MOLECULAR MODELING AND BIOCHEMICAL EFFECTS OF A GLYCINE DELETION IN WATERHEMP PROTOPORPHYRINOGEN OXIDASE. Patrick J. Tranel, Ryan M. Lee, Franck E. Dayan, Stephen O. Duke, Pankaj R. Daga, and Robert J. Doerksen, Professor and Postdoctoral Research Assistant, Department of Crop Sciences, University of Illinois, Urbana, IL 61801, Research Leader and Research Plant Physiologist, USDA-ARS National Center for Natural Products Research, University, MS 38677, Graduate Research Assistant and Assistant Professor, Department of Medicinal Chemistry, University of Mississippi, University, MS 38677.

Resistance to PPO-inhibiting herbicides in waterhemp previously was determined to be due to a deletion of a glycine codon (ΔG210) in the *PPX2* gene. Despite being an unusual mutation, it has been identified in multiple waterhemp populations with resistance to these herbicides and, in fact, is the only mechanism thus far identified for resistance to PPO inhibitors in waterhemp. Biochemical experiments with mutant and wild-type enzyme were conducted to determine the effects of the ΔG210 mutation on enzyme kinetics. Consistent with whole-plant data, the mutant enzyme conferred an approximately 200-fold level of resistance to PPO inhibitors in vitro. Although the mutation did not affect binding affinity for the substrate, it decreased the catalytic efficiency approximately 10-fold. Unexpectedly, the mutation also changed herbicidal inhibition from competitive to mixed-type inhibition. Molecular modeling and molecular dynamics simulation revealed that the ΔG210 mutation destabilized the capping region of an alpha helix that is in close proximity to the active site. This was predicted to cause an increase in the distance between bound substrate and the isoalloxazine ring of the FAD cofactor, which could account for the decreased reaction rate, and an increase in the size of the active site, which could account for the change to mixed-type – rather than strictly competitive – inhibition. These results provide further insights as to why the ΔG210 mutation appears to be the most favored evolutionary path to achieve resistance to PPO inhibitors in waterhemp.