HERBICIDE RESISTANCE ACTION COMMITTEE

Monitoring and Mitigation of Herbicide Resistance: Global Herbicide Resistance Committee (HRAC) Perspectives

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I. Executive Summary

Managing the risk of herbicide resistance (HR) is an area of strategic importance for leading herbicide technology providers and is the focus of the Global Herbicide Resistance Action Committee (HRAC), an organization comprised of 8 major companies working as a part of Crop Life International. Early detection of HR, understanding the scope of HR in a defined area, and potential mitigation of resistance through efforts to limit its spread are important aspects of managing the risk of HR. Monitoring for HR populations has been employed by public and private weed scientists for both early detection and defining the scope of resistance. The primary methods used to monitor for resistance include:

1) field surveys where seed from putative resistant plants are collected and tested in a controlled environment using bioassay procedures,
2) market research surveys of farmers and weed management experts, and
3) tracking farmer performance inquiries with appropriate follow up field evaluation and testing.

The most common monitoring method is the use of field surveys designed to either qualitatively (i.e., determine whether the level of resistance is high, medium, or low) or quantitatively (i.e., determine the area infested with HR populations) define existing HR. The primary method to detect resistance in new species and in new geographies is to track farmer performance inquiries.

Once resistance is detected, steps may be taken to mitigate its impact. A critical aspect to mitigation is the implementation of best management practices (BMPs) which is facilitated by effective education and training programs. Education efforts can be enhanced with information obtained from monitoring studies and early detection of resistant populations using appropriate monitoring methods can improve the outcome of mitigation efforts.

The following is a summary of HRAC's perspective on monitoring and mitigation goals and methods.

1) Monitoring HR using qualitative approaches, such as surveys, is useful to understand and enhance awareness of the scope of the problem and improve adoption of HR best management practices (BMPs).
4) Monitoring HR for quantitative purposes is not a cost effective use of limited resources in most cases, nor is it necessary to meet the main goal of encouraging greater farmer adoption of BMPs.
5) Routine periodic monitoring of known resistant weeds is of limited value and is not a cost effective use of limited resources. Monitoring studies should be justified and resourced on a case-by-case basis to address specific needs.
6) Proactive HR field surveys (either directed at discovery of resistance in a new species or for known resistant species in a new geography) are resource intensive and have a low probability of success.
7) Monitoring herbicide performance inquiries for possible cases of resistant weeds can be an effective means to facilitate the detection of resistance. However, this method is subjective in nature and may initially overestimate

resistance.

8) Better procedures need to be developed in order to facilitate earlier communication between herbicide providers, academics, consultants, and farmers regarding cases of resistance. However, this must be balanced with the need for accuracy.
9) The primary goal of mitigation programs is to contain or slow the spread of resistant populations. Only in rare cases, can 'eradication' be a goal. Effective mitigation is accomplished through enhanced farmer awareness and implementation of resistance BMPs.
10) Cases of resistance in a new species may warrant special mitigation actions when, based on careful evaluation, uncontained resistance would significantly and negatively impact farm production. Probability of success needs to be carefully evaluated prior to taking action.
11) Resistance monitoring processes for weeds are fundamentally different from those for insects or diseases. Methods such as baseline monitoring that are used for these other pests are not readily transferable to weeds.
12) All parties involved in weed management have responsibility for the early detection, monitoring and mitigation of HR. HRAC recommends that the following guidelines be used to delineate primary responsibility for various activities:
    a. Individual active ingredient registrants should primarily be responsible for the collection, handling, and timely communication of performance inquiries being investigated and those confirmed as resistant.
    b. Mitigation programs should be the joint responsibility of the primary registrant for an active ingredient and the local extension weed management experts. Registrant product stewardship plans can provide general guidance for developing specific mitigation plans.
    c. Weed science organizations (e.g. WSSA) should ensure coordination across the network of those providing technical assistance to farmers.
    d. Monitoring programs that define the scope and spread of resistance should continue to be proposed and implemented by either public or private weed scientists, on a case by case basis, and resourced through normal public and private funding opportunities.

II. Introduction

Herbicides are a primary tool for the control of weeds in modern agricultural production, providing a means to achieve optimum crop yields and enabling the adoption of environmentally friendly practices such as conservation tillage. In most of the world's major crop production areas the evolution of weed populations with resistance to one or more herbicides is a serious concern. Herbicide resistance (HR) is defined by the WSSA as the acquired ability of a weed population to survive an herbicide application that previously was known to control the population (www.WSSA.net). HR is a natural response of certain weed species to the use of herbicides and can be mitigated using recognized best management practices.

Surveying fields and testing for possible herbicide resistance is an important best management practice (Norsworthy et al. 2012). In addition, the close monitoring of weed populations and early detection of HR are crucial to avert economic losses (Burgos et al. 2013). The early detection of resistance and its extent within defined geographies has been the objective of numerous resistance monitoring projects in the U.S., Canada, Australia, and Europe. Much has been learned about the design and the value of such studies. Furthermore, researchers continue to use them to evaluate the spread of resistance within the context of specific farming practices. This knowledge can help facilitate farmer resistance education and training programs.

Regulatory authorities around the world have been considering monitoring and testing for HR as part of the regulatory approval process for herbicides. In part, they are attempting to apply the models developed for fungicide and insecticide resistance to herbicide resistance. However, herbicide resistance is fundamentally different and should be treated differently, for reasons which are discussed later.

Recent US-EPA registration approvals have included requirements for the reporting of incidents of suspected resistance discovered via investigation of product performance complaints. Once identified, the registrant is required to work with the affected farmer to develop an appropriate mitigation plan. Indications are that similar requirements will be imposed on other registrants as they request approval for new products, reregistration of existing products, or new uses of existing active ingredients. Additionally, the EPA has recently requested input from the Weed Science Society of America (WSSA) on how to generate validated information that quantitatively estimates the extent and location of resistant weeds for mechanisms of action where resistance is already widespread.

In the European Union, discussions have centered on risk assessments (and associated data requirements) that describe the potential for resistant populations to evolve in response to the use of a given herbicide in the context of various risk management strategies. Lengthy discussions have also centered on the generation of data to demonstrate the sensitivity of a species to an active ingredient before and after resistance has evolved.

The purpose of this paper is to review the scientific literature related to resistance monitoring, mitigation, and sensitivity/baseline testing, as well as discuss the value of various methodologies and present HRAC positions on these topics.

III. Herbicide Resistance Monitoring

Broadly defined, HR monitoring encompasses activities designed to identify the absence or presence of resistance across defined geographical areas at a single point in time or across multiple years. To date, monitoring of HR has primarily been conducted to define the scope and spread of resistance across a region, province, state, or other defined local area (Baumgartner et al. 1999; Beckie et al. 1999; Beckie et al. 2001; Beckie et al. 2008; Beckie et al. 2013; Davis et al. 2008; Falk et al. 2005; Kruger et al. 2009; Legleiter and Bradley, 2008; Lewellyn and Powles, 2001, Tucker et al.2006, Owen et al. 2007, Henriet and Marechal 2009). This work sometimes also measures resistance relative to crop production variables in order to better understand farming practices associated with resistance development (Hanson et al. 2009; Legere et al. 2008). Some studies focused on ALS and ACCase inhibitors with the objective of understanding the relative abundance of known resistance mechanisms.

There are a number of surveying methods and designs that have been used in monitoring programs (Burgos et al. 2013; Beckie et.al. 2000). *Development of a monitoring program and selection of the survey method depends upon the goals of the monitoring effort, available resources, and the reliability of available methods.* Indeed, it is important to clearly understand the goal of a monitoring program and the reliability of tools to meet that goal before a program can be justified and resources allocated. Monitoring goals. Monitoring can be viewed broadly as reactive or proactive, each with different goals and challenges.

Reactive monitoring *is the case where monitoring is conducted for the purpose of documenting the incidence and spread of existing HR weed populations. This has been the most common type of monitoring conducted to date.* It can be useful in helping educate farmers on the scale and intensity of the issue with the intent to increase adoption of resistance best management practices (BMPs). This type of monitoring can also be used to correlate HR with weed management and general farming practices which would assist in the development of BMPs tailored to a specific geography and/or weed resistance situation.

Reactive monitoring, when implemented widely, can be useful to quantify resistance (i.e., estimate number of acres/hectares) if the resistant biotype is present at relatively high levels and random survey methods are used. Reactive monitoring on a more localized basis can also be effective in determining the spread of resistance if the intensity of sampling is sufficiently high to detect relatively rare resistant individuals. In general, the sampling methods and level of resources required to accomplish the goal of reactive monitoring will depend on the specific objectives and degree of accuracy desired. Because reactive monitoring is generally very resource intensive, a cost/benefit analysis of the various alternatives needs to be thoroughly evaluated.

In general, reactive monitoring to qualitatively estimate the intensity of HR (i.e., low, moderate, high level) and relative shifts in resistance can be more cost effective and thus can be more readily justified than reactive monitoring to quantify HR in terms of area (i.e., acres or hectares) infested. In addition, reactive monitoring to quantify the infested area provides no significant benefits compared to reactive monitoring conducted to gain a qualitative understanding of resistance in a given geography.

Review of reactive monitoring studies. Experience from reactive monitoring studies has provided a greater understanding of the logistics, costs and reliability of HR monitoring. Both large (across a region) and small scale (localized within a state or province) reactive monitoring studies have been conducted. The largest monitoring in North America was conducted by weed scientists in Canada. Periodic monitoring across a broad area of the Canadian Prairies was conducted from the early 1990s through 2009 in order to document the presence and change in resistance to ACCase and ALS inhibiting herbicides over time (Beckie et al. 1999; Beckie et al. 2001; Beckie et al. 2008; Beckie et al. 2013). While these broad-area surveys were discontinued after 2009 the authors indicated smaller scale monitoring might be conducted as needed in select situations (Beckie et al. 2013). These surveys were found to be helpful in determining which farming practices were most correlated with the incidence of resistance (Legere et al.2000). Similarly, in Western Australia, a large scale random resistance monitoring study was conducted over a 5-week period in 2003 for the purpose of understanding the spread of single and multiple HR in *Lolium rigidum* (Owen et al. 2007). Medium scale (e.g. state-wide) reactive monitoring programs have been conducted in several U.S. states (Baumgartner et al. 1999; Davis et al. 2008; Falk et al. 2005; Kruger et al. 2009; Tucker et al., 2006). For example, in Indiana, scientists monitored for the presence of glyphosate resistant *Conyza spp*. across large areas of the state (Kruger et al. 2009). Surveys were conducted in 978 Indiana soybean fields across 3 years using random and non-random sampling methods to estimate the number of acres infested and the overall distribution of resistant *Conyza spp*. This work was justified on the basis of directing farmer education programs and as an early warning system in areas that had not yet reported resistant populations. The fact that no further surveys of this type have been conducted in Indiana since the 2008 publication of the initial work points to the fact that these efforts are difficult to sustain.

Medium scale HR monitoring studies with a focus on the distribution of select species have been completed in many world areas. Two examples are blackgrass (*Alopecurus myosuroides*) resistance to ACCase inhibitors and ALS inhibitors in Europe (Delye et al. 2006; Henriet and Mareschal 2009) and ALS inhibitor and auxin resistance in *Raphanus raphanistrum* in Australia (Walsh et al. 2007).

Monitoring studies have also been used to understand the distribution of resistance mechanisms. The objective of a German monitoring study was to determine the ratio of metabolic resistance to ACCase target-site resistance in black grass (Drobny et al. 2006). The findings of this study were used to refine the best management practices for this weed. Limited scale reactive monitoring has also been conducted to document the spread of resistance from a single field source. Examples include a 3-year Arkansas study to determine distribution of glyphosate resistant Johnsongrass (*Sorghum halepense*) around a field with documented resistance (Riar et al. 2011) and a 2011 survey in southern Alberta to determine the spread of glyphosate resistant kochia population (*Kochia scoparia*) first discovered in summer of 2011 (Beckie et al. 2013). However, results of this type of monitoring may not definitively indicate the spread of resistance genes from a single source. An ongoing study of ALS and ACCase resistant blackgrass distribution in small landscapes in Germany has shown that spatially-close fields (even those adjacent) can develop very different herbicide resistance profiles (based upon target site mutations and occurrence of non-target site resistance). This suggests independent evolution of resistance (Hess et al. 2012, Hermann et al. 2014). In another study of over 100 fields in Illinois with glyphosate-resistant waterhemp (Amaranthus spp.), resistance status of fields was not related with proximity to resistant fields, but rather was found to be better correlated with the number of mechanisms of action applied annually (Evans et al., 2015). The latter studies demonstrate that the weed management program of individual fields can have more influence on the status of resistance than distance from other resistant fields. It is therefore possible that a resistance “outbreak” within a geographical region may actually be related more to the uniform adoption of similar or identical weed control practices that lack diversity than to gene flow through movement of seed or pollen.

Proactive monitoring. Proactive monitoring is defined as a means to detect resistance before it becomes dominant in a field or widespread across a geography. In theory this type of monitoring could be of value in slowing the development and spread of resistance. However, the utility of proactive monitoring depends on two key questions: 1) Can practical sampling schemes be resourced that will have a reasonable probability of detecting rare resistant individuals and 2) Can testing programs actually detect resistant biotypes at a point before resistance becomes broadly established?

Accurate estimates of resistance can require sampling many sites and testing large numbers of samples, which may be impractical. The need large sample sizes (sites and plants) is due to the fact that resistant alleles occur at estimated frequencies ranging from 10^{-4} to 10^{-12} or lower for some herbicide sites of action (Powles et al. 1997; Jander et al. 2003; Duke and Powles 2009; Neve et al. 2011). In short, one would be looking for a ‘needle in a haystack’ requiring extensive sampling and testing given that in many species there is a large amount of genetic variation between and within weed populations. Also in cases where resistance is multigenic, resistance would be more difficult to detect at the time the proactive monitoring was initiated.

Proactive monitoring is difficult to justify because of its many challenges and low probability for success. There are no published studies documenting that random resistance surveys prior to the presence of widespread resistance have been successful in detecting previously unknown resistance.

Estimating the number of samples needed to find a single resistant individual: If one assumes a resistance gene frequency of 1×10^{-8}, the resistance gene would occur in 1 of every 100,000,000 plants. Assuming an initial weed density (seed bank x % emergence) of approximately 25 plants per square meter for a given species and 90% control (resulting in an average of 2.5 surviving plants per square meter), an end of season survey would have to sample 4,000 hectares (about 10,000 acres) in order to find the resistant individual for a single species. This effort would also require greenhouse testing of the progeny from 100,000,000 plants per species. Even at later stages of resistance development it would be necessary to sample and test hundreds of thousands of plants in order to find resistant individuals purely through random sampling. This example assumes perfect sampling and collection of representative individuals. Actual sampling numbers would likely need to be larger to account for less than ideal sampling schemes.

Sampling and testing methods. Effective methods used to monitor for resistance may include; 1) collection (random or non-random) and testing of seed using bioassays, 2) research surveys of farmers or technical experts, and 3) evaluation of product performance inquiries.

Collecting and testing of seed. Sampling sites can be separated into random (i.e., sites and/or plant selected randomly) and non-random (i.e., sites and plants selected based on predefined criteria) methods. Random sampling requires collection from many field sites and many individuals per site in order to quantify the level of existing resistance. Nonrandom sampling is more appropriate to identify (and qualify) the presence of existing resistance at a relatively high level of occurrence (Beckie et al. 2000; Davis 2008). The number of sites required in a field survey to meet a particular monitoring objective is also related to the biology of weeds. Those exhibiting a large degree of phenotypic variability in response to an herbicide application will invariably require more sites and samples. The classic greenhouse bioassay method of testing is labor and resource intensive and, as noted by Burgos et al. (2013), is not generally practical for use in testing programs that are 'large scale'. Analytical tests for detection of specific resistance mutations are more rapid but are often not available for new or complex types of resistance. (Note: Burgos et al 2013 provide a thorough review of quick tests developed for the detection of resistance to various herbicides.)

Market Research. Farmer-focused market research studies require fewer resources than the collection and testing approach and are relatively easy to implement. However, their reliability in meeting monitoring goals is limited. For example, in a 2007 Monsanto market research study U.S. farmers in the North Central Corn Belt were asked which weeds were resistant to glyphosate (Table 1). The survey results indicated much higher resistance than was likely present at that time. Further, farmers indicated resistance in lamb's quarters (*Chenopodium album*), nightshade (*Solanum spp.),* and morning glory (*Ipomea spp.)* populations, even though resistance to glyphosate had not been confirmed in those species.

Another example of the challenges in obtaining reliable data from market research is found in an article published in *The Western Producer* (Amason, 2013) about glyphosate resistant (GR) kochia in the Canadian Prairies (The Western Producer, May 13, 2013 by Robert Amason). A market research study of farmers by Stratus Agri-Marketing estimated there were 651,000 acres of GR kochia in the region. Field survey and testing data from Dr. Hugh Beckie and weed scientists with Agriculture Canada, suggested the infestation to be about 8,000 acres. A spokesperson for Stratus acknowledged that their number was too high because of farmer confusion about the cause of poor performance (i.e., poor performance of glyphosate on kochia is often related to stage of growth at time of application) and Dr. Beckie, in the article, indicated that his estimated number was probably too low because of their limited sampling plan. Regardless of the number of estimated acres, the fact remained that GR kochia was spreading and was an issue that needed prompt attention. This overall, qualitative understanding of the problem did allow Industry and Ag Canada to work together on education, training, and incentive programs to help farmers address the problem.

Farmer-focused Market Research can be more reliable and thus of greater value in understanding HR when the resistance is wide spread and more easily recognized by farmers than in situations where resistance is not well established nor recognized. For example, Stratus Ag Research published the results of a 2012 U.S. farmer market research study that estimated the number of acres infested with glyphosate resistant weeds was 61.2 million acres in the U.S. (www.stratusresearch.com, January 25, 2013 blog). This information provided an idea of the magnitude of the issue and helped frame discussions on glyphosate resistance, regardless of the accuracy of the absolute number of acres.

Table 1. Weeds Resistant to Herbicide Product June 2007 farmer market research sponsored by Monsanto Company

Farmers responding to the question, "What specific weeds have developed resistance to (herbicide)?"

	Glyphosates	Roundup		
	Overall (n=27)	Overall (n=118)	North Central (n=49)	South (n=69)
Marestail	65%	37%	31%	64%
Pigweed	21%	12%	6%	35%
Lamb's Quarters	19%	27%	32%	3%
Nightshade	17%	-	2%	6%
Waterhemp	12%	38%	46%	5%
Ragweed	5%	19%	23%	4%
Morning glory	5%	-	2%	4%
Other	18%	19%	18%	22%

Note: 'Glyphosates' refers to any single active ingredient commercial product containing glyphosate. "n" is the total number of farmers in each region ('North Central' or 'South') or collectively ('Overall') responding to the question.

Conducting surveys of public and/or private weed scientists or agronomists can be useful in addition to farmer surveys (Riar et al. 2013; Culpepper 2006). These experts interact with a large network, including farmers, retailers, dealers, herbicide company representatives, etc. They also are in a position to compile and interpret local information. However, there are challenges with the reliability of such information. For example, technical experts are often called upon to help in situations where there are significant problems and rarely when weed control programs are working well. This can sometimes skew their perspective and result in resistance estimates that are more extensive than is actually the case.

Additionally, an expert's previous experience with a particular type of resistance may be outdated or limited, in which case, there could be a tendency to either underestimate or overestimate the current level of resistance. An example of this is an Arkansas study published in 2014 which mapped the distribution of herbicide resistant Johnsongrass (Johnson et al. 2014). Populations of Johnsongrass in 14 counties along the Mississippi river were sampled and tested for resistance to glyphosate, ACCase and ALS inhibiting herbicides. Whereas ACCase resistance was believed by many to be very common across this area before the introduction of glyphosate tolerant crops, none of the 141 accessions tested were ACCase resistant and only one accession was ALS resistant.

Performance Inquiry monitoring. Monitoring Performance Inquiries is a process where an evaluation for herbicide resistance is made within the complaint management process of a company. Companies will learn of the issue from direct contact from the farmer, or more often through a consultant, retailer, dealer, or state extension specialist. Follow up with the farmer is usually a coordinated effort by the company and the other party(s) involved.

Reliance on Performance Inquiries as the method to identify resistance may have a greater probability of success and a better return on investment than other monitoring options even though there are challenges to early identification of resistance.

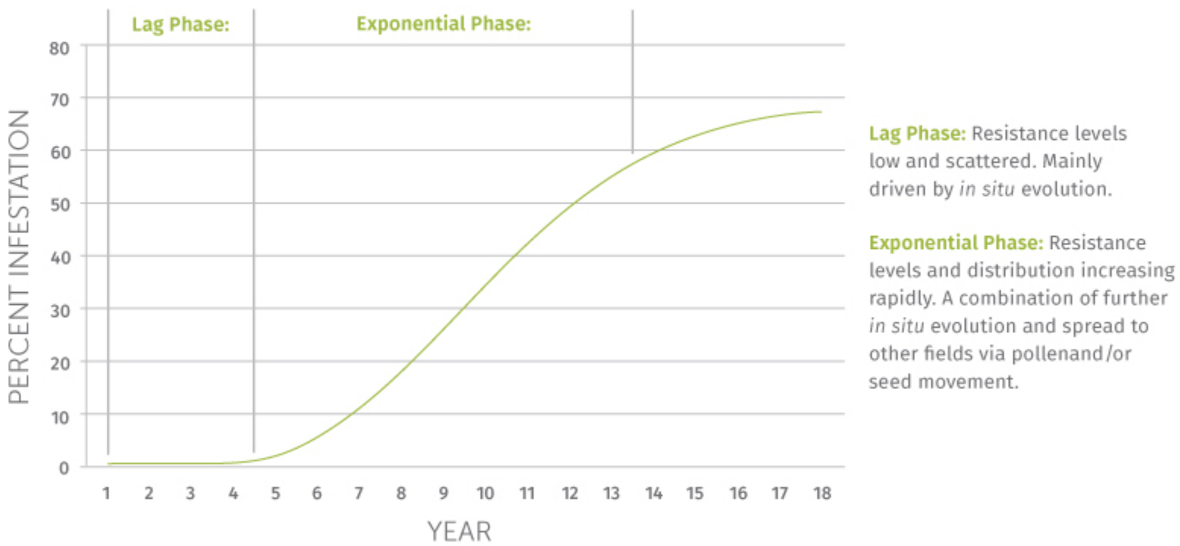
Monitoring Performance Inquiries has been a primary means by which resistance in a new species or in a new geography is identified. This process is also an important avenue for experts to be able to qualitatively understand the intensity and distribution of resistance within their geographical area. Recently US EPA imposed a registration requirement on Dow AgroSciences' Enlist Duo™ herbicide that placed a greater emphasis on registrant investigation and reporting of performance complaints that are potentially a result of resistance.

While Performance Inquiries can be a workable method for gathering indications of resistance, there are challenges to using Performance Inquiries as an avenue for "early" detection of herbicide resistant weed populations. For herbicides, there are often uncertainties about the cause of a performance inquiry. Herbicide performance is frequently influenced by weather and application issues. Prior to the widespread presence of a new resistant species, most reports of performance failures in that species cannot be attributed to resistance, but rather are related to agronomic practices.

Distinguishing between resistance, weather, or application errors requires a person with considerable experience. This is especially true when there is no known resistance in a species or when resistance in a species is not known to be present in a geographical area. In new cases, the initial, subjective evaluation needs to confirm resistance through testing which can take several months.

Consider the theoretical example illustrated in Figure 1. Development of resistance starts with rare individuals that can withstand applications of an herbicide. Models of resistance development generally predict that the proportion of resistant individuals in a field will be well below 10% for the first 4-6 years of continuous selection pressure given an initial resistance gene frequency of around 10-8 (Neve et al. 2011; Maxwell et al. 1990). During this time, farmers are generally not concerned about reduced herbicide performance and therefore sampling cannot be guided by farmer reports of nonperformance. In addition, it is unlikely that proactive random sampling will detect resistant individuals in the early years of this lag phase. In general, farmers do not report a performance issue until the poor performance impacts a significant portion of a field. Experience suggests that action does not occur until at least 20% to 30% of the field is affected. In short, small patches of uncontrolled weeds have not traditionally been a major farmer concern. Year-to-year variability in herbicide performance can be an issue and makes it easy to rationalize these escapes as the result of weather or application errors. Thus, there can be a delay of 3 or more years, depending upon the species, from the first selection of resistance in a field until a Performance Inquiry is lodged and further delay thereafter before resistance can be confirmed and broadly communicated.

Figure 1: Theoretical example of herbicide resistance development after resistance has evolved.



Monitoring programs that identify situations as 'likely resistant'. Recent USEPA registration actions require the registrant to evaluate farmer performance complaints for cases of 'likely resistance'. This determination is made based on visual criteria suggestive of resistance but before actual confirmation in the lab or greenhouse. The visual criteria suggested are; 1) failure to control a weed species normally controlled by the herbicide at the dose applied, especially if control is achieved on adjacent weeds: 2) a spreading patch of non-controlled plants of a particular weed species, and 3) surviving plants mixed with controlled individuals of the same species (Norsworthy et al 2012).

While these criteria can help provide experts and farmers with an indication of possible resistance, they are a subjective evaluation that needs to be followed by confirmation testing. In short, only a fraction of these observations of performance failure may ultimately be confirmed as resistant. Thus, the process would probably overestimate the actual presence of resistance.

An example to illustrate this point is as follows: During the first few years after commercial launch of any new herbicide or new use of an herbicide (e.g., 2,4-D and dicamba used in appropriately tolerant soybeans and cotton) there are often a number of performance inquiries as farmers become familiar with using the technologies. For the auxins this will involve farmers learning the growth stage limits for effective control of many species. In this case it is highly likely that there will be a number of performance inquiries that meet one or more of the aforementioned criteria, but will not necessarily be related to resistance. For example, Figure 2 is a photo of an early 2,4-D research plot where the variable control observed was a function of the growth stage of the horseweed. Using the aforementioned criteria this could also have been suggestive of resistance. In this example, and for other cases involving a new herbicide active ingredient or new use pattern, resistance is improbable during the first few years after introduction due to the time required for resistant populations to evolve.

While it does appear that visual criteria such as these are of value in the early identification of resistance, the above example leads us to assert that the criteria should not be the sole basis for any official regulatory action.

Figure 2. Field plot showing variable control of horseweed biotypes after application of 2,4-D plus glyphosate to weeds beyond the recommended growth stage.

Other factors should also be considered during these field investigations of possible resistance. Cases of suspected resistance should be evaluated in the framework of field history (i.e., number of applications of a given herbicide and the degree of diversity of weed management practices) as well as presence of resistance in nearby fields.

Monitoring summary. Given the wide range of objectives, biological diversity of weeds, and differences in farming practices, monitoring for herbicide resistance is very situational. Evaluating non-performance inquires for possible resistance may be the most practical (though imperfect) means of providing an early indication of resistance issues with a given active ingredient. A reasonable, qualitative understanding of herbicide resistance may be gained through various other means such as market surveys, seed collections, or some combination thereof. Resistance monitoring can best be justified when it is used to facilitate farmer education and training programs and improve BMPs. These objectives can be accomplished with qualitative studies designed to gain an understanding of the relative levels of resistance.

Communication of monitoring results. Communication of monitoring results is an important aspect of monitoring programs. Timely communication of confirmed resistance to farmers and experts in the agricultural community can facilitate better adoption of preventative BMPs, more active consideration of ways to mitigate a particular type of resistance, and better vigilance for the resistance.

Resistance in a new species or in an existing species in a new geography is often not communicated publically until confirmation testing has been completed. Broad communication often begins with a public announcement by a university researcher and/or the registrant. In addition, new species and new geographies are posted on the International Survey of Herbicide-Resistant Weeds database, a global website found at www.weedscience.com.

However, information about resistance cases may not be communicated as quickly as needed to allow initiation of early mitigation efforts. It is not uncommon for the time from an initial investigation of a field performance complaint until resistance confirmation on posting to the Resistant Weeds database to take a year or more (Ian Heap, pers. comm.). This delay, plus the fact that resistance may have been present 3 or more years in the field before it was investigated, often mean that resistance was already broadly distributed from a source field by the time its presence was publically communicated.

Closing the communication gap from researchers to farmers and within the research/technical community is an effort that needs attention. However, the need to increase speed of communication must be tempered with the need to be as accurate as possible in what is communicated. There are also privacy and confidentiality issues relative to farmers, academics and companies that would need to be addressed, as new procedures are evaluated.

IV. Comparison to insecticide and fungicide resistance monitoring

In some cases, regulatory authorities have attempted to use monitoring programs established for insecticides and fungicides as a template for developing programs for herbicides. A discussion between regulatory authorities and the weed science community (industry and academics) in Europe has focused on the development of a testing plan(s) to determine the sensitivity of target weeds to an active ingredient. The purported advantages of this are that it would provide the means of measuring the original level of sensitivity before the weed is subjected to the active ingredient (in which case this would be considered as baseline data) in order to identify resistant species and populations in laboratory studies, and to monitor any shifts in response following widespread use (European and Mediterranean Plant Protection Organization, PP1/213(3) http://pp1.eppo.int/getnorme.php?id=260).

Proposed methods for generating sensitivity or baseline data include the collection of seed before commercial launch of an active ingredient or retrieval of seed from a seed bank that has not been exposed to the active ingredient and evaluation of the sensitivity of such seed using bioassay procedures in a controlled environment.

While the objectives for resistance monitoring for herbicides, insecticides and fungicides are generally similar (i.e., early detection of resistance and its extent) the most effective and most practical cost effective approaches to monitoring differ. The main biological factors contributing to the differences are the mobility (insects), inheritance of resistance gene(s) (dominant or recessive), and the time between generations for both insects and fungi (multiple generations over a short time period). Monitoring for insecticide resistance is often accomplished by collecting targeted insects in various locations subsequent to a product's introduction and then comparing their sensitivity to a reference population collected and maintained in a laboratory prior to widespread use of the insecticide in question. The mobile nature of many insect species allows the initial sampling of reference populations and subsequent sampling of test insects to be fairly representative of overall species sensitivity. Monitoring for fungicide resistance is similar in that disease propagules can be spread over wide geographies.

In contrast, weed populations within a given species evolve and adapt in response to local environmental conditions. This results in a large amount of genetic diversity that can affect response to herbicides. Therefore, the reference population used for a resistance determination should come from a source as close as possible to the putative resistant sample location. Unlike insects or pathogenic fungi, the establishment of lab-reared "baseline" populations for the purpose of comparison to wild populations is largely impractical for weeds.

The selection of reference populations and rates for testing are important considerations for evaluation of putative resistance in weeds. Resistance as defined by the WSSA is the failure of an herbicide to control a population of a species that was previously controlled by a given rate. This reference dose is usually the commercially acceptable rate as defined in development field testing programs that encompass several years and many environments. How and when reference weed populations are selected has been the subject of debate within the technical community. Burgos et al. (2013) published an excellent review on the subject. In this paper the authors indicate that one should evaluate multiple 'susceptible' populations to generate comparative herbicide sensitivity data as well as providing an indication of the natural variability within the species.

Ultimately, development of broadly-applicable sensitivity baselines for herbicide-weed combinations would be difficult. This is a key difference between resistance testing for herbicides and that often conducted for insecticides or fungicides.

V. Mitigation of Herbicide Resistance

In its broadest sense, mitigation is defined as the action of reducing the severity, seriousness, or painfulness of something. In the context of herbicide resistant weed populations, mitigation can encompass a wide range of activities meant to contain or slow the spread of resistant individuals. For example, early detection of small patches of weeds that escape an herbicide application may allow follow up applications of alternative mechanism of action herbicides or mechanical removal of these escapes before they can produce seed. This may also result in a change in farmer weed management practices in an effort to prevent further spread and development.

Mitigation is can be contrasted with "remediation" which is sometimes equated with "eradication" of the resistant individuals within the area of detection. Given weed seedbank dynamics and seed dormancy, weed scientists agree that eradication is not feasible for most weed species.

Factors affecting the ability to mitigate resistance. The ability to implement an effective mitigation program is dependent upon the ability to identify resistance early and to have cost effective tools and strategies to manage, contain, or (in rare cases) eliminate the resistant population before it spreads. This ability is also influenced by: 1) the number of trained individuals monitoring for resistance; 2) the biological characteristics (reproduction, longevity of seed, etc.) of the species; and 3) how quickly one can identify a truly resistant population.

Some species will be extremely difficult to contain because of their reproductive characteristics. Palmer amaranth (*Amaranthus palmeri*) and common waterhemp (*Amaranthus rudis*), resistant genes are spread long distances via pollen, a process that is difficult to prevent. Likewise, it is difficult to contain species whose seed moves with the wind (e.g., *Conyza* spp.) or is disseminated through movement of the whole plant (e.g., kochia or tumbleweed).

Seed dormancy and seed longevity can also have a significant impact on the ability to mitigate a situation. For some weeds where seed longevity is relatively short (i.e., 3 to 5 years) achieving the goal of mitigation may be feasible assuming further additions to the soil seed bank are prevented. However, for other weed species whose seed can remain viable for many years the ability to mitigate may be low. Another important biological factors includes fitness penalties conferred by the resistant gene(s). Resistance that confers a fitness penalty could increase mitigation effectiveness.

Early detection of resistance. Another factor that significantly impacts efforts to mitigate resistance is the ability to detect resistance early before it can significantly spread. As discussed previously, early detection is impacted by farmer actions or reactions to herbicide performance issues in their fields. Experience indicates that a farmer does not generally recognize individual plants as being an agronomic problem (and potentially resistant) until one to two years after the season in which a few individuals (less than 10-30% of a population) are not effectively controlled by a herbicide (Gressel and Segel 1990). However, this delay in detection does not preclude implementation of effective mitigation plans. For some species with limited outcrossing, pollen dispersal, or seed dispersal, mitigation can be effective even if resistance is detected several years after it first occurs in a field. For example, Johnsongrass has limited ability to outcross within the species and the seed is not easily dispersed via natural means such as wind (Paterson et al. 1995). Therefore, it may represent a species where effective mitigation may be possible.

Mitigation efforts. We know of no published studies focused specifically on mitigation, but herbicide manufactures and public institutions, often in cooperation, have undertaken some efforts to contain resistance. These efforts have met with mixed results due primarily to the biological attributes of the weed species or the lack of an organized effort to address the issue more broadly than in a single field. Examples of mitigation in two cases involving resistance to glyphosate are briefly described below:

1) The first documented case of GR Johnsongrass was identified in an isolated soybean field in northeastern Arkansas. University of Arkansas weed scientists were first called to investigate the field in the fall of 2007 (Riar et al. 2013). From 2008 to 2010, the University of Arkansas and Monsanto Company had collaborative efforts to contain the population. A survey for glyphosate resistance in the surrounding area was initiated in 2008 and continued into 2009 and 2010 (Johnson et al. 2014). Widespread resistant Johnsongrass was not found with only a single population out of 141 populations showing resistance to glyphosate. It appears that mitigation in the source field may have helped to limit spread of the resistant biotype.
2) In contrast to the first example, GR kochia was first discovered in 2011 in Alberta, 2013 in Saskatchewan and 2014 in Manitoba. GR kochia had been reported in the United States prior to these occurrences in Canada. Kochia is a weed that disperses seed through plant movement by wind. Canadian provincial, Agriculture Canada, and Monsanto weed scientists worked together to on a survey to determine how far the resistance may have spread (Beckie et al. 2013 Blackshaw et al. 2013). At the same time, Monsanto Company in cooperation with BASF Corporation developed and launched incentive programs to encourage farmers to use more diversified management programs in their fallow weed control programs. While industry and public organizations responded quickly, it was apparent that resistance had spread well beyond the first fields that were investigated. For this effort on this species, containment was not possible despite rapid response by industry and local experts. However, the early effort was beneficial since it quickly raised awareness and encouraged the initiation of early actions to address the situation.

While there are several factors to consider before efforts to mitigate a situation can be justified, the ability to mitigate should be considered in all 'first time' situations (i.e., resistance in a new species or new geography). Early intervention is warranted when there is a reasonable chance of success and/or when the impact of uncontained resistant weeds is especially significant to farm production.

VI. Conclusions

All parties involved in weed management have responsibility for the early detection, monitoring and mitigation of HR. Individual active ingredient registrants should primarily be responsible for the collection, handling, and timely communication of performance inquiries being investigated and those confirmed as cases of resistance.

Monitoring programs that define the scope and spread of resistance should continue to be proposed and implemented by either public or private weed scientists, on a case by case basis, and resourced through normal public and private funding opportunities.

Mitigation programs with realistic goals should be the joint responsibility of the primary registrant for an active ingredient and the local extension weed management experts. Registrant product stewardship plans can provide general guidance for developing specific mitigation plans.

Coordination between these stakeholders can be fostered through professional weed science organizations (e.g. WSSA), advancing the overall goal of sustainable weed management.

VII. Literature Cited

Amason, R. 2013. Data on glyphosate resistant kochia surprises experts. The Western Producer http://www.producer.com/2013/05/data-onglyphosate-resistant-kochia-surprises-experts/ (accessed January, 2015)

Baumgartner, J. R., K. Al-Khatib, and R. S. Currie. 1999. Survey of common sunflower (*Helianthus annuus*) resistance to imazethapyr and chlorimuron in northeast Kansas. Weed Technology. 13:510–514.

Beckie, H. J., A. G. Thomas, and A. Le´ge`re. 1999. Nature, occurrence, and cost of herbicide-resistant green foxtail (*Setaria viridis*) across Saskatchewan ecoregions. Weed Technology. 13:626–631.

Beckie, H. J., I. M. Heap, R. J. Smeda, and L. M. Hall. 2000. Screening for herbicide resistance in weeds. Weed Technology. 14:428–445.

Beckie, H. J., A. G. Thomas, and F. C. Stevenson. 2001. Survey of herbicide resistant wild oat (*Avena fatua*) in two townships in Saskatchewan. Can. J. Plant Sci. 82:463–471.

Beckie, H. J., J. Y. Leeson, A. G. Thomas, C. A. Brenzil, L. M. Hall, G. Holzgang, C. Lozinski, and S. Shirriff. 2008. Weed Resistance Monitoring in the Canadian Prairies. Weed Technology 22:530–543.

Beckie, H. J., C. Lozinski, S. Shirriff, and C. A. Brenzil. 2013. Herbicide-resistant weeds in the Canadian prairies: 2007-2011. Weed Technology 27:171-183.

Beckie, H. J., R. E. Blackshaw, R. Low, L. M. Hall, C. A. Sauder, S. Martin, R. N. Brandt, and S. W. Shirriff. 2013. Glyphosate- and Acetolactate Synthase Inhibitor– Resistant Kochia (*Kochia scoparia*) in Western Canada. Weed Science 61:310–318.

Blackshaw, B., H. Beckie, H. L. Hall, and R. Low. 2013. Glyphosate-resistant kochia. Presentation found at www.agric.gov.ab.ca.

Burgos, N. R., P. J. Tranel, J. C. Streibig, V. M. Davis, D. Shaner, J. K. Norsworthy, and C. Ritz. 2013. Review: Confirmation of Resistance to Herbicides and Evaluation of Resistance Levels. Weed Science 61:4– 20.

Culpepper, A. S. 2006. Glyphosate-Induced Weed Shifts. Weed Technology 20:277–281.

Davis, V. M., K. D. Gibson, and W. G. Johnson. 2008. A field survey to determine distribution and frequency of glyphosate-resistant horseweed (*Conyza canadensis*) in Indiana. Weed Technology. 22:331–338.

Delye, C., Y. Menchari, J.P. Guillemin, A. Matejicek, S. Michel, C. Camilleri, and B. Chauvel. 2006. Status of black- grass (*Alopecurus myosuroides*) resistance to acetyl-coenzyme A carboxylase inhibitors in France. Weed Research 47:95-105.

Drobny J.G., M. Salas and J.P. Claude. 2006. Management of metabolic Resistant black-grass (*Alopecurus myosuroides* Huds.) populations in Germany challenges and Opportunities. Journal of Plant Diseases and Protection 65-72.

Duke, S.O. and S. B. Powles. 2009. Glyphosate-resistant crops and weeds: now and in the future. AgBioForum 12:346–357.

Evans, J.A., P. J. Tranel, A. G. Hager, B. Schutte, C. Wu, L. A. Chatham, Davis and A. S. 2015. Managing the evolution of herbicide resistance. Pest Management Science (in press).

Falk, J. S., D. E. Shoup, K. Al-Khatib, and D. E. Peterson. 2005. Survey of common waterhemp (*Amaranthus rudis*) response to protox- and ALS-inhibiting herbicides in northeast Kansas. Weed Technology. 19:838–846.

Gressel, J. and L. A. Segel. 1990. Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. Weed Technology. 4:186–198.

Hanson, B. D., A. Shrestha, and D. L. Shaner. 2009. Distribution of Glyphosate-Resistant Horseweed (*Conyza canadensis*) and Relationship to Cropping Systems in the Central Valley of California. Weed Science 57:48–53.

Henriet, F. and P.Y. Marechal. 2009. Black-Grass Resistance to Herbicides: Three years of Monitoring in Belgium. Comm. Appl. Biol. Sci. Ghent University 74/2:1-8.

Hermann, J., M. Hess, T. Schubel, H. Strek, O. Richter and R. Beffa, 2014. Spatial and temporal development of ACCase and ALS resistant black-grass (*Alopecurus myosuroides* Huds.) populations in neighboring fields in Germany. Julius-Kühn-Archiv 443:274-279 DOI: http://dx.doi.org/10.5073/jka.2014.443.034

Hess, M., R. Beffa, J. Kaiser, B. Laber, H. Menne and H. Strek. 2012. Status and development of ACCase and ALS Inhibitor resistant black-grass (*Alopecurus myosuroides* Huds.) in neighboring fields in Germany. Julius-Kühn Archive 434:163-170.DOI: http://dx.doi.org/10.5073/jka.2012.434.019

Jander, G., S. R. Baerson, J. A. Hudak, K. A. Gonzalez, K. J. Gruys, and R. L. Last. 2003. Ethylmethanesulfonate Saturation Mutagenesis in Arabidopsis to Determine Frequency of Herbicide Resistance. Plant Physiology Vol. 131, pp. 139–146.

Johnson, D. B., J. K. Norsworthy, and R. C. Scott. 2014. Distribution of Herbicide-Resistant Johnsongrass (*Sorghum halepense*) in Arkansas. Weed Technology 28:111-121.

Kruger, G. R., V. M. Davis, S. C. Weller, J. M. Stachler, M. M. Loux, and W. G. Johnson. 2009. Frequency, Distribution, and Characterization of Horseweed (*Conyza canadensis*) Biotypes with Resistance to Glyphosate and ALS-Inhibiting Herbicides. Weed Science 57:652–659.

Legleiter, T. R. and K. W. Bradley. 2008. Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Science. 56:582–587.

Legere, A., H. J. Beckie, F. C. Stevenson, and A. G. Thomas. 2000. Survey of management practices affecting the occurrence of wild oat (*Avena fatua*) resistance to acetyl-CoA carboxylase inhibitors. Weed Technology. 14:366–376.

Llewellyn, R. S. and S. B. Powles. 2001. High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheatbelt of Western Australia. Weed Technology. 15:242–248.

Maxwell, B. D., M. L. Rousch, and S. R. Radosevich. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technology. 4:2-13.

Neve, P., J. K. Norsworthy, K. L. Smith, and I. A. Zelaya. 2011a. Modelling evolution and management of glyphosate resistance in *Amaranthus palmeri*. Weed Research. 51:99–112.

Norsworthy, J. K., S. M. Ward, D. R. Shaw, R. S. Llewellyn, R. L. Nichols, T. M. Webster, K. W. Bradley, G. Frisvold, S. B. Powles, N. R. Burgos, W. W. Witt, and M. Barrett. 2012. Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Science. Special Issue. Pp. 31–62.

Owen, M. J. and S. B. Powles. 2010. Glyphosate Resistant Rigid Ryegrass (*Lolium rigidum*) Populations in the Western Australian Grain Belt. Weed Technology: February-April, Vol. 24, No. 1, pp. 44-49.

Owen, M. J., M. J. Walsh, R. S. Llewellyn, and S. B. Powles. 2007. Widespread occurrence of multiple herbicide resistance in Western Australian annual ryegrass (*Lolium rigidum*) populations. Australian Journal of Agricultural Research, 58:711-718.

Paterson, A. H.T., K. F. Schertz T, Y. Lin, S. Liu, And Y. Chang. 1995. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of Johnsongrass, Sorghum halepense (L.) Pers. Proc. Natl. Acad. Sci USA Vol 92, ppl 6127-6131.

Powles, S. B., C. Preston, I. B. Bryan, and A. R. Jutsum. 1997. Herbicide resistance: impact and management. Advances in Agronomy, Volume 58, Copyright 1997 by Academic Press. Inc.

Riar, D. S., J. K. Norsworthy, D. B. Johnson, R. C. Scott, and M. Bagavathiannan. 2011. Glyphosate Resistance in a Johnsongrass (*Sorghum halepense*) Biotype from Arkansas. Weed Science 59:299-304.

Riar, D. S., J. K. Norsworthy, L. E. Steckel, D. O. Stephenson, IV, T. W. Eubank, and R. C. Scott. 2013. Assessment of Weed Management Practices and Problem Weeds in the Midsouth United States— Soybean: A Consultant's Perspective. Weed Technology, 27(3):612-622.

Tucker, K. P., G. D. Morgan, S. A. Senseman, T. D. Miller, and P. A. Bauman. 2006. Identification, distribution, and control of Italian ryegrass (*Lolium multiflorum*) ecotypes with varying levels of sensitivity to triasulfuron in Texas. Weed Technology. 20:745–750.

Walsh, M.J., M.J. Owen, S.B. Powles. 2007. Frequency and distribution of herbicide resistance in Rahphanus raphanistrum population randomly collected across the Western Australian wheatbelt. Weed Research 47:542- 550.

Westhoven, A. M., V. M. Davis, K. D. Gibson, S. C. Weller, and W. G. Johnson. 2008. Field Presence of Glyphosate-Resistant Horseweed (*Conyza canadensis*), Common Lamb's Quarters (*Chenopodium album*), and Giant Ragweed (*Ambrosia trifida*) Biotypes with Elevated Tolerance to Glyphosate. Weed Technology 22:544-548.

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