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Short title: Gene stacking: Sustainable?

Ecological, evolutionary, and management implications of sugarbeet cropping systems with three transgenic herbicide resistance traits

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Abstract

A three-way transgenic sugarbeet cultivar, engineered for resistance to glyphosate, glufosinate, and dicamba (referred to as '*triple-stacked*,' henceforth), is anticipated to be commercialized by mid-2020s. While offering potential benefits for growers facing glyphosate resistance, two of three herbicides (dicamba and glyphosate) to be utilized with *triple-stacked* sugarbeet (*Beta vulgaris* L.) have previously been used on major weeds in western United States cropping systems, raising concerns about pre-existing resistance to these active ingredients. We conducted a field survey in sugarbeet-growing counties of South-East Montana and North-West Wyoming in fall 2021, prior to the sugarbeet harvest. We collected kochia [*Bassia scoparia* (L.) A. J. Scott], redroot pigweed (*Amaranthus retroflexus* L.), and common lambsquarters (*Chenopodium album* L.) populations and screened them for glyphosate, glufosinate, and dicamba resistance in greenhouse conditions. Our results showed two-way resistance (glyphosate and dicamba) in 32% of *B. scoparia* populations and 78% populations of *C. album* with reduced susceptibility to glyphosate. Additionally, we conducted a greenhouse experiment to assess the emergence patterns of collected populations. Phylogenetically closely related *B. scoparia* and *C. album* showed higher resemblance in emergence pattern than distant relative *A. retroflexus*. While the majority of *B. scoparia* and *C. album* populations emerged in <20 day(s) (d) [time required to reach 90% emergence (E_{90}) < 20 d], *A. retroflexus* populations required >30 d to reach E_{90}. Widespread glyphosate and dicamba resistance in *B. scoparia* populations raises concerns about the long-term feasibility of a *triple-stacked* sugarbeet cultivar. Furthermore, the delayed emergence of *A. retroflexus* may enable it to evade early-season weed management.

Keywords: *Amaranthus retroflexus, Bassia scoparia, Chenopodium album*, dicamba, emergence dynamics, glyphosate, glufosinate, phylogenetic niche conservatism

Introduction

Genetically modified herbicide-resistant crop cultivars have been widely adopted shortly after commercialization, covering 71.5 million ha in the United States alone (Brunharo et al. 2022). These crop cultivars facilitated consistent and improved weed management, enhanced crop safety, and promoted no-till farming for increased soil and water conservation (Duke 2015; Green 2012). However, the widespread adoption of herbicide-resistant cultivars has led to a reduction in the diversity of herbicide usage and other non-chemical weed management tactics (Beckie and Hall 2014; Brunharo et al. 2022; Duke 2012). As a consequence, weed management protocols have become monotonous, relying on a single or a few sites of action (Brunharo et al. 2022; Mortensen et al. 2024). Repeated applications of the same site of action exert selective pressure on weed populations and ultimately result in the selection of herbicide-resistant biotypes (Heap 2014; Mortensen et al. 2024; Vencill et al. 2012), including many cases of multiple herbicide resistance (Bagavathiannan and Davis 2018; Menalled et al. 2016). For example, worldwide, more than 534 unique cases of herbicide resistance have been identified across 273 weed species, with more than 100 of those species showing multiple herbicide resistance (Heap 2025).

Presently, glyphosate-resistant crops in the United States encompass 95% of soybean [*Glycine max* (L.) Merr.], 70% of cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.), and 100% of sugarbeet (*Beta vulgaris* L.) cultivation (Beckie and Hall 2014; McGinnis et al. 2010). Within the Northern Great Plains, herbicide-resistant alfalfa (*Medicago sativa* L.), corn, canola, and sugarbeet are regularly cultivated. Among these crops, the most commonly prevalent trait is glyphosate resistance, colloquially referred to as ‘Roundup Ready’ (Dill 2005). Glyphosate-resistant sugarbeet was first commercialized in 2008 in the United States after being deregulated in 2005 (Morishita 2018). Before the commercialization of glyphosate-resistant sugarbeet, no effective herbicide without crop phytotoxicity was labeled for satisfactory weed management (Morishita 2018). Glyphosate usage has also increased approximately ten-fold in the past 15 years with the enhanced adoption of glyphosate-resistant sugarbeet and other crop cultivars (USGS 2023). As a result, there has been a notable upsurge in the selection of glyphosate-resistant weeds, increasing from two to 48 species in two decades (Heap 2025), thus threatening the sustainability of this technology.

Due to the widespread prevalence of glyphosate-resistant weed populations, there has been a significant push toward developing two- or three-way herbicide-resistant crop cultivars using gene-stacking techniques (Thornby et al. 2018). In the short term, these genetically modified cultivars may enable the use of multiple herbicide sites of action, a practice previously avoided due to the risk of phytotoxicity (Gressel et al. 2017; James 2010; Manalil et al. 2015). However, while cultivars with stacked traits increase herbicide diversity, the propensity of weeds to evolve multiple herbicide resistance impedes their long-term utilization (Menalled et al. 2016). Therefore, the sustainable adoption of cultivars with stacked traits demands careful consideration, including the current herbicide resistance status in weeds, crop rotations, application of herbicide mixtures and labeled rates, and integration of non-chemical weed management approaches (Beckie and Hall 2014; Gressel et al. 2017). A three-way (glyphosate-, glufosinate-, and dicamba-resistant) transgenic sugarbeet cultivar (referred to as '*triple-stacked*,' henceforth) is expected to be commercialized by 2027. Two of the three herbicide (glyphosate and dicamba) sites of action have been extensively used for decades and account for more than half of the overall herbicide market in the United States (Beckie and Hall 2014; Duke 2012). Hence, the current herbicide selection pressure, coupled with the presence of multiple herbicide-resistant biotypes, raises questions about the utility of these cultivars even before their release (Mortensen et al. 2024).

A cross-pollinated kochia [*Bassia scoparia* (L.) A. J. Scott] and two self-pollinated [common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.)] weed species are often seen to survive early season weed management (e.g., herbicide and pre-plant tillage) in Northern Great Plains. Besides herbicide resistance, intraspecific shifts and interspecific differences in emergence could play a significant role in enabling weed species to escape or survive early season weed management, potentially determining the success or failure of *triple-stacked* sugarbeet. As an evolutionary trait maintained through stabilizing selection, weed emergence may be phylogenetically conserved, influencing long-term management decisions. For example, previous studies have documented bet-hedging adaptation in weed emergence patterns due to constant selective pressure to avoid local extinction (Davis et al. 2013; Schutte et al.

2012). Considering the economic significance of sugarbeet cultivation (Soltani et al. 2018), it is crucial to jointly evaluate the current herbicide resistance status and the shifts in emergence patterns of these weed species before adopting *triple-stacked* sugarbeet.

To assess the feasibility of a *triple-stacked* cultivar, we conducted two complementary field and greenhouse studies. First, we performed a late-season survey of the spread and abundance of herbicide resistance in three dominant species: *B. scoparia*, *A. retroflexus,* and *C. album* across sugarbeet-growing counties of South-Eastern (SE) Montana and North-Western (NW) Wyoming. Second, we complemented our survey with an evaluation of the emergence patterns of the target weed species. These two studies allowed us to (1) quantify the occurrence of glyphosate, glufosinate, and dicamba resistance in *B. scoparia*, *A. retroflexus*, and *C. album* populations in SE Montana and NW Wyoming and (2) evaluate the underlying attributes enabling these species to evade early-season management. Our research questions were (1) How do the frequency and spread of herbicide resistance differ between tested weed species? (2) Is there any correlation between phylogenetic distance and the emergence pattern of tested weed species? and (3) Which evolutionary adaptation could contribute to the escape of tested weed species from early-season management in sugarbeet? Based on these questions, we hypothesized that (1) the frequency and spread of herbicide resistance differ between self-pollinated and cross-pollinated weed species, (2) weed emergence pattern is a phenological trait that phylogenetically closely related species have conserved during the evolutionary process, and (3) the escape of weed species from early-season management in sugarbeet could be attributed to the dual evolutionary adaptations of herbicide resistance plus delayed and/or extended emergence.

Materials and Methods

Field survey

A field survey was conducted before the sugarbeet harvest in SE Montana (Big Horn, Carbon, Custer, Rosebud, Stillwater, Treasure, and Yellowstone counties) and NW Wyoming in August 2021 (Big Horn, Hot Springs, Park, and Washakie counties) (Figure 1A). Prior to the survey, the coordinates of sugarbeet fields were acquired from local agronomists and loaded into a handheld GPS device (Garmin GPSmap® 76C, Olathe, KS, United States). The northernmost sugarbeet field (46.299637, -107.226228) was visited first, and weed populations were collected from adjacent sugarbeet fields every 8 km. If no sugarbeet field was found at the 8 km mark, weed populations were collected from the nearest sugarbeet field. The agronomists also indicated fields with suspected herbicide resistance; therefore, weed populations were collected from these fields, even if they did not fall in the survey design. This systematic collection approach enabled us to sample across the survey area, capturing a representative distribution of weed populations without excessive sampling. Based on the infestation level, the occurrence of weed populations was visually classified into four different categories within each field: (A) weed-free (i.e., no visible weeds), (B) isolated (i.e., singular plant in 5 m diameter), (C) clustered (i.e., multiple plants in 5 m diameter), and (D) widespread (i.e., plants were spread across the field) (Figure 2).

Seed collection and storage

The seeds of three major weed species, *B. scoparia*, *A. retroflexus*, and *C. album*, and three minor species, wild oats (*Avena fatua* L.), green foxtail [*Setaria viridis* (L.) P. Beauv.], and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], were collected by detaching inflorescence with a pruning shear and placing them in paper bags. Major and minor species were classified based on their abundance and impact. For each weed species, seeds were collected from at least two to 30 individuals within fields and along the field margins. Seeds collected from fields and margins were kept in separate bags. Seeds from each individual within the population were bulked to achieve a representative seed lot. All within-field collections were conducted leaving ≥50 m from field margins. While seeds were collected by walking in a zig-zag pattern across the sugarbeet fields for weed populations that showed a clustered and widespread dispersal, a targeted collection was used for isolated populations. Seed bags were kept in the hot air oven at 35 C for a week to prevent mold development due to excessive moisture. Mature seeds were threshed, cleaned, and stored in paper bags at 15-17 C in dark conditions for ~1-2 years at the greenhouse facility of Southern Agricultural Research Center (SARC), Huntley, MT, until use.

Glyphosate, glufosinate, and dicamba screening

To answer our first question, all herbicide screenings were conducted in a greenhouse at SARC, Huntley, MT, from July 2022 to August 2023. Seeds of *B. scoparia*, *A. retroflexus*, *C. album*, *A. fatua*, *S. viridis*, and *E. crus-galli* populations were individually sown on the surface of thermoformed trays (53 × 28 cm,1020 Heavy duty, CN-FLHD-X1, Greenhouse

Megastore, Danville, IL, USA) filled with potting mix (Pro-Mix BX™, Premier Brands, New York, USA). Seedlings of individual populations, upon emergence, were transplanted into plastic trays (53 × 28 cm) containing 32 sub-units, with one seedling per sub-unit and one species per tray. In addition, seeds of *B. scoparia*, *A. retroflexus,* and *C. album* susceptible to glyphosate, dicamba, and glufosinate were sown and transplanted following the method described above. The seedlings were irrigated daily and fertilized weekly [Miracle-Gro water-soluble fertilizer (24N:8P:16K), Scotts Miracle-Gro Products, Marysville, OH, USA]. Throughout the study, the greenhouse was maintained at 25/20 ± 2 C day/night and a 16/8 hour(s) (h) photoperiod. These conditions closely mimic the environmental characteristics of SE Montana and NW Wyoming during late May and early June, which is the usual timeframe for the first herbicide application.

Individual trays were treated with either glyphosate (Roundup PowerMax®, Bayer CropScience, St. Louis, MO), glufosinate (Liberty® 280 SL, BASF Corporation, Research Triangle Park, NC), or dicamba (Clash®, Nufarm American Inc., Alsip, IL) to assess herbicide resistance. Herbicides were uniformly applied to 5-10 cm tall seedlings using a stationary spray cabinet (Research Track Sprayer, De Vries Manufacturing, Hollandale, MN, USA), calibrated to deliver 187 L ha^{-1} through flat fan nozzles (TeeJet XR 8002VS, Spraying System, Wheaton, IL, USA). Given the documented existence of resistance (Hall et al. 2014; Kumar et al. 2014), glyphosate was applied at 1.3 kg ae ha^{-1} for *B. scoparia,* whereas a 0.8 kg ae ha^{-1} rate was used for *A. retroflexus* and *C. album*. Glufosinate at 0.6 kg ai ha^{-1} and dicamba at 0.6 kg ae ha^{-1} were applied to all dicot weed species. Monocot weed species were excluded from dicamba screening. Glyphosate applications were made at 0.9, 0.5, and 0.7 kg ae ha^{-1} for *A. fatua*, *S. viridis,* and *E. crus-galli*, respectively. Glufosinate was applied at 0.6 kg ai ha^{-1} for all monocot species. Ammonium sulfate (20 g L^{-1}) was added as an adjuvant to glyphosate and glufosinate, and a non-ionic surfactant (Induce®, BASF Corporation, 26 Davis Drive, Research Triangle Park) (0.25 % v/v) + ammonium sulfate (20 g L^{-1}) was added to dicamba. Plants were not watered for 12 h following herbicide applications to meet the required rainfast period.

Given the humidity-dependent nature of glufosinate (Coetzer et al. 2001), greenhouse relative humidity was elevated to 60-80% using an automated misting system (Homenote, Guangzhou, China) before spraying. Plants treated with glufosinate were maintained under elevated humidity conditions throughout the entire experimental period. Specifically, following a 12 h rainfast period, the greenhouse misting system was set to activate at 10-minute intervals to maintain >60% humidity throughout the study.

The number of surviving plants (i.e., with green tissue) per population was recorded and converted into percentages to represent survival rates at 28 days after glyphosate and glufosinate applications, and 35 days after dicamba application. The populations were subsequently classified into three different categories based on their survival percentages: (1) susceptible (0%), (2) developing resistance (1-20%), and (3) resistant (>20%) [scale modified from Owen et al. (2007)]. All herbicide screenings were repeated three times. The screening protocol was collaboratively designed by our team following Simões Araujo et al. (2024).

Seedling emergence dynamics

To address our second and third questions, a greenhouse experiment was conducted at SARC from March to May 2023 in a randomized complete block design with four replications, each consisting of 13, 11, and 4 field and margin populations of *B. scoparia*, *A. retroflexus*, and *C. album*, respectively. The populations were selected based on the presence of field and margin pairs across the surveyed area. The greenhouse was maintained at 22/17 ± 2 C day/night temperatures and a 16/8 h photoperiod. These conditions closely mimic the environmental characteristics of SE Montana and NW Wyoming during late May and early June, which is the usual timeframe for the first herbicide application.

Twenty-five seeds of each population were manually counted and stored in paper envelopes. Thermoformed trays (53 × 28 cm,1020 Heavy duty, CN-FLHD-X1, Greenhouse Megastore, Danville, IL, USA) with eight sub-units were filled with the same potting mix used in herbicide screening. Each sub-unit represented one replication, and each tray contained eight different populations. Twenty-five seeds per population were uniformly placed on the surface of a sub-unit filled with the potting mix and slightly incorporated. Seeds were not buried into the potting mix due to the surface-emerging nature of all tested species.

Seedling emergence counts were recorded daily for 40 day(s), and emerged seedlings were removed using forceps. Seedlings were classified as ‘emerged’ when their plumules were visible. Daily emergence counts were aggregated to derive cumulative emergence counts, from which the cumulative emergence percentage (CE%) was calculated (Equation 1).

$$\text{CE\%} = \left(\frac{\sum_{i=1}^{t} E_i}{N}\right) \times 100 \ (1)$$

where 'CE%' is the cumulative emergence percentage. '$\sum_{i=1}^{t} E_i$' is the sum of the emerged seedling(s) from day 1 to day t. 'N' represents the total number of seeds per experimental unit (i.e., 25) at the start of the experiment. The second run was repeated 20 days after the completion of the first run. Each run was concluded upon the emergence of all seedlings (i.e., 100% emergence).

Statistical analysis

The data were analyzed in R Studio (v. 4.2.1., R Development Core Team). For herbicide screening, survival (%) data were plotted against the populations of three major weed species. A three-parameter log-logistic regression (Equation 2) was fitted to model the cumulative emergence using the 'drm' function from the 'drc' package (Knezevic et al. 2007). No violations, including monotonicity and homoscedasticity, were observed in diagnostic plots; therefore, non-transformed data were used for analysis. Data were pooled across two runs, as no significant difference was observed between them. Species differed in their emergence response; therefore, the model was computed separately for each species to capture the unique characteristics of their emergence patterns. In these models, the response, predictor, and categorical variables were CE%, days after sowing (DAS), and population, respectively.

$$Y = \frac{d}{1 + exp[b(\log x - log \mathrm{E}_{50})]} \ (2)$$

where 'Y,' 'x,' 'b,' and 'E_{50}' represent CE%, DAS, the slope of the regression curve (rate of emergence), and 'x' value (DAS) required to reach 50% emergence, respectively. A smaller 'b' value indicates a slow emergence rate. The upper limit 'd' was fixed at '100' because the emergence of all populations was 100%. Additionally, the DAS required for 10% and 90% emergence (E_{10} and E_{90}, respectively) were quantified with a 95% confidence interval using the 'ED' function of the 'drc' package. The Akaike Information Criterion (AIC) was used for model selection. A lack-of-fit test ($P>0.05$) was performed using the 'modelFit' function to determine whether the selected model adequately described the emergence response. The parameters estimated by the three-parameter log-logistic model were compared based on an approximate *t*-test using the ‘CompParm’ and ‘EDcomp’ functions.

To assess our second question, the relationship between phylogenetic distance and emergence patterns of *A. retroflexus*, *B. scoparia*, and *C. album*, a phylogenetic tree was generated using the ‘V.PhyloMaker2’, a package that uses three databases (i.e., TPL, LCVP, and WP) to create large phylogenies for vascular plants (Jin and Qian 2022). In conjunction, the ‘cophenetic.phylo’ function in the ‘ape’ package was used to compute pairwise interspecific phylogenetic distance (referred to as ‘*InterspecificPD*,’ henceforth) based on the sum of branch lengths of the shortest path. E_{10}, E_{50}, and E_{90} data of the three tested weed species were subjected to a one-way analysis of variance (ANOVA) using the ‘aov’ function. Mean emergence parameters were separated across the three weed species based on Fisher’s protected least significant different test ($\alpha = 0.05$). Results of one-way ANOVA and phylogenetic analysis were plotted adjacent to each other using the ‘phytools’ package.

Results and Discussion

Weed species richness and infestation pattern

A total of 72 sugarbeet fields were visited (Figure 1B), of which 25% were weed-free, 31% were infested by all three major weed species (*B. scoparia*, *A. retroflexus*, and *C. album*), while 25% and 7% had infestations of two and one of three major species, respectively (Figure 1B). Among the surveyed fields, 60%, 56%, and 38% were infested by *A. retroflexus*, *B. scoparia*, and *C. album*, respectively. In 4 fields (3%), the three other weed species, *S. viridis*, *E. crus-galli*, or *A. fatua*, were observed (Figure 1B).

For *B. scoparia*, 57.5% of the field populations were isolated, 17.5% were clustered, and 25% were widespread (Figure 1C). *Amaranthus retroflexus* field populations were present in isolated (37%) and clusters (30%) conditions, while 33% of populations were present in widespread (Figure 1D). For *C. album*, 63% of the field populations occurred in isolation, 26% in clusters, and 11% in widespread conditions (Figure 1E). These categories provide useful insights into the spatial distribution of weed species, which is crucial for designing effective management strategies. The observed differences in infestation levels between species hold broader ecological and management implications, discussed in ‘Management implications of triple-stacked sugarbeet systems’ section. The presence of these weed species before sugarbeet harvesting could be attributed to two different evolutionary adaptations: herbicide resistance and/or a temporal shift in emergence dynamics (Essman et al. 2021), as well as application conditions such as drought stress and/or herbicide application error.

Glyphosate, glufosinate, and dicamba resistance

Bassia scoparia

Out of the 16 populations found in field and margin pairs, 25% showed two-way resistance (glyphosate + dicamba), and 50% of pairs had one-way resistance to glyphosate (Figures 3A and 3B). Among 24 populations found only from fields, 46% and 42% of them displayed two-way resistance (glyphosate + dicamba) and one-way resistance to glyphosate, respectively (Figures 3A and 3B). Two-way resistance (glyphosate + dicamba) and one-way glyphosate resistance were observed in 17% and 33% of populations found only from margins, respectively.

Population K13 exhibited >20% survival after being sprayed with glyphosate and glufosinate (Figure 3A). To our knowledge, there is no documented evidence of glufosinate-resistant *B. scoparia*. Therefore, pending inheritance studies, dose-response, and molecular validation, this could be the first glufosinate-resistant *B. scoparia* case. Additionally, field populations K11, K44, K17, K20, and K23 demonstrated a low level of survival (3-9%) to glufosinate, suggesting that a few individuals in these populations may have resistance to glufosinate (Figure 3A), with further investigation of heritability and dose-response required.

The prevalence of glyphosate and dicamba resistance in margin populations, where herbicides are not usually applied, suggests pollen- and seed-mediated gene flow (Beckie et al. 2016; Jhala et al. 2021; Sarangi et al. 2017; Stallings et al. 1995). In *B. scoparia,* pollen-mediated gene flow can occur up to 100 m due to its highly cross-pollinated nature and protogynous flowers (Beckie et al. 2016). Also, seed-mediated gene flow can reach up to 1000 m in *B. scoparia,* attributed to its tumbling characteristic (Beckie et al. 2016). In addition, glyphosate-resistant populations K02 and K55 and two-way resistant (glyphosate and dicamba) populations K30 were found only from the margins (Figure 3B). Due to the existence of pollen- and seed-mediated gene flow of *B. scoparia*, herbicide-resistant traits present in margins plants could infest sugarbeet fields over time. These results highlight the importance of managing *B. scoparia* on field margins to minimize the spread of resistant biotypes.

In our study, while glyphosate resistance was observed in 93% of *B. scoparia* populations, 40% of populations exhibited resistance to dicamba (Figure 3A). These data indicate a rapid evolutionary ability as glyphosate and dicamba resistance were reported in ~30% and ~8% of *B. scoparia* populations, respectively, in a survey conducted across Colorado from 2012 to 2014 (Westra et al. 2019). To date, dicamba resistance has been reported in the United States and Canada in six different dicot weed species, including *B. scoparia* (Heap 2025). Although synthetic auxins such as dicamba have been utilized for >70 years, resistance to this herbicide class is not as widely documented as other modes of action (e.g., ALS, PSII, and EPSPS inhibitors). Relative to glyphosate, the limited distribution of dicamba resistance could be attributed to the associated fitness penalty. For example, in Montana, reduced germination and 39% lower fecundity were observed in the dicamba-resistant *B. scoparia* compared to the dicamba-susceptible population, suggesting a potential fitness penalty, which may have restricted the spread of dicamba resistance in SE Montana and NW Wyoming (Kumar and Jha 2016). However, *B. scoparia* does not necessarily express such fitness penalties in field situations (Menalled and Smith 2007), suggesting that other demographic mechanisms could also be conditioning the dynamics of dicamba resistance in the region.

Amaranthus retroflexus

Despite the occurrence of *A. retroflexus*, no populations were identified as resistant to glyphosate, glufosinate, and dicamba (Figure 4A). However, 27% of the 44 populations showed developing resistance (1-20% survival) to glyphosate (Figure 4A). Out of the total populations, 7% showed one-way (dicamba) and two-way (glyphosate and dicamba) developing resistance (Figure 4A). We did not detect herbicide resistance in margin populations (*n=16*) (data not shown).

Chenopodium album

In *C. album*, 78% of the 27 sampled populations showed reduced susceptibility to glyphosate (Figure 4B). However, glyphosate resistance in *C. album* has not been formally reported, but many populations across the United States showed inconsistent responses to glyphosate (Kniss et al. 2007; Owen 2008; Westhoven et al. 2008). No population survived glufosinate application. Population L14 showed developing resistance to dicamba, with 19% survival (Figure 4B). All margin populations (*n* = 4) were susceptible to glyphosate, glufosinate, and dicamba, except for L03, which displayed dicamba resistance (>35%) (data not shown). *Chenopodium album* predominantly undergoes autogamy (self-pollination), with a minimal propensity for allogamous (cross-pollination) events (Gasquez 1985; Holm et al. 1977). As a result, the pollen-mediated gene flow is restricted to only 15 m, and even within this range, the occurrence rate is 0.2% (Yerka et al. 2012). Therefore, in contrast to *B. scoparia*, all margin populations of *C. album* were glyphosate-susceptible despite many field populations surviving glyphosate application.

No herbicide resistance was recorded in *A. fatua* (*n* = 1), *E. crus-galli* (*n* = 2), and *S. viridis* (*n* = 3) populations (data not shown). For *B. scoparia*, which undergoes cross-pollination, a significant portion of populations collected from margins, as well as those collected in the fields, exhibited herbicide resistance. Conversely, for *C. album*, a self-pollinated species, reduced susceptibility to glyphosate was widely observed in field populations but notably absent in margin populations. In *A. retroflexus,* another self-pollinated species, herbicide resistance was not observed in margin populations, and most of the field populations were also found to be susceptible. Addressing our first research question and in accordance with our first hypothesis, our results suggest that the evolution and spread of herbicide resistance in self-pollinated and cross-pollinated species follow distinct patterns due to the differential gene flow rate.

Seedling emergence dynamics

The CE% response of *B. scoparia*, *A. retroflexus*, and *C. album* was adequately described by the three-parameter log-logistic model ($p > 0.05$) (Table 1). No consistent emergence response was observed among populations collected from fields and margins for all tested species. All *B. scoparia* and *C. album* populations reached 10% emergence (E_{10}) within 4-6 d (Table 1). In contrast, 4-22 d were required by *A. retroflexus* populations to reach 10% emergence (Table 1). Similarly, E_{50} values for *B. scoparia* and *C. album* populations ranged from 2 to 11 d, while *A. retroflexus* populations exhibited a wider range, requiring 8-32 d (Table 1). Except for K23-field, K32-field, and K47-margin *B. scoparia* populations, all populations reached E_{90} within 20 d. Similarly, *C. album* populations required <20 d to achieve E_{90}. In contrast, all *A. retroflexus* populations, except P56-field, took >25 d to reach E_{90}. These results suggest that a large proportion of *A. retroflexus* seedlings tend to emerge later compared to the other two weed species. The delayed and/or extended emergence response of *A. retroflexus* populations was also reflected by the smaller '*b*' values, ranging from -2.1 to -17.1. Similar emergence patterns were also documented in giant ragweed (*Ambrosia trifida* L.) populations growing under the selective pressure of repeated cultivation (Davis et al. 2013; Hovick et al. 2018; Schutte et al. 2008).

Based on the phylogenetic tree (Figure 5), the *InterspecificPD* between *B. scoparia* and *C. album* and *B. scoparia*/*C. album* and *A. retroflexus* are 80.5 and 87.3, respectively. These data suggest that *B. scoparia* and *C. album* are more closely related compared to *A. retroflexus*. In response to our second research question and in agreement with our second hypothesis, we detected a higher resemblance of emergence patterns between *B. scoparia* and *C. album* compared to *A. retroflexus* (Figure 5 and Table 1), showing evidence of phylogenetic niche conservatism (i.e., a positive relationship between phylogenetic distance and functional dissimilarity). Despite the positive relationship between the phylogenetic distance and dissimilarity in emergence patterns across three tested species, the phylogenetic niche conservatism hypothesis is not ubiquitous in trait and lineage and may not be universally justified (Crisp and Cook 2012; E-Vojtkó et al. 2023). Therefore, a comprehensive analysis exploring the relationship between various weed emergence (dis)similarities and phylogenetic relatedness would provide in-depth insights into weed management, plant systematics, and ecology.

Unlike *B. scoparia* and *C. album*, herbicide resistance was not as prevalent in *A. retroflexus* (Figure 4A). However, there was a late-season occurrence of 43 populations, potentially reflecting the delayed and extended emergence of the *A. retroflexus* populations. Additionally, the temperature increases in Montana and Wyoming in late June and early July favor the rapid emergence of *A. retroflexus*, given its ability to germinate at a higher rate at 35/30 C (Guo and Al-Khatib 2003). These findings address our third research question and support the third hypothesis, suggesting that a dual evolutionary adaptation—herbicide resistance and delayed/extended emergence—can enable weed species to escape early-season weed management in sugarbeet cropping systems.

Most of the *B. scoparia* populations reached E_{90} within 20 d, except K23-field, K32-field, and K47-margin, which required 31, 24, and 29 d, respectively (Table 1). However, they reached E_{10} within 4 d, indicating an extended emergence period. The smaller '*b*' values associated with the K23-field (-3.9), K32-field (-3.6), and K47-margin (-3.4) further support the extended emergence of these populations. Given the prevalent herbicide resistance observed (Figure 3) and the extended emergence of these *B. scoparia* populations, our results support the possibility of dual evolutionary adaptation. In *C. album*, no population exhibited a temporal shift in emergence (Table 1). Nevertheless, 78% of *C. album* populations showed reduced susceptibility to glyphosate (Figure 4B), explaining the late-season detection of 27 populations.

The delayed and extended emergence of *A. retroflexus* and the prolonged emergence period of some *B. scoparia* populations observed in this study could be a form of phenological adaptation (Délye et al. 2013; Kumar et al. 2018; Mortimer 1997; Recasens et al. 2005) that allows these weed species to evade the burndown herbicide applications, pre-planting tillage operations, and in-crop herbicide applications. Therefore, attention should also be given to late-season weed management strategies to manage and prevent seedbank replenishment from the late-emerging cohorts (Kumar and Jha 2015; Wilson and Sbatella 2011). Considering the short stature of sugarbeet, weed electrocution could be a potential non-chemical method for late-emerging and herbicide-resistant weeds (Schreier et al. 2022; Slaven et al. 2023). For example, in sugarbeet, Peters et al. (2020) reported 80% and 76% control of escaped waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] and *B. scoparia* using weed electrocution, respectively.

From an eco-evolutionary perspective (Neve et al. 2009), the extended emergence patterns of some *B. scoparia* populations could lead to sympatric speciation over time, a process by which a new species forms when barriers to gene flow, such as reproductive isolation, develop between groups of populations. Later-emerged *B. scoparia* seedlings may occupy niches at different times, indicating temporal niche divergence (Vaissi and Rezaei 2022), which could lead to reproductive isolation (Rundle and Nosil 2005). From a weed management perspective, the extended emergence pattern could potentially be exploited by adjusting the crop planting dates to promote size-asymmetric competition, i.e., the unequal distribution of resources among consumer species (Weiner 1990) that usually occurs due to initial size advantage and over-topping (Freckleton and Watkinson 2001). Early planting may enhance crop competitiveness as crops gain an initial size advantage and significantly suppress the growth of later-emerged weed seedlings (Beckie et al. 2018).

Management implications of triple-stacked sugarbeet systems

Sustainable management of herbicide resistance relies on minimizing herbicide usage by integrating other tactics (Hawes 2003). In this context, the prevention of weed seedbank replenishment is a fundamental approach for effective herbicide-resistant weed management (Schwartz-Lazaro and Copes 2019). During the survey, a large fraction of herbicide-resistant *B. scoparia* (57.5%) and *C. album* (63%) populations were present in isolation (Figures 1C and 1E). Given the greater fecundity of *B. scoparia* and *C. album*, these isolated populations could be precursors to pervasive infestation if not managed at an early stage. It would be of utmost importance for sugarbeet growers to manually uproot these populations or conduct precision applications of alternative herbicides to prevent herbicide-resistant seedbank replenishment.

The clusters of *B. scoparia* (17.5%) and *C. album* (26%) (Figures 1C and 1E) could be problematic hotspots due to the accumulation of herbicide-resistant biotypes, suggesting a localized gene flow. Late-season herbicide-based rescue treatments, mechanical removal, and electric weeding could be effective management strategies to prevent the spread of resistance alleles to adjacent fields. Within the sugarbeet fields, several *B. scoparia* populations, namely K07, K17, K23 K47, K54, K59, K68, and K69, existed in clustered and widespread conditions. Interestingly, these populations demonstrated developing resistance to dicamba with <20% survival, suggesting that only a few plants within these populations can produce dicamba resistance progenies. Fields with widespread herbicide-resistant *B. scoparia* (25%) and *C. album* (11%) populations may exceed economic thresholds sooner compared to those with clustered and isolated populations. These fields need immediate interventions and an integrated weed management approach (e.g., crop rotations, multiple tillage operations, layered herbicide application, and late-season rescue treatment) to ensure long-term and profitable sugarbeet productions. Even though herbicide resistance is not as prevalent in *A. retroflexus* as *B. scoparia* and *C. album*, fields with widespread infestation of *A. retroflexus* require season-long management due to its delayed and extended emergence to secure production goals.

The introduction of the glyphosate-resistant sugarbeet cultivar provided short-term efficient weed control. Consequently, there has been a shift in philosophy from managing weeds based on an economic threshold (Wilkerson et al. 2002) to zero-tolerance for weed seedbank replenishment (Brunharo et al. 2022). Almost 100% sugarbeet production in SE Montana and NW Wyoming is ‘Roundup ready.’ Glyphosate is the predominant herbicide in the ‘Roundup ready’ sugarbeet. Other post-emergent herbicides [Phemedipham + desmedipham (discontinued in the USA), clopyralid, ethofumesate, and triflusulfuron] are rarely used (Peter et al. 2024). These changes in weed management principles led to over-reliance on glyphosate usage, which escalated the evolution of glyphosate-resistant *B. scoparia* populations (Kumar et al. 2019). In accordance with our results, the majority of *B. scoparia* populations possess some level of glyphosate resistance in the Great Plains region (Godar et al. 2015; Kumar et al. 2019; Wiersma et al. 2015). No effective post-emergent options are available for *B. scoparia* resistant to glyphosate and triflusulfuron (Lawrence and Kniss 2021). Our study also highlights the widespread occurrence of dicamba resistance in 88% of populations of *B. scoparia*, including the resistance recorded at the developing stage. In the Northern Great Plains, sugarbeet is typically cultivated in a three-year rotation cycle with corn. While dicamba is not a widely used herbicide in sugarbeet, it has been one of the major herbicides used to manage glyphosate-resistant *B. scoparia* in corn for >30 years (Wicks et al. 1993), resulting in the evolution of two-way (glyphosate + dicamba) resistance.

This study determined that a few *B. scoparia* populations displayed developing resistance to glufosinate (Figures 3), an herbicide not extensively used in the surveyed area. A low level of glufosinate resistance in *B. scoparia* populations suggests that glufosinate might still be effective against two-way (glyphosate and dicamba) resistant *B. scoparia* populations if applied as a standalone herbicide in *triple-stacked* sugarbeet. However, previous studies have shown that glufosinate requires ≥60% relative humidity for optimum activity (Coetzer et al. 2001; Ramsey et al. 2002). In the Northern Great Plains, where relative humidity often ranges from 25% to 35% during summer, sub-optimal weed management following glufosinate application is a recurrent issue. Therefore, the long-term stewardship of glufosinate requires integrated weed management that encompasses, but is not limited to, the adoption of novel application technologies, crop rotations, pre-planting tillage, herbicide diversification, weed electrocution, rescue herbicide treatment, and manual uprooting. In conclusion, widespread two-way (glyphosate and dicamba) herbicide resistance, developing glufosinate resistance in *B. scoparia*, and the humidity-dependent nature of glufosinate raise ecological, evolutionary, and management concerns about the long-term suitability of the *triple-stacked* sugarbeet cultivar in semiarid regions of the Northern Great Plains.

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Competing Interests

No professional competing interests exist.

References:

Bagavathiannan MV, Davis AS (2018) An ecological perspective on managing weeds during the great selection for herbicide resistance. Pest Manag Sci 74:2277-2286

Beckie H, Blackshaw R, Leeson J, Stahlman P, Gaines T, Johnson E (2018) Seedbank persistence, germination and early growth of glyphosate-resistant *Kochia scoparia*. Weed Res 58:177-187

Beckie HJ, Blackshaw RE, Hall LM, Johnson EN (2016) Pollen-and seed-mediated gene flow in kochia (*Kochia scoparia*). Weed Sci 64:624-633

Beckie HJ, Hall LM (2014) Genetically-modified herbicide-resistant (GMHR) crops a two-edged sword? An Americas perspective on development and effect on weed management. Crop Prot 66:40-45

Brunharo CA, Gast R, Kumar V, Mallory-Smith CA, Tidemann BD, Beckie HJ (2022) Western United States and Canada perspective: are herbicide-resistant crops the solution to herbicide-resistant weeds? Weed Sci 70:272-286

Coetzer E, Al-Khatib K, Loughin TM (2001) Glufosinate efficacy, absorption, and translocation in amaranth as affected by relative humidity and temperature. Weed Sci 49:8-13

Crisp MD, Cook LG (2012) Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? New Phytologist 196:681-694

Davis AS, Clay S, Cardina J, Dille A, Forcella F, Lindquist J, Sprague C (2013) Seed burial physical environment explains departures from regional hydrothermal model of giant ragweed (*Ambrosia trifida*) seedling emergence in US Midwest. Weed Sci 61:415-421

Délye C, Menchari Y, Michel S, Cadet E, Le Corre V (2013) A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. Ann Bot 111:681-691

Dill GM (2005) Glyphosate-resistant crops: history, status and future. Pest Manag Sci 61:219-224

Duke SO (2012) Why have no new herbicide modes of action appeared in recent years? Pest Manag Sci 68:505-512

Duke SO (2015) Perspectives on transgenic, herbicide-resistant crops in the United States almost 20 years after introduction. Pest Manag Sci 71:652-657

E-Vojtkó A, de Bello F, Lososová Z, Götzenberger L (2023) Phylogenetic diversity is a weak proxy for functional diversity but they are complementary in explaining community assembly patterns in temperate vegetation. J Ecol 111:2218-2230

Essman AI, Loux MM, Lindsey AJ, Ackley BA, Regnier EE (2021) A survey evaluating the spatial and temporal distribution of horseweed (*Conyza canadensis*) late season in Ohio soybean fields from 2013 to 2017. Weed Sci 69:719-728

Freckleton R, Watkinson A (2001) Asymmetric competition between plant species. Func Ecol 15:615-623

Gasquez J (1985) Breeding system and genetic structure of a *Chenopodium album* population according to crop and herbicide rotation. Pages 57-66 *in* Adv Research Workshop NATO, Jacquard P. (ed.), Springer-Verlag, Berlin

Godar AS, Stahlman PW, Jugulam M, Dille JA (2015) Glyphosate-resistant kochia (*Kochia scoparia*) in Kansas: EPSPS gene copy number in relation to resistance levels. Weed Sci 63:587-595

Green JM (2012) The benefits of herbicide-resistant crops. Pest Manag Sci 68:1323-1331

Gressel J, Gassmann AJ, Owen MD (2017) How well will stacked transgenic pest/herbicide resistances delay pests from evolving resistance? Pest Manag Sci 73:22-34

Guo P, Al-Khatib K (2003) Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). Weed Sci 51:869-875

Hall LM, Beckie HJ, Low R, Shirriff SW, Blackshaw RE, Kimmel N, Neeser C (2014) Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. Can J Plant Sci 94:127-130

Hawes C, Haughton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.R. and Champion, G.T. (2003) Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide–tolerant crops. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 358:1899-1913

Heap I (2014) Global perspective of herbicide-resistant weeds. Pest Manag Sci 70:1306-1315

Heap I (2025) International herbicide-resistant weed database. https://www.weedscience.org/Pages/filter.aspx. Accessed January 29, 2025

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977) The world's worst weeds. Distribution and biology: University Press of Hawaii.

Hovick SM, McArdle A, Harrison SK, Regnier EE (2018) A mosaic of phenotypic variation in giant ragweed (*Ambrosia trifida*): Local-and continental-scale patterns in a range-expanding agricultural weed. Evol Appl 11:995-1009

James C (2010) A global overview of biotech (GM) crops: adoption, impact and future prospects. GM crops 1:8-12

Jhala AJ, Norsworthy JK, Ganie ZA, Sosnoskie LM, Beckie HJ, Mallory-Smith CA, Liu J, Wei W, Wang J, Stoltenberg DE (2021) Pollen-mediated gene flow and transfer of resistance alleles from herbicide-resistant broadleaf weeds. Weed Technol 35:173-187

Jin Y, Qian H (2022) V. PhyloMaker2: An updated and enlarged R package that can generate very large phylogenies for vascular plants. Plant Diver 44:335-339

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840-848

Kniss AR, Miller SD, Westra PH, Wilson RG (2007) Glyphosate susceptibility in common lambsquarters (*Chenopodium album*) is influenced by parental exposure. Weed Sci 55:572-577

Kumar V, Jha P (2015) Influence of herbicides applied postharvest in wheat stubble on control, fecundity, and progeny fitness of *Kochia scoparia* in the US Great Plains. Crop Protection 71:144-149

Kumar V, Jha P (2016) Differences in germination, growth, and fecundity characteristics of dicamba-fluroxypyr-resistant and susceptible *Kochia scoparia*. PLoS One 11:e0161533

Kumar V, Jha P, Dille JA, Stahlman PW (2018) Emergence dynamics of kochia (*Kochia scoparia*) populations from the US Great Plains: a multi-site-year study. Weed Sci 66:25-35

Kumar V, Jha P, Jugulam M, Yadav R, Stahlman PW (2019) Herbicide-resistant kochia (*Bassia scoparia*) in North America: a review. Weed Sci 67:4-15

Kumar V, Jha P, Reichard N (2014) Occurrence and characterization of kochia (*Kochia scoparia*) accessions with resistance to glyphosate in Montana. Weed Technol 28:122-130

Lawrence N, Kniss AR(2021) Herbicide options for control of glyphosate-resistant weeds in sugarbeet. Nebraska Extension Publications. 4 p

Manalil S, Werth J, Jackson R, Hereward J, Thornby D, Charles G, Cook T, Chauhan BS, Preston C (20Is Australia ready for triple gene herbicide stack technology? *In* 2nd Aus Cot Res Conf, Toowoomba.

McGinnis EE, Meyer MH, Smith AG (2010) Sweet and sour: a scientific and legal look at herbicide-tolerant sugar beet. The Plant Cell 22:1653-1657

Menalled FD, Peterson RK, Smith RG, Curran WS, Páez DJ, Maxwell BD (2016) The eco-evolutionary imperative: revisiting weed management in the midst of an herbicide resistance crisis. Sustainability 8:1297

Menalled FD, Smith RG (2007) Competitiveness of herbicide-resistant and herbicide-susceptible kochia (*Kochia scoparia* [L.] Schrad.) under contrasting management practises. Weed Bio and Manag 7:115-119

Morishita DW (2018) Impact of glyphosate-resistant sugar beet. Pest Manag Sci 74:1050-1053

Mortensen DA, Ryan MR, Smith RG (2024) Another step on the transgene-facilitated herbicide treadmill. Pest Manag Sci 80:4145-4149

Mortimer AM (1997) Phenological adaptation in weeds—an evolutionary response to the use of herbicides? Pest Sci 51:299-304

Neve P, Vila-Aiub M, Roux F (2009) Evolutionary-thinking in agricultural weed management. New Phytologist 184:783-793

Owen MD (2008) Weed species shifts in glyphosate-resistant crops. Pest Manag Sci: formerly Pestic Sci 64:377-387

Owen MJ, Walsh MJ, Llewellyn RS, Powles SB (2007) Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. Aus J of Agric Res 58:711-718

Peters TJ, Borgen RM, Lystad AL (2020) Weed control using high voltage electricity. Sugarbeet Research and Education Board **Incomplete Reference**

Peters TJ, Lystad AL, Khan M, Boetel MA (2024) Survey of weed control and production practices on sugarbeet in western North Dakota and Eastern Montana in 2021. Sugarbeet Research and Education Board - **Incomplete Reference**

Ramsey R, Stephenson G, Hall J (2002) Effect of relative humidity on the uptake, translocation, and efficacy of glufosinate ammonium in wild oat (*Avena fatua*). Pestoc Biochem and Physiol 73:1-8

Recasens J, Calvet V, Cirujeda A, Conesa JA (2005) Phenological and demographic behaviour of an exotic invasive weed in agroecosystems. Biological Invasion 7:17-27

Rundle HD, Nosil P (2005) Ecological speciation. Ecol Letters 8:336-352

Sarangi D, Tyre AJ, Patterson EL, Gaines TA, Irmak S, Knezevic SZ, Lindquist JL, Jhala AJ (2017) Pollen-mediated gene flow from glyphosate-resistant common waterhemp (*Amaranthus rudis* Sauer): consequences for the dispersal of resistance genes. Sci Rep 7:44913

Schreier H, Bish M, Bradley KW (2022) The impact of electrocution treatments on weed control and weed seed viability in soybean. Weed Technol 36:481-489

Schutte BJ, Regnier EE, Harrison SK (2012) Seed dormancy and adaptive seedling emergence timing in giant ragweed (*Ambrosia trifida*). Weed Sci 60:19-26

Schutte BJ, Regnier EE, Harrison SK, Schmoll JT, Spokas K, Forcella F (2008) A hydrothermal seedling emergence model for giant ragweed (*Ambrosia trifida*). Weed Sci 56:555-560

Schwartz-Lazaro LM, Copes JT (2019) A review of the soil seedbank from a weed scientists perspective. Agronomy 9:369

Simões Araujo AL WE, Shergill L, Gaines TA (2024) Baseline survey reveals glyphosate and dicamba resistance in broadleaf weeds before sugar beet trait introduction. Weed Technol 38:e87

Slaven MJ, Koch M, Borger CP (2023) Exploring the potential of electric weed control: A review. Weed Sci 71:403-421

Soltani N, Dille JA, Robinson DE, Sprague CL, Morishita DW, Lawrence NC, Kniss AR, Jha P, Felix J, Nurse RE (2018) Potential yield loss in sugar beet due to weed interference in the United States and Canada. Weed Technol 32:749-753

Stallings GP, Thill DC, Mallory-Smith CA, Shafii B (1995) Pollen-mediated gene flow of sulfonylurea-resistant kochia (*Kochia scoparia*). Weed Sci 43:95-102

Thornby D, Werth J, Hereward J, Keenan M, Chauhan BS (2018) Herbicide resistance evolution can be tamed by diversity in irrigated Australian cotton: a multi-species, multi-herbicide modelling approach. Pest Manag Sci 74:2363-2375

USGS (2023) Pesticide national synthesis project. https://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php. Accessed August 16, 2023

Vaissi S, Rezaei S (2022) Niche divergence at intraspecific level in the Hyrcanian wood frog, *Rana pseudodalmatina*: a phylogenetic, climatic, and environmental survey. Front in Ecol and Evol 10:774481

Vencill WK, Nichols RL, Webster TM, Soteres JK, Mallory-Smith C, Burgos NR, Johnson WG, McClelland MR (2012) Herbicide resistance: toward an understanding of resistance development and the impact of herbicide-resistant crops. Weed Sci 60:2-30

Weiner J (1990) Asymmetric competition in plant populations. Trends in Eco and Evo 5:360-364

Westhoven AM, Stachler JM, Loux MM, Johnson WG (2008) Management of glyphosate-tolerant common lambsquarters (*Chenopodium album*) in glyphosate-resistant soybean. Weed Technol 22:628-634

Westra EP, Nissen SJ, Getts TJ, Westra P, Gaines TA (2019) Survey reveals frequency of multiple resistance to glyphosate and dicamba in kochia (*Bassia scoparia*). Weed Technol 33:664-672

Wicks GA, Martin AR, Mahnken GW (1993) Control of triazine-resistant kochia (*Kochia scoparia*) in conservation tillage corn (*Zea mays*). Weed Sci 41:225-231

Wiersma AT, Gaines TA, Preston C, Hamilton JP, Giacomini D, Robin Buell C, Leach JE, Westra P (2015) Gene amplification of 5-enol-pyruvylshikimate-3-phosphate synthase in glyphosate-resistant *Kochia scoparia*. Planta 241:463-474

Wilkerson GG, Wiles LJ, Bennett AC (2002) Weed management decision models: pitfalls, perceptions, and possibilities of the economic threshold approach. Weed Sci 50:411-424

Wilson RG, Sbatella GM (2011) Late-season weed control in glyphosate-resistant sugarbeet. Weed Technol 25:350-355

Yerka MK, de Leon N, Stoltenberg DE (2012) Pollen-mediated gene flow in common lambsquarters (*Chenopodium album*). Weed Sci 60:600-606

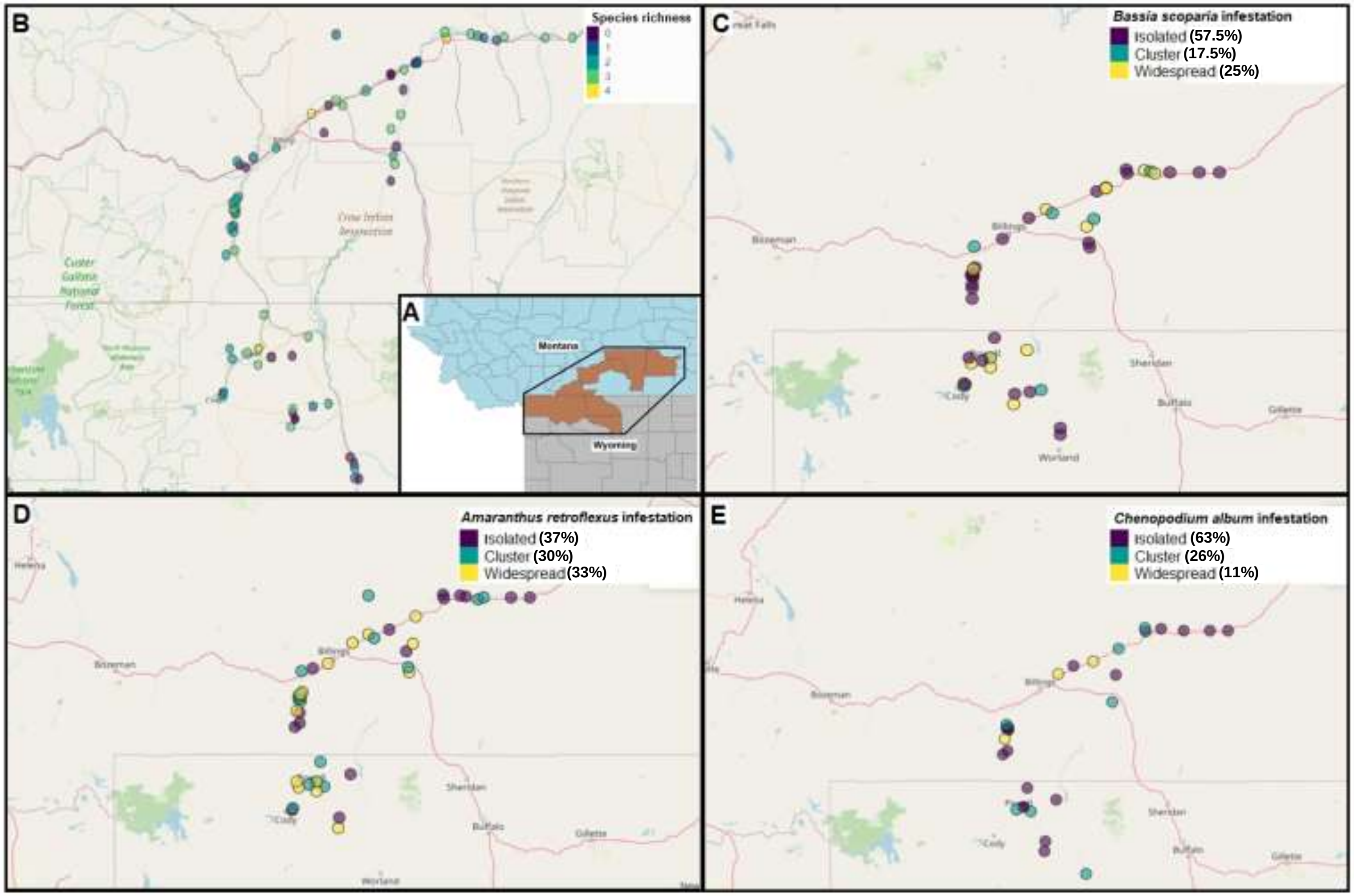

Figure 1: Infestation pattern of the weed species during an August 2021 survey within the sugarbeet cropping systems in SE Montana and NW Wyoming: (A) surveyed counties highlighted in orange color; (B) number of weed species present (species richness); infestation level and spatial pattern of (C) *Bassia scoparia*; (D) *Amaranthus retroflexus*; and (E) *Chenopodium album*.

Figure 2: Categories of sugarbeet fields based on weed infestation visually identified during a survey conducted in South-East Montana and North-West Wyoming counties: (A) weed-free, (B) isolated, (C) clustered, and (D) widespread. Black arrows indicate isolated and clustered infestations of weeds.

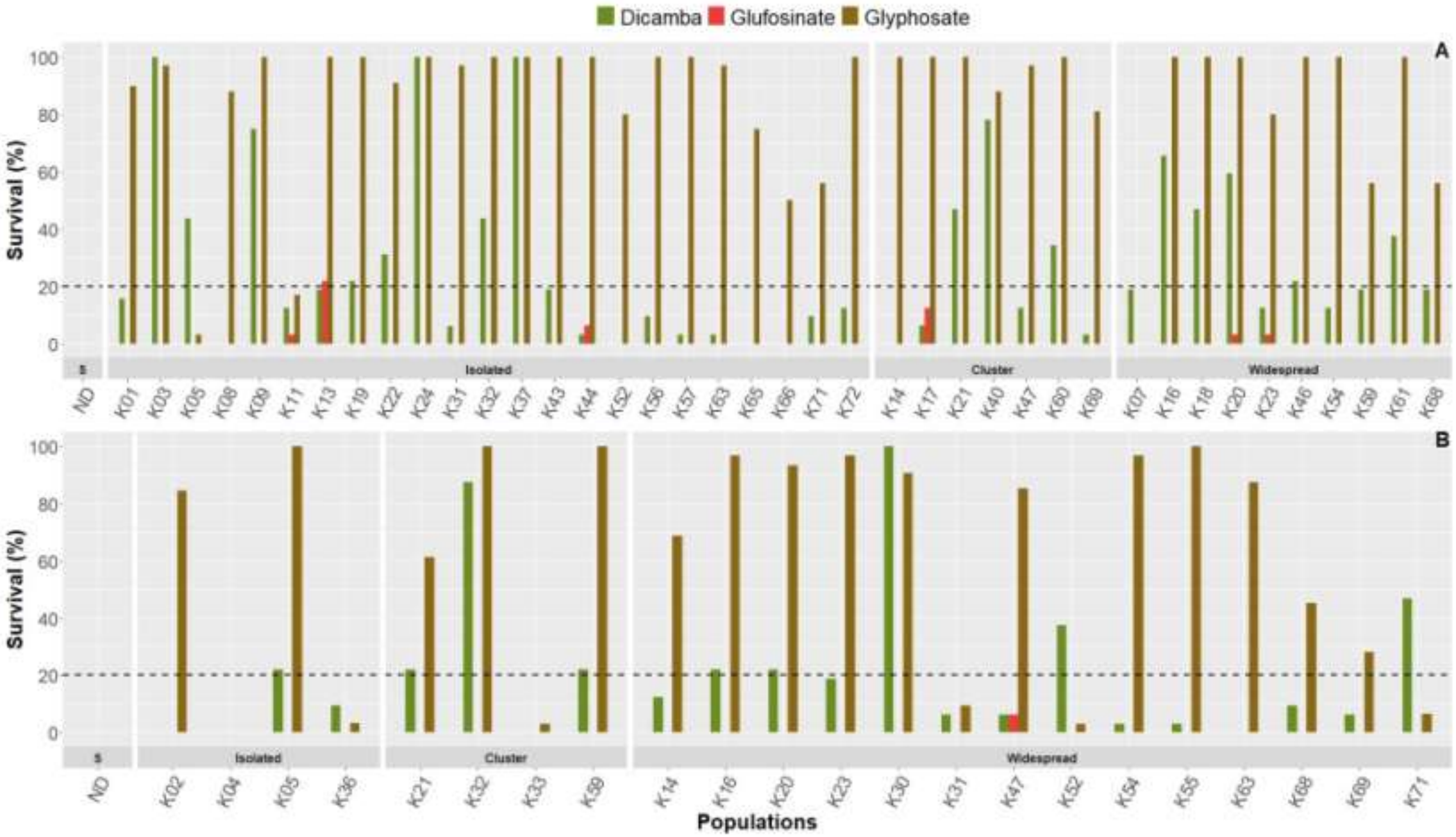

Figure 3: Percentage survival of *Bassia scoparia* populations from (A) fields and (B) margins collected during August 2021 when sprayed with dicamba (0.6 kg ae ha^{-1}), glufosinate (0.6 kg ae ha^{-1}) and glyphosate (1.3 kg ae ha^{-1}). The numerical values preceded by the letter K represent the surveyed field number. The horizontal truncated black line differentiates populations based on developing resistance (1-20%) and resistance (20%). Isolated, cluster, and widespread indicate the infestation level during the August 2021 survey, while 'S' represents the susceptible populations from North Dakota used as a reference line.

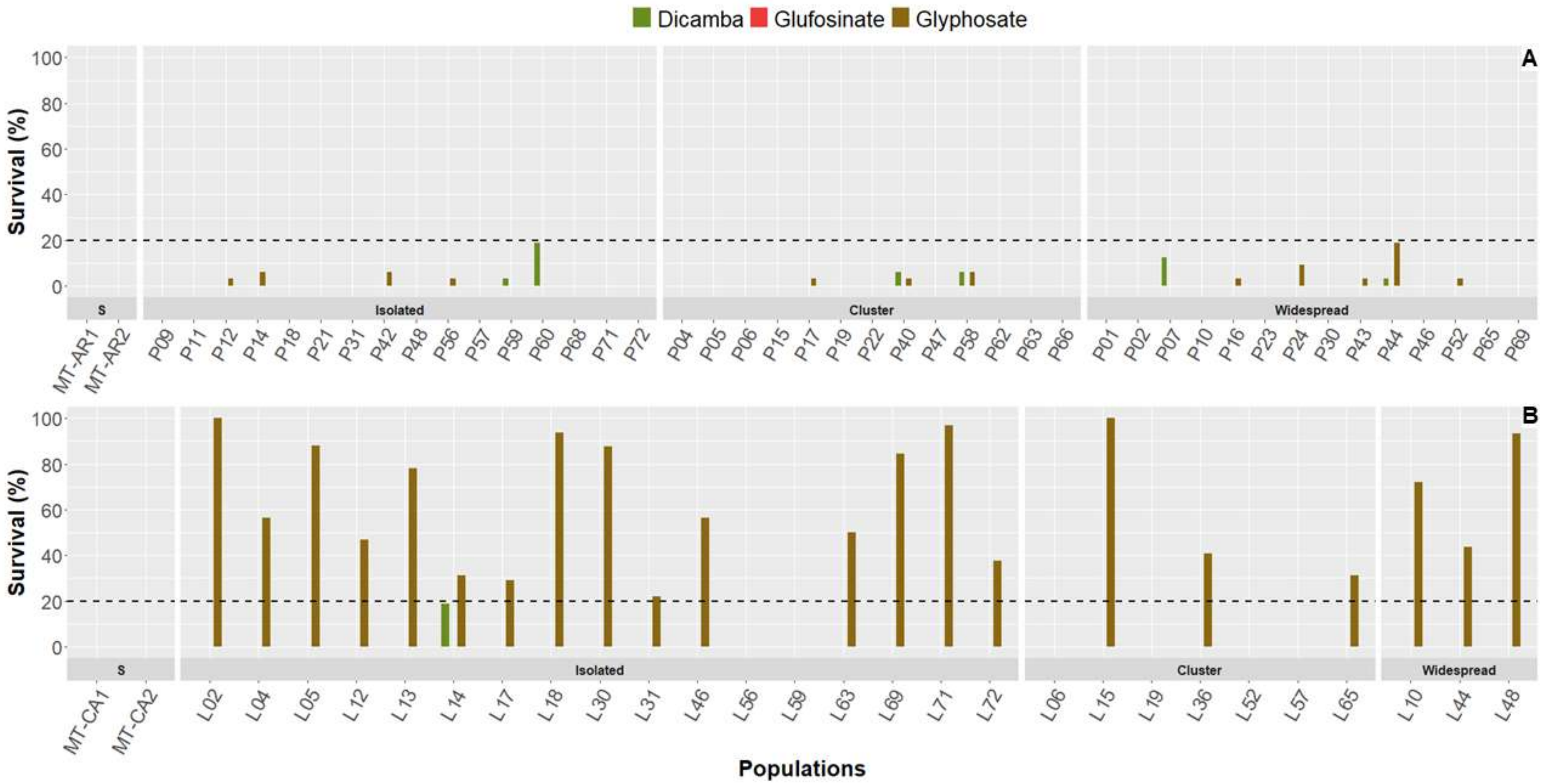

Figure 4: Percentage survival of (A) *Amaranthus retroflexus* and (B) *Chenopodium album* populations collected from sugarbeet fields during the August 2021 survey when sprayed with dicamba (0.6 kg ae ha^{-1}), glufosinate (0.6 kg ae ha^{-1}) and glyphosate (0.8 kg ae ha^{-1}). The numerical values preceded by the letters P or L represent the surveyed field number. The horizontal truncated black line differentiates populations based on developing resistance (1-20%) and resistance (20%). Isolated, cluster, and widespread indicate the infestation level during the August 2021 survey, while ‘S’ represents the susceptible populations from Montana used as a reference line.

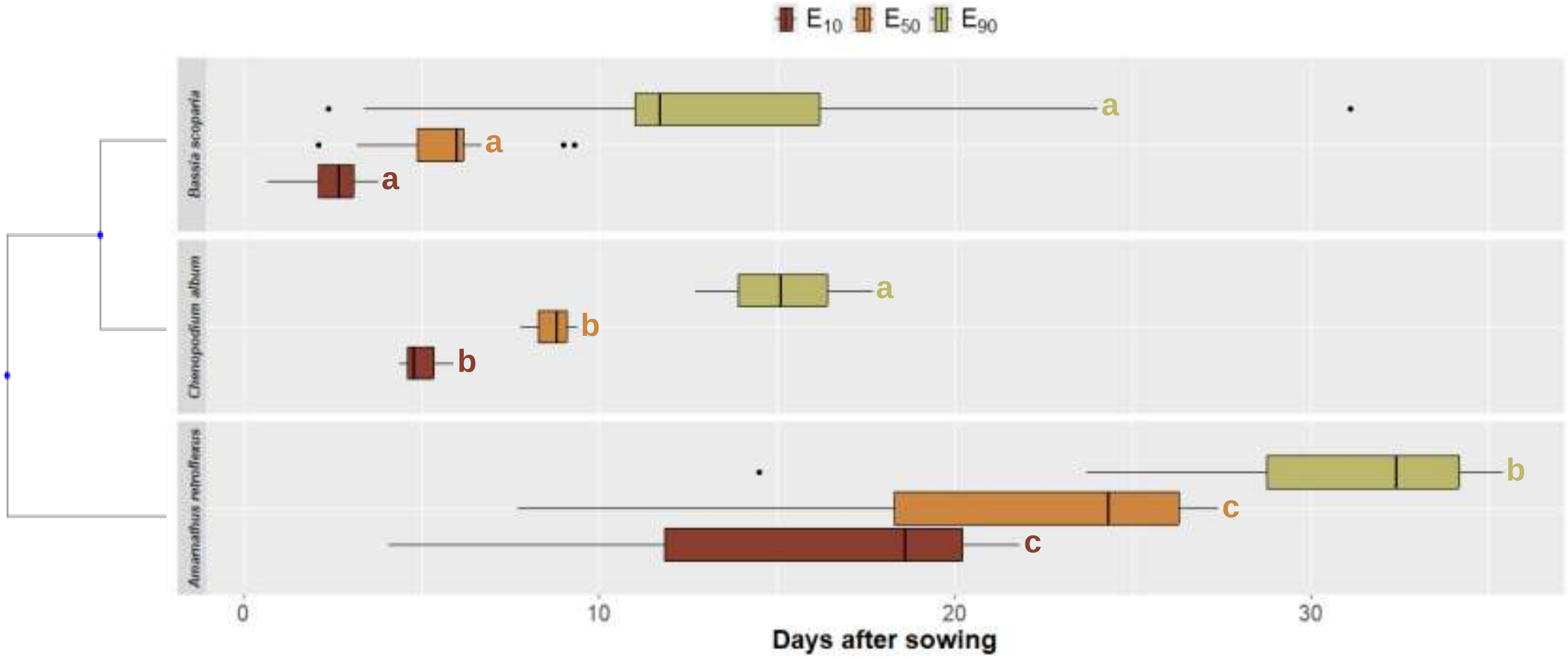

Figure 5: Phylogenetic relatedness and (dis)similarity of emergence pattern across *Bassia scoparia*, *Chenopodium album*, and *Amaranthus retroflexus*. Different colored boxes represent E_{10}, E_{50}, and E_{90}, which are days required to reach 10%, 50%, and 90% emergence, respectively, estimated by a three-parameter log-logistic model at 22/17 ± 2 C day/night temperatures and a 16/8 h photoperiod. Similar letters denoting nonsignificant differences based on Fisher's protected least significant different test (α=0.05) are for E_{10} across species, E_{50} across species, and E_{90} across species.

Table 1: Regression parameters estimated by a three-parameter log-logistic model $\{Y = d/1 + \exp[b(\log x - \log E_{50})]\}$ for cumulative emergence percentage of *Bassia scoparia*, *Amaranthus retroflexus*, and *Chenopodium album* populations collected in South-East Montana and North-West Wyoming, USA.

Population(s)[a]	Field				Margin			
	Regression parameters (SE)[b]							
	B[c]	E_{10}[c]	E_{50}[c]	E_{90}[c]	B[c]	E_{10}[c]	E_{50}[c]	E_{90}[c]
Bassia scoparia								
K14	-2.1 (0.1)	3.3 (0.2)	6.0 (0.1)	11.0 (0.3)	-1.3 (0.1)	1.1 (0.1)	2.7 (0.1)	6.7 (0.4)
K16	-3.2 (0.2)	2.2 (0.1)	6.0 (0.2)	16.7 (0.5)	-1.3 (0.1)	1.1 (0.1)	6.0 (0.1)	6.7 (0.4)
K20	-2.8 (0.1)	2.7 (0.1)	5.8 (0.1)	12.4 (0.4)	-2.7 (0.1)	1.6 (0.1)	3.9 (0.1)	9.6 (0.4)
K21	-1.2 (0.1)	1.9 (0.1)	2.1 (0.5)	2.4 (1.3)	-2.4 (0.1)	1.9 (0.1)	4.1 (0.1)	8.9 (0.3)
K23	-3.9 (0.2)	2.8 (0.2)	9.3 (0.2)	31.1 (1.0)	-3.2 (0.2)	2.7 (0.1)	5.5 (0.1)	19.9 (0.3)
K32	-3.6 (0.2)	3.4 (0.1)	9.0 (0.2)	24.0 (0.7)	-3.0 (0.2)	2.3 (0.1)	5.4 (0.1)	12.7 (0.4)
K47	-2.8 (0.1)	3.8 (0.2)	6.7 (0.1)	11.7 (0.4)	-3.4 (0.1)	4.4 (0.2)	11.3 (0.2)	29.0 (0.8)
K54	-2.5 (0.1)	2.1 (0.2)	4.9 (0.5)	11.3 (1.2)	-3.2 (0.1)	0.6 (0.1)	3.4 (0.1)	18.3 (0.9)
K59	-2.8 (0.1)	3.1 (0.1)	6.0 (0.1)	11.8 (0.4)	-1.9 (0.2)	2.3 (0.1)	4.4 (0.1)	8.5 (0.6)
K63	-2.9 (0.1)	0.7 (0.1)	3.4 (0.1)	16.2 (1.3)	-2.0 (0.2)	2.2 (0.1)	4.3 (0.1)	8.5 (0.3)
K68	-1.4 (0.1)	2.9 (0.1)	6.2 (0.1)	3.4 (0.4)	-2.5 (0.2)	1.3 (0.1)	3.4 (0.1)	9.1 (0.4)
K69	-2.0 (0.1)	1.1 (0.1)	3.2 (0.1)	9.0 (0.4)	-2.5 (0.1)	1.2 (0.1)	3.3 (0.1)	9.2 (0.4)
K71	-2.2 (0.1)	2.3 (0.1)	5.1 (0.1)	11.3 (0.4)	-1.6 (0.2)	1.9 (0.1)	3.9 (0.1)	8.0 (0.3)
Amaranthus retroflexus								
P11	-3.5 (0.2)	5.3 (0.3)	11.2 (0.3)	23.7 (0.8)	-4.4 (0.3)	14.2 (0.6)	23.3 (0.3)	38.3 (1.0)
P14	-8.3 (0.5)	18.6 (0.4)	24.3 (0.2)	31.6 (0.5)	-8.4 (0.5)	22.4 (0.4)	29.1 (0.2)	37.6 (0.6)
P16	-7.1 (0.5)	16.1 (0.4)	21.9 (0.2)	29.1 (0.6)	-3.8 (0.3)	12.4 (0.6)	22.2 (0.4)	39.7 (1.3)
P17	-9.1 (0.6)	20.0 (0.4)	25.5 (0.2)	32.4 (0.5)	-17.1 (1.3)	28.2 (0.3)	32.1 (0.2)	36.5 (0.4)
P19	-8.6 (0.6)	20.3 (0.4)	26.2 (0.2)	33.9 (0.5)	-13.8 (1.0)	27.4 (0.4)	32.2 (0.2)	37.7 (1.0)
P22	-7.8 (0.5)	20.1 (0.5)	26.7 (0.2)	35.4 (0.6)	-8.8 (0.7)	21.3 (0.5)	25.9 (0.2)	31.6 (0.5)
P43	-7.0 (0.5)	17.4 (0.5)	23.7 (0.3)	32.5 (0.6)	-3.2 (0.2)	9.4 (0.5)	18.6 (0.3)	36.9 (1.2)
P47	-8.3 (0.5)	20.3 (0.4)	26.4 (0.2)	34.4 (0.6)	-11.1 (1.0)	23.0 (0.5)	29.5 (0.2)	37.9 (0.7)
P56	-2.9 (0.1)	4.1 (0.2)	7.7 (0.2)	14.5 (0.6)	-3.5 (0.2)	10.1 (0.4)	19.1 (0.3)	35.9 (1.0)
P66	-3.3 (0.2)	7.6 (0.4)	14.7 (0.3)	28.4 (0.8)	-2.3 (0.1)	3.8 (0.2)	9.9 (0.3)	25.9 (1.0)

P72	-9.6 (0.6)	21.8 (0.4)	27.4 (0.2)	34.5 (0.5)	-2.1 (0.1)	3.5 (0.2)	10.2 (0.3)	29.3 (1.2)
Chenopodium album								
L13	NA	NA	NA	NA	-3.5 (0.8)	3.4 (0.2)	8.4 (0.2)	12.1 (1.1)
L14	-4.6 (0.2)	4.8 (0.3)	7.8 (0.2)	12.7 (1.6)	-2.4 (0.1)	6.1 (0.2)	8.1 (0.1)	10.9 (0.4)
L69	-4.8 (0.1)	4.4 (0.2)	8.8 (0.3)	17.7 (1.8)	NA	NA	NA	NA
L71	-4.8 (0.3)	5.9 (0.2)	9.4 (0.2)	15.1 (1.2)	NA	NA	NA	NA

[a] Letters represent the common names of the weed species, e.g., ‘K,’ ‘P,’ and ‘L’ denote kochia, redroot pigweed, and common lambsquarters, respectively. The numerical values preceded by letters represent the surveyed field number; for instance, K14 was collected from field 14 and the margins of field 14.

[b] Values in parentheses are standard errors of the mean.

[C] ‘*b*’; the slope of the curve indicating the emergence rate of populations over time. ‘E_{10}’, ‘E_{50}’, and ‘E_{90}’ represent d required to reach 10%, 50%, and 90% emergence, respectively.