

Dinitroaniline herbicide resistance evolution in *Lolium rigidum*

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Summary Dinitroaniline herbicides have been used for pre-emergence weed control for decades. Trifluralin is widely used in Australia as one of the most important pre-emergence herbicides to control annual ryegrass (*Lolium rigidum*) populations. However, periodic herbicide resistance surveys in southern Australia indicate that trifluralin resistance evolution is on the increase but is relative slow compared to resistance to major post-emergence herbicides. Like resistance to other herbicide modes of action, both target-site and non-target-site resistance mechanisms have been identified in some trifluralin-resistant ryegrass populations. A recent resistance mechanism survey with more than 20 field-evolved resistant populations from Western Australia suggests target-site resistance to trifluralin is more prevalent than non-target-site resistance. Based on this survey, novel resistance mutations of I235M, L238L, R243S and V252M have also been identified and functionally characterized in transgenic rice, in addition to previously identified resistance mutations of V202F, T239I, R243M and R243K in ryegrass. Among them, the V202F mutation exhibiting a fitness advantage is the most

frequent one and resistance with this mutation is inherited as a recessive trait. Other resistance mutations occur at low frequencies, particularly the R243M mutation causing plant helical growth that is nearly lethal. Based on our work, relatively slow evolution of high-level dinitroaniline herbicide resistance in ryegrass is likely associated with recessive target-site resistance due to the existence of multiple target tubulin isoforms, and low frequencies of multiple resistance alleles possibly due to associated fitness costs. Other contributing factors slowing resistance include plants surviving the pre-emergence herbicide treatment early in the season controlled by the application of post-emergence herbicides as well as by non-chemical control measures, and use of mixtures of pre-emergence herbicides of different modes of action that significantly improves weed control efficacy. Together, these factors help delay and mitigate trifluralin resistance evolution.

Keywords Dinitroaniline herbicides, trifluralin, ryegrass, tubulin mutations, genetic transformation, fitness advantage/cost