doi:10.20944/preprints202201.0329.v1

Article

Transpiration responses of herbicide-resistant and -susceptible Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] to progressively drying soil

Koffi Badou-Jeremie Kouame[1], Mary C. Savin[1], Gulab Rangani[1], Thomas R. Butts[2], Matthew B. Bertucci[3], and Nilda Roma-Burgos[1]*

[1] Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR 72704; kbkouame@uark.edu (KB-JK); msavin@uark.edu (MCS); grangani@uark.edu (GR); nburgos@uark.edu (NR-B)
[2] Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Lonoke, AR 72086; tbutts@uada.edu (TRB)
[3] Department of Horticulture, University of Arkansas, Fayetteville, AR 72701; bertucci@uark.edu (MBB)
* Correspondence: nburgos@uark.edu

Abstract: Drought events are predicted to become more prevalent in the future. Evaluating the performance of herbicide-resistant and susceptible weed ecotypes to progressive drought can provide insights into whether resistance trait(s) increased or reduced the fitness of a resistant population. Two experiments were conducted in the greenhouse between January and May 2021 to evaluate drought tolerance differences between Palmer amaranth accessions resistant to *S*-metolachlor or glyphosate and their susceptible counterparts. The accessions used were: *S*-metolachlor-resistant (17TUN-A), a susceptible standard (09CRW-A), and glyphosate-resistant (22 to 165 EPSPS copies) and glyphosate-susceptible (3 to 10 EPSPS copies) plants from accession 16CRW-D. Daily transpiration of each plant was measured. The daily transpiration rate was converted to normalized transpiration ratio (NTR) using a double-normalization procedure. The daily soil water content was expressed as a fraction of transpirable soil water (FTSW). The threshold FTSW (FTSWcr), after which NTR decreases linearly, was estimated using a two-segment linear regression analysis. The data showed differences between *S*-metolachlor- resistant and -susceptible accessions ($P \leq 0.05$). The FTSW remaining in the soil at the breakpoint for the *S*-metolachlor-susceptible accession (09CRW-A) was 0.17±0.007. The FTSW remaining in the soil at the breakpoint for the *S*-metolachlor-resistant accession (17TUN-A) was 0.23±0.004. Although the mechanism endowing resistance to *S*-metolachlor might have contributed to increased drought tolerance, follow-up experiments are needed to verify this finding. Increased *EPSPS* copy number did not improve drought tolerance of Palmer amaranth.

Keywords: metabolic resistance, drought tolerance, threshold FTSW, stomatal closure, EPSPS gene amplification, *S*-metolachlor resistance, glyphosate resistance

1. Introduction

Drought can negatively affect physiological and biochemical processes and cause yield reduction [1]. As the world's largest exporter of major grain and oilseed crops, the United States accounted for 49% and 46% of total global exports of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.], respectively, between 2008 and 2010 [2]. However, the 'flash drought' event in the US in 2012 disrupted the production of major crops and affected international grain markets [2]. This most detrimental drought of the past century in the US Midwest had greater impact on agricultural systems (crop and grassland) than on forests [3]. Drought frequency and severity are likely to increase in the future [3-5]. Consequently, yields of crops such as corn and soybean are predicted to decline by 8-21% [6]. Under rainfed conditions, seasonal water supply and soil water holding capacity are major determinants of crop productivity [7]. The largest corn and soybean production area, the US Corn Belt, is 92% rainfed; thus, vulnerable to drought occurrences [8]. Also, under irrigated conditions, irrigation does not always fully supply crop water demand during drought [9].

Evapotranspiration (ET), the sum of water used by plants via transpiration and direct soil water loss via evaporation, informs us about soil moisture availability [10]. Plant transpiration is a key component of soil water consumption; it is a crucial physiological process that is strongly related to biomass production [11]. Under drought, plants can sense water stress around the roots and respond by sending chemical signals such as increased abscisic acid (ABA) production to close the stomates [12]. The determination of the threshold value for the initiation of stomatal closure is critical for understanding plant physiological responses to drought [13]. A major challenge in studying plant responses to soil drying is the ability to characterize soil water content in a way that is relevant to biological processes [14]. The simple method of volumetric measure of soil water content was reported to be a good predictor of physiological response in plants [15-19]].

One useful parameter to monitor soil drying and corresponding plant response to progressive drought stress is the fraction of transpirable soil water (FTSW). This is the amount of water available to plants at any given time in the drying cycle relative to the total amount of water available for transpiration at the pot-holding capacity. Plant transpiration in response to a drying soil has been well characterized by previous research and reported to display two phases: (1) the initial plateau where transpiration is optimal and (2) a linear decline in response to a drying soil. These phases are connected by a breakpoint also known as threshold value for the initiation of stomatal closure [15,16,18,19]. The threshold value (FTSWcr) is a crucial parameter for comparing populations, ecotypes, or genotypes. Differences in threshold values at which transpiration begins to decline can provide valuable information about plant water use pattern and stomatal closure during a drying cycle [16,17,19]. Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] has the ability to adapt to various stress conditions [20]. It uses osmoregulation to keep stomates open during drought to continue carbon fixation [21]. This trait may be modified by biochemical, physiological, or structural modifications in the plant associated with resistance to herbicides.

Previous research documented the association of herbicide resistance traits with fitness cost and competitive disadvantages in the absence of herbicide selection pressure that favors resistant plants [22,23]. Additionally, gene families (P450s and GSTs) involved in non-target site resistance (NTSR) to herbicides play a role in plant protection against, and survival in, adverse environmental conditions [24]. In Arkansas, Palmer amaranth has evolved target-site resistance to glyphosate due to EPSPS (5-enolpyruvyl-shikimate-3-phosphate synthase) gene amplification [25] and NTSR to *S*-metolachlor via upregulation of GSTs [26,27]. Harboring these mechanisms may impart some latent benefits such as increased tolerance to abiotic stress. One indicator of this is adjustment in transpiration rate under drought stress. The objective of this research was to quantify the transpiration changes that occur in herbicide-resistant and- susceptible Palmer amaranth accessions submitted to a progressive drying cycle.

2. Materials and Methods

Two experiments were conducted in the greenhouse from January to May 2021 at the Milo J. Shult Agricultural Research & Extension Center, University of Arkansas, Fayetteville and were repeated in time. The experiments involved accessions resistant and susceptible to *S*-metolachlor as well as accessions resistant and susceptible to glyphosate. Resistance to *S*-metolachlor was due to GST overexpression [27] and resistance to glyphosate was due to EPSPS amplification [25].

2.1. Comparison of transpiration rate between glyphosate-resistant and -susceptible plants that differ in EPSPS copy number

Plant material preparation

Glyphosate-resistant Palmer amaranth accession 16CRW-D was collected from Crawford County (Arkansas) in 2016. A total of 100seeds were planted in two, 50-cell trays filled with Sunshine ® Premix #1 (Sun Gro Horticulture, Bellevue, WA). All healthy seedlings (8-cm tall) were transplanted to 13 cm x 11 cm (diameter-by-depth) pots filled with the same soil. Plants were fertilized weekly using a water-soluble, all-purpose plant food (Scotts Miracle-Gro Products, Marysville, OH) containing 24% N, 8% P, and 16% K.

Leaf tissue was collected from each plant to determine the relative *EPSPS* gene copy number using the method described by [25]. Briefly, genomic DNA was extracted from approximately 100 mg of leaf tissue using a modified CTAB protocol and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). The *EPSPS* copy number was determined by quantitative real-time polymerase chain reaction (qPCR) relative to the A36 gene [25]. The primers were: A36_F244 (5′TTGGAACTGTC AGAGCAACC3′) and A36_R363 (5′GAACCCACTT CCA CCAAAAC3′) for the reference gene and EPSPS1CNF (5′ATGTTGGACGCT CTCAGAACTCTTGGT3′) and EPSPS1CNR (5′TGAATTTCCTCCAGCAACGGCAA3′) for *EPSPS*. Two technical replicates of genomic DNA template (20 ng) were amplified in a 10-μL reaction volume using Sybr-Green master mix (Bio-Rad, Hercules, CA). For the qPCR, the 10-μL reactions were prepared using a Master-mix composed of 5 μL SYBR-itaq (Bio-Rad, Hercules, CA), 0.5 μL of 5 μM forward primer, 0.5 μL of 5 μM reverse primer, 3 μL water and 1 μL gDNA. The thermoprofile was 95°C for 15 min, then 30 cycles of 95°C for 30 s and 60°C for 1 min. Melt-curve analysis was conducted by holding the samples at 95°C for 5 min, then reducing the temperature to 55°C for 5 min, followed by increasing the temperature by 0.5°C every 10 s up to 95°C.

Twenty-four plants of similar size (25 cm tall; 12 with increased *EPSPS* copy) were transplanted into pots, 19 cm diameter x 17 cm deep, at the same depth of 11 cm. Each pot contained 2.5 kg of 1:1 mixture of field soil and Sunshine ® Premix #1 (Sun Gro Horticulture, Bellevue, WA). The field soil, a Roxana silt loam (USDA-SCS, 1979) had pH 7.3, 112 μmhos/cm EC, 0.04% total N and 0.4% total C, was collected from the Vegetable Research Station of the University of Arkansas, Kibler, Arkansas.

2.2. Differences in drought tolerance between S-metolachlor-susceptible and -resistant accessions

Plant material preparation

The resistant and susceptible accessions referred to as 17TUN-A and 09CRW-A, respectively, were used for the experiment. Accession 17TUN-A showed resistance to *S*-metolachlor and resistant plants required up to 5 times more *S*-metolachlor to reduce seedling emergence 50% (Kouame et al., unpublished). Seeds of accessions 09CRW-A and 17TUN-A were collected in Crawford County (Arkansas) in 2009 and Tunica County (Mississippi) in 2017, respectively. One hundred seeds of each accession, 09CRW-A and 17TUN-A, were grown in 50-cell tray using commercial soil as described in experiment 1. Seedlings (8-cm tall) were transplanted and maintained as described previously. Later, plants of similar size (25 cm tall) were transplanted into larger pots, 19-cm diameter x 17-cm deep, filled with 2.5 kg of 1:1 mixture of field soil and Sunshine ® Premix #1.

2.3. Dry-down treatment

The experiment was conducted as a completely randomized design with six replications (Figure 1) and pots were rerandomized every other day during the experiment.

Figure 1. Set-up of greenhouse experiment to evaluate the transpiration responses of herbicide-resistant and -susceptible Palmer amaranth [*Amaranthus palmeri* (S.) Wats.] to progressively drying soil, at the Milo J. Shult Agricultural Research & Extension Center, University of Arkansas, Fayetteville in 2021.

The drought factor had two levels (well-watered and water-deficit). The method used was adapted from previous research [28]. The plants were watered daily and weekly fertilized with a water-soluble, all-purpose plant food (Scotts Miracle-Gro Products, Marysville, OH) until dry down imposition. The evening before starting dry down, pots were saturated and allowed to drain overnight. The pots were enclosed in black plastic bags [19,29] and each bag opening was sealed around the plant stem with twist ties to minimize evaporation. A 6-mL syringe barrel was inserted between the base of the plant and the plastic bag for water replenishment. Newly bagged pots were weighed to obtain an estimate of gravimetric water content at water holding capacity. The pots were weighed daily at 4 pm and in the same order for the duration of the experiment. Daily transpiration was calculated as the difference in mass of each pot on successive days. To maintain well-watered conditions but prevent anaerobic conditions in the control pots [18], the plants were maintained at 80% of well-watered pot-capacity weight. For the water stress treatments, the six plants of each accession were watered to a target level of 50 mL below the amount of water lost via transpiration in the past 24 h, starting at the beginning of drought stress treatment. Five pots with water-saturated soil in the greenhouse, without plants, were used to estimate daily evaporation rate.

2.3.1. Daily transpiration and normalized transpiration ratio

The transpiration data were analyzed using a double normalization procedure [15,18]. A first normalization minimized the influence of daily fluctuations in transpiration on the transpiration rate (TR) of stressed plants across days [15,19], by normalizing daily transpiration rates for the stressed plants divided by the average for non-stressed plants for the same day [18] using equation 1:

TR of stressed plant = (Transpiration of stressed plant)/(Average transpiration of control plant)

[1]

The second normalization, normalized transpiration ratio (NTR), allowed the normalized transpiration rate of each plant to be centered on a value of 1. A mean TR was calculated for each plant for the first 3 d of the experiment when the soil water content in each pot was high [15,19]. The daily TR for each stressed plant was divided by the mean TR of the same plant during the well-watered stage to give a daily normalized transpiration ratio (NTR) [17-19] as shown in equation 2.

NTR = (Daily TR)/(Average TR of the first 3 d) [2]

The treatments were maintained for each resistant or susceptible accession or plant until NTR value dropped below 0.1, defined as the endpoint of the drying cycle [15,19].

2.3.2. Drought stress level in the root zone

The FTSW was defined as the amount of gravimetric water available for plant transpiration at any given time during the drying cycle relative to the total amount of water available for transpiration at the pot-holding capacity. Determination of the FTSW was accomplished using equation 3:

daily FTSW = (Daily pot weight-Final pot weight)/(Initial pot weight-Final pot weight) [3]

Calculations of FTSW were done using the daily average of the beginning and ending interval pot weights [28,30].

2.4. Data analysis

The relationship between NTR and FTSW were quantified using a two-segment linear regression analysis [16,18,19]. The NTR calculated for each pot on each day was plotted for each accession versus the corresponding FTSW. The two-segment linear regression analysis was accomplished, for the six drying pots studied for the *S*-metolachlor-resistant and susceptible accessions and the glyphosate- resistant and susceptible plants, using nonlinear least squares regression (nls) of R version 4.0.0 [31]. The intersection of the two linear regressions is the FTSW at the breakpoint in the soil drying cycle. The resulting R^2 for the regression analysis and breakpoint values for the NTR for each accession were determined and differences between breakpoints were compared using confidence intervals ($\alpha = 0.05$) [14,30,32,33].

3. Results

3.1. Differential response of S-metolachlor-resistant and -suceptible accessions to progressive drying

The two-segmented linear regression analysis [34] was used to relate NTR to FTSW for plants submitted to progressive drying treatment (Figure 2).

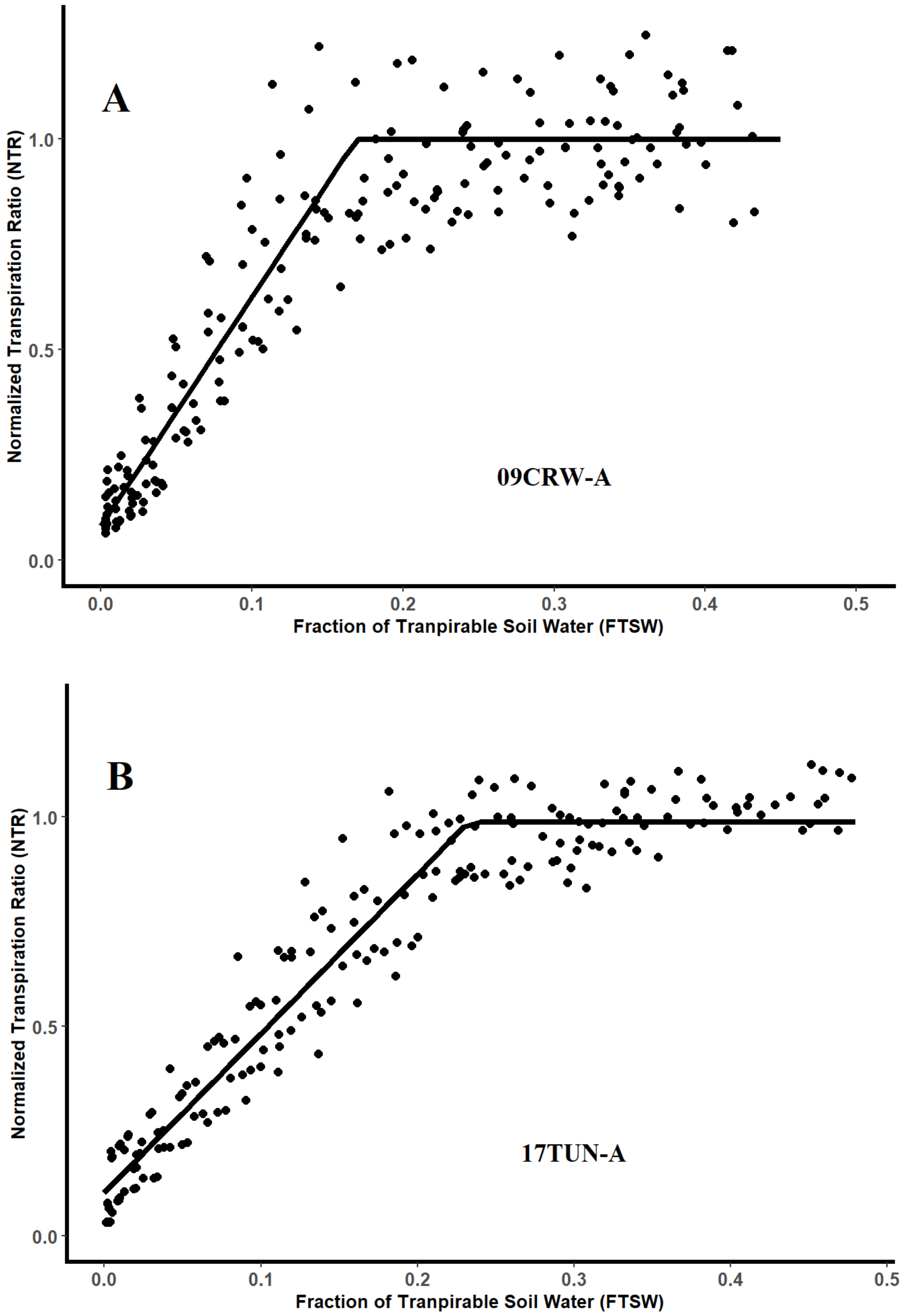

Figure 2. Relationship between normalized transpiration ratio (NTR) and fraction of transpirable soil water (FTSW) during soil drying cycle for *S*-metolachlor- susceptible (09CRW-A) (panel A) and resistant (17TUN-A) (panel B) accessions of Palmer amaranth; greenhouse experiment, Milo J. Shult Agricultural Research & Extension Center, University of Arkansas, Fayetteville in 2021.

The breakpoints at which each accession initiated stomatal closure were compared using their respective confidence intervals. The NTR response of *A. palmeri* to progressive drying soil followed the two-segmented linear regression with R^2 values ranging between 0.85 and 0.93 (Table 1).

Table 1. Breakpoint (threshold value for the initiation of stomatal closure), standard error (SE), R^2, and confidence intervals for the plateau regression analysis used to evaluate differences in drought tolerance between *S*-metolachlor- susceptible and -resistant Palmer amaranth accessions submitted to progressive drought; greenhouse experiment conducted at the Milo J. Shult Agricultural Research & Extension Center, University of Arkansas, Fayetteville in 2021.

Accessions	Breakpoint[a]	SE	R^2	Confidence intervals[b]	
09CRW-A	0.17a	0.007	0.85	0.15	0.19
17TUN-A	0.23b	0.004	0.93	0.22	0.25

[a] Means within a column, followed by different letters are different ($P \leq 0.05$).

[b] 95% confidence intervals of breakpoints

The FTSWcr of the two accessions differed ($P \leq 0.05$) (Table 1), but no differences existed between breakpoints for the same accession across runs ($P > 0.05$); therefore, data were pooled across runs for each accession. The *S*-metolachlor-resistant accession 17TUN-A had a greater FTSWcr than the *S*-metolachlor-susceptible accession 09CRW-A, indicating that the *S*-metolachlor-resistant accession started reducing its transpiration at higher threshold levels of 0.23 ±0.004 with an R^2 value of 0.93 than the susceptible plants. The *S*-metolachlor-susceptible accession 09CRW-A started reducing its transpiration at a lower FTSWcr of 0.17±0.007 with an R^2 value of 0.85.

3.2. Threshold value for stomatal closure of glyphosate-resistant and -susceptible plants

The glyphosate-susceptible and -resistant plants were chosen from one accession, 16CRW-D, based on EPSPS copy number. It was determined previously that resistance to glyphosate in this population is due to increased production of the target protein, EPSPS. The field population consisted of resistant and susceptible plants; the genomic diversity among these plants would be minimal, except for the traits contributing to glyphosate resistance. The relative *EPSPS* gene copy number detected in 16CRW-D ranged between 3 and 226. Twelve plants with *EPSPS* copy number between 22 and 165 (considered resistant) and 12 plants with <10 EPSPS copy number, which were considered susceptible [25], were used for each run. The FTSWcr between plants with increased *EPSPS* copy number and plants with low gene copy number did not differ ($P > 0.05$). The NTR response of *A. palmeri* to progressive drying soil followed the two-segmented linear regression with R^2 values ranging between 0.90 and 0.91 (Table 2). The presence of more *EPSPS* copies in accession 16CRW-D did not change the breakpoint ($P > 0.05$) (Figure 3; Table 2). In other words, increasing the production of this key enzyme in the shikimate pathway had no effect on the initiation of stomatal closure under drought.

Table 2. Breakpoint (threshold value for the initiation of stomatal closure), standard error (SE), R^2, and confidence intervals for the plateau regression analysis used to evaluate differences in drought tolerance between glyphosate-susceptible and -resistant Palmer amaranth differing in *EPSPS* gene copy number; greenhouse experiment conducted at the Milo J. Shult Agricultural Research & Extension Center, University of Arkansas, Fayetteville in 2021.

Gene copy number	Breakpoint[a]	SE	R^2	Confidence intervals[b]	
Increase	0.25a	0.007	0.90	0.23	0.26
No increase	0.25a	0.008	0.91	0.23	0.25

[a] Means within a column, followed by the same letter are not different ($P > 0.05$)

[b] 95% confidence intervals of breakpoints

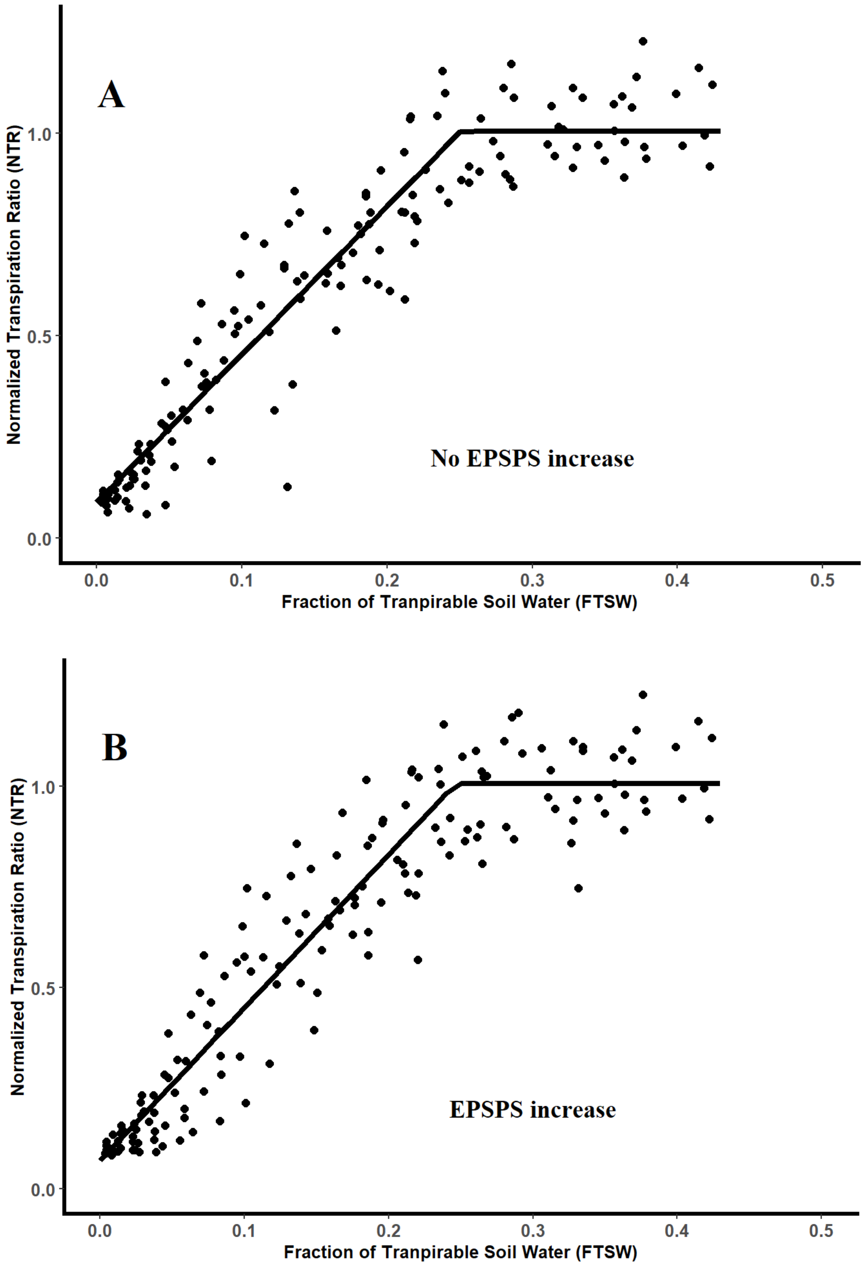

Figure 3. Relationship between normalized transpiration ratio (NTR) and fraction of transpirable soil water (FTSW) during soil drying cycle for glyphosate- resistant and susceptible Palmer amaranth accessions differing by the number of *EPSPS* gene copy number; greenhouse experiment conducted at the Milo J. Shult Agricultural Research & Extension Center (University of Arkansas, Fayetteville) in 2021. A: plants with <10 *EPSPS* copy number; B: *EPSPS* copy number between 22 and 165.

4. Discussion

4.1. Differential response of S-metolachlor-resistant and -suceptible accessions to daily tranpiration

The advantages of early and late breakpoints are interpreted diversely by authors and depend on drought scenarios [35,36]. With smaller FTSWcr in this study, the *S*-metolachlor-susceptible accession is likely to sustain its normal transpiration and prevent growth reduction during short-term water stress [35]. On the contrary, the *S*-

doi:10.20944/preprints202201.0329.v1

metolachlor-resistant accession with greater FTSWcr has an advantage under long-term water stress and drier conditions [32]. Greater FTSWcr means early stomatal closure with respect to the intiation of water deficit. By doing this, the plant conserves water and delays desiccation or mitigates drought stress; thereby enhancing the plant's survival under prolonged drought [13,14,19,37]. In nature, this would increase the probability of survival until the next rain event.

S-metolachlor resistance reported in Arkansas is attributed to an increase in metabolism of the herbicide in the plants catalyzed by glutathione *S*-transferases (GSTs) [27]. As multifunctional enzymes encoded by large gene families, GSTs are known to have a protection role against different biotic and abiotic stresses including xenobiotics and oxidative stress [24,38]. An overexpression of GST genes might have improved drought tolerance in the resistant accession. GSTs have been used to confer drought tolerance to transgenic tobacco [39-41] and transgenic Arabidopsis plants [42,43]. Data from previous and current experiments on *S*-metolachlor-resistant Palmer amaranth collectively indicate that the GST-mediated resistance mechanism could increase tolerance to drought in resistant plants. We observed this expected latent effect in this current study; however, we could not attribute increased drought tolerance solely to the *S*-metolachlor NTSR mechanism because the reference susceptible plants did not come from the same population as the resistant plants. The baseline tolerance to drought could be different between resistant and susceptible populations coming from different localities. Also, the resistance profile of the 17TUN-A has not been fully characterized yet. If this population also happens to be resistant to other herbicide modes of action, there may be different NTSR mechanisms associated with other herbicide modes of action that could also contribute to drought tolerance in this population.

4.2. No difference in threshold value for stomatal closure between glyphosate-resistant and -susceptible plants

It has been hypothesized that amplification of *EPSPS* gene copy could divert a significant fraction of fixed carbon resource away from the normal metabolic processes toward producing more of the *EPSPS* protien. If so, then this mechanism of resistance could have negative physiological consequences that could reduce plant fitness. However, researchers had reported no effect of *EPSPS* gene amplification on fitness of glyphosate-resistant kochia [45] and Palmer amaranth [44,46]. It can be argued that fitness cost might not have been detected in these past studies because the plants were not exposed to certain stress factor(s), with or without competition. In the current study, Palmer amaranth with high EPSPS copy number also did not show fitness penalty when exposed to progressive drying. In this case, fitness was assessed in terms of the ability to mitigate desiccation from water deficit by curbing transpiration sooner after the onset of drought stress. Glyhosate-resistant and -susceptible plants from the same field population exhibited the same response to drought stress. Being the key enzyme in the shikimate synthesis pathway, *EPSPS* does not have a role in drought stress mitigation.

Fitness penalty may also be manifested in terms of reduced fecundity, seed germination capacity, seedling vigor, competitive ability, or even the ability to recover from stress. It is interesting to note that NTSR mechanism involving reduced absorption and translocation of glyphosate in rigid ryegrass (*Lolium rigidum* Gaud.) resulted in reduced fitness with or without competition with wheat [47,48]. The fitness cost was manifested in terms of reduced seed production of resistant plants compared to susceptible ones with low-density wheat competition and without wheat competiton. In this case, the evolved resistance mechanism came with a fitness penalty, unlike the *EPSPS* gene amplification resistance mechanism and the GST-associated resistance mechanism discussed previously. Several aspects of plant physiology and growth need to be investigated to fully understand the effect of herbicide resistance mechanisms on weediness and fitness.

5. Conclusion

The GST-mediated resistance to *S*-metolachlor may impart some tolerance to drought stress. This is a logical consequence of the diverse roles of GSTs in general stress mitigation or intrinsic plant protection. This can be verified by developing near isolines of resistant and susceptible plants and conducting stress experiments on these materials. In contrast, *EPSPS* gene amplification does not affect the response of Palmer amaranth to drought stress with respect to reducing water loss. As droughts are predicted to increase in frequency and severity, these results suggest that *S*-metolachlor-resistant and glyphosate-resistant Palmer amaranth populations will not be at a competitive disadvantage compared to susceptible biotypes. Alternative and diverse management strategies will be required for effective Palmer amaranth control regardless of herbicide resistance status.

Author Contributions:
Conceptualization, Nilda Roma-Burgos; Data curation, Koffi Badou-Jeremie Kouame and Nilda Roma-Burgos; Formal analysis, Koffi Badou-Jeremie Kouame; Funding acquisition, Nilda Roma-Burgos; Investigation, Koffi Badou-Jeremie Kouame and Gulab Rangani; Methodology, Koffi Badou-Jeremie Kouame and Nilda Roma-Burgos; Project administration, Mary Savin and Nilda Roma-Burgos; Resources, Nilda Roma-Burgos; Supervision, Nilda Roma-Burgos; Validation, Koffi Badou-Jeremie Kouame, Gulab Rangani and Nilda Roma-Burgos; Visualization, Koffi Badou-Jeremie Kouame; Writing – original draft, Koffi Badou-Jeremie Kouame; Writing – review & editing, Nilda Roma-Burgos, Mary C. Savin, Thomas R. Butts, Koffi Badou-Jeremie Kouame, and Matthew Bertucci.

Acknowledgments:

Financial support for this research was provided by Arkansas soybean producers through check-off funds administered by the Arkansas Soybean Promotion Board. The authors thank the University of Arkansas System Division of Agriculture for all other personnel support and facilities. The authors also thank Pâmela Carvalho-Moore and Fidel Gonzalez-Torralva for their assistance in lab-related work.

Conflicts of Interest:

The authors declare no conflict of interest.

References

1. Khan, A.; Pan, X.D.; Najeeb, U.; Tan, D.K.Y.; Fahad, S.; Zahoor, R.; Luo, H.H. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biological Research* **2018**, *51*, doi:10.1186/s40659-018-0198-z.
2. Boyer, J.S.; Byrne, P.; Cassman, K.G.; Cooper, M.; Delmer, D.; Greene, T.; Gruis, F.; Habben, J.; Hausmann, N.; Kenny, N.; Lafitte, R.; Paszkiewicz, S.; Porter, D.; Schlegel, A.; Schussler, J.; Setter, T.; Shanahan, J.; Sharp, R.E.; Vyn, T.J.; Warner, D.; Gaffney, J. The US drought of 2012 in perspective: A call to action. *Global Food Security-Agriculture Policy Economics and Environment* **2013**, *2*, 139-143, doi:10.1016/j.gfs.2013.08.002.
3. Jin, C.; Luo, X.; Xiao, X.M.; Dong, J.W.; Li, X.M.; Yang, J.; Zhao, D.Y. The 2012 Flash drought threatened US Midwest agroecosystems. *Chinese Geographical Science* **2019**, *29*, 768-783, doi:10.1007/s11769-019-1066-7.
4. Liu, L.; Basso, B. Impacts of climate variability and adaptation strategies on crop yields and soil organic carbon in the US Midwest. *Plos One* **2020**, *15*, doi:10.1371/journal.pone.0225433.

5. Lobell, D.B.; Deines, J.M.; Di Tommaso, S. Changes in the drought sensitivity of US maize yields. *Nature Food* **2020**, *1*, 729-735, doi:10.1038/s43016-020-00165-w.
6. Bowling, L.C.; Cherkauer, K.A.; Lee, C.I.; Beckerman, J.L.; Brouder, S.; Buzan, J.R.; Doering, O.C.; Dukes, J.S.; Ebner, P.D.; Frankenberger, J.R.; Gramig, B.M.; Kladivko, E.J.; Volenec, J.J. Agricultural impacts of climate change in Indiana and potential adaptations. *Climatic Change* **2020**, *163*, 2005-2027, doi:10.1007/s10584-020-02934-9.
7. Kropff, M.; van Laar, H. *Modelling crop-weeds interactions*; CAB International: 1993.
8. Kimm, H.; Guan, K.Y.; Gentine, P.; Wu, J.; Bernacchi, C.J.; Sulman, B.N.; Griffis, T.J.; Lin, C.J. Redefining droughts for the US Corn Belt: The dominant role of atmospheric vapor pressure deficit over soil moisture in regulating stomatal behavior of Maize and Soybean. *Agricultural and Forest Meteorology* **2020**, *287*, doi:10.1016/j.agrformet.2020.107930.
9. Grassini, P.; Torrion, J.A.; Yang, H.S.; Rees, J.; Andersen, D.; Cassman, K.G.; Specht, J.E. Soybean yield gaps and water productivity in the western US Corn Belt. *Field Crops Research* **2015**, *179*, 150-163, doi:10.1016/j.fcr.2015.04.015.
10. Yang, Y.; Anderson, M.C.; Gao, F.; Johnson, D.M.; Sun, L.; Dulaney, W.; Hain, C.R.; Otkin, J.A.; Prueger, J.; Meyers, T.P.; Bernacchi, C.J.; Moore, C.E. Phenological corrections to a field-scale, ET-based crop stress indicator: An application to yield forecasting across the US Corn Belt. *Remote Sensing of Environment* **2021**, *257*, doi:10.1016/j.rse.2021.112337.
11. Li, L.J.; Song, X.Y.; Xia, L.; Fu, N.; Feng, D.; Li, H.Y.; Li, Y.L. Modelling the effects of climate change on transpiration and evaporation in natural and constructed grasslands in the semi-arid Loess Plateau, China. *Agriculture Ecosystems & Environment* **2020**, *302*, doi:10.1016/j.agee.2020.107077.
12. Saradadevi, R.; Palta, J.A.; Siddique, K.H.M. ABA-mediated stomatal response in regulating water use during the development of terminal drought in wheat. *Frontiers in Plant Science* **2017**, *8*, doi:10.3389/fpls.2017.01251.
13. Sinclair, T.R. Is transpiration efficiency a viable plant trait in breeding for crop improvement? *Functional Plant Biology* **2012**, *39*, 359-365, doi:10.1071/fp11198.
14. Cathey, S.E.; Kruse, J.K.; Sinclair, T.R.; Dukes, M.D. Transpiration and visual appearance of warm season turfgrasses during soil drying. *Environmental and Experimental Botany* **2013**, *89*, 36-43, doi:10.1016/j.envexpbot.2012.12.004.
15. Sinclair, T.; Ludlow, M. Influence of soil-water supply on the plant water-balance of 4 tropical grain legumes. *Australian Journal of Plant Physiology* **1986**, *13*, 329-341, doi:10.1071/PP9860329.
16. Ray, J.; Gesch, R.; Sinclair, T.; Allen, L. The effect of vapor pressure deficit on maize transpiration response to a drying soil. *Plant and Soil* **2002**, *239*, 113-121, doi:10.1023/A:1014947422468.
17. Ray, J.; Samson, B.; Sinclair, T. Vegetative growth and soil water extraction of two maize hybrids during water deficits. *Field Crops Research* **1997**, *52*, 135-142, doi:10.1016/S0378-4290(96)01066-0.
18. Ray, J.; Sinclair, T. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *Journal of Experimental Botany* **1998**, *49*, 1381-1386, doi:10.1093/jexbot/49.325.1381.
19. Ray, J.; Sinclair, T. Stomatal closure of maize hybrids in response to drying soil. *Crop Science* **1997**, *37*, 803-807, doi:10.2135/cropsci1997.0011183X003700030018x.
20. Bravo, W.; Leon, R.; Ferrell, J.; Mulvaney, M.; Wood, C. Evolutionary adaptations of Palmer amaranth (*Amaranthus palmeri*) to nitrogen fertilization and crop rotation history affect morphology and nutrient-use efficiency. *Weed Science* **2018**, *66*, 180-189, doi:10.1017/wsc.2017.73.
21. Ehleringer, J. Ecophysiology of *Amaranthus palmeri:* A Sonoran desert summer annual. *Oecologia* **1983**, *57*, 107-112, doi:10.1007/BF00379568.
22. Butts, T.R.; Vieira, B.C.; Latorre, D.O.; Werle, R.; Kruger, G.R. Competitiveness of herbicide-resistant waterhemp (*Amaranthus tuberculatus*) with soybean. *Weed Science* **2018**, *66*, 729-737, doi:10.1017/wsc.2018.45.
23. Tardif, F.J.; Rajcan, I.; Costea, M. A mutation in the herbicide target site acetohydroxyacid synthase produces morphological and structural alterations and reduces fitness in *Amaranthus powellii*. *New Phytologist* **2006**, *169*, 251-264, doi:10.1111/j.1469-8137.2005.01596.x.

24. Montellano, P. *Cytochrome P450: Structure, mechanism, and biochemistry*; 3rd, Ed.; Kluwer Academic/Plenum Publishers New York, 2005.
25. Singh, S.; Singh, V.; Lawton-Rauh, A.; Bagavathiannan, M.; Roma-Burgos, N. EPSPS gene amplification primarily confers glyphosate resistance among Arkansas Palmer amaranth (*Amaranthus palmeri*) populations. *Weed Science* **2018**, *66*, 293-300, doi:10.1017/wsc.2017.83.
26. Brabham, C.; Norsworthy, J.; Houston, M.; Varanasi, V.; Barber, T. Confirmation of *S*-metolachlor resistance in Palmer amaranth (*Amaranthus palmeri*). *Weed Technology* **2019**, *33*, 720-726, doi:10.1017/wet.2019.44.
27. Rangani, G.; Noguera, M.; Salas-Perez, R.; Benedetti, L.; Roma-Burgos, N. Mechanism of resistance to *S*-metolachlor in Palmer amaranth. *Frontiers in Plant Science* **2021**, *12*, 13, doi:10.3389/fpls.2021.652581.
28. King, C.; Purcell, L. Evaluation of methods for estimating transpiration response to soil drying for container-grown plants. *Crop Science* **2017**, *57*, 2143-2148, doi:10.2135/cropsci2016.12.1000.
29. Schmidt, J.; Blankenship, E.; Lindquist, J. Corn and velvetleaf (*Abutilon theophrasti*) transpiration in response to drying soil. *Weed Science* **2011**, *59*, 50-54, doi:10.1614/WS-D-10-00078.1.
30. King, C.; Purcell, L.; Brye, K. Differential wilting among soybean genotypes in response to water deficit. *Crop Science* **2009**, *49*, 290-298, doi:10.2135/cropsci2008.04.0219.
31. R Core Team, R. *R: A language and environment for statistical computing*, 4.0.0; R Core Team (2020): Vienna, Austria, 2020.
32. Sinclair, T.R.; Manandhar, A.; Belko, N.; Riar, M.; Vadez, V.; Roberts, P.A. Variation among cowpea genotypes in sensitivity of transpiration rate and symbiotic nitrogen fixation to soil drying. *Crop Science* **2015**, *55*, 2270-2275, doi:10.2135/cropsci2014.12.0816.
33. Sinclair, T.R.; Shekoofa, A.; Isleib, T.G.; Balota, M.; Zhang, H. Identification of Virginia-type peanut genotypes for water-deficit conditions based on early decrease in transpiration rate with soil drying. *Crop Science* **2018**, *58*, 2607-2612, doi:10.2135/cropsci2018.05.0293.
34. Devi, M.J.; Reddy, V. Cotton genotypic variability for transpiration decrease with progressive soil drying. *Agronomy-Basel* **2020**, *10*, doi:10.3390/agronomy10091290.
35. Fuentealba, M.P.; Zhang, J.; Kenworthy, K.; Erickson, J.; Kruse, J.; Trenholm, L. Transpiration responses of warm-season turfgrass in relation to progressive soil drying. *Scientia Horticulturae* **2016**, *198*, 249-253, doi:10.1016/j.scienta.2015.11.042.
36. Miller, G.L. Physiological response of bermudagrass grown in soil amendments during drought stress. *Hortscience* **2000**, *35*, 213-216, doi:10.21273/hortsci.35.2.213.
37. Devi, M.J.; Sinclair, T.R.; Vadez, V.; Krishnamurthy, L. Peanut genotypic variation in transpiration efficiency and decreased transpiration during progressive soil drying. *Field Crops Research* **2009**, *114*, 280-285, doi:10.1016/j.fcr.2009.08.012.
38. Lee, J.; Jo, H.; Kong, K. A Plant-specific Tau class glutathione *S*-transferase from *Oryza sativa* having significant detoxification activity towards chloroacetanilide herbicides. *Bulletin of the Korean Chemical Society* **2011**, *32*, 3756-3759, doi:10.5012/bkcs.2011.32.10.3756.
39. George, S.; Venkataraman, G.; Parida, A. A chloroplast-localized and auxin-induced glutathione *S*-transferase from phreatophyte *Prosopis juliflora* confer drought tolerance on tobacco. *Journal of Plant Physiology* **2010**, *167*, 311-318, doi:10.1016/j.jplph.2009.09.004.
40. Ji, W.; Zhu, Y.M.; Li, Y.; Yang, L.A.; Zhao, X.W.; Cai, H.; Bai, X. Over-expression of a glutathione *S*-transferase gene, GsGST, from wild soybean (*Glycine soja*) enhances drought and salt tolerance in transgenic tobacco. *Biotechnology Letters* **2010**, *32*, 1173-1179, doi:10.1007/s10529-010-0269-x.
41. Liu, D.; Liu, Y.; Rao, J.; Wang, G.; Li, H.; Ge, F.; Chen, C. Overexpression of the glutathione *S*-transferase gene from *Pyrus pyrifolia* fruit improves tolerance to abiotic stress in transgenic tobacco plants. *Molecular Biology* **2013**, *47*, 515-523, doi:10.1134/s0026893313040109.

42. Xu, J.; Zheng, A.Q.; Xing, X.J.; Chen, L.; Fu, X.Y.; Peng, R.H.; Tian, Y.S.; Yao, Q.H. Transgenic *arabidopsis* plants expressing grape glutathione *S*-transferase gene (VvGSTF13) show enhanced tolerance to abiotic stress. *Biochemistry-Moscow* **2018**, *83*, 755-765, doi:10.1134/s0006297918060135.
43. Yang, Q.; Liu, Y.J.; Zeng, Q.Y. Overexpression of three orthologous glutathione *S*-transferases from *Populus* increased salt and drought resistance in *Arabidopsis*. *Biochemical Systematics and Ecology* **2019**, *83*, 57-61, doi:10.1016/j.bse.2019.01.001.
44. Giacomini, D.; Westra, P.; Ward, S.M. Impact of genetic background in fitness cost studies: an example from glyphosate-resistant Palmer amaranth. *Weed Science* **2014**, *62*, 29-37, doi:10.1614/ws-d-13-00066.1.
45. Osipitan, O.A.; Dille, J.A. No Impact of Increased EPSPS gene copy number on growth and fecundity of glyphosate-resistant kochia (*Bassia scoparia*). *Weed Science* **2019**, *67*, 22-28, doi:10.1017/wsc.2018.82.
46. Vila-Aiub, M.M.; Goh, S.S.; Gaines, T.A.; Han, H.P.; Busi, R.; Yu, Q.; Powles, S.B. No fitness cost of glyphosate resistance endowed by massive EPSPS gene amplification in *Amaranthus palmeri*. *Planta* **2014**, *239*, 793-801, doi:10.1007/s00425-013-2022-x.
47. Lorraine-Colwill, D. F.; Powles, S. B.; Hawkes, T. R.; Hollinshead, P. H.; Warner, S. A. J.; Preston, C. Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. *Pesticide Biochemistry* and *Physiology* **2003**, 74(2), 62–72.
48. Pedersen, B.P.; Neve, P; Andreasen, C.; Powles, S.B. Ecological fitness of a glyphosate-resistant *Lolium rigidum* population: Growth and seed production along a competition gradient. *Basic and Applied Ecology* **2007**, 8, 258-268.