CHARACTERIZATION OF PROTOPORPHYRINOGEN OXIDASE (PPO) HERBICIDE RESISTANCE IN TALL WATERHEMP (*AMARANTHUS TUBERCULATUS*)

by

Brent C. Mansfield

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Botany & Plant Pathology

West Lafayette, Indiana

August 2021

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Bryan G. Young, Chair
Department of Botany and Plant Pathology

Dr. William G. Johnson
Department of Botany and Plant Pathology

Dr. Michael V. Mickelbart
Department of Botany and Plant Pathology

Approved by:
Dr. Christopher J. Staiger

Dedicated to my parents

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor, Dr. Bryan Young, as well as my committee members, Drs. William Johnson and Michael V. Mickelbart. Completing my Master's has been a long drawn out process to say the least. I want to especially thank Dr. Young for his endless patience and guidance during this process. Thanks to Dr. Johnson and Mickelbart for their support, advice, and thesis edits throughout my time as a graduate student. Having the three of you serving on my committee has given me a much greater appreciation of faculty and what you go through on a day to day basis.

During my graduate work, I spent countless hours in the greenhouse and lab either transplanting tall waterhemp seedlings or extracting DNA and preparing qPCR plates. If I had to guess, I spent more time in the greenhouse and lab than in my actual office. I wouldn't have been able to complete this work without the help of an army of undergraduate, graduate students, and other department labs. There are too many people to list, but you all know who you are. However, I want to give a special shout out to Marcelo Zimmer for being a great friend and gym partner. I also want to thank Stephanie DeSimini for her friendship by letting me live with her a couple short months when my apartment lease was up. Two additional people that deserve endless thanks and were vital to the successful completion of my Master's degree are Julie Young and Dr. Haozhen Nie. Thank you, Julie, for being so organized and professional all the time. You provide a solid foundation to set up students for success although I know it comes with a lot of headache. I greatly appreciate you teaching me how to conduct high-quality greenhouse research. Thank you, Haozhen, for your friendship and teaching me your excellent molecular techniques.

Lastly, I want to show my appreciation to my loving family. Thank you, mom and dad, for instilling in me my work ethic and always pushing me to succeed. I know my graduate school process has not been the easiest for the two of you, but you never once gave up on me. Thank you, Brad, Jennifer, Brian, and Kate for always cheering me on as well. I love and look up to all of you!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Tall waterhemp management in agronomic crops continues to be an increasing problem due to widespread resistance to herbicides, including protoporphyrinogen oxidase (PPO)-inhibitors. With limited effective postemergence herbicides, especially in soybeans, research to further understand the selection of PPO-resistant (PPO-R) tall waterhemp and identification of new herbicide resistance mechanisms is crucial for improving weed management decisions in order to slow selection for herbicide resistance and prolong the effectiveness of PPO-inhibiting herbicides.

Previous research has shown that soil-applied applications of PPO-inhibiting herbicides can increase the frequency of the PPO resistance trait (ΔG210) in surviving tall waterhemp plants, even when applied in combination at the same ratio with the very long chain fatty acid inhibitor (VLCFA), *s*-metolachlor. Field experiments were conducted to determine if selection for tall waterhemp resistant individuals to PPO-inhibitors could be reduced when the soil residual activity of *s*-metolachlor persisted longer than the PPO-inhibitor herbicide. The frequency of ΔG210 in surviving individual plants increased as the fomesafen rate increased, but was independent of the rate of *s*-metolachlor. Additionally, heterozygosity of ΔG210 in surviving individuals did not change with any rate or combination of fomesafen and *s*-metolachlor. However, saflufenacil, standard PPO-inhibitor with relatively short soil residual activity, applied alone increased the number of homozygous PPO-R tall waterhemp by 15% compared to the high rate of *s*-metolachlor and the combination of saflufenacil and *s*-metolachlor. Furthermore, this research demonstrated that end of season control of tall waterhemp plays a more vital role in delaying a large-scale shift towards herbicide resistance through reduced seed production. This can be achieved through the combination of multiple effective herbicide sites of action, including soil residual PPO-inhibitors. Tall waterhemp control and density were greatest with the high rates of fomesafen plus *s*-metolachlor, which resulted in the lowest number of PPO-R tall waterhemp that survived herbicide treatment at the end of season.

Prior to the research conducted in this thesis, the only known resistance mechanism to PPO-inhibiting herbicides in tall waterhemp has been the ΔG210 target site mutation. A previously developed TaqMan assay used to determine the presence or absence of the ΔG210 mutation has allowed accurate, high throughput screening of this mutation. However, suspected

PPO-R tall waterhemp do not always receive positive confirmation indicating the presence of an alternative resistance mechanism. Identification of additional resistance mechanisms can provide valuable insight in regards to resistance to PPO-inhibiting herbicides as well as cross resistance to other herbicide modes of action, which can lead to improved tall waterhemp management decisions. Of 148 tall waterhemp populations collected across the Midwestern U.S., 84% of the populations sampled contained at least one PPO-R biotype with the ΔG210 mutation, although several individual plants across the Midwest U.S. exhibited phenotypic resistance to fomesafen that could not be explained by ΔG210. The percentage of PPO-R tall waterhemp without ΔG210 was 19, 5, 2, 1, and 2% for Iowa, Illinois, Indiana, Minnesota, and Missouri, respectively. Following the initial greenhouse screening, subsequent tall waterhemp populations were selected that exhibited low-, mid-, and high-level resistance to fomesafen that resulted in resistance ratios from 0.6 to 17X in response to fomesafen. This research documents the variability in fomesafen response to multiple tall waterhemp populations in addition to revealing the presence of additional resistance mechanism(s), other than the previously known ΔG210 mutation that has been the benchmark for resistance to PPO-inhibiting herbicides in tall waterhemp.

Lastly, greenhouse and lab experiments were conducted to investigate the role of antioxidant enzymes with PPO-R tall waterhemp via ΔG210. The objectives of this research were to determine if the variability in resistance ratios for PPO-R tall waterhemp documented in greenhouse and field scenarios could be due to an enhanced antioxidant enzyme pathway. Basal levels of antioxidant enzymes in PPO-S populations were not different from PPO-R populations when pooled together by respective phenotype. However, enzyme activity of tall waterhemp populations varied at the individual level, but independent of the ΔG210 mutation. This indicates that an inherent enhanced antioxidant enzyme pathway does not cause the variability in fomesafen response in tall waterhemp. With the exception of glutathione reductase, antioxidant enzyme activity following fomesafen application was generally the same for PPO-R and PPO-S populations by increasing, decreasing, or remaining unchanged. Glutathione reductase activity in PPO-S populations decreased compared to PPO-R populations from 9 to 36 HAT. By 36 HAT, all antioxidant enzyme activity for PPO-S populations was lower compared to PPO-R populations most likely a consequence of more lipid peroxidation. This research shows that antioxidant enzyme activity correlated with fomesafen application and documents the variability observed within tall waterhemp populations with and without the ΔG210 mutation.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Tall Waterhemp

Tall waterhemp (*Amaranthus tuberculatus*) is a dicot, summer annual weed belonging to the Amaranthaceae family also known as the pigweed family. Tall waterhemp and common waterhemp (*Amaranthus rudis*) have been documented as two closely related species that are able to hybridize with each other due to their dioecious nature (Pratt and Clark 2001). Hybridization of the two species caused great difficulty in distinguishing them when the infestation of these weeds in the U.S. merged geographically. Eventually, weed scientists were encouraged to consider both species synonymous and adopt the scientific name for tall waterhemp as [*Amaranthus tuberculatus* (Moq.) Sauer (syn. *rudis*)] (Pratt and Clark 2001). Before the two species hybridized, common waterhemp was native to the western Corn Belt region of present-day Nebraska and Kansas and south to Texas. Tall waterhemp was native further east in present-day Indiana and Ohio (Pratt and Clark 2001). Today, the species is present throughout Midwest U.S. and continues to present challenges in agronomic crop production. Midwest U.S. refer to the following twelve states: Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

1.2 Problematic Characteristics of Tall Waterhemp

A survey in 2016 listed waterhemp as the number one most common and troublesome weed in soybeans (Van Wychen 2016). Tall waterhemp also presents challenges in corn production and was listed as third most common and second most troublesome in corn in a 2017 survey (Van Wychen 2017). Tall waterhemp exhibits numerous traits that contribute to management challenges in agronomic crop production.

The discontinuous germination of tall waterhemp allows for multiple germination and emergence events throughout a prolonged growing season (Hartzler et al. 2004; Steckel and Sprague 2004a; Leon and Owen 2006). Delayed emergence creates more difficulty during the growing season because it increases the chance of tall waterhemp missing an herbicide application that typically occur early in the season. Cost of herbicide inputs also increase for growers if an herbicide application is necessary to control late emerged tall waterhemp. According to the

findings of Sellers et al. (2003), emergence of common waterhemp was approximately two weeks later than other *Amaranthus* species.

Another significant trait of tall waterhemp is the dioecious nature of the species meaning male and female reproductive structures are located on separate plants. The capability of outcrossing provides ease to increasing genetic diversity as well as transferring resistance traits.

Tall waterhemp also is capable of producing millions of seeds plant^{-1} depending on location and growing conditions (Hartzler et al. 2004; Wu and Owen 2014). Sellers et al. (2003) concluded common waterhemp produced approximately 250,000 seeds plant^{-1} and produced the largest number of seeds gram^{-1} of plant dry matter relative to other *Amaranthus* species.

Seed longevity is another weedy trait of tall waterhemp making crop rotations in the Midwest U.S. ineffective for reducing the soil seedbank (Buhler and Hartzler 2001). Research has shown that seed germination can remain as high as 10% after three years (Steckel 2007). Unfortunately, today's dominant cropping system in Midwest U.S. consists of corn and soybean production in a two-year rotation. In 2016 and 2017, Midwest states accounted for 82.3% and 81.3% of total U.S. acres planted to corn and soybeans, respectively (USDA-NASS 2016; USDA-NASS 2017).

One of the most undesired traits of tall waterhemp is the ability to highly compete for water and nutrients with other crops (Horak and Loughin 2000; Cordes et al. 2004). Hager et al. 2002b reported soybean yield losses of 43% with 200 plants m^{-2}. Previous research also revealed that only 8 plants m^{-1} of row in soybean resulted in a 56% yield loss (Bensch et al. 2003). In corn, common waterhemp reduced corn yield 74% when left uncontrolled during the entire growing season (Steckel and Sprague 2004b). Researching and fully understanding the troublesome characteristics of tall waterhemp can help with developing effective management strategies in agronomic crop production and limit potential yield loss.

1.3 Management of Tall Waterhemp

Tall waterhemp can be effectively managed using a variety of tools ranging from cultural, mechanical, and chemical control. Cultural practices for crops to help prevent infestations of tall waterhemp comprise planting date, planting population, and row spacing. Mechanical control uses the concept of tillage to disturb the soil destroying any emerged seedlings and burying weed seeds on the surface. Chemical control using herbicides is by far the primary means of weed control. In

soybeans alone, 95% of planted acres across 16 states, which is a total of 90.1 million acres, received an application of an herbicide (USDA-NASS 2017). Herbicides allow for less tillage passes in agronomic crops and can help prevent erosion and soil runoff.

There are two primary types of herbicides: foliar- and soil-applied. Foliar-applied herbicides control weeds that have already emerged. Soil-applied (soil residual) herbicides control weeds before they emerge by having a phytotoxic effect on the seed or seedling in the soil profile. Soil residual activity of these herbicides can range from days to weeks depending on the herbicide's chemical properties. Some foliar-applied herbicides also provide soil residual activity and vice versa; however, soil-applied herbicides that have foliar activity cannot always be applied directly to the crop because it will result in a phytotoxic crop response. Effective herbicide programs consist of a burndown, preplant herbicide application, or mechanical tillage followed by a preemergence and postemergence herbicide application of which all can use a mix of soil- and/or foliar-applied herbicides. Preemergence applications are herbicide applications that occur prior to crop and/or weed emergence while postemergence applications occur after crop and/or weed emergence. Utilizing a mechanical approach would simply replace the burndown or preplant herbicide application because both strategies have the same goal of removing weeds before planting.

Weed Science Society of America (WSSA) herbicide sites of action commonly used for tall waterhemp management in corn and soybean production include acetolactate synthase (ALS)-inhibitors (group #2), synthetic auxins (group #4), photosystem II (PSII)-inhibitors (group #5), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor (group #9), glutamine synthetase inhibitor (group #10), protoporphyrinogen oxidase (PPO)-inhibitors (group #14), very long chain fatty acid (VLCFA)-inhibitors (group #15), and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitors (group #27). Multiple studies have shown excellent control of common waterhemp with the herbicide sites of action listed above (Mayo et al. 1995; Sweat et al. 1998; Hager et al. 2002a). Unfortunately, tall waterhemp management still presents some problems. Four out of the eight herbicide groups available require a genetically modified soybean variety of which two groups have only recently been developed. Soybean varieties tolerant to group #4 herbicides have only been commercialized since the 2016 growing season and varieties tolerant to group #27 herbicides are currently unavailable commercially. Evolution of herbicide resistance has also affected the herbicide options available.

1.4 Protoporphyrinogen Oxidase Inhibiting Herbicides

Protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides (WSSA group #14; HRAC Group E) were introduced to agronomic crop production in the 1960s. Protoporphyrinogen oxidase is an enzyme in the last step of the tetrapyrrole biosynthesis pathway that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX; Proto) (Figure 1.1) (Jacobs and Jacobs 1984; Duke et al. 1991). PPIX is a precursor for chlorophyll in photosynthesis and heme production for electron transfer chains. Previously, three genes were hypothesized to encode for PPO in plants, *PPX1, PPX2S*, and *PPX2L*, and that resistant plants did not possess *PPX2S* (Patzoldt et al. 2006). However, previous research discovered only *PPX1* and *PPX2L* are responsible for production of PPO in plants (Lee et al. 2008).

Inhibition of PPO results in accumulation of PPIX because PPGIX overflows into the thylakoid membrane and oxidizes to PPIX. Production of PPIX in the thylakoid membrane is separated from Mg chelatase and other enzymes that help regulate overproduction. Excess PPIX readily absorbs light and results in the production of reactive oxygen species (ROS) (Duke et al. 1991). ROS are highly unstable and reactive causing damage to cell membranes and cell leakage eventually resulting in cell death by lipid peroxidation. Chemical families of PPO-inhibiting herbicides include diphenylethers, N-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyrimidindiones, thiadiazoles, and triazolinones.

PPO-inhibiting herbicides were predominantly used for control of tall waterhemp prior to the adoption of glyphosate-resistant soybeans (Mayo et al. 1995; Sweat et al. 1998; Hager et al. 2002a). Adoption of glyphosate-resistant corn and soybeans led to increased usage of the non-selective herbicide glyphosate, which eventually led to widespread weed resistance to glyphosate. With already widespread resistance to ALS-inhibitors and now glyphosate, the use of PPO-inhibitors increased substantially following the glyphosate era (USDA-NASS 2017) (Table 1.1).

1.5 Herbicide Resistance

Herbicide resistance can be broken down into two categories: target and non-target site resistance. Target site resistance refers to gene mutations that occur in target sites of the herbicide (Figure 1.2) (Preston 2014). Non-target site resistance includes mechanisms such as enhanced metabolism and reduced translocation. Enhanced metabolism refers to a plants ability to detoxify

the herbicide before it reaches the target site resulting in no herbicide activity in the plant. Enhanced metabolism is the most common type of non-target site resistance. Reduced translocation sequesters the herbicide in a vacuole or elsewhere in the plant and does not reach or has a reduced concentration of the herbicide at the target site. Target-site resistance is the most common type of resistance mechanism known to researchers primarily because it is rather simple to identify this mechanism using DNA sequencing. The advancement in molecular techniques has allowed scientists to sequence an entire genome of a plant within days. Scientists are able to utilize this information by comparing the base pairs of a DNA sequence among individual plants, which in return can assist with the identification of a resistant plant if there is a change in the base pairs or mutation within the sequence identified. Non-target site mutations are more complex mechanisms than target site mutations involving a multitude of biological processes creating difficulty for researchers to identify and fully understand the resistance mechanism. Resistance mechanisms can take decades to fully understand and vary among herbicide sites of action presenting an even greater challenge for weed scientists as new mutations arise. Herbicide resistance is present in over 255 weed species globally with a total of 495 unique cases. A unique case of herbicide resistance is defined as a weed resistant to a new herbicide site of action that was not previously identified. In the Midwest U.S., there are 225 unique cases of herbicide-resistant weeds (Heap 2020). Tall waterhemp accommodates for at least one unique case of herbicide resistance in all Midwest states with 43 total unique cases (Figure 1.3) (Heap 2020). Due to tall waterhemp being an obligate outcrossing species, many of the unique cases involve multiple herbicide resistance (Patzoldt et al. 2005; Legleiter and Bradley 2008; Schultz et al. 2015; Schwartz-Lazaro et al. 2017). In addition, tall waterhemp is the only weed species in the U.S. to evolve resistance to six different herbicide sites of action in the same plant (Shergill et al. 2018).

1.6 Detection of Herbicide Resistance

Common practices to identify herbicide-resistant weed populations starts by conducting surveys to collect plants and/or their seeds that survived an herbicide application. Numerous surveys have been conducted in previous years to characterize herbicide resistance, especially in *Amaranthus* species (Thinglum et al. 2011; Schultz et al. 2015; Varanasi et al. 2018).

Two general methods of evaluating putative herbicide-resistant weed populations are implemented. A whole-plant dose response can be conducted in a greenhouse to compare relative

herbicide efficacy to a known herbicide susceptible biotype and determine the resistant biotype's magnitude of resistance (MOR). The MOR for a weed biotype refers to the ratio of resistant and susceptible (R:S) GR_{50} values where GR_{50} is defined as the herbicide dose that results in 50% growth response relative to the nontreated control. The data used for evaluations are typically visual control estimates and/or shoot biomass that are then used to calculate GR_{50} values. Plants with GR_{50} values that are statistically different from a known susceptible biotype are considered resistant (Burgos et al. 2013).

The second method for confirming herbicide resistance in suspected biotypes involves using molecular based DNA assays. Molecular assays are more convenient and take less time compared to whole-plant dose responses because there is no need to grow plants and spray them with herbicides. Leaf tissue can be collected from suspected resistant plants, extracted for DNA, and assayed all in the same day. Molecular assays, however, are specific to single target site mutations and have the potential to underrepresent any other resistance mechanisms that may be present but have not been identified. Molecular assays also have not been developed for rapid identification of non-target site mutations due to the complexity of the resistance mechanism.

1.7 Resistance Mechanisms for PPO-Inhibiting Herbicides

Thirteen weed species have evolved resistance to PPO-inhibiting herbicides (Heap, 2020). The first weed to evolve PPO resistance was in a tall waterhemp population in Kansas (Shoup et al. 2003). Three distinct resistance mechanisms by target site mutation confer resistance to PPO-inhibiting herbicides of which two were discovered in *Amaranthus* species. A codon deletion at the 210th position in *PPX2* leading to the loss of a glycine (ΔG210) was first discovered in tall waterhemp, but also exhibited in Palmer amaranth (Patzoldt et al. 2006; Salas et al. 2016). The second target site mutation is a substitution of an arginine for leucine in *PPX2* in common ragweed (Rousonelos et al. 2012). More recently, two new target site mutations of *PPX2* (R98G and R98M) were discovered in Palmer amaranth at the same relative site that confers PPO resistance in common ragweed (Giacomini et al. 2017). No known fitness costs are associated with PPO resistance. However, a resistant and susceptible tall waterhemp population have been observed displaying equal growth patterns (Duff et al. 2009).

1.8 Selection for Resistance to PPO-Inhibiting Herbicides

Herbicide resistant biotypes surviving foliar-applied herbicides is common knowledge. However, soil-applied herbicides remain effective on herbicide resistant weed populations (Harder et al. 2012). Substantial selection pressure from increased usage of PPO-inhibiting herbicides garnered questions regarding selection of PPO-resistant biotypes exerted from soil-applied PPO-inhibiting herbicides. Previous research from Wuerffel et al. (2015) revealed that soil residual PPO-inhibiting herbicides indeed increase selection pressure of the ΔG210 mutation in waterhemp. Wuerffel also discovered that adding an additional site of action with a PPO-inhibitor did not decrease the frequency of resistance in tall waterhemp with the ΔG210 mutation but rather delayed resistance from occurring (Wuerffel et al. 2015). Wuerffel et al. (2015) was the first to investigate selection of PPO-R tall waterhemp with ΔG210 from fomesafen (Group #14 herbicide) and *s*-metolachlor (Group #15 herbicide). While Wuerffel et al. (2015) provided new insight, the research only evaluated two herbicides applied at different rates but at the same ratio of soil residual activity based on the commercially available premixed herbicide Prefix® (Syngenta Crop Protection, LLC, Greensboro, North Carolina 27419). Further research is justified to understand the influence of an alternative site of action applied at different ratios so that the alternative site of action persists the longest in the soil. Soil-applied herbicides remain crucial to weed management programs especially in the case of multiple herbicide resistance. Loss of soil-applied herbicides would be detrimental to agronomic crop production and potentially cause crop production land to become unsuitable for corn and soybean production.

1.9 Reactive Oxygen Species

Reactive oxygen species (ROS) are detrimental to plant survival as previously mentioned above. Examples of ROS species include singlet oxygen ($^1O_2$), hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), and superoxide radical (O_2·⁻). Generation of ROS result from various biotic and abiotic stresses such as salinity, drought, heavy metals, temperature, nutrient deficiencies, and herbicides (Gill and Tuteja 2010). Similar to humans, plants have ROS defense mechanisms that help detoxify ROS following an oxidative stress event. ROS defense mechanisms include enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD; GP), ascorbate peroxidase (APX),

monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Non-enzymatic antioxidants consist of ascorbic acid (AsA), glutathione (GSH), α-tocopherol, proline, carotenoids, and flavonoids (Gill and Tuteja 2010). The seven enzymatic antioxidants can be grouped into three categories for their roles in detoxifying ROS. The first group is SOD, which is responsible for catalyzing the dismutation of $O_2^{\cdot -}$ to H_2O_2 and O_2. The second group is CAT and POD, which are responsible for converting H_2O_2 to water. The third group consisting of APX, MDHAR, DHAR, and GR make up the ascorbate-glutathione cycle (AsA – GSH cycle) (Figure 1.4) (Harre et al. 2018).

While antioxidants are vital to protecting plants from excessive amounts of ROS leading to death, this also has implications on effective weed management. Multiple herbicide modes of action result in the production of ROS species. Limited research has been conducted to determine the impact of antioxidants on herbicide efficacy or resistance. A few cases have been documented suggesting increased levels of certain enzymatic antioxidants following herbicide application play a role in herbicide resistance by safening plant tissue from destructive ROS (Chiang et al. 2008; Harre et al. 2018). Currently, no research has been performed investigating the influence of enzymatic antioxidants on PPO-inhibiting herbicides. This research could help explain the variable responses in herbicide efficacy by PPO-inhibiting herbicides documented in greenhouse and field experiments.

1.10 Summary and Justification of Research

Tall waterhemp continues to be one of the most problematic weeds in corn and soybean production throughout Midwest U.S. Herbicides are currently the primary means of controlling tall waterhemp. Widespread resistance to the non-selective herbicide glyphosate has increased the use of PPO-inhibiting herbicides in glyphosate-tolerant corn and soybean. PPO-inhibiting herbicides are also heavily relied on in non-gmo corn and soybean due to the prevalence of resistance to acetolactate synthase (ALS)-inhibiting herbicides. This increase in PPO-inhibiting herbicides causes tremendous selection pressure for PPO-resistant biotypes. Research to understand the current PPO-resistance mechanism and identification of novel mechanisms is crucial in order to develop improved management strategies for PPO-resistant tall waterhemp, slow selection for PPO-R biotypes, and prolong the effective lifespan of PPO-inhibiting herbicides

in order to lessen the detrimental impact of tall waterhemp on corn and soybean yields. In order to address the knowledge gap, the following research objectives have been made.

Chapter 2:

1. Measure herbicide efficacy of soil-applied PPO-inhibiting herbicides on tall waterhemp populations with the ΔG210 mutation.
2. Determine the frequency of the ΔG210 mutation in surviving tall waterhemp following an application of fomesafen and *s*-metolachlor applied alone and in combination at different ratios relative to their soil residual activity in populations containing PPO-resistant individuals.

Chapter 3:

1. Quantify the response of tall waterhemp populations from five Midwest U.S. states to fomesafen.
2. Determine which, if any, tall waterhemp populations exhibit a resistance response unexplained by the ΔG210 mutation.

Chapter 4:

1. Establish basal levels of enzymatic antioxidants as well as protein and malonyldialdehyde (MDA) content in tall waterhemp populations resistant and susceptible to PPO-inhibiting herbicides.
2. Determine if increased levels of enzymatic antioxidants following an application of fomesafen could contribute to reduced sensitivity to the herbicide in a PPO-R biotype.

1.11 Literature Cited

Bensch CN, Horak MJ, Peterson D (2003) Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. Weed Sci 51:37–43

Buhler DD, Hartzler RG (2001) Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. Weed Sci 49:230–235

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK, Ritz C (2013) Review: confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4–20

Chiang Y, Wu Y, Chiang M, Wang C (2008) Role of antioxidative system in paraquat resistance of tall fleabane (*Conyza sumatrensis*). Weed Sci 56:350–355

Cordes JC, Johnson WG, Scharf P, Smeda RJ (2004) Late-emerging common waterhemp (*Amaranthus rudis*) interference in conventional tillage corn. Weed Technol 18:999–1005

Duff MG, Al-khatib K, Peterson DE (2009) Relative competitiveness of protoporphyrinogen oxidase-resistant common waterhemp (*Amaranthus rudis*). Weed Sci 57:169–174

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen Jr. LP, Matsumoto H (1991) Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 39:465–473

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri.* Pest Manag Sci 73:1559–1563

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Hager AG, Wax LM, Bollero GA, Simmons FW (2002a) Common waterhemp (*Amaranthus rudis* Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.). Crop Prot 21:277–283

Hager AG, Wax LM, Stoller EW, Bollero GA (2002b) Common waterhemp (*Amaranthus rudis*) interference in soybean. Weed Sci 50:607–610

Harder DB, Nelson KA, Smeda RJ (2012) Management options and factors affecting control of a common waterhemp (*Amaranthus rudis*) biotype resistant to protoporphyrinogen oxidase-inhibiting herbicides. Int J Agron 7

Harre NT, Nie H, Jiang Y, Young BG (2018) Differential antioxidant enzyme activity in rapid-response glyphosate-resistant *Ambrosia trifida*. Pest Manag Sci 74:2125–2132

Hartzler RG, Battles BA, Nordby D (2004) Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci 52:242–245

Heap I (2020) The international survey of herbicide resistant weeds. https://www.weedscience.org. Accessed December 4, 2020

Horak MJ, Loughin TM (2000) Growth analysis of four *Amaranthus* species. Weed Sci 48:347–355

Jacobs JM, Jacobs NJ (1984) Protoporphyrinogen oxidation, an enzymatic step in heme and chlorophyll synthesis: partial characterization of the reaction in plant organelles and comparison with mammalian and bacterial systems. Arch Biochem Biophys 229:312–319

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci 56:371–375

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587

Leon RG, Owen MDK (2006) Tillage systems and seed dormancy effects on common waterhemp (*Amaranthus tuberculatus*) seedling emergence. Weed Sci 54:1037–1044

Mayo CM, Horak MJ, Peterson DE, Boyer JE (1995) Differential control of four *amaranthus* species by six postemergence herbicides in soybean (*Glycine max*). Weed Technol 9:141–147

Patzoldt WL, Hager AG, Mccormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. PNAS 103:12329–12334

Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30–36

Pratt DB, Clark LG (2001) *Amaranthus rudis* and *A. tuberculatus*, One Species or Two? Torrey Bot Soc 128:282–296

Preston (2014) The mechanisms of herbicide resistance. Grains Research and Development Corporation. GRDC code: UA00124

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS-and PPO-inhibiting herbicides. Weed Sci 60:335–344

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72:864–869

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336–345

Schwartz-Lazaro LM, Norsworthy JK, Scott RC, Barber LT (2017) Resistance of two Arkansas Palmer amaranth populations to multiple herbicide sites of action. Crop Prot 96:158–163

Sellers BA, Smeda RJ, Johnson WG, Kendig JA, Ellersieck MR (2003) Comparative growth of six *Amaranthus* species in Missouri. Weed Sci 51:329–333

Shergill LS, Barlow BR, Bish MD, Bradley KW (2018) Investigations of 2,4-D and multiple herbicide resistance in a Missouri waterhemp (*Amaranthus tuberculatus*) population. Weed Sci 66:386–394

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145–150

Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567–570

Steckel LE, Sprague CL (2004a) Late-season common waterhemp (*Amaranthus rudis*) interference in narrow- and wide-row soybean. Weed Technol 18:947–952

Steckel LE, Sprague CL (2004b) Common waterhemp (*Amaranthus rudis*) interference in corn. Weed Sci 52:359–364

Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) Herbicide efficacy on four *Amaranthus* species in soybean (*Glycine max*). Weed Technol 12:315–321

Thinglum KA, Riggins CW, Davis AS, Bradley KW, Al-khatib K, Tranel PJ (2011) Wide distribution of the waterhemp (*Amaranthus tuberculatus*) ΔG210 *PPX2* mutation, which confers resistance to PPO-inhibiting herbicides. Weed Sci 59:22–27

USDA-NASS (United States Department of Agriculture - National Agricultural Statistics Service) (2016) Agricultural chemical use database. https://quickstats.nass.usda.gov. Accessed December 11, 2018

USDA-NASS (United States Department of Agriculture - National Agricultural Statistics Service) (2017) Agricultural chemical use database. https://quickstats.nass.usda.gov. Accessed December 11, 2018

Van Wychen L (2016) Survey of the Most Common and Troublesome Weeds in Broadleaf Crops, Fruits & Vegetables in the United States and Canada. WSSA. http://wssa.net/wssa/weed/surveys/. Accessed December 11, 2018

Van Wychen L (2017) Survey of the Most Common and Troublesome Weeds in Grass Crops, Pasture and Turf in the United States and Canada. WSSA. http://wssa.net/wssa/weed/surveys/. Accessed December 11, 2018

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2018) A statewide survey of PPO-inhibitor resistance and the prevalent target-site mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 66:149–158

Wu C, Owen MDK (2014) When is the best time to emerge: reproductive phenology and success of natural common waterhemp (*Amaranthus rudis*) cohorts in the Midwest United States? Weed Sci 62:107–117

Wuerffel RJ, Young JM, Tranel PJ, Young BG (2015) Soil residual protoporphyrinogen oxidase–inhibiting herbicides influence the frequency of associated resistance in waterhemp (*Amaranthus tuberculatus*). Weed Sci 63:529–538

Table 1.1. Fomesafen and sulfentrazone usage in soybeans in the U.S.

Herbicide	Soybean acres planted[a]		
	2000	2005	2017
		%	
fomesafen	7	3	19
sulfentrazone	4	2	22

[a] Reference: USDA-NASS (2017).

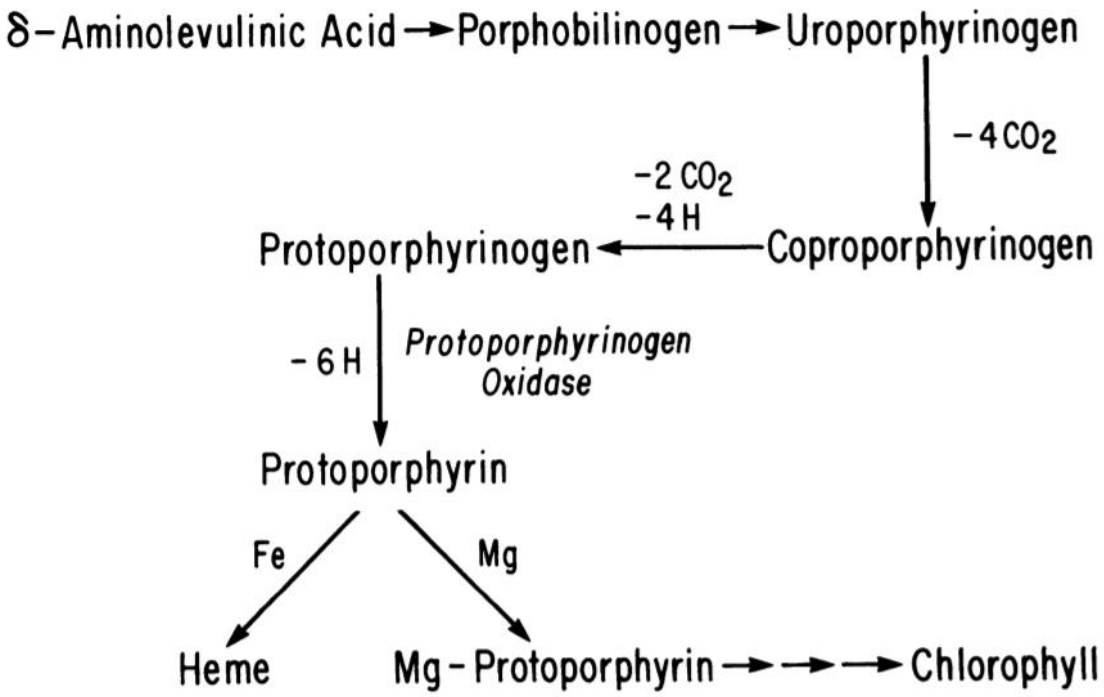

Figure 1.1. Pathway of heme and chlorophyll synthesis (From Jacobs and Jacobs 1984).

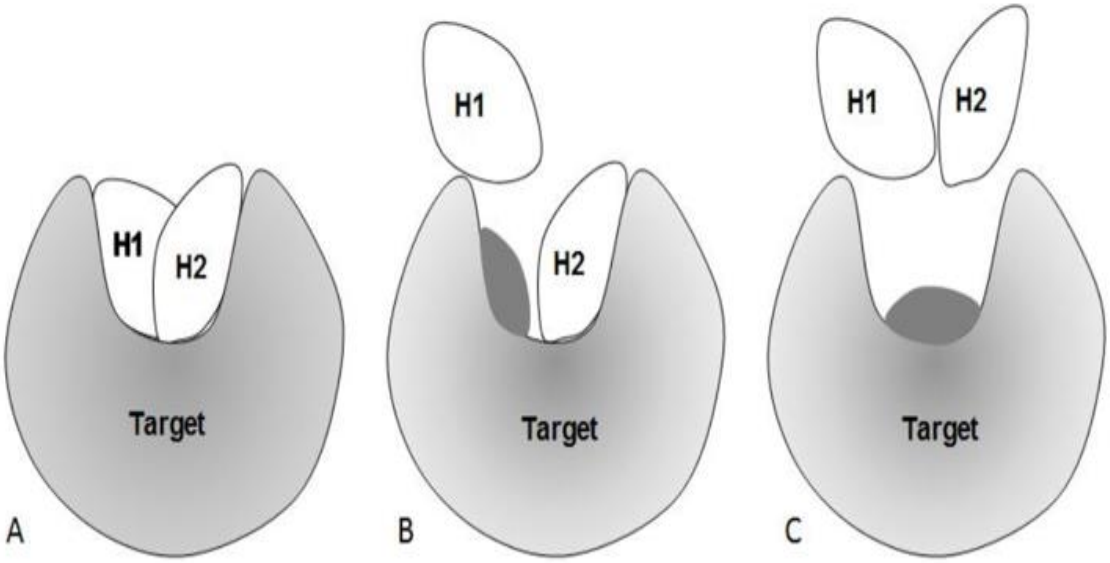

Figure 1.2. Different mutations within a target site can result in different patterns of resistance. A target has a binding site where two chemically different herbicides, H1 and H2, can bind (A). The herbicides bind to different parts of the binding site. A mutation within the target site (B) may stop binding of one herbicide, but not the other. A different mutation elsewhere within the target site (C) may stop both herbicides from binding (Preston 2014).

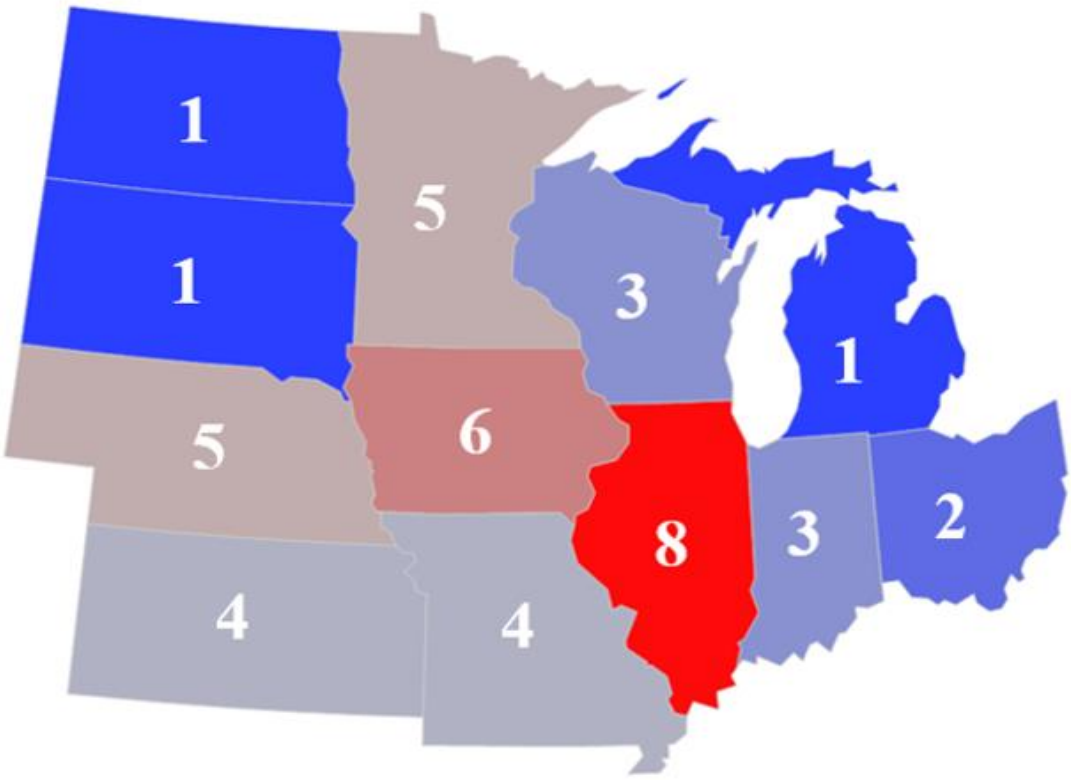

Figure 1.3. Unique cases of herbicide resistance in tall waterhemp in Midwest U.S. (Adapted from Heap 2020).

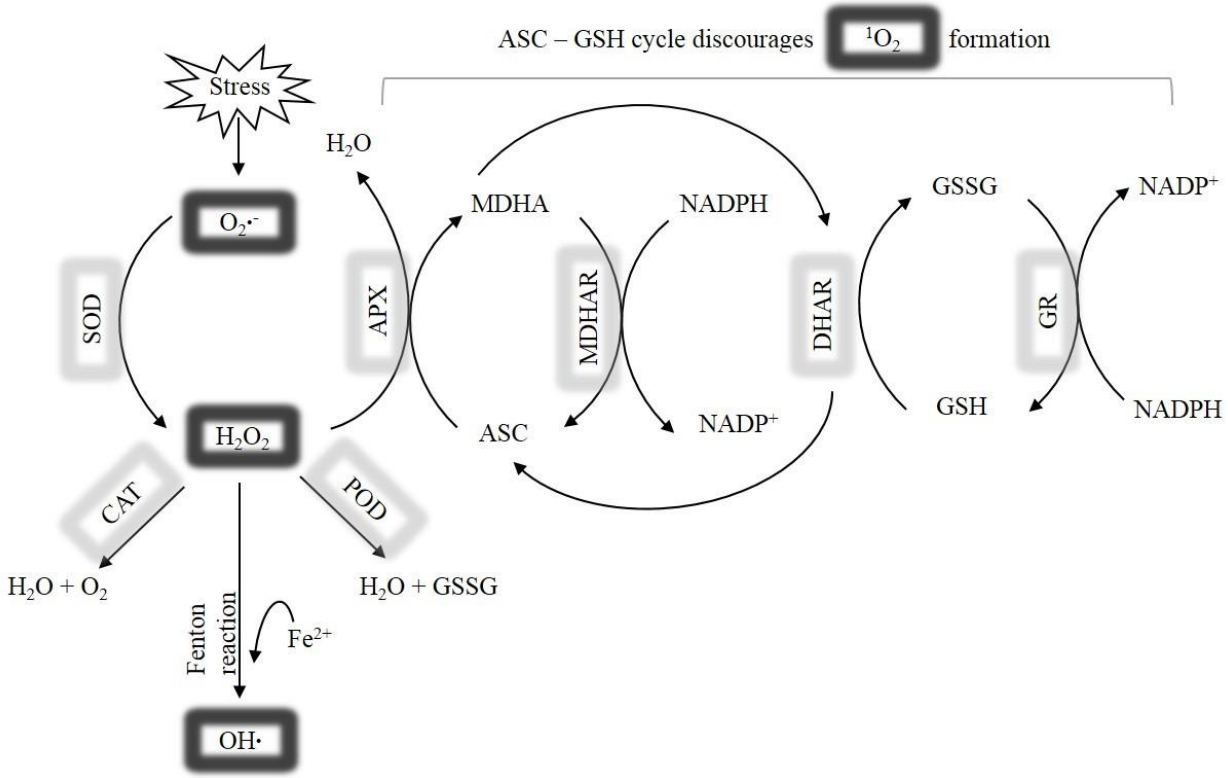

Figure 1.4. Generalized scheme for oxidative stress protection found in plants via enzymatic antioxidants. The four primary reactive oxygen species are outlined in black: $O_2\bullet^-$, superoxide; H_2O_2, hydrogen peroxide; OH•, hydroxyl radical; and $^1O_2$, singlet oxygen. Antioxidant enzymes are outlined in gray: SOD, superoxide dismutase; CAT, catalase; POD, guaiacol peroxidase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; and GR, glutathione reductase. The action of the ascorbate (ASC)–glutathione (GSH) cycle discourages $^1O_2$ formation by sustaining excess energy dissipation from photosystems (Harre et al. 2018).

CHAPTER 2. INFLUENCE OF A SOIL RESIDUAL PPO-INHIBITING HERBICIDES APPLIED ALONE AND IN COMBINATION WITH AN ALTERNATIVE HERBICIDE SITE OF ACTION ON THE SELECTION PRESSURE FOR THE ΔG210 MUTATION

2.1 Abstract

Previous research has demonstrated that the use of soil residual PPO-inhibiting herbicides, including fomesafen, can increase the frequency of the PPO resistance trait (ΔG210 mutation) in the proportion of tall waterhemp plants that escape the residual herbicide. In addition, combining *s*-metolachlor as an alternative site of action with fomesafen did not affect this increase in the PPO resistance trait when the rate of the two herbicides were applied at a constant ratio. A hypothesis was formed that the length of effective soil residual activity of the alternate herbicide site of action relative to the length of soil residual from fomesafen will influence the frequency of the PPO resistance trait in the surviving weed population. A field experiment was conducted over three site-years to investigate the hypothesis in a population of tall waterhemp containing the ΔG210 mutation. The experimental design consisted of a factorial of three rates each of fomesafen (66, 132, 264 g ai ha^{-1}) and *s*-metolachlor (335, 710, 1420 g ai ha^{-1}) applied preemergence to a weed-free seedbed as well as a nontreated control. Comparison herbicide treatments of saflufenacil at 25 g ai ha^{-1} and sulfentrazone at 280 g ai ha^{-1} applied alone and with *s*-metolachlor at 1420 g ha^{-1} were also included to provide further insight of selection pressure of PPO-R from PPO-inhibiting herbicides with different levels of soil residual activity. The greatest herbicide efficacy, in terms of visual control and reductions in tall waterhemp density, resulted from the combination of fomesafen and *s*-metolachlor applied at the highest rates. The frequency of the ΔG210 mutation was not influenced as the rate of *s*-metolachlor increased relative to fomesafen, regardless of how long each herbicide contributed to the length of soil residual tall waterhemp control. However, an increase in the frequency of the ΔG210 mutation was observed in the surviving plants as the rate of fomesafen increased, independent of *s*-metolachlor rate. Additionally, saflufenacil alone increased the number of homozygous PPO-R plants compared to *s*-metolachlor at 1420 g ha^{-1} and saflufenacil plus *s*-metolachlor. Interestingly, the number of individual tall waterhemp plants with PPO-R surviving the herbicide treatment was lower for fomesafen applied at the highest rate even though this treatment increased the frequency of the ΔG210 mutation. This research further

supports that both the extent of weed control and the frequency of resistance traits in the surviving weed population must be considered in determining the value of herbicide combinations for herbicide resistance management.

2.2 Introduction

Tall waterhemp presents major challenges in agronomic crop production, especially in soybean, primarily through evolved herbicide resistance specifically to herbicides that inhibit the protoporphyrinogen oxidase (PPO) enzyme, in addition to herbicide resistance to glyphosate and ALS-inhibiting herbicides (Foes et al. 1998; Legleiter and Bradley 2008; Shoup et al. 2003). Tall waterhemp was the first weed to evolve resistance to PPO-inhibiting herbicides in 2001 (Shoup et al. 2003). Currently, 13 weeds worldwide have evolved resistance to PPO-inhibiting herbicides (Heap 2020). Three mechanisms of resistance have been identified to confer resistance to PPO-inhibiting herbicides. The first being a codon deletion of *PPX2* that leads to a loss of a glycine at the 210th position (ΔG210) found in tall waterhemp and Palmer amaranth (Patzoldt et al. 2006). The second resistance mechanism occurred first in common ragweed which results in a substitution of an arginine for leucine at the 98th position (R98L) of *PPX2* (Rousonelos et al. 2012). Palmer amaranth was discovered with two similar target site mutations more recently in 2017 resulting in a substitution of an arginine for glycine (R128G) or a substitution of an arginine for methionine (R128M) (referred to as R98 in Giacomini et al. 2017) at the 128th position of *PPX2* (Giacomini et al. 2017).

Herbicides that inhibit the PPO enzyme are commonly applied for soil residual and foliar control of tall waterhemp in agronomic crop production (Hager et al. 2002; Mayo et al. 1995; Sweat et al. 1998). Specifically in the Midwest, tall waterhemp has garnered much attention in soybeans due to the evolution of resistance to seven herbicide sites of action in addition to multiple resistance to six sites of action in a single tall waterhemp plant (Heap 2020; Shergill et al. 2018). Tall waterhemp exhibits resistance to foliar-applied PPO-inhibiting herbicides (Falk et al. 2006; Patzoldt et al. 2005; Wuerffel et al. 2015a). However, soil-applied PPO-inhibiting herbicides still remain effective on tall waterhemp populations possessing the ΔG210 mutation (Falk et al. 2006; Shoup et al. 2003; Wuerffel et al. 2015b).

Previous research in greenhouse settings documented that selection pressure for PPO-resistant (PPO-R) waterhemp maybe greater than PPO-susceptible (PPO-S) among the first

emerging plants as fomesafen, lactofen, and acifluorfen diminish in the soil (Falk et al. 2006). Wuerffel et al. (2015b) was the first to quantify the observed increase in FOR in waterhemp for the ΔG210 mutation with applications of fomesafen, flumioxazin, and sulfentrazone in a greenhouse and field. Additionally, Wuerffel et al. (2015b) discovered the combination of *s*-metolachlor with fomesafen did not decrease the frequency of resistance (FOR). This research demonstrated that the improved overall efficacy of the herbicide combinations reduced the number of surviving tall waterhemp plants, thereby reducing the number of surviving PPO-R individuals, and ultimately improving herbicide resistance management. However, this research only investigated herbicide treatments where fomesafen and *s*-metolachlor were applied at the same ratio as the commercially premixed formulation of fomesafen + *s*-metolachlor (Prefix®, Syngenta Crop Protection). Even though two herbicides may be applied at the same ratios this does not mean the lengths of soil residual activity for both herbicides are the same initially at application. Additionally, the biologically effective dose or herbicide concentration required to control tall waterhemp through soil residual may be a greater factor for differences in efficacy from fomesafen and *s*-metolachlor.

Theoretically, selection pressure of the ΔG210 mutation in tall waterhemp should exist when the PPO-inhibiting herbicide persists in the soil at a discriminating dose for a longer period than the alternative herbicide site of action applied in combination, thereby providing a PPO-inhibitor filter for weed seedling survival. This theory depends on the individual herbicide persistence in the soil relative to the herbicide applied in the combination. With the exception of environmental factors at play, the length of soil residual activity can be estimated prior to herbicide application based on herbicide soil adsorption properties and half-life. This provides insight into which herbicide will more likely last longer in the soil and thus, determine selection for herbicide resistance traits. Soil adsorption properties (K_d and K_{oc}) and half-life indicate that sulfentrazone persists the longest in the soil followed by *s*-metolachlor, fomesafen, and saflufenacil (Table 2.1) (Papiernik et al. 2012; WSSA 2014). Field research conducted in Tennessee confirmed that saflufenacil, fomesafen, and sulfentrazone had half-lives of 21.4, 45.6, and 70.8 days, respectively (Mueller et al. 2014). Of course, the rate of soil dissipation is no indication of the herbicide concentration necessary to induce a lethal biological effect on weed seedlings.

Multiple authors in previous years have discussed the concern and documented the influence of soil residual herbicides on selection for herbicide resistant biotypes (Falk et al. 2006;

Norsworthy et al. 2012; Taylor-Lovell et al. 1996; Wrubel and Gressel 1994; Wuerffel et al. 2015b). However, no research has addressed how herbicides with different levels of soil residual activity, especially when applied in herbicide combinations, influence selection pressure for herbicide-resistant biotypes. Therefore, the objectives of this research were to measure herbicide efficacy and the selection of the ΔG210 mutation in surviving tall waterhemp plants from soil residual applications of PPO-inhibiting herbicides applied alone and in mixture with *s*-metolachlor.

2.3 Materials and Methods

2.3.1 Trial Establishment

Field experiments targeting tall waterhemp with the ΔG210 mutation were conducted in 2016 and 2017 at the Meigs Purdue Agriculture Center in Lafayette, Indiana and in 2017 at Davis Purdue Agriculture Center (DPAC) in Farmland, Indiana. The experiment was in the same field each year, but in different specific locations to avoid confounding with previous herbicide applications. The Lafayette trials were conducted on a Camden silt loam in 2016 and a Starks-Fincastle complex in 2017. Organic matter and pH across both soil types in Lafayette were approximately 2% and 6.5, respectively. The soil type in Farmland was a Pewamo silty clay loam with 4% organic matter and pH of 6.3. Existing weed vegetation received a burndown application of paraquat (Gramoxone® SL 2.0, Syngenta Crop Protection, LLC, Greensboro, NC 27419) prior to trial initiation to ensure weed-free conditions. A clethodim application (Select Max®, Valent U.S.A. Corporation, Walnut Creek, CA 94596) was used mid-season to control grass weeds in order to optimize germination and growth of tall waterhemp.

2.3.2 Herbicide Application and Experimental Design

Herbicide treatments included a factorial of three rates each of fomesafen (Flexstar®, Syngenta Crop Protection, LLC, Greensboro, NC 27419) (66, 132, 264 g ai ha^{-1}) and *s*-metolachlor (Dual II Magnum®, Syngenta Crop Protection, LLC, Greensboro, NC 27419) (355, 710, 1420 g ai ha^{-1}). Two additional soil residual PPO-inhibiting herbicides, saflufenacil applied at 25 g ai ha^{-1} (Sharpen®, BASF Corporation, Research Triangle Park, NC 27709) and sulfentrazone (Spartan® 4F, FMC Corporation, Philadelphia, PA 19104) applied at 280 g ai ha^{-1} were included as comparison treatments with and without the addition of *s*-metolachlor at 1420 g ha^{-1}. Saflufenacil

was chosen because of the relatively short soil half-life expected to be similar to 66 g ha^{-1} of fomesafen while sulfentrazone has a relatively long soil half-life and was expected to be similar to 264 g ha^{-1} of fomesafen (Table 2.1) (WSSA 2014). Wuerffel et al. (2015b) investigated two out of the three herbicide families used in this experiment in addition to flumioxazin, which belongs to the *N*-phenylphthalimide chemical family (Table 2.1). However, all of the herbicides used in their experiment had relatively long soil residual activity. Therefore, saflufenacil provided further insight into how PPO-inhibiting herbicides with relatively short soil residual activity influence selection pressure for PPO-R biotypes. The experimental design also included a nontreated control with no herbicides applied. Plot size measured 3 by 7.6 m with the treated area measuring 2 m wide, allowing for nontreated strips on each side of the plot. Herbicide applications were performed using a CO_2-pressurized backpack sprayer and a 4-nozzle boom with 50-cm nozzle spacing calibrated to deliver 140 L ha^{-1} at a pressure of 207 kPa with XR8002VS flat fan nozzles. The experimental design was a randomized complete block (RCB) with four replications.

2.3.3 Genotypic Analysis of Tall Waterhemp

Tall waterhemp leaf tissue was collected on emerged plants twice during the experiment for genotypic analysis in order to assess the frequency of resistance (FOR) for the ΔG210 mutation, which has been the most prevalent herbicide resistance mechanism conferring resistance to PPO-inhibiting herbicides in tall waterhemp (Thinglum et al. 2011; Mansfield et al. 2017; Nie et al. 2019). Thus, only selection pressure of the ΔG210 mutation was investigated and no other PPO target site mutations were found at the research locations. The first plant material collection for genotyping was designed to have the highest selection pressure for PPO-R biotypes since the collection consisted of the first 25 tall waterhemp plants to emerge following herbicide application (i.e. plants surviving the highest concentrations of the soil residual herbicides). The second collection consisted of an additional 25 plants that occurred five to seven weeks after the completion of the first collection (Table 2.2) to represent late weed escapes after herbicide dissipation. Hypothetically, a larger increase in frequency of resistance should be observed initially due to higher fomesafen concentrations compared with end-of-season fomesafen concentrations following herbicide degradation in the soil. The youngest leaf tissue was collected for all plant samples and stored in a -20 C freezer until processed for DNA extraction using a modified

cetyltrimethylammonium bromide (CTAB) method originally designed by Saghai-Maroof et al. (1984).

2.3.4 Data Collection

Visual assessments of overall herbicide control per plot relative to the nontreated check were recorded weekly from 14 to 42 days after treatment (DAT) using a scale of 0 to 100% with 0% defined as no herbicide effect and 100% defined as no plant emergence. Tall waterhemp density was recorded at 28 and 56 DAT using 0.1-m^2 quadrats with four quadrats per plot. Tall waterhemp tissue was subjected to real-time polymerase chain reaction (RT-qPCR) to determine the presence or absence of the ΔG210 mutation. Frequency of resistance (FOR) in tall waterhemp was calculated by dividing the total number of heterozygous and homozygous resistant individuals with ΔG210 to the total number of tall waterhemp plants collected as described by Wuerffel et al. (2015b). In order to gain further insight regarding FOR, an additional variable (end-of-season PPO-R plants) was created by multiplying FOR by emerged plant density in each plot to compare differences among projected surviving PPO-R tall waterhemp at the end of the growing season.

2.3.5 Statistical Analysis

Farmland in 2017 marked the first year Purdue weed science trials were initiated at the Davis Purdue Agriculture Center resulting in an unknown tall waterhemp soil seedbank. Unfavorable field conditions prior to trial initiation caused a late spring burndown application potentially excluding the first major emergence event of tall waterhemp from being included in the research. Even though adequate rainfall (data not shown) was received following trial establishment, limited tall waterhemp emergence occurred throughout the rest of the growing season resulting in difficulty making accurate conclusions from the data; therefore, Farmland data were excluded in the results. Tall waterhemp control, density (presented as a percentage of the nontreated plots), FOR, and surviving PPO-R plants were subjected to a two-way mixed effect ANOVA using PROC GLIMMIX (SAS 9.4) for the two main factors, fomesafen and *s*-metolachlor. Data were pooled over year at the Lafayette site since no significant herbicide treatment by year interaction was identified for any data variables. The FOR values were combined over collection timings due to a non-significant P-value ($p > 0.05$) in the ANOVA. Model

assumptions of normality and homogeneity of variance were tested and data were arcsine (density and surviving PPO-R plants) or square root (FOR) transformed prior to analysis. Data are presented as back-transformed means for ease of interpretation. Means were separated using Tukey's honest significant difference (HSD) test at alpha = 0.05. Orthogonal contrast statements within PROC GLIMMIX were used to compare tall waterhemp control, density, FOR, and surviving PPO-R plants for the PPO herbicides saflufenacil and sulfentrazone relative to the fomesafen and *s*-metolachlor treatments.

2.4 Results and Discussion

The trends in herbicide treatment differences for tall waterhemp control at 28 and 42 DAT were similar; therefore, only the evaluations taken at 42 DAT are presented since these should be a greater reflection of the soil residual activity of the herbicides (Table 2.3). An interaction of the two main factors, fomesafen and *s*-metolachlor, was observed for tall waterhemp control. More specifically, the influence of *s*-metolachlor rate on control of tall waterhemp diminished as the rate of fomesafen increased. For instance, control of tall waterhemp at 42 DAT when applied with fomesafen at 66 g ha^{-1} increased from 23 to 64% as the rate of *s*-metolachlor increased from 335 to 1420, respectively. However, control of tall waterhemp did not increase as the rate of *s*-metolachlor increased in combination with 132 and 264 g ha^{-1} of fomesafen. The high rates of fomesafen alone were effective enough to achieve good control, and therefore did not benefit with the addition of *s*-metolachlor. This interaction demonstrates that fomesafen and *s*-metolachlor were contributing similar levels of efficacy at the low rates of fomesafen. The interaction was not surprising due to the baseline FOR (5%) in Lafayette observed from preliminary genotypic analysis prior to trial establishment in 2016 (unpublished data). Due to the low FOR, fomesafen still resulted in high levels of tall waterhemp efficacy in a soil residual application at this field location. Palmer amaranth is a similar species as tall waterhemp and resulted in 80 to 98% control from fomesafen applied preemergence at 280 g ha^{-1} (Barkley et al. 2016). End of season tall waterhemp control from tank mixes of fomesafen and *s*-metolachlor at the high rates in this study also align with previous research where common waterhemp control at preharvest was 78% or greater with similar rates of fomesafen plus *s*-metolachlor applied preemergence (Duff et al. 2008).

Similar to the control data, the trends in herbicide treatment differences for tall waterhemp plant density at 28 and 56 DAT were similar; therefore, only the evaluations taken at 56 DAT are

presented since these should also be a greater reflection of the soil residual activity of the herbicides (Table 2.4). An interaction between fomesafen and *s*-metolachlor was not observed with tall waterhemp plant density data; therefore, data was pooled over *s*-metolachlor rate since fomesafen was the only significant main effect. Tall waterhemp density at 56 DAT decreased as fomesafen rate increased, with incremental reductions in tall waterhemp density for each increase in fomesafen rate. Thus, tall waterhemp density data suggest that fomesafen was a greater determinant of tall waterhemp efficacy than *s*-metolachlor, which is in slight contrast to the visual control data that would account for a combination of both plant biomass and the number of surviving plants.

The majority of tall waterhemp plants possessed the ΔG210 mutation in the heterozygous form (≥93% of emerged individuals) (Table 2.5). The nontreated plots served as the benchmark for the soil seedbank and the ΔG210 mutation was heterozygous in all resistant individuals, as no homozygous individuals were identified (data not shown). Similar to tall waterhemp plant density, only fomesafen influenced the FOR in the plants surviving the soil residual herbicide applications (Table 2.5). When pooled over *s*-metolachlor, the FOR increased from 8% for no fomesafen to 13% for the highest rate of fomesafen (264 g ha^{-1}). No change in the frequency of surviving individuals that were heterozygous or homozygous for ΔG210 were observed. The end of season frequency of PPO-R individuals, as predicted by the weed density and allele frequencies, was reduced by 14% as the rate of fomesafen was increased. This can be attributed to the greater level of efficacy achieved with the higher rates of fomesafen, which limited the number of surviving individuals even though the FOR in those surviving tall waterhemp individuals was higher. This same result was observed by Wuerffel et al. (2015b) where the highest rate of fomesafen relative to the nontreated control increased the FOR in waterhemp by 70% in a greenhouse and 20% in field experiments. However, tall waterhemp emergence were markedly reduced with combinations of fomesafen and *s*-metolachlor resulting in a reduction of PPO-R individuals, and ultimately seed, at the end of the season (Wuerffel et al. 2015b).

The fomesafen rate was largely influencing the soil residual efficacy (control and density data) and the FOR in tall waterhemp for the ΔG210 mutation when applied in combination with *s*-metolachlor. However, to more completely address our research objectives we included two other commercial herbicides, saflufenacil and sulfentrazone, that varied in the extent of soil residual activity. Our hypothesis was the longer the soil residual activity of the PPO-inhibiting herbicide in

the soil, the more persistent the selection for the ΔG210 mutation in the surviving tall waterhemp population. Thus, the greater soil residual activity from a PPO-inhibiting herbicide for control of tall waterhemp, the greater the FOR for the ΔG210 mutation.

Fomesafen applied at the low rate (66 g ha^{-1}) and saflufenacil resulted in similar control of tall waterhemp at 42 DAT (17 and 14%, respectively) and plant density at 56 DAT (45 and 68%, respectively) (Table 2.6). Conversely, fomesafen applied at the high rate (264 g ha^{-1}) and sulfentrazone both resulted in higher levels of soil residual herbicide efficacy on tall waterhemp, with 90% or greater reduction in tall waterhemp plant density. Thus, saflufenacil resulted in relatively short residual control of tall waterhemp compared with the longer soil residual control from sulfentrazone, and these corresponded to the activity observed with the low and high application rates of fomesafen. Orthogonal contrasts confirmed the low rate of fomesafen resulted in less tall waterhemp control and plant density than sulfentrazone and the high rate of fomesafen resulted in greater tall waterhemp control and plant density than saflufenacil. This research establishes that the soil residual activity of the low and high rates of fomesafen were similar to saflufenacil and sulfentrazone, respectively, and shared similar dissipation of the biologically effective dose in the soil. The addition of *s*-metolachlor resulted in greater control of tall waterhemp when applied with saflufenacil, but not sulfentrazone (Table 2.6). As previously mentioned in the factorial analysis, the addition of *s*-metolachlor to fomesafen resulted in greater control of tall waterhemp when the lowest rate of fomesafen (66 g ha^{-1}) was applied.

Orthogonal contrasts of the FOR data provided little differences between PPO-inhibiting herbicides. An increase in the FOR from 12 to 23% was observed with sulfentrazone compared to fomesafen (66 g ha^{-1}). Similar to fomesafen results, the addition of *s*-metolachlor to saflufenacil and sulfentrazone also did not influence FOR (Table 2.7). Wrubel and Gressel (1994) stated that selection of weed resistance to soil residual ALS-inhibiting herbicides can be reduced if the soil residual activity of the alternative herbicide is equal to or greater than the ALS-inhibiting herbicide. Common theory has evolved to suggest the practice of using alternative herbicides with longer persistence of the active above the biologically effective dose for the weed species would be an effective tactic to apply to weed resistance management for other herbicide mode of action groups. However, our data contradict this theory as even a short persistence of the biologically effective dose (BED) of saflufenacil combined with *s*-metolachlor had a similar impact on the FOR for the ΔG210 mutation as a longer persistence of the BED with sulfentrazone plus *s*-metolachlor.

When comparing percentage of homozygous PPO-R tall waterhemp (RR), no differences were observed between PPO-inhibiting herbicides. Interestingly, the addition of *s*-metolachlor to saflufenacil reduced the homozygous PPO-R genotype from 19% to 4% (Table 2.7). Although there were no differences in FOR when comparing these treatments, this result suggests saflufenacil can influence the ratio of homozygous and heterozygous PPO-R tall waterhemp plants. The same result was observed in tall waterhemp investigating selection pressure of ALS-inhibiting herbicides (Boe et al. 2017). This shift in heterozygosity is detrimental for management of resistance to PPO-inhibiting herbicides by limiting the number of PPO-S tall waterhemp in future progeny within the population. Research from Patzoldt et al. (2006) showed the ΔG210 mutation has incomplete dominance and that a differential response to foliar applications of lactofen exists between heterozygous and homozygous PPO-R plants. Differences in heterozygosity for the ΔG210 mutation from saflufenacil could be explained from differences in binding affinity compared to fomesafen and sulfentrazone, which could result in more selection pressure of saflufenacil if the homozygous PPO-R plants are not being controlled at the same level as heterozygous PPO-R plants (Grossman et al. 2011).

As previously mentioned, we demonstrated surviving PPO-R tall waterhemp at the end of season can be reduced by using the higher rate of fomesafen due to greater herbicide efficacy. This result was further supported by the efficacy achieved by sulfentrazone, which provided similar residual activity to the high rate of fomesafen, resulting in a 67% reduction of PPO-R plants compared to saflufenacil. Furthermore, *s*-metolachlor applied with either saflufenacil or sulfentrazone did not influence the surviving PPO-R tall waterhemp at a low FOR level in the field (Table 2.7). With the exception of saflufenacil, which is not recommended for soil residual control of tall waterhemp, this data provides evidence that PPO-R plants at the end of the season can be reduced greater with fomesafen at the higher rate and sulfentrazone and ultimately reduce the amount of PPO-R seed contributed to the soil seedbank.

In conclusion, these data support previous evidence that soil residual PPO-inhibiting herbicides control a portion of the PPO-R tall waterhemp individuals with the ΔG210 mutation (Wuerffel et al. 2015b). In addition, control of PPO-R tall waterhemp can be improved by combining a PPO-inhibiting herbicide with an alternative herbicide site of action, such as group #15 herbicides like *s*-metolachlor. However, the latter conclusion is dependent on the length of soil residual control provided by each component in the herbicide combination. Group #15

herbicides such as *s*-metolachlor may have limited utility in mitigating an increase in the frequency of PPO-R individuals if the PPO-inhibiting herbicide applied in the combination has similar or longer residual activity in the soil. However, this research only investigated the use of *s*-metolachlor, which is not representative of other group #15 herbicides. Pyroxasulfone is generally recognized as having longer soil residual activity than *s*-metolachlor and thus, may provide a greater reduction in selection pressure. Overall, the addition of a group #15 herbicide that improves overall herbicide efficacy and reduces the number of surviving tall waterhemp plants with the PPO-R trait can limit seed rain and future infestation of PPO-R individuals. This research also demonstrated that selection pressure for the ΔG210 mutation from PPO-inhibiting herbicides is not dependent on the length of soil residual activity. For instance, saflufenacil and sulfentrazone resulted in no differences in FOR. Furthermore, the shift in heterozygosity for the PPO resistance trait from saflufenacil also reveals the potential for increasing FOR and suggests selection pressure of PPO-R biotypes depends more on the specific active ingredient within the group #14 herbicides. Future research on herbicide resistance selection with similar herbicide sites-of-action, such as HPPD inhibiting herbicides could be useful for delaying the increase in target-site resistance to these herbicides.

2.5 Acknowledgements

The authors extend appreciation to Julie Young and Dr. Haozhen Nie for their technical assistance with this research as well as undergraduate and graduate members of the Purdue Weed Science lab for their labor and support. Additional thanks goes to the Purdue Statistical Consulting Service. No conflicts of interest have been declared.

2.6 Literature Cited

Barkley SL, Chaudhari S, Jennings KM, Schultheis JR, Meyers SL, Monks DW (2016) Fomesafen Programs for Palmer Amaranth (*Amaranthus palmeri*) Control in Sweetpotato. Weed Technol 30:506-515

Boe JE, Nie H, Young BG (2017) Interaction of application timing, herbicide active ingredient, and specific target-site mutation on the selection of ALS-inhibitor resistant horseweed and tall waterhemp. Page 96 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, Missouri

Duff MG, Al-Khatib K, Peterson DE (2008) Efficacy of preemergence application of *s*-metolachlor plus fomesafen or metribuzin as an element in the control of common waterhemp (*Amaranthus rudis* Sauer) in soybeans. Trans Kans Acad Sci 111:230–238

Falk JS, Shoup DE, Peterson DE (2006) Protox-resistant common waterhemp (*Amaranthus rudis*) response to herbicides applied at different growth stages. Weed Sci 54:793–799

Foes MJ, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 46:514–520

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559–1563

Grossmann K, Hutzler J, Caspar G, Kwiatkowski J, Brommer CL (2011) Saflufenacil (Kixor™): biokinetic properties and mechanism of selectivity of a new protoporphyrinogen IX oxidase inhibiting herbicide. Weed Sci 59:290–298

Hager AG, Wax LM, Bollero GA, Simmons FW (2002) Common waterhemp (*Amaranthus rudis* Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.). Crop Prot 21:277–283

Heap I (2020) The international survey of herbicide resistant weeds. https://www.weedscience.org. Accessed September 15, 2020

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587

Mansfield BC, Nie H, Young JM, Bradley KW, Young BG (2017) A multi-state survey to determine the potential for resistance to PPO-inhibiting herbicides in tall waterhemp beyond the G210 target site mutation. Page 62 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, Missouri

Mayo CM, Horak MJ, Peterson DE, Boyer JE (1995) Differential control of four *Amaranthus* species by six postemergence herbicides in soybean (*Glycine max*). Weed Technol 9:141–147

Mueller TC, Boswell BW, Mueller SS, Steckel LE (2014) Dissipation of fomesafen, saflufenacil, sulfentrazone, and flumioxazin from a Tennessee soil under field conditions. Weed Sci 62:664–671

Nie H, Mansfield BC, Harre NT, Young JM, Steppig NR, Young BG (2019) Investigating target-site resistance mechanism to the PPO-inhibiting herbicide fomesafen in waterhemp and interspecific hybridization of *Amaranthus* species using next generation sequencing. Pest Manag Sci 75: 3235-3244

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci:31–62

Papiernik SK, Koskinen WC, Barber BL (2012) Low Sorption and fast dissipation of the herbicide saflufenacil in surface soils and subsoils of an eroded prairie landscape. J Agric Food Chem 60:10936–10941

Patzoldt WL, Hager AG, Mccormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. PNAS 103:12329–12334

Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30–36

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS-and PPO-inhibiting herbicides. Weed Sci 60:335–344

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Shergill LS, Barlow BR, Bish MD, Bradley KW (2018) Investigations of 2,4-D and multiple herbicide resistance in a Missouri waterhemp (*Amaranthus tuberculatus*) population. Weed Sci 66:386–394

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145–150

Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) Herbicide efficacy on four *Amaranthus* species in soybean (*Glycine max*). Weed Technol 12:315–321

Taylor-Lovell S, Wax LM, Horak MJ, Peterson DE (1996) Imidazolinone and sulfonylurea resistance in a biotype of common waterhemp (*Amaranthus rudis*) I. Weed Sci 44:789–794

Thinglum KA, Riggins CW, Davis AS, Bradley KW, Al-khatib K, Tranel PJ (2011) Wide distribution of the waterhemp (*Amaranthus tuberculatus*) ΔG210 *PPX2* mutation, which confers resistance to PPO-inhibiting herbicides. Weed Sci 59:22–27

Wrubel RP, Gressel J (1994) Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technol 8:635–648

WSSA, America WSS of (2014) Herbicide handbook. 10th ed. Lawrence, KS: Weed Science Society of America

Wuerffel RJ, Young JM, Matthews JL, Young BG (2015a) Characterization of PPO-inhibitor–resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting herbicides. Weed Sci 63:511–521

Wuerffel RJ, Young JM, Tranel PJ, Young BG (2015b) Soil residual protoporphyrinogen oxidase–inhibiting herbicides influence the frequency of associated resistance in waterhemp (*Amaranthus tuberculatus*). Weed Sci 63:529–538

Table 2.1. Herbicide characteristics in the soil.

Herbicide	Chemical family	Water solubility	K_{oc}[a]	K_d[b,c]	Soil half-life
		mg L^{-1}		mL g^{-1}	day
fomesafen	diphenylether	600,000 (salt at pH 7, 25 C)	60	1.11 to 12.76	100
saflufenacil	pyrimidindione	2100 (pH 7)	9 to 56	0.02 to 0.2	1 to 36
sulfentrazone	triazolinone	780 (pH 7)	43	< 1	121 to 302
s-metolachlor	chloroacetamide	488 (20 C)	200	1.869	124

[a] K_{oc}: soil/water partition coefficient - defined as the tendency of the herbicide to bind to soil by organic matter. A small value means the herbicide will less likely be adsorbed to the soil and thus more mobile in the soil.

[b] K_d: soil sorption index - defined as the ratio of the herbicide amount in soil compared to the amount in water. A small value means a greater herbicide concentration in water.

[c] References: (Papiernik et al. 2012); (WSSA 2014).

Table 2.2. Herbicide treatment application and tall waterhemp collection dates for genotyping in all trials conducted in Farmland and Lafayette, IN.

Site year	Application date	Collection 1	Collection 2
Lafayette 2016	June 3	June 30 to July 21[a]	August 4 to August 12
Lafayette 2017	May 26	June 22 to July 9	August 15
Farmland 2017	June 3	July 17 to July 29	-[b]

[a] Collection dates define the range of days when plants were collected.

[b] Tall waterhemp plant tissue for the second collection period was not performed due to low tall waterhemp emergence.

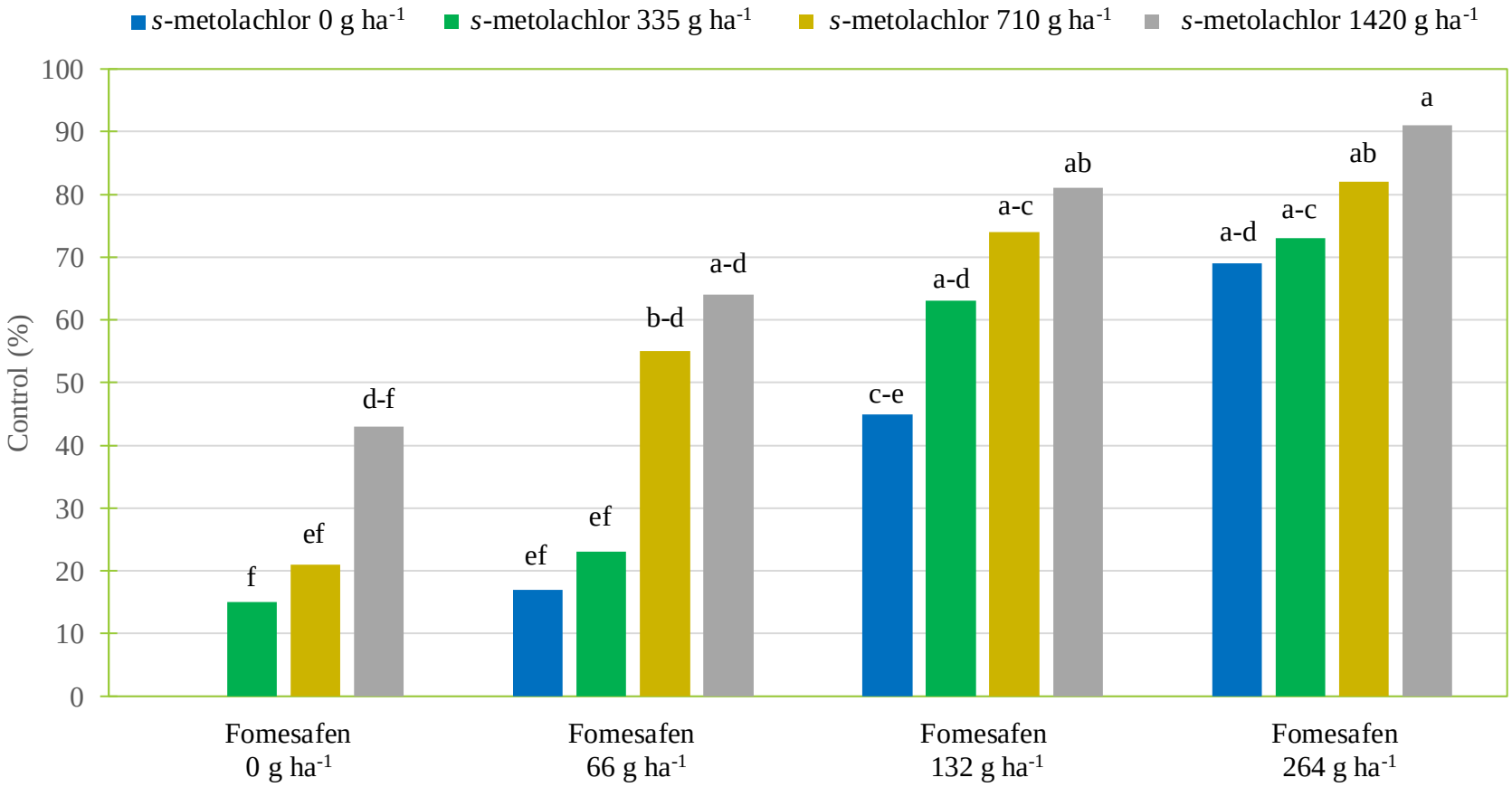

Figure 3.1. Control of tall waterhemp recorded 42 days after treatment in Lafayette, IN in 2016 and 2017. Means followed by the same letter are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

Table 2.3. Plant density of tall waterhemp recorded at 56 days after treatment, pooled over 2016 and 2017 at the Lafayette, IN field site.

Fomesafen[a]	Plant density[b]
g ai ha^{-1}	% of the nontreated
0	86 a
66	49 b
132	23 c
264	11 d

[a] Fomesafen data were analyzed with rate pooled over *s*-metolachlor.

[b] Plant density presented as percent of the nontreated control. Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

Table 2.4. Frequency of resistance (FOR) and projected end of season surviving PPO-resistant (PPO-R) tall waterhemp in Lafayette, IN in 2016 and 2017.

Fomesafen[a]	FOR[b]	RR[c]	Surviving PPO-R[d,e]
g ai ha^{-1}	----------%----------		Plant m^{-2}
0	8 b	1 a (99)	18 a
66	11 ab	5 a (95)	14 ab
132	11 ab	6 a (94)	7 bc
264	13 a	7 a (93)	4 c

[a] Fomesafen data were analyzed with rate pooled over *s*-metolachlor. Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

[b] FOR values were combined over collection timings due to no significant difference. FOR in the nontreated control was 7%.

[c] Percentage of RR tall waterhemp were calculated from the total percentage of PPO-R tall waterhemp. Numbers in parenthesis represents percentage of heterozygous PPO-R plants.

[d] Surviving PPO-R tall waterhemp calculated by multiplying FOR by density recorded 56 DAT.

[e] Abbreviations: PPO, protoporphyrinogen oxidase; RR, homozygous PPO-R tall waterhemp.

Table 2.5. Comparison of saflufenacil and sulfentrazone to rates of fomesafen for tall waterhemp control and plant density, pooled over 2016 and 2017 at the Lafayette, IN site.

Contrast[a,b]	Rate	Control at 42 DAT	Plant density at 56 DAT
	g ai ha^{-1}	%	% of nontreated
fomesafen vs saflufenacil	66 vs 25	17 vs 14	45 vs 68
fomesafen vs saflufenacil	264 vs 25	**69 vs 14*** **	**10 vs 68*** **
fomesafen vs sulfentrazone	66 vs 280	**17 vs 86*** **	**45 vs 11** **
fomesafen vs sulfentrazone	264 vs 280	69 vs 86	10 vs 11
saflufenacil vs sulfentrazone	25 vs 280	**14 vs 86*** **	**68 vs 11*** **
saflufenacil vs *s*-metolachlor	25 vs 1420	**14 vs 43** **	68 vs 79
sulfentrazone vs *s*-metolachlor	280 vs 1420	**86 vs 43*** **	**11 vs 79*** **
saflufenacil vs saflufenacil + *s*-metolachlor	25 vs 25 + 1420	**14 vs 76*** **	68 vs 56
sulfentrazone vs sulfentrazone + *s*-metolachlor	280 vs 280 + 1420	86 vs 96	11 vs 5.8

[a] Bolded contrasts with an asterisk(s) denote significance at $P \leq 0.05$ (*), ≤ 0.01 (**), ≤ 0.001 (***). P-values were adjusted using the Bonferroni adjustment.

[b] Abbreviations: fom, fomesafen; safl, saflufenacil; sulf, sulfentrazone; smeto, *s*-metolachlor; DAT, days after treatment.

Table 2.6. Comparison of saflufenacil and sulfentrazone to rates of fomesafen for the frequency of resistance (FOR) for the ΔG210 mutation and projected number of surviving tall waterhemp resistant to PPO-inhibiting herbicides (PPO-R), pooled over 2016 and 2017 at the Lafayette, IN site.

Contrast[a]	Rate	FOR[b]	RR[c]	Surviving PPO-R[d,e]
	g ai ha^{-1}	----------%----------		Plant m^{-2}
fomesafen vs saflufenacil	66 vs 25	12 vs 14	5 (95) vs 19 (81)	16 vs 24
fomesafen vs saflufenacil	264 vs 25	13 vs 14	10 (90) vs 19 (81)	**2 vs 24****
fomesafen vs sulfentrazone	66 vs 280	**12 vs 23***	5 (95) vs 6 (94)	16 vs 8
fomesafen vs sulfentrazone	264 vs 280	13 vs 23	10 (90) vs 6 (94)	2 vs 8
saflufenacil vs sulfentrazone	25 vs 280	14 vs 23	19 (81) vs 6 (94)	**24 vs 8***
saflufenacil vs *s*-metolachlor	25 vs 1420	14 vs 7	**19 (81) vs 1 (99)****	24 vs 13
sulfentrazone vs *s*-metolachlor	280 vs 1420	**23 vs 7****	6 (94) vs 1 (99)	8 vs 13
saflufenacil vs saflufenacil + *s*-metolachlor	25 vs 25 + 1420	14 vs 13	**19 (81) vs 4 (96)***	24 vs 15
sulfentrazone vs sulfentrazone + *s*-metolachlor	280 vs 280 + 1420	23 vs 18	6 (94) vs 1 (99)	8 vs 3

[a] Bolded contrasts with an asterisk(s) denote significance at $P \leq 0.05$ (*), ≤0.01 (**), ≤0.001 (***). P-values were adjusted using the Bonferroni adjustment.

[b] FOR values were combined over collection timings due to no significant difference. FOR in the nontreated control was 7%.

[c] Percentage of RR tall waterhemp are calculated from the total percentage of PPO-R tall waterhemp. Numbers in parenthesis represents percentage of heterozygous PPO-R plants.

[d] Surviving PPO-R tall waterhemp calculated by multiplying FOR by density recorded 56 DAT.

[e] Abbreviations: PPO, protoporphyrinogen oxidase; RR, homozygous PPO-R tall waterhemp.

CHAPTER 3. CHARACTERIZATION OF TALL WATERHEMP RESISTANCE TO PPO-INHIBITING HERBICIDES ACROSS FIVE MIDWEST U.S. STATES

3.1 Abstract

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides are frequently used in soybean production to control tall waterhemp (*Amaranthus tuberculatus*). Tall waterhemp resistance to PPO-inhibitors has been confirmed in eight Midwest states to date. The only previously known mechanism of resistance has been a target site mutation resulting in deletion of a glycine at position 210 of *PPX2*. However, tall waterhemp tissue samples submitted to university labs suspected to be resistant to PPO-inhibiting herbicides do not always receive positive confirmation of the ΔG210 mutation. A multi-state survey was conducted to determine the potential for alternative resistance mechanisms in tall waterhemp beyond the ΔG210 mutation. Whole-plant greenhouse screening indicated that 126 out of 148 populations from Illinois, Indiana, Iowa, Minnesota, and Missouri contained plants displaying phenotypic resistance to PPO-inhibitors. Furthermore, 125 (84%) populations contained plants with the ΔG210 mutation. Individual tall waterhemp plants from all Midwest states sampled exhibited a resistance response without the ΔG210 mutation. Approximately 5, 2, 19, 1, and 2% of tall waterhemp plants demonstrating phenotypic resistance to PPO-inhibitors did not possess the ΔG210 mutation in Illinois, Indiana, Iowa, Minnesota, and Missouri, respectively. Further investigation into these populations led to the discovery of five novel R128 codons of which three conferred fomesafen resistance.

3.2 Introduction

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have been used for over 30 years in row-crop production in the Midwest U.S. for foliar and soil residual control of broadleaf weeds (Lee and Oliver 1982; Minton et al. 1989; Niekamp et al. 1999; Stephenson IV et al. 2004). The introduction of glyphosate-resistant crops in the 1990s led to a dramatic increase in glyphosate usage for in-season herbicide applications (Young 2006). Subsequently, use of PPO inhibitors declined due to the effectiveness, timeliness, and economic benefits provided by glyphosate. However, the decrease in diversity of herbicide sites of action from relying solely on glyphosate

for weed management quickly resulted in glyphosate-resistant weeds (Davis et al. 2008; Pollard et al. 2004; VanGessel 2001; Westhoven et al. 2008). Herbicide resistance to ALS-inhibiting herbicides and glyphosate, particularly in tall waterhemp, has created additional problems due to the limited number of effective herbicide sites of action for soybean production (Foes et al. 1998; Legleiter and Bradley 2008). Therefore, current weed management decisions have relied more heavily on the use of PPO herbicides for control of tall waterhemp resulting in concerns of increasing selection pressure for herbicide resistance to PPO-inhibiting herbicides (USDA-NASS 2017).

Thirteen weed species worldwide have been confirmed resistant to PPO-inhibiting herbicides (Heap 2020). Tall waterhemp was the first weed to evolve resistance to PPO-inhibiting herbicides in a Kansas population in 2001 (Shoup et al. 2003). Currently, tall waterhemp has developed resistance to PPO-inhibitors in eight U.S. states as well as Ontario, Canada (Heap 2020). The only known mechanism of resistance prior to 2016 was a target site mutation resulting in a glycine deletion at the 210th position of *PPX2* (ΔG210) (Patzoldt et al. 2006).

Confirmation of herbicide resistance can be difficult and a labor-intensive task due to the number of plant samples tested across multiple species and/or herbicides. Multiple review papers have discussed the specific protocols for confirming herbicide resistance (Beckie et al. 2000; Burgos et al. 2013; Délye et al. 2015). In short, confirmation of herbicide-resistant weeds begins with identifying weeds within a population that survived an herbicide application whereas the rest of the population did not survive in the absence of environmental factors, herbicide applicator errors, or missed application timing. Based on the procedures described in the above review papers, two methods can be implemented to identify herbicide-resistant plants or populations: discriminating herbicide dose and full dose response experiments. The full dose response experiment is required to quantify the resistance ratio of populations; ideally populations that are no longer segregating for the resistance trait to eliminate susceptible plants from confounding the analysis. An alternative to the full dose response experiment is the use of a few discriminating herbicide doses that allow for separation of plants that are sensitive (death from the herbicide dose) versus resistant plants (survival at the herbicide dose). This discriminating dose method would be preferred in segregating populations since individual plant survival is an indicator of the presence of a resistance trait and would be a significant observation. A low frequency of resistant plants in a segregating population would not easily be identified in a dose-response regression analysis

where multiple plants are involved in the analysis instead of individual plants. Molecular DNA assays with known markers for herbicide-resistant alleles can also be conducted for herbicide resistance mechanisms that have been well characterized. These assays require less labor and only leaf tissue eliminating the need to conduct a whole-plant herbicide assay. Although this process takes less time, the cost of molecular assays can be high for reagents and having access to the necessary lab equipment. These assays also are specific to target site resistance mechanisms and create the potential of missing non-target site resistance or other mutations related to the target site.

The ΔG210 mutation in tall waterhemp can be identified using a TaqMan qPCR assay in addition to determining the heterozygosity of the DNA (Lee et al. 2008; Wuerffel et al. 2015a). Multiple surveys in the Midwest U.S. have been conducted in previous years to characterize herbicide resistance in tall waterhemp to PPO-inhibiting herbicides as well as identify the underlying resistance mechanism. Molecular analysis of tall waterhemp DNA in certain surveys confirmed that only the ΔG210 mutation was present and likely the only mechanism of resistance in field populations (Bell et al. 2013; Lee et al. 2008; Schultz et al. 2015; Wuerffel et al. 2015a). Two surveys identified tall waterhemp plants exhibiting resistance phenotypically without possessing the ΔG210 mutation (Murphy et al. 2019; Thinglum et al. 2011). Thinglum et al. (2011) reported only 1 of 35 resistant plants did not have the ΔG210 mutation indicating that the primary resistance mechanism was not the ΔG210 mutation. Murphy et al. (2019) observed several instances (43% of resistant plants) where lactofen resistance was not explained by a known resistance mechanism; however, their research group was not confident in their classification of those plants as resistant.

In addition to research-based field surveys, herbicide resistance screening is available to the public as a service from select universities. Tall waterhemp plants suspected with resistance to PPO-inhibiting herbicides do not always receive a positive confirmation of the ΔG210 mutation, which may indicate the presence of another resistance mechanism. The previous discussed surveys were conducted in Illinois, Kansas, Missouri, and Ohio, but do not indicate the presence of the ΔG210 mutation in other U.S. states (Bell et al. 2013; Lee et al. 2008; Murphy et al. 2019; Schultz et al. 2015; Thinglum et al. 2011; Wuerffel et al. 2015a). Expanding the current knowledge of the ΔG210 mutation presence as well as discovering any new resistance mechanisms could lead to improved weed management decisions. Therefore, the objectives of this research were to characterize the general response of tall waterhemp populations to fomesafen across multiple

Midwest U.S. states and survey for the ΔG210 mutation. Following the initial screening, a second objective was to conduct a full dose response experiment on tall waterhemp populations to quantify resistance ratios (R:S) and identify any alternative resistance mechanisms.

3.3 Materials and Methods

3.3.1 Plant Propagation

Tall waterhemp seed from mature plants were collected from 113 fields in Illinois, Indiana, Iowa, and Minnesota during the 2016 growing season. An additional 35 populations from a field survey conducted in Missouri were provided by the University of Missouri (K. Bradley). Soybean fields were sampled based on the presence of tall waterhemp escapes or targeted due to a history of PPO-inhibiting herbicide applications or the failure of these herbicides postemergence. No more than four populations were collected per county and the minimum distance between populations was two miles to eliminate the potential of cross-pollination between populations. Information regarding these fields were obtained through samples submitted to universities for resistance testing, cooperator/retailer support, and support from industry representatives. Tall waterhemp plants were collected from within a soybean field, excluding field borders, to ensure plants survived herbicide applications. At least 80% of the seed heads were removed from 10 to 15 female tall waterhemp plants per field. Seed heads were placed in a single large paper bag to prevent trapping moisture and mold formation. Paper bags were labeled with GPS coordinates of the collection site, county and state of collection, and the name of the collector. Sample bags were stored in a greenhouse and mixed daily until tall waterhemp plants were dry. Plants were threshed by hand and cleaned using a series of woven wire screens from 0.02 to 0.15 mm in diameter and placed in cold storage at 5 C.

Tall waterhemp seed was treated with a 10% sodium hypochlorite solution with deionized water for 10 min and then rinsed with deionized water to break dormancy. Tall waterhemp seeds were sown in greenhouse flats at a depth of 3 mm using 100% commercial potting mix (Sun Gro seedling mix, Sun Gro Horticulture, Bellevue, WA, 98008) and transplanted later to 3.8-cm diameter plastic tubes using a 2:1 blend of commercial potting mix to sand. Plants were transplanted at the one- to two-leaf stage with one plant per tube. Plants were kept in a greenhouse with day and night temperatures of 30 and 25 C, respectively, with natural lighting supplemented

using high-pressure sodium bulbs delivering 1,100-umol m^{-2} s^{-1} photon flux density set to a 16-h photoperiod. Plants were watered daily and a standard fertilizer plus water solution (Jack's Classic Professional (20-20-20), JR Peters Inc., Allentown, PA 18106) was applied as a drench once weekly starting at two days after transplanting.

Two PPO-susceptible (PPO-S) (known tall waterhemp populations without the ΔG210 mutation) and two PPO-resistant (PPO-R) (known populations with high frequency of the ΔG210 mutation) populations were grown as negative and positive controls, respectively. Tall waterhemp seed for the known populations were collected from mother plants previously screened and documented for susceptibility and resistance with confirmation of ΔG210 mutation using RT-qPCR.

3.3.2 Discriminating Dose-Response with Fomesafen

3.3.2.1 Herbicide Application and Experimental Design

When tall waterhemp plants reached four to five leaves (4 to 7 cm), fomesafen (Flexstar®, Syngenta Crop Protection, LLC, Greensboro, NC 27409) was applied at 13, 52, and 416 g ai ha^{-1}. Crop oil concentrate (Prime Oil®, Winfield Solutions, LLC, St. Paul, MN 55164) was applied with fomesafen at 1% v/v to maximize fomesafen penetration and uniform coverage of leaf surfaces. All experiments were conducted twice using a randomized complete block (RCB) design with eight replications. A single tall waterhemp plant was considered an experimental unit. Plants were sprayed approximately at noon in an automated spray chamber in a greenhouse with consistent application times for all experiments. Application parameters were 140 L ha^{-1}, 276 kPa, 3.46 km hr^{-1}, 2 m width, and XR8002E nozzle with 50 mesh screen.

3.3.2.2 Data Collection and Analysis

Tall waterhemp control was recorded at 3, 7, and 14 days after treatment (DAT) using a scale of 0 to 100% (0% = no injury; 100% = complete death). Leaf tissue was only collected from tall waterhemp that survived fomesafen applications at 14 DAT. Plant "survival" was determined as plants with new leaf growth at 14 DAT. Fomesafen applied at 13 g ha^{-1} generally provided good separation of PPO-S and PPO-R populations based on visual control. However, plant survivorship remained high making it difficult to identify true resistant plants (data not shown). Therefore, we

excluded the 13 g ha^{-1} fomesafen rate for estimating the number of PPO-R plants without the ΔG210 mutation to avoid inclusion of any false positives. Plants were classified as PPO-R if control was ≤ 80% when applied with 52 g ha^{-1} as well as the presence of new leaf growth following applications of fomesafen at 416 g ha^{-1}. Tissue collections were stored at -20 C until ready for processing and then ground for DNA extraction using a modified cetyltrimethylammonium bromide (CTAB) method originally designed by Saghai-Maroof et al. (1984). Tissue collections were subjected to RT-qPCR to determine the presence or absence of the ΔG210 mutation. Data were subjected to ANOVA in PROC MIXED (SAS 9.4) and pooled across experimental runs. Visual control was analyzed using custom hypothesis tests using the LSMESTIMATE statement in PROC MIXED (SAS 9.4) of survey populations to the four control (positive and negative) populations. Means of the two PPO-S and PPO-R with ΔG210 control populations were combined into one PPO-S and PPO-R group in order to increase statistical power. Replication and experimental run were considered random effects while the fixed effect was tall waterhemp population. Survival data were analyzed using contingency tables to determine differences of expected and observed frequencies among tall waterhemp populations (JMP 13). Frequency of resistance (FOR) in surviving tall waterhemp plants was determined by dividing the number of homozygous and heterozygous resistant individuals with the ΔG210 mutation by the total number of tall waterhemp plants genotyped.

3.3.3 Dose-Response with Fomesafen

3.3.3.1 Seed Source

As previously stated, the objective of our research was to further characterize PPO-R tall waterhemp populations showing variability in response to fomesafen by quantification of R:S ratios in addition to identifying any alternative resistance mechanisms. Tall waterhemp populations were categorized into three groups based on their response to fomesafen (Table 3.1). Group A included resistant populations with more sensitivity to fomesafen than a population with ΔG210, and yet not include or have a low frequency of ΔG210. This group considered fomesafen application rates of 13 and 52 g ha^{-1}, with the 13 g ha^{-1} rate as the established rate to provide near complete control of susceptible plants. The criteria for identifying populations in group A included plant survival number, FOR, and comparison of visual control to the known PPO-S and known

PPO-R controls. Group B populations exhibited a similar response to fomesafen as the known PPO-R controls, yet lacked any plants with the ΔG210 mutation or had a low frequency of the ΔG210 mutation in surviving plants. Group C included plant responses that demonstrated less sensitivity to fomesafen than the known PPO-R controls. This group considered fomesafen application rates of 52 and 416 g ha^{-1}. The criteria for identifying populations in group C included plant survival number (relative and greater than PPO-R controls) and comparison of visual control (relative to PPO-R controls). Using this selection criteria, twenty-nine tall waterhemp populations out of the original 148 populations were further evaluated in a full dose-response experiment. Tall waterhemp populations in group A (9) were primarily found in Iowa with one exception in Missouri. Group B (1) and C (19) populations were found in Missouri and primarily the southern regions of Illinois and Indiana.

3.3.3.2 Herbicide Application and Experimental Design

Application parameters and the experimental design followed the same methods as the discriminating dose-response experiments except for replication number (10 vs 8). The fomesafen rate titration included 0, 0.081, 0.41, 2.0, 10, 51, 254, and 1270 g ha^{-1}. Two PPO-S (known tall waterhemp populations without the ΔG210 mutation) and two PPO-R (known populations with high frequency of the ΔG210 mutation) populations were included as controls.

3.3.3.3 Data Collection and Analysis

Visual control, survival data, and validation of tall waterhemp genotype followed the same method as the discriminating dose-response experiment. Plant biomass were collected at 14 DAT and dried in an oven at 45 C until weight remained constant. Data were subjected to ANOVA in PROC MIXED (SAS 9.4) and pooled across experimental runs. Replications and experimental runs were considered random variables. Dry weight were converted to a percentage of the non-treated control and used to calculate GR_{50} values of each tall waterhemp population via non-linear regression using PROC NLIN (SAS 9.4). Regression parameters were assessed using a three-parameter Weibul model (Equation 1) as described by Price et al. (2012).

$$f(x) = d * \exp(-\exp(b * (logx - \log(i50)))) \quad [1]$$

In this equation, d = the upper limit, b = the slope of the curve around i50, x = fomesafen dose, and i50 = the fomesafen dose required to achieve 50% growth reduction relative to the nontreated control.

3.4 Results and Discussion

3.4.1 Discriminating Dose-Response with Fomesafen

Tall waterhemp populations were collected throughout the five survey states as described previously, with failed control of tall waterhemp in soybean at crop maturity frequently observed during the collection process (Figure 3.1, Appendix Table B.1). The two known PPO-S tall waterhemp populations resulted in an average (pooled over population) control of 90, 98, and 100% when applied with 13, 52, and 416 g ha^{-1} of fomesafen, respectively (data not shown). Thus, the two highest rates resulted in near complete plant death, while the lowest rate allowed for a low level of plant survival. This low level of plant survival was critical in identifying surviving plants with 90% or greater growth reduction, but still had green living tissue. The two known PPO-R populations with the ΔG210 mutation resulted in an average of 40, 75, and 98% (data not shown). Thus, the highest dose of fomesafen resulted in near complete plant death of known ΔG210 plants and any populations with plants surviving this dose would be categorized as populations that may contain an enhanced resistance mechanism(s).

Tall waterhemp populations were considered phenotypically resistant if control was less than the known PPO-S controls at the discriminating fomesafen rate of 13 g ha^{-1}. All survey states contained tall waterhemp plants resistant to PPO-inhibiting herbicides. Iowa populations had the lowest FOR with 80% of the populations resulting in greater than 74% control at 14 DAT with 13 g ha^{-1}. Indiana and Minnesota populations contained a mix of resistant and susceptible plants that were distributed evenly across populations with the exception of two counties in Indiana (Figure 3.2, Table 3.1). Illinois and Missouri contained the highest frequency of populations with PPO-R.

The ΔG210 mutation was discovered in at least one population in every state. Every population sampled in Illinois, Minnesota, and Missouri had some degree of resistance to PPO-inhibitors conferred by ΔG210. The frequency of the ΔG210 mutation was greater than 74% in 52 and 63% of populations from Illinois and Missouri, respectively (Table 3.1). The frequency of individual plants with the ΔG210 mutation in each population was relatively low (less than 25%)

for the majority of the Iowa populations (86%), based on fomesafen at 13 g ha^{-1} (Table 3.1). The evolution of resistance to PPO-inhibitors in tall waterhemp was first confirmed in 2002 and 2005 for Illinois and Missouri, respectively, followed by Iowa in 2009 (Heap 2020; Li et al. 2003; Patzoldt et al. 2005). Indiana and Minnesota were the last states in this survey to report resistance to PPO-inhibitors in tall waterhemp (Heap 2020). Therefore, Illinois and Missouri have had a longer history of managing resistance to PPO-inhibitors, which would lead to greater selection pressure.

Further investigation into the genotypic analysis of the ΔG210 mutation revealed a range in heterozygosity throughout the surveyed tall waterhemp populations. Tall waterhemp populations with the highest number of homozygous resistant (RR) individuals for the ΔG210 mutation were located in Illinois and Missouri. The frequency of the wild type allele was less than 25% in screened plants for 58 and 74% of Illinois and Missouri populations, respectively (Table 3.2). Similar to the control and overall frequency of resistance values, heterozygosity was mostly mixed for tall waterhemp populations in Indiana and Minnesota and not weighted towards either genotype. Iowa populations consisted primarily homozygous susceptible and heterozygous resistant for the ΔG210 mutation, but the latter at relatively low frequencies. Only 33% of Iowa populations contained heterozygous resistant (Rr) plants in a frequency greater than 24% of the screened plants (Table 3.2). A high frequency of RR individuals in Illinois and Missouri is highly concerning for tall waterhemp management due from the lack of susceptible alleles and reveals a genotypic shift towards the RR state has occurred in these regions. Furthermore, the ΔG210 mutation in tall waterhemp does not possess a fitness cost for plant growth associated with fomesafen resistance, and therefore, will likely remain in established populations in the absence of selection pressure from PPO-inhibiting herbicides (Duff et al. 2009; Wu et al. 2018).

The ΔG210 mutation explained the majority of tall waterhemp resistance to PPO-inhibiting herbicides in Iowa, Illinois, Indiana, Minnesota, and Missouri. Of the 953 plants expressing a phenotypic resistance to fomesafen following application rates of 52 and 416 g ha^{-1}, 96% possessed the ΔG210 mutation. Thus, only 4% of the resistant plants could not be explained by the ΔG210 mutation (Table 3.3). Interestingly, all states included at least one fomesafen-resistant plant that could not be explained by the ΔG210 mutation. The high prevalence of the ΔG210 mutation in plants causes difficulty in identifying the true frequency of additional resistance mechanisms because we are unable to differentiate them from plants that already contain ΔG210. Therefore,

plants with the ΔG210 mutation should not be regarded as having only the ΔG210 mutation suggesting the percentage of PPO-R plants with unknown resistance mechanisms could be greater in frequency than reported in this research.

Since the ΔG210 mutation was the first mutation discovered that confers resistance to PPO-inhibiting herbicides, we assumed the evolution of PPO resistance began with this mutation (Patzoldt et al. 2006). Following the evolution of the ΔG210 mutation, we would expect to see other resistance mechanisms evolve as selection pressure continues. The results of Iowa, however, suggest other mutations evolved concurrently with the ΔG210 mutation, but may be less robust for survival. Future identification of these purported mutations could be missed due to the masking effect caused from increased frequencies of the ΔG210 mutation and our ability to confirm ΔG210 using molecular techniques.

3.4.2 Dose-Response with Fomesafen

The ΔG210 mutation has been the most common mechanism for resistance to PPO-inhibiting herbicides in tall waterhemp. The two known resistant populations with ΔG210 resulted in R:S ratios of 4.4X (IL-CAR) and 5.9X (IN-DAV). Similar results were observed with foliar applied applications of fomesafen in previous research with R:S ratios ranging from 6.2X to 8.3X (Shoup et al. 2003; Patzoldt et al. 2005). In contrast, Wuerrfel et al. (2015b) reported a R:S ratio of 38X from fomesafen in tall waterhemp. However, the latter study concluded differences in R:S ratios between experiments could be due to the population variability in inherent sensitivity to PPO-inhibiting herbicides, climatic and plant growth factors in a greenhouse, the possible presence of an alternative resistance mechanism, or the tall waterhemp population used for the susceptible control.

Results from the dose-response experiment revealed a range in R:S ratios from 0.6X to 17X for the three tall waterhemp response groups (Table 3.4). Group A and B resistant populations all resulted in R:S ratios similar to the known PPO-S control populations, IL-DSO and IN-KNOX7. Even though group A and B resistant populations were considered susceptible by definition of the dose regression analysis, all contained plants with the ΔG210 mutation but at relatively low frequencies. With the exception of three, all populations classified in group C resulted in similar R:S ratios as the known resistant populations with a high frequency of ΔG210. Populations IL-WAS and IN-DUB resulted in R:S ratios of 16X and 17X, respectively (Table 3.4).

This increase in R:S ratios compared to the known ΔG210 populations indicates another resistance mechanism maybe present. Interestingly, IL-RAN was classified as high-level resistant based on discriminating dose results and possessed the ΔG210 mutation in 76% of surviving plants in the full dose experiment, but still resulted in a low R:S ratio (2.7X) (Table 3.4). This result could be explained by the frequency of PPO-S plants (24%) within the population that creates variability in plant sensitivity to fomesafen. Unlike a discriminating dose experiment that investigates herbicide resistance at the plant level, full dose response experiments evaluate multiple plants at the population level, which allows for the potential to overlook a resistance mechanism with a low frequency in the population. Group C resistant populations varied in frequency of the ΔG210 mutation, but all had a frequency of the wild type allele in surviving plants less than 25% (Table 3.4). Linear regression of the FOR for ΔG210 and GR_{50} values showed a positive correlation (R^2=0.5486), indicating that the ΔG210 mutation remains to be the dominant mutation conferring resistance in tall waterhemp to PPO-inhibiting herbicides in the Midwest U.S.

3.4.3 Implications and Conclusions

This research adds to several previous surveys where survival of *Amaranthus* species following foliar applications of lethal doses of PPO-inhibiting herbicides could not be explained by the ΔG210 mutation (Copeland et al. 2018; Murphy et al. 2019; Salas-Perez et al. 2017; Thinglum et al. 2011; Varanasi et al. 2018). In addition, R:S ratios showed wide variability among resistant tall waterhemp populations even though these populations had similar proportions of plants without the ΔG210 mutation. In other research, two new mutations at the R128 position (referred to as R98 by Giacomini et al. 2017) of *PPX2* in Palmer amaranth were identified that conferred resistance to PPO-inhibiting herbicides (Giacomini et al. 2017). Further investigation of the same site in tall waterhemp populations with resistant plants without the ΔG210 mutation resulted in the discovery of five novel R128 codons of which three conferred fomesafen resistance in a bacterial system (Nie et al. 2019). Future research will be necessary to characterize the level of resistance of these mutations as well as determine if these mutations can be controlled with soil-applied applications of PPO-inhibiting herbicides in order to improve tall waterhemp management decisions. Interestingly, the two high-level resistant populations, IL-WAS and IN-DUB, did not possess any R128 mutations suggesting another mutation in addition to the ΔG210 mutation may be present (Nie et al. 2019).

These results emphasize the importance of using the proper screening approach for identification of herbicide resistance mechanisms. A discriminating dose screen for identifying mutations in segregating populations can highlight individual plant responses for low frequency traits compared with relying on full dose response experiments that emphasize the overall population response using regression and GR_{50} values. This research also revealed tall waterhemp resistant to PPO-inhibiting herbicides is widespread throughout the Midwest emphasizing the need for effective, alternative herbicide modes of action or the integration of non-chemical weed management practices in the future. In regions of no or low frequencies of PPO resistance, foliar applications of PPO-inhibiting herbicides still are a viable option for tall waterhemp control. However, the evolution of PPO resistance in tall waterhemp continues to increase as weed management relies on the use of PPO-inhibiting herbicides especially in soybean production. Previous research has shown that soil applied applications of PPO-inhibiting herbicides can control PPO-R tall waterhemp with the ΔG210 mutation, but this may not be the case as new mutations arise (Wuerffel et al. 2015c). Overall, the dominant PPO resistance mechanism in the Midwest for tall waterhemp continues to be the ΔG210 mutation although there is evidence of new mutations present among certain populations (Nie et al. 2019), which was a product of our research performing the initial phenotypic and genotypic characterization relative to the ΔG210 mutation.

3.5 Acknowledgements

The authors extend appreciation to Syngenta for providing partial funding and coordinating seed collection in Iowa and Minnesota. Thank you to Dr. Kevin Bradley's lab at the University of Missouri for collecting the Missouri tall waterhemp seed samples. Thanks also goes to Julie Young and Dr. Haozhen Nie for their technical assistance with this research as well as undergraduate and graduate members of the Purdue Weed Science lab for their labor and support. No conflicts of interest have been declared.

3.6 Literature Cited

Beckie HJ, Heap IM, Smeda RJ, Hall LM (2000) Screening for herbicide resistance in weeds. Weed Technol 14:428–445

Bell MS, Hager AG, Tranel PJ (2013) Multiple resistance to herbicides from four site-of-action groups in waterhemp (*Amaranthus tuberculatus*). Weed Sci 61

Burgos NR, Tranel PJ, Streibig JC, Davis VM, Shaner D, Norsworthy JK, Ritz C (2013) Review: confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4–20

Copeland JD, Giacomini DA, Tranel PJ, Montgomery GB, Steckel LE (2018) Distribution of *PPX2* mutations conferring PPO-inhibitor resistance in Palmer amaranth populations of Tennessee. Weed Technol 32:592–596

Davis, V. M., K. D. Gibson, T. T. Bauman, S. C. Weller, and W. G. Johnson. 2008. A field survey to determine the distribution and frequency of glyphosate-resistant horseweed (*Conyza canadensis*) in Indiana. Weed Technol. 22:331-338

Délye C, Duhoux A, Pernin F, Riggins CW, Tranel PJ (2015) Molecular mechanisms of herbicide resistance. Weed Sci:91–115

Duff MG, Al-khatib K, Peterson DE (2009) Relative competitiveness of protoporphyrinogen oxidase-resistant common waterhemp (*Amaranthus rudis*). Weed Sci 57:169–174

Foes MJ, Tranel PJ, Wax LM, Stoller EW (1998) A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. Weed Sci 46:514–520

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559–1563

Heap I (2020) The international survey of herbicide resistant weeds. https://www.weedscience.org. Accessed September 15, 2020

Lee RM, Hager AG, Tranel PJ (2008) Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). Weed Sci 56:371–375

Lee SD, Oliver LR (1982) Efficacy of acifluorfen on broadleaf weeds. Times and methods for application. Weed Sci 30:520–526

Legleiter TR, Bradley KW (2008) Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci 56:582–587

Li J, Smeda RJ, Dayan FE (2003) Two Missouri common waterhemp (*Amaranthus rudis*) biotypes resistant to protoporphyrinogen oxidase herbicides. Proc. Weed Sci. Soc. Am. 43:32

Minton BW, Kurtz ME, Shaw DR (1989) Barnyardgrass (*Echinochloa crus-galli*) control with grass and broadleaf weed herbicide combinations. Weed Sci 37:223–227

Murphy BP, Larran AS, Ackley B, Loux MM, Tranel PJ (2019) Survey of glyphosate-, atrazine- and lactofen- resistance mechanisms in Ohio waterhemp (*Amaranthus tuberculatus*) populations. Weed Sci 67:296–302

Nie H, Mansfield BC, Harre NT, Young JM, Steppig NR, Young BG (2019) Investigating target-site resistance mechanism to the PPO-inhibiting herbicide fomesafen in waterhemp and interspecific hybridization of *Amaranthus* species using next generation sequencing. Pest Manag Sci 75:3235–3244

Niekamp JW, Johnson WG, Smeda RJ (1999) Broadleaf weed control with sulfentrazone and flumioxazin in no-tillage soybean (*Glycine max*). Weed Technol 13:233–238

Patzoldt WL, Hager AG, Mccormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. PNAS 103:12329–12334

Patzoldt WL, Tranel PJ, Hager AG (2005) A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. Weed Sci 53:30–36

Pollard J, Sellers B, Smeda R (2004) Differential response of common ragweed to glyphosate. Page 27 Proc 59th Annu Meet North Cent Weed Sci Soc

Price WJ, Shafii B, Seefeldt SS (2012) Estimation of dose-reponse models for discrete and continuous data in weed science. Weed Technol 26:587-601

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Salas-Perez RA, Burgos NR, Rangani G, Singh S, Refatti JP, Piveta L, Tranel PJ, Mauromoustakos A, Scott RC (2017) Frequency of Gly-210 deletion mutation among protoporphyrinogen oxidase inhibitor-resistant Palmer amaranth (*Amaranthus palmeri*) populations. Weed Sci 65:718–731

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in missouri waterhemp (*Amaranthus rudis* Sauer) populations. Weed Sci 63:336–345

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145–150

Stephenson IV DO, Patterson MG, Faircloth WH, Lunsford JN (2004) Weed management with fomesafen preemergence in glyphosate-resistant cotton. Weed Technol 18:680–686

Thinglum KA, Riggins CW, Davis AS, Bradley KW, Al-khatib K, Tranel PJ (2011) Wide distribution of the waterhemp (*Amaranthus tuberculatus*) ΔG210 *PPX2* Mutation, which confers resistance to PPO-Inhibiting herbicides. Weed Sci 59:22–27

Vangessel MJ (2001) Glyphosate-resistant horseweed from Delaware. Weed Sci 49:703–705

Varanasi VK, Brabham C, Norsworthy JK, Nie H, Young BG, Houston M, Barber T, Scott RC (2018) A statewide survey of PPO-inhibitor resistance and the prevalent target-site mechanisms in Palmer amaranth (*Amaranthus palmeri*) accessions from Arkansas. Weed Sci 66:149–158

Westhoven AM, Davis VM, Gibson KD, Weller SC, Johnson WG (2008) Field presence of glyphosate-resistant horseweed (*Conyza canadensis*), common lambsquarters (*Chenopodium album*), and giant ragweed (*Ambrosia trifida*) biotypes with elevated tolerance to glyphosate. Weed Technol 22:544–548

Wu C, Davis AS, Tranel PJ (2018) Limited fitness costs of herbicide-resistance traits in *Amaranthus tuberculatus* facilitate resistance evolution. Pest Manag Sci 74:293–301

Wuerffel RJ, Young JM, Lee RM, Tranel PJ, Lightfoot DA, Young BG (2015a) Distribution of the ΔG210 protoporphyrinogen oxidase mutation in Illinois waterhemp (*Amaranthus tuberculatus*) and an improved molecular method for detection. Weed Sci 63:839–845

Wuerffel RJ, Young JM, Matthews JL, Young BG (2015b) Characterization of PPO-inhibitor resistant waterhemp (*Amaranthus tuberculatus*) response to soil-applied PPO-inhibiting herbicides. Weed Sci 63:511–521

Wuerffel RJ, Young JM, Tranel PJ, Young BG (2015c) Soil-Residual Protoporphyrinogen Oxidase–Inhibiting Herbicides Influence the Frequency of Associated Resistance in Waterhemp (*Amaranthus tuberculatus*). Weed Sci 63:529–538

Young BG (2006) Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. Weed Technol 20:301-307

Table 3.1. Criteria for categorizing tall waterhemp populations into different response groups based on frequency of ΔG210, plant survival number, and control from fomesafen at 14 days after treatment in a greenhouse.

Response group	Fomesafen rate	Criteria[a]
A	13 and 52 g ai ha^{-1}	More sensitivity to fomesafen than a population with ΔG210, and yet not include or have a low frequency of ΔG210.
B	13 and 52 g ai ha^{-1}	Similar response to fomesafen as the known PPO-R controls, yet lacked any plants with the ΔG210 mutation or had a low frequency of the ΔG210 mutation in surviving plants.
C	52 and 416 g ai ha^{-1}	Less sensitivity to fomesafen than the known PPO-R controls.

[a] Abbreviations: ΔG210, glycine deletion at position 210 of *PPX2*

Table 3.2. Distribution of tall waterhemp populations for control, frequency of resistance, and survival at 14 days after treatment from three discriminating fomesafen rates in a greenhouse.

		Fomesafen rate (g ai ha^{-1})								
		13			52			416		
State[a]	Quartile[b]	Control	FOR[d]	Survival[c]	Control	FOR	Survival	Control	FOR	Survival
	%	No. of populations								
IA	75-100	29	1	32	36	7	4	36	11	0
	50-74	6	2	4	0	6	5	0	0	1
	25-49	1	2	0	0	4	15	0	1	2
	0-24	0	31	0	0	17	12	0	2	33
IL	75-100	4	16	31	20	26	21	31	27	1
	50-74	12	6	0	11	3	5	0	0	10
	25-49	14	7	0	0	1	4	0	0	12
	0-24	1	2	0	0	1	1	0	0	8
IN	75-100	14	12	20	21	19	11	31	16	0
	50-74	7	4	9	6	3	5	0	0	1
	25-49	8	3	2	4	0	5	0	0	12
	0-24	2	12	0	0	6	10	0	0	18
MN	75-100	6	3	15	14	8	4	15	10	0
	50-74	7	5	0	1	3	6	0	0	0
	25-49	2	4	0	0	3	5	0	0	5
	0-24	0	3	0	0	1	0	0	0	10
MO	75-100	2	22	35	27	28	23	35	31	2
	50-74	24	7	0	8	6	9	0	2	8
	25-49	9	5	0	0	0	3	0	0	14
	0-24	0	1	0	0	1	0	0	0	11

[a] IA: n=36; IL: n=31; IN: n=31; MN: n=15; MO: n=35

[b] Quartile represents the number of tall waterhemp populations that fall within 0-24, 25-49, 50-74, or 75-100% for control, FOR, and survival.

[c] Percent survival was calculated out of 16 total plants.

[d] Abbreviations: FOR, frequency of resistance for ΔG210 mutation.

Table 3.3. Distribution of tall waterhemp populations by genotypic frequencies for the ΔG210 mutation from plants surviving fomesafen in the greenhouse.[a]

	Genotype for ΔG210 mutation[b]																	
	RR[d]						Rr						rr					
Quartile[c]	IA	IL	IN	MN	MO	Total	IA	IL	IN	MN	MO	Total	IA	IL	IN	MN	MO	Total
%	No. populations																	
75-100	0	3	3	0	3	9	0	0	0	0	0	0	24	2	9	2	0	37
50-74	1	7	4	2	7	21	3	10	7	4	16	40	9	4	5	5	4	27
25-49	0	9	7	1	12	29	9	13	11	8	15	56	2	7	3	4	5	21
0-24	35	12	17	12	13	89	24	8	13	3	4	52	1	18	14	4	26	63

[a] Plant data were pooled over three rates of fomesafen applied in the discriminating dose experiment (13, 52, and 416 g ai ha^{-1}).

[b] IA: n=36; IL: n=31; IN: n=31; MN: n=15; MO: n=35

[c] Quartile represents the number of tall waterhemp populations that fall within 0-24, 25-49, 50-74, or 75-100% for control, FOR, and survival.

[d] Abbreviations: RR, homozygous PPO-resistant tall waterhemp; Rr, heterozygous PPO-resistant tall waterhemp; rr, homozygous PPO-susceptible tall waterhemp; PPO, protoporphyrinogen oxidase; ΔG210, glycine deletion at position 210 of *PPX2*.

Table 3.4. Number of tall waterhemp plants resistant to PPO-inhibiting herbicides with and without the ΔG210 mutation following applications of 52 and 416 g ai ha^{-1} of fomesafen 14 days after treatment.

State[c]	Total individual plants genotyped[a]	PPO-R with ΔG210	PPO-R without ΔG210
	----------No. plants----------		
IA	58	47	11
IL	301	287	14
IN	205	201	4
MN	76	75	1
MO	313	307	6
Total	953	917	36

[a] Tall waterhemp plants were classified resistant if control was ≤ 80% (known PPO-S resulted in ≥ 98%) or new leaf growth (no survivors for known PPO-S) was observed following fomesafen applications of 52 and 416 g ha^{-1}, respectively.

[b] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; PPO-R, PPO-resistant; PPO-S, PPO-susceptible; PPO, protoporphyrinogen oxidase; ΔG210, glycine deletion at position 210 of *PPX2*.

Table 3.5. Dose-response analysis with fomesafen resulting in 50% reduction of shoot dry weight (GR_{50}) in multiple tall waterhemp populations resistant to PPO-inhibiting herbicides as well as genotypic frequencies for ΔG210 from surviving plants sprayed with 10, 51, 254, and 1270 g ai ha^{-1} fomesafen. Populations are in order of their R/S ratio.

Tall waterhemp population[a,d]	Response group[b]	GR_{50} (SE)	R/S[c]	Surviving plants	rr	Rr	RR
		g ha^{-1}		No.	---------%---------		
IL-DSO	Known susceptible	2.1 (0.2)	-	29	100	0	0
IN-KNOX7	Known susceptible	3.3 (0.6)	-	32	97	3	0
IL-CAR	Known resistant (ΔG210)	12 (2.2)	4.4X	79	9	44	47
IN-DAV	Known resistant (ΔG210)	16 (2.8)	5.9X	70	10	51	39
IL-PEO	B	1.6 (0.3)	0.6X	33	70	27	3
IA-340	A	2.7 (0.4)	1X	19	95	0	5
IA-293	A	3.0 (0.6)	1.1X	28	64	11	25
IA-369	A	3.1 (0.6)	1.1X	25	92	8	0
IA-358	A	3.3 (0.4)	1.2X	24	96	4	0
IA-152	A	3.6 (0.7)	1.3X	30	60	37	3
MO-45	A	3.9 (0.8)	1.4X	34	38	47	15
IA-332	A	4.2 (1.0)	1.6X	26	88	12	0
IA-315	A	4.5 (1.0)	1.7X	20	80	15	5
IL-CLT3	A	4.5 (0.7)	1.7X	29	76	14	10
IL-RAN	C	7.3 (2.0)	2.7X	49	24	45	31
IL-SANG2	C	12 (2.7)	4.4X	52	6	48	46
IL-CLT1	C	13 (3.3)	4.8X	50	8	44	48
IL-CLT2	C	14 (3.5)	5.2X	46	9	54	37
IL-MAR	C	14 (3.3)	5.2X	43	9	28	63
MO-10	C	16 (3.8)	5.9X	58	3	16	81
IL-BRO1	C	18 (5.3)	6.7X	49	4	6	90
IL-WHT	C	18 (4.2)	6.7X	48	0	35	65
MO-9	C	23 (5.6)	8.5X	42	0	52	48
IL-JAS	C	24 (3.9)	8.9X	52	0	46	54
MO-22	C	24 (7.7)	8.9X	53	6	53	42

Table 3.4 continued

IN-PIKE1	C	25 (4.6)	9.3X	58	5	36	59
IN-SPEN	C	25 (5.1)	9.3X	63	6	40	54
IL-BRO2	C	27 (6.3)	10X	51	2	12	86
IL-SCT	C	27 (6.8)	10X	54	2	2	96
MO-53	C	28 (5.6)	10X	50	12	26	62
IL-BDW	C	31 (8.3)	11X	65	12	25	63
IL-WAS	C	43 (7.4)	16X	54	0	11	89
IN-DUB	C	45 (9.1)	17X	56	11	26	63

[a] n = 160 plants for all populations except known controls. n = 240 plants for known controls.

[b] Response groups are described in Table 3.1.

[c] Resistance ratios were calculated by dividing the GR_{50} of the PPO-R tall waterhemp population by the average of the GR_{50} of the PPO-S tall waterhemp populations.

[d] Abbreviations: PPO, protoporphyrinogen oxidase; ΔG210, glycine deletion at position 210 of *PPX2*; PPO-R, PPO-resistant; PPO-S, PPO-susceptible; SE, standard error; rr, homozygous PPO-S tall waterhemp; Rr, heterozygous PPO-R tall waterhemp; RR, homozygous PPO-R tall waterhemp.

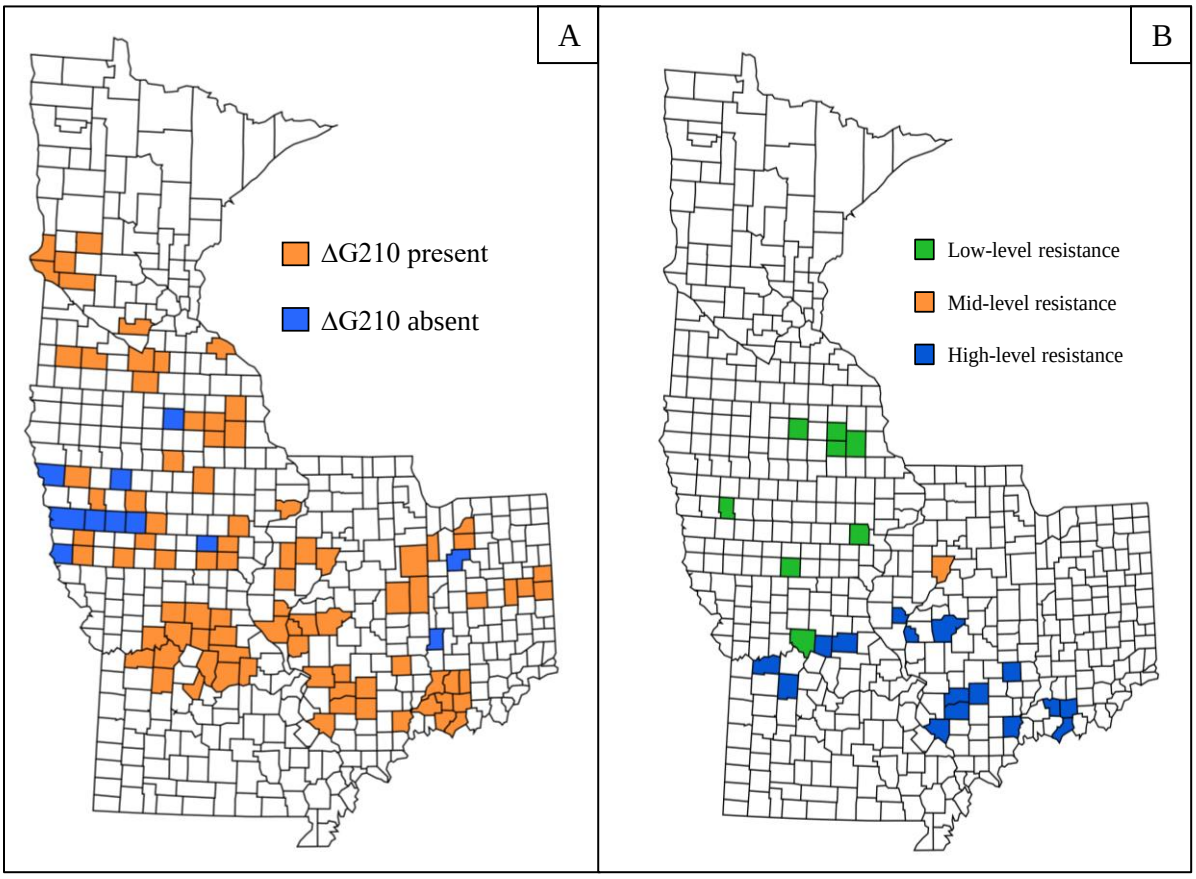

Figure 3.1. (A) Results from discriminating dose response experiments revealing distribution of tall waterhemp populations in the Midwest U.S. resistant to PPO-inhibiting herbicides via the ΔG210 mutation. (B) Results from dose response experiments of tall waterhemp populations categorized by response group.

CHAPTER 4. ROLE OF REACTIVE OXYGEN SPECIES DEGRADATION IN TALL WATERHEMP RESISTANCE TO PPO-INHIBITING HERBICIDES

4.1 Abstract

Reactive oxygen species (ROS) result from oxidative stress in plants, such as those induced by herbicide treatment. Over-accumulation of ROS in plants results in lipid peroxidation and is among the most destructive cellular processes in living organisms. The primary defense mechanisms in plants to detoxify ROS are enzymatic and non-enzymatic antioxidants. Although enhanced antioxidant enzyme activity is beneficial for plants enduring oxidative stress, these pathways also have negative implications for the efficacy of herbicides that generate ROS as part of the mode of action, such as protoporphyrinogen oxidase (PPO)-inhibitors. Furthermore, the role of these ROS degradative pathways in tall waterhemp populations with resistance to PPO-inhibiting herbicides has not been discerned. Greenhouse experiments have shown variable resistance ratio values in tall waterhemp populations resistant to PPO-inhibiting herbicides that contain the same target site mutation. Thus, a hypothesis was formed that enzymatic antioxidant activity in tall waterhemp resistant to PPO-inhibiting herbicides may contribute to the overall variability in herbicide response. Greenhouse and lab experiments were conducted to measure the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) in 20 PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) tall waterhemp populations. Lipid peroxidation occurred in all tall waterhemp populations, but more severe in PPO-S populations. Although differences at the population level were observed, basal levels of antioxidant enzymes did not correlate phenotypically or genotypically with resistance to PPO-inhibiting herbicides. Following fomesafen application, antioxidant enzymes in PPO-R and PPO-S populations either increased, decreased, or remained relatively stable. Responses of antioxidant enzyme activity correlated with fomesafen application and do not appear to compliment resistance to PPO-inhibiting herbicides in tall waterhemp.

4.2 Introduction

Protoporphyrinogen IX oxidase (PPO)-inhibiting herbicides (WSSA group #14; HRAC Group E) were introduced to agronomic crop production in the 1960s. Protoporphyrinogen oxidase is an enzyme in the last step of the tetrapyrrole biosynthesis pathway that oxidizes protoporphyrinogen IX (PPGIX) to produce protoporphyrin IX (PPIX; Proto) (Jacobs and Jacobs 1984; Duke et al. 1991). Protoporphyrin IX is a precursor for chlorophyll in photosynthesis and heme production for electron transfer chains. Inhibition of PPO results in accumulation of PPIX because PPGIX overflows into the thylakoid membrane and oxidizes to PPIX. Production of PPIX in the thylakoid membrane is separated from Mg chelatase and other enzymes that help regulate overproduction. Excess PPIX readily absorbs light and results in the production of reactive oxygen species (ROS) (Duke et al. 1991). Reactive oxygen species are highly unstable causing damage to cell membranes and cell leakage eventually resulting in cell death.

Prior to the adoption of glyphosate-resistant soybeans, postemergence PPO-inhibiting herbicides were predominantly used for control of tall waterhemp (Mayo et al. 1995; Sweat et al. 1998; Hager et al. 2002). Adoption of glyphosate-resistant corn and soybeans increased the frequency and amount of glyphosate applied for weed management in these major agronomic crops, eventually leading to extensive weed resistance to glyphosate. With already widespread resistance to ALS-inhibitors and now glyphosate, the use of PPO-inhibitors increased substantially following the glyphosate era (USDA-NASS 2017).

Thirteen weed species have evolved resistance to PPO-inhibiting herbicides (Heap, 2020). The first weed to evolve PPO resistance was in a tall waterhemp population in Kansas (Shoup et al. 2003). Three distinct resistance mechanisms by target site mutation confer resistance to PPO-inhibiting herbicides of which two were discovered in *Amaranthus* species. A codon deletion at the 210th position in *PPX2* leading to the loss of a glycine (ΔG210) was first discovered in tall waterhemp, but also exhibited in Palmer amaranth (Patzoldt et al. 2006; Salas et al. 2016). The second target site mutation is a substitution of an arginine for leucine in *PPX2* in common ragweed (Rousonelos et al. 2012). More recently, two new target site mutations of *PPX2* (R98G and R98M) were discovered in Palmer amaranth at the same relative site that confers PPO resistance in common ragweed (Giacomini et al. 2017). No known fitness costs are associated with PPO resistance as resistant and susceptible tall waterhemp populations have been observed displaying equal growth patterns (Duff et al. 2009).

Reactive oxygen species (ROS) are very detrimental to plant survival as previously mentioned above. Examples of ROS species include singlet oxygen ($^1O_2$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), and superoxide radical ($O_2^{\cdot-}$). Various biotic and abiotic stresses such as salinity, drought, heavy metals, temperature, nutrient deficiencies, and herbicides result in the production of ROS (Gill and Tuteja 2010). Similar to humans, plants have ROS defense mechanisms that help prevent the inevitable death that follows oxidative stress. ROS defense mechanisms include enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD; GP), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Non-enzymatic antioxidants consist of ascorbic acid (AsA), glutathione (GSH), α-tocopherol, proline, carotenoids, and flavonoids (Gill and Tuteja 2010). The seven enzymatic antioxidants can be grouped into three categories for their roles in detoxifying ROS. The first group is SOD, which is responsible for catalyzing the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2. The second group is CAT and POD, which are responsible for converting H_2O_2 to water. The third group consisting of APX, MDHAR, DHAR, and GR play a role in the ascorbate-glutathione cycle (AsA – GSH cycle) responsible for detoxifying O_2.

While antioxidants are vital to protecting plants from excessive amounts of ROS leading to death, this also has implications on effective weed management. Multiple herbicide modes of action such as EPSP synthase inhibitors, Glutamine synthase inhibitors, PPO-inhibitors, and PSI electron diverter result in the production of ROS species. Limited research has been conducted to determine the impact of antioxidants on herbicide efficacy or weed resistance to herbicides. A few cases have been documented suggesting increased levels of certain enzymatic antioxidants following herbicide application play a role in herbicide resistance by safening plant tissue from destructive ROS (Chiang et al. 2008; Harre et al. 2018). Currently, no research has been performed investigating the influence of enzymatic antioxidants on PPO-inhibiting herbicides. This research could help explain the variable responses in herbicide efficacy by PPO-inhibiting herbicides documented in greenhouse and field experiments. Therefore, the objectives of this research were to 1) measure basal levels of SOD, CAT, AP, and GR activity and 2) evaluate the change in these antioxidant enzymes over time following an application of a PPO-inhibiting herbicide.

4.3 Materials and Methods

4.3.1 Plant Propagation

Tall waterhemp seed from twenty populations representing PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) biotypes were used from a previous seed collection survey (Table 4.1) (Mansfield et al. 2017). A population was defined as all plants collected within the same field. The selected populations chosen represent a mix of phenotypes and genotypes to encompass the wide diversity of population genetics throughout the Midwest U.S. Tall waterhemp seeds were treated with a 10% sodium hypochlorite solution to reduce seed dormancy. Seeds were stirred continuously in sodium hypochlorite solution for 10 min and then rinsed with deionized water prior to planting. Seeds were planted in greenhouse flats using 100% commercial potting mix (Sun Gro propagation mix, Sun Gro Horticulture, Bellevue, WA, 98008) at a depth of 3 mm. Tall waterhemp seedlings were transplanted at the one to two true-leaf growth stage to 3.8 cm diameter plastic tubes filled with a 2:1 blend of commercial potting mix to sand. Plants were maintained in a greenhouse with day and night temperatures of 30 and 25 C, respectively, with natural lighting supplemented using high-pressure sodium bulbs delivering 1,100-umol m^{-2} s^{-1} photon flux density set to a 16-hr photoperiod. Plants were watered daily and a standard fertilizer plus water solution (Jack's Classic Professional (20-20-20), JR Peters Inc., Allentown, PA 18106) was applied as a drench once weekly starting at 2 d after transplanting.

4.3.2 Herbicide Application and Experimental Design

Fomesafen (Flexstar®, Syngenta Crop Protection, LLC, Greensboro, NC 27409) was applied at 342 g ha^{-1} (equivalent to 1X field use rate) in solution with deionized water to tall waterhemp with 10 to 14 true leaves (13 to 20 cm). The size of the plants sprayed were larger than the common size recommendation (2 to 6 true leaves) found on the Flexstar® herbicide label in order to ensure there was enough leaf tissue for analysis of antioxidant enzymes. Crop oil concentrate (Prime Oil®, Winfield Solutions, LLC, St. Paul, MN 55164) was applied with fomesafen at 1% v/v to maximize fomesafen foliar uptake and promote uniform spray coverage of leaf surfaces. Plants were sprayed at approximately 8:00 AM in an automated spray chamber in a greenhouse with consistent application times for both experimental runs. Application parameters were 140 L ha^{-1} of spray carrier at 276 kPa with a XR8002E spray tip. All experiments were

conducted twice and organized as a split-plot arrangement in a randomized complete block (RCB) design with three replications. The main plot was timing of greenhouse leaf tissue collection following herbicide application while the subplot was tall waterhemp population, as defined by individual populations or phenotypic classification as resistant or susceptible to fomesafen. One tall waterhemp plant was considered an experimental unit and the 0 HAT collection timing represented the nontreated control.

4.3.3 Data Collection

Visual estimates of control for tall waterhemp plants were recorded at 0, 3, 6, 9, 12, 24, and 36 hours after treatment (HAT) using a scale from 0 to 100% (0% = no injury; 100% = complete death). Subsequently, levels of SOD, CAT, APX, GR, total protein content, and MDA were determined from leaf tissue collected from the fifth node and higher on the main stem. Leaves were cut at the end of the petiole excluding axillary bud growth to prevent confounding results by including leaf tissue that may have not encountered fomesafen during application. Leaves from each individual tall waterhemp plant were combined into one sample, frozen in 50-ml polypropylene conical tubes with liquid nitrogen immediately following cutting, and kept in a -80 C freezer until further processing. A secondary leaf tissue collection was performed and ground for DNA extraction using a modified cetyltrimethylammonium bromide (CTAB) method originally designed by Saghai-Maroof et al. (1984). The presence or absence of the ΔG210 mutation was determined using RT-qPCR.

4.3.4 Preparation of Enzyme Extract

Liquid nitrogen was added to leaf tissue samples and ground with a mortar and pestle. Approximately 0.2 g of leaf tissue was added to 1.2 ml of potassium phosphate buffer (PBS, pH =7.8) containing 50 mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1% polyvinylpyrrolidone (PVP). The mixture was vortexed for 1 min and centrifuged at 15,000 g at 4 C for 15 min. All of the supernatant was collected and used as the enzyme extract for all enzymatic antioxidant assays, measurement of total protein content, and determination of malondialdehyde. Absorbance was recorded using a Genesys 10 Bio UV-Visible Spectrophotometer (Thermo Fisher Scientific, Waltham, MA 02451).

4.3.5 Lipid Peroxidation

Malondialdehyde (MDA) was measured using a modified protocol from Zhang and Kirkham (1996). A solution of 2 ml of 20% trichloroacetic acid (TCA)/0.5% thiobarbituric acid (TBA) was combined with 500 µl of enzyme extract in a 15-ml tube. Tubes were heated at 95 C for 30 min in a hot water bath, immediately cooled in an ice bath for 5 min, and centrifuged at 10,000 g for 10 min. In a 3-mL plastic cuvette, absorbance was recorded at 532 and 600 nm. Nonspecific absorption at 600 nm was subtracted from the reading at 532 nm and the MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

4.3.6 Total Protein Content

Total protein content was measured with slight modifications following the methods of Bradford (1976) using bovine serum albumin (BSA) as a standard. The standard test tube protocol was used to prepare a set of protein standards. To 25 ul of enzyme extract, 75 ul of PBS (pH=7.8) was added to a plastic cuvette and mixed by vortexing. To the mixture, 2.9 ml Coomassie reagent was added, vortexed, and placed inside a cabinet to avoid light for a minimum of 10 min, but no later than 60 min. Protein measurements were recorded using a 3-ml plastic cuvette by measuring the absorbance at 595 nm.

4.3.7 Enzymatic Antioxidant Assays

Superoxide dismutase (SOD) was measured in a 3-ml plastic cuvette containing 75 µM *p*-nitro blue tetrazolium chloride (NBT), 2 µM riboflavin, 13 mM methionine, 0.1 mM ethylenediaminetetraacetic (EDTA), 50 mM PBS (pH=7.8), and 10 to 15 µl of enzyme extract. Riboflavin was added last to initiate the reaction. The samples were placed under fluorescent lamps at 4,000 lux for 10 min prior to recording the absorbance at 560 nm. One unit of SOD activity is equal to the amount of enzyme necessary to cause 50% inhibition of NBT reduction at 560 nm (Giannopolitis and Ries 1977).

Catalase (CAT) was measured in a 3-ml UV cuvette containing 50 mM PBS (pH=7.0), 15 mM hydrogen peroxide (H_2O_2), and 100 ul of enzyme extract. Enzyme extract was added last to initiate the reaction. The activity of CAT was measured following the decrease in absorbance at

240 nm for 1 min and calculated using an extinction coefficient of 39.4 M^{-1} cm^{-1} (Nelson and Kiesow 1972).

Ascorbate peroxidase (APX) was measured in a 3-ml UV cuvette containing 0.5 mM Ascorbic acid (AsA), 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM PBS (pH=7.0), and 50 µl of enzyme extract. The reaction was initiated by adding H_2O_2 last. Activity of APX was measured following the decrease in absorbance at 290 nm for 1 min and calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} (Nakano and Asada 1981).

Glutathione reductase (GR) was measured in a 3-ml UV cuvette containing 1 mM EDTA, 1 mM oxidized glutathione (GSSG), 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), 100 mM PBS (pH=7.8), and 75 µl of enzyme extract. The reaction was initiated by adding GSSG last. The activity of GR was measured following the decrease in absorbance at 340 nm for 1 min and calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1} (Cakmak et al. 1993).

4.3.8 Statistical Analysis

Data for visual control, protein, MDA, SOD, CAT, APX, and GR activity were analyzed with ANOVA using PROC GLIMMIX (SAS 9.4) with Tukey's honest significant difference (HSD) test at alpha = 0.05. Main effects consisted of tall waterhemp population and genotype while random effects were experimental run and replication. To gain broader insight across populations, a second main effect was created by pooling populations confirmed as phenotypically resistant and susceptible to fomesafen. Populations were labeled resistant or susceptible based on comparison of overall visual control of fomesafen at 52 g ha^{-1} in a greenhouse. The susceptible phenotype included populations in which visual control was not different from a known PPO-S population and the resistant phenotype was defined by less control than a known PPO-S population. Normality and homogeneity of variance were tested using PROC UNIVARIATE and Levene's test in PROC GLM. Data transformations (log, square root, or arcsine) did not improve model assumptions; therefore, untransformed data were used for analysis. Pearson correlation tests were performed using PROC CORR to evaluate the relationship between basal levels of antioxidant enzyme activity to the following background phenotypic and genotypic information of each population previously collected in the greenhouse: frequency of resistance (FOR) with the ΔG210 mutation, control, survival, and R:S ratio (Mansfield et al. 2017; Steppig et al. 2017) (Table 4.1). Coefficient of variation (CV) values were generated using PROC MEANS (SAS 9.4) for each population in

addition to overall CV of populations pooled together for each respective antioxidant enzyme at 0 HAT.

4.4 Results and Discussion

All data were analyzed by sample timing due to a significant interaction with population. In general, antioxidant enzyme activity was not associated with genotype and differences observed were indicative from the resulting lipid peroxidation caused by the fomesafen application. Therefore, data presented below is represented by tall waterhemp population or phenotype. A significant interaction of population by experimental run and phenotype by experimental run were observed for certain sample times for visual control, MDA, and all antioxidant enzymes; however, this is likely due to the genetic variability in tall waterhemp as well as environmental conditions during herbicide application. The interaction of experimental run with population or phenotype did not alter conclusions made about the main hypothesis; therefore, data were pooled across experimental runs.

4.4.1 Visual Control and MDA Content

Symptoms of fomesafen injury on the upper portion of tall waterhemp plants characterized by water soaked lesions and necrotic spots on leaf tissue were first observed as early as 6 HAT for PPO-S and PPO-R phenotypes, but the latter with less severity (Figure 4.1A). By 36 HAT, visual control of PPO-S phenotypes were approximately twice the injury compared to PPO-R. Visual control of PPO-R populations plateaued from 12 to 36 HAT and never exceeded 25%. Similar to visual control, MDA content increased starting at 6 HAT for PPO-S populations and continued to increase in incremental amounts at every sample time (Figure 4.1B). Visual injury directly correlated with MDA content due to the resulting lipid peroxidation as part of the mode of action for PPO-inhibiting herbicides. The PPO-R populations had a delayed response with increases in MDA content beginning at 9 HAT. Over the course of the experiment, MDA content remained lower in the PPO-R populations resulting in a 40% reduction compared to PPO-S populations.

4.4.2 Enzymatic Antioxidants

4.4.2.1 SOD

Multiple isozymes of SOD exist within plants that are classified by their active site metal ion: copper/zinc, manganese, and iron. Data presented in this research are for all forms of SOD; therefore, we were not able to differentiate between SOD isozyme(s) responsible for any observed changes in SOD activity. Basal levels of SOD in tall waterhemp plants were variable at the population level showing minimal differences, but did not segregate by phenotype (Figure 4.2A). Pearson correlation supported these results by revealing a weak correlation between basal levels of SOD activity and FOR (r^2 = 0.04772; p-value = 0.6047) and R:S ratios (r^2 = 0.16946; p-value = 0.2206). In addition, basal levels of SOD activity were weakly correlated with background levels of overall control (r^2 = -0.12512; p-value = 0.1733) and survival (r^2 = 0.13970; p-value = 0.1281). Furthermore, the coefficient of variation (CV) values for PPO-S populations ranged from 8 to 28 compared to 9 to 26 for PPO-R populations. The CV results support our conclusion that there are no inherent differences in SOD levels between PPO-S and PPO-R tall waterhemp populations in regards to the ΔG210 mutation. Our results of basal levels of SOD are similar to other research where SOD activity in resistant and susceptible giant ragweed biotypes were similar in the absence of glyphosate (Harre et al. 2018). Furthermore, additional studies have reported higher basal levels of SOD following paraquat application in resistant versus susceptible biotypes in various weed species (Harper and Harvey 1978; Pyon et al. 2004; Shaaltiel and Gressel 1986). Following fomesafen application, SOD remained relatively unchanged from 0 to 36 HAT for PPO-S and PPO-R phenotypes although MDA content increased indicating the generation of ROS species (Figure 4.2B). The role of SOD in detoxification of ROS is to convert $O_2 \cdot^-$ to H_2O_2 (Gill and Tuteja 2010). No changes in SOD activity suggest $O_2 \cdot^-$ was not being generated or were present in a low enough concentration that does not overwhelm the SOD defensive mechanism resulting in increased activity. This result may be attributed to the mode of action for PPO-inhibiting herbicides that result in accumulation of protoporphyrin IX (Proto) where in the presence of oxygen and light generates $^1O_2$ (Duke et al. 1991). In other antioxidant enzyme research involving PPO-inhibiting herbicides, SOD activity were reported higher in wild-type plants of rice compared to the transgenic PPO-R line following applications of acifluorfen, oxyfluorfen, carfentrazone-ethyl, and oxadiazon (Jung et al. 2008). Increased SOD activity following glyphosate and paraquat

applications have also been reported in resistant giant ragweed and hairy fleabane, respectively (Harre et al. 2018; Shaaltiel and Gressel 1986).

4.4.2.2 CAT

Basal levels of CAT activity were not different between PPO-R and PPO-S tall waterhemp populations (Figure 4.3A). Pearson correlation revealed a weak correlation between basal levels of CAT activity and FOR (r^2 = -0.12655; p-value = 0.1684), R:S ratios (r^2 = -0.01013; p-value = 0.9420), control (r^2 = 0.01837; p-value = 0.8422), and survival (r^2 = -0.05594; p-value = 0.5439) in PPO-R and PPO-S populations. Similar to SOD, CV values for PPO-S populations ranged from 6 to 30 compared to 6 to 24 for PPO-R populations. The CV results support our conclusion that there are no inherent differences in CAT levels between PPO-S and PPO-R tall waterhemp populations in regards to the ΔG210 mutation. In regards to other weed species, basal activity of CAT in horseweed resistant to paraquat has been documented higher in resistant plants than susceptible plants (Pyon et al. 2004). Following fomesafen application, CAT activity did not increase above basal levels and generally declined throughout the length of the experiments for PPO-S and PPO-R phenotypes (Figure 4.3B). Differences in CAT activity between phenotypes were detected from 9 to 36 HAT with greater reductions observed in PPO-S. Higher CAT activity in PPO-R populations could suggest that CAT activity is providing resistance through greater detoxification of H_2O_2; however, the differences observed were not due to increases in CAT activity, but rather greater reductions in the PPO-S populations. The activity of CAT in response to oxidative stress is known to be variable with increases, decreases, or no changes being observed (Jiang and Huang 2001; Jung et al. 2008; Zhang and Kirkham 1996). Previous research in waterhemp has reported no differences in CAT activity between nontreated and treated leaves following lactofen application regardless of population or genotype (Wuerffel 2014). However, the differences in the two studies is most likely due from the sample time following herbicide application where the previous study only evaluated CAT activity after 4 HAT. Regardless, the present study supports the previous conclusions made suggesting CAT activity is not involved with resistance to PPO-inhibiting herbicides.

4.4.2.3 APX

Basal levels of APX activity slightly differed between tall waterhemp populations, but did not segregate by phenotype (Figure 4.4A). Pearson correlation revealed a weak correlation between basal levels of APX activity and FOR (r^2 = -0.08395; p-value = 0), R:S ratios (r^2 = -0.18505; p-value = 0.1804), control (r^2 = 0.10091; p-value = 0.2728), and survival (r^2 = -0.08943; p-value = 0.3314) in PPO-R and PPO-S populations. Similar to SOD and CAT, CV values for PPO-S populations ranged from 6 to 20 compared to 9 to 13 for PPO-R populations. The CV results support our conclusion that there are no inherent differences in APX levels between PPO-S and PPO-R tall waterhemp populations in regards to the ΔG210 mutation. Results of APX activity are similar to previous research reported in giant ragweed (Harre et al. 2018). However, activity of APX has been reported higher in biotypes of *Conyza bonariensis* resistant to paraquat (Ye and Gressel 1994). Similar to CAT, activity of APX in PPO-S never increased above basal levels and declined from 9 to 36 HAT following fomesafen application (Figure 4.4B). However, activity of APX remained relatively stable for PPO-R phenotypes throughout the entire experiment.

4.4.2.4 GR

Basal levels of GR activity were more variable than SOD, CAT, and APX in tall waterhemp populations, but still did not segregate by phenotype (Figure 4.5A). Pearson correlation revealed a weak correlation between basal levels of GR activity and R:S ratios (r^2 = -0.16012; p-value = 0.2474), control (r^2 = 0.10095; p-value = 0.2726), and survival (r^2 = -0.14361; p-value = 0.1176) in PPO-R and PPO-S populations. Activity of GR was negatively correlated with FOR (r^2 = -0.33596; p-value = 0.0002) indicating GR activity in tall waterhemp plants decreases as FOR for the ΔG210 mutation increases in populations. Similar to SOD, CAT, and APX, CV values for PPO-S populations ranged from 6 to 19 compared to 5 to 25 for PPO-R populations. The CV results support our conclusion that there are no inherent differences in GR levels between PPO-S and PPO-R tall waterhemp populations in regards to the ΔG210 mutation. In order for GR to be associated with resistance to PPO-inhibiting herbicides, we would expect higher GR activity in resistant plants to help overcome the buildup of ROS. This was not the case in the present study, which suggests GR activity does not provide an advantage in PPO-R populations prior to herbicide

application. In comparison to SOD, CAT, and APX, only GR activity increased above basal levels progressively after herbicide application for PPO-R and PPO-S phenotypes (Figure 4.5B). Interestingly, the response in GR activity was relatively similar between PPO-R and PPO-S phenotypes despite lower levels of MDA content in PPO-R. In contrast, GR activity has been found to complement glyphosate resistant Palmer amaranth and giant ragweed as well as paraquat resistant tall fleabane (Chiang et al 2008, Harre et al 2018, Maroli et al 2015).

4.4.3 Total Protein Content

Total protein content varied across tall waterhemp populations regardless of phenotype at each sample time, but remained relatively unchanged throughout the experiment. Protein levels in PPO-R and PPO-S phenotypes were not different from 0 to 12 HAT although changes in antioxidant enzyme activity occurred during this time (data not shown). By 36 HAT, the PPO-S populations as a whole were slightly more elevated than PPO-R populations. However, this result is in contrast to the observed decreases in CAT and APX activity at 36 HAT indicating that total protein variation in tall waterhemp is not largely affected by changes in antioxidant enzymes following fomesafen application.

4.4.4 Conclusions

This research documents the response of SOD, CAT, APX, and GR enzymes following an application of fomesafen in a greenhouse in addition to investigating the relationship of antioxidant enzymes with resistance to PPO-inhibiting herbicides. Basal levels of antioxidant enzymes varied among tall waterhemp populations, but did not correlate with the presence of the ΔG210 or R128G mutation. Furthermore, there were no correlation with antioxidant enzyme activity and the average fomesafen control and survival among populations prior to fomesafen application. These results indicate that the variability observed with fomesafen control is not because of an enhanced antioxidant enzyme system. With the exception of GR activity, all antioxidant enzymes remained relatively stable or decreased in the PPO-S populations following fomesafen application. In terms of herbicide resistance, these results indicate PPO-R biotypes do not inherently have higher antioxidant enzyme levels that can provide an advantage with detoxifying ROS compared to PPO-

S biotypes as well as suggests the absence of an enhanced antioxidant enzyme system in tall waterhemp following fomesafen application.

4.5 Acknowledgements

The authors extend appreciation to Julie Young and Dr. Haozhen Nie for their technical assistance with this research as well as undergraduate and graduate members of the Purdue Weed Science lab for their labor and support. Special thanks goes to Dr. Yiwei Jiang for providing advice and training of the antioxidant assays in addition for the use of his lab at Purdue University. No conflicts of interest have been declared.

4.6 Literature Cited

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cakmak I, Strbac D, Marschner H (1993) Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. J Exp Bot 44:127–132

Chiang Y, Wu Y, Chiang M, Wang C (2008) Role of Antioxidative system in paraquat resistance of tall fleabane (*Conyza sumatrensis*). Weed Sci 56:350–355

Duff MG, Al-khatib K, Peterson DE (2009) Relative competitiveness of protoporphyrinogen oxidase-resistant common waterhemp (*Amaranthus rudis*). Weed Sci 57:169–174

Duke SO, Lydon J, Becerril JM, Sherman TD, Lehnen Jr. LP, Matsumoto H (1991) Protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 39:465–473

Giacomini DA, Umphres AM, Nie H, Mueller TC, Steckel LE, Young BG, Scott RC, Tranel PJ (2017) Two new *PPX2* mutations associated with resistance to PPO-inhibiting herbicides in *Amaranthus palmeri*. Pest Manag Sci 73:1559–1563

Giannopolitis CN, Ries SK (1977) Superoxide dismutases. I. Occurrence in higher plants. Plant Physiol 59:309–314

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Hager AG, Wax LM, Bollero GA, Simmons FW (2002) Common waterhemp (*Amaranthus rudis* Sauer) management with soil-applied herbicides in soybean (*Glycine max* (L.) Merr.). Crop Prot 21:277–283

Harper DB, Harvey BMR (1978) Mechanism of paraquat tolerance in perennial ryegrass. Plant Cell Environ 1:211–215

Harre NT, Nie H, Jiang Y, Young BG (2018) Differential antioxidant enzyme activity in rapid-response glyphosate-resistant *Ambrosia trifida*. Pest Manag Sci 74:2125–2132

Heap I (2020) The international survey of herbicide resistant weeds. https://www.weedscience.org. Accessed September 15, 2020

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Jacobs JM, Jacobs NJ (1984) Protoporphyrinogen oxidation, an enzymatic step in heme and chlorophyll synthesis: partial characterization of the reaction in plant organelles and comparison with mammalian and bacterial systems. Arch Biochem Biophys 229:312–319

Jiang Y, Huang B (2001) Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci 41:436–442

Jung H Il, Kuk YI, Back K, Burgos NR (2008) Resistance pattern and antioxidant enzyme profiles of protoporphyrinogen oxidase (Protox) inhibitor-resistant transgenic rice. Pestic Biochem Physiol 91:53–65

Mansfield BC, Nie H, Young JM, Bradley KW, Young BG (2017) A multi-state survey to determine the potential for resistance to PPO-inhibiting herbicides in tall waterhemp beyond the G210 target site mutation. Page 62 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis, Missouri

Maroli AS, Nandula VK, Dayan FE, Duke SO, Gerard P, Tharayil N (2015) Metabolic profiling and enzyme analyses indicate a potential role of antioxidant systems in complementing glyphosate resistance in an *Amaranthus palmeri* biotype. J Agric Food Chem 63:9199–9209

Mayo CM, Horak MJ, Peterson DE, Boyer JE (1995) Differential control of four *Amaranthus* species by six postemergence herbicides in soybean (*Glycine max*). Weed Technol 9:141–147

Nakano Y and Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Nelson DP and Kiesow LA (1972) Enthalpy of decomposition of hydrogen peroxide by catalase at 25 C (with molar extinction coefficients of H_2O_2 solutions in the UV). Anal Biochem 49:474–478

Patzoldt WL, Hager AG, Mccormick JS, Tranel PJ (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. PNAS 103:12329–12334

Pyon JY, Piao RZ, Roh SW, Shin SY, Kwak SS (2004) Differential levels of antioxidants in paraquat-resistant and -susceptible *Erigeron canadensis* biotypes in Korea. Weed Biol Manag 4:75–80

Rousonelos SL, Lee RM, Moreira MS, VanGessel MJ, Tranel PJ (2012) Characterization of a common ragweed (*Ambrosia artemisiifolia*) population resistant to ALS-and PPO-inhibiting herbicides. Weed Sci 60:335–344

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Salas RA, Burgos NR, Tranel PJ, Singh S, Glasgow L, Scott RC, Nichols RL (2016) Resistance to PPO-inhibiting herbicide in Palmer amaranth from Arkansas. Pest Manag Sci 72:864–869

Shaaltiel Y, Gressel J (1986) Multienzyme oxygen radical detoxifying system correlated with paraquat resistance in *Conyza bonariensis*. Pestic Biochem Physiol 26:22–28

Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci 51:145–150

Steppig NR, Mansfield BC, Nie H, Young JM, Young BG (2017) Presence of an alternative mechanism of resitance to PPO-inhibiting herbicides in tall waterhemp populations from Indiana, Illinois, Iowa, Missouri, and Minnesota. Page 63 *in* Proceedings of the 72nd Annual Meeting of the North Central Weed Science Society. St. Louis. Missouri

Sweat JK, Horak MJ, Peterson DE, Lloyd RW, Boyer JE (1998) Herbicide efficacy on four *Amaranthus* species in soybean (*Glycine max*). Weed Technol 12:315–321

USDA-NASS (United States Department of Agriculture - National Agricultural Statistics Service) (2017) Agricultural chemical use database. https://quickstats.nass.usda.gov. Accessed December 11, 2018

Wuerffel RJ (2014) A characterization of selection for evolved resistance to protoporphyrinogen oxidase (PPO)-inhibiting herbicides in *Amaranthus tuberculatus*. PhD dissertation. Carbondale, IL: Southern Illinois University Carbondale. Pp 1–301

Ye B, Gressel J (1994) Constitutive variation of ascorbate peroxidase activity during development parallels that of superoxide dismutase and glutathione reductase in paraquat-resistant *Conyza*. Plant Sci 102:147–151

Zhang J, Kirkham MB (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol 132:361–373

Table 4.1. Background information and sources of tall waterhemp populations used for evaluation of malondialdehyde and antioxidant enzyme assays following a fomesafen application in a greenhouse.

County (population ID)[c]	State	Phenotype[a]	Genotype[b]		Control	Survival	R/S[c]
			ΔG210	R128G			
			------ % individuals-----		----------%----------		
Cerro Gordo (IA-340)	IA	Susceptible	0	0	97	31	1X
Chickasaw (IA-369)	IA	Susceptible	0	0	90	94	1.1X
Greene (IA-157)	IA	Susceptible	0	0	99	19	-
Champaign (IL-CHAM)	IL	Susceptible	8	0	98	19	-
Iroquois (IL-IRO1)	IL	Susceptible	7	0	97	31	-
Boone (IN-BNE2)	IN	Susceptible	0	0	99	6	-
Randolph (IN-RAN1)	IN	Susceptible	0	0	99	6	-
Sibley (MN-388)	MN	Susceptible	2	0	98	31	-
Carroll (MO-58)	MO	Susceptible	30	0	89	56	-
Chariton (MO-45)	MO	Susceptible	39	0	89	63	1.4X
Lafayette (MO-47)	MO	Susceptible	43	0	96	44	-
Brown (IL-BRO2)	IL	Resistant	100	0	65	94	10X
Randolph (IL-BDW)	IL	Resistant	95	0	57	100	11X
Dubois (IN-DUB)	IN	Resistant	97	0	48	94	17X
Gibson (IN-GIB5)	IN	Resistant	0	100	61	100	19X
Pike (IN-PIKE1)	IN	Resistant	95	0	41	94	9.3X
Cottonwood (MN-395)	MN	Resistant	90	0	76	81	-
Stevens (MN-401)	MN	Resistant	45	0	87	50	-
Lafayette (MO-53)	MO	Resistant	87	0	68	100	10X
Montgomery (MO-39)	MO	Resistant	89	0	70	88	-

[a] Phenotype was defined susceptible if overall population control by fomesafen (52 g ha^{-1}) in a greenhouse was similar to a known PPO-susceptible (PPO-S) population and resistant if the overall population control was less than a known PPO-S population.

[b] Genotype, control, and survival data were recorded from a discriminating dose of fomesafen (52 g ha^{-1}) in a greenhouse (Mansfield et al. 2017).

[c] Data for R/S ratios derived from a full dose response of fomesafen in a greenhouse (Mansfield et al. 2017; Steppig et al. 2017).

[d] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; ΔG210, glycine deletion at position 210 of *PPX2*; R128G, substitution of an arginine for glycine at position 128 of *PPX2*.

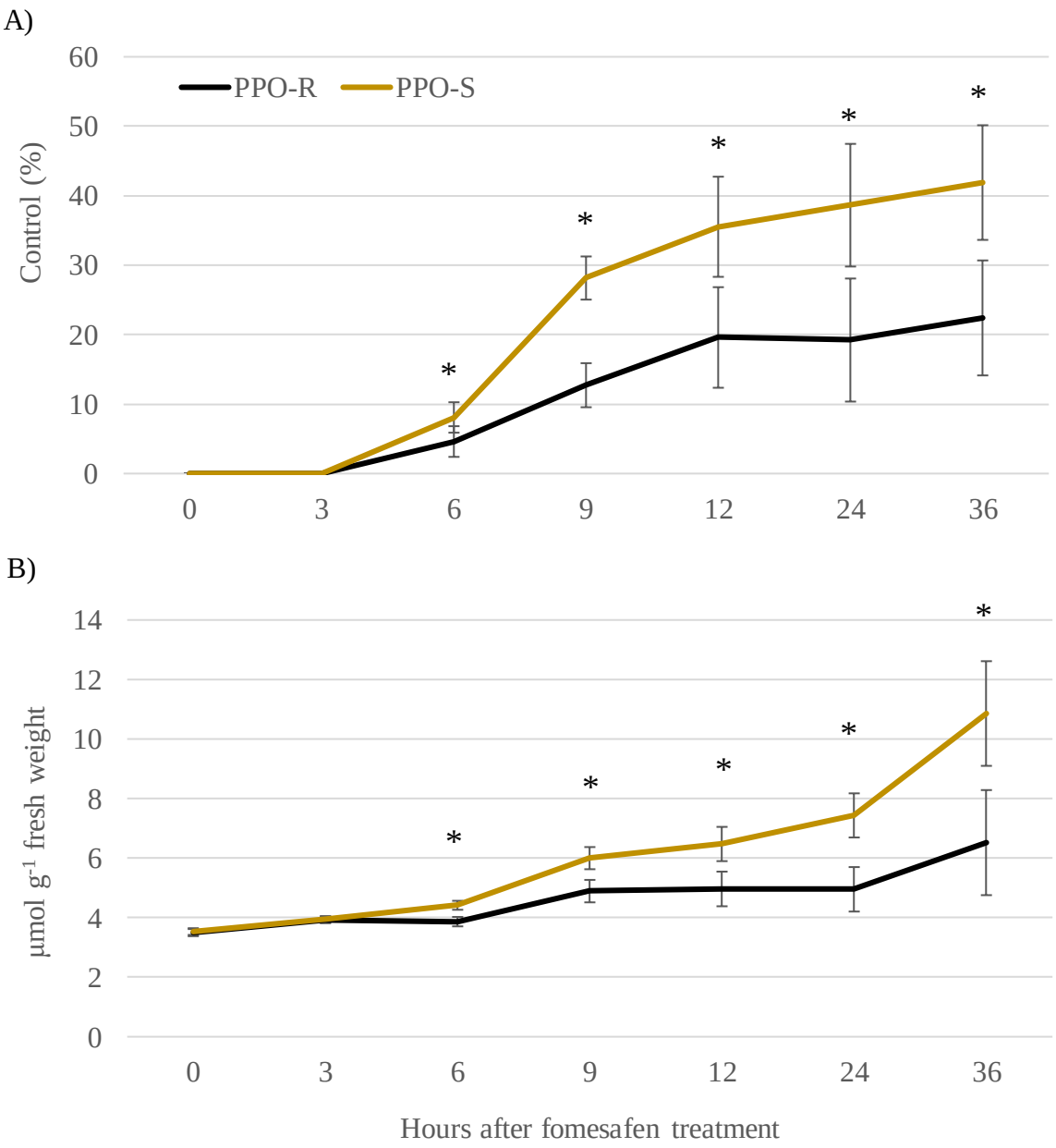

Figure 4.1. Visual control (A) and malondialdehyde levels (B) of PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) tall waterhemp populations following application of fomesafen applied at 342 g ai ha^{-1}. Vertical bars represent standard of the mean (n=66 for PPO-S; n=54 for PPO-R). An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0) within each collection timing.

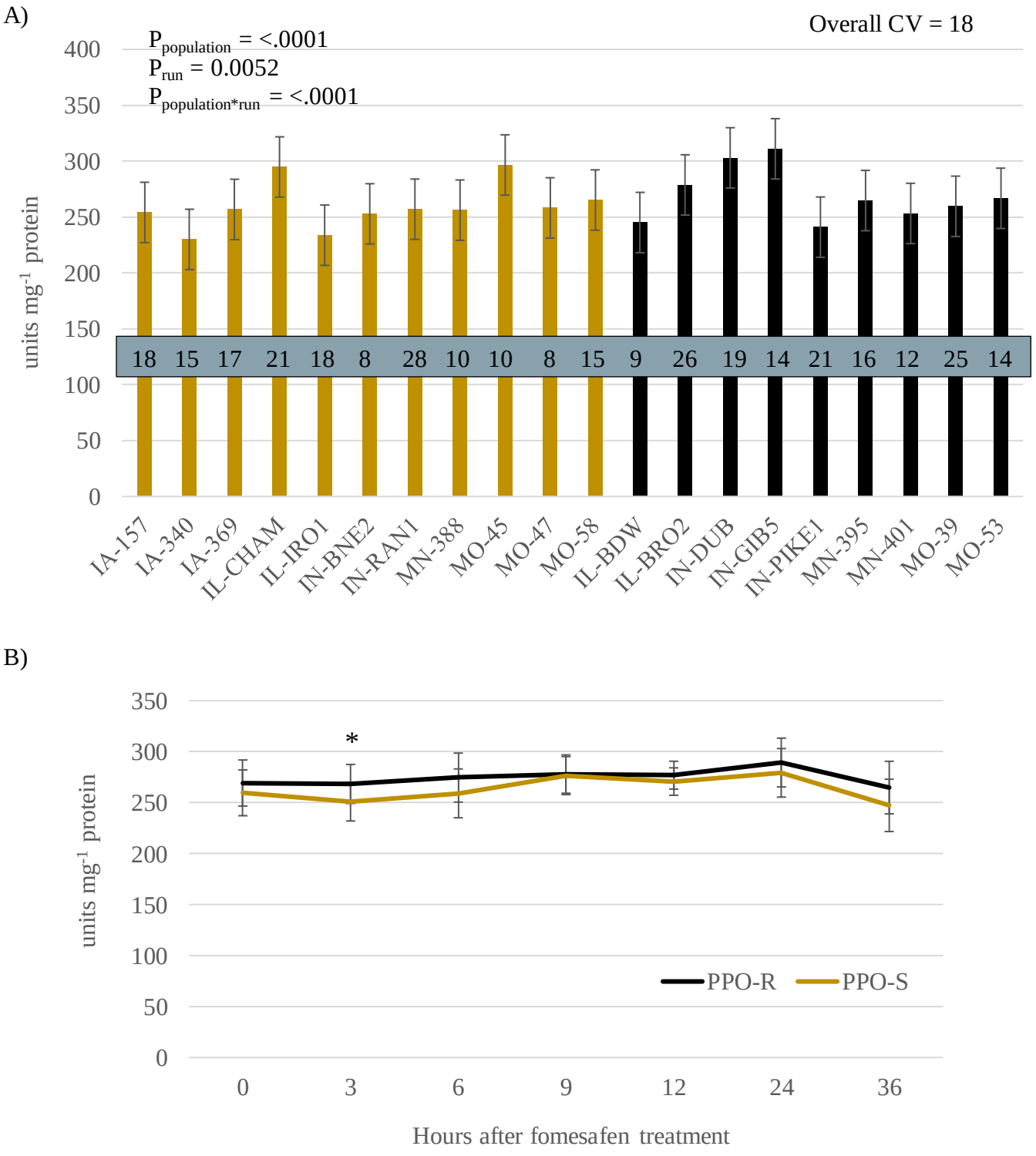

Figure 4.2. Superoxide dismutase basal levels (A) and response following fomesafen application on PPO-susceptible (gold) and PPO-resistant (black) tall waterhemp populations (B). Vertical bars represent standard error of the mean (n=66 for PPO-S; n=54 for PPO-R). Coefficient of variation (CV) listed for each population in gray box. An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0.05) within each collection time.

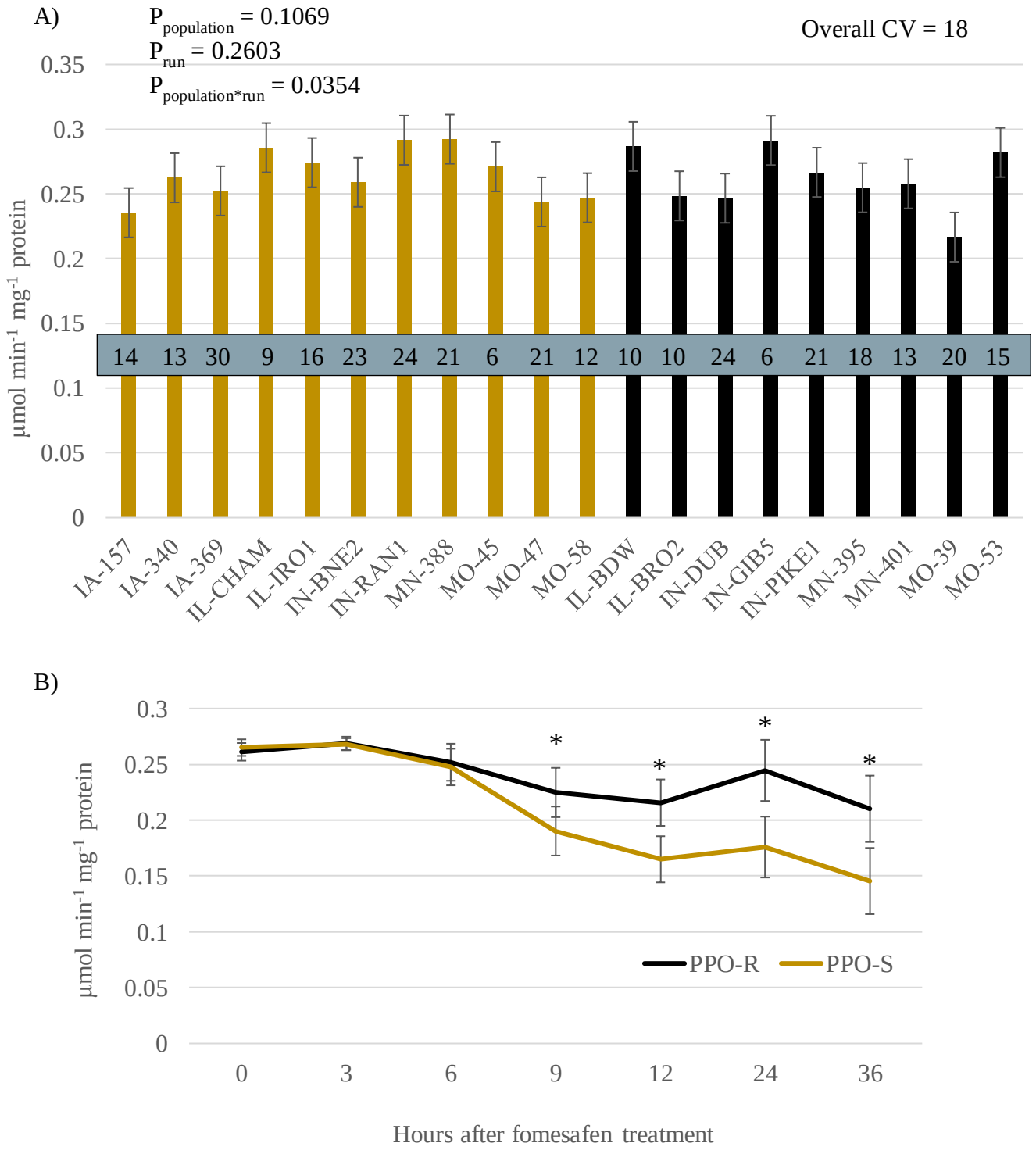

Figure 4.3. Catalase basal levels (A) and response following fomesafen application on PPO-susceptible (gold) and PPO-resistant (black) tall waterhemp populations (B). Vertical bars represent standard error of the mean (n=66 for PPO-S; n=54 for PPO-R). Coefficient of variation (CV) listed for each population in gray box. An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0.05) within each collection timing.

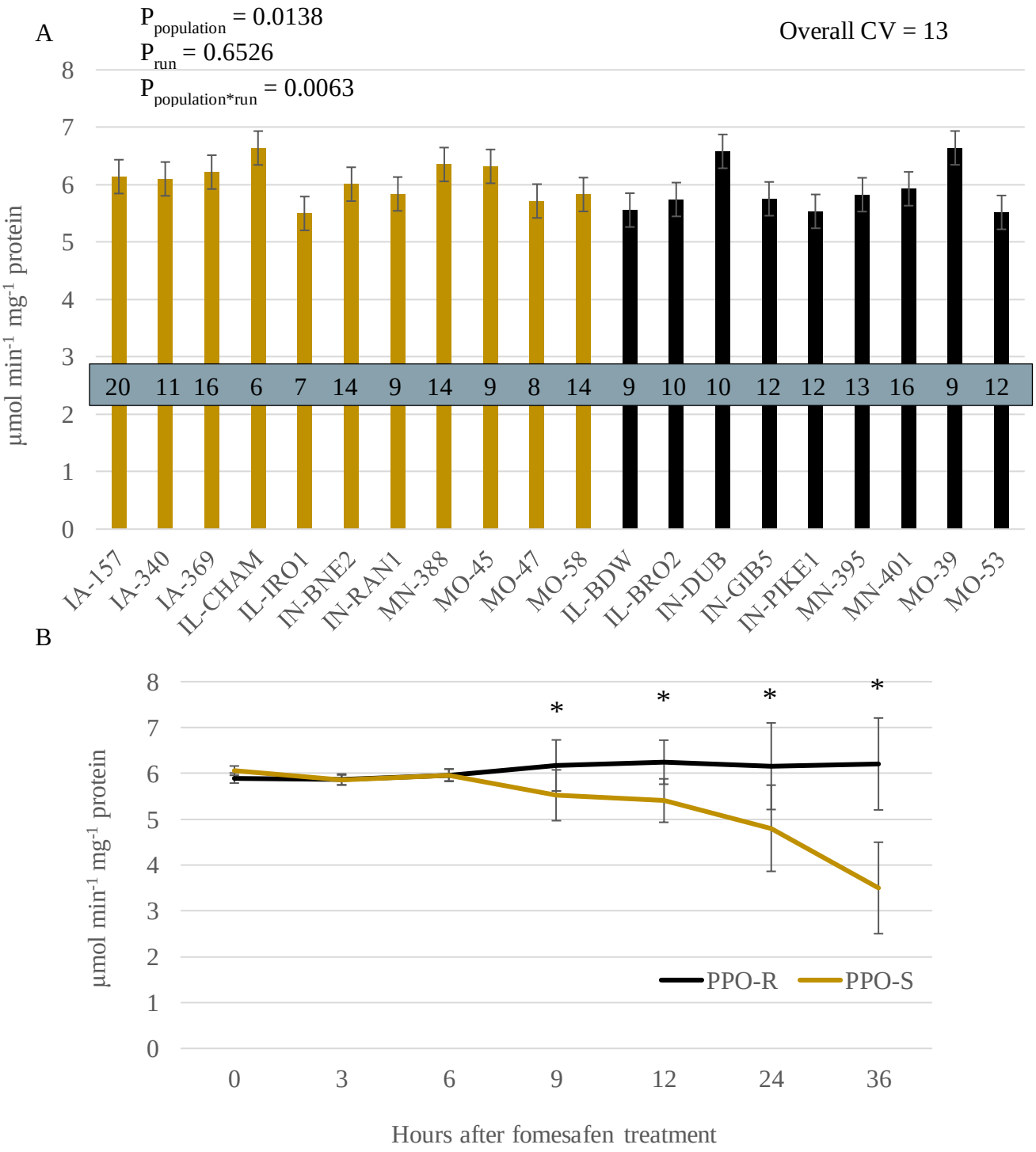

Figure 4.4. Ascorbate peroxidase basal levels (A) and response following fomesafen application on PPO-susceptible (gold) and PPO-resistant (black) tall waterhemp populations (B). Vertical bars represent standard error of the mean (n=66 for PPO-S; n=54 for PPO-R). Coefficient of variation (CV) listed for each population in gray box. An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0.05) within each collection timing.

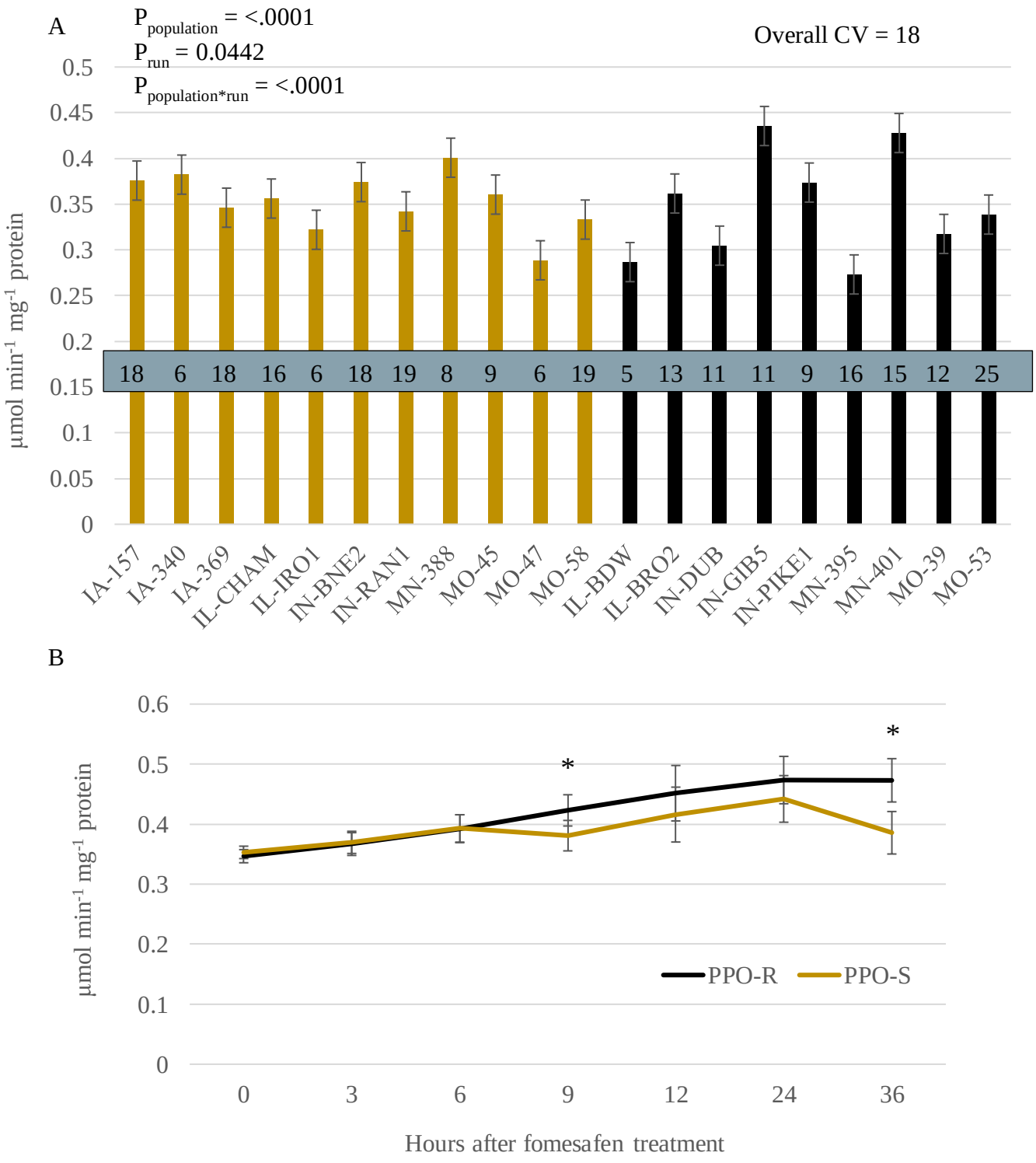

Figure 4.5. Glutathione reductase basal levels (A) and response following fomesafen application on PPO-susceptible (gold) and PPO-resistant (black) tall waterhemp populations (B). Vertical bars represent standard error of the mean (n=66 for PPO-S; n=54 for PPO-R). Coefficient of variation (CV) listed for each population in gray box. An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0.05) within each collection timing.

APPENDIX A. CHAPTER 2 SUPPLEMENTARY DATA

Table A.1 Real-time polymerase chain reaction (RT-qPCR) results for the glycine deletion at position 210 of *PPX2* (ΔG210) in tall waterhemp resistant to PPO-inhibiting herbicides for all samples collected in herbicide treatments and the nontreated control in Farmland in 2017 and Lafayette, IN in 2016 and 2017.

Site year	Homozygous susceptible	Heterozygous resistant	Homozygous resistant	Total samples	FOR[a,b]
		#			%
Lafayette 2016	3126	258	18	3402	8
Lafayette 2017	3039	514	41	3594	15
Farmland 2017	1518	215	30	1763	14

[a] FOR calculated by dividing the sum of heterozygous and homozygous resistant tall waterhemp by the total number of tall waterhemp sampled.

[b] Abbreviations: FOR, frequency of resistance; PPO, protoporphyrinogen oxidase.

Table A.2 Weekly rainfall for Farmland and Lafayette, IN.

	Lafayette		Farmland
	2016	2017	2017
Rainfall Interval	Precipitation		
DAT[a]	----------cm----------		
-7 to -1	0.20	7.67	2.08
0 to 6	3.35	1.63	0.41
7 to 13	1.37	0.00	2.34
14 to 20	2.24	6.60	4.19
21 to 27	3.12	2.97	8.46
28 to 34	2.54	2.29	3.78
35 to 41	0.79	5.08	3.12
42 to 48	5.38	13.28	2.11
49 to 55	0.08	0.74	3.99
56 to 62	0.00	10.11	1.14
63 to 69	0.00	0.53	0.00
70 to 76	4.60	1.14	1.02
77 to 83	0.94	0.41	1.09
Total	24.61	52.45	33.73

[a] Abbreviations: DAT, days after treatment.

Table A.3 Two-way mixed effect ANOVA of tall waterhemp resistant to PPO-inhibiting herbicides control 28 days after treatment (DAT) in Lafayette, IN in 2016. Data subjected to an arcsine transformation.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	38	121.25	<.0001
smeto	3	38	43.90	<.0001
fomesafen*smeto	8	38	8.77	<.0001

[a] Replication was considered a random variable in PROC GLIMMIX.

[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.4. Two-way mixed effect ANOVA of tall waterhemp resistant to PPO-inhibiting herbicides control 28 days after treatment (DAT) in Lafayette, IN in 2017. Data subjected to an arcsine transformation.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	41	21.22	<.0001
smeto	3	41	49.84	<.0001
fomesafen*smeto	8	41	3.27	0.0057

[a] Replication was considered a random variable in PROC GLIMMIX.

[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.5. Two-way mixed effect ANOVA of tall waterhemp resistant to PPO-inhibiting herbicides control 28 days after treatment in Farmland, IN in 2017. Data subjected to an arcsine transformation.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	42	1.24	0.3084
smeto	3	42	1.34	0.2745
fomesafen*smeto	8	42	2.68	0.0180

[a] Replication was considered a random variable in PROC GLIMMIX.

[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.6. Tall waterhemp resistant to PPO-inhibiting herbicides control 28 days after treatment (DAT) in Farmland, IN in 2017.

fomesafen	*s*-metolachlor	Control[a,b]
g ai ha^{-1}		%
0	0	
66	0	90 a
132	0	94 a
264	0	97 a
0	335	95 a
0	710	97 a
0	1420	98 a
66	335	96 a
66	710	98 a
66	1420	92 a
132	335	98 a
132	710	92 a
132	1420	96 a
264	335	96 a
264	710	97 a
264	1420	92 a

[a] Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$). Control presented as percent of nontreated control.

[b] Abbreviations: PPO, protoporphyrinogen oxidase.

Table A.7. Two-way mixed effect ANOVA of tall waterhemp resistant to PPO-inhibiting herbicides density 28 days after treatment (DAT) in Farmland, IN in 2017. Data was subjected to an arcsine transformation.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	42	2.21	0.1012
smeto	3	42	0.94	0.4286
fomesafen*smeto	8	42	1.90	0.0857

[a] Replications were considered random variables in PROC GLIMMIX.

[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.8. Two-way mixed effect ANOVA of tall waterhemp resistant to PPO-inhibiting herbicides density 56 days after treatment (DAT) in Farmland, IN in 2017.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	42	1.17	0.3317
smeto	3	42	1.38	0.2628
fomesafen*smeto	8	42	1.26	0.2875

[a] Replications were considered random variables nested within site year in PROC GLIMMIX.
[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.9. Tall waterhemp resistant to PPO-inhibiting herbicides density recorded at 28 and 56 days after treatment (DAT) in Farmland, IN in 2017.

Herbicide	Rate[a,b]	Density	
		28 DAT	56 DAT
	g ai ha^{-1}	---------- % ----------	
fomesafen			
	0	10 A	31 A
	66	27 A	58 A
	132	14 A	43 A
	264	13 A	53 A
s-metolachlor			
	0	26 a	59 a
	335	11 a	48 a
	710	10 a	29 a
	1420	17 a	49 a

[a] No observed significant interaction of the two main factors, fomesafen and s-metolachlor; therefore, data was analyzed separately for each factor with rate pooled over the second factor. Data represents trials conducted in Lafayette in 2016 and 2017. Farmland data in 2017 is excluded due to a significant herbicide treatment by site interaction in the ANOVA. Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

[b] Abbreviations: PPO, protoporphyrinogen oxidase.

Table A.10. Two-way mixed effect ANOVA of frequency of ΔG210 for tall waterhemp resistant to PPO-inhibiting herbicides in Farmland, IN in 2017.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	45	1.00	0.4030
smeto	3	45	1.64	0.1934
fomesafen*smeto	9	45	0.58	0.8082

[a] Replications were considered random variables nested within site year in PROC GLIMMIX.
[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.11. Two-way mixed effect ANOVA of frequency of tall waterhemp heterozygous for ΔG210 in Farmland, IN in 2017.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	37	1.32	0.2828
smeto	3	37	1.15	0.3424
fomesafen*smeto	9	37	2.24	0.0408

[a] Replications were considered random variables nested within site year in PROC GLIMMIX.
[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom;

Table A.12. Two-way mixed effect ANOVA of frequency of tall waterhemp homozygous for ΔG210 in Farmland, IN in 2017. Data subjected to an arcsine transformation.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	37	1.04	0.3842
smeto	3	37	0.56	0.6423
fomesafen*smeto	9	37	2.15	0.0497

[a] Replications were considered random variables nested within site year in PROC GLIMMIX.
[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom.

Table A.13. Frequency of tall waterhemp heterozygous or homozygous for ΔG210 in Farmland, IN in 2017. Data subjected to an arcsine transformation.

fomesafen	*s*-metolachlor	Rr	RR
g ai ha^{-1}		%	
0	0	92 a	8 a
66	0	100 a	0 a
132	0	86 a	14 a
264	0	94 a	6 a
0	335	100 a	0 a
0	710	83 a	17 a
0	1420	46 a	54 a
66	335	67 a	33 a
66	710	92 a	8 a
66	1420	79 a	21 a
132	335	94 a	6 a
132	710	100 a	0 a
132	1420	100 a	0 a
264	335	75 a	25 a
264	710	67 a	33 a
264	1420	97 a	3 a

[a] Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

[b] Abbreviations: Rr, heterozygous PPO-resistant; RR, homozygous PPO-resistant

Table A.14. Two-way mixed effect ANOVA of projected end of season surviving PPO-resistant tall waterhemp in Farmland, IN in 2017.

Effect[a]	Num DF[b]	Den DF	F-value	P-value
fomesafen	3	45	0.25	0.8627
smeto	3	45	2.42	0.0786
fomesafen*smeto	9	45	0.91	0.5279

[a] Replications were considered random variables nested within site year in PROC GLIMMIX.
[b] Abbreviations: Num, numerator; Den, denominator; DF, degrees of freedom; PPO, protoporphyrinogen oxidase.

Table A.15. Frequency of resistance (ΔG210) and projected end of season surviving PPO-resistant (PPO-R) tall waterhemp in Farmland, IN in 2017.

Herbicide	Rate[a]	FOR[c]	Rr	RR	Surviving PPO-R[b]
	g ai ha^{-1}	----------%----------			#
fomesafen					
	0	13 A	80 A	20 A	3 A
	66	10 A	84 A	16 A	4 A
	132	15 A	95 A	5 A	3 A
	264	17 A	83 A	17 A	4 A
s-metolachlor					
	0	15 a	93 a	7 a	6 a
	335	12 a	84 a	16 a	4 a
	710	18 a	86 a	14 a	2 a
	1420	9 a	80 a	20 a	2 a

[a] No observed significant interaction of the two main factors, fomesafen and s-metolachlor; therefore, data was analyzed separately for each factor with rate pooled over the second factor. Percentage of RS and RR tall waterhemp are calculated from the total percentage of PPO-R tall waterhemp. Data represents trials conducted in Lafayette in 2016 and 2017. Farmland data in 2017 is excluded due to a significant herbicide treatment by site interaction in the ANOVA. Data subjected to an arcsine transformation. Means followed by the same letter within a column are not significantly different according to Tukey's honest significant difference (HSD) test ($P \leq 0.05$).

[b] Surviving PPO-R calculated by multiplying FOR by density recorded 56 DAT. Collection timings were pooled together for FOR.

[c] Abbreviations: FOR, frequency of resistance; Rr, heterozygous PPO-R tall waterhemp; RR, homozygous PPO-R tall waterhemp; PPO, protoporphyrinogen oxidase.

Table A.16. Contrasts of tall waterhemp control and density in Farmland, IN in 2017.

		Control	Density	
Contrast[a,b]	Rate	28 DAT	28 DAT	56 DAT
	g ai ha^{-1}	----------%----------		
fom vs safl	66 vs 25	90 vs 96	47 vs 34	78 vs 74
fom vs sulf	264 vs 280	97 vs 98	17 vs 0	54 vs 5
fom vs fom + smeto	66 vs 66 + 1420	**90 vs 98***	47 vs 6	78 vs 66
safl vs safl + smeto	25 vs 25 + 1420	96 vs 98	34 vs 9	74 vs 49
fom vs fom + smeto	264 vs 264 + 1420	97 vs 98	17 vs 0	54 vs 9
sulf vs sulf + smeto	280 vs 280 + 1420	98 vs 99	0 vs 2	5 vs 5

[a] Bolded contrasts with an asterisk(s) denote significance at P ≤ 0.05 (*), ≤0.01 (**), ≤0.001 (***). Bonferroni adjustment. Data represents trials conducted in Lafayette in 2016 and 2017. Data subjected to an arcsine transformation. Control and density represent percent of the nontreated control.

[b] Abbreviations: fom, fomesafen; safl, saflufenacil; sulf, sulfentrazone; smeto, *s*-metolachlor; DAT, days after treatment.

Table A.17. Contrasts of frequency of resistance (ΔG210) and projected number of surviving tall waterhemp resistant to PPO-inhibiting herbicides in Farmland, IN in 2017.

Contrast[a,d]	Rate	FOG[c]	Rr	RR	Surviving PPO-R
	g ai ha^{-1}	----------%----------			#
fom vs safl	66 vs 25	8 vs 21	100 vs 96	0 vs 4	7 vs 10
fom vs sulf	264 vs 280	19 vs 15	94 vs 100	6 vs 0	6 vs 0.3
fom vs fom + smeto	66 vs 66 + 1420	8 vs 18	100 vs 79	0 vs 21	7 vs 7
safl vs safl + smeto	25 vs 25 + 1420	21 vs 12	96 vs 100	4 vs 0	10 vs 4
fome vs fom + smeto	264 vs 264 + 1420	19 vs 14	94 vs 97	6 vs 3	6 vs 0.5
sulf vs sulf + smeto	280 vs 280 + 1420	15 vs 15	100 vs 94	0 vs 6	0.3 vs 0.3

[a] Bolded contrasts with an asterisk(s) denote significance at P ≤ 0.05 (*), ≤0.01 (**), ≤0.001 (***). Bonferroni adjustment. Data represents trials conducted in Lafayette in 2016 and 2017. FOG subjected to a square root transformation. Rr, RR, and SRTW subjected to an arcsine transformation.

[b] Percentage of Rr and RR tall waterhemp are calculated from the total percentage of PPO-R tall waterhemp.

[c] Surviving PPO-R calculated by multiplying FOG by density recorded 56 DAT. Collection timings were pooled together for FOG.

[d] Abbreviations: fom, fomesafen; safl, saflufenacil; sulf, sulfentrazone; smeto, *s*-metolachlor; FOR, frequency of resistance; PPO-R, PPO-resistant; Rr, heterozygous PPO-R tall waterhemp; RR, homozygous PPO-R tall waterhemp; PPO, protoporphyrinogen oxidase.

Table A.18. Dates of tall waterhemp collections and frequency of resistance (ΔG210) at Lafayette in 2016.

Herbicide	Rate	Total reps	Collection 1			Collection 2			Collection 1 + 2
	g ai ha^{-1}		Total Plants Sampled	DAT[a] Collected	FOR[b]	Total Plants Sampled	DAT[c] Collected	FOR	FOR
nontreated		4	99	27-37	0.02	98	62-70	0.02	0.02
fomesafen	66	4	99	27-37	0.04	100	62	0.04	0.04
	132	4	99	27-37	0.11	99	62	0.05	0.08
	264	2	50	27-48	0.10	42	62-70	0.15	0.12
fomesafen + *s*-metolachlor	66 + 335	4	99	27-37	0.05	97	62	0.01	0.03
	66 + 710	4	99	27-41	0.05	100	62	0.06	0.06
	66 + 1420	3	75	35-48	0.04	71	62	0.02	0.03
	132 + 335	4	82	27-48	0.10	90	62	0.06	0.08
	132 + 710	4	97	27-48	0.17	97	62	0.17	0.17
	132 + 1420	4	97	27-48	0.06	99	62	0.09	0.08
	264 + 335	4	51	37-48	0.08	81	62-70	0.04	0.05
	264 + 710	4	34	37-48	0.11	107	62-70	0.09	0.09
	264 + 1420	4	11	48	0.19	70	62-70	0.14	0.17
s-metolachlor	335	4	100	27-37	0.02	99	62	0.03	0.03
	710	4	100	27-37	0.05	100	62	0.06	0.06
	1420	4	97	27-41	0.03	98	62	0.03	0.03
sulfentrazone	280	4	26	37-48	0.27	119	62-70	0.23	0.23
sulfentrazone + *s*-metolachlor	280 + 1420	4	23	48	0.22	101	62-70	0.11	0.12
saflufenacil	25	4	100	27-37	0.12	98	62	0.08	0.10
saflufenacil + *s*-metolachlor	25 + 1420	4	100	37-48	0.11	98	62	0.12	0.12

[a] Represents the range in days of when tall waterhemp were collected.

[b] Frequency of resistance calculated by dividing the sum of heterozygous and homozygous resistant tall waterhemp by the total number of tall waterhemp sampled per treatment. Value equals average FOR per replication.

[c] Abbreviations: DAT, days after treatment; FOR, frequency of resistance; reps, replications.

Table A.19. Dates of tall waterhemp collections and frequency of resistance (ΔG210) at Lafayette in 2017.

Herbicide	Rate	Total reps	Collection 1			Collection 2			Collection 1 + 2
	g ai ha^{-1}		Total Plants Sampled	DAT[a] Collected	FOR[b]	Total Plants Sampled	DAT[c] Collected	FOR	FOR
nontreated		4	95	27	0.12	96	81	0.10	0.12
fomesafen	66	4	95	27	0.22	93	81	0.18	0.20
	132	4	96	27	0.17	97	81	0.12	0.14
	264	4	93	27	0.15	74	81	0.15	0.15
fomesafen + *s*-metolachlor	66 + 335	4	96	27	0.12	96	81	0.13	0.12
	66 + 710	4	97	27	0.18	92	81	0.14	0.16
	66 + 1420	4	85	27-44	0.18	90	81	0.23	0.21
	132 + 335	4	99	27	0.15	83	81	0.19	0.17
	132 + 710	4	94	27-44	0.11	85	81	0.11	0.11
	132 + 1420	4	92	27-44	0.04	96	81	0.14	0.09
	264 + 335	4	98	27	0.14	70	81	0.07	0.11
	264 + 710	4	95	27-44	0.13	72	81	0.10	0.14
	264 + 1420	4	86	27-44	0.17	74	81	0.26	0.20
s-metolachlor	335	4	92	27	0.15	98	81	0.13	0.14
	710	4	97	27	0.12	90	81	0.10	0.11
	1420	4	90	27	0.08	101	81	0.14	0.11
sulfentrazone	280	4	93	27-44	0.26	63	81	0.21	0.23
sulfentrazone + *s*-metolachlor	280 + 1420	4	74	27-44	0.27	93	81	0.23	0.25
saflufenacil	25	4	97	27	0.16	85	81	0.22	0.19
saflufenacil + *s*-metolachlor	25 + 1420	4	92	27-44	0.19	90	81	0.10	0.15

[a] Represents the range in days of when tall waterhemp were collected.

[b] Frequency of resistance calculated by dividing the sum of heterozygous and homozygous resistant tall waterhemp by the total number of tall waterhemp sampled per treatment. Value equals average FOR per replication.

[c] Abbreviations: DAT, days after treatment; FOR, frequency of resistance; reps, replications.

Table A.20. Dates of tall waterhemp collections and frequency of resistance (ΔG210) at Farmland in 2017.

Herbicide	Rate	Total reps	Collection 1		
	g ai ha^{-1}		Total Plants Sampled	DAT[a,c] Collected	FOR[b]
nontreated		4	83	44	0.14
fomesafen	66	4	94	44	0.08
	132	4	98	44-56	0.19
	264	4	95	44-56	0.19
fomesafen + *s*-metolachlor	66 + 335	4	92	44	0.08
	66 + 710	4	88	44-56	0.10
	66 + 1420	4	89	44-56	0.18
	132 + 335	4	94	44-56	0.12
	132 + 710	4	80	44-56	0.14
	132 + 1420	4	93	44-56	0.15
	264 + 335	4	68	44-56	0.17
	264 + 710	4	85	44-56	0.26
	264 + 1420	4	95	44-56	0.14
s-metolachlor	335	4	93	44-56	0.07
	710	4	96	44	0.04
	1420	4	87	44-56	0.12
sulfentrazone	280	4	84	44-56	0.15
sulfentrazone + *s*-metolachlor	280 + 1420	4	78	44-56	0.15
saflufenacil	25	4	98	44	0.21
saflufenacil + *s*-metolachlor	25 + 1420	4	73	44-56	0.12

[a] Represents the range in days of when tall waterhemp were collected.

[b] Frequency of resistance calculated by dividing the sum of heterozygous and homozygous resistant tall waterhemp by the total number of tall waterhemp sampled per treatment. Value equals average FOG per replication.

[c] Abbreviations: DAT, days after treatment; FOR, frequency of resistance; reps, replications.

Figure A.1. Modified CTAB protocol using centrifuge tubes.

Plant Tissue Sampling

1. Collect plant tissue from the youngest leaf possible, near the apical or axillary meristems.
2. Use forceps or scissors to cut a piece of leaf half the size of a dime and place in a 2.0 ml centrifuge tube. This is the amount of tissue required for DNA extraction.

DNA Extraction

1. Place 5 glass beads per 2.0 ml centrifuge tube and add 600 ul CTAB.
2. Grind samples in centrifuge tubes for 4 min using a beadbeater.
3. Place centrifuge tubes in 65°C hot water bath for 20 to 40 min.
4. Add 600 ul chloroform to each centrifuge tube, mix by shaking, and centrifuge at 13,000 RPM for 10 min.
5. Add 350 ul isopropyl alcohol to a new 1.5 ml centrifuge tube. Transfer upperphase of each 2.0 ml centrifuge tube to the new 1.5 ml centrifuge tube. Mix by shaking and keep in -20°C freezer for 20 min.
6. Remove centrifuge tubes from freezer and centrifuge at 13,000 RPM for 10 min.
7. Decant the liquid, then wash DNA pellet by adding 1 ml of 75% EtOH and mix by shaking.
8. Dry DNA pellet by decanting the EtOH and using a 100 ul pipette to remove any excess EtOH in the centrifuge tube. Be careful when decanting as the DNA pellet can easily slide out of the tube or get stuck in the lid of the centrifuge tube.
9. Leave centrifuge tubes under hood overnight to dry with the caps open.
10. Dissolve the DNA pellet by adding 100 ul of ddH_2O to each centrifuge tube and keep in -20°C freezer until ready to use.

APPENDIX B. CHAPTER 3 SUPPLEMENTARY DATA

Table B.1. State, county, GPS coordinates, and ΔG210 mutation presence of surveyed tall waterhemp populations from three discriminating fomesafen rates in a greenhouse.

State[c]	County (population ID)	GPS coordinates	ΔG210 mutation presence[a]	Total plants assayed[b]
				No.
IA	Adair (IA-120)	41.46191, -94.45240	no	18
IA	Cass (IA-136)	41.47580, -95.06046	no	16
IA	Chickasaw (IA-142)	42.91418, -92.14091	yes	16
IA	Clarke (IA-143)	41.03020, -93.60565	yes	16
IA	Crawford (IA-147)	42.06777, -95.18628	no	18
IA	Dallas (IA-149)	41.68330, -94.02320	yes	16
IA	Fayette (IA-152)	42.66942, -91.80488	yes	26
IA	Greene (IA-157)	42.03446, -94.32979	no	11
IA	Hardin (IA-167)	42.50201, -93.38091	yes	15
IA	Monona (IA-191)	42.06025, -95.71059	no	13
IA	Pottawattamie (IA-207)	41.27985, -95.79265	no	13
IA	Jefferson (IA-268)	40.9641405, -91.961655	yes	16
IA	Wapello (IA-277)	41.004567, -92.289074	no	18
IA	Davis (IA-279)	40.741558, -92.457611	yes	36
IA	Madison (IA-284)	41.382237, -93.968134	no	11
IA	Warren (IA-288)	41.37102, -93.426054	yes	25
IA	Scott (IA-291)	41.517147, -90.715529	yes	36
IA	Washington (IA-293)	41.32198, -91.717324	yes	7
IA	Adair (IA-308)	41.49207, -94.29404	no	16
IA	Crawford (IA-309)	42.03572, -095.42629	yes	15
IA	Ringgold (IA-310)	40.711107, -94.356907	yes	31
IA	Audubon (IA-315)	41.55040, -095.00926	yes	16
IA	Pottawattamie (IA-316)	41.41.123, -95.41.026	no	14
IA	Crawford (IA-319)	42.04.492, -095.11.293	no	15
IA	Wapello (IA-322)	41.013224, -92.486456	no	18
IA	Fremont (IA-325)	40.736642, -95.629661	no	8
IA	Montgomery (IA-326)	40.994768, -95.109785	yes	12
IA	Bremer (IA-332	42.833000, -92.356233	yes	21
IA	Tama (IA-338)	42.09.498, -92.54.229	yes	23
IA	Cerro Gordo (IA-340)	43.17.122, -93.20.368	no	21
IA	Winneshiek (IA-347)	43.211434, -91.904431	yes	11
IA	Wayne (IA-358)	40.754844, -93.286675	yes	21
IA	Page (IA-364)	40.742825, -95.384242	yes	22
IA	Floyd (IA-368)	43.17.021, -92.59.077	yes	20
IA	Chickasaw (IA-369)	43.04.682, -92.38.010	yes	32
IA	Van Buren (IA-378)	40.740164, -91.975201	yes	18
IL	Randolph (IL-BDW)	-	yes	43

IL	Bond (IL-BON)	38.838015, -89.575211	yes	30
IL	Brown (IL-BRO1)	40.0894086, -90.8063438	yes	35
IL	Brown (IL-BRO2)	39.958231, -90.792862	yes	39
IL	Champaign (IL-CHAM)	40.02439, -88.104442	yes	12
IL	Clinton (IL-CLT1)	38.542013, -89.232057	yes	34
IL	Clinton (IL-CLT2)	38.724448, -89.463295	yes	30
IL	Clinton (IL-CLT3)	38.534966, -89.666236	yes	20
IL	Greene (IL-GRE1)	39.262422, -90.447107	yes	26
IL	Greene (IL-GRE2)	39.411406, -90.464981	yes	30
IL	Iroquois (IL-IRO1)	40.873534, -87.9815777	yes	12
IL	Iroquois (IL-IRO2)	40.925594, -87.784689	yes	25
IL	Jasper (IL-JAS)	38.878349, -88.06147	yes	33
IL	Jefferson (IL-JEF)	38.432481, -89.144108	yes	29
IL	Knox (IL-KNX1)	40.968526, -90.02476	yes	32
IL	Knox (IL-KNX2)	40.914839, -90.227174	yes	20
IL	Madison (IL-MAD)	38.75562, -89.664156	yes	24
IL	Marion (IL-MAR)	38.519513, -88.997927	yes	32
IL	McDonough (IL-MCD)	40.512656, -90.799845	yes	20
IL	Morgan (IL-MOR)	39.593745, -90.024613	yes	12
IL	Peoria (IL-PEO)	40.782961, -89.898621	yes	27
IL	Pike (IL-PIKE)	39.508528, -90.65704	yes	29
IL	Randolph (IL-RAN)	38.089926, -89.783632	yes	36
IL	Sangamon (IL-SANG1)	39.873573, -89.310282	yes	23
IL	Sangamon (IL-SANG2)	39.816556, -89.449075	yes	28
IL	Sangamon (IL-SANG3)	39.584327, -89.814509	yes	23
IL	Scott (IL-SCT)	39.52856, -90.520856	yes	35
IL	Vermillion (IL-VER)	40.1523894, -87.9027141	yes	32
IL	Warren (IL-WAR)	40.877142, -90.6606	yes	39
IL	Washington (IL-WAS)	38.432254, -89.628843	yes	38
IL	White (IL-WHT)	38.250583, -88.149406	yes	39
IN	Boone (IN-BNE1)	40.078718, -86.30595	yes	23
IN	Boone (IN-BNE2)	40.12766, -86.470528	no	8
IN	Daviess (IN-DAV)	38.726778, -87.008947	yes	30
IN	Delaware (IN-DEL1)	40.282138, -85.236919	no	11
IN	Delaware (IN-DEL2)	40.139059, -85.234616	yes	10
IN	Delaware (IN-DEL3)	40.200731, -85.253519	no	7
IN	Delaware (IN-DEL4)	40.246496, -85.267301	yes	11
IN	Dubois (IN-DUB)	38.49709, -86.980942	yes	32
IN	Gibson (IN-GIB1)	38.392559, -87.497924	no	14
IN	Gibson (IN-GIB2)	38.347142, -87.353813	yes	34
IN	Gibson (IN-GIB3)	38.327556, -87.419757	yes	24
IN	Gibson (IN-GIB4)	38.475899, -87.567134	yes	10
IN	Jay (IN-JAY)	40.436486, -85.021052	yes	31
IN	Knox (IN-KNX)	38.537486, -87.492467	yes	22
IN	Madison (IN-MAD)	39.992534, -85.713543	yes	9
IN	Martin (IN-MAR)	38.645861, -86.885278	yes	30

IN	Newton (IN-NWT)	40.7513889, -87.469722	yes	17
IN	Pike (IN-PIKE1)	38.435626, -87.133492	yes	32
IN	Pike (IN-PIKE2)	38.46436, -87.26557	yes	25
IN	Pulaski (IN-PUL)	41.126111, -86.756666	yes	13
IN	Randolph (IN-RAN1)	40.227965, -84.928576	no	9
IN	Randolph (IN-RAN2)	40.235501, -84.862482	no	9
IN	Randolph (IN-RAN3)	40.219851, -85.193507	yes	34
IN	Spencer (IN-SPEN)	37.990722, -87.165278	yes	36
IN	Starke (IN-STAR)	41.220278, -86.688611	yes	11
IN	Vigo (IN-VIGO)	39.503067, -87.4339	no	11
IN	Warrick (IN-WAR1)	37.976, -87.369	yes	28
IN	Warrick (IN-WAR2)	38.092184, -87.31348	yes	37
IN	Warrick (IN-WAR3)	38.043074, -87.464632	yes	23
IN	Warrick (IN-WAR4)	38.004248, -87.416078	yes	31
IN	White (IN-WHTE)	40.7138889, -86.813888	no	12
MN	Sibley (MN-388)	44.672281, -94.110130	no	21
MN	Cottonwood (MN-389)	43.947794, -94.876541	yes	19
MN	Sibley (MN-390)	44.600054, -94.505978	yes	20
MN	Sibley (MN-391)	44.526958, -94.064502	yes	18
MN	Wabasha (MN-392)	44.144276, -92.110447	yes	30
MN	Fairbault (MN-393)	43.703922, -93.810803	yes	34
MN	Murray (MN-394)	43.856774, -96.043206	yes	23
MN	Cottonwood (MN-395)	43.862492, -94.876541	yes	31
MN	Waseca (MN-396)	44.026473, -93.523443	yes	22
MN	Blue Earth (MN-397)	43.977346, -93.989847	yes	31
MN	Swift (MN-400)	45.25371, -95.87197	yes	29
MN	Stevens (MN-401)	45.64653, -96.19244	yes	23
MN	Douglas (MN-404)	45.92306, -95.60369	yes	14
MN	Traverse (MN-405)	45.97141, -96.55617	yes	25
MN	Big Stone (MN-406)	45.45428, -96.39049	yes	24
MO	Monroe (MO-1)	39.565256, -92.045217	yes	29
MO	Randolph (MO-2)	39.51725, -92.10419	yes	27
MO	Carroll (MO-3)	39.466108, -92.139435	yes	27
MO	Boone (MO-4)	38.859156, -93.249793	yes	29
MO	Lafayette (MO-5)	39.422387, -92.665526	yes	35
MO	Callaway (MO-6)	39.086504, -93.20287	yes	29
MO	Montgomery (MO-7)	39.085236, -92.042029	yes	27
MO	Randolph (MO-9)	38.761576, -93.418081	yes	35
MO	Monroe (MO-10)	39.4013436, -91.8542871	yes	35
MO	Monroe (MO-12)	38.996953, -93.593918	yes	26
MO	Pettis (MO-16)	38.8975404, -92.4648105	yes	20
MO	Saline (MO-20)	39.749962, -92.138328	yes	32
MO	Audrain (MO-21)	39.571275, -93.440469	yes	27
MO	Pettis (MO-22)	39.077694, -91.576638	yes	31
MO	Monroe (MO-23)	39.175351, -92.283134	yes	34
MO	Lafayette (MO-25)	38.889542, -93.21513	yes	25

MO	Boone (MO-27)	39.519075, -92.755828	yes	24
MO	Shelby (MO-28)	38.704166, -91.614379	yes	29
MO	Montgomery (MO-39)	39.075688, -93.816818	yes	36
MO	Pettis (MO-43)	39.048557, -91.547891	yes	33
MO	Chariton (MO-45)	39.496647, -92.356569	yes	10
MO	Montgomery (MO-46)	39.012111, -94.077887	yes	24
MO	Lafayette (MO-47)	39.488783, -92.440819	yes	17
MO	Montgomery (MO-48)	38.99185, -93.684145	yes	19
MO	Randolph (MO-49)	39.069035, -91.935659	yes	32
MO	Randolph (MO-50)	38.817242, -93.294266	yes	30
MO	Lafayette (MO-53)	39.227817, -93.522571	yes	40
MO	Audrain (MO-55)	38.754443, -92.415291	yes	33
MO	Pettis (MO-56)	38.704012, -91.805789	yes	31
MO	Carroll (MO-58)	39.854945, -93.115613	yes	22
MO	Moniteau (MO-59)	38.779274, -92.496542	yes	42
MO	Linn (MO-61)	39.09656, -91.627843	yes	22
MO	Moniteau (MO-66)	39.539335, -92.44824	yes	31
MO	Randolph (MO-74)	39.607532, -92.466388	yes	17
MO	Macon (MO-75)	39.436417, -92.691834	yes	27

[a] Counties were labeled yes if at least one tall waterhemp survivor possessed the ΔG210 mutation.

[b] n = 48

[c] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; ΔG210, glycine deletion at position 210 of *PPX2*.

Table B.2. Control, frequency of resistance (ΔG210), and survivorship of tall waterhemp 14 days after treatment from three discriminating fomesafen rates in a greenhouse.

Tall waterhemp population[a,d]	Fomesafen rate (g ai ha^{-1})								
	13			52			416		
	Control[b]	FOR	Survival[c]	Control	FOR	Survival	Control	FOR	Survival
	%		No.	%		No.	%		No.
IA-Known PPO-R	37	63	16 (32)	84	66	15 (28)	98	88	5 (9)
IA-Known PPO-S	92	0	12 (21)	99	0	2 (3)	99	-	0 (0)
IA-120	90**	0	16 (13)	98**	0	5** (4)	99**	0	1 (1)
IA-136	86**	0	15 (14)	99**	0	5** (2)	99**	-	0** (0)
IA-142	91**	8	12 (12)	98**	25	4** (4)	99**	-	0** (0)
IA-143	86**	15	14 (13)	98**	67	4** (3)	99**	-	0** (0)
IA-147	80**	0	16 (16)	99**	0	3** (2)	99**	-	1 (0)
IA-149	84**	8	13 (13)	99**	33	5** (3)	99**	-	0** (0)
IA-152	**76*** **	**13**	**16 (16)**	88*	63	11* (8)	99**	100	2 (2)
IA-157	96**	0	10** (8)	99**	0	3** (3)	99**	-	0** (0)
IA-167	91**	0	14 (12)	99**	0	2** (2)	99**	-	0** (0)
IA-191	85**	0	13 (13)	99**	-	0** (0)	99**	-	0** (0)
IA-207	94**	0	9** (8)	98**	0	4** (4)	99**	-	0** (0)
IA-268	80**	0	12 (11)	96**	25	7** (4)	99**	100	1 (1)
IA-277	89**	0	15 (14)	98**	0	4** (4)	99**	-	0** (0)
IA-279	68***	60	15 (15)	89*	92	13* (13)	98*	100	6* (6)
IA-284	86**	0	13 (10)	99**	0	2** (1)	99**	-	0** (0)
IA-288	82**	13	16 (16)	96**	57	8** (7)	99***	100	2 (2)
IA-291	49*	87	16 (15)	93***	100	13* (11)	98*	100	10* (8)
IA-293	**79*** **	**17**	**16 (6)**	99**	0	5** (1)	99**	-	2 (0)
IA-308	91**	0	14 (13)	98**	0	3** (3)	99**	-	1 (0)
IA-309	87**	9	13 (11)	98**	67	3** (3)	99**	100	2 (1)
IA-310	64***	53	16 (15)	92***	77	13* (13)	99***	100	4 (3)
IA-315	**73*** **	**25**	**14 (12)**	97**	50	4** (4)	99**	-	0** (0)

IA-316	84**	0	14 (13)	99**	0	1** (1)	99**	-	0** (0)
IA-319	84**	0	13 (13)	98**	0	2** (2)	99**	-	0** (0)
IA-322	84**	0	15 (15)	98**	0	3** (3)	99**	-	0** (0)
IA-325	96**	0	10** (8)	99**	-	0** (0)	99**	-	0** (0)
IA-326	87**	13	13 (8)	95**	75	5** (4)	99**	-	0** (0)
IA-332	**78*** **	**7**	**15 (14)**	96**	17	6** (6)	99**	0	2 (1)
IA-338	80**	7	14 (14)	91***	86	10* (7)	99**	100	2 (1)
IA-340	**76*** **	**0**	**16 (16)**	97**	0	5** (5)	99**	-	0** (0)
IA-347	92**	10	10** (10)	99**	0	1** (1)	99**	-	0** (0)
IA-358	**71*** **	**15**	**14 (13)**	93***	38	8** (8)	99**	-	0** (0)
IA-364	67***	43	15 (14)	96**	57	9*** (7)	99**	100	2 (1)
IA-368	90**	20	14 (15)	98**	100	5** (3)	99**	100	2 (2)
IA-369	**68*** **	**0**	**16 (16)**	**90*** **	**0**	**15* (13)**	99**	33	3 (3)
IA-378	80**	17	13 (12)	97**	75	5** (4)	99**	100	3 (2)
IL-Known PPO-R	30	60	16 (30)	85	81	14 (27)	98	60	10 (13)
IL-Known PPO-S	92	0	14 (22)	98	0	3 (4)	99	-	0 (0)
IL-BDW	43*	100	16 (15)	**57*** **	**94**	**16*** (16)	96*	92	15* (12)
IL-BON	47***	80	15 (15)	90	100	12* (10)	98	100	7* (5)
IL-BRO1	31*	94	16 (16)	**61*** **	**100**	**16*** (16)	97	100	7* (3)
IL-BRO2	47***	100	16 (16)	**65*** **	**100**	**15*** (15)	97	100	5* (5)
IL-CHAM	93**	0	14 (10)	98**	100	3** (2)	99	-	0** (0)
IL-CLT1	53***	77	16 (13)	74*	87	16* (15)	**94*** **	**100**	**9*** (6)
IL-CLT2	52***	69	15 (13)	86*	58	13* (12)	**95*** **	**100**	**6*** (5)
IL-CLT3	**56*** **	**29**	**15** (14)	90	83	8 (6)	99	-	1** (0)
IL-GRE1	56***	42	16 (12)	83*	82	12* (11)	98	100	5* (2)
IL-GRE2	42*	87	16 (15)	80*	100	12* (12)	98	100	5* (3)
IL-IRO1	84**	18	13 (11)	97	100	5** (1)	99	-	0** (0)
IL-IRO2	73***	36	16 (14)	92	78	11* (9)	98	100	2** (2)
IL-JAS	47***	92	15 (13)	**72*** **	**100**	**14*** (14)	98	100	9* (6)
IL-JEF	37*	62	16 (13)	76*	91	14* (11)	97*	100	10* (5)
IL-KNX1	61***	81	16 (16)	85*	93	14* (14)	99	100	3** (2)
IL-KNX2	67***	46	13 (13)	96	50	6** (4)	98	100	4 (3)
IL-MAD	54***	67	16 (15)	89	75	8 (8)	99	-	5* (0)

IL-MAR	24*	86	16 (14)	**63*** **	**92**	**15*** (13)	97	100	10* (5)
IL-MCD	80**	27	13 (11)	91	67	6** (6)	99	100	4 (3)
IL-MOR	84**	30	15 (10)	98	0	5** (1)	99	100	1** (1)
IL-PEO	66***	36	14 (14)	**82***	**27**	**10*** (10)	98	100	3** (2)
IL-PIKE	58***	50	16 (14)	86*	75	13* (12)	99	100	6* (1)
IL-RAN	27*	88	16 (16)	**71*** **	**86**	**15*** (14)	98	100	9* (6)
IL-SANG1	67***	56	15 (9)	85*	78	12* (9)	98	100	6* (5)
IL-SANG2	43*	100	15 (12)	**73*** **	**100**	**16*** (11)	98	100	10* (5)
IL-SANG3	70***	69	15 (13)	95	100	10* (9)	99	100	3** (1)
IL-SCT	48***	100	16 (15)	**69*** **	**100**	**16*** (14)	97	100	11* (6)
IL-VER	35*	93	16 (15)	79*	100	13* (11)	97	100	8* (6)
IL-WAR	33*	100	16 (16)	76*	100	16* (16)	96*	100	7* (7)
IL-WAS	26*	92	16 (13)	**66*** **	**87**	**16*** (15)	96*	100	11* (10)
IL-WHT	38*	100	15 (14)	**61*** **	**100**	**16*** (16)	97	100	8* (8)
IN-Known PPO-R	38	73	16 (29)	62	67	15 (28)	98	67	4 (5)
IN-Known PPO-S	86	0	10 (17)	99	0	4 (4)	99	-	0 (0)
IN-BNE1	77**	30	12 (10)	78***	100	11* (9)	98	100	4 (4)
IN-BNE2	88**	0	10** (8)	99**	-	1** (0)	99	-	0 (0)
IN-DAV	47*	85	15 (13)	65*	100	14* (14)	97*	100	4 (3)
IN-DEL1	90**	0	13 (11)	99**	-	0** (0)	99	-	0 (0)
IN-DEL2	93**	25	8** (8)	98**	50	2** (2)	99	-	0 (0)
IN-DEL3	93**	0	7** (7)	99**	-	0** (0)	99	-	0 (0)
IN-DEL4	60***	71	14 (7)	85***	100	9** (4)	99	-	3 (0)
IN-DUB	25*	100	15 (13)	**48*** **	**100**	**15*** (13)	**95*** **	**100**	**7*** (5)
IN-GIB1	79**	0	12 (12)	98**	0	3** (2)	99	-	1 (0)
IN-GIB2	49*	87	16* (15)	68*	93	16* (15)	98	100	3 (3)
IN-GIB3	63***	92	13 (13)	82***	100	9** (9)	99	100	5* (2)
IN-GIB4	85**	0	10** (6)	97**	0	6** (3)	99	-	0 (0)
IN-JAY	45*	93	14 (14)	73*	100	12* (12)	98	100	5* (4)
IN-KNX	59***	62	13 (13)	81***	100	9** (7)	99	100	1 (1)
IN-MAD	91**	20	7** (5)	96**	75	5** (4)	99	-	1 (0)
IN-MAR	60***	92	13 (12)	76***	100	12* (12)	97	100	7* (6)
IN-NWT	67***	36	13 (11)	88**	67	7** (6)	99	-	0 (0)

IN-PIKE1	23*	100	16* (10)	**41*** **	**100**	**15*** (14)	97	100	8* (8)
IN-PIKE2	45*	90	13 (10)	88**	100	11* (11)	97*	100	3 (4)
IN-PUL	84**	11	9** (9)	96**	75	4** (4)	99	-	0 (0)
IN-RAN1	92**	0	9** (8)	99**	0	1** (1)	99	-	0 (0)
IN-RAN2	87**	0	8** (7)	98**	0	3** (2)	99	-	0 (0)
IN-RAN3	35*	100	16* (15)	49*	100	15* (15)	98	100	4 (4)
IN-SPEN	24*	88	16* (16)	**40*** **	**94**	**16*** (16)	98	100	4 (4)
IN-STAR	91**	13	10** (8)	98**	67	2** (2)	99	-	0 (0)
IN-VIGO	90**	0	8** (8)	98**	0	3** (3)	99	-	0 (0)
IN-WAR1	38*	83	16* (12)	58*	100	16* (10)	98	100	6* (5)
IN-WAR2	30*	88	16* (16)	51*	100	15* (13)	97	100	7* (5)
IN-WAR3	71***	50	15 (14)	90**	100	7** (7)	99	100	4 (2)
IN-WAR4	56***	64	15 (14)	70*	77	13* (13)	98	100	7* (4)
IN-WHTE	94**	0	11 (10)	99**	0	2** (2)	99	-	0 (0)
MN-Known PPO-R	32	64	16 (31)	68	77	15 (29)	97	75	6 (9)
MN-Known PPO-S	80	0	14 (23)	98	0	3 (3)	99	-	0 (0)
MN-388	83**	0	16 (16)	98**	0	5** (5)	99	-	0** (0)
MN-389	75**	50	12 (10)	90**	75	9** (8)	97*	100	1 (1)
MN-390	78**	50	14 (14)	94**	83	7** (6)	99	-	0** (0)
MN-391	76**	25	14 (12)	95**	25	6** (4)	99	100	3 (2)
MN-392	64***	67	15 (12)	79***	85	15* (13)	98	100	5* (5)
MN-393	62***	79	14 (14)	73*	92	13* (13)	96*	100	7* (7)
MN-394	76**	25	16 (16)	95**	67	6** (6)	99	100	4 (1)
MN-395	49***	100	16 (15)	76*	100	13* (12)	98	100	6* (4)
MN-396	67**	43	16 (14)	94**	50	8** (8)	99	-	0** (0)
MN-397	44*	87	16 (15)	80***	77	14* (13)	99	100	4 (3)
MN-400	72**	19	16 (16)	91**	30	11* (10)	99	100	3 (3)
MN-401	71**	56	16 (16)	87***	100	8** (7)	99	-	1 (0)
MN-404	89**	9	13 (11)	99**	33	4** (3)	99	-	0** (0)
MN-405	64***	60	15 (15)	95**	75	8** (8)	99	100	3 (2)
MN-406	74**	33	15 (15)	89**	67	8** (6)	99	100	3 (3)
MO-Known PPO-R	40	69	16 (32)	64	66	16 (32)	98	78	8 (11)
MO-Known PPO-S	89	0	14 (23)	99	50	2 (3)	99	-	0 (0)

MO-1	62***	80	16 (15)	91**	92	12* (12)	99	100	3 (2)
MO-2	58***	100	16 (12)	86**	100	13* (12)	99	100	3 (2)
MO-3	58***	40	16 (15)	83***	89	11* (9)	99	100	5* (3)
MO-4	64***	57	15 (14)	86***	63	11* (8)	98	71	8* (7)
MO-5	36*	86	16 (14)	72*	100	15* (14)	97*	100	11* (7)
MO-6	60***	64	16 (14)	83***	90	15* (10)	97*	100	11* (5)
MO-7	74**	62	14 (13)	92**	100	13* (11)	99	100	4 (3)
MO-9	49*	79	16 (14)	73*	100	16* (15)	**96*** **	**100**	**7*** (6)
MO-10	53*	100	16 (14)	78***	91	15* (11)	**97*** **	**100**	**12*** (9)
MO-12	74**	85	14 (13)	92**	100	10*** (10)	99	100	4 (3)
MO-16	74**	69	14 (13)	98**	80	7** (5)	99	100	2 (1)
MO-20	46*	80	16 (15)	83***	100	14* (14)	99	100	4 (2)
MO-21	60***	73	16 (15)	87**	100	9*** (9)	99	100	4 (3)
MO-22	33*	90	16 (10)	72*	100	15* (13)	**96*** **	**100**	**10*** (8)
MO-23	44*	100	14 (14)	81***	87	16* (15)	97*	100	6* (4)
MO-25	84**	75	14 (12)	89**	90	11* (10)	99	100	4 (3)
MO-27	53*	92	16 (13)	86***	100	13* (7)	98	100	10* (4)
MO-28	43*	83	16 (12)	69*	93	14* (14)	98	100	6* (3)
MO-39	33*	100	16 (15)	70*	100	14* (13)	98	100	8* (8)
MO-43	31*	94	16 (16)	82***	83	13* (12)	98	100	6* (5)
MO-45	**61*** **	**20**	**16 (5)**	89**	50	10*** (4)	98	100	3 (1)
MO-46	72***	75	14 (12)	87**	90	10*** (10)	99	50	3 (2)
MO-47	59***	42	15 (12)	96**	0	7** (4)	99	100	1** (1)
MO-48	54*	82	14 (11)	87**	67	9*** (6)	99	100	3 (2)
MO-49	53*	79	15 (14)	61*	92	15* (12)	98	100	7* (6)
MO-50	58***	93	16 (15)	94**	100	13* (13)	99	100	4 (2)
MO-53	56***	80	15 (15)	68*	87	16* (15)	**97*** **	**100**	**10*** (10)
MO-55	54*	92	16 (13)	70*	100	14* (13)	98	100	7* (7)
MO-56	57***	60	16 (15)	83***	82	14* (11)	98	100	6* (5)
MO-58	75**	25	16 (12)	89**	63	9*** (8)	99	-	2 (0)
MO-59	45*	94	16 (16)	79***	88	16* (16)	97*	100	12* (10)
MO-61	55*	64	14 (11)	85***	88	13* (8)	99	100	3 (2)
MO-66	61***	43	16 (14)	80***	73	13* (11)	98*	100	10* (5)

MO-74	69***	40	15 (15)	93**	50	5** (2)	99	-	1** (0)
MO-75	51*	93	16 (14)	83***	91	14* (11)	99	100	3 (2)

[a] Bolded tall waterhemp populations represent selected populations that fit all three criteria for exhibiting low, mid, or high level resistance.

[b] Control data analyzed using custom t-tests to compare unknown tall waterhemp populations versus known PPO-R and known PPO-S controls with significance followed according to the unadjusted P-value. Survival data analyzed using contingency tables with significance followed according to Fisher's Exact two-tail test. One asterisk (*) denotes significance compared to the known PPO-S control. Two asterisks (**) denote significance compared to the known PPO-R control. Three asterisks (***) denote significance compared to both known PPO-R and PPO-S controls. Alpha = 0.05. Data pooled over experimental runs.

[c] Survival number for surveyed tall waterhemp populations represent sum of total surviving plants from both experimental runs. Survival number for the known PPO-R and PPO-S controls represents the average survival number of both experimental runs. Number in parenthesis indicates number of samples subjected to qPCR for PPO-resistance quantification by ΔG210 for all tall waterhemp populations including the known controls.

[d] Abbreviations: PPO, protoporphyrinogen oxidase; PPO-R, PPO-resistant; PPO-S, PPO susceptible; FOR, frequency of resistance; ΔG210, glycine deletion at position 210 of *PPX2*; IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri.

Table B.3. Genotypic frequencies of tall waterhemp populations for the ΔG210 mutation from three discriminating rates of fomesafen in a greenhouse.

Tall waterhemp population[a]	Fomesafen rate (g ai ha^{-1})											
	13				52				416			
	RR	Rr	rr	Samples	RR	Rr	rr	Samples	RR	Rr	rr	Samples
	%			No.	%			No.	%			No.
IA-Known PPO-R	25	38	38	32	21	39	39	28	67	22	11	9
IA-Known PPO-S	0	0	100	21	0	0	100	3	-	-	-	0
IA-120	0	0	100	13	0	0	100	4	0	0	100	1
IA-136	0	0	100	14	0	0	100	2	-	-	-	0
IA-142	0	8	92	12	0	25	75	4	-	-	-	0
IA-143	0	15	85	13	0	67	33	3	-	-	-	0
IA-147	0	0	100	16	0	0	100	2	-	-	-	0
IA-149	0	8	92	13	0	33	67	3	-	-	-	0
IA-152	6	6	88	16	13	50	38	8	0	100	0	2
IA-157	0	0	100	8	0	0	100	3	-	-	-	0
IA-167	0	0	100	12	0	0	100	2	-	-	-	0
IA-191	0	0	100	13	-	-	-	0	-	-	-	0
IA-207	0	0	100	8	0	0	100	5	-	-	-	0
IA-268	0	0	100	11	0	25	75	4	0	100	0	1
IA-277	0	0	100	14	0	0	100	4	-	-	-	0
IA-279	0	60	40	15	15	77	8	13	67	33	0	6
IA-284	0	0	100	10	0	0	100	1	-	-	-	0
IA-288	0	13	88	16	29	29	43	7	0	100	0	2
IA-291	47	40	13	15	55	45	0	11	50	50	0	8
IA-293	17	0	83	6	0	0	100	1	-	-	-	0
IA-308	0	0	100	13	0	0	100	3	-	-	-	0
IA-309	0	9	91	11	0	67	33	3	0	100	0	1
IA-310	7	47	47	15	0	77	23	13	0	100	0	3
IA-315	0	25	75	12	0	50	50	4	-	-	-	0
IA-316	0	0	100	13	0	0	100	1	-	-	-	0
IA-319	0	0	100	13	0	0	100	2	-	-	-	0
IA-322	0	0	100	15	0	0	100	3	-	-	-	0
IA-325	0	0	100	8	-	-	-	0	-	-	-	0
IA-326	0	13	88	8	25	50	25	4	-	-	-	0
IA-332	0	7	93	14	0	17	83	6	0	0	100	1
IA-338	0	7	93	14	0	86	14	7	0	100	0	1
IA-340	0	0	100	16	0	0	100	5	-	-	-	0
IA-347	0	10	90	10	0	0	100	1	-	-	-	0
IA-358	0	15	85	13	0	38	63	8	-	-	-	0
IA-364	0	43	57	14	0	57	43	7	0	100	0	1
IA-368	7	13	80	15	0	100	0	3	0	100	0	2

IA-369	0	0	100	16	0	0	100	13	0	33	67	3
IA-378	0	17	83	12	0	75	25	4	0	100	0	2
IL-Known PPO-R	30	30	40	30	26	52	22	27	38	31	31	13
IL-Known PPO-S	0	0	100	22	0	0	100	4	-	-	-	0
IL-BDW	73	27	0	15	69	25	6	16	83	8	8	12
IL-BON	20	60	20	15	20	80	0	10	60	40	0	5
IL-BRO1	88	6	6	16	88	13	0	16	100	0	0	3
IL-BRO2	69	31	0	16	80	20	0	15	80	20	0	5
IL-CHAM	0	0	100	10	0	100	0	2	-	-	-	0
IL-CLT1	0	77	23	13	13	73	13	15	67	33	0	6
IL-CLT2	23	46	31	13	8	50	42	12	80	20	0	5
IL-CLT3	14	14	71	14	33	50	17	6	-	-	-	0
IL-GRE1	8	33	58	12	18	64	18	11	50	50	0	2
IL-GRE2	40	47	13	15	58	42	0	12	100	0	0	3
IL-IRO1	0	18	82	11	100	0	0	1	-	-	-	0
IL-IRO2	29	7	64	14	33	44	22	9	100	0	0	2
IL-JAS	38	54	8	13	43	57	0	14	50	50	0	6
IL-JEF	15	46	38	13	27	64	9	11	100	0	0	5
IL-KNX1	19	63	19	16	29	64	7	14	0	100	0	2
IL-KNX2	23	23	54	13	50	0	50	4	33	67	0	3
IL-MAD	13	53	33	15	38	38	25	8	-	-	-	0
IL-MAR	64	21	14	14	69	23	8	13	60	40	0	5
IL-MCD	0	27	73	11	17	50	33	6	0	100	0	3
IL-MOR	10	20	70	10	0	0	100	1	0	100	0	1
IL-PEO	7	29	64	14	0	20	80	10	50	50	0	2
IL-PIKE	36	14	50	14	33	42	25	12	100	0	0	1
IL-RAN	25	63	13	16	29	57	14	14	17	83	0	6
IL-SANG1	11	44	44	9	22	56	22	9	20	80	0	5
IL-SANG2	67	33	0	12	36	64	0	11	40	60	0	5
IL-SANG3	15	54	31	13	11	89	0	9	100	0	0	1
IL-SCT	100	0	0	15	100	0	0	14	100	0	0	6
IL-VER	27	67	7	15	45	55	0	11	67	33	0	6
IL-WAR	100	0	0	16	100	0	0	16	100	0	0	7
IL-WAS	69	23	8	13	60	27	13	15	90	10	0	10
IL-WHT	57	43	0	14	56	44	0	16	100	0	0	8
IN-Known PPO-R	17	55	28	29	29	36	36	28	20	40	40	5
IN-Known PPO-S	0	0	100	17	0	0	100	4	-	-	-	0
IN-BNE1	0	30	70	10	56	44	0	9	75	25	0	4
IN-BNE2	0	0	100	8	-	-	-	0	-	-	-	0
IN-DAV	31	54	15	13	29	71	0	14	100	0	0	3
IN-DEL1	0	0	100	11	-	-	-	0	-	-	-	0
IN-DEL2	0	25	75	8	0	50	50	2	-	-	-	0

IN-DEL3	0	0	100	7	-	-	-	0	-	-	-	0
IN-DEL4	0	71	29	7	25	75	0	4	-	-	-	0
IN-DUB	77	23	0	13	77	23	0	13	80	20	0	5
IN-GIB1	0	0	100	12	0	0	100	2	-	-	-	0
IN-GIB2	27	60	13	15	53	40	7	15	100	0	0	3
IN-GIB3	46	46	8	13	56	44	0	9	100	0	0	2
IN-GIB4	0	0	100	6	0	0	100	3	-	-	-	0
IN-JAY	57	36	7	14	46	54	0	13	75	25	0	4
IN-KNX	15	46	38	13	29	71	0	7	100	0	0	1
IN-MAD	0	20	80	5	0	75	25	4	-	-	-	0
IN-MAR	67	25	8	12	50	50	0	12	50	50	0	6
IN-NWT	0	36	64	11	0	67	33	6	-	-	-	0
IN-PIKE1	90	10	0	10	79	21	0	14	75	25	0	8
IN-PIKE2	40	50	10	10	27	73	0	11	75	25	0	4
IN-PUL	0	11	89	9	0	75	25	4	-	-	-	0
IN-RAN1	0	0	100	8	0	0	100	1	-	-	-	0
IN-RAN2	0	0	100	7	0	0	100	2	-	-	-	0
IN-RAN3	67	33	0	15	80	20	0	15	100	0	0	4
IN-SPEN	50	38	13	16	69	25	6	16	100	0	0	4
IN-STAR	0	13	88	8	33	33	33	3	-	-	-	0
IN-VIGO	0	0	100	8	0	0	100	3	-	-	-	0
IN-WAR1	33	50	17	12	30	70	0	10	40	60	0	5
IN-WAR2	44	44	13	16	46	54	0	13	60	40	0	5
IN-WAR3	21	29	50	14	0	100	0	7	0	100	0	2
IN-WAR4	7	57	36	14	38	38	23	13	50	50	0	4
IN-WHTE	0	0	100	10	0	0	100	2	-	-	-	0
MN-Known PPO-R	26	39	35	31	21	55	24	29	44	44	11	9
MN-Known PPO-S	0	0	100	23	0	0	100	3	-	-	-	0
MN-388	0	0	100	16	0	0	100	5	-	-	-	0
MN-389	0	50	50	10	25	50	25	8	0	100	0	1
MN-390	7	43	50	14	0	83	17	6	-	-	-	0
MN-391	0	25	75	12	0	25	75	4	0	100	0	2
MN-392	33	33	33	12	38	46	15	13	60	40	0	5
MN-393	36	43	21	14	69	23	8	13	86	14	0	7
MN-394	6	19	75	16	17	50	33	6	0	100	0	1
MN-395	53	47	0	15	67	33	0	12	25	75	0	4
MN-396	0	43	57	14	0	50	50	8	-	-	-	0
MN-397	13	73	13	15	31	46	23	13	33	67	0	3
MN-400	13	6	81	16	20	10	70	10	100	0	0	3
MN-401	6	50	44	16	29	71	0	7	-	-	-	0
MN-404	0	9	91	11	0	33	67	3	-	-	-	0
MN-405	13	47	40	15	25	50	25	8	50	50	0	2

MN-406	0	33	67	15	0	67	33	6	100	0	0	3
MO-Known PPO-R	28	41	31	32	28	38	34	32	18	45	36	11
MO-Known PPO-S	0	0	100	23	0	33	67	3	-	-	-	0
MO-1	27	53	20	15	33	58	8	12	50	50	0	2
MO-2	58	42	0	12	33	67	0	12	50	50	0	2
MO-3	7	33	60	15	44	44	11	9	100	0	0	3
MO-4	7	50	43	14	25	38	38	8	29	43	29	7
MO-5	43	43	14	14	43	57	0	14	57	43	0	7
MO-6	14	50	36	14	10	80	10	10	40	60	0	5
MO-7	31	31	38	13	9	91	0	11	33	67	0	3
MO-9	36	43	21	14	27	73	0	15	67	33	0	6
MO-10	64	36	0	14	64	27	9	11	78	22	0	9
MO-12	31	54	15	13	40	60	0	10	67	33	0	3
MO-16	0	69	31	13	20	60	20	5	0	100	0	1
MO-20	20	60	20	15	43	57	0	14	100	0	0	2
MO-21	20	53	27	15	22	78	0	9	33	67	0	3
MO-22	40	50	10	10	46	54	0	13	38	63	0	8
MO-23	73	27	0	15	53	33	13	15	75	25	0	4
MO-25	8	67	25	12	30	60	10	10	33	67	0	3
MO-27	38	54	8	13	43	57	0	7	100	0	0	4
MO-28	42	42	17	12	50	43	7	14	100	0	0	3
MO-39	87	13	0	15	69	31	0	13	88	13	0	8
MO-43	44	50	6	16	25	58	17	12	60	40	0	5
MO-45	0	20	80	5	25	25	50	4	0	100	0	1
MO-46	42	33	25	12	20	70	10	10	50	0	50	2
MO-47	17	25	58	12	0	0	100	4	0	100	0	1
MO-48	9	73	18	11	17	50	33	6	50	50	0	2
MO-49	57	21	21	14	92	0	8	12	83	17	0	6
MO-50	47	47	7	15	38	62	0	13	100	0	0	2
MO-53	33	47	20	15	40	47	13	15	100	0	0	10
MO-55	38	54	8	13	69	31	0	13	43	57	0	7
MO-56	13	47	40	15	27	55	18	11	20	80	0	5
MO-58	0	25	75	12	25	38	38	8	-	-	-	0
MO-59	75	19	6	16	69	19	13	16	90	10	0	10
MO-61	9	55	36	11	38	50	13	8	50	50	0	2
MO-66	14	29	57	14	36	36	27	11	40	60	0	5
MO-74	0	40	60	15	50	0	50	2	-	-	-	0
MO-75	57	36	7	14	73	18	9	11	50	50	0	2

[a] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; PPO-R, PPO-resistant; PPO-S, PPO-susceptible; PPO, protoporphyrinogen oxidase; ΔG210, glycine deletion at position 210 of *PPX2*.

Table B.4. ANOVA of data analysis for fomesafen full-dose response experiment on 29 tall waterhemp populations resistant and susceptible to PPO-inhibiting herbicides.

Population[a]	Source	DF	SS	MS	F-value	P-value
IL – DSO	Rate	7	301208.1852	43029.7407	371.00	<.0001
Known PPO-S	Rep	9	1447.6563	160.8507	1.39	0.1957
	Run	2	1324.0464	662.0232	5.71	0.0039
	Rate*Run	14	3438.2065	245.5862	2.12	0.0122
IN – KNOX7	Rate	7	288719.8606	41245.6944	170.31	<.0001
Known PPO-S	Rep	9	4792.7067	532.5230	2.20	0.0234
	Run	2	13393.2475	6696.6238	27.65	<.0001
	Rate*Run	14	16007.3983	1143.3856	4.72	<.0001
IL – CAR	Rate	7	270309.8159	38615.6880	148.92	<.0001
Known PPO-R	Rep	9	1687.4619	187.4958	0.72	0.6875
	Run	2	5583.2837	2791.6419	10.77	<.0001
	Rate*Run	14	8535.8109	609.7008	2.35	0.0049
IN – DAV	Rate	7	311649.4945	44521.3564	117.64	<.0001
Known PPO-R	Rep	9	15303.4720	1700.3858	4.49	<.0001
	Run	2	1752.7566	876.3783	2.32	0.1012
	Rate*Run	14	2977.2947	212.6639	0.56	0.8923
IA – 152	Rate	7	187281.1942	26754.4563	140.98	<.0001
	Rep	9	3726.4837	414.0537	2.18	0.0269
	Run	1	1345.8570	1345.8570	7.09	0.0087
	Rate*Run	7	3644.4295	520.6328	2.74	0.0108
IA – 293	Rate	7	198669.8435	28381.4062	94.56	<.0001
	Rep	9	6319.8381	702.2042	2.34	0.0175
	Run	1	52.6084	52.6084	0.18	0.6761
	Rate*Run	7	1542.7264	220.3895	0.73	0.6432
IA – 315	Rate	7	183152.3750	26164.6250	101.65	<.0001
	Rep	9	3420.5347	380.0594	1.48	0.1629
	Run	1	1970.9519	1970.9519	7.66	0.0065
	Rate*Run	7	5274.9568	753.5653	2.93	0.0070
IA – 332	Rate	7	241227.7116	34461.1017	39.98	<.0001
	Rep	9	14711.0736	1634.5637	1.90	0.0575
	Run	1	105.8048	105.8048	0.12	0.7266
	Rate*Run	7	3374.9310	482.1330	0.56	0.7878
IA – 340	Rate	7	217774.5765	31110.6538	189.69	<.0001

	Rep	9	5985.6508	665.0723	4.06	0.0001
	Run	1	995.6058	995.6058	6.07	0.0150
	Rate*Run	7	1434.1444	204.8778	1.25	0.2805
IA – 358	Rate	7	195482.8971	27926.1282	265.33	<.0001
	Rep	9	2571.8286	285.7587	2.72	0.0061
	Run	1	683.3774	683.3774	6.49	0.0120
	Rate*Run	7	2146.5497	306.6500	2.91	0.0072
IA – 369	Rate	7	189268.5153	27038.3593	148.66	<.0001
	Rep	9	6181.5615	686.8402	3.78	0.0003
	Run	1	5165.8265	5165.8265	28.40	<.0001
	Rate*Run	7	2706.3036	386.6148	2.13	0.0450
IL – CLT3	Rate	7	198102.1507	28300.3072	167.60	<.0001
	Rep	9	4329.3974	481.0442	2.85	0.0042
	Run	1	58.7526	58.7526	0.35	0.5563
	Rate*Run	7	2870.5686	410.0812	2.43	0.0225
MO – 45	Rate	7	198429.9892	28347.1413	83.20	<.0001
	Rep	9	8737.3317	970.8146	2.85	0.0042
	Run	1	105.5469	105.5469	0.31	0.5787
	Rate*Run	7	1118.4542	159.7792	0.47	0.8556
IL – PEO	Rate	7	167629.6458	23947.0923	148.38	<.0001
	Rep	9	2481.3261	275.7029	1.71	0.0928
	Run	1	346.9032	346.9032	2.15	0.1449
	Rate*Run	7	569.2756	81.3251	0.50	0.8303
IL – BDW	Rate	7	148710.8748	21244.4107	83.01	<.0001
	Rep	9	3181.1937	353.4660	1.38	0.2026
	Run	1	1571.3249	1571.3249	6.14	0.0145
	Rate*Run	7	2749.9004	392.8429	1.53	0.1606
IL – BRO1	Rate	7	166984.2032	23854.8862	69.27	<.0001
	Rep	9	5104.9790	567.2199	1.65	0.1080
	Run	1	4476.3354	4476.3354	13.00	0.0004
	Rate*Run	7	3753.8367	536.2624	1.56	0.1534
IL – BRO2	Rate	7	164431.4392	23490.2056	108.75	<.0001
	Rep	9	4358.5971	484.2886	2.24	0.0229
	Run	1	107.2757	107.2757	0.50	0.4822
	Rate*Run	7	413.8942	59.1277	0.27	0.9632
IL – CLT1	Rate	7	171920.7430	24560.1061	85.34	<.0001
	Rep	9	7265.4156	807.2684	2.81	0.0048

	Run	1	419.3134	419.3134	1.46	0.2295
	Rate*Run	7	2689.7253	384.2465	1.34	0.2385
IL – CLT2	Rate	7	199783.4431	28540.4919	64.70	<.0001
	Rep	9	13896.5273	1544.0586	3.50	0.0006
	Run	1	2367.1531	2367.1531	5.37	0.0220
	Rate*Run	7	3085.9388	440.8484	1.00	0.4346
IL – JAS	Rate	7	215458.0291	30779.7184	112.41	<.0001
	Rep	9	3491.9084	387.9898	1.42	0.1867
	Run	1	353.0082	353.0082	1.29	0.2582
	Rate*Run	7	901.1812	128.7402	0.47	0.8548
IL – MAR	Rate	7	193239.9370	27605.7053	82.18	<.0001
	Rep	9	8524.0020	947.1113	2.82	0.0046
	Run	1	1033.1749	1033.1749	3.08	0.0817
	Rate*Run	7	2546.9118	363.8445	1.08	0.3776
IL – RAN	Rate	7	163239.7188	23319.9598	78.18	<.0001
	Rep	9	4493.8858	499.3206	1.67	0.1011
	Run	1	18.5474	18.5474	0.06	0.8035
	Rate*Run	7	1124.3817	160.6260	0.54	0.8041
IL – SAN2	Rate	7	181844.4638	25977.7805	118.78	<.0001
	Rep	9	4393.1993	488.1333	2.23	0.0235
	Run	1	7362.1276	7362.1276	33.66	<.0001
	Rate*Run	7	4100.4783	585.7826	2.68	0.0125
IL – SCT	Rate	7	177850.8825	25407.2689	91.27	<.0001
	Rep	9	13849.9847	1538.8872	5.53	<.0001
	Run	1	1520.6396	1520.6396	5.46	0.0209
	Rate*Run	7	2638.3528	376.9075	1.35	0.2300
IL – WAS	Rate	7	201772.3879	28824.6268	99.96	<.0001
	Rep	9	6654.3333	739.3704	2.56	0.0094
	Run	1	125.4355	125.4355	0.43	0.5107
	Rate*Run	7	3631.1491	518.7356	1.80	0.0924
IL – WHT	Rate	7	178301.5284	25471.6469	102.17	<.0001
	Rep	9	8322.6361	924.7373	3.71	0.0003
	Run	1	1388.6218	1388.6218	5.57	0.0197
	Rate*Run	7	1500.5067	214.3581	0.86	0.5402
IN – DUB	Rate	7	179212.7550	25601.8221	103.46	<.0001
	Rep	9	10442.1343	1160.2371	4.69	<.0001
	Run	1	1541.9750	1541.9750	6.23	0.0138

	Rate*Run	7	3020.8467	431.5495	1.74	0.1040
IN – PIK1	Rate	7	190522.7582	27217.5369	119.45	<.0001
	Rep	9	2965.6544	329.5172	1.45	0.1746
	Run	1	914.5098	914.5098	4.01	0.0471
	Rate*Run	7	864.1616	123.4517	0.54	0.8015
IN – SPEN	Rate	7	225493.2858	32213.3265	72.57	<.0001
	Rep	9	18586.4808	2065.1645	4.65	<.0001
	Run	1	48.4291	48.4291	0.11	0.7417
	Rate*Run	7	2060.7079	294.3868	0.66	0.7028
MO – 10	Rate	7	177704.3064	25386.3295	85.02	<.0001
	Rep	9	5362.2291	595.8032	2.00	0.0444
	Run	1	893.7610	893.7610	2.99	0.0859
	Rate*Run	7	1864.3993	266.3428	0.89	0.5148
MO – 22	Rate	7	194220.5254	27745.7893	43.46	<.0001
	Rep	9	35217.0907	3913.0101	6.13	<.0001
	Run	1	1718.7440	1718.7440	2.69	0.1032
	Rate*Run	7	3307.8226	472.5461	0.74	0.6383
MO – 53	Rate	7	181894.2876	25984.8982	110.38	<.0001
	Rep	9	7427.7799	825.3089	3.51	0.0006
	Run	1	4.7765	4.7765	0.02	0.8869
	Rate*Run	7	3765.3598	537.9085	2.28	0.0313
MO – 9	Rate	7	184582.0208	26368.8601	73.43	<.0001
	Rep	9	4782.3946	531.3772	1.48	0.1615
	Run	1	637.4437	637.4437	1.77	0.1850
	Rate*Run	7	3514.7712	502.1102	1.40	0.2111

[a] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MO, Missouri.

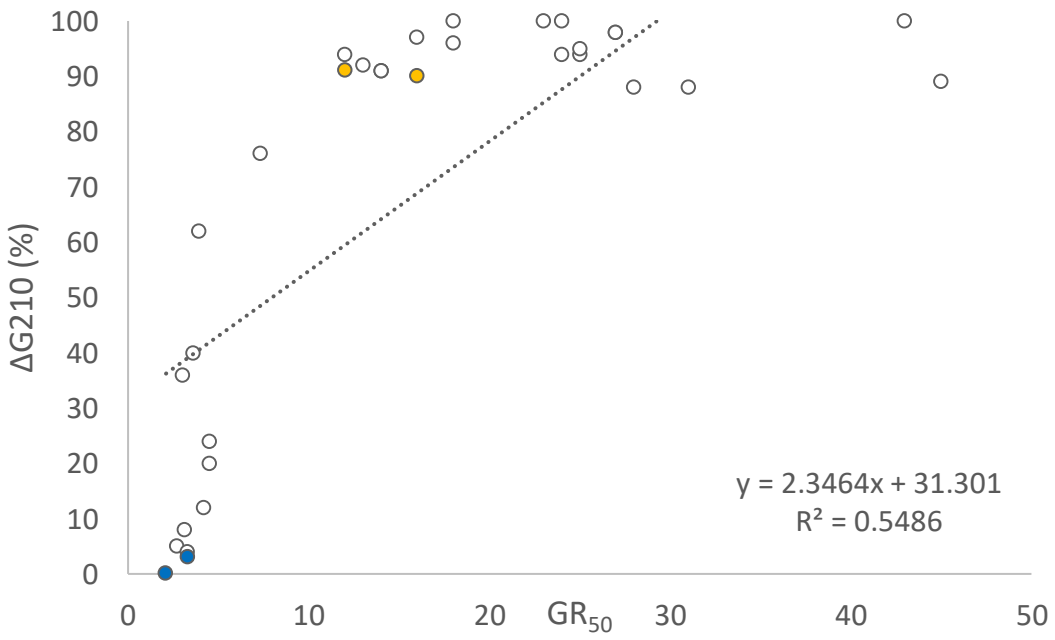

Figure B.1. Linear regression of percent ΔG210 versus GR_{50} in multiple tall waterhemp populations resistant and susceptible to PPO-inhibiting herbicides. Blue dots represent PPO-susceptible and orange dots represent PPO-resistant populations.

Figure B.2. Modified 96-well plate DNA extraction protocol.

Plant Tissue Sampling

1. Collect plant tissue from the youngest leaf possible, near the apical or axillary meristems.
2. Use forceps or scissors to cut a piece of leaf half the size of a dime and place in a 2.0 ml centrifuge tube. This is the amount of tissue required for DNA extraction.

DNA Extraction

1. Put leaf tissue and 1 BB gun bead into each 1.2 ml well of a 96-well plate.
2. Add 500 ul CTAB, then seal the rubber mat firmly on top of 1.2 ml 96-well plate.
3. Grind samples in plate for 4 min using a bead beater. Be sure that you seal the rubber mat to avoid contamination from well leakage.
4. Place 1.2 ml 96-well plate in 65°C water bath for 20 to 40 min and cool down for 5 to 10 min.
5. Remove rubber mat and add 400 ul chloroform to 1.2 ml 96 well plate.
6. Place new rubber mat on 1.2 ml 96-well plate and gently mix by shaking plate. Do not invert plate to avoid well leakage.
7. Centrifuge 1.2 ml 96-well plate at 3500 RPM for 15 min.
8. Add 150 ul 100% EtOH to a clean 0.3 ml 96-well plate.
9. Carefully transfer 120 ul supernatant from 1.2 ml 96-well plate to clean 0.3 ml 96-well plate and mix using pipette.
10. Centrifuge 0.3 ml 96-well plate for 35 min at 3500 RPM, decant liquid by inversion.
11. Leave 0.3 ml 96-well plate under hood until dry to remove any excess EtOH.
12. Add 100 ul ddH_2O to each well, seal with plastic film, and place in -20°C freezer until ready to use.

APPENDIX C. CHAPTER 4 SUPPLEMENTARY DATA

Table C.1. Genotypic frequencies of tall waterhemp samples evaluated for enzymatic antioxidant activity prior to and following an application of fomesafen.

Population	Phenotype[a,b]	Run	HAT	Total Samples	No ΔG210	Rr	RR	ΔG210
					No. individual plants			%
IA-157	Susceptible	1	0	3	3	0	0	0
IA-157	Susceptible	2	0	3	3	0	0	0
IA-157	Susceptible	1	3	3	3	0	0	0
IA-157	Susceptible	2	3	3	3	0	0	0
IA-157	Susceptible	1	6	3	3	0	0	0
IA-157	Susceptible	2	6	3	3	0	0	0
IA-157	Susceptible	1	9	3	3	0	0	0
IA-157	Susceptible	2	9	2	2	0	0	0
IA-157	Susceptible	1	12	3	3	0	0	0
IA-157	Susceptible	2	12	3	3	0	0	0
IA-157	Susceptible	1	24	3	3	0	0	0
IA-157	Susceptible	2	24	3	3	0	0	0
IA-157	Susceptible	1	36	3	3	0	0	0
IA-157	Susceptible	2	36	3	3	0	0	0
IA-340	Susceptible	1	0	3	3	0	0	0
IA-340	Susceptible	2	0	3	3	0	0	0
IA-340	Susceptible	1	3	3	3	0	0	0
IA-340	Susceptible	2	3	3	3	0	0	0
IA-340	Susceptible	1	6	3	3	0	0	0
IA-340	Susceptible	2	6	2	2	0	0	0
IA-340	Susceptible	1	9	3	3	0	0	0
IA-340	Susceptible	2	9	3	3	0	0	0
IA-340	Susceptible	1	12	3	3	0	0	0
IA-340	Susceptible	2	12	3	3	0	0	0
IA-340	Susceptible	1	24	3	3	0	0	0
IA-340	Susceptible	2	24	3	3	0	0	0
IA-340	Susceptible	1	36	3	3	0	0	0
IA-340	Susceptible	2	36	3	3	0	0	0
IA-369	Susceptible	1	0	3	3	0	0	0
IA-369	Susceptible	2	0	3	3	0	0	0
IA-369	Susceptible	1	3	3	3	0	0	0
IA-369	Susceptible	2	3	3	3	0	0	0
IA-369	Susceptible	1	6	3	3	0	0	0
IA-369	Susceptible	2	6	3	3	0	0	0
IA-369	Susceptible	1	9	3	3	0	0	0
IA-369	Susceptible	2	9	3	3	0	0	0

IA-369	Susceptible	1	12	3	3	0	0	0
IA-369	Susceptible	2	12	3	3	0	0	0
IA-369	Susceptible	1	24	3	3	0	0	0
IA-369	Susceptible	2	24	1	1	0	0	0
IA-369	Susceptible	1	36	3	3	0	0	0
IA-369	Susceptible	2	36	3	3	0	0	0
IL-Cham	Susceptible	1	0	3	2	1	0	33
IL-Cham	Susceptible	2	0	3	2	1	0	33
IL-Cham	Susceptible	1	3	3	3	0	0	0
IL-Cham	Susceptible	2	3	3	3	0	0	0
IL-Cham	Susceptible	1	6	3	3	0	0	0
IL-Cham	Susceptible	2	6	2	1	1	0	50
IL-Cham	Susceptible	1	9	3	3	0	0	0
IL-Cham	Susceptible	2	9	3	3	0	0	0
IL-Cham	Susceptible	1	12	3	3	0	0	0
IL-Cham	Susceptible	2	12	3	3	0	0	0
IL-Cham	Susceptible	1	24	3	3	0	0	0
IL-Cham	Susceptible	2	24	2	2	0	0	0
IL-Cham	Susceptible	1	36	3	3	0	0	0
IL-Cham	Susceptible	2	36	3	3	0	0	0
IL-Iro1	Susceptible	1	0	3	3	0	0	0
IL-Iro1	Susceptible	2	0	3	3	0	0	0
IL-Iro1	Susceptible	1	3	3	2	1	0	33
IL-Iro1	Susceptible	2	3	3	3	0	0	0
IL-Iro1	Susceptible	1	6	3	2	1	0	33
IL-Iro1	Susceptible	2	6	3	3	0	0	0
IL-Iro1	Susceptible	1	9	3	3	0	0	0
IL-Iro1	Susceptible	2	9	3	3	0	0	0
IL-Iro1	Susceptible	1	12	3	3	0	0	0
IL-Iro1	Susceptible	2	12	3	3	0	0	0
IL-Iro1	Susceptible	1	24	3	2	1	0	33
IL-Iro1	Susceptible	2	24	3	3	0	0	0
IL-Iro1	Susceptible	1	36	3	3	0	0	0
IL-Iro1	Susceptible	2	36	3	3	0	0	0
IN-Bne2	Susceptible	1	0	3	3	0	0	0
IN-Bne2	Susceptible	2	0	3	3	0	0	0
IN-Bne2	Susceptible	1	3	3	3	0	0	0
IN-Bne2	Susceptible	2	3	3	3	0	0	0
IN-Bne2	Susceptible	1	6	3	3	0	0	0
IN-Bne2	Susceptible	2	6	3	3	0	0	0
IN-Bne2	Susceptible	1	9	3	3	0	0	0
IN-Bne2	Susceptible	2	9	3	3	0	0	0
IN-Bne2	Susceptible	1	12	3	3	0	0	0
IN-Bne2	Susceptible	2	12	3	3	0	0	0
IN-Bne2	Susceptible	1	24	3	3	0	0	0

IN-Bne2	Susceptible	2	24	2	2	0	0	0
IN-Bne2	Susceptible	1	36	3	3	0	0	0
IN-Bne2	Susceptible	2	36	3	3	0	0	0
IN-Ran1	Susceptible	1	0	3	3	0	0	0
IN-Ran1	Susceptible	2	0	3	3	0	0	0
IN-Ran1	Susceptible	1	3	3	3	0	0	0
IN-Ran1	Susceptible	2	3	3	3	0	0	0
IN-Ran1	Susceptible	1	6	3	3	0	0	0
IN-Ran1	Susceptible	2	6	3	3	0	0	0
IN-Ran1	Susceptible	1	9	3	3	0	0	0
IN-Ran1	Susceptible	2	9	2	2	0	0	0
IN-Ran1	Susceptible	1	12	3	3	0	0	0
IN-Ran1	Susceptible	2	12	3	3	0	0	0
IN-Ran1	Susceptible	1	24	3	3	0	0	0
IN-Ran1	Susceptible	2	24	2	2	0	0	0
IN-Ran1	Susceptible	1	36	3	3	0	0	0
IN-Ran1	Susceptible	2	36	2	2	0	0	0
MN-388	Susceptible	1	0	3	2	1	0	33
MN-388	Susceptible	2	0	3	3	0	0	0
MN-388	Susceptible	1	3	3	3	0	0	0
MN-388	Susceptible	2	3	3	3	0	0	0
MN-388	Susceptible	1	6	3	3	0	0	0
MN-388	Susceptible	2	6	3	3	0	0	0
MN-388	Susceptible	1	9	3	3	0	0	0
MN-388	Susceptible	2	9	3	3	0	0	0
MN-388	Susceptible	1	12	3	3	0	0	0
MN-388	Susceptible	2	12	3	3	0	0	0
MN-388	Susceptible	1	24	3	3	0	0	0
MN-388	Susceptible	2	24	3	3	0	0	0
MN-388	Susceptible	1	36	3	3	0	0	0
MN-388	Susceptible	2	36	3	3	0	0	0
MO-45	Susceptible	1	0	3	2	1	0	33
MO-45	Susceptible	2	0	3	2	1	0	33
MO-45	Susceptible	1	3	3	3	0	0	0
MO-45	Susceptible	2	3	3	1	1	1	67
MO-45	Susceptible	1	6	3	1	2	0	67
MO-45	Susceptible	2	6	3	2	1	0	33
MO-45	Susceptible	1	9	3	3	0	0	0
MO-45	Susceptible	2	9	2	0	1	1	100
MO-45	Susceptible	1	12	3	2	1	0	33
MO-45	Susceptible	2	12	3	1	1	1	67
MO-45	Susceptible	1	24	3	3	0	0	0
MO-45	Susceptible	2	24	3	1	1	1	67
MO-45	Susceptible	1	36	3	2	1	0	33
MO-45	Susceptible	2	36	3	2	1	0	33

MO-47	Susceptible	1	0	3	2	1	0	33
MO-47	Susceptible	2	0	3	1	2	0	67
MO-47	Susceptible	1	3	3	2	1	0	33
MO-47	Susceptible	2	3	3	2	1	0	33
MO-47	Susceptible	1	6	3	1	1	1	67
MO-47	Susceptible	2	6	3	1	2	0	67
MO-47	Susceptible	1	9	3	2	1	0	33
MO-47	Susceptible	2	9	3	3	0	0	0
MO-47	Susceptible	1	12	3	2	1	0	33
MO-47	Susceptible	2	12	3	1	1	1	67
MO-47	Susceptible	1	24	3	1	2	0	67
MO-47	Susceptible	2	24	3	2	1	0	33
MO-47	Susceptible	1	36	3	2	1	0	33
MO-47	Susceptible	2	36	3	2	1	0	33
MO-58	Susceptible	1	0	3	2	1	0	33
MO-58	Susceptible	2	0	3	2	1	0	33
MO-58	Susceptible	1	3	3	3	0	0	0
MO-58	Susceptible	2	3	2	2	0	0	0
MO-58	Susceptible	1	6	3	1	2	0	67
MO-58	Susceptible	2	6	3	2	1	0	33
MO-58	Susceptible	1	9	3	1	2	0	67
MO-58	Susceptible	2	9	3	2	0	1	33
MO-58	Susceptible	1	12	3	3	0	0	0
MO-58	Susceptible	2	12	3	2	1	0	33
MO-58	Susceptible	1	24	3	2	1	0	33
MO-58	Susceptible	2	24	2	1	0	1	50
MO-58	Susceptible	1	36	3	2	1	0	33
MO-58	Susceptible	2	36	3	3	0	0	0
IL-BDW	Resistant	1	0	3	0	0	3	100
IL-BDW	Resistant	2	0	3	0	0	3	100
IL-BDW	Resistant	1	3	3	0	0	3	100
IL-BDW	Resistant	2	3	2	0	0	2	100
IL-BDW	Resistant	1	6	3	0	1	2	100
IL-BDW	Resistant	2	6	3	1	0	2	67
IL-BDW	Resistant	1	9	2	0	0	2	100
IL-BDW	Resistant	2	9	3	0	1	2	100
IL-BDW	Resistant	1	12	3	1	0	2	67
IL-BDW	Resistant	2	12	3	0	2	1	100
IL-BDW	Resistant	1	24	3	0	0	3	100
IL-BDW	Resistant	2	24	3	0	1	2	100
IL-BDW	Resistant	1	36	3	0	1	2	100
IL-BDW	Resistant	2	36	3	0	1	2	100
IL-Bro2	Resistant	1	0	3	0	1	2	100
IL-Bro2	Resistant	2	0	3	0	1	2	100
IL-Bro2	Resistant	1	3	3	0	0	3	100

IL-Bro2	Resistant	2	3	3	0	0	3	100
IL-Bro2	Resistant	1	6	3	0	0	3	100
IL-Bro2	Resistant	2	6	3	0	0	3	100
IL-Bro2	Resistant	1	9	3	0	0	3	100
IL-Bro2	Resistant	2	9	3	0	1	2	100
IL-Bro2	Resistant	1	12	3	0	0	3	100
IL-Bro2	Resistant	2	12	3	0	1	2	100
IL-Bro2	Resistant	1	24	3	0	0	3	100
IL-Bro2	Resistant	2	24	2	0	0	2	100
IL-Bro2	Resistant	1	36	3	0	0	3	100
IL-Bro2	Resistant	2	36	2	0	0	2	100
IN-Dub	Resistant	1	0	3	0	1	2	100
IN-Dub	Resistant	2	0	3	0	2	1	100
IN-Dub	Resistant	1	3	3	0	3	0	100
IN-Dub	Resistant	2	3	3	0	3	0	100
IN-Dub	Resistant	1	6	3	0	2	1	100
IN-Dub	Resistant	2	6	3	0	1	2	100
IN-Dub	Resistant	1	9	3	1	1	1	67
IN-Dub	Resistant	2	9	3	0	0	3	100
IN-Dub	Resistant	1	12	3	0	0	3	100
IN-Dub	Resistant	2	12	3	0	3	0	100
IN-Dub	Resistant	1	24	3	0	1	2	100
IN-Dub	Resistant	2	24	1	0	0	1	100
IN-Dub	Resistant	1	36	3	0	2	1	100
IN-Dub	Resistant	2	36	2	0	1	1	100
IN-Gib5	Resistant	1	0	3	3	0	0	0
IN-Gib5	Resistant	2	0	3	3	0	0	0
IN-Gib5	Resistant	1	3	3	3	0	0	0
IN-Gib5	Resistant	2	3	3	3	0	0	0
IN-Gib5	Resistant	1	6	3	3	0	0	0
IN-Gib5	Resistant	2	6	3	3	0	0	0
IN-Gib5	Resistant	1	9	3	3	0	0	0
IN-Gib5	Resistant	2	9	3	3	0	0	0
IN-Gib5	Resistant	1	12	3	3	0	0	0
IN-Gib5	Resistant	2	12	3	3	0	0	0
IN-Gib5	Resistant	1	24	3	3	0	0	0
IN-Gib5	Resistant	2	24	0	0	0	0	-
IN-Gib5	Resistant	1	36	3	3	0	0	0
IN-Gib5	Resistant	2	36	3	3	0	0	0
IN-Pike1	Resistant	1	0	3	0	2	1	100
IN-Pike1	Resistant	2	0	3	0	1	2	100
IN-Pike1	Resistant	1	3	3	0	2	1	100
IN-Pike1	Resistant	2	3	3	0	2	1	100
IN-Pike1	Resistant	1	6	3	0	2	1	100
IN-Pike1	Resistant	2	6	3	0	1	2	100

IN-Pike1	Resistant	1	9	3	0	0	3	100
IN-Pike1	Resistant	2	9	3	0	2	1	100
IN-Pike1	Resistant	1	12	3	0	0	3	100
IN-Pike1	Resistant	2	12	3	1	2	0	67
IN-Pike1	Resistant	1	24	3	1	0	2	67
IN-Pike1	Resistant	2	24	1	0	0	1	100
IN-Pike1	Resistant	1	36	3	0	3	0	100
IN-Pike1	Resistant	2	36	2	0	0	2	100
MN-395	Resistant	1	0	3	0	3	0	100
MN-395	Resistant	2	0	3	0	1	2	100
MN-395	Resistant	1	3	3	0	1	2	100
MN-395	Resistant	2	3	3	1	1	1	67
MN-395	Resistant	1	6	3	0	0	3	100
MN-395	Resistant	2	6	3	0	1	2	100
MN-395	Resistant	1	9	3	0	2	1	100
MN-395	Resistant	2	9	3	1	2	0	67
MN-395	Resistant	1	12	3	0	2	1	100
MN-395	Resistant	2	12	3	1	1	1	67
MN-395	Resistant	1	24	3	0	0	3	100
MN-395	Resistant	2	24	3	0	1	2	100
MN-395	Resistant	1	36	3	1	2	0	67
MN-395	Resistant	2	36	1	0	0	1	100
MN-401	Resistant	1	0	3	1	1	1	67
MN-401	Resistant	2	0	3	2	1	0	33
MN-401	Resistant	1	3	3	0	1	2	100
MN-401	Resistant	2	3	3	2	1	0	33
MN-401	Resistant	1	6	2	1	0	1	50
MN-401	Resistant	2	6	3	2	1	0	33
MN-401	Resistant	1	9	3	3	0	0	0
MN-401	Resistant	2	9	3	1	1	1	67
MN-401	Resistant	1	12	3	2	1	0	33
MN-401	Resistant	2	12	2	0	1	1	100
MN-401	Resistant	1	24	3	2	1	0	33
MN-401	Resistant	2	24	2	1	0	1	50
MN-401	Resistant	1	36	3	2	0	1	33
MN-401	Resistant	2	36	2	2	0	0	0
MO-39	Resistant	1	0	3	0	1	2	100
MO-39	Resistant	2	0	2	0	1	1	100
MO-39	Resistant	1	3	3	1	1	1	67
MO-39	Resistant	2	3	3	0	2	1	100
MO-39	Resistant	1	6	3	0	1	2	100
MO-39	Resistant	2	6	3	0	1	2	100
MO-39	Resistant	1	9	3	1	0	2	67
MO-39	Resistant	2	9	3	1	0	2	67
MO-39	Resistant	1	12	1	0	0	1	100

MO-39	Resistant	2	12	2	0	1	1	100
MO-39	Resistant	1	24	3	0	0	3	100
MO-39	Resistant	2	24	3	0	1	2	100
MO-39	Resistant	1	36	3	1	0	2	67
MO-39	Resistant	2	36	3	0	0	3	100
MO-53	Resistant	1	0	3	1	1	1	67
MO-53	Resistant	2	0	3	0	0	3	100
MO-53	Resistant	1	3	3	1	0	2	67
MO-53	Resistant	2	3	3	0	2	1	100
MO-53	Resistant	1	6	3	1	1	1	67
MO-53	Resistant	2	6	3	1	1	1	67
MO-53	Resistant	1	9	3	1	0	2	67
MO-53	Resistant	2	9	2	0	0	2	100
MO-53	Resistant	1	12	3	0	2	1	100
MO-53	Resistant	2	12	2	0	1	1	100
MO-53	Resistant	1	24	3	0	2	1	100
MO-53	Resistant	2	24	3	0	1	2	100
MO-53	Resistant	1	36	3	0	1	2	100
MO-53	Resistant	2	36	2	0	1	1	100

[a] Phenotype was defined susceptible if overall population control by fomesafen (52 g ha^{-1}) in a greenhouse was similar to a known PPO-susceptible (PPO-S) population and resistant if the overall population control was less than a known PPO-S population.

[b] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; HAT, hours after treatment; ΔG210, glycine deletion at position 210 of *PPX2*; Rr, heterozygous PPO-resistant tall waterhemp; RR, homozygous PPO-resistant tall waterhemp.

Table *C.2*. P-values of main effects and interactions for analysis of injury, malondialdehyde (MDA), and enzymatic antioxidants following application of fomesafen.

	P-value (F-value)[a,b]					
Source	Injury	MDA	SOD	CAT	AP	GR
Population	**<.0001 (42.83)**	**<.0001 (15.74)**	**<.0001 (10.46)**	**<.0001 (10.30)**	**<.0001 (10.13)**	**<.0001 (12.95)**
Run	**0.0004 (119.46)**	**0.0004 (118.30)**	**0.0044 (33.65)**	**0.0003 (129.37)**	**0.0004 (127.49)**	**0.0006 (100.09)**
HAT	**<.0001 (239.73)**	**<.0001 (215.98)**	**<.0001 (11.83)**	**<.0001 (103.67)**	**<.0001 (21.65)**	**<.0001 (27.12)**
Population*Run	**<.0001 (6.31)**	**<.0001 (5.12)**	**<.0001 (15.54)**	**<.0001 (7.08)**	**<.0001 (3.47)**	**<.0001 (8.66)**
Population*HAT	**<.0001 (3.89)**	**<.0001 (3.44)**	**0.0010 (1.53)**	**<.0001 (2.27)**	**<.0001 (2.60)**	**0.0001 (1.65)**
Population*Run*HAT	**<.0001 (3.64)**	**<.0001 (2.05)**	0.3334 (1.06)	**0.0103 (1.37)**	**<.0001 (1.99)**	**0.0309 (1.29)**
Phenotype	**<.0001 (283.49)**	**<.0001 (183.15)**	**0.0001 (14.70)**	**<.0001 (100.64)**	**<.0001 (87.92)**	**<.0001 (16.00)**
Run	**0.0004 (117.57)**	**0.0008 (85.66)**	**0.0044 (33.64)**	**0.0004 (117.15)**	**0.0004 (119.15)**	**0.0013 (63.45)**
HAT	**<.0001 (110.47)**	**<.0001 (161.84)**	**<.0001 (6.94)**	**<.0001 (76.28)**	**<.0001 (13.72)**	**<.0001 (18.62)**
Phenotype*Run	0.5380 (0.38)	**0.0028 (9.02)**	0.7488 (0.10)	**0.0027 (9.07)**	0.3461 (0.89)	0.4714 (0.52)
Phenotype*HAT	**<.0001 (11.30)**	**<.0001 (31.10)**	0.7190 (0.61)	**<.0001 (14.06)**	**<.0001 (21.62)**	**0.0017 (3.56)**
Phenotype*Run*HAT	**<.0001 (5.42)**	**<.0001 (10.50)**	0.6582 (0.79)	**<.0001 (3.50)**	**<.0001 (7.11)**	**0.0429 (1.81)**
Genotype	**<.0001 (175.48)**	**<.0001 (91.86)**	0.0669 (2.71)	**<.0001 (36.16)**	**<.0001 (65.40)**	0.1213 (2.12)
Run	**0.0005 (110.31)**	**0.0037 (37.16)**	**0.0068 (26.43)**	**0.0016 (57.46)**	**0.0009 (76.21)**	**0.0036 (37.76)**
HAT	**<.0001 (69.35)**	**<.0001 (66.91)**	**<.0001 (5.43)**	**<.0001 (36.22)**	0.2253 (1.37)	**<.0001 (14.77)**
Genotype*Run	0.1045 (2.27)	**0.0045 (5.44)**	0.0907 (2.41)	**0.0138 (4.31)**	0.6005 (0.51)	0.8421 (0.17)
Genotype*HAT	**<.0001 (8.69)**	**<.0001 (17.09)**	0.3456 (1.11)	**<.0001 (7.41)**	**<.0001 (15.27)**	0.1550 (1.41)
Genotype*Run*HAT	**0.0001 (3.31)**	**<.0001 (7.22)**	0.5099 (0.96)	**0.0068 (2.03)**	**<.0001 (4.41)**	0.2331 (1.23)

[a] Numbers in bold represent P-values <0.05.

[b] Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; HAT, hours after treatment.

Table *C.3*. P-values of main effects and interactions for analysis of injury, malondialdehyde (MDA), and enzymatic antioxidants following application of fomesafen.

Time (HAT)	Source	P-value[a,b]					
		Injury	MDA	SOD	CAT	APX	GR
0	Population	-	**0.0068**	**<.0001**	0.1069	**0.0138**	**<.0001**
	Run	-	0.4194	**0.0052**	0.2603	0.6526	**0.0442**
	Population*Run	-	**<.0001**	**<.0001**	**0.0354**	**0.0063**	**<.0001**
3	Population	-	**0.0200**	**<.0001**	**0.0216**	**0.0199**	**<.0001**
	Run	-	0.2846	**0.0022**	0.6370	0.2358	**0.0182**
	Population*Run	-	**<.0001**	**<.0001**	**0.0140**	**0.0003**	**<.0001**
6	Population	**<.0001**	**0.0388**	**<.0001**	0.0575	**0.0201**	**0.0001**
	Run	**0.0043**	0.1501	**0.0007**	**0.0168**	0.3737	**0.0280**
	Population*Run	**<.0001**	**0.0277**	**<.0001**	**0.0359**	0.3452	**0.0009**
9	Population	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0187**	**<.0001**
	Run	**0.0238**	**0.0171**	**0.0382**	**0.0023**	**0.0032**	**0.0233**
	Population*Run	**0.0461**	**<.0001**	**<.0001**	**<.0001**	0.0769	**<.0001**
12	Population	**<.0001**	**<.0001**	**0.0004**	**<.0001**	**0.0021**	**0.0111**
	Run	**0.0003**	**0.0250**	**0.0215**	**0.0203**	0.0816	**0.0219**
	Population*Run	**<.0001**	**0.0117**	**<.0001**	0.1751	**0.0497**	**0.0061**
24	Population	**<.0001**	**<.0001**	**0.0002**	**<.0001**	**0.0001**	**0.0160**
	Run	**0.0055**	**0.0375**	**0.0012**	**0.0183**	**0.0152**	**0.0233**
	Population*Run	**<.0001**	**0.0412**	**0.0036**	0.0505	0.1369	0.6961
36	Population	**<.0001**	**<.0001**	**0.0192**	**<.0001**	**<.0001**	**<.0001**
	Run	**0.0006**	**0.0018**	0.1658	**0.0013**	**0.0017**	**0.0133**
	Population*Run	**0.0019**	0.7108	**0.0055**	**0.0384**	0.1072	**0.0029**

[a] Numbers in bold represent P-values <0.05.

[b] Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; HAT, hours after treatment.

Table *C.4*. P-values of main effects and interactions for analysis of injury, malondialdehyde (MDA), and enzymatic antioxidants following application of fomesafen.

Time (HAT)	Source	P-value[a,b]					
		Injury	MDA	SOD	CAT	APX	GR
0	Phenotype	-	0.8566	0.2115	0.6610	0.2419	0.5835
	Run	-	0.4465	**0.0054**	0.2853	0.6316	0.2160
	Phenotype*Run	-	**0.0435**	0.7890	0.4063	0.6664	0.3696
3	Phenotype	-	0.9357	**0.0259**	0.9240	0.9362	0.8537
	Run	-	0.5558	**0.0080**	0.7606	0.3474	0.0747
	Phenotype*Run	-	0.0554	0.7170	0.8601	0.6325	0.2519
6	Phenotype	**0.0003**	**0.0009**	**0.0252**	0.6278	0.9876	0.9780
	Run	**0.0091**	0.2067	**0.0024**	**0.0287**	0.4034	**0.0407**
	Phenotype*Run	0.5870	0.1652	0.3176	**0.0182**	0.3747	0.1757
9	Phenotype	**<.0001**	**<.0001**	0.8378	**<.0001**	**0.0007**	**0.0206**
	Run	**0.0465**	**0.0222**	**0.0397**	**0.0065**	**0.0039**	**0.0450**
	Phenotype*Run	0.8316	0.1894	0.5909	0.3253	0.3358	0.0521
12	Phenotype	**<.0001**	**<.0001**	0.3939	**<.0001**	**0.0005**	0.0775
	Run	**0.0028**	**0.0298**	**0.0212**	**0.0186**	0.0643	**0.0223**
	Phenotype*Run	0.8335	0.2974	0.5720	**0.0179**	0.4710	0.4962
24	Phenotype	**<.0001**	**<.0001**	0.1705	**<.0001**	**<.0001**	0.1599
	Run	**0.0060**	**0.0418**	**0.0031**	**0.0192**	**0.0164**	**0.0258**
	Phenotype*Run	0.1045	**0.0197**	0.5728	0.3921	0.1647	0.4291
36	Phenotype	**<.0001**	**<.0001**	**0.0265**	**<.0001**	**<.0001**	**<.0001**
	Run	**0.0011**	**0.0023**	0.1669	**0.0025**	**0.0028**	**0.0383**
	Phenotype*Run	0.7736	0.1873	0.6528	0.6052	0.9500	0.2029

[a] Main effects and interactions without a number are not applicable. Numbers in bold represent P-values <0.05.

[b] Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; HAT, hours after treatment.

Table *C.5*. P-values of main effects and interactions for analysis of injury, malondialdehyde (MDA), and enzymatic antioxidants following application of fomesafen.

Time (HAT)	Source	P-value[a,b]					
		Injury	MDA	SOD	CAT	APX	GR
0	Genotype	-	0.3307	0.6059	0.4084	0.4994	**0.0017**
	Run	-	0.3680	**0.0077**	0.3618	0.3494	0.3472
	Genotype*Run	-	0.5146	0.5079	0.8947	0.1395	0.6289
3	Genotype	-	0.9428	0.1276	0.3576	0.4908	0.1250
	Run	-	0.8106	**0.0170**	0.9848	0.4254	0.1096
	Genotype*Run	-	0.2364	0.2787	0.8963	0.6805	0.9211
6	Genotype	**0.0102**	0.2904	0.9019	0.6797	0.9568	0.2424
	Run	**0.0090**	0.2571	**0.0047**	0.0997	0.5630	0.0550
	Genotype*Run	0.3842	0.7963	0.6198	**0.0422**	0.4044	0.8113
9	Genotype	**<.0001**	**0.0004**	0.5578	**0.0127**	**0.0070**	0.3312
	Run	0.1046	0.0568	0.0822	**0.0357**	**0.0092**	**0.0485**
	Genotype*Run	0.5951	0.9977	0.2250	0.3037	0.9559	0.4150
12	Genotype	**<.0001**	**<.0001**	**0.0216**	**<.0001**	**0.0007**	0.2022
	Run	**0.0062**	0.1006	**0.0283**	**0.0232**	0.1583	**0.0232**
	Genotype*Run	0.1389	0.7664	0.1926	0.1910	0.3609	0.3183
24	Genotype	**<.0001**	**<.0001**	0.0971	**<.0001**	**<.0001**	0.4893
	Run	**0.0102**	0.1960	**0.0080**	**0.0406**	**0.0301**	0.0786
	Genotype*Run	0.2317	0.1341	0.7717	0.4857	0.6128	0.4191
36	Genotype	**<.0001**	**<.0001**	0.7541	**<.0001**	**<.0001**	0.2568
	Run	**0.0021**	**0.0106**	0.2414	**0.0068**	**0.0058**	0.2157
	Genotype*Run	0.9926	0.0835	0.4350	0.9820	0.9692	0.4744

[a] Main effects and interactions without a number are not applicable. Numbers in bold represent P-values <0.05.

[b] Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; HAT, hours after treatment.

Table *C.6.* Visual control of tall waterhemp applied with fomesafen at 342 g ai ha^{-1} in a greenhouse.

		Control										
		Combined Runs	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
County (population ID)	Phenotype	3 HAT	6 HAT		9 HAT		12 HAT		24 HAT		36 HAT	
		%										
Cerro Gordo (IA-340)	Susceptible	0 a	10 b	7 a-c	35 ab	28 a-c	55 a	25 b-e	67 a	43 ab	52 ab	27 b-g
Chickasaw (IA-369)	Susceptible	0 a	7 b	8 a-c	35 ab	33 a-c	38 a-e	32 a-d	47 a-d	17 c-e	47 ab	37 a-c
Greene (IA-157)	Susceptible	0 a	3 b	12 a	37 ab	45 a	43 a-c	45 ab	55 ab	45 ab	50 ab	52 a
Champaign (IL-CHAM)	Susceptible	0 a	8 b	3 a-c	38 ab	30 a-c	48 ab	20 c-e	37 b-f	15 c-e	52 ab	35 a-d
Iroquois (IL-IRO1)	Susceptible	0 a	8 b	3 a-c	35 ab	23 a-c	43 a-c	35 a-c	40 a-f	50 a	57 a	37 a-c
Boone (IN-BNE2)	Susceptible	0 a	7 b	2 bc	23 a-c	10 bc	47 ab	27 b-e	37 b-f	25 b-e	52 ab	22 b-g
Randolph (IN-RAN1)	Susceptible	0 a	25 a	7 a-c	35 ab	15 a-c	48 ab	22 b-e	52 a-c	22 c-e	55 a	22 b-g
Sibley (MN-388)	Susceptible	0 a	5 b	10 ab	38 ab	38 ab	43 a-c	53 a	60 ab	25 b-e	50 ab	40 ab
Carroll (MO-58)	Susceptible	0 a	15 ab	7 a-c	23 a-c	23 a-c	42 a-d	28 b-e	48 a-d	30 a-d	47 ab	53 a
Chariton (MO-45)	Susceptible	0 a	8 b	7 a-c	23 a-c	10 bc	37 b-f	10 de	43 a-e	32 a-c	40 ab	28 b-f
Lafayette (MO-47)	Susceptible	0 a	13 ab	3 bc	17 bc	23 a-c	27 c-f	13 c-e	55 ab	7 e	48 ab	22 b-g
Brown (IL-BRO2)	Resistant	0 a	3 b	1 c	5 c	12 bc	20 f-h	7 e	27 c-f	15 c-e	32 a-c	12 e-g

Randolph (IL-BDW)	Resistant	0 a	12 ab	0 c	12 c	5 c	23 e-g	4 e	37 b-f	5 e	27 bc	7 fg
Dubois (IN-DUB)	Resistant	0 a	3 b	0 c	17 bc	3 c	5 h	7 e	15 f	6 e	35 a-c	5 g
Gibson (IN-GIB5)	Resistant	0 a	3 b	3 a-c	17 bc	12 bc	38 a-e	5 e	23 d-f	17 c-e	37 a-c	17 c-g
Pike (IN-PIKE1)	Resistant	0 a	8 b	2 bc	8 c	6 bc	8 gh	23 b-e	23 d-f	10 de	32 a-c	12 e-g
Cottonwood (MN-395)	Resistant	0 a	7 b	2 bc	12 c	13 a-c	38 a-e	18 c-e	18 ef	17 c-e	30 a-c	13 d-g
Stevens (MN-401)	Resistant	0 a	13 ab	4 a-c	40 a	8 bc	42 a-d	12 c-e	55 ab	18 c-e	42 ab	33 a-e
Lafayette (MO-53)	Resistant	0 a	3 b	4 a-c	22 a-c	12 bc	25 d-g	25 b-e	17 ef	12 c-e	12 c	12 e-g
Montgomery (MO-39)	Resistant	0 a	10 b	3 a-c	12 c	15 a-c	38 a-e	13 c-e	18 ef	13 c-e	33 a-c	15 c-g

[a] Abbreviations: IA, Iowa; IL, Illinois; IN, Indiana; MN, Minnesota; MO, Missouri; HAT, hours after treatment.

Table *C.7*. Correlation of basal levels of total protein, MDA, SOD, CAT, APX, and GR with frequency of resistance (FOR) via ΔG210 mutation, control, survival, and R/S ratios in tall waterhemp resistant and susceptible to PPO-inhibiting herbicides.

	FOR[a,b]		Control		Survival		R/S ratio	
Parameters	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
Total protein	0.09660	0.2939	0.07725	0.4017	0.01624	0.8603	-0.08721	0.5306
MDA	-0.01708	0.8563	-0.01018	0.9140	-0.00856	0.9277	-0.01472	0.9183
SOD	0.04772	0.6047	-0.12512	0.1733	0.13970	0.1281	0.16946	0.2206
CAT	-0.12655	0.1684	0.01837	0.8422	-0.05594	0.5439	-0.01013	0.9420
APX	-0.08395	0.3620	0.10091	0.2728	-0.08943	0.3314	-0.18505	0.1804
GR	**-0.33596**	**0.0002**	0.10095	0.2726	-0.14361	0.1176	-0.16012	0.2474

[a] FOR, control, survival, and R/S ratios represent background information of populations determined from 52 g ai ha^{-1} of fomesafen (Mansfield et al. 2017). Control and survival data was recorded 14 days after treatment.

[b] Abbreviations: PPO, protoporphyrinogen oxidase; ΔG210, glycine deletion at position 210 of *PPX2*; R/S ratio, ratio of GR_{50}-PPO-resistant to GR_{50}-PPO-susceptible tall waterhemp; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase.

Table *C.8* Coefficient of Variation of tall waterhemp populations for SOD, CAT, AP, and GR basal levels following fomesafen application at 342 g ai ha^{-1} in a greenhouse.

	Phenotype[a]		Genotype			
Antioxidant Enzyme[b]	PPO-R	PPO-S	No ΔG210	Rr	RR	Overall
SOD	19	17	17	19	18	18
CAT	17	19	18	18	16	18
AP	13	13	13	13	13	13
GR	21	16	17	18	16	18

[a] Phenotypes were classified resistant or susceptible based on the overall population response to fomesafen relative to known PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) controls.

[b] Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; ΔG210, glycine deletion at position 210 of *PPX2*; Rr, heterozygous PPO-resistant tall waterhemp; RR, homozygous PPO-resistant tall waterhemp.

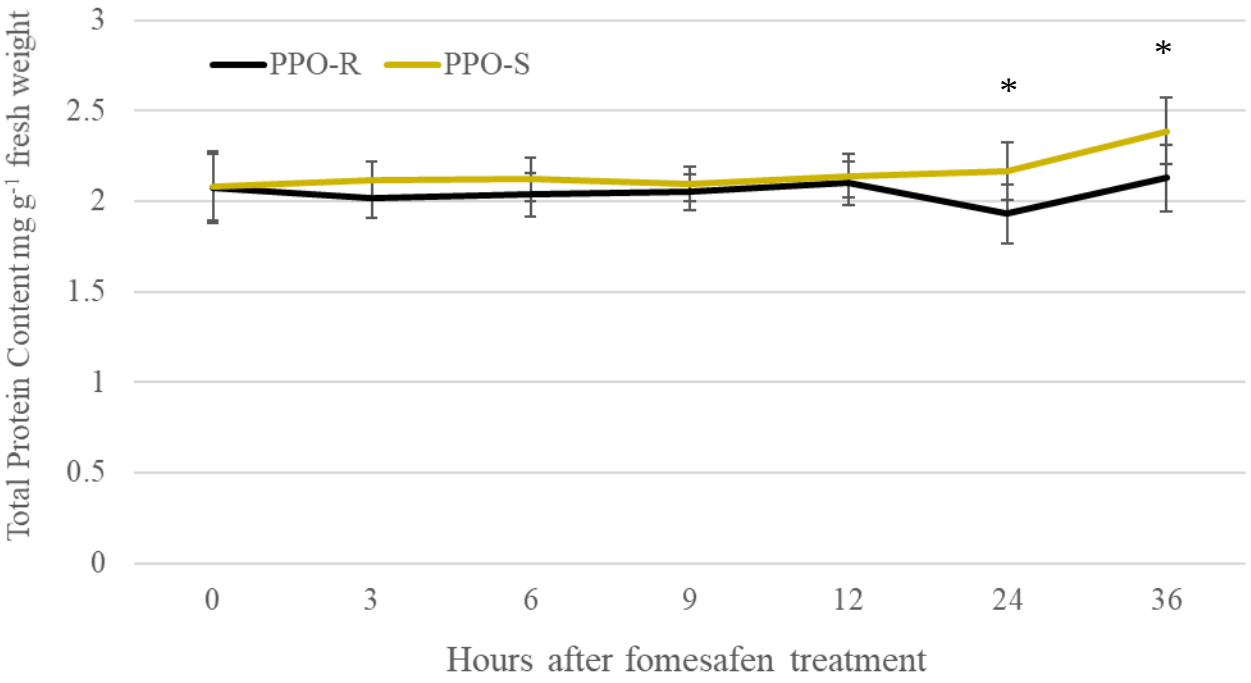

Figure *C.1*. Measurement of total protein content in PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) tall waterhemp biotypes. Phenotypes were classified resistant or susceptible based on the overall population response to fomesafen relative to known PPO-R and PPO-S controls. Vertical bars represent standard error of the mean. An asterisk (*) indicates significance according to Tukey's HSD (P-value ≤ 0.05).

VITA

Brent C. Mansfield

EDUCATION

M.S., Weed Science, Purdue University, West Lafayette, IN Aug 2021
Advisor: Dr. Bryan Young
Thesis: Characterization of Protoporphyrinogen Oxidase Herbicide Resistance in Tall Waterhemp (*Amaranthus Tuberculatus*)

B.S., Crop Sciences, University of Illinois, Urbana-Champaign, IL May 2016
Concentration: Integrated Pest Management
Minor: Food and Agribusiness Management

A.S., Agriculture, John Wood Community College, Quincy, IL May 2014

WORK EXPERIENCE

Purdue University, West Lafayette, IN March 2020 – present
Research Associate

- Lead and evaluate field research trials in regards to weed control in corn and soybean production
- Perform all required tasks for research trials including field preparation, trial establishment, spraying, data collection, data analysis, and data reporting
- Train and manage summer interns assisting with field research trials

Exacto Inc., Sharon, WI June 2019 – September 2019
Research Lab Agronomist

- Support Exacto's mission of providing sound chemical solutions in the form of adjuvants to aid in all types of pesticide applications by conducting field and greenhouse research trials to evaluate new products for turf, row crops, and industrial vegetation management
- Conduct field and greenhouse research trials to aid development of biostimulant products in specialty crops as well as row crops
- Collaborate with Tria Global Solutions by performing research trials for products related to soil sustainability and water management
- Collaborate with universities and contract research companies to conduct off-site research trials to aid in the evaluation of new adjuvant products
- Perform all required tasks for research trials including field preparation, treatment design, trial establishment, spraying, data collection, data analysis, and data reporting
- Present results of research trials in the form of a poster or paper at conferences including but not limited to the North Central Weed Science Society
- Train and supervise interns assisting with field and greenhouse research trials

Purdue University, West Lafayette, IN May 2016 – May 2019
Graduate Research Assistant

- Manage and evaluate field and greenhouse herbicide efficacy trials and weed management programs for herbicide resistant weeds.
- Perform laboratory procedures for herbicide resistance testing in addition to upholding proper lab etiquette and safety practices.
- Train and supervise undergraduate students and interns assisting with field, greenhouse, and laboratory research trials.
- Collaborate in organizing, conducting, and rating multiple large-scale industry field trials involving dicamba volatility and drift research.
- Create herbicide symptomology plots for graduate students in preparation for the annual North Central Weed Science Society weed contest and the Purdue Crop Diagnostic Training and Research Center for training purposes.

Graduate Teaching Assistant

- AGRY 399, Investigate Crop Weed and Grain (August 2016 –December 2017)
  - Reestablished the Purdue University Crop Judging Team.
  - Prepared study material for undergraduates to compete in the annual Collegiate Crop Judging and North American Colleges and Teachers of Agriculture Contests.
- BTNY 304, Introduction to Weed Science (August 2017 – December 2017)
  - Lectured undergraduate students during the lab section of the course in basic weed science principles including weed identification, herbicide leaching, and herbicide formulations.

BASF Midwest Research Farm, Seymour, IL May 2015 – November 2015
Field Biology Intern

- Assisted with implementation and completion of small-plot field trials related to herbicide, fungicide, and insecticide research.
- Operated farm machinery for trial establishment, planting, tillage, and mowing.

Prairieland FS, Inc., Jacksonville, IL May 2014 – August 2014
Sales Marketing Intern

- Assisted with establishment of corn and soybean variety trials.
- Conducted a customer survey throughout IL in order to increase business relations.

Monsanto, Jerseyville, IL May 2011 – August 2013
Temporary Farm Employee

- Assisted with implementation, management, and data collection of small-plot research corn breeding trials across IL.

Mansfield Acres, White Hall, IL January 2004 – present
Owner/Operator

- Perform key business decisions for the production of field corn and soybeans including soil fertility, seed selection, crop protection, and tillage
- Make market decisions for the sale of field corn and soybeans

- Operate and perform general maintenance of farm machinery related to all tasks involved in the production of field corn and soybeans

TECHNICAL SKILLS

Field

- Safe operation and general maintenance of various sizes of farm machinery involving planting, harvesting, tillage, chemical applicators, and grain transportation
- Establishment of small- and large-scale research trials
- Pesticide applications using a CO_2-pressurized backpack sprayer
- Data collection for research trials involving herbicides, fungicides, and insecticides

Greenhouse

- Large-scale genotypic and phenotypic screens of herbicide resistant weed populations
- Whole-plant bioassays for determination of GR_{50} values in tall waterhemp

Laboratory

- Genomic DNA extraction using a modified CTAB method
- Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)
- Plant oxidative stress assays using a Genesys 10 Bio UV-Visible Spectrophotometer
  - Measured total protein content, malondialdehyde for detection of lipid peroxidation, and enzymatic antioxidant levels

Information Technology

- ARM 2009: protocol development, treatment formation, and data entry and analysis
- Microsoft Office: generation of written reports and presentations
  - Excel, Outlook, Powerpoint, Word
- JMP v.13: data management and analysis
- SAS 9.4: data management and analysis
- SigmaPlot 13.0: data analysis and graphical representation

PROFESSIONAL DEVELOPMENT

- Member – Weed Science Society of America (August 2018 – present)
- Member – North Central Weed Science Society (May 2016 – present)
- Member – Botany and Plant Pathology Graduate Student Organization (August 2016 – May 2019)
- Botany and Plant Pathology Senator – Purdue Graduate Student Government (May 2017 – May 2018)
- Vice president – Field and Furrow Club, University of Illinois (December 2014 – May 2016)

- Member – American Society of Agronomy (August 2014 – May 2016)
- President – Phi Theta Kappa Honor Society, John Wood Community College (January – December 2013)
- Member – National Professional Agriculture Student Organization (August 2012 – May 2014)
- Member – Agriculture Club, John Wood Community College (August 2012 – May 2014)
- Member – Lions Clubs International, White Hall, IL (December 2012 – August 2020)

PROCEEDINGS

Oral Presentations

- **Mansfield BC**, Nie H, Young JM, Young BG (2018) Do Varying Antioxidant Enzyme Levels Following a PPO Herbicide Application Help Explain the Resulting Variable Herbicide Response in Tall Waterhemp? Page NA *in* Proceedings of the North Central Weed Science Society. Milwaukee, WI: North Central Weed Science Society.
- **Mansfield BC**, Nie H, Young JM, Bradley KW, Young BG (2017) A multi-state survey to determine the potential for resistance to PPO-inhibiting herbicides in tall waterhemp beyond the G210 target site mutation. Page 14 *in* Proceedings of the North Central Weed Science Society. St. Louis, MO: North Central Weed Science Society.
- **Mansfield BC**, Nie H, Young BG (2016) Interaction of soil residual PPO-inhibiting herbicides and *s*-metolachlor on selection of PPO-resistant waterhemp. Page 9 *in* Proceedings of the North Central Weed Science Society. Des Moines, IA: North Central Weed Science Society.

Poster Presentations

- **Mansfield BC**, Nie H, Young JM, Bradley KW, Young BG (2018) A Multi-State Survey of Tall Waterhemp Discovers a Broad Range of Sensitivity to PPO-Inhibiting Herbicides and Points to Mechanisms Other than the ΔG210 Target Site Mutation. Page NA *in* Proceedings of the North Central Weed Science Society. Milwaukee, WI: North Central Weed Science Society.
- **Mansfield BC**, Nie H, Young JM, Young BG (2017) Tall waterhemp resistance to PPO-inhibiting herbicides: does *s*-metolachlor reduce selection pressure, decrease overall survivorship, or both? Page 7 *in* Proceedings of the North Central Weed Science Society. St. Louis, MO: North Central Weed Science Society.
- **Mansfield BC**, Moretti ML, Young BG (2016) Simulated tank contamination of 2,4-D with combinations of dicamba and glyphosate applied to dicamba tolerant soybeans. Page 5 *in* Proceedings of the North Central Weed Science Society. Des Moines, IA: North Central Weed Science Society.

AWARDS

- North Central Weed Science Society
  - 1st place graduate team, NCWSS Weed Science Contest (2018)
  - 2nd place graduate team, NCWSS Weed Science Contest (2017)
  - 1st place, NCWSS Poster Contest (2018)

- 2nd place, NCWSS Poster Contest (2017)

- Illinois Soybean Association Scholar (2014)
- Honorary Macebearer – John Wood Community College (May 2014)
- 2014 USA Today All-USA Community College Academic Team Nominee – Phi Theta Kappa Honor Society, John Wood Community College
- Outstanding Returning Student – Student Government Association, John Wood Community College (2013)
- George and Sharon Borrowment Scholarship – John Wood Community College (2013)
- American FFA Degree (2013)
- Silver Medallion Award – Lewis and Clark Community College (2012)