

Evaluation of integrated weed management techniques and their nuances in Virginia crop production

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ABSTRACT

Herbicide resistant weeds are driving implementation of integrated weed management (IWM). A new tactic to manage weeds is harvest weed seed control (HWSC), which targets weed seeds retained on the plant at crop harvest and either destroys, removes, or concentrates them. Research is limited on the effectiveness of HWSC in US cropping systems. For HWSC to be effective it is important to know when and how many seed are shed from a weed species in relation to crop harvest. Research was conducted to quantify when weed seed are shattered from 6 economically important weed species, four broadleaf (redroot pigweed, common ragweed, common lambsquarters, and common cocklebur) and two grass species (large crabgrass and giant foxtail). Results indicate that among summer annuals, broadleaf species retain larger proportions of their seed compared to grass species at the first opportunity for soybean harvest. As harvest was delayed, more seeds shattered from all species evaluated, indicating timely harvest is critical to maximizing HWSC effectiveness. Studies were conducted on grower fields in Virginia to evaluate the effectiveness of HWSC (field residue and weed seed removal). Results indicate that HWSC can significantly reduce populations of Italian ryegrass in wheat and common ragweed in soybean in the next growing season, but reductions were not observed for Palmer amaranth in soybean. Investigating IWM system for common ragweed control in soybean, HWSC was found to be less effective than soybean planting date (i.e. double cropping after wheat) at reducing common ragweed populations. However, the effectiveness of HWSC

varied by location. If HWSC adoption were to become widespread, weeds could adapt by shedding seed earlier in the season. Research was conducted by growing Palmer amaranth populations from across the eastern US in a common garden. Currently there are differences in flowering time and seed shatter among Palmer amaranth populations based on the location of the maternal population, indicating potential for adaptation. This research demonstrates that HWSC is a viable option for weed management in US cropping systems but needs to be stewarded like any other weed management tool.

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General Audience Abstract

Herbicide resistance in weeds is a growing problem in the US and around the world. Alternative methods of weed control must be adopted to maintain crop yields in the presence of herbicide-resistant weeds. Researchers and extension specialists strongly advise growers to adopt an integrated weed management (IWM) approach. Integrated weed management involves implementing multiple weed control tactics during a growing season. By using multiple methods of weed control within a given season the chances of weeds becoming resistant or adapting to any single tactic is reduced.

Harvest weed seed control (HWSC) is a new tactic developed in Australia in response to herbicide resistance. HWSC targets weed seeds retained on the plant at crop harvest. In a normal crop harvest, the combine removes the grain and spreads crop residues (leaves, stalks, and other plant parts), including weed seeds, back across the field. When HWSC is implemented, weed seeds are destroyed (narrow windrow burning, cage mills) or concentrated and potentially removed from the field (chaff carts, direct bale, chaff lining). Thus, HWSC limits the number of weed seeds returned to the soil seed bank. There is limited research on HWSC and its integration with other tactics, in US cropping systems.

For HWSC to be effective it is necessary for weed seeds to be retained on the mother plant in sufficient quantities at crop harvest. Research was conducted in Virginia to determine when weed seeds are shattered during the soybean growing season for 6 economically important weed species, four broadleaf (redroot pigweed, common ragweed, common lambsquarters, and

common cocklebur) and two grass species (large crabgrass and giant foxtail). The broadleaf species retained >85% of their seed until the first opportunity for soybean harvest (mid-October). In the grass species, more seed shattered prior to soybean harvest with 50% of large crabgrass and 74% of giant foxtail seed being retained at the first opportunity for soybean harvest. When harvest was delayed seed continued to shatter and less was captured using HWSC. This research indicates broadleaf species are more suitable candidates for HWSC than grass species, among summer annuals. Further research on the ability of seed to germinate in relation to when seeds were shed was conducted on redroot pigweed, common ragweed and common lambsquarters. Results indicate that there are variable effects on germination of these species depending on when they were shed.

HWSC was implemented on grower fields to assess the impact on weed populations of 3 weed species (Italian ryegrass, common ragweed, and Palmer amaranth). These experiments compared conventional harvest and HWSC (field residue and weed seed removal) when all other management strategies were the same within that field. Italian ryegrass tiller density in wheat varied by location but was reduced up to 69% in the spring following implementation of HWSC. By wheat harvest, HWSC reduced Italian ryegrass seed head density 67% at one location compared to conventional harvest. In soybean, common ragweed densities were reduced by 22 and 26% prior to field preparation and postemergence herbicide applications, respectively, in the HWSC plots compared to the conventional harvest plots. No differences were observed in common ragweed density by soybean harvest. No differences were observed with Palmer amaranth densities at any point during the soybean growing season. This research show that HWSC can reduce weed populations but is variable and additional research is still needed.

IWM experiments were established across Virginia to compare soybean planting date (full season or double cropped), \pm cover crop (cereal rye/wheat or no cover), and \pm HWSC (field residue removal) to evaluate the best management strategy for common ragweed in soybean. Across all locations, double cropping soybean behind wheat had the greatest impact on common ragweed densities at the end of the first season. The impact of double cropping soybeans on common ragweed population is due to the emergence pattern of common ragweed; majority of common ragweed emerges prior to planting double cropped soybean (mid-June to early-July). HWSC was variable and only reduced common ragweed density at one of three locations.

Widespread adoption of HWSC could place a selection pressure on weeds to shatter seed earlier in the season. A common garden experiment was conducted in Blacksburg, VA to assess Palmer amaranth populations collected from central Florida to southern Pennsylvania for differences in flowering time, time to seed shatter, and other phenotypic traits. Results indicate that latitude of the maternal population influences time to first flower with a 0.53 d reduction in flowering time for every degree north in latitude the maternal population was collected from. The strongest predictor of Palmer amaranth flowering time was emergence date/daylength. For every day emergence was delayed the time to first flower was reduced by 0.31 and 0.24 d for female and male plants, respectively. Time from emergence or first flower to first seed shatter was reduced by 0.48 or 0.17 d, respectively, for each day emergence was delayed. These results indicate that differences exist currently among Palmer amaranth populations and the selection pressure of HWSC could push these populations to flower and shatter seed early.

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Seed retention and shatter phenology of six economically important weed species in Virginia

Annual weed species rely on yearly additions to the soil seed bank to ensure survival. Weed management tactics that target weed seed are gaining popularity. For these tactics to be successful it is necessary to understand the timing and quantity of seed shattered or retained on the mother plant, especially with respect to timing of crop harvest. Studies were conducted in Blacksburg, Virginia in 2016 and 2017 to assess seed shatter and retention of redroot pigweed, common ragweed, common lambsquarters, common cocklebur, large crabgrass, and giant foxtail. Additionally, studies were conducted to assess the germinability of seed shattered at different time points during the soybean growing season for: redroot pigweed, common ragweed, and common lambsquarters. Seed retention of the broadleaf weed species at the first opportunity for soybean harvest was greater than that of the grass weed species. Redroot pigweed and common ragweed retained 91.9 and 91.3%, respectively, of their seed at the first opportunity for soybean harvest (mid-October). Seed retention varied between years in both common lambsquarters and common cocklebur. At the first opportunity for soybean harvest common lambsquarters seed retention was 96.5 and 91.5% in 2016 and 2017, respectively and 85.7 and 98.4% in common cocklebur in 2016 and 2017 respectively. Seed retention for large crabgrass also varied by year with 52 and 50% of seed retained in 2016 and 2017, respectively, at the first opportunity for soybean harvest. Giant foxtail seed retention was 73.7% at the first opportunity for soybean harvest across both years. Seed germinability was highly variable across years and species. Redroot pigweed seed germinability decreased as seed shattered later in the season in 2016 and increased in 2017. Common ragweed seed germinability decreased as seed shattered later in both years of the study. Common lambsquarters seed germinability increased as seed

shattered later in the growing season in both 2016 and 2017. Based on these data in soybean, summer annual broadleaf weed species are better candidates than summer annual grass weed species to be managed with tactics that target retained seed at crop harvest.

Nomenclature: Common cocklebur, *Xanthium strumarium* L. XANST; common lambsquarters, *Chenopodium album* L. CHEAL; common ragweed, *Ambrosia artemisiifolia* L. AMBEL; giant foxtail, *Setaria faberi* Herrm. SETFA; large crabgrass, *Digitaria sanguinalis* (L.) Scop. DIGSA; redroot pigweed, *Amaranthus retroflexus* L. AMARE; soybean, *Glycine max* (L.) Merr.

Keywords: Germinability, seed bank.

Introduction

Annual weed species rely on seeds to ensure survival in future years. These seeds, once mature, shatter and enter the soil seed bank where they can emerge in future growing seasons. The seed is a vulnerable growth stage of annual weed species since it is vital for the survival of the species and is exposed to pathogens and predators while in the soil (Tidemann et al. 2016). Since seeds are necessary for survival in annual species, this makes the seed a weak point in the life cycle that hasn't been fully exploited for weed control (Norris 2007; Walsh et al. 2013). If fewer seeds are allowed to return to the soil seed bank each year than enter, over time the soil seed bank will shrink (Ball and Miller 1989). Tactics that reduce or eliminate seed inputs to the soil seed bank can therefore serve to manage weeds (Norris 2007).

Weeds that emerge early in the growing season and pose a threat to crop yield are often controlled, and therefore do not return seeds to the soil seed bank. However, late-emerging weeds that do not pose a threat to crop yield are often left uncontrolled. Even though these late-emerging weeds produce fewer seeds than earlier emerging weeds (Bagavathiannan et al. 2011; Bosnic and Swanton 1997; Steckel and Sprague 2004), they are still capable of maintaining or increasing the soil seed bank when left unmanaged (Bagavathiannan and Norsworthy 2012). To limit seed inputs to the soil seed bank, seed maturation and shattering phenology must be understood (Norris 2007; Norsworthy et al. 2012).

Many weed species have adapted their lifecycle to be concurrent with many crops. One such adaptation includes retaining large proportions of seed on the plant until grain harvest when the seeds are then spread by the combine harvester (Shirtliffe and Entz 2005). Research indicates that some weed seeds (5 to 23%) will shatter prior to crop harvest (Burton et al. 2016; Goplen et al. 2016; Schwartz et al. 2016; Walsh and Powles 2014; Walsh et al. 2018). In wheat production

systems, it has been found that rigid ryegrass (*Lolium rigidum* Gaud.), wild radish (*Raphanus raphanistrum* L.), brome grass (*Bromus spp.*), and wild oat (*Avena fatua* L.) retain 85, 99, 77, and 84% of their seed, respectively, at the time of wheat harvest (Walsh and Powles 2014). Burton et al. (2016) found that wild mustard (*Sinapis arvensis* L.) and green foxtail (*Setaria viridis* (L.) P. Beauv) retain >95% of their seed at the time of wheat harvest in Canada. Even though these species retain most of their seed at grain harvest, seed shatter continues if crop harvest is delayed (Goplen et al. 2016). Since a high proportion of seeds are retained at crop harvest, this presents a unique opportunity to control these weed seeds at the same time as the harvest operation via harvest weed seed control (Walsh et al. 2013).

In addition to seed retention at harvest, rate of seed shatter is also important to consider. Goplen et al. (2016) found that giant ragweed (*Ambrosia trifida* L.) had similar rates of seed shatter among field margins as within a soybean field, despite producing more seed per plant (1,420) in soybeans than along field margins (826). Approximately 0.75 seed plant⁻¹ day⁻¹ shattered beginning in September and lasting through October. At soybean harvest, 80% of giant ragweed seed were retained on the plant (Goplen et al. 2016).

Closely related weed species can have dissimilar fecundity and retention under the same conditions (Norris 2007). One such group is the pigweeds (*Amaranthus spp.*), among which there are significant differences in growth rate, biomass accumulation, and seed production (Sellers et al. 2003). Of these species, Palmer amaranth (*Amaranthus palmeri* S. Wats.) is the fastest growing compared to redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus* L.), common waterhemp (*Amaranthus rudis* L.), spiny amaranth (*Amaranthus spinosus* L.) and tumble pigweed (*Amaranthus albus* L.). As well, all of these species except spiny amaranth and tumble pigweed produce >250,000 seed plant⁻¹ (Horak and

Loughin 2000; Sellers et al. 2003). Research has shown that Palmer amaranth and tall waterhemp retain >95 and 99%, respectively, of their seed at the time of soybean harvest (Schwartz et al. 2016).

Another component of weed seed shattering phenology is relative germinability of seed that are shed at different times during the growing season. There is limited published data on this topic. However, Moss (1983) reported differential germinability in blackgrass (*Alopecurus myosuroides* Huds.) in the United Kingdom. Seed shed in the middle of the season had greater germinability than seed shed earlier or later in the season. This difference may be due to differences in pollination efficiency at different points in the season (Moss 1983). Differences in seed fitness (i.e. germinability of seed) shed at different points in the growing season could have weed management implications, such as the efficacy of HWSC and other tactics that target additions to the soil seed bank.

Ideally, weeds should be controlled early so that they do not compete with the crop and have the opportunity to produce seed, but this does not always occur. With growing interest in using integrated weed management (IWM) tactics such as harvest weed seed control it is important to understand the seed shattering phenology of weed species and associated seed fitness. The objectives of this research are to (i) understand the seed shattering phenology characteristics of six agronomically important weed species in soybean production and (ii) understand if there is differential germinability of seeds that are shattered at different points during the growing season.

Materials and Methods

Seed shattering phenology. Field experiments were established at Kentland Farm in Blacksburg, VA on June 22, 2016 and May 30, 2017. Soybeans were planted on 76 cm rows at 296,520 and 407,550 seed ha⁻¹ in 2016 and 2017, respectively. Glyphosate was applied at 1.26 kg ha⁻¹ at planting and the field was maintained as a no-till system. Six species of endemic economically important weeds including redroot pigweed (*Amaranthus retroflexus* L.), common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), common cocklebur (*Xanthium strumarium* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and giant foxtail (*Setaria faberi* Herrm.) were allowed to germinate along with the soybean crop. Weeds that did not emerge from the soil seed bank were either seeded or transplanted into the crop. Transplanted weeds were of the same growth stage as those in the study field to mimic if it had germinated with the soybean crop. Other weeds were controlled using glyphosate at 1.26 kg ae ha⁻¹ or hand weeding as needed throughout the growing season. Twenty-four plants of each weed species were selected to track seed shatter phenology throughout the growing season. Each plant was its own replicate for a total of 24 replicates per species. The plants of each species were grown between the crop rows and located randomly throughout the field. In 2017, some of the selected weeds died after the initiation of the study so in all species, except common cocklebur, there were less than 24 replicates. The final number replicates for redroot pigweed, common ragweed, common lambsquarters, giant foxtail, and large crabgrass was 21, 10, 18, 11, and 15, respectively.

The weed species were allowed to grow in competition with the crop until reproductive structures began to appear. Once reproductive structures were observed on each species the soybean crop was removed from an approximately 1 m² area around each weed and four slotted greenhouse trays measuring 43 cm by 53 cm were placed around the base of each plant. The

trays were secured to the ground using landscape staples. Each tray was lined with landscape fabric that was secured to the tray with adhesive ensuring there were no gaps between the trays and the fabric lining. To help ensure trays captured shattered seed, during the course of the study if a plant spread over the outer edges of the trays it was trained using twine and stakes to keep the entire plant over the trays. The trays were monitored weekly for the presence of shed seed. Once seed shatter began, the seed were collected weekly from the trays surrounding each plant using a handheld vacuum, except for common cocklebur where the burs were removed from the trays by hand. Weekly seed collections continued until 3 wk after the soybean crop had reached approximately 15% moisture, which represented the first possible harvest date. This additional 3 weeks simulated a delay in harvest that a grower might experience due to weather, equipment, or logistical considerations. The soybean crop reached 15% moisture on October 20, 2016 and October 26, 2017. Final seed collections occurred on November 9, 2016 and November 16, 2017. Following the final weekly seed collection, the aboveground biomass of each plant was harvested and then dried.

Data collected for each species included the number of seeds shattered each week, total seed shattered and collected, seed retained on the mother plant at the end of the experiment, and aboveground biomass at the end of the experiment. Weekly seed collection samples were cleaned of large debris by sieving. Mother plant samples were air dried and weighed. Once the plants were dry, they were threshed and cleaned of large debris by sieving. To quantify the seed in the weekly samples the sample was first weighed then a half gram aliquot was weighed and seeds counted; if the whole sample was less than half of a gram, the whole sample was counted. For seed samples that were retained on the mother plant, a half gram aliquot was weighed and the seed in that aliquot counted. This process was repeated 3 times, averaged and then the whole

seed sample was weighed. The total number of seeds in all samples were then calculated using the following formula:

$$Y = (A \times B) / 0.5 \quad [1]$$

Where Y= total number of seeds in the sample, A= total weight of the sample, B= average number of seeds per 0.5-gram aliquot. For common cocklebur, bur number was calculated by counting 25 burs and weighing them and repeating three times and taking the average weight of the 25 burs and then the whole sample was weighed. Total bur number was calculated using the following formula:

$$Y = (25 \times A) / B \quad [2]$$

Where Y= total number of burs in the sample, A= total weight of the sample, B= average weight of the 25-bur sample.

Data were analyzed using JMP Pro 13 (SAS Institute, Cary, NC). The data were subjected to ANOVA with collection date and year considered fixed effects and plant a random effect. The data were tested for interactions between seed shatter and year. When significant interactions were observed data are presented by year. A generalized linear model with a Poisson distribution and log link was used to find the overall trends in seed shattering phenology of the six species. The model fit an equation in the form:

$$Y = e^{(mx + b)} \quad [3]$$

Where Y is the number of seed shattered by a plant in the week prior to collection, and x is the Julian date of seed collection.

Seed germinability. Weekly collected seed samples and the seed retained on the plant at the end of the experiment of redroot pigweed and common lambsquarters were placed in 10 cm petri dishes and common ragweed in 6 cm petri dishes. The dishes were prepared by placing 2 layers

of filter paper (Fisherbrand™ qualitative grade plain filter paper circles – p5 grade, Fisher Scientific Co. L.L.C, Pittsburg, PA) of the corresponding size into each petri dish. Eighty seed of each plant from each collection were placed in the dish. The 10 cm dishes had 5 mL of deionized water and the 6 cm dishes had 3 mL of deionized water placed on the filter paper. Once water was added the dishes were sealed with parafilm. Each petri dish was placed under grow lights with a 12 h photoperiod at 25 C for 3 wk then moved to a darkened cold room at 4.4 C for 3 wk (Baskin and Baskin 1977; Grime et al. 1981; Schonbeck and Egley 1980; Taylorson and Hendricks 1971; Willemsen 1975. The dishes were exposed to light for 3 cycles. During germination portions of each cycle the dishes were evaluated weekly for germinated seed. Seed were considered to have germinated when a radicle was visible. Germinated seed were counted and removed from the dish and water was added as needed to keep the filter paper moist.

Data were analyzed using JMP pro 13 (SAS Institute Inc., Cary, NC). Standard linear regression models were constructed to evaluate germination rates as a function of time of seed shatter. Linear models were selected based on lower AICc values compared to other nonlinear models tested. Year and growing degree day base 10 C (GDD_{10}) were considered fixed effects and plant was considered a random effect. The interaction between year and GDD_{10} was tested and when significant, data were analyzed by year.

Results and Discussion

Seed shattering phenology. With the seed collection methodology utilized in this study there is the potential that some shattered seed may not have landed in the trays below the plant. However, plants that stretched further than the trays were trained to be entirely over the trays. Interactions were observed between seed shatter and year for common lambsquarters, common

cocklebur, and large crabgrass; data for these species are presented by year. For redroot pigweed, common ragweed, and giant foxtail, no significant interactions were observed and the data are presented pooled across years.

Redroot pigweed was one of the first weed species to start shattering seed in both years of the study. The first seed shattered was observed 6 wk before the first opportunity for soybean harvest in both years of the study. Prior to soybean harvest, an average of 5,583 seed plant⁻¹ or 8% of the total redroot pigweed seed production were shed (Table 1). During the simulated harvest delay, an additional 9,964 seed plant⁻¹ or 13.9% of the total redroot pigweed seed production were shed. An average of 112,218 seed plant⁻¹ were retained when the plants were harvested at the end of the study each year; therefore, 92.0% of the seed were retained on the mother plant at the first opportunity for soybean harvest. Seed shatter rate for redroot pigweed increased throughout the soybean growing season; however, the rates were higher during the harvest delay as compared to prior to the first opportunity for soybean harvest (Figure 1). Prior to soybean harvest an average of 133 seed plant⁻¹ d⁻¹ were shed whereas an average of 474 seed plant⁻¹ d⁻¹ were shed during the harvest delay, emphasizing the need to harvest soon after soybean reach a harvestable moisture. Total seed production of redroot pigweed was 127,547 seed plant⁻¹ and the dry aboveground biomass was 153.4 g plant⁻¹ (Table 2). Total seed production was well correlated with aboveground biomass (correlation coefficient = 0.752). Total seed production in the current study was less than previous reports. Sellers et al. (2003) reported redroot pigweed plants producing 291,570 seed plant⁻¹. These data are similar to Schwartz-Lazaro et al. (2017) that reported Palmer amaranth retained 98% of its seed at soybean maturity. It has also been reported that another *Amaranthus* sp., tall waterhemp (*Amaranthus tuberculatus*) retained 98 to 100% of its seed at soybean maturity (Schwartz et al. 2016).

Common ragweed seed shatter was observed 3 wk prior to first opportunity for soybean harvest in both years of the study. Prior to the first opportunity for soybean harvest, an average of 145 seed plant⁻¹ were shed or 8.7% of total seed production (Table 1). During the simulated harvest delay, an average of 958 seed plant⁻¹ were shed or 31.5% of total seed production. There was an average of 1,408 seed plant⁻¹ retained when the plants were harvested at the end of the study. Therefore, at the time of first opportunity for soybean harvest, 91.3% of the seed were retained on the mother plant. Rate of seed shatter increased throughout the season (Figure 2). Prior to soybean harvest, an average of 7 seed plant⁻¹ d⁻¹ were shed whereas an average of 46 seed plant⁻¹ d⁻¹ were shed during the harvest delay. Total seed production of common ragweed was 2,511 seed plant⁻¹ and the dry aboveground biomass was 67.1 g plant⁻¹ (Table 2). Total seed production was well correlated with aboveground biomass (correlation coefficient = 0.731). Common ragweed seed retention was greater at soybean harvest than what has been reported in giant ragweed. Goplen et al. (2016) reported 75.3% seed retention in giant ragweed when 75% of soybean had been harvested in Minnesota. Total seed production of common ragweed has been reported to be approximately 1,200 seed plant⁻¹ when plants emerge and grow in competition with soybean all season which is lower than in the current study (Simard and Benoit 2012). When allowed to grow all season without competition common ragweed can produce up between 32,000 and 62,000 seed plant⁻¹ (Jordan et al. 2010).

A significant year by collection date interaction was observed with common lambsquarters, so data are presented by year. Seed shatter was observed 3 and 5 wk prior to first opportunity for soybean harvest in 2016 and 2017, respectively. In 2016, prior to first opportunity for soybean harvest, an average of 1,709 seed plant⁻¹ were shed or 3.5% of total seed production (Table 3). During the simulated harvest delay, an average of 25,186 seed plant⁻¹ were

shed or 43% of total seed production. An average of 34,261 seed plant⁻¹ were retained when the plants were harvested at the end of the study each year. At the time of first opportunity for soybean harvest in 2016, 96.5% of the seed were retained on the mother plant. Rates of seed shatter increased throughout the season and were greatest during the simulated harvest delay (Figure 3). Prior to first opportunity for soybean harvest, an average of 81 seed plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 1,199 seed plant⁻¹ d⁻¹ were shed. Total seed production of common lambsquarters was 61,156 seed plant⁻¹ and the dry aboveground biomass was 56.5 g plant⁻¹ (Table 4). Total seed production was highly correlated with mother plant biomass (correlation coefficient = 0.927).

In 2017, prior to first opportunity for soybean harvest, an average of 7,877 common lambsquarters seed plant⁻¹ were shed or 8.5% of total seed production (Table 3). During the simulated harvest delay an average of 22,474 seed plant⁻¹ were shed or 25.5% of total seed production. There was an average of 73,885 seed plant⁻¹ retained when the plants were harvested at the end of the study each year. At the time of first opportunity for soybean harvest in 2017, 91.5% of the seed were retained on the mother plant. Rate of seed shatter increased throughout the season and were greatest during the simulated harvest delay (Figure 3). Prior to first opportunity for soybean harvest, an average of 225 seed plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 1,070 seed plant⁻¹ d⁻¹ were shed. Total seed production of common lambsquarters was 104,236 seed plant⁻¹ and the aboveground biomass was 247 g plant⁻¹ (Table 4). Total seed production was correlated with aboveground biomass (correlation coefficient = 0.594). Seed retention of common lambsquarters has been reported at 90% (Beckie et al 2018; Walsh et al. 2018). This value is very similar to the seed retention observed in the current study. Total seed production in the current study was less than what has been reported. Colquhoun et

al. (2001) reported seed production of common lambsquarters to be between 75,000 and 150,000 seed plant⁻¹.

A significant year by collection date interaction was observed with common cocklebur, so data are presented by year. Bur shed began 3 wk prior to first opportunity for soybean harvest in both years of the study. In 2016, prior to first opportunity for soybean harvest, an average of 165 burs plant⁻¹ were shed or 14.3% of total bur production (Table 3). During the simulated harvest delay, an average of 624 burs plant⁻¹ were shed or 44.6% of total bur production. There was an average of 515 burs plant⁻¹ retained when the plants were harvested at the end of the study in 2016. At the time of first opportunity for soybean harvest in 2016, 85.7% of the burs were retained on the mother plant. Rate of bur shatter increased throughout the season and was greatest during simulated harvest delay (Figure 4). Prior to first opportunity for soybean harvest an average of 7.9 burs plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 29.7 burs plant⁻¹ d⁻¹ were shed. Total bur production of common cocklebur was 1,303 burs plant⁻¹. Assuming there were two seeds in every bur (Holm et al. 1977) this would be 2,606 seed plant⁻¹. The aboveground biomass was 201 g plant⁻¹ (Table 4). Total bur production was highly correlated with aboveground biomass (correlation coefficient = 0.865).

In 2017, prior to first opportunity for soybean harvest an average of 29 burs were shed or 1.6% of total bur production (Table 3). During the simulated harvest delay, an average of 196 burs plant⁻¹ were shed or 12% of total bur production. There was an average of 1,659 burs plant⁻¹ retained when the plants were harvested at the end of the study in 2017. At the time of first opportunity for soybean harvest in 2017, 98.4% of the burs were retained on the mother plant. Rate of bur shatter increased throughout the season and was greatest during simulated harvest delay (Figure 4). Prior to first opportunity for soybean harvest an average of 1.3 burs plant⁻¹ d⁻¹

were shed whereas during the harvest delay an average of 79 burs plant⁻¹ d⁻¹ were shed. Total bur production of common cocklebur was 1,883 burs plant⁻¹. Assuming there were two seeds in every bur (Holm et al. 1977) this would be 3,766 seed plant⁻¹. The aboveground biomass was 448.2 g plant⁻¹ (Table 4). Total bur production was highly correlated with aboveground biomass (correlation coefficient = 0.921). Total bur production of common cocklebur was lower in both years of the current study compared to what has been reported. Bararpour and Oliver (1998) reported bur production of common cocklebur to be 4,469 burs plant⁻¹.

A significant year by collection date interaction was observed with large crabgrass, so data are presented by year. Seed shatter was observed 6 and 5 wk prior to first opportunity for soybean harvest in 2016 and 2017, respectively. In 2016, prior to first opportunity for soybean harvest, an average of 34,622 seed plant⁻¹ were shed or 48% of total seed production (Table 3). During the simulated harvest delay, an average of 11,617 seed plant⁻¹ were shed or 16.3% of total seed production. There was an average of 33,872 seeds plant⁻¹ retained when the plants were harvested at the end of the study in 2016. At the time of first opportunity for soybean harvest, 52.1% of the seed were retained on the mother plant. Unlike the broadleaf weed species, rate of seed shatter decreased throughout the season with the highest rates of seed shatter being prior to ideal soybean harvest in 2016 (Figure 5.). Prior to first opportunity for soybean harvest, an average of 824 seed plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 553 seed plant⁻¹ d⁻¹ were shed. The aboveground biomass was 93.5 g plant⁻¹ (Table 4). Total seed production was highly correlated with aboveground biomass (correlation coefficient = 0.939).

In 2017, prior to first opportunity for soybean harvest, an average of 4,200 large crabgrass seed were shed or 49.6% of total seed production (Table 3). During the simulated harvest delay, an average of 3,795 seed plant⁻¹ were shed or 27.1% of total seed production. An

average of 5,083 seed plant⁻¹ were retained when the plants were harvested at the end of the study in 2017. At the time of first opportunity for soybean harvest 50.4% of the seed were retained on the mother plant. Rate of seed shatter increased slightly throughout the soybean growing season with the greatest rates of seed shatter occurring during the simulated harvest delay in 2017 (Figure 5). Prior to first opportunity for soybean harvest an average of 120 seed plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 181 seed plant⁻¹ d⁻¹ were shed. The aboveground biomass was 19.8 g plant⁻¹ (Table 4). Total seed production was highly correlated to aboveground biomass (correlation coefficient = 0.969).

No significant year by collection date interaction was observed with giant foxtail, so data presented are pooled across years. Seed shatter was observed 5 and 3 wk prior to first opportunity for soybean harvest in 2016 and 2017, respectively. Prior to first opportunity for soybean harvest, an average of 4,266 seed plant⁻¹ were shed or 26.3% of total seed production (Table 1). During the simulated harvest delay, an average of 4,446 seed were shed or 27.4% of total seed production. There was an average of 9,726 seed plant⁻¹ retained when the plants were harvested at the end of the study. At the time of first opportunity for soybean harvest 73.7% of the seed were retained on the mother plant. The rate of seed shatter stayed relatively constant throughout the season (Figure 6). Prior to first opportunity for soybean harvest, an average of 122 to 203 seed plant⁻¹ d⁻¹ were shed whereas during the harvest delay an average of 211 seed plant⁻¹ d⁻¹ were shed. The aboveground biomass was 159.5 g⁻¹ (Table 2). Total seed production was highly correlated with aboveground biomass (correlation coefficient = 0.894). Giant foxtail seed retention was much lower in the current study than has been previously reported in other *Setaria* spp. Green foxtail (*Setaria viridis*) has been reported to retain 94% of its seed as compared to 74% in the current study (Beckie et al. 2018; Burton et al. 2016; Walsh et al. 2018).

Seed germinability. A significant year by GDD₁₀ interaction was observed in redroot pigweed and common lambsquarters so the data presented are pooled by years. No significant interaction was observed in common ragweed so data are pooled across year. The germinability of seed collected from individual plants within each week and retained on the mother plant was highly variable. Average differences in germination were 23.4, 27.4, 21.4, 13.8, and 66.4% from the onset of seed shatter to final collection for redroot pigweed, common ragweed, and common lambsquarters in 2016 and 2017, respectively. In 2016, redroot pigweed germinability decreased as GDD₁₀ increased. Conversely in 2017, germinability increased as GDD₁₀ increased for redroot pigweed (Figure 7). In common ragweed, the regression was not significant however, the overall trend was decreased germinability as GDD₁₀ increased (Figure 8). In common lambsquarters, the regression in 2016 was not significant, but germinability generally increased as GDD₁₀ increased (Figure 9). The regression in 2017 was significant and germinability increased as GDD₁₀ increased. The fit of the lines for each regression were also highly variable (r^2 : redroot pigweed 2016 = 0.334, redroot pigweed 2017 = 0.308, and common lambsquarters 2017 = 0.624). The trends with redroot pigweed in 2017 and common lambsquarters in both years are similar to what has been reported in other weed species. Moss (1983) reported that in blackgrass seed viability increased in the middle of the season and dropped as seed were shed late in the growing season, potentially due to different environmental conditions during pollination. Tidemann et al. (2017) also reported increasing viability of wild oat (*Avena fatua* L.), cleavers (*Galium spurium* L.), and canola (*Brassica napus* L.) as GDD increased, which means that seed shed later in the growing season were more likely to be viable.

Research Implications. Based on this research, the four broadleaf weed species, redroot pigweed, common ragweed, common lambsquarters, and common cocklebur, are good

candidates for HWSC since they all shed <10% of their seed prior to the first opportunity for soybean harvest. The two grass species in this study, large crabgrass and giant foxtail, are not as good candidates for HWSC due to the large proportions of seed (48.6 and 26.3%, respectively) that are shed prior to soybean harvest. Tidemann et al. (2016) reported in wild oat that for HWSC to be effective at reducing weed populations over time, >80% of seed must be removed with the harvest operation. Even though many weed species retain a large proportion of their seed at crop harvest and could be targeted with HWSC, some do not. Weed species like horseweed, (*Conyza canadensis* L. Cronquist) are adapted to have their seed dispersed via wind and seed that are shed can rapidly move long distances away from the mother plant (Andersen 1993; Dauer et al. 2006; Shields et al. 2006), making them poor candidates for HWSC. All of the species used in this study shed between 13.9 and 35.5% of the total seed production during a simulated three-week harvest delay, making timely harvest of the crop critical for success of HWSC. Seed germinability is highly variable based on when it shatters and between species, which could have potential impacts on seedbank dynamics if HWSC were to be implemented.

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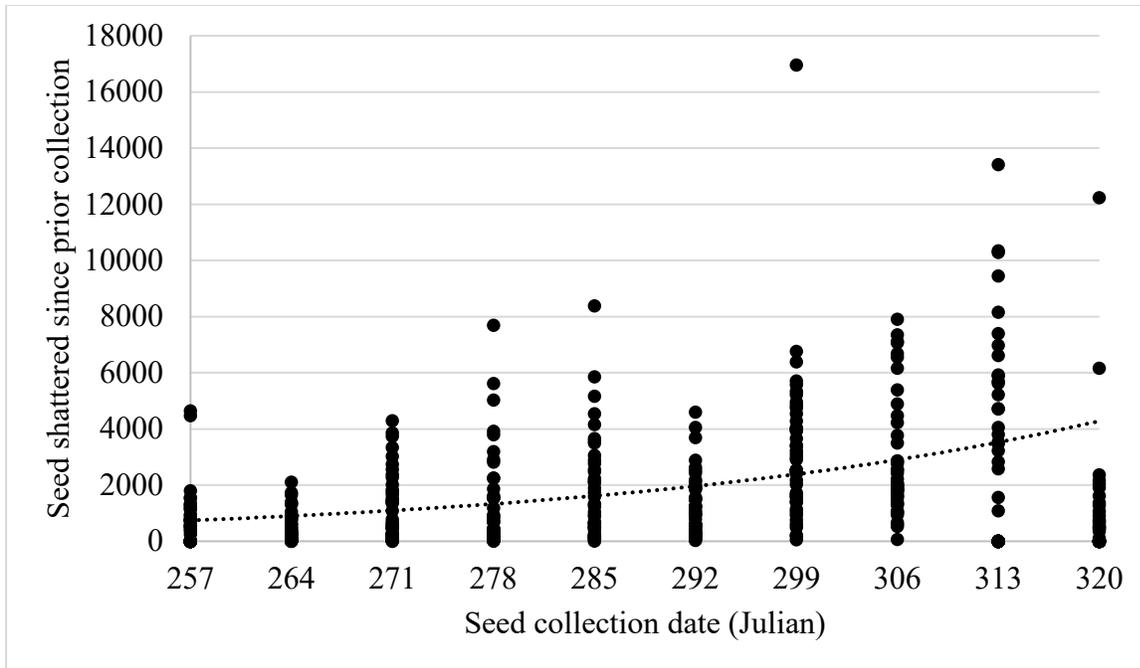


Figure 1. Number of redroot pigweed seed shed at each collection date (black circles) combined across 2016 and 2017 in Blacksburg, VA. The regression line is described by the equation

$$Y = e^{(0.0279x-0.5515)}.$$

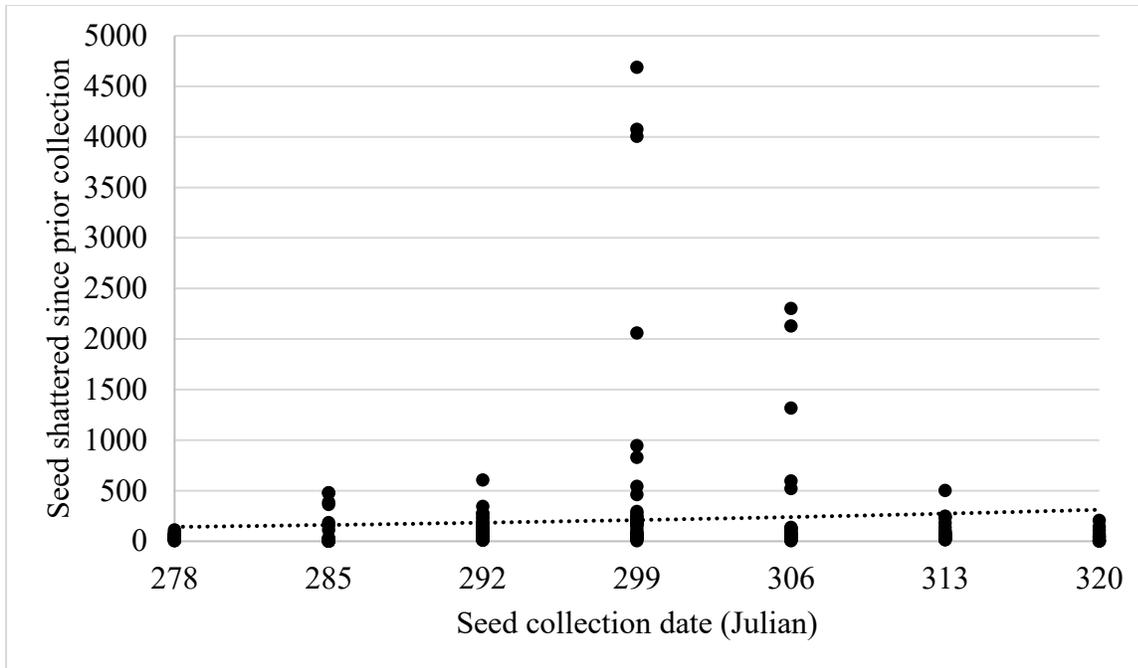


Figure 2. Number of common ragweed seed shed at each collection date (black dots) combined across 2016 and 2017 in Blacksburg, VA. The regression line is described by the equation $Y = e^{(0.0189x - 0.2927)}$.

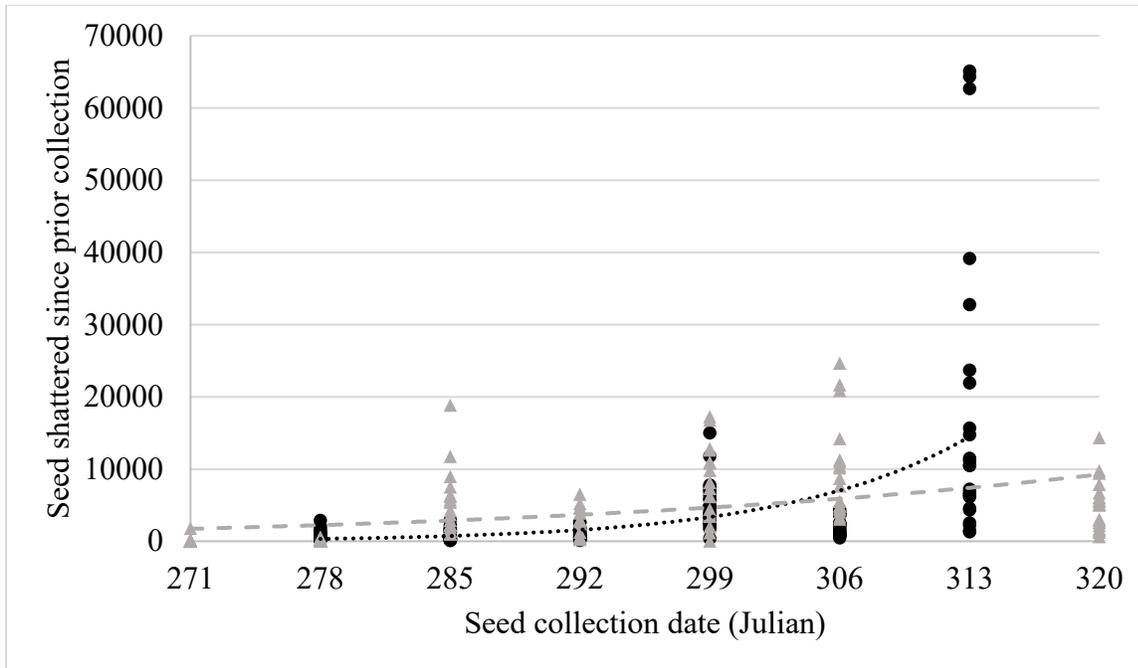


Figure 3. Number of common lambsquarters seed shed at each collection date for 2016 (black dots) and 2017 (gray triangles) in Blacksburg, VA. The regression line is described by the equation $Y = e^{(0.1082x-24.7575)}$ for 2016 data and $Y = e^{(0.0342x-1.8109)}$ for 2017 data.

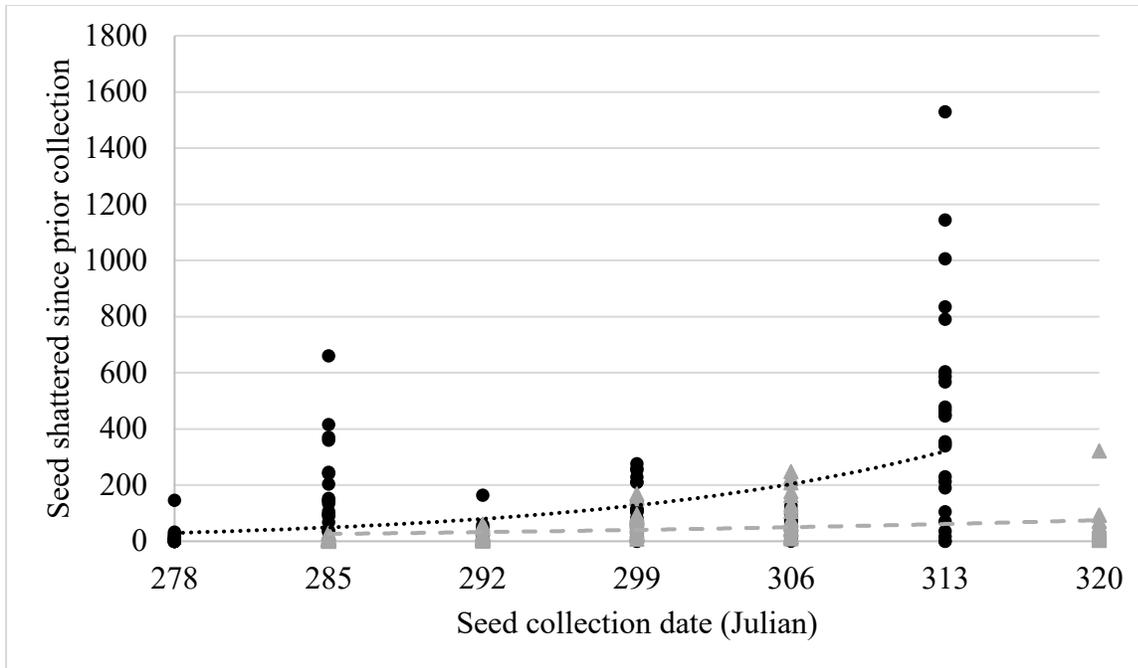


Figure 4. Number of common cocklebur seed shed at each collection date for 2016 (black dots) and 2017 (gray triangles) in Blacksburg, VA. The regression line is described by the equation $Y = e^{(0.0679x-15.4662)}$ for 2016 data and $Y = e^{(0.0302x-5.3635)}$ for 2017 data.

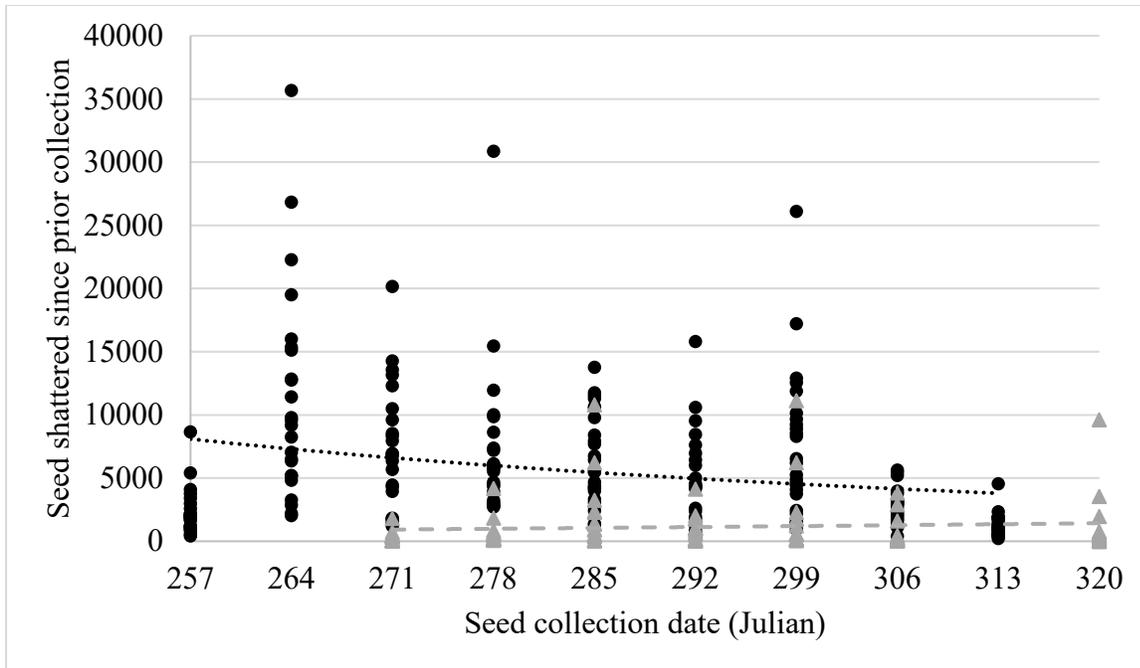


Figure 5. Number of large crabgrass seed shed at each collection date for 2016 (black dots) and 2017 (gray triangles) in Blacksburg, VA. The regression line is described by the equation $Y = e^{-(0.0134x+12.4575)}$ for 2016 data and $Y = e^{(0.0089x+4.4188)}$ for 2017 data.

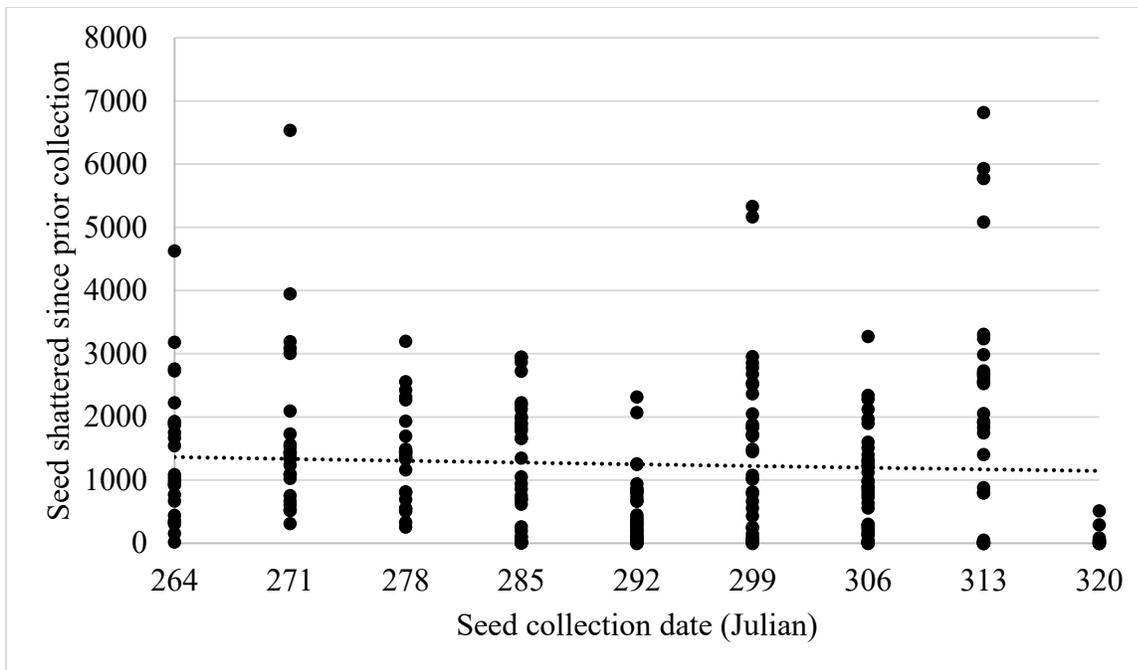


Figure 6. Number of giant foxtail seed shed at each collection date (black dots) combined across 2016 and 2017 in Blacksburg, VA. The regression line is described by the equation

$$Y = e^{(-0.0031x+8.0441)}$$

Table 1. Amount of seed shattered and seed retained for redroot pigweed, common ragweed, and giant foxtail in Blacksburg, VA pooled across 2016 and 2017. All values are shown by mean \pm the standard error.

Species	Seed shattered prior to harvest	Seed shattered during harvest delay	Total seed shattered	Seed retained at end of experiment	Seed shattered prior to harvest	Seed shattered during harvest delay	Total seed shattered	Seed retained at first opportunity for soybean harvest
							-----%-----	
Redroot pigweed	5,583 \pm 766	9,964 \pm 926	15,547 \pm 1,470	112,218 \pm 17,803	8.05 \pm 1.48	13.94 \pm 1.74	22 \pm 2.88	91.95 \pm 1.48
Common ragweed	145 \pm 38	958 \pm 272	1,103 \pm 304	1,408 \pm 259	8.69 \pm 2.59	31.54 \pm 4.31	40.23 \pm 5.31	91.31 \pm 2.59
Giant foxtail	4,266 \pm 593	4,446 \pm 619	8,712 \pm 1,147	9,276 \pm 1,346	26.33 \pm 3.52	27.35 \pm 2.38	53.69 \pm 3.24	73.67 \pm 3.52

Table 2. Total seed production, aboveground biomass of the mother plants, and their correlation for redroot pigweed, common ragweed, and giant foxtail in Blacksburg, VA pooled across 2016 and 2017. Total seed per plant and aboveground biomass of the mother plants shown as means \pm the standard error.

Species	Total seed plant ⁻¹	Mother plant aboveground biomass (g)	Equation	Correlation
Redroot pigweed	127,547 \pm 18,521	153.36 \pm 24.14	y=39325 + 576.7x	0.565
Common ragweed	2,511 \pm 383	67.07 \pm 15.07	y=1265 + 18.58x	0.534
Giant foxtail	17,988 \pm 2,381	159.53 \pm 24.97	y=4379 + 85.31x	0.800

Table 4. Total seed production, aboveground biomass of the mother plants, and correlation equations for common lambsquarters, common cocklebur, and large crabgrass in Blacksburg, VA split across 2016 and 2017. Total seed per plant and aboveground biomass of the mother plants shown as means \pm the standard error.

Species	Total seed plant ⁻¹		Mother plant aboveground biomass		Equation		Correlation	
	2016	2017	2016	2017	2016	2017	2016	2017
Common lambsquarters	61,156 \pm 11,323	104,236 \pm 13,196	56.48 \pm 11.26	246.97 \pm 21.15	y=931.92x+8517.31	y=370.89x+12636.76	0.859	0.353
Common cocklebur	1,303 \pm 150	1,883 \pm 167	200.68 \pm 22.94	448.22 \pm 40.89	y=5.64x+170.03	y=3.76x+199.89	0.748	0.848
Large crabgrass	80,110 \pm 11,009	13,078 \pm 5,323	93.45 \pm 14.79	19.83 \pm 6.28	y=698.95x+14790.81	y=821.29x-3205.24	0.881	0.939

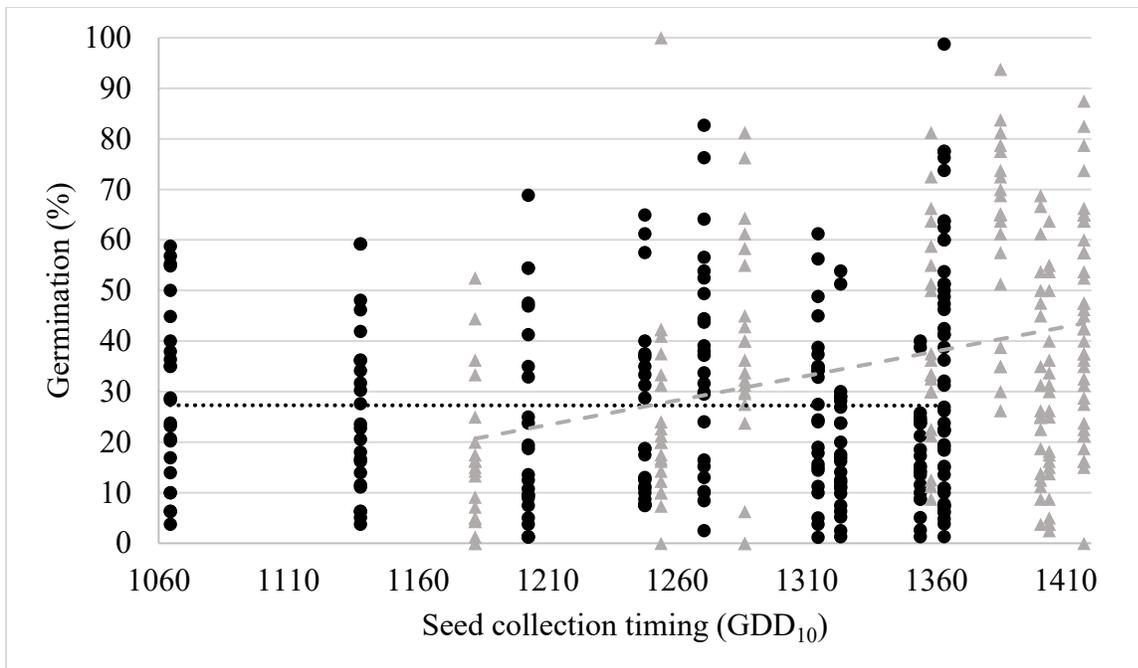


Figure 7. Redroot pigweed germination split by year, 2016 (black dots) and 2017 (gray triangles), from seed shattered throughout the soybean growing season. The regression line for 2016 is described by the equation $Y = -0.0005x + 27.5804$ with $r^2 = 0.334$. The line for 2017 is described by the equation $Y = 0.0969x - 94.0991$ with $r^2 = 0.308$.

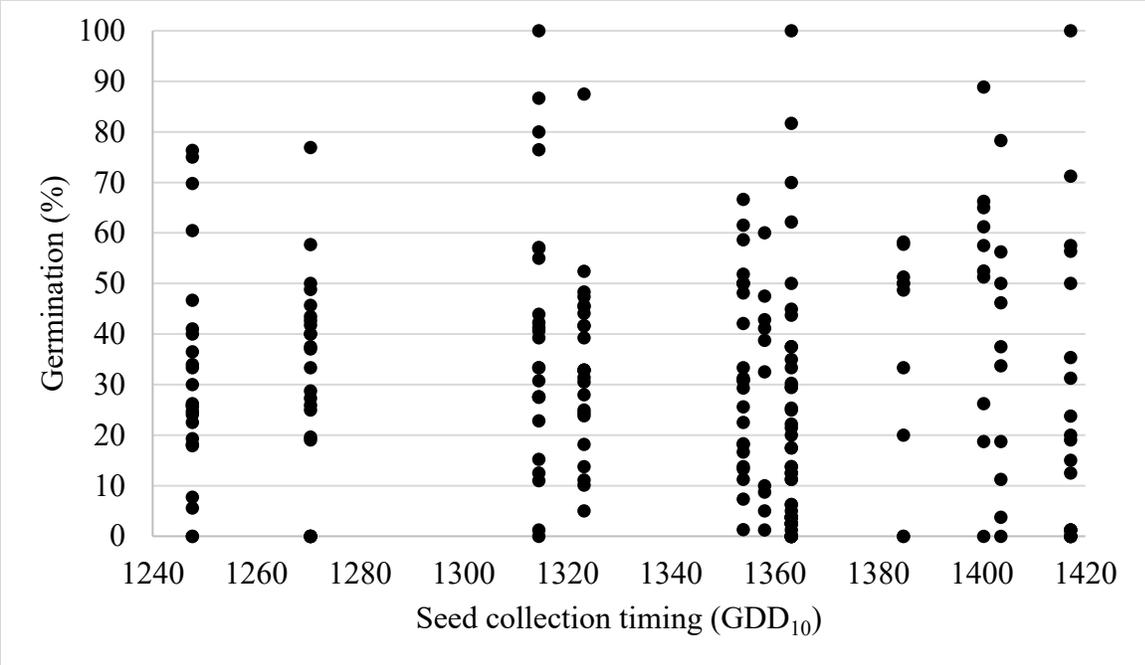


Figure 8. Common ragweed germination combined across 2016 and 2017, from seed shattered throughout the soybean growing season.

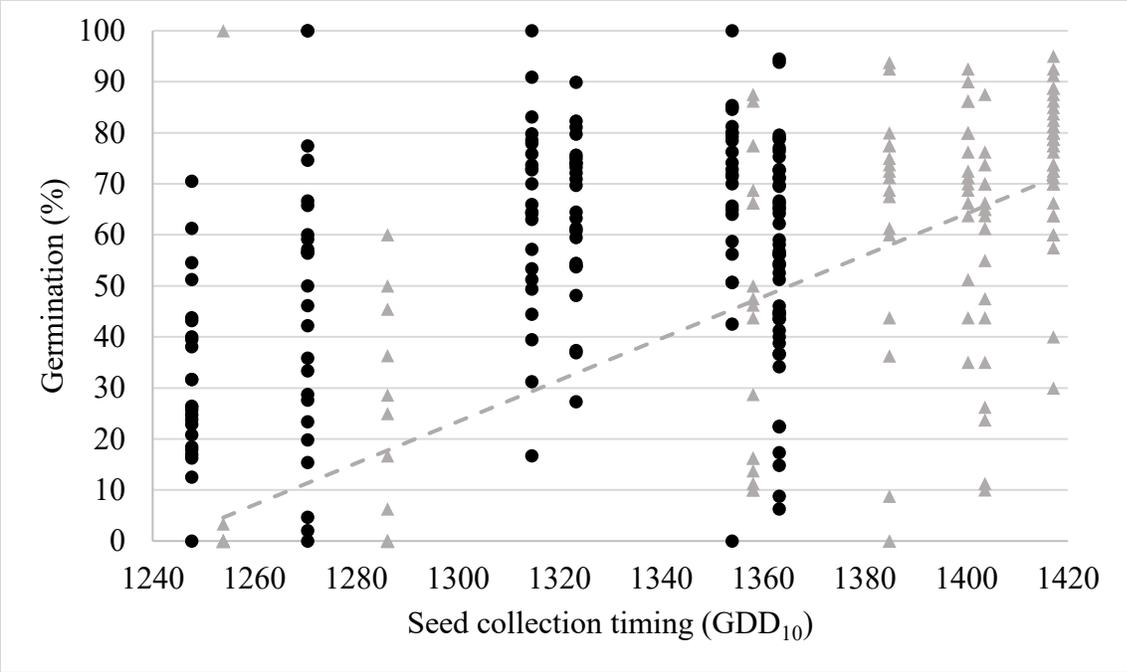


Figure 9. Common lambsquarters germination split by year, 2016 (black) and 2017 (gray), from seed shattered throughout the soybean growing season. The line for 2017 is described by the equation $Y = 0.4079x - 506.1176$ with $r^2 = 0.624$.

Harvest Weed Seed Control of Italian Ryegrass (*Lolium perenne* ssp *multiflorum*), Common Ragweed (*Ambrosia artemisiifolia*), and Palmer Amaranth (*Amaranthus palmeri*)

Herbicide resistance is a major problem in US and global agriculture, driving farmers to consider other methods of weed control. One of these methods is harvest weed seed control (HWSC), which has been demonstrated to be effective in Australia. HWSC studies were conducted across Virginia in 2017 and 2018 targeting Italian ryegrass in continuous winter wheat as well as common ragweed and Palmer amaranth in continuous soybean. These studies assessed the impact of HWSC (via weed seed removal) on weed populations in the next year's crop compared to conventional harvest (weed seeds returned). HWSC reduced Italian ryegrass tillers compared to the conventional harvest at two locations in April (29 and 69%), but no difference was observed at a third location. At wheat harvest, HWSC at one location reduced Italian ryegrass seed heads (41 seed heads m⁻²) compared to conventional harvest (125 seed heads m⁻²). In soybean, prior to preplant herbicide applications and postemergence (POST) herbicide applications, HWSC reduced common ragweed densities by 22 and 26%, respectively, compared to the conventional harvest plots. By soybean harvest, no differences in common ragweed density, seed retention, or crop yield were observed, due to effectiveness of POST herbicides. No treatment differences were observed at any evaluation timing for Palmer amaranth, which is attributed to farmer weed management (i.e. effective herbicides) and low weed densities making any potential treatment differences difficult to detect. Across wheat and soybean, there were no differences observed in crop yield between treatments. Overall, HWSC was demonstrated to be a viable method to reduce Italian ryegrass and common ragweed populations.

Nomenclature: Common ragweed, *Ambrosia artemisiifolia* L. AMBEL; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot LOLMU; Palmer amaranth, *Amaranthus palmeri* S. Wats AMAPA; soybean, *Glycine max* (L.) Merr.; winter wheat, *Triticum aestivum* L.

Keywords: Density, integrated weed management, seed retention, weed seed production.

Herbicide resistance is a current and growing problem in the United States (US) and around the world. Currently there are approximately 500 unique cases of herbicide resistance worldwide (Heap 2019). As a result, effective herbicide options are decreasing. New herbicide sites of action (SOA) are increasingly difficult to commercialize (Stubler and Streck 2016), necessitating adoption of integrated weed management (IWM), which relies on the use of multiple, different strategies to control weeds (Swanton and Weise 1991; Thill et al. 1991). Using IWM places multiple selection pressures on weeds, which reduces the likelihood that resistance will develop to any single management practice (Thill et al. 1991). One such IWM technique is harvest weed seed control (HWSC), which was pioneered in Australia in response to widespread herbicide resistance. In Australian wheat production, rigid ryegrass (*Lolium rigidum* Guad.) and wild radish (*Raphanus raphanistrum* L.) are major problems due to multiple resistance to up to 7 and 4 herbicide SOA, respectively.

Harvest weed seed control (HWSC) targets weed seed at harvest, reducing soil seed bank inputs (Walsh et al. 2013). Methods of HWSC include narrow windrow burning, chaff lining, chaff tramlining, chaff carts, bale-direct, and seed destructors (Walsh et al. 2012, 2017a; Walsh and Newman 2007; Walsh and Powles 2007). Walsh et al. (2018) provided an excellent explanation of these systems. Walsh et al. (2017b) reported an average of 60% reduction in rigid ryegrass populations in the season after HWSC implementation, regardless of system (i.e. seed destructor, chaff cart, narrow windrow burning) used. There are few data on the efficacy of chaff lining on limiting the emergence of weeds, but preliminary data shows rigid ryegrass emergence can be reduced by as much as 80% when the seed are in field residues from either wheat, barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.), or lupins (*Lupinus albus* L.) (Condon 2018).

In Australia as of 2014, 43% of farmers surveyed practice some form of HWSC. When respondents were asked about future use of the technique, 82% said they would implement some form of HWSC in their operation by 2019. The most common method of HWSC that respondents report using is narrow windrow burning (30%) and the least used being chaff carts (3%) (Walsh et al. 2017a). In US cropping systems, research on the effectiveness of HWSC systems is limited. Norsworthy et al. (2016) reported that field residue removal and narrow windrow burning can reduce Palmer amaranth densities (37 to 90%) when used with various herbicide programs. However, efficacy of HWSC systems can be variable based on Palmer amaranth density and soil seed bank size (Norsworthy et al. 2016; Walsh et al. 2017b).

The efficacy of HWSC is reliant on high proportions of weed seed production being retained at crop maturity. Research has demonstrated that several agronomically important weed species in US and Australian cropping systems retain large proportions of their seed at crop maturity. Notably, Walsh and Powles (2014) reported that rigid ryegrass retained 85% of its seed at the time of wheat harvest in Australia. It has been reported that Italian ryegrass seed retention is 58% at the time of wheat harvest in the US (Walsh et al. 2018). In US soybean production systems, it has been reported that Palmer amaranth and tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer] retain greater than 95 and 99%, respectively, of their seed at soybean harvest (Schwartz et al. 2016). In their 2018 publication, Walsh et al. provide a complete list of weed species with reported seed retention values in multiple cropping systems around the world. Even though these weed species retain most of their seed at crop maturity, delays in crop harvest can result in fewer weed seeds being captured due to seed shatter. Rates of seed shatter range from 0.75 to 721 seed d⁻¹ for giant ragweed, barnyardgrass, and Palmer amaranth thereby, reducing the efficacy of HWSC if harvest is delayed (Goplen et al. 2016;

Schwartz-Lazaro et al. 2017). Tidemann et al. (2016) report seed retention needs to be greater than 80% at crop harvest for HWSC to be effective. Seed retention of greater than 80% at crop maturity in many agronomically important weed species creates the unique opportunity to target these weed seed to prevent their input to the weed seed bank.

Weeds such as Palmer amaranth, common ragweed, and Italian ryegrass are major problems in crops across the United States (Webster 2012, 2013); biotypes of these weeds are resistant to eight, four, and six SOA, respectively. Additionally, there are biotypes with multiple resistance to three SOA in each of these species except Italian ryegrass for which there is a population with multiple resistance to four SOA (Heap 2019). These weeds, or their relatives, retain much of their seed on the plant at harvest, making them excellent candidates for HWSC.

Even though HWSC systems have been demonstrated to be effective at reducing weed densities in Australia, there is limited research on the efficacy of HWSC in US cropping systems. Therefore, our objective was to determine the effect of HWSC on Italian ryegrass in winter wheat and common ragweed and Palmer amaranth in soybean.

Materials and Methods

Field studies were initiated on production fields in Virginia in 2017 and continued into 2018 (Table 1). In selected fields, the dominant weed was either Italian ryegrass, common ragweed, or Palmer amaranth. Three wheat fields infested with Italian ryegrass were selected in Lanexa, Cape Charles, and Painter, Virginia (Table 5). The sites in Lanexa and Cape Charles had plots measuring 9 by 30 m and the site in Painter had plots measuring 4.5 by 30 m. For the Palmer amaranth and common ragweed sites, four soybean fields for each weed species were selected in Southside Virginia (Table 5). Each soybean field had plots measuring 9 by 30 m. At

all sites, experiments were arranged as a randomized complete block design with four replications.

Treatments at all locations consisted of either conventional harvest or HWSC at grain harvest in 2017. Conventional harvest was conducted with a commercial combine that returned all field residues and weed seed exiting the combine to the respective plot. The HWSC treatments were implemented using a Wintersteiger Classic plot combine (Wintersteiger AG, Reid im Innkreis, Austria) modified with a trailer, which captured all weed seeds and field residues exiting the combine. All field residues and weed seeds were then dumped outside of the field, removing them from the plot. All sites were no-tillage production, so the only soil disturbance was the planting operation. Wheat row spacing was 15 cm while soybean row spacing was 76 cm. The farmer at each location was responsible for all other management decisions and practices including planting date, fertility, crop variety, and herbicides.

Italian ryegrass study. Italian ryegrass plant density counts were recorded at harvest in 2017 and then again in April 2018 (the Lanexa site was lost after data collection on April 12, 2018, prior to harvest when the farmer terminated wheat to plant corn), initial plant densities were determined by counting plants in six random 0.25-m² quadrats per plot immediately prior to harvest on June 14, 2017. The number of seed heads per quadrat were counted instead of individual plants, due to the difficulty of distinguishing large tillers from whole Italian ryegrass plants. In the subsequent production season, tillers were counted from eight random 0.25-m² quadrats per plot on April 12, 2018. Numbers of seed heads were counted immediately prior to wheat harvest on June 20, 2018 as described for the 2017 harvest.

Common ragweed and Palmer amaranth studies. At the common ragweed and Palmer amaranth locations, data collected included initial weed density from six random 0.25-m²

quadrats per plot at the 2017 harvest. Common ragweed density was measured in late-April to early-May 2018, prior to preplant herbicide application in preparation for soybean planting. Density was determined by counts from eight random 0.25-m² quadrats per plot. Palmer amaranth density was not measured at this timing due to lack of germination across all study locations at this time of the year. Density was determined again from eight random 0.25-m² quadrats per plot in June or July 2018 prior to POST herbicide applications to control both common ragweed and Palmer amaranth. End of season weed density was assessed immediately prior to soybean harvest at all locations between October 1 and November 15, 2018. The number of subsamples ranged from eight 0.25-m² quadrats per plot to full-plot counts and were adjusted depending on weed density to ensure an accurate census.

Across all study locations and species, the quantity of seed retained was determined at the initial (2017) and subsequent (2018) harvest, by collecting seed heads or whole weed plants from one 0.25 m² quadrat per plot. To quantify the seed number, samples were dried, threshed, and sieved to remove large plant material. After cleaning, the entire sample was weighed. Then a 0.5 g aliquot of the sample (seed and fine chaff) was weighed, and the number of seeds counted; this process was done three times per sample and the number of seeds were averaged. The total number of seed ha⁻¹ was calculated on the basis of the triplicate 0.5-g aliquot average using the following formula:

$$Y = [(A * B / C) * D] * 10,000 \quad [1]$$

Where Y is the number of seeds ha⁻¹, A is the average number of seed g⁻¹, B is the total seed and fine chaff sample weight (in grams), C is the number of seed heads for Italian ryegrass or plants for common ragweed and Palmer amaranth from which the seeds were collected, D is the average seed head or plant density m⁻² determined within each plot, and 10,000 is a conversion

factor for m^2 to ha. For Palmer amaranth, the number of plants was divided by 2 to account for an assumed 1:1 male-to-female ratio (Rottenberg 1998). To determine the weight of seed ha^{-1} , an estimate of 493,835 seed kg^{-1} was used for Italian ryegrass (Lacefield et al. 2003), 224,719 seed kg^{-1} for common ragweed (Guillemin and Chauvel 2011), and 2,204,620 seed kg^{-1} for Palmer amaranth (Jha 2008). Grain yield was assessed in all crops by harvesting a single pass (46.5 m^2) from each plot at the time of treatment implementation in 2017 and at the conclusion of the study in 2018.

All data were analyzed in JMP Pro 14 (SAS Institute Inc., Cary, NC) with density, seed retained at harvest, and grain yield subjected to ANOVA with main model effects of treatment, location, block, and interaction of treatment by location. Treatment and location were considered to be fixed effects in the model and block was considered a random effect. When the model was significant, means were separated using Fisher's Protected LSD ($P=0.05$). When a significant location by treatment effect was observed, the data were analyzed and presented by location.

Results and Discussion

Italian ryegrass. In 2018 after treatments were applied at 2017 harvest, there were significant treatment by location interactions at all sites ($P=0.001$, and $P < 0.001$ for April and harvest censuses, respectively). Therefore, all locations are presented separately. Initial Italian ryegrass densities ranged from 88 to 115 seed heads m^{-2} across all locations (Table 5). In April, Italian ryegrass tillers were reduced in the HWSC plots at both the Lanexa and Painter locations (Table 6). In Lanexa, average tiller densities in the HWSC and conventional harvest plots were 175 and 245 m^{-2} , respectively, a 29% reduction. At Painter, HWSC (46 tillers m^{-2}) reduced Italian

ryegrass tillers 69% compared to conventional harvest (149 tillers m⁻²). At the final density measurement just before wheat harvest in 2018, only two locations were assessed; the location in Lanexa was lost due to the farmer deciding to terminate the wheat crop and plant corn instead. In Painter, seed head density was less in HWSC plots compared to conventional harvest: 41 and 125 seed heads m⁻², respectively, a 67% reduction. These reductions in Italian ryegrass populations are similar to what has been observed with rigid ryegrass populations in Australia.

Walsh et al. (2017b) reported that following a one-time HWSC treatment, rigid ryegrass populations were reduced by an average of 60% compared to the nontreated control when assessed prior to POST herbicide application. In our study, reductions in Italian ryegrass populations ranged from 30 to 69%, which is similar to the observed variability of 37 to 90% reduction found by Walsh et al. (2017b). This variability in efficacy of HWSC can be attributed to differences in seed retention as well as the number of seeds in the soil seed bank in a particular field (Walsh et al. 2017b). Italian ryegrass is a species that does not form a very persistent soil seed bank (Ghersa and Martinez-Ghersa 2000). Ichihara et al. (2009) reported that 89.3 and 96.8 % of Italian ryegrass seed on the soil surface did not emerge after 100 d. By 300 d, 98.3% did not emerge, and buried seed had germination of 61 and 72% at 300 d. Most Italian ryegrass seed will be on the soil surface in a no-tillage production system, so a large proportion of seed would not germinate and become a problem in the subsequent crop. However, seed that is buried can persist and become a problem. This means that HWSC would need to be successfully implemented for at least two consecutive seasons to substantially deplete Italian ryegrass seedbank populations.

At wheat harvest in 2018, no differences in wheat yield were observed between treatments. Yield at the Cape Charles location was 3,642 and 3,581 kg ha⁻¹ in the HWSC and

conventional harvest plots, respectively. At Painter, wheat yield was 3,085 and 2,834 kg ha⁻¹ in the HWSC and conventional harvest plots, respectively. Yield differences were not reported in previous HWSC research. Wheat yield response is variable to Italian ryegrass density, ranging from 19 to 39% yield loss from 39 to 107 plants m⁻² (Appleby et al. 1976) to no yield loss with no control of Italian ryegrass (Ritter and Menbere 2002).

HWSC has the capability of removing large quantities of weed seeds with the harvest operation. The potential number of seeds that could be removed by a HWSC operation in 2017 ranged from 7,559 to 11,095 seed m⁻² (Table 5) across locations. The amount of Italian ryegrass seed that could be removed by HWSC before treatment (153 to 225 kg ha⁻¹) implementation was approximately 6.7 to 6.8 times the seeding rate of Italian ryegrass for pastures, which is between 22.4 and 33.6 kg ha⁻¹ (Lacefield et al. 2003). When Italian ryegrass is seeded into fields for weed science studies it is seeded between 8 and 9 kg ha⁻¹ (MJ VanGessel, personal communication) or a 19- to 25-fold reduction than what was observed in the present study.

Similar to the tiller and seed head density data, total seed production at the Cape Charles location, in the HWSC plots, was not different from the conventional harvest plots (309 and 348 seed m⁻², respectively) (Table 7). At the Painter location, seed production in the HWSC plots was less than in the conventional harvest plots (1,027 and 5,866 kg seed m⁻², respectively). Seed retention in these studies is similar to that reported by Walsh and Powles (2014), between 4,029 and 15,913 seed m⁻² of rigid ryegrass was retained. Since some seed may have shattered from the plant prior to harvest and, therefore, sampling, these data are not an estimate of total fecundity or fraction of seeds retained at harvest. It has been reported that Italian ryegrass retains approximately 58% of its seed at crop harvest in Washington State, US (Walsh et al. 2018).

Common ragweed. Initial common ragweed densities across all locations ranged from 4.1 to 24 plants m⁻² (Table 5). At all other timepoints, no significant treatment by location interactions were observed (P=0.186, 0.515, and 0.274 at preplant, POST, and harvest censuses, respectively), so data were pooled across all locations for analyses. Before preplant herbicide applications in spring 2018 common ragweed density in the HWSC plots was lower than the conventional harvest plots: 94 and 120 plants m⁻², respectively (Table 8), representing a 22% reduction. When common ragweed density was assessed again prior to POST herbicide applications, the HWSC treatment had lower density compared to the conventional harvest plots (31 and 42 plants m⁻², respectively, approximately a 26% reduction).

At soybean harvest in 2018, no differences in common ragweed density or seed retention were observed between treatments, which we attribute to effective postemergence herbicide programs applied by the farmers. Since this was the case, it is not surprising that no significant differences in soybean yield were observed between the HWSC (2,648 kg ha⁻¹) and conventional harvest (2,452 kg ha⁻¹) plots. The critical weed free period for soybeans falls between V2 to R3 growth stages (Van Acker et al. 1993). Since weed density was reduced following POST herbicide applications, common ragweed competition with the crop was greatly reduced, leading to similar soybean yield at the end of the season.

There is limited research on seed retention and efficacy of HWSC in common ragweed. However, different HWSC techniques have been demonstrated to be effective at removing or destroying broadleaf weed seeds in Australia and the US. In wild radish, 95% of seed was removed via chaff carts and 93% killed using a Harrington Seed Destructor (HSD) (Walsh and Powles 2007; Walsh et al. 2012). In the United States, Schwartz-Lazaro et al. (2017) reported 100% destruction of giant ragweed seed using an integrated Harrington Seed Destructor (iHSD).

These high levels of removal or destruction of broadleaf weed species demonstrate how effective these systems can be at limiting additions to the soil seed bank. As demonstrated with rigid ryegrass in Australia, different HWSC systems were comparable at reducing weed populations following HWSC implementation (Walsh et al. 2017b). Thus, it is likely that using the iHSD or other HWSC system would provide similar results to those observed in the current study.

At soybean harvest in 2017, the total number of common ragweed seed that could potentially be removed by HWSC ranged from 2,126 to 31,602 seed m⁻² or 95 to 1,406 kg seed ha⁻¹ (Table 5) across all locations. At soybean harvest in 2018, similar to the common ragweed density data, no differences were observed in total common ragweed seed retention between the HWSC and conventional harvest plots for total common ragweed seed retention. Seed retention ranged 23 and 27 seed m⁻² or 1.0 and 1.2 kg seed ha⁻¹ (Table 8). Goplen et al. (2016) reported that giant ragweed retained 80% of its seed at the time of soybean harvest. Since common ragweed and giant ragweed are closely related, common ragweed is likely to have similar levels of seed retention, as has been seen with other closely related species such as Palmer amaranth and tall waterhemp (Schwartz et al. 2016).

Palmer amaranth. Initial Palmer amaranth density ranged from 4.0 to 9.9 plants m⁻² (Table 5) across all locations. At all other timepoints, no significant treatment by location interaction was observed (P=0.831 and 0.423 at POST and harvest censuses, respectively), so data were pooled across all locations for analyses. At all data collection dates in 2018 no differences between treatments were observed, likely due to the use of effective PRE and POST herbicide applications which led to better Palmer amaranth control across the study locations making any potential treatment differences difficult to detect. There was little to no emergence of Palmer amaranth prior to field preparation for soybean planting in 2018, so no data were collected at that

timing. Palmer amaranth does not typically emerge until after full season soybean planting in Virginia. Prior to POST herbicide application only the sites in McKenny and Blackstone were included in the analysis due to low weed densities at the other locations. Palmer amaranth density was 126 plants m⁻² in the HWSC plots and 131 plants m⁻² in the conventional harvest plots (Table 9). At soybean harvest in 2018, Palmer amaranth density was 0.25 plants m⁻² in the HWSC plots and 0.32 plants m⁻² in the conventional harvest plots. Palmer amaranth has been demonstrated to retain 95 to 100% of its seed at soybean harvest across many different environments (Schwartz et al. 2016). Norsworthy et al. (2016) reported reductions in Palmer amaranth density compared to conventional harvest; however, the effects from HWSC treatments, including field residue removal and narrow windrow burning, were variable. This is different than the current study that saw no differences in Palmer amaranth density between HWSC and conventional harvest. Lack of differentiation can be attributed to effective management by the farmers. The farmers were able to achieve high levels of palmer amaranth control in 2018 through the use of timely and effective POST plus residual herbicide applications. Schwartz-Lazaro et al. (2017) reported 100% destruction of Palmer amaranth seed when passed through an iHSD. It is likely that Palmer amaranth populations can be reduced through HWSC due to the efficacy of the iHSD at destroying seed. However, the magnitude of the effects observed in subsequent seasons can be influenced by the size of the residual soil seed bank (Walsh et al. 2017b). As with common ragweed, at soybean harvest in 2018, no significant differences in yield were observed between treatments at the Palmer amaranth locations. Soybean yield in the HWSC plots was 3,349 kg ha⁻¹ while yield in the conventional harvest plots was 3,269 kg ha⁻¹.

Herbicide resistance is one of the biggest threats to advancing crop production and as it continues to grow it will be critical to continue to adopt additional weed management tactics to control troublesome weeds like Italian ryegrass, common ragweed, and Palmer amaranth (Swanton and Weise 1991; Thill et al. 1991). HWSC shows promise as a tool to reduce weed populations with up to 70% Italian ryegrass and 21 and 28%, common ragweed reductions, and control of Palmer amaranth that was equal to best management practices with current, effective, herbicides. However, differences between HWSC and conventional harvest were not detected when weed seed densities were low or where weeds were well controlled with other tactics. Reductions in weed density and therein subsequent seed production, can help reduce weed populations to manageable levels.

The current study observed variability in HWSC effectiveness, this suggests that additional research needs to be conducted. Such research should validate HWSC methods in US winter wheat and soybean production systems and additional weed species. The impact of prolonged use of HWSC on soil seed banks should also be evaluated.

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Table 5. Study locations, including weed species, closest town, GPS coordinates, initial density, and initial seed retention at 2017 harvest.

Crop	Weed	Location	Closest town	GPS coordinates ^a	Initial density ^b		Initial seed retention	
					seed heads m ⁻²	plants m ⁻²	seed m ⁻²	kg seed ha ⁻¹
	Italian					N/A		
Wheat	ryegrass	1	Lanexa	37.540670, -76.892974	112		11,095	225
		2	Cape Charles	37.258540, -75.957963	115	N/A	7,559	153
		3	Painter	37.587877, -75.827716	88	N/A	9,128	185
	common					5.3		
Soybean	ragweed	1	South Hill	36.808536, -78.128693	N/A		15,979	711
		2	Alberta 1	36.891993, -77.942714	N/A	4.1	31,602	1,406
		3	Alberta 2	36.890098, -77.939677	N/A	24	2,126	95
		4	Powelton	36.688721, -77.756751	N/A	20	5,580	48
	Palmer					9.9		
Soybean	amaranth	1	McKenny	37.048327, -77.786347	N/A		210,083	953
		2	Blackstone	37.063479, -77.831805	N/A	4.8	20,777	94.2
		3	Red Oak	36.859520, -77.919192	N/A	5.5	17,770	80.6
		4	South Hill	36.816356, -78.138689	N/A	4	5,863	26.6

^a Abbreviations: GPS, global positioning system; N/A, not applicable.

^b Initial densities were taken at crop harvest 2017.

Table 6. Italian ryegrass tiller and seed head density in 2018 following 2017 harvest treatment application.

Treatment ^a	April tiller density ^b			Seed heads at wheat harvest ^b		
	Lanexa	Cape Charles	Painter	Lanexa	Cape Charles	Painter
	-----m ⁻² -----					
HWSC ^b	175 b	27	46 b	- ^c	15	41 b
Conventional	245 a	35	149 a	-	14	128 a
P-value for treatment	<0.001	0.221	<0.001		0.749	<0.001

^a Abbreviation: -, no data; HWSC, harvest weed seed control.

^b Means within a column followed by the same letter are not different according to Fisher's Protected LSD (P=0.05).

^c This site was lost after April data collection, prior to harvest when the farmer terminated wheat to plant corn.

Table 7. Italian ryegrass seed retention at wheat harvest in 2018 following 2017 harvest treatment application.

Treatment ^a	Seed retention ^b			
	Cape Charles		Painter	
	seed m ⁻²	kg seed ha ⁻¹	seed m ⁻²	kg seed ha ⁻¹
HWSC	309	6.3	1027 b	21
Conventional	348	7.0	5866 a	119
P-value for treatment	0.844		0.013	

^a Abbreviation: HWSC, harvest weed seed control.

^b Means within a column followed by the same letter are not different according to Fisher's Protected LSD (P=0.05).

Table 8. Common ragweed density and seed retention in 2018, following 2017 harvest treatment application.

Treatment ^a	Before preplant	Before POST	Soybean	Seed retention at	
	herbicide	herbicide	harvest	harvest	
	application ^b	application ^b	2018	seed m ⁻²	kg seed ha ⁻¹
	-----plants m ⁻² -----				
HWSC ^b	94 b	31 b	0.05	27	1.2
Conventional	120 a	42 a	0.3	23	1.0
P-value for					
treatment	0.011	0.003	0.152	0.696	

^a Abbreviation: HWSC, harvest weed seed control.

^b Means within a column followed by the same letter are not different according to Fisher's Protected LSD (P=0.05).

Table 9. Palmer amaranth density and seed retention in 2018, following 2017 harvest treatment application.

Treatment ^a	Before POST ^b Soybean harvest 2018		Seed retention at harvest	
	-----plants m ⁻² -----		seed m ⁻²	kg seed ha ⁻¹
HWSC	126	0.25	561	2.5
Conventional	131	0.32	1560	7.1
P-value for treatment	0.688	0.218	0.261	

^a Abbreviation: HWSC, harvest weed seed control.

^b Only the McKenny and Blackstone locations were analyzed due to minimal Palmer amaranth presence at the other two locations at this rating timing.

Integrated weed management systems to control common ragweed (*Ambrosia artemisiifolia* L.) in soybean

As herbicide resistance continues to become a larger problem in the US, growers are looking for other ways to control weeds. An integrated weed management (IWM) study was conducted at three locations across Virginia from 2016 to 2019. The factorial study evaluated: 1) soybean planting date (early or late planted) 2) \pm winter cover (cereal rye/wheat or no cover), and 3) \pm HWSC (via field residue removal). Prior to soybean planting, winter cover resulted in a 22% reduction in common ragweed density compared to no cover. At soybean harvest in the first year, the overall lowest common ragweed densities were in the late planted plots following winter wheat with densities of 1.5, 0.5, and 3.4 plants m^{-2} at the three locations. At soybean harvest, common ragweed aboveground biomass was reduced by 46 and 22% at two locations in late planted compared to early planted soybean. To evaluate the impact of the first year's treatments and HWSC, full season soybean were planted in May across the trial in year two. Prior to soybean planting in the second year, common ragweed density was reduced by 83% at one location in plots that had been late planted in the first year. However, no significant reductions in common ragweed density were observed at the other two locations. When comparing winter cover to no cover, common ragweed densities were reduced by 31 and 49% at two locations and densities were similar at the third location. Harvest weed seed control reduced common ragweed density by 43% at one location compared to the conventional harvest plots but no significant reductions were observed at the other two locations. At POST herbicide application in the second year, HWSC was not significant. However, there was a significant location by planting date by winter cover interaction and the overall lowest common ragweed

densities (4.1 to 10.3 plants m⁻²) were in the late planted plots with winter cover. While variability across locations was observed, overall studies indicated that IWM approaches can reduce common ragweed populations and the most important factor tested is planting time.

Nomenclature: Common ragweed, *Ambrosia artemisiifolia* L. AMBEL; cereal rye, *Secale cereale* L.; soybean *Glycine max* (L.) Merr.; winter wheat, *Triticum aestivum* L.

Keywords: Cover crops, density, double crop soybean, harvest weed seed control, seed retention.

As herbicide resistance in weed species continues to grow (Heap 2019) there is a need to develop new integrated strategies for weed control around the world. Integrated weed management (IWM) is an approach to weed management that relies on using multiple different strategies to control weeds (Swanton and Weise 1991; Thill et al. 1991). A multi-tactic weed control plan is necessary to control troublesome weeds that can quickly adapt when a single tactic is used (Norsworthy et al. 2012; Thill et al. 1991). To combat the growing problem of herbicide resistance, many more acres of farmland will need to implement IWM strategies (Redlick et al. 2017). Weed control tactics that can be components of an IWM system include using cover crops, tillage, sound cultural practices, harvest weed seed control (HWSC), and herbicide programs, among others (Swanton and Weise 1991).

Common ragweed is a major problem in the mid-Atlantic soybean production region (Scruggs et al. 2019) due to resistance to 4 different sites of action (SOA) including groups 2, 5, 9, and 14 (Heap 2019). There are also biotypes reported to be multiple resistant to groups 2 and 9, 2 and 14, and 2, 9, and 14 in several states around the US (Heap 2019). Challenges in controlling common ragweed, partly due to herbicide resistance, is why it is listed as the number 9 most common and troublesome weed in all broadleaf crops, and the number 7 most troublesome weed in soybeans by the Weed Science Society of America (WSSA) (Van Wychen 2016). It has been reported that even low common ragweed pressure, 4 plants 10 m^{-1} row, is enough to reduce soybean yield up to 132 kg ha^{-1} . When common ragweed is left uncontrolled all-season, soybean yield can be reduced up to 62%, with densities of up to $160 \text{ plants m}^{-2}$ (Coble et al. 1981). Therefore, an IWM approach for common ragweed is necessary.

Weed scientists in the US are following the lead of Australia and starting to develop IWM strategies using multiple tactics for weed control including harvest weed seed control

(HWSC) (Beam et al. 2019; Norsworthy et al. 2016). HWSC removes or kills seed that are retained on the weed mother plant with harvest operations (Walsh et al. 2013). There are several HWSC systems including narrow windrow burning, direct bale, chaff removal, chaff lining, and cage mills (i.e. the Harrington Seed Destructor, integrated Harrington Seed Destructor, and Seed Terminator) (Walsh et al. 2013). If seed has already shattered and is on the ground, HWSC is not effective (Walsh and Powles 2014). All of these systems are being used commercially in Australia (Walsh et al. 2017a) and some are being used on an experimental basis by early adopters in the US.

There are various HWSC techniques that do not kill weed seed, but instead concentrate them in certain areas of the field or remove them from the field. One in particular is chaff removal. In this method, a cart is pulled by the combine and the chaff is collected into the cart. Chaff cart use is limited due to the volume of chaff produced, which can be as high as 100 m³ ha⁻¹ for wheat (Walsh and Powles 2007). Matthews et al. (1996) found that of the rigid ryegrass (*Lolium rigidum* Gaud.) seeds that enter the combine between 75 and 85% were collected in the chaff cart and up to 94% of wild mustard (*Sinapis arvensis* L.) seeds were captured. In this method, the seed is still viable; so, there is potential for these seed to infest other fields depending on where the chaff is dumped. Beam et al. (2019) reported 22 to 26% reductions in common ragweed density in the growing season following HWSC.

Sound cultural practices are critical to reducing yield loss due to weeds. To give crops an advantage against weeds, well adapted genetics and agronomic practices need to be utilized. Planting crops at different times during the growing season can lead to reduced weed densities based on weed emergence. Amuri et al. (2010) reported weed densities were lower in a double-crop soybean system when wheat residues are left on the soil surface than when it was burned or

tilled. Common ragweed has been demonstrated to have a shortened emergence window in the spring of the year compared to some weed species. The majority of common ragweed emergence (>90%) occurs by mid-May in many regions of the US (Barnes et al. 2017; Myers et al. 2014; Werle et al 2014), which is prior to when double crop soybean is planted. Due to this early germination window, double-cropping soybean behind wheat is likely a tool that can be utilized to manage common ragweed in the mid-Atlantic region. To further aid in crop competitiveness, planting crops with a narrow row spacing (≤ 38 cm) allows the crop canopy to close faster allowing less time for weeds to germinate and establish before they are shaded (Norsworthy et al. 2012). Chandler et al. (2001) reported that weeds growing in narrow or twin row soybeans produced fewer seed than weeds growing in wide row (76 cm) soybeans. Crops often have a greater competitive advantage by germinating before the emergence of weeds from under a cover crop (O'Donovan et al. 2013; Olsen et al. 2012).

Cover crops are traditionally planted for soil and water quality improvement, nutrient cycling, and other ecosystem benefits (Snapp et al. 2005). Weed suppression is possible with cover crops through various means including light exclusion, reducing soil temperature, immobilizing nitrogen, and providing a physical barrier for weed seedlings (Mirsky et al. 2013). The amount of biomass needed to suppress weeds after cover crop termination is 8000 kg ha^{-1} (Teasdale and Mohler 1993). Cereal rye has been shown to consistently produce the most biomass of fall-planted grass cover crops (Finney et al. 2009; Mirsky et al. 2013). Teasdale and Mirsky (2015) reported that hairy vetch reduced common ragweed and giant foxtail emergence 84 and 71%, respectively, when the cover crop was terminated with a roller crimper. However, cover crop suppression of summer annual weeds can be variable based on cover crop biomass and weed species (Wallace et al. 2018).

Cover crops and HWSC, have been demonstrated as effective but there is limited research on how various techniques will work together in an IWM system with agronomically important weeds (Hay et al. 2019; Norsworthy et al. 2016) despite numerous calls for such research (Harker and O'Donovan 2013; Swanton and Weise 199; Swanton et al. 2008; Thill et al. 1991). The objective of this research was to evaluate integrated common ragweed management strategies including planting time, use of a cover crop, and HWSC.

Materials and Methods

A study was conducted at three locations in Virginia over two years at each location. The locations included Kentland Farm in Blacksburg, a grower field in Lawrenceville, and at the Southern Piedmont Agricultural Research and Extension Center in Blackstone to evaluate integrated approaches to common ragweed management in soybean. The Blacksburg location (37.93958, -80.571234) was on a Ross loam (Fine-loamy, mixed, superactive, mesic Cumulic Hapludolls) with pH 6.6 and 3.4% organic matter. The Lawrenceville location (36.650324, -77.82617) was on an Emporia sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Haludults) with pH 5.48 and 0.9% organic matter and the Blackstone location (37.083214, -77.972078) was on an Appling sandy loam (Fine, kaolinitic, thermic, Typic Kanhapludults) with pH 6.42 and 3% organic matter. The studies were a factorial design with 3 factors, each with 2 levels and 5 replications and arranged as a randomized complete block. Factors included (1) soybean planting date, (2) \pm winter cover, and (3) \pm HWSC. Soybean planting dates were in May, to represent early planted soybean or early July to represent late planted soybean. Winter cover was either cereal rye, planted in the fall prior to early planted soybean, or wheat planted in the fall and harvested prior to late planted soybean. Plots without winter cover were left fallow

over the winter. HWSC was implemented at the end of the soybean growing season as described by Beam et al. 2019. All crop residues and weed seeds contained therein were removed from the plot. Plots without HWSC had crop residues distributed back across the plot like a standard harvest operation. There were eight treatments total. Fertility, herbicide programs, planting dates, row spacing, crop varieties, and other practices were selected to mimic standard production practices for the region and are described below and in Table 10. All herbicide applications were made using a 6-nozzle boom with 45.72 cm nozzle spacing equipped with XR11002 nozzles calibrated to apply 140 L ha⁻¹ of spray solution. All plots measured 4.57 by 7.62m.

Year one. *Early planted soybean.* Cereal rye, variety not stated (Southern States Cooperative, Richmond VA), was drilled on 16.5 cm spacing at 134 kg ha⁻¹. Cereal rye planting date, along with other termination, planting, and harvesting dates are located in Table 10. Cereal rye was terminated 2 wk before soybean planting using a roller crimper and glyphosate (Roundup Powermax, Monsanto Co, St. Louis, MO) at 1126 g ae ha⁻¹ plus 2,4-D (Shredder Amine 4, WinField Solutions LLC, St. Paul, MN) at 532 g ae ha⁻¹ plus flumioxazin (Valor SX, Valent USA Corp., Walnut Creek, CA) at 89.25 g ai ha⁻¹. Early planted soybean plots that had no winter cover received the same herbicide application as the plots with cereal rye. Soybeans were planted into the early planted plots in rows on 76 cm centers, at 407,550 seed ha⁻¹ (AG48X7 in 2017 and AG56X8 in 2018, Monsanto Co., St. Louis MO) with 6 rows per plot. At planting, glufosinate (Liberty 280 SL, Bayer CropScience LP, Research Triangle Park, NC) was applied at 59.38 g ai ha⁻¹ plus ammonium sulfate at 1.68 kg ha⁻¹ (Spray Grade Ammonium Sulfate, DSM Chemicals North America, Inc., Augusta, GA) and crop oil concentrate (Crop Oil Concentrate, Southern States Cooperative, Richmond, VA) at 1% v v⁻¹. Early planted plots both with and

without winter cover residue were fertilized at soybean planting with 56 kg ha⁻¹ of P₂O₅ and 56 kg ha⁻¹ of K₂O. When common ragweed average height reached 30 cm tall in the no cover plots, a POST application of glyphosate plus fomesafen (Flexstar GT 3.5, Syngenta Crop Protection LLC, Greensboro, NC) at 1,107 g ae plus 274 g ai ha⁻¹ plus nonionic surfactant (Scanner, Loveland Products, Greeley, CO) at 0.25% v v⁻¹ was made. The POST herbicide application timing was late by design. It ensured that not all common ragweed plants were controlled by the herbicide program, allowing all treatment effects to be measured, but was also realistic and similar to what often occurs in farmer fields. Early planted soybean harvest occurred and HWSC treatments implemented in the fall of the year. Yield, however, was not measured due to poor soybean stand from drought and deer herbivory.

Late planted soybean. Winter wheat was drilled (SS8340 in 2017, Southern States Cooperative, Richmond VA and Hilliard in 2018, Featherstone Seed, Amelia VA) at 134 kg ha⁻¹, on 16.5 cm spacing, see Table 10 for dates. Plots with a wheat cover crop had 56 kg ha⁻¹ of N and thifensulfuron (Harmony SG, Corteva, Indianapolis, IN) applied at 26.25 g ai ha⁻¹ plus nonionic surfactant at 0.25% v v⁻¹ in late winter. Wheat was harvested in June of each year (Table 1).

Late planted plots that had a wheat cover or that had been left fallow had glufosinate applied at 65.52 g ai ha⁻¹ plus flumioxazin at 89.25 g ai ha⁻¹ plus crop oil concentrate at 1% v v⁻¹ immediately after wheat harvest. Late planted soybean were drilled at 494,000 seed ha⁻¹ in rows on 33 cm centers with 15 rows per plot. Glufosinate was applied again at 65.52 g ai ha⁻¹ plus crop oil concentrate at 1% v v⁻¹ following drilling soybean. Late planted plots, both with and without winter wheat, were fertilized at soybean planting with 56 kg ha⁻¹ of P₂O₅ and 56 kg ha⁻¹ of K₂O. A postemergence application of glyphosate plus fomesafen plus nonionic surfactant at

0.25% v v⁻¹ was made when common ragweed average height was 30 cm tall in the no cover plots, to mimic late applications that often occur in farmer fields.

Year two. The second year of the study was used to evaluate the effect of different IWM tactics (soybean planting time, winter cover, and HWSC) on common ragweed populations in the following growing season. Following soybean harvest in the first year of the study, the site was left fallow over the winter. In year two, the entire study at each site was planted full season soybean (early planted) using the same herbicide program, fertility, planting rate, and row spacing as previously described, with the exceptions of soybean variety (AG56X8 and AG41X8 in 2018 and 2019, respectively).

Site specific management. Blacksburg and Blackstone sites did not have a naturalized common ragweed population, so these sites were over seeded with common ragweed prior to the initiation of the experiment. These seeds were harvested from the Lawrenceville location in an area outside but adjacent to the study. This population of common ragweed was 30% glyphosate resistant, based on glyphosate response in replicated research trials adjacent to this study. Common ragweed seed were spread using a rotary spreader at approximately 11.6 million seed ha⁻¹ on November 11, 2016 and November 15, 2017 at Blacksburg and Blackstone, respectively. This rate of common ragweed seed was used to ensure a dense uniform stand and account for potential low germination of ripe dormant seed (Baskin and Baskin 1977; Willemsen 1975). Blacksburg also contained large crabgrass (*Digitaria sanguinalis* (L.) Scop), giant foxtail (*Setaria faberi* Herrm.), and johnsongrass (*Sorghum halepense* (L.) Pers.) that was controlled with sethoxydim (Poast, BASF Corp., Research Triangle Park, NC) at 315 g ai ha⁻¹ plus crop oil concentrate at 1% v v⁻¹ on June 17, 2017 in the full season soybean plots and August 16, 2017 in the double crop soybean plots. The Lawrenceville site was previously in tobacco and the soil pH

was low, slowing the growth of both the cereal rye and winter wheat. To help correct this problem and get sufficient biomass for weed control the field was fertilized with 50.4 kg of N, 16.8 kg of P₂O₅, and 67.2 kg of K₂O ha⁻¹ plus 560 kg ha⁻¹ of lime (as per soil test recommendation) in mid-February 2017. The Blackstone site contained large crabgrass that was controlled with sethoxydim at 315 g ai ha⁻¹ plus crop oil concentrate at 1% v v⁻¹ on June 12, 2018.

Data collection and analyses. Common ragweed density measurements were conducted at preplant herbicide application, at POST herbicide application and harvest in two random 0.25 m² quadrats per plot. Height data were collected by measuring 10 random common ragweed plants per plot at cereal rye termination or wheat harvest for both the winter cover and no cover plots for each planting timing and again just prior to the POST herbicide application. At soybean harvest, common ragweed density measurements were taken in two random 0.25 m² quadrats per plot and four representative common ragweed plants were hand harvested and air dried. The samples were weighed and then threshed to determine total seed remaining on the plant at the time of soybean harvest. Using the density at soybean harvest and the average number of seeds per plant the total number of seeds that could be impacted by HWSC was calculated. Data collected in the second year of the experiment included common ragweed density and height, as described for year one.

All data were analyzed in JMP Pro 14 (SAS Institute Inc., Cary, NC) with a model that included main effects of location, planting date, winter cover, HWSC, block, and interactions with all main effects, excluding block. All model effects were considered to be fixed effects. The models were reduced using stepwise model selection to remove non-significant interactions.

Main model terms were never removed. Means were separated using Fisher's Protected LSD (P=0.05).

Results and Discussion

Year one. Cereal rye biomass was assessed just prior to termination and was variable with location. Cereal rye biomass was 5,940, 2,205, and 1,508 kg ha⁻¹ at Blacksburg, Lawrenceville, and Blackstone, respectively. These biomass levels are below the 8,000 kg ha⁻¹ threshold that has been reported for summer annual weed suppression (Teasdale and Mohler 1993). At wheat harvest, most of the wheat residue was removed from the plots with the harvest operation. The remaining wheat residue was approximately 15 to 20 cm in height.

Common ragweed density was measured at cereal rye termination in both the winter cover and no cover plots in the early planted half of the experiment and prior to wheat harvest in the winter cover and no cover plots in the late planted half of the experiment. For initial common ragweed density, there was a significant location by planting time interaction (Table 11). Common ragweed density at Blacksburg in the late planted timing was 0 plants m⁻² compared to all other locations and planting timings which had similar common ragweed densities of 92.4 to 116.8 plants m⁻² (data not shown). Winter cover as a main effect alone was significant for common ragweed density prior to soybean planting. In the plots that had a winter cover, either cereal rye or winter wheat, common ragweed density was 77.2 plants m⁻² compared to the no cover plots which had a density of 99 plants m⁻² (data not shown).

At POST herbicide application (6 WAP), there was a significant location by planting time by winter cover interaction for common ragweed density (Table 11). Overall, the Lawrenceville location had greater common ragweed densities than either Blacksburg or Blackstone. Both the

Blacksburg and Blackstone locations had similar common ragweed densities for each treatment. Across all three locations, the late planted plots with winter cover had the least common ragweed with 7.6, 5.4, and 5 plants m^{-2} at Blacksburg, Lawrenceville, and Blackstone, respectively, (Figure 10A). The greatest common ragweed densities were both at the Lawrenceville location in both treatments that did not have winter cover with 26 and 20.6 plants m^{-2} (Figure 10A) in the early planted without cover and the late planted without cover treatments, respectively. In most instances, plots with cover had lower common ragweed densities compared to the no cover plots for both soybean planting times.

At harvest, a significant location by planting time by winter cover interaction was observed for common ragweed density (Table 11). Common ragweed densities at harvest were again overall greater at the Lawrenceville location and densities were similar at both the Blacksburg and Blackstone locations. The treatments with the greatest overall density were the late planted no cover treatments with 20.8 and 22.5 plants m^{-2} (Figure 10B) at Lawrenceville and Blackstone, respectively. Comparing the winter cover and no cover treatments within the late planting timing, there was a 98% reduction in common ragweed densities at Lawrenceville and 85% at Blackstone. At the Blacksburg location, regardless of winter cover, the common ragweed densities in the late planted treatments were the same at 1.5 plants m^{-2} (Figure 10B).

It has been reported that common ragweed has a short germination window in the spring compared to many summer annual weeds. Barnes et al. (2017) reported that 90% of common ragweed emergence occurs around the first to middle of May in Nebraska. In Pennsylvania, Delaware, and New Jersey, it has been reported that 95% of common ragweed emergence for the growing season occurs around mid-April to the first of May (Myers et al. 2004). Werle et al. (2014) reported that 90% of cumulative common ragweed emergence occurs around mid-May in

Iowa. Common ragweed emergence patterns are similar across a wide area of the United States. These dates of common ragweed emergence are similar to the current study which saw little common ragweed emergence occurring after preplant herbicide application in the treatments that were late planted either behind wheat (what is known as a double-crop soybean) or had been left fallow until late soybean planting timing and planted in late June to early July. Amuri et al. (2010) reported that overall weed densities in double-crop soybean following winter wheat were lower when residues were left on the soil surface instead of burning. This trend of lower weed densities in late planted with soybean with winter cover is similar to what was observed in the current study.

Initial common ragweed height was measured prior to wheat harvest in the late planted soybean treatments. In the late planted treatments, a significant location by winter cover interaction was observed (Table 12). At all locations, common ragweed height was reduced in the late planted treatments with winter cover (60, 50, and 29% at Blacksburg, Lawrenceville, and Blackstone, respectively) compared to the no cover treatments (Figure 11). The wheat growing in competition with common ragweed resulted in shorter plants at the time of wheat harvest than where left fallow.

Common ragweed heights at POST herbicide applications showed a significant location by planting time by winter cover interaction (Table 12), similar to the density data. Common ragweed height in early planted treatments was similar regardless of whether there was winter cover or not at the Lawrenceville and Blackstone locations, with heights ranging from 31.7 to 38 cm (Figure 12). At the Blacksburg location, the common ragweed plants in the early planted, winter cover treatments were shorter than the common ragweed plants in the early planted, no cover treatments with heights of 28.9 and 35.5 cm, respectively, a 19% reduction. The

difference in location was likely the result of greater cereal rye biomass in Blacksburg location compared to the other locations. In most instances, common ragweed was shorter in the early planted treatments when compared to late planted treatments regardless of winter cover. At the Blacksburg location, there was no difference in common ragweed height in either late planted treatment. At Lawrenceville, common ragweed was 21% shorter in the late planted, winter cover treatment (56.3 cm) compared to the late planted, no cover treatment (71.6 cm). At Blackstone, a similar trend was seen with plants in the late planted, winter cover treatment (28.4 cm) being shorter than the late planted no cover treatment (45.9 cm), by 38% (Figure 12). Competition from a cover crop has been shown to reduce the height of Palmer amaranth. Hay et al. (2019) reported a 26 to 40% reduction in Palmer amaranth height when grown in competition with winter wheat compared to no cover crop. This is similar to the current study where a 19 to 38% reduction in common ragweed height was observed with winter cover compared to no cover crop.

Common ragweed aboveground biomass and seed retention data were collected just prior to soybean harvest. A significant location by planting time interaction was observed with common ragweed aboveground biomass (Table 13). At the Blacksburg location, the common ragweed biomass was less in the late planted treatments (22.6 g plant⁻¹) compared to the early planted treatments (42.3 g plant⁻¹), a 46% reduction (Figure 13). At the Lawrenceville and Blackstone locations, biomasses were similar across both soybean planting timings with biomasses ranging from 26.6 to 35.4 g plant⁻¹.

A significant location by planting time by winter cover interaction was observed for common ragweed seed retention at soybean harvest (Table 13). Common ragweed seed retention was variable across the three locations. Total seed retention ranged from 836 to 3,611 seed plant⁻¹

¹. Although not always significantly different from other treatments, the numerically greatest seed retention was in the Lawrenceville early planted no cover treatment and the Blackstone late planted no cover treatment with 3,609 and 3,611 seed plant⁻¹, respectively (Figure 14). Common ragweed that emerges later in the growing season and grown in competition with a soybean crop are smaller and produce less aboveground biomass and seed (Dickerson and Sweet 1971; Simard and Benoit 2012). Simard and Benoit (2012) reported that common ragweed produced 3,694 seed plant⁻¹ when grown in competition with soybean. This is similar to what was observed in the current study, however, in the current study only seed retained at harvest were recorded.

Year two. Common ragweed density in the second year of the experiment was collected prior to preplant herbicide application in the spring and again at POST herbicide application when common ragweed reached 30 cm in height. There were multiple significant interactions observed for common ragweed density at the start of the second year of the experiment. These interactions include location by planting time, location by winter cover, and location by HWSC (Table 11). At the Blacksburg location, common ragweed densities were 83% lower in the late planted treatments from year one compared to the early planted treatments (Figure 15). At the Lawrenceville and Blackstone locations, densities were similar between the early and late planted treatments with densities of 83 and 81.2 and 52.8 and 46.8 plants m⁻², respectively.

When comparing treatments across location based on winter cover, common ragweed densities at the Blacksburg location were similar between the winter cover and no cover treatments with densities of 41.2 and 44.6 plants m⁻², respectively (Figure 15). At the Lawrenceville location, common ragweed density in the winter cover treatments (55.1 plants m⁻²) were 50% lower than in the no cover treatments (109.1 plants m⁻²). At the Blackstone location, similar to the Lawrenceville location, the common ragweed density in the winter cover

treatments (40.7 plants m⁻²) were 31% lower than in the no cover treatments (58.9 plants m⁻²).

The effect of HWSC on common ragweed density was only different at Lawrenceville where HWSC significantly reduced common ragweed density compared to the conventional harvest treatments with densities of 59.7 and 104.5 plants m⁻², respectively, a 43% reduction. Prior research on HWSC has demonstrated that it can be variable on a species such as common ragweed. Beam et al. (2019) demonstrated that a one-time implementation of HWSC can reduce common ragweed density by 22% in the spring of the following year prior to preplant herbicide application, similar to the Lawrenceville location in the current study. Norsworthy et al. (2016) reported variability in the effect of HWSC at reducing weed density when using field residue removal with Palmer amaranth (*Amaranthus palmeri* S. Wats.). The efficacy of HWSC can be influenced by weed seed retention and the size of soil seedbank in a given field (Walsh et al. 2017b). The Lawrenceville location had greater seed retention than the other sites which likely explains why HWSC had a greater effect at this location compared to Blacksburg or Blackstone.

At POST herbicide application, in the second year of the study, a significant location by planting time by winter cover interaction was observed (Table 11). Similar to in the first year of the study, the late planted, winter cover treatments had less common ragweed compared to the other treatments across all locations with 4.7, 6.7, and 10.3 plants m⁻² at Blacksburg, Lawrenceville, and Blackstone, respectively (Figure 16). While not significantly greater than some treatments, the greatest common ragweed densities were in the Blacksburg early planted, no cover treatment and the Blackstone late planted, no cover treatment with 29.2 and 30.1 plants m⁻², respectively. There was no significant effect of HWSC on common ragweed density at POST herbicide application (Table 11). The effect of HWSC on weed populations can be variable depending on the quantity of seed shattered prior to HWSC implementation, efficacy of

subsequent herbicide applications, and the size of the soil seedbank (Beam et al. 2019; Norsworthy et al. 2016; Tidemann et al. 2016; Walsh et al. 2017b).

Herbicide resistance is a growing problem and diversifying weed control strategies into an IWM system is a necessity. The current study demonstrates that using multiple methods including planting timing, winter cover, and HWSC can help reduce common ragweed populations in one year. It appears that the planting timing (late planting or double-cropping soybean after winter wheat compared to early planted) reduces common ragweed populations more than other treatments tested in this study. Therefore, double-cropping soybeans after wheat is a recommended strategy for integrated common ragweed management, where feasible. This system should be used in conjunction with crop rotation and other weed management techniques to keep common ragweed densities at manageable levels. Including a winter cover (wheat or cereal rye) resulted in similar or reduced common ragweed density and reduced common ragweed heights across locations at soybean planting. The effectiveness of HWSC was variable but reduced common ragweed densities at one of three locations. Variability in HWSC has been demonstrated in other research (Beam et al. 2019; Norsworthy et al. 2016; Walsh et al. 2017b) and highlights the need for additional research. The impact of planting timing and HWSC and its effect on soil seedbanks should be evaluated further.

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Table 10. Dates for cover crop and soybean planting, cover crop termination, POST herbicide application, and soybean harvest for all locations and years of the experiment.

Field operation	Blacksburg		Lawrenceville		Blackstone	
	2016-2017	2018	2016-2017	2018	2017-2018	2019
Cereal rye planted	October 10	--	November 8	--	November 16	--
Winter wheat planted	October 19	--	November 8	--	November 16	--
Winter wheat N application	March 13	--	March 8	--	February 28	--
Winter wheat POST herbicide application	February 20	--	March 8	--	February 28	--
Cereal rye terminated/early planted burndown and residual herbicide application	May 3	--	May 9	--	May 9	--
Early soybean planting ^a	May 18	May 28	May 26	May 22	May 23	May 22
Wheat harvested	June 27	--	June 22	--	June 18	--
Late planting burndown and residual herbicide application	June 27	--	June 22	--	June 18	--
Late soybean planting ^a	July 6	--	June 28	--	July 3	--
Early planted POST herbicide application	June 29	July 9	July 7	July 3	July 3	June 19
Late planted POST herbicide application	August 18	--	August 6	--	August 13	--
Soybean harvest	November 14	October 29	November 7	October 25	October 24	

^a Additional burndown applied at planting.

Table 11. Effects table for common ragweed density across all locations for years one and two of field experiments at Blacksburg and Lawrenceville, VA in 2017-2018 and Blackstone, VA in 2018-2019.

Model effects ^a	Year 1			Year 2	
	At soybean planting	At POST	Harvest	At soybean planting	At POST
	-----P-values-----				
Block	0.321	0.032	0.002	0.798	<0.001
Location	<0.001	<0.001	<0.001	<0.001	0.809
Planting time	0.003	<0.001	0.797	<0.001	<0.001
Cover	0.031	<0.001	<0.001	<0.001	0.028
HWSC	--	--	--	<0.001	0.070
Location by planting time	<0.001	0.013	<0.001	<0.001	<0.001
Location by cover	.	<0.001	<0.001	<0.001	0.106
Location by HWSC	--	--	--	<0.001	.
Planting time by cover	.	0.705	<0.001	.	0.016
Planting time by HWSC	--	--	--	.	.
Cover by HWSC	--	--	--	.	.
Location by planting time by cover	.	<0.001	0.001	.	0.006
Location by planting time by HWSC	--	--	--	.	.
Location by cover by HWSC	--	--	--	.	.
Planting time by cover by HWSC	--	--	--	.	.
Location by planting time by cover by HWSC	--	--	--	.	.
Global ANOVA	<0.001	<0.001	<0.001	<0.001	<0.001

^a --, effect not included in the model; ., effect removed from the model using stepwise selection.

Table 12. Effects table for initial common ragweed height and at POST herbicide application across all locations for year one of the field experiment at Blacksburg and Lawrenceville, VA in 2017 and Blackstone, VA in 2018.

Model effects ^a	Initial	At POST
	Double crop	
	-----P-values-----	
Block	<0.001	0.801
Location	<0.001	<0.001
Planting time	--	<0.001
Cover	<0.001	<0.001
Location by planting time	--	<0.001
Location by cover	<0.001	<0.001
Planting time by cover	--	<0.001
Location by planting time by cover	--	<0.001
Global ANOVA	<0.001	<0.001

^a --, effect not included in the model.

Table 13. Effects table for common ragweed biomass and seed retention at soybean harvest across all locations for year 1 of the field experiment at Blacksburg and Lawrenceville, VA in 2017 and Blackstone, VA in 2018.

Model effects ^a	Biomass (g plant ⁻¹)	Retention (seed plant ⁻¹)
	-----P-values-----	
Block	0.035	0.037
Location	0.563	0.157
Planting time	0.011	0.729
Cover	0.002	0.183
Location by planting time	0.009	0.215
Location by cover	--	0.912
Planting time by cover	--	0.977
Location by planting time by cover	--	0.025
Global ANOVA	<0.001	0.040

^a --, effect not included in the model.

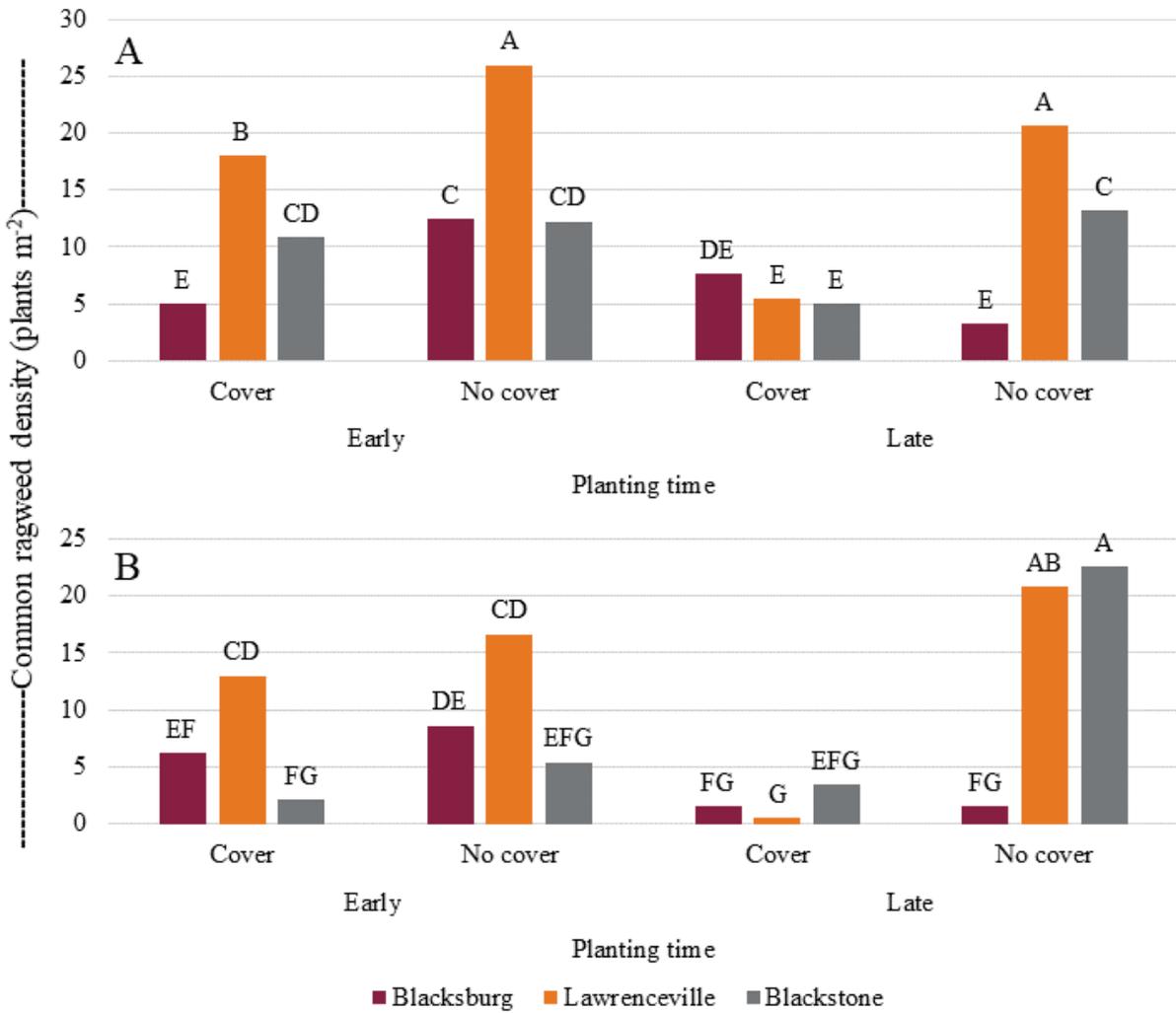


Figure 10. Common ragweed density A) at POST herbicide application (6 wk after planting) and B) at soybean harvest by location, planting time, and \pm winter cover in the first year of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD ($P=0.05$).

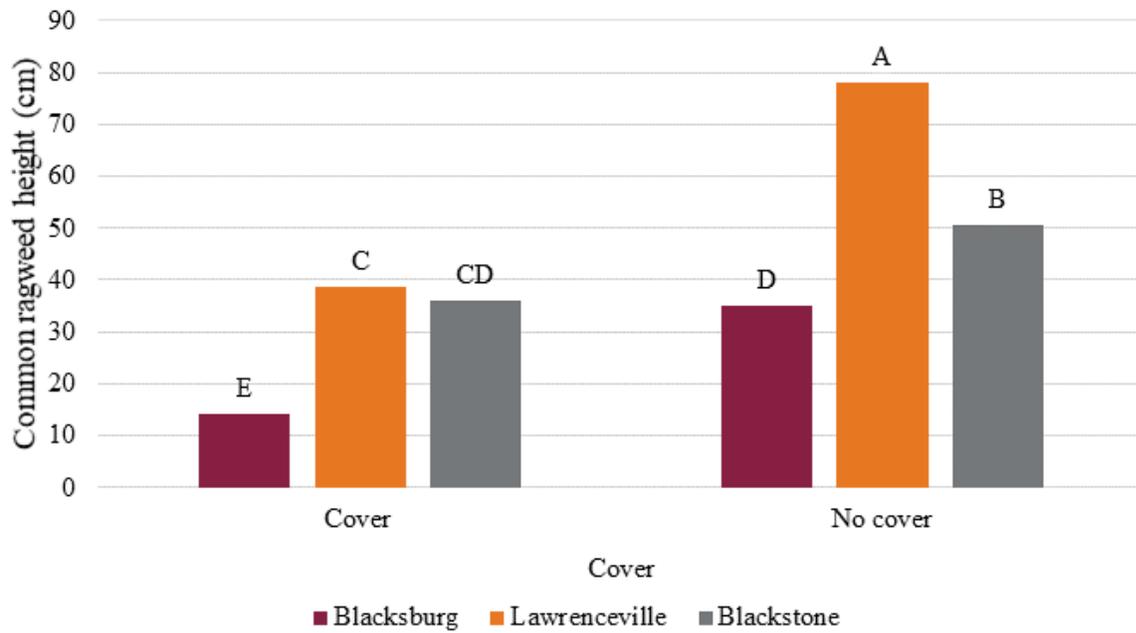


Figure 11. Common ragweed height at late planting across all locations in year one of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD ($p=0.05$).

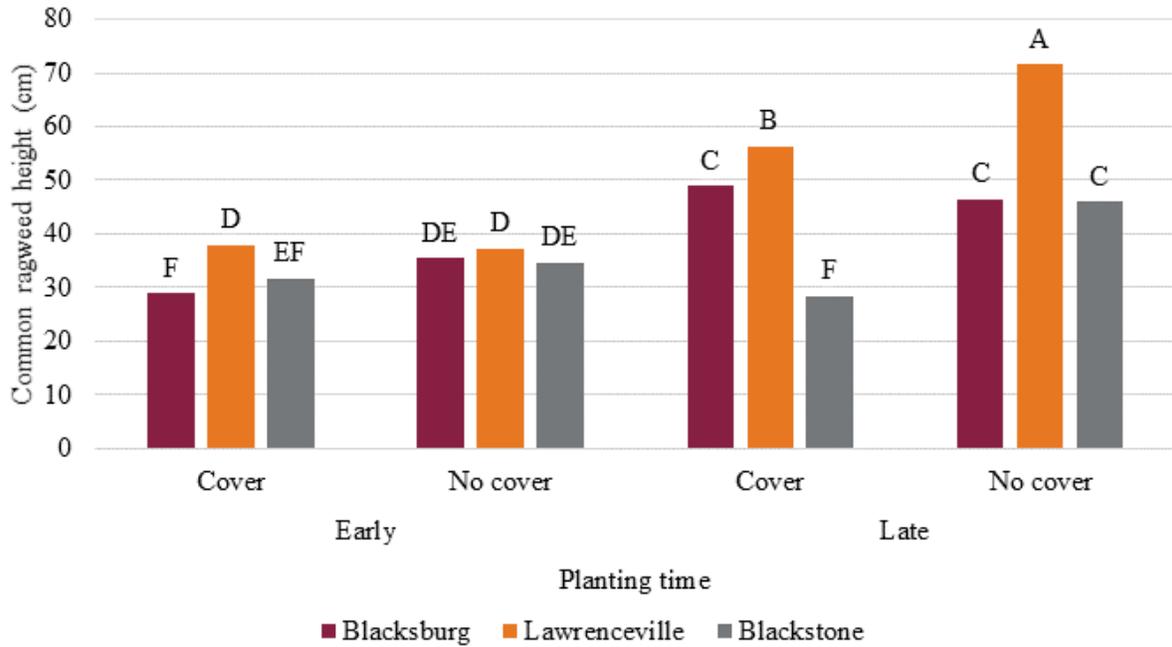


Figure 12. Common ragweed height at POST herbicide application (6 wk after planting) by location, planting time, and ± winter cover (cereal rye for early planting and winter wheat for late planting) in year one of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher’s Protected LSD ($P=0.05$).

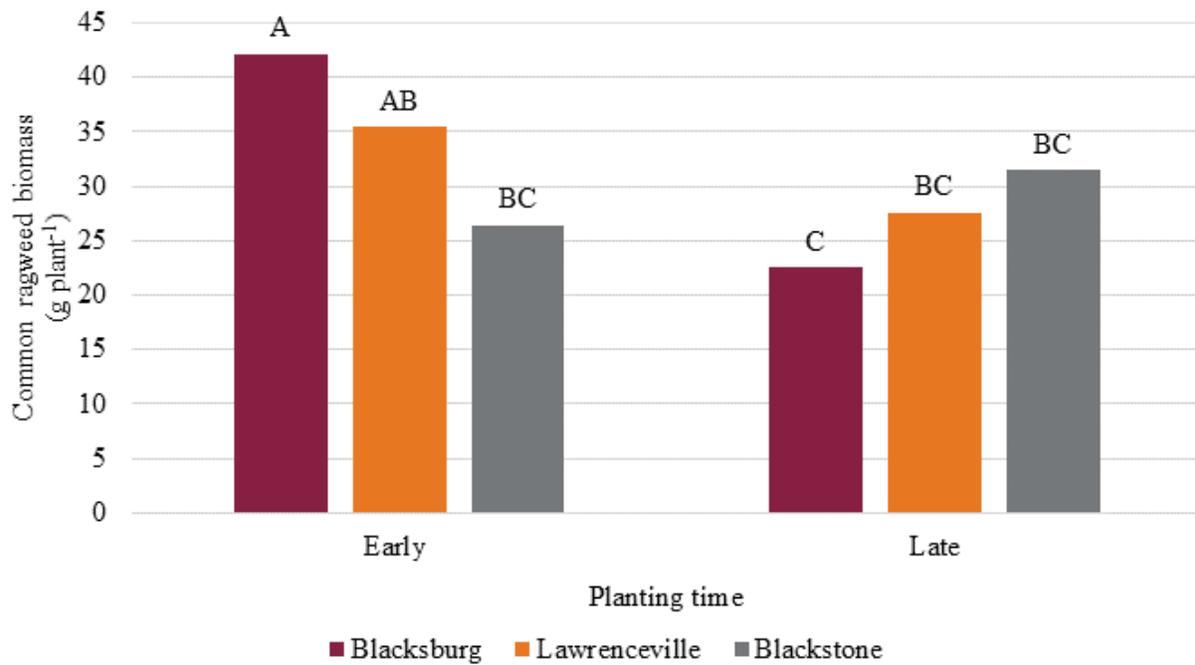


Figure 13. Common ragweed biomass at soybean harvest by location and planting time for the first year of the field experiment. Means are different when they do not share a letter according to Fisher's Protected LSD ($P=0.05$).

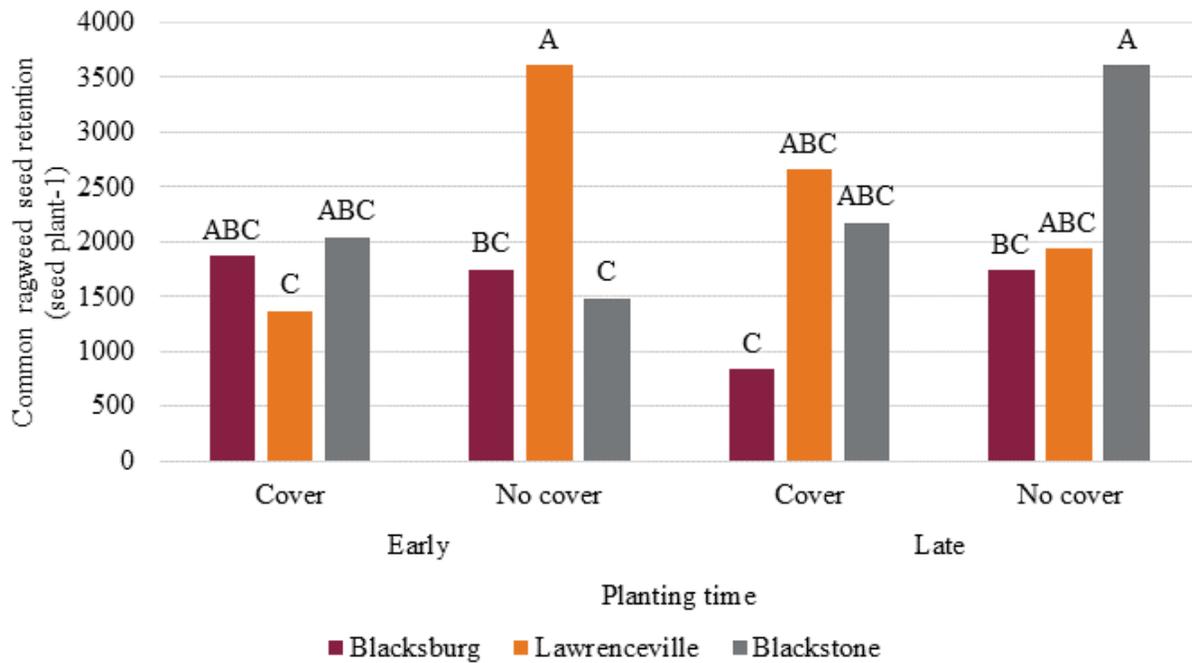


Figure 14. Common ragweed seed retention at soybean harvest by location, planting time, and cover (cereal rye for early planting and winter wheat for late planting) for the first year of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD ($p=0.05$).

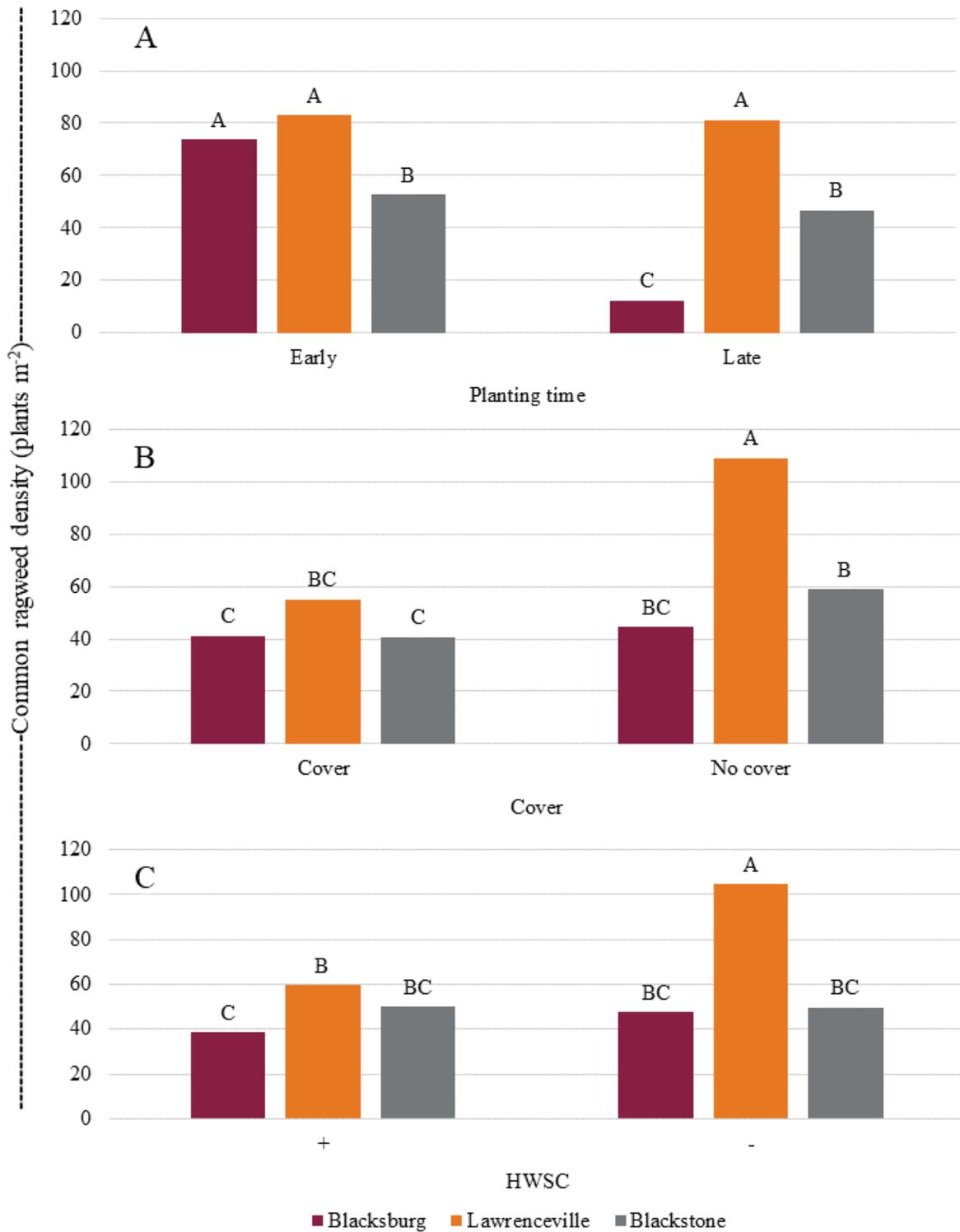


Figure 15. Common ragweed density at preplant herbicide application in year 2 of the experiment A) by location and planting time B) by location and \pm winter cover and C) by

location and \pm harvest weed seed control (HWSC) prior to preplant herbicide application. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD ($P=0.05$).

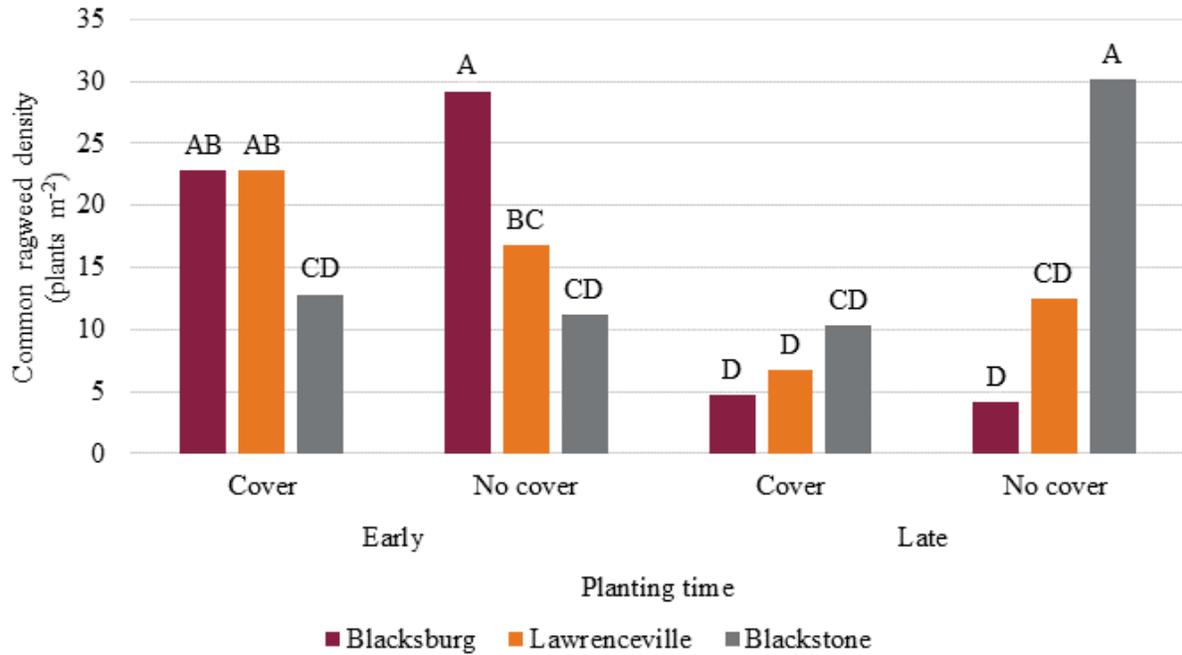


Figure 16. Common ragweed density at POST herbicide application by location, planting time, and \pm winter cover (cereal rye for early planting and winter wheat for late planting) in year two of the experiment. POST herbicide applications were made when common ragweed reached 30 cm in height. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD ($P=0.05$).

Phenology of Palmer amaranth (*Amaranthus palmeri*) populations from the eastern United States

Palmer amaranth is a highly adaptive weed species that retains large proportions of its seed at crop harvest. Widespread adoption of harvest weed seed control could place selection pressure on weed species to adapt (e.g. earlier seed shattering) to this tactic. Since no information is available, a common garden experiment was conducted in Blacksburg, Virginia in 2018 to assess the differences among Palmer amaranth populations in flowering time, seed shatter phenology, and other phenotypic traits. Palmer amaranth seeds were collected from 30 crop fields from central Florida to southern Pennsylvania in 2017, to capture populations that had differences in flowering time and presumably seed shatter based on being adapted to particular latitudes. Maternal population collection latitude had no effect on seed shatter date and on the time from first flower to first seed shatter, which averaged 34.2 d. Both maternal latitude and daylength/emergence date influenced flowering date. In female plants, time to first flower was reduced 0.53 d for every degree of latitude further north the maternal population was collected from. The biggest driver of flowering time in Palmer amaranth was daylength. As plants emerged later in the season, the time to flower was reduced by 0.31 and 0.24 d for each day emergence was delayed for female and male plants respectively. Time from first flower and emergence to first seed shatter was reduced by 0.48 and 0.17 d, respectively, for each day emergence was delayed. Since latitude of maternal population only influenced time from emergence to first flower, this suggests that genetic variability exists and plays a role for this duration, making it a heritable trait that can adapt to HWSC. Other factors such as height at first flower, end of experiment height and biomass, terminal inflorescence length and diameter were

also evaluated. Further research is ongoing which will evaluate the underlying genetics among these populations that are likely driving these differences in phenology and phenotype. With selection pressure from HWSC, populations could shift to flower earlier and shed seed earlier in the season, thus avoiding the HWSC operation.

Nomenclature: Palmer amaranth, *Amaranthus palmeri* S. Watson

Keywords: Adaptation, flowering time, height, biomass, seed shatter, inflorescence length, inflorescence diameter.

Weeds can adapt to many different control measures. When a selection pressure is placed on a weed species, the adaptive traits required to survive become more prevalent within the population (Barrett 1983; Norsworthy et al. 2012; Reddy and Norsworthy 2010). One example of this adaptation is mimicry in barnyardgrass [*Echinichloa crus-galli* (L.) P. Beauv. var. *oryzicola* (Vasinger) Ohwi]; this species has adapted to closely mimic cultivated rice to evade hand removal (Barrett 1983.). Another example is herbicide resistance now observed in many weeds (Heap 2019).

Palmer amaranth (*Amaranthus palmeri* S. Watson) is a prime example of a weed species with adaptive traits. It has been observed to develop resistance to multiple herbicide modes of action and has become endemic across a large geographic range (Ward et al. 2013). Palmer amaranth has been found in 39 states across the US (Kniss 2018). Herbicide resistance has been reported to 8 individual sites of action (SOA) and some populations have been reported to be resistant to up to 5 SOA. Herbicide resistant populations are widespread and have been documented in 29 states (Heap 2019). For these reasons, Palmer amaranth is ranked as one of the most troublesome and common weeds in corn, cotton, and soybeans (Van Wychen 2016, 2017).

Palmer amaranth is successful at overcoming herbicidal controls in part due to dioecious nature and prolific fecundity. Palmer amaranth is dioecious and as such, is an obligate outcrosser (Franssen et al. 2001; Sauer 1957). Pollen of Palmer amaranth is spread by wind which helps maintain genetic diversity with populations as well as transfer resistance and other adaptive traits across the agricultural landscape. Sosnoskie et al. (2012) reported that glyphosate resistance was transferred via pollen of resistant male to susceptible females up to 300 m from the pollen source. It has been predicted that based on the aerodynamics of pollen grains of

Palmer amaranth, that pollen could potentially move up to 46 km. However, Palmer amaranth pollen loses viability after 240 min of being shed (Sosnoskie et al. 2007). Palmer amaranth is a prolific seed producer with reports of up to 600,000 seed per female plant (Jha et al. 2008; Keeley et al. 1987; Massinga et al. 2001; Sellers et al. 1983).

These traits also make Palmer amaranth able to quickly adapt to other control measures. Hand hoeing and hand removal of weeds can lead to plants being cut or broken off at different heights above the soil line. Sosnoskie et al. (2014) reported that Palmer amaranth plants that were cut at different heights above the soil line were able to regrow and produce seed. Plants cut at 0, 3, and 15 cm above the soil line had 95, 64, and 35% mortality, respectively, and produced 690, 27,560, and 116,420 seed plant⁻¹, respectively (Sosnoskie et al. 2014). This adaptive ability of Palmer amaranth, both to chemical and non-chemical methods of weed control, demonstrates that other methods of weed control could be ineffective with intensive selection pressure.

As new methods of weed control are developed, new selection pressures will be placed on weed species, which can lead to resistance. Harvest weed seed control (HWSC) is a nonchemical method of weed management that targets weed seeds retained on the plant at the time of crop harvest. Seeds shattered prior to harvest are not subjected to HWSC. There are several different HWSC techniques, including chaff removal, chaff lining, narrow windrow burning, among others, that have been developed in Australia in response to widespread herbicide resistance in weeds like rigid ryegrass (*Lolium rigidum* Gaud.) and wild radish (*Raphanus raphanistrum* L.) (Walsh et al. 2018). As herbicide resistance becomes a larger problem in the US, there is more interest in using HWSC as part of an integrated weed management system. However, if these techniques are widely implemented, a selection pressure will be placed on weeds. There are several conceivable ways a weed or weed population could

adapt to HWSC, including increased seed density, shift to weeds with perennial lifecycles, vegetative propagation, wind dispersed seed, prostrate growth habit, and others (Haring and Flessner 2018). Perhaps the most straightforward adaptation for a species is to shatter seed earlier in the growing season (Walsh et al. 2018). It has been demonstrated in wild radish that selecting for flowering time can greatly alter the time from emergence to first flower within a population. Ashworth et al. (2016) reported that by selecting the earliest and latest flowering individuals within a population over successive generations, flowering time was cut in half in five generations and doubled in three generations. These changes in flowering time lead to differences in phenotypes of wild radish and, while not directly measured, could have the potential to change when seed shatter during the growing season (Ashworth et al. 2016; Taghizadeh et al. 2012). Plants that flowered earlier had reduced height and biomass at maturity whereas the later flowering plants were taller and had greater biomass (Ashworth et al. 2016). Similar trends in flowering time have been reported in wild mustard in response to drought (Franke et al. 2006; Franks 2011; Franks et al. 2007). Selection pressure from HWSC could lead to phenological and phenotypic differences within plant populations that could be canalized to heritable genetic changes.

Palmer amaranth has been demonstrated to retain >95% of its seed at soybean harvest, making it an excellent candidate for HWSC (Schwartz et al. 2016). But at the same time, this seed retention level results in HWSC potentially placing an intense selection pressure on this species to shatter before crop harvest if HWSC were widely used. In *Amaranthus* spp. each seed is contained within a papery utricle. An abscission layer forms along the seam of the utricle and allows the seed to shatter from the inflorescence (Brenner 2002). It has been observed in populations of Powell's amaranth (*Amaranthus powellii* S. Watson) that the utricle is non-

circumscissile, leading to lower levels of seed shatter in this species (Brenner 2002; Sauer 1967). Jain et al. (1986) described this trait using Dh and dh to describe a dominant circumscissile and recessive non-circumscissile allele, respectively, in *Amaranthus hypochondriacus*. HWSC could act on populations of Palmer amaranth to select for the dominant circumscissile allele and result in earlier seed shatter.

Flowering time is also known to vary between populations of a species based on a latitudinal gradient. It has also been found that common ragweed populations from more northern latitudes (44.48° N) flower approximately 10 d sooner than common ragweed populations from more southern latitudes (40.71° N) (Stinson et al. 2016). This natural variability in flowering time demonstrates that there is the potential for selection of genotypes that flower earlier. It is conceivable that earlier flowering could result in earlier seed shattering in weed species, however, this has not been observed in response to a particular management tactic/selection pressure (Taghizadeh et al. 2012).

The objective of this research was to assess differences in Palmer amaranth phenology and phenotype in order to gain a better understanding of its potential to adapt and overcome HWSC.

Materials and Methods

Palmer amaranth seed collection. Palmer amaranth seed were collected from populations along the eastern United States. Seed were harvested in late summer 2017 from crop fields in 30 locations ranging from central Florida to southern Pennsylvania (Table 14), a span of approximately 1,252 km. Populations were selected along a latitudinal gradient to capture differences in flowering time, and presumable timing of seed shatter, in these populations based

on local environmental conditions and daylengths. That is, we assumed Palmer amaranth was locally adapted and was genetically and phenotypically disposed to those conditions for our primary traits of interest, flowering time and seed shatter. At each harvest location, seed from up to 30 plants were collected (n=854). To harvest seed, the terminal inflorescence of each randomly selected maternal plant was removed and bagged individually. The inflorescences were allowed to air dry for 7 d at 22 C. The seed were then threshed and sieved to remove large debris and stored at 4° C until sowing.

Common garden. A common garden was established at the Glade Road Research Facility (37.234153, -80.436371) in Blacksburg, VA on May 29, 2018. The site had no prior history of Palmer amaranth infestation. The site was prepared by killing the existing sod with two applications of glyphosate (Roundup Powermax, Bayer CropScience, St. Louis, MO) at 1.26 kg ae ha⁻¹. The site was then disked and subsequently tilled to prepare an appropriate seed bed. This seed bed was allowed to stay fallow for approximately one month prior to planting. Prior to sowing Palmer amaranth seed, the area was sprayed with paraquat (Gramoxone SL 2.0, Syngenta Crop Protection, LLC, Greensboro, NC) at 280 g ai ha⁻¹ with 1% crop oil concentrate v v⁻¹ so that area was weed free at planting. The study was arranged as a completely randomized design. Approximately 100 Palmer amaranth seed from each maternal line were sown in each plot. Each maternal line was represented two times in the field, with the intent to have one male and one female plant from each maternal line. As the seeds germinated, the seedlings were thinned and one plant was left per plot. Plots measured 0.37 m², however, not all plots had Palmer amaranth emerge. The plots were maintained weed free by applying clethodim (SelectMAX, Valent U.S.A. Corporation, Walnut Creek, CA) at 136 g ai ha⁻¹ with 1% crop oil concentrate v v⁻¹ and hand weeding throughout the growing season.

Data collection and analysis. Phenotypic data were recorded for each plant in the garden. These data included date of emergence, date of first flower, date of first seed shatter, plant height at flowering, end-of-season height, end-of-season biomass, terminal inflorescence length and maximum diameter, and 100 seed weight. Date of first seed shatter was determined by placing mesh bags (white organza bags, S-12429, ULINE, Pleasant Prairie, WI) over the terminal inflorescence of each female plant within 2 wk of first flower and monitoring for loose seed. The bags were secured to the plant by tying it to the stem of the plant; care was taken not to girdle the inflorescence. Data were collected multiple times a week throughout the growing season and data collection ended (mid-October) when the plants were harvested for end of experiment height and biomass as the plants started to drop leaves due to the first frost.

Data were analyzed in JMP Pro 14 (SAS Institute Inc., Cary, NC). Due to the dioecious nature of Palmer amaranth, all data analyses were split by plant sex. Data were subjected to ANOVA followed by linear regression to determine if latitude of maternal population and emergence date and resulting daylength was significant for time of emergence to first flower, first flower to first seed shatter, emergence to first seed shatter, height at first flower, end-of-season height, end-of-season aboveground biomass, inflorescence length, inflorescence diameter, and 100 seed weight.

Results and Discussion

Time to First Flower. No significant interactions between maternal population latitude and emergence date/daylength were observed for any analysis. However, all analyses were split by either latitude or emergence date/daylength due to the objective of determining if maternal population alone explained differences between Palmer amaranth populations. Palmer amaranth

emergence was prolonged as is commonly observed with this species (Keeley et al. 1987). Emergence began on June 2 (Julian day 153) and continued throughout the summer. Tracking of emergence stopped for plants that emerged after August 6 (Julian day 218), due to the changing environmental factors (daylength, temperature, and rainfall). Plant emergence date did not correlate with latitude of the maternal plant, suggesting that genetic differences between populations were not driving emergence or that similar variability existed in all populations. Across all 30 populations, there was a significant but very weak trend ($p < 0.001$, $r^2 = 0.04$) that for every degree in latitude further north, approximately 111 km, the days from emergence to flower was reduced by 0.53 d for female plants (Figure 17a). No such trend was observed for male plants ($p = 0.941$). The average number of days from emergence to first flower was 34.9 d for female plants and 28.7 d for male plants (Table 15). These data suggest that genetic differences among populations play a role in time from emergence to first flower and if populations are moved could eventually adapt and reproduce in areas with growing seasons of different lengths.

No significant trends ($p = 0.1524$ and 0.0706) were observed for time from first flower to first seed shatter and from emergence to first seed shatter among female plants across all populations, which averaged 34.2 and 69 d, respectively. This suggests genetic differences among populations do not play a role in time from first flower to first seed shatter or from emergence to first seed shatter. It has been reported in other *Amaranthus* spp. that the latitude of the parental population can play a role in time to flower when grown under the same conditions (Iamónico 2010; McWilliams et al. 1966; Weaver and McWilliams 1980). Stinson et al. (2016) reported that common ragweed populations showed a difference in emergence to first flower of 10 d less in populations from 44.48° N compared to populations from 40.71° N, or approximately 418 km south.

Emergence date and daylength. The largest driver of Palmer amaranth flowering was daylength/emergence date as indicated by ANOVA. There was a significant trend for both female and male plants that the later the plants emerged the less time it took for the plants to flower ($p < 0.001$, $r^2 = 0.28$ and $p < 0.001$, $r^2 = 0.21$ for female and male plants, respectively). In female plants, for every day emergence was delayed the time to first flower was reduced by 0.31 d (Figure 17b). In male plants, for every day emergence was delayed time to first flower was reduced by 0.24 d (Figure 17c). Most *Amaranthus* spp. are short day plants and floral initiation begins more quickly when daylengths are less than 12 hr (Assad et al. 2017; Fuller 1949; Huang et al. 2000). However, Allard and Garner (1940) reported *Amaranthus* spp. were indeterminate in their flowering habits. In redroot pigweed (*Amaranthus retroflexus* L.), photoperiods of 8 to 12 hours had a similar response and the initiation of flowering began at 21.1 to 21.7 d after emergence (DAE). Photoperiods of 14 and 16 hr resulted in plants flowering in 25 and 53.1 d, respectively (Huang et al. 2000), which is similar to what was observed in the current study; that as daylengths got shorter the time to flowering was reduced.

While no trend was observed in the time from first flower to first seed shatter across latitudes of the maternal populations, there was a significant trend in time from emergence date to first seed shatter based on maternal population latitude ($p < 0.001$ and $r^2 = 0.12$). The average time from first flower to first seed shatter was 34.2 d (Table 15), and was reduced by 0.17 d for every day emergence was delayed (Figure 18a). A significant trend was observed from emergence to first seed shatter ($p < 0.001$ and $r^2 = 0.42$). Time to first seed shatter was reduced 0.48 d for every day emergence was delayed (Figure 2b). The average time from emergence to first seed shatter was 69 d. Schwartz-Lazaro et al. (2017) reported that seed began to shatter approximately 160 days after planting Palmer amaranth in competition with soybean. This time

is longer than the current study where time from emergence to first seed shatter ranged from 39 to 101 DAE. Huang et al. (2000) reported redroot pigweed photoperiod sensitivity was observed through the end of seed maturity. This finding is similar to the current study where daylength appears to have an influence on the timing of seed shatter in Palmer amaranth.

Other Characteristics. Similar to first flower, there was a significant yet weakly correlated trend ($p=0.033$ and $r^2=0.02$) in height of female Palmer amaranth at first flower as the populations from more northern latitudes were shorter, 1.4 cm for every degree of latitude further north (Figure 19). Average height at first flower was 96.8 cm for female plants and 75.7 cm for male plants (Table 15). There was no trend observed for height at first flower with male plants ($p=0.978$). Additionally, there was no significant trend between emergence date and height at first flower with female or male plants ($p=0.070$ and 0.406 , respectively). There were no differences in final Palmer amaranth height, taken at the end of the growing season when the plants were harvested, around mid-October, for female or male plants ($p=0.322$ and 0.289 , respectively) when comparing populations across latitude. However, end-of-season height by emergence date was significant for both female and male plants ($p<0.001$ and $r^2=0.1$, $p<0.001$ and $r^2=0.11$, respectively). For female plants there was a reduction in final height of 0.87 cm for every day emergence was delayed (Figure 20a), and for male plants a 0.94 cm reduction (Figure 20b). The average height at the conclusion of the experiment for female and male plants was 177.2 and 157.7 cm, respectively (Table 15).

There was a significant and weakly correlated trend of reduced end-of-season aboveground biomass with latitude ($p=0.005$ and $r^2=0.03$), a reduction of 24.1 g for every degree in latitude further north, in female Palmer amaranth plants (Data not shown). No trends were observed in male plants when comparing across latitude. When comparing across emergence

date, significant trends were observed with both female and male Palmer amaranth plants ($p < 0.001$ and $r^2 = 0.19$, $p < 0.001$ and $r^2 = 0.23$, respectively). Aboveground biomass was reduced by 14.4 g and 8.2 g for every day emergence was delayed in female and male plants, respectively (Figure 21). Average end-of-season aboveground biomass of female and male plants was 503.7 and 253.8 g, respectively (Table 15). Due to the longer daylengths earlier in the growing season, the plants take longer to initiate flowering allowing the weed to spend more time growing vegetatively, growing taller and accumulating more biomass (Costea et al. 2004; Huang et al. 2000). This finding is similar to the current study where plants were shorter at first flower and at the end of the season, and had lower aboveground biomass as they emerged later in the growing season.

No trends were observed in Palmer amaranth inflorescence length and diameter for female and male plants across latitude ($p = 0.322$, $p = 0.954$ and $p = 0.289$, $p = 0.384$, respectively). When comparing across emergence date, there were significant trends in inflorescence length and diameter ($p < 0.001$, $r^2 = 0.14$ and $p = 0.003$, $r^2 = 0.05$) for female Palmer amaranth plants. Female inflorescence length and diameter were reduced by 0.27 and 0.005 cm, respectively, for every day emergence was delayed (Figure 22a, 22b). In male plants, the same trends in inflorescence length and diameter ($p < 0.0001$, $r^2 = 0.32$ and $p < 0.0001$, $r^2 = 0.14$, respectively) were observed. In male Palmer amaranth plants, inflorescence length and diameter were reduced by 0.49 and 0.006 cm, respectively for every day emergence was delayed (Figure 22c, 22d).

Mass of 100 seeds was measured for every female plant grown. There were no trends observed in seed mass when comparing across latitude or the date of emergence ($p = 0.285$ and $p = 0.322$, respectively). The 100 seed weight (40 mg) was consistent across all the plants (Table

15). Costea et al. (2004) reported that in redroot pigweed seed weight was positively associated with latitude. However, this trend was not observed in the current study.

Research Implications. This study was the first to examine the relationship between flowering time and seed shatter. Flowering time is dependent on both emergence date/daylength and latitude of maternal population. The time from first flower to first seed shed is only dependent on emergence date/daylength, indicating that environment governs this duration and not genetic differences. Further research is ongoing which will evaluate the underlying genetics among these populations that are likely driving these differences in phenology and phenotype. With the known adaptive ability of Palmer amaranth, if HWSC is widely utilized, adaptation (i.e. earlier seed shattering) could occur. As the push for IWM systems continues to grow, it will be important to steward these technologies so that they are not lost as valuable weed management tools.

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Table 14. Palmer amaranth collection locations, coordinates, and crop from which the plants were collected.

State	Coordinates		Plants collected	Crop
	Latitude	Longitude		
Florida	29.334515	-82.567988	30	Peanut
Florida	29.334903	-82.573790	30	Peanut
Florida	29.194124	-82.560098	30	Peanut
Florida	30.306509	-82.902295	7	Peanut
Georgia	31.361466	-83.553502	30	Cotton
Georgia	31.723343	-82.451983	26	Cotton
Georgia	32.143094	-82.347170	30	Cotton
Georgia	32.176958	-82.333334	25	Soybean
Georgia	32.145212	-82.294024	30	Cotton
South Carolina	32.903562	-80.851750	30	Corn
South Carolina	33.353080	-80.829504	30	Soybean
South Carolina	33.894825	-80.070074	30	Soybean
South Carolina	34.487417	-79.304893	30	Cotton
North Carolina	34.731485	-78.794342	30	Soybean
North Carolina	35.021660	-78.279916	30	Sweetpotato
North Carolina	35.322407	-77.984601	30	Soybean
North Carolina	35.806195	-77.829664	30	Cotton
North Carolina	36.310658	-77.590151	30	Soybean
Virginia	36.832135	-78.141909	29	Soybean
Virginia	36.985104	-77.245732	30	Soybean
Virginia	37.728189	-77.100729	30	Soybean
Virginia	37.901595	-76.519247	30	Soybean
Virginia	37.475449	-75.895362	29	Soybean
Maryland	38.156785	-75.690891	30	Soybean
Delaware	38.987342	-75.396225	30	Soybean
Delaware	38.505731	-75.516292	30	Soybean
Delaware	39.211578	-75.614714	30	Soybean
New Jersey	39.731220	-75.352108	30	Asparagus
Pennsylvania	40.468161	-76.228775	18	Soybean
Pennsylvania	40.438500	-76.469139	30	Soybean

Table 15. Palmer amaranth phenotypic data mean (\pm standard error), median, and range for female and male plants in a common garden experiment in Blacksburg, VA 2018.^a

Parameter	Female				Male			
	Mean	Median	Range		Mean	Median	Range	
			minimum	maximum			minimum	maximum
Days from emergence to first flower	34.8 \pm 0.54	35	15	63	28.7 \pm 0.49	26	6	53
Days from first flower to first seed shatter	34.2 \pm 0.47	35	9	58	--	--	--	--
Days from emergence to first seed shatter	69 \pm 0.69	68	39	101	--	--	--	--
Height at first flower (cm)	96.8 \pm 2.41	91.4	25.4	213.4	75.7 \pm 2.13	71.1	8.9	190.5
End-of-season height (cm)	177.2 \pm 2.55	182.9	71.1	317.5	157.7 \pm 2.74	165.1	71.1	254
End-of-season aboveground biomass (g)	503.7 \pm 30.46	350.7	2.8	2,823	253.8 \pm 16.86	171	3.6	1,345
Inflorescence length (cm)	28.8 \pm 0.67	27.3	7.6	76.2	31.6 \pm 0.84	29.2	5.1	71.1
Inflorescence diameter (cm)	1.4 \pm 0.02	1.3	0.8	3.2	1.1 \pm 0.02	1.1	0.48	1.9
100 seed weight (mg)	40 \pm 0.5	39	22	60	--	--	--	--

^a --, no data

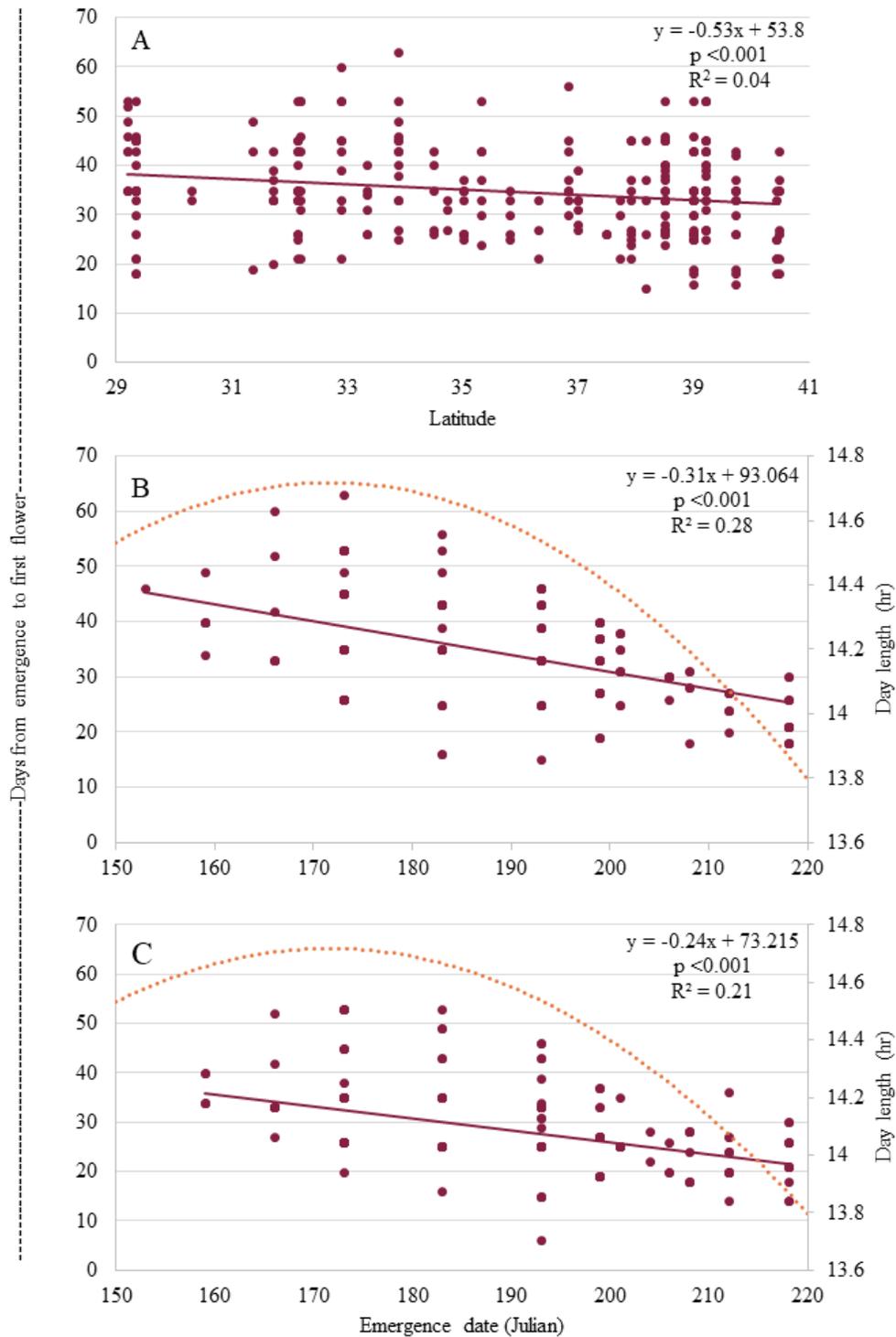


Figure 17. Palmer amaranth days from emergence to first flower A) female plants based on latitude of maternal populations, B) female plants based on emergence date, C) male plants based on emergence date in a common garden experiment in Blacksburg, VA 2018. Points are

observations and solid maroon lines are linear regressions. Plotted on the secondary axis is daylength across the range of emergence dates (dotted orange line).

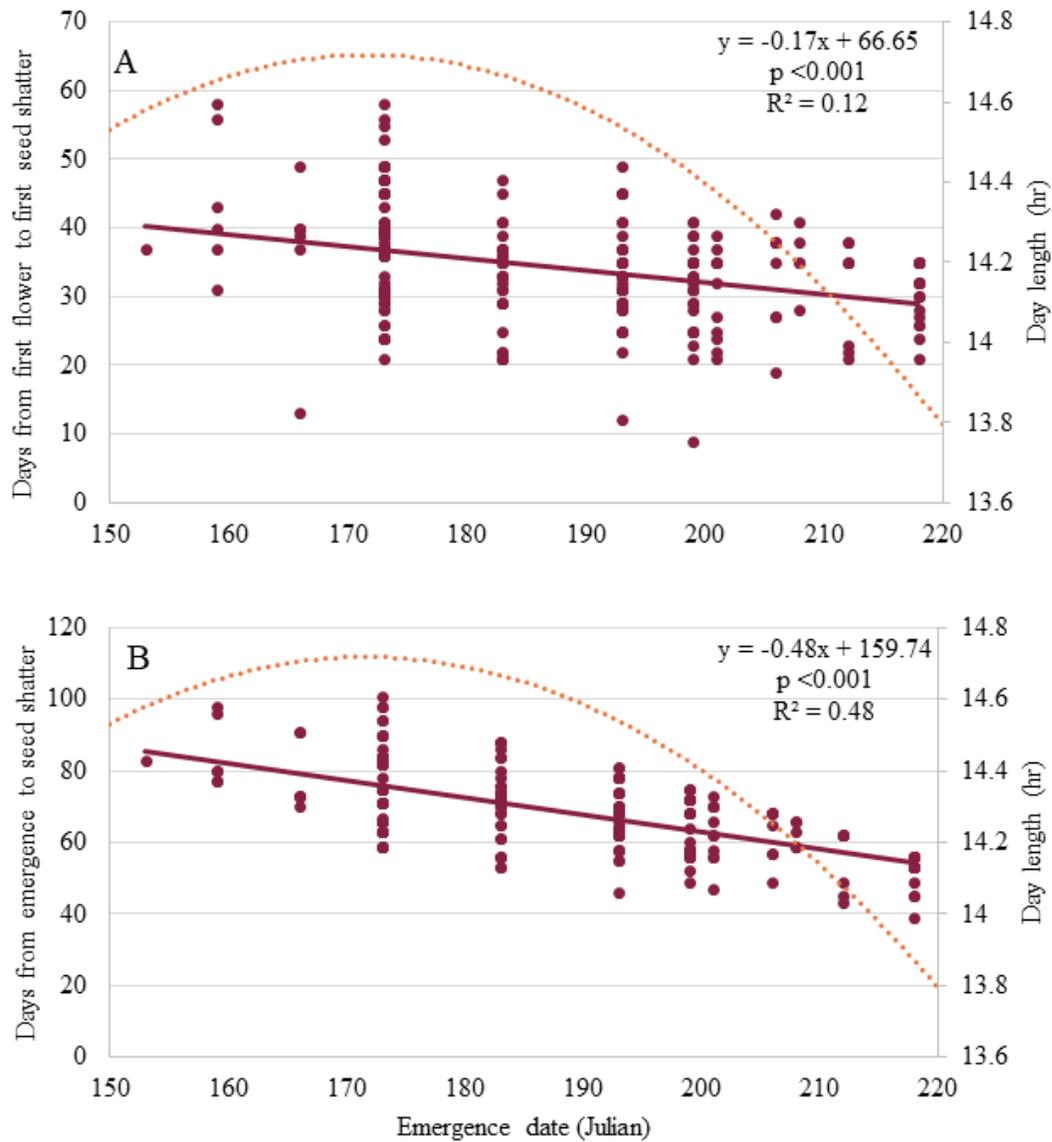


Figure 18. Palmer amaranth emergence date influence on, A) days from first flower to first seed shatter and B) days from emergence to first seed shatter in a common garden experiment in Blacksburg, VA 2018. Points are observations and solid maroon lines are linear regressions. Plotted on the secondary axis is daylength across the range of emergence dates (dotted orange line).

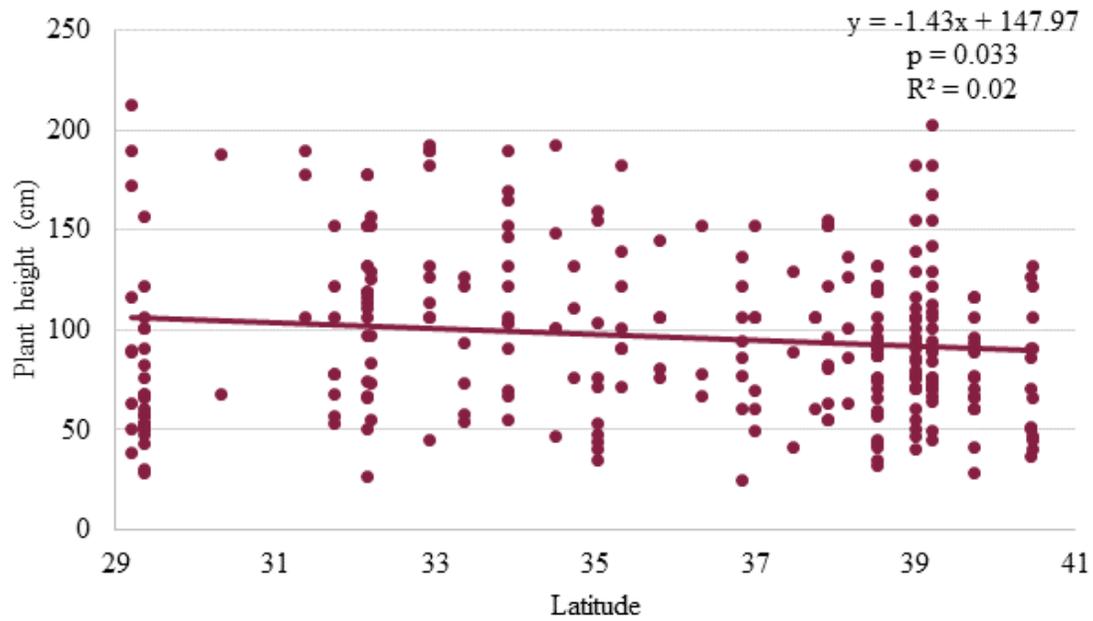


Figure 19. Female Palmer amaranth height at first flower plotted against latitude of the maternal population in a common garden experiment in Blacksburg, VA 2018. Points are observations and the solid maroon line is the linear regression.

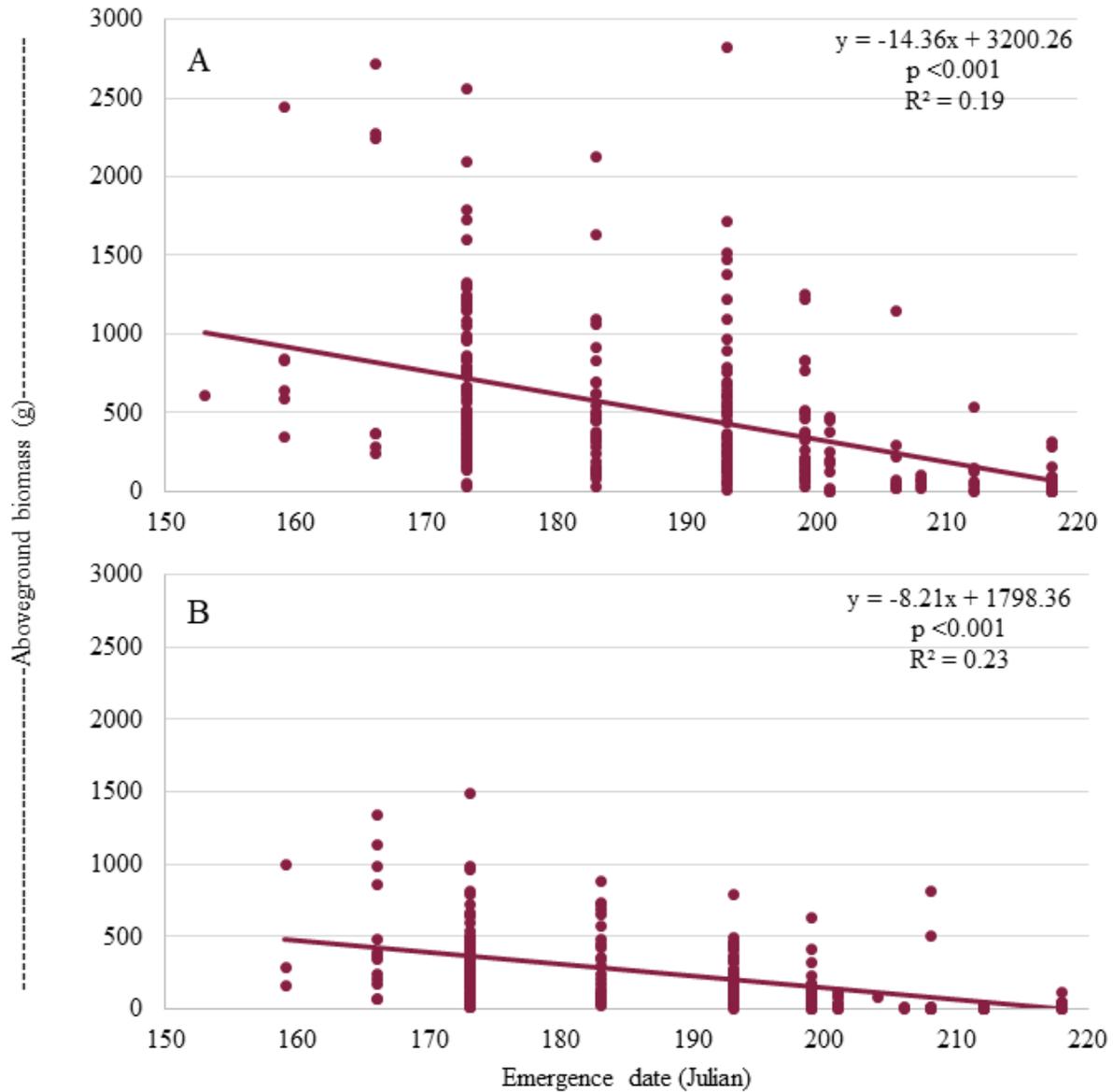


Figure 20. Relationship between end-of-season Palmer amaranth plant height by emergence date, A) female plants, B) male plants in a common garden experiment in Blacksburg, VA 2018.

Points are observations and solid maroon lines are linear regressions.

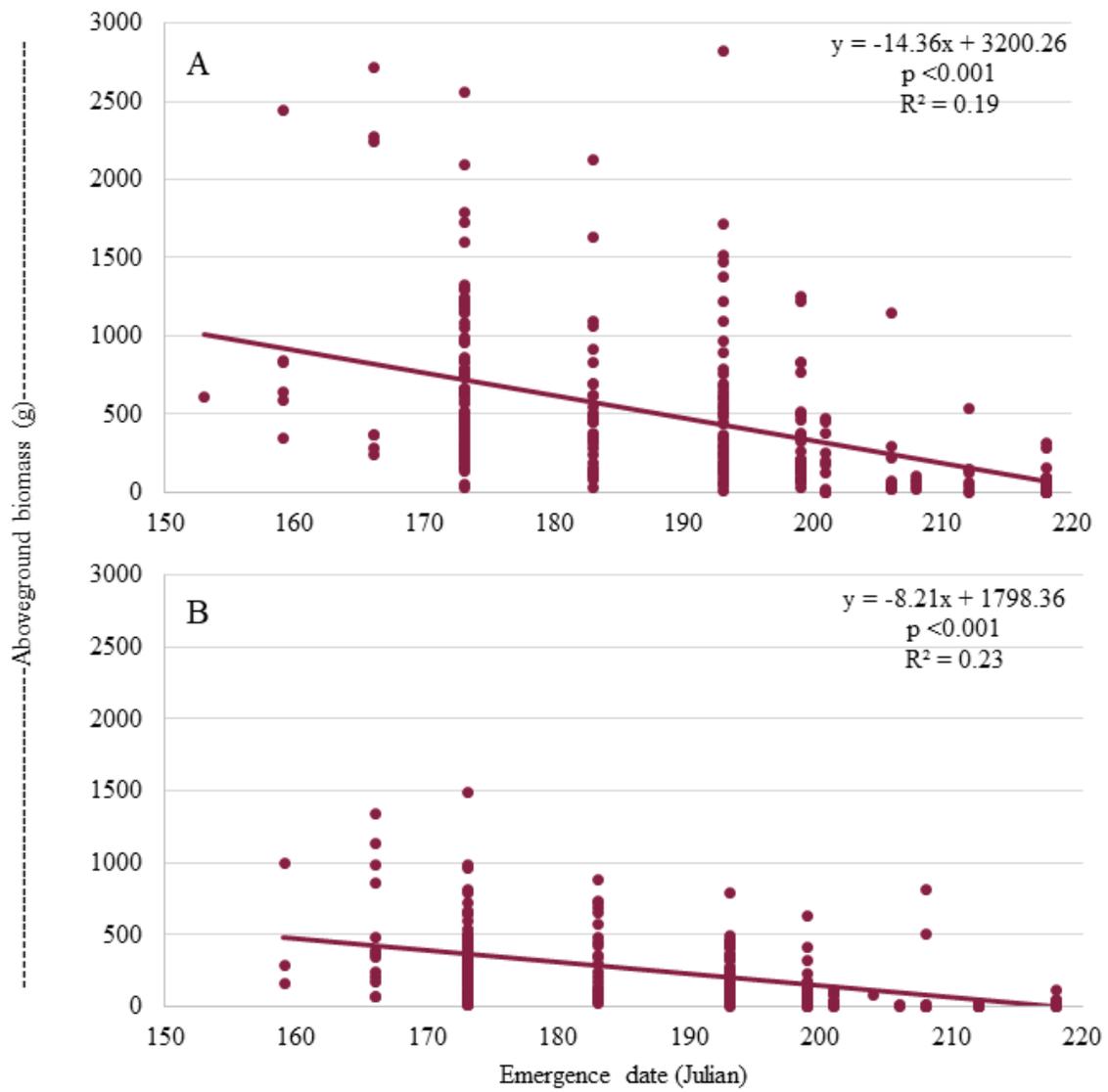
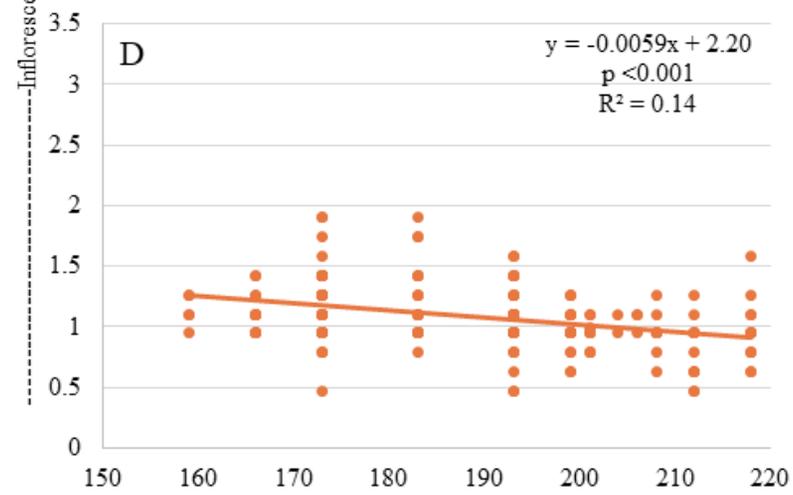
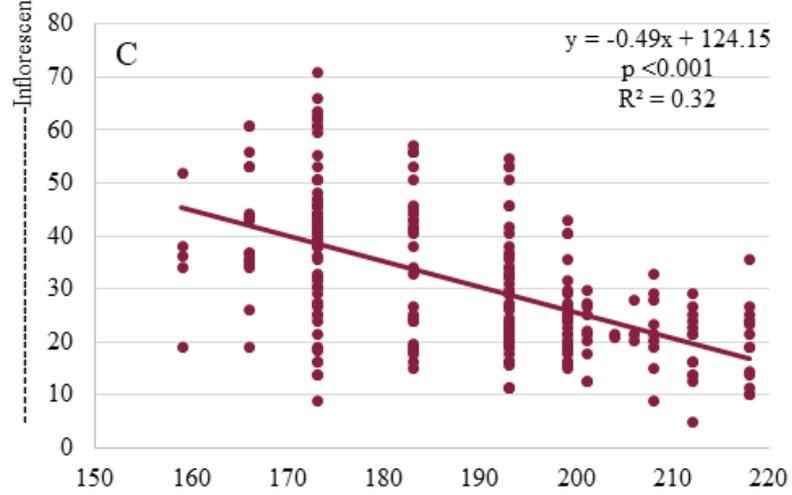
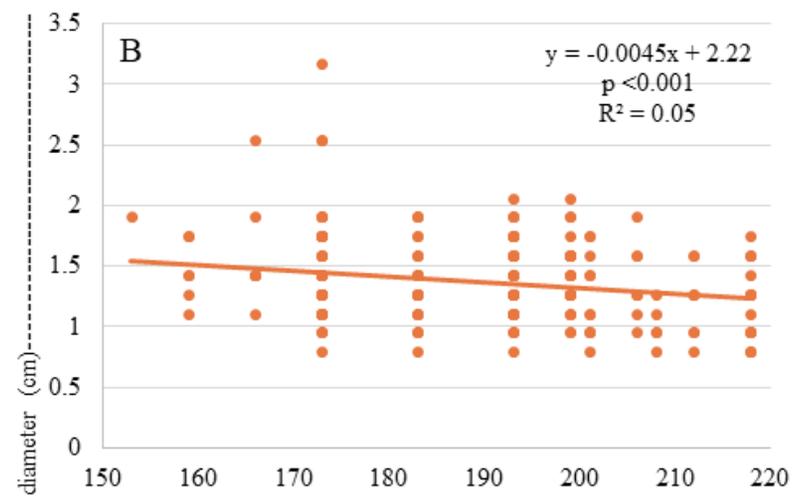
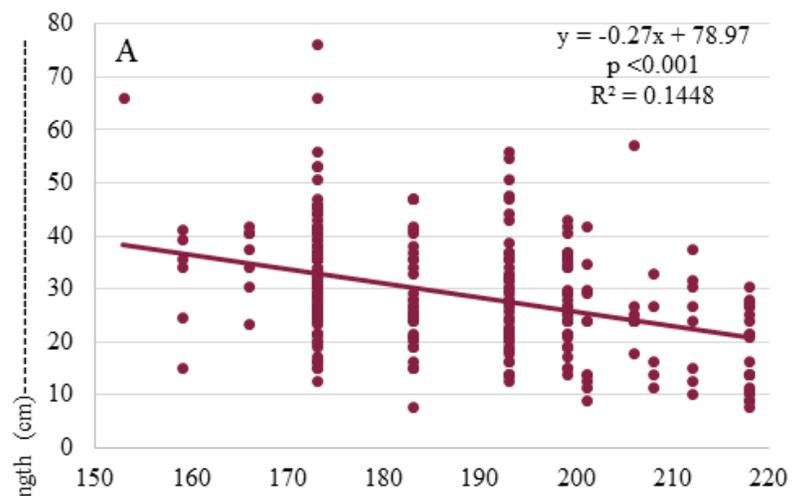


Figure 21. End-of-season Palmer amaranth aboveground biomass by emergence date, A) female plants, B) male plants in a common garden experiment in Blacksburg, VA 2018. Points are observations and solid maroon lines are linear regressions.



-----Date of emergence (Julian)-----

Figure 22. Palmer amaranth mature terminal inflorescence measurements by emergence date, A) female terminal inflorescence length, B) female plant terminal inflorescence diameter, C) male plant terminal inflorescence length, D) male plant terminal inflorescence diameter in a common garden experiment in Blacksburg, VA 2018. Points are observations and solid lines are linear regressions.