the northern
HERBICIDE RESISTANCE Reporter

A Newsletter keeping you up to date with research and development in herbicide resistance in the Northern Region

Current Status of Herbicide Resistance in the Northern Grain Region

The GRDC-funded project 'Detection, monitoring and management of herbicide resistance – northern region' (UQ 138) commenced in 2001. The agronomy component of this project, which has been using random survey throughout the northern region to detect resistant weeds, concluded on 30th June 2004. The free herbicide resistance testing service at UQ offered to farmers and advisors also concluded on this date. Between October 2001 and June 2004, over 600 weed populations from the northern region were collected and screened for resistance, including 56 populations sent by farmers and agronomists **(see Map 1).**

Field surveys

The random surveys were conducted towards the end of the summer and winter cropping periods when weed seed was mature. Most of the sampling was from in-crop paddocks and, because glyphosate resistance poses a major threat to fallow weed control in this region, fallow paddocks were included in the sampling **(Figure 1).**

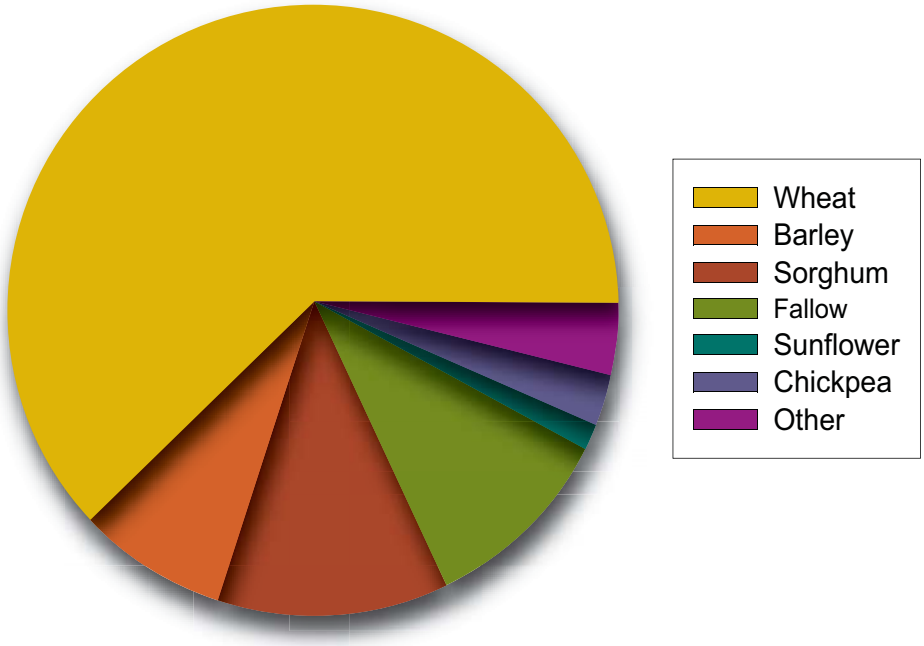

Figure 1: Crops and fallows sampled throughout the northern region for herbicide resistant weeds

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LOOK OUT FOR ...

Herbicide resistance preventive strategies being published soon for key at risk weeds in southern Queensland, central Queensland and northern New South Wales.

RESISTANCE is FUTILE

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Seed was collected from representative samples of the most abundant mature weed species in each paddock. Wild oats accounted for 50% of the species that were collected and screened. Approximately 95% of the wild oats were *Avena sterilis* ssp. *ludoviciana* and the remaining were *Avena fatua*.

Another seven species accounted for 35% of the remaining total; Common sowthistle (14%), African turnip weed (4%), Bladder ketmia (4%), Barnyard grass (4%), Turnip weed (3%), Black bindweed (2%), Annual ryegrass (2%), and Liverseed grass (2%). The remaining 15% consisted of a large range of grass and broadleaf weeds **(Figure 2).** The large percentage of wild oats that were sampled was primarily due to their abundance within wheat and barley paddocks but could also have been slightly biased by the fact that wild oats exist as different aged cohorts within a crop and, therefore, mature over a relatively long period. For other species the timing of a collection survey must coincide within a much narrower window in order to collect mature seed.

NEXT ISSUE

The **March 2005 Issue** will focus on the molecular results of **project UQ 138** and will also wrap up the **long-term field trials** that have been testing herbicide resistance preventive strategies.

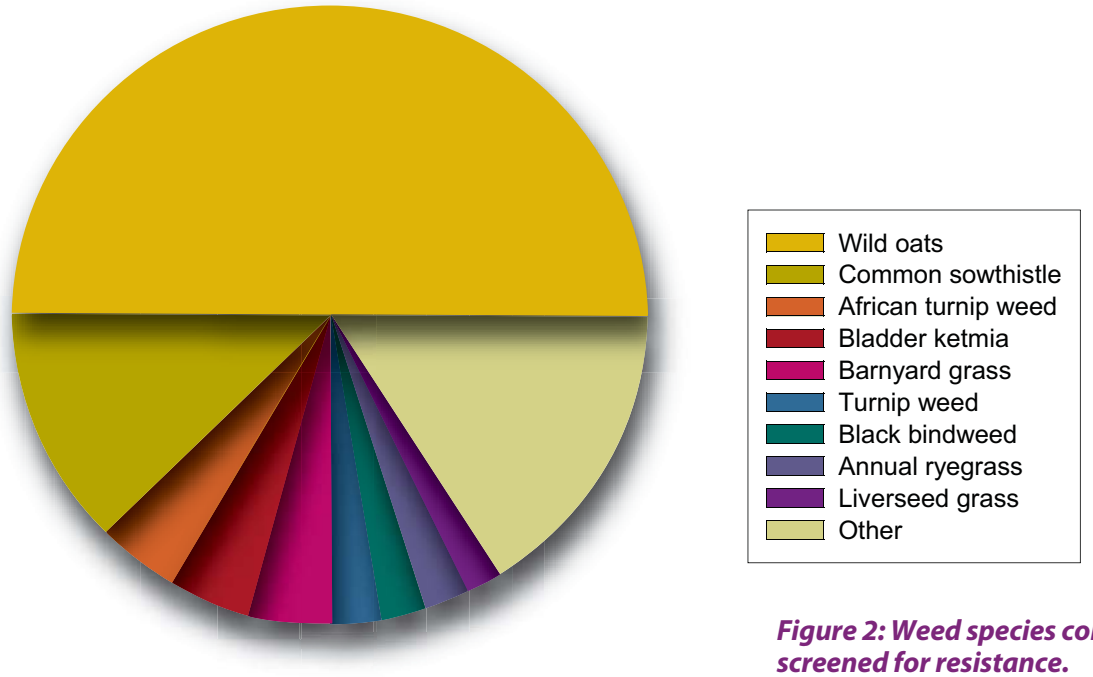
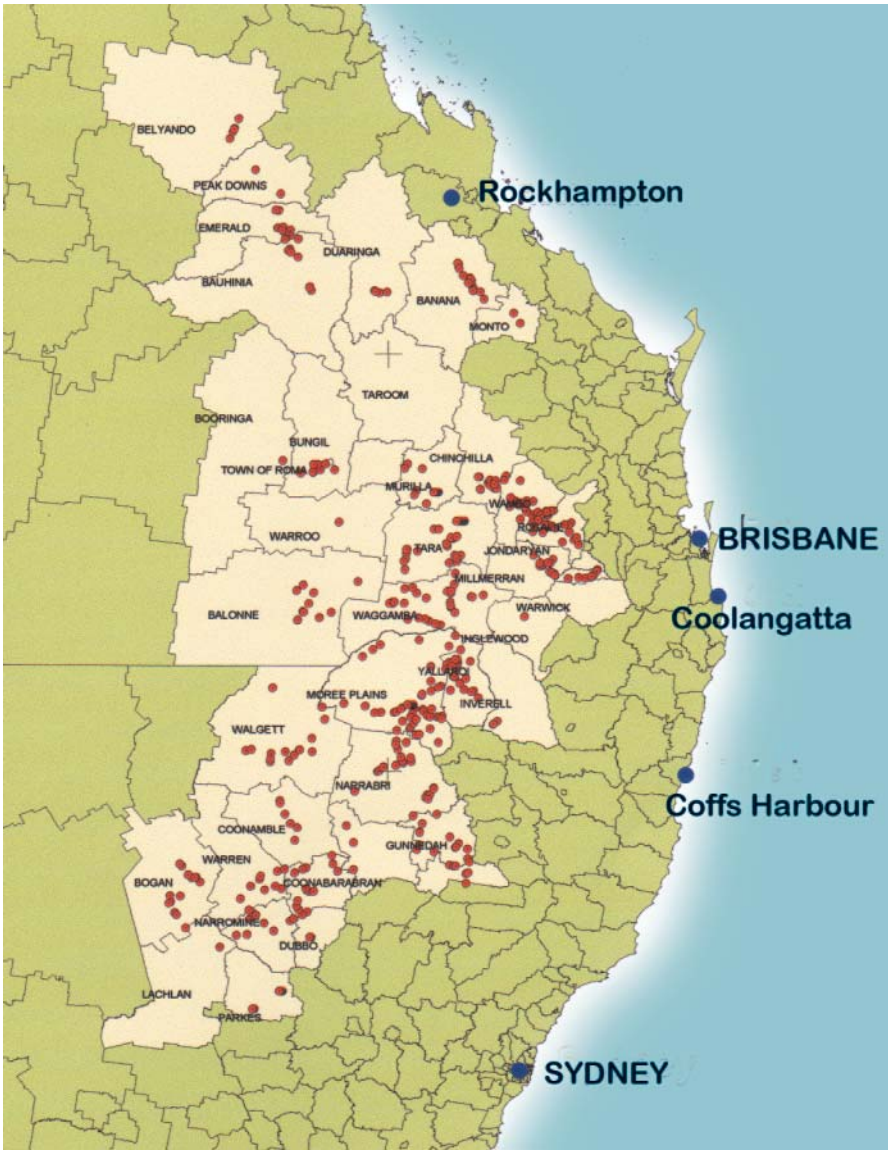


Figure 2: Weed species collected and screened for resistance.

Screening techniques

The randomly collected wild oat populations were screened with a petri dish bioassay called the Rothamsted Rapid Resistance Test (RRRT) that was developed by Dr Stephen Moss at the Rothamsted Research Station in the UK. This test imbibes the wild oat seed in petri dishes, which have the Group A 'fop' herbicide (fenoxaprop-p-ethyl eg. Wildcat) added to the filter papers at a very low concentration (7.5 ppm). If the wild oat seed can germinate and produce at least 1 cm of green shoot material on the herbicide solution, then this indicates resistance. A comparison is made against two reference populations, one being a known resistant population and the other being a known susceptible population, to determine the level, if any, of herbicide resistance. A susceptible population is one that is adequately controlled or killed by the herbicide.

When the plant response to herbicides is absolute, interpretation of the result is relatively straightforward. That is, if 100% of plants are killed then the population is susceptible, and if 100% survive then the population is resistant. This test also classifies populations as 'partially resistant' and 'strongly resistant', but these terms have been combined to 'resistant' in this report and accompanying maps. The RRRT takes 14 days to complete but requires the wild oat seed to have little or no dormancy in order to obtain acceptable germination percentages. This means that the test cannot usually be conducted on freshly collected seed as this



UQ 138 Sampling Locations 2001 - 2004

Map 1

Sampling locations throughout the northern grain region where 608 weed populations were obtained for the GRDC – funded project, UQ 138 between 2001 and 2004.

Sampling Locations

seed normally has high levels of dormancy. This necessitates the pre-requisite step of placing the wild oat seed in an incubator at a constant temperature (35°C) for 4 – 6 weeks until the dormancy level has declined sufficiently.

We found that the RRRT had a slight tendency to overestimate the level of resistance. That is, it would sometimes return a 'false positive' result, and indicate that a population had a low level of resistance or insensitivity when it was actually susceptible. For this reason, populations that were assessed as resistant were often re-screened with a standard pot test to confirm results.

All wild oat seed sent in by growers and agronomists was screened for Group A resistance using standard pot tests. These populations were screened with the same chemical that farmers had used in the paddocks and failed to get satisfactory weed control with. Some populations were subjected to dose-response trials using seven application rates of ¼, ½, 1, 2, 4, 8, and 16 times the recommended rate. These trials were conducted in the wind tunnel laboratory with precision application equipment.

All other species were tested using standard pot tests.

Resistance status of grass weeds

There were 28 wild oat populations that were resistant to Group A herbicides **(Map 2)**, which is approximately 10% of the samples. Eleven of the 28 resistant populations were very strongly resistant and had 100% survival at 16 times the recommended application rate.

Two of the Group A resistant wild oat populations were resistant to both 'fop' and 'dim' chemical classes, and 2 populations were cross resistant to the recommended rate of flamprop-m-methyl (eg. Mataven), a Group K herbicide.

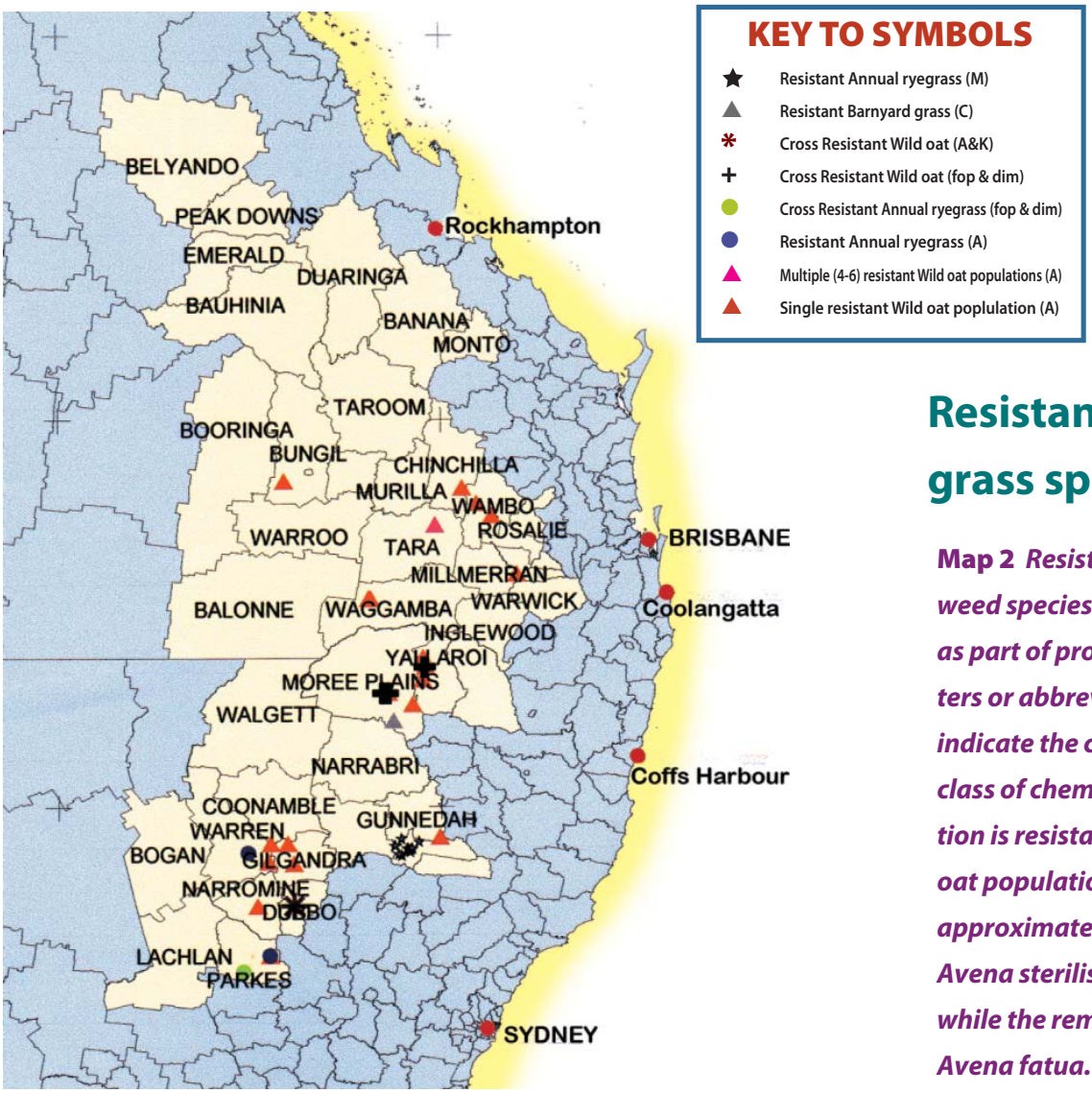
Two populations of annual ryegrass were resistant to Group A herbicides with one population being both 'fop' and 'dim' resistant.

The glyphosate resistant (Group M) annual ryegrass populations in the Quirindi shire were detected and confirmed by staff from NSW DPI.

As well, 1 strongly resistant barnyard grass population to atrazine, a Group C herbicide, was found in the Moree Plains Shire in northern NSW.

Resistance status of broadleaf weeds

Four broadleaf weed species, common sowthistle, turnip weed, African turnip weed,



Resistance status - grass species

Map 2 *Resistance status of grass weed species that were screened as part of project UQ 138. Letters or abbreviations in brackets indicate the chemical group or class of chemical that the population is resistant to. Of the 300 wild oat populations that were tested, approximately 95% of these were Avena sterilis ssp. ludoviciana while the remaining 5% were Avena fatua.*

and charlock, were found to be resistant to Group B herbicides. Most of these resistant weeds were from Queensland, predominantly in the Waggamba, Wambo and Tara shires **(see Map 3)**. There were only two resistant broadleaf weed populations from New South Wales, a resistant charlock population from Narrabri shire and one resistant common sowthistle population from the Yallaroi shire. This pattern is somewhat different to what had been detected in earlier projects (UQ 35NR and UQ 103), which detected a total of 39 resistant broadleaf weed populations and 22 of these were from the Moree Plains and Yallaroi shires of New South Wales.

Mechanism of Group A resistance

The mechanism for the strongly resistant wild oat populations has been characterised by the molecular biologist in this project, Ms Wenjie Liu, as 'target site' resistance. Target site resistance results from a genetic change so that the site where the herbicide molecule binds or acts within the plant is changed and the herbicide is no longer recognised.

The molecular characterisation has detected the mutation site, not previously found in other resistant wild oats. Ms Liu has subsequently developed a PCR-based molecular diagnostic test for this mutation. These discoveries were presented at the recent Australian Weeds Conference at Wagga Wagga **(see Wenjie's abstract on page 7).**

Other resistance mechanisms are 'enhanced metabolism' where a resistant plant is able to degrade a herbicide to non-phytotoxic metabolites at a faster rate than a sensitive plant and 'enhanced sequestration' where the herbicide is inactivated either through binding (often to a sugar) or is removed from metabolically active regions of the cell, often into a vacuole.

The molecular biology component of project UQ 138 is continuing until the end of 2004 and when completed this work should provide a valuable tool to assist in the early detection of resistant weeds in the northern grain region. An update on this work will be provided in the next issue of the Herbicide Resistance Reporter.

Summary

Prior to 1993, when the first resistance screening project (UQ35NR) commenced, there

KEY TO SYMBOLS

- Resistant Charlock (B)
- Resistant Turnip weed (B)
- Resistant African turnip weed (B)
- Resistant Common sowthistle (B)

Resistance status - broadleaf weeds

Map 3

Resistance status of broadleaf weed species that were screened as part of project UQ 138. Letters in brackets indicate the chemical group that the population is resistant to.

were no confirmed instances of herbicide resistance in the northern region. In a period of just over 10 years, a total of 110 weed populations have been confirmed as resistant **(see Map 4)**. This rapid increase in the incidence of herbicide resistant weed populations is consistent with the world-wide trend **(Figure 3)** and there are presently no signs that it is about to level out.

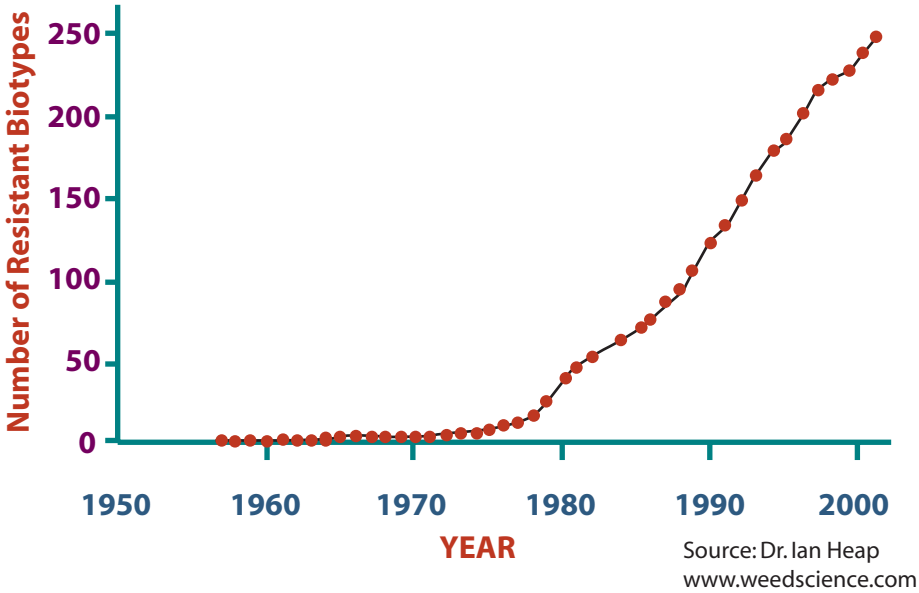
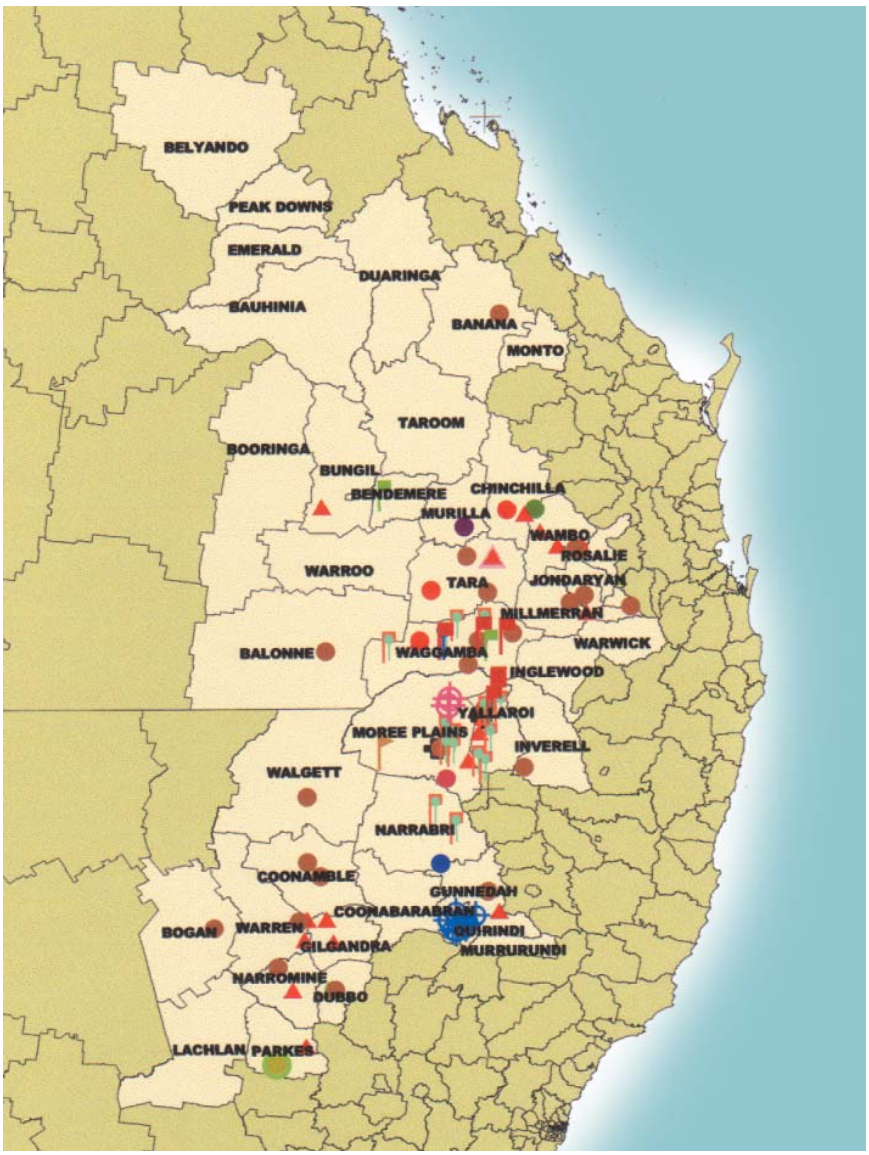


Figure 3. Worldwide increase in the number of herbicide resistant weed biotypes since the late 1950s



Resistance - Northern Grain Region
(based on results from current and former projects)

Map 4

Summary map showing all known resistant populations from UQ 138 and also from two former GRDC-funded projects; Project UQ 35NR (1993 – 1997) and project UQ 103 (1997 to 2000). Symbols prefaced by an asterisk (*) indicate resistant populations that were detected as part of projects UQ 35NR or UQ 103.

Herbicide Resistance Message Spread at Wagga Wagga by Northerners

Scientists associated with herbicide resistance projects in the northern grain region recently presented their findings at the 14th Australian Weeds Conference in Wagga Wagga. Below are the abstracts of the papers that were presented. If you wish to know more about any of these papers, please contact the primary author.

Preventing glyphosate resistance in weeds of the northern grain region

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Summary

Herbicide resistance is an increasing challenge across Australia's northern grain belt. Already 10 weeds have been confirmed as herbicide resistant in various parts of this region, and more have been identified at risk of developing resistance, particularly to glyphosate. Apart from common sowthistle (*Sonchus oleraceus* L.) and summer grasses, the weed threats differ across the region along with the farming systems. Mostly summer weeds are at risk in central Queensland, whereas a mix of summer and winter weeds are at risk in southern Queensland and northern New South Wales. The risk is greatest for the cropping systems using zero tillage with only limited rotation between summer and winter crops. Preventive strategies are being tested currently in a series of long-term field experiments in this diverse cropping region.

The proposed strategies to conserve glyphosate susceptibility use alternatives to glyphosate and/or stopping seed set in each weed flush during one in 4-5 seasons. These strategies will help ensure maximum effective life of important herbicides, as well as ensuring the continuing viability of conservation cropping in this region.

Keywords **Herbicide resistance, glyphosate.**

Molecular characterisation of resistance to ACCase-inhibiting herbicides in wild oat in the northern grain-growing region of Australia

Wenjie Liu[1], Dion Harrison[1], Chris O'Donnell[2], Steve Adkins[2] and Richard Williams[1]

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Summary

The molecular basis of resistance to ACCase-inhibiting herbicides was investigated for wild oat (*Avena* spp.) biotypes in the northern grain-growing region of Australia. Of 14 wild oat populations assessed, two resistant populations (UQT and UQM) were identified. UQT was resistant to the aryloxyphenoxypropionate, fenoxaprop-p-ethyl, but not to the cyclohexanedione, sethoxydim. By comparison, UQM was resistant to both herbicides. This indicates that the molecular basis of resistance to ACCase-inhibiting herbicides differs for the two populations. DNA sequencing of the genes encoding plastidic ACCase of UQT plants revealed a single nucleotide mutation resulting in an Ile-Asn substitution homologous to that recently found in *Alopecurus myosuroides* Hudson (black grass) and *Lolium rigidum* (annual ryegrass) populations which were also resistant to aryloxyphenoxypropionate but not cyclohexanedione herbicides. Our study is the first to detect this mutation in wild oat. We have developed a PCR-based molecular diagnostic test for this mutation. Future work will involve determining the genetic basis of resistance in the UQM population.

Keywords **Wild oat, ACCase-inhibitors, ACCase, herbicide resistance.**

Glyphosate-resistant annual ryegrass (Lolium rigidum Gaudin) from Tamworth, Australia, has upregulated EPSPS expression

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Summary

We have developed a TaqMan real-time PCR assay to quantify expression in annual ryegrass (*Lolium rigidum* Gaudin), of the EPSPS gene which codes for the enzyme targeted by glyphosate. Using this assay, we found that EPSPS gene expression was three to four fold higher in a glyphosate resistant biotype from Tamworth when compared to susceptible plants. These results are consistent with Baerson *et al*. (2002) who used Northern blotting and enzymes assays to estimate a two to three fold increase in EPSPS gene expression and enzyme activity, respectively, in a glyphosate-resistant *L. rigidum* biotype from Echuca. Hence, upregulation of the EPSPS genes and greater production of the EPSPS enzyme appears to be a common component of the glyphosate-resistance mechanism in annual ryegrass from Australia.

Keywords **Glyphosate, herbicide resistance, *Lolium rigidum*, annual ryegrass, EPSPS, shikimate pathway, TaqMan, real-time PCR.**

Reference

Baerson, S.R., Rodriguez, D.J., Biest, N.A., Tran, M., You, J., Kreuger, R.W. and Dill, G.M. (2002). Investigating the mechanism of glyphosate resistance in rigid ryegrass (Lolium rigidum). Weed Science 50, 721-30.

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