American Journal of Plant Sciences, 2017, 8, 1499-1510
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Morphological Characterization of *Amaranthus palmeri* x *A. spinosus* Hybrids

William T. Molin*, Vijay K. Nandula

Crop Production Systems Research Unit, U.S. Department of Agriculture-Agricultural Research Service, Stoneville, MS, USA
Email: *william.molin@ars.usda.gov

How to cite this paper: Molin, W.T. and Nandula, V.K. (2017) Morphological Characterization of *Amaranthus palmeri* x *A. spinosus* Hybrids. *American Journal of Plant Sciences*, **8**, 1499-1510.
https://doi.org/10.4236/ajps.2017.86103

Received: April 7, 2017
Accepted: May24, 2017
Published: May 27, 2017

Abstract

The growth of clones of seven *Amaranthus palmeri* x *A. spinosus* hybrids was compared to type specimens of *A. palmeri* and *A. spinosus*. The hybrids came from the field where they were originally discovered and clones of the type specimens and hybrids were established under greenhouse conditions and used to compare growth rates. *A. palmeri* had the highest growth rate and *A. spinosus* the lowest growth rate based on height, node counts, and dry weight accumulation. *A. palmeri* also had the greatest number of days to flowering and *A. spinosus* the fewest. Hybrids had intermediary growth rates and days to flowering, but differed from each other with regard to sex identity. The hybrids were either dioecious like *A. palmeri* or, if monoecious, had patterns unlike *A. spinosus*. Spine length and texture also varied in hybrids and some were without spines. Hybrid 16Ci was short compared to all others and had succulent leaves and stems, which easily separated from the plant body. These hybridizations resulted in morphologically distinct types with acquisition of physical traits intermediate to the type specimens which may drive evolution of these species.

Keywords

Herbicide, Hybrid, Palmer Amaranth, Resistance, Spiny Amaranth

1. Introduction

Interspecific hybridization between *Amaranthus palmeri* S. Wats. (Palmer amaranth) and *A. spinosus* L. (spiny amaranth) in which glyphosate resistance was transferred from *A. palmeri* into *A. spinosus* x *A. palmeri* hybrids was reported [1]. This was the first report of interspecies transfer of glyphosate resistance in *Amaranthus* under field conditions. Subsequently, acetolactate synthase (ALS) inhibitor resistance, confirmed by resistance to pyrithiobac, was also present in these hybrids [2] indicating that the parental *A. palmeri* may have had multiple

resistances. Interspecific hybridization between *A. palmeri* and *A. spinosus* was previously demonstrated when these species were grown in close proximity to each other under controlled conditions [3]. *A. palmeri* also hybridized with *A. hybridus* and *A. tuberculatus* Moq. Sauer, with the transfer of glyphosate resistance [3], and with *A. tuberculatus*, with the transfer of ALS resistance [4]. Transfer of ALS resistance from *A. tuberculatus* to *A. hybridus* has also been confirmed [5].

The hybrids were discovered in a production cotton (*Gossypium hirsutum* L.)/soybean (*Glycine max* Merr. L.) field in Mississippi, USA in 2011. The production field had *A. palmeri* growing along its roadside edges and just across a narrow two lane road was a pasture with populations of *A. spinosus*. Putative hybrids were collected from 2012 through 2015 and maintained in the greenhouse. Using specific probes to intron 1 of *EPSPS* from each species, about one quarter of the collected pigweeds were determined to be hybrids [6]. Plants identified as hybrids were generally similar in stature to *A. palmeri*, but varied with regard to the presence and length of spines. Some plants were clearly dioecious, but among monoecious types, there were diminutive female flowers at the leaf axils with uniformly male or female flower spikes.

Hybrids from the field may be original first filial generation (F1s), second filial generation (F2s), backcrosses with either parent, or subsequent generations from the F1 generation itself. F1 hybrids generated at Stoneville, Mississippi, using glyphosate-resistant (GR) male *A. palmeri* and glyphosate-sensitive (GS) emasculated *A. spinosus* as parents, were 95% dioecious GR males, although a few monecious F1s were identified (unpublished data). Reciprocal crosses were not made. These results may indicate that the monoecious condition may be rare in nature following such crosses. If monoecious hybrids are self-fertile, the proximity to neighboring weeds would not necessarily limit formation of next year's seed production.

These hybrids persisted in the field for several years following their initial discovery. The reoccurrence, year after year, may indicate the presence of a large seed bank created from the first occurrence of hybridization or repeated occurrences both with traits sufficient for continuous establishment. Although these hybrids were growing in competition with a crop, whether the hybrids will be as competitive as *A. palmeri* in crops is unknown. If these interspecific hybrids among weedy amaranths retain growth rates similar to *A. palmeri*, they may survive and propagate, thereby, increasing genetic diversity within this population. Whether the hybrids favor either type specimen or represent a population with blended morphologies is unknown. Characterizing the relative growth potential of these hybrids and determining whether clones of the hybrids respond in similar fashion was a first step in assessing future competitive interactions. The objective of this research was to evaluate the relative growth of GR-hybrids of *A. spinosus* and *A. palmeri* compared to GS *A. spinosus* and GR-*A. palmeri*.

2. Materials and Methods

The *A. palmeri* × *A. spinosus* hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13, and 9Ci)

used in this study were collected in 2013, 2014, and 2015 from a farmer's field north of Water Valley, MS, USA, the field of origin (34.23450 N, 89.63433 W). *A. spinosus* (35SA) was collected from a field adjacent to above field. *A. palmeri* (13PA) was collected from USDA-ARS farm in Stoneville, MS. Hybrids were confirmed using previously established procedures [7]. Briefly, genomic DNA was isolated from each species or hybrid from 1 - 2 leaves, depending on size. Leaf tissue was homogenized in an extraction buffer (12) using a mortar and pestle. The homogenate was transferred to a microcentrifuge tube and incubated at 65˚C for 1 h. Cellular debris was collected by centrifugation at 13000 rpm for 4 min, and 266 μL of the supernatant was transferred to a fresh tube. The DNEasy Plant Mini kit (Qiagen, Valencia, CA) protocol was followed from this point forward. Quality and quantity of DNA were assessed by an *A*260/280 reading and by visualization of the DNA on a 1% agarose 1× TAE (0.04M Tris-acetate and 0.001M EDTA) gel stained with ethidium bromide.

For *A. palmeri, A. spinosus* and hybrids, intron 1 of EPSPS from *A. palmeri* was amplified from genomic DNA with primer pairs GTTGTGAGTTCGATACACTGC and CAGTAGGTAAACCGTGTTG, and for *A. spinosus* with primer pairs GAGAAGATTGATTTGGTCGTG and GAGTCAAAGAGATACTGTATGACG as previously described (6). PCR was performed using the Takara LA PCR kit v.2.1 (Clontech, Mountain View, CA) as follows: ~50 ng of DNA, 200 nM of primers, 2.5 mM of Mg^{2+}, 1× buffer, 400 μM of dNTPs, 5 units of polymerase and H_2O to 50μL. Cycle conditions were as follows for *A. palmeri* and *A. spinosus*: 94˚C for 4 min; 30 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 2 min; 72˚C for 5 min; and a 4˚C hold. Intron 1 PCR product size was 697 base pairs for *A. palmeri* and 1335 base pairs for *A. spinosus*.

Clones of the field hybrids and species were created by rooting axillary branches from parent plants using established protocols [7]. Briefly, an axillary branch, approximately 4 cm long, was cut from the stem and lateral leaves removed leaving 4 leaves per stalk. The cut end was lightly coated with Rootone rooting hormone (TechPac, Lexington, KY, USA) and placed in moist, growth media (Metro-Mix 360, Sun Gro Horticulture, Bellevue, WA, USA). The plantlets were kept in indirect sunlight for 3 weeks, then transplanted into larger pots of growth media. The success rate for formation of viable plantlets was very high (> 90%).

All research reported here was conducted at the USDA-ARS Crop Production Systems Research Unit laboratory at Stoneville, MS. The design was a completely randomized design with 12 replications and the experiment was repeated. Growth (height) was measured every week and plants were checked daily for initiation of flowering. At eight weeks after initiation, final height (distance between soil surface and highest node/leaf) was measured, nodes were counted and plants were evaluated for monoecious or dioecious nature and presence of spines. Plants were severed from the root system and dried at 49˚C in open paper bags for two weeks. Mean comparisons were performed for dry weight,

number of nodes, plant height over an eight week period, the days to flowering, and length of the terminal flower spike. Regression parameters were computed for relative growth rate and number of flowering plants in response to weeks and days after initiation, respectively. All statistical parameters were computed using SigmaPlot (v.11.0; Systat Software, Inc., San Jose, CA, USA).

3. Results

The growth of the hybrids relative to *A. palmeri* and *A. spinosus* is shown in Figure 1 and Table 1).

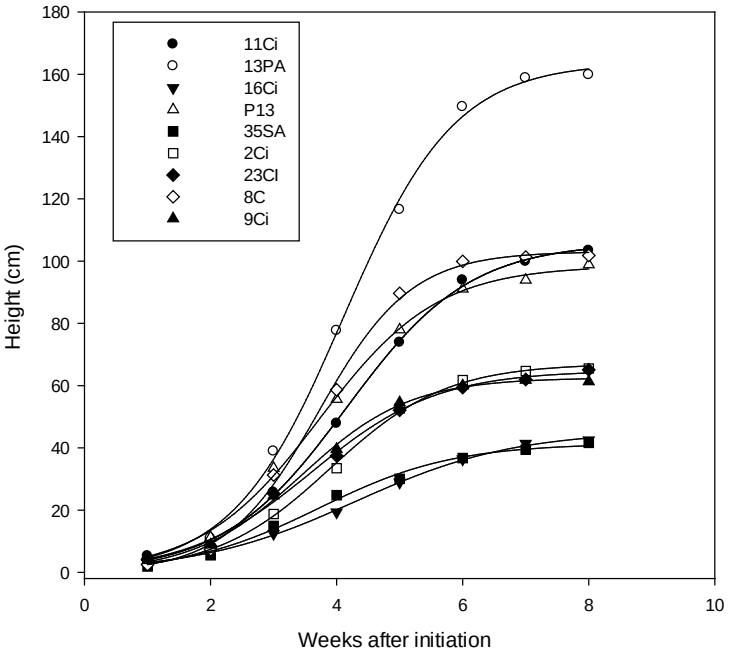

Figure 1. Relative growth of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13 and 9Ci) from days after initiation of the experiment. The total measurement period was 8 weeks.

Table 1. Regression parameters for Figure 1.

$$y = \frac{a}{1 + e^{-\left(\frac{x - x0}{b}\right)}}$$

Hybrid	a	X0	b	F	P	R^2
13PA	163.7	4.1078	0.8853	1817	< 0.0001	0.99
8C	103.1	3.7116	0.7337	1218	< 0.0001	0.99
11CI	105.9	4.1004	0.9784	3750	< 0.0001	0.99
P13	98.5	3.7259	0.9384	1502	< 0.0001	0.99
2Ci	67.1	3.9060	0.9152	1467	< 0.0001	0.99
23Ci	64.8	3.6145	0.9894	685	< 0.0001	0.99
9Ci	62.5	3.4442	0.8569	1545	< 0.0001	0.99
35SA	41.4	3.7250	1.0612	411	< 0.0001	0.99
16Ci	45.4	4.2945	1.2646	2257	< 0.0001	0.99

A. palmeri (13PA) had the greatest growth rate and hybrid 16Ci was similar to 35SA (*A. spinosus*) with both having the lowest growth rate. The remaining hybrids separated into two distinct groups, both intermediate to *A. palmeri* and *A. spinosus*. The distinction in growth rates observed in Figure 1 was reflected in the final dry weights (Figure 2) and height to node ratios (Figure 3).

Nodes per plant (Figure 4) also followed a similar trend with the exception that 2Ci was more like the group containing 11Ci, 8C, and P13.

The weight to height ratio (Figure 5) indicated that, overall, the plant structure of the hybrids was more like *A. palmeri* with the exception of *A. spinosus*, which had a greater g/cm ratio than the other plants.

Inflorescence lengths varied among the hybrids and were different from the type specimens (Figure 6). Hybrids were either dioecious like *A. palmeri* (Figures 7(a) and Figure 7(b)), or monoecious like *A. spinosus* (Figure 7(c)). The inflorescences of the hybrids were also more like *A. palmeri* in that the terminal stalk consisted of either male or female flowers (Table 2). Furthermore, the terminal flower stalks of *A. spinosus* (Table 2) typically had proximal female flowers and distal male flowers roughly in equally proportions. For those hybrids that were monoecious, the flower arrangement on the stalk was considerably altered. For those with a prominent male sex identity, the female flowers were clustered at the leaf axils. For those with a prominent female sex identity, the

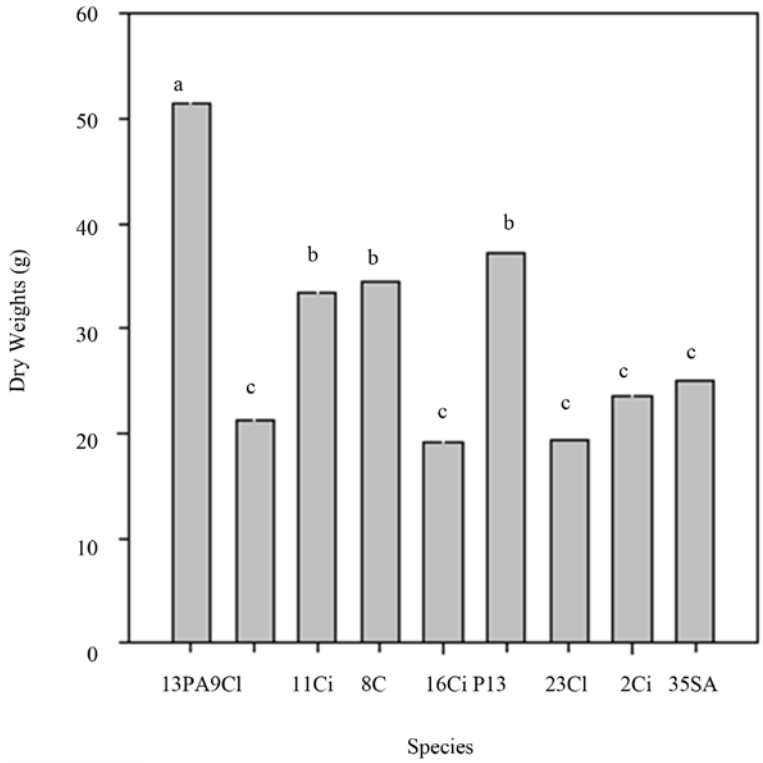

Figure 2. Mean dry weights of *A. Spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13 and 9Ci) at 8 weeks after initiation of the experiment. Values designated with the same letter are not significantly different from each other based on differences of least square means ($P \leq 0.05$).

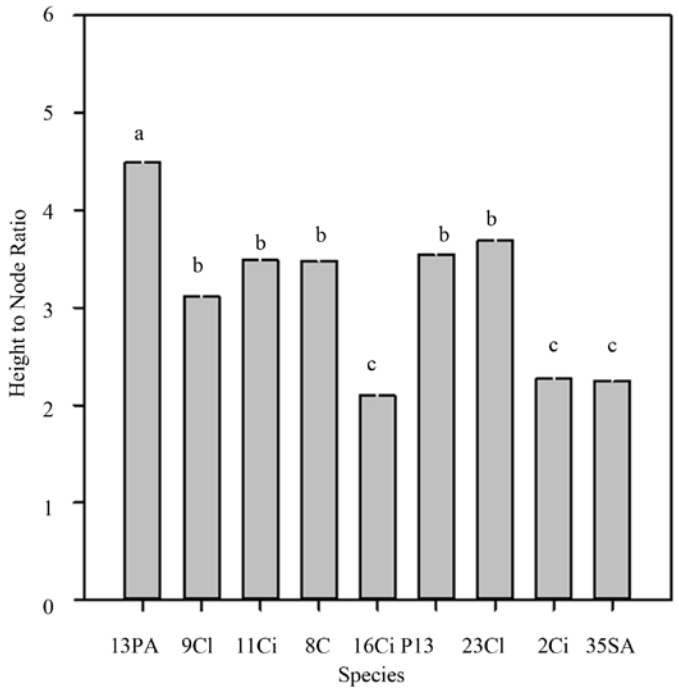

Figure 3. Mean Height to Node Ratios of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13and 9Ci) at 8 weeks after initiation of the experiment. Values designated with the same letter are not significantly different from each other based on differences of least square means ($P \leq 0.05$).

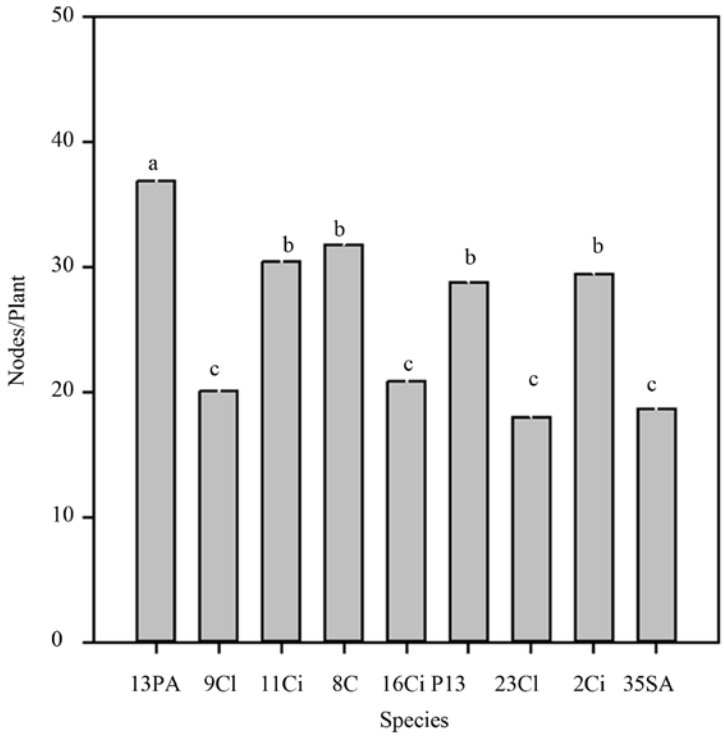

Figure 4. Mean Nodes per Plant of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13 and 9Ci) at 8 weeks after initiation of the experiment. Values designated with the same letter are not significantly different from each other based on differences of least square means ($P \leq 0.05$).

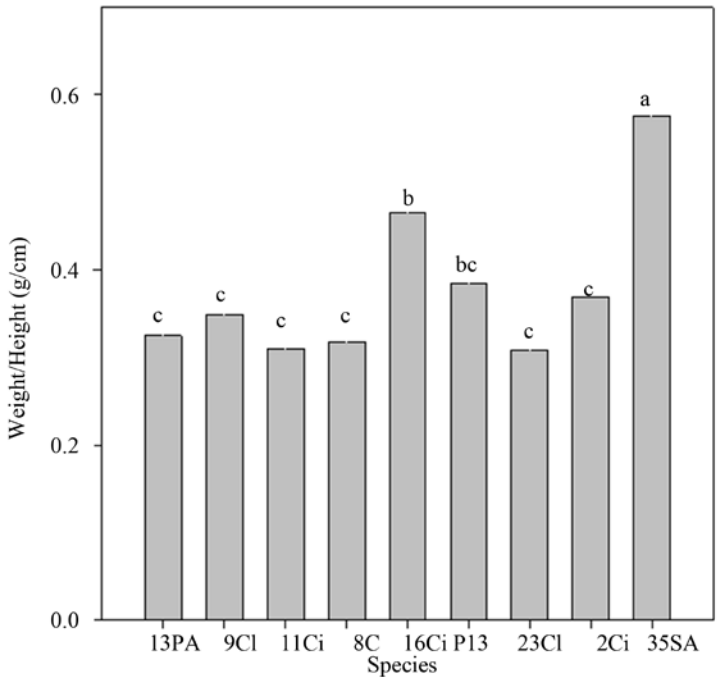

Figure 5. Mean Weight to Height ratios of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13, and 9Ci) at 8 weeks after initiation of the experiment. Values designated with the same letter are not significantly different from each other based on differences of least square means ($P \leq 0.05$).

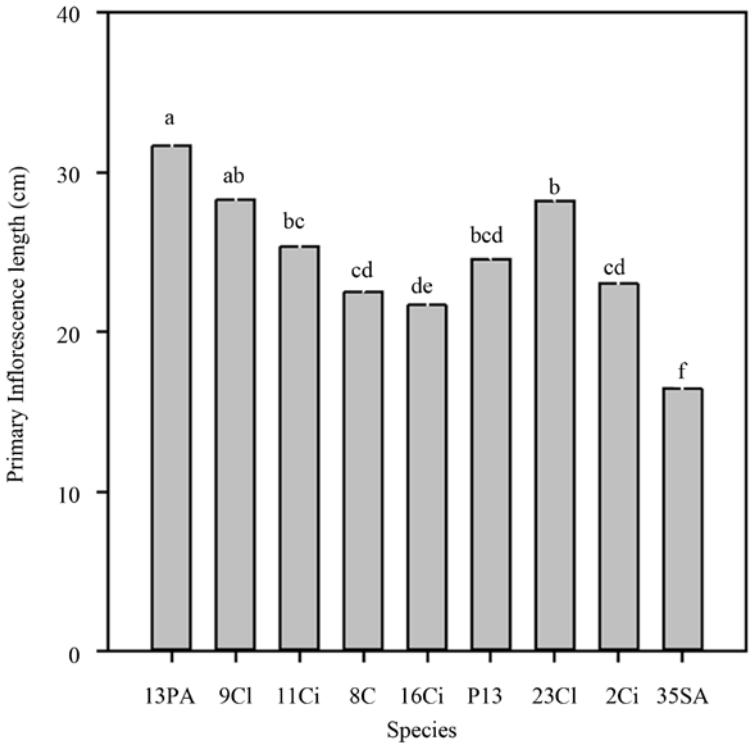

Figure 6. Mean length of the Primary Inflorescence of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13, and 9Ci) at 8 weeks after initiation of the experiment. Values designated with the same letter are not significantly different from each other based on differences of least square means ($P \leq 0.05$).

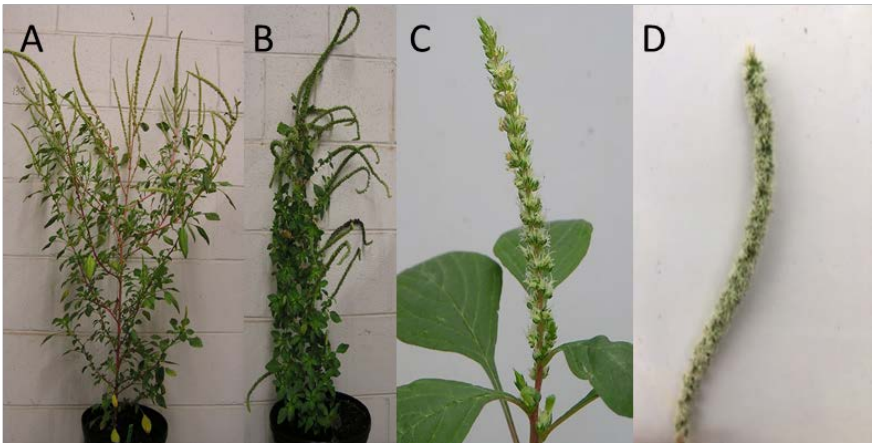

Figure 7. Dioecious (A, male and B female), monoecious (C, proximal female and distal male flowers in equal proportion), and mixed (D, disproportionate proximal female and distal male) inflorescences of *A. palmeri* (13PA), *A. spinosus* (35SA), and hybrid (8C), respectively.

Table 2. Presence and texture of spines, flower morphology and sex identity of species and hybrids.

Designation	Species	Spines (length texture)	Flower Morphology and Sex Identity
13PA	*A. palmeri*	None	Dioecious 26♀
11Ci	Hybrid	2, soft	Mixed mono and di, 19♀, 5♀♂
9Ci	Hybrid	1, oft	Monoecious, terminal ♂ inflorescences , ♀♂ at axils
16Ci	Hybrid	1, soft	Dioecious 30♂
P13	Hybrid	3, stiff, sharp	Dioecious 24♂
2Ci	Hybrid	2, soft	Monoecious 30-♀ at axils, ♂inflorescence
23Ci	Hybrid	None	Dioecious 18-♀ at axils,
8C	Hybrid	2, soft	Monoecious inflorescence ♀, ♂at tips
35SA	*A. spinosus*	3 to 4, stiff, sharp	Monoecious

male flowers were clustered at the very distal end of the flower stalk (**Figure 7(d)**).

All of the hybrids initiated flower development earlier than *A. palmeri* (**Figure 8**, **Table 3**).

Flower initiation was complete by 3.5 weeks after initiation of the experiment, although inflorescences continued to expand and elongate throughout the 8 week test period. Plant height continued to increase until 7 weeks. The groupings observed among hybrids in **Figure 1** based on growth rate were not observed for flower initiation. The time to reach 50% flower initiation (estimated from **Figure 8**) was different for type specimens and hydrids.

4. Discussion

The relative growth curves, dry weights, height to node ratios, nodes per plant,

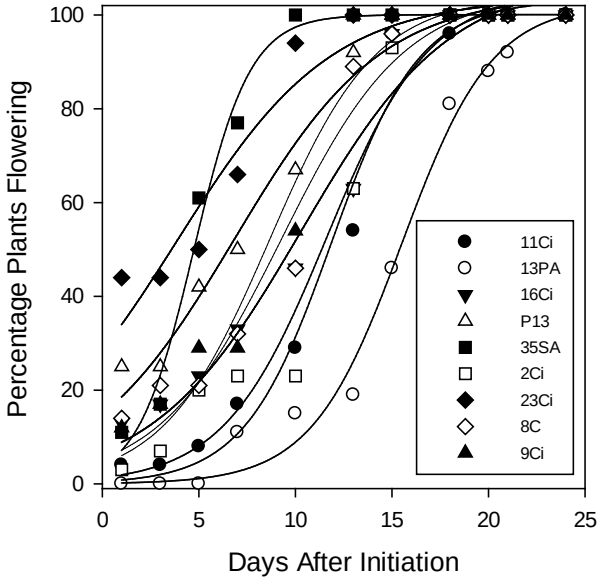

Figure 8. Percentage of *A. spinosus* (35SA), *A. palmeri* (13PA), and hybrids (11Ci, 16Ci, 2Ci, 23 Ci, 8C, P13, and 9Ci) producing terminal flowers from days after initiation of the experiment. The measurement period was 3.5 weeks.

Table 3. Regression parameters for **Figure 8**.

$$y = \frac{a}{1 + e^{-\left(\frac{x - x0}{b}\right)}}$$

Hybrid	a	X0	b	F	P	R^2
13PA	102.5	15.5	2.3408	360	< 0.0001	0.98
8C	104.9	9.2918	3.1948	135	< 0.0001	0.97
11CI	103.1	11.8685	2.2637	203	< 0.0001	0.98
P13	104.4	6.8287	3.8188	219	< 0.0001	0.98
2Ci	105.2	11.6205	2.6668	122	< 0.0001	0.96
23Ci	103.7	3.8753	4.0001	68	< 0.0001	0.94
9Ci	104.0	8.6419	2.7556	110	< 0.0001	0.98
35SA	100.1	4.7406	1.4629	350	< 0.0001	0.98
16Ci	108.0	10.3015	3.865	152	< 0.0001	0.97

weight to height ratios, and inflorescence lengths presented here for the *A. palmeri* x *A. spinosus* hybrids in most cases ranked between the high and low values measured for the type specimens. Each hybrid, except for 16Ci, had upright stems which easily supported branches, leaves, and inflorescences. However, hybrid 16Ci appeared to have a fitness penalty. It had fleshy, drooping, decumbent stems and leaves, perhaps, indicating a lignin deficiency, but still grew at the same rate as *A. spinosus*. The long term consequences of these hybridization events, such as enhanced gene flow and accelerated evolution, will likely remain unknown because considerable effort was made by the farmer to eradicate these weeds. A greater number of hybrids may have revealed greater diversity amongst

the specimens.

The presence of both glyphosate and ALS resistance within this population of hybrids (which was unknown at the time these hybrids were discovered) may have contributed to their survival by making it possible to tolerate applications of these herbicides. One of the herbicides used to address this resistance issue was Envive®, which is a mixture of flumioxazin, chlorimuron, and thifensulfuron. With the presence of ALS resistance in the hybrids and *A. palmeri*, the two sulfonylurea herbicides, chlorimuron and thifensulfuron, likely contributed little to their control. Without a complete understanding of the resistances present, timely application of more appropriate herbicides may have been missed. Consequently, it could be argued that these resistant hybrids represent new or invasive weeds because they survived herbicide treatments, failed to respond to current management practices, and thus may continue to multiply and spread unabated.

The consequences of added resistance traits in these hybrids above and beyond the obvious increased tolerance to herbicides is uncertain. Sometimes a mutation in one gene has unexpected consequences in other physiological/biochemical systems or developmental processes. For example, investigations into the pleiotropic effects of three acetyl coA carboxylase alleles (Leu1781, Gly 2078, and Asn2041) differing in frequency resulted in changes in the early life cycle stages of *Alopecurus myosuroides* Huds. (seed survival in the soil, seed germination, and seedling emergence) [8]. One mutation had no significant effects on germination. Mutation Gly2078 decreased the time to reach 50% germination and Leu1781 caused a delay in germination. These consequences of hybridization were on non-target processes. If these *A. palmeri* x *A. spinosus* persist in the environment, they may alter weed evolution by introducing new genes, gene combinations, and altered regulatory mechanisms into the plant life cycle.

The flowering pattern in these hybrids was also altered compared to their parent species. This implies a change in internal regulatory mechanism(s) as well as environmental cues affecting flowering, which in turn, may lead to new regulatory systems affording adaption to new ecological niches [9]. The appearance of the monoecious condition in hybrids with *A. palmeri*-like characteristics may signal a dramatic change in *A. palmeri* or *A. spinosus* evolution and create an opportunity for increased invasiveness. Monoecious plants may have efficient self-fertilization eliminating the need for outcrossing and long distance pollination. The reproductive capacity of the hybrids also may have been altered, such as in another species, *Raphanus sativus* L., where a greater duration of seed production was observed [10]. These consequences of hybridization have not been extensively investigated in the *Amaranthus* hybrids. Thus, it is important to perform weed biology experiments for any weed species exhibiting resistance or undergoing hybridization [11].

Weed resistance to herbicides, resulting from their introduction and repeated and/or excessive use, is a consequence of anthropogenic habitat disturbance. The

continued use of herbicides provides a stage for selection of genes already present in populations or newly arisen through mutation. Herbicides have acted as a selective force sufficient to drive introgression of resistance alleles from resistant to sensitive plants within species and thus represents a mechanism for rapid evolution. Currently, there are 252 weed species (147 dicots and 105 monocots) that are resistant to 161 different herbicides in 91 crops in 68 countries [12]. The appearance of glyphosate resistance in plants should raise serious concern in the agricultural community, as in the case of *A. palmeri*, these resistant forms have displaced sensitive forms. When glyphosate resistant weedy *Amaranthus* species with characteristics common to *A. spinosus* and *A. palmeri* were identified and subsequently shown to be hybrids, greater alarm should have been raised. These hybrids represent a population in which the reproductive barrier has been crossed and the fitness of plants (here meaning survival over several years under production practices) appears to have been maintained. These hybrids were further shown to have resistance to ALS-inhibiting herbicides, unbeknownst, when these hybrids were first discovered. The morphological variability present within this small sample of glyphosate and ALS resistant hybrids demonstrates a blending and acquisition of traits between species. Above results clearly indicate that the new 2,4-D + glyphosate and dicamba formulations registered for use on 2,4-D and dicamba-resistant soybean, respectively, provide a very viable alternative for controlling GR as well as wild types of troublesome pigweeds such as Palmer amaranth and tall waterhemp, and other broadleaf weeds such as common ragweed. Further, the new 2, 4-D + glyphosate formulation does not significantly impact the activities of soil microorganisms linked to nutrient cycling in the soybean rhizosphere.

5. Conclusion

Hybridizations between *A. palmeri* and *A. spinosus* resulted in offspring with intermediary traits differentiating them from parent types. The acquired characteristics may further enrich morphological diversity in parent species should subsequent backcrossing with parent types occur. The hybrids had acquired resistance to two major herbicide classes which upon backcrossing could enrich these traits in *A. spinosus* thereby spreading resistance. This potential movement of resistant genes is silent to the casual observer in that it is completely unexpected, unknown and not the result of herbicide application. These trait transfers may complicate future control efforts for new weeds emerging with resistance. Transfer of *A. spinosus* characteristics into *A. palmeri* may result in plants that are monoecious and have spines which could reduce their level of predation by cattle or other herbivores.

Acknowledgements

The technical assistance of R. E. Gordon and A. A. Wright is very much appreciated. This work was supported by the United States Department of Agriculture–Agricultural Research Service project number 6066-21000-060-00D.

References

[1] Nandula, V.K., Wright, A.A., Bond, J.A., Ray, J.D., Eubank, T.W. and Molin, W.T. (2014) *EPSPS* Amplification in Glyphosate-Resistant Spiny Amaranth (*Amaranthus spinosus*): A Case of Gene Transfer via Interspecific Hybridization from Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*). *Pest Management Science,* **70**, 1902-1909. https://doi.org/10.1002/ps.3754

[2] Molin, W.T., Nandula, V.K., Wright, A.A. and Bond, J.A. (2016) Transfer and Expression of ALS Inhibitor Resistance from Palmer Amaranth (*Amaranthus palmeri*) to an *A. spinosus* X *A. palmeri* Hybrid. *Weed Science,* **64**, 240-247. https://doi.org/10.1614/WS-D-15-00172.1

[3] Gaines, T.A., Ward, S.M., Bukun, B., Preston, C., Leach, J.E. and Westra, P. (2011) Interspecific Hybridization Transfers a Previously Unknown Glyphosate Resistance Mechanism in *Amaranthus* Species. *Evolutionary Applications,* **5**, 29-38. https://doi.org/10.1111/j.1752-4571.2011.00204.x

[4] Wetzel, D.K., Horak, M.J., Skinner, D.Z. and Kulakow, P.A. (1999) Transferal of Herbicide Resistance Traits from *Amaranthus palmeri* to *Amaranthus rudis*. *Weed Science,* **47**, 538-543.

[5] Trucco, F., Jeschke, M.R., Rayburn, A.L. and Tranel, P.J. (2005) *Amaranthus hybridus* can be Pollinated Frequently by *A. tuberculatus* Under Field Conditions. *Heredity,* **94**, 64-70. https://doi.org/10.1038/sj.hdy.6800563

[6] Wright, A.A., Molin, W.T. and Nandula V.K. (2016) Distinguishing between Weedy *Amaranthus* Species Based on Intron 1 Sequences from the 5-Enolpyruvylshikimate-3-Phosphate Synthase Gene. *Pest Management Science,* **72**, 2347-2354.

[7] Hoagland, R.E., Jordan, R.H. and Teaster, N.D. (2013) Bioassay and Characterization of Several Palmer Amaranth Biotypes with Varying Tolerances to Glyphosate. *American Journal of Plant Sciences,* **4**, 1029-1037. https://doi.org/10.4236/ajps.2013.45127

[8] Délye, C., Jasieniuk, M. and Le Corre, V. (2013) Deciphering the Evolution of Herbicide Resistance in Weeds. *Trends in Genetics,* **29**, 649-658. https://doi.org/10.1016/j.tig.2013.06.001

[9] Anderson, E. and Stebbins, G.L. (1954) Hybridization as an Evolutionary Stimulus. *Evolution,* **8**, 378-388. https://doi.org/10.2307/2405784

[10] Klinger, T. and Ellstrand, N.C. (1994) Engineered Genes in Wild Populations: Fitness of Weed-Crop Hybrids of *Raphanus sativus*. *Ecological Applications,* **4**, 117-120. https://doi.org/10.2307/1942121

[11] Ward, S.M., Cousens, R.D., Bagavathiannan, M.V., Barney, J.N., Beckie, H.J., Busi, R., Davis, A.S., Dukes, J.S., Forcella, F., Freckleton, R.P., Gallandt, E.R., Hall, L.M., Jasieniuk, M., Lawton-Rauh, A., Lehnhoff, E.A., Liebman, M., Maxwell, B.D., Mesgaran, M.B., Murray, J.V., Neve, P., Nuñez, M.A., Pauchard, A., Queenborough, S.A. and Webber, B.L. (2014) Agricultural Weed Research: A Critique and Two Proposals. *Weed Science,* **62**, 672-678. https://doi.org/10.1614/WS-D-13-00161.1

[12] International Survey of Herbicide Resistant Weeds. (2017) http://www.weedscience.org/Summary/home.aspx