HGCA

PROJECT REPORT No. 225

TOWARDS A DIAGNOSTIC TEST FOR HERBICIDE RESISTANCE IN GRASSES

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TOWARDS A DIAGNOSTIC TEST FOR HERBICIDE RESISTANCE IN GRASSES

by

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This is the final report of two HGCA-funded projects. The first project (no. 1466 - £111,992) started in February 1996 and lasted for three years. The second project (no. 2068 - £66,583) started February 1999 and lasted for one year.

CONTENTS Page

ABSTRACT

Black-grass (*Alopecurus myosuroides* Huds.) is a major problem weed in winter cereal crops in the UK. Its presence causes reduction in both crop quality and yield. Chemical control (by selective graminicides) has proved useful but the occurrence of herbicide-resistant biotypes has resulted in variable control in recent years. Although herbicide resistance in black-grass was first reported in Essex in the early 1980s, more than 750 cases, from 30 English counties, have now been reported. An important aspect of black-grass control is therefore determining whether poor control is due to resistance, so that suitable management measures can be adopted. Current tests for resistance either involve transplanting plants to a glasshouse, where herbicide spray trials are carried out, or collection of seed prior to crop harvest for plate-based growth analysis. These tests are both expensive and time-consuming. A better test would be cheap, quick and would ideally provide results before the application of post-emergent herbicides, so alternative strategies could be adopted where necessary.

This study has focused on an enzyme GST (glutathione *S*-transferase) that is more active in the resistant biotype Peldon. Raised activities have been demonstrated in other resistant biotypes and a correlation between GST activity and fenoxaprop resistance demonstrated. A GST polypeptide has been purified from a susceptible biotype (Herbiseed) and an additional GST polypeptide, found in Peldon only, has also been purified. Monoclonal antisera raised against the Peldon polypeptide have been used to develop an ELISA (enzyme-linked immunosorbent assay) for quick detection of GST abundance in black-grass plants. This has confirmed that resistant biotypes possess increased abundance of GST polypeptide as well as increased GST activity. This ELISA test has been used to assess plants surviving herbicide treatment and to predict field performance at sites where black-grass control has been poor. As the test is carried out on plants at 2-3 leaf stage and gives a result in 3 days, it can provide useful information prior to post-emergent herbicide application. Such a test will aid resistance diagnosis and allow resistance management strategies to be started earlier in the season.

The authors are grateful to the HGCA for funding and Novartis CP UK for seeds of novel biotypes and for access to field sites of known agronomy and herbicide history.

SUMMARY

1. BACKGROUND

Black-grass (*Alopecurus myosuroides* Huds.) is a problem annual weed associated with cereals in the UK and northern Europe. It is a particular problem in winter cereals as its period of main germination coincides with crop drilling. Presence of black-grass reduces both crop quality and yield. Heavy infestation can reduce yield by up to 45% and in particularly bad cases complete crop loss may occur. Black-grass can also harbour ergot and can increase crop lodging. Due to its high seed return (up to 300 seed per head) it is vital that black-grass is controlled, as a few remaining plants can lead to a large population in a few years. Black-grass control relies on a combination of chemical and cultural means. Both the ureas and the 'fops' have been used extensively as herbicides, with good results. However, extensive use of herbicides of a single type (or mode of action), combined with monoculture of cereals and low tillage, has resulted in the appearance of black-grass populations showing herbicide resistance. Herbicide resistance in black-grass was first reported in the UK in Peldon, Essex in the early 1980s. Today, more than 750 herbicide-resistant black-grass populations have been characterized in 30 counties in the UK. Although resistance to a single herbicide is reported, cross- and multiple resistance are also very common. Cross-resistance is resistance to two or more herbicides due to a single resistance mechanism. Multiple resistance is the presence of more than one resistance mechanism. Both altered target site and enhanced metabolism appear to be mechanisms of resistance in black-grass.

A resistance test is necessary where poor black-grass control is noted, so that appropriate resistance management schemes can be adopted where resistance is confirmed. Ideally this test would be quick, cheap and would provide results before the application of post-emergent herbicides, so alternative action can be taken where resistance is indicated. Current tests either involve transplanting to a glasshouse, where herbicide screens are carried out, or growth of seed in the presence of herbicides. Due to the length of time required for glasshouse study, results from these tests are rarely available before post-emergent herbicide treatment. Seed tests necessitate the collection of seed prior to crop harvest, and hence only allow alternative control strategies to be adopted in subsequent seasons. The aim of this study has been to develop a resistance test that is quick, cheap and can be carried out in the autumn, on black-grass plants at GS12/13 (prior to post-emergent treatment).

2. GST ACTIVITY IN BLACK-GRASS BIOTYPES

Glutathione *S*-transferases (GSTs) are a family of enzymes found throughout the animal and plant kingdoms. They have a variety of functions including the metabolism of various xenobiotics and in the dissipation of active oxygen species. GSTs were extracted and assayed as follows: Black-grass plants (2-3 leaf stage) were ground to a powder in liquid nitrogen, thawed in buffer and homogenized. After clarification by centrifugation the extracts were buffer exchanged. GST activities were then assayed using the model substrate CDNB (1-chloro-2,4-dinitrobenzene).

The black-grass biotype Peldon, which is resistant to a variety of herbicides including chlortoluron, isoproturon and fenoxaprop, was demonstrated to have approximately double the GST activity of susceptible biotypes. This higher activity is constitutive, i.e not requiring herbicide application to be expressed. As GSTs have been implicated in herbicide metabolism in various plant systems, it is possible that the raised GST activities in Peldon biotype are responsible for the observed herbicide resistance. In order to further investigate this novel observation, GST activities were studied in fourteen black-grass biotypes (a kind gift from Novartis CP UK) that showed a range of responses to herbicide treatments. Five biotypes showing resistance to fenoxaprop [Cheetah] (but not clodinafop [Topik]) possessed higher GST activities and a correlation between resistance to fenoxaprop and GST activity in some biotypes is shown in Figure 1.

Four resistant biotypes that had lower GST activities were also identified. These demonstrated resistance to both Cheetah and Topik and there is evidence to suggest that these have altered target site resistance to 'fops', in which case elevated GST activities would be of no benefit. As these results are from plants that have not been treated with herbicide it appears that GST activities may give an insight into how a particular biotype will respond to herbicide treatment.

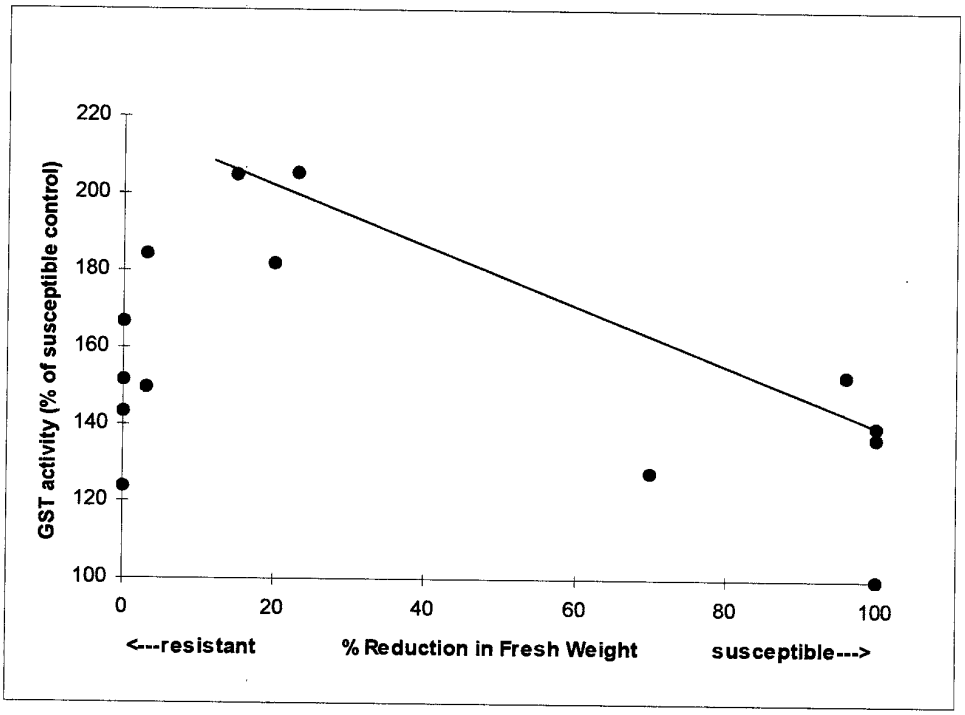

Figure 1. GST activity (% of susceptible control) against reduction in fresh weight (% of control) after Cheetah treatment. Each point is a separate biotype.

3. PURIFICATION OF GST POLYPEPTIDES

In order to further study the roles of GSTs in herbicide resistance, it was necessary to purify the GST polypeptides. As GSTs had not been previously purified from black-grass, a purification process was developed. Tissue was ground to a powder in liquid nitrogen, homogenized in buffer and clarified by centrifugation. After protein harvest by ammonium sulphate precipitation, proteins were separated by ion-exchange chromatography. The semi-purified GSTs were buffer exchanged by gel filtration and further purified by affinity chromatography on a glutathione-agarose column. Purified GSTs were analysed by SDS-PAGE (SDS polyacrylamide gel electrophoresis). A 27.5 kDa polypeptide possessing GST activity was purified from the susceptible biotype Herbiseed. Purification of GSTs from the resistant biotype Peldon revealed the 27.5kDa polypeptide present in greater abundance and a 30kDa polypeptide that was undetectable in the susceptible biotype.

4. RAISING MONOCLONAL ANTISERA

Antisera were raised to the 30kDa polypeptide purified from Peldon biotype. After successful screening of the polyclonal serum against Herbiseed and Peldon extracts (by dot-blot and

ELISA), *in vitro* cell lines producing antisera were produced. These were screened and further purified until a cell line that produced a satisfactory difference between Herbiseed and Peldon was obtained. Table 1 shows the final 6 selected cell-lines, selected from over 200. Cell line 2E3 was selected for further study. This was then fused to form an immortal cell line and the monoclonal antisera tested again (by ELISA) before it was used in all subsequent study.

Table 1. ELISA responses for final selected cell lines. 2E3 was selected and fused for all subsequent studies.

	ELISA response (arbitrary units)			
Cell line	Peldon	Herbiseed	Difference	% increase
2E3	0.615	0.483	0.132	27
2H3	1.031	0.903	0.128	14
2D6	0.905	0.804	0.101	12
2G1	0.847	0.713	0.134	19
2F4	0.886	0.783	0.103	13
2G2	0.645	0.526	0.119	23

5. ELISA DEVELOPMENT

The basic ELISA procedure used for all subsequent development and field-testing was as follows:

1) Black-grass protein binding: Proteins were bound at a concentration of 1μg per 50μl to 96 well plastic microplates. This allowed ten plants to be assayed per plate (at n=5), along with the necessary blanks. Binding was carried out overnight at 4°C.
2) Site blocking using milk powder: This was carried out for 3 hours at 37°C using milk protein. It was necessary in order to prevent unspecific binding of the antisera to the microplate.
3) Incubation with primary antisera: This incubation was carried out, using undiluted antisera, overnight at 4°C.
4) Wash step one.
5) Incubation with secondary antisera (containing marker enzyme): This incubation was carried out for 2 hours at 21°C, with agitation. Antibody labeled with alkaline phosphatase was chosen as horseradish peroxidase resulted in excessive background in blank samples (possibly due to peroxidase activity in black-grass extracts).

6) Wash step two.

7) Visualization of protein-antibody conjugate: Incubation with a developer solution resulted in the production of a coloured product. The reaction was stopped after 2 hours and the amount of coloured product was measured. This reading was proportional to the amount of GST polypeptide in the plant extract.

6. FIELD-TESTING

In order to assess whether either GST activity (measured against CDNB) or GST abundance (determined by ELISA) was a useful indicator of resistance in the field, trials were carried out at sites in the East Midlands (courtesy of Novartis CP UK). The first set of trials involved the comparison of GST activity and abundance in plants surviving various herbicide treatments compared to those in untreated plots. These trials were carried out during 1998/1999. The second set of trials tested black-grass plants both before and after treatment to assess whether GST activity or abundance was an accurate predictor of herbicide efficacy. These trials were carried out during1999/2000. Ten plants were randomly sampled from each test plot and proteins extracted as detailed above. GST activity was assayed against CDNB and GST abundance was measured using the ELISA.

Year one (1998/1999)

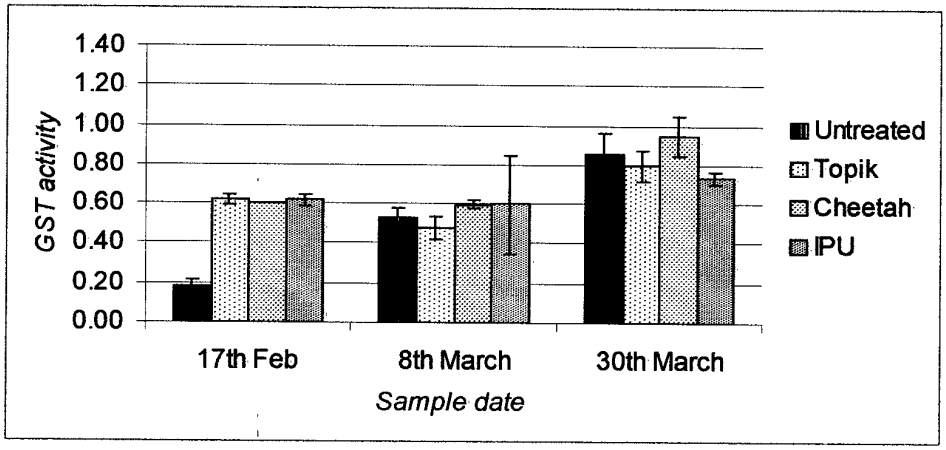

Figure 2. Average GST activity (μmol CDNB min^{-1} mg^{-1} fresh weight, n=10) for 4 plots receiving different treatments at site 2. Data is presented for 3 time-points during year one.

Figure 2 shows the GST activities obtained for one site between February and May 1999. This site had not received herbicide treatment during 1998/1999, but had received the same

treatments for the previous six years, inducing a heavy selection pressure for individuals resistant to the particular herbicides. On the first sample date (17th Feb) GST activity for the control population was approximately a third of that of any of the treated plots (Figure 2). By sample point two (8th March) all activities were similar and remained so through to May. This suggests that in younger plants there is a difference in GST activities between the control and treated plots.

ELISA data (Figure 3) displays a similar pattern for the abundance of GST polypeptides.

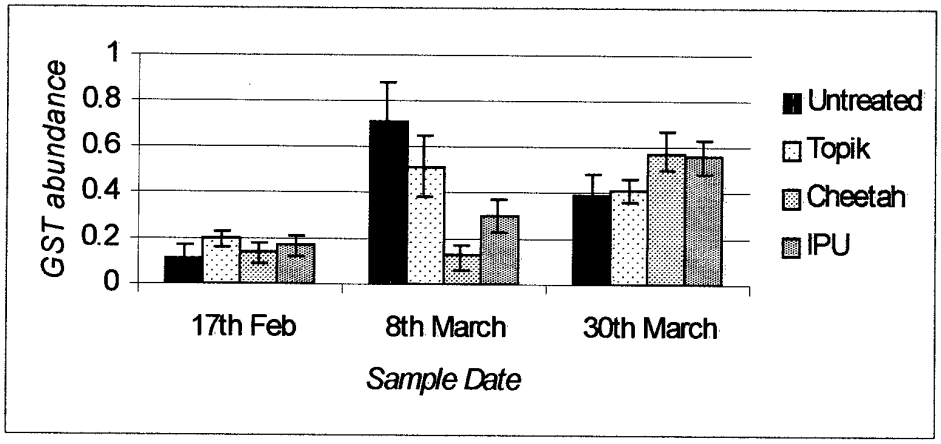

Figure 3. Average GST abundance (OD (405nm) g^{-1} fresh weight, n=10) for 4 plots receiving different treatments at site 2. Data is presented for 3 time-points during year one.

The conclusion from the data obtained in year one is that a population composed of individuals that are all herbicide resistant has a different profile, with respect to GST activity and abundance, from a population composed of both resistant and susceptible individuals. This was felt to be a strong basis for a test based either on GST activity or on ELISA.

Year two (1999/2000)

Field trials similar to those detailed above were carried out during 1999/2000. However, all plots were sampled before treated plots received herbicide and counts of black-grass plants were carried out to compare with the black-grass GST profiles obtained. Figure 4 shows the number of plants in the plots during the period of study. Figure 5 and 6 show GST activity and ELISA response for the plots.

The conclusion from the data obtained in year two is that the GST profile of a population before treatment can be useful in predicting how the black-grass will be controlled by

herbicides. This seems to be the case for all the herbicides studied (Cheetah, Topik and IPU). The data is also useful in predicting an ELISA cut-off above which plants can be considered to be a resistance problem.

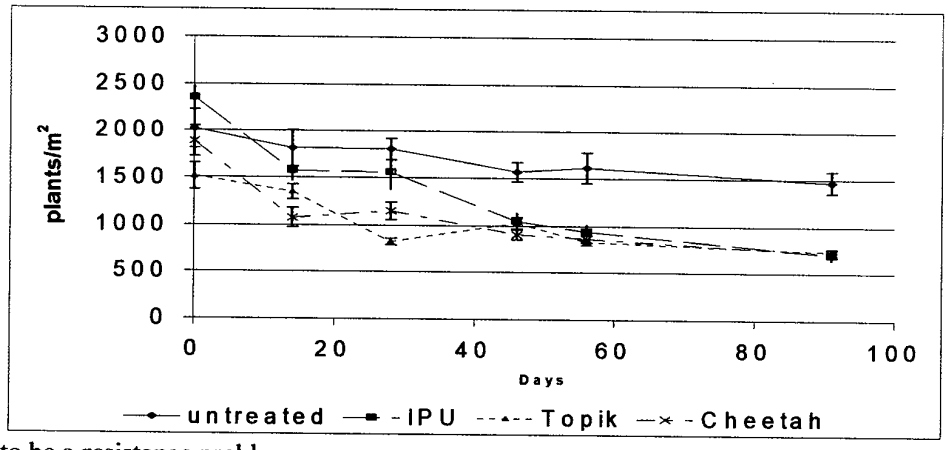

Figure 4. Average number of black-grass plants m^{-2} for 4 plots receiving different treatments at site 1 (year 2).

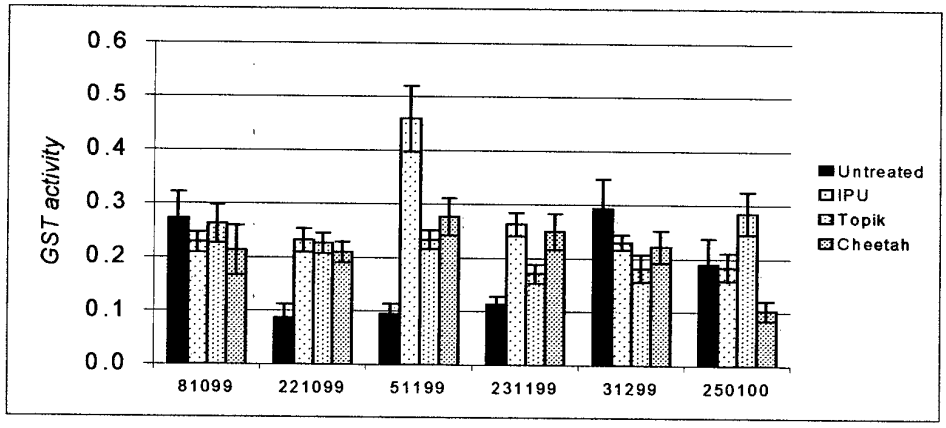

Figure 5. Average GST activity (μmol CDNB min^{-1} mg^{-1} fresh weight, n=10) for 4 plots receiving different treatments at site 1 (year 2).

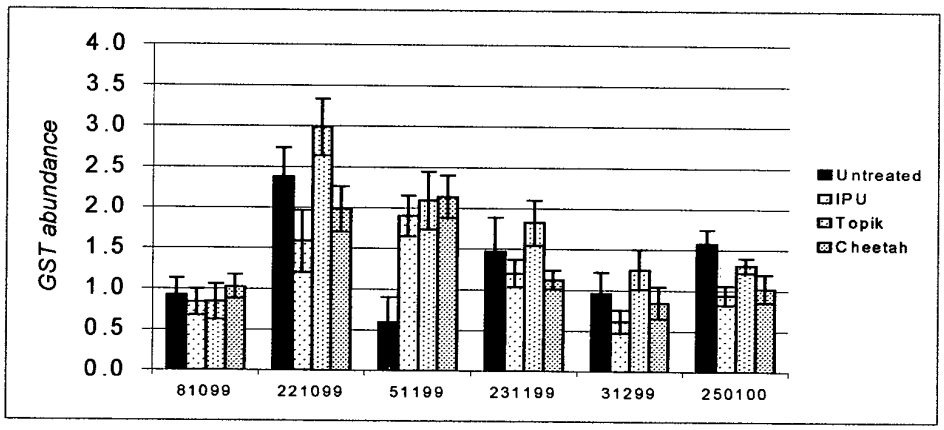

Figure 6. Average GST abundance (OD (405nm) g^{-1} fresh weight, n=10) for 4 plots receiving different treatments at site 1 (year 2).

7. CONCLUSIONS

From the data obtained thus far it is clear that GST activity or GST abundance (measured by ELISA) can give an indication of the level of herbicide resistance in a field population of black-grass. The ELISA test is, at present, laboratory based and takes three days. This can be reduced to one day by carrying all steps out at 37°C. Potentially an ELISA can be reduced to a few hours and may not have to be laboratory based. Data to date suggests that the test gives a good indication of the extent of resistance in a black-grass population. As plants must be tested early in the season this gives an indication of resistance before application of post-emergent herbicides, allowing alternative strategies to be adopted where resistance is suspected before crop quality and yield have been affected. Further study needs to be carried out to rigorously field test the ELISA and to automate the procedure for ease of use and faster throughput.

8. FURTHER DEVELOPMENTS

Although HGCA funding has now ceased the project continues at HAUC and further field and glasshouse data are being gathered. Initial observations with other grasses are in progress, e.g. *Lolium* and *Avena*. Furthermore, a patent has been taken out on the ELISA test and further technical refinements are in progress.

9. TECHNOLOGY TRANSFER

Technology transfer has been achieved via conferences, presentations and papers (details are shown below). In addition, 3 articles in popular press (2 in Farmers Weekly, 1 in The Furrow) and one radio interview (Farm Gate, Radio Shropshire) increased public awareness of the scope and progress of the project.

1. Andy H Cobb, Catherine R Sharples, John PH Reade and Mark R Hull. (1996). A role for glutathione-S-transferase in herbicide resistance in black-grass (*Alopecurus myosuroides* Huds.).*WSSA Abstracts*,1997 Meeting of the Weed Science Society of America, Feb 3-6, 1997, Clarion Plaza Hotel, Orlando, Florida, Volume 37, 1997.

2. Cobb AH, Sharples CR, Reade JPH and Hull MR. (1997). A role for glutathione-S-transferase in herbicide resistance in black-grass (*Alopecurus myosuroides* Huds.). Resistance '97.

3. Reade JPH, Hull MR and Cobb AH. (1997). A role for glutathione-S-transferase in herbicide resistance in black-grass (*Alopecurus myosuroides*). *BCPC-weeds*, 777-782.

4. Reade JPH and Cobb AH. (1998). Glutathione-S-transferases in black-grass (*Alopecurus myosuroides* Huds.): properties and involvement in herbicide resistance. 9th IUPAC international congress of pesticide chemistry.

5. A test for herbicide resistance in black-grass. Poster at Cereals, 98.

6. Reade JPH and Cobb AH. (1999). Glutathione *S*-transferases in black-grass (*Alopecurus myosuroides* Huds.): properties and involvement in herbicide resistance. *Pesticide Science* **55**, 349-351.

7. Reade JPH and Cobb AH. (1999). Purification, characterisation and comparison of Glutathione *S*-transferases from black-grass (*Alopecurus myosuroides* Huds.) biotypes. *Pesticide Science* **55**, 993-999.

8. Milner LJ, Reade JPH and Cobb AH. (1999). An investigation of glutathione *S*-transferase activity in *Alopecurus myosuroides* Huds. (black-grass) in the field. *The Brighton conference-Weeds*, 173-178.

9. Reade JPH, Belfield JL and Cobb AH. (1999). Rapid tests for herbicide resistance in black-grass based on elevated glutathione *S*-transferase activity and abundance. *The Brighton Conference-Weeds*, 185-190.

10. Milner LJ, Belfield JL, Reade JPH and Cobb AH. (1999). An investigation of the detoxification of active oxygen species in black-grass (*Alopecurus myosuroides*) plants susceptible and resistant to herbicides. *The Brighton Conference-Weeds*, 561-562

TECHNICAL DETAILS

1. INTRODUCTION

Black-grass (*Alopecurus myosuroides* Huds.) is a major problem weed in winter cereal crops in the UK. Its presence causes reduction in both crop quality and yield. Selective graminicides are routinely used for black-grass control. However, the occurrence of herbicide-resistant biotypes mean satisfactory control is not always obtained. Herbicide resistance in black-grass was first reported in the UK in the early 1980s (Moss & Cussans, 1985). Today more than 750 cases, from 30 English counties, have been reported. Hence, an important aspect of black-grass control is determining whether poor control is due to resistance, in order that resistance management measures can be appropriately adopted. Current tests involve either transplanting plants to a glasshouse (Boutsalis, 1999), where herbicide spray trials are carried out, or collection of seed prior to crop harvest for plate-based growth analysis (Moss, 1999). These tests are both expensive and time-consuming. A better test would be cheap, quick, and would ideally provide results before the application of post-emergent herbicides (so alternative strategies could be adopted where necessary). This study focuses on an enzyme, GST (glutathione *S*-transferase), that has increased activity in the resistant biotype Peldon (Reade *et al*, 1997). Raised activities have been demonstrated in other resistant biotypes, and a correlation between GST activity and fenoxaprop resistance is demonstrated. A GST polypeptide has been purified from the susceptible biotype Herbiseed (Reade & Cobb, 1999) and a GST polypeptide found in Peldon, but not Herbiseed, has also been purified. Monoclonal antisera raised against the Peldon polypeptide has been used to develop an ELISA (enzyme-linked immunosorbent assay) for quick detection of GST abundance in black-grass plants. This has confirmed that resistant biotypes possess increased abundance of GST polypeptide as well as increased GST activity. This ELISA test has been used to predict field performance at sites where black-grass control has been poor. As the test is carried out on plants at 2-3 leaf stage, and gives results in days, it is felt that it could provide useful information prior to post-emergent herbicide application. Such a test will aid resistance diagnosis and allow resistance management strategies to be started earlier in the season.

2. MATERIALS AND METHODS

Glasshouse study

Black-grass seeds were sown in multipurpose peat-based compost, watered from below and grown to 2-3 leaf stage. All above ground biomass was sampled.

Field study

Black-grass plants were harvested from various field trials (courtesy of Novartis CP UK), where they had either survived herbicide trearment or were from control plots. Details of field trial sites are given in Table 1. All above ground biomass was harvested and frozen on dry-ice for transportation to the laboratory. Growth stage was recorded and plant counts were also carried out at the field trial sites.

Table 1. Details of field trial plots for 1998/1999 and 1999/2000

	Date of treatment	Plot 1	Plot 2	Plot 3	Plot 4
Year 1 Site 1	04/11/98	Untreated	IPU	Diclofop	----------
Year 1 Site 2 *	n/a	Untreated	IPU	Clodinafop	Fenoxaprop
Year 1 Site 3	16/12/98	Untreated	IPU	Diclofop	----------
Year 2 Site1	22/10/99	Untreated	IPU	Clodinafop	Fenoxaprop
Year 2 Site 2	16/12/99	Untreated	IPU	Clodinafop	Fenoxaprop
Year 2 Site 3	26/01/00	Untreated	IPU	Clodinafop	Fenoxaprop

* no treatments during year of study, but listed treatment carried out for the previous six years.

Protein extraction and GST activity assay

Proteins were extracted and GST activity assayed as detailed in Reade *et al* (1997). Protein concentration of extracts was determined according to Bradford (1976).

ELISA detection of GST polypeptides

ELISA detection of GST polypeptides was carried out as described in Reade and Cobb, 2000. Black-grass extracts were diluted to a concentration of 1µg/50µl in ELISA buffer (0.14M NaCl containing 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4. pH 7.4) and loaded on to a 96 well microtitre plate overnight at 4°C. The plate was emptied by inversion and all available protein binding sites were blocked by incubation with blocking buffer (ELISA buffer containing 3% (w/v) milk powder) for 3 hrs at 37°C. After wells were emptied by inversion the plate was incubated with primary antisera (clone 2E3) overnight at 4°C. Wells were emptied by inversion and washed with 3 changes of wash buffer (ELISA buffer containing 0.1% (v/v) Tween 20) and two changes of distilled water. Plates were then incubated with second antisera (goat anti-mouse alkaline phosphotase, GARAP) diluted 1:1000 in blocking buffer, for 2 hrs with shaking. The wash step was repeated and

polypeptide-antibody conjugate was visualized by incubation with developer (100µl p-nitrophenol phospate [1mg/ml] in 0.1M glycine buffer, pH 10.4, containing 1mM $ZnCl_2$, 1mM $MgCl_2$). This reaction was stopped once sufficient colour was obtained, by addition of 50µl of 3M NaOH. Plates were subsequently read in a BioRad microplate reader.

Protein purification and antisera production

GST polypeptides were purified as detailed in Reade and Cobb (1999). Purified polypeptides from the resistant biotype Peldon were resolved on SDS-polyacrylamide gels and the 30kDa polypeptide band excised and electroeluted. The purified 30kDa polypeptide was used to raise antisera in mice. Cell cultures producing antisera were prepared and screened against crude extracts of biotypes Herbiseed and Peldon. Successful cell cultures were subcultured and 6 were chosen for further study as they gave best differential results with Herbiseed and Peldon (Table 2). After further subculturing and screening one cell culture, 2E3, was selected for all subsequent field study.

Table 2. ELISA responses for final selected cell lines. 2E3 was selected and fused for all subsequent study.

	ELISA response			
Cell line	Peldon	Herbiseed	Difference	% increase
2E3	0.615	0.483	0.132	27
2H3	1.031	0.903	0.128	14
2D6	0.905	0.804	0.101	12
2G1	0.847	0.713	0.134	19
2F4	0.886	0.783	0.103	13
2G2	0.645	0.526	0.119	23

3. RESULTS

Year one

All samples taken from the sites during year one were taken post-treatment, so represent populations of plants that have survived herbicide treatments. Differences in GST activity between control plots (no treatment) and surviving populations were noted at all sites (Table 3). These differences were not due to physiological response to treatment as they were noted in populations at site 2, where no treatment was carried out during the season of study, but the populations had received heavy selection pressure from treatment during the previous 6 years.

Where sampling was repeated these differences were found to be absent in more mature plants, suggesting that there are developmental changes with respect to GST activity (for further details of this, see Milner *et al*, 1999). ELISA detection of GST polypeptides revealed a similar situation, with greater responses in populations that had survived herbicide treatment. This difference was absent from subsequent samplings, suggesting that more mature plants in the control populations had increased GST abundance, similar to those in treated plots (Figure 1).

Table 3. Mean GST activities in plants sampled in February 1999, expressed as μmol CDNB $min^{-1}g^{-1}$ fresh weight. n=10 except * where n=7. Figures in brackets represent the ratio (treated:untreated)

Site	Untreated	IPU	Diclofop	Clodinafop	Fenoxaprop
1	0.44	0.90 * (2.05)	0.74 (1.68)	--------	--------
2	0.18	0.62 (3.44)	--------	0.62 (3.44)	0.60 (3.33)
3	0.34	0.99 (2.91)	0.80 * (2.35)	--------	--------

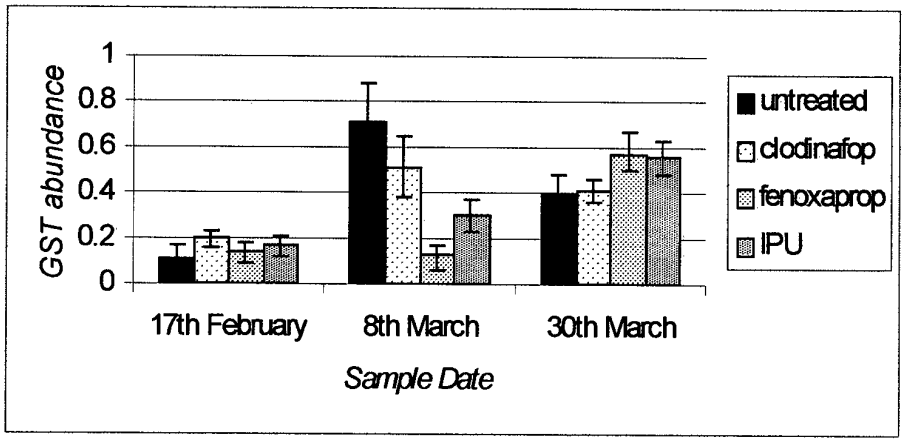

Figure 1. ELISA responses (OD (405nm) g^{-1} fresh weight) from four plots (site 2) revealing the differences between treated and untreated plots on 17th February (absent from subsequent samplings).

Year two

All three sites sampled during the second year of field study were sampled both pre- and post-treatment. Data presented here is from site 1. GST activity during the period of study is

shown in Figure 2. As previously observed, plants surviving herbicide applications possess higher average GST activities than control plot plants. This difference was noted up to growth stage 24, when GST activity in control plants was found to have risen to a similar level to that in treated plot plants. ELISA detection of GST polypeptides in these plants revealed that more detectable GST polypeptide was present in surviving plants up to GS 24. Although there were only slight differences in mean ELISA responses, careful study of the data revealed that the untreated plot contained plants that gave very low ELISA responses. No plants from treated plots gave these low responses (Figure 3). It hence appears that populations composed of individuals surviving herbicide treatment have a different ELISA profile to that of the population before treatment. By assessing the proportion of a population with high ELISA responses, it should be possible to predict how the population will respond to herbicide treatment.

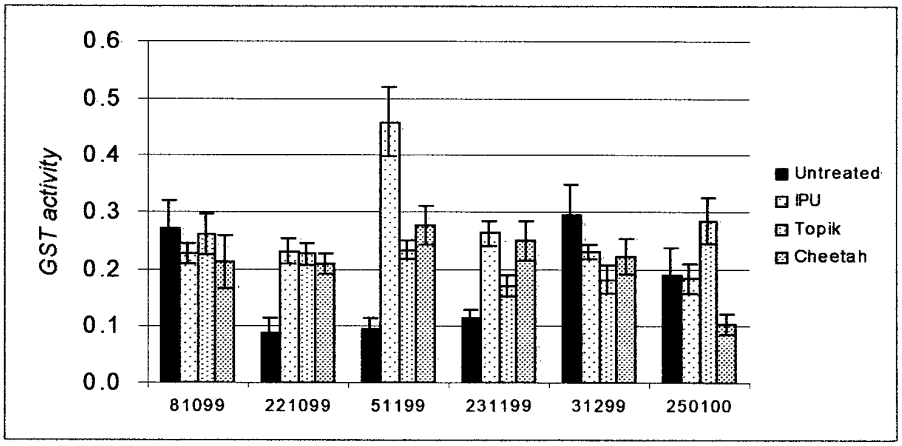

Figure 2. Mean GST activities (μmol CDNB $min^{-1}g^{-1}$ fresh weight) in plants sampled during year 2 of field study, expressed as μmol CDNB min^{-1} g^{-1} fresh biomass.

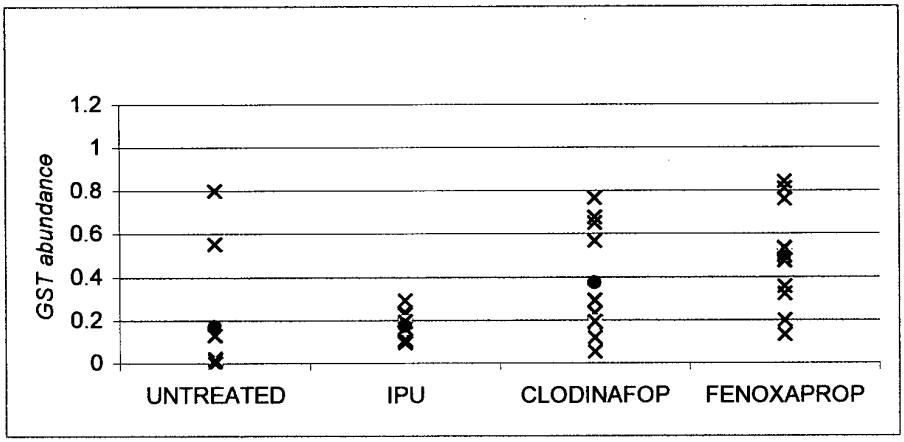

Figure 3. ELISA response (OD (405nm) g^{-1} fresh weight) for plants from the four field test plots (site 4). Each symbol (X) represents a single plant. Mean values for each plot (•) are also shown. Data is from 5th November but is typical of post-treatment data up to GS 24. Five of the nine plants from the untreated plot had ELISA responses below 0.025.

4. DISCUSSION

Herbicide resistance in black-grass is found on over 750 farms in England. It seems likely that this figure will continue to rise. In order to adopt successful resistance management and control strategies it is necessary to be able to test for resistance in black-grass populations. Currently available tests either involve transplantation of black-grass to a glass house (Boutsalis, 1999), where test sprayings are carried out, or collection of black-grass seed, prior to crop harvest, to carry out petri-dish grown tests (Moss, 1999). Both these methods allow resistance to be diagnosed, but only give results after post-emergent herbicides have been applied and poor control has taken place. Research presented here was carried out to try and develop a quick, simple test for resistance in black-grass that could be carried out prior to herbicide application, so that alternative control measures could be adopted where resistance was indicated.

Initial research indicated that the resistant biotype Peldon contained approximately double the activity of the enzyme glutathione S-transferase compared to the susceptible biotype Herbiseed. This raised activity was constitutive, not requiring herbicide treatment to be expressed (Reade *et al*, 1997). Study of other black-grass biotypes with characteristic resistance profiles indicated that GST activity could be correlated with resistance to the graminicide fenoxaprop (Reade *et al*, 1997), suggesting that GST activity could form the basis of a quick test for resistance in black-grass. Initial field-tests suggested that populations surviving herbicide resistance consisted of individuals that possessed higher GST activities

when compared to control (untreated) populations (Reade *et al*, 1999). This would suggest that the proportion of individuals in a black-grass population possessing high GST activities would be a useful indication of the population's resistance to herbicides. This appeared to be the case for all herbicides studied (IPU, diclofop, fenoxaprop, clodinafop) regardless of whether GSTs have been implicated in metabolism of the particular herbicide in plant systems. Further field studies, involving sampling of plots before and after herbicide application, backed up initial observations. It appears that the observed differences between populations was present up to GS 24, when GST activities in control plot had risen to the levels found in treated plots.

Assaying for GST activity requires expensive equipment and can be time-consuming. In order to further develop a test it was decided to raise antisera to a GST polypeptide in order to study whether GST abundance followed similar patterns to GST activity. In order to do this a purification procedure was developed, details of which are given in Reade & Cobb (1999). Purification of GSTs from the herbicide susceptible biotype Herbiseed resulted in one, 27.5 kDa polypeptide. Purification from the resistant biotype Peldon revealed the 27.5kDa polypeptide, present in greater abundance, together with a 30kDa polypeptide that was not observed in the susceptible biotype. Monoclonal antisera, raised against the 30kDa polypeptide, could distinguish between extracts of Herbiseed and Peldon biotype in an ELISA test. Further study of field samples with the ELISA showed that GST abundance followed a similar pattern to GST activity, with a difference between treated and untreated populations being observed up to GS 24. At present the ELISA test is carried out over three days. This can be reduced to one day in our laboratory. Commercialization of ELISA tests is commonly carried out and can reduce the time between sample collection and result to under one hour. Even utilizing the three-day test allows results to be obtained prior to post-emergent herbicide application.

It is felt that the ELISA test will provide farmers with very important information that will enable them to make better decisions with regard to black-grass control measures. The cost of the test will be more than balanced by the improved yields obtained through better black-grass control. Further field testing will confirm the reliability of the data obtained.

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