Criteria for Confirmation of Herbicide-Resistant Weeds - with specific emphasis on confirming low level resistance.

Ian Heap, Organizer of the "International Survey of Herbicide-Resistant Weeds".
Web: www.weedscience.com,
Email: IanHeap@Weedscience.org
2005.

This paper outlines the criteria used by the "International Survey of Herbicide-Resistant Weeds" to document new cases of weed resistance on the www.weedscience.com web site. The intent of the "International Survey of Herbicide-Resistant Weeds" is to document practical cases of field selected, genetically inherited resistant weed biotypes that survive a rate of herbicide to which the indigenous population was controlled. This information assists farmers and academics in the development of effective weed control systems for the field and assists herbicide manufacturers in the development of appropriate stewardship programs for their products.

For a weed biotype to be listed on the site it must meet all of these criteria.

1. Fulfillment of the WSSA definition of resistance and the survey's definition of a herbicide-resistant weed.
2. Data confirmation using acceptable scientific protocols.
3. The resistance must be heritable
4. Demonstration of practical field impact
5. Identification as a problem weed to species level, not the result of deliberate/artificial selection.

Failure to conform to any one of these criteria will prevent a case from being posted.

Criteria 1. Fulfillment of the WSSA and International Survey of Herbicide-Resistant Weeds definition of resistance.

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".

Note that herbicide-resistant weeds fall within this definition. However not all "herbicide-resistant plants" are herbicide-resistant weeds; they may be herbicide-resistant crops or laboratory creations.

For the purposes of the survey, herbicide resistance is defined as "The evolved capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Source: Heap and Lebaron. 2001 in Herbicide Resistance and World Grains).

Criteria 2. Data Confirmation of Resistance

Resistance must be confirmed by an unbiased scientist through comparison of resistant and susceptible plants of the same species in a replicated and scientifically sound trial. The Herbicide Resistance Action Committee (HRAC) has prepared a publication that specifically deals with the detection of herbicide resistance that can be found online at http://www.plantprotection.org/hrac/detecting.html (HRAC 1999).

Initial Characterization of Resistance vs. Routine Screening

There is a big difference between testing for the initial characterization of a putative resistant weed biotype and the routine testing of hundreds of samples that may follow after the initial discovery. The literature is full of herbicide resistance tests, such as Petri dish bioassays, shoot assays, target enzyme assays, and fluorescence tests (Beckie et al. 2000). However for the initial characterization of a putative resistant weed biotype the most preferred test is a dose response experiment under controlled conditions (growth cabinet, glasshouse etc) using whole plants. Almost all other tests have been devised as quicker, and/or less expensive to facilitate routine screening of hundreds of samples. To confirm a new case of resistance the aim is to mimic, as closely as possible, the normal field application conditions in a controlled environment (growth cabinet, greenhouse etc) to determine the GR_{50} (dose required to reduce shoot weight by 50% relative to untreated plants) of the resistant and susceptible populations. A range of herbicide doses are used that encompass sub-lethal and lethal doses for both resistant and susceptible populations (Heap, 1994). Resistance in this experiment is confirmed if there is a statistical difference in response to the herbicide between the putative resistant population and the susceptible population (note: this is the scientific definition referred to below). Non-linear regression models are used to compare biotypes (Streibig, 1988; Brain and Cousens 1989, Seefelt et al. 1995). If the regressions are statistically different then resistance is usually reported as a ratio based on the GR_{50}'s of the resistant biotype compared to the susceptible.

Typically it is a relatively straight forward process to document resistance when the level of resistance exhibited is clear cut with an R/S resistance ratio (based on GR_{50}'s) of greater than 10 fold. However the confirmation of low level resistance is much more difficult and in some cases subjective. 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. The most important point of this paper is to clarify what we consider the cut off point for low level resistance and the requirements needed to test such cases.

Low Level Resistance

At what level do we declare a weed to be resistant and place it on the list of resistant weeds? This question is not easily answered. It is intriguing to me that many of us that have been involved in identifying herbicide resistance have an intuitive feel for true cases of resistance; however it is extremely difficult to put a clear cut definition down on paper.

There is a scientific definition and an agricultural field definition of resistance. Neither of them alone is ideal for the purpose of this survey, which is to accurately reflect the occurrence of a herbicide-resistant weed problem. There are strengths and weaknesses for each definition, and the International Survey of Herbicide-Resistant Weeds relies upon a combination of the two. Below is a description of each definition, along with the problems that the definition presents, and the final combination of the two.

1. **Scientific definition.**
   From a scientific view point resistance can be defined as a genetically inherited statistical difference in herbicide response between two weed populations of the same species.

   The problems with the scientific definition.
   The scientific definition does not take into account the recommended rate of a herbicide. Although two populations may statistically differ in their response to a herbicide it does not necessarily follow that the herbicide will not kill the most resistant of them at the recommended rate. A problem arises when a weed population is declared resistant under the scientific definition, but it is killed by the recommended rate of the herbicide under field conditions. Weed populations taken from different regions are likely to vary in their GR_{50} values for a herbicide, some of them being clearly statistically different than others. This is natural variation and does not warrant listing as a herbicide-resistant weed. 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. A better scientific view point would be that a population differs significantly in response to a herbicide when compared to the average response from numerous populations.

2. **Agricultural field definition.**
   Using this definition a classification of "resistant" requires that the resistant population must survive the recommended rate of herbicide under normal field conditions.

   The problems with the agricultural field definition.
   The problem that scientists have with using the recommended rate as a yard stick is that the recommended rate is a subjective rate that may vary from region to region depending on the crop or situation, or even economics of a herbicide. Thus it would be possible to define the same weed population as resistant in one crop and susceptible in another. (In addition the recommended rate is usually targeted at the most difficult to control weed. Thus some weed species may evolve a 4 fold level of resistance to a herbicide yet still be killed by the "recommended rate".
   Using the recommended rate as a measure of resistance without consideration for relative resistance to a susceptible control can be misleading. Application of the recommended rate on the same biotype will give varying results depending on the conditions. The environment (weather, soil type, growing conditions, stress etc) all play a big role in the effect of a herbicide on a weed. For marginal cases of resistance, a population that typically survives a field application may succumb to the recommended rate under greenhouse conditions. This result may be because the recommended rate under greenhouse or growth room conditions is often much more effective on weeds than when the herbicide

is applied under field conditions. Alternatively, it may indicate that further field testing is necessary to discern whether environmental conditions were originally at play. Using the recommended rate alone is not a sufficient criterion for classification of resistance.

3. **Practical definition of resistance.** If we were to rely upon a scientific definition alone then the survey would be clogged with many cases of "resistance" or varied tolerance that were of no practical consequence whatsoever. If we were to rely solely upon an agricultural field definition then we would be relying on a relatively subjective approach that is likely to change from crop to crop and region to region. A combination of both a scientific and agricultural perspective is necessary to provide a practical definition of resistance. Clearly the scientific definition is the lowest hurdle, and any population that does not pass this definition cannot be listed. For the purpose of the survey, the requirement to demonstrate a practical impact of the case of resistance is that the resistant population has caused a problem of control in the field when the herbicide is used at the recommended field rate. When investigating a case of low level resistance, it is necessary to provide much more experimental evidence than for high-level resistance (i.e., at least 10 fold). One dose response experiment will not suffice for cases that exhibit less than 10 fold resistance. It will require both greenhouse dose response experiments and field experiments using susceptible and potentially resistant plants of similar size and location. Ideally field experiments should be replicated on more than one site and over more than one year.

Criteria 3: The resistance must be heritable
In some cases scientists have tested for resistance by removing plants from the field, potting them up, and then running dose response experiments on them. This may be a quick test to determine if further trials should be conducted, but it is not acceptable for confirmation of a new case of resistance. The R (potentially resistant) and S (susceptible) 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. Fortunately this is not usually an issue, as the testing procedures usually require the collection of seed from resistant and susceptible populations. For the purpose of listing a biotype in the survey, testing with collected seed is generally required for sexually propagated species. 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.

Criteria 4. Demonstration of practical field impact
The survey is intended for practical relevance rather than to document natural variations in herbicide response between weed populations. 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. Although valid scientific arguments may be made for the occurrence of low level resistance even when the weed is controlled by the field rate under field conditions, for the purposes of a practical survey, the weed must present a problem to the farmer when using the herbicide at the recommended rate. This criterion becomes even more critical when low level resistance is involved.

Criteria 5: Be a weed and identified to species level and not be the result of deliberate/artificial selection.

To be classified as a herbicide-resistant weed, the plant in question must be a weed and identified down to species level. Cases of deliberate selection for herbicide resistance, including herbicide-resistant crops as volunteers, are not included in the survey.

Conclusion
Listing a weed biotype in the survey must be conducted with prudence to ensure the claim is accurate. It should also be timely to allow appropriate guidance for herbicide manufacturers and growers. These criteria are intended to facilitate that end through a combination of objectivity, transparency and consistency that is critical to the scientific method and the practical application of expert experience.

LITERATURE CITED

Brain, P. and R. Cousens. 1989. An equation to describe dose responses where there is stimulation of growth at low doses. Weed Res. 29:93-96.

Beckie, Hugh J., Heap, Ian M., Smeda, Reid J., Hall, Linda M. Screening for Herbicide Resistance in

Weeds. Weed Technology 2000 14: 428-445
HRAC 1999. Detecting Herbicide Resistance Guidelines for conducting diagnostic tests and interpreting results. June 1999. Online at http://www.plantprotection.org/hrac/detecting.html.

Heap, I. M. 1994. Identification and documentation of herbicide resistance. Phytoprotection 75 (Suppl.):85-90.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technol. 9:218-227.

Streibig, J. C. 1988. Herbicide bioassay. Weed Res. 28:479-484.
Tardif, F. J., J.A.M. Holtum, and S. B. Powles. 1993. Occurrence of a herbicide resistant acetyl-coenzyme A carboxylase mutant in annual ryegrass (*Lolium rigidum*)

Additional References that may assist in Diagnosing Resistance.

Clarke, J. H., A. M. Blair, and S. R. Moss. 1994. The testing and classification of herbicide resistant *Alopecurus myosuroides* (black-grass). Asp. Appl. Biol. 37:181-188.

Dupont, S., C. Biesenthal, and M. D. Devine. 1997. In vivo diagnostic of grass weed resistance to ACCase-inhibitor herbicides. Weed Sci. Soc. Amer. Abst. 37:274.

Gerwick, B. C., L. C. Mireles, and R. J. Eilers. 1993. Rapid diagnosis of ALS/AHAS-resistant weeds. Weed Technol. 7:519-524.

Hensley, J. R. 1981. A method for identification of triazine resistant and susceptible biotypes of several weeds. Weed Sci. 29:70-73.

Letouzé, A. and J. Gasquez. 1999. A rapid reliable test for screeningaryloxyphenoxypropionic acid resistance within *Alopecurus myosuroides* and *Lolium*spp. populations. Weed Res. 39:37-48.

Letouzé, A., J. Gasquez, and D. Vaccara. 1997. Development of new reliable quick tests and state of grass-weed herbicide resistance in France. Brighton Crop Prot. Conf. - Weeds Vol 1. pp. 325-330.

Lovell, S. T., L. M. Wax, D. M. Simpson, and M. McGlamery. 1996. Using the in vivo acetolactate synthase (ALS) assay for identifying herbicide-resistant weeds. Weed Technol. 10:936-942.

Moss, S. R. and G. W. Cussans. 1987. Detection and practical significance of herbicide resistance with particular reference to the weed *Alopecurus myosuroides* (black-grass). *In* M. Ford, D. Hollomon, B. Khambay, and R Sawicki, eds. Biological and Chemical Approaches to Combating Resistance in Xenobiotics. Chichester, UK: Ellis Horwood. pp. 200-213.

Murray, B. G., L. F. Friesen, K. J. Beaulieu, and I. N. Morrison. 1996. A seed bioassay to identify acetyl-CoA carboxylase inhibitor resistant wild oat (*Avena fatua*) populations. Weed Technol. 10:85-89.

Norsworthy, J. K., R. E. Talbert, and R. E. Hoagland. 1998. Chlorophyll fluorescence for rapid detection of propanil-resistant barnyardgrass (*Echinochloa crus-galli*). Weed Sci. 46:163-169.

O'Donovan, J. T., A. Rashid, H. V. Nguyen, J. C. Newman, A. A. Khan, C. I. Johnson, R. E. Blackshaw, and K. N. Harker. 1996. A seedling bioassay for assessing the response of wild oat (*Avena fatua*) populations to triallate. Weed Technol. 10:931-935.

Rashid, A., J. T. O'Donovan, A. A. Khan, M. P. Sharma, and H. V. Nguyen. 1997. Response of triallate-resistant and -susceptible wild oat (*Avena fatua*) populations to difenzoquat and EPTC in a seedling bioassay. Weed Technol. 11:527-531.

Singh, B. K. and D. L. Shaner. 1998. Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. Weed Technol. 12:527-530.

Singh, B. K., M. A. Stidham, and D. L. Shaner. 1988. Assay of acetohydroxyacid synthase. Anal. Biochem. 171:173-179.

Van Oorschot, J. L. P. and P. H. Van Leeuwen. 1992. Use of fluorescence induction to diagnose resistance of *Alopecurus myosuroides* Huds. (black-grass) to chlorotoluron. Weed Res. 32:473-482.

Yanase D. and A. Andoh. 1993. The paraquat bioassay to evaluate photosynthesis inhibition. *In* P. Boger and G. Sandmann, eds. Target Assays For Modern Herbicides and Related Phytotoxic Compounds. Boca Raton, FL: Lewis Publ. pp. 257-262.