Gene amplification delivers glyphosate-resistant weed evolution

Stephen B. Powles

Western Australian Herbicide Resistance Initiative, School of Plant Biology, University of Western Australia M086, Perth, Western Australia 6009, Australia

In a world of more than 6 billion people, humans heavily rely on the dependable cultivation of the global food grain crops: rice, wheat, soybeans, maize (and cotton for fiber). History shows that threats to food production have major repercussions, including famine, war, and civil unrest. A major threat to food production occurs every single growing season, when wild plant species (weeds) infest crop fields. Humans have battled since the dawn of agriculture to control weeds and to minimize their negative influence on food production. Modern herbicides have largely replaced human labor as the primary tool for weed control, and this has contributed significantly to the productivity of world cropping. However, despite the success of herbicides, weeds remain a primary challenge to food production, in part because selection pressure from herbicides has resulted in the evolution of herbicide resistance in weeds. A current and important example is evolved resistance to the world's most important herbicide, glyphosate (1). Glyphosate resistance evolution is a major adverse development because glyphosate is a one in a 100-year discovery that is as important for reliable global food production as penicillin is for battling disease. The report by Gaines et al. (2) in this issue of PNAS shows how one economically important weed species has evolved glyphosate resistance via gene amplification.

In the past decade there has been a revolution in world cropping with the advent and widespread adoption in the Americas of transgenic soybean, maize, cotton, and canola crops (3). In these crops engineered to be glyphosate resistant, this herbicide removes infesting weeds without any damage to the crop. The massive adoption of transgenic glyphosate-resistant crops has meant excessive reliance on glyphosate for weed control across vast areas. In evolutionary terms, widespread and persistent glyphosate use without diversity in weed control practices is a strong selection pressure for weeds able to survive glyphosate. Genes endowing glyphosate resistance are initially very rare; however, repeated use of glyphosate without diversity selects for such rare glyphosate resistance genes. This is occurring particularly in areas with transgenic glyphosate-resistant crops,
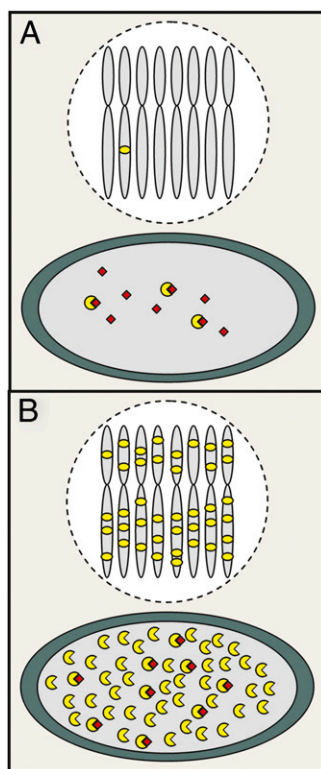

Fig. 1. Glyphosate resistance resulting from gene amplification. In a susceptible individual (*A*), the normal number of *EPSPS* gene copies (yellow dots on chromosomes) produce EPSPS (yellow) in leaf chloroplasts that is targeted and overwhelmed by the normal usage rate of glyphosate (red), and the plant dies. In a resistant individual (*B*) with amplified *EPSPS* gene copies present on multiple chromosomes, there is increased EPSPS, and the normal glyphosate rate cannot inhibit all of the available EPSPS.

where glyphosate is repeatedly used as the only weed control measure. Inevitably, glyphosate resistance is evolving in many important weed species (1). In some but not all parts of the world, glyphosate resistance evolution can no longer be prevented, and therefore resistance must be managed. Globally, no weed control tools are as good as glyphosate, and its potential widespread loss because of resistance is a looming threat to global cropping and food production.

In plants, glyphosate is toxic because it inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). Thus far, glyphosate-resistant weed plants exhibit either a mutation of the EPSPS

gene or, more commonly, a gene trait (unknown) that restricts glyphosate movement within resistant plants so that it does not reach EPSPS at toxic levels (4). Now an important new resistance mechanism (2) is evident in glyphosate resistant populations of the particularly damaging weed species, *Amaranthus palmeri* (5). This weed infests large areas of US crop land, can devastate crop yield, and, together with some other *Amaranthus* species, must be controlled to ensure productivity of global crops. The report by Gaines et al. (2) in this issue of PNAS documents that this weed species has shown yet another evolutionary tool, gene amplification, to resist an herbicide. Although gene amplification is a well-characterized phenomenon in plant evolution (6), here we see this response evolving in plants under anthropogenic selection pressures. Massive overproduction of EPSPS produced by the additional EPSPS gene copies allows the plant to cope despite the presence of glyphosate (Fig. 1), essentially acting as a molecular sponge to soak up the herbicide and allowing normal metabolic functions to continue.

Even more fascinating is that this gene amplification may have occurred via a mobile genetic element, as chromosome images show that the gene amplification appears on nearly every chromosome throughout the genome. How could such an extraordinarily large gene amplification occur in the first place and be maintained through subsequent generations? What does it mean for a plant to produce 20 times higher levels of an important metabolic enzyme? The EPSPS enzyme targeted by glyphosate is in the shikimate pathway, linking carbohydrate metabolism to the synthesis of aromatic compounds in microorganisms and plants (7). Both the energetic expense of producing extra EPSPS and the potential consequences of increased activity of this enzyme could have fitness costs for the resistant populations as well as other evolutionary consequences.

Insects have displayed the capacity to evolve resistance to insecticides through

amplification of genes that detoxify insecticides (8), but the example of *A. palmeri* involves amplification of the target site gene itself. A further risk is resistance endowing mutations in EPSPS could accumulate without detrimental effects, due to the buffering presence of multiple functional copies, and a highly glyphosate-resistant EPSPS could evolve. It could be possible that other genes have also been amplified in this species, potentially a source of genetic diversity for response to future selection pressures.

Clearly, nature can and will evolve in response to modern agricultural practices.

Recurrent selection with herbicides at sublethal rates, including glyphosate, can lead to rapid resistance evolution. Gene amplification can now be added to the category of potential evolutionary paths by which weeds can combat human attempts to control them. The importance of the report by Gaines et al. (2) is that dramatic gene amplification can occur as an evolutionary response to herbicide selection pressure. Aside from contribution to our understanding of evolution this example may reveal novel molecular genetic processes in plants that could be manipulated for targeted gene amplification in genetic

engineering. With this development, we have an even stronger basis to urge world agriculture to use glyphosate-resistant crop technology more wisely than has occurred until now. Indeed, the precious herbicide glyphosate is at risk for being driven into redundancy because of overuse without diversity in weed control practices. It is not an exaggeration to state that the potential loss of glyphosate to significant areas of world cropping is a threat to global food production. To avert this situation requires that glyphosate be used more judiciously and with more diversity than is currently the case.

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