EFFECT OF HERBICIDES ON THE MEMBRANE POTENTIAL OF COLEOPTILE CELLS FROM SUSCEPTIBLE AND CROSS-RESISTANT BIOTYPES OF ANNUAL RYEGRASS (*LOLIUM RIGIDUM*)

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INTRODUCTION

Throughout Australia biotypes of annual ryegrass (*Lolium rigidum*) have developed resistance to the aryloxyphenoxypropionate herbicide diclofop-methyl (cf. 7). All biotypes documented to date also exhibit resistance to other aryloxyphenoxypropionate graminicides. Many also exhibit cross-resistance to a variety of cyclohexanedione, sulfonylurea, imidazolinone and/or dinitroaniline herbicides (2, 3). The mechanisms of resistance and cross-resistance are unknown.

Although the aryloxyphenoxypropionate and cyclohexanedione graminicides are known to inhibit the plastidic enzyme acetyl CoA carboxylase (ACCase) no differences in herbicide sensitivity have been observed for this enzyme from susceptible and resistant ryegrass (5). The sulfonylurea herbicide chlorsulfuron is more rapidly detoxified in resistant plants than in susceptible plants (1) but biotype-specific differences in the capacity to deactivate diclofop are small (4).

In addition to effects on ACCase the aryloxyphenoxypropionate graminicide diclofop and the cyclohexanedione graminicide sethoxydim can depolarise plant cell membranes (11, 12). We report here on the capacity of susceptible and resistant biotypes of *L. rigidum* to respond to membrane depolarisation.

RESULTS AND DISCUSSION

The effect of aryloxyphenoxypropionates and cyclohexanediones on membrane potentials of susceptible and resistant ryegrass coleoptiles.

Microelectrodes, inserted into individual cells of peeled ryegrass coleoptiles, were used to measure membrane potentials. The overall membrane potentials (most probably between vacuole and external solution) of populations of coleoptile cells were measured whilst bathing the coleoptile with either a standard solution or solutions containing herbicides. In both the susceptible and the resistant biotype (VLRS 1 and SR 31) diclofop acid, the herbicidally active form of diclofop-methyl, rapid depolarised membrane potentials. The putatative diffusion potential of -30 to -40 mV was approached within 10 to 20 minutes (Fig. 1). A striking, biotype-dependent response followed the removal of diclofop acid from the external solution. The membranes in the susceptible plants remained depolarised (Fig. 1 A) for at least 90 min whereas the membranes in the resistant biotype were capable of recovering full polarity. Repolarisation occurred within 15 min (Fig. 1 B). Essentially similar biotype-specific patterns of recovery were observed for the aryloxyphenoxypropionates haloxyfop acid and fluazifop acid, and for the cyclohexanedione sethoxydim (Fig. 2).

For diclofop acid a concentration of 1 and 3 to 5 μM was required for half maximum depolarisation of plasmamembranes from the resistant and susceptible biotype, respectively. Maximum depolarization was observed at 50 μM.

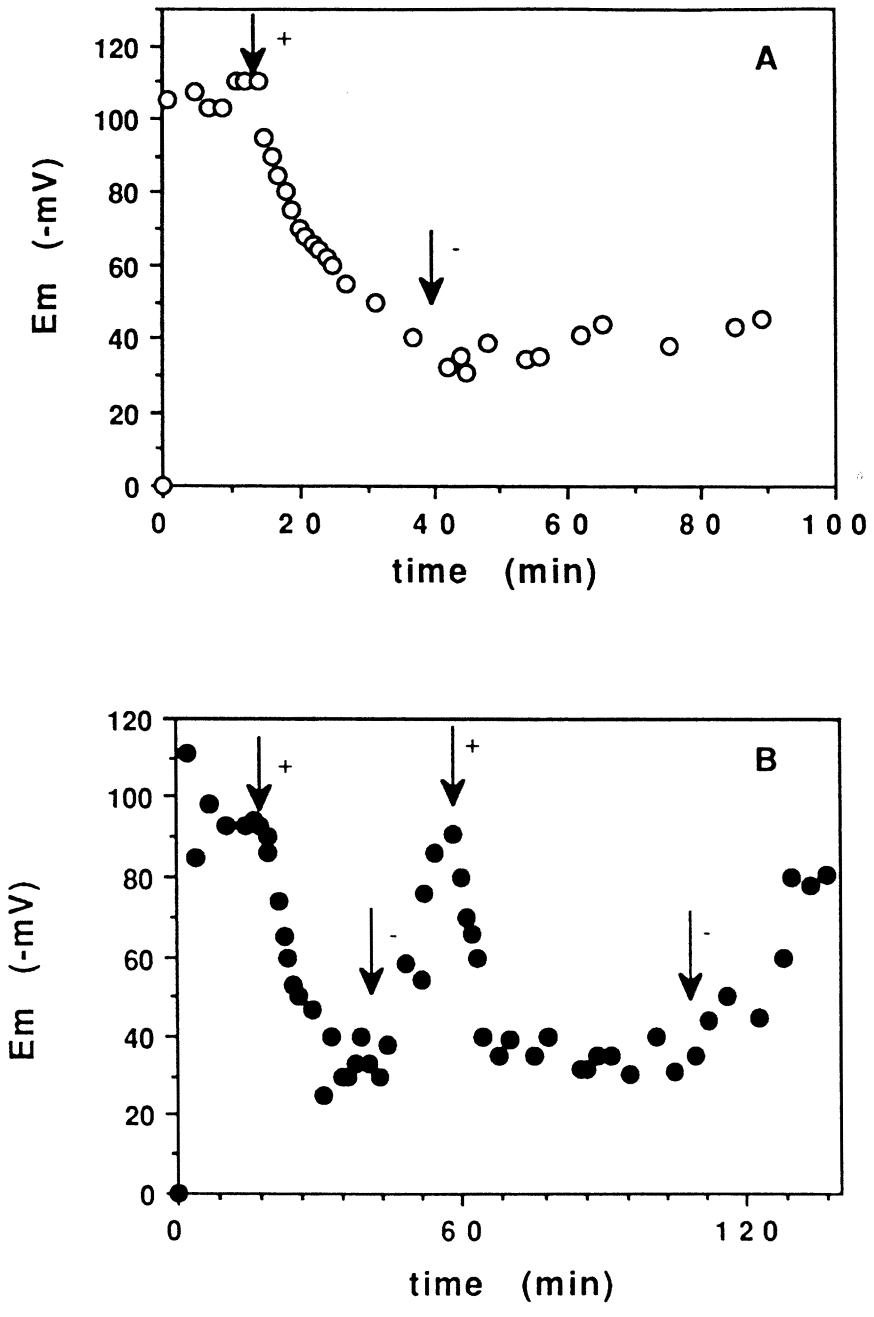

Figure 1. The effect of 50 μM diclofop acid on the membrane potential (Em) of parenchyma cells from coleoptiles of a susceptible (A) and a resistant (B) *L. rigidum* biotype. Each circle represents an individual cell of one peeled coleoptile. Arrows combined with + or - indicate the addition or removal of the herbicide solution.

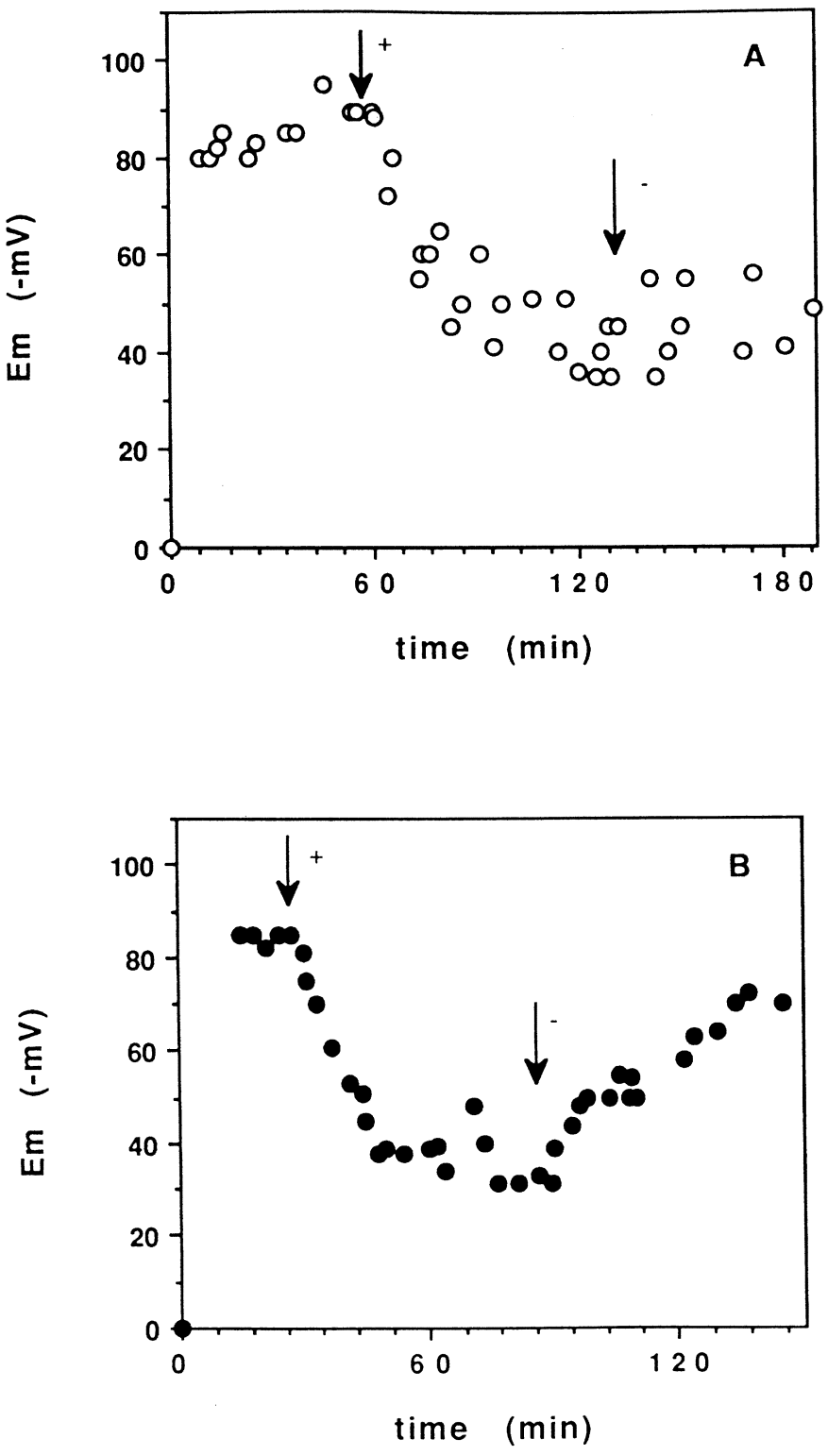

Figure 2. The effect of 100 µM sethoxidim on the membrane potential (Em) of parenchyma cells from coleoptiles of a susceptible (A) and a resistant (B) *L. rigidum* biotype. For further information see Fig. 1.

The effect of (+) and (-) enantiomers of diclofop-acid on the plasma membranes from ryegrass coleoptiles.

Aryloxyphenoxypropionate herbicides such as diclofop and haloxyfop consist of a racemic 50:50 mixture of (-) and (+) enatiomers. The (+) enantiomer not only exhibits greater herbicidal activity at the whole plant level (6) but is a more potent inhibitor of ACCase (8, 9, 10). We investigated the effect of the (+) and (-) enantiomers on the membrane potential of susceptible and resistant ryegrass coleoptiles. Both enantiomers are capable of depolarizing membranes. However, once again a striking biotype-specific response of membrane potentials occurred following the removal of the herbicide. Membranes from both biotypes recovered after treatment with the (-) enantiomer whereas only membranes from the resistant biotype repolarised after treatment with the (+) enantiomer. We conclude that not only do both enantiomers exert a protonophoric action, as was proposed by Wright and Shimabukuro for the racemic mixture (12), but that a stereospecific effect of the (+) enantiomer on membranes from ryegrass coleoptiles also exists.

It is probable that the biotype-dependent, stereospecific (in the case of the aryloxyphenoxypropionates) recovery from membrane depolarisation is be involved in the mechanism of resistance in *L. rigidum* to the aryloxyphenoxypropionate and cyclohexanedione herbicides. The reader is referred to reports in this volume by Christopher *et al.* (1) and Holtum *et al.* (4) for further discussion on the phenomenon of herbicide resistance in *L. rigidum.*

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