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Herbicide Resistances in *Amaranthus tuberculatus*: A Call for New Options

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ABSTRACT: *Amaranthus tuberculatus* is a major weed of crop fields in the midwestern United States. Making this weed particularly problematic to manage is its demonstrated ability to evolve resistance to herbicides. Herbicides to which *A. tuberculatus* has evolved resistance are photosystem II inhibitors, acetolactate synthase inhibitors, protoporphyrinogen oxidase inhibitors, and glyphosate. Many populations of *A. tuberculatus* contain more than one of these resistances, severely limiting the options for effective herbicide control. A survey of multiple-herbicide resistance in *A. tuberculatus* revealed that all populations resistant to glyphosate contained resistance to acetolactate synthase inhibitors, and 40% contained resistance to protoporphyrinogen oxidase inhibitors. The occurrences of multiple-herbicide resistances in *A. tuberculatus* illustrate the need for continued herbicide discovery efforts and/or the development of new strategies for weed management.

KEYWORDS: *Amaranthus tuberculatus*, *Amaranthus rudis*, waterhemp, multiple resistance, glyphosate, acetolactate synthase, protoporphyrinogen oxidase, triazine

INTRODUCTION

Large-scale agronomic crop production systems currently depend on herbicides for weed management. A weakness in this approach lies in its strength: because herbicides are so effective, they exert tremendous selection pressures that, over time, result in resistant weed populations as natural outcomes of the evolutionary process.[1,2] There are now nearly 350 herbicide-resistant weed biotypes worldwide, and the number continues to increase.[3] Despite this, herbicides continue to be utilized intensively for weed management.

Several herbicides are labeled for use in most crops and, therefore, a herbicide-resistant weed population typically can be managed with an alternative herbicide to which the weed population has not yet evolved resistance. Furthermore, many cases of herbicide-resistant weeds are isolated occurrences that affect relatively few fields.[3] Although one species in a field may be resistant to a particular herbicide, other species in the same field remain sensitive to it. This fact, together with the often isolated nature of herbicide-resistant weed biotypes, means that resistance to a particular herbicide does not necessarily negate use of that herbicide. For example, resistance to triazine herbicides (such as atrazine) initially was documented 40 years ago,[4] and there are now 68 weed species resistant to triazines;[3] yet atrazine continues to be used on the majority of corn hectares in the United States.[5] Thus, although the threat of herbicide-resistant weeds is real, for the most part they have posed a manageable problem to date. We argue, however, that burgeoning multiple-herbicide resistance (the occurrence of resistance to more than one herbicide group in a weed biotype) in significant weed species has the potential to become an unmanageable problem with currently available tools.

Amaranthus tuberculatus (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif (common waterhemp) is a significant weed of corn and soybean throughout much of the midwestern United States. Although considered native to the region, it has been only during the past two decades that *A. tuberculatus* has risen to prominence as a Midwest weed.[6−8] Its high reproductive potential (hundreds of thousands of seeds per plant) provides a wealth of genetic variants on which herbicide selection acts, promoting evolution of resistance. As a dioecious species, and thus an obligate outcrosser, it is ideally suited for evolving multiple-herbicide resistance by sharing resistance genes among populations and biotypes via wind pollination.[6−8] In this paper, we briefly review cases of herbicide resistance in *A. tuberculatus* and present new data on the occurrence of multiple-herbicide resistance in this species. Herbicide resistance in *A. tuberculatus* appears to be on the threshold of becoming an unmanageable problem in soybean.

RESISTANCE TO PHOTOSYSTEM II (PSII)-INHIBITING HERBICIDES

Biotypes of *A. tuberculatus* resistant to PSII-inhibiting herbicides were first identified in Nebraska in 1990 [9] (Figure 1) and subsequently in other midwestern states and Canada.[3] Chlorophyll fluorescence and gene sequence data identified an altered D1 protein as a mechanism of triazine resistance in *A. tuberculatus*.[8,10] More recently it was reported that triazine resistance in *A. tuberculatus* alternatively can be conferred by a nontarget-site mechanism.[11] Segregation within half-sib populations (i.e., populations from single female plants) arbitrarily collected throughout Illinois indicated that the nontarget-site rather than the

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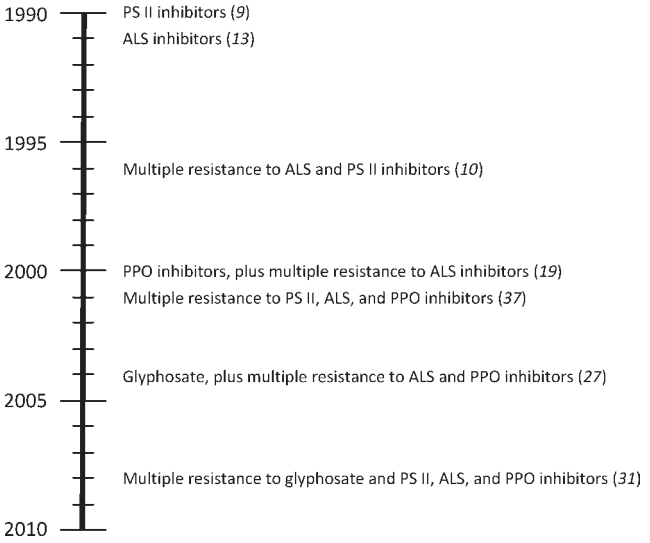

Figure 1. Chronology of resistance and multiple resistance to herbicide or herbicide groups in *Amaranthus tuberculatus*. References are shown in parentheses.

target-site mechanism was predominant among triazine-resistant *A. tuberculatus* populations in Illinois.[12]

The relatively isolated occurrence and slow spread of the triazine-resistant *A. tuberculatus* biotype were attributed to the target-site-based mechanism of resistance.[8] Specifically, this resistance mechanism is maternally inherited and, therefore, unable to be widely transmitted by pollen, and it typically has an associated fitness penalty. In contrast, the nontarget-site mechanism can be transmitted via both seed and pollen and, therefore, is expected to disseminate more rapidly. Importantly, pollen dissemination of the nontarget-site triazine resistance mechanism will enable sharing of resistance genes among *A. tuberculatus* populations, thereby fostering the evolution of multiple-herbicide resistances.

RESISTANCE TO ACETOLACTATE SYNTHASE (ALS)-INHIBITING HERBICIDES

Resistance to herbicides that inhibit ALS was identified in *A. tuberculatus* in 1991 and, within about a decade, became the norm rather than the exception within populations of this species.[12−14] In Illinois, for example, we estimate over half of *A. tuberculatus* plants in any given field are resistant to ALS inhibitors.[12] Extensive use of ALS-inhibiting herbicides, high initial frequencies of resistance-conferring mutations, and low associated fitness penalties likely contributed to widespread resistance to these herbicides in *A. tuberculatus* as well as in other weed species.[15] Furthermore, the dioecious nature of *A. tuberculatus* likely enabled particularly rapid dissemination of the resistance trait in this species.

As is the case with most weeds resistant to ALS inhibitors, resistance in *A. tuberculatus* is conferred by mutations in the *ALS* gene.[10,16] The Trp574Leu ALS mutation is most common in *A. tuberculatus*, although mutations also have been identified at the Ser653 position.[17] The Trp574Leu mutation confers broad cross-resistance among the ALS inhibitors (e.g., sulfonylureas, imidazolinones, and other classes). Because sulfonylurea and imidazolinone herbicides were widely used in midwestern corn and soybean production, it is not surprising that the Trp574Leu mutation predominates over other mutations (such as Ser653 mutations), which generally confer narrower cross-resistance patterns.[15]

RESISTANCE TO PROTOPORPHYRINOGEN OXIDASE (PPO)-INHIBITING HERBICIDES

Diphenyl ether herbicides, which inhibit PPO, were used for postemergence control of broadleaf weeds in soybean in the 1980s. Use of these herbicides in soybean greatly escalated in the 1990s, however, and was driven at least in part by the widespread occurrence of resistance to ALS inhibitors in *A. tuberculatus*.[18]

An *A. tuberculatus* biotype resistant to PPO inhibitors was identified in Kansas in 2000, representing the first case of evolved resistance to this group of herbicides.[19] Soon thereafter, other *A. tuberculatus* populations from Missouri, Illinois, and Iowa were identified with resistance to PPO inhibitors.[3]

The mechanism of resistance to PPO inhibitors was first elucidated in an Illinois biotype. Resistance was shown to result from a deletion of an amino acid codon for a glycine residue at position 210 (ΔG210) of the *PPX2* gene.[20] This gene encodes the mitochondrial isoform of PPO and, via a 5′ extension encoding a chloroplastic targeting sequence, can also encode a plastid-targeted PPO. The ΔG210 mutation was subsequently identified in other Illinois *A. tuberculatus* populations resistant to PPO inhibitors and in resistant populations from Kansas and Missouri.[21,22] Despite being an unusual mutation (a codon deletion rather than a single-nucleotide substitution), the ΔG210 mutation is the only mechanism identified to date for resistance to PPO inhibitors in *A. tuberculatus*.

Biochemical analysis and molecular modeling of the resistant enzyme provided insights into the favorable attributes of the ΔG210 mutation.[23,24] In particular, the mutation reduced herbicide sensitivity 100-fold while not appreciably affecting substrate affinity and only modestly decreasing (about 10-fold) catalytic turnover. It is thought that a short microsatellite repeat spanning the wild-type G210 codon enables the codon deletion to occur.[20,25] The presence of this microsatellite repeat and the favorable enzymatic consequences of the ΔG210 mutation may account for this mutation being, at least to date, the exclusive mechanism of resistance to PPO inhibitors in *A. tuberculatus*.

Diphenyl ether herbicides were once widely used for postemergence control of *A. tuberculatus* and other broadleaf weeds in soybean. However, when *A. tuberculatus* populations resistant to the PPO inhibitors were first reported, use of these herbicides had already declined dramatically with the widespread adoption of glyphosate-resistant soybean.[5] Thus, it is somewhat surprising that the frequency of resistance to PPO inhibitors in *A. tuberculatus* continues to increase. In Kansas, for example, a survey conducted in 2002 found resistance to PPO inhibitors in *A. tuberculatus* from over a third of sites sampled within a 16 km radius of the original site where the resistance was identified.[14] In Illinois, *A. tuberculatus* biotypes with resistance to PPO inhibitors have now been confirmed from over 30 counties (B. Young and P. Tranel, unpublished data). Continued use of PPO inhibitors applied preemergence, along with an apparent lack of associated fitness penalty,[26] likely contributes to the maintenance of the resistance allele. Regardless, it is apparent that had glyphosate-resistant soybean not become available, resistance to PPO inhibitors, coupled with the already widespread resistance to ALS inhibitors in *A. tuberculatus*, would have eliminated all

effective postemergence herbicide options available at that time for control of this species in soybean.

RESISTANCE TO GLYPHOSATE

Since farmers began relying on glyphosate in conjunction with glyphosate-resistant crops for weed control, the number of glyphosate-resistant weeds has steadily increased.[3] Glyphosate-resistant *A. tuberculatus* was identified in Missouri in 2004, at a site grown in soybean and exposed to glyphosate for at least six consecutive years.[27] Glyphosate-resistant *A. tuberculatus* biotypes have now been confirmed additionally from Illinois, Kansas, Minnesota, Iowa, and Mississippi.[3] As with other glyphosate-resistant weeds, the level of resistance in *A. tuberculatus* is modest (about 10- vs >100-fold for many other types of herbicide resistance). This fact, along with *A. tuberculatus*'s naturally variable responses to many herbicides, has sometimes blurred the distinction between glyphosate resistance and tolerance. For example, Zelaya and Owen [28] reported an *A. tuberculatus* population that was not effectively controlled by glyphosate in 1998, the first year the producer adopted glyphosate-resistant soybean. Whether this population had evolved glyphosate resistance in response to selection or was naturally tolerant to glyphosate is unclear. Subsequent selection experiments, however, demonstrated that the decreased glyphosate sensitivity was genetically inherited, although likely as a multigenic trait.

Even among *A. tuberculatus* biotypes that likely evolved glyphosate resistance in response to selection, the mechanisms underlying resistance are not known, nor is the nature of inheritance clear. Preliminary data [29] suggest the glyphosate-resistant biotype identified in Missouri contains increased copy numbers of *EPSPS* (the glyphosate target-site gene), although not to the extent that has been reported in glyphosate-resistant *Amaranthus palmeri*.[30] More research is needed to fully understand the glyphosate resistance mechanism in *A. tuberculatus*. Regardless of the underlying resistance mechanism, it is clear that glyphosate-resistant *A. tuberculatus*, as well as other glyphosate-resistant weeds, poses a serious threat to current cropping systems, which are dominated by glyphosate-resistant crops and heavily dependent on glyphosate for weed control.

Of the herbicide resistances that have evolved in *A. tuberculatus*, resistance to glyphosate is the only one to evolve in a transgenic-based cropping system (e.g., glyphosate-resistant crops).[27] The other herbicide resistances were selected in non-transgenic crops. From a management standpoint, however, this distinction likely is of little significance. Regardless of the system in which resistance was selected, the end result is one less alternative for managing the weed.

MULTIPLE-HERBICIDE RESISTANCE

Resistance in a weed population to one herbicide (or to a group of herbicides having the same site of action) typically is overcome by using a herbicide having a different site of action. For example, as stated previously, resistance to ALS inhibitors in *A. tuberculatus* was largely managed by replacing these herbicides with PPO inhibitors. Iteration of this process, however, can lead to the accumulation within a population of different resistance alleles for different herbicides. As a consequence, effective herbiscide options become more and more limited.

Subsequent to the identification of *A. tuberculatus* biotypes resistant to either PSII inhibitors or ALS inhibitors, all new cases of resistance in this species have been associated with multiple resistance (Figure 1). A biotype resistant to both ALS and PSII inhibitors was identified in 1996.[10] When resistance to PPO inhibitors was identified in *A. tuberculatus*, the population was also found to have resistance to ALS inhibitors.[19] Similarly, when the first glyphosate-resistant *A. tuberculatus* biotype was identified, the population contained resistance to ALS and PPO inhibitors.[27] In the most extreme case to date with *A. tuberculatus*, a population was identified in Illinois with resistance to four different herbicides/herbicide groups: PSII inhibitors, ALS inhibitors, PPO inhibitors, and glyphosate.[31] Using combinations of herbicide efficacy studies and molecular assays for resistance genes, it was further determined that some plants within this population contain all four resistances. In other words, all four resistances are present not only within the population but also within individual plants. This is significant in that if the four resistances were only at the population and not at the individual plant level, then a tank mix of multiple herbicides could provide effective control. In the case of this particular population, however, even a tank mix of herbicides from the four different sites of action would not provide effective control.

The occurrence of multiple resistance in *A. tuberculatus* particularly threatens the ability of producers to manage this weed in soybean. Although there remain several soybean preemergence herbicides that are effective on *A. tuberculatus*, continuing seedling emergence of this species relatively late into the growing season often necessitates the use of postemergence herbicides for adequate control.[32−34] Herbicides that can be used postemergence in soybean and may potentially control *A. tuberculatus* are limited to diphenyl ethers (e.g., lactofen), ALS inhibitors (e.g., imazethapyr), glyphosate, and glufosinate (the latter two requiring the use of resistant crop varieties). Of these, glufosinate is the only one for which resistance in *A. tuberculatus* has not yet been reported.

Farmers currently rely largely on glyphosate for postemergence control of *A. tuberculatus* in soybean. As glyphosate-resistant *A. tuberculatus* becomes more common, we expect this approach to result in an increasing number of control failures. With this in mind, we conducted research to examine the occurrence of multiple resistance within glyphosate-resistant *A. tuberculatus* populations. The specific practical question we wanted to address was, if glyphosate fails to adequately control a specific *A. tuberculatus* population, what is the likelihood that alternative postemergence options (ALS inhibitors or diphenyl ethers) would be effective? We did not consider glufosinate in this research, because resistance has not yet evolved to this herbicide. Plus, in a current-year rescue situation, glufosinate would not be an option on glyphosate-resistant soybean.

A total of 93 individual plants were assayed from 18 fields (1 from Missouri, 17 from multiple counties in Illinois). Glyphosate-resistant *A. tuberculatus* had been confirmed or was suspected (on the basis of glyphosate efficacy) in most of these fields. Tissue samples from plants were either collected by ourselves or provided to us by producers or weed management professionals. DNA extracted from the tissue samples was subjected to molecular assays for resistance to ALS inhibitors (Trp574Leu *ALS* mutation), PPO inhibitors (ΔG210 *PPX2* mutation), and glyphosate (*EPSPS* amplification), essentially following published procedures.[21,30,35] Resulting data will underestimate resistance to any one of the herbicide/herbicide groups if a resistance mechanism other than the one assayed was present within the populations. The trade-off for this limitation is that the molecular assays required only leaf tissue (which is relatively

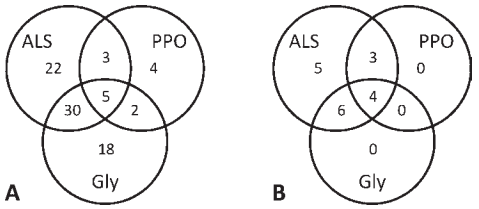

Figure 2. Venn diagrams illustrating multiple-herbicide resistance in *Amaranthus tuberculatus*. Ninety-three individual plants collected from 18 fields were screened for resistance to ALS inhibitors, PPO inhibitors, or glyphosate using molecular assays. The numbers of plants resistant to each of the three herbicide/herbicide groups and their combinations are shown in panel **A**. The occurrence of resistance/multiple resistance at the field population level is shown in panel **B**. Note that in panel **B**, a field population was scored, for example, as having multiple resistance to both ALS and PPO inhibitors if at least one plant from the population was identified as resistant to each of these two herbicide groups, regardless of whether the two resistances were present in the same plant.

insensitive to storage and shipping conditions) as sample material, and they allowed for simultaneous detection of the three different resistances within single plants. Furthermore, on the basis of information that was discussed above, we expect the molecular alterations assayed to comprise the majority of resistance cases for each of the three respective herbicide/herbicide groups.

Results of the assays for multiple resistance are summarized in Figure 2. Eight of the fields were found to not contain glyphosate-resistant *A. tuberculatus*. Although this might indicate there is an alternative glyphosate-resistance mechanism in this species, we have found it not uncommon that a producer suspects resistance when, in fact, poor control was due to other factors. As expected, a majority of the plants contained resistance to ALS inhibitors. Resistance to this group of herbicides was present in every field and, therefore, an ALS-inhibiting herbicide would not be an effective option on a glyphosate-resistant *A. tuberculatus* population. Resistance to PPO inhibitors was found in 14% of the plants, which came from seven different fields. Four of these fields were the same as those that contained glyphosate-resistant *A. tuberculatus*. On the basis of these findings, the odds are nearly 50−50 that a glyphosate-resistant *A. tuberculatus* population from Illinois will also contain resistance to PPO inhibitors. Overall, only 9 of the 93 total plants were identified as being sensitive to all three herbicides, and 5 were identified that were resistant to all three herbicides.

The multiple-resistance survey data just described illustrate the problem of multiple herbicide resistance in *A. tuberculatus*. Glufosinate may soon be the only effective postemergence herbicide option for *A. tuberculatus* control in soybean, and this herbicide has several limitations. These limitations include contact-like activity (making efficacy dependent on thorough spray coverage), lack of soil residual activity, and restrictive application timing relative to weed size.[36] Thus, a species such as *A. tuberculatus*, which exhibits rapid growth rates, a prolonged seedling emergence pattern, and high population densities, can be challenging to effectively control with glufosinate. Furthermore, on the basis of *A. tuberculatus*'s history, there is no reason to expect it will not evolve resistance to glufosinate if this herbicide is widely used. If this happens, and no new soybean postemergence herbicides are commercialized, soybean production may not be practical in many Midwest U.S. fields.

To be sure, *A. tuberculatus* is not the only weed to have evolved multiple-resistant populations and biotypes. Other members of the *Amaranthus* genus, *Lolium* spp., *Avena fatua*, and *Kochia scoparia*, are but a few of the others.[3] Although the affected crops and specific herbicides would vary, ongoing evolution of multiple-herbicide resistance in these weeds would lead us to the same conclusion. We urgently need new herbicide options or a new weed management paradigm if we are to maintain our ability to effectively manage weeds such as *A. tuberculatus* in large-scale crop production systems.

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ABBREVIATIONS USED

ALS, acetolactate synthase; PPO, protoporphyrinogen oxidase; PSII, photosystem II.

REFERENCES

(1) Maxwell, B. D.; Roush, M. L.; Radosevich, S. R. Predicting the evolution and dynamics of herbicide resistance in weed populations. *Weed Technol.* **1990**, *4*, 2–13.

(2) Jasieniuk, M.; Brûlé-Babel, A. L.; Morrison, I. N. The evolution and genetics of herbicide resistance in weeds. *Weed Sci.* **1996**, *44*, 176–193.

(3) Heap, I. The International Survey of Herbicide Resistant Weeds, available at http://www.weedscience.com (accessed June 7, 2010).

(4) Ryan, G. F. Resistance of common groundsel to simazine and atrazine. *Weed Sci.* **1970**, *18*, 614–616.

(5) National Agricultural Statistics Service Agricultural Chemical Use Database, available athttp://www.pestmanagement.info/nass/ (accessed June 7, 2010).

(6) Costea, M.; Weaver, S. E.; Tardif, F. J. The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif. *Can. J. Plant Sci.* **2005**, *85*, 507–522.

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

(8) Tranel, P. J.; Trucco, F. 21st-century weed science: a call for *Amaranthus* genomics. In *Weedy and Invasive Plant Genomics*; Stewart, C. N., Jr., Ed.; Blackwell: Ames, IA, 2009; pp 53−81.

(9) Anderson, D. D.; Roeth, F. W.; Martin, A. R. Occurrence and control of triazine-resistant common waterhemp (*Amaranthus rudis*) in field corn (*Zea mays*). *Weed Technol.* **1996**, *10*, 570–575.

(10) Foes, M. J.; Liu, L.; Tranel, P. J.; Wax, L. M.; Stoller, E. W. A biotype of common waterhemp (*Amaranthus rudis*) resistant to triazine and ALS herbicides. *Weed Sci.* **1998**, *46*, 514–520.

(11) Patzoldt, W. L; Dixon, B. S.; Tranel, P. J. Triazine resistance in *Amaranthus tuberculatus* (Moq) Sauer that is not site-of-action mediated. *Pest Manag. Sci.* **2003**, *59*, 1134–1142.

(12) Patzoldt, W. L.; Tranel, P. J.; Hager, A. G. Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. *Crop Prot.* **2002**, *21*, 707–712.

(13) Horak, M. J.; Peterson, D. E. Biotypes of Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*) are resistant to imazethapyr and thifensulfuron. *Weed Technol.* **1995**, *9*, 192–195.

(14) Falk, J. S.; Shoup, D. E.; Al-Khatib, K.; Peterson, D. E. Survey of common waterhemp (*Amaranthus rudis*) response to protox- and ALS-inhibiting herbicides in northeast Kansas. *Weed Technol.* **2005**, *19*, 838–846.

(15) Tranel, P. J.; Wright, T. R. Resistance to ALS-inhibiting herbicides: what have we learned. *Weed Sci.* **2002**, *50*, 700–712.

(16) Woodworth, A. R.; Rosen, B. A.; Bernasconi, P. Broad range resistance to herbicides targeting acetolactate synthase (ALS) in a field isolate of *Amaranthus* sp. is conferred by a Trp to Leu mutation in ALS gene. *Plant Physiol.* **1996**, *111*, 1353.

(17) Patzoldt, W. L.; Tranel, P. J. Multiple ALS mutations confer herbicide resistance in waterhemp (*Amaranthus tuberculatus*). *Weed Sci.* **2007**, *55*, 421–428.

(18) McGlamery, M. D.; Knake, E. L. 50 years of weed science in Illinois. *1998 Proceedings of the Illinois Agricultural Pest Conference*; University of Illinois College of Agricultural, Consumer and Environmental Sciences Cooperative Extension Service: Urbana, IL, **1998**, pp 81−86.

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

(20) Patzoldt, W. L.; Hager, A. G.; McCormick, J. S.; Tranel, P. J. A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12329–12334.

(21) Lee, R. M.; Hager, A. G.; Tranel, P. J. Prevalence of a novel resistance mechanism to PPO-inhibiting herbicides in waterhemp (*Amaranthus tuberculatus*). *Weed Sci.* **2008**, *56*, 371–375.

(22) Thinglum, K. A.; Riggins, C. W.; Tranel, P. J.; Bradley, K. W.; Al-Khatib, K. Preponderance of the protoporphyrinogen oxidase glycine deletion in waterhemp resistant to PPO inhibitors. *North Central Weed Sci. Soc. Abstr. [CD-ROM Computer File]* **2009**, *64*, 96.

(23) Hao, G. F.; Zhu, X. L.; Ji, F. Q.; Zhang, L.; Yang, G. F.; Zhan, C. G. Understanding the mechanism of drug resistance due to a codon deletion in protoporphyrinogen oxidase through computational modeling. *J. Phys. Chem. B* **2009**, *113*, 4865–4875.

(24) Dayan, F. E.; Daga, P. R.; Duke, S. O.; Lee, R. M.; Tranel, P. J.; Doerksen, R. J. Biochemical and structural consequences of a glycine deletion in the α-8 helix of protoporphyrinogen oxidase. *Biochim. Biophys. Acta* **2010**, *1804*, 1548–1556.

(25) Gressel, J.; Levy, A. A. Agriculture: the selector of improbable mutations. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12215–12216.

(26) Duff, M. G.; Al-Khatib, K.; Peterson, D. E. Relative competitiveness of protoporphyrinogen oxidase-resistant common waterhemp (*Amaranthus rudis*). *Weed Sci.* **2009**, *57*, 169–174.

(27) Legleiter, T. R.; Bradley, K. W. Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. *Weed Sci.* **2008**, *56*, 582–587.

(28) Zelaya, I. A.; Owen, M. D. K. Differential response of *Amaranthus tuberculatus* (Moq ex DC) JD Sauer to glyphosate. *Pest Manag. Sci.* **2005**, *61*, 936–950.

(29) Bell, M. S.; Tranel, P. J.; Riggins, C. W. Glyphosate resistance in waterhemp: inheritance and EPSPS copy number. *North Central Weed Sci. Soc. Abstr. [CD-ROM Computer File]* **2009**, *64*, 100.

(30) Gaines, T. A.; Zhang, W.; Wang, D.; et al. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 1029–1034.

(31) Bell, M. S.; Tranel, P. J.; Hager, A. G. Introducing quad-stack waterhemp: populations containing individuals resistant to four herbicide modes of action. *North Central Weed Sci. Soc. Abstr. [CD-ROM Computer File]* **2009**, *64*, 40.

(32) Hartzler, R. G.; Buhler, D. D.; Stoltenberg, D. E. Emergence characteristics of four annual weed species. *Weed Sci.* **1999**, *47*, 578–584.

(33) Hartzler, R. G.; Battles, B. A.; Nordby, D. Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. *Weed Sci.* **2004**, *52*, 242–245.

(34) Vyn, J. D.; Swanton, C. J.; Weaver, S. E.; Sikkema, P. H. Control of herbicide-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) with pre- and post-emergent herbicides in soybean. *Can. J. Plant Sci.* **2007**, *87*, 175–182.

(35) Foes, M. J.; Liu, L.; Vigue, G.; Stoller, E. W.; Wax, L. M.; Tranel, P. J. A kochia (*Kochia scoparia*) biotype resistant to triazine and ALS-inhibiting herbicides. *Weed Sci.* **1999**, *47*, 20–27.

(36) Green, J. M.; Owen, M. D. K. Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. *J. Agric. Food Chem.* DOI: 10.1021/jf101286h.

(37) Patzoldt, W. L.; Tranel, P. J.; Hager, A. G. A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. *Weed Sci.* **2005**, *53*, 30–36.