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What is the distribution of single and multiple herbicide resistance in waterhemp populations across the state of Illinois?

Jared Worthle[1]
[1]Research Apprentice Program Student Participant

Advisors for research: Jung Eun Song, Patrick Tranel*
*Correspondence; tranel@illinois.edu

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ABSTRACT

The current trend in controlling waterhemp (*Amaranthus tuberculatus*) populations using herbicides has led to concern about herbicide resistance. The distribution of herbicide resistance in waterhemp populations was investigated. Waterhemp leaf tissue samples were obtained from farmers and weed management practitioners and the DNA was extracted and used for herbicide-resistance assays. Gel/PCR experiments were conducted in order to determine protoporphyrinogen oxidase (PPO) inhibiting herbicide resistance and qPCR experiments were conducted to determine glyphosate resistance. Results showed that the majority of field samples received were resistant to both PPO inhibitors and to glyphosate. Farmers should become aware of the distribution of herbicide resistance in waterhemp populations and change their control tactics to better manage this weed.

INTRODUCTION

Weeds reduce crop yields by using up available sunlight, water, space, and nutrients. Through agricultural innovation, herbicides were created and have been widely used to control weed populations. More than ninety percent of the acreage of common U.S. crops are being sprayed with herbicide on a regular basis (Gianessi and Sankula 2003). The use of herbicides reduces the need of physical labor using mechanical tools. After the mass adoption of herbicides, crop yields increased tremendously in the U.S. (Gianessi and Sankula 2003).

Waterhemp is usually found in the Midwest, being indigenous to the state of Illinois. It is part of the Amaranth family. It thrives in the wettest parts of fields, but it can easily adapt to a wide range of conditions. This weed has caused many problems in the past two decades for soybean and corn farmers in the Midwest. There are many reasons why waterhemp is becoming such a threat to farmers. Waterhemp plants have the ability to produce >300,000 seeds in unfavorable conditions and up to 5,000,000 seeds in favorable conditions. Recent studies show that common waterhemp is the most problematic weed in the states of Missouri and Illinois for corn farmers (Hager et al., 2002). Germination rates of these mass-produced seeds are high and the seeds are able to germinate throughout the growing season. This requires the farmer to make multiple herbicide applications. The small seeds produced from the plant favor a no-till field because the seeds are near the top of soil allowing them to germinate quicker (Buhler and Hartzler, 2001). These weeds have a higher relative rapid growth rate than most other weeds. They can grow up to 3 cm/day in the growing season, allowing these weeds to compete effectively for available sunlight hindering other weeds. Soybean fields containing 200 plants per m^2 can have reduced yields by 44% (Steckel and Sprague, 2004). The weed emerges early in the growing season of a crop, and an even higher percentage emerges later in the season allowing the plant to avoid pre-emergent herbicides and can flourish after post emergent herbicides, such as glyphosate, are applied. Waterhemp has both female and male flowers on separate plans meaning it is dioecious. Because of this, waterhemp has much greater genetic diversity than most other problematic weeds. This allows the weed to have a better chance of evolving, which allows the spread of new herbicide resistance genes and other ecological traits that improve waterhemp survival in agronomic systems.

Waterhemp's biology allows it to have an extraordinary ability to adapt to control methods. Through evolution, waterhemp has become resistance to six herbicide classes (Group 5, Group, 2, Group 14, Group 9, Group 27, and Group 4) (Heap, 2014). In this study, we focused on resistance to glyphosate (Group 9) and PPO inhibiting (Group 14) herbicides in waterhemp populations throughout the state of Illinois. We hypothesized that many fields contain waterhemp resistant to both PPO-inhibiting herbicides and glyphosate due to waterhemp's genetic diversity and farmers reliance on using these herbicides in the past decade.

METHODOLOGY

DNA Extraction of leaf tissue

Waterhemp leaf samples brought in from local farmers and weed management practitioners of Illinois were used in this study. The recommended number of samples from a single field was five. A small leaflet was removed from the whole sample and placed into a tube. Tubes were labeled appropriately and then placed in a freezer for later DNA extraction (Doyle and Doyle, 1990). The tissues were pulverized in liquid nitrogen and then 600 µl of CTAB (cetyltrimethylammonium bromide) solution was added to each tube. The tubes were then incubated for 20 minutes at 65 C in a water bath. The tubes were mixed by inversion at 5-minute intervals. 400 µl of chloroform was added to each tube and mixed by inversion. The tubes were then placed in a Centrifuge 5424 R for 5 minutes at 15,000 revolutions per minute. The upper layer of the extraction was transferred into newly labeled tubes and 500 µl of isopropanol. The new tubes were then centrifuged for 10 minutes at 15,000 revolutions per minute. The tubes were then decanted and 250 µl of 80% ethyl alcohol was added to each tube. The tubes were centrifuged for 2 minutes at 15,000 revolutions per minute. The tubes were then decanted and 250 µl of 95% ethyl alcohol was added to each tube. The tubes were centrifuged for 2 minutes at 15,000 revolutions per minute. The tubes were then decanted and dried in a Savant DNA SpeedVac concentrator at medium heat for 7 minutes. 75 µl of ultra-pure water was added to the tubes and the pellet was resuspended and later placed in a refrigerator. The DNA was then diluted depending on the quality of the DNA after using the Nanodrop.

Resistance Assays

DNA samples were used in PCR-based assays to test for a mutation conferring resistance to PPO inhibitors and to test for amplification of the EPSPS gene, which confers glyphosate resistance (Tranel et al., 2011). PPO resistance was scored based on presence/absence of an amplicon after PCR using gel electrophoresis. Samples were scored as glyphosate-resistant if qPCR indicated a relative EPSPS copy number of 3-fold or more. If a field contained at least one sample that was resistant then the whole field was considered resistant for the particular herbicide.

FINDINGS

Samples were evaluated from 94 fields thus far in 2014. The percentages of the fields that were resistance to just glyphosate, just PPO inhibitors, both herbicides, or neither herbicide are shown in Figure 1. It can be seen that fields with both glyphosate and PPO-inhibitor resistance are the most common. Counties with glyphosate resistance as of 2014 are shown in Figure 2.

Figure 1. Percentage of fields with no resistance, resistance to PPO-inhibiting herbicides, resistance to glyphosate, or resistance to both herbicides.

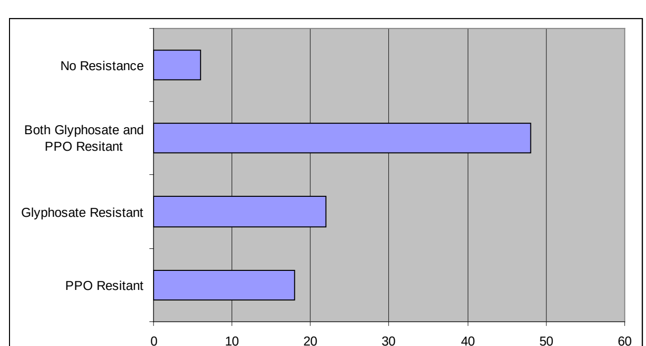
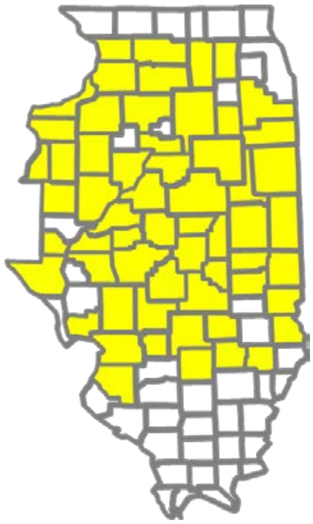


Figure 2. Counties (in yellow) in which glyphosate-resistant waterhemp has been identified based on grower-submitted samples 2010-2014.



The results from this study indicate that fields containing both PPO-inhibitor and glyphosate resistant waterhemp are the most prevalent. These two herbicide groups are the most commonly used for postemergence control of waterhemp in soybean. These results agree with past studies (Rosenbaum and Bradley, 2013) showing the growing distribution of herbicide resistance due to evolution and farmers consistent use of PPO-inhibitors and glyphosate herbicides. The findings suggests that farmers need to rely on and use preemergence herbicides and use other classes of herbicides in order to control waterhemp populations instead of glyphosate and PPO-inhibiting herbicides that have been heavily used in the past decade.

CONCLUSIONS

This study determined that waterhemp populations that are resistant to both PPO inhibitors and to glyphosate are common across the state of Illinois. The direct application of this study is that it allows farmers who submitted samples to know whether or not their waterhemp population is resistant to PPO inhibitors and/or glyphosate, encouraging them to change their control method to have an end result of a greater weed control. Future studies should emphasize on the need of using different classes of herbicides and reducing the use of PPO inhibitors and glyphosate herbicides. The results from this study will allow farmers to gain a better insight on the rapid spread and massive distribution of PPO and glyphosate resistant waterhemp population in the state of Illinois. This study also allows farmers to become more aware of how fast herbicide resistant traits can evolve in weeds.

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