Tall Waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) Seed Production and Retention at Soybean Maturity

Lauren M. Schwartz, Jason K. Norsworthy, Bryan G. Young, Kevin W. Bradley, Greg R. Kruger, Vince M. Davis, Larry E. Steckel, and Michael J. Walsh*

Two of the most problematic *Amaranthus* species in soybean production today are tall waterhemp and Palmer amaranth. This study determined the percentage of tall waterhemp and Palmer amaranth seed that was retained by the weed at soybean maturity to assess the likelihood of using at-harvest weed seed control tactics for soil seedbank management. Palmer amaranth plants were collected from fields in Arkansas, Tennessee, Illinois, Missouri, and Nebraska, and tall waterhemp plants were collected from fields in Nebraska, Missouri, Wisconsin, and Illinois. Collected plants were assessed for at-harvest weed seed retention in 2013 and 2014. Within 1 wk of soybean maturity, *Amaranthus* plants were harvested and the loose soil and debris beneath the plants were swept into a pan with a hand broom to collect any shattered seed. Percent seed retention ranged from 95 to 100% for all states both years, regardless of species. There was a strong correlation between weed biomass (g) and total seed production (no. plant^{-1}) in that the larger the plant, the more seeds it produced. However, there was no correlation between percent seed retention and weed biomass, which indicates that regardless of plant size and likely time of emergence, seed retention is high at the time of crop maturity. Overall, this study demonstrated that there is great opportunity for Palmer amaranth and tall waterhemp seed capture or destruction at soybean harvest. It is likely that nearly all of the seeds produced for both *Amaranthus* species passes through the combine during harvest to be returned to the soil seedbank. Thus, there is continued need for research focused on developing and testing harvest weed seed control tactics that aim at reducing the soil seedbank and lowering risks for evolution of herbicide resistance.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Wats.; tall waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; soybean, *Glycine max* (L.) Merr.

Keywords: *Amaranthus*, harvest weed seed control, soil seedbank, weed biology, weed ecology.

Dos de las especies de *Amaranthus* más problemáticas en la producción de soja, hoy en día, son *Amaranthus tuberculatus* y *Amaranthus palmeri*. Este estudio determinó el porcentaje de semilla de *A. tuberculatus* y *A. palmeri* que fue retenido por la maleza al momento de la madurez de la soja, para evaluar la probabilidad de usar tácticas para el control de semilla de malezas durante la cosecha para el manejo del banco de semillas. Plantas de *A. palmeri* fueron colectadas en campos en Arkansas, Tennessee, Illinois, Missouri, y Nebraska, y plantas de *A. tuberculatus* fueron colectadas en campos en Nebraska, Missouri, Wisconsin, e Illinois. Las plantas colectadas fueron evaluadas por su retención de semilla al momento de la cosecha en 2013 y 2014. A la semana de la madurez de la soja, las plantas de *Amaranthus* fueron cosechadas y el suelo suelto y los residuos vegetales debajo de las plantas fueron removidos con una escoba de mano y fueron depositados en un contenedor para colectar semilla que hubiera caído al suelo antes de la cosecha. El porcentaje de retención de semilla varió de 95 a 100% en todos los estados y en ambos años, sin importar la especie. Hubo una correlación alta entre la biomasa de la maleza (g) y el total de semilla producida (no. planta^{-1}), así entre más grande la planta, más semilla produjo. Sin embargo, no hubo una correlación entre el porcentaje de retención de semilla y la biomasa de la maleza, lo que indica que sin importar el tamaño de la planta y el momento de emergencia, la retención de la semilla es alta al momento de la madurez del cultivo. En general, este estudio demostró que existe una gran oportunidad para capturar o destruir la semilla de *A. palmeri* y *A. tuberculatus* durante la cosecha de la soja. Es probable que casi toda la semilla producida por ambas

especies de *Amaranthus* pase por la cosechadora al momento de la cosecha y que sea retornada al banco de semillas del suelo. Por esta razón, existe una necesidad de investigación que se enfoque en el desarrollo y evaluación de tácticas de control de semillas de malezas durante la cosecha con el objetivo de reducir el banco de semillas del suelo y a su vez disminuir el riesgo de evolución de resistencia a herbicidas.

The *Amaranthus* species are among the most troublesome weeds in row-crop production. Specifically, tall waterhemp and Palmer amaranth are the most problematic *Amaranthus* species in the United States (Johnson 2000; Norsworthy et al. 2014). These dioecious, summer annual forbs have many characteristics that make them ideal agricultural weeds, such as environmental adaptability, discontinuous emergence pattern, rapid growth, and evolved resistance to multiple herbicide modes of action (Heap 2014; Horak and Loughin 2000; Sellers et al. 2003). These two *Amaranthus* species, through morphological and physiological means, are an increasing threat to agricultural systems across the Midwest and southern United States (Johnson 2000; Norsworthy et al. 2014).

Weeds and crops interact primarily by competing for shared resources such as light, nutrients, water, and space. The competition effect is further driven by ecological factors such as weed emergence relative to the crop (Dieleman et al. 1996; Knezevic et al. 1994), the effects of weed density (Dunan et al. 1995), the duration of competition (Swanton et al. 2015), and the morphology and life history of the weed species (Davis et al. 2015). For example, Palmer amaranth and tall waterhemp have the ability to grow 2 to 3 m tall (Horak and Loughin 2000; Trucco and Tranel 2011) and have an extended period of seedling emergence, which can continue late into the row-crop growing season (Hartzler et al. 1999). A density of only 8 Palmer amaranth plants m^{-2}, present at soybean emergence, reduced soybean grain yield by 78% compared with 56% yield loss in soybeans competing with same density of tall waterhemp plants established at crop emergence (Bensch et al. 2003). Tall waterhemp reduced soybean yields by as much as 56% when it emerged with the crop, and 10% when it emerged as late as the V4 soybean stage (Steckel and Sprague 2004a; Steckel et al. 2004, 2008).

Weed-crop competition is not limited to only one growing season. The high fecundity and seed dormancy of Palmer amaranth and tall waterhemp ensure carryover to subsequent year(s). For example,

without competition, tall waterhemp and Palmer amaranth can produce up to 1 million seeds per plant (Keeley et al. 1987; Nordby et al. 2007), whereas when in competition with soybean, the *Amaranthus* species can produce up to 500,000 seeds per plant (Sellers et al. 2003; Ward et al. 2013). *Amaranthus* species seed are predominantly gravity-dispersed, but can also be spread by water, birds or animals, and through agricultural management practices such as plowing, mowing, harvesting, and spreading compost (Costea et al. 2005; Norsworthy et al. 2009; Ward et al. 2013). Although these seeds lack specialized dispersal mechanisms, strong winds can move seeds over 300 m and the pollen over 500 m (Norsworthy et al. 2012; Sosnoskie et al. 2012).

Today, many of the *Amaranthus* species have evolved resistance to multiple herbicide modes of action (Heap 2014; Zimdahl 2004), and the concurrent maturation of crops and annual weed species has resulted in the unintentional "harvesting" of the weed seeds when commercial harvesting occurs (Walsh et al. 2013). Furthermore, the weed seeds can be redistributed on the soil surface, causing further spread, as well as increasing the soil seedbank (Walsh and Powles 2014). Thus, alternative nonchemical weed control practices are needed to control the herbicide-resistant or escaped weeds (Walsh et al. 2013). Harvest weed seed control (HWSC) tactics have been developed that include various cultural and mechanical management practices to decrease the number of weed seeds replenishing the soil seedbank. These management practices include the use of chaff carts (Walsh et al. 2012), narrow-windrow burning (Walsh and Newman 2007), the Harrington Seed Destructor (Walsh et al. 2012), bale-direct systems (Walsh and Powles 2007), and other means of targeting the chaff during harvest (Gill 1996; Walsh and Powles 2007).

There are many challenges associated with preventing inputs to the soil seedbank, but the biggest is preventing seed return over a large area and having a diverse weed seedbank (Norsworthy et al. 2012; Swanton and Weise 1991). The length of

time that seeds remains viable in the soil seedbank determines how long best management practices (BMP) must be used to reduce the seedbank and any herbicide-resistant seed (Norsworthy et al. 2012). Seed retention levels for Palmer amaranth and tall waterhemp in soybean fields across the United States have not been investigated and therefore, the potential efficacy of HWSC systems on these species remains unknown. Thus, the objective of this study was to determine the percentage of total tall waterhemp and Palmer amaranth seed production that was retained on the plant at soybean maturity, and therefore assess the likelihood of using HWSC tactics for soil seedbank management.

Materials and Methods

Data Collection. In 2013 and 2014, Palmer amaranth plants were collected from fields in Arkansas, Tennessee, Illinois, Missouri, and Nebraska, and tall waterhemp plants were collected from Nebraska, Missouri, Wisconsin, and Illinois. Additionally, Palmer amaranth was collected in Arkansas in 2012 to act as a preliminary study (data not shown). In each state, five separate commercial soybean fields with tall waterhemp and an additional five separate fields with Palmer amaranth were chosen. Field histories were not possible to collect due to the random sampling across the states. Five seed-producing *Amaranthus* plants were collected at soybean maturity from each field for a total of 25 plants per species per state. Soybean maturity was similar across fields within a state. The date of collection was recorded and the number of days until or following soybean maturity ($\pm$ 7 d) was visually estimated. Plants were selected from fields that had a low density of *Amaranthus* plants (< 5 plants m^{-2}) because seed loss from shattering was determined by sweeping up all of the seed on the soil surface beneath individual *Amaranthus* plants. It was unknown if the collected plants were herbicide-resistant or from a late-emerging flush. A hand broom was used to sweep the seed, loose debris, and loose soil into a dustpan, which was then transferred into a bag for further analysis. In addition, each *Amaranthus* plant was clipped at the soil surface and bagged to obtain aboveground dry weights (the entire plant was oven dried for 72 h at 55 C). Plants were then threshed and 0.5 g of seed from the seedhead were counted and weighed, and used to determine the total number of seeds on the plant.

The bagged seed and soil mixtures were thoroughly mixed in 36 by 48 by 5 cm trays at an approximate 1 : 1 ratio (v/v) of commercial potting mix and sample retrieved from the soil surface beneath each plant. These samples were placed into a greenhouse in early January each year at the University of Arkansas to evaluate seedling emergence over two separate 1-mo periods. Emerged seedlings were counted and removed after 1 mo, and then samples were placed outside for a further month-long cold stratification period, followed by a return of the samples to the greenhouse to monitor emergence for an additional month. There were few plants emerging by the end of evaluation, but it is possible that some viable seed might have remained dormant. Hence, the exhaustive germination technique only allowed for an estimate of the seed that had shattered prior to soybean maturity. The number of germinated seedlings were summed and used to determine the fraction of seed that shattered prior to soybean maturity. Percent seed retention was then determined by the following equation:

$$\% \text{ seed retention} = \left(\text{total no. of seed} \middle/ \left(\text{total no. of seed} + \text{germinated seed in soil} \right) \right) *100 \quad [1]$$

Statistical Analyses. Data (biomass in g), estimated total number of seeds plant^{-1}, seedling emergence, and percent seed retention) were analyzed with a three-way ANOVA using the PROC MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Species, year, and state were the fixed effects, and site location was a random effect in the model. Significance was assessed at $P < 0.05$. A first-order autoregressive covariance structure (type = ar(1) in PROC MIXED) was used in the model because it returned the lowest Akaike's Information Criteria (AIC) fit statistic compared with unstructured or compound symmetry. Means separation of significant interactions and, when appropriate, main effects, were based on least square means (LSMeans) tests. To determine the effects of state and species on percent seed retention, a correlation analysis was

Table 1. Mean (± SE) Palmer amaranth and tall waterhemp characteristics from each state in 2013 and 2014. Sites per species in each state were pooled. Different letters indicate significant differences within species between years and among states.

Species	State	Biomass		Seed production		Seed retention	
		2013	2014	2013	2014	2013	2014
		—g plant^{-1}—		—no. plant^{-1}—		—%—	
Palmer amaranth	AR	92.13 ± 9.94 b	77.45 ± 11.01 b	50,022 ± 8,209 b	33,915 ± 5,775 c	99.98 ± 0.00 a	99.85 ± 0.05 a
	IL	64.64 ± 9.17 c	—	26,038 ± 3,753 cd	—	99.95 ± 0.03 a	—
	NE	86.24 ± 12.81 bc	77.74 ± 8.71 b	36,978 ± 5,399 c	58,004 ± 9,434 b	98.89 ± 0.23 a	99.93 ± 0.02 a
	MO	206.05 ± 35.20 a	195.07 ± 73.88 a	13,384 ± 27,363 a	60,221 ± 21,991 a	99.98 ± 0.00 a	99.67 ± 0.20 a
	TN	80.75 ± 15.54 bc	—	22,833 ± 4,914 d	—	99.96 ± 0.01 a	—
Tall waterhemp	IL	34.88 ± 7.01 b	24.96 ± 6.10 c	25,649 ± 5,800 b	11,833 ± 2,277 d	99.98 ± 0.01 a	94.98 ± 0.94 b
	NE	129.51 ± 14.44 a	70.48 ± 9.32 a	60,228 ± 8,348 a	82,811 ± 15,051 a	99.99 ± 0.00 a	99.63 ± 0.10 a
	MO	26.2 ± 3.66 c	26.17 ± 4.84 c	19,727 ± 2,493 c	23,787 ± 4,200 c	100.00 ± 0.00 a	99.84 ± 0.04 a
	WI	30.78 ± 4.18 bc	41.38 ± 5.96 b	17,459 ± 2,625 c	38,221 ± 7,956 b	99.96 ± 0.01 a	98.80 ± 0.30 a

performed. Additionally, multiple linear regression analysis was performed to determine if a combination of variables affected seed retention.

Results and Discussion

Biomass (g) for Palmer amaranth plants, regardless of state or year (2012 to 2014), ranged from 7.69 to 1,143.3 g (mean of 64.64 ± 9.17 to 206.05 ± 35.20). In comparison, tall waterhemp plant weights (2013 to 2014) had a range of 24.96 to 129.51 g (mean of 24.96 ± 6.10 to 129.51 ± 14.44). Palmer amaranth plants produced seeds ranging from 1,011 to 796,135 seeds per plant (mean 13,384 ± 27,363 to 60,221 ± 21,991) across locations and years. Furthermore, tall waterhemp plants produced seeds ranging from 135 to 305,831 seeds per plant (mean 11,833 ± 2,277 to 82,811 ± 15,051) across locations and years. Total plant seed production in this study was generally lower than values previously documented in the literature (600,000 seeds plant^{-1}) for plants grown in the absence of soybean interference (Keeley et al. 1987; Norsworthy et al. 2012; Sellers et al. 2003). However, in some instances, especially for Palmer amaranth, plant seed production was close to values reported in previous studies (Dalley et al. 2004; Jha et al. 2008). Variation in plant seed production can result from weed control tactics, time of emergence, local and regional climatic differences, and soybean crop competition. Varying management practices by individual growers is known to alter the emergence of the weed species, which in turn can influence seed production potential (Norsworthy et al. 2012). Late-emerging plants are smaller and produce less seed per plant than earlier-emerging plants.

Seed retention was consistently very high across all regions. Percent seed retention of Palmer amaranth and tall waterhemp ranged from 94.98 to 100% and 98.89 to 99.98%, respectively. Illinois was the only state that exhibited a significant difference in average seed retention from 2013 (99.9%) to 2014 (94.9%) for tall waterhemp (Table 1). The lower retention in 2014 in Illinois was likely a result of a 1- to 2-wk delay in collecting samples as compared to 2013. Even so, the values in this study are as high or higher than what has been reported in Australia for rigid ryegrass (*Lolium rigidum* Gaudin), wild radish (*Raphanus raphanistrum* L.), brome grass (*Bromus* spp.), and wild oat (*Avena*
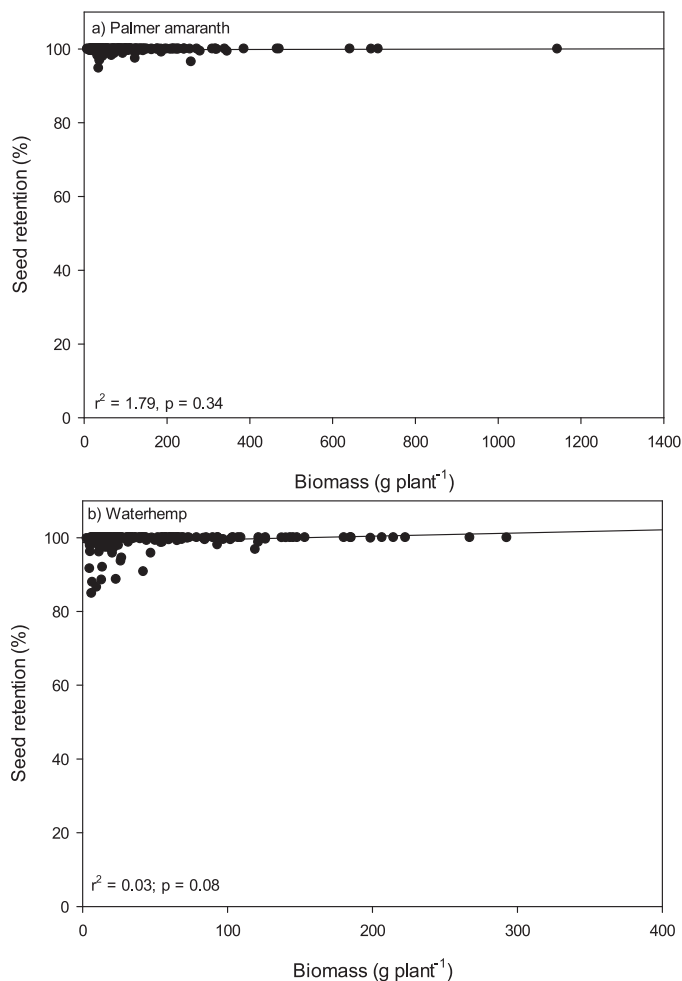
Figure 1. Correlation between seed retention (%) and biomass (g plant^{-1}) for (a) Palmer amaranth (n = 228) and (b) tall waterhemp (n = 193) in 2013 and 2014.

Figure 2. Correlation between biomass (g plant^{-1}) and total seed production per plant for (a) Palmer amaranth in 2012 to 2014 (n = 352) and (b) tall waterhemp in 2012 to 2013 (n = 193).

fatua L.), which retained 85, 99, 77, and 84% of seed until crop maturity, respectively (Walsh and Powles 2014). Similarly, perennial ryegrass (*Lolium perenne* L.) had a 96% seed retention in wheat (*Triticum aestivum* L.) fields (Blanco-Moreno et al. 2004). Tall waterhemp and Palmer amaranth seed retention in all of the remaining states that were studied varied by no more than 0.01% between years, which means that seed retention was stable and a high percentage was available for HWSC. Furthermore, biomass and percent seed retention were not correlated or significant (Figure 1), which indicates that regardless of plant size, and possibly time of emergence, seed retention remained high and close to maximum.

Aboveground biomass and total seed production was highly correlated for both species (Figure 2).
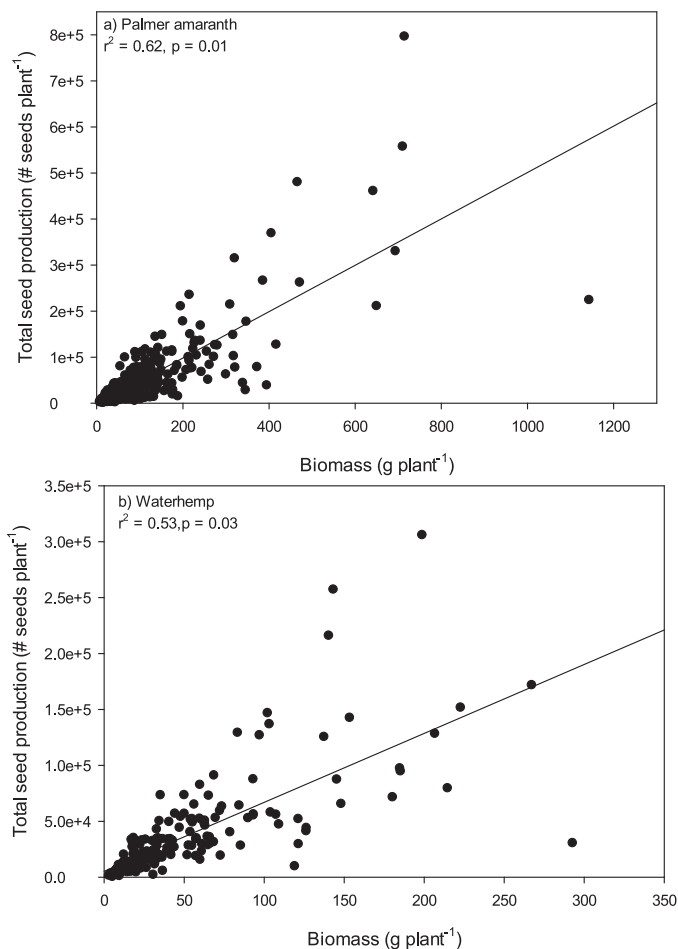
Regardless of species, the majority of smaller plants had low seed production, indicating that these plants were late-emerging cohorts (Zimdahl 2004), possibly after the final herbicide applications. Additionally, it was apparent that many of the larger Palmer amaranth and tall waterhemp plants occurred in areas having a low population or weaker soybean plants. A strong correlation between biomass production and seed production has been well established for many weeds (Harrison 1990; Norris 2007; Steckel and Sprague 2004b; Webster and Grey 2015).

Seedling emergence (data not shown), which was conducted as an exhaustive grow-out from the soil and seeds that were collected from underneath each plant, was not reflective of the other characteristics (i.e., biomass, seed production, seed retention) and

appeared to be solely a function of the number of seeds per plant or percent seed retention. Differences among states and years were not significant (P < 0.07). The differences that did occur could have been a result of specific weather events (i.e., rainfall, strong winds) in the areas where the samples were collected.

Palmer amaranth and tall waterhemp are two of the most prominent agriculture weeds in the United States today. In most cases, farmers will harvest soybean fields with some mature weeds present, most likely due to the continual evolution of resistance to various herbicide modes of action. Thus, replenishment of the soil seedbank is common. Understanding more about weed seed retention at crop harvest is imperative to developing and utilizing nonchemical management practices to control weed species at that point. Various HWSC tactics, such as chaff carts (Walsh et al. 2012), narrow-windrow burning (Walsh and Newman 2007), the Harrington Seed Destructor (Walsh et al. 2012), and a bale-direct system (Walsh and Powles 2007) can be used to prevent weed seeds from entering into the soil seedbank. Additionally, understanding the quantity of weed seed added to the soil seedbank and knowledge of weed seed retention is necessary to develop tactics that decrease additions to the soil seedbank. Furthermore, this knowledge aids our understanding of the consequences of not targeting weed seed production at crop harvest. However, more research is still needed on these mechanisms to fully understand how to reduce the amount of weed seeds that are being retained in the soil seedbank as well as the implication of delayed harvest on seed retention.

Acknowledgments

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