Detecting Herbicide Resistance

Guidelines for conducting diagnostic tests and interpreting results. June 1999

Reliable tests for resistance are an essential pre-requisite for the rational implementation of effective integrated control strategies (See HRAC Guideline to the Management of Herbicide Resistance). Ideally diagnostic tests should be rapid, accurate, cheap, readily available and give a reliable indication of the likely impact of resistance on herbicide activity in the field.

Initial suspicion of resistance usually results from unsatisfactory weed control following a herbicide application. Resistance should not be assumed to be the cause, and other reasons should be investigated first. Resistance should be considered as a possible cause when other factors have been eliminated. This leaflet summarises the key principles involved in detecting resistance.

THE MOST IMPORTANT FACTOR DETERMINING THE EASE OF DETECTING RESISTANCE IS THE DEGREE OF INSENSITIVITY.

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When resistance is absolute, and a herbicide has no visible effect at the recommended rate, detection is easy. With partial resistance, when some herbicidal effects are seen, detection is more difficult as resistance is only one of many factors that can reduce herbicide performance.

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1 Field observation

Accurate field observation is important so that any reduction in herbicide efficacy can be detected. This *may* indicate developing resistance. However, many other factors, apart from resistance, may be responsible for poor herbicide performance.

These include:

a *Herbicide application factors*: e.g. inappropriate dose or timing; faulty spraying.

b *Soil conditions*: e.g. soil moisture; seedbed quality; adsorption.

c *Climatic conditions*: e.g. rainfall patterns; temperature.

d *Weed factors*: e.g. size of weeds; subsequent germination; very high infestation.

Because so many factors may be responsible for inadequate herbicide performance, it is often difficult to determine the exact cause of herbicide failure in the field. Although it is rarely possible to confirm resistance solely on the basis of field observation and consideration of field records, several factors will point in this direction.

Living plants next to dead plants may indicate developing resistance

These are:

a *The level of weed control of other susceptible species*. If these have been controlled effectively, then resistance is a distinct possibility.

b *The presence of alive plants adjacent to dead individuals*. This may indicate the presence of resistant individuals, although such situations can arise through variations in weed growth stage,

Dense patches of weeds may indicate localised resistance

incorrect application or through crop shielding.

c *Past experience*. If the surviving species has been controlled successfully by the same treatment in the past, or a gradual decline in control has been noticed over a period of years, resistance may be responsible.

d *Herbicide history*. The repeated annual use of the same herbicide, or herbicides with the same mode of action, favours selection for resistance (See HRAC Classification of Herbicides according to Modes of Action).

e *Occurrence of resistance in the vicinity*. If resistance in the same weed and involving the same herbicide has been positively identified in adjacent fields or farms, then there is a high probability that resistance is implicated.

If resistance is suspected, a sample of seeds (or plants) should be collected from the suspected resistant weed population for a resistance confirmation test.

2 Seed collection

The reliability of results based on plant assays is largely dependent on the quality of the seed sample from which they are grown. Poor quality seeds will often have a low % germination or produce poor plants with consequent variable response to herbicides.

- **collect seeds when the majority are mature.** Collecting too early or too late is likely to lead to samples with low viability. With grass-weeds, e.g. wild-oats (*Avena* spp.), rye-grass (*Lolium* spp.), the best time is when about 20% of seeds have already been shed.

- **collect ripe seeds by gently rubbing inflorescences over a bag or tray.** Seeds of tall weeds, such as wild-oats, are most easily collected by holding inflorescences inside a large bag and shaking vigorously. The best technique will vary with species. With grass-weeds it is usually best to try to collect seeds directly in the field, rather

Tests for resistance require seed samples which must be of good quality. Plastic bags are good for seed collection purposes but do not *store* seeds in them

Seed samples should be clean and free from debris. Neither of these wild-oat samples has been cleaned, but that on the left was collected in the correct manner, that on the right was not.

than collect inflorescences.

- **aim to collect over an area of at least 100m by 50m** within the main problem area, unless the problem is confined to one or more smaller, very distinct patches. Avoid obvious unsprayed areas. The sample needs to be representative of the problem field or area, so a few seeds from lots of heads should be collected. Make a sketch map of area sampled.
- **quality is more important than quantity.** Aim to collect at least a volume of 250ml of seeds of grass-weeds such as rye-grass to allow for losses during cleaning. The amount of seed to collect of other weeds will vary with seed size and ease of collection, but the aim must be to collect an adequate (several 1000 seeds) sample of ripe seeds.
- **do not collect in wet conditions.** Collection is harder and seeds of some species can become very dormant.
- **beware of rapid heating of freshly collected samples – do not store in polythene bags.** Seeds are best kept in paper envelopes for transport and storage. Staple side and bottom seams of paper envelopes to prevent them coming unstuck due to moisture from seeds. Label envelope with name of field, farm and date of collection.
- **air dry seeds as soon as possible after collection.** Small samples can be dried in the envelopes by simply standing them on end with the flap open, and shaking the envelope daily. Larger samples are best dried in trays placed in a dry, well ventilated, but not windy, environment. Seeds of most species should be dry within about a week.

The quality of seed samples can be greatly improved by the use of an air column cleaner.

- **clean samples to remove poor quality seeds.** The best technique for cleaning samples will vary with species but sieving to remove large pieces of plant debris and air flow to remove lighter seeds are appropriate for many species.

3 Whole plant pot assays

The most widely used test for resistance involves growing plants from seeds collected from the suspect field, and spraying them with herbicides applied either at a single discriminating dose, or a range of doses. Such assays are usually conducted in a glasshouse or controlled environment chamber. Assessments usually involve visual assessments of mortality or plant vigour, or measurements of fresh or dry weight of foliage.

- **An essential component of all resistance assays is the inclusion of an appropriate susceptible reference population.** Susceptible standards should be chosen with care, to ensure that they are truly representative, and not atypically sensitive or insensitive to the herbicide under evaluation. Inclusion of several susceptible standards is recommended, especially when resistance is partial, as this will provide information on the background range of responses to herbicides.
- **Statistical advice should be sought to ensure that the experiment design and replication is appropriate.** Experiments which include populations with varying levels of resistance, often introduce a large amount of variability into the resulting data.

Resistance screening tests in progress in a glasshouse.

DOSE RESPONSE EXPERIMENTS

- **In initial studies it is preferable to use a range of doses to obtain a response curve.** This enables the degree of resistance to be better quantified by calculating the ratio of doses required to produce the same effect in resistant and susceptible populations.

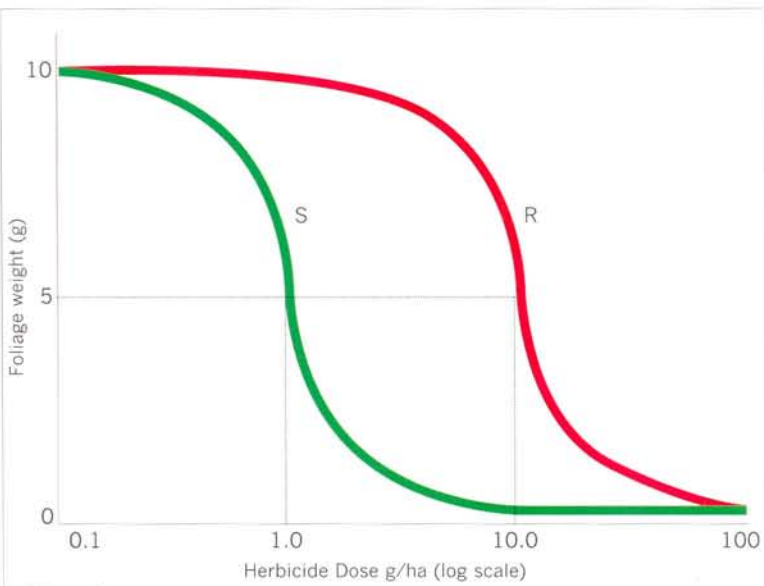


Figure 1.
Dose response curves for a Susceptible (S) and a Resistant (R) population

ED_{50} (susceptible) = 1.0 ED_{50} (resistant) = 10.0

$$\text{Resistance Index} = \frac{ED_{50}\text{ (resistant)}}{ED_{50}\text{ (susceptible)}} = \frac{10}{1} = 10$$

Usually the dose required to give a 50% reduction in the measured parameter (usually foliage weight or number of surviving plants), relative to the untreated control is determined (Figure 1).

• Ratios of these estimates, (variously termed ED50, GR50, LD50 or I50), relative to that of a susceptible population, provide a resistance index (RI) which enables the degree of resistance to be described relatively simply.

• To obtain a good estimate of ED50 the dose range should be relatively wide and at least six doses are needed. It is usually best that each dose is twice the preceding dose in the range (e.g. 10, 20, 40, 80, 160, 320 g a.i./ha). The dose range used should include doses both below and above the field recommended rate as herbicides are normally more active under greenhouse conditions.

Resistance is not always absolute as shown in this *Alopecurus myosuroides* herbicide test. The Rothamsted population is known to be susceptible and has been well controlled. The Peldon population is showing no herbicidal symptoms as it is highly resistant. The Faringdon plants are alive, but show severe herbicide effects. Particular care must be exercised in interpreting such marginal effects; subsequent studies showed that the level of resistance seen in the Faringdon population can reduce the performance of herbicides in the field.

SINGLE DOSE RESISTANCE ASSAYS

• Once dose response information has been obtained, it is often possible to use a single (or two or three) discriminating dose(s) in future screening assays, which allows many more populations to be tested as fewer pots per population are needed. With some forms of resistance, such as most cases of resistance to triazine herbicides, resistance tends to be absolute. In such cases, resistance is easy to identify and choice of dose is not critical – so long as it kills susceptible plants. When resistance is partial, more care is required in choosing the most appropriate single dose.

A 'ring test' involving 16 organisations in 8 European countries has recently been undertaken to evaluate the consistency of resistance screening tests in order to improve the standardisation of testing procedures (Moss *et al.*, 1998). As a consequence of this study, the following recommendations were made:

RECOMMENDATIONS

1 **Ensure adequate seed supplies are available and clean them to remove poor quality seeds.** Poor quality, insufficient, seed samples are likely to result in poor quality plants which may be more, or less, susceptible to herbicides.

2 **Prior to spraying achieve well matched plants in terms of growth stage and vigour** by sowing pre-germinated seeds or by sowing plenty of seeds and thinning down to a constant number per pot.

3 **Do not rely solely on sub-irrigation for watering if soil-acting herbicides are being used** as this will prevent herbicides being moved down into the plant rooting zone.

4 **If a single dose assay is used, the best single herbicide dose is likely to vary between individual testing centres** and can only be determined by *preliminary experimentation*. Herbicide activity will be affected by numerous factors, but the most important factors are likely to be the soil organic matter level (for soil acting herbicides) and the growing conditions (especially light and temperature).

5 **Use susceptible and resistant standard reference populations in every assay.** Ideally, different testing centres should use identical standards for each species. Do not assume that all susceptible populations are equally susceptible to all herbicides.
Choose standards carefully and consider availability of seeds in the longer term.

6 **In single dose assays, aim to achieve an 85-95% reduction in foliage fresh weight for the susceptible standard.** Too high or low a level will reduce the sensitivity of the assay.

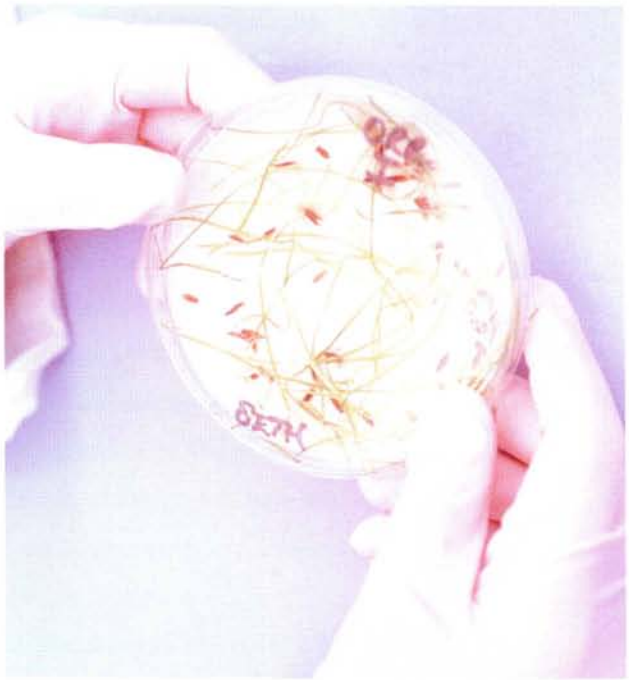
7 **Aim for <50% reduction in foliage fresh weight for any resistant standard.** If appropriate, include both a highly resistant (expected 0% reduction) and partially resistant (about 50% reduction) standards. Inclusion of only a highly resistant standard will not allow the relative herbicide efficacy between subsequent assays tests to be determined.

8 **Ideally record foliage fresh weight as an objective assessment of herbicide activity, when full effects of the herbicide are evident on the susceptible standard.** The time from spraying to assessment will vary with herbicide used, weed species and environmental conditions. With many weeds and herbicides, a three week time span between spraying and assessment is appropriate for plants kept in glasshouse conditions.

9 **Visual assessments may be a suitable alternative** and are certainly much quicker than weight assessments. If visual assessments alone are conducted, record foliage weights for the susceptible and resistant standard reference populations. This data can be used to check on the accuracy of the visual assessments and the consistency of results between subsequent assays.

10 Regardless of how the screening assay is conducted, the basis on which resistance is assigned should be stated. This is particularly important where populations show marginal or partial resistance.

11 Comparison of results obtained from different testing centres should be done with care, especially when resistance is partial, rather than absolute. Consistency between assays conducted at any one centre is likely to be better than between centres.



In Petri-dish assays, seeds are germinated in the presence of a dilute herbicide solution and subsequent growth is assessed. Petri-dish assays can be more convenient, cheaper and give quicker results than pot tests, but are not suitable for all weeds and herbicides.

4 Other diagnostic techniques

Other diagnostic techniques have been developed for detecting specific forms of resistance. These include pots tests using field collected plants, Petri-dish germination assays, chlorophyll fluorescence, leaf disc flotation and enzyme sensitivity assays. These have been reviewed by Moss (1995). Most of the principles outlined above are also relevant to these other techniques. However, the glasshouse pot assay is likely to remain the most appropriate single test for resistance as herbicide application and activity mimic what happens in the field. In addition pot assays can detect resistance regardless of mechanism – a very important attribute.

More specific assays may be quicker and more precisely identify the mechanisms responsible, but their very precision may be a limitation, especially where multiple mechanisms of resistance exist. In addition, care must be taken in interpreting results from methods which involve using herbicides in ways totally different to field applications.

5 Interpretation of results

It is important to recognise the fact that plants or seeds collected for resistance tests usually represent a biased sample. How representative they are of the entire field depends on the method of sampling and the proportion of plants which survived treatment in the field. If seed samples were collected from a few surviving resistant plants, when the majority of susceptible plants were killed, then any test result will overstate the degree of resistance currently present in the entire field population. **This should not be viewed as a limitation of diagnostic assays, but a positive attribute, as it enables resistance to be detected at an early stage of development,** when it is easier to take action to prevent the situation getting worse.

• With results from dose response experiments, the higher the resistance index (ratios of ED50 values relative to that of a susceptible population), the greater the level of resistance (Table 1). Small resistance indices (e.g 2-3) can occur between normal susceptible populations, so these should be interpreted with care, regardless of statistical significance. With highly resistant populations it may not be possible to obtain an ED50 value and so a precise resistance index cannot be calculated.

Table 1. Results of a glasshouse dose response investigating the effect of fenoxaprop on four populations of *Alopecurus myosuroides*.

Population	ED50 value (g a.i./ha)	Resistance Index
A (susceptible)	38	1.0
B	1022	27.0
C	184	4.8
D	76	2.0

Interpretation: Population B had a resistance index (RI) of 27.0 indicating a high level of resistance. Population C, with a RI of 4.8, showed partial resistance, which is likely to have some impact in the field. The marginal insensitivity of population D, with a RI of 2.0 may, or may not be of significance in the field. Further studies would be essential before any firm conclusion could be made.

• When resistance is absolute, interpretation is relatively easy as plants are either likely to be alive (resistant) or dead (susceptible) over a wide dose range. In such situations simply expressing the proportion of plants surviving treatment is likely to be appropriate, although how representative the tested sample is of the entire

Table 2. Results of a glasshouse pot screening assay in which a single dose of fenoxaprop (55 g a.i./ha) was applied to four *Avena fatua* populations.

Population	% reduction in foliage weight*
W (susceptible)	93%
X	7%
Y	68%
Z	84%

* = relative to untreated control pots for same population

Interpretation: The susceptible standard, population W, was well controlled by this dose of herbicide. Control of population X was very poor indicating that is was resistant. Population Y was partially controlled, indicating partial resistance. There appeared to be a marginal difference between the susceptible standard (W) and population Z. Further studies would be needed to determine whether this difference had any relevance in the field.

field population must be born in mind. When resistance is partial, interpretation is more difficult (Table 2). Statistical comparisons, while essential for research studies, are not necessarily appropriate in routine screening tests.

- With single dose assays, one classification system that can be used to assign different degrees of resistance is a * rating system which encompasses the concept of varying degrees of resistance at the population level. The original system required the inclusion of three reference populations, but the revised system (Clarke, Blair & Moss, 1994) requires the inclusion of only two reference populations, one susceptible and one resistant, which are included in every test.

- Results from resistance screening experiments should be related to the herbicide performance in the sampled fields. It then becomes possible to use diagnostic test results to predict, at least to some degree, the likely impact of resistance on herbicide performance elsewhere.

6 Conclusions and references

One of the primary aims of integrated weed control must be to try to prevent herbicide-resistance developing. However, if this is unsuccessful, it is vital that resistance to herbicides is detected as early as possible so that resistance management strategies can be implemented. If resistance becomes an acute, whole farm problem, then control options are more limited and greater expense and effort will be almost inevitable. Confirmation of resistance can result in substantial changes to the farming system e.g. changes to crop rotation, cultivation practices and the use of more expensive herbicides. Therefore it is essential that resistance tests are conducted properly if reliable and meaningful results are to be obtained. It is hoped that these guidelines will help achieve this goal.

HRAC publications

All HRAC publications are accessible on the internet at:
http://www.plantprotection.org/HRAC/
Requests for reprints can be made from the HRAC publicity officer or from the HRAC display at major weed-related conferences.

- *Monograph 1
A review of graminicide resistance
Dr A.M. Mortimer, University of Liverpool, August 1993
- Monograph 2
Herbicide cross-resistance and multiple resistance in plants. Dr S Powles and Dr C Preston, University of Adelaide, February 1995
- **How to minimise resistance risk and how to respond to cases of suspected and confirmed resistance*
- *Classification of herbicides according to mode of action* 1998 (periodically updated)
- *Partnership in the management of resistance* 1997
- *Guidelines to the management of herbicide resistance* 1998
- *The role of HRAC in the management of weed resistance.* Dr D Nevill, D Cornes and S Howard *Pesticide Outlook*, August 1998

* *No reprints available*

International Survey of Herbicide-Resistant Weeds Ian Heap, Oregon 1997
Accessible on internet at:
http://www.weedscience.com/

OTHER REFERENCES

Clarke, J.H., Blair, A.M. & Moss, S.R. (1994) The testing and classification of herbicide resistant *Alopecurus myosuroides* (black-grass). *Association of Applied Biologists Aspects of Applied Biology 37, Sampling to Make Decisions*, 181-188.

Moss, S.R. (1995) Techniques for determining herbicide resistance. *Proceedings of the Brighton Crop Protection Conference – Weeds*, 547-556.

Moss, S.R. et al. (1998) Screening for herbicide resistance in black-grass *(Alopecurus myosuroides)*: a 'ring test'. *Proceedings of the 50th International Symposium on Crop Protection, Gent, Belgium, Part III*, 671- 679.

Herbicide Resistance Action Committee

MISSION

We will facilitate the effective management of herbicide resistance by fostering understanding, co-operation and communication between industry, government and farmers.

AIMS

To foster a responsible attitude to herbicide use.

To support and participate in research, conferences and seminars which serve to increase our understanding of herbicide resistance.

To promote a better understanding of the causes and results of herbicide resistance.

To communicate herbicide resistance management strategies and support their implementation through practical guidelines.

To seek active collaboration with public and private researchers, especially in the areas of problem identification and devising and implementing management strategies.

To facilitate communication between industry representatives.

ORGANISATION

HRAC is an industry-based group supported by GCPF (Global Crop Protection Federation). Its members are AgrEvo, American Cyanamid, BASF, Bayer, Dow Elanco, DuPont, FMC, Monsanto, Novartis, Rohm and Haas, Rhône-Poulenc, Tomen and Zeneca. In order to ensure effective co-operation and communication, its working groups are divided on a regional basis (Europe, NAFTA and Rest of World). These regional groups interact with an informal network of country committees which are often indirectly linked to HRAC and often led by government researchers. There is no set structure but our co-operation is fuelled by our common aim – to manage resistance.

FURTHER INFORMATION

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