International survey of herbicide-resistant weeds – survey results and criteria to add cases

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Summary ALS and ACCase inhibitor-resistant weeds currently account for the greatest area and economic impact of herbicide-resistant weeds globally. Academics, farmers, and industry have been concerned about the potential impacts of glyphosate-resistant weeds since the introduction of GMO crops in 1996, and they have been actively searching for new cases. Thirteen new cases of glyphosate resistant weeds have been identified. It can be difficult to distinguish evolved low level glyphosate resistance from natural pre-selection variation in susceptibility to glyphosate between weed populations. This has challenged scientists to fulfil the criteria (such as the requirement to show impact under field conditions) needed to add a new case to the survey. These criteria are presented in this paper.

Keywords Resistance, ALS inhibitors, ACCase inhibitors, glyphosate, *Lolium*, *Avena*.

GLOBAL STATUS OF HERBICIDE-RESISTANT WEEDS

The 'International Survey of Herbicide-Resistant Weeds' (ISHRW http://www.weedscience.org) recorded approximately nine new cases of herbicide-resistant weeds annually from 1978 to 2007 (Figure 1). The tailing off of new cases in the last two years is an artefact as it usually takes two years of research to confirm resistance, thus some cases investigated in 2005 to 2007 may not be posted.

The importance of resistance cases is based on estimates made by researchers. These estimates are prone to a very wide margin of error but still give an indication of the number of sites and hectares infested. Many of the 315 cases of herbicide resistance recorded in 59 countries are scientific curiosities rather than major agronomic problems. Of the top 25 worst herbicide-resistant weed species ten are grasses, and six are pigweed species (*Amaranthus* spp.). The most problematic species world wide is *Lolium rigidum*, which has been identified as resistant in 18 countries, has evolved resistance to nine MOAs, occurs in six cropping regimes, infests over 9000 farms and 840,000 hectares. *Avena fatua*, *Amaranthus retroflexus*, *Chenopodium album*, and *Setaria viridis* are sequentially the next most important herbicide resistant weeds globally.

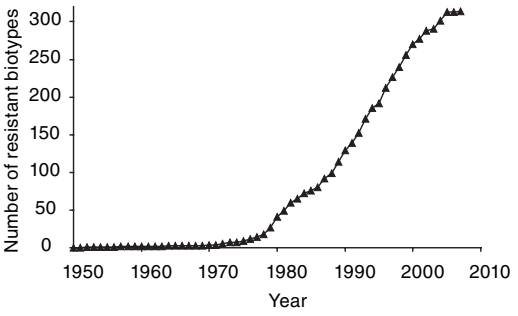

Figure 1. The increase in new cases of herbicide resistant weeds globally.

Some herbicide modes of action are more prone to the problem of resistance than others (Figure 2). The two major factors that contribute to the differences in the shape of the curves in Figure 2 are:

1. *The difference in the pre-selection proportions of resistant individuals in weed populations for each Mode of Action (MOA).* For instance the proportion of resistant individuals in weed populations that have not been exposed to herbicides is greater for ALS inhibitor herbicides than for synthetic auxin herbicides.
2. *The total number of weeds treated by the mode of action.* This is a factor of the total area treated with the MOA per year, the number of years that the herbicide MOA has been used, and the number of weed species that the herbicide MOA targets.

ALS inhibitors Ninety-five weed species have evolved resistance to ALS inhibitor herbicides, more than for any other herbicide MOA (Figure 2). This is

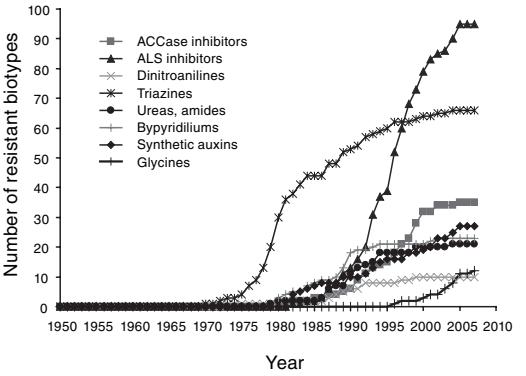

Figure 2. The increase in new cases of herbicide resistant weeds by mode of action.

partly but not solely due to the high initial proportion of ALS-resistant individuals in weed populations. The many different registered ALS inhibitor herbicides collectively target a very wide spectrum of broadleaf and grass species, and the popularity of these herbicides has ensured that a massive area globally has been treated with ALS inhibitor herbicides annually over the past 25 years. The ALS inhibitor herbicides still command a high market share globally and approximately five new ALS inhibitor-resistant species per year are expected to be identified into the next decade. ALS inhibitor-resistant weeds are of major importance globally.

Triazines Sixty-six weed species have evolved resistance to PSII inhibitor herbicides. The number of triazine-resistant weeds climbed most rapidly from 1975 to 1985, a period in which triazines dominated the herbicide market (Figure 2). In the last decade less than one new triazine-resistant species per year was identified. A few factors account for the levelling off of the triazine curve. First, most of the key weeds of maize that are targeted by triazines have already been identified as triazine-resistant – we are running low on new candidates. Secondly, newer herbicides, such as the ALS and ACCase inhibitors (along with the introduction of Roundup Ready crops) have undoubtedly controlled some of the new cases of triazine-resistant weeds. Thirdly, farmers, extension agents, and researchers are more likely to assume triazine-resistance and they do not bother to do the research to confirm the new species. Triazine-resistant weeds have moved from major importance in the 1970s and 80s to moderate to low importance today – farmers have learned to deal with them by adding other MOAs to their weed control program.

ACCase inhibitors Thirty-five grass weeds have evolved resistance to ACCase inhibitors. In 2001 the number of new species evolving resistance to ACCase inhibitors annually declined, primarily due to the fact that there are relatively few key grass weeds left to add to the list. Even so the area infested with ACCase inhibitor-resistant grasses is second only to that of the ALS inhibitors and continues to grow at a rapid rate. ACCase inhibitor-resistant species are of major importance.

Dinitroanilines Ten dinitroaniline-resistant weeds have been identified, and these were of most significance in the mid-1980s to the mid-1990s. Farmers have learnt to deal with most of them and their economic impact on crop production has waned.

Ureas and amides Twenty-one species have evolved resistance to ureas and amides. They have been used as long as triazines but have been used on far fewer hectares per year. Propanil-resistant *Echinochloa* species are still of major global importance in rice and account for the majority of hectares infested by weeds resistant to this MOA.

Bypiridiliums Together paraquat and diquat target a wide spectrum of weeds and were used extensively in the 1960s to the 1980s. Twenty-three weeds have developed resistance to bypiridiliums. In the last 15 years their importance has declined.

Synthetic auxins Synthetic auxins have been used for longer and over a greater area than any other herbicide MOA, yet only twenty-five weed species have evolved resistance to them. In addition few of the 25 reported synthetic auxin-resistant weed species have gone on to infest large areas or presented a major economic impact on crop production. Synthetic auxins are very low risk herbicides.

Glycines Glyphosate targets a very wide spectrum of weeds, has been used for over 30 years, and has been used over a very large acreage for over 20 years. Given this it is truly surprising that we have only 13 weeds that have evolved resistance to glyphosate, and only a few of these cover more than 100 hectares. In addition there have been a few cases of recurrent selection resulting in glyphosate resistance such as *Lolium perenne* in Ireland, and *Lotus corniculatus* in the USA (Boerboom *et al*. 1991, D. Johnson pers. comm.). Deliberately selected cases are not included in the International Survey of Herbicide Resistant Weeds. Glyphosate is a very low risk herbicide, yet it is clear that the number of glyphosate-resistant weeds will increase commensurate with its usage. The introduction of Roundup Ready crops in the mid 1990s has rapidly increased the acreage and intensity of usage which will accelerate the number of new glyphosate-resistant weeds identified.

Glyphosate resistant weeds currently have the least economic impact when compared to weeds resistant to the other MOAs discussed and paradoxically have the potential to have the greatest economic impact in the future. Farmers will deal with glyphosate-resistant weeds in a similar fashion to how they dealt with triazine-resistant weeds. They will continue to use glyphosate and add other MOAs to their program. This strategy effectively mitigated the impact of triazine-resistant weeds because many new herbicide MOAs became available in the 1980s and 90s. Few new herbicide MOAs are being developed today, hence the high level of concern by farmers, academics and industry that this strategy may not be as effective in mitigating the economic impact of glyphosate-resistant weeds in the future.

CRITERIA FOR POSTING HERBICIDE-RESISTANT WEEDS

The criteria for adding herbicide-resistant weeds (Heap 2005) to the 'International Survey of Herbicide-Resistant Weeds' (ISHRW) is posted at http://weedscience.org. The Weed Science Society of America (WSSA) defines herbicide resistance as '*the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis*'.

For a weed biotype to be added to the ISHRW database it must meet this WSSA definition of resistance **and in addition must be** identified as a problem weed to species level, confirmed using acceptable scientific protocols, heritable, and demonstrated to have a practical field impact on herbicide efficacy. Failure to conform to any one of these criteria will prevent a case from being added to the database. Few researchers have issue with identifying a weed to species level, and fortunately the literature is full of acceptable scientific protocols to confirm the 'first case' of resistance (Beckie *et al*. 2000, Heap 1994, HRAC 1999). The two criteria that present most difficulty to scientists are heritability and demonstration of resistance in the field.

Confirmation of heritability does NOT require detailed genetic studies The heritability criteria was included because some scientists have tested weeds for resistance by removing plants from the field, potting them up, and then running dose response experiments on them. This is not acceptable for confirmation of a 'new case' of resistance as the R and S populations may be at different growth stages, or may already have been exposed to a herbicide in the field, which can severely affect the outcome of the experiment.

Whilst not absolute evidence of heritability, testing with collected seed is sufficient for the purposes of the survey. It is also preferred, but not absolutely required, that second generation seed from greenhouse grown plants of R and S populations are collected and tested for resistance.

Low level resistance and the requirement to demonstrate resistance in the field Disputes over the definition of resistance primarily result from differing view points on what constitutes natural variation in weed populations and what is classified as low level resistance. Weed populations taken from different regions are likely to have natural variation in their GR_{50} (herbicide dosage required to result in a reduction in growth of 50%) values for a herbicide prior to herbicide selection pressure. Though these small differences may be statistically significant they are due to natural variation in herbicide response and listing the population as a herbicide-resistant weed is not warranted. Companies already know this variation exists and set their recommended rate for a particular weed based on widespread trials that encompass this naturally occurring diverse response to a herbicide. If we were to rely upon a scientific definition alone (a statistical difference in dose responses between two populations) then the survey would be clogged with many cases of 'resistance' that are of no practical consequence whatsoever, devaluing the survey.

The survey is intended for practical relevance rather than to document natural variations in herbicide response between weed populations. If greenhouse dose response experiments reveal less than a 10 fold level of resistance ($GR_{50}R/GR_{50}S$), which is the case for many of the glyphosate-resistant weeds recently identified, then we require scientific confirmation of the impact of resistance under field conditions. Unfortunately some researchers do not take this next step and valid cases of resistance may go un-posted for several years until another researcher performs the test. That is the price we pay to ensure the integrity of the survey. If there is no detectable difference in control of the weed under field conditions at the recommended rate then it will not be added to the survey.

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