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Transgenic Maize

Transgenic maize for commercial production currently confers either insect resistance or herbicide tolerance or a combination of these traits. The introduction of transgenic maize has resulted in an increase in maize production. Effects of these transgenic plants on non-target insects, soil, and animals consuming them have been studied, and in general these effects are small. The economic impact of transgenic maize into the global market has been tremendous because maize can no longer be marketed as a simple commodity. Identity preservation and tracking systems are now required to ensure that maize meets the tolerance levels set by different countries for content of transgenic maize.

Keywords: Bt corn; Herbicide tolerant corn; Biotechnology

1 Introduction

Maize is an important commodity with a global market. Eighty percent of the starch produced in the world is derived from maize. Forty percent of the maize produced in the U.S. is transgenic, and this percentage is predicted to increase both in the U.S. and in other countries. The development of transgenic maize and the introduction of this product into the global market have made a tremendous impact on maize production, transport and marketing procedures. Because different countries have different regulations about production and importation of transgenic maize, segregation of commodity maize into lots with known content of transgenic grain has become necessary. Identity preservation and tracking transgenic grain is a tremendous undertaking considering that more than 700,000,000 mt of maize were produced globally in 2004 [1].

The topic of transgenic maize is very broad, and cannot be addressed comprehensively in this review. We provide an overview of the aspects of transgenic maize that we think are the most important for scientists not directly involved with maize breeding and production to understand. Thus, we will summarize how transgenic maize is produced and discuss the transgenic maize products currently on the market. We will next describe comparisons between transgenic plants and their non-transgenic counterparts. Finally we will present some of the issues that the introduction of transgenic maize to the world market has caused.

2 How Transgenic Maize is Produced

Transgenes are introduced into the maize genome through a process called transformation. Several methods for transformation of maize have been developed, including microprojectile bombardment [2], whiskers-mediated transformation [3], and *Agrobacterium tumefaciens* -mediated transformation [4, 5]. These methods all involve introduction of foreign DNA including a selectable marker gene into the nucleus of cultured maize callus cells. The selectable marker gene is frequently a herbicide resistance gene. In addition to the selectable marker gene, the foreign DNA can include genes that confer other traits of interest. Transgenes may include DNA from different sources, for example, a specific promoter may be chosen to confer a desired pattern of developmental or tissue specificity. The foreign DNA integrates at a low frequency into the maize genomic DNA by recombination. When the selectable marker gene is expressed properly, it allows selection of cells in which the transgenes have integrated into the genome. These cells give rise to recombinant callus which is regenerated to give transgenic plants.

A transformation event occurs when the foreign DNA is stably incorporated in the genome of the recipient cells and the phenotypes of those cells are transformed to herbicide resistance (hence the term “transformation”). The concept of a transformation event is important because each transformation event is unique and is tracked by pedigree through the breeding process. Approvals for commercial release are granted on the basis of the transformation event that gave rise to the plants to be released. Thus, an approval covers any hybrid containing a particular event. Approval is based not only on the predicted product of the transgene, but also on the structure of the genome at the transgene integration site for a particular

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event. Thus, one event containing a particular transgene may be approved for commercial use while another event containing the same transgene may not.

The position of a transgene in the genome can influence the expression of the transgenes and the expression of endogenous genes at or near the insertion site. Thus, the same transgene performs differently in different transformation events. In addition, varieties containing different transformation events can exhibit different characteristics depending on whether endogenous genes were disrupted by the transgene. Hence, to identify lines with desired transgene performance and minimal adverse effects, thousands of transformation events are screened prior to commercialization of a transgene. All transgenic maize commercially grown to date has been developed by transgene integration at random positions in the genome, with each transformation event having a unique transgene integration site.

Clues to the molecular processes occurring during transformation have been obtained by examination of transgene loci. Transgene integration sites generated by microprojectile bombardment have been sequenced in rice [6] and oat [7]. These studies indicated that transgene loci are likely to be generated by illegitimate recombination and can be complex, containing multiple copies of the transgene and transgene fragments interspersed with genomic DNA. These complex integration sites could potentially contain genes encoding novel proteins with unknown functions, thus supporting the need to grant approval on the basis of transformation events rather than on the basis of the transgene alone. To avoid potential problems resulting from complex integrations, events, that contain simple integration sites are normally selected for commercialization.

The ability to control the insertion site of transgenes is one of the main hurdles facing plant biotechnologists. Natural homologous recombination systems such as those found in fungi and bacteria appear to be absent or function at a very low frequency in plants. Progress has been made in controlling the site of integration of the transgene by incorporating specific recombination systems into plants [8].

Relatively few varieties of maize can be transformed efficiently, and those that can be transformed efficiently are not competitive commercially. Thus, prior to deployment, a transgene must be incorporated into elite, commercially viable germplasm. This is normally done using backcross breeding and this process is frequently referred to as "conversion". The non-transgenic elite inbred line to be converted is referred to as the recurrent parent. Conversion is accomplished by crossing the transgene donor to the recurrent parent. Repeated cycles of selection for the transgene and crossing selected individuals to the recurrent parent are conducted until an inbred line containing the transgene with traits very similar to the recurrent parent is developed. It may take seven generations to accomplish this, but the process is accelerated by the use of molecular markers and nurseries around the world allowing up to three generations to be grown each year.

3 Transgenic Maize Currently on the Market

In the USA, a transgenic variety must be deregulated by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service prior to commercialization. Depending on the trait, other regulatory agencies may be involved as well, for example the Environmental Protection Agency regulates transgenic plants insect resistance and the Food and Drug Administration is involved when the trait is a pharmaceutical product. The traits conferred by currently deregulated events are listed in Tab. 1. It is striking to note that all of the deregulated events confer insect resistance, herbicide resistance, male sterility, or a combination of these traits. The U.S. land area on which these traits are grown is shown in Fig. 1. The insect resistant events produce an insecticidal protein in the tissues of the plant. Because this protein is derived from the bacterium *Bacillus thurengiensis,* plants carrying these transgenes are referred to as Bt corn, and are marketed under the trade names "Yield Guard™", "Knockout™", or "Herculex ™". The herbicide resistant events on the market confer resistance to either glyphosate or glufosinate and are marketed as either "Roundup Ready™" or "Liberty Link™", respectively.

For a given trait, certain events are used more widely than others. The event Mon810, conferring insect resistance, was released in 936 hybrids, more than double the number of the next most prevalent event (Tab. 2). It is also interesting to compare events that have been approved by the E.U. For example, several individual events are approved in the E.U., while these approved events in combination are not.

Tab. 1. Traits conferred by deregulated events.

Trait	Number of events
Insect resistance	6
Herbicide tolerance	5
Male sterility	1
Herbicide tolerance and insect resistance	3
Male sterility and herbicide tolerance	4

Source: USDA-APHIS

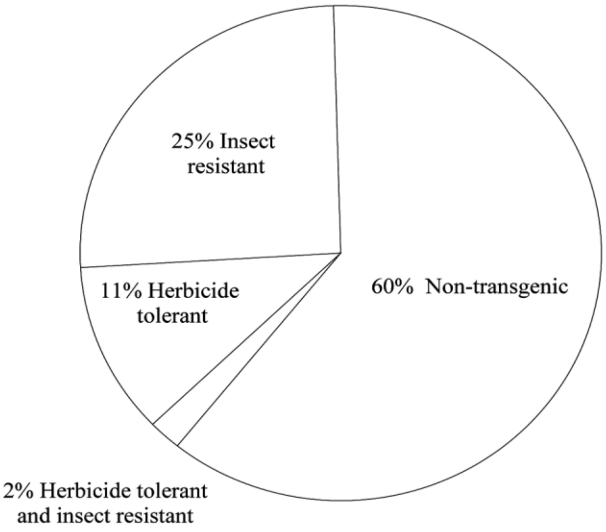

Fig. 1. U.S. land area planted to corn in 2003. Source: National Agricultural Statistics Service, Agricultural Statistics Board, USDA.

Tab. 2. Commercially available events for 2004.

Event	Trait[1]	Approved in E.U.	Number of hybrids
Bt11	I,H	Yes	60
Bt176	I	Yes	3
TC1507	I,H	No	34
Mon810	I	Yes	936
Mon863	I	No	260
MonGA21	H	No	387
Nk603	H	Yes	383
T25	H	Yes	83
Mon810+GA21	I,H	No	202
Mon810+Nk603	I,H	No	203
Mon810+T25	I,H	No	4
Mon863+GA21	I,H	No	21
Mon 863+Nk603	I,H	No	43

[1] I, Insect resistance; H, Herbicide tolerance

Sources: National Corn Growers Association, E.U. Health and Consumer Protection DG, Food and Feed Safety

Insect resistance and herbicide tolerance are classified as "input traits" because their primary purpose is to reduce the cost of production of maize. This benefits consumers, but the benefit is not readily apparent to most consumers. Output traits, on the other hand, are defined as those which impact the quality or value of the product. Examples include increased nutritional properties or longer shelf life. In general, the benefit of output traits is more apparent to consumers because output traits often result in a product with improved quality.

In addition to deregulated transgenic maize, maize has been produced in the U.S. under regulated status for production of high value products such as enzymes and antibodies as well as for basic scientific studies and for development of new transgenic varieties. If grown outside, production of this maize is monitored by the U.S. Animal and Plant Health Inspection Service. An approved protocol for minimizing the possibility of accidental release of this material must be followed by the grower. The land area devoted to this production is small relative to the amount of deregulated maize produced. The list of applications for regulated field release is available from the U.S. Animal and Plant Health Inspection Service. Examination of this list reveals that several types of output traits are in the pipeline (Fig. 2). Common output traits include modifications to amino acid balance, oil composition, and starch biosynthesis.

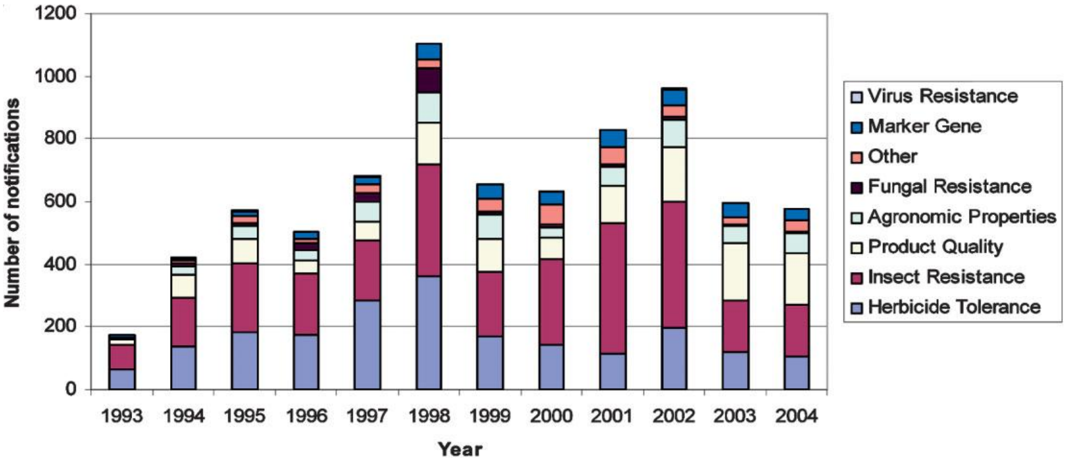

Fig. 2. Notifications of release of experimental maize varieties. Source: Animal and Plant Health Inspection Service, Biotechnology Division, USDA.

4 Comparisons to Conventional Maize

4.1 Intended benefits

European corn borer is an insect pest that is one of the main yield-limiting factors in the U.S. Corn Belt. This pest can reduce yields by as much as 30% in some areas in some years, although in most cases it is less. The primary benefit of Bt corn is that it confers resistance to the European corn borer. A meta-study of the benefits of Bt corn concluded that the benefit of Bt corn thus depends on the severity of the European corn borer infestation but on average the yield increase is small but significant, and in most cases Bt corn is profitable for farmers [9].

Herbicide-tolerant crops allow the use of post-emergence herbicides glyphosate or glufosinate in place of pre-emergence herbicides such as atrazine or alachlor. Lower application rates are required with glyphosate and glufosinate, and these herbicides are less persistent in the environment as well. Herbicide tolerant crops probably do not result in an increased grain yield, but may reduce herbicide usage and persistent chemicals in the environment.

4.2 Breeding considerations

Plant transformation allows genes to be transferred between different species. This allows breeders to incorporate a variety of traits into maize that would be difficult or impossible to develop using maize genes alone. However, for many traits there is sufficient natural variation in maize making it is possible for breeders to develop varieties with the desired trait through conventional breeding practices. For example, breeders have developed varieties of maize that are resistant to European corn borer [10], have herbicide resistance [11], or have improved nutritional properties [12], all without the use of transgenes.

Nonetheless, there are difficulties with these approaches that are overcome in part by using transgenes. For example, European corn borer resistance is a complex trait, so different levels of resistance are conferred by different combinations of genes, and it is difficult to transfer all of the required genes into established inbred lines by backcross breeding. Traits conferred by single gene mutations can be transferred to elite inbreds by backcross breeding, but they can have other problems. For example, the *opaque2* (*o2)* mutation that confers improved nutritional quality also confers poor agronomic traits on the grain, such as soft kernels that are prone to insect attack [12]. In addition, most mutations are recessive, while transgenes normally are dominant. Dominant genes simplify the breeding process because normally selection for a dominant gene is easier in backcross breeding.

Another reason seed companies are attracted to traits conferred by transgenes is that it is possible to use backcross breeding to convert many of their inbred lines in parallel, something which is not possible with traits with more complex genetics. A problem with this method of deploying transgenes is that backcross breeding requires effort, that would otherwise be devoted to developing improved inbred lines and hybrids. Also, the time it takes for backcross breeding creates a delay between when an inbred line is ready for release and when its transgenic counterpart will be ready for release. This delay can be minimized by starting the backcross program early in the development of an inbred, but this is risky and can result in wasted effort if the inbred turns out to be unsuitable for commercialization.

4.3 Effect of Bt corn on non-target insects

In reports of experiments conducted by *Losey* et al. [13] and later by *Jesse* and *Obrycki* [14] on the harmful effects of pollen from Bt corn on a non-target insect of great public interest, the monarch butterfly (*Danaus plexippus* L.), the authors cautioned that more extensive evaluation of the ecological effects was needed before transgenic crops with insecticidal properties were used widely. These studies were followed by widespread publicity and discussion in both the general public and scientific community, especially after the publication of the first report. The scientific community responded by developing a comprehensive research project to use scientific-based risk assessment to fully understand the situation. The research conducted by the consortium [15–19] led to the conclusion that with current Bt hybrids, risk to the monarch butterfly was negligible.

Studies of the effect of pollen from Bt corn on another non-target insect of public interest, the black swallowtail (*Papilio polyxenes*), led to a similar conclusion but generated less controversy. The initial report with this insect [20] found no impact of Bt pollen on larvae in laboratory and field studies. A subsequent report [21] found deleterious effects on swallowtail larvae for transgenic corn pollen expressing Cry1Ab endotoxin. *Hellmich* et al. [15] also reported deleterious effects on monarch larvae from Cry1Ab event 176, because it had much higher concentrations of endotoxins expressed in pollen than other Bt events.

4.4 Development of insect resistance

Bt corn has been a useful tool for farmers, allowing them to avoid a reduction in their profitability from insect damage, but this tool would be lost if insects develop resistance to the Bt toxin. Thus far, resistance to Bt in maize in the field has not been reported. Development of resistance is a concern because selection for resistance has been accomplished in the laboratory or greenhouse in at least seven strains of three pests [22]. Refuges, or non-Bt plantings near Bt crops, have been mandated as a strategy to slow or prevent the occurrence of resistance. Non-Bt plantings ensure a large population of susceptible insects, and a rare resistant insect would be much more likely to mate with a susceptible insect than with another rare resistant insect. Their heterozygous offspring would then be killed by feeding on the Bt crop. *Chilcutt* and *Tabashnik* [23] have reported low to moderate Bt toxin levels in refuge plants due to gene transfer through pollen, and recommended modification of the refuge strategy to decrease the chances of developing resistance. This would involve planting the non-Bt refuge corn so that it is not adjacent to Bt corn. Changing refuge strategies to minimize the occurrence of plants producing low toxin levels could increase the numbers of susceptible insects and thus decrease the survival level of any insects heterozygous for resistance genes that would result from matings of insects that happened to develop resistance.

4.5 Mycotoxin effects

Fusarium ear rot is a fungal disease in maize that is important because the fungal pathogens that cause this condition produce mycotoxins called fumonisons that may cause cancer and neurological diseases in animals and humans that consume infected corn. Damaged kernels are much more susceptible to fungal infection and insect feeding is a major cause of kernel damage. There is evidence to suggest that Bt corn has reduced susceptibility to infection by *Fusarium* species [24], presumably as an indirect effect of its reduced insect feeding. The reduction of fumonison concentrations varies with the insect infestation [25].

4.6 Impact on soil and related microbial communities

There has been concern about unintended harmful effects of transgenic maize, especially on the release of Bt hybrids, on soil and soil organisms. Although any transgene may have an effect, Bt has been especially worrisome because root exudates and decaying Bt plant tissue release more Cry endotoxin into the soil than do naturally occurring soil *Bacillus thuringiensis* [26]. In a review of the literature regarding environmental effects of transgenic crops on soil microbially- mediated plant-nutrient transformations, *Motavalli* et al. [27] found no conclusive evidence that current transgenic crops are causing significant direct effects in the field. They cautioned that evaluating environmental effects is difficult for several reasons, including: expression dependence on factors such as soil type, weather, and crop variety; lack of long-term baseline information in diverse agroecosystems; and lack of knowledge about the diversity of soil microorganisms. Assessing environmental effects of hybrids with multiple transgenes, and those under development, that will produce industrial products or pharmaceuticals, will be challenging. Another review by *Dunfield* and *Germida* [28] also emphasized that environmental effects of transgenic plants on plant-associated microbial communities are dependent on many factors. They emphasized the need for further research on long-term effects of growing transgenic crops compared to such agroecosystem changes as growing a crop altered by traditional breeding or an alternate agronomic practice rather than just comparing a transgenic crop to its normal counterpart.

Two papers looking at the effect of Bt toxin from transgenic corn on soil microbial ecology reported small and insignificant effects, although longer-term studies may be necessary. *Blackwood* and *Buyer* [26], in comparing Bt plants expressing the Cry1 toxin for European corn borer resistance with non-transgenic isolines growing in three soil types in a growth chamber, found a small Bt effect in high clay soil. This was likely due to the ability of clay to increase retention of Cry protein in the soil [29]. *Devare* et al. [30] found little difference in microbial measures among Bt corn expressing the Cry3Bb toxin for corn rootworm resistance (*Diabrotica* spp.; Coleoptera: Chrysomelidae), non-Bt isolines, and isolines treated with the insecticide tefluthrin (Force G™; Dow Elanco, St. Louis, MO) in a field of gravelly loam soil.

4.7 Nutrition studies with transgenic maize

The majority of maize produced worldwide is used for food or animal feed. For this reason studies on the nutritional impact of Bt maize in animal feed are particularly important. A review of transgenic plants in animal feed has recently been conducted [31].

Maize grain is an important component of the diets of monogastric animals. Studies of Bt maize used for swine nutrition show that transgenic maize does not produce significantly different results in weight gain [32] or composition of fecal matter [33]. Herbicide tolerant and normal corn were compared in a broiler chicken feeding study [34]. No significant differences in body weight and other growth related characteristics were observed between the two treatments. Similarly, no significant differences in body weight were observed in a feeding study comparing Bt maize to normal maize [35].

A number of studies have addressed the forage quality of transgenic maize, including the grain and other parts of the plant, in the diets of ruminant animals. Consistent differences were not found in several compositional traits related to forage quality when Bt and their non- Bt counterpart hybrids were compared [36]. In most cases, dairy cattle fed silage derived from commercial transgenic maize hybrids did not have significantly different milk production compared to cattle fed non-transgenic silage [37, 38, 39]. Bt maize silage plus grain did not have a consistent effect on weight gain in steers relative to the non-Bt versions of the same hybrids [39]. Weight gain in dairy cows was not different between cows fed Bt and non-Bt silage, however, the cows consumed more of the Bt feed. Milk quality was not different between the two groups. Digestibility of either Bt or non-Bt silage was not different in sheep [40]. Feed intake and milk production were not effected by herbicide tolerant or corn rootworm resistant maize when these varieties were included as silage in the diets of diary cattle [41].

On the other hand, *Saxena* and *Stotzky* [42] found the content of relatively indigestible lignin to be significantly higher in ten Bt hybrids representing three transformation events as compared to their non-Bt isolines. The increase in lignin content can have both beneficial ecological implications such as reduced susceptibility to mold and detrimental implications such as reduced digestion of the plant material when used as cattle feed. A more recent report suggests that this result is due to a flawed experimental design, and that no significant difference in lignification or digestibility exists between Bt and non-Bt hybrids [43].

While the majority of the evidence indicates that currently commercially available transgenic maize varieties do not have a great effect on animal nutrition, experimental varieties designed to have improved nutrition have been produced but have not been grown commercially. Increases in grain total protein content [44] and improved amino acid balance [45] have been reported.

5 Tracking Transgenes

5.1 Adventitious presence

Maize is a monoecious species, with each plant producing a male inflorescence called a tassel and a female inflorescence called a silk. In order to ensure efficient pollination, a large amount of pollen must be shed by each tassel and this pollen must be carried by gravity and wind to the silk. A consequence of this process is that it is difficult to ensure that pollen from a given maize field does not pollinate other fields of maize, and thereby result in adventitious presence of unwanted genes in the harvested crop. This has been problematic in several situations involving transgenic maize.

Maize likely originated in Mexico from teosinte, a wild relative still growing there. Mexico, as a center of origin for maize, is the source of many unique landraces still grown by farmers [46]. Maize played a significant role in many of the Mexican civilizations, and is still important in the Mexican culture and diet [47]. Gene flow between landraces and improved hybrids has likely occurred in Mexico since improved hybrids were first used there and may have contributed to genetic diversity [48].

The North American Free Trade Agreement (NAFTA) negotiated in 1992–93 among the United States, Canada and Mexico opened Mexico to maize importation from the United States. Because of this, Mexico's importation from the USA has increased markedly. The maize imported to Mexico for industry and consumption includes transgenics, but a 1998 government moratorium disallows transgenic maize to be grown there commercially. In 2001, *Quist* and *Chapela* [49] reported finding transgenes in landraces collected in remote mountainous areas of Mexico. Both the team of *Quist* and *Chapela* and the Mexican environmental ministry have reported finding small plots of transgenic maize growing in mountainous areas of Mexico, and have attributed pollen contamination from those small plots as the source of transgene contamination in the land races [50]. *Quist* and *Chapela*'s conclusions created controversy on both sides of the transgenic debate, leading to reports questioning their methods [51, 52]. *Nature* concluded that the evidence presented did not warrant their publication of the 2001 paper [53].

Experiences in the USA have also demonstrated the impact of contamination from transgenic to other varieties or hybrids, that do not contain transgenes. The most prominent case of contamination concerned maize containing the StarLink™ trait, and adversely affected producers and growers of transgenic and non-transgenic maize alike. StarLink™ maize is a type of Bt maize containing the insecticidal protein Cry9C. The U.S. Environmental Protection Agency approved the use of StarLink™ grain in animal feed but not in human food because of concerns over allergenicity of the Cry9C protein. Producers of StarLink™ were not required to control the pollen of their maize. StarLink™ DNA was detected in Kraft maize food products, spurring a recall by the U.S. Food and Drug

Administration. Although StarLink™ maize has not been linked to any cases of food allergies [54], a huge and costly effort was required to purge the U.S. seed production system of StarLink™ seed.

Another example of adventitious presence of transgenes has been in organic maize. Demand for organic food has been increasing about 20% per year. Many export markets and food manufacturers have an allowable tolerance for transgenic contamination, but the U.S. National Organic Standards allow no transgenic tolerance in order to be certified organic. Although the rules allow organic farmers with unintentionally contaminated maize to still call their crop "organic", their organic food or feed customers often require testing to show zero contamination. Rejection of their crops as organic is very costly to the farmers who bear the entire cost of avoiding transgenic contamination because they lose their substantial premiums.

It will be necessary to strictly control adventitious presence of transgenic maize containing regulated products for the pharmaceutical or chemical industries. These concerns have led to a number of studies of transmission of genes through maize pollen [reviewed in 55]. Several methods have been proposed for controlling the spread of transgenes through pollen, including growing mixtures of male-sterile transgenic plants with male-fertile non-transgenic pollinators [56] and a system in which pollen carrying the gene of interest can only successfully pollinate plants which carry a specific recovering gene [57, 58]. If the product is expressed and can be extracted from kernels produced by transgenic female plants crossed with non-transgenic males, standard detasseling of females and larger than normal isolation distances can be used if care is taken to remove tassels so no pollen is produced [59].

5.2 Metabolic fate of ingested transgenes

In addition to the nutritional impacts of transgenic maize in food and feed, it is important to consider the metabolic fate of the ingested transgenes themselves. Perhaps the greatest concern is that transgenes ingested by an animal or human will be taken up by microorganisms in the gut. For example, an antibiotic resistance gene could potentially be transferred from a transgenic crop to a bacterium resulting in an antibiotic resistant strain of bacteria. Transgene DNA was identified in the stomachs and crops of chickens fed transgenic grain, but could not be detected in the intestines [60]. In addition, neither endogenous maize DNA nor transgene DNA was detectable in chicken muscle using a highly sensitive PCR assay [61]. In contrast, transgene and endogenous maize DNA were detected in the intestinal contents of swine [62] and in the rumen and rectal contents of calves fed transgenic maize [63].

The possibility of transgenes from transgenic plants being taken up by intestinal microflora, leading to transfer of antibiotic resistance genes, was studied in humans with ileostomies, and others with intact intestinal tracts. The Roundup Ready™ transgene from transgenic soybean survived in the digesta of the ileostomists, but not in that of the people with intact systems. There was also some evidence of preexisting gene transfer in the small intestine. It was concluded that although the observed gene transfer would be highly unlikely to pose a risk to human health, safety assessments of transgenic foods should take into account survival of transgenic DNA during passage through the upper digestive tract [64].

5.3 Fate of transgenes in maize products

Production of fuel ethanol is increasing for environmental and economic reasons and most is produced from corn grain. Bt and non-Bt hybrid pairs were compared to determine the fate of Bt protein after wet milling and dry grind for ethanol production [65]. After wet milling the Bt hybrids, Bt protein was found in the germ, gluten, and fiber fractions, but not found after liquefaction in the dry grind process. No differences were found between Bt and non-Bt hybrid pairs for yield of ethanol in the dry grind process.

6 Conclusions

Transgenic maize currently produced commercially is designed for improved insect resistance or herbicide tolerance. The majority of studies comparing transgenic and non-transgenic grain fail to find significant differences between the two. There is potential to use this technology to develop grain with significant physical differences, that will make different types of grain suitable for different end uses. In order to capture the value in these products, sophisticated identity preservation and grain handling systems will be needed, requiring additional changes to maize production, handling, testing and marketing procedures.

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data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may be suitable.

References

[1] FAOSTAT data, http://apps.fao.org/, last accessed December 2004.

[2] W. J. Gordon-Kamm, T. M. Spencer, M. L. Mangano, T. R. Adams, R. J. Daines, W. G. Start, J. V. O'Brien, S. A. Chambers, W. R. Adams Jr, N. G. Willetts, T. B. Rice, C. J. Mackey, R. W. Krueger, A. P. Kausch, P. G. Lemaux: Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell* **1990**, *2*, 603–618.

[3] B. R. Frame, P. R. Drayton, S. V. Bagnall, C. J. Lewnau, W. P. Bullock, H. M. Wilson, J. M. Dunwell, J. A. Thompson, K. Wang: Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation. *Plant J.* **1994**, *6*, 941–948.

[4] B. R. Frame, H. Shou, R. K. Chikwamba, Z. Zhang, C. Xiang, T. M. Fonger, S. E. K. Pegg, B. Li, D. S. Nettleton, D. Pei, K. Wang: Agrobacterium tumefaciens-mediated transformation of maize embryos using a standard binary vector system. *Plant Physiol.* **2002**, *129*, 13–22.

[5] J. Gould, M. Devey, O. Hasegawa, E. C. Ulian, G. Peterson, R. H. Smith: Transformation of zea mays L. using Agrobacterium tumefaciens and the shoot apex. *Plant Physiol.* **1991**, *95*, 426–432.

[6] M. Takano, H. Egawa, J.-E. Ikeda, K. Wakasa: The structures of integration sites in transgenic rice. *Plant J.* **1997**, *11*, 353–361.

[7] I. Makarevitch, S. K. Svitashev, D. A. Somers: Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. *Plant Mol. Biol.* **2003**, *52*, 421–432.

[8] D. W. Ow: Recombinase-directed plant transformation for the post-genomic era. *Plant Mol. Biol.* **2002**, *48*, 183–200.

[9] M. Marra, P. Pardey, J. Alson: The payoffs of transgenic field crops: An assessment of the evidence. *AgBioForum* **2002**, *5*, 43–50.

[10] C. A. Abel, L. M. Pollak, W. Salhuana, M. P. Widrlechner, R. L. Wilson: Registration of GEMS-0001 maize germplasm resistant to leaf blade, leaf sheath, and collar feeding by European corn borer. *Crop Sci.* **2001**, *41*, 1651–1652.

[11] K. Newhouse, B. Singh, D. Shaner, M. Stidham: Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides. *Theor. Appl. Genet.* **1991**, *83*, 65–70.

[12] S. K. Vasal: High quality protein corn, in *Specialty Corns*, 2nd ed. (Ed. A. R. Hallauer) CRC Press Boca Raton, Fl., **2001**, p. 85–129.

[13] J. E. Losey, L. S. Rayor, M. E. Carter: Transgenic pollen harms monarch butterfly. *Nature* **1999,** *399*, 214.

[14] L. C. H. Jesse, J. J. Obrycki: Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia* **2000**, *125*, 241–248.

[15] R. L. Hellmich, B. D. Siegfried, M. K. Sears, D. E. Stanley-Horn, M. J. Daniels, H. R. Mattila, T. Spencer, K. G. Bidne, L. C. Lewis: Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11925–11930.

[16] K. S. Oberhauser, M. Prysby, H. R. Mattila, D. E. Stanely-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W.-K. F. Lam, R. L. Hellmich: Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci. USA*. **2001**, *8*, 11937–11942.

[17] J. M. Pleasants, R. L. Hellmich, G. Dively, M. K. Sears, D. E. Stanley-Horn, H. R. Mattila, J. E. Foster, P. L. Clark, G. D. Jones: Corn pollen deposition on milkweeds in and near cornfields. *Proc Natl. Acad. Sci. USA* **2001**, *98*, 11919–11924.

[18] M. K. Sears, R. L. Hellmich, D. E. Stanley-Horn, K. S. Oberhauser, J. M. Pleasants, H. R. Matilla, B. D. Siegfried, G. P. Dively: Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11937–11942.

[19] D. E. Stanley-Horn, G. P. Dively, R. L. Hellmich, H. R. Mattila, M. K. Sears, R. Rose, L. C. H. Jesse, J. E. Losey, J. J. Obrycki, L. Lewis: Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11931–11936.

[20] C. L. Wraight, A. R. Zangeri, M. J. Carroll, M. R. Berenbaum: Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7700–7703.

[21] A. R. Zangerl, D. McKenna, C. L. Wraight, M. Carroll, P. Ficarello, R. Warner, M. R. Berenbaum: Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11908–11912.

[22] B. E. Tabashnik, Y. Carriére, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, J.-Z. Zhao: Insect resistance to transgenic Bt crops: lessons from the laboratory and field. *J. Econ. Entomol.* **2003**, *96*, 1031–1038.

[23] C. F. Chilcutt, B. E. Tabashnik: Contamination of refuges by *Bacillus thuringensis* toxin genes from transgenic maize. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 7526–7529.

[24] G. P. Munkvold, R. L. Hellmich, W. B. Showers: Reduced fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. *Phytopathology* **1997,** *87*, 1071–1077.

[25] M. J. Clements, K. W. Campbell, C. M. Maragos, C. Pilcher, J. M. Headrick, J. K. Pataky, D. G. White: Influence of Cry1Ab protein and hybrid genotype on fumonisin contamination and fusarium ear rot of corn. *Crop Sci.* **2003**, *43*, 1283–1293.

[26] C. B. Blackwood, J. S. Buyer: Soil microbial communities associated with Bt and non-Bt corn in three soils. *J. Environ. Qual.* **2004**, *33*, 832–836.

[27] P. P. Motavalli, R. J. Kremer, M. Fang, N. E. Means: Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. *J. Environ. Qual.* **2004**, *33*, 816–824.

[28] K. E. Dunfield, J. J. Germida: Impact of genetically modified crops on soil- and plant-associated microbial communities. *J. Environ. Qual.* **2004**, *33*, 806–815.

[29] D. Saxena, S. Flores, G. Stotzky: Vertical movement in soil of insecticidal Cry1Ab protein from*Bacillus thuringensis*. *Soil Biol. Biochem.* **2002**, *34*, 111–120.

[30] M. H. Devare, C. M. Jones, J. E. Thies: Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. *J. Environ. Qual.* **2004**, *33*, 837–843.

[31] A. Aumaitre, K. Aulrich, A. Chesson, G. Flachowsky, G. Piva: New feeds from genetically modified plants: substantial equivalence, nutritional equivalence, digestibility, and safety for animals and the food chain. *Livestock Production Science* **2002**, *74*, 223–238.

[32] T. Reuter, K. Aulrich, A. Berk: Investigations on genetically modified maize (Bt-maize) in pig nutrition: Fattening perfor-

mance and slaughtering results. *Archives of Animal Nutrition-Archiv Für Tierernährung* **2002**, *56*, 319–326.

[33] T. Reuter, K. Aulrich, A. Berk, G. Flachowsky: Investigations on genetically modified maize (Bt-maize) in pig nutrition: Chemical composition and nutritional evaluation. *Archives of Animal Nutrition-Archiv Für Tierernährung* **2002**, *56*, 23–31.

[34] R. S. Sidhu, B. G. Hammond, R. L. Fuchs, J.-N. Mutz, L. R. Holden, B. George, T. Olson: Glyphosate-tolerant corn: The composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2305–2312.

[35] J. Brake, D. Vlachos: Evaluation of transgenic event 176 "Bt" corn in broiler chickens. *Poultry Sci.* **1998**, *77*, 648–653.

[36] H. G. Jung, C. C. Sheaffer: Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for silage. *Crop Sci.* **2004**, *44*, 1781–1789.

[37] W. J. Cox, D. J. R. Cherney: Influence of brown midrib, leafy, and transgenic hybrids on corn forage production. *Agronomy J.* **2001**, *93*, 790–796.

[38] S. S. Donkin, J. C. Velez, A. K. Totten, E. P. Stanisiewski, G. F. Hartnell: Effects of feeding silage and grain from glyphosate-tolerant or insect-protected corn hybrids on feed intake, ruminal digestion, and milk production in dairy cattle. *J. Dairy Sci.* **2003**, *86*, 1780–1788.

[39] J. D. Folmer, R. J. Grant, C. T. Milton, J. Beck: Utilization of Bt corn residues by grazing beef steers and Bt corn silage and grain by growing beef cattle and lactating dairy cows. *J. Animal Sci.* **2002**, *80*, 1352–1361.

[40] Y. Barriere, R. Verite, P. Brunschwig, F. Surault, J. C. Emile: Feeding value of corn silage estimated with sheep and dairy cows is not altered by genetic incorporation of Bt176 resistance to *Ostrinia nubilalis*. *J. Dairy Sci.* **2001**, *84*, 1863–1871.

[41] R. J. Grant, K. C. Fanning, D. Kleinschmit, E. P. Stanisiewski, G. F. Hartnell: Influence of glyphosate-tolerant (event nk603) and corn rootworm protected (event MON863) corn silage and grain on feed consumption and milk production in Holstein cattle. *J. Dairy Sci.* **2003**, *86*, 1707–1715.

[42] D. Saxena, G. Stotzky: Bt corn has a higher lignin content than non-Bt corn. *Am. J. Bot.* **2001**, *88*, 1704–1706.

[43] H. G. Jung, C. C. Sheaffer: Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for silage. *Crop Sci.* **2004**, *44*, 1781–1789.

[44] J. Yu, P. Peng, X. Zhang, Q. Zhao, D. Zhy, X. Sun, J. Liu, G. Ao: Seed-specific expression of a lysine rich protein sb401 gene significantly increases both lysine and total protein content in maize seeds. *Mol. Breed.* **2004**, *14*, 1–7.

[45] S. Huang, W. R. Adams, Q. Zhou, K. P. Malloy, D. A. Voyles, J. Anthony, A. L. Kriz, M. H. Luethy: Improving nutritional quality of maize proteins by expressing sense and antisense zein genes. *J. Agric. Food Chem.* **2004**, *52*, 1958–1964.

[46] G. Wilkes: Corn, strange and marvelous: but is a definite origin known? in *Corn: Origin, History, Technology, and Production* (Ed. C. W. Smith) John Wiley & Sons, Inc., New York, **2004**.

[47] A. Nadal: Mexican corn: genetic variability and trade liberalization. *Programa Sobre Ciencia, Tecnología y Desarrollo Documento de Trabajo* **2000** No. 1–06. El Colegio de México, México D.F.

[48] B. M. Baltazar, J. B. Schoper: Crop-to-crop gene flow: dispersal of transgenes in maize during field tests and commercialization, in *Proc. 7th Int. Symp. on the Biosafety of Genetically Modified Organisms*. **2002** Beijing, China. (http//www.bba.de/gentech/isbgmo.pdf).

[49] D. Quist, I. H. Chapela: Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* **2001**, *414*, 541–543.

[50] R. Dalton: Transgenic corn found growing in Mexico. *Nature* **2001**, *413*, 337.

[51] M. Metz, J. Fütterer: Suspect evidence of transgenic contamination. *Nature* **2002**, *416*, 600–601.

[52] N. Kaplinsky, D. Braun, D. Lisch, A. Hay, S. Hake, M. Freeling: Maize transgene results in Mexico are artifacts. *Nature* **2002**, *416*, 601–602.

[53] Editor: Editorial note. *Nature* **2002**, *416*, 600.

[54] Centers for Disease Control: *Investigation of human health effects associated with potential exposure to genetically modified corn*, A report to the U.S. Food and Drug Administration from the Centers for Disease Control and Prevention, June 11, **2001**.

[55] D. E. Aylor, N. P. Schultes, E. J. Shields: An aerobiological framework for assessing cross-pollination in maize. *Agric. For. Meteorol.* **2003**, *119*, 111–129.

[56] B. Feil, U. Weingartner, P. Stamp: Controlling the release of pollen from genetically modified maize and increasing its grain yield by growing mixtures of male-sterile and male-fertile plants. *Euphytica* **2003**, *130*, 163–165.

[57] V. Kuvshinov, A. Anissimov, B. M. Yahya: Barnase gene inserted in the intron of GUS–a model for controlling transgene flow in host plants. *Plant Sci.* **2004**, *167*, 173–182.

[58] V. Kuvshinov, K. Koivu, A. Kanerva, E. Pehu: Molecular control of transgene escape from genetically modified plants. *Plant Sci.* **2001**, *160*, 517–522.

[59] W. E. Stevens, S. A. Berberich, P. A. Sheckell, C. C. Wiltse, M. E. Halsey, M. J. Horak, D. J. Dunn: Optimizing pollen confinement in maize grown for regulated products. *Crop Sci.* **2004**, *44*, 2146–2153.

[60] P. A. Chambers, P. S. Duggan, J. Heritage, J. M. Forbes: The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. *J. Antimicrob. Chemother.* **2002**, *49*, 161–164.

[61] J. C. Jennings, L. D. Albee, D. C. Kolwyck, J. B. Surber, M. L. Taylor, G. F. Hartnell, R. P. Lirette, K. C. Glenn: Attempts to detect transgenic and endogenous plant DNA and transgenic protein in muscle from broilers fed Yield Gard corn borer corn. *Poultry Sci.* **2003**, *82*, 371–380.

[62] E. H. Chowdhury, O. Mikami, Y. Nakajima, H. Kuribara, A. Hino: Detection of genetically modified maize DNA fragments in the intestinal contents of pigs fed StarLink (TM) CBH351. *Vet. Hum. Toxicol.* **2003**, *45*, 95–96.

[63] E. H. Chowdhury, O. Mikami, H. Murata, P. Sultana, N. Shimada, M. Yoshioka, K. S. Guruge, S. Yamamoto, S. Miyazaki, N. Yamanaka: Fate of maize intrinsic and recombinant genes in calves fed genetically modified maize Bt11. *J. Food Prot.* **2004**, *67*, 365–370.

[64] T. Netherwood, S. M. Martín-Orú, A. G. O'Donnell, S. Gockling, J. Graham, J. C. Mathers, H. J. Gilbert: Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnol.* **2004**, *22*, 204–209.

[65] B. S. Dien, R. J. Bothast, L. B. Iten, L. Barrios, S. R. Eckhoff: Fate of Bt protein and influence of corn hybrid on ethanol production. *Cereal Chem.* **2002**, *79*, 582–585.

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