Seed sampling for resistance tests

Most resistance tests rely on pot or Petri-dish assays using seed samples typically collected from weeds surviving herbicide treatment in the field. Some tests are also available using plants collected from the field. However, remember....

- **Resistance tests are only as good as the sample collected and the extent to which it represents the suspect population**
- **Poor quality or immature seed = poor or no test result, which can be misleading and potentially worse than useless.**

How to collect a quality seed sample

- Collect samples when the majority of seeds are mature and 10-20% have already shed. Collecting too early or too late is likely to lead to samples with low viability
- Do not simply collect intact heads, as many seeds on those will be immature.
- Ensure samples are representative of the problem field by collecting seeds from many plants in an area of about 100m by 2-3 tramlines, unless the problem occurs in a distinct, smaller patch.
- For all species, allow seeds to air-dry in a shallow open container for a few days and then transfer to paper envelopes for storage and transport (never store in polythene bags).
- Send samples to the testing centre as soon as possible after collection.

Why have weed seed or plant samples from your fields tested?

- To know what you are dealing with – will herbicides work on your weeds?
- Avoid potentially wasteful use of herbicides - save money and prevent unnecessary use.
- Help assess the threat posed by resistance on newly acquired blocks of land.
- Monitor the success (or otherwise) of long-term resistance management strategies.
There are several testing methods available:

- **Glasshouse pot tests using seeds.** Seeds are sown in pots and established plants are treated with herbicide (typically at 2-3 leaf stage for post-emergence applications) and assessed 3-4 weeks later;

- **Glasshouse pot tests using plants (e.g. Syngenta Quick Test).** Surviving plants are collected from the field, transferred to pots (after trimming), allowed to regrow, sprayed, assessed.

- **Petri-dish assays.** Seeds are germinated in herbicide solutions and seedling growth recorded after 14 days.

- **Molecular tests.** Leaves are tested in a laboratory for specific mutations known to confer target site resistance.

- **Radio-labelled lab tests.** Plants are collected from the field and the rate of metabolism of radio-labelled herbicide in leaves is measured.

Keep a record of both the weed infestation level and the area sampled (either a sketch map or a GPS reading). This information greatly enhances the value of any resistance test result.

**Black-grass** – in winter cereals collect seeds in early July – early August. Never collect in June - too early. Collect seeds on dry days by gently rubbing heads over a bag or tray so only ripe seeds fall off. Aim to collect an amount equivalent to a cupfull of seeds if possible.

**Italian rye-grass and wild-oats** – in winter cereals collect seeds in late July/early August by holding heads or panicles inside a large polythene bag and shaking vigorously. Samples collected in the correct manner will consist almost entirely of seeds, with few stalks and little chaff or other debris. Aim to collect an amount equivalent to a mugful of seeds if possible.

**Broad-leaved weeds.**

**Poppy** capsules can be collected when mature in late July/August and seeds shaken out either in the field, or later. **Chickweed** plants can be pulled up when seeds start shedding in June/July and placed upside down in paper bags – seeds will fall out naturally. **Mayweed** heads should be collected when mature, in August, and seeds rubbed out later.
### The advantages and disadvantages of different resistance testing methods

<table>
<thead>
<tr>
<th></th>
<th>Answer in same crop year</th>
<th>Suitable for all weed species</th>
<th>Mimics field conditions</th>
<th>Suitable for all herbicides</th>
<th>Detects resistance regardless of mechanism</th>
<th>Test duration</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasshouse pots – seeds from field</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Slow</td>
<td>Medium</td>
</tr>
<tr>
<td>Glasshouse pots – plants from field</td>
<td>Yes/No potently</td>
<td>Yes</td>
<td>Yes</td>
<td>Most (not pre-em)</td>
<td>Yes</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Petri-dish germination</td>
<td>No</td>
<td>No/Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Fast</td>
<td>Low</td>
</tr>
<tr>
<td>Molecular lab assays</td>
<td>Yes/No potently</td>
<td>No/Yes</td>
<td>No/Yes (ACCase and ALS only)</td>
<td>No (TSR only)</td>
<td>Very fast</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Radio-labelled lab assays</td>
<td>Yes/No potently</td>
<td>No/Yes</td>
<td>No/Yes (EMR only)</td>
<td>No (TSR only)</td>
<td>Very Fast</td>
<td>Very High</td>
<td></td>
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</tbody>
</table>

TSR = target site resistance  
EMR = enhanced metabolism resistance

Glasshouse pot assays are the most robust method for detecting herbicide resistance

### Interpretation of test results

It is desirable that any resistance test:

- **Includes susceptible and resistant reference populations.** These are populations with known response to herbicides. Ideally their resistance mechanisms will be known. These helps validate the testing methodology and greatly help in the interpretation of results.

- **Provides photos of pots or dishes for both the test and reference populations** as these can make more impact than written results or 'R' ratings alone (See below).

- **States the assessment method and basis on which resistance is assigned.** This is particularly important where populations show marginal or partial resistance.

- **Incorporates the 'R' resistance rating system which has been widely used in the UK** to assist in interpreting the results of resistance tests and relating these to the likely impact on herbicide performance.

<table>
<thead>
<tr>
<th>Resistance rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRR</td>
<td>Resistance confirmed, highly likely to reduce herbicide performance</td>
</tr>
<tr>
<td>RR</td>
<td>Resistance confirmed, probably reducing herbicide performance</td>
</tr>
<tr>
<td>R?</td>
<td>Early indications that resistance may be developing, possibly reducing herbicide performance</td>
</tr>
<tr>
<td>S</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

RRR = Resistance confirmed, highly likely to reduce herbicide performance  
RR = Resistance confirmed, probably reducing herbicide performance  
R? = Early indications that resistance may be developing, possibly reducing herbicide performance  
S = Susceptible
The benefits and limitations of resistance tests

- Seeds or plants collected for resistance tests usually represent a biased sample as they have (usually) been collected from survivors of herbicide treatment. Hence, resistance test results will tend to exaggerate the level of resistance in the field, bearing in mind that there will be many older seeds in the soil seed bank which may produce plants that are still susceptible. Thus, the detection of resistance does not mean that herbicides will necessarily fail in the field.

- This bias in resistance testing should not be viewed as a limitation, but as a positive attribute, as confirmation of resistance provides an early warning sign of greater potential problems ahead. Resistance rarely decreases – it’s a one-way ticket – so this early warning should be heeded and steps taken to reduce the resistance risk.

- Resistance test results should be related to the sample location, the weed infestation and herbicide performance. The relevance of the resistance test result in terms of its applicability to the entire field depends on the area sampled, the weed infestation and distribution, and the proportion of plants that survived treatment in the field. This information helps in assessing the likely impact of resistance on herbicide performance. The higher the weed density, the sooner major problems are likely if resistance has been confirmed.

Acknowledgements
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For more information on herbicide resistance testing, visit the WRAG website:

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