

Palmer amaranth (*Amaranthus palmeri*) adaptation to agroecosystems

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2 ABSTRACT

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INTRODUCTION

Palmer amaranth (*Amaranthus palmeri* S. Watson) is currently considered one of the most economically damaged weed species to cropping systems in the United States. Unmanaged Palmer amaranth in competition for water, light and nutrients can drastically impact on crop yields (Berger et al., 2015). For example, Palmer amaranth is documented with potential to reduce 91%, 68%, and 54% of corn (Massinga et al., 2001), soybean (Klingaman and Oliver, 1994), and cotton (Morgan et al., 2001) yields, respectively. Moreover, Palmer amaranth has showed a remarkable capacity to evolve resistance to herbicides. To date, Palmer amaranth has evolved resistance to eight herbicide sites of action (Heap, 2021), increasing the weed management complexity (Lindsay et al., 2017). Thus, Palmer amaranth poses an economical risk to sustainable agriculture.

Palmer amaranth is a fast growing summer annual forb indigenous to Sonoran Desert (Sauer, 1957). The species would eventually emerge as a threat to US agriculture in the 1990s. Palmer amaranth weediness is likely a result of human-assisted selection in combination with species biology. Farm mechanization, conservation agriculture (e.g., no-till), and reliance on herbicides for weed management are the main human-mediated selection of Palmer amaranth into cropping systems (Ward et al., 2013). On the other hand, Palmer amaranth is a prolific seed producer with a C4 photosynthetic apparatus (Wang et al., 1992). With a dioecy nature, Palmer amaranth male and female plants are obligate outcrosser species, increasing the chances of exchanging adaptive traits among plants (Oliveira et al., 2018). Also, Palmer amaranth small seed size (e.g, 1 mm) tend to thrive in no-tillage systems (Price et al., 2011), and spread across locations through farm equipment (Sauer, 1972), manure (Hartzler and Anderson, 2016), animals (Farmer et al.,

26 2017), and plant propagules (Yu et al., 2021). The dispersal capacity of Palmer amaranth make the species
27 one of the most successful cases of weed adaption to cropping systems.

28 Light and temperature are likely the main environment requirements for Palmer amaranth successful
29 grow and development. Palmer amaranth is reported with an extended germination period (Jha et al., 2010).
30 Germination of Palmer amaranth is triggered by 18 C soil temperature (Keeley et al., 1987), and optimal
31 germination and biomass production occur at 35/30 C day and night temperatures (Guo and Al-Khatib,
32 2003). Palmer amaranth germination is also human-mediated by tillage timings and preemergence-applied
33 herbicides (Chahal et al., 2021). Water has not shown to limit Palmer amaranth fitness. Under continuous
34 water stress, Palmer amaranth survived and produced at least 14000 seeds plant-1 (Chahal et al., 2018).
35 Seeds from Palmer amaranth growing with limited water conditions were heavier, less dormant, and
36 prompt for germination (Matzrafi et al., 2021). Also, growing conditions and management can impact
37 sex dimorphism and flowering pattern in Palmer amaranth (Korres et al., 2017; Rumpa et al., 2019). The
38 continuous global temperature warming can impact agriculture and promote niches for Palmer amaranth
39 invasion/adaptation into new environments. Currently, it is estimated that the greatest climatic risk of
40 Palmer amaranth establishment are agronomic crops in Australia and Sub-Saharan Africa (Kistner and
41 Hatfield, 2018). Temperature is a key factor limiting Palmer amaranth expansion to cooler geographies
42 (Briscoe Runquist et al., 2019); however, under future climate change Palmer amaranth is likely to expand
43 northward into Canada and Northern Europe (Kistner and Hatfield, 2018; Briscoe Runquist et al., 2019).

44 Palmer amaranth is already found in agronomic crops of South America (Larran et al., 2017; Küpper
45 et al., 2017) and Southern Europe (Milani et al., 2021). In the US, Palmer amaranth is well established
46 at cotton fields (Garetson et al., 2019) and non-crop areas (Bagavathiannan and Norsworthy, 2016) in
47 the southern United States but its range is expanding northward. For example, herbicide resistant Palmer
48 amaranth is widespread in Nebraska (Oliveira et al., 2021), Michigan (Kohrt et al., 2017), Connecticut
49 (Aulakh et al., 2021), and potential damaged to soybean fields in Illinois is reported (Davis et al., 2015).
50 In Iowa, a study showed that Palmer amaranth is not well adapted compared to waterhemp (*Amaranthus*
51 *tuberculatus*) (Baker, 2021). Successful cases of Palmer amaranth invasion and near to eradication is
52 documented in Minnesota (Yu et al., 2021). No Palmer amaranth actively growing was found in Canada;
53 however, Palmer amaranth seeds was detected in sweet potato slips (Page et al., 2021). Nonetheless, it
54 seems fated the need to manage Palmer amaranth in agronomic crops throughout multiple environments
55 in the near future. Strategies on Palmer amaranth management should encompass the agroecosystem
56 level but not only attempts to eradicate the weed. Most tactics to manage Palmer amaranth are based on
57 technology fixes (Scott, 2011), which are short-term (e.g., herbicide and/or tillage) rather than long-term
58 weed management. Palmer amaranth management should be built on minimizing the species ability to
59 adapt, grow and develop into agroecossystems.

60 In the southeastern US, early growing Palmer amaranth is well known to have higher impact on cotton
61 yields than late established plants (MacRae et al., 2013). In the northern states, Palmer amaranth impact
62 on the agroecosystem is recent. Studies investigating Palmer amaranth in those locations is limited due
63 to the plant classification as noxious weed species (Yu et al., 2021). Nonetheless, the continuous Palmer
64 amaranth dispersal and potential establishment across northern United States is concerning and warrant
65 investigations on species morphology in such environments. Understanding Palmer amaranth biology and
66 growing strategies under different agroecosystems can enhance our knowledge on species adaptation. It
67 can also aid on designing proactive and ecological tactics to limit the species range expansion, reduce its
68 negative impact, and design resilient and sustainable farming systems (MacLaren et al., 2020). Therefore,
69 the objective of this study was to investigate the flowering pattern, biomass production, and height of

70 Palmer amaranth growing under corn, soybean and fallow at two timings across five locations in the United
71 States Midwest.

MATERIAL AND METHODS

72 Plant material and growing conditions

73 The study was performed with a Palmer amaranth accession (Per1) from Perkins County, Nebraska.
74 Per1 accession collection is documented with no reported herbicide resistance (Oliveira et al., 2021).
75 Three weeks prior to the field experiment, seeds were planted in plastic trays containing potting-mix.
76 Emerged seedlings (1 cm) were transplanted into 200 cm⁻³ plastic pots (a plant pot-1). Palmer amaranth
77 seedlings were supplied with adequate water and kept under greenhouse conditions at Arlington, Clay
78 Center, Lincoln, and Macomb; and kept outdoors in Grant. Palmer amaranth seedlings were kept under
79 greenhouse/outdoors until the onset of the experiment (2-3 leaf stage/5 to 8 cm height).

80 Field study

81 The experiment was conducted in 2018 and 2019 under field conditions at five locations: Arlington
82 (Washington County, Wisconsin), Clay Center (Clay County, Nebraska), Grant (Perkins County, Nebraska),
83 Lincoln (Lancaster County, Nebraska), and Macomb (McDonough County, Illinois).

84 A glyphosate-resistant soybean cultivar (DSR-1950 R2Y at 296,400 seeds ha⁻¹), and a corn hybrid were
85 planted at

86 Monthly mean air temperature and sum precipitation were obtained using Daymet weather data from
87 June through September across the five locations in 2018 and 2019 (Correndo et al., 2021) (Figure 1)

88 The field experimental unit were six adjacent 9.1 m wide (12 rows at 72.2 cm row spacing) by 10.7
89 m long. Each experimental unit was planted with corn or soybean (DSR-1950 R2Y at 296,400 seeds ha
90 ⁻¹), or under fallow condition. Palmer amaranth seedlings (potting mix + two seedlings) were and gently
91 transferring to the ground (6 cm deep and 8 cm wide). Twenty-four plants were equidistantly placed (0.76
92 m apart) between rows within each agroecosystems. After a week, one was eliminated and one was kept.
93 There were two transplant timing: first (June 1) and second (July 1). There were 24 Palmer amaranth plants
94 in each experimental unit, with a total of 144 plants for each location. The study was repeated twice.

95 After transplanting, Palmer amaranth flowering was monitored until the end of the study. When a plant
96 started flowering, the day was recorded, plant sex was identified as male or female, and plant height
97 was measured from soil surface to the plant top. Then, aboveground plant biomass was harvest near soil
98 surface and oven dried at 65 C until reaching constant weight before the weight of biomass (g plant⁻¹) was
99 recorded.

100 Statistical analyses

101 The statistical analyses were performed using R statistical software version 4.0.1. Data across locations
102 and year were combined.

103 The cumulative Palmer amaranth flowering estimation was determined using a asymmetrical three
104 parameter log logistic Weibull model of the drc package (Ritz et al., 2015).

$$Y(x) = 0 + (d - 0)\exp(-\exp(b(\log(x) - e)))$$

105 In this model, Y is the Palmer amaranth cumulative flowering, d is the upper limit (set to 100), and e is the
106 XXX, and x day of year (doy).

107 The day for 10, 50, and 90% Palmer amaranth cumulative flowering were determined using the *ED*
108 function of drc package. Also, the 10, 50, and 90% Palmer amaranth cumulative flowering were compared
109 among agroecosystems and timings using the *EDcomp* function of drc package. The EDcomp function
110 compares the ratio of cumulative flowering using t-statistics, where P-value < 0.05 indicates that we fail to
111 reject the null hypothesis.

112 Palmer amaranth gender was fitted to a binary logistic regression (Bangdiwala, 2018). Binary logistic
113 regression is used for predicting binary classes, such gender of Palmer amaranth plants. Prior to the analysis,
114 all missing values were removed from the dataset. Also, data from Grant was not used in this analysis due
115 to the uniform plant harvesting at that location. The whole dataset was splitted into 80% train and 20% test
116 data. The 80% train is used for the model training and the rest 20% is used for checking how the model
117 generalized on unseen dataset. With 80% dataset, a binary response variable, male (0) and female (1), was
118 fitted to a generalized linear model (*glm* function) including day of year harvest, height, weight, crop and
119 month as independent variables. The model family was binomial with a logit function. The model fit was
120 assessed through pseudo R squared values (McFadden, Cox and Snell, Cragg and Uhler) and likelihood
121 ratio using *nagelkerke* function (“rcompanion” package). The marginal effects computation was performed
122 with Average Marginal Effects at every observed value of x and average across the results (AMEs) (Leeper,
123 2017) using *margins* function from “margins” package. The rest 20% dataset was predicted using *predict*
124 function with a cutoff estimation for male or female using *performance* function. The model quality
125 prediction from the classification algorithm was measure with precision (*precision* function), recall (*recall*
126 function) and F1 score (*f_meas* function) using “yardstick” package. The precision determines the accuracy
127 of positive predictions (female plants), recall determines the fraction of positives that were correctly
128 identified, and F1 score is a weighted harmonic mean of precision and recall with the best score of 1 and
129 the worst score of 0. F1 score conveys the balance between the precision and the recall.

130 Palmer amaranth height and biomass were performed with a linear mixed model using *lmer* function from
131 “lme4” package (Bates et al., 2015). Plant height and biomass were transformed to meet model assumption
132 of normality. In the model, agroecosystem (crop, soybean, fallow) was the fixed effect and year nested with
133 location the random effects. Analysis of variance was performed with *anova* function from “car” package
134 (Fox and Weisberg, 2018). Marginal means and compact letter display were estimated with *emmeans* and
135 *cld* from packages “emmeans” and “multcomp” (Hothorn et al., 2008).

RESULTS

136 Palmer amaranth height and biomass

137 Palmer amaranth accumulated more biomass when growing in fallow compared to Palmer amaranth
138 growing in soybean and corn (Figure 3A). At first cohort time, Palmer amaranth biomass was 75.5 g plant-1,
139 28.3 g plant-1 and 16.3 g plant-1 in fallow, soybean and corn, respectively. At second cohort timing, Palmer
140 amaranth produced 62.6 g plant in fallow, followed by 6.3 g plant in soybean, and 1.4 g plant in corn.

141 Palmer amaranth height was more uniform across cohort timings, except when growing in corn (Figure
142 3B). Palmer amaranth achieve achieve 69.2 cm tall when growing at first cohort timing in bareground,
143 which was not different to 70.7 cm tall at second cohort timing (P = 0.74). In addition, no difference in
144 Palmer amaranth height (69.3 cm) was found in soybean at first cohort compared to fallow (P > 0.75). At
145 second cohort in soybean, Palmer amaranth was near to 10 cm lower compared to its first cohort time (P =
146 0.04). The tallest and smallest Palmer amaranth plants were found in corn. Palmer amaranth reached 85.2
147 cm tall at first cohort and 38.2 cm at second cohort timings.

148 Palmer amaranth cumulative flowering

149 Palmer amaranth growing in corn resulted in a longer flowering pattern compared to fallow and soybean
150 at first cohort (Figure 2A). Nonetheless, the 10% cumulative Palmer amaranth flowering in soybean, fallow
151 and corn occurred at the end of June. Palmer amaranth reached 10% flowering in soybean at doy 180, which
152 was slightly different from fallow (doy 180.9; $P = 0.01$) and corn (doy 181.7; $P = 0.00$). The 50% Palmer
153 amaranth cumulative flowering occurred in July. For example, Palmer amaranth reached 50% flowering in
154 fallow at doy 193.4, followed by soybean (doy 194.8), corn (doy 206.6). Similar trend was observed at
155 90% Palmer amaranth cumulative flowering. Palmer amaranth growing in corn reached 90% flowering
156 at doy 252.6 (early September), which was 37.8 and 32.2 days after Palmer amaranth 90% flowering in
157 fallow and soybean, respectively.

158 Palmer amaranth cumulative flowering at second cohort ranged from mid July to mid September (Figure
159 2B). Palmer amaranth growing in fallow resulted in earlier flowering time compared to soybean and corn.
160 Palmer amaranth growing in fallow reached 10%, 50%, and 90% flowering time at day 203.8, 214.4, and
161 232.2, respectively. Palmer amaranth growing in soybean reached 10% flowering at doy 210.9, which
162 was 6 days prior to corn (P -value = 0.00). Similar trend was observed at 50% flowering, whereas Palmer
163 amaranth reached 50% flowering in corn (doy 233.0) 4 days after soybeans (doy 228.9; $P = 0.00$). The 90%
164 Palmer amaranth cumulative flowering occurred at same day in corn (260.9) and soybean (260.5; $P = 0.66$).
165

Palmer amaranth gender

166 The model fit was 0.11, 0.14, 0.18 with using pseudo R squared test from McFadden, Cox and Snell, and
167 Cragg and Uhler, respectively. The likelihood ratio test showed a p-value of < 0.00. The average marginal
168 effects showed that increasing a weight unit by 1 g increases the probability of having a female plant by
169 0.08% ($P = 0.01$). Similar trend is observed to height as well as doy, whereas the probability of being
170 female increase by 0.2% ($P = 0.01$) and 0.4% ($P = 0.00$) when a unit of height (cm) and day increases,
171 respectively.

172 The model accuracy evaluation accuracy in the unseen 20% dataset was 0.61 with a cutoff value for
173 female and male plants of 0.49. The model classification showed a precision of 0.59, recall of 0.76, and a
174 F1 score of 0.66. In addition, the area under the curve was 0.61.

DISCUSSION

175 Our study showed that Palmer amaranth biomass, height, flowering pattern and gender varied within
176 agroecosystems and cohort timings. In general, Palmer amaranth produced more biomass and taller plants
177 when growing at first cohort rather than second cohort. At first cohort, resources (e.g., soil nutrients) and
178 conditions (e.g., light) were more timely available for both species, crop and weed. High biomass and
179 taller Palmer amaranth plants are likely a weed strategy to compete for light in between crop rows in
180 absence of canopy. In such condition, Palmer amaranth showed an extraordinary plasticity to adapt upon
181 the agroecosystem. This is evident when comparing Palmer amaranth canopy shape, and its extended
182 flowering pattern when growing into corn compared to soybean. The Palmer amaranth competition strategy
183 was to mimic the crop grow and development (Figure 4). These results suggests that Palmer amaranth
184 can fast evolve life-history traits to adapt into agroecosystems and cultural practices, including nitrogen
185 fertilization (Bravo et al., 2018). Our results highlight the Palmer amaranth threat to agroecosystems as
186 breeding more competitive crop varieties is likely to select more competitive biotypes (Bravo et al., 2017).

187 Palmer amaranth grow and development at second cohort was limited due to the crop competitive ability
188 at advanced development stages. Palmer amaranth was transplanted when corn canopy was nearly closed,
189 which reduced Palmer amaranth competitiveness. As a result, Palmer amaranth height and biomass was

lower compared to its first cohort. Under crop canopy (e.g., second cohort), Palmer amaranth flowering pattern was near to similar in corn and soybean. Palmer amaranth growing without crop competition produced highest amounts of biomass and less extended flowering pattern. The Palmer amaranth strategy in bareground was to invest biomass in growing plant width and height. Nonetheless, Palmer amaranth produced 21% less biomass in second cohort compared to first cohort timing. In a bareground study, early emerged Palmer amaranth without competition was 50% taller than late emerged plants (Webster and Grey, 2015). These results suggests that crop competition is not the only factor limiting late Palmer amaranth establishment. The limited growth of Palmer amaranth at second cohort is likely a reduced plant response to thermal units (e.g, growing degree days). It is hypothesize that reduced day length contributed to smaller plants at second cohort as well as shorter flowering period. A study in North Carolina and Illinois predicted that less than 10% Palmer amaranth seedlings emergence occurred after June (Piskackova et al., 2021). In addition, Palmer amaranth negative impact on soybean (Korres et al., 2020) and cotton (Webster and Grey, 2015) yields was higher when plants were established near to crop planting.

Seed production was not evaluated due to plant harvest at initiation of flowering. Nonetheless, it is well documented a strong positive correlation between Palmer amaranth biomass and seed production (Schwartz et al., 2016; Spaunhorst et al., 2018). In our study, Palmer amaranth growing at first cohort accumulated an overall 36% more biomass when compared to second cohort. Therefore, Palmer amaranth plants growing in the second cohort is likely to produce less seeds regardless the agroecosystem. Our observation is consistent with the findings that first Palmer amaranth cohort produced 50% more seeds per plant than Palmer amaranth plants established six weeks later in bareground (Webster and Grey, 2015). Still, seed production at second cohort is likely to replenish the soil seedbank. Seed production and deposition in the seedbank is also a key factor for species perpetuation (Menges, 1987). Palmer amaranth can produce hundred thousands seeds per plant (Schwartz et al., 2016; Keeley et al., 1987), and stay viable buried in the seedbank for at least 36 months (Sosnoskie et al., 2013). Therefore, preventing Palmer amaranth seed production or/and seed migration to its non-native habitat is an essential strategy to minimize weed impact into agroecosystem (Davis et al., 2015).

An ecological approach to reduce seed production in Palmer amaranth is understanding its flowering pattern. Our study suggests that Palmer amaranth flowering pattern was slightly influenced by cropping systems and cohort timings. Palmer amaranth growing in fallow and corn resulted in the overall shortest and longest flowering pattern, respectively. When growing in soybean, Palmer amaranth flowering pattern was similar to bareground at first cohort but similar to corn at second cohort timing. Plant flowering initiation is complex and it depends on biological and ecological factors (Lang, 1965). We hypothesize that when growing in high competition (e.g., second cohort), Palmer amaranth plants tend to initiate flowering early, as well as having an extended flowering pattern. Early flower initiation is plant strategy when growing in stress conditions. For example, when growing under water stress, early flowering in Palmer amaranth resulted in a mismatch between female and male plants by seven days (Mesgarian et al., 2021). A mismatch in Palmer amaranth male and female flowering period can minimize plant outcross, and thus reduce plant seed production. Sex dimorphism manipulation is considered a potential ecological pest control (McFarlane et al., 2018; Schliekelman et al., 2005).

The mechanisms of sex-determination in plant species is intriguing and arouse the curiosity of many scientists, including Darwin (Darwin, 1888). In our study, the gender model performance was decent considering the biology of plant flowering. A 1:1 male and female sex ratio is a general evolutionary stable strategy for plant species perpetuation (Fisher, 1930). However, a slight deviation from 1:1 sex ratio might occur in some dioecious species. For example, the dioecious *Halophila stipulacea* is a female-biased plant

234 in its native habitat, but the naturalized *H. stipulacea* have a 1:1 ratio (Nguyen et al., 2018). Naturalized of
235 *H. stipulacea* reduced female-male ratio to expand into its non-native habitat (Nguyen et al., 2018). Also,
236 biotic and/or abiotic stress can influence plant sex determination. Palmer amaranth male-to-female ratio was
237 greater under high plant densities (Korres and Norsworthy, 2017) and after herbicide application (Rumpa
238 et al., 2019). Our model estimated that late flowering, heavier and taller Palmer amaranth plants deviated
239 from 1:1 ratio in favor to female plants. It was reported that female Palmer amaranth plants invested more
240 in height, stem and biomass while male invested more in leaf area and leaf dry weight under nutrient
241 deficiency (Korres et al., 2017). Our model also estimated more female plants in soybean and bareground
242 compared to corn, which might be a result of plant biomass in each agroecosystem. Our results showed the
243 influence of life-history and ecological traits on sexual dimorphism in Palmer amaranth. Sexual dimorphism
244 is documented in other dioecious species (Barrett and Hough, 2013). For example, stronger female plant
245 competition and greater male tolerance to herbivory was reported in *Spinacia oleracea* (Pérez-Llorca
246 and Sánchez Vilas, 2019). Research on candidate genes for sex determination in *Amaranthus* species are
247 currently underway but it is far to complete (Montgomery et al., 2021, 2019). Further studies are also
248 needed to understand the ecological basis of Palmer amaranth flowering, including the plant behavior under
249 climate change.

250 Our study demonstrated the short-term Palmer amaranth plasticity to grow and develop into cropping-
251 systems. Palmer amaranth management priority should focus on minimizing Palmer amaranth dispersal
252 into new geographies. Flower manipulation is a promising ecological Palmer amaranth but it is not well
253 understood. Nonetheless, early-season management programs would have a large negative effect in Palmer
254 amaranth growth and development. Tactics that promote early-season crop advantage against Palmer
255 amaranth, including early crop planting, crop rotation (Oliveira et al., 2021), plant width, preemergence
256 applied herbicide (Sanctis et al., 2021), and crop residue (e.g. cover crops) would minimize the negative
257 impact of Palmer amaranth in agroecosystems.

DISCLOSURE/CONFLICT-OF-INTEREST STATEMENT

258 The authors declare that the research was conducted in the absence of any commercial or financial
259 relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

260 RW: designed the experiments; AJ, CP, MB, MO, and SS: conducted the experiments; MO: analyzed the
261 data and wrote the manuscript; AJ, CP, MB, MO, SS, and RW: conceptualized the research. All authors
262 reviewed the manuscript.

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1 SUPPLEMENTAL DATA

265 Supplementary Material should be uploaded separately on submission, if there are Supplementary Figures,
266 please include the caption in the same file as the figure. LaTeX Supplementary Material templates can be
267 found in the Frontiers LaTeX folder

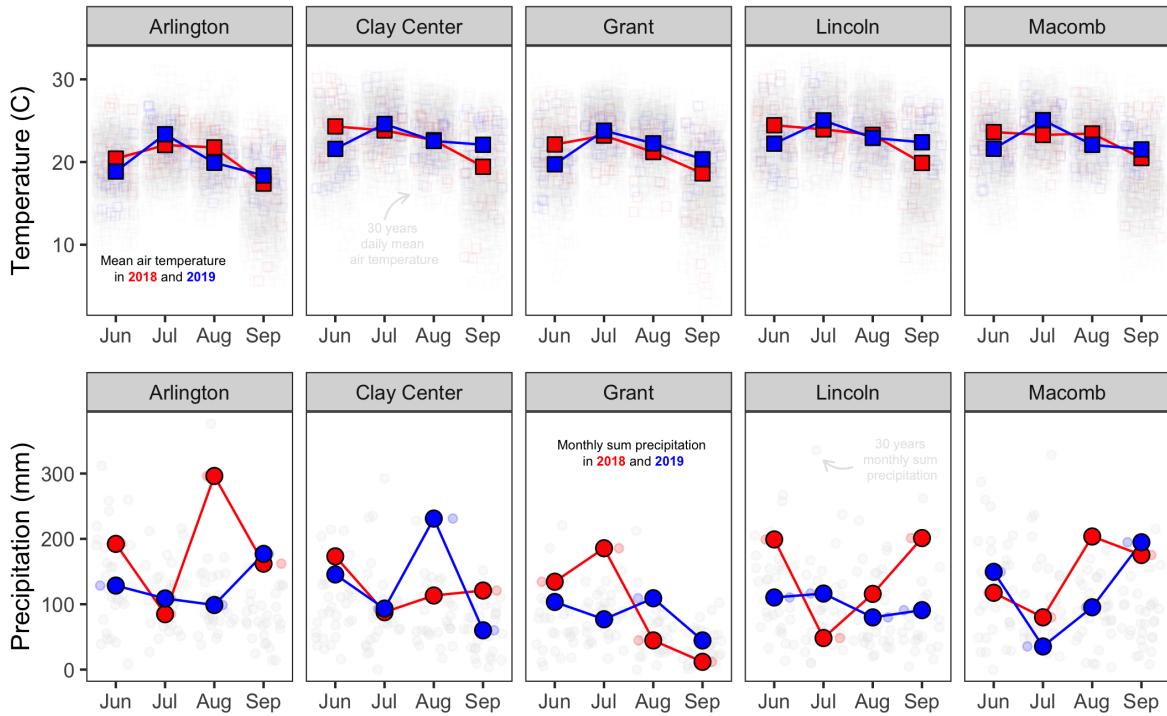


Figure 1. Mean average temperature (C) and monthly sum precipitation (mm) at Arlington, WI, Clay Center, NE, Grant, NE, Lincoln, NE and Macomb, IL

2 REFERENCES

268 A reference list should be automatically created here. However it won't. Pandoc will place the list of
 269 references at the end of the document instead. There are no convenient solution for now to force Pandoc to
 270 do otherwise. The easiest way to get around this problem is to edit the LaTeX file created by Pandoc before
 271 compiling it again using the traditional LaTeX commands.

FIGURES

- 272 Aulakh, J. S., Chahal, P. S., Kumar, V., Price, A. J., and Guillard, K. (2021). Multiple herbicide-resistant
 273 Palmer amaranth (*Amaranthus palmeri*) in Connecticut: Confirmation and response to POST herbicides.
 274 *Weed Technology* 35, 457–463. doi:10.1017/wet.2021.6.
- 275 Bagavathiannan, M. V., and Norsworthy, J. K. (2016). Multiple-Herbicide Resistance Is Widespread in
 276 Roadside Palmer Amaranth Populations. *PLOS ONE* 11, e0148748. doi:10.1371/journal.pone.0148748.
- 277 Baker, R. (2021). Comparative analysis of Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*A.
 278 Tuberculatus*) in Iowa. doi:10.31274/etd-20210609-11.
- 279 Bangdiwala, S. I. (2018). Regression: Binary logistic. *International Journal of Injury Control and Safety
 280 Promotion* 25, 336–338. doi:10.1080/17457300.2018.1486503.
- 281 Barrett, S. C. H., and Hough, J. (2013). Sexual dimorphism in flowering plants. *Journal of Experimental
 282 Botany* 64, 67–82. doi:10.1093/jxb/ers308.
- 283 Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using
 284 Lme4. *Journal of Statistical Software* 67, 1–48. doi:10.18637/jss.v067.i01.

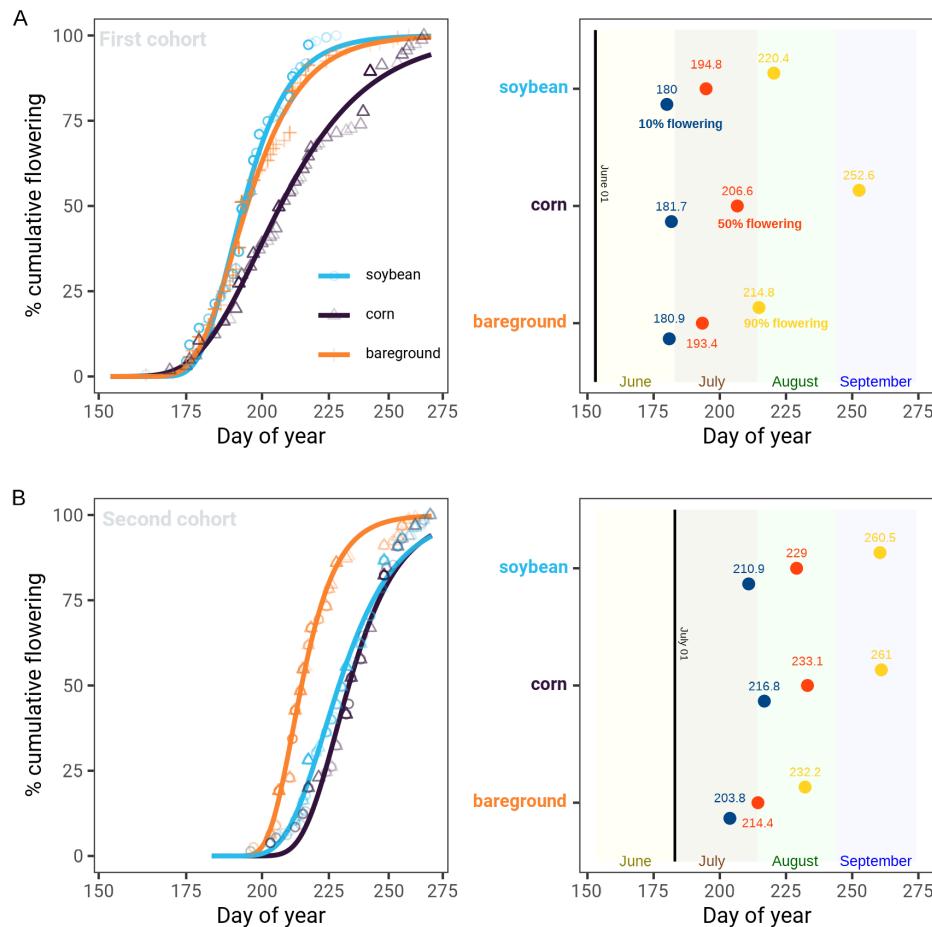


Figure 2. Cumulative flowering of Palmer amaranth at first and second transplant timing (A) and day of year of 10, 50, and 90 cumulative flowering at first and second transplant timing (B)

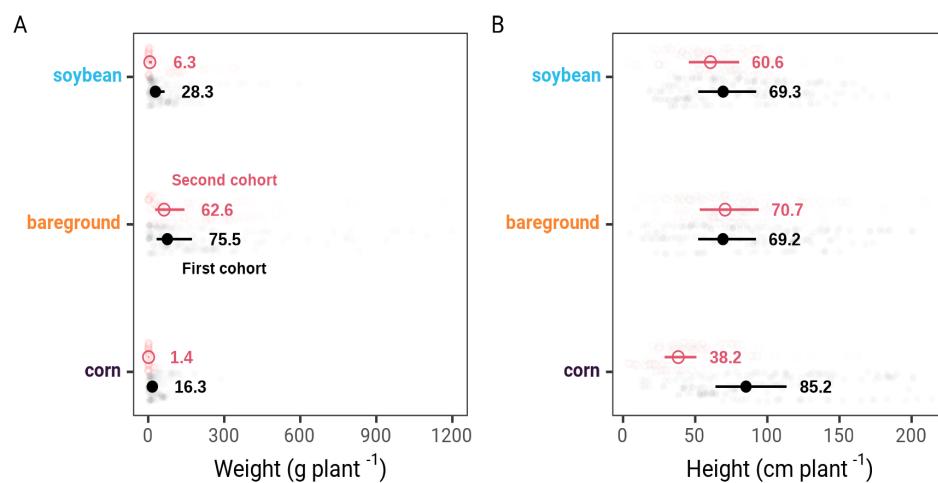


Figure 3. Palmer amaranth biomass (A) and height (B) growing in corn, fallow, and soybean across Arlington, Clay Center, Grant, Lincoln, and Macomb



Figure 4. Harvest Palmer amaranth plants at 40 days after first transplant timing. From left to right, Palmer amaranth growing in fallow, soybean and corn in Arlington, Wisconsin

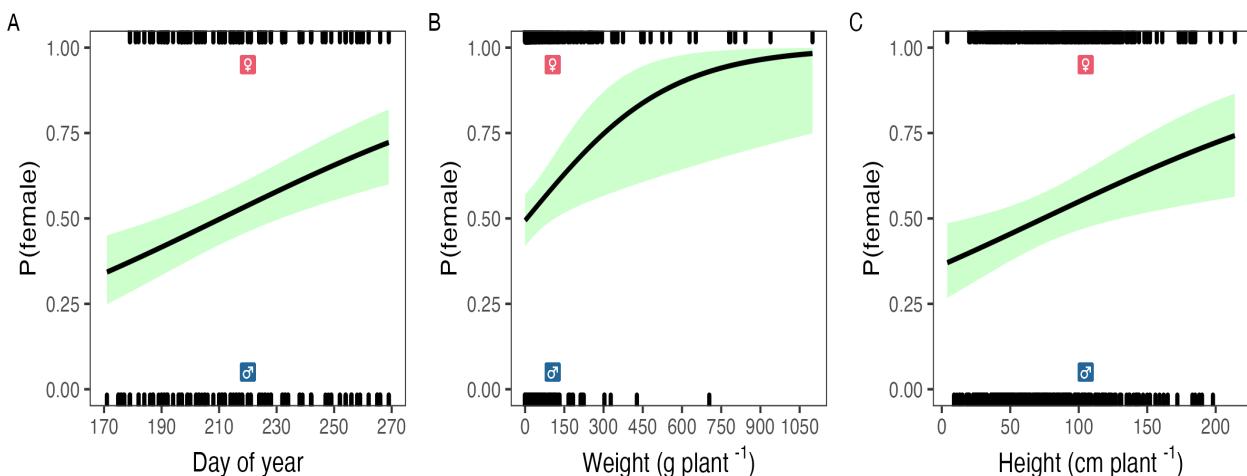


Figure 5. Cumulative flowering of Palmer amaranth at first and second transplant timing (A) and day of year of 10, 50, and 90 cumulative flowering at first and second transplant timing (B)

- 285 Berger, S. T., Ferrell, J. A., Rowland, D. L., and Webster, T. M. (2015). Palmer Amaranth (*Amaranthus*
286 *palmeri*) Competition for Water in Cotton. *Weed Science* 63, 928–935. doi:10.1614/WS-D-15-00062.1.
- 287 Bravo, W., Leon, R. G., Ferrell, J. A., Mulvaney, M. J., and Wood, C. W. (2017). Differentiation of
288 Life-History Traits among Palmer Amaranth Populations (*Amaranthus palmeri*) and Its Relation to
289 Cropping Systems and Glyphosate Sensitivity. *Weed Science* 65, 339–349. doi:10.1017/wsc.2017.14.
- 290 Bravo, W., Leon, R. G., Ferrell, J. A., Mulvaney, M. J., and Wood, C. W. (2018). Evolutionary Adaptations
291 of Palmer Amaranth (*Amaranthus palmeri*) to Nitrogen Fertilization and Crop Rotation History Affect
292 Morphology and Nutrient-Use Efficiency. *Weed Science* 66, 180–189. doi:10.1017/wsc.2017.73.
- 293 Briscoe Runquist, R. D., Lake, T., Tiffin, P., and Moeller, D. A. (2019). Species distribution models
294 throughout the invasion history of Palmer amaranth predict regions at risk of future invasion and reveal

- 295 challenges with modeling rapidly shifting geographic ranges. *Sci Rep* 9, 2426. doi:10.1038/s41598-
296 018-38054-9.
- 297 Chahal, P. S., Barnes, E. R., and Jhala, A. J. (2021). Emergence pattern of Palmer amaranth (*Amaranthus*
298 *palmeri*) influenced by tillage timings and residual herbicides. *Weed Technology* 35, 433–439.
299 doi:10.1017/wet.2020.136.
- 300 Chahal, P. S., Irmak, S., Jugulam, M., and Jhala, A. J. (2018). Evaluating Effect of Degree of Water Stress
301 on Growth and Fecundity of Palmer amaranth (*Amaranthus palmeri*) Using Soil Moisture Sensors.
302 *Weed Science* 66, 738–745. doi:10.1017/wsc.2018.47.
- 303 Correndo, A. A., Moro Rosso, L. H., and Ciampitti, I. A. (2021). Retrieving and processing agro-
304 meteorological data from API-client sources using R software. *BMC Research Notes* 14, 205.
305 doi:10.1186/s13104-021-05622-8.
- 306 Darwin, C. (1888). *The Different Forms of Flowers on Plants of the Same Species*. J. Murray Available at:
307 <http://books.google.com?id=7uMEAAAAAYAAJ>.
- 308 Davis, A. S., Schutte, B. J., Hager, A. G., and Young, B. G. (2015). Palmer Amaranth (*Amaranthus palmeri*)
309 Damage Niche in Illinois Soybean Is Seed Limited. *Weed Science* 63, 658–668. doi:10.1614/WS-D-14-
310 00177.1.
- 311 Farmer, J. A., Webb, E. B., Pierce, R. A., and Bradley, K. W. (2017). Evaluating the potential for weed seed
312 dispersal based on waterfowl consumption and seed viability. *Pest Management Science* 73, 2592–2603.
313 doi:10.1002/ps.4710.
- 314 Fisher, R. A. (1930). The genetical theory of natural selection. *Eugen Rev* 22, 127–130. Available at:
315 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2984947/> [Accessed August 12,
316 2021].
- 317 Fox, J., and Weisberg, S. (2018). *An R Companion to Applied Regression*. SAGE Publications Available at:
318 <http://books.google.com?id=uPNrDwAAQBAJ>.
- 319 Garetson, R., Singh, V., Singh, S., Dotray, P., and Bagavathiannan, M. (2019). Distribution of herbicide-
320 resistant Palmer amaranth (*Amaranthus palmeri*) in row crop production systems in Texas. *Weed
321 Technology* 33, 355–365. doi:10.1017/wet.2019.14.
- 322 Guo, P., and Al-Khatib, K. (2003). Temperature effects on germination and growth of redroot pigweed
323 (*Amaranthus retroflexus*), Palmer amaranth (A. *Palmeri*), and common waterhemp (*A. rudis*). *Weed
324 Science* 51, 869–875. doi:10.1614/P2002-127.
- 325 Hartzler, B., and Anderson, M. (2016). Palmer amaranth: It's here, now what? 10.
- 326 Heap, I. (2021). Internation Herbicide-Resistant Weed Database. Available at: <http://www.weedscience.org/Home.aspx> [Accessed July 26, 2021].
- 328 Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous Inference in General Parametric Models.
329 *Biometrical Journal* 50, 346–363. doi:10.1002/bimj.200810425.
- 330 Jha, P., Norsworthy, J. K., Riley, M. B., and Bridges, W. (2010). Annual Changes in Temperature and Light
331 Requirements for Germination of Palmer Amaranth (*Amaranthus palmeri*) Seeds Retrieved from Soil.
332 *Weed Science* 58, 426–432. doi:10.1614/WS-D-09-00038.1.

- 333 Keeley, P. E., Carter, C. H., and Thullen, R. J. (1987). Influence of Planting Date on Growth of Palmer
334 Amaranth (*Amaranthus palmeri*). *Weed Science* 35, 199–204. doi:10.1017/S0043174500079054.
- 335 Kistner, E. J., and Hatfield, J. L. (2018). Potential Geographic Distribution of Palmer
336 Amaranth under Current and Future Climates. *Agricultural & Environmental Letters* 3, 170044.
337 doi:10.2134/ael2017.12.0044.
- 338 Klingaman, T. E., and Oliver, L. R. (1994). Palmer Amaranth (*Amaranthus palmeri*) Interference in
339 Soybeans (*Glycine max*). *Weed Science* 42, 523–527. doi:10.1017/S0043174500076888.
- 340 Kohrt, J. R., Sprague, C. L., Nadakuduti, S. S., and Douches, D. (2017). Confirmation of a Three-Way
341 (Glyphosate, ALS, and Atrazine) Herbicide-Resistant Population of Palmer Amaranth (*Amaranthus*
342 *palmeri*) in Michigan. *Weed Science* 65, 327–338. doi:10.1017/wsc.2017.2.
- 343 Korres, N. E., and Norsworthy, J. K. (2017). Palmer Amaranth (*Amaranthus palmeri*) Demographic and
344 Biological Characteristics in Wide-Row Soybean. *Weed Science* 65, 491–503. doi:10.1017/wsc.2017.12.
- 345 Korres, N. E., Norsworthy, J. K., FitzSimons, T., Roberts, T. L., and Oosterhuis, D. M. (2017). Differential
346 Response of Palmer Amaranth (*Amaranthus palmeri*) Gender to Abiotic Stress. *Weed Science* 65,
347 213–227. doi:10.1017/wsc.2016.34.
- 348 Korres, N. E., Norsworthy, J. K., Mauromoustakos, A., and Williams, M. M. (2020). Soybean density and
349 Palmer amaranth (*Amaranthus palmeri*) establishment time: Effects on weed biology, crop yield, and
350 economic returns. *Weed Science* 68, 467–475. doi:10.1017/wsc.2020.41.
- 351 Küpper, A., Borgato, E. A., Patterson, E. L., Netto, A. G., Nicolai, M., Carvalho, S. J. P. de, Nissen, S.
352 J., Gaines, T. A., and Christoffoleti, P. J. (2017). Multiple Resistance to Glyphosate and Acetolactate
353 Synthase Inhibitors in Palmer Amaranth (*Amaranthus palmeri*) Identified in Brazil. *Weed Science* 65,
354 317–326. doi:10.1017/wsc.2017.1.
- 355 Lang, A. (1965). “Physiology of flower initiation,” in *Differenzierung und Entwicklung / Differentiation
356 and Development* Handbuch der Pflanzenphysiologie / Encyclopedia of Plant Physiology., ed. A. Lang
357 (Berlin, Heidelberg: Springer), 1380–1536. doi:10.1007/978-3-642-50088-6_39.
- 358 Larran, A. S., Palmieri, V. E., Perotti, V. E., Lieber, L., Tuesca, D., and Permingeat, H. R. (2017). Target-site
359 resistance to acetolactate synthase (ALS)-inhibiting herbicides in *Amaranthus palmeri* from Argentina.
360 *Pest Management Science* 73, 2578–2584. doi:10.1002/ps.4662.
- 361 Leeper, T. J. (2017). Interpreting Regression Results using Average Marginal Effects with R’s margins. 31.
- 362 Lindsay, K., Popp, M., Norsworthy, J., Bagavathiannan, M., Powles, S., and Lacoste, M. (2017). PAM:
363 Decision Support for Long-Term Palmer Amaranth (*Amaranthus palmeri*) Control. *Weed Technology*
364 31, 915–927. doi:10.1017/wet.2017.69.
- 365 MacLaren, C., Storkey, J., Menegat, A., Metcalfe, H., and Dehnen-Schmutz, K. (2020). An ecological
366 future for weed science to sustain crop production and the environment. A review. *Agron. Sustain. Dev.*
367 40, 24. doi:10.1007/s13593-020-00631-6.
- 368 MacRae, A. W., Webster, T. M., Sosnoskie, L. M., Culpepper, A. S., and Kichler, J. M. (2013). Cotton
369 Yield Loss Potential in Response to Length of Palmer Amaranth (*Amaranthus palmeri*) Interference.
370 17, 6.
- 371 Massinga, R. A., Currie, R. S., Horak, M. J., and Boyer, J. (2001). Interference of Palmer amaranth in corn.
372 *Weed Science* 49, 202–208. doi:10.1614/0043-1745(2001)049[0202:IOPAIC]2.0.CO;2.

- 373 Matzrafi, M., Osipitan, O. A., Ohadi, S., and Mesgaran, M. B. (2021). Under pressure: Maternal effects
374 promote drought tolerance in progeny seed of Palmer amaranth (*Amaranthus palmeri*). *Weed Science*
375 69, 31–38. doi:10.1017/wsc.2020.75.
- 376 McFarlane, G. R., Whitelaw, C. B. A., and Lillico, S. G. (2018). CRISPR-Based Gene Drives for Pest
377 Control. *Trends in Biotechnology* 36, 130–133. doi:10.1016/j.tibtech.2017.10.001.
- 378 Menges, R. M. (1987). Weed Seed Population Dynamics during Six Years of Weed Management Systems
379 in Crop Rotations on Irrigated Soil. *Weed Science* 35, 328–332. Available at: <http://www.jstor.org/stable/4044593>.
- 380
- 381 Mesgaran, M. B., Matzrafi, M., and Ohadi, S. (2021). Sex dimorphism in dioecious Palmer amaranth
382 (*Amaranthus palmeri*) in response to water stress. *Planta* 254, 17. doi:10.1007/s00425-021-03664-7.
- 383 Milani, A., Panizzo, S., Farinati, S., Iamonico, D., Sattin, M., Loddo, D., and Scarabel, L. (2021). Recent
384 Discovery of *Amaranthus palmeri* S. Watson in Italy: Characterization of ALS-Resistant Populations
385 and Sensitivity to Alternative Herbicides. *Sustainability* 13, 7003. doi:10.3390/su13137003.
- 386 Montgomery, J. S., Giacomini, D. A., Weigel, D., and Tranel, P. J. (2021). Male-specific Y-chromosomal
387 regions in waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*). *New*
388 *Phytologist* 229, 3522–3533. doi:10.1111/nph.17108.
- 389 Montgomery, J. S., Sadeque, A., Giacomini, D. A., Brown, P. J., and Tranel, P. J. (2019). Sex-specific
390 markers for waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*). *Weed*
391 *Science* 67, 412–418. doi:10.1017/wsc.2019.27.
- 392 Morgan, G. D., Baumann, P. A., and Chandler, J. M. (2001). Competitive Impact of Palmer Amaranth
393 (*Amaranthus palmeri*) on Cotton (*Gossypium hirsutum*) Development and Yield. *Weed Technology* 15,
394 408–412. doi:10.1614/0890-037X(2001)015[0408:CIOPAA]2.0.CO;2.
- 395 Nguyen, H. M., Kleitou, P., Kletou, D., Sapir, Y., and Winters, G. (2018). Differences in flowering sex
396 ratios between native and invasive populations of the seagrass *Halophila stipulacea*. *Botanica Marina*
397 61, 337–342. doi:10.1515/bot-2018-0015.
- 398 Oliveira, M. C., Gaines, T. A., Patterson, E. L., Jhala, A. J., Irmak, S., Amundsen, K., and Knezevic, S.
399 Z. (2018). Interspecific and intraspecific transference of metabolism-based mesotrione resistance in
400 dioecious weedy *Amaranthus*. *The Plant Journal* 96, 1051–1063. doi:10.1111/tpj.14089.
- 401 Oliveira, M. C., Giacomini, D. A., Arsenijevic, N., Vieira, G., Tranel, P. J., and Werle, R. (2021).
402 Distribution and validation of genotypic and phenotypic glyphosate and PPO-inhibitor resistance in
403 Palmer amaranth (*Amaranthus palmeri*) from southwestern Nebraska. *Weed Technology* 35, 65–76.
404 doi:10.1017/wet.2020.74.
- 405 Page, E. R., Nurse, R. E., Meloche, S., Bosveld, K., Grainger, C., Obeid, K., Filotas, M., Simard, M.-J., and
406 Laforest, M. (2021). Import of Palmer amaranth (*Amaranthus palmeri* S. Wats.) Seed with sweet potato
407 (*Ipomea batatas* (L.) Lam) slips. *Can. J. Plant Sci.*, CJPS-2020-0321. doi:10.1139/CJPS-2020-0321.
- 408 Pérez-Llorca, M., and Sánchez Vilas, J. (2019). Sexual dimorphism in response to herbivory and
409 competition in the dioecious herb *Spinacia oleracea*. *Plant Ecol* 220, 57–68. doi:10.1007/s11258-
410 018-0902-7.

- 411 Piskackova, T. A. R., Reberg-Horton, S. C., Richardson, R. J., Jennings, K. M., Franca, L., Young, B. G.,
412 and Leon, R. G. (2021). Windows of action for controlling palmer amaranth (*Amaranthus palmeri*)
413 using emergence and phenology models. *Weed Research* 61, 188–198. doi:10.1111/wre.12470.
- 414 Price, A. J., Balkcom, K. S., Culpepper, S. A., Kelton, J. A., Nichols, R. L., and Schomberg, H. (2011).
415 Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. *Journal of Soil and Water
416 Conservation* 66, 265–275. doi:10.2489/jswc.66.4.265.
- 417 Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. (2015). Dose-Response Analysis Using R. *PLOS ONE*
418 10, e0146021. doi:10.1371/journal.pone.0146021.
- 419 Rumpa, M. M., Krausz, R. F., Gibson, D. J., and Gage, K. L. (2019). Effect of PPO-Inhibiting Herbicides
420 on the Growth and Sex Ratio of a Dioecious Weed Species *Amaranthus palmeri* (Palmer Amaranth).
421 *Agronomy* 9, 275. doi:10.3390/agronomy9060275.
- 422 Sanctis, J. H. S. de, Barnes, E. R., Knezevic, S. Z., Kumar, V., and Jhala, A. J. (2021). Residual herbicides
423 affect critical time of Palmer amaranth removal in soybean. *Agronomy Journal* 113, 1920–1933.
424 doi:10.1002/agj2.20615.
- 425 Sauer, J. (1957). Recent Migration and Evolution of the Dioecious Amaranths. *Evolution* 11, 11–31.
426 doi:10.2307/2405808.
- 427 Sauer, J. D. (1972). The dioecious amaranths: A new species name and major range extensions. *Madroño*
428 21, 426–434. Available at: <http://www.jstor.org/stable/41423815>.
- 429 Schliekelman, P., Ellner, S., and Gould, F. (2005). Pest Control by Genetic Manipulation of Sex Ratio.
430 *Journal of Economic Entomology* 98, 18–34. doi:10.1093/jee/98.1.18.
- 431 Schwartz, L. M., Norsworthy, J. K., Young, B. G., Bradley, K. W., Kruger, G. R., Davis, V. M., Steckel,
432 L. E., and Walsh, M. J. (2016). Tall Waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth
433 (*Amaranthus palmeri*) Seed Production and Retention at Soybean Maturity. *Weed Technology* 30,
434 284–290. doi:10.1614/WT-D-15-00130.1.
- 435 Scott, D. (2011). The Technological Fix Criticisms and the Agricultural Biotechnology Debate. *J Agric
436 Environ Ethics* 24, 207–226. doi:10.1007/s10806-010-9253-7.
- 437 Sosnoskie, L. M., Webster, T. M., and Culpepper, A. S. (2013). Glyphosate Resistance Does Not
438 Affect Palmer Amaranth (*Amaranthus palmeri*) Seedbank Longevity. *Weed Science* 61, 283–288.
439 doi:10.1614/WS-D-12-00111.1.
- 440 Spaunhorst, D. J., Devkota, P., Johnson, W. G., Smeda, R. J., Meyer, C. J., and Norsworthy, J. K. (2018).
441 Phenology of Five Palmer amaranth (*Amaranthus palmeri*) Populations Grown in Northern Indiana and
442 Arkansas. *Weed Science* 66, 457–469. doi:10.1017/wsc.2018.12.
- 443 Wang, J. L., Klessig, D. F., and Berry, J. O. (1992). Regulation of C4 Gene Expression in Developing
444 Amaranth Leaves. *The Plant Cell* 4, 173–184. doi:10.1105/tpc.4.2.173.
- 445 Ward, S. M., Webster, T. M., and Steckel, L. E. (2013). Palmer Amaranth (*Amaranthus palmeri*): A Review.
446 *Weed Technology* 27, 12–27. doi:10.1614/WT-D-12-00113.1.
- 447 Webster, T. M., and Grey, T. L. (2015). Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*)
448 Morphology, Growth, and Seed Production in Georgia. *Weed Science* 63, 264–272. doi:10.1614/WS-D-
449 14-00051.1.

- 450 Yu, E., Blair, S., Hardel, M., Chandler, M., Thiede, D., Cortilet, A., Gunsolus, J., and Becker, R. (2021).
451 Timeline of Palmer amaranth (*Amaranthus palmeri*) invasion and eradication in Minnesota. *Weed*
452 *Technology*, 1–31. doi:10.1017/wet.2021.32.