Uncovering the Mechanism of Resistance to Propanil in Ricefield Bulrush (Schoenoplectus mucronatus (L.) Palla) from Rice Fields of California.

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Schoenoplectus mucronatus (L.) Palla (ricefield bulrush; SCPMU) is a problematic annual weed (Cyperaceae) of rice in 43 countries. In California, SCPMU management was complicated by the evolution of resistance to acetolactate-synthase (ALS)-inhibiting herbicides in 1997; ALS-resistant (R) populations are now widespread throughout CA rice fields. In the wake of resistance to ALS inhibitors, applications of the post emergent photosystem II (PSII)-inhibiting herbicide propanil (3, 4-dichlopropionanilide) were increased to control ALS-R SCPMU and other weeds of rice. Lack of proper control following propanil spraying was detected in 2012 suggesting resistance to this herbicide might have also evolved in some SCPMU populations. The objectives of this research were to confirm resistance to propanil, ascertain resistance levels, and establish the underlying mechanisms of resistance in SCPMU biotypes collected in rice fields of California. Our results indicate biotypes derived from field-collected populations displayed a high level of resistance to propanil (R/S ratio equaled 6.5). When rice cv. M-206 and propanil-susceptible (S) and –R SCPMU were sprayed with propanil jointly with the insecticide carbaryl (a known propanil synergist that inhibits propanil degradation in plants), all plant species except propanil-R SCPMU experienced significant growth suppression, suggesting propanil metabolism is not the mechanism of resistance in the R biotypes used. Afterwards, experiments were conducted to determine whether or not P450 monooxygenases and esterases are involved as a mechanism of resistance to propanil. Since such enzymes and inhibited by the organophosphate insecticide malathion, propanil was sprayed jointly with this herbicide onto rice cv. M-206 and propanil-R and –S SCPMU biotypes. Results indicated a 48% decrease in the resistance level of R biotypes (which was not detected in S biotypes or rice) and thus suggested involvement of either P450s or esterases; inhibition of these enzymes, however, did not yield results of similar magnitude to those reported for other propanil-R weeds displaying metabolic resistance, and could be a secondary resistance mechanism. Interestingly, propanil-R biotypes were found to be cross-resistant to other PSII-inhibiting herbicides (diuron, atrazine, bromoxynil, and metribuzin), although resistance to atrazine is weak. These results suggested propanil resistance might involve the PSII-inhibitor binding site at the target protein D1 of PSII. Therefore, we sequenced the herbicide-binding region of the chloroplast \textit{psbA} gene, which codes for propanil’s target site (e.g. the D1 protein), where a valine to isoleucine substitution at amino acid residue 219 was identified. This mutation had already been identified in \textit{Poa annua} biotypes resistant to diuron and metribuzin and in propanil-R \textit{Cyperus difformis} from California, and is not associated with resistance to atrazine in agreement with our results. Therefore, unlike resistance in grasses and selectivity in rice - at which resistance is attributed to enhanced propanil degradation, the mechanism of resistance to propanil in SCPMU from CA resembles propanil resistance recently discovered in another weedy sedge (\textit{Cyperus difformis}) and is endowed by a single mutation at the D1 protein, which affects binding of propanil at its target-site. For control of propanil-R SCPMU (and given the widespread resistance to ALS inhibitors in
CA rice fields), it is thus necessary to switch herbicide modes of action away from PSII and ALS inhibitors, and prevent spread of resistant populations by preventing seed contamination by performing proper cleaning of tillage and harvest machinery. Further research has also indicated that other herbicides used in rice are effective against propanil-R SCPMU, such as carfentrazone, benzobicyclon, and thiobencarb. Since applications of malathion and propanil in combination decreased the biomass of propanil-R SCPMU but not rice cv. M-206, future research will be carried out in the field to evaluate the feasibility of use of this mixture as an option for management of propanil-R ricefield bulrush.