FINAL PROJECT REPORT

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Project title: Understanding the level of resistance in Palmer amaranth, waterhemp and kochia populations to key corn herbicides

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Background

Palmer amaranth, waterhemp, and kochia are emerging troublesome weeds in corn-based production systems in Texas. These three weed species are widely known to be resistance-prone due to their unique biological characteristics. Palmer amaranth is native to the southwestern US deserts, but has shown tremendous potential to adapt to a range of cropping situations. Since the first report of glyphosate resistance in Palmer amaranth in 2005, this species has been rapidly spreading across the US. As of now, Palmer amaranth has spread to more than 24 US states and is resistant to five different herbicide mechanisms of action. In corn production, herbicide-resistant Palmer amaranth has been reported in several states, including Mississippi, Missouri, Illinois, Indiana, Nebraska, Kansas, Georgia, Florida, and South Carolina, with resistance to atrazine, glyphosate, ALS-inhibitors, and HPPD-inhibitors. In some areas, multiple-resistant Palmer amaranth have also been reported. In 2009, a Palmer amaranth population collected in a corn field in Kansas showed three-way resistance to ALS-inhibitors (thifensulfuron-methyl), Photosystem II-inhibitors (atrazine), and HPPD-inhibitors (mesotrione, pyrasulfotole, tembotrione, and topramezone). More recently, a Palmer amaranth population with multiple resistance to photosystem-II inhibitors (atrazine) and HPPD-inhibitors (mesotrione, tembotrione, and topramezone) were documented in a seed corn production field in Nebraska. A survey conducted by the PI in eastern Arkansas during 2012 showed that the majority of surveyed Palmer amaranth populations were resistant to ALS-inhibitors and glyphosate, and also exhibited high variability for survival to HPPD-inhibitors.

Waterhemp is also an Amaranthaceous plant, with similar life history characteristics as Palmer amaranth. Although waterhemp is predominant in the US Midwest region, it is also a problematic weed in several other regions including Texas. Waterhemp is currently resistant to seven different herbicide modes of action. In corn-based production systems, waterhemp resistance to atrazine, glyphosate, ALS-inhibitors, and HPPD-inhibitors is common. Multiple herbicide resistance is also a growing issue in waterhemp. In 2009, three-way resistance to ALS-inhibitors, photosystem-II inhibitors, and HPPD-inhibitors were confirmed in waterhemp samples collected from seed corn production fields in Iowa and Illinois. In 2011, a waterhemp population with multiple resistance to four herbicide modes of action (ALS-inhibitors, photosystem-II inhibitors, HPPD-inhibitors, and glyphosate) was confirmed in corn-soybean system in Iowa.

Kochia is an early-emerging summer annual plant commonly found in the western regions. Kochia is widely resistant to ALS-inhibitors (imidazolinones and sulfonyl ureas) throughout the US in small grains production. In corn-based systems, atrazine-resistant kochia has been reported in Kansas, Colorado, Wyoming, Wisconsin, and North Dakota. The first glyphosate-resistant kochia population in the US was reported from Kansas in 2007 in a corn-based system and currently glyphosate-resistant kochia is spreading in at least eight US states. Kochia has also developed resistance to synthetic auxins such as dicamba in several states, including Montana, North Dakota, Idaho, Colorado, and Nebraska. Multiple herbicide-resistant kochia populations have also been emerging. For example in corn-wheat system in Illinois, a kochia population has developed multiple resistance to photosystem-II inhibitors (atrazine) and ALS-inhibitors (sulfonyl ureas).

Although anecdotal evidence suggests that herbicide resistance is a growing issue in Texas corn, little has been documented on the prevalence of occurrence and resistance profiles of some of the most problematic weed species such as Palmer amaranth, waterhemp, and kochia to important corn herbicides. In 1993, a Palmer amaranth population with resistance to atrazine was reported from Hale County, TX, and it is likely that there are more such cases since then. Glyphosate-resistant Palmer amaranth and waterhemp have been known to occur in a number of counties from central Texas to Gulf Coast through Southeast Texas. However, there has been a lack of systematic survey in Texas corn in recent times to better understand the current status of resistance to important corn herbicides.

With financial support from Texas Corn Producers Board, an extensive survey was initiated during the 2015 field season with an objective of understanding the response to important corn herbicides of Palmer amaranth, waterhemp and kochia populations in Texas corn production.

Methodology

A semi-stratified survey design was implemented to collect seed samples in the major corn producing regions in Texas. The survey area was grouped into four major regions: Panhandle, Rio Grande Valley, Coastal, and Central Texas. The methodology involves random assignment of travel route and survey sites in a spatial map without prior knowledge of the occurrence of study populations. In each survey region, approximately 100 to 150 survey locations were randomly assigned such that they are separated by at least 5 miles from each other in all directions (Figure 1). A Google map® and GIS integrated software (ITNConverter) was used to assign survey points and co-ordinate them with a GPS device for navigation.

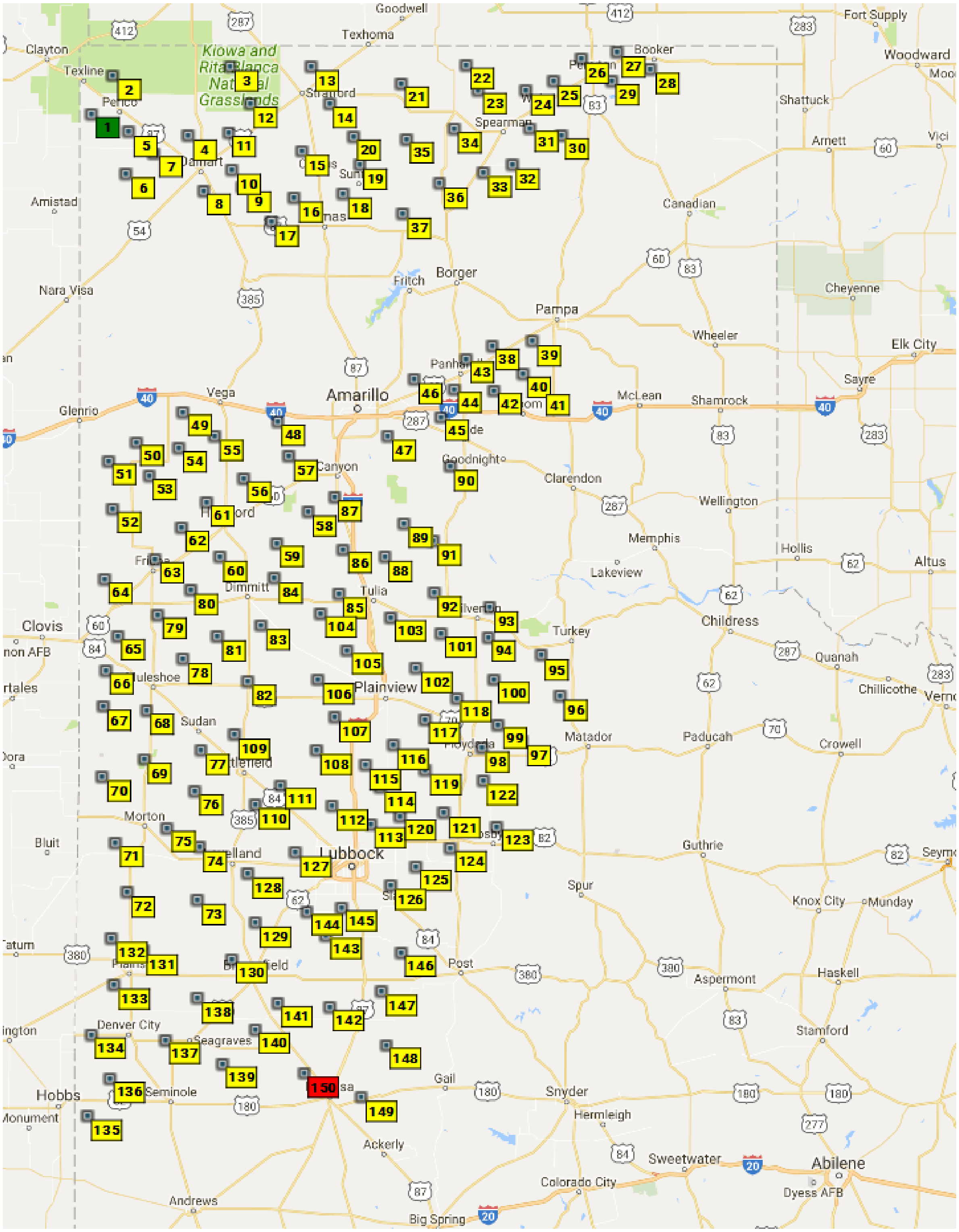

Figure 1. Example of random assignment of survey sites in the Panhandle region using the ITNConverter software

In each survey site, seed samples were collected from 10-15 individual plants and pooled into a single sample. Samples were dried in an oven at 50 C for 72 hours and thrashed manually. Herbicide screening was carried out in the greenhouse under standard environmental conditions (30/25 C day/night temperature regime and 16h photoperiod). Seed were planted in plastic trays (25 cm x 25 cm) and thinned to a density of approx. 50 seedlings per tray. Herbicide applications were carried out at 3 to 4 leaf seedling stage using a spray chamber. Seedling survival (%) as well as control ratings (%) were carried out at 21 days after treatment. Survival was measured as the number of surviving seedling out of total seedlings treated with the herbicide. Control ratings were carried out on a scale of 0 to 100, with 0 representing no injury compared to non-treated plants and 100 indicating complete plant death. All herbicide resistance screenings were carried out at the Texas A&M University – Norman Borlaug Center for Southern Crop Improvement greenhouse facilities.

Progress made and ongoing tasks

Given the nature of this project, all the activities could not be completed at this point. The original surveys started during late summer/fall 2015 and the seeds were thrashed and ready to be planted by December 2015. Waterhemp seeds did not germinate and required a cold treatment for a month to break dormancy. Standardization of experimental methodology also consumed a month period. Active mass screening of the samples started in January 2016. A vast majority of Palmer amaranth and waterhemp samples were already screened for resistance to Roundup. Screening for resistance to atrazine and Laudis are on the way for both Palmer amaranth and waterhemp. Detailed greenhouse dose-response assays will be carried out during early fall.

Additionally, the survivors will be tested for molecular mechanisms of resistance. Plant tissue samples were collected and stored in -20C for DNA extraction and subsequent molecular analysis. Some of the Palmer amaranth samples collected in Central Texas region had very poor germination and the seed supply was not sufficient. A follow-up survey is planned for the Central Texas region in late August 2016. All screening, dose-response assays and molecular characterization of the samples will be complete by December 2016.

An MS student was hired to oversee the survey and screening of the samples. Russ Garetson started his MS program in summer 2015 and is on track to graduate in spring 2017. His thesis has two chapters with survey and confirmation of herbicide resistance in Palmer amaranth and waterhemp in Texas. In addition, two undergraduate student workers were routinely involved in assisting with sample thrashing, planting, and other project activities.

Results obtained

Surveys revealed the distribution of Palmer amaranth predominantly in the Panhandle, Rio Grande Valley and Central Texas, whereas waterhemp was found in the Coastal and Central Texas regions. Palmer amaranth and waterhemp co-occurred around Corpus Christi, but waterhemp was the dominant weed east of Corpus Christi and all the way up to Beaumont in the Coastal region. Palmer amaranth was rarely noticed in the Coastal region east of Corpus Christi. Palmer amaranth was also found north of Corpus Christi and around San Antonio, up to Temple. Palmer amaranth and waterhemp co-occurred in some areas north of Austin to Temple. However, waterhemp was predominantly found in the Blacklands region from Temple to Commerce. Kochia was noticed in the counties bordering Oklahoma in the Panhandle region. However, not enough samples were available to conduct a detailed herbicide assay.

Palmer amaranth and waterhemp samples were screened for resistance to glyphosate (Roundup), atrazine POST and tembotrione (Laudis) (see Table 1 for herbicides and application rates). Samples survived for atrazine POST were screened for atrazine PRE using field soil. Tembotrione was included as a herbicide of interest for screening, given the current use of HPPD-inhibitor herbicides in corn and likely availability of HPPD-tolerant cotton in the near future. A sub-sample will also be screened for resistance to ALS-inhibitor herbicides.

Table 1. Herbicide and application rates used in the screening of Palmer amaranth and waterhemp samples

Herbicide	Active ingredient	1X rate (g ai ha^{-1})	Adjuvant (1% v/v)
RoundUp®	glyphosate	140.4	N/A
Aatrex 4L®	atrazine	181.6	COC
Laudis®	tembotrione	14.8	MSO

Palmer amaranth screening results

A total of 117 palmer amaranth populations have been screened up to the date of this report (Table 2, figures 1a, b). These samples are grouped into four separate regions of Texas: The Panhandle, Valley, Central, and Coastal. The majority of samples collected in the Panhandle region exhibited resistance to glyphosate. Of the 51 samples screened for glyphosate, 43 survived herbicide application. Twenty-six of the 43 surviving populations showed a survival rate of 80% or higher, suggesting that resistance evolution is in its advanced stages and glyphosate is no longer useful as a management tool. The other 17 populations showed much lower survival rates and indicated that resistance evolution is in its early stages and shift to diversified management options is vital before it is too late. A sub-sample of Palmer amaranth populations from the Panhandle region was screened for atrazine, but none of the samples survived the herbicide. Additionally, two populations were screened for resistance to tembotrione. One of the populations showed individuals that survived tembotrione (Figure 2). We are continuing to screen more populations, but observation of resistance to tembotrione in one of the two screened populations is alarming.

Figure 1a. Image showing the experimental setup of greenhouse screening for herbicide resistance in Palmer amaranth. This picture shows survivors to glyphosate 21 days after application in samples collected from the Panhandle region.

Figure 1b. Image showing the experimental setup of greenhouse screening for herbicide resistance in Palmer amaranth. This picture shows survivors to glyphosate 21 days after application in samples collected from the Valley region.

The Valley region includes samples collected from around the Brownsville and McAllen area. Twenty-seven populations from this area were screened for glyphosate and 16 of those found to show resistance. Only one population showed survival >80%. Four of the populations from the Valley were screened for resistance to atrazine, and none survived. None of the samples from this region were screened with tembotrione thus far. The Central region includes San Antonio/Temple area. Eighteen populations from this region were screened for glyphosate and 5 of them showed resistance. Only one of the resistant ones showed survival rating >80%. None of the central populations were screened with atrazine or tembotrione due to seed limitations. Palmer amaranth samples from the Coastal area is centered around Corpus Christi. A total of nine populations from this area were screened for glyphosate, and all nine of them survived. Only three of the nine were rated with a survival of >80%. One population from the Coastal region was screened for atrazine and it was susceptible. Three Palmer amaranth populations from the Coastal region were screened with tembotrione, and two survived with moderate injury.

Figure 2. Image of Palmer amaranth plants surviving tembotrione (Laudis) application

Table 2. Results of Palmer amaranth populations screened for resistance to glyphosate, atrazine and tembotrione

	Total Populations	Resistant Populations	>80% survival
Glyphosate			
Panhandle	51	43 (84%)	26 (51%)
Rio Grande Valley	27	16 (59%)	1 (4%)
Central	18	5 (28%)	1 (6%)
Gulf Coast	9	9 (100%)	3 (33%)
Atrazine			
Rio Grande Valley	4	0	0
Coast	1	0	0
Panhandle	2	0	0
Tembotrione			
Panhandle	2	1 (50%)	0
Gulf Coast	3	2 (60%)	0

Waterhemp screening results:

A total of 61 common waterhemp populations were screened with glyphosate (Table 3). Samples were collected from two regions: Coastal (from Corpus Christi to Beaumont along the Coast) and Central Texas. For the Coastal region, a total of 44 populations have been screened with glyphosate, and 39 survived the application. Among these, 10 populations showed a survival rating of >80%. In the Central region, 17 populations were screened, with 4 showing survival to glyphosate. Three out of the four surviving populations showed >80% survival rate. None of the common waterhemp populations have been screened for atrazine or tembotrione thus far.

Table 3. Results of common waterhemp populations screened for resistance to glyphosate

Region	**Total Populations**	**Resistant Populations**	**>80% Survival**
Coastal Texas	44	39 (89%)	10 (23%)
Central Texas	17	4 (24%)	3 (18%)

Outreach activities:

A factsheet is being prepared and is expected to be published in fall 2016 after all screening is complete. Drs. Paul Baumann, Peter Dotray, and Josh McGinty are actively involved in the dissemination of the findings through various outreach activities.

Summary:

Glyphosate resistance is widespread in Palmer amaranth and waterhemp throughout Texas. Glyphosate resistant Palmer amaranth is particularly prevalent in the Panhandle region, whereas glyphosate resistant waterhemp is prevalent in the Coastal region. The majority of Palmer amaranth populations in the Panhandle region showed very high levels of resistance, suggesting that resistance is in its advanced stages. However, the majority of Palmer amaranth populations in the Valley and Central Texas showed lower levels of resistance (i.e. high % survivors among the individuals), meaning that resistance has been developing within the past few years. This knowledge can be of great use for producers. In regions where resistance is high, glyphosate is no longer effective, whereas in areas where resistance is in its early stages of development, growers need to employ more diversified options in order to protect the utility of glyphosate in their farms. The widespread glyphosate resistance in Palmer amaranth and waterhemp indicates the intensity of glyphosate use without much mechanism of action diversity. If this trend continues, we will lose herbicide options rather quickly.

The confirmation of resistance to tembotrione (HPPD inhibitor) is a serious concern. HPPD-inhibitors are among the newest herbicide chemistries available for broadspectrum weed control. Resistance to HPPD-inhibitors has already been confirmed in other states, but data from our screening reveals that HPPD-inhibitor resistance is more prevalent than we previously thought. Although none of the screened populations thus far showed resistance to atrazine, it is very likely to be found in our screening of samples in the Panhandle region. Overall, the outcomes of this research are highly valuable for sustaining the utility of current and future weed management technologies.

Acknowledgment

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