Characterizing Herbicide Resistance in Southwestern U.S. and Invasive California Populations of *Amaranthus palmeri*

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INTRODUCTION

Palmer amaranth (*Amaranthus palmeri*) is a dioecious C_4 annual weed from the family Amaranthaceae (Fig 1). Palmer amaranth is native to northern Mexico and the southwestern U.S. The species originally grew primarily in arid regions and was consumed by Native American tribes. Palmer amaranth began to spread out of its native range in the early 20th century, possibly due to the spread of human activity (Sauer, 1955). Agricultural expansion and seed transportation may have led to the species being introduced to suitable environments that were not originally spatially accessible (Ward et al., 2013). While the spread of Palmer amaranth was recognized in the early 20th century, the plant wasn't considered a serious agricultural pest until the late 1980's (Sauer, 1972). As an agricultural pest, Palmer amaranth can make harvesting crops more difficult, decrease crop yields from 50 to 80 percent, and delay crop germination time (Davis et al., 2015). Due to continuous application and overuse of herbicides, populations of Palmer amaranth are strongly selected for herbicide resistance. Multiply-herbicide-resistant populations of Palmer amaranth have been discovered in several invaded Midwestern and Eastern U.S. states (Bagavathiannan and Norsworthy, 2016; Kohrt et al., 2017).

Recently, populations of Palmer amaranth have been appearing throughout Central California in agricultural areas. It is important to determine whether these populations carry herbicide resistance genes to determine the most effective management strategies for Palmer amaranth. At least one California population is hypothesized to be resistant to glyphosate (the widely-used chemical RoundUp®) based on field trials (Rios et al. 2016). We also hypothesize that some populations in the native Southwestern range of Palmer amaranth may be independently developing herbicide resistance, because the species has been appearing more frequently in desert agricultural environments. To test these hypotheses, we surveyed 29 populations from the Southwest and Central California for four different types of herbicide resistance, using both genetic and greenhouse assays.

METHODS

Sample collection and DNA extraction: Leaf tissue and seeds were gathered from twenty individual plants from 29 different populations, carefully chosen to cover both the native and invaded California range of Palmer amaranth, and both crop and non-crop field sites (Fig. 2). DNA was extracted from leaf tissue by being frozen with liquid nitrogen and ground in a mortar, and then extracted with an Omega Bio-Tek E.Z.N.A. Plant DNA kit. The seeds were used for greenhouse herbicide spraying experiments.

Herbicide resistance screening: After quality testing, DNA was tested for acetolactate synthase (ALS) inhibitor herbicide resistance. ALS-inhibiting herbicides are commonly used throughout the United States, and the most common mutation that leads to ALS resistance (a single base substitution, Trp574Leu) provides a simple and robust way of testing for the evolution of herbicide resistance (Kohrt et al., 2017). Individuals containing this allele for ALS-resistance are easily detected with a polymerase chain reaction (PCR) to amplify a large section of the acetolactate synthase gene. Then, a restriction digest containing enzymes that will cleave only herbicide resistant copies of the ALS gene is applied, and the products are visualized using agarose gel electrophoresis. At least two individuals per population were also Sanger sequenced to survey for other mutations in this ALS gene region. DNA was also tested for glyphosate resistance using quantitative PCR (qPCR). qPCR allows us to determine whether the EPSPS gene (the target of glyphosate) is amplified in copy number relative to a housekeeping gene (9240): this is the most common type of glyphosate resistance in Palmer amaranth (Délye et al. 2015). Herbicide resistance screening was also performed in greenhouse assays. 20 plants per population were included in an initial resistance screening, where plants were separated into 4 groups and treated with different herbicides. From these groups, any surviving populations were tested further with dose-response assays.

Figure 1 – Pictures of both sexes of Palmer amaranth, male on left, female on right, growing as a cotton field weed in Marana, Arizona. (Photos by K. Waselkov)

RESULTS / DISCUSSION

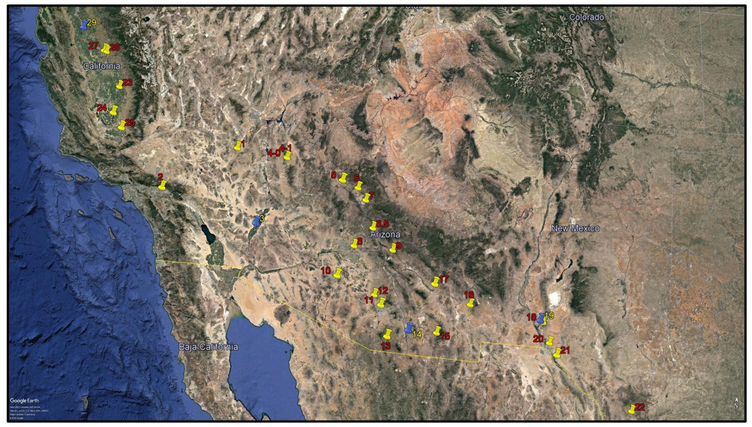

Figure 2 – Population sample map showing 28 of this study's collection points for Palmer amaranth in the western and southwestern U.S. These points include agricultural and non-agricultural roadside populations, as well as some wild desert populations. Not included on the map is population 26 – a population grown from seeds from the USDA ARS seedbank (originally from Arizona). Populations 3, 14, 19 and 29 – Blythe, CA, Fairbank, AZ, Las Cruces, NM, and Hilmar, CA, respectively – are marked in blue and labeled in yellow to indicate that herbicide resistance was confirmed at these sites.

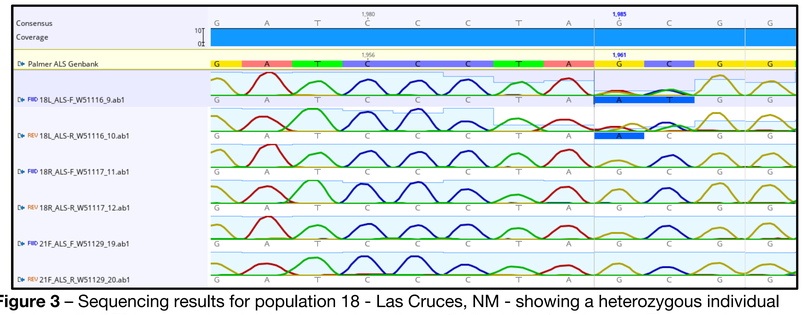

Figure 3 – Sequencing results for population 18 - Las Cruces, NM - showing a heterozygous individual carrying a Ser653Asn mutation that leads to imidazolinone (ALS-inhibitor) resistance. This was the only individual found to carry target-site genetic resistance to ALS-inhibiting herbicides.

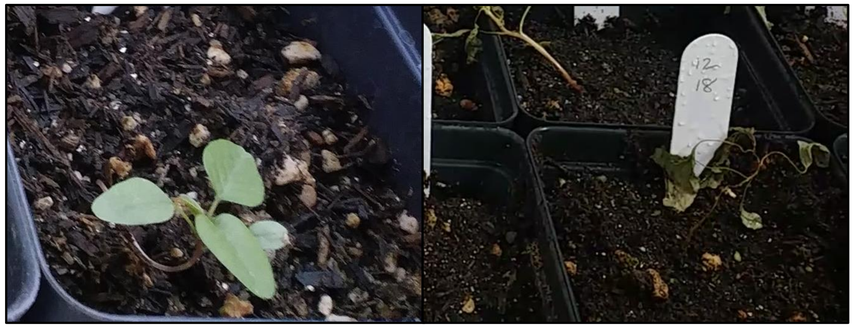

Figure 4 - After herbicide treatment, a resistant plant from population 10 compared to a susceptible plant from population 12. After herbicide treatments, 10 populations (2, 5, 10, 11, 13, 14, 17, 24, 25, 27) showed significant levels of ALS-inhibitor resistance. All other herbicide treatments totally killed off all treated plants.

Genetic Herbicide Resistance Testing: For all 29 populations, 20 individuals were tested for the most common form of ALS resistance. After visualizing DNA, any individual with potential positives were sequenced, alongside several other individuals per population. One individual from Las Cruces, NM (population 18) carried an ALS-inhibitor resistance mutation. 18L carried a Ser653Asn mutation that imparts partial resistance to ALS inhibitors. These results are interesting - combined with our greenhouse testing, there is a discrepancy between genetic herbicide resistance and demonstrated resistance *in vivo*. One of the issues may be that we did not sequence the entire ALS coding region. The *ALS* gene in our populations may carry other mutations throughout the sequence that could lead to herbicide resistance. It is also possible that these individuals are using some form of non-target site herbicide resistance. These possibilities require deeper study.

RESULTS / DISCUSSION CONTINUED

Conversely, Population 29 (**Table 1**) revealed a high level of genetic resistance with low levels of greenhouse resistance. This discrepancy may be due to changes in germination rhythms due to glyphosate resistance, which may have led to selection against resistant seeds in greenhouse testing. It is also possible our seeds carry too few copies of the EPSPS gene to be resistant, as the inheritance mechanisms of glyphosate resistance are not well understood.

Whatever the case, there is genetic evidence of Palmer amaranth growing in Central California that carries multiple copies of the EPSPS gene. It is important to continue to survey these populations, as this data can be used to inform agricultural communities of resistance profiles, and help them to create weed management plans.

Table 1 – qPCR data for 8 samples from population 29 – a known glyphosate-resistant population. Average EPSPS/9240 are threshold values (cycles when samples first fluoresced strongly enough to be quantified). Std – standard deviations. dCt – change in Ct values. ddCt – change in Ct values compared to a working standard (working standard used was 29I). xfold – the fold difference between the gene of interest and the housekeeping gene. Numbers in bold red text indicate ~5 or more copies of EPSPS are present in the plant, and it is probably glyphosate-resistant. Low/high – Fold difference plus or minus a standard deviation.

Sample	Avg EPSPS (Ct)	Std	Avg 9240 (Ct)	std	dCt	dCt std	ddCt	xfold	low	high
29A	20.6	1.4	18.2	0.2	2.5	1.4	1.4	0.4	0.1	1
29B	16.9	0.5	18.2	0.8	-1.3	0.9	-2.3	**4.8**	2.5	9.1
29C	23.5	0.3	19.8	0.6	3.7	0.7	2.7	0.2	0.1	0.2
29D	19.9	0.3	18.9	0.1	0.9	0.3	-0.1	1	0.8	1.3
29F	15.3	0	19.2	0	-3.9	0	-4.9	**29.9**	29.9	29.9
29H	25.8	1.1	23.3	1.5	2.5	1.9	1.5	0.3	0.1	1.2
29I	20	0.6	19	0.2	1	0.6	0	1	0.6	1.6
29J	17.1	0	18.8	0.5	-1.7	0.5	-2.7	**6.5**	4.5	9.4

FUTURE WORK

PPO-resistance screening – Populations will be tested for protoporphyrinogen oxidase (PPO) inhibitor herbicide resistance. This is a PCR-based resistance confirmation like ALS-inhibitor resistance.

Genotyping-by-sequencing: Currently, the origins of the Central California invasive populations of Palmer amaranth (whether a human-mediated invasion from the Eastern U.S., or an independent invasion from the Southwestern U.S.) are unknown. In order to determine the origins of these populations, we plan to use genotyping-by-sequencing (GBS), a cost-effective, accurate and effective method of generating large amounts of useful genetic data (Andrews et al., 2016). GBS uses a restriction enzyme to cleave DNA samples into small fragments. These fragments will be sequenced, and, when aligned and analyzed using various statistical programs, the SNP data generated will give insights into how the spread of Palmer amaranth outside of its native range occurred and mechanisms of biological invasions.

Further testing of our sampled populations: While our results are interesting, our samples require further testing to truly determine the current levels of herbicide resistance in our sampled areas. More in-depth sequencing, specifically of the ALS gene within the 10 populations that showed significant levels of herbicide resistance, as well as more qPCR testing of population 29 is required.

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