Open Access

Do Environmental Effects of Herbicide-Resistant GM Plants Differ from Effects of Other Herbicide Resistant Plants?

Esa Tyystjärvi*

Department of Biology, University of Turku, FI-20014 Turku, Finland

Abstract: Genetic modification of crop plants has promoted concerns about potential environmental effects of this new technology. In this essay, I will discuss the environmental effects of genetic modification of crop plants using herbicide resistance as an example. Considering herbicide resistance as an old agricultural trait, it is difficult to find any reason to assess the environmental risks of genetically modified herbicide resistant plants differently from the risks of traditionally bred herbicide resistant plants.

INTRODUCTION

Genetically modified organisms are defined in different ways by different authorities. The Cartagena Protocol on Biosafety [1] defines "modified organism" as an organism that results from the use of modern biotechnology. In European legislation, the term genetically modified (GM) organism refers to an organism whose genome has been changed in a way that would not occur naturally (EU directive 2001/18/EC). In practice, the European legislation, too, refers to molecular biological methods used to change the genome, as the term "would not occur naturally" cannot be defined accurately.

The possible environmental problems related to GM plants can be classified to three categories. Firstly, the GM plant in itself can be harmful to environment or agriculture. Secondly, cultivation of the GM plant can lead to environmentally unacceptable agricultural practices. Thirdly, the spread of the genetic material of the GM plant to other organisms can be harmful.

I will consider the risks of GM plants with emphasis on comparison between GM plants and non-GM plants that are currently cultivated or occur in the nature. Comparison between the properties that plants can obtain *via* biotechnology and properties of plants found in the nature can give us a realistic view of the risks related to the current large-scale cultivation of GM plants. I will focus on herbicide resistant plants because herbicide resistance is the most important genetic modification in crop plants [2].

HERBICIDE RESISTANCE IN TRADITIONAL CROPS, WEEDS AND GM PLANTS

The most widely used herbicide resistance genes of GM plants are those conferring resistance against glyphosate and phosphinothricin (gluphosinate ammonium) (see [3]). Transgenic plants resistant to several other herbicides, including the PSII herbicide bromoxynil and herbicides like imidazolinone and chlorimuron that inhibit acetolactate synthase (ALS) have also been produced. Plants resistant to the auxin analog dicamba are being developed, and new cultivars are often resistant against several herbicides, starting with glyphosate and phosphinothricin. The cross-resistant cultivars are often obtained simply by crossing two existing cultivars.

In public discussions, it is often assumed that the practice of growing plants resistant to a particular herbicide was born with plant biotechnology. However, herbicide resistance has always been an important agricultural trait. Before the biotechnology era, herbicide resistance was usually described as a property of the selective herbicide, not as a property of the resistant plant.

Phylogenetically related groups of plants often have detoxification mechanisms that make the whole group resistant to certain herbicides. Monocots are resistant to auxin analog herbicides like 2,4-D, possibly because the detoxification of 2,4-D in monocots proceeds *via* irreversible conjugation while dicots have reversible conjugation mechanisms [4]. Maize, sorghum and sugar cane are able to detoxify the Photosystem II inhibiting herbicide atrazine [5]. Wheat and several other grasses tolerate the acetyl-CoA carboxylase inhibiting herbicide diclofop-methyl; in wheat the tolerance is caused by an efficient detoxification mechanism [6]. Sugarbeet tolerates phenmedipham and desmedipham, also due to a detoxification mechanism [7]. Natural herbicide resistance is not always based on a detoxification mechanism. The paraquat resistant weed *Conyza bonariensis* is apparently able restrict the translocation of the herbicide to its site of action in the chloroplast [8], and sensitivity to bialophos varies because different plant species do not equally efficiently convert bialophos to the active toxin, phosphinothricin [4]. Antioxidative defense mechanisms partially explain resistance to herbicides that cause oxidative damage [4].

In many cases, a plant is naturally herbicide resistant because the site of action of the herbicide is tolerant. Dicots are resistant to cyclohexanedienones and aryloxyphenoxypropionates because the dicot form of the target enzyme, acetyl-coenzyme A carboxylase, is herbicide tolerant. Triazine resistance of several weeds in families

*Address correspondence to this author at the Department of Biology, University of Turku, FI-20014 Turku, Finland; E-mail: esatyy@utu.fi

1874-7612/09

Amaranthus, *Solanum*, *Brassica* and *Phalaris* is a result of a serine to glycine mutation in the chloroplast gene that codes for the target protein of the herbicide [9]. Acetolactate synthase (ALS) is a highly flexible enzyme, as resistance to ALS inhibiting herbicides like imidazolinones and sulfonylureas in weeds can be the result of several different single nucleotide modifications in the *ALS* gene [10]. However, the natural resistance of crop plants against these herbicides is based on detoxification mechanisms [4].

The basis of herbicide resistance in GM crops does not qualitatively differ from natural herbicide resistance. Resistance to glyphosate is typically obtained by transforming the plant with a bacterial gene encoding a glyphosate tolerant form of 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme functioning in both plants and bacteria in the pathway producing aromatic amino acids. In some constructs, a bacterial glyphosate oxidoreductase gene is used in addition to the glyphosate resistant *EPSPS* gene. Resistance to phosphinothricin, in turn, is obtained with a bacterial phosphinothricin acetyltransferase gene that confers a detoxification mechanism. Similarly, resistance to bromoxynil can be obtained with a bacterial nitrilase gene and resistance to dicamba with a bacterial dicamba monooxygenase gene. Most herbicide resistant transgenic plants do not carry antibiotic resistance genes because the herbicide itself has been used as a selection marker.

Due to the flexibility of the ALS enzyme, also wheat, sunflower, canola and rice varieties have been made resistant to ALS inhibitors using mutation breeding (see [11]) that apparently leads to alterations in the target site of the herbicide. In this method, a genotoxic agent, ethylmethanesulfonate, is used to cause mutations, and the herbicide is applied to obtain resistant plants for further breeding. Because mutation breeding is not legally considered as gene manipulation, the resulting plants are not GM plants and the new herbicide resistant varieties do not need to pass similar risk assessment as GM crops. In some transformed plants (e.g. the Pioneer maize variety [12]), an imidazolinone/chlorimuron tolerant form of ALS has been obtained by changing the amino acid sequence of an endogenous gene of the same plant and transforming the plant with a construct containing the changed gene. Because molecular biological methods were used, such a plant is classified as GM.

With regard to the herbicide resistance mechanisms and genes conferring the resistance, it is hard to find any obvious difference that would make herbicide-resistant GM plants behave in the nature in differently from herbicide resistant non-GM plants. This does not mean that all herbicide resistant GM plants are inherently environmentally safe, but the risk assessment of herbicide resistance should be based on similar criteria, including genetic and ecological aspects, whether a new herbicide resistant cultivar is GM or not.

DO HERBICIDE RESISTANT GM PLANTS PROMOTE UNACCEPTABLE AGRICULTURAL PRACTICES?

The possibility that the use of herbicide resistant plants could lead to environmentally unacceptable agricultural practices, behaves closer inspection. The history of agriculture knows cases of environmentally questionable agricultural practices that have been adopted because a herbicide resistant plant has been available. Maize is an example. The natural atrazine resistance of maize has promoted the use of atrazine in maize for tens of years all over the world. Atrazine persists long in waters [13] and acts as a herbicide on phytoplankton; mainly for this reason the herbicide was recently banned in EU. The massive use of atrazine has led to the development of a large number of atrazine resistant weeds; Heap [14] lists 67 species.

To avoid promoting unwanted agricultural practices, development of herbicide resistant GM plants has focused on low-risk herbicides, especially glyphosate and gluphosinate ammonium, two herbicides that are harmless to non-target organisms [15, 16] and degrade rapidly in the soil [17, 18]. However, due to the inevitable evolution of herbicide-resistant weeds, application of only one herbicide is never a viable long-term strategy in agriculture. The expansion of the use of glyphosate due to introduction of glyphosate-resistant crops is the most probable reason why reports about glyphosate resistant weeds are rapidly increasing. International survey of herbicide resistant weeds [14] lists 15 species of glyphosate-resistant weeds, and most recent occurrences are from USA and Latin America where the large scale cultivation of glyphosate-resistant crops has led to increase in the use of glyphosate.

The light side of agricultural practices related to herbicide-resistant crops is that although they have promoted the use of some herbicides, the total consumption of herbicides may decrease. For example, use of Roundup Ready (R) maize decreased the use of active ingredients of herbicides by 40 % on area basis in US agriculture in 2006 [19]. Herbicide resistance also facilitates no-tillage agriculture that saves energy and causes less erosion than traditional methods.

IS A HERBICIDE RESISTANT GM PLANT A POTENTIAL NEW WEED?

Herbicide resistant crop species itself can be agronomically harmful if the resistant species germinates as a crop volunteer before the sown plants germinate, or if the herbicide resistant plant occurs as a weed on another field. The weediness of cultivated plants depends on the combination of species and climate. Many species cultivated far from the origin of the species, like most cereals of the temperate zone, are seldom found as weeds. Conversely, other crop species like canola (*Brassica napus*) in Canada [20, 21], sunflower (*Helianthus annuus*) in USA [22] and pearl millet (*Pennisetum glaucum*) in North Africa [23] are weedy. It is obvious that resistance against herbicides that are widely used on other fields in the area should be avoided in weedy crop species, and herbicides should be rotated even in cultivation of herbicide resistant plants. However, these safety measures are equally important for crop plants whether their herbicide resistance is a property of the species, was obtained by mutation breeding, or was obtained by gene technology.

GENE FLOW FROM GM PLANTS TO WILD RELATIVES

Transfer of genetic material between cultivated plants and wild relatives occurs continuously, and crop-weed hybrids are known from several major crop species, including the main cereals wheat, rice and maize [24]. Genes conferring herbicide resistance genes are not exceptions. However, reported cases in which herbicide resistance has appeared as a result of a crop-wild hybridization are uncommon. Cross resistance to glyphosate, gluphosinate ammonium and imidazolinone herbicides in escaped, weedy canola populations in Canada [21] show that escape is possible. In this case, the herbicide-resistant transient roadside populations consist of progeny of cultivated plants and the resistance genes against glyphosate and gluphosinate apparently come from GM plants and the altered ALS gene from an imidazolinone resistant non-GM variety.

Instead of appearing as a result of gene flow, herbicide resistant weeds have usually evolved due to the mere presence of the herbicide selection pressure. Different herbicides promote resistance at different rates. For example, resistance to paraquat, glyphosate and acetyl-CoA carboxylase (ACCase) inhibiting herbicides was reported in ryegrass (*Lolium rigidium*) from a South African farm in which paraquat had been used for 40 years, glyphosate for 25 years and ACCase inhibiting herbicides for 3 years [25]. ALS inhibiting herbicides are particularly prone to rapid evolution of resistant weeds (see e.g [26]).

The history of agriculture contains continuous development of new plant cultivars. Each new cultivar possesses new genes, these genes may spread to the environment and may have effects on the environment. For example, wheat, barley, oats and rye cultivars with genes conferring resistance against rust diseases caused by fungi of the family *Puccinia* have been cultivated in Australia for 40 years [27]. No safety precautions have been applied, and therefore the resistance genes have had the possibility of escaping to natural Australian populations of wild barley grass (*Hordeum* spp.) which is known to be susceptible to at least one *Puccinia* strain that also infects barley and wheat [28]. Rust-resistant cereals are only one example of traditional cultivars with genes conferring obvious fitness advantage if transferred to a wild relative. Similarly, herbicide resistance genes of cultivated species have had the possibility of escaping during the history of agriculture. More research on the effects of genes transferred in the past from crop species to wild relatives is needed for proper understanding of the effects of gene flow from GM plants to wild plants.

In public discussion, GM crops are sometimes classified to a new category whose potential hazardous properties can only be assessed after an extended period of time (see e.g. [29]). But what would make the difference between, for example, a Clearfield (R) cultivar produced in a traditional way by inducing mutations with ethylmethanesulfonate treatment and selecting plants that tolerate an ALS inhibiting herbicide and a plant that was made resistant to the same herbicide by transferring a gene with the same mutation? From the plant physiologist's viewpoint it is difficult to understand how a risk assessment tool could to be able to predict the long-term effect of the mutated gene but would not be able to predict the effect of the same gene as a transgene.

FUTURE PROSPECTS IN GENE CONTAINMENT PROCEDURES

Like all agriculture, use of herbicide resistant GM crops can be environmentally harmful. The potential negative environmental effects are related to the action of the products of the transgenes and to the effects on agricultural practices. However, the history of commercial GM crops has not revealed a single case of negative environmental impact caused by the fact that the cultivar was produced by genetic manipulation. On the contrary, biotechnology allows the use of methods to slow down the flow of particular genes to the environment. Recombinases [30] or ribonuclease genes [31] with tissue-specific promoters can be used to inhibit the formation of transgenic pollen, to prevent flower formation [32] or to prevent germination [33]. Targeting the transgenes to the chloroplast genome instead of the nuclear genome [34] would greatly slow down the rate of escape of crop-specific genes to natural populations. Both recombinases [30] and chloroplast transformation are routine in plant biotechnology (for a commercial cultivar in which the Cre recombinase has been used to remove a marker gene, see [35]).

In the future, it may be possible to use constructs that contain, in addition to a gene conferring an agronomically important property, a gene that lowers the plant's fitness in natural conditions while being neutral or beneficial from the agricultural viewpoint. If the chloroplast is the target of the gene transfer, both genes can be in the same operon; this would slow the segregation of the traits down to the level of mutation frequency. More complicated constructs, with several different containment factors, have been developed [36].

Risk assessment of new cultivars should not start when a cultivar is ready for market. Instead, ecological and evolutionary aspects of new plant varieties, whether GM or not, must be considered already during breeding in order to make well founded decisions about gene containment procedures and other environmental issues.

ACKNOWLEDGEMENTS

Taina Tyystjärvi is thanked for valuable comments. The author was supported by Academy of Finland.

REFERENCES

[1] Cartagena Protocol on Biosafety to the Convention of Biological Diversity. Montreal; 2000.

[2] Dill GM, CaJacob CA, Padgette SR. Glyphosate-resistant crops: adoption, use and future considerations. Pest Manag Sci 2008; 64: 326-31.

[3] GMO Compass. http://www.gmo-compass.org. Accessed: 1 May 2009.

[4] Sherman TD, Vaughn KC, Duke SO. Mechanisms of action and resistance to herbicides. In: Duke SO, Ed. Herbicide-Resistant Crops. Agricultural, Environmental, Regulatory, and Technical Aspects. Boca Raton: CRC Press 1996: 13-35.

[5] Shimabukuro RG. Atrazine metabolism in resistant corn and sorghum. Plant Physiol 1968; 43: 1925-30.

[6] Shimabukuro RH, Walsh WC, Hoerauf RA. Metabolism and selectivity of diclofop-methyl in wild oat and wheat. J Agric Food Chem 1979; 27: 615-23.

[7] Hendrick LW, Meggitt WF, Penner D. Basis of selectivity of phenmedipham and desmedipham on wild mustard, redroot pigweed, and sugar beet. Weed Sci 1974; 22: 179-84.

[8] Vaughn KC, Vaughan MA, Camilleri P. Lack of cross-resistance of paraquat-resistant hairy fleabane (*Conyza bonariensis*) to other toxic oxygen generators indicates enzymatic protection is not the resistance mechanism. Weed Sci 1989; 37: 5-11.

[9] Hirschberg J, Yehuda AB, Pecker I, Ohad N. Mutations resistant to Photosystem II herbicides. In: von Wettstein D, Chua N-M, Eds. Plant Molecular Biology. New York: Plenum Press 1987: 357-66.

[10] Tranel PJ, Wright TR, Heap IM. ALS mutations from herbicide-resistant weeds. 2009. http://www.weedscience.com. Accessed: 12 April, 2009.

[11] BASF website. http://www.agproducts.basf.us. Accessed: 1 May 2009.

[12] Summary of the application for authorisation of genetically modified 98140 maize derived food and feed in accordance with regulation (EC) 1829/2003. 2008. http://www.gmo-compass.org/pdf/regulation/ maize/98140_mais_application_foodfeed.pdf. Accessed: 1 May 2009.

[13] Müller SR, Berg M, Ulrich MM, Schwarzenbach RP. Atrazine and its primary metabolites in Swiss lakes: Input characteristics and long-term behavior in the water column. Environ Sci Technol 1997; 31: 2104-13.

[14] Heap IM. International survey of herbicide resistant weeds. 2009. http://www.weedscience.org. Accessed: 26 April 2009.

[15] McComb BC, Curtis L, Chambers CL, Newton M, Bentson K. Acute toxic hazard evaluations of glyphosate herbicide on terrestrial vertebrateds of the Oregon coast range. Environ Sci Pollut Res 2008; 15: 266-72.

[16] Dinehart SK, Smith LM, McMurry ST, Anderson TA, Smith PN, Haukos DA. Toxicity of a glufosinate- and several glyphysate-based herbicides to juvenile amphibians from the Southern High Plains, USA. Sci Total Environ 2008; 407: 1065-71.

[17] Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH. Fate and availability of glyphosate and AMPA in agricultural soil. J Environm Sci Health 2008; B43: 36-375.

[18] Faber MJ, Stephenson GR, Thompson DG. Persistence and leachability of glufosinate-ammonium in a Northern Ontario terrestrial environment. J Agric Food Chem 1997; 45: 3672-6.

[19] Johnson SR, Strom S, Grillo K. Quantification of the impacts on US agriculture of biotechnology-derived crops planted in 2006. Washington, DC: National Center for Food and Agriculture Policy 2007. http://www.ncfap.org/documents/2007biotech_report/Quantification_of_the_Impacts_on_US_Agriculture_of_Biotechnology.pdf. Accessed: May 1st, 2009.

[20] Orson J. Gene stacking in herbicide tolerant oilseed rape: lessons from the North American experience. English Nature Research Reports No. 443. England, Peterborough: English Nature 2002.

[21] Knispel AL, McLachlan SM, Van Acker RC, Firesen LF. Gene flow and multiple herbicide resistance in escaped canola populations. Weed Sci 2008; 56: 72-80.

[22] Kane NC, Rieseberg LH. Genetics and evolution of weedy *Helianthus annuus* populations: adaptation of an agricultural weed. Mol Ecol 2008; 17: 384-94.

[23] Miura R, Terauchi R. Genetic control of weediness traits and the maintenance of sympatric crop-weed polymorphism in pearl millet (*Pennisetum glaucum*). Mol Ecol 2005; 14: 1251-61.

[24] Ellstrand NC. Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives. Baltimore, USA: Johns Hopkins Univ Press 2003.

[25] Yu Q, Cairns A, Powles S. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 2007; 225: 499-513.

[26] Schmitzer PR, Eilers RJ, Czéke C. Lack of cross-resistance of imazaquin-resistant *Xanthium strumarium* acetolactate synthase to flumetsulam and chlorimuron. Plant Physiol 1993; 103: 281-3.

[27] Park RF. Breeding cereals for rust resistance in Australia. Plant Pathol 2008; 57: 591-602.

[28] Wellings CR, Burdon JJ, McIntosh RA, Wallwork H, Raman H, Murray GM. A new variant of *Puccinia striiformis* causing stripe rust on barley and wild *Hordeum* species in Australia. Plant Pathol 2000; 49: 803.

[29] Guarnieri V, Benessia A, Camino E, Barbiero G. The myth of natural barriers. Is transgene introgression by genetically modified crops an environmental risk? Riv Biol 2008; 101: 195-214.

[30] Gidoni D, Srivastava V, Carmi N. Site-specific excisional recombination strategies for elimination of undesirable transgenes from crop plants. In Vitro Cell Dev Biol Plant 2008; 44: 457-67.

[31] Burgess DG, Ralston EJ, Hanson WG, *et al.* A novel, two-component system for cell lethality and its use in engineering nuclear male-sterility in plants. Plant J 2002; 31: 113-25.

[32] Lännenpää M, Hassinen M, Ranki A, *et al.* Prevention of flower development in birch and other plants using *BpFULL1::BARNASE* construct. Plant Cell Rep 2005; 24: 69-78.

[33] Oliver MJ, Quisenberry JE, Trolinder NLG, Kleim DL. Washington, D.C.: U.S. Patent and Trademark Office, Patent No. 5,273,765, 1998.

[34] Daniell H, Khan MS, Allison L. Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology. Trends Plant Sci 2002; 7: 84-91.

[35] Application for authorization to place on the market LY038 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed. 2006. http://www.gmo-compass.org/pdf/regulation/maize/LY38_maize_application.pdf. Accessed: 1 May 2009.

[36] Mascia PN, Flavell RB. Safe and acceptable strategies for producing foreign molecules in plants. Curr Opin Plant Biol 2004; 7: 189-95.

Received: May 4, 2009 Revised: July 7, 2009 Accepted: July 8, 2009