

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

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 *A. palmeri* accession (Per1) from Perkins County, Nebraska. Per1
74 accession collection is documented with no reported herbicide resistance (Oliveira et al., 2021). Three
75 weeks prior to the field experiment, seeds were planted in plastic trays containing potting-mix. Emerged
76 seedlings (1 cm) were transplanted into 200 cm⁻³ plastic pots (a plant pot-1). Palmer amaranth seedlings
77 were supplied with adequate water and kept under greenhouse conditions at Arlington, Clay Center, Lincoln,
78 and Macomb; and kept outdoors in Grant. Palmer amaranth seedlings were kept under greenhouse/outdoors
79 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⁻¹), or under fallow condition. Palmer amaranth seedlings (potting mix + two seedlings) were and gently
90 transferring to the ground (6 cm deep and 8 cm wide). Twenty-four plants were equidistantly placed (0.76
91 m apart) between rows within each agroecosystems. After a week, one was eliminated and one was kept.
92 There were two transplant timing: first (June 1) and second (July 1). There were 24 Palmer amaranth plants
93 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 cumulative flowering

137 Palmer amaranth growing in corn resulted in a longer flowering pattern compared to fallow and soybean
138 at first cohort (Figure 2A). Nonetheless, the 10% cumulative Palmer amaranth flowering in soybean, fallow
139 and corn occurred at the end of June. Palmer amaranth reached 10% flowering in soybean at day 180, which
140 was slightly different from fallow (day 180.9; $P = 0.01$) and corn (day 181.7; $P = 0.00$). The 50% Palmer
141 amaranth cumulative flowering occurred in July. For example, Palmer amaranth reached 50% flowering in
142 fallow at day 193.4, followed by soybean (day 194.8), corn (day 206.6). Similar trend was observed at
143 90% Palmer amaranth cumulative flowering. Palmer amaranth growing in corn reached 90% flowering
144 at day 252.6 (early September), which was 37.8 and 32.2 days after Palmer amaranth 90% flowering in
145 fallow and soybean, respectively.

146 Palmer amaranth cumulative flowering at second cohort ranged from mid July to mid September (Figure
147 2B). Palmer amaranth growing in fallow resulted in earlier flowering time compared to soybean and corn.
148 Palmer amaranth growing in fallow reached 10%, 50%, and 90% flowering time at day 203.8, 214.4, and

149 232.2, respectively. Palmer amaranth growing in soybean reached 10% flowering at doy 210.9, which
150 was 6 days prior to corn (P -value = 0.00). Similar trend was observed at 50% flowering, whereas Palmer
151 amaranth reached 50% flowering in corn (doy 233.0) 4 days after soybeans (doy 228.9; P = 0.00). The 90%
152 Palmer amaranth cumulative flowering occurred at same day in corn (260.9) and soybean (260.5; P = 0.66).

153 **Palmer amaranth gender**

154 The model fit was 0.11, 0.14, 0.18 with using pseudo R squared test from McFadden, Cox and Snell, and
155 Cragg and Uhler, respectively. The likelihood ratio test showed a p-value of < 0.00. The average marginal
156 effects showed that increasing a weight unit by 1 g increases the probability of having a female plant by
157 0.08% (P = 0.01). Similar trend is observed to height as well as doy, whereas the probability of being
158 female increase by 0.2% (P = 0.01) and 0.4% (P = 0.00) when a unit of height (cm) and day increases,
159 respectively.

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

163 **Palmer amaranth height and biomass**

164 Palmer amaranth accumulated more biomass when growing in fallow compared to Palmer amaranth
165 growing in soybean and corn (Figure 3A). At first cohort time, Palmer amaranth biomass was 75.5 g plant-1,
166 28.3 g plant-1 and 16.3 g plant-1 in fallow, soybean and corn, respectively. At second cohort timing, Palmer
167 amaranth produced 62.6 g plant in fallow, followed by 6.3 g plant in soybean, and 1.4 g plant in corn.

168 Palmer amaranth height was more uniform across cohort timings, except when growing in corn (Figure
169 3B). Palmer amaranth achieve achieve 69.2 cm tall when growing at first cohort timing in bareground,
170 which was not different to 70.7 cm tall at second cohort timing (P = 0.74). In addition, no difference in
171 Palmer amaranth height (69.3 cm) was found in soybean at first cohort compared to fallow (P > 0.75). At
172 second cohort in soybean, Palmer amaranth was near to 10 cm lower compared to its first cohort time (P =
173 0.04). The tallest and smallest Palmer amaranth plants were found in corn. Palmer amaranth reached 85.2
174 cm tall at first cohort and 38.2 cm at second cohort timings.

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. Palmer amaranth was taller and thinner
183 when growing within corn. The Palmer amaranth competition strategy was to mimic the crop grow and
184 development (Figure 4). These results suggests that Palmer amaranth can fast evolve life-history traits
185 to adapt into agroecosystems and cultural practices, including nitrogen fertilization (Bravo et al., 2018).
186 Our result highlight the threat of Palmer amaranth to agroecosystems as breeding more competitive crop
187 varieties is likely to select more competitive biotypes (Bravo et al., 2017).

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

lower compared to its first cohort. Under crop canopy, 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 a key 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 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 under stress. For example, when growing under water stress, early flowering in Palmer amaranth resulted in a mismatch between female and male plants by seven days (Mesgaran 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

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

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

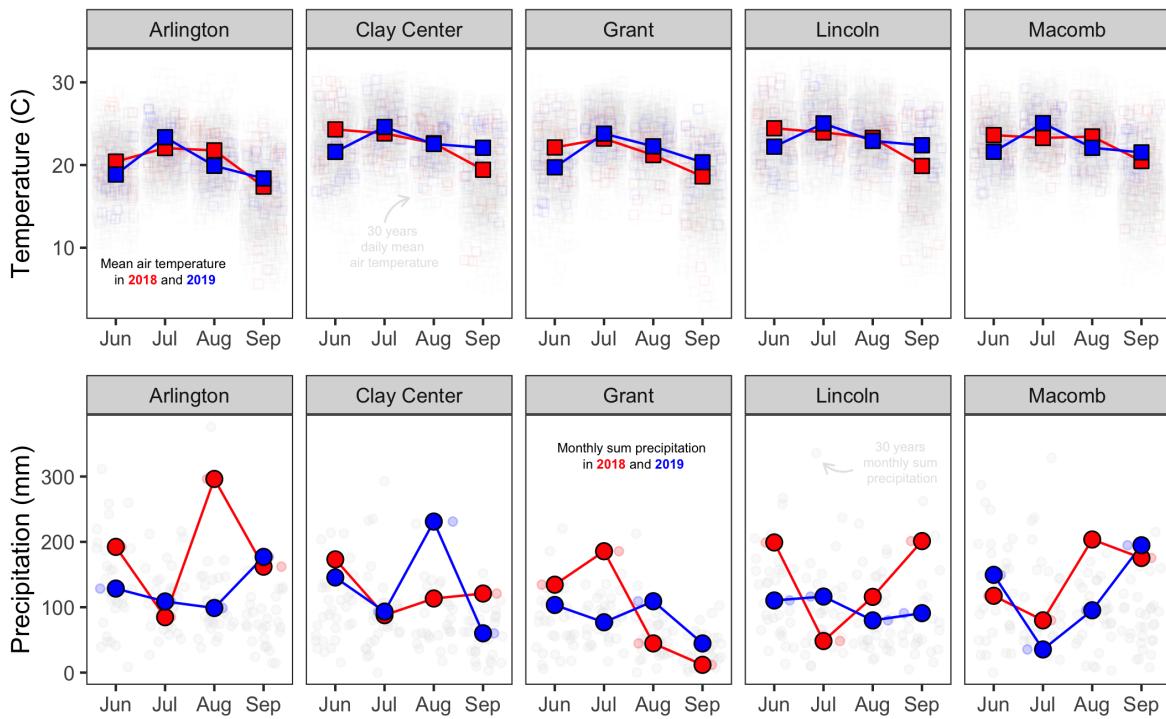


Figure 1. Mean average temperature (C) and monthly sum precipitation (mm) at Arlington, WI, Clay Center, NE, Grant, NE, Lincoln, NE and Macomb, IL

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

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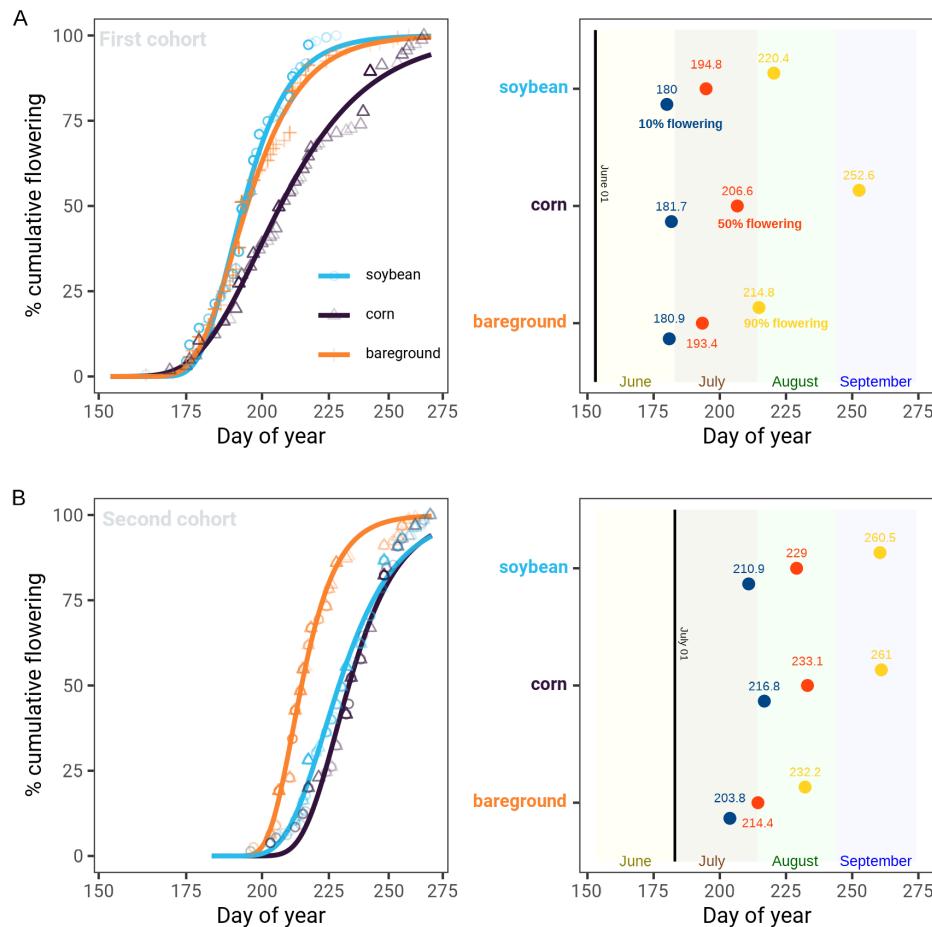


Figure 2. Cumulative flowering of Palmer amaranth at first and second transplant timing (A) and day of year of 10, 50, and 90 cumulative flowering at first and second transplant timing (B)

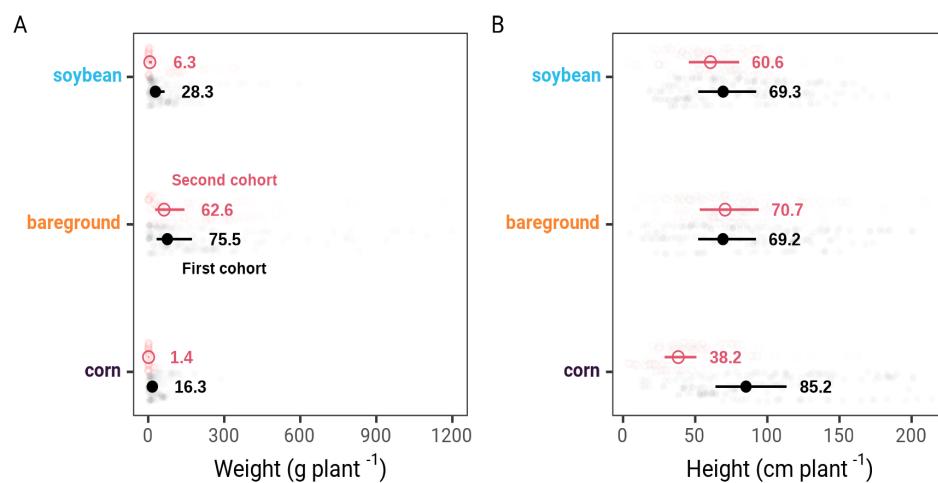


Figure 3. Palmer amaranth biomass (A) and height (B) growing in corn, fallow, and soybean across Arlington, Clay Center, Grant, Lincoln, and Macomb



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

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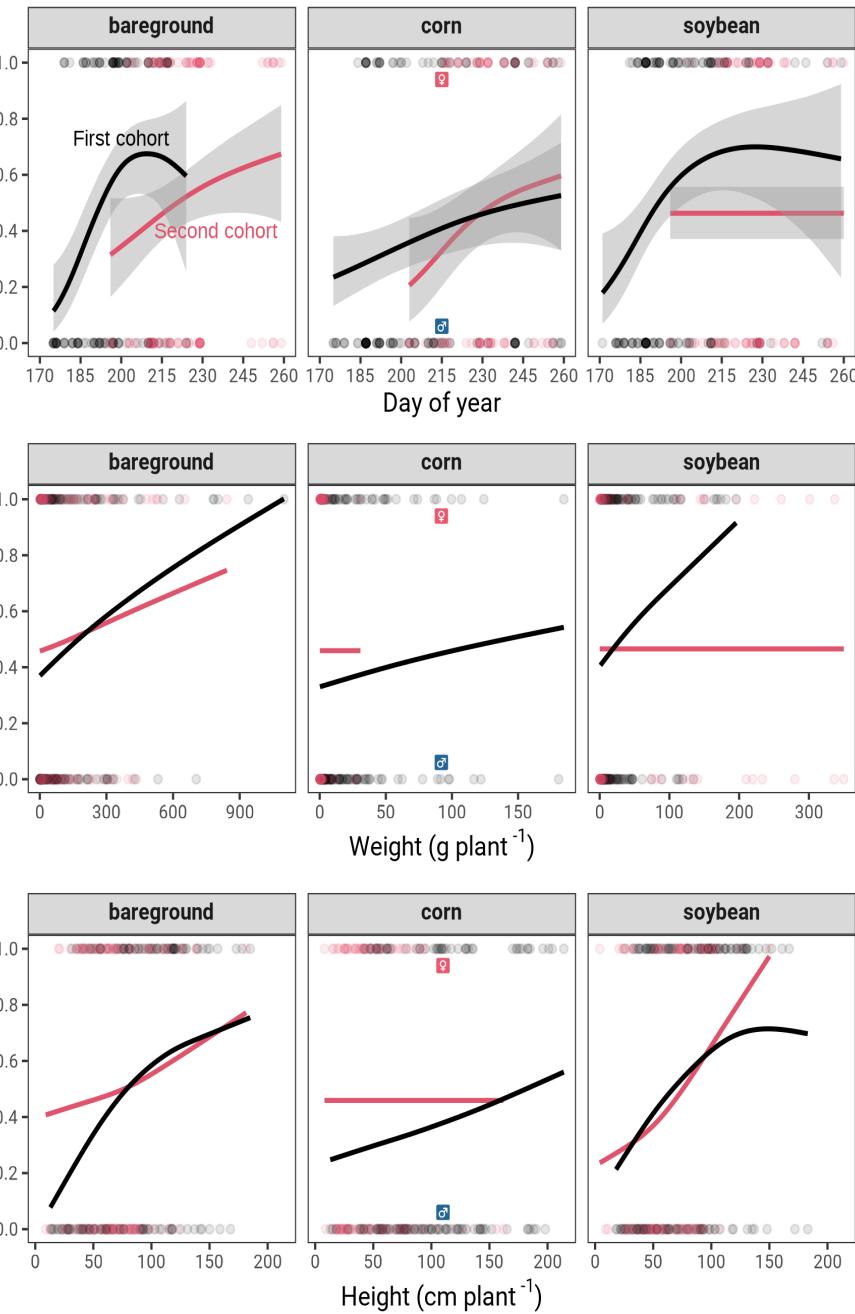


Figure 5. Cumulative flowering of Palmer amaranth at first and second transplant timing (A) and day of year of 10, 50, and 90 cumulative flowering at first and second transplant timing (B)

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