European Guidelines to conduct herbicide resistance tests

Contents

Resistance to herbicides is undoubtedly one of the primary concerns in modern agriculture. Accurate and timely diagnosis is critical to resistance management and mitigation. Reliable tests for resistance are essential in enabling the rational implementation of effective integrated weed control strategies. Diagnostic tests should be rapid, accurate, cheap, and 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 summarizes the types of diagnostic tests used for detecting resistance.

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.

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. 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, 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

Collect history of the field

- Cropping for the last 5 years
- Cultivation techniques – for those crops
- Applications made in the past 5 years
- Any decline in activity seen on target weed

Any resistance seen on adjacent farms

Observed failure in the field.

2. Seed Collection

Information, sampling and seed shipment

As part of the implementation of a monitoring programme to follow the performance of plant protection products and the evolution of annual grass populations, the following process and a sampling procedure is recomended.

I- Information

Fill out an information sheet relating to the location.

This information will allow a population(s) to be tracked over time.

Information:

- Name/Plot Reference :
- *Address :*
- Crop :
- Trial number (if applicable) :
- Treatment date :
- Weed target :
- Weed stage t application (BBCH) :
- Crop stage at application (BBCH) :
- Spray volume - l/ha :
- Temperature (C°) and % RH at application :
- Product mixture : Yes or No
  Which product ?
  Crop history :
- Treatments and dose rates used in previous seasons(where known) :
- Final efficacy recorded with the product and dose rate used (g ai/Ha) :

II- Sampling.

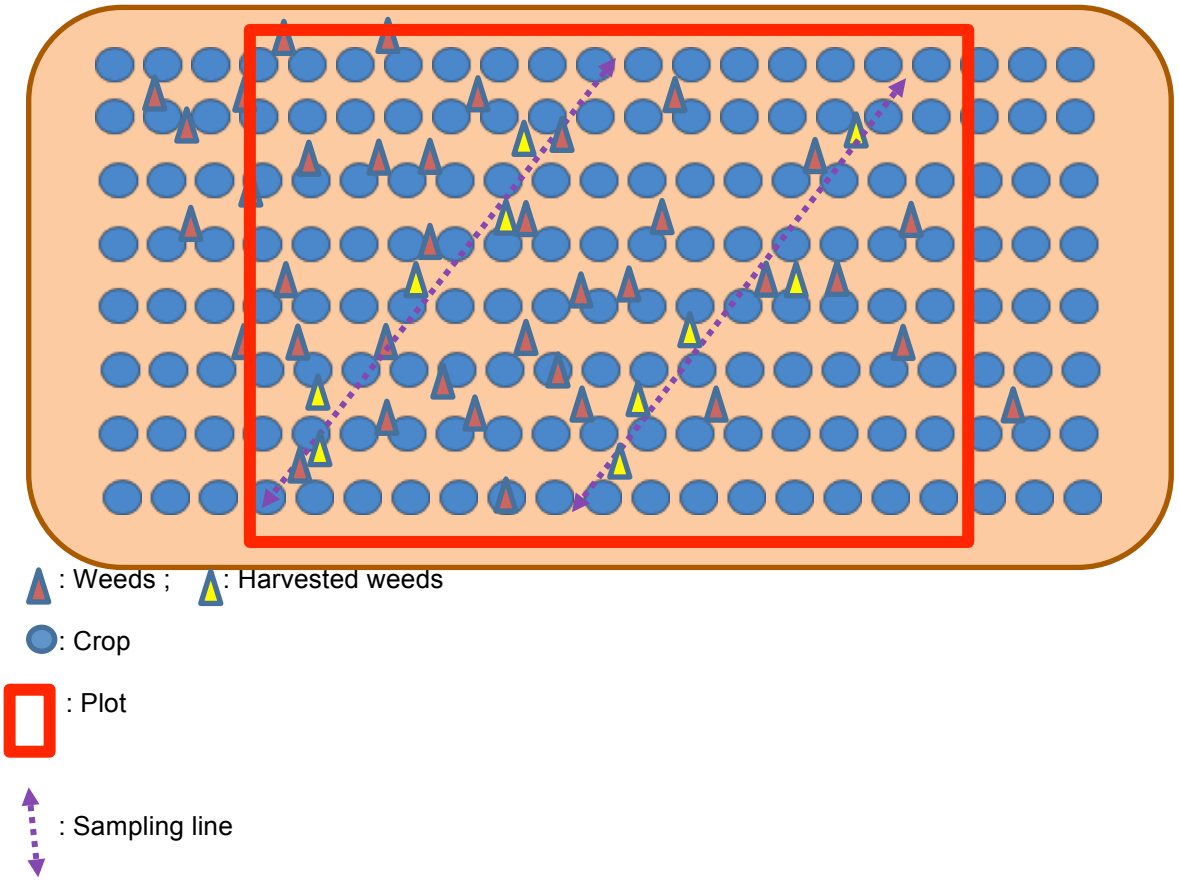
An appropriate process to collect seeds from putative resistance (R) and susceptible (S) plants for herbicide assays is critical. How to collect and how many plants will be sampled should be decided carefully. Seed heads or seeds must be collected at a timing that maximizes the number of viable seeds collected for use in whole-plant assays, unless other assays that only require plant tissue collection are available. Weeds are generally diverse in their maturation time and often weed maturation is aligned just prior to crop maturation.

Sampling : hundreds of seeds or around one hundred mature ears (one ear per plant) are gently rubbed above a kraft bag to obtain a quantity equivalent to a standard cup or a kraft bag

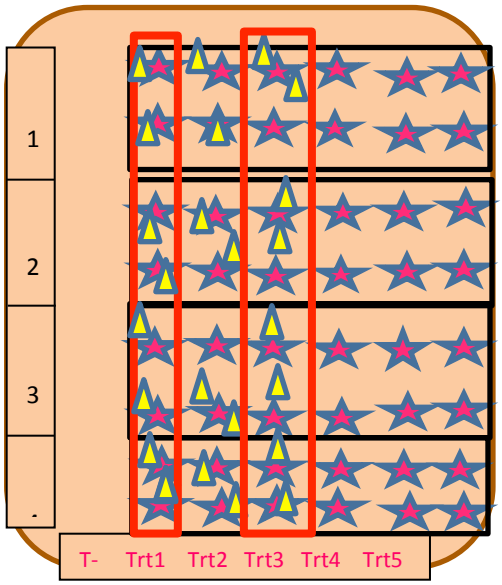
Farmer plot : Sample diagonally (2 or 3)

Trial plot : Sample in untreated and

Plot sampling example :



Trial sampling example :



: replicates

: sampling areas

T- : Untreated
Trt : Treatments with the same active substance at several dose rates.
: Weed seeds harvested

Watchout : samples must be harvested perfectly ripe and dried and sent directly to the responsible.

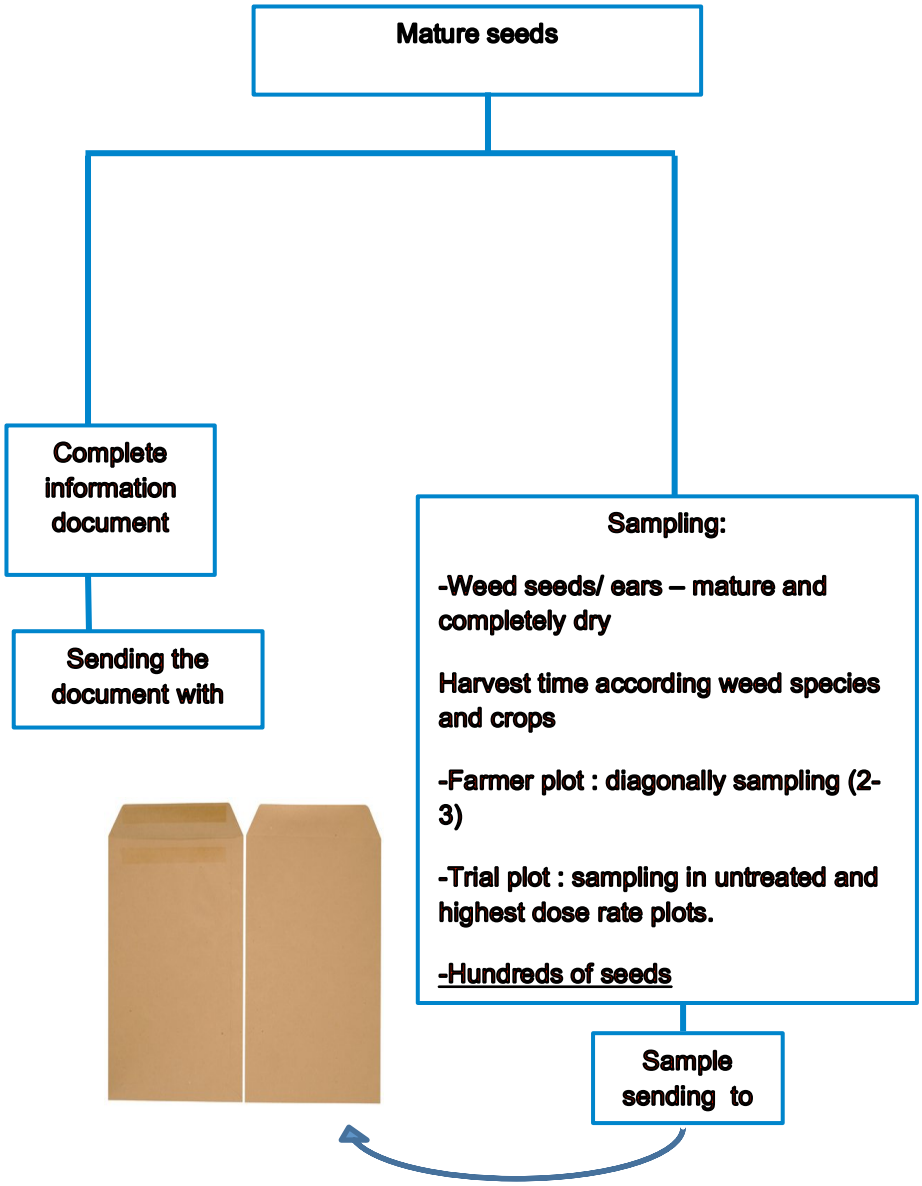
Especially avoid storage in damp and poorly ventilated.

Sending.

Samples of dried seeds and ears should be placed in an paper envelope clearly labelled with:

- Species harvested
- Type of sampling (seeds / ears)
- Date of sampling
- Plot reference (farmer or trial)
- Plot address
- Name of the local responsible person
- Treatment (active substance +dose rate)


Plot reference
Weed
Type of sampling (seeds, ears)
Date of sampling
Address
Local person reference
Company responsible
Full address


Mature seeds
Complete information document
Sending the document with
Sampling:
-Weed seeds/ ears – mature and completely dry
Harvest time according weed species and crops
-Farmer plot : diagonally sampling (2-3)
-Trial plot : sampling in untreated and highest dose rate plots.
-Hundreds of seeds
Sample sending to

3.Whole plant pot assays

The most widely used and recognized resistance tests are whole plant pot assays. In these studies, plants are grown from seed collected from locations where a herbicide is suspected to have failed due to resistance. They are then sprayed with the herbicide in question at either a range of doses (a 'dose response' study) or a single discriminating dose and then compared with the performance of other herbicides and with biotypes of known resistance status, including susceptible biotypes. Such assays are usually conducted in a glasshouse or controlled environment chamber, but may also be conducted in larger pots placed outside if consistent conditions can be achieved. Assessments usually involve visual assessments of mortality or plant vigor, or measurements of fresh or dry weight of foliage.

An essential component of all resistance assays is the inclusion of an appropriate **standard sensitive reference population**. Susceptible standards should be chosen with care to ensure that they are truly representative of the species and are 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. Consideration should be taken with regard to the long term availability and reliability of seed samples if embarking on a significant series of studies.

Standard sensitive reference populations (and standard resistant reference populations in some cases) can be sourced from:

- Adjacent fields, nearby locations: samples from areas where the herbicide in question is still fully effective
- Recognized suppliers: bulked samples of known sensitive biotypes available for purchase
- Other reliable sources: confirmed sensitive samples determined either through experimentation or taken from reference fields that have never been treated with a herbicide

Statistical advice, where available, should be sought to ensure that the experimental design is appropriate before proceeding. Experiments which include populations with varying levels of resistance often introduce a large amount of variability into the final dataset. A robust dataset is more likely to be achieved using a higher number of replicates and/or by using more individual doses. As the design and execution of experiments are refined through experience, variability might be expected to be reduced and the statistical power of the experiment therefore increased.

3.1 Dose response studies

Design

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. If possible, it is best to include both a highly resistant and a partially resistant standard. Inclusion of only a highly resistant standard may not allow the relative herbicide efficacy between subsequent assays to be determined.

Studies will typically aim to determine the dose required to give a 50% reduction in the measured parameter (usually fresh/dry foliage weight or visual assessments of injury/mortality) relative to the untreated control (Figure 1). Ratios of these estimates, variously termed ED50 (estimated dose giving 50% control), GR50 (growth reduction~) or LD50 (lethal dose~) relative to that of a standard sensitive population provide a Resistance Index (RI) value. These enable the degree of resistance to be described relatively simply.

To obtain a good estimate of ED50, the dose range should be relatively wide, with around six doses and 4-5 replicates considered ideal. The dose range used should include doses both below and above the field recommended rate (referred to variously as '1x', 'x', 'n', etc) as herbicides are normally more active under glasshouse conditions.

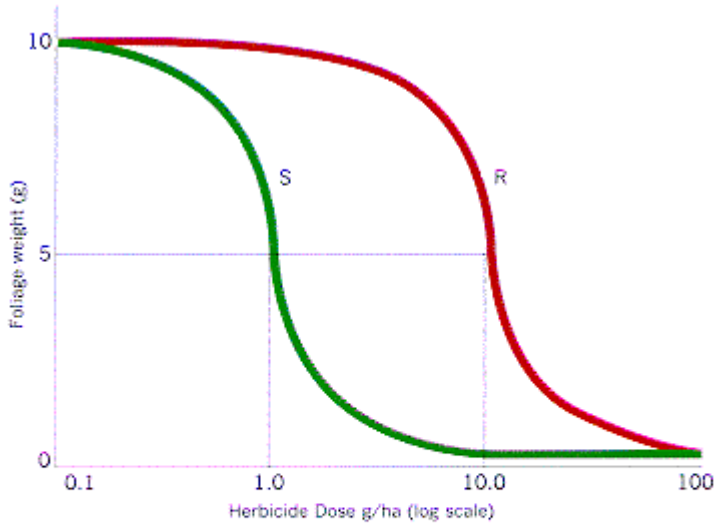


Figure 1.
Dose response curves for a Susceptible (S) and a Resistant (R) population

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

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

Key design features:

- Six doses relative to the 'x' field rate of the herbicide, such as: 0.125x, 0.25x, 0.5x ,1x, 2x, 6x
- 4-5 replicates (pots), with multiple individual seedlings per pot
- Include a standard of a different mode of action (where available) at 1x field rate for a given species or situation under investigation
- Follow the product label recommendation with regard to adjuvant and water volume, but ensure a consistent approach between tests; use tap water

Preparation

The first step in setting up a study is to ensure that good quality seed is available in adequate quantities. Clean seed samples prior to sowing, as poor quality seeds are likely to result in poor quality plants; these in turn may vary in their susceptibility to herbicides.

The next stage is to determine the mean % germination of a seed sample; this is of particular importance if a pre-emergence study is planned so as to ensure a sufficient number of seedlings germinate and emerge in the untreated control. Germination can initially be quantified in petri dish assays, but ultimately it is advisable to confirm this in the soil that will be used in the study. The soil used should be a medium soil type and sandy or heavy soils should be avoided.

The % germination of a sample can be improved by various techniques, such as freezing (e.g. some grasses), boiling water treatment (e.g. some hard-coated broadleaf seeds), mechanical abrasion (e.g. *Avena* spp) or soaking in dilute ammonium nitrate solution (e.g. many grasses) or other inorganic materials such as dilute sodium hydroxide. Some experimentation may be needed to determine the optimum method.

Pre-emergence studies: pre-germinated seeds should be sown into moist soil at a depth of 1-2cm at seeding rates designed to deliver a robust number of emerged seedlings in each pot (typically 5-10 individuals, depending on species), based on the known mean % germination rate.

Post-emergence studies: seeding rates should aim to consistently deliver a minimum of 4-5 emerged seedlings per pot and can be achieved most easily by using a higher seeding rate and thinning the number of seedlings down after emergence. Allow at least 5 days before spraying for plants to re-establish. Alternatively, various published methods outline the use of cuttings or seedling samples taken directly from the field and replanted in the glasshouse for resistance testing (e.g. Boutsalis, 2001).

Application/Maintenance

Applications should be made at weed growth stages which are consistent between replicates, treatments and across a series of studies.

Pre-emergence studies: applications should be made at a true pre-emergence timing - no later than 1 day after seeding has occurred. Applications should be made to moist soil.

Post-emergence studies: applications should be made at the 2-leaf stage for the majority of grass and broadleaf weeds; an exception are rosette forming species such as *Conyza* spp., where a diameter of 5cm should be targeted.

Applications should be made using a tracksprayer or boom sprayer using standard nozzles and pressures. Doses should be applied from the lowest to the highest to minimise the risk of carryover in the spray equipment.

Following treatment, pots should be laid out in a randomised complete block design or completely randomised fashion in the glasshouse, controlled conditioned environment or outdoor location. When watering, avoid contact with the foliage for at least 24-48 hours after application. Do not rely solely on sub-irrigation for watering if soil-acting herbicides are being used as this may prevent herbicides being moved down into the plant rooting zone. Monitor general plant health in terms of water status, nutrition and pest infestation; manage pests accordingly but avoid maintenance applications for the first 7 days after application.

Assessment

The most appropriate timing to assess post-emergence studies is 28-35 days after application (DAA), whereas for pre-emergence studies an interval of 35-42 DAA is recommended. However, depending on the herbicide used, the species being tested and environmental conditions, variation of assessment timing may be possible. The most important factor is to be sure that the full effects of the herbicide are visible on the standard sensitive reference population.

Non-destructive, subjective assessment methods based on the observed visual percentage reduction in biomass combined with other symptomology assessments, have been shown to be quite accurate depending on the experience of the assessor (Moss et al, 1998) and are quicker to conduct. It is still recommended to record foliage fresh weight assessments for the standard sensitive and resistant reference populations to ensure that visual assessments of biomass reduction are broadly aligned.

The most objective assessment however is to record foliage fresh and/or dry weight for all replicates of all treatments for each biotype tested.

The dataset is then in a form by which an analysis of variance (ANOVA) can be performed, together with the generation of efficacy plots and subsequent calculation of ED50 values for each biotype. The **Resistance Index** of a

given biotype can then be simply calculated by dividing the ED50 of a test biotype with the ED50 of the standard sensitive reference population. See Figure 1 earlier in this section for an example.

A resistance index >1 implies resistance to some degree, but the statistical significance of such differences is important and the figures derived for the standard resistant reference population(s) included in the study provides good guidance. Typically, arbitrary bands of resistance indices can be established to interpret the data, e.g. S, R, RR, RRR etc. These methods and their interpretation are covered in Section 5 below – 'Interpretation of Results.' The basis on which resistance is being assigned should *always* be stated.

3.2 Single dose experiments

Once dose response information has been obtained, it is often possible to use a single or lower number of 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 the choice of dose is less critical, as long as it kills the susceptible plants. When resistance is partial, more care is required in choosing the most appropriate single dose.

In single dose assays, it is important to aim to achieve a minimum 85-95% reduction in foliage fresh weight for the standard sensitive reference population; major deviations from this will reduce the sensitivity of the assay.

If a single dose assay is used, the best single herbicide dose is likely to vary depending on who and where the study is being conducted 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.

As for dose response studies, the basis on which resistance is assigned should always be stated, particularly where populations show marginal or partial resistance.

3.3 Rapid whole-Plant Assay for Post-Applied herbicides.

The Syngenta Quick test was developed for grass species, specifically rigid ryegrass (*Lolium rigidum*) and black-grass (*Alopecurus miosuroides)*[1]where vegetative tillers are separated then the shoots are cut and potted. The regenerated cuttings can be sprayed with foliar herbicides after at least 1 week from transplanting. ALS inhibitor herbicides, ACCase inhibitor herbicides and Photosymtem II inhibitors herbicides have been tested on the indicated grass weeds producing robust confirmation of the resistance to these herbicides. This assay can be used on some broadleaf weeds[2], but need to be pretested for applicability to other species.

Another quick test to detect resistance in the season was developed by DuPont[3] for black-grass (*Alopecurus myosuroides*) using agar-based medium for seedlings. With this test farmers and agronomists will be quickly and accurately informed about the level and type of grass weed resistant populations present in their fields, and will permit to put in place during the crop season the most adequate resistant weed management programs to preventatively assess the status of their fields and/or to stop the spread of resistance.

1 Syngenta quick test: A rapid whole plant test for herbicides resistance. 2001. Weed technology 15:257-263
2 High frequency of chlosulfuron resistant wild radish (Raphanus raphanistrum L.) populations nacross the western Australia wheatbelt. 2001. Walsh et al., Weed Research 47:542-550
3 Quick Test to identify herbicide resistant blackgrass (Alopecurus myosuroides). 1999. Salas et al., 11th EWRS (European Weed Research Society) Symposium, Basel

Seedlings plants collected from fields are transplanted to agar plates and place in the growth chamber or greenhouse until new shoots or roots develop. Plants are sprayed with discriminating doses of herbicides and evaluated 14 days after treatment.

Recently, anin-season quick resistance test (RISQ) was developed to test resistance to ACCase and ALS inhibitor herbicides amoung grass species, including blackgrass (*Alopecurus*), foxtails (*Setaria*), canarygrasses (*Phalaris*, ryegrasses (*Lolium*) and wild oats (*Avena fatua* L.) in agar medium[4].This test requires at least a benchtop under grow lights, petri plates, agarose or agar. One or three leaf seedlings are placed horizontally on the agar medium containing a discriminating dose of the herbicide, with the root in full contact with the agagr. The petri plates are then incubated for 10 to 14 days. Resistant plants develop new leaves and roots at the discriminating dose, but the susceptible plants do not.

Farmers can use information from all of these in-season assays to make in-season weed management decision.

4. Other diagnostic techniques

Other diagnostic techniques have been developed for detecting specific forms of resistance. These include, petri-dish resistance assays, chlorophyll fluorescence[5], leaf disc flotation[6,7] and enzyme sensitivity assays[8].

4.1 Petri-dish resistance assays

These tests include the possibility to determine if a weed population is resistant using seeds in petri dishes. There are several tests that can be carried out based on the type of herbicide to test. S. Moss on 2007[9] has published the methodology to detect resistance to blackgrass to ALS inhibitor herbicides. This test can be used as an indicator of ALS target site resistance using herbicides such as sulfometuron ('Oust®') and mesosulfuron + iodosulfuron ('Atlantis® WG') on seed samples. Sulfometuron tends to give the most reliable results. The test is unlikely to reliably detect partial resistance due to enhanced metabolism. The best doses to discriminate between resistant and susceptible black-grass populations are confirmed as 1 ppm for sulfometuron and 0.1 ppm for mesosulfuron + iodosulfuron.

Other herbicides different that ALS inhibitors herbicides can also be used on this type of assays. That is the case of ACCase inhibitor herbicides[10,11] where products like clodinafop at 7.5 ppm, tralkoxydim at 3ppm, fenoxaprop 10 ppm, sethoxydim 10 ppm and cycloxydim at 1 ppm were used to detect resistance. Also herbicide like pendimethalin at 10 ppm could be used

There are also petri dish tests using seeds develop for poppies[12] (*Papaver rhoeas*).

---

[4] Syngenta RISQ test: a novel in-season method for detecting resistance to post-emergence ACCase and ALS inhibitor herbicies in grass weeds. 2011. Weed research 51:284-293

[5] Chlorophyll fluorescence imaging: a new method for rapid detection of herbicide resistance *Alopecurus myosuroides*. 2013. Kaiser et al., Weed Research

6 Rapid diagnosis of ALS/AHAS-resistant weeds. 1993. Gerwick et al., Weed Technology 7:519-524

7 A rapid invivo shikimate accumulation assay with excised leaf discs. 2005. Shaer et al., Weed Science 53:769-774

[8] Assay of acetohydroxyacid synthase. 1988. Singh, et al., . Anal. Biochem. 171, 173-179

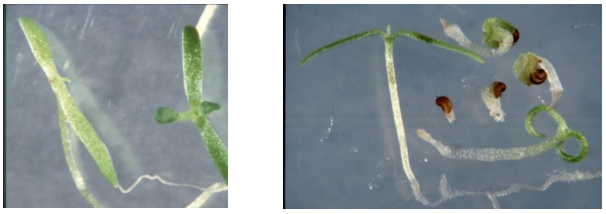
[9] WRAG: 2007 Rothamsted Rapid Resistance test for ALS inhibiting herbicides: Black-grass (*Alopecurus myosuroides*). R. Hull & S. Moss Rothamsted research

[10] WRAG 2003 Rothamsted rapid resistance test in ray-grass. S. Moss. Rothamsted research

[11] WRAG 1999 Rothamsted rapid resistance test in black-grass, wild oats and ray-grass. S. Moss. Rothamsted research

[12] A qualitative quick-test for detection of herbicide resistance to tribenuron-methyl in Papaver rhoeas. 2001. Cirujeda et al., Weed Research 41, 523-534

In the figure below can be observed results this types of tests



ALS target site resistant standard population left dish = untreated,
right dish = 0.1 ppm mesosulfuron+iodosulfuron

For other weed species it is necessary prior to the test to determine the best single dose to use.
For each weed population being tested, include always at least one and preferably two, susceptible reference populations; if possible, also include an ALS-resistant reference population. Two weeks after the seeds have been exposed to the herbicides, measure shoot length for each germinated seed (to the nearest mm) and calculate total shoot length per dish for each untreated and herbicide treated dish for all populations.

Calculate the % reduction in total shoot length relative to the untreated value for the same population.

% reduction = (Total shoot length in untreated dishes – Total shoot length in treated dishes) x 100 / Total shoot length in NIL dishes

Although those testing techniques are relevant the pot assay under controlled conditions, greenhouse or growth chamber, 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.

4.2 DNA analysis

Bioassays are generally not quick enough to permit an adaptation of the spraying program during the growing season when resistance is detected. However, when resistance genes have been identified, development of fast and accurate DNA-based diagnosis tools is possible DNA analysis is becoming more and more routine and offers a quick tool to detect resistance to those herbicides where the resistance mechanism is linked to a mutation at the target site enzyme. When point mutations in genes encoding herbicide target enzymes have been identified, the polymerase chain reaction (PCR) technique can be exploited to magnify and ultimately detect resistant genotypes.

Thus, PCR is a highly effective tool used for the specific detection of resistance genes, but should be coupled with bioassays to conduct optimal resistance management strategies. Several scientific publications are available to conduct such analyses[13,14]

[13] Molecular tools for the diagnosis of resistance to herbicides inhibiting acetyl-CoA carboxylase in three grass weeds. Deley et al., *Thirteenth Australian Weeds Conference.*

[14] Molecular Biology and Genomics: New Tools for Weed Science. 2009. Tranel & Horvarth, BioScience 59(3): 207-215.

4.3 Leaf Disc Assays

Various leaf disc assays have been developed over the last 30 years to screen weed populations for resistance to herbicides with different mechanisms of action. Leaf disc assays have the advantage of being rapid, mechanism of action-specific and nondestructive. As the assays use leaves, it is critical that the appropriated tissue is selected and that plants are healthy and vigorously growing. For some type of herbicides young leaves are required. The strength of the leaf disc assays is the rapid turnover. In many cases, initial determination of resistance can be made within 24 to 48 hours. However, such assays can be labor intensive and the interpretation of the results need to be carried out with cautioun.

5. Interpretation of results

ssample. 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 ED_{50} 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 ED_{50} 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 (RI)
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 field population may be not easy to say. 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.

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.

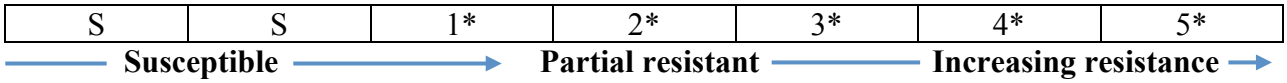
Population	% reduction in foliage weight*
W(susceptible)	93%
X	7%
Y	68%
Z	84%

* = 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 it 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.

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[15] 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.



S, 1* : S- (0.5(S-R)/4; 2*: S-((S-R)/4); 3*: S-(2(S-R)/4); 4*: S-(3(S-R)/4)
S=% reduction in fresh weight of the susceptible population
R= % reduction in fresh weight of the resistant population.
Fig. 1.Proposed resistance classification system

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.

[15] Testing and classification of herbicide resistance A*lopecurus mysuroides* (black-grass). 1994. Clark et al., Aspect of Applied biology. 37.