Herbicide Tolerant Genes, Part 3

"Super Weed" Myths and Kryptonite Remedies

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If ubiquity on hardware store shelves is any indication of product popularity, then homeowners love Roundup (formulated glyphosate). The ready-to-use formulation has such a low toxicity and hazard for eye and skin irritation that it's hard to believe it can injure anything. Yet, squirt it on young plants growing in the cracks and crevices of your driveway and sidewalk, and you will not have any weed problems for the rest of the summer. Owing to glyphosate's systemic abilities, it readily moves from the leaves to all parts of the plant, effectively eliminating any regrowth. But glyphosate's effectiveness is deceptive, giving some people the mistaken impression that it will kill every plant it comes in contact with. The truth is, older plants are much less susceptible to the effects of glyphosate than younger plants, and certain plants like mature woody brush may not be effectively controlled. And once glyphosate hits the ground, it tightly binds to soil, and its phytotoxic capabilities disappear.

But the myth of glyphosate as a macho, kill-anything herbicide has crossed paths with the concerns over the planting of millions of acres of Roundup Ready (RR) canola, corn, cotton, and soybeans. One fear, expressed particularly in the United Kingdom, is that glyphosate is so effective at weed control, that all forage plants used by songbirds will disappear in the wake of mass plantings of RR canola.

Meanwhile, the concern expressed most in North America is about glyphosate-susceptible "wimpy" weeds evolving into glyphosate-resistant "super" weeds. In crop rotations, the glyphosate-resistant volunteers will be uncontrollable and will run wild over the susceptible rotational crop. Perhaps songbirds will thrive, but those "uncontrollable super weeds" and resistant volunteer plants will devastate crop production, not to mention out-compete and therefore supplant the native vegetation.

And there are other concerns about herbicide-resistant crops. In a nutshell, they can be boiled down to five broad hypotheses:

1. Changes in landscape ecology will wipe out wildlife (the U.K. concern);
2. Repeated, widespread use of glyphosate will select for resistance in weeds, making them impossible to control;
3. Resistant genes will flow from RR crops to weedy species, creating uncontrollable super weeds;
4. Herbicide-tolerant crops will become weeds in fields where rotational crops are grown; and
5. Glyphosate is so toxic to soil microorganisms and invertebrates that soil fertility will be adversely affected.

Of course, these and other concerns regarding glyphosate use and Roundup Ready crops have already been reviewed through agencies including the United States Department of

Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) and the U.S. Environmental Protection Agency (EPA). Nevertheless, the concerns have not died, and will only be voiced louder as crops resistant to other herbicides enter production. RR crops, having become a huge commercial success, are the guinea pigs for examining the possible ecological threats of transgenic plant protection technology.

In Part 1 of this series ("Squaring Up Roundup Ready Crops," *AENews* No. 173, Sept. 2000), I discussed the basics of transgenic engineering; in Part 2 ("Giddy 'bout Glyphosate," *AENews* No. 175, Nov. 2000), I looked at the fears surrounding the use of glyphosate itself. In this essay, I will concentrate on dispelling the myth of "super weeds," addressing the second (resistance), third (gene flow), and fourth (volunteer crops) items above. I will also advocate the need for integrated weed management whether or not herbicide-tolerant transgenic crops are cultivated.

Resistance Preceded Transgenics

Over the last five years the planting of herbicide-tolerant crops has rapidly expanded to over 75 million acres worldwide (13). As of 1998, herbicide-tolerant transgenic crops were grown on 18, 26, and 44% of U.S. corn, cotton, and soybean acreage, respectively (6). In Canada, over 70% of canola acreage is now planted with herbicide-tolerant varieties (some transgenic, some nontransgenic). But herbicide-resistant weeds had become a big problem long before the advent of transgenic crop technology.

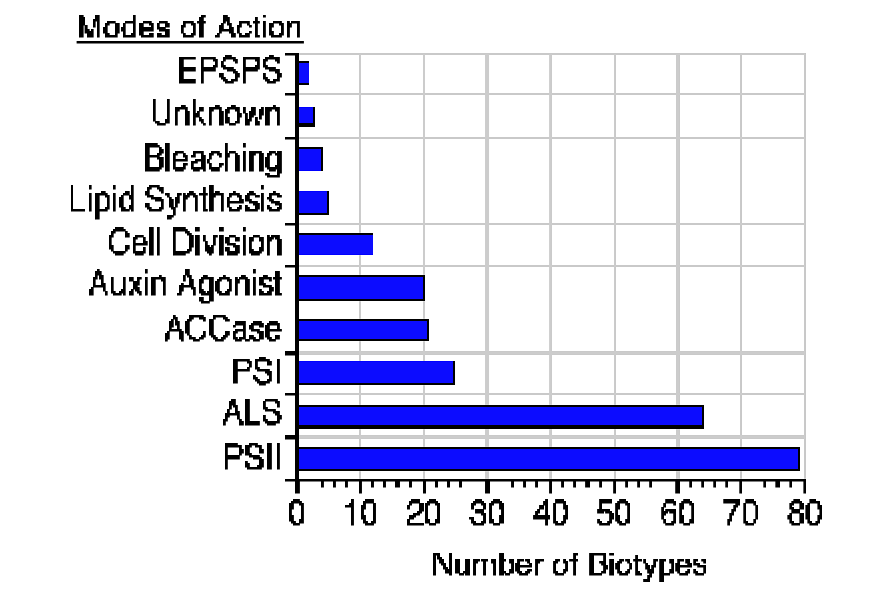
In the world today, about 235 weed biotypes have developed resistance to one or more herbicides; about 80 resistant biotypes have been found in the United States (9). About 22 biotypes have been documented as herbicide-resistant in Washington State. To date, none of the reported incidences of resistant weeds is related to the introduction of a herbicide-resistant crop, whether transgenic or not.

Like insects, weeds can develop resistance when continually selected by a single herbicide or group of herbicides having the same mechanism of toxic action. Weeds develop resistance in one of two ways. First, a few individuals in a population may possess a gene that enhances metabolic detoxification reactions, thereby breaking down the herbicide fast enough to avoid its phytotoxicity. The second and more prevalent method is the occurrence of some individuals with a gene that alters the herbicide's biochemical target site, making the plant resistant to injury. In either case, if these infrequent individuals escape control and successfully go to seed, comparatively more of these resistant individuals will occur in the population during the next growing cycle. Eventually, most of the population will be resistant to the herbicide.

Most cases of herbicide resistance are caused by a gene that produces an insensitive target site. Usually, that target site is an enzyme. The vast majority of weed resistance has developed to herbicides that specifically inhibit the synthesis of amino acids or photosynthesis (Figure 1). For example, herbicides that inhibit the enzyme acetolactate synthase (ALS) kill plants by shutting down branched-chain amino acid synthesis. Sulfonylurea and imidazolinone herbicides are two distinctly different chemical classes that inhibit ALS. Animals lack ALS and the ability to synthesize leucine, isoleucine, and valine (the branched-chain amino acids), so ALS-inhibiting herbicides are of very low hazard to animals. Sixty-three biotypes of weeds worldwide have developed resistance to the effects of ALS inhibitors. The triazine herbicides are one chemical class of several that can inhibit photosynthesis at a specific reaction center, photosystem II, which resides in the plant cell chloroplast, the chlorophyll-containing organelles that make a plant green. Seventy-eight weed biotypes have developed resistance to herbicides inhibiting photosystem II.

FIGURE 1

Number of plant biotypes resistant to herbicides, categorized by mode of herbicide action.



PSII, photosystem II inhibition; ALS, acetolactate synthase inhibition; PSI, diversion of electrons at photosystem I; ACCase, inhibition of lipid biosynthesis by blocking acetyl co-enzyme A carboxylase; EPSPS, glyphosate resistance caused by inhibition of enolpyruvyl shikimate phosphate synthase.

Since the overwhelming majority of the herbicide-tolerant crops worldwide are RR, most concerns focus on glyphosate. After 26 years of commercial use, glyphosate resistance has only been documented for two weed species, annual rigid ryegrass *(Lolium rigidum)* and goosegrass *(Eleusine indica)* (7, 8). Glyphosate-resistant ryegrass has been confirmed in Australia and California (wheat production), and resistant goosegrass was observed in Malaysia (oil palm production). In both cases, resistance occurred after 10-15 years of intensive glyphosate use (>2 applications per site per season). RR crops, especially soybeans, have been planted on vast acreage for five years now, and no reports of glyphosate-resistant weeds have surfaced.

Promiscuous Plant Phenomena: Gene Flow, Hybridization, Introgression

In addition to direct selection of resistant weeds by repeated application of herbicides with the same mechanism of action, some scientists have hypothesized that transgenes (modified genes) might move from the resistant crop to weeds. Termed "gene flow," the movement of genes between closely related plant species is quite natural and has been occurring ever since flowering plants evolved (14). Gene flow occurs when pollen from one plant species fertilizes flowers of a different but compatible species. The compatible species is usually closely related to the first species. (Gene flow can also occur between populations of the same species.) The offspring resulting from the mating, called hybrids, may then mate via pollen exchange with the wild-type (original) plants. This subsequent mating is called backcrossing or introgression. Introgression causes the hybrid in subsequent generations to become more like the wild-type plant in character and genetic makeup, but it also results in the cultivated plant's genes being stably incorporated into the genome of the wild-type plant. Naturally, a crop plant can receive pollen from a wild-type plant, but chances for successful establishment of this hybrid is poor considering most field crops are grown from certified seed.

A number of cultivated crops, including canola, sugar beet, and wheat, are grown in close proximity to their related wild-type ancestors (14). A few cases of gene flow have been observed under field conditions. On the other hand, corn, soybean, and cotton have no closely related compatible wild-type relatives in the United States or Canada (14). Thus, the likelihood of gene flow from these crops to noncrop species is nil.

Perhaps the most studied examples of gene flow have been in sugar beet *(Beta vulgaris)* and in canola *(Brassica napus)*. Both of these crops are plagued by the presence of related weedy wild-type plants that cannot be differentially controlled by a herbicide. In France, adventitious beet weeds were hypothesized to be hybrids resulting from pollen transfer between nonweedy wild-type beets and cultivated seed production sugar beets (1). Control of the adventitious beets could be accomplished by introduction of herbicide-tolerant cultivated sugar beets. However, the hybrid weed problem must also be managed by ensuring that seed production sugar beets are not grown in areas where the wild type exists.

Canola (originally a marketing name registered by the Western Canadian Oilseed Crushers Association for food-quality rapeseed oil) was developed from a cross of two wild-type ancestors, Brassica campestris and B. oleracea (11). *B. campestris* is a common weed in rape production and is not easily controlled with herbicides. Controlled field experiments and genetic analysis of field-collected *B. campestris* showed that high levels of hybridization occurred between *B. campestris* and *B. napus*. Backcrossing (introgression) of *B. campestris* with itself stabilized the genes of *B. napus* in the hybrids. But gene flow also occurred from *B. campestris* to *B. napus*. Such gene flow possibilities are quite natural, given the close relationship between cultivated oilseed rape and its wild, weedy relative.

Will Resistance Flow with Genes?

Given the ease with which genes can be exchanged between crop plants and their wild relatives (which are weeds in some cases) concern would naturally surface over the potential exchange of herbicide-resistant traits. In such an event, the weedy relatives have been perceived as uncontrollable. Furthermore, there is concern that a transgene will somehow endow a weed with even more aggressive traits (i.e., a "weedier" weed) such as increased seed production. For example, some believe that Johnson grass, a Corn Belt weed, became more fit after hybridization with corn (14).

Ironically, specialists in sugar beet and canola production have eagerly awaited the introduction of herbicide-tolerant cultivars that would enable susceptible weedy relatives to be easily controlled in the same field (1, 11). (See **EDITOR'S NOTE**; this paragraph originally contained an error.) In Canada, four systems of herbicide-tolerant canola have been approved and commercially introduced. No herbicide-resistant weedy relatives have been reported where these tolerant cultivars are grown.

Controlled experiments, however, have proved that herbicide resistance traits can flow to weedy relatives. Whether the trait will be stable over many generations in the weed is still unknown. A laboratory experiment was used to determine the ability of glufosinate (Basta herbicide) resistant transgenic *B. napus* to transfer its resistance character (known as the bar gene) to five related species in the family Brassicae (12). While the gene was detected in a number of hybrids, it wasn't always expressed, especially when the mating of the wild type was with the cultivated parent having multiple copies of the bar gene. The gene may have integrated in a place on the chromosomes (i.e., the genome) where gene expression was suppressed.

In another experiment, rape with Basta resistance was crossed with wild radish (Raphanus raphanistrum) (2). The resulting hybrid seeds were then planted in a field of wild radish and studied for four generations. Predictably, the bar gene showed up in half of the hybrids. After four generations of introgression, the chromosome number and morphology of the hybrids were similar to the wild-type radish, but the frequency of Basta resistance was reduced by one half. Given the optimal conditions for pollen transfer in this experiment (i.e., hybrids located in a solid planting of wild radish), the authors of the study concluded that the occurrence of transgene introgression under actual field conditions is probably rare and would occur very slowly.

Few experiments to date have measured flow of herbicide resistance traits between closely related species. Nevertheless, the National Academy of Sciences supports making predictions of the likelihood and consequences of commercial scale, crop-to-wild gene flow from pest-protected plants on the basis of general ecological and agricultural knowledge (14).

Factors affecting the successful establishment of hybrids resulting from gene flow have been reviewed and serve as a useful guide for regulatory questions (3, 14). The likelihood of gene flow between crops and weeds depends on the coexistence of the crop and wild relatives within a distance pollen may travel; on the simultaneous timing of flowering of the various species and their mating compatibility; on the survival and reproductive ability of the hybrids; and on the fate of the genes in wild populations (3). The greater the distance pollen travels, the more likely a wild-type compatible plant will be fertilized. For certified seed production, therefore, definitive distances are set by the USDA to isolate seed crops from potential cross-pollination with different varieties (14). Similar buffer zones could be imposed if transgenic gene flow was determined to be a significant threat.

Immigration of susceptibility genes into a weed population is also possible. In any case, for a hybrid to become established and crop genes be introgressed into the wild-type weed, gene flow of susceptible or resistant genes must be associated with some fitness advantage. In the absence of herbicide selection or other fitness trait such as increased fertility, there is no reason that a weedy wild-type relative outside of the cultivated field would present a problem.

Resistant Crops As Weeds

Many crops are rotated annually with unrelated crops. Prior to planting the rotational crop, the field is cultivated to prepare a new seed bed and/or treated with herbicide either before or after emergence of the new crop. This combination of treatments is likely to kill any volunteer crops, herbicide-tolerant or not. However, in fields with little or no tillage, concern has been expressed that the transgenic crop might not be easily controlled, especially if effective herbicide choices were limited by the resistance trait. Furthermore, the crop might become a "super weed" if it "escapes" from the field and establishes on uncultivated land. The premise for these concerns is that somehow the transgenic character endows the crop with the characteristics of a weed. However, this is a specious argument because herbicide tolerance is conferred by a single gene coding for an altered form of a target site that already exists in the plant. Furthermore, many crops naturally tolerate certain herbicides quite well, yet those crops do not turn into uncontrollable "super weeds."

Prolific seed production is one characteristic of weediness. Increased reproduction of RR crops has not occurred, according to studies showing that yields of transgenic and native cultivars are not significantly different (4). Even if volunteers of rotational crops need to be controlled, there are still many herbicides available with a different mode of action than the one to which the subject plant possesses tolerance. For example, canola in Canada is resistant to three herbicides acting at the biochemical target sites of ALS, EPSPS, or photosystem II. But volunteer canola can still be controlled by 2,4-D (5).
Finally, few crops survive outside of the cultivated field. "Escapees" of corn, cotton, or soybean are unlikely to survive in the wild (14). Canola is a bit different, since it is actually a hybrid of a closely related wild species; it could perhaps survive on uncultivated land. But the ability to survive intact with a herbicide resistance gene doesn't give canola any fitness advantage in the absence of herbicide applications. Outside a cultivated field, it would lose its "incentive" to retain the gene.

IPM: Kryptonite for "Super Weeds"

Until recently, environmental advocacy groups (EAGs) seemed about as interested in herbicide-resistant weeds as they were in insecticide-resistant insect pests. With the commercial introduction of herbicide-resistant crops, EAGs seem to have found a reason to be intolerant of herbicide-tolerant crops. As discussed earlier, however, resistant weeds have been around a long time. As a result, so have strategies to manage resistance.
With or without transgenic crops, resistance development remains a threat if chemical control is not carefully managed and integrated with nonchemical methods. The Herbicide Resistance Action Committee (HRAC), a consortium of agrichemical manufacturers who provide information about resistance cases and strategies for management, has developed a herbicide risk assessment table to help growers and crop protection specialists determine the probability that certain practices will lead to resistance (Table 1) (10). By conducting weed control using practices listed in the lowest risk category column, growers can minimize herbicide resistance regardless of its cause.

TABLE 1

Risk assessment decision chart for determining probability of herbicide resistance development (10).

	Low	Moderate	High
Herbicide Mix or Rotation	>2 MOA*	2 MOA	1 MOA
Weed Control Method	Cultural, Mechanical, Chemical	Cultural, Chemical	Chemical
Use of Same MOA* Per Season	Once	More Than Once	Many Times
Cropping System	Full Rotation	Limited Rotation	No Rotation
Resistance Status to MOA*	Unknown	Limited	Common
Weed Infestation	Low	Moderate	High
Control Over Last 3 Years	Good	Declining	Poor

***MOA=Mode of Action, Mechanism of Action**

Integrated weed management relies on the same principles as any other integrated pest management (IPM) strategy:

- Identify the weed species.
- Tailor the weed control program to weed densities and/or economic thresholds.
- Use a combination of weed control techniques, including physical (e.g., tillage), cultural (e.g., cover crops), and chemical practices.
- If using chemical controls, rotate herbicides on the basis of mode of action (MOA) and/or use mixtures where appropriate.
- Follow recommend use rates and application timing listed on the label.
- Routinely monitor results of herbicide applications to prevent escapes and seeding of weeds.
- Maintain detailed field records of the species present, control efficacy, and herbicides used (including rates and timing).

In summary, weeds have been developing resistance to various herbicides for quite a few years. Two types of toxicity in particular, ALS and photosystem II inhibition, seem to be the biggest problems. Only two weed species have developed glyphosate resistance after a quarter century of commercial use. Nevertheless, weed resistance due to widespread cultivation of herbicide-tolerant crops (whether transgenic or not) may develop as a result of direct selection pressure from the herbicide or as a result of gene flow. Gene flow among closely related species occurs naturally; however, gene flow from corn, cotton, and soybean to weedy relatives will not occur in North America owing to the absence of the wild-type species. Even with species like canola that are cultivated in proximity to their wild ancestors, hybridization and introgression of herbicide-resistant genes under natural field conditions seems to be of low probability. Even if herbicide-resistant genes do "escape" and establish in weeds, implementation of integrated weed management can ensure long-term effective control of these species.

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