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Glyphosate Resistance in Weeds

The Transgenic Treadmill

Glyphosate resistant weeds may spell the end of patented herbicide tolerant crops, but can farmers exit the transgenic treadmill that's very profitable for Monsanto? Prof. Joe Cummins

An evolving problem

Glyphosate herbicide was patented and sold by Monsanto corporation since 1974 under the trade name and proprietary formulation Roundup. The herbicide has been used widely in agriculture, forestry, aquaculture, alongside roads and highways, and in home gardening. Glyphosate is a broad-spectrum herbicide that poisons many plant species so it is frequently used to 'burn down' weeds on a field prior to the planting or emergence of crops.

Before 1996, weeds were not observed to have evolved resistance to glyphosate in the field, but since then, the introduction of transgenic glyphosate tolerant crops has led to evolution of a number of resistant weeds as the result of the greatly increased use of the herbicide particularly during the post-emergent growth of the crops. Glyphosate reisistant Asiatic dayflower (*Commelina cumminus* L) common lambsquarters (*Chenopodium album* L) and wild buckwheat (*Polygonum convolvulus* L) are reported to be increasing in prominence in some agro ecosystems as are populations of horseweed (*Conyza canadensis* (L) Cronq) [1].

In regions of the USA where transgenic glyphosate resistant crops dominate, there are now evolved glyphosate-resistant populations of the economically damaging weed species *Ambrosia artemissifolia* (rag weed), *Ambrosia trifida* L.(great ragweed), palmer pigweed (*Amaranthus palmeri*), common water hemp (*Amaranthus rudis*) , rough fruit amaranth (*Amaranthus tuberculatus*) and various *Conyza* (horse weed) and *Lolium* (rye grass) species.

Likewise, in areas of transgenic glyphosate resistant crops in Argentina and Brazil, there are now evolved glyphosate resistant populations of Johnson grass (*Sorghum halepense*) and Mexican fireplant (*Euphorbia heterophylla*) [2]. These herbicide resistant weeds pose a clear threat to the transgenic crops dominating North and South America [3].

Can resistance be managed?

There is no simple remedy for the evolution of resistance to glyphosate. Interestingly, the inventor of both glyphosate and the herbicide tolerant crops, Monsanto Corporation, does not appear to be engaged in finding remedies for the invasion of resistant weeds. There has been an effort to remedy the invasion of resistant weeds by the academic community. Simulation modeling has been developed. Glyphosate use for weed control prior to crop emergence is associated with low risks of resistance. These models are based on assumptions that low risks can be further reduced by applying glyphosate in sequence with other broad-spectrum herbicides prior to crop seeding [4]. Post-emergence glyphosate use, however, associated with glyphosate-tolerant crops, very significantly increases the risks of resistance evolution. Annual rotation with conventional crops reduces these risks, but the proportion of resistant populations can only be reduced to close to zero by mixing two of three post-emergence glyphosate

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applications with herbicides that have alternative modes of action. Weed species that are prolific seed producers with high seed bank turnover rates are most at risk of glyphosate resistance evolution. The model is especially sensitive to the initial frequency of resistance alleles, and other genetic and reproductive parameters, including weed breeding system, dominance of the resistance trait and relative fitness, influence rates of resistance. (Although these assumptions may be quite irrelevant in view of numerous physiological mechanisms of the 'fluid genome' that can produce resistant mutations in plants exposed to non-lethal levels of glyphosate, as discussed in [5] (GM Crops Facing Meltdown in the USA, *SiS* 46).

Over the past decade, the most problematic weeds in agronomic cropping systems have shifted away from perennial grass and perennial broad leaf weeds to primarily annual broadleaf weeds, although the glyphosate resistance mechanisms in weeds are currently poorly understood [6]. It appears that post-emergent use of glyphosate may be a main contributor to evolved glyphosate resistance, but eliminating post-emergent herbicide treatment practically eliminates all the advantages of and hence the need for herbicide tolerant crops.

Evaluation of herbicide programs for the management of glyphosate-resistant waterhemp (*Amaranthus rudis*) in maize involved pre-emmergent application with the herbicide glufosinate followed by a post-emmergent treatment of the transgenic maize with glyphosate which controlled water hemp better than pre and post emmergent treatment with glyphosate alone [7].

Maize growing in the EU27 increased to over 13 million ha in 2007, most of which (>80 percent) grown in just eight countries (France, Romania, Germany, Hungary, Italy, Poland, Spain and Bulgaria). The number of herbicides used to control the wide spectrum of weeds occurring in all these countries is likely to decline in the future, and care need to be taken to manage potential weed shifts to more difficult-to-control species and to reduce the risk of selection for glyphosate-resistant weeds [8].

In 2002, a glyphosate resistant Johnson grass (*Sorghum halepense*) appeared in Argentina and now covers at least 10 000 ha. The introduction of novel genetically modified crops promoted the use of more herbicides. This in turn reinforces the emergence of herbicide-resistant weeds, constituting a new phenomenon of intensification, the "transgenic treadmill" [9].

Many herbicide tolerant crops become "volunteer" weeds that infest crop rotations. Those weeds complicate cultivation, contaminate crops, and enhance gene transfer to weedy relatives. A transgenically mitigated (TM), dwarf, herbicide-resistant construct using a gibberellic acid-insensitive (Deltagai) gene in the *B. napus* crop was effective in offsetting the risks of transgene establishment in volunteer populations of *B. napus* (oilseed rape) [10]. Dwarfing the transgenic crop did not hurt yield of the oil seeds, but controlled the volunteer weeds because they were shaded by a taller crop. Mitigation by dwarfism worked well in greenhouse experiments but failed in field experiments. The proposed mitigation increased escape and persistence of transgenic weeds [11]. An alternative mitigation strategy for transgenic rice involved the introduction of a potpourri of traits including dwarfism, non-shattering, no secondary dormancy and herbicide sensitivity [12].

The companies selling herbicides and herbicide tolerant transgenic crops are in no hurry to control herbicide resistant weeds; for them, the multiple herbicides used to control transgenic weeds may come as an economic blessing. It is the farmers and the rest of society that suffer.

Genetic basis of glyphosate tolerance

Glyphosate kills plants by interfering with the synthesis of the amino acids phenylalanine, tyrosine and tryptophan. It does this by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenol pyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (ESP). ESP is subsequently dephosphorylated to chorismate, an essential precursor in plants for the aromatic amino acids: phenylalanine, tyrosine and tryptophan. These amino acids are used as building blocks in peptides, and to produce secondary metabolites such as folates, ubiquinones and naphthoquinone. X-ray crystallographic studies of glyphosate and EPSPS show that glyphosate functions by occupying the binding site for phosphoenol pyruvate.

Some micro-organisms have a version of 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS) that is resistant to glyphosate inhibition. The version used in genetically modified crops was isolated from *Agrobacterium* strain CP4 (CP4 EPSPS) that was resistant to glyphosate. The CP4 EPSPS gene was engineered for plant expression by fusing the 5' end of the gene to a chloroplast transit peptide derived from the petunia EPSPS. This transit peptide was used because it had shown previously an ability to deliver bacterial EPSPS to the chloroplasts of other plants The chloroplasts of higher plants synthesize amino acids phenylalanine, tyrosine and tryptophan [13].

The genetic basis of many of the glyphosate resistant weeds remains unknown; but those studied in detail show that there is no single genetic alteration responsible in all of the resistant weeds. Some populations of goosegrass from Malaysia, rigid ryegrass from Australia,and Italian ryegrass from Chile exhibit target site-based resistance to glyphosate through changes at amino acid 106 of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene. Mutations change amino acid 106 from proline to either serine or threonine, resulting in an EPSPS weakly resistant to glyphosate. The moderate level of resistance is sufficient for commercial failure of the herbicide to control these plants in the field. Other mechanisms of resistance include a nontarget site resistance mechanism has been documented in glyphosate-resistant populations of horseweed and rigid ryegrass from the United States and Australia, respectively. In these resistant plants, there is reduced translocation of glyphosate to meristematic tissues. Both of these mechanisms are inherited as a single, nuclear gene trait [14].

EHSPS gene amplification has been found to lead to glyphosate resistance in *Amaranthus palmeri* populations from Georgia, in comparison with normally sensitive populations. EPSPS enzyme activity from resistant and susceptible plants was equally inhibited by glyphosate. Genomes of resistant plants contained from 5-fold to more than160-fold more copies of the EPSPS gene than did genomes of susceptible plants. Quantitative RT-PCR on cDNA revealed that EPSPS expression was positively correlated with genomic EPSPS relative copy number. The amplified genes were not clustered on the chromosomes but distributed among all of the chromosomes [15, 16]. These results suggest that the EPSPS genes were amplified through mobile genetic elements (jumping genes). Interestingly, in a laboratory experiment with alfalfa cells in culture reported eight years before the *Amaranthus* investigation gradual stepwise increases in glyphosate in culture medium led to gene amplification of the EPSPS gene `

[17]. Another evolutionary glyphosate resistance mechanism was observed in the horse weed. The mutant weeds rapidly pumped the herbicide into vacuoles preventing contact of the herbicide with the chloroplast [18].

Conclusion

The evolution of glyphosate resistance among weeds that interfere with the productivity of crops is approaching catastrophic proportions. The evolutionary process leading to the resistant weeds has been described as a “transgenic treadmill” that renders current use of transgenic crops unsustainable. As current transgenic crops are rendered obsolete through weed resistance, the crops will be replaced with new transgenic varieties made available at higher prices to the farmers [19] (GM Crops Increase Herbicide Use in the United States , *SiS* 45) followed by another round of weed evolution to resistance. The only escape from this treadmill is to shift comprehensively to organic agriculture [5], as farmers have discovered in India [20] (Farmer Suicides and Bt Cotton Nightmare Unfolding in India, *SiS* 45).

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In my understanding herbicides and the subsequent development of genetic modifications in crop plants in order to render them herbicide resistant are both the natural consequence of viewing Nature as wholly separate from ourselves. This view is completely erroneous. We are part of Nature. A natural by product of this erroneous view is an exploitative as opposed to a cooperative approach toward Nature. I think the likes of companies such as Monsanto and their corporate behaviours exemplify this erroneous view.

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