SIMAZINE RESISTANCE IN *LOLIUM RIGIDUM*

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Abstract. A biotype of annual ryegrass, *Lolium rigidum* Gaud., is resistant to amitrole, triazine herbicides, metribuzin and substituted urea herbicides. Resistance occurred after exposure to amitrole and atrazine in combination for ten years as part of a program of weed control on railway lines. Studies of the degree of resistance of this biotype to various herbicides revealed that resistance to atrazine was relatively low (four-fold increase in L.D. 50 when compared with the susceptible biotype of *L. rigidum*) whereas resistance to simazine, a herbicide to which the biotype had not been exposed, was relatively higher (eight-fold increase in L.D. 50). Simazine differs from atrazine in that simazine has two ethylamino side-chains while atrazine has an ethylamino and an isopropylamino side-chain. Previous studies on the metabolism of triazine herbicides indicated that the ethylamino sidechain was more readily cleaved than the isopropylamino sidechain. It was hypothesized that the greater resistance to simazine than atrazine in this biotype of annual ryegrass could be due to the greater number of more easily cleaved side-chains in simazine.

The triazine herbicides are inhibitors of photosystem II and resistance to these herbicides is commonly caused by an alteration of the target site in photosystem II. Changes in the target site in photosystem II can be detected by observing the effect of the herbicides on oxygen evolution *in vitro*. Thylakoids isolated from the resistant biotype displayed no decline in sensitivity to simazine or atrazine when compared with those from the susceptible biotype, suggesting that the basis of triazine resistance is not related to changes in the target site for these herbicides.

Resistance mechanisms other than changes at the target site include reduced uptake and translocation or enhanced metabolism of the herbicide. The uptake of ^{14}C labelled simazine from nutrient solution by susceptible and resistant plants was found to be similar and was related to transpiration rates. The distribution of label between roots and shoots was found to be the same in both biotypes. In pulse-chase experiments 4-5% of total activity recovered from the plants was found in the roots and this did not change with time after the end of the pulse, indicating that a proportion of absorbed simazine is bound in the roots in both biotypes. Studies on the distribution of simazine within the shoot tissue are yet to be made.

The metabolism of ^{14}C labelled simazine was studied using two methods of plant dosing. In the first dosing method the plants were exposed to nutrient solution containing 24 µM simazine for the full twelve hours of their photoperiod. The roots of the plants were then washed and plants were harvested at regular intervals after the start of the pulse. Plants were ground in liquid nitrogen and extracted with methanol/water. The extracts were dried and resuspended in 5% acetonitrile in water. Metabolites were separated and quantified using reverse phase HPLC with an on-line radioactivity monitor. The resistant plants produced an intermediately polar primary metabolite (putatively de-ethylsimazine) and a polar metabolite (putatively dide-ethylsimazine) and other unidentified polar metabolites at a greater rate than the susceptible biotype. The major metabolites are tentatively identified on the basis of cochromatography with chemically synthesised simazine metabolites. At least ten different labelled peaks have been observed indicating that many detoxification reactions may occur to a minor extent. The second dosing method used continual exposure to 3 µM simazine (a sub-lethal concentration for the resistant plants) over six days. Harvests were made 12, 24, 48, 72, 96, 120 and 144 hours after the start of dosing. Uptake of simazine during this period was linear with time in both biotypes. In susceptible plants the amount of radioactivity recovered as simazine increased with time while in the resistant plants the amount of unmetabolised simazine remained lower and constant. This result demonstrates that minor differences in rates of metabolism can lead to large differences in levels of active herbicide remaining within the plant.

From these results it is apparent that there is no difference between the susceptible and resistant biotypes in the effect of simazine on the target site. There is also no difference in the uptake of the herbicide. There is, however, an increase in the rate of metabolism of simazine in the resistant plants and we suggest that this is the major component of the resistance mechanism.