Rothamsted Rapid Resistance Test

Rye-grass

Revised protocol for testing Italian rye-grass *(Lolium multiflorum)*

(Refer to the full protocol leaflet for further details)

1. Seed collection

It is essential that both the **quality** and **quantity** of the seed sample is adequate. In winter cereals, the best time to collect rye-grass seeds is usually **mid July**. Collect the **equivalent of a mug-full** of ripe seeds by *gently* rubbing heads inside a small polythene bag. Do not store seeds in polythene bags. Air dry seeds as soon as possible and store in paper envelopes.

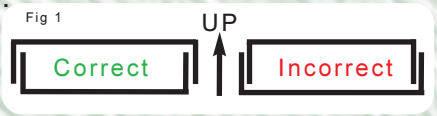
2. Seed cleaning and dormancy breaking storage treatment

When seeds are dry, clean sample by removing any pieces of stem or panicle. Rye-grass seeds have less dormancy than black-grass, so there is no need for storage at elevated temperatures. Seeds are best stored at 15-20°C for 2-4 weeks before testing.

3. Preparing Petri-dishes

Mix each seed sample thoroughly, and then for **each** population count out exactly **50 seeds into each of eight Petri-dishes (9 cm) containing four filter papers** (three cellulose and one glass-fibre filter paper on top). **Ensure dishes are used correct way up to avoid paper drying out (see Fig 1). Label the lid of each dish with its population name**. For each set of eight dishes, label two 'NIL', two 'DIC', two 'TRAL' and two 'CYC'. For each pair, label one rep 1, the other rep 2. Always include a commercial Italian rye-grass cultivar as a standard susceptible reference population. Preferably, also include a standard resistant population.

Fig 1



4. Preparing and adding herbicides to dishes

Herbicides should only be handled by people trained in their safe use and disposal.

Prepare a 1 litre solution of potassium nitrate in de-ionised (or distilled) water (2 g/litre).

Diclofop (DIC)

Half fill a **250 ml measuring cylinder with de-ionised water**, then measure out as accurately as possible **EITHER 0.83 ml of a product containing 378 g diclofop-methyl/litre (*e.g.* '*Hoegrass*'), OR 1.25 ml of a product containing 250 g diclofop-methyl + 20 g fenoxaprop-P-ethyl/litre (*e.g.* '*Tigress-Ultra*')**. Add this to the cylinder and make up to 250 ml with more water to form a stock solution of diclofop-methyl. Mix thoroughly and label.

Half fill a **second 250 ml** measuring cylinder with **potassium nitrate** solution, then measure out exactly **1.5 ml of either of the diclofop stock solutions**, add to cylinder and make up to 250 ml with more potassium nitrate solution. Mix thoroughly and label. This solution contains 7.5 ppm diclofop-methyl.

Add **7 ml** of the 7.5 ppm diclofop solution to the filter papers in each of the DIC labelled Petri-dishes.

Tralkoxydim (TRAL)

Use **1.25 ml of a product containing 250 g tralkoxydim/litre (*e.g. 'Grasp'*)** to make up the **250 ml** stock solution. Use **0.6 ml of this stock solution in 250 ml potassium nitrate** to produce a 3 ppm tralkoxydim solution. Add **7 ml** of the 3 ppm tralkoxydim solution to the filter papers in each of the TRAL labelled Petri-dishes.

Cycloxydim (CYC)

Use **0.78 ml of a product containing 200 g cycloxydim/litre (*e.g. 'Laser'*)** to make up the **250 ml** stock solution. Use **0.4 ml of this stock solution in 250 ml potassium nitrate** to produce a 1 ppm cycloxydim solution. Add **7 ml** of the 1 ppm cycloxydim solution to the filter papers in each of the CYC labelled Petri-dishes.

Controls (NILS)

Add **7 ml of the potassium nitrate solution** to the filter papers in each of the NIL labelled Petri-dishes.

Place dishes in clear polythene bags. Keep herbicide treatments and replicates separate. Place an empty Petri-dish containing filter papers on the top of each stack and seal the bags with a rubber band. Place stacks of dishes in an incubator set to provide a 17°C, 14 hour day (light on) and a 10°C, 10 hour night (light off). Move stacks around within incubator every 1-2 days.

5. Assessing Petri-dishes (Petri-dishes should be ready for assessing after 2 weeks)

Count and record the number of seeds which have germinated in each NIL (control) dish. 'Germinated' means that an emerging root or shoot is visible.

For all NIL **and** herbicide treatments, count and record the number of shoots in each dish that are 1 cm or more in length (touching lid, or would have touched lid if vertical).

For the assessments of germination (NILS only) and numbers of shoots over 1 cm (NILS and herbicide treatments), add the two replicate values together, to obtain a % figure.

Calculate the % of germinated seeds which have shoots over 1 cm for the NIL dishes. Typically it should be 80-90%.

For all populations and herbicides, calculate the % reduction in number of shoots over 1 cm relative to the NIL values for the same population (see assessment sheets).

6. Interpretation of results

See page 14 of the full protocol leaflet for assignment of resistance '*' and 'R' resistance ratings, and interpretation of results. The system is the same as that described for black-grass, but the value for the Italian rye-grass susceptible standard is used instead of that for the ROTH susceptible black-grass standard.

Dr Stephen Moss
Rothamsted Research
14 April 2003

Tel: (01582) 763133 ext. 2521
E-mail: Stephen.Moss@bbsrc.ac.uk