from each of the artificial seedbanks designated for moisture measurements. Moisture levels were determined with sensors (EC-5 Soil Moisture Sensor, Decagon Devices, Inc., 2365 NE Hopkins Court, Pullman, WA 99163) equipped with data loggers (HOBO Micro Station, Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532) programmed to record soil conditions every 60 min simultaneously. Prior to use in the field, soil moisture sensors were calibrated with the use of artificial seedbanks hydrated to specific moisture levels.

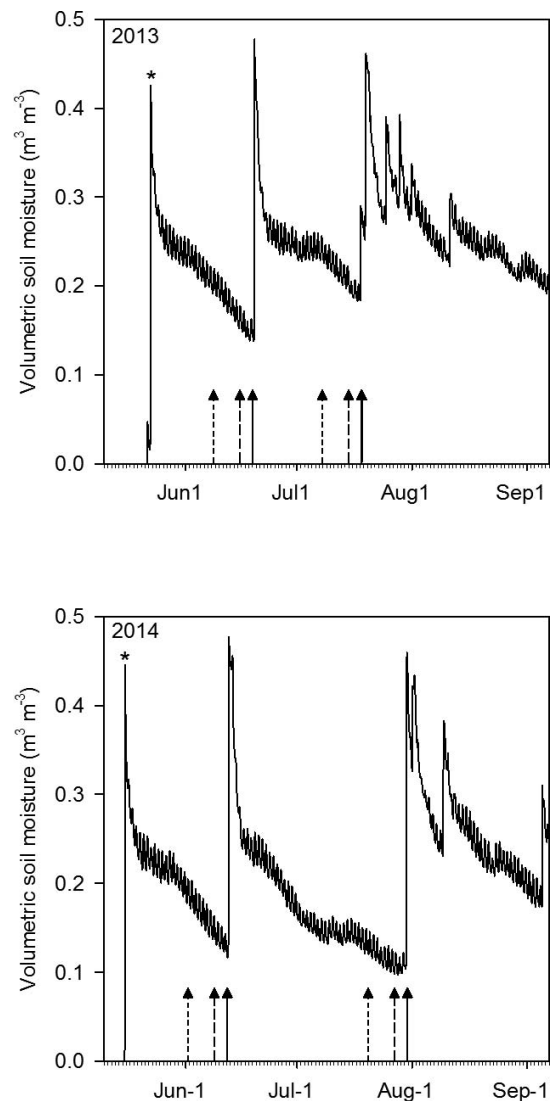


Figure 1. Volumetric soil moisture contents in artificial seedbanks during 2013 and 2014. Asterisks indicate irrigations conducted prior to disturbance treatments. Arrows along the *x* axes indicate dates on which disturbance treatments were implemented. Short-dashed arrows denote soil disturbances 10 d prior to irrigation. Long-dashed arrows show soil disturbances 3 d prior to irrigation. Solid arrows indicate soil disturbances 0 d prior to irrigation. Large increases in soil moisture after the final irrigations were caused by late-summer precipitation events.

Treatments included nondisturbed controls and soil disturbances 10, 3, or 0 d prior to irrigation. Each soil disturbance treatment was applied twice to a given seedbank. To ensure soil hydration sufficient for light-induced termination of seed dormancy (Gallagher and Cardina 1997), artificial seedbanks were irrigated prior to the initial disturbance treatment. Soil moisture levels and dates on which soil disturbances took place are indicated in Figure 1. Soil disturbance was implemented using a sequence of steps, with each step performed in the field. First, a pipe was lifted from the ground and

Table 1. Soil moisture treatments for laboratory study.

Treatment	Soil moisture content	Soil water potential
	g H ₂ O g ⁻¹ dry soil	kPa
1	0.63	Flood ^a
2	0.53	0
3	0.37	-30
4	0.27	-60
5	0.23	-180

^a Soil moisture treatment “flood” consisted of saturated soil under 1 cm of standing water.

the soil within the pipe was poured into a plastic bin. Next, soil was hand mixed for 15 s and then returned to the pipe that was again incorporated into the ground as described above. All soil disturbances occurred within 45 min of solar noon.

Soil disturbance treatments were structured to mimic a series of stale seedbeds implemented during summer fallow. Stale seedbeds are a set of practices that first stimulates weed seed germination through tillage and/or irrigation and then eliminates subsequent seedlings with nonselective control prior to cash crop planting (Johnson and Mullinix 1995). In this study, timetables for soil disturbance were developed with the use of weather forecasts and real-time information on soil friability. Specifically, soil disturbances were scheduled to occur prior to late-summer precipitation events and were initiated when soil in pipes was dry enough to be easily crumbled. To determine the friability of soil in pipes, the study included extra pipes that were used for this purpose only. Hand mixing was employed because this method for soil disturbance was optimal for the artificial seedbank system that enabled precise measurements of seedbank persistence. Previous studies used hand mixing to simulated tillage (Calderon et al. 2000; Davis and Liebman 2003; Schutte et al. 2014a); however, the relationship between hand mixing and tillage is poorly understood.

Prior to the initial irrigation in early summer, seedbank persistence was determined with the use of seedbanks designated for this purpose only (1 seedbank species⁻¹ replicate⁻¹). Because seed recovery and viability tests were destructive (described below), no further data were collected from the seedbanks used in early summer persistence measurements. The following data were collected from seedbanks not used in early summer persistence measurements: (1) the number of emerged seedlings after irrigations, and (2) the number of viable seeds present at the end of summer. Emerged seedlings

were counted and removed at 4 to 7-d intervals. Viable seeds were recovered with the use of an elutriation procedure in which each soil seedbank was immersed in 2 L of a 1.5% aqueous solution of sodium hexametaphosphate. The soil suspension was agitated for 20 min with the use of a reciprocating shaker and was then poured through a 425- μ m sieve cloth. Collected material was dried for 48 h at 35 C. Following manual removal from dry soil matter, seeds were assayed for viability with the use of the tetrazolium staining assay procedures described above.

With the use of end-of-summer seed viability data, disturbance-induced reductions in seedbank persistence (DR) were determined:

$$DR_{nr} = \frac{UND_r - DIST_{nr}}{UND_r}$$

where DR_{nr} is the disturbance-induced reduction in seedbank persistence for disturbance timing treatment *n*, replicate *r*, UND_{*r*} is the number of viable seeds recovered from the nondisturbed seedbank in replicate *r*, DIST_{nr} is the number of viable seeds recovered from disturbance timing treatment *n*, replicate *r*. Seed viability data from both the end of summer and prior to the initial irrigation were used to determine rates of seedbank persistence, which were the number of viable seeds recovered expressed as percentages of the number of seeds buried.

Laboratory Experiment. To identify soil moisture levels that cause seedbank reductions under flood irrigation, soil seedbanks were hydrated to specific soil moisture levels and then incubated in a growth chamber. Treatments were soil moisture levels (Table 1) that were expected to be unfavorable for seedbank persistence (Schutte et al. 2008). The study consisted of two runs separated in time. Each run was arranged in a randomized complete-block design with three replications.

Experimental units were seedbank mesocosms designed to maintain desired soil matric potentials over a prolonged period and prevent the development of artificially anaerobic conditions. Soil for mesocosms was a Belen clay loam collected from LPSC. Soil characteristics were described above (see “Field experiment”). Prior to use in the laboratory experiment, soil was passed through a 2-mm sieve cloth to remove large clods and rocks. Soil for this experiment was not autoclaved. Also prior to the laboratory experiment, seeds for mesocosms were subjected to winter field conditions (burial in mesh packets at LPSC [10-cm depth] for 3 months

beginning in December) so that levels of physiological seed dormancy were reduced (Baskin and Baskin 2014). Mesocosms were assembled by burying overwintered seeds to the 1-cm depth in soil that was contained in polyvinyl chloride pipes (6 cm diameter, 7 cm height). During mesocosm assembly, the bottoms of polyvinyl chloride pipes were wrapped with loose-woven cotton cloths (cheesecloth, grade 40). Cotton cloths were removed after mesocosms were hydrated to desired moisture content but before mesocosms were wrapped in polyethylene film (described below). A single mesocosm contained seeds for one of the species in this study. Seed densities were 50 seeds seedbank⁻¹ for Palmer amaranth and junglerice, 30 seeds seedbank⁻¹ for yellow foxtail.

After seed burial, mesocosms were subirrigated to saturation and then dried to the specific moisture contents that corresponded with the desired soil water potentials (Table 1). Drying requirements for desired soil moisture potentials were determined with the use of a standard function for the relationship between gravimetric soil water content and soil water potential. This function was developed with the use of a pressure-plate extractor in accordance with the procedures of Dane and Hopmans (2002). Once specific amounts of water evaporated from soil, as determined by changes in mesocosm weight, mesocosms were wrapped entirely in a single layer of low-density polyethylene film (Glad Cling Wrap, The Glad Products Company, 1221 Broadway, Oakland, CA 64612). Mesocosms for the soil moisture treatment “flooded” were not wrapped in polyethylene film.

Hydrated mesocosms were placed in a growth chamber set to 35 C day/25 C night, 12-h photoperiods; conditions considered favorable for germination of the study species (Baskin and Baskin 2014). Growth chambers were humidified by placing water-filled, stainless-steel trays on interior shelves. At 3 to 5-d intervals throughout the 35-d incubation period, mesocosms were monitored for seedling emergence. When seedlings were observed, mesocosms were temporarily unwrapped and seedlings were removed with forceps. Soil moisture levels were regularly assessed by determining mesocosm weights. The mean percent change from original seedbank weight was $0.35 \pm \text{SE } 0.05\%$, which indicated that moisture levels in soil mesocosms were maintained over the course of the experiment. Water was not added to mesocosms after initial hydration.

At the conclusion of the incubation period, seeds were recovered and assessed for viability by the elutriation and tetrazolium staining assay procedures described in the Field Experiment section. Seed viability data were used to determine rates of seedbank persistence, which were the number of viable seeds recovered expressed as percentages of the number of seeds buried. The availability of oxygen in seedbanks was assessed by determining Fe²⁺ concentrations. An accumulation of Fe²⁺ indicates development of hypoxic conditions because certain anaerobic microorganisms use Fe³⁺ as an oxidant in respiration (Lovley 1991). Levels of Fe²⁺ were measured with a modified ferrozine method (Lovley and Phillips 1987). Specifically, iron was extracted by gentle mixing of soil with 0.4 M HCl (5 ml/0.5 g dry soil) for 10 min and extract was filtered through a 0.2 μm Millipore filter. A 250-μl aliquot of extract was added to a 96-well plate, mixed with 15 μl of 4-mM color reagent (3-[2-Pyridyl]-5,6-di[2-furyl]-1,2,4-triazine-5',5''-disulfonic acid disodium salt) and incubated for 10 min. Then, 30 μl of 4-M ammonium acetate buffer (pH 9.2) was added and incubation continued for 30 more min. Absorbance was determined at 590 nm.

Data Analysis. All statistical analyses were performed with the use of the open source statistical software program R (v.3.0.1, The R Foundation for Statistical Computing, <http://www.r-project.org>).

For the field experiment, disturbance timing effects on DR were determined with linear mixed-effects models developed for each species with the R package *nlme*. In these models, disturbance timing treatments were fixed effects. Random effects were the hierarchical structures of sampling, year and year(replicate). Following development of linear mixed-effects models, the R package *multcomp* was used to generate Tukey all-pairs comparisons for the factor “disturbance timing.” Linear mixed-effects models with Tukey comparisons were also used to compare rates of seedbank persistence among the three general seedbank types: seedbanks recovered prior to initial irrigations, nondisturbed seedbanks recovered at the end of summer and disturbed seedbanks recovered at the end of summer. Relationships between rates of seedbank persistence and rates of seedling emergence (percentages of buried seeds that produced seedlings) were evaluated with Pearson Correlation coefficients.

Agreement between simultaneous moisture measurements in artificial seedbanks and surrounding soil was determined with the root-mean-square

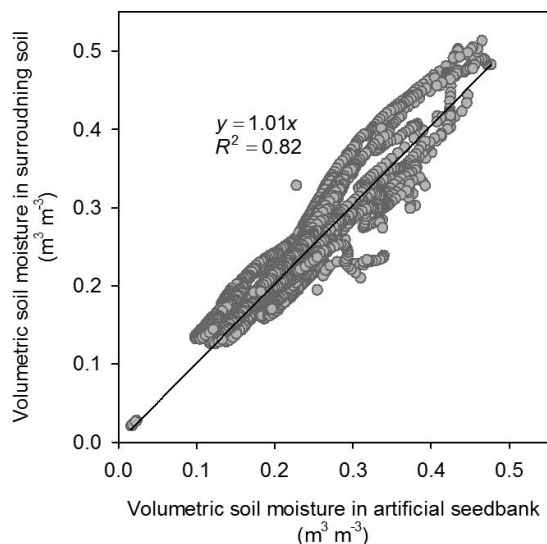


Figure 2. Volumetric soil moisture measured simultaneously in artificial seedbanks and surrounding soil. The linear regression was fit with the intercept forced to zero.

error (RMSE) (Mayer and Butler 1993). The RMSE provided a measurement of the typical difference between the artificial seedbank moisture levels and the natural soil moisture levels in units of volumetric water content. The degree to which moisture levels in artificial seedbanks represented natural soil was also evaluated by plotting natural soil moisture measurements as functions of the concurrent measurements in artificial seedbanks. To this plot, a linear regression was fit with the intercept forced to zero. The slope of the regression line provided information on the tendencies in moisture level differences between artificial seedbanks and natural soil.

For the laboratory study, soil moisture level effects on seedbank persistence were assessed with linear mixed-effects models that featured soil moisture level and species as the fixed effects; run and run(replicate) as the random effects. Following

development of linear mixed-effects models, the R package *multcomp* was used to generate Tukey all-pairs comparisons for the factor “soil moisture level.”

Results and Discussion

Field Experiment. Seedlings did not emerge and seeds were not recovered from pipes that were filled with only autoclaved soil (data not shown). Thus, inadvertent movement of seeds into artificial seedbanks did not occur in this study. Moisture measurements taken concurrently in artificial seedbanks and soil outside of artificial seedbanks showed that, in general, artificial seedbanks tended to be drier than surrounding soil (Figure 2). This was consistent with a study by Leon and Owen (2004), who suggested that soil water retention is reduced in artificial seedbanks compared to natural soil. However, in the current study, the RMSE for comparing moisture measurements between artificial seedbanks and natural soil was $0.02 \text{ m}^3 \text{ m}^{-3}$, indicating that the two environments were generally similar with respect to soil moisture. Calibrations in the laboratory indicated that volumetric soil moisture at saturation was $0.45 \text{ m}^3 \text{ m}^{-3}$. In the field, artificial seedbanks were saturated for 15 h in 2013, and 26 h in 2014 (Figure 1).

Bartlett’s test for homogeneity of variance (Zar 1999) indicated equal variances in seedbank persistence and DR data between years, and therefore, seedbank persistence and DR data were pooled across years. Losses from seedbanks primarily occurred after irrigations, as indicated by the relatively high rates of persistence prior to the initial irrigations and the low rates of persistence at the end of summer (Table 2). Soil conditions at the times of disturbances were conducive to light-induced seed dormancy termination because soil moisture levels

Table 2. Rates of persistence in Palmer amaranth, junglerice, and yellow foxtail seedbanks that were either recovered at different times or subjected to different regimes of soil disturbance. Lower-case letters within species denote significant differences ($\alpha = 0.05$).

Species	Seedbank recovery time	Disturbance regime	Seedbank persistence
			%
Palmer amaranth	Before initial irrigation	Nondisturbed	81 a
	End of summer	Nondisturbed	47 b
	End of summer	Disturbed	13 c
Junglerice	Before initial irrigation	Nondisturbed	79 a
	End of summer	Nondisturbed	27 b
	End of summer	Disturbed	24 b
Yellow foxtail	Before initial irrigation	Nondisturbed	84 a
	End of summer	Nondisturbed	10 b
	End of summer	Disturbed	6 b

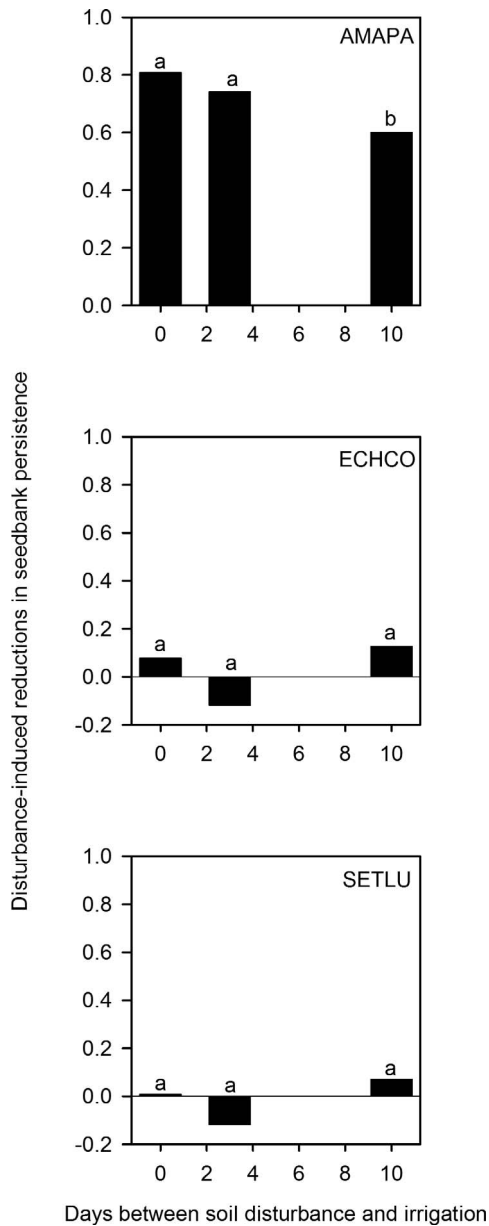


Figure 3. Disturbance-induced reductions (DR) in seedbank persistence for soil seedbanks disturbed at different days before irrigation. Bars are means of 16 replications. Within species, bars with different lowercase letters are significantly different at $\alpha = 0.05$. DR scaled such that DR = 1 indicates that disturbance eliminated all seeds and DR = 0 indicates that disturbance did not affect persistence. Negative values for DR indicate that disturbance promoted persistence relative to nondisturbed seedbanks. Abbreviations: AMAPA, Palmer amaranth; ECHCO, junglerice; SETLU, yellow foxtail.

at these times were greater than $0.10 \text{ m}^3 \text{ m}^{-3}$ (Figure 1), which was previously shown to be a threshold below which light-induced dormancy termination is inhibited (Gallagher and Cardina 1997). Soil disturbances reduced seedbank persistence of Palmer amaranth but did not affect seedbank persistence of junglerice and yellow foxtail

(Table 2). For Palmer amaranth, DR decreased with increasing days from disturbance to irrigation (Figure 3). For junglerice and yellow foxtail, DR was not affected by the number of days between disturbance and irrigation.

Disturbance effects on Palmer amaranth seedbanks were consistent with the combined results from previous studies that indicated that Palmer amaranth seed dormancy is terminated by light in a manner consistent with phytochrome regulation (Jha et al. 2010), light is an environmental cue that causes seed dormancy termination during soil disturbance (Scopel et al. 1991) and germination is central to seedbank depletion (Forcella 2003). The effect of irrigation timing on Palmer amaranth seedbank persistence was congruent with a previous study that found that, for species with positively photoblastic seeds, postponing irrigation after soil disturbance reduced population densities of seedling flushes occurring after irrigation (Botto et al. 2000). This gradual loss of the disturbance stimulus on emergence was attributed to the reversion of phytochrome to the physiologically inactive form (Botto et al. 2000).

Botto et al. (2000) presented a conceptual framework for understanding seedling emergence responses to irrigation timing relative to soil disturbance. The current study provides empirical evidence that extends the conceptual framework for understanding irrigation timing effects on disturbed soil seedbanks to include seedbank persistence. Such an extension required direct measurements of seed survival because seedbank persistence is not unequivocally correlated with seedling emergence (Schutte et al. 2014b), reflecting the fact that mortality might occur during pre-emergence seedling growth (Forcella 2003). In the current study, Palmer amaranth seedbank persistence was correlated with the percentage of seeds that produced seedlings after disturbance ($r = 0.41$, $P = 0.004$), but seedbank persistence for this species was not correlated with the percentage of seeds that produced seedlings over the entire season ($r = 0.16$, $P = 0.28$).

For yellow foxtail, the results from the current study were consistent with previous studies that indicated that, for this species, seed dormancy termination is not influenced by light (Steel et al. 1983) and seedling emergence is not promoted by tillage (Myers et al. 2005). However for junglerice, the absence of disturbance effects on seedbank persistence was unexpected and did not agree with Chauhan and Johnson (2009b), who identified positive photoblastism in junglerice seeds under laboratory conditions. The lack of disturbance

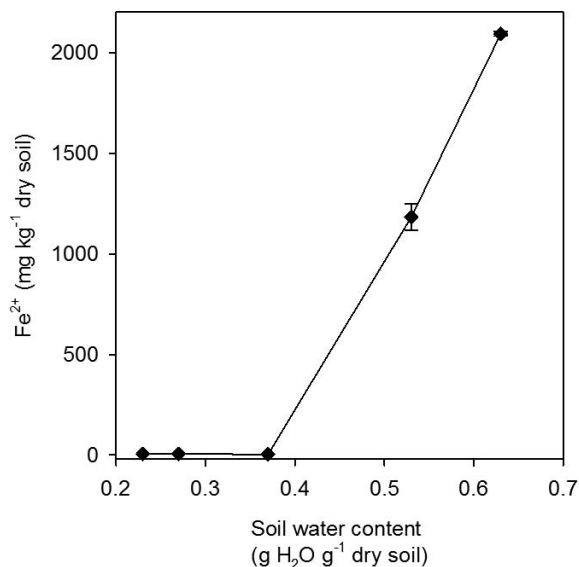


Figure 4. The availability of oxygen in soil mesocosms indicated by Fe^{2+} concentrations. Under hypoxic conditions, anaerobic microorganisms use Fe^{3+} as an oxidant in respiration. Thus, accumulation of Fe^{2+} indicates development of low oxygen conditions.

effects on seedbank persistence for junglerice might have been caused by the seasonality in seedbank sensitivity to soil disturbance (Roberts and Potter 1980) that is mediated by the species-specific changes over time in light sensitivity in seeds (Jha et al. 2010; Milberg and Andersson 1997). Although Chauhan and Johnson (2009b) identified light requirements for junglerice germination in the laboratory, Chauhan and Johnson (2009a) determined that junglerice seedling emergence was greater under no-till compared to conventional and minimum tillage, which suggests that light requirements for germination under laboratory conditions are not obligate signals for disturbance-induced seedbank losses under field conditions.

It should be noted that the autoclave procedure that was necessary for removing indigenous soil seedbanks of the study species also adversely affected soil microbial communities. Because of its effects on soil microorganisms, the autoclave procedure might have enhanced absolute rates of seedbank persistence (Wagner and Mitschunas 2008). Further, autoclaving soil might have lessened disturbance-induced reductions in seedbank persistence because germination was potentially suppressed by the absence of ethylene produced by soil microorganisms (Hilhorst and Darssen 2000).

Laboratory Experiment. After 35 days of incubation, saturated seedbanks (flooded and 0 kPa treatments) were characterized by reduced oxygen

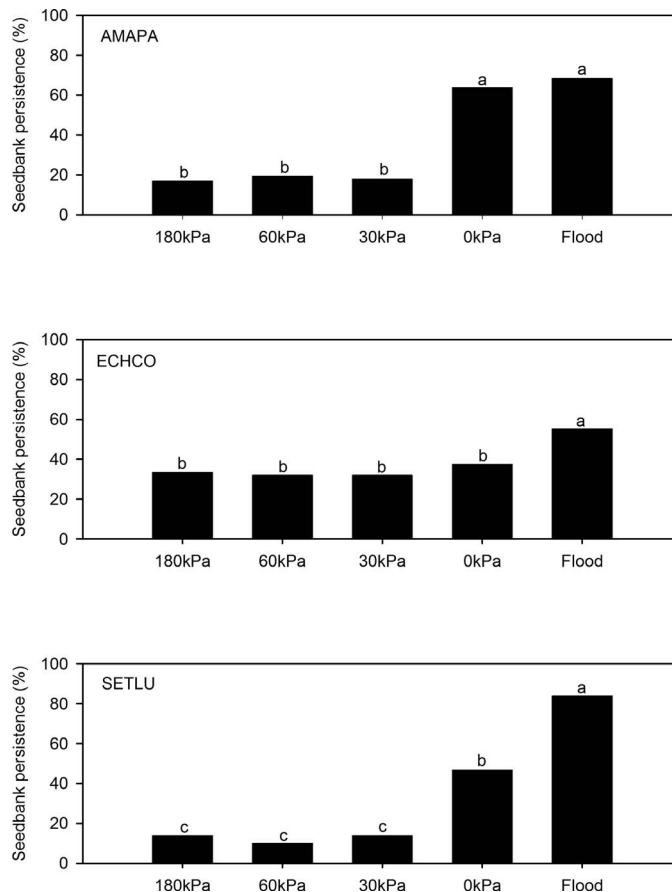


Figure 5. Seedbank persistence in soil mesocosms hydrated to different moisture levels and incubated for 35 d under 35 C day/25 C night, 12-h photoperiods. Bars are means of six replications. Within species, bars with different lowercase letters are significantly different at $\alpha = 0.05$. Abbreviations: AMAPA, Palmer amaranth; ECHCO, junglerice; SETLU, yellow foxtail.

availability, whereas aerobic conditions were maintained in seedbanks at -30 kPa, -60 kPa, and -180 kPa (Figure 4). Hypoxia became more severe with time because preliminary experiments indicated that Fe^{2+} concentrations gradually increased in flooded and 0-kPa seedbanks over the time course of the experiment (data not shown).

Bartlett's test for homogeneity of variance indicated equal variances in rates of seedbank persistence between runs, and thus, seedbank persistence data were pooled across runs. The effect of soil moisture on seedbank persistence was influenced by an interaction between species and soil moisture level ($P < 0.01$). However, for all species in this study, the greatest rates of persistence were observed for seeds in the flooded treatment, whereas seedbank persistence was reduced in soil moisture treatments -30 kPa, -60 kPa, and -180 kPa (Figure 5). In the 0-kPa treatment, Palmer

amaranth seedbank persistence was equal to the flooded treatment, junglerice seedbank persistence was equal to the reduced soil moisture treatments (−30 kPa, −60 kPa, −180 kPa), and yellow foxtail seedbank persistence was greater than reduced soil moisture treatments, but not as great as the flooded treatment. Differences among species in their responses to the moisture gradient were consistent with previous studies that determined that soil moisture level effects on seedbank dynamics were species-specific (Bekker et al. 1998; Keddy and Constabel 1986; Schutte et al. 2008).

Waterlogged soil induces seed mortality in species including downy brome (*Bromus tectorum* L.), smooth crabgrass [*Digitaria ischaemum* (Schreb.) Muhl.], spreading dogbane (*Apocynum androsaemifolium* L.), and prickly lettuce (*Lactuca serriola* L.) (Comes et al. 1978). For some species, seed death by flooding occurs in as little as 2 d (Sarlistyaningsih et al. 1995). Possible mortality mechanisms for seeds in waterlogged soils include an accumulation of toxic metabolites resulting from accelerated glycolysis under hypoxia (Crawford 1977), infection from soil microorganisms (Drew and Lynch 1980; Schafer and Kotanen 2003) and lethal organic acids that form from decomposing plant residues in saturated soil (Drew and Lynch 1980). Despite the hazards in hypoxic, waterlogged soil; many plant species produce persistent seedbanks in environments characterized by frequent and prolonged flooding (Baskin and Baskin 2014; Bekker et al. 1998). Because Palmer amaranth, junglerice, and yellow foxtail seeds are capable of persistence in waterlogged soil, the seedbank losses observed in the field study were not consequences of flooding itself. Therefore, field study findings on irrigation timing effects on disturbed soil seedbanks are generally applicable to all types of irrigation water delivery systems, including those that reduce the amount of water applied to cropland compared to flood irrigation.

Implications for Seedbank Management. A number of methods for targeting weed seedbanks are potentially available to crop producers. These methods include, but are not limited to biocontrol with seed predators (Menalled et al. 2006), soil heating (Egley 1983), windrow flaming (Walsh and Newman 2007) and removal by crop harvest machinery (Walsh et al. 2013). Perhaps the most readily adoptable seedbank depletion method is the stale seedbed (Caldwell and Mohler 2001; Johnson and Mullinix 1995; Lonsbary et al. 2003). Stale seedbed practices can be optimized with knowledge

of the species-specific interactions between irrigation timing and persistence in disturbed soil seedbanks. For species for which delays in irrigation diminish the seedbank reduction potentials of soil disturbance events, stale seedbeds are more efficacious if irrigation occurs soon after soil disturbance. Irrigation quickly after soil disturbance will likely affect seedbanks of species with light requirements for dormancy termination. However, expectations for improvement in stale seedbed efficacy might first require studies that confirm synchronicity between positive photoblastism and soil disturbance because light requirements for dormancy termination can be variable over time (Jha et al. 2010; Milberg and Andersson 1997).

Palmer amaranth is an economically important agricultural weed that affects numerous cropping systems and regions (reviewed by Ward et al. 2013). Management difficulties for Palmer amaranth are partly attributable to this species' high levels of seed production and its propensity for evolving resistance to herbicides (Ward et al. 2013). To prevent the development and spread of herbicide-resistant biotypes, experts encourage broadening conventional weed control programs to include strategies for reducing seedbank densities (Norsworthy et al. 2012). This is because large numbers of seeds in soil increase the risk of herbicide resistance evolution (Neve et al. 2011). Accordingly, the results of the current study indicate that herbicide-resistance mitigation strategies for Palmer amaranth can be enhanced by reducing the amount of time between soil disturbance and irrigation or precipitation events.

In addition to reducing the number of seeds in soil, weed seedbank management involves control on the population densities of weed seedling flushes. Management factors that affect the percentages of buried seeds that produce seedlings include timings of tillage and planting (Johnson and Holm 2010; Spandl et al. 1998), tillage system (Spandl et al. 1998; Verdu and Mas 2004), crop residue type (Didon et al. 2014) and crop residue quantity (Chauhan and Abugho 2013). To the list of factors that influence weed seedling densities, irrigation timing after soil disturbance can be added. Delayed irrigation after soil disturbance might be a means for improving subsequent weed control tactics that are negatively affected by increases in weed seedling density. Such tactics include POST herbicide applications (Dieleman et al. 1999), cultivation (Davis and Williams 2007; Dieleman et al. 1999), and hand weeding (Melander and Rasmussen

2001). To understand the potential for improvement in weed control provided by changes in irrigation timing after soil disturbance, further research on this topic is needed in conditions that closely resemble crop production environments.

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