THE MODE OF ACTION, METABOLISM AND SELECTIVITY OF DICLOFOP-METHYL BETWEEN RESISTANT AND SUSCEPTIBLE PLANTS

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Abstract. Aryloxyphenoxy alkanoic acid esters and related analogues are post-emergence herbicides that are selective for grasses (monocots) in broad-leaved (dicots) crops. Diclofop-methyl (DM) is an exception that controls wild oat (*Avena fatua*) and other grasses in wheat (*Triticum aestivum*). Annual ryegrass (*Lolium rigidum*) is also controlled in wheat but biotypes resistant to DM have appeared in Australia and are becoming widespread. The development of cross resistance by these ryegrass biotypes towards other related and unrelated herbicides is becoming a serious problem.

There are two mechanisms for DM selectivity between plants: (a) differential metabolism and detoxification (unrelated to mechanisms of action), and (b) site-based resistance (directly related to mechanisms of action). DM is rapidly hydrolyzed to the acid, diclofop, in both resistant and susceptible species. Aryl hydroxylation catalysed by a cytochrome P-450 mono-oxygenase and subsequent glucosylation constitutes the primary mechanism for detoxification of DM in resistant species such as wheat. In susceptible grasses such as wild oat diclofop is rapidly conjugated to a glucose ester conjugate that is susceptible to hydrolysis intracellularly. The hydrolysis of the ester conjugate regenerates the phytotoxic diclofop. Therefore, in susceptible plants metabolism of diclofop is not a true detoxification mechanism. Resistant broad-leaved plants are site-resistant to both the biophysical mechanism (depolarization of the plasmalemma) and inhibition of fatty acid synthesis (biochemical mechanism - inhibition of acetyl-CoA carboxylase). Therefore, dicots are totally resistant to DM and related analogues irrespective of their metabolism of the graminicides.

Two mechanisms of action have been proposed for DM: (1) a biophysical mechanism involving the pertubation of the transmembrane proton gradient and (2) a biochemical mechanism involving the inhibition of acetyl-CoA carboxylase (ACCase) which affects lipid biosynthesis. The biophysical mechanism affects the functional aspects of the plasmalemma by interfering with the energy transduction mechanism that is vital to the growth and maintenance of all plant cells. The interference with the proton gradient also affects short-term auxin mediated cell responses. The biochemical mechanism affects the structural aspects of the membrane by interfering with the synthesis of new membranes and maintenance of existing membranes.

The meristematic zones of grasses (root and shoot apex, intercalary meristem) are the sensitive sites of action for DM. Rapid growth inhibition together with chlorosis of mature green tissues are the symptoms of early phytoxicity by DM. The most characteristic response to DM and its related analogues in susceptible grasses is the reversal or antagonism of the phytotoxic herbicide action by IAA and auxinic compounds such as 2, 4-D, MCPA and dicamba. Intact plants sprayed with DM will recover if chlorosis is not accompanied by growth inhibition since newly emerging leaves are usually unaffected. The application of 2, 4-D with DM at herbicidal field rates overcomes the growth inhibition but not the chlorosis to allow plants to recover and survive the action of DM.

Both the R(+) and S(-) enantiomers of DM are herbicidally active when applied pre- and post-emergence to intact susceptible grasses. However, in intact oat plants and oat root bioassay the S(-) enantiomer was less active than the R(+) enantiomer at lower field rates and physiological concentrations, respectively. Both enantiomers actively depolarized (biophysical) the membrane potential of oat root tip cells, whereas only the R(+) and not the S(-) enantiomer inhibited ACCase. Only the R(+) and not the S(-) enantiomer caused chlorosis of mature green tissues. Both enantiomers inhibited oat seedling growth when applied in close proximity to the apical meristems, but the S(-) enantiomer was not as inhibitory as the R(+) enantiomer at equimolar concentrations. Growth inhibition by both enantiomers in intact plants was overcome by 2, 4-D, but chlorosis due to the R(+) enantiomer was not prevented by 2, 4-D. The results indicate

that the R(+) enantiomer interferes with both mechanisms but only the biophysical mechanism is affected by the S(-) enantiomer.

Growth inhibition is probably due to the biophysical mechanism whereas chlorosis is due to the biochemical mechanism. Lipid biosynthesis is localised primarily in chloroplasts and plastids of non-green tissues and the chloroplasts are the organelles most sensitive to ultrastructural damage due to DM. Both mechanisms interact in the cells of sensitive meristematic zones when plants are treated with the racemic mixture to produce the severe phytotoxic response observed in susceptible plants. The biophysical or the biochemical mechanism alone cannot account for the severe phytotoxicity of DM.

Mung bean root tip cells are insensitive to the depolarizing activity of DM. ACCase from dicots are also reported to be insensitive to inhibition by the R(+) enantiomer. Therefore, dicots are site insensitive to both the biophysical and biochemical mechanisms. Growth inhibition and chlorosis are not observed in resistant broad-leaved plants.

The metabolism and detoxification of DM does not differ greatly between resistant and susceptible biotypes of annual ryegrass. The sensitivity to inhibition by diclofop of ACCase from susceptible and resistant ryegrass biotypes is reportedly similar. If so, DM resistance in ryegrass is not metabolism- or site-based resistance. However, both biotypes of ryegrass are susceptible to the depolarisation of the plasma membrane by diclofop. The recovery or repolarisation response occurs more readily in the resistant than in the susceptible biotype upon removal of the herbicide. The membrane potential of depolarised root tip cells of the susceptible biotype recovered just as rapidly as the resistant biotype if 2, 4-D was added immediately upon the removal of diclofop. The application of 2, 4-D with DM reversed the phytotoxic action of DM on intact susceptible ryegrass plants. It is yet unknown if the ability to restore the membrane to its functional state is a significant factor in the resistance of the ryegrass biotype to DM.

The coleoptile parenchyma cells and root tips of oat may be treated with [^{14}C] acetate to monitor simultaneously the effects of the biophysical and biochemical mechanisms. The uptake of acetate is dependent on pH at the membrane surface which is influenced by the effect of diclofop on the transmembrane proton gradient (biophysical mechanism). The incorporation of absorbed [^{14}C] acetate into the lipid fraction is a measure of the inhibition of ACCase by diclofop (biochemical mechanism). The ACCase is very sensitive to diclofop *in vitro* but in intact tissues the biophysical and biochemical effects do not seem to differ greatly in sensitivity. Significant effects on inhibition of acetate uptake and incorporation into lipids are observed only between 50 and 100µm concentrations of diclofop. The inhibition of lipid biosynthesis appears to recover more readily within a 3 hour period whereas the effects of the biophysical mechanism are maintained over a similar period. The interaction between the two mechanisms in young, actively-growing cells in the meristems of susceptible plants will require further study.