Symposium

Genetic Aspects of Herbicide-Resistant Weed Management[1]

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Abstract: Weed populations develop herbicide resistance when they evolve due to selection pressure. Mutations and gene flow contribute to genetic variability and provide resistant alleles. The speed of resistance gene frequency increase is determined by the inheritance of resistance alleles relative to wild-type susceptibility and is influenced by the interaction between gene expression and selection. The goal of herbicide resistance management is to minimize selection pressure while maintaining adequate weed control. However, the specific nature of each herbicide, weed, and resistance combination determines the practices that optimize undesirable selection pressure. Therefore, generalized management strategies should be recommended with caution and must not be mandated without thorough evaluation on a case-by-case basis.
Additional index words: Evolution, selection.
Abbreviations: DNA, deoxyribonucleic acid.

INTRODUCTION

The development of an herbicide-resistant weed population is an example of evolution. Evolution occurs when gene frequencies within a population change as a result of selection, mutation, migration, or random drift. The driving force behind evolution toward herbicide resistance is typically selection pressure imparted by frequent use of one or more herbicides with the same site of action. Plant scientists have experience in using evolution to benefit humanity (e.g., plant breeding) but are not as experienced in stopping or slowing the process when it is detrimental. The management of herbicide resistance in weeds is an example where genetic theories, often used to study or enhance evolution, must now be applied in an effort to hinder evolution (i.e., keep weeds susceptible to practical control measures for as long as possible). This paper discusses the relationship between genetics and herbicide resistance management, both potential and realized. It is not a review of specific herbicide resistance cases.

Genetics is the study of variation and heritability. Both play major roles in the evolution of herbicide-resistant weeds and must be considered when developing management strategies. Variation is essential in the initial stages of selection, because at least one resistant plant must exist within a population before selection can

act. Subsequently, the heritability of resistance influences the rate at which selection will increase the frequency of resistance. These genetic aspects remain important after a resistant population develops, as they determine the likelihood of reversion to susceptibility. Genetic techniques may also be used to identify and characterize a resistant population for effective management.

Evolution toward resistance in response to herbicide selection is influenced by several factors. The specific genetic aspects of each herbicide, weed, and resistance combination will greatly influence the effectiveness of management strategies. Often these specifics are unknown, so a fair amount of extrapolation is required to make management recommendations. However, the genetics of weed populations are likely to be as complex as genetic phenomena documented in other systems. Therefore, caution is warranted when making generalized management recommendations.

GENETIC VARIABILITY

Herbicide resistance alleles are first required within a weed population before selection can increase their frequency. These alleles may enter a population either as new mutations or may immigrate through seed, pollen, or other propagules. Although either mutation or immigration is capable of increasing the genetic variability within one field, the ultimate source of this variation is mutagenesis.

Mutations are considered random and not directed to-

ward specific goals, so the amount of raw diversity they produce is important in determining adaptability. Because only a small portion of all mutations will provide positive adaptive value, the rate at which they occur is a critical factor in determining the likelihood of creating a resistance allele. Mutation rates may differ among and within species; may be influenced by plant age, tissue type, and perhaps environment; and also may vary depending on the genome and locus involved. For example, mutations in the chloroplast genome are believed to occur about half as frequently as in the nucleus, and mitochondrial mutation rates are less than a third of that for the chloroplast (Wolfe et al. 1987). In some cases, the presence of a mutator gene may increase the rate of mutation (Prina 1992; Taddei et al. 1997).

The frequency of conservation for these mutations is also an important factor because they may be reduced or lost from a population through genetic drift, selection, or reversion to susceptible forms. In particular, mutations within structural genes that already are optimized for a specific function may be deleterious and selected against, perhaps leading to low diversity at that locus.

The specific nature of the mutation necessary to confer a particular type of resistance is also an important consideration. Is one specific amino acid within a protein responsible for susceptibility, with any deviation from it providing resistance? Or is the situation reversed so that resistance requires a mutation coding for a specific amino acid among the 20 possibilities? Is the point mutation of a single base sufficient to create resistance (i.e., the codons for the amino acids differ minimally by a single base) or are two or three base substitutions necessary? Even the rate of different point mutations at the same locus may vary, as transitions (purine to purine or pyrimidine to pyrimidine) are more likely than transversions (purine to pyrimidine or vice versa) (Kimura 1980).

It is conceivable that alterations other than point mutations may impart resistance, and if so, their frequency of occurrence is also important to resistance evolution. Possible alterations include translocations, duplications, deletions, epimutations such as changes in deoxyribonucleic acid (DNA) methylation, and other phenomena not easily classified, including the activity of transposable elements. Multigenic resistance is also possible, as alleles carried at different gene loci may contribute to overall resistance. The polyploid nature of some weed species also complicates resistance because specific mutations would have the opportunity to occur in two or more genomes.

Mutations conferring resistance need not originate within the weed population of concern but may enter by gene flow via seed, pollen, or vegetative parts from an external population. This will increase in importance as more resistant populations evolve, thereby providing a source of these alleles. A further consideration is the possible introgression of resistance genes from other species, including genetically engineered crops. Whatever the source of alleles, the importance of gene flow is best judged by comparing its frequency to the rate of spontaneous mutations giving rise to similar alleles within a population. Good estimates of gene flow and mutation rates are often difficult to obtain and must be made on a case-by-case basis.

How do these genetic variability concepts affect management decisions? Is it possible to limit the presence of resistance alleles within a population? Although mutation rates are mostly out of the control of managers, certainly good weed control practices that keep populations low will minimize the presence of individual plants that either produce a resistance mutation or that carry a resistance allele descendant from a previous event.

Can mutation rates themselves be affected by management practices? For the most part it is assumed that management does not influence mutation. But basic genetic research has suggested that stresses may increase mutation rates (Bridges 1997; Mikula 1995), and herbicide treatment is certainly a stress to target weeds. Of course, induced mutations are not important if weeds are killed prior to reproducing. However, the possible effects of nonlethal herbicide treatments on classical genetic and epigenetic mutation rates should not be discounted and are topics for future study.

Good herbicide resistance management includes limiting gene flow into a field. Restricting the introduction of seeds and vegetative propagules is recommended, although restricting the immigration of pollen is difficult to manage. There may be some risk with the intentional introduction of resistant crops (both engineered for resistance and naturally tolerant), either through subsequent resistant volunteer plants or the introgression of resistance into weed species. But these risks must be weighed against the benefits of resistant and tolerant crops in an overall weed management strategy. Not utilizing all available weed control and cropping options may carry a weed management risk as well.

GENE EXPRESSION, HERITABILITY, AND SELECTION

The baseline gene frequencies within a population may be altered by herbicide selection through preferen-
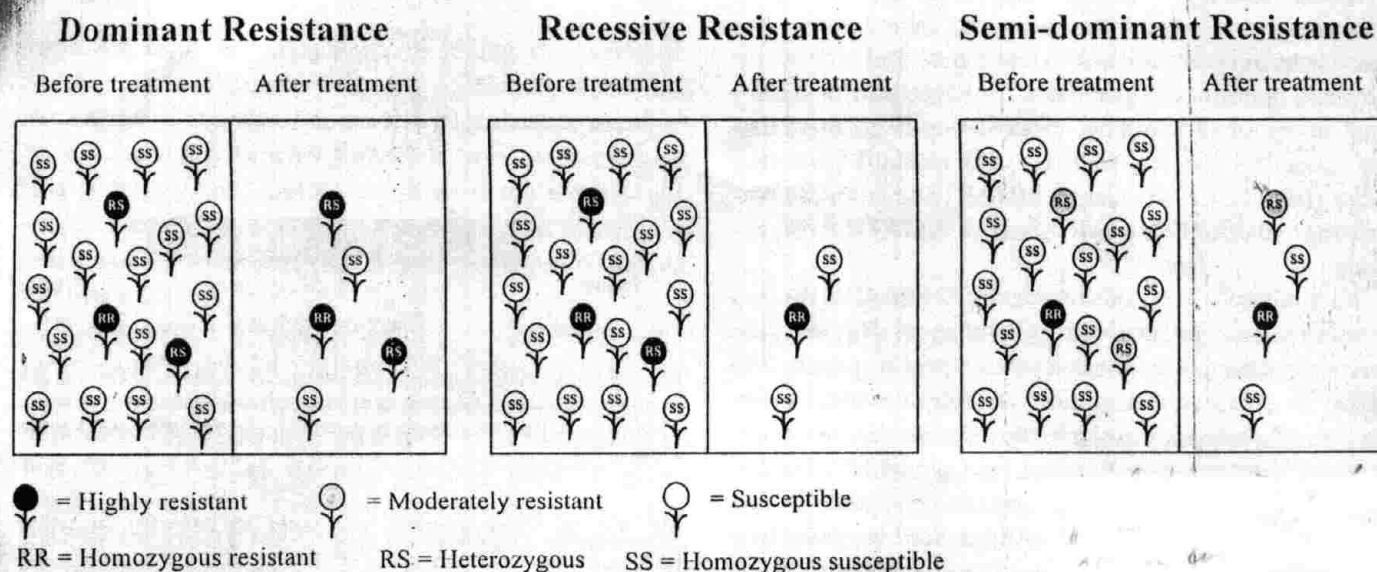
Dominant Resistance
Before treatment | After treatment

Recessive Resistance
Before treatment | After treatment

Semi-dominant Resistance
Before treatment | After treatment

● = Highly resistant   ◯ = Moderately resistant   ◯ = Susceptible

RR = Homozygous resistant   RS = Heterozygous   SS = Homozygous susceptible

Figure 1. Herbicide selection with single-gene dominant, recessive, and semidominant resistance gene expression; hypothetical examples illustrating how dominance, recessivity, and semidominance affect selection within each category. All highly resistant plants and half of moderately resistant plants are shown as surviving treatment, and a few susceptible plants escape control in these examples.

tial survival and inheritance of resistance alleles. A prerequisite to inheritance is that resistance alleles must be present within germ cells. Second, resistance must be expressed during the time of herbicide application. Although neutralist theory may discount the importance of gene expression to evolution at many loci (Kimura 1983), a selectionist view (see reviews by Gillespie 1991; Li 1997) is more relevant here because the expression of resistance is fundamental to the evolution of a resistant weed population. Third, the herbicide rate must be selective and reveal susceptible and resistant phenotypes. For example, a relatively low herbicide rate that does not control susceptible plants will not preferentially eliminate susceptible alleles. Likewise, a relatively high herbicide rate might not be selective if it succeeds in controlling both susceptible and resistant plants.

Resistance may be expressed as a qualitative or quantitative difference in herbicide absorption, translocation, metabolism, or affinity at the site of action when compared to wild-type susceptibility. Various cross- and multiple-resistance patterns are also possible. Although the attributes of the specific alleles involved are major determinants of these features, other factors of genotype and environment may influence the expression of resistance and therefore its inheritance and response to selection.

The level of dominance associated with the resistance trait is one of these factors (Figure 1), although typically not applicable when resistance is maternally inherited. In its simplest form, dominant resistance coded by a single nuclear gene is expressed regardless of homozygosity or heterozygosity so that selection may always act. Conversely, a recessive trait will not be expressed in a heterozygous state. A plant may carry an allele for recessive resistance while not displaying a resistant phenotype, which negates any potential selection. Resistance may also be expressed in a semidominant fashion, where heterozygous plants may display a level of resistance intermediate between homozygous genotypes.

The level of dominance is often a function of the gene itself, depending on whether survival under herbicide pressure is the result of a gain of resistance (likely to be dominant) or a loss of susceptibility (likely to be recessive) (see reviews by Gould 1995; Keightley 1996). However, dominance may also be influenced by the environment and, most importantly for this discussion, by the herbicide treatment itself. For example, a relatively low herbicide rate may succeed in controlling susceptible weeds, and perhaps a single copy of a resistance allele would be sufficient for survival. In this case, resistance is at least partially dominant. However, a higher herbicide rate may necessitate two copies of a resistance allele for survival, which means resistance is expressed as a recessive trait.

Because the level of dominance is most important when a plant is heterozygous for resistance, the frequency of heterozygosity within a weed population is a critical component of its response to selection. Heterozygote frequency is largely determined by the breeding system

of the weed as measured by its level of outcrossing. The difference between dominant and recessive resistance is minimized in populations of primarily inbred weeds because heterozygotes are rare. Dominance and recessivity are most important in populations of weeds with significant levels of outcrossing, especially self-incompatible and dioecious species, such as wild mustard [*Brassica kaber* (DC.) L. C. Wheeler #[3] SINAR] and common waterhemp (*Amaranthus rudis* Sauer # AMATA), respectively.

An additional level of complexity is added when two or more genes are involved in resistance. The multiple gene combinations possible may result in a quantitative trait with continuous variation whereby common intermediate phenotypes cannot easily be classified as either susceptible or resistant. Because environmental influences can also result in similar continuous variation, the inheritance of these traits is best measured by their 'heritability,' here limited to 'narrow-sense heritability' (Falconer 1989). This is a statistical measure of the contribution that parental alleles make to the overall variation of progeny. Traits with high heritabilities are most likely to respond to selection.

Resistant weeds that are treated with herbicide are more fit, with a higher fecundity, than susceptible plants. However, mutations conferring resistance may result in decreased fitness compared to susceptible types when the relevant herbicide is not applied; therefore, selection favors susceptibility. Under selectionist theory, decreased fitness with resistance would typically be the case because if the resistance allele were most fit, it would already be the wild-type allele. Neutralist theory, however, argues that there may be little difference in fitness between alleles (again, without herbicide application) and that wild-type susceptibility may be by chance. Equal fitness might particularly be true if the site-of-action enzyme does not represent a rate-limiting step in a metabolic function that contributes to fitness.

It is also worth noting that genes for resistance may be genetically linked to genes coding for traits unrelated to resistance, resulting in cosegregation. A particular trait may appear to be the result of resistance, when perhaps its gene is only genetically linked to resistance. Other plants with similar resistance may by chance carry alternative alleles at the linked gene, so caution is warranted when using small plant numbers to draw conclusions about traits associated with resistance.

Successful herbicide resistance management requires

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[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available only on computer disk from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.
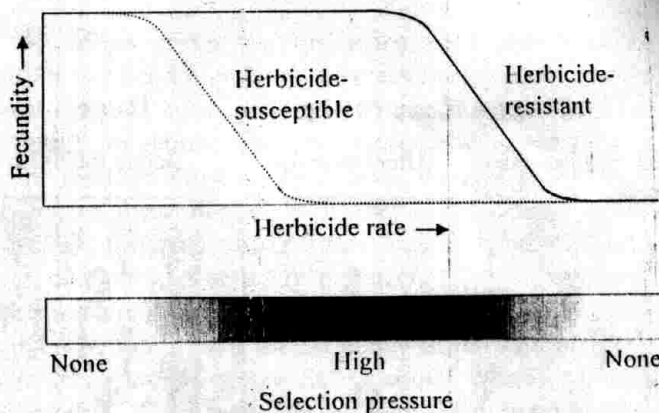

Figure 2. An example of herbicide-susceptible and -resistant weeds with parallel dose-responses as measured by fecundity. Selection pressure is positively correlated with the difference in fecundity between susceptible and resistant weeds.

the use of these genetic concepts to prevent or more likely reduce the overall selection for resistance. Although resistance management is simply a matter of using practices that keep selection pressure to a minimum, knowing what these practices are in each individual situation and implementing them while still obtaining adequate weed control is difficult. A good estimate of selection pressure is also necessary to predict the speed at which resistance, if present within a population, may evolve to economically important levels.

Any weed control practice that controls susceptible and resistant phenotypes equally eliminates selection pressure (Figure 2). Therefore, managers should not automatically reduce treatments such as herbicide rates and adjuvants that enhance herbicide efficacy in an effort to reduce resistance evolution. Practices that represent the minimum inputs to control susceptible weeds are more likely than higher levels of control to select for resistance. This concept is used in medicine to manage antibiotic resistance by recommending that dosages always be taken in full (see review by Levy 1998). Of course, when it takes impractical herbicide rates to control resistant weeds, this concept is not applicable.

Recessive resistance within an outbreeding weed species may be slow to evolve when compared to dominant resistance because the trait is not expressed in heterozygotes. This means that recessive resistance alleles can be removed from a population when in the heterozygous state. Therefore, an advantage may be gained from a resistance management standpoint by leaving a certain number of susceptible outcrossed plants uncontrolled. This is similar to the management recommendations for insect resistance to *Bacillus thuringiensis* toxin (McGaughey et al. 1998). However, the adverse effect of this

approach must be considered, including the economic consequences of purposefully not controlling weeds and the increased chance of an undesired resistance mutation or fertilization with immigrating resistant pollen. Again, this strategy would not be effective if the weed is primarily inbred (few heterozygotes in the first place) or if resistance is dominant (the heterozygotes will survive anyway).

For outcrossing weeds having semidominant resistance expression and initially low resistance gene frequencies, most resistant plants would be heterozygotes with moderate resistance. Herbicide rates that would allow for survival of these moderately resistant plants would increase the likelihood of crosses between them, thereby increasing the frequency of weeds homozygous for resistance at high levels. A similar situation may exist if resistance is multigenic or the weed is polyploid, such as wild oat (*Avena fatua* L. # AVEFA). In these cases, plants carrying resistance alleles at only a few of several possible gene loci may have intermediate resistance and survive low herbicide pressure. A polyploid carrying resistance in only one genome may also be intermediately resistant compared to a weed carrying resistance in multiple genomes (this assumes the increased gene dosage results in increased resistance).

Thus, moderately resistant plants carrying some but not all resistance alleles may eventually recombine to create a weed with a higher level of resistance than previously. In a worst case scenario, this weed would carry all possible resistance alleles for quantitative resistance or carry resistance alleles in all genomes. This type of recombination is minimized in inbreeding weeds but can still occur, especially after selection has increased the frequency of plants with intermediate resistance. Once again, decreased weed control that allows moderately resistant plants to survive may be detrimental to resistance management.

Resistance evolution may occur more slowly but probably will not be prevented by yearly rotation of weed control methods with different mechanisms of resistance, including the use of herbicides with different sites of action. Different weed control strategies may also be used within the same year, but they must complement each other so that weeds missed due to resistance to one of the treatments are targeted and controlled by the other treatment. A tank-mix using herbicides with different resistance mechanisms is a good way to reduce selection pressure for resistance. However, combinations of weed control methods will still select for multiple-resistant types if they exist within a population. The use

of relatively low rates of individual herbicides within a tank-mix may also be a concern for reasons discussed previously.

MANAGEMENT OF RESISTANT POPULATIONS

The first course of action when a resistant population evolves into an economic problem is to find an alternative means of weed control. As previously discussed, classification of the specific nature of resistance within a population is important. This is particularly meaningful when considering alternative herbicides because cross- and multiple-resistance might be involved.

However, successful control of resistant weeds does not in itself reverse the evolution of resistance; it only halts selection so that the resistant gene frequency in a population remains the same rather than increasing. Is it possible to reduce the frequency of resistance alleles? One way is to remove resistant plants in small patches by hand or other means. Alternatively, reducing the frequency of resistance requires selection for susceptibility by providing an environment whereby susceptible plants are more fit than those carrying resistance. Any lack of fitness directly related to the resistance mutation, such as the reduced photosynthetic capability of many triazine-resistant weeds, would be most useful. Fitness differences may be large or subtle, so the rate at which a population shifts back to susceptibility may vary. Of course there is no guarantee a mutant resistance allele will be less fit than the wild-type. Resistance mutations may also revert to susceptible in individual plants, especially if the mutation is not particularly stable.

A resistant weed population may be susceptible to alternative control measures, but may begin to evolve resistance when the alternatives are used. Genetic variability is again important to the adaptability of a resistant weed population to alternative control measures. In theory, however, variability may be compromised as a result of selection. During the initial stages of selection for a particular type of resistance, only a few plants or perhaps just one might be resistant. Evolution of a resistant population descending from these few plants might decrease genetic diversity. In outcrossed species, selection for resistance might also lead to an atypical level of deleterious inbreeding between relatives with a negative effect on fitness. Therefore, a resistant population may have unpredictable weaknesses that might be exploited, especially if these weaknesses are genetically linked to the resistance gene.

INFORMATION AND MANAGEMENT DECISIONS

What genetic information is helpful toward making good herbicide resistance management decisions? First, consideration should be given to the resistant gene frequency in the weed population of concern and to the possibility of new resistance allele introductions. Resistant gene frequency information is useful in judging both potential and realized resistance problems and in determining possible management regimes. Although resistant gene frequencies may be measured by sampling, they may also be estimated by roughly predicting mutation rates based on the molecular nature of resistance alleles. Weeds screened by herbicide treatment allow the estimation of resistant gene frequencies only when the expression of the resistance trait is understood (dominant, recessive, quantitative, etc.) and the frequencies of heterozygotes are known.

Here it is important to reiterate the possibility that a wide variety of molecular events may provide resistance to a given herbicide. Each molecular event may need to be considered independently due to different frequencies of occurrence, cross- and multiple-resistance patterns, and other genetic characteristics. Therefore, identification of the specific resistance allele or alleles of concern may be helpful and perhaps crucial in some instances. Other disciplines, especially those in the medical field, have successfully used DNA technologies such as genetic testing to classify disease alleles (Holtzman et al. 1997). Genetic testing holds tremendous potential as a tool for herbicide resistance management by allowing quick identification and classification of resistance alleles.

The expression and inheritance of resistance and the fitness of resistant weeds are critical to predicting selection pressure and evolution under various management schemes. The many complex genetic, biological, and environmental factors discussed above will influence resistance development. Thus, effective management systems may need to be specific to each weed, herbicide, environment, and resistance type. The information important to management decisions is often lacking. The particular circumstances are not known that will provide the optimum, undesired resistance selection pressure in each situation. Nor is identification of specific resistance alleles always possible.

Although most generalized strategies to reduce, not prevent, selection pressure (rotation of herbicides with different sites of action, tank-mixes, etc.) are sound, we must realize that these strategies are not the final answer. Researchers throughout science are coming to grips with the complexities of managing biological systems. "Fail-ure isn't what it used to be ... but neither is success" has been used to describe acquired immune deficiency syndrome research when the limitations of new treatments are realized (Cohen 1998). Society is bettered by the current arsenal of weed control technologies, including herbicides. But management practices designed to delay or remedy herbicide resistance will be influenced by unforeseen complexities. It is important that the complex and variable genetic aspects of weed populations and herbicide resistance be considered when recommending management strategies. It is even more critical that management practices not be mandated without ample evidence that they will be helpful in specific resistance scenarios. Potentially harmful or needless burdens on growers must be avoided.

ACKNOWLEDGMENTS

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