Resistance is Growing

GM herbicide tolerant crops and resistance in weeds

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This briefing examines the development of GM herbicide tolerant (GMHT) crops and how early predictions that weeds would become resistant to the herbicides used with them have turned out to be correct. It examines the implications of the herbicide "arms race" and the alternatives that exist for managing weeds in arable fields.

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Background

Herbicide tolerant crops existed before genetically modified herbicide tolerant (GMHT) crops were developed. Broad leaf weeds in cereal crops are routinely controlled using hormone weed killers, such as MCPA, without the growing crop being killed. However, controlling weeds which are similar to crop plants, for example grass weeds in cereal crops or broad leafed weeds in oilseed rape, proved more difficult. The agro-chemical companies, having developed exceedingly profitable herbicides, such as glyphosate, then began to engineer crops that could tolerate them, so the crops can carry on growing but the weeds are killed. This opened up the possibility of total weed control even when the crop and weed were from the plant sub class or family. The agro-chemical companies used herbicide tolerance as a means to develop themselves into global seed and biotechnology businesses. These companies are now able to sell GMHT seeds linked to sales of their own-brand of herbicides (marketed as Roundup Ready). This not only increases sales of both, as a package protected by intellectual property rights, but has also prevented rivals from taking a major share in the market after patents on the herbicides themselves had expired.

The terms "tolerance" and "resistance" are often used interchangeably. This report applies tolerance to crops and resistance to weeds unless quoting directly.

Monsanto have been most successful in this process following the discovery of a gene able to render the glyphosate molecule ineffective as a weedkiller (the seeds are marketed as Roundup Ready(RR)). As a result, they have managed to increase sales of their herbicide Roundup (active ingredient, glyphosate) despite patents ending in 2000 (see below). Bayer CropScience have developed their own GMHT crops using glufosinate ammonium tolerant genes extracted from a soil bacterium (marketed as Liberty Link).

Since artificial pesticides were first used in the 1940s, the pests targeted by them began to develop resistance to these chemicals. The first herbicide resistant weed was found in Canada in 1952 (wild carrot resistant to 2,4D). This evolutionary process applies to all types of crop pest – weeds, fungal diseases, insects and molluscs. The rate of resistance development depends on how the pesticides are used. In any population of pests there are likely to be a few individuals who will be resistant to the pesticide as a result of a naturally occurring gene(s) or by chance mutation. Long term dependence on one pesticide means that these few individuals survive to reproduce and become dominant in the population which over time becomes resistant to the pesticide.

Thus from the late 1940s, pesticide chemists and farmers have been engaged in a "pesticides arms race" to attempt to keep ahead of the pests. Success in this race depends on new active ingredients being developed all the time. Each new active ingredient brings with it a new toxicological profile and interaction with the crops, pests and general environment. These now require the agrochemical corporations to produce extensive sets of safety data to meet the requirements of pesticide regulations before approval for commercial use can be obtained. The products also have to be proved effective against the pest they target

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(ie, herbicides have to be able to kill their target weeds to get approval). The costs of developing a pesticide to the point they are approved for marketing is high – said to be £140 million[1] by the agrochemical industry.

The agro-biotech industry thus aims to maximise the sales of each product to recoup the costs of development and licensing. Patents on GM technologies, GM traits and chemicals, as well as plant varieties protection legislation (to ensure royalties on seed sales and farm saved seeds[2]) are used to prevent other companies profiting by copying their developments.

Another factor in the development of herbicide tolerant crops has been the increasing weed pressure in some crops grown in intensive arable systems, for instance oilseed rape (canola) in Canada, because "(*a) number of weeds are not effectively controlled by the available herbicides and changes in tillage and cultural practices are resulting in weed population shifts thus increasing the weed management problem*"[3].

Thus there are two changes occurring in weeds. One is "weed shifts" whereby the make of arable weeds changes toward species which are more tolerant of the herbicides being used. The second is the development of resistance within species when a few individuals (biotypes) are able to withstand the herbicide doses applied and consequently increase in number due to selection pressure.

Herbicide tolerant crops

Herbicide tolerant crops enable the crop to be sprayed for weed control without the growing crop being killed.

A number of herbicide tolerant (HT) crops have been developed without using genetic modification, but the vast majority of HT seeds currently sold on the global market used the genetic engineering technique. Table1 shows the types of herbicide tolerant crops that have been developed.

Table 1 Herbicide tolerant crops

Herbicide	GM	Commercial HT Crops global	Experimental HT crops global	Herbicide brand names (company)
Glyphosate	Yes	Roundup ready (RR) Soya, maize, cotton, sugar beet and oilseed rape		Roundup (Monsanto)
Glufosinate ammonium	Yes	Liberty link (LL), soya, maize, oilseed rape and cotton.	Beet, cereals	Liberty (Bayer CropScience)
Imidazolinone	No	Clearfield maize, wheat, oilseed rape, lentils, peanuts		Pursuit/Odessy (BASF)
2,4 D	Yes		Soya, cotton, maize	(Dow)
Sulfonylurea	Both	Soya (GM and non-GM), cotton (GM), flax(GM)		Synchrony (Du Pont)
Sethoxydim	No	Maize		(BASF)
Haloxyfop ("fop" herbicides)	Yes		Soya, maize	Verdict,(Dow), Hoegrass (Bayer), Laser (BASF)
Triazines (atrazine and simazine	No	Oilseed rape (canola), maize		Widely produced
Dicamba[4]	Yes		Soya	(Monsanto and Dow)

Another justification for developing herbicide tolerant crops is as a means of protecting growing crops from being damaged by spray drift of herbicides applied to neighbouring land:

> "*Commercial use of transgenic, 2,4-D-tolerant cotton has the potential to greatly reduce problems of 2,4-D damage in cotton from accidental spray drift and herbicide residues in spraying equipment, where plants are predominantly exposed to low rates of 2,4-D*[5]*.*"

This is a poignant example of the "need" for a technical fix to rectify the problems caused by bad management of a previous technical fix.

Herbicide tolerance and resistance in weeds

The terms herbicide tolerance and herbicide resistance need a short explanation. Herbicide tolerance is the ability of weeds *"to survive and reproduce with a herbicide treatment at a normal use rate[6]"*. Higher doses of the same herbicide may therefore kill the plant, unlike resistant individuals which can withstand much higher amounts.

There are three ways in which herbicide resistance can be acquired: natural selection: out crossing to wild crop relatives; and GM volunteers plants in following crops.

1. By the regular use of a particular herbicide leading to the natural selection of resistant individual plants which are already present in the weed population, either naturally or through new random genetic mutations, which can then multiply and eventually become dominate in the weed population if the same chemical is used long enough.

Monsanto describe this process thus:

> *"Herbicide resistance is the genetic ability of a weed to survive and reproduce after a normally lethal herbicide application. Weeds with a wide genetic diversity all have naturally occurring resistant biotypes. So, even before a herbicide has been introduced, it's possible that a resistant weed biotype lays waiting in the soil. Depending on the herbicide application practices and weed species, it may be just a matter of time before a resistant biotype germinates, survives the herbicide application and reproduces seed[7]."*

Weeds with resistance to a particular herbicide are known as 'biotypes'.

Resistance to a single herbicide with a particular mode of action may mean that the biotype can also be resistant to all the other herbicides within that herbicide group with the same mode of action (called cross resistance). Some plants may develop resistance to several groups of herbicides with different modes of action (called multiple resistance). A biotype of Rigid Ryegrass (*Lolium rigidum*) in South Australia has evolved resistance to 7 different herbicide modes of action[8].

In the UK, there are examples of where overuse of particular herbicides has resulted in a weed shift followed by resistance developing into a persistent and troublesome weeds. For example, Black Grass (*Alopecurus myosuroides)* is now a major weed in arable fields and has resistance to two herbicide modes of action. Resistant biotypes have been found on 2000 farms in 32 English counties and recently the first cases were reported in Scotland[9].

2. Out crossing to wild relatives of the crop by wind or insect borne pollination (gene transfer).

Not all crops have sexually compatible wild relatives, or relatives sufficiently nearby, with which they can cross. For instance, maize has no plant relatives in Europe, but there are plenty in Mexico (the source of maize and an area of huge genetic diversity) where out crossing to landraces of maize has been detected[10]. Oilseed rape and beet both have sexually compatible wild species in Europe. Oilseed rape has been found to cross with wild turnip, wild radish and charlock; and beet with sea beet/weed beet. Crosses have already been recorded: following UK field trials of GM oilseed rape when up to 48.5% GM seeds were detected in wild turnip seeds(*Brassica rapa)[11];* in wild radish hybridisation experiments using male sterile plants in the field yielded a low percentage of crosses[12] ; and a single case with charlock (*Arvensis sinapis*) during the Farm Scale Evaluations[13]. Crossing of GM sugar beet to weed beet has also been demonstrated[14].

3. By HT crops becoming herbicide resistant weeds in following crops.

HT seeds spilled in fields before or during harvest can germinate in following seasons to become "volunteers" in other crops which follow in the same field. The potential for long seed dormancy of oilseed rape means that GMHT volunteers can appear for at least 15 years after the crop was harvested[15].

Sugar beet, which is a biennial crop, is normally harvested after one season for its root (the beet). However a small minority of plants flower (bolt) in the first year rather than in the second and, if pollinated, they set seeds which can become "weed beet". If GM beet were allowed to flower and set seed, they would present a difficult weed control problem, especially if, over time, additional herbicide resistance genes were acquired through cross pollination. Weed beet is already a significant problem in UK sugar beet fields, where it is found in 60% of fields[16].

Field scale research in France has confirmed that resistance genes were transferred from the crop to neighbouring weed beet at 112 metres distance[17], emphasising the very real potential for resistance to spread if bolting plants are not controlled. Cross-pollination can occur over considerable distances by wind and insects (honey bees have been found to forage up to 9.5km from their hive[18]), so it is possible for HT seeds to be formed by a cross with a non-HT crop.

At present the main chemical means to control bolting beet is by a tractor dragging a glyphosate-soaked wick across the foliage of the flowering plants. If GMHT beet tolerant to Roundup were approved, this method of control could be lost and farmers would have to use other control methods, including hand pulling, which is expensive and time consuming.

Human activity, such as sharing of farm equipment between farms, can move HT seeds and produce HT volunteers in new areas where HT crops have never been grown or feral populations outside arable fields. Spillage during transport of harvested crops or seeds can also spread HT seeds widely:

> *"The intentional movement of seeds by human beings during commerce results in an essentially limitless dispersal capability. The seed handling system is 'leaky', and seed loss can occur at any point from planting to final sale. The more steps that occur during the production and post-harvest operation, the more opportunities there are for gene flow"*[19].

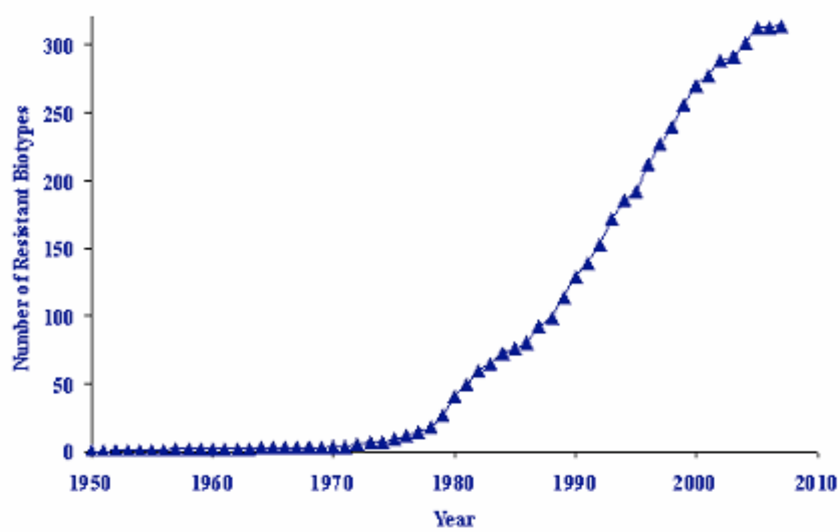
Research on oilseed rape in Canada found that volunteers acquired genes resistant to three different herbicides over several seasons by cross breeding and a failure to control volunteers – a process known as gene stacking[20].

Evidence of herbicide resistance

Weed resistance to herbicides began to increase in the mid 1970s, and since 1990 the number of biotypes exhibiting resistance has trebled[21]. At present resistant biotypes exist for herbicides with 16 different modes of action. In all, 331 biotypes covering 189 weed species (113 dicots(broad leaf) and 76 monocots(grasses /cereals) have been confirmed in over 300,000 fields[22] around the world. Every temperate and tropical continent has resistant weeds present.

Figure 1 shows the rapid escalation in herbicide resistance since the 1980s.

Figure 1: World wide chronological increase in the number of herbicide resistant weeds.



[Source: Heap, I. M. 2007[23]]

Weed resistance has been recorded in five out of eight groups of herbicides for which herbicide tolerant crops have been developed (listed in Table 1 above).

Table 2 gives details of the resistance that has developed for herbicides where GM and non-GM tolerant crops have been developed.

Table 2 Crop resistance to groups of pesticides

Herbicide	Mode of Action	Number of resistant biotypes to herbicide group	Countries where resistance to herbicide has been found	Examples of weeds showing resistance
Glyphosate	Inhibition of EPSP synthase	16	USA, Canada Argentina, Chile, S Africa, Australia, Indonesia	Johnsongrass, Rigid Rye Grass, Italian Rye Grass, Horseweed
2,4 D	Synthetic auxins (action like indoleacetic acid)	27	USA, Canada, Spain, UK, France, Hungary, Australia, New Zealand, Indonesia, Philippines, Malaysia, Thailand	Field Bindweed, Wild Carrot, Creeping thistle
Sulfonylurea	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)	101	USA, Canada, Brazil, Paraguay, Argentina, Australia, New Zealand, Japan, Sth Korea, Indonesia, UK, France, Spaon, Portugal, Sweded, Italy,Belgium, Poland , Austria, Denmark, Greece, Ireland	Black grass, Wild Oat, various Amaranths
Triazines (eg atrazine and simazine	Inhibition of photosynthesis at photosystem II	68	USA, Canada, Australia, New Zealand, Japan, China, S Africa, UK, Portugal, Spain, France, Belgium, Germany, The Netherlands, Luxemburg Italy, Greece, Poland, Czech Rep, Austria, Bulgaria, Slovenia, Yugoslavia, Israel	Fat Hen, Pineapple Mayweed, Black Grass, various Amaranths
"Fop" and "dim" herbicides (eg Haloxyfop-methyl ,diclofop-methyl and cycloxdim)	ACCase inhibitor	36	UK, Germany, France, Belgium, The Netherlands, Denmark, Turkey, Australia, S Africa,,Canada, USA, Chile, Mexico, Iran, Israel, Italy, Brazil, Costa Rica, Thailand, S Korea, Malaysia, Spain, Saudi Arabia, Tunisia, India, Ethiopia, Greece, Bolivia	Johnsongrass, Wild Oat, Black Grass, Perennial Ryegrass, Italian Ryegrass, Rigid Ryegrass,

Note: Table derived from the International Survey of Herbicide Resistant Weeds [24]

Roundup resistance

Roundup is the world's best-selling herbicide. It is a broad spectrum herbicide, capable of killing any plant. Its active ingredient is glyphosate, which was developed by Monsanto. Roundup is the brand name for Monsanto products containing glyphosate mixed with other chemicals, or adjuvants, which allow the product to stick to the leaves or other parts of the plant and help the active ingredients to enter the plant cells. Glyphosate-based herbicides are also made by other companies using different formulations, as Monsanto's patent on glyphosate expired in 2000.

Glyphosate has been claimed to be a "once-in-a-century herbicide"[25] because of its ability to kill the whole plant and because its unique mode of action[26] made weed resistance less likely:

> *"It's the best way to kill weeds the entire season, year after year. By wiping out weeds throughout the season, you'll prevent them from setting seed. And with no seeds, you minimize the risk of weed resistance.*
>
> *"Glyphosate, the active ingredient in Roundup, has a unique mode of action and makes the chances of weed resistance to Roundup agricultural herbicide less likely than other chemistries.*
>
> *"After nine years of commercial use in the United States, 12 weeds were resistant to the active ingredient in Pursuit® Herbicide, while only two weeds have been confirmed resistant to glyphosate in Roundup Ready cropping areas[27]."*

The expiry of the patent could have exposed Monsanto to serious competition from other manufacturers who could then freely produce the chemical without paying royalties and potentially undercut the company's prices. The response from Monsanto was produce GM crops with the Roundup tolerant gene marketed as "Roundup Ready" (RR). The gene comes from a soil bacterium and the GM trait is patented by Monsanto. This allows Roundup to be used on the growing crop in a way that would have been impossible 20 years ago.

The company's systematic acquisition of seed companies around the globe in the late 1990s and 2000s allowed them to develop RR varieties in soya, maize, cotton, oilseed rape, sugar beet and alfalfa. Farmers buying RR seeds are contractually obliged to buy Roundup from Monsanto. Thus they have secured a substantial part of the market for Roundup (still Monsanto's star product), excluding many other glyphosate manufacturers from around the world.

RR soya has been taken up by the largest number of farmers in North and South America, attracted by promises of cheaper and easier weed control, no till cultivation and higher yields. However, the RR trait is not a yield-enhancing gene *per se,* and a recently published review concluded:

> *"GE soybeans have not increased yields, and GE corn has increased yield only marginally on a crop-wide basis. Overall, corn and soybean yields have risen substantially over the last 15 years, but largely not as result of the GE traits. Most of the gains are due to traditional breeding or improvement of other agricultural practices[28]."*

Industry sources say that around 70% of global soya production is currently RR soya, mainly in three countries (US, Argentina and Brazil[29]). Smaller areas are grown in Canada, South Africa, Chile, Paraguay, Uruguay, Bolivia and Mexico. GMHT Oilseed rape (canola) is grown in Canada, USA and Chile. Small areas of RR sugar beet have been grown in the US and Canada.

In 2008 a quarter of maize grown globally was GM, with most with most being grown in the USA, where over half is tolerant to herbicides (Roundup or Liberty/glufosinate ammonium)[30]. GMHT maize is also grown commercially in Canada, Argentina, and Brazil, with far smaller areas in Chile, Honduras, Spain, Romania, Portugal, Czech Republic, Poland, Slovenia and Egypt. France and Germany banned cultivation of GM maize in 2008 and 2009 respectively. The practice of deliberately "stacking" traits is common in maize, where varieties often containing herbicide tolerant and insect resistant genes[31] combined by the crossbreeding of different GM lines containing different GM genes (see for example Syngenta's application for a GM maize submitted to the EU in 2009, which contains three insect resistant genes and two herbicide tolerant genes[32]).

No RR crops or other GMHT crops can be commercially grown in Europe or Asia. Applications for commercial approval of GMHT oilseed rape and beet in the EU were rejected following the large-scale Farm Scale Evaluations in the UK. These found that the management of glufosinate ammonium tolerant oilseed rape crops and glyphosate tolerant beet crops significantly reduced food supply for farmland birds by reducing the amount seeds shed from broad leafed weeds which is a vital source of food for the birds in winter[33]. India and China have so far blocked the commercial cultivation of RR crops.

Roundup remains the world's best-selling herbicide, and Monsanto has become a major player in the global seed industry. Following RR crop introductions in the USA, glyphosate use grew 15 fold in the period 1994 to 2005 and a further 28% in the following year. Similar trends have followed the introductions of RR soya in Argentina and Brazil. Roundup sales are essential for Monsanto's strength – sales were 48% of the total corporate sales in the first quarter of 2008[34].

Thus Roundup is now used extensively as the main herbicide (and often the only one) in soya, maize, oilseed rape and cotton to the point when weed resistance is a major issue in North and South America. The lag time before weed resistance and herbicide resistant volunteers first appear is long enough to have enabled GMHT seeds to become the dominant type for some crops in a few countries. However, the initial enthusiasm for the technology as means of cost effective weed control (which leads to quick adoption by farmers in the first place) is soon dampened as these resistance problems develop. Farmers are back to square one in about a decade.

Table 3 shows the weeds that have acquired Roundup resistance and an indication extent of the problem which results in older, more toxic products, such as 2,4 D and paraquat, being re- introduced.

Table 3 Weeds with acquired resistance to Roundup

Resistant weed	**Country**	**Maximum area affected acres in ha**	**Crop affected**	**Year Roundup resistance first detected**
Palmer amaranth *Amaranthus palmeri*	USA	100000-1m	Maize, cotton and soya	2005
Common water hemp *Amaranthus rudis*	USA	51-100	Corn and soya	2007
Common ragweed *Ambrosia artemisiifolia*	USA	51-100	Soya	2004
Hairy Fleabane *Conyza bonariensis*	USA, Colombia, Brazil, Spain,	1001-10000	Roadsides, fruit and orchards, maize, soya	2000
Horseweed *Conyza canadensis*	USA, Brazil, Spain, China, Czech Rep	>2m	Fruit, orchards, soya, cotton, maize, rice	2000
Sourgrass *Digitaria insularis*	Paraguay, Brazil	10000-1m	Soya	2006
Junglerice *Echinochloa colona*	Australia	11-50	Cropland	2007
Goosegrass *Eleusine indica*	Colombia Malaysia	101-500	Cropland	1997
Wild poinsettia *Euphorbia heterophylla*	Brazil	101-500	Soya	2006
Italian ryegrass *Lolium multiflorium*	Chile, brazil, USA, Spain, Argentina	1001-1000	Cropland, orchards, fruit, cotton, soya, wheat	2001
Rigid ryegrass *Lolium rigidia*	Italy, Spain, France, South Africa, Australia, USA	101-500	Orchard and vineyards, asparagus, cereals, wheat, almonds, cropland, sorghum	1996

Table 3 cont.				
Ragweed Parthenium *Parthenium hysterophorus*	Colombia	51-100	Fruit	2004
Buckhorn Plantain *Plantago lanceolata*	South Africa	11-50	Orchards and vineyards	2003
Johnsongrass *Sorghum halepense*	USA, Argentina	10001-100000	Soya	2005
Liverseedgrass *Urochloa panicoides*	Australia	6-10	Sorghum, wheat	2008

Note: Table derived from the International Survey of Herbicide Resistant Weeds[35] - figures for infestation areas show the range of areas recorded.

Table three does not include resistant crop volunteers of oilseed rape in Canada.

Twenty one other weeds in Argentina have been listed as "just barely controlled by glyphosate" and "might be the next to upgrade to full resistance by another evolutionary step"[36]. These include Field Bindweed *(Convolvulus arvensis),* Curled Dock *(Rumex crispus)* and Morning Glory *(Impomoea purpurea*)

The extent of glyphosate resistance is growing in terms of the number of biotypes and of the area infested. Alarm bells are being loudly sounded by scientists involved in weed science:

- "*Because glyphosate is the herbicide most often used in no-till and minimum-till systems, GR* [glyphoste resistant] *volunteer crop plants and glyphosate-resistant or tolerant weeds will jeopardize the sustainability of those systems"*[37].
- *"…the evolution of glyphosate-resistance in S,halepense is a major threat to glyphosate-resistant soybean productivity in northern fields of Argentina"*[38].
- *"…more worrisome are glyphosate-resistant populations of far more economically damaging weed species"*[39].

Crops modified to be tolerant to Roundup feature strongly in those affected by resistant weeds (ie, soya, maize and cotton): *"Most of the documented cases of evolved GR weeds in the past 6 years have been in GR crops"*[40]. Glyphosate (Roundup) resistant volunteers are also becoming a real problem for farmers who have to use a range of techniques to ensure the volunteers do not become a major weed problem, including cultivation and additional herbicide use (for instance applying a mixture of paraquat and diuron[41] and 2,4D and MCPA[42]). Some of the weeds resistant to glyphosate have multiple resistance making control by herbicides more difficult and increasing costs and herbicide use thus increasing the toxic load on the environment[43].

Table 4 shows the extent of multiple resistance in weeds that already have glyphosate (Roundup) resistance.

Table 4 Weeds with multiple pesticide resistance in addition to glyphosate resistance

Weed with existing glyphosate resistance	**2,4D resistance (crops and country)**	**Sulfonylurea resistance (crops and country)**	**Triazines resistance (crops and country)**	**Fop and Dim resistance**
Palmer amaranth *Amaranthus palmeri*	No	Yes (cropland USA)	Yes (corn, grain sorghum USA)	No
Common water hemp *Amaranthus rudis*	No	Yes (soya, maize cropland USA)	Yes (soya, maize, cropland grain sorghum USA soya Canada	No

Table 4 cont.				
Common ragweed *Ambrosia artemisiifolia*	No	Yes (soya USA)	Yes (maize, nurseries USA maize and cropland Canada)	No
Hairy Fleabane *Conyza bonariensis*	No	Yes (forest and industrial sites Israel)	Yes (orchards Spain)	No
Horseweed *Conyza canadensis*	No	Yes (soya, USA railways, Poland)	Yes (blueberry USA maize, orchard and roadsides Spain, cropland France, Roadsides Railways Cz Rep)	No
Sourgrass *Digitaria insularis*	No	No	No	No
Junglerice *Echinochloa colona*	Yes (rice ,Colombia)	Yes (rice , Costa Rica)	Yes (grain sorghum Australia)	No
Goosegrass *Eleusine indica*	No	Yes (industrial sites Costa Rica)	No	Yes (soya Brazil, orchards Malaysia)
Wild poinsettia *Euphorbia heterophylla*	No	Yes (Soya and maize Brazil)	No	No
Italian ryegrass *Lolium multiflorium*	No	Yes (lentils, peas, wheat, roadsides, wheat, Chile)	No	Yes (canola, lupins, wheat Chile, wheat France, Italy durum wheat, canola ,cereals and wheat UK, wheat, lentils, peas USA).
Rigid ryegrass *Lolium rigidia*	No	Yes (wheat, barley, canola in South Africa, Australia and France)	Yes (orchards and roadside Israel, cropland, pasture seed, railways, roadsides, and triazine-tolerant canola, Australia)	Yes (barley, wheat, canola, pastures and cropland Australia) Canola, cereals, lupins and wheat Chile, wheat France, wheat Greece, wheat Iran, Cereals and wheat Saudi Arabia, wheat and vineyards S Africa, wheat Spain, cereals Tunisia)
Ragweed Parthenium *Parthenium hysterophorus*	No	Yes (soya Brazil)	No	No
Buckhorn Plantain *Plantago lanceolata*	No	No	No	No

Table 4 cont.				
Johnsongrass *Sorghum halepense*	No	Yes (corn and soya USA)	No	Yes (soya, cotton,cropland USA, Cotton (Greece, Italy, Israel, tomatoes (Italy)
Liverseedgrass *Urochloa panicoides*	No	No	Yes (Cropland Australia)	No

This table is compiled using data from the International Survey of Herbicide Resistant Weeds[44]

From table 4 it is clear that some weeds resistant to glyphosate have already develop multiple resistance or have the potential to do so. Such resistance can arise naturally by selection of resistant individuals rather than through gene transfer from GMHT or non-GM HT crops. Such gene transfers, when they occur, are likely to make the problems of weed control for farmers worse.

However, recent research on the awareness of farmers to the problems of Roundup resistance in the USA suggests that many believe "it won't happen here":

> *"A survey of farmers from six U.S. states (Indiana, Illinois, Iowa, Nebraska, Mississippi, and North Carolina) was conducted to assess the farmers' views on glyphosate-resistant (GR) weeds and tactics used to prevent or manage GR weed populations in genetically engineered (GE) GR crops. Only 30% of farmers thought GR weeds were a serious issue. Few farmers thought field tillage and/or using a non-GR crop in rotation with GR crops would be an effective strategy. Most farmers did not recognize the role that the recurrent use of an herbicide plays in evolution of resistance. A substantial number of farmers underestimated the potential for GR weed populations to evolve in an agroecosystem dominated by glyphosate as the weed control tactic"[45].*

Others have confirmed that farmers do not start to manage for resistant weeds until they have appeared in their fields [46]:

> "*Part of the reason growers do not manage proactively is because most know other options are still available and expect companies to continue to provide new technology. Unfortunately, companies are not being as successful in discovering selective herbicides with new modes of action as they have been in the past"[47].*

Thus the reliability of chemical herbicides cannot be guaranteed in the future. The potency of Roundup is under threat from natural evolution of resistance especially in the areas where RR crops dominate.

Toxicity of Roundup and other herbicides

Like all herbicides, Roundup is designed to kill living tissue. Its active ingredient, glyphosate, is less toxic to mammals than some other pesticides however there is evidence showing that Roundup formations are much more toxic to mammals than glyphosate alone because of the action of other chemicals in the formulated product which allows it to stick to leaves more effectively allowing the active ingredient greater chance of penetrating the cells. Researchers concluded that the presence of adjuvants changes the permeability of human cells to Roundup and amplifies the toxicity of glyphosate
, *"…the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from R* (Roundup) *formulation-treated crops." eve*n when very dilute doses were applied to human umbilical, embryonic, and placental cells[48]. Glyphosate's healthy image has also been undermined by numerous research papers and the chemical has been linked to several serious conditions including an increase in cases on non-Hodgkin's lymphoma[49]. Roundup has also been linked to the reduction in amphibians and direct toxic effects have been demonstrated[50].

In order to facilitate the introduction of RR soya the maximum residue level for glyphosate allowed in crops for export was increased by two hundred times[51]. The use of glyphosate to desiccate cereal crops in the UK just prior to harvest produced frequent residues in grain, flour and bread. Data on residues in RR soya based products is not easy to obtain. However, soya based tofu produced using Brazilian soya (origin of raw materials unknown) and purchased in the UK contained residues[52] .

Other herbicides with resistance problems also have been shown to be toxic including 2,4D[53], atrazine[54] , chlorsulfuron (a sulphonylurea herbicide listed for reproductive effects[55]) and dicamba (also listed for reproductive effects[56]).

Strategies to avoid resistance developing

There are clear steps farmers can take to slow (but not prevent) the development of herbicide resistance in weed population in arable fields where herbicides are routinely used. These methods are well documented[57 and 58] and many are advocated for organic/agroecological farming systems where no herbicide are used:

- Vigilance and quick control of resistant populations.
- Crop rotation and avoidance of monoculture forces different weed populations and densities thus preventing the establishment of a resistant population.
- Crop breaks – fallow or temporary grazing systems.
- Hand or mechanical weeding.
- Cultivating soil to kill weed seedlings.
- Mulching using cover crop or weed residues to reduce weed populations.
- Rotations of herbicides with different modes of action.
- Using mixture of herbicides in one spay ("tank mixes").
- Using herbicides covering several modes of action.
- Using herbicides that do not persist.
- Combining control methods.

All these measures work towards reducing the selection pressure on individual resistant plants to prevent their biotype becoming dominant in the population. In the case of non-chemical interventions used in agroecological systems the issue of resistances does not arise because the selection pressure for resistance to develop is removed.

The weed control and monoculture systems adopted for GMHT crops ignore these good agricultural principles and practices despite the fact that "*farmers who practice continuous cropping, or intensive cropping, run a much greater risk of developing resistance*"[59].

The introduction of GMHT crops has meant that many farmers adopted continuous, intensive cropping relying on one herbicide with a single mode of action - exactly what they should not be doing if they wish to avoid resistance developing. A wholesale change in approach is needed away from farming systems that so deeply rely on herbicides and for herbicides to be effective. A switch is needed towards agroecological systems to substantially reduce dependence on herbicides. Research programmes across the world need to change to place far greater emphasis on the development crop management techniques over chemical interventions.

Industry solutions

The answer to the problem of weed resistance from the agri-biotech companies is two-fold – both involve the use of chemical herbicides. The first is to produce seeds with several herbicide tolerant genes (gene stacking) by crossing GMHT varieties so different herbicides can be used in rotation this avoiding dependency on one product. However, the options of which herbicides which can be stacked are already limited by the existence of multiple resistant weeds (see table 4 above).

The second approach is to use herbicides which remain active in the soil (residual herbicides or residuals) which kill seedling weeds as soon as they germinate. Again this approach has serious limitations because of weed resistance to residual herbicides and the need to avoid damaging the crop following the application of the residual herbicide.

Many residuals already have weeds resistant to them already and HT crops which tolerate them have already been developed. Thus over reliance on certain residuals already runs a serious risk of developing resistant biotypes. Residual herbicides also bring their own set of problems with crop management. Very persistent herbicides can damage following crops and long re-cropping intervals are recommended by the manufactures on labels to prevent this type of damage[60]. Crop damage occurs when residual persist in the soil at concentrations that can harm susceptible crops as they germinate. There are many factors, some of which are outside the control of farmers, which can increase the persistence of residuals in the soil and lead to unexpected crop damage. These include the soil type (organic and clay contents), pH, temperature, rainfall and moisture content all of which can impact on the rate residuals degrade in the soil through microbial activity, chemical action or photo-degradation[61]. Residuals damage to seedling crops can be confused with other factors such as nutrient deficiency or water stress meaning the problems can be missed and repeated. Farmers are faced with a difficult balancing act of ensuring that the concentrations of residuals in the soil are sufficient and available to kill off weed seedlings but low enough to protect crops seedlings. Simple errors such as overlapping passes with spraying equipment so that part of the field get too much spray can throw out calculations, even without the help of the weather.

Some residuals can also be used on herbicide tolerant crops, ie 2,4D, sulfonylureas and triazines. Thus overuse of herbicides on HT crops could generate resistance in weeds and preclude that herbicide being used as a residual backup. Multiple resistant weeds would make weed control even more complicated for farmers as they attempt to juggle different products, in different mixes to cope with resistant biotypes.

Table 5 shows the main residual herbicides with an indication of known resistance problems, HT crops tolerant to the same product and crops which may be susceptible to damage. It is clear that there is considerable overlap between herbicides used on HT crops and those used as residuals.

Table 5 Common Residual herbicides and some factors which limit their effectiveness in controlling herbicide resistant weeds

Residual herbicide (Mode of action)	Residuals half-life in soil – known range	Herbicide tolerant crops for same herbicide	Number of known resistant biotypes	Examples of main crops susceptible to residual
Sulfonylureas (ALS inhibitors)	Less than 1 yr to 2years (if pH >7)	Yes	101	Legumes ,oilseeds and some barley
Sulphonamides (ALS inhibitors)	2 weeks to 4 months (less persistent in soils pH>7)	Yes	Ditto	No data found
Imidazolinones (ALS inhibitors)	3-12 months	Yes	Ditto	Oilseeds
Triazines (Photosystem II inhibitors)	2-18 months	Yes	68	Cereals
Trifluralin (Microtubule assembly inhibition)	3-6 months	No	10	Wheat, oats lentils
Isoxazoles (Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase)	10-39 days	No	None	Legumes and pastures
Metolachlor (Inhibition of very long chain fatty acids)	2-12months plus	No	3	Oilseeds rape (canola)
Phenoxys (synthetic auxins)	< 1 month to 2 years	Yes	27	Autumn crops, oilseed s and legumes

Note: this table was compiled from the following sources Bayer[62], Lehmann et al[63], Rouchaud et al[64] and the International Survey of Herbicide Resistant Weeds [65]

The value placed on residual herbicides in maintaining the viability of RR soya and cotton crops faced with glyphosate resistant Palmer Amaranth (*Amaranthus palmeri*) was set out very clearly by Dr Ken Smith of the Southeast Research and Extension Center, Monticello, University of Arkansas in an on-line video[66]:

> *"..we can no longer farm like we have in the past. Weed control will never be as easy as it was in 2003. Now soil residuals both in soya beans and cotton are going to be essential for controlling this pest. In cotton, we can never allow it to germinate. We must keep a soil residual herbicide in place*

and active at all times. If it ever germinates, we have no products that we can come over the top, of small cotton particularly, and take out glyphosate resistant Palmer Armaranth".

Palmer Amaranth resistant to glyphosate is already present in six states in the USA. In particular, in Tennessee where it infests between 100000 and one million acres of maize, cotton and soya[67]. The options for residual herbicides are already restricted because resistant biotypes have already been confirmed which resist three different herbicide modes of action in addition to glyphosate, including multiple resistance to Glyphosate and pyrithiobac-Na in Mississippi[68].

The dual approach of gene stacking and residual herbicides will only serve to deepen chemical dependency in arable farming. Recent experience in North and South America suggest that chemical- based prevention strategies only need to breakdown on a few fields to allow resistant biotypes to spread. The dual approach just postpones the day when alternative approaches will have to be adopted whilst also adding to the toxic burden on the environment.

RR Crops and resistance: Two case studies

Johnsongrass (*Sorghum halepense)*
Johnsongrass is "one of the world's worst weeds[69]". The plant originated in the Mediterranean region and was widely introduced as a forage crop to North and South America, from early in the 19th and 20th centuries, respectively. In both continents it was soon identified as a highly invasive weed spread by rhizomes and seeds. It reaches over 2 metres in height and forms dense clumps in crops. It was banned from agriculture in Argentina in 1936[70], and 19 states in the USA classify it as a "noxious" weed[71].

The introduction of GMHT soya in Argentina in 1996 enabled farmers to get control over Johnsongrass for the first time since it escaped into arable land and established itself as a weed. One farmer reported, ''Glyphosate becomes the essential tool for fallow-land and soybean cultivation in 1996. Johnsongrass practically disappeared from the rolling Pampas, except from patches on uncultivated land. . .but none in agricultural land"[72].

Argentinian glyphosate consumption has rocketed in the past two decades, from 1 million litres in 1991 to 18 million litres in 2007[73]. It is largely used on the RR soya crops, which occupied 16 million ha in 2007 producing 48 million tonnes of soya, nearly all of which was exported for animal feed, vegetable oil and biodiesel.

This mode of production was the ideal recipe for weed resistance to develop, and the first reports of a Johnsongrass biotype with resistance to Roundup (GR) came in 2003. However Monsanto only confirmed resistance in 2005. This delay may have been critical in allowing the resistant biotype to become established in soya fields. Farmers who raised concerns in the Las Lijatas area were re-assured :

> *"Little attention was paid to the uncontrolled lumps and the farmer complained that no effective action was taken because they were mislead by technical advisors who stated that the situation was not problematic. This year, upset by the increasing severity of the resistance problem, he decided to speak up and talk to the local press to raise awareness and speed up proper action"[74].*

By 2007, all Argentinian provinces growing soya were infested with GR Johnsongrass and it covered 10,000 ha in Northern Argentina, although throughout the country the area may be as high as 100,000ha[75].

The first case of Roundup resistance in Johnsongrass in the USA was reported in 2007 in a soya bean crop in Arkansas.

In Argentina, control measures have mainly been additional spraying of other herbicides in tank mixes of glyphosate with MSMA, 2,4-D, cletodim or haloxifop, post-emergence graminicides (eg, Micosulfuron, Imazethapir) or for use in fallow fields (eg, atrazine, paraquat, 2,4-D, metsulfuron metil). A tank mix involving 2,4D and glyphosate has been reported to increase production costs for soya by 19.3%[76]per hectare. Other commentators believe herbicide cost may double in the wake of Johnsongrass resistance developing.

Exposure of the rural population to aerial spraying of herbicides is a major health concern, particularly in the light of recent research[77] highlighting how much more toxic Roundup is (with its mixture of wetting agents which help the product stick to plants and penetrate plant tissue) than glyphosate alone.

The rapid development of Johnsongrass resistance in Argentina is a fine example of how over-reliance on a single herbicide accelerates the development of resistance. Johnsongrass has already developed resistance to three other herbicide groups in the USA[78]. Thus it is at the forefront of the "pesticides arms race".

Horseweed (*Conyza canadensis*)
Horseweed is a member of the Daisy family that spreads via wind-blown seeds produced in large numbers (up to 2,000,000 per plant). It is a native of North America. It overwinters as a seedling and bolts in the spring to flower in July when the plant reach 1.7m in height. Infestation of soya crops with Horseweed can reduce the yield by 83% if untreated[79]. Globally horseweed is a major resistance problem, with the presence of 41 biotypes and resistance to five groups of herbicides. The earliest recorded resistance was to paraquat in Japan in 1980. Since then resistant plants have been found in Europe, North America, South America, China and Israel. Twenty-three biotypes with Roundup resistance have been found. Four cases of multiple-resistance have been recorded[80], two of these cases include glyphosate. Horseweed has already shown resistance to three herbicide groups for which HT crops have been developed. In Tennessee, over 2 million acres of glyphosate resistant Horseweed have been recorded on over 500 farms.

US weed scientists have suggested that the rapid spread of glyphosate resistance in Horseweed can be traced to the use of no tillage in RR crops:

> "*No-tillage corn (Zea mays L.) and soybean [Glycine max (L.) Merr.] production has been widely accepted in the mid-Atlantic region, favoring establishment of horseweed [Conyza canadensis (L.) Cronq.]. Within 3 yr of using only glyphosate for weed control in continuous glyphosate-resistant soybeans, glyphosate failed to control horseweed in some fields. Seedlings originating from seed of one population collected in Delaware were grown in the greenhouse and exhibited 8- to 13-fold glyphosate resistance compared with a susceptible population[81].*"

Conclusions

Herbicide resistance is a global problem and is accelerating – brought about by over-reliance on chemical weed control and poor management. The development of GMHT crops resulted in good weed control in the early years after their introduction in 1996, but the farming techniques employed by farmers (monocultures, zero tillage and almost total dependence on Roundup for weed control) have provided an ideal breeding ground for resistance to develop.

The promises of the agri-biotech companies that GMHT would mean simpler weed control and cost savings look difficult to justify as resistant weeds and volunteers are developing quickly under GMHT crop management. The solution from industry is to use different herbicides and staked herbicides tolerant genes in crops and residual soil-acting herbicides. Solution that will increase the toxic burden on the environment and that requires new herbicidal chemicals to be discovered regularly. Indeed without additional chemical herbicides being developed, farmers may have to adopt quite different approaches to weed control including crop rotation, crop breaks and mechanical weed control. The problem facing farmers is neatly summed in this quote:

> *"The problem with this attitude is that no new herbicides with novel mechanisms of action are in the latter stages of development, and no herbicides with new modes of action have been released since 1990. Because development time of a new pesticide is at least 11 yr and the cost is greater than $190 million, it is unlikely that herbicides with new modes of action will become available to farmers in the next 5 yr or longer[82]."*

The GMHT model of farming has proven to be another leg of the "pesticides arms race" that began in the 1950s when the first weeds resistant to 2.4D were discovered. The model does not fit into the agroecological vision of multifunctional farming set out in the report of the *International Assessment of Agricultural Knowledge, Science and Technology for Development*[83], which saw no long-term future for farming systems reliant on artificial inputs based on fossil-fuels. Governments around the world need to recognise the fact that the "pesticide arms race", like other arms races, is not ultimately winnable, and they therefore need to invest in research and development of alternative weed control techniques based on sound agroecological principles of crop rotation, crops breaks, weed suppression and mechanical weed control methods. In the absence of a lead by Governments and scientists in this transition, farmers need to act themselves, including the recovery of previously used weed control methods that have been abandoned since the advent of the GMHT model. Otherwise they risk being overwhelmed by the weed resistance problem in the next 20 to 30 years.

Notes

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