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Glyphosate-resistant crops: history, status and future†

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Abstract: The commercial launch of glyphosate-resistant soybeans in 1996 signaled the beginning of a new era in weed management in row crops. Today, over 80% of the soybeans grown in the USA are glyphosate resistant. Since that time, many crops have been transformed that have allowed crop applications of many classes of herbicide chemistries. Crops currently under production include maize, soybean, cotton and canola. Transformation technology and selection methods have improved and the rate of development as well as the breadth of crops being considered as commercial targets has increased. On the basis of recent adoption rates by growers around the world, it appears that glyphosate-resistant crops will continue to grow in number and in hectares planted. However, global public acceptance of biotechnology-derived products will continue to impact the rate of adoption of this and other new innovations derived from transformation technology.

Keywords: glyphosate; glyphosate-resistant crop; EPSPS; CP4; herbicide-resistant crop

1 INTRODUCTION

Glyphosate is the herbicidal active ingredient in Roundup® and many other herbicide brands that control a broad spectrum of plant species. Its mechanism of action targets an enzyme found only in plants and certain bacteria. Because of this, it has an excellent toxicological and environmental profile.[1] However, it also destroys crop species on application, and thus its traditional use has been in non-crop and orchard production systems. The ability to transform plants using molecular biology has allowed the transfer of a glyphosate-insensitive gene into crop species, so allowing glyphosate use 'in crop' and signaling a new era in weed management. Today, glyphosate resistance has been introduced into several major crop species and is being grown on an increasing number of hectares of soybean, maize, canola and cotton. This paper will describe some of the historical events leading to the introduction of glyphosate-resistant crops, the status of this technology globally and provide a speculative discussion of its future use.

2 History of glyphosate resistance

The history of the development of glyphosate-resistant crops has been reviewed previously.[1,2] Both publications provide an excellent review of the literature as well as insight into the different approaches undertaken to achieve resistance to glyphosate in crop plants. The present discussion will briefly summarize and update the findings described in these reviews. There are three basic strategies that have been evaluated in order to introduce glyphosate resistance into crop species: over-expression of the sensitive target enzyme, detoxification of the glyphosate molecule and expression of an insensitive form of the target enzyme.

Before discussing approaches to attaining glyphosate resistance, a brief discussion of glyphosate's herbicidal mechanism of action is appropriate. 5-Enylpyruvylshikimate-3-phosphate synthase (EPSPS) is the enzyme inhibited by glyphosate.[3] The enzyme catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to shikimate-3-phosphate (S3P). This is a key step in the synthesis of aromatic amino acids and, ultimately, hormones and other critical plant metabolites, including flavonoids, lignins and other phenolic compounds. The active site of the EPSPS enzyme in higher plants is very highly conserved.[2] The mechanism of inhibition is also unique in that the binding site for glyphosate is reported to overlap closely with the binding site of PEP.[1] Glyphosate is competitive with respect to PEP binding to EPSPS but uncompetitive with respect to S3P and the resulting glyphosate:EPSPS:S3P complex is very stable, has a very slow reversal rate and serves essentially as a 'dead-end' for EPSPS. A diagram of the

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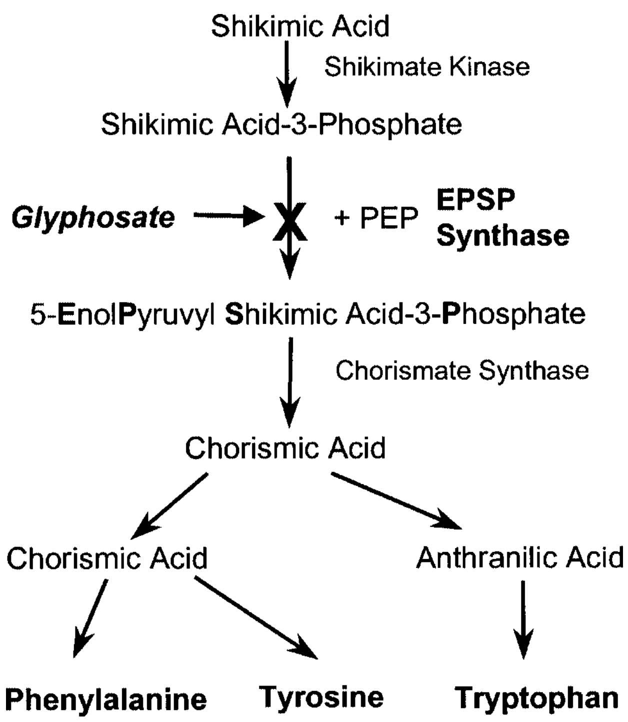

Figure 1. Glyphosate mode of action.

shikimate pathway and glyphosate's inhibition point is shown in Fig 1.

The strategy of over-expressing the EPSPS protein in the hope of overcoming the herbicidal effects of glyphosate has been attempted in both cell and whole plant systems. Amplification of the endogenous EPSPS gene has been accomplished in cell culture of several species including *Aerobacter aerogenes* Biejer, *Daucus carota* L and *Nicotiniana tobacum* L. EPSPS activity that has been elevated up to 800-fold has been shown in *Nicotiniana* cell culture.[4] However, glyphosate-resistant whole plants have not been generated from any of these cell lines to date. The alternative approach of genetically engineering over-expression of native EPSPS in a variety of systems has also been attempted with limited success. Petunia plants were generated that could withstand a fourfold dose of glyphosate.[5] However, these plants also exhibited significantly reduced growth rates compared to wild-type. No glyphosate-resistant crop species are marketed today using over-expression of native EPSPS as the mechanism of resistance.

Detoxification of the glyphosate molecule is also a strategy that has been employed to confer glyphosate resistance. Glyphosate detoxification has been demonstrated via two routes, one resulting in the formation of phosphate and sarcosine, while the other results in the formation of aminomethylphosphonic acid (AMPA) and glyoxylate, and is referred to as glyphosate oxidase (GOX).[2] As stated by previous authors, neither of these mechanisms has been shown to occur in higher plants to a significant degree. While GOX is employed in glyphosate-resistant canola, it is used in combination with a glyphosate-insensitive EPSPS. This approach was necessary, as using the detoxification mechanism alone provided insufficient resistance to glyphosate in commercial applications.

The method that resulted in commercial glyphosate resistance and is marketed in multiple crops under the Roundup Ready® brand was the introduction of an insensitive EPSPS. The strategy employed in the development of these crops is shown in Fig 2. Several approaches to attaining this result have been tried. Treating *Arabidopsis thaliana* Heynhoe with ethanemethosulfate was attempted by several laboratories without generating a glyphosate-resistant mutant.[6] Extensive functional mutagenesis of bacterial and plant EPSPS enzymes has also failed to produce a commercially resistant EPSPS. In fact, some studies have indicated that the level of resistance afforded by single-point mutations in the EPSPS molecule would be unlikely to produce commercially acceptable levels of glyphosate resistance.[7] Because of the close overlap of the binding sites of PEP and glyphosate on the EPSPS enzyme, and the highly conserved sequence found in that binding domain, obtaining altered target sites that will bind PEP, exclude glyphosate, result in commercial levels of glyphosate resistance and result in plants that develop normally has been difficult. Kinetic data for a select group of EPSPS enzymes are shown in Table 1.[2] The data show that the two single-point mutations substituting glycine with alanine at

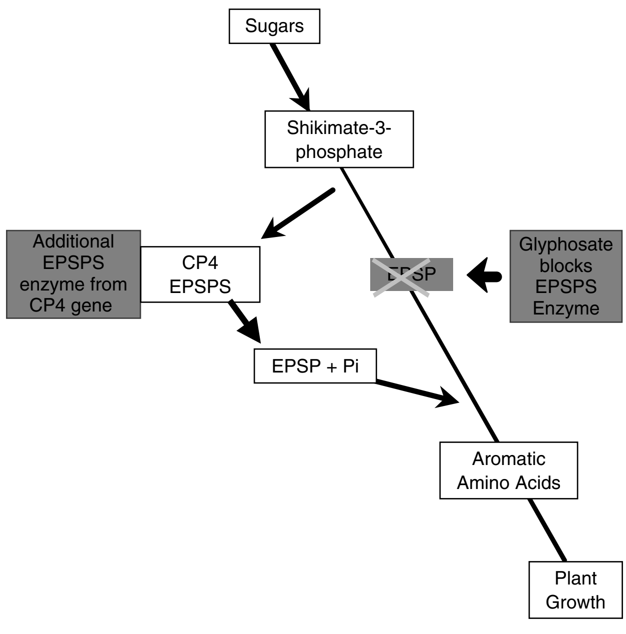

Figure 2. Strategy for the development of glyphosate-resistant crops.

Table 1. Kinetic properties for selected EPSPS enzymes

Enzyme source	K_m (PEP) (μM)	K_i (glyphosate) (μM)	K_i/K_m
Petunia (wild type)	5.0	0.4	0.08
Theoretical Ideal	<15	~1500	100
G101A	210	2000	9.5
T102I/P106S	10.6	58	5.5
P106S	17	1	0.06
Agrobacterium sp CP4	12	2720	227

position 101 (G101A) or substituting proline with serine at position 106 (P106S) have enzyme kinetics that do not meet the theoretical ideal, and result in commercially unacceptable levels of glyphosate resistance in plants. Only a single multiple missense mutation in endogenous maize EPSPS has been utilized to date to generate commercial glyphosate resistance. The mutation was generated via site directed mutagenesis of a maize cell line.[8] This variant of maize EPSPS is a transgene with substitution of threonine at position 102 with isoleucine and substitution of proline at position 106 with serine that is presently sold commercially in some maize hybrids and known as GA21.[9] The vast majority of the commercial glyphosate-resistant products on the market today contain the bacterial EPSPS known as CP4. The CP4 enzyme was isolated from *Agrobacterium* sp and is insensitive to glyphosate (Table 1). The substrate and glyphosate binding region of CP4-EPSPS is identical to the substrate and glyphosate binding region of sensitive EPSPS found in most plant species. The CP4-EPSPS protein overall is 50.1% similar and only 23.3% identical to native maize EPSPS. This suggests that binding of glyphosate is excluded by conformational changes resulting from those amino acid sequence changes outside the glyphosate/PEP binding region. As shown in Table 1, CP4-EPSPS combines a high affinity for PEP coupled with a very high tolerance for glyphosate. The result is an ability to 'bypass' the endogenous EPSPS system with the CP4-EPEPS insertion that allows the shikimate pathway to function normally (Fig 2). The CP4-EPSPS enzyme is employed in nearly all glyphosate resistant crops currently sold.

3 STATUS

Glyphosate-resistant soybean was the first crop launched and marketed under the Roundup Ready brand in the USA in 1996. Since introduction, herbicide-resistant soybeans, the overwhelming majority of which are glyphosate resistant, have been adopted at a very rapid pace. In 2004 85% of all soybeans grown in the USA were herbicide resistant as well as 60% of all cotton and 18% of all maize.[10] Table 2 shows the total hectares of herbicide-resistant crops planted globally in 2003 and the percentage of total crop plantings that this represents.[11] In 2003, 98% of all soybean plantings in Argentina were glyphosate resistant and estimates indicate up to 3 million ha of the same technology were planted in Brazil.[11] Globally in 2003, herbicide-resistant soybeans occupied >41 million ha and represented 61% of all transgenic crop plantings in that year.[11] Other herbicide-resistant crops include maize, cotton and canola that were planted on 5, 4 and 2.5 million ha respectively in 2003. Again, the vast majority of these plantings were glyphosate resistant. Adoption rates of glyphosate-resistant crops since introduction are depicted in Fig 3. Adoption in cotton and canola has been rapid as in soybeans.

Table 2. Hectares of herbicide-resistant crops planted globally in 2003 and the percentage of total crop plantings represented

Crop	Million hectares	% of transgenic crop plantings
Herbicide-resistant soybeans	41.4	61
Herbicide-resistant maize	6.4	10
Herbicide-resistant cotton	4.1	6
Herbicide-resistant canola	3.6	5

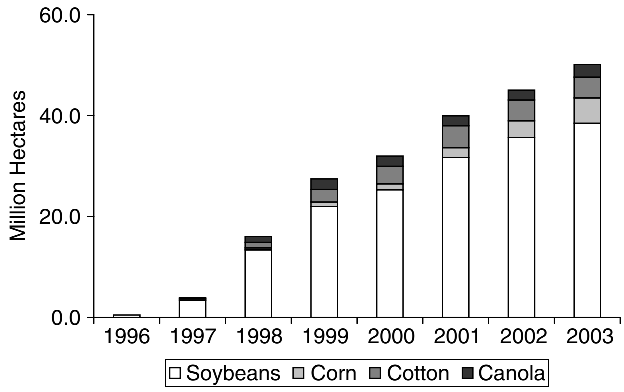

Figure 3. Global adoption rates of glyphosate-resistant crops since introduction.

Economics, availability of alternative weed-control options and availability of export markets all play a significant role in the rate extension of herbicide-resistant technology. Economics and convenience were likely more important in determining the rate of adoption of glyphosate-resistant soybeans, while superior weed control compared with existing alternative technology likely had a larger impact in cotton and canola. Several competitive and economical weed-control alternatives are available to maize growers, which may account for the slower adoption rate than for soybeans, cotton and canola. It is possible that the recent approval of glyphosate-resistant maize for import into the EU will accelerate the adoption of glyphosate-resistant maize.

The rapid adoption rates of these technologies were driven by several factors. Economic benefits, production efficiency and flexibility, and facilitation of conservation tillage have been cited as reasons for the success of glyphosate-resistant crops in the market place. Market research data have shown that weed-management costs have been reduced by up to $10 per acre when comparing 1996 with 2002 weed-control costs in soybeans (Fig 4). These data represent constant dollars and account only for seed and weed-control chemistry costs, and do not account for potential savings in production practices. Adoption of conservation tillage results in less fuel burned with fewer tillage trips with lower horse-power requirements, less capital equipment required, reduction in top soil loss, improved water use efficiency and improved organic matter content.[12] While precise

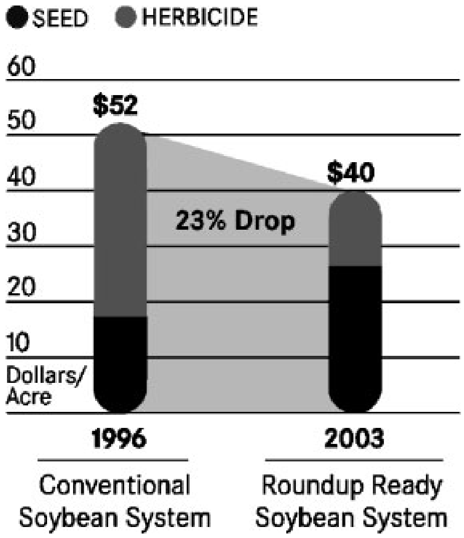

Figure 4. Since Roundup Ready Soybeans were introduced in 1996, total weed control input costs have dropped over $10 per acre. Source: Doane Market Research, 452 farmers in 19 states.

Table 3. Global area of transgenic crops planted in 2003 by country

Country	Million hectares	%
USA	42.8	63
Argentina	13.9	21
Canada	4.4	6
Brazil	3.0	4
China	2.8	4
South Africa	0.4	1
Australia	0.1	<1
India	0.1	<1
Romania	<0.1	<1
Uruguay	<0.1	<1
Spain	<0.1	<1
Mexico	<0.1	<1
Philippines	<0.1	<1
Colombia	<0.1	<1
Bulgaria	<0.1	<1
Honduras	<0.1	<1
Germany	<0.1	<1
Indonesia	<0.1	<1
Total	67.7	100

estimates of cost are difficult due to the range of production practices that exist, it has been estimated that complete conversion to no-till can save as much as 53 liter ha^{-1} in fuel alone, depending on the number of trips eliminated from production practices.[12] This saving, combined with labor and equipment, can lead to significant savings per hectare when conservation tillage practices are adopted. One other observation worth mention is the trend in altered row spacing. The use of glyphosate as an 'in-crop' broad-spectrum herbicide in soybeans can eliminate the need for cultivation for weed control. This allows farmers to reduce row spacing when planting soybeans from 76 to 33 cm and less. The closer row spacing results in faster canopy closure by the crop, effectively providing an earlier crop canopy which provides added competition to weeds. In other words, the crop is more competitive with weed species in the field and helps provide its own weed control.

Although the bulk of the adoption of transgenic crops has been in industrialized countries, growth has begun in less developed countries. Table 3 shows current global plantings of transgenic crops, the bulk of which are herbicide resistant.[11] The growth in area planted of all transgenic crops between 2002 and 2003 was almost the same in developing countries (4.4 million ha) as in industrialized nations (4.6 million ha).[11]

4 FUTURE

Several factors will determine the future adoption rates of current and new glyphosate-resistant crops that may be introduced in the coming years. While this discussion is speculative, it is based in experience with several successful glyphosate-resistant crop introductions and 30 years experience with the glyphosate herbicide molecule. It is likely that the major factors influencing this technology will include commodity pricing and grower economics, regulatory requirements, acceptance by grower groups and the general public, and the agronomic performance of the glyphosate-resistant system in individual cropping situations.

The economics around commodity prices will continue to drive grower decisions about which crops are to be planted. As has been the case with glyphosate-resistant soybeans, should production and weed control costs be competitive with other available technologies, ease of use and flexibility in timing of weed management will likely drive adoption of glyphosate-resistant crops. As patents on glyphosate (the herbicide molecule itself) expire, generic glyphosate products will continue to put downward pressure on the price of glyphosate. This reduction in price will also drive the adoption of glyphosate-resistant crops.

Current regulatory requirements dictate that the time interval between gene discovery and product launch for transgenic products can range between 8 to 10 years and cost from *ca* $50 to $100 million dollars (Monsanto data). Given the commitment necessary to commercialize these products by companies, it is not surprising that commodity industry and public acceptance are keys to successful and timely technology introductions. A case in point is glyphosate-resistant sugarbeet. This product, after meeting all regulatory requirements for product safety and quality, is currently registered with the USDA and EPA, offers growers a superior weed-control system as well as a more economical weed-management system than currently exists in the market. However, the manufacturers who use the refined sugar product for packaged food goods are unwilling to chance potential consumer non-acceptance of the resulting food product, and the technology remains unused.

A second example would be glyphosate-resistant wheat. In May 2004, Monsanto announced that it would defer further efforts to introduce glyphosate-resistant spring wheat. Working with an industry advisory committee composed of growers, grain handlers, millers, bakers, food companies and other experts involved in wheat or its resulting food products, six commercial milestones were identified that should be met before glyphosate-resistant wheat could be introduced. The milestones included regulatory approvals in the USA, Canada and Japan; approvals or marketing arrangements in place in major export markets; grain handling, sampling and detection methods implemented; comprehensive stewardship programs in place; quality varieties that meet end-use need; and buyers identified. While these milestones have been met for traits in other crops (ie glyphosate-resistant maize), given the percentage of the industry preferring to wait on the introduction of glyphosate resistance and near-term opportunities elsewhere, Monsanto made the decision to defer development of glyphosate-resistant spring wheat.

The potential for rejection of products derived from biotechnology in the EU and other countries has been one of the underlying factors in the reluctance of growers, millers, grain handlers and others to hesitate in the adoption of these technologies. However, to date no scientific advisory panel in any country evaluating the safety of these products has given any concern as to the safety of biotechnology derived products. Glyphosate-resistant soybeans and maize as well as insect-protected maize are approved and imported into the EU. It should also be noted that transgenic crops are grown in both Spain and Germany (Table 3). While it is unfortunate that opinion and politics rather than scientific data can impact the availability of technology to growers, application of the best scientific knowledge and methods in evaluating new technology remain the means to advance the science of biotechnology in agriculture.

Agronomic performance of glyphosate-resistant crops to date has been excellent as evidenced by adoption across large hectares over a relatively short period. Increasing adoption and use of glyphosate in cropping systems has led to speculation around the durability of the system and that resistant weed populations could make glyphosate-resistant crops less attractive with time. Thirty years of experience with the molecule, coupled with its mechanism of action and other chemical properties, indicate that development of weeds resistant to glyphosate has been and will continue to be slower than other chemical classes.[13] To date, biotypes of six species have been reported resistant to glyphosate.[14] *Lolium rigidum* (Gaud) was the first species reported to be resistant to glyphosate in Australia.[15,16] Although much research has been done on this biotype, the exact mechanism of resistance has yet to be defined.[17,18] It has been hypothesized that interference with the transport of glyphosate to the target site could be involved in the resistance mechanism.[17] Resistance to glyphosate in *Eleusine indica* (L) Gaertner was reported to be due to a mutation in the EPSPS target site at position 106-proline to serine.[19] Mechanistic studies in *Conyza bonariensis* (L) Cronq, *Lolium multiflorum* Lam and *Plantago lanceolata* L have not been reported to date.

Glyphosate-resistant *Conyza canadensis* (L) Cronq was reported in the USA, and has been labeled the first weed to evolve resistance in a glyphosate-resistant crop (soybeans).[20] However, as *Conyza* is largely treated prior to planting, it should be noted that exposure to glyphosate began before introduction of glyphosate-resistant soybeans, and attributing this resistance solely to the introduction of glyphosate-resistant soybeans is not completely accurate. Growers now manage this weed through the combination of glyphosate and auxinic herbicides prior to planting glyphosate-resistant soybeans. Control of glyphosate-resistant *C canadensis* in glyphosate-resistant soybeans is achieved utilizing glyphosate in combination with ALS inhibitors. While tillage and rotation away from glyphosate-resistant soybeans are options that growers can choose, no drop in acres planted to glyphosate-resistant soybeans has been observed.[10] Recent reports suggest that translocation of glyphosate in the resistant *Conyza* biotype was reduced and that this could be contributing to the observed resistance.[21] Based on observations in *L rigidum* and *C canadensis*, the authors suggest that, while reduced translocation is not a common mechanism of weed resistance, it might be a common mechanism in glyphosate-resistant weeds.

As the use of glyphosate increases, the number of occurrences of glyphosate-resistant weeds will increase. In the event that weed resistance to glyphosate does develop, it is likely farmers will continue to use glyphosate-resistant cropping systems with added control measures for resistant species. Difficult-to-control weeds or situations where production practices dictate alternative weed-control measures will also require additions to glyphosate as a weed-control tool. Future weed-management strategies in glyphosate-resistant crops will include control of weeds resistant to other chemistries as well as management of glyphosate-resistant weeds in the event that glyphosate resistance is encountered. Generally, glyphosate alone, in tank mix or sequence with other chemistries or rotations with cropping systems which employ other weed-management systems will all be used as dictated on a local basis by grower needs. In addition, growers will supplement glyphosate with other chemistries based on weed-management needs, farm size and economics.

In summary, glyphosate resistance has been sold commercially in many crops since 1996 and is the major weed-management system employed in soybeans, maize, cotton and canola globally. Indications are that glyphosate-resistant crop plantings will continue to increase. The system has shown broad adaptability to manage general weed control, tough weed-control situations and weed resistance that has evolved to other chemistries. The flexibility and economic benefits of the system will continue to drive adoption and likely result in glyphosate resistance becoming the base element in the weed-management strategies of the major grain and fiber cropping systems grown globally.

REFERENCES

1 Franz JE, Mao MK and Sikorski JA, *Glyphosate: a unique global herbicide*, ACS Monograph No 189, American Chemical Society, Washington, DC, pp 617–642 (1997).

2 Cajacob CA, Feng PC, Heck GR, Murtaza FA, Sammons RD and Padgette SR, Engineering resistance to herbicides, in *Handbook of biotechnology*, ed by Christou P and Klee H, Chapter 1, John Wiley & Sons, Chichester, UK, pp 353–372 (2004).

3 Amrhein N, Deus B, Gehrke P and Steinrucken HC, *Plant Physiol* **66**:830–834 (1980).

4 Widholm JM, Chinnala AR, Ryu JH, Song HS, Egett T and Brotherton JE, Glyphosate selection of gene amplification in suspension cultures of three plant species. *Physiol Plant* **112**:540–545 (2001).

5 Shah DM, Horsch RB, Klee HJ, Kishore GM, Winter JA, Tumer NE, Hironaka CM, Sanders PR, Gasser CS, Ayken S, Siegel NR, Rogers SG and Fraley RT. Engineering herbicide tolerance in transgenic plants. *Science (Washington)* **233**:478–481 (1986).

6 Jandern G, Baerson SR, Hudak JA, Gonzalez KA, Gruys KJ and Last RL, Ethanemethosulfate saturation mutagenesis in *Arabidopsis* to determine frequency of herbicide resistance. *Plant Physiol* **131**:139–146 (2003).

7 Padgette SR, Re DB, Gasser CS, Eicholtz DA, Frazier RB, Hironaka CM, Levine EB, Shah DM, Fraley RT and Kishore GM, Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimatephoshphate synthase active site. *J Biol Chem* **266**:22 364–22 369 (1991).

8 Lebrun M, Sailland A, Freyssinet G and Degryse E, Mutated 5-enolpyruvylshikimate-3-phosphate synthase, gene coding for said protein and transformed plants containing said gene, US patent 6 566 587 (2003).

9 Sidhu RS, Hammond BJ, Fuchs RL, Mutz JN, Holden LR, George B and Olsen T, Glyphosate tolerant corn: the composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays*). *J Agric Food Chem* **48**:2305–2312 (2000).

10 Economic Research Service, Adoption of genetically engineered crops in the US, USDA, Washington, DC, can be found at http://www.ers.usda.gov/Data/BiotechCrops/.

11 James C, ISSA Briefs: Preview, Global Status of Commercialized Transgenic Crops, in *ISAA Briefs* **30,** ISAA, Ithica, NY (2003).

12 Fawcett R and Towery D, Conservation tillage and plant biotechnology: how new technologies can improve the environment by reducing the need to plow, can be found at the Conservation Technology Information Center, http://www.ctic.purdue.edu/CTIC/BiotechPaper.pdf.

13 Benbrook C, Racing against the clock. *Agrichem Age* **35**:30–33 (1991).

14 Heap I, International Survey of Herbicide Resistant Weeds, www.weedscience.org/in.asp (2004).

15 Pratley J, Urwin N, Stanton R, Baines P, Broster K, Cullis D, Schafer D, Bohn J and Krueger R, Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. *Weed Sci* **47**:405–411 (1999).

16 Powles S, Lorraine-Colwill D, Dellow J and Preston C, Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci* **46**:604–607.

17 Loraine-Colwill D, Powles S, Hawkes T, Hollinshed P, Warner S and Preston C, Investigations into the mechanism of glyphosate resistance in *Lolium rigidum*. *Pestic Biochem Physiol* **74**:62–72 (2003).

18 Baerson S, Rodriguez D, Biest N, Tran M, You J, Krueger R, Dill G, Pratley J and Gruys K, Investigating the mechanism of glyphosate resistance in rigid ryegrass (*Lolium rigidum*) *Weed Sci* **50**:721–730 (2002).

19 Baerson S, Rodriguez D, Tran M, Feng Y, Biest N and Dill G, Glyphosate resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Plant Physiol* **129**:1265–1275 (2002).

20 VanGessel M, Glyphosate-resistant horseweed from Delaware. *Weed Sci* **49**:705–703 (2001).

21 Feng P, Tran M, Chiu T, Sammons R, Heck G and Cajacob C, Investigations into glyphosate-resistant horseweed (*Conyza canadensis*): retention, uptake, translocation and metabolism. *Weed Sci* **52**:498–505 (2004).