TOWARDS AN UNDERSTANDING OF THE MECHANISMS OF RESISTANCE TO ARYLOXYPHENOXYPROPIONATE (APP) HERBICIDES IN *ALOPECURUS MYOSUROIDES* (BLACK-GRASS)

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Summary. Resistant biotypes Lincs. E1 and Peldon A1 have fenoxaprop ED_{50} values 27 and 4 times greater than the susceptible (Rothamsted) biotype, respectively. Lincs. E1 and Peldon A1 are 10 and 13 fold more resistant to diclofop, respectively. The target site of these herbicides is acetyl-CoA carboxylase (ACCase). Affinity of ACCase for the substrate acetyl-CoA was similar for all biotypes. ACCase extracted from Peldon A1 and Rothamsted was more inhibited by fenoxaprop and diclofop than ACCase from Lincs. E1. Differences in ACCase inhibition were due to a subset comprising approximately 15% of the Lincs. E1 population. ACCase extracted from this subset was less sensitive to inhibition by diclofop. Absorption and translocation experiments showed that Rothamsted absorbed fenoxaprop-ethyl faster than both the resistant biotypes but there were no significant differences in translocation. Rothamsted contained more herbicidally active fenoxaprop than resistant biotypes 6 to 48 HAT (hours after treatment). The reasons for the lower fenoxaprop content in the resistant plants are under investigation but probably accounts for their ability to survive fenoxaprop treatment.

INTRODUCTION

Populations of *Alopecurus myosuroides* in the United Kingdom have developed multiple herbicide resistance to herbicides in unrelated chemical groups with different modes of action (8,9). In one population (Peldon A1) the major mechanism of resistance to chlorotoluron is enhanced herbicide metabolism (6). Two multiple-herbicide resistant populations, from Essex (Peldon A1) and Lincolnshire (Lincs. E1), show high levels of resistance to herbicides in the APP family of herbicides. The mechanism(s) of herbicide resistance of these biotypes to APP herbicides is the subject of this investigation.

The APP herbicides, along with cyclohexanedione (CHD) herbicides, inhibit the enzyme ACCase. Several mechanisms which contribute to APP resistance have been reported. Resistant plants may contain a modified ACCase which is less inhibited by herbicides (1,10). APP resistance has also been correlated with rapid cell membrane repolarisation after herbicide removal (3), however the mechanistic basis of this response and its role in whole plant resistance is poorly understood (4). A population of *Lolium rigidum* has developed high levels of resistance to APP and CHD herbicides not based on target site mutations (7). This population has multiple mechanisms, including enhanced metabolism of diclofop (5) and cell membrane repolarisation (3), one or both of which can contribute to an individual's resistance.

Uptake, translocation and metabolism of fenoxaprop and inhibition of ACCase by APP and CHD herbicides are compared in two resistant biotypes of *A. myosuroides* and a susceptible to determine the mechanism(s) of resistance.

METHODS

Seeds were collected in fields in Hertfordshire (Rothamsted), Essex (Peldon A1) and Lincolnshire (Lincs. E1), UK. The Rothamsted biotype was collected from an area untreated with herbicides. Peldon A1 and Lincs. E1 biotypes were collected from fields where herbicides had failed to control *A. myosuroides*. Methods used to compare resistance to herbicides at the whole plant level have been described previously (8).

Inhibition of ACCase activity by five herbicides, diclofop, fenoxaprop, fluazifop, sethoxydim and tralkoxydim, at final concentrations between 0 and 100 µM was measured as previously described (10) and the affinity of ACCase for acetyl CoA in extracts from the three biotypes measured at concentrations between 0 and 600 µM acetyl CoA. To determine if the small differences in ACCase inhibition by diclofop between Lincs. E1 and Rothamsted was characteristic of the whole population, seeds of Lincs. E1 were germinated on 6% (w/v) agar supplemented with 100 µM fluazifop and herbicide inhibition profiles of ACCase extracted from survivors were compared with those of the bulk of the Lincs. E1 population.

To measure the absorption and translocation of fenoxaprop, ^{14}C fenoxaprop-ethyl (spec. act. 971 MBq/ g) was applied to the second leaf of three leaf stage plants and 12, 24, 48, 72 or 96 HAT, unabsorbed herbicide washed from the leaves, plants sections oxidised separately and uptake and translocation quantified by liquid scintillation counting (2).

Metabolism of fenoxaprop-ethyl was determined by applying a 2 µL drop of ^{14}C fenoxaprop-ethyl to the leaf axis, washing off unabsorbed herbicide 3, 6, 12, 24, 48, 72 and 96 HAT and extracting the herbicide and metabolites with 80% (v/v) methanol. Parent herbicides and metabolites were separated by HPLC and the quantity of herbicide and metabolites expressed as a percent of total radioactivity in the extract.

RESULTS AND DISCUSSION

Effect of Inhibitors of ACCase on growth of intact plants. Peldon A1 and Lincs. E1 show varying levels of resistance to both APP and CHD herbicides compared to the susceptible Rothamsted. Lincs. E1 was more resistant than Peldon A1 to fenoxaprop (ED_{50} ratios to Rothamsted of 27 and 4) but showed similar resistance to diclofop (13 and 10), quizalofop (8 and 6); fluazifop (7 and 6); and the CHD herbicides sethoxydim (2 and 1.5) and tralkoxydim (16 and 13). There was no direct relationship between levels of resistance and the family of herbicide, APP or CHD.

ACCase Activity. Affinity of ACCase extracted from the three biotypes for acetyl-CoA was similar. Fifty percent of maximum enzyme activity was obtained at 70, 71 and 81 µM acetyl-CoA in Lincs. E1, Peldon A1 and Rothamsted, respectively.

ACCase activity of all three biotypes was inhibited by APP herbicides, diclofop, fenoxaprop and fluazifop and CHD herbicides, tralkoxydim and sethoxydim. Lincs. E1 shows consistently less inhibition of ACCase than Rothamsted, the susceptible biotype. If ACCase insensitivity in Lincs. E1 was a property of the whole population, it would be unlikely to protect plants from herbicide toxicity. While Lincs E1 may show small differences in inhibition kinetics, it is also possible some individuals in the population have an insensitive ACCase while the majority of this field collected population does not. A subset of approximately 15% of the population

selected on 100 µM fluazifop contained ACCase less inhibited by diclofop than that of the unselected population. Continued selection of Lincs. E1 with APP herbicides would be expected to increase the frequency in the population of this less sensitive ACCase.

Absorption and Translocation of Fenoxaprop-ethyl. Absorption of ^{14}C fenoxaprop-ethyl was low, with an average of 29% after 12 hours, increasing to 44% 72 HAT. Translocation from the applied leaf was limited, reaching an average of 4% 72 HAT. Rothamsted absorbed more ^{14}C fenoxaprop-ethyl, but there were no significant differences between biotypes in amount of herbicide translocated from the site of application. Differences in uptake or translocation of APP herbicides have not been previously shown to contribute to herbicide resistance.

Metabolism of Fenoxaprop-ethyl. Extracts from both resistant biotypes contained a smaller proportion of herbicidally active fenoxaprop than extracts from susceptible 6 to 48 HAT. Reduced fenoxaprop content in the resistant biotypes may be due to slower fenoxaprop-ethyl conversion to fenoxaprop, or to enhanced binding of fenoxaprop-ethyl, making it unavailable for breakdown to fenoxaprop, or to enhanced detoxification of fenoxaprop to inactive conjugates. These possibilities are under investigation.

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