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When is the Best Time to Emerge—II: Seed Mass, Maturation, and Afterripening of Common Waterhemp (*Amaranthus tuberculatus*) Natural Cohorts

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Field studies were conducted to determine the effect of emergence timing on the fitness of the next generation as represented by seed mass, maturation, and afterripening of common waterhemp cohorts. Five natural cohorts were documented both in 2009 and 2010. Different maternal environments resulting from varied cohort emergence timings did not influence seed maturation time and seed mass, but had an inconsistent effect on seed afterripening. Here are our major findings. (1) Waterhemp cohorts needed similar amounts of time to generate viable seeds (20 to 27 d after flower initiation) and the seeds produced were of similar size (2.0 to 2.35 g), and (2) waterhemp has strong primary dormancy that may be released within 4 mo during the afterripening process, depending on the dormancy level. Seeds produced by later cohorts were more sensitive to the afterripening period, suggesting more flexibility in life strategy. Seeds from the 2009 cohorts had similar afterripening patterns; newly harvested seeds had strong primary dormancy (<10% germination), which was gradually released during dry storage and reached the maximum germination (>80%) rate 4 mo after harvest (MAH). However, germination then dropped to 40% 6 and 8 MAH, suggesting the induction of secondary seed dormancy. Strong primary dormancy at harvest for 2010 seeds was sustained in dry afterripening, perhaps because of higher dormancy level, which was the result of less-favorable parental environments brought by 10 to 30 times higher population densities and 2.5 to 5 times higher accumulative precipitation than in 2009 (see Wu and Owen 2014). We also tested the soil seed-bank seed population densities for each waterhemp cohort and found that early cohorts greatly influenced the seed population densities at the soil surface level and the turnover rate of the soil seed bank. Results from this research will provide insights into better management of waterhemp, targeting a better understanding of the seed bank.

Nomenclature: Common waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer.

Key words: Common waterhemp, seed afterripening, seed mass, seed maturation.

Common waterhemp is a strong competitor to crops throughout the midwestern United States. The troublesome weed is known for an opportunistic emergence pattern, aggressive vegetative growth, high seed production, and moderately long seed viability in the soil seed bank (Bensch et al. 2003; Hager et al. 2002). Among the weediness characteristics mentioned above, the opportunistic seedling emergence contributes most to the difficulties in effective control of waterhemp. In the past decade, much effort has been expended to increase our knowledge of the importance of emergence timing on waterhemp ecological fitness and adaptation to current crop production systems. In previous studies, reproductive traits such as flowering phenology and seed production were thought to be good indicators of fitness (Hartzler et al. 2004; Steckel and Sprague 2004). Plant fitness can be also be characterized by the ability of plants to produce offspring and the number of genes the plant can contribute to the total population gene pool (Radosevich et al. 1997). In our previously published paper (Wu and Owen 2014), we described a 2-yr field study comparing the fitness of different natural waterhemp cohorts. Our cohort fitness study showed that waterhemp is a facultative short day plant with a multimodal flowering pattern tailoring flowering to variable environmental conditions and high seed production throughout the growing season. However, plant fitness depends not only on the current generation seed production, but also on the characteristics of the seeds, such as seed mass, dormancy, and afterripening patterns, which influence the success of future generations and impact adaptation of to the agricultural systems (Pedersen et al. 2007).

Seed mass is of great ecological significance, because it connects reproduction with vegetative growth and life strategy, and influences seed viability, germination, seed-bank longevity, dormancy, and seedling establishment, and thus the fate of future generations (Castro 1999; Hodkinson et al. 1998; Leishman et al. 2000; Norden et al. 2009; Tripathi and Khan 1990).

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Seed dormancy, which describes the condition when viable seeds fail to germinate under favorable environmental conditions, allows weeds to escape management strategies by emerging when the weed control tactics are not in place, and thus is a crucial survival strategy of many weedy plants (Foley 1994). Afterripening (AR) is a complex biochemical process that breaks physiological dormancy, such as the primary dormancy of newly mature seeds, of many species (Baskin and Baskin 2004; Stanisavljevic et al. 2010). Generally, weed seeds with a longer AR period and prolonged germination pattern cause more management problems for growers than those with short AR periods and more concentrated germination patterns (Tarasoff et al. 2007). Seed characteristics are greatly influenced by the environmental factors prevalent during seed development, such as soil and air temperatures, moisture conditions, and day length (Baskin and Baskin 1998; Fenner 1991). Maternal plant environments affect seed development, seed dormancy, and seed mass (Alexander and Wulff 1985; Allen and Meyer 2002; Contreras et al. 2008; El-Keblawy and Al-Ansari 2000; Eslami et al. 2010; Gutterman 1992). According to these studies, maternal plants grown in adverse environments, such as higher temperatures, water stress, and shorter days, are more likely to produce seeds with lower dormancy and higher seed mass than plants grow at lower temperatures, long days, and favorable moisture conditions.

Seed characteristics have also been widely studied in many *Amaranthus* species, including waterhemp. A study has also been conducted to test if seeds produced at different times during the growing season differ in germination and found that later smooth pigweed [syn. *Amaranthus quitensis* (Kunth.)] cohorts produced seeds with lower levels of dormancy (Faccini and Vitta 2005). The effect of different emergence timing on seed characteristics is in part attributable to different maternal environments during the growing season. Seeds that developed from parents under drought and medium nitrogen levels were found to have higher germination in redroot pigweed (*Amaranthus retroflexus* L.) (Karimmojeni et al. 2014). Canopy shade experienced by the maternal parents influenced seed dormancy but had no effect on Powell amaranth (*Amaranthus powellii* S. Wats.) seed weight (Brainard et al. 2005). Secondary dormancy in seeds could be induced if the seeds are exposed to undesirable environments such as high temperatures, as was found in love-lies-bleeding (*Amaranthus caudatus* L.) (Kepczyński and Bihun 2002). Proteome analysis showed that waterhemp biotypes of different geographical origins differed in dormancy regulation mechanism (Leon et al. 2006). Schutte and Davis (2014) evaluated common waterhemp biotypes from similarly managed fields and found no maternal effects on emergence period. However, relatively few studies have investigated how different emergence timings influence waterhemp seed characteristics, which greatly influences the fitness of future generations.

Because seed maturation time, seed mass, seed dormancy, and AR patterns all contribute to or directly influence plant emergence timing and establishment, an understanding of these seed characteristics may help farmers predict the population dynamics of subsequent weed generations and contribute to the development of more effective weed control strategies (Tarasoff et al. 2007). Because maternal environments vary throughout the growing season, it would be very interesting to test if seeds that mature under different environmental conditions differed in seed mass, dormancy, and AR requirements. As a follow-up to our first paper, the focus of this article was on seed characteristics that impact next-generation fitness. The specific questions of interest to us were: How does maternal environment influence seed characteristics and ultimately the fitness of the next waterhemp generation? Do waterhemp cohorts that emerge later in the growing season produce seeds faster? Are the late-produced seeds heavier or less dormant and thus contribute more to the next generation? How does each waterhemp cohort influence the soil seed bank?

Materials and Methods

Establishment of Cohort Studies and Measurements of Environmental Conditions. Common waterhemp cohort studies were conducted in 2009 and 2010 at the Iowa State University Curtiss Farm (42.03°N, 93.61°W) in Ames, IA. The soil type was a Canisteo, Nicollet, Clarion loam (fine loamy, mixed, mesic Typic Haplaquall) with 5% organic matter (Taylor and Hartzler 2000). The experimental design was a complete randomized design because of the scattered distribution of waterhemp in the study field. In this study, a *cohort* was defined as waterhemp seedlings that emerged within a few days following favorable environmental conditions, such as rain. Cohorts were established at random locations, with the highest population densities within the same field at the designated emergence timings following a rain event. Each cohort was established by counting and marking all waterhemp

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seedlings with full cotyledon expansion, indicating the same emergence time within a single 5 by 5–m quadrat in 2009 and three 3 by 3–m quadrats in 2010. The study sites of the 2009 and 2010 studies were within the same field, but different, as the 2010 location had a considerably larger waterhemp seed bank. Common waterhemp seedlings and other weeds that emerged later within an established cohort plot were removed by hand throughout the rest of the growing season. After establishing the first cohort plots, glyphosate (1.54 kg ai ha^{-1}) was applied to the area designated for later cohort plots with the use of a backpack sprayer with a carrier volume of 13.05 L per acre and XR11003VS nozzle tips. Common waterhemp seedlings and other weeds emerging within an established cohort plot were removed by hand throughout the rest of the growing season. Daily precipitation, air temperature, and soil temperature at 2-cm soil depth were measured by with the use of a rain gauge (TE525WS-L Sensor, Texas Electronics) and a 107-L sensor (Campbell Scientific), respectively. Data were collected on site with a data logger (CR23X, Campbell Scientific).

Seed Maturation Time and Seed Mass of Cohorts. Seed maturation time was defined as the time required from flowering initiation to when seeds became viable and could germinate. Five female plants were randomly chosen from each cohort as soon as flowers were initiated, and the flower initiation date was noted for each plant. Seeds were collected from each female plant when the plants had developed visible spikelets and were ready for pollination. The pollination initiation date was approximately 2 wk after flowering and was designated as day 1 of the seed developmental process. To minimize the damage to the plant on which we conducted repeated sampling, we collected a minimal number seeds for a germination test, including three replications with 10 seeds per replication. Based on the knowledge that waterhemp seeds become viable on the 9th to the 12th day after pollination (Bell and Tranel 2010), two apical spikelets from the five chosen plants were collected 5, 7, 9, 11, 13, 15 d after the designated initial flowering date for each cohort. Seeds were threshed by hand and cleaned with an air-column seed separator (Seedburo Equipment Co., (2293 South Mountain Prospect Road, (Des Plaines, IL 60018); seeds were then used to determine seed maturation time in a 14-d germination test conducted in the laboratory (Wu and Owen 2014). Mature seeds were indicated by successful germination. The germination protocol used was as follows: Seeds were placed in a six-well cell culture cluster plate with 10 seeds per well with 1.0 ml deionized water. The cell culture cluster plates were then placed inside a germination cabinet and subjected to white light (200 $\mu E\ s^{-1}\ m^{-2}$ for 16 h, dark for 8 h) and a temperature regime of 31.3 C for 16 h and 21.5 C for 8 h. Germination percentage was recorded and germinated seeds were removed daily for the duration of the study. A seed was considered germinated when radical protrusion was observed. At the end of the experiment, nongerminated seeds were air dried and subjected to a seed crush test to determine viability (Rothrock et al. 1993; Sawma and Mohler 2002). The seed maturation time was calculated with the use of the following formula

$$\text{seed maturation time} = \text{time from flower initiation to pollination initiation} + \text{earliest seed collection date that lead to germination (D5 to D15).} \quad [1]$$

Common waterhemp seed mass was determined with the use of seeds from five female plants from each cohort 40 d after flowering was initiated. Seeds were cleaned as described. With the use of a commercial seed counter, 10,000 mature (i.e., shiny black) seeds were counted and weighed to determine the 10,000-seed mass, which reduced errors in weighing extremely small seeds such as waterhemp.

Afterripening of Seeds from Different Cohorts. The importance of AR on seed dormancy was assessed from seeds collected from another five randomly chosen female plants from each cohort 40 d after flowering was initiated. Seeds were threshed and cleaned as described, and air dried at room temperature (20 to 25 C) for 1 wk. The cleaned seeds were then stored in plastic containers at room temperature (20 to 25 C) with 30 to 50% relative humidity until the start of the germination tests. A series of germination tests were conducted 4 mo after harvest (MAH), 6 MAH, and 8 MAH, with the use of the germination test protocol described above. Each germination test lasted 14 d, and at the conclusion of the experiment, a seed crush test was conducted to determine the viability of nongerminated seeds (Rothrock et al. 1993; Sawma and Mohler 2002).

Determination of Seed Population Densities in Soil Seed Bank after Cohort Harvest. The contribution of each waterhemp cohort to the soil seed bank

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Table 1. Meteorological data and corresponding common waterhemp (*Amaranthus tuberculatus*) cohort for 2009 and 2010 at the Curtiss Farm, Ames, IA.

Year	Month	Corresponding cohort	Accumulative precipitation (mm)	Average maximum air temperature	Average minimum air temperature	Average maximum soil temperature	Average minimum oil temperature
2009	May	Cohorts 1 and 2	110	22.7	9.8	24.8	13.1
	June	Cohorts 3 and 4	101	18.4	6.3	17.9	9.0
	July	Cohort 5	56	27.6	14.7	33.3	20.4
	August		88	27.2	15.0	31.7	20.0
	Average		89	24	11	27	16
2010	May	Cohorts 1 and 2	92	22.8	9.9	23.8	13.8
	June	Cohorts 3 and 4	272	28.0	16.3	28.6	19.9
	July	Cohort 5	121	29.8	18.7	34.2	23.4
	August		312	30.1	18.0	29.2	22.2
	Average		199	28	16	29	20

was determined by soil samples collected from every cohort plot on October 19, 2009 and October 14, 2010, respectively, after all the female plants were harvested. Each plot was evenly divided into four quadrats (microplots), and five soil cores (4.1-cm diameter) were collected from each microplot and subdivided into 0 to 2, 2 to 5, 5 to 15–cm depths. Individual soil cores from each microplot were pooled by depth in a composite sample. Soil samples were placed in strainers lined with 0.5-mm stainless-steel screens and then elutriated for 2 h (Wiles et al. 1996). After elutriation, samples were dried at 35 C for 24 h and waterhemp seeds were identified and counted and a crush test was conducted to test seed viability, as described above.

Statistical Analyses. Statistical analyses of data were performed with the use of the SAS software GLIMMIX procedure (Version 9.4, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513). Seed maturation time data were log transformed, and seed germination data were arcsine transformed, because the assumptions of homogeneity of variances were not met (Shapiro and Wilk 1965). The interactions between cohort and year effects were tested, and based on the results, the seed mass and seed maturation time data were pooled. However, the AR data from each year were analyzed separately. For seed maturation and seed mass data, year was a random effect in the statistical model and an individual plant was the experimental unit. Differences in seed mass, seed maturation time, and seed germination during AR were tested with analysis of variance (ANOVA). Pairwise comparisons such as flowering initiation time between male and female plants across all the cohorts were subjected to the Tukey-Kramer method at $P = 0.05$.

Results and Discussion

Although there are many reports on the effect of emergence timing on plant fitness (Hartzler et al. 2004; Steckel and Sprague 2004; Steckel et al. 2007; Uscanga-Mortera et al. 2007), few studies have been conducted to determine if this effect continues to the next generation (Mulugeta and Stoltenberg 1998). In this 2-yr field study, we investigated the effects of mother-plant emergence timing on subsequent seed maturation time, seed mass, and AR pattern. Five waterhemp cohorts were identified each year, and emergence occurred from late May until early July. More detailed information about the environmental conditions and vegetative and reproductive biology, as well as life phenology of each cohort is summarized in Table 1. Importantly, 2010 had two times higher accumulative precipitation than 2009 and slightly higher air and soil temperatures. In accordance, we saw large year-to-year variability in weed population densities, with 2010 cohorts having 20 times greater population densities than 2009, which we believe was attributable in part to the different environmental conditions. Seed maturation and seed mass data were pooled over years and seed AR data was presented by year after checking the interaction between year and cohort effects (Table 2). Seed maturation data was analyzed on log scale because of lack of variance.

Seed Maturation Time and Seed Mass. Common waterhemp emergence timing does not have a significant effect on seed maturation time or seed mass (Table 2, $P = 0.1188$ and $P = 0.3044$). Common waterhemp cohorts produced viable seeds in early through late August (Table 3), which was 50 to 80 d after plant emergence. Cohorts did not differ in their time requirement to generate viable

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Table 2. Analysis of variance comparing common waterhemp (*Amaranthus tuberculatus*) seed maturation time, mass, and germination as influenced by cohort emergence and afterripening time in 2009 and 2010.

Parameters in comparison	Year	Source of variance	DF	*F* value	Pr > *F*
Seed maturation time	Pooled, year as random effect	Cohort	4	4.82	0.1188
Seed mass		Cohort	4	2.3	0.3044
Seed germination during afterripening	2009	Cohort	4	0.47	0.7549
		Seed afterripening time	3	26.76	< 0.0001**
		Cohort * afterripening time	12	0.71	0.7289
	2010	Cohort	4	6.79	0.0049**
		Seed afterripening time	3	2.51	0.0778
		Cohort * afterripening time	12	1.81	0.0932
Soil seed-bank population density (seeds/m^3)	Pooled, year as random effect	Cohort	4	25.23	< 0.0001
		Depth	2	52.89	< 0.0001
		Cohort * depth	8	8	< 0.0001

** Represents significant difference at P = 0.05 level.

seeds, Seed maturation time for each cohort varied from 20 to 27 d after flowering initiation or the equivalent of 6 to 13 d after pollination initiation (Table 3). Bell and Tranel (2010) noted that waterhemp seeds matured 7 to 9 d after pollination in the greenhouse. Our field study confirmed that waterhemp had the ability to reproduce quickly, which can be a great advantage for surviving unpredictable field conditions, as well as late-season agronomic practices.

The 10,000 seed weight for waterhemp varied from 2.01 to 2.35 g, but did not differ among cohorts (Table 3). Seed mass is one of the most important traits influencing the early phases of the plant life cycle. Larger seeds are considered as ecologically beneficial, because larger seeds can produce larger seedlings, which have better chance to avoid size-dependent mortality (Quero et al. 2007). The factors that influence seed mass vary among different plant species. In some species, larger seeds were associated with adverse establishment conditions experienced by the mother plant (Armstrong and Westoby 1993; Buckley 1982; Grime and Jeffrey 1965; Krannitz et al. 1991), whereas in other species, adverse growing conditions resulting from late emergence timing led to smaller seed sizes and faster maturation (Eslami et al. 2010; Roach 1986). Common waterhemp overcomes the disadvantage of later emergence by transitioning into flowering faster, which supports adequate seed development and the opportunity to produce seeds with equal quality as found in early cohorts (Wu and Owen 2014).

Interestingly, when data were analyzed separately, later waterhemp cohorts in 2009 produced larger seeds, whereas no significant differences in seed mass among cohorts were detected in 2010 (data not shown). Because 2009 cohorts experienced only half of the accumulative precipitation as the 2010 cohorts (Table 1), and 2010 cohorts had 20 times higher population densities than 2009, we suspect that seed mass of waterhemp is sensitive to moisture condition and population densities. The reason for the lack of significant difference for seed mass in 2010 was likely the advantage of better growing conditions for early emergence, which was compromised by high intraspecific competition resulting from high plant population densities.

Table 3. Comparison of common waterhemp (*Amaranthus tuberculatus*) seed maturation time and 10,000-seed mass of different cohorts.

Cohort	Cohort emergence time	Seed maturation time	Estimated seed maturation date	10,000 seed mass
		d		g
1	Late May	27.7 ± 1.2a[a]	Early August	2.09 ± 0.17a
2	Late May/Early June	21.4 ± 1.2a	Early August	2.01 ± 0.17a
3	Mid June	22.7 ± 1.1a	Mid August	2.12 ± 0.17a
4	Late June	22.2 ± 1.2a	Mid August	2.06 ± 0.17a
5	Early July	20.6 ± 1.2a	Late August	2.35 ± 0.17a

[a] Seed maturation data were analyzed on log scale.

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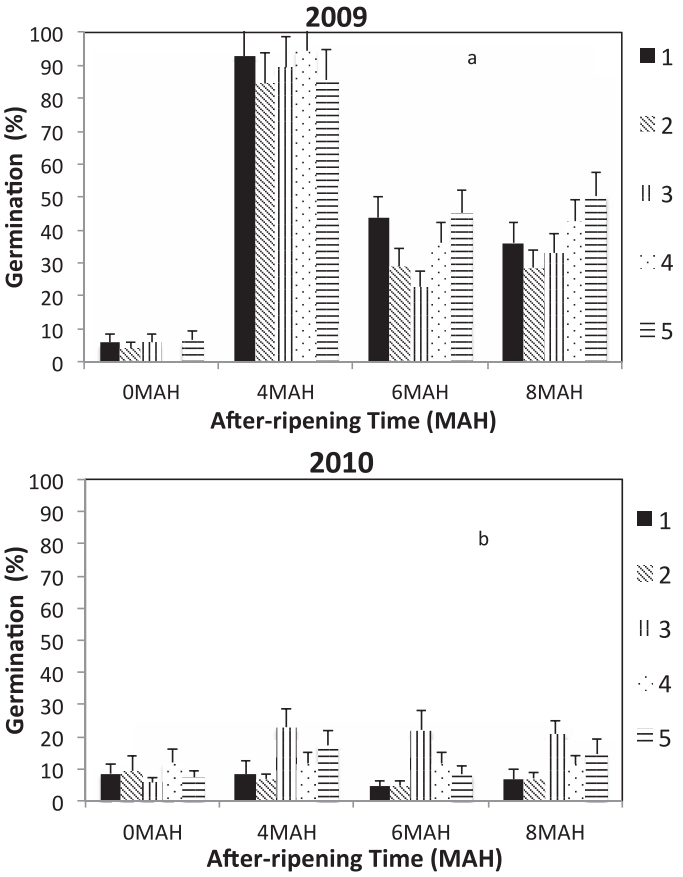

Figure 1. Comparison of mean common waterhemp (*Amaranthus tuberculatus*) cohort accumulative seed germination at different after-ripening times in 2009 (a) and 2010 (b). The x-axis is seed after-ripening time (months after harvest, MAH), the y-axis is the germination percentage. Each column represents the mean germination percentage of four randomly chosen plants from each cohort. The legend corresponds to each cohort as shown at the right side of the figure. The error bars represent standard error.

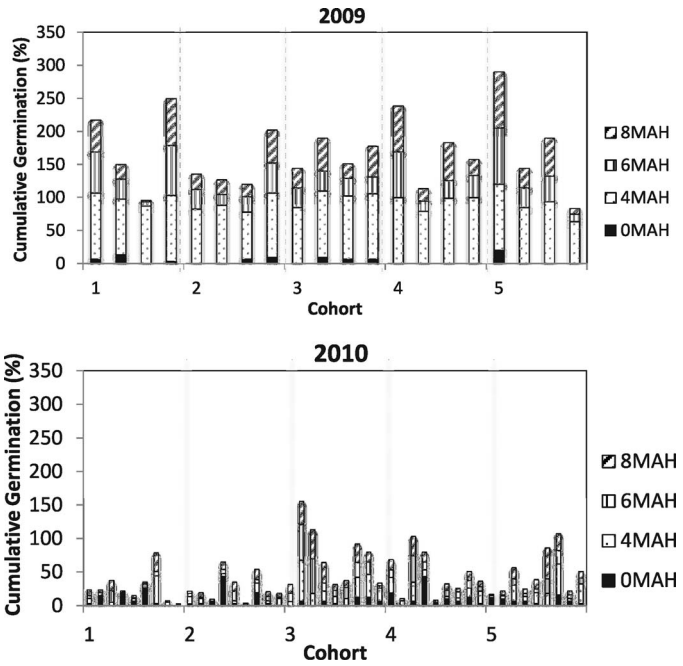

Figure 2. Germination of common waterhemp (*Amaranthus tuberculatus*) seeds from individual plants within each cohort for different after-ripening periods in 2009 (a) and 2010 (b). Each bar represents final germination percentage of seeds from an individual plant. The x-axis is the cohort, with duplicate plants in each cohort (4 plants in 2009 and 9 plants in 2010). The y-axis is the cumulative final germination percentage after a 14 day germination test with 16h/8h light/dark and alternating temperature of 22°C/16°C. The error bars represent standard error.

Seed AR Pattern of Cohorts. Common waterhemp seeds showed strong primary dormancy, with up to 90% of newly harvested waterhemp seeds (0 MAH) failing to germinate even though the seeds were viable as determined from the crush test conducted on the nongerminated seeds (Figure 1). However, the dormancy release process differed between years. In 2009, cohorts showed similar AR patterns and the length of AR time had a significant effect on common waterhemp seed dormancy release. Four months of dry AR increased waterhemp seed germination from ~ 10% to more than 70%, indicating loss of primary dormancy. However, germination then dropped to 40% at 6 MAH and 8 MAH, indicating that secondary dormancy was probably induced (Figures 1 and 2). In contrast, AR periods did not differ in their effect on 2010 waterhemp seed germination, and cohorts behaved similarly during the AR. In 2010, high primary dormancy at seed dispersal was sustained during dry AR conditions and seeds exhibited low germination (< 20%) throughout the AR periods (Figures 1 and 2). Moreover, the later cohorts (3 to 5) tended to be more sensitive to the dormancy-releasing processes in 2010; however, the difference was barely significant ($P = 0.0449$, Table 2). We also found that even with relatively similar emergence timings, waterhemp could still produce seeds that varied in dormancy levels (Figure 2). The high individual variance between and within waterhemp cohorts could lead to more varied dormancy levels in the soil seed bank, which could contribute significantly to the extended emergence pattern typically demonstrated by waterhemp.

Primary seed dormancy plays a critical role in the extended emergence patterns of *Amaranthus* species, because it has the ability to maximize the probability of seedling survival (Baskin and Baskin 1985; Cristaudo et al. 2007). AR periods strongly influence seed germination in some *Amaranthus* species but not others (Cristaudo et al. 2007). Our study confirmed that waterhemp has strong primary dormancy and the dormancy could be released through AR processes depending on the level of

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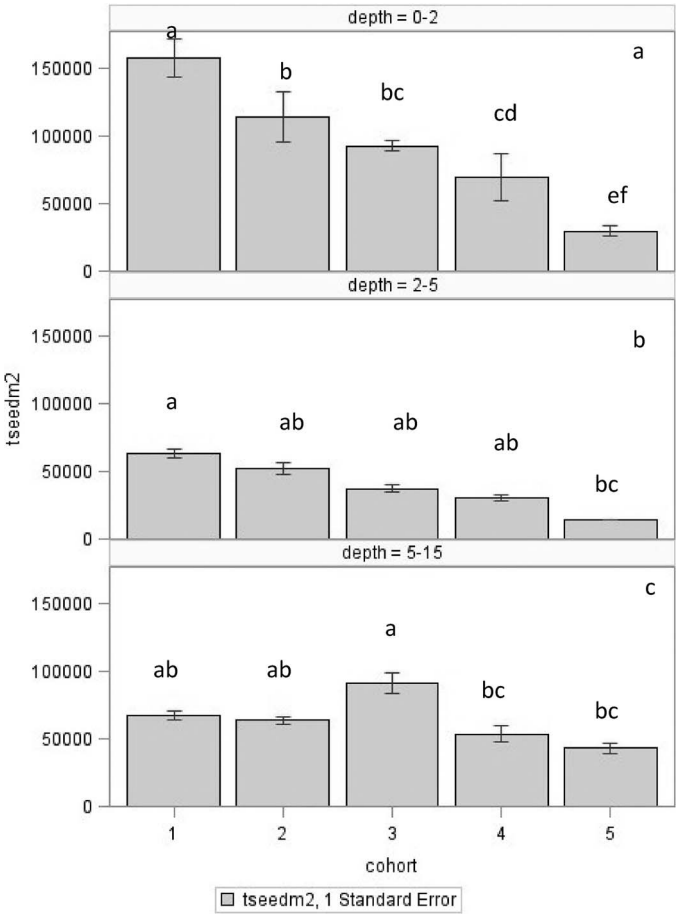

Figure 3. Seed population densities for different common waterhemp (*Amaranthus tuberculatus*) cohorts at different soil depths 0–2 cm (a), 2–5 cm (b) and 5–15 cm (c). The x-axis is the cohort number and y-axis is the total seed number (seeds). The grey bars represent the seed population densities (seeds m^{-2}) of each cohort at each soil depth (averaged from soil samples within the plots designated for a cohort, 1 plot $cohort^{-1}$ in 2009 and 3 plots $cohort^{-1}$ in 2010) in the soil seed bank. The error bars represent the standard error.

dormancy. Seed dormancy represents an interaction between genetics and environmental factors and is influenced by the maternal growth environment (i.e., temperature, day length, light, water, and nutrient levels). However, how seed dormancy responds to environmental factors varied among different plant species. In some species, lower dormancy was associated with high temperatures, short days, red light, drought, and high nitrogen levels during seed development (Fenner 1991). However, in other plant species, adverse environments induced heritable genetic traits stored as seed memory, which manifests in seed dormancy (Jurado and Flores 2005). In our study, the lack of AR effect in 2010 may be correlated with a higher amount of rainfall as well as intraspecific competition of parent plants, which could result in much deeper physiological dormancy, as reported in other species (Leishman et al. 2000; Platenkamp and Shaw 1993). Therefore, the moisture conditions experienced by maternal plants might induce high seed dormancy in waterhemp. The extended germination pattern exhibited by waterhemp is probably a result of different seed dormancy levels within a population. The extended germination patterns demonstrated in waterhemp populations allows seedlings to experience different environments that occur during the growing season, resulting in more variation of dormancy levels within populations, which can be ecologically advantageous (Leon and Owen 2006).

A simulation study assessing variability in Powell amaranth fecundity suggested that seed production occurs in relatively few high seed production environments, and seeds produced from those high-fecundity years have critical influence on the characteristics of the Powell amaranth seed bank (Brainard and Bellinder 2004). Given the much higher seed production reported (Wu and Owen 2014) and high levels of dormancy we observed in the current study, waterhemp seeds from 2010 cohorts are more likely to become part of the persistent soil seed bank than those produced in 2009, and thus will be more of a concern to farmers with regard to long-term waterhemp management.

Soil Seed Bank as Influenced by Establishment of Different Cohorts. Seed population densities in the waterhemp soil seed bank differed significantly among cohorts, as well as among soil depths (Table 2). A significant interaction between cohort and soil depth was detected (Table 2). When comparing cohort influence on the soil seed bank at each soil depth, the main differences are for the 0 to 2–cm depth (Figure 2). Little differences were found at deeper soil depths for the soil seed bank, suggesting that all cohort plots had similar initial soil seed-bank population densities for the 2 to 15 cm depths. (Figure 3). The seed population density at soil surface is a function of seed predation, decay, and weed emergence, and augmented by newly produced seeds. Assuming that seed predation and decay are similar for all the cohorts, the only factors that influence soil surface seed-bank densities are weed emergence and new seed production.

The season-long emergence pattern demonstrated by waterhemp serves to deplete the shallow (active) soil seed bank, and newly produced seeds serve to replenish the soil seed bank. Our results showed that early cohorts depleted the soil seed bank because of more abundant

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emergence at the beginning of the growing season, but these cohorts also returned more seeds to the soil, indicating a critical role of the early cohorts on the soil seed bank (Figure 3). We noted previously that plants from early cohorts that survive herbicide exposure could pass the resistant alleles to later cohorts that flower relatively faster and have the opportunity to produce viable seeds (Wu and Owen 2014). Here we extend our assumption that, besides contributing herbicide-resistant alleles that can become a feature of the soil seed bank, early cohorts also influence the turnover rate of soil seed bank, which can act as a buffer affecting weed population shifts, and this could impact herbicide resistance evolution (Gressel and Segel 1990). The critical question is the number of seeds that carry herbicide resistance alleles relative to the number of seeds that have a herbicide sensitive phenotype; if a higher proportion of the sensitive phenotype replenishes the soil seed bank, the greater the buffering capacity delaying a population shift to the resistant phenotype. Regardless, as a prolific seed producer, it is important for producers to target the waterhemp soil seed bank in order to develop long-lasting and robust weed control tactics (Steckel et al. 2007).

To conclude, emergence timing resulting in exposure to different maternal environments (e.g., water, nutrient resources, and competition level) had little effect on waterhemp seed mass and seed maturation, and an inconsistent effect on AR patterns; these effects were largely dependent on the yearly fluctuating environmental conditions. The ecological consequences of the prolonged emergence patterns of waterhemp are likely more complicated than what we can see on the surface: (1) the plasticity in flowering phenology of later cohorts offered the opportunities to incorporate the genetic information from the survivors to the gene pool, (2) the high variance on dormancy both between and within cohorts helps to maintain the cycling of the prolonged emergence patterns, and (3) the impact of the early cohorts on the soil seed bank may buffer against the evolution of herbicide resistance. More research needs to be conducted to confirm the scientific assumptions about the ecological benefits of continuous emergence patterns of common waterhemp, so as to help farmers develop more effective weed control strategies that target depletion of the soil seed bank.

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