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Seedbank Persistence of Palmer Amaranth (*Amaranthus palmeri*) and Waterhemp (*Amaranthus tuberculatus*) across Diverse Geographical Regions in the United States

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Abstract

Knowledge of the effects of burial depth and burial duration on seed viability and, consequently, seedbank persistence of Palmer amaranth (*Amaranthus palmeri* S. Watson) and waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] ecotypes can be used for the development of efficient weed management programs. This is of particular interest, given the great fecundity of both species and, consequently, their high seedbank replenishment potential. Seeds of both species collected from five different locations across the United States were investigated in seven states (sites) with different soil and climatic conditions. Seeds were placed at two depths (0 and 15 cm) for 3 yr. Each year, seeds were retrieved, and seed damage (shrunken, malformed, or broken) plus losses (deteriorated and futile germination) and viability were evaluated. Greater seed damage plus loss averaged across seed origin, burial depth, and year was recorded for lots tested at Illinois (51.3% and 51.8%) followed by Tennessee (40.5% and 45.1%) and Missouri (39.2% and 42%) for *A. palmeri* and *A. tuberculatus*, respectively. The site differences for seed persistence were probably due to higher volumetric water content at these sites. Rates of seed demise were directly proportional to burial depth ($\alpha = 0.001$), whereas the percentage of viable seeds recovered after 36 mo on the soil surface ranged from 4.1% to 4.3% compared with 5% to 5.3% at the 15-cm depth for *A. palmeri* and *A. tuberculatus*, respectively. Seed viability loss was greater in the seeds placed on the soil surface compared with the buried seeds. The greatest influences on seed viability were burial conditions and time and site-specific soil conditions, more so than geographical location. Thus, management of these weed species should focus on reducing seed shattering, enhancing seed removal from the soil surface, or adjusting tillage systems.

Introduction

The high fecundity of Palmer amaranth (*Amaranthus palmeri* S. Watson) (Korres 2018; Korres and Norsworthy 2017) and waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] (Hartzler et al. 2004; Heneghan and Johnson 2017) and the relatively high frequency of evolving herbicide resistance (Heap 2017; Jhala et al. 2014; Molin et al. 2016; Vencill et al. 2008) are major reasons why these species have become two of the most problematic weeds in U.S. cropping systems (Riar et al. 2013; Webster and Nichols 2012). The excessive proliferation of these species can rapidly enrich the soil seedbank, the persistence of which is the driving force for future weed infestations in agricultural production systems. Seed persistence in soil seedbanks counteracts the effects of unfavorable environmental conditions for seed germination over long periods (Gutterman 1994; Holmgren et al. 2006) and increases the possibility that viable seeds are

available when conditions for seed germination and seedling recruitment are optimal (Holmgren et al. 2006).

Weed species that form persistent seedbanks are a concern for future weed management. The persistence of viable seeds in the soil seedbank depends on a wide range of interacting biotic and abiotic factors. These include germination cues, seed dormancy, seed size (Honda 2008; Hulme 1998; Ooi et al. 2007; Thompson et al. 1994), physiological age, predation, and microbial decay, along with environmental conditions, burial depth, and burial duration (Davis et al. 2005; Liebman et al. 2001). High mortality occurs at the seed stage owing to high seed losses and fatal germination (i.e., the condition wherein seeds germinate but fail to emerge) (Cavers 1983; Forcella 2003; Forcella et al. 1992). Consequently, reducing the number of germinable seeds will decrease the number of individuals that will be subjected to weed management operations and the number of escapees that could replenish the soil seedbank. Longevity of seeds in the soil is the most determinant factor for the success of this approach.

The first crucial phase in the formation of a persistent soil seedbank is burial (Fenner and Thompson 2005). Whether buried seeds contribute to soil seedbank persistence and consequently to weed population regeneration depends mainly on the depth from which the seeds are able to germinate (Baker 1989). The persistence and viability of some weed species after long burial periods is well documented (Conn et al. 2006; Telewski and Zeevaart 2002). However, the majority of weed species lose seed viability at relatively short periods after burial (Burnside et al. 1996; Conn et al. 2006; Egley and Chandler 1983; Lutman et al. 2002), particularly small-sized seeds such as *A. palmeri* (Jha et al. 2014; Sosnoskie et al. 2013), which are more likely to become buried (Peart 1984; Thompson et al. 1994). Omami et al. (1999) found changes in redroot pigweed (*Amaranthus retroflexus* L.) viability for seeds placed on the soil surface compared with seeds buried at various depths up to 10 cm. He reported that the decline in viability was most rapid for the seeds on the soil surface compared with buried seeds. Schweizer and Zimdahl (1984) discussed the persistence of *Amaranthus* species seedbank due to longevity of seeds, which, based on the literature, seems to vary considerably from a 12-mo period (Horng and Leu 1974; Omami et al. 1999) up to 4 (Jha et al., 2014; Steckel et al. 2007), 10 (Burnside et al. 1981; Toole and Brown 1946), or even 40 yr (Kivilaan and Bandurski 1981; Quick 1961).

Recent research has sought to address the influence of climate in relation to plant biological characteristics on the establishment and persistence of plant populations (Scott et al. 2014). As stated by Ooi (2012), expanding our knowledge of the response and adaptability of seedbanks to environmental and climatic factors will provide the basis for accurate predictions of species occurrence and future distribution, especially in ecosystems that are exposed to temporarily irregular disturbances.

As mentioned previously, the persistence of viable seeds in the seedbank is affected by a wide range of interacting biotic and abiotic factors that in turn depend on the position of seeds in the soil profile (Omami et al. 1999) and geographic location (Warr et al. 1993). Knowledge of the effects of burial depth and burial duration on long-term seed viability and, subsequently, seedbank persistence of *A. palmeri* and *A. tuberculatus* populations originating from different locations and dispersed among diverse regions with different soil and climatic conditions can be used for the development of efficient weed management approaches. This is of particular interest given the high fecundity of both species and their high seedbank replenishment potential.

The aim of this study, therefore, was to assess *A. palmeri* and *A. tuberculatus* seed persistence at two soil burial depths over a 3-yr period at various locations by testing the following hypotheses: (1) Was seed viability of *A. palmeri* and *A. tuberculatus* affected when seeds were exposed to diverse soil surface and subsurface environments at different experimentation sites? (2) Was seed viability of *A. palmeri* and *A. tuberculatus*, hence seedbank persistence of these species, reduced as burial depth and burial duration increased?

Materials and Methods

Seed Material and Seedbank Establishment

Seeds of *A. palmeri* and *A. tuberculatus* ecotypes, originating from five different locations (i.e., *A. palmeri* from Arkansas, Indiana, Missouri, Nebraska, and Tennessee; *A. tuberculatus* from Indiana, Missouri, Nebraska, Ohio, and Wisconsin), were collected as they matured between mid-September to late October 2013 and sent to the University of Arkansas, Fayetteville, for further processing. Approximately 1 mo after the plant material was collected, a cleaned seed sample from each seed lot was sent to seven experimental sites (i.e., Arkansas, Illinois, Indiana, Mississippi, Missouri, Tennessee, and Wisconsin) for the establishment of the seed burial trials (Figure 1).

At each site, seeds of each species under investigation were buried using polyethylene mesh bags (64 cm^2), with 500-micron pore openings (Elko Filtering, Miami, FL). More specifically, 100 seeds from each seed lot were counted and thoroughly mixed with approximately 20 g of soil collected from the burial site and known to be free of both weeds. Soil placed in the bags was sieved though a 1.4-mm (14 mesh) screen to ensure that no alien seeds would be enclosed in the polyethylene bag. The use of the polyethylene bags, particularly for weeds with small-sized seeds such as *A. palmeri* and *A. tuberculatus*, ensures that seeds could be retrieved on any sampling occasion. Wijayratne and Pyke (2012) adopted the same approach when investigating the persistence of the small-sized seeds of big sagebrush (*Artemisia tridentata* Nutt.).

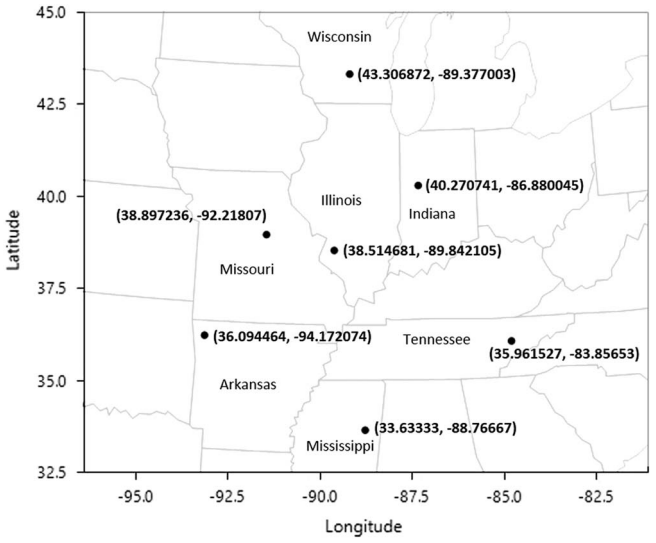

Figure 1. Experimental sites across Midsouth United States, where *Amaranthus palmeri* and *Amaranthus tuberculatus* seed material was exposed to burial trials for a period of 1 to 3 yr before viability test evaluation. Numbers in parentheses represent the latitude and longitude of the experimental sites.

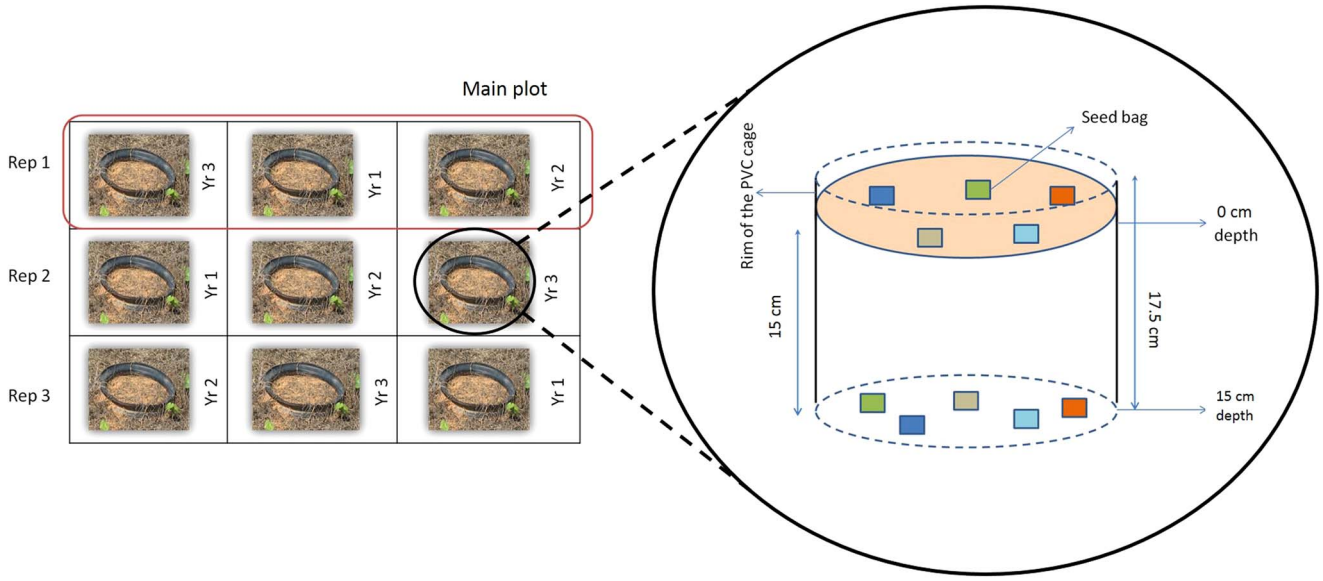

Figure 2. Experimental layout in which the randomized arrangement of main plots (i.e., retrieval year), subplots (colored seed bags representing the site by ecotype treatments), and sub-subplot (i.e., burial depth treatment) are depicted along with details for seedbank establishment (dimensions and burial depth of PVC cage).

Soil was excavated at each experimental site to a 15-cm depth, and polyvinyl chloride (PVC) plastic cylindrical pipe cages (60-cm diameter by 17.5-cm height with openings at both ends; Figure 2) were placed in the opening and filled with excavated soil after installation of the polyethylene bags within the cage. One polyethylene bag containing seeds from one location of origin and one species (one ecotype) was placed in each cage at the 15-cm depth. Polyethylene bags were also placed at the soil surface (0 cm) after the cage was filled with excavated soil, for a total of 10 polyethylene bags. The remaining 2.5 cm of the rim of the cage remained above the soil surface to prevent off-site movement of seed-containing bags but also to ensure that potential stagnant water could percolate through the soil profile. The cages were covered with wire mesh to prevent possible damage of seed bags by rodents and birds. Three replicates were used for this experimental setup for each of *A. palmeri* and *A. tuberculatus* species. Eighteen cages (nine for each species) were used in the study in each experimental location (Figure 2).

Seed Germination

Germination tests on *A. palmeri* and *A. tuberculatus* seed samples before burial (December 2013) and after the completion of the experiment (December 2017) were conducted as described by Jha et al. (2014), with four replications for each combination of species and ecotype. For the duration of the experiment, seed samples were stored at 4 C with approximately 25% to 30% relative humidity.

For the germination evaluations, a seed lot of 100 seeds per species from each ecotype were placed in separate 9-cm-diameter plastic petri dishes (Fisher Scientific, Suwanee, GA, USA) lined with two layers of Whatman filter paper (Whatman's No. 1, Fisher Scientific), and moistened with 5 ml of 1% (v/v) captan fungicide (Captan 4-L, Drexel Chemical, Memphis, TN, USA) solution in deionized water. These were incubated for 18 d with a 14-h photoperiod at 30 C, which is the optimum temperature for *Amaranthaceae* germination (Steckel et al. 2004). Deionized water was added when necessary to maintain adequate moisture for the incubated seeds. Seed germination was assessed every 6 d, with germination determined by radicle protrusion of at least 1 mm. Nongerminated seeds were checked for viability using both a tetrazolium test, as described below, and a seed crush test (Borza et al. 2007; Sawna and Mohler 2002). The viability of the untreated seed material from each location was evaluated separately; however, germination and viability test results were combined to estimate viability of each seed lot.

Seed Retrieval and Viability (Tetrazolium) Test

Each November, the bags pertaining to the retrieval timing at both depths were extracted by carefully unearthing the PVC cages. The retrieved seed bags from all sites were sent to Fayetteville, AR, where seeds were carefully retrieved and subjected to the tetrazolium test for seed viability evaluation. Soil that had been earlier added to the bags was gently rinsed with tap water, and the remaining content of the bag was placed in a 9-cm-diameter petri dish from which the seeds were retrieved using a pair of forceps (Becton, Dickinson, Franklin Lakes, NJ, USA) and visually inspected using a dissection Accu-Scope 3055 LED Stereo microscope (Accu-Scope, Commack, NY, USA). All seeds were counted and classified as damaged or intact.

Damaged seeds (%) were estimated based on Equation 1:

$$DS = 100 - IS_{t_i},\ i = 1, 2, \text{ or } 3 \qquad [1]$$

where DS is damaged seeds + seed losses, IS is intact seeds found in the polyethylene seed bag at retrieval year t_i, 100 is the total number of seeds placed in the polyethylene seed bag at the beginning of the experiment, and t_i is retrieval year. Damaged seeds included broken, shrunk, or malformed seeds (Figure 3) and those lost due to deterioration and futile germination. Any other debris was also discarded.

To facilitate tetrazolium straining, the undamaged seeds were initially placed in petri dishes between two Whatman filter papers that were moistened with 2.5 ml of deionized water for 24 h at room temperature. Immediately after this period, 2.5 ml of 1% w/w solution of 2, 3, 5-triphenyltetrazolium chloride was added to the petri dish to ensure that the seeds were well imbibed into the

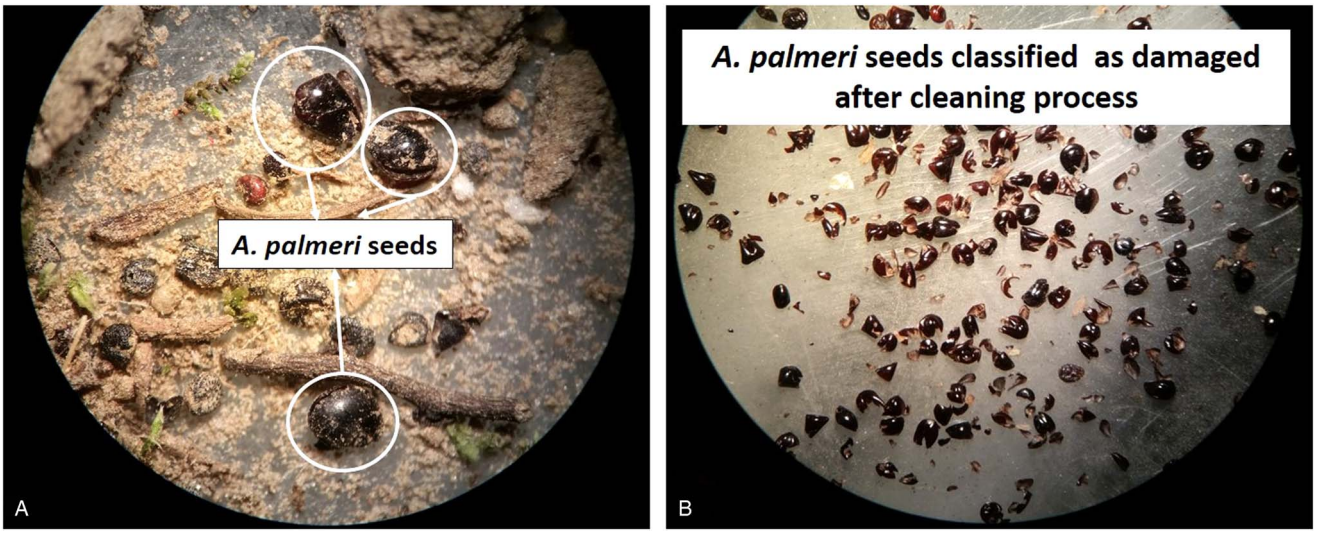

Figure 3. *Amaranthus palmeri* seeds as they appeared under a dissection microscope (A) before the seed retrieval and cleaning processes and (B) after the cleaning process. The same criteria were used for *A. tuberculatus* seeds.

solution. Seeds remained at room temperature for a 24-h staining period (Forcella et al. 2003). Seeds were then removed from the petri dish and gently crushed and classified as viable if the entire embryo was stained (Association of Official Seed Analysts 1970; Forcella et al. 2003; International Seed Testing Association 1985; Price et al. 2010).

The percentage of viable seeds (VS) after tetrazolium staining was calculated as the total number of viable seeds, which was the sum of germinated seeds (G) plus those that tested positive with tetrazolium (T) divided by the total number of seeds placed in the polyethylene seed bag (N) and multiplied by 100 (Equation 2) (Borza et al. 2007).

$$\text{VS} = \frac{G+T}{N} \times 100 \quad [2]$$

Damaged seeds were expressed as a percentage of the total seeds found in the polyethylene mesh bags at each retrieval time.

Soil Temperature and Volumetric Water Content

Minimum/maximum soil temperature and volumetric water content (i.e., soil moisture content) were recorded every 15 min using Onset HOBO U12 (Onset Computer, Bourne, MA, USA) data loggers with a soil-temperature probe (TMC6-HD, Onset Computer) and a soil-moisture probe (Onset S-SMD-M005 10HS, Onset Computer) placed at the soil surface and 15 cm below the soil surface throughout the entire experimental period. Data from the data loggers were downloaded to a laptop unit every 6 mo. Logger batteries were checked and were replaced, when necessary, every year.

Experimental Design and Data Analysis

The experiment was conducted as a split-split-plot design with three replications, where year of retrieval was the main plot factor, site by location of origin (ecotype) were subplot factors, and burial depth was the sub-subplot factor. The main plot treatments were randomly assigned, and they were permanent throughout the period of the study. The plot area was 1-m wide by 3-m long, and the subplot was 1 m^2, although the experiment was limited to a PVC cage, and the sub-subplot was limited within the PVC cage (i.e., 60-cm diameter) (Figure 2).

Seed viability, expressed as a percentage viability of the initial 100-seed population placed in the polyethylene mesh bags between sites by origin locations, years, and burial depths, was analyzed as fixed effects by ANOVA using JMP v. 13.1.0 Pro software (SAS Institute, Cary, NC, USA), whereas replication was set as a random effect. Species were analyzed separately due to different collection origins. Seed damage was expressed as a percentage of the remaining seed population at each retrieval time and was analyzed using the same methods as those used for seed viability data.

Results and Discussion

Viability of Initially Harvested and Stored Seeds

The viability of the seeds from each location of origin was evaluated at the beginning and at the end of the experimental period

Table 1. Percentage of *Amaranthus palmeri* and *Amaranthus tuberculatus* viable seeds before (2013) and at the end of the experimental period (2017) of stored seed lots.

Weed species	Origin	Seed viability[a]	
		2013	2017
		————%————	
A. palmeri	Arkansas	97.7	92.5
	Indiana	91.4	89.2
	Missouri	94.0	88.5
	Nebraska	97.1	92.4
	Tennessee	94.0	92.5
A. tuberculatus	Indiana	98.0	92.2
	Missouri	97.3	94.5
	Nebraska	92.0	89.2
	Ohio	88.2	90.7
	Wisconsin	91.9	90.2

[a]Germinated and nongerminated but viable seeds were summed as viable seeds for both species.

using germination and tetrazolium tests. Both results were averaged and expressed as percentage viability. The percent viability ranged from 88.5% to 92.5% in 2013 and 89.2% to 94.5% in 2017 for *A. palmeri* and 88.5% to 94.5% in 2013 and 90.2% to 94.5% in 2017 for *A. tuberculatus* (Table 1). Barton (1961) reported 25% germination of *A. retroflexus* seed when stored on moist glass wool at constant temperatures over a period of 8 yr, but 100% viability and germination for the remaining seeds when these were exposed to suitable temperature and relative humidity environments.

Effects of Site and Seed Origin (Ecotype) on Seed Damage and Seed Viability

Significant differences ($\alpha = 0.0001$) for seed damage and viability were recorded for both species due to burial site. More specifically, higher seed damage averaged across ecotype, burial treatment, and retrieval year was recorded for the seed that originated from Illinois (51.3% and 51.8%) followed by Tennessee (40.5% and 45.1%), and Missouri (39.2% and 42%) for *A. palmeri* and *A. tuberculatus*, respectively. Pakeman et al. (2012) reported that increases in soil moisture resulted in increases of the rate of seed mortality. This might be attributed to the activity of fungal pathogens during moist or flooded conditions (Fogliatto et al. 2010; Liebman et al. 2014). The average volumetric water content values for Illinois and Tennessee were recorded at 0.39 and 0.38, respectively, and were the highest levels among all sites, whereas that of Missouri was at a moderate level equal to 0.22 (Figure 4).

Volumetric water content at field capacity varies among soils, with values ranging from 0.1 or less for sandy soils to 0.4 for clay soils (Sinclair and Bennet 1998). The Indiana and Mississippi sites exhibited high viability, averaged across burial treatments, years, and ecotypes, for both species (i.e., 33.8% and 19.5% for *A. palmeri* and 33.4% and 27.8% for *A. tuberculatus*, respectively). Both the Indiana and Mississippi locations, despite their differences in soil temperature, exhibited moderate average volumetric water content, compared with values recorded for Illinois or Tennessee, at 0.32 and 0.29, respectively (Figure 4).

Despite the similarities of soil texture between the experimental sites (Table 2), the moderately higher percentage of sand content at Indiana and Arkansas followed by Tennessee and Mississippi is noticeable. Nevertheless, further research is required to clarify

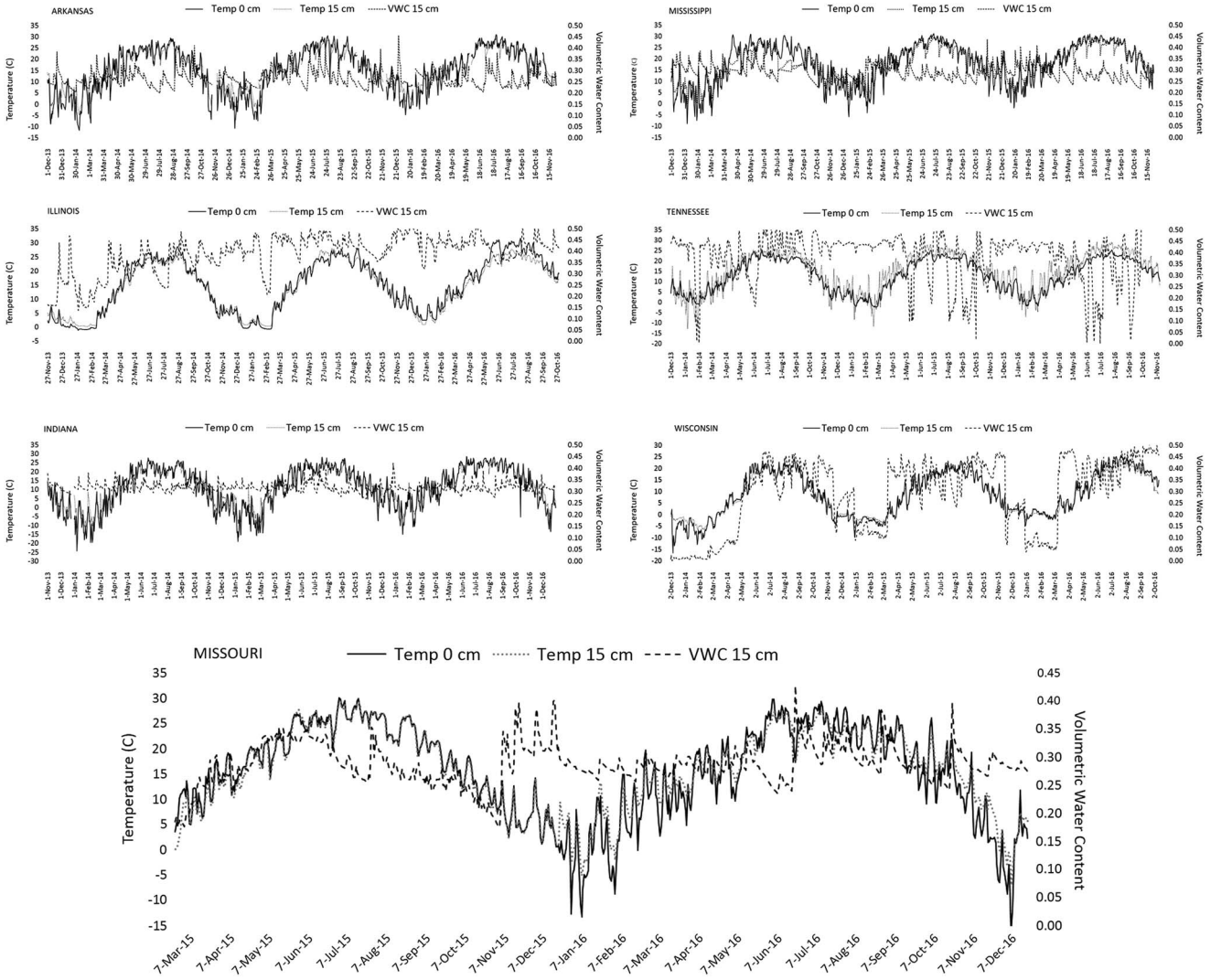

Figure 4. Monthly soil temperature (averaged over a 15-min concurrent recording period on a daily basis for the entire experimental period) at the top and at 15 cm below the soil surface (left *y*-axis) and monthly soil volumetric water content at 15 cm below soil surface (right *y*-axis) for each of the seven sites where the seed material of *Amaranthus palmeri* and *Amaranthus tuberculatus* was exposed to burial conditions during 2014–2016. Data presented for Missouri include only two experimental years (2015 and 2016).

Table 2. Soil series, texture class, and related particle percentages along with organic matter (OM) content and pH for each of the experimental sites where seeds of *Amaranthus palmeri* and *Amaranthus tuberculatus* were exposed to burial treatments.

State	Soil series	Soil texture	Sand	Silt	Clay	OM	pH
			——————————%——————————				
Arkansas	Typic Albaqualts	Silty loam	34	53	13	1.5	6.9
Illinois	Bethalto	Silty loam	10	72.5	17.5	2	6.2
Indiana	Starks-Fincastle complex	Loam	40.7	38.6	20.7	2.3	6.5
Mississippi	Catalpa	Silty clay loam	18	52	30	1.25	7.2
Missouri	Mexico	Silty loam	10	67.5	22.5	2.2	5.8
Tennessee	Sequatchie	Loam	24	58	18	1.3	6.7
Wisconsin	PnB-Plano	Silty loam	10	68	22	3.5	6.5

possible relationships among soil type structure, volumetric water content, and seed damage or viability.

Annual mean soil temperature at soil surface and 15 cm below the soil surface was recorded at 12.6 and 13.9 C for the Indiana site and at 17.8 and 18.2 C for the Mississippi site, respectively. Webb et al. (1987) reported that amaranth seedling emergence increased as temperature increased from 15.3 to 21.3 C under controlled environmental conditions. He also mentioned that these results corroborate earlier research under field conditions when mean spring soil temperatures were considered.

Larcher (1980) reported that the movement of water through the soil profile and into plant tissue are soil-temperature dependent; water can be extracted more readily from warm than cold soils. The relatively low average soil temperatures (13.5 C) in combination with the low average soil volumetric water content recorded at Missouri (equal to 0.22) possibly resulted in low seed viability for both *A. palmeri* and *A. tuberculatus* (Figure 4). High soil moisture levels deplete soil oxygen, causing hypoxic conditions (Wesseling and van Wijk 1957). Orthodox seeds, such as these of the *Amaranthaceae* family (Hong et al. 1996), maintain their longevity under aerobic conditions and permissible moisture levels; otherwise, seed viability will show the maximum rate of deterioration at a given temperature (Roberts and Ellis 1989).

The effects of site by seed origin (ecotype) averaged over years and burial depth on seed viability were found to be different ($\alpha = 0.001$) for both species (Figure 5; Supplementary Tables S1 and S2). Independently, the origin of the seed, hence the conditions of maternal environment under which the seeds were produced, influences the ability of these species to form a persistent seedbank, even though persistence varies by site (Figure 5; Supplementary Tables S1 and S2). This facilitates the occurrence and distribution of these species, particularly *A. palmeri*, over a wide range of habitats (Korres et al. 2015) or soil characteristics (Korres et al. 2017). Nevertheless, Penfield and MacGregor (2017) reported that seed-production environment effects are multifaceted and involve a complex and overlapping gene network that acts independently on fruit, seed coat, or zygotic tissues, which can be analyzed through careful physiological, molecular, and genetic approaches.

Seed Burial Effects

Burial depth affected ($\alpha = 0.001$) seed damage plus loss for both species. Seeds placed on the soil surface had increased damage and loss compared with seeds buried at 15 cm, independent of the experimental site. Increased damage and loss of unburied seeds at all sites within the same year ranged from 3% to 42% for *A. palmeri* and 10% to 62% for *A. tuberculatus* (Figure 6; Supplementary Tables S1 and S2). The rate of unburied seed damage/loss versus that of buried seeds, between consecutive years, was greater for the unburied seeds, particularly for 2014 and 2015 (Figure 6; Supplementary Tables S1 and S2), for both species. As reported by Hulme (1998), seed burial protects seeds from insect predation; however, seeds are susceptible to fungal or bacterial pathogen infection (Blaney and Kotanen 2001; Leishman et al. 2000). In addition, burial of seeds can amend unfavorable environmental effects, reducing seed weathering and increasing seed longevity (Facelli et al. 2005; Wijayratne and Pyke 2012).

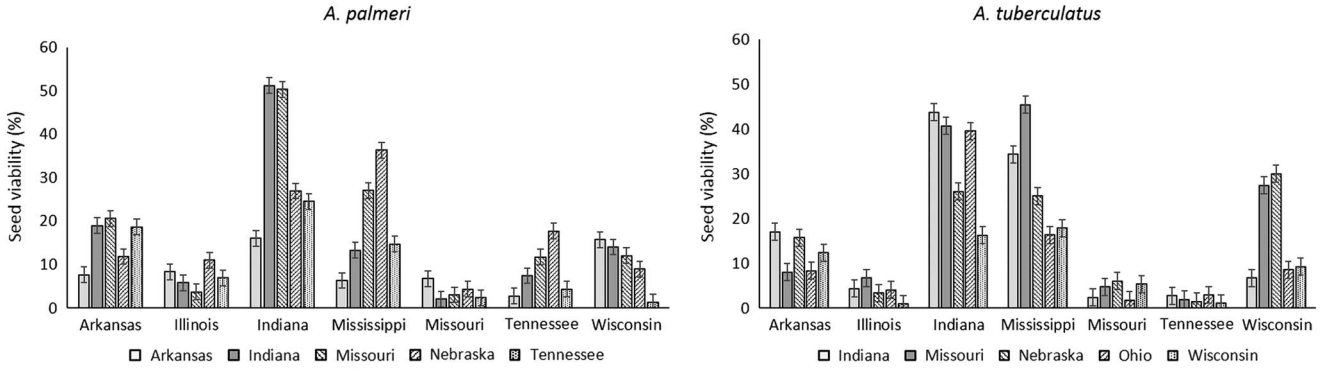

Figure 5. Interaction of experimental site by ecotype on seed viability for *Amaranthus palmeri* and *Amaranthus tuberculatus* averaged across 2014–2016. Vertical bars represent ± standard error of the mean. Supplemental information is also provided in Supplementary Tables S1 and S2, where the actual values averaged across three replications per treatment and five locations of seed material origins are shown.

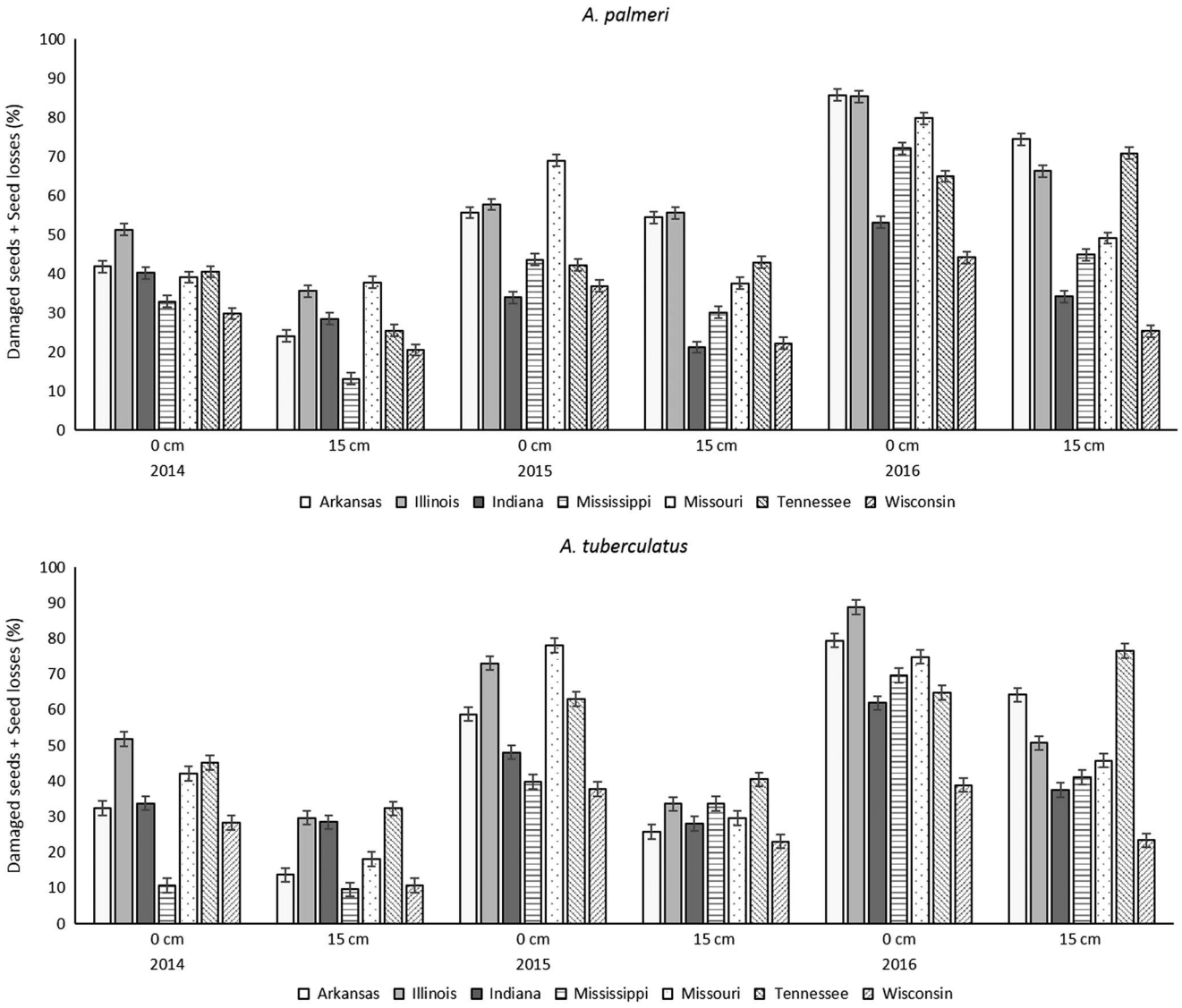

Figure 6. Interaction of experimental site by burial depth on percentage seed damage and loss averaged across 2014–2016 for *Amaranthus palmeri* and *Amaranthus tuberculatus*. Damaged seeds include broken, shrunk, or malformed seeds and those lost due to deterioration or futile germination, which were impossible to count. Vertical bars represent ± standard error of the mean. Supplemental information is also provided in Supplementary Tables S1 and S2, where the actual values averaged across three replications per treatment and five locations of seed material origins are shown.

Crist and Friese (1993) reported that seed decomposition and fungal pathogens were the major factors for the greatest decline of seeds deposited on soil surface compared with buried seeds. Nevertheless, susceptibility of weed seeds to decay by soil microorganisms is species dependent (Chee-Sanford et al. 2006), particularly in regard to the soil microbial community. The manipulation of soil fertility by the incorporation of organic amendments into the soil and/or the choice of cropping system that can influence the composition of fungal and bacterial communities (Davis 2007; Davis et al. 2006; Ullrich et al. 2011) might also affect seedbank longevity and persistence (De Cauwer et al. 2011).

Seeds that were not placed in the field but remained under storage conditions had an average viability of 94.8% and 91.1% (±1.3 SE) for *A. palmeri* and 93.5% and 91.4% (±1.3 SE) and *A. tuberculatus* for 2013 and 2017, respectively (Table 1). On the contrary, viability of intact seeds rapidly declined the first 12 mo by 80% and 85% for buried and unburied *A. palmeri* seeds, respectively (Figure 7). Likewise, the percentage loss of viability for buried and unburied *A. tuberculatus* seeds for the first 12 mo was 78.2% and 84.6%, respectively. Loss of seed viability continued to decrease for both species between 12 and 24 mo burial, reaching the lowest level, at which burial depth had no influence, by the end of the 36-mo experimental period (Figure 7).

The rate at which *A. palmeri* and *A. tuberculatus* seeds lost viability over time depended on whether seeds were placed on the soil surface or buried, and it was found to vary among sites. A clear trend in reduction of seed viability was observed for Arkansas, Illinois, Indiana, Missouri, and Tennessee ecotypes compared with Mississippi and Wisconsin ecotypes in the case of *A. palmeri* (Figure 8). Viability of intact *A. palmeri* seeds on the soil surface was lower for the entire duration of the study but with some exceptions, as in the case of *A. tuberculatus* from the Indiana experimental site (Figure 8). Seed burial seemed to act as a long-term conservation mechanism for seedbank persistence in most sites for both species. *Amaranthus palmeri* seed viability was

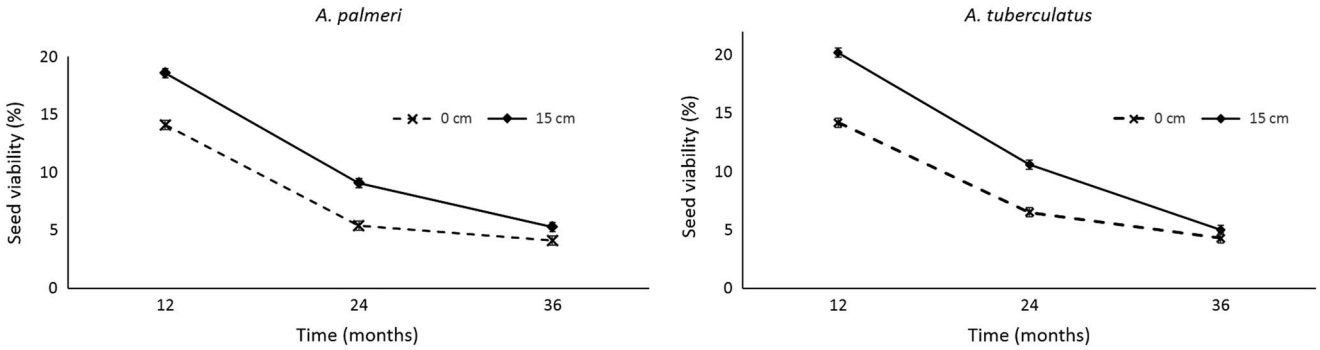

Figure 7. Percentage seed viability as affected by burial depth and retrieval time (in months) for *Amaranthus palmeri* and *Amaranthus tuberculatus*. Vertical bars represent ± standard error of the mean (i.e., 0.612 and 0.649 for *A. palmeri* and *A. tuberculatus*, respectively).

reduced in Arkansas between 2014 and 2016 for unburied seeds (90.3%) compared with buried seeds (79.4%). Similarly, seed viability declined by 88.7% and 68.4% in Illinois, by 82.4% and 69.2% in Indiana, by 63.1% and 62.2% in Mississippi, and by 60.6% and 43.8% in Wisconsin for unburied and buried seeds, respectively. Conversely, viability was reduced at a slower rate for unburied seeds than for buried seeds in Missouri and Tennessee. Similar trends were recorded for *A. tuberculatus* (Figure 8).

The effects of burial depth and burial duration on deterioration of seed viability and seedbank longevity have been demonstrated for a range of weed species, such as wild oat (*Avena fatua* L.) (Miller and Nalewaja 1990), kochia [Bassia *scoparia* (L.) A. J. Scott] (Zorner et al. 1984), ripgut brome (*Bromus diandrus* Roth) (Gleichsner and Appleby 1989), weedy rice (*Oryza* sp.) (Chauhan 2012), giant ragweed (*Ambrosia trifida* L.) (Harrison et al. 2007), and *A. palmeri* (Sosnoskie et al. 2013). All of these studies reported

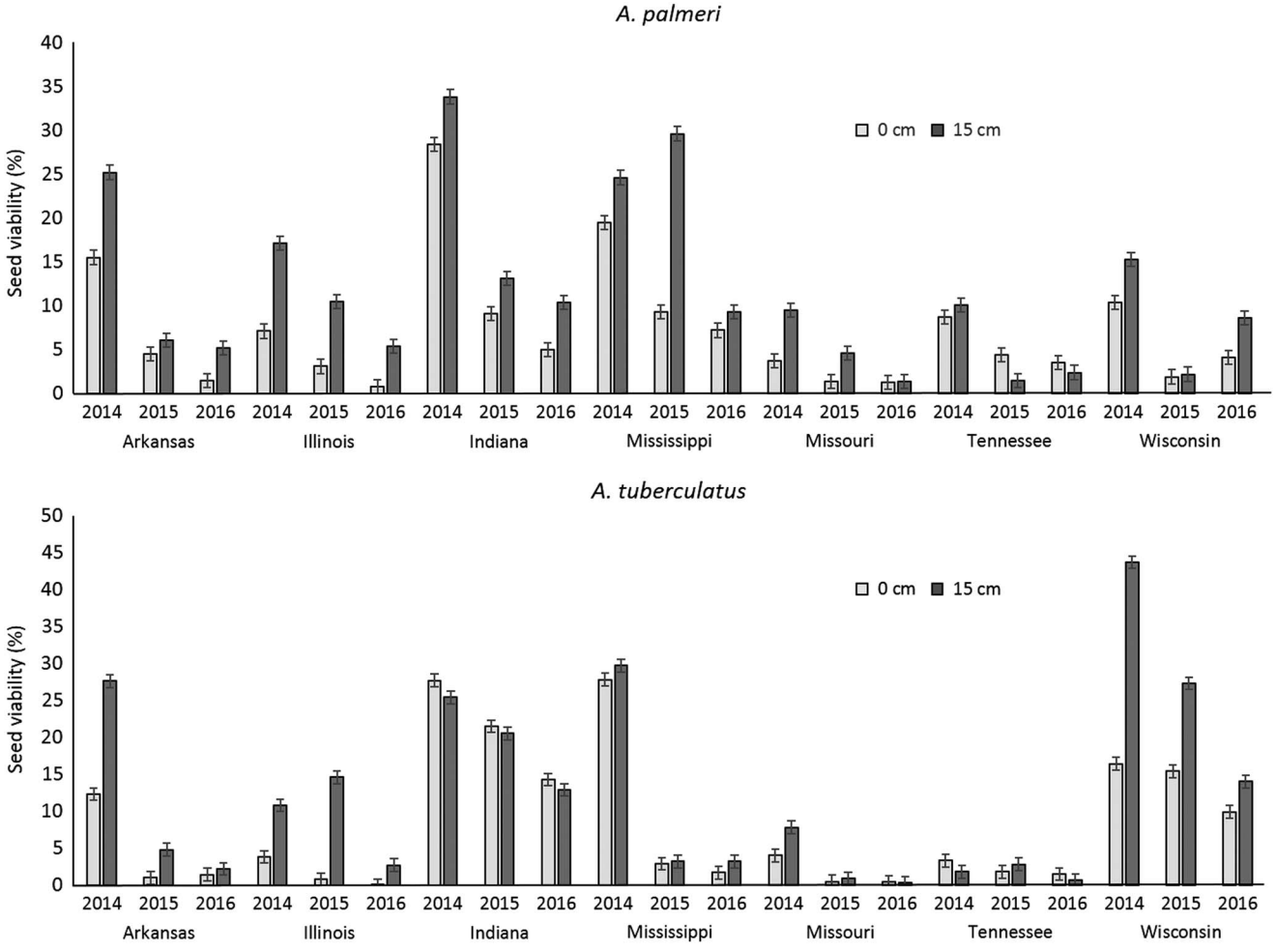

Figure 8. Effects of experimental site by burial depth by year on percentage seed viability for *Amaranthus palmeri* and *Amaranthus tuberculatus*. Vertical bars represent ± standard error of the mean.

findings similar to the work presented here and highlighted the importance of burial duration and depth on seed longevity, seed viability, and seedbank persistence; the deeper the seed burial (up to 30 cm), the greater the seedbank persistence (up to 36 mo burial duration).

As mentioned previously, the first important phase for the development of a persistent soil seedbank is burial (Fenner and Thompson 2005). Small seeds are more likely to become buried, thereby reinforcing the selective advantage of a small seed size, as is found in *A. palmeri* and *A. tuberculatus*, two very prolific weed species. The importance of tillage practices was mentioned as a primary tool for the depletion of seedbank persistence (Clements et al. 1996; Cousens and Moss 1990). Various studies (Blackshaw et al. 1994; Ominski and Entz 2001) reported that conservation or zero-tillage systems resulted in reductions of weed populations and seedbank depletion, an approach that could be proven to be quite suitable for *A. palmeri* and *A. tuberculatus*. Accumulation of seeds on soil surface in reduced-tillage cropping systems could increase seed mortality due to increased seed predation (Hossain and Begum 2015). Lack of soil disturbance via tillage could also encourage higher predator populations, as it enhances the number, diversity, and/or activity of seed-consuming habitat (Blubaugh and Kaplan 2015). In addition, the removal of weed before seed-set or harvesting weed seed could serve as a prevention method in reducing soil seedbank inputs and depleting weed seedbanks (Walsh et al. 2012, 2013), including those of Amaranthaceae weed species (Norsworthy et al. 2016). The results presented here indicate that the greatest influence of seed viability was burial conditions, time, and site-specific soil conditions, more so than geographical location. Hence, management of these weed species should focus on reducing seed shattering, removing the seed from the soil surface where germination may occur for prolonged periods, enhancing seed predation, or adjusting tillage systems.

Supplementary Materials. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2018.27

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