OPINION

Confronting the Gordian knot

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Galvanizing plant science in Europe will depend on an overhaul of the tangle of indefensible regulations themselves, not on the advent of new plant breeding technologies that may escape existing rules.

Rachel Carson's *Silent Spring* espouses the application of new biological technologies to address environmental and agricultural challenges facing humanity. SOURCE: Houghton Mifflin

> "Any society goes through social movements or fads, in which economically useless things become valued or useful things devalued temporarily. Nowadays, when almost all societies on Earth are connected to each other, we cannot imagine a fad's going so far that an important technology would actually be discarded. A society that temporarily turned against a powerful technology would continue to see it being used by neighboring societies and would have the opportunity to reacquire it by diffusion (or would be conquered by neighbors if it failed to do so)."
>
> Jared Diamond, *Guns, Germs and Steel*[1]

An array of approaches is becoming available for manipulating the genetic content of plants and animals. Such approaches are gaining attention from regulators, particularly in Europe, where the question is whether new technologies should fall under the same restrictive regulatory framework as plants modified using the traditional transgenic approaches[2]. This is of central importance because restrictive European regulations have not only had pernicious effects on applied plant science throughout Europe, but have also been a factor in the closure of major R&D facilities of European agrochemical companies[3]. Even if legislative loopholes could be found that would allow biotech plant products produced by new technologies to move forward outside of existing regulations, we argue here that the Gordian knot binding European plant science through continuing policy failure and political timidity will remain uncut.

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Regulatory myopia

It is, in general, highly commendable (and all too rare) that regulators look ahead with the intent to ensure that their mechanisms for oversight and safety assessment/assurance are appropriate to anticipated developments. But much as armies are often judged for preparing to fight the last war rather than the one that looms, so, too, regulators must do more than look a year or three down the road to prepare for the future. One of the most important, and most often mishandled challenges facing regulators, is the need frequently to recalibrate the level of scrutiny they apply to a class of products so that it is defensible in the light of the actual hazard intrinsic to a product.

The reasons this challenge is often mishandled are legion: not only is it philosophically difficult to re-evaluate one's presuppositions, but vested interests and institutional imperatives create inertia and sometimes overt obstacles to change. But the world has seldom seen a greater discrepancy between the inherent hazard of a product and the level of regulatory burden imposed on it than exists today for crops improved through biotech. It is important, here, to be very clear: there is no basis in science for regulation specific to crops and foods improved through biotech or 'GMOs'[4–7].

Looking back

In 1953, James Watson and Francis Crick divined the structure of DNA, noting wryly near the end of their paper that "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."[8] Over the subsequent several decades, an army of researchers discovered and illuminated the numerous mechanisms by which DNA is recombined in nature, and learned how to use those techniques in the laboratory. They figured out how to harness these natural processes to create old medicines in new ways and to impart new characteristics to plant varieties in a fraction of the time it previously took, using techniques discovered by evolution before the dawn of humanity. As a consequence, crops improved through biotech—we purposely avoid 'genetically modified (GM) crops' as a term as it does little more than reinforce ignorance of the fact that all crop improvement is mediated by genetic modification—have been grown by now on well over a billion hectares in more than 30 countries by nearly 17 million farmers, 15 million of whom are resource-poor smallholders in developing countries[9].

The economic and environmental impacts of biotech crops have been overwhelmingly positive. "In 2009, the direct global farm income benefit from biotech crops was $10.8 billion. This is equivalent to having added 5.8% to the value of global production of the four main crops of soybeans, maize, canola and cotton. Since 1996, farm incomes have increased by $64.7 billion…in 2009, 53.1% of the farm income benefits have been earned by developing country farmers…. Over the fourteen years, 1996 to 2009, the cumulative farm income gain derived by developing country farmers was also 49.2% ($31.85 billion). Since 1996, the use of pesticides on the biotech crop area was reduced by 393 million kg of active ingredient (8.7% reduction) and the environmental impact associated with herbicide and insecticide use on these crops, as measured by the EIQ (environmental impact quotient) indicator, has fallen by 17.1%"[10].

Looking forward

The increased production of food and feed derived from these crop varieties has comprised billions upon billions of meals eaten by humans and livestock around the world.

Agbiotech 2.0

As parts of the developing world embrace biotech, the focus is shifting from food production to fuels, industrial chemicals and even drugs. Daniel Grushkin investigates.

As Europe increasingly becomes a genetically modified (GM)-free zone, countries in Asia and South America are embracing the technology. Even African states are beginning to come around. Only five days before last year's vote in the European Parliament to give individual member countries the right to ban GM crops on the grounds of environmental and health concerns, Kenya became the fourth African country to approve the import and production of GM crops.

The thawing environment for transgenic products outside of Europe partly reflects a realization that grain commodity prices are threatening food security and that, according to the United Nations, agricultural production will need to rise by 70% by 2050 to meet the needs of the world's growing population[1]. To stave off a hunger pandemic and dire projections about the wilting effects of climate change on agriculture, new agbiotech tools and applications will be a key part of the solution. As a result, multinational companies are quickening the pace and widening the variety of innovation they are undertaking, not only to compete with each other, but also to outpace low-cost competitors in emerging economies that are producing innovations of their own. Thus, begins Agbiotech 2.0.

The end of hegemony?

Despite controversy about efficacy and safety, the adoption of the limited variety of first-generation GM crops has been remarkably widespread. Since their commercialization in 1996, crops transgenic for *Bacillus thuringiensis* (*Bt*) toxin or herbicide resistance now cover 160 million hectares and are used by 15.4 million farmers in 29 countries, according to the International Service for the Acquisition of Agri-biotech Applications, based in Ithaca, New York (**Fig. 1**).

And although the majority of research and seed production comes out of agrochemical corporations in the developed world, these multinational operations are no longer the only game in town. The country with the second-highest acreage of GM crops is now Brazil. And it is projected that developing nations, which grow half of the world's GM crops, will be growing the majority in 2012. Although most of those crops are now patented by seed giants such as Monsanto of St. Louis and Pioneer Hi-Bred (a DuPont business in Johnston, Iowa). local seed companies popping up in developing countries are bound to bite into the multinational market share.

For example, China's largest GM seed company is Shenzhen-based Biocentury Transgene, a state-supported company whose cotton seed incorporating the *Bt* gene is grown on 90% of Chinese cotton plantations. In the Indian market, Biocentury now goes toe to toe with Monsanto, which cut its prices by nearly half to compete with the Chinese company.

By 2015, some 34 GM crops will have moved into advanced development in Asia, compared with only 26 in the US and Europe, according to a 2009 report by the European Union Joint Research Centre[2] (http://www.nature.com/nbt/journal/v28/n1/extref/nbt0110-23b-S2.xls). In the short term, these crops aren't a technological challenge. The technology the companies are adding to their crops has trailed the giants, and those crops are slated to be grown domestically. But according to the report, "in [the] future the adoption pattern may change fundamentally, with more new GM crops being adopted first in Asia (and then potentially spreading from there)."

Hello, generics

The seed hegemony will further be challenged in 2014, when the early patents on GM crops expire. As many as 29 seed patents could be on the chopping block, but the most important among them is the last of Monsanto's Roundup Ready soy seed, which contains a gene resistant to the company's herbicide Roundup. Herbicide-resistant soy occupies more acreage worldwide than any other GM crop. Just as generic versions of Roundup originating from China have wedged into the company's chemical herbicide business, cut-rate seed companies producing generics are also likely to rush in to challenge the giant for market share.

The expiration of these patents marks a turning point for GM crops. Whereas for the past 16 years, GM crops have exploited exclusively foreign genes to kill pests and tolerate herbicides, the next generation of biotech crops from the major seed companies goes much further. They have engineered seed that increases yield and addresses stressors from climate change: drought, heat stress and even the salinity in the soil. The giants have had no choice but to innovate.

Agro-genomics

A decade ago, Ceres, an agbiotech based in Thousand Oaks, California, adopted the tools of the Human Genome Project to study plants. It was the beginning of a revolution in agbiotech. In one project, the company upregulated 10,000 genes in *Arabidopsis thaliana* to test how the new GM plants would react under various conditions. It was act of random searching: "You look at the 20,000-odd genes that are in the plant and you say, 'I'll take this half,' " says CSO Richard Flavell. In the process, Ceres screened hundreds of thousands of plants

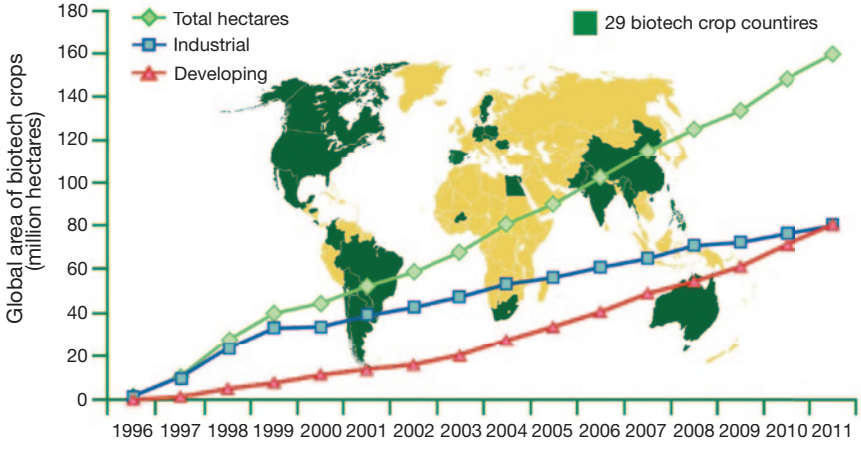

Figure 1 GM crops around the world. (Source: Clive James, ISAAA, 2012.)

for traits that might be useful. "That was our source of variation and we could do it at a scale that was essentially bigger than anyone else could do at the time," he says.

The project had the random quality of the experiments that plant breeders performed in the first half of the twentieth century. Leading up to the birth of genetic engineering, scientists zapped seeds with X-rays or dipped them in chemicals to induce mutations. If the mutant produced a useful trait, they'd breed the plant. The difference here was that Ceres could identify the gene and the mutation producing a particular effect. Instead of playing genetic potluck, they were slowly building a data set that could start to answer questions about an individual gene's relationships to plant physiology.

The trick to connecting genotype with phenotype was having sophisticated screening techniques and the tools of computational biology. The work gave birth to a five year $137-million product-discovery and development deal with Monsanto in 2002 and a stream of imitators. Although the technology under license has not been commercialized to date, through this process, agricultural-genetics research has begun to form a shadowy picture of the network of genes that determine certain plant traits. Last year, Ceres reported on two genes regulated by the *Arabidopsis* circadian clock that affect flowering. When upregulated, the genes (*At5g52250* and *At5g23730*) produced early flowering. The genetic alteration could one day be carried into food crops to increase yield[3] (see p. 215).

The widespread adoption of next-generation sequencing has now begun to fill in the blanks bit by bit. "DNA sequencing has evolved at a speed nobody could have predicted a few years ago. This is absolutely essential for going forward," says Michael Metzlaff, research manager at Bayer CropScience, headquartered in Monheim am Rhein, Germany.

Researchers have even begun to sequence multiple strains of crops to understand the relationships of various alleles to traits, something that would have been prohibitively expensive just a few years ago. One team in China, for example, has sequenced 14 domestic and 17 wild varieties of soybean to find specific genetic variants between wild-type and cultivated strains[4]. "Once you've got a conventional genome assembly, then to reassemble against an existing skeleton is very cheap and quick," says Jim Dunwell, a plant biologist at the University of Reading, UK.

In addition, marker-assisted breeding—in which short DNA sequences, associated with genes of interest can be used to determine whether a seed is likely to possess a desirable trait, without the need to grow a mature plant—has become a mainstay of major seed companies. For example, Pioneer Hi-Bred has developed a 120-watt laser to score a thin slice off of a seed in order to sample its genes without destroying it. According to Metzlaff, the use of molecular markers cuts breeding time in half.

Box 1 Pipeline to regulatory limbo?

Deregulation has always been a convoluted process in the US. A new transgenic crop might pass through three agencies—the US Food and Drug Administration, the US Environmental Protection Agency and US Department of Agriculture (USDA)—before being approved for commercialization.

Now, according to the Washington, DC–based Biotechnology Industry Organization (BIO), deregulation is more arduous than ever. In the USDA, the average processing time has risen from 140 days in 1996 to nearly 1,200 last year. Seed companies have paid on average $35 million in expenses associated with deregulating individual crops according to a September Crop Life International survey of seed companies.

The delay has coincided with a glut of new GM crop varieties. In 2008, there were 33 GM crops in worldwide commercial circulation. That number will reach 124 by 2015, according to a 2009 report from the Joint Research Centre (JRC) of the European Union[2]. Coupled with increasingly shrill debate over GM crops, the surge has created a regulatory logjam, both domestically and internationally. Alexander Stein, author of the 2009 JRC report, points to the controversy in India over brinjal (eggplant) engineered with a *Bt* gene. "When India posted a moratorium on eggplant, the whole Indian pipeline came to a stop. It's the politics involved," he says.

In the US, industry has blamed the backlog of 20 crops awaiting deregulation on a series of lawsuits made by public interest groups, particularly over alfalfa and the sugar beets modified with the Roundup Ready gene. The lawsuit over GM sugar beets, for example, hinged on whether the USDA had done a proper environmental impact study before approval. Last August, Jeffrey White, a district judge in San Francisco, declared that it had not.

"The whole system has been screwed up as a consequence of harassment of lawsuits filed by activists," says Val Giddings, a senior fellow at the Information Technology & Innovation Foundation in Washington, DC. For its part, the Washington, DC–based US Center for Food Safety, which filed the suit, complains that officials have never evaluated the crops in good faith. "Our experience is that the USDA fundamentally views all biotech products as a good thing, and their job is just to rubber-stamp approvals," says Bill Freese, Center for Food Safety science policy analyst.

To expedite the process, last April the USDA introduced a pilot program allowing seed companies to write their own environmental impact studies on new crops (or hire a third-party company). The announcement produced a new round of uproar over conflicts of interest.

If there's one thing that the both sides agree on, it's that regulation needs an overhaul. The USDA has been trying to update its regulations since 2004, with little headway. Until now, agencies have evaluated GM crops on the basis of the dangers associated with the genes' organism of origin or the vector by which they're inserted. "We have to start regulating by the properties of the crop, not the techniques by which it was modified, which is what we're doing now," says Nina Fedoroff, former science and technology advisor to the US Secretary of State.

Ironically, the next generation of gene-editing technologies may sidestep the regulatory process entirely. The USDA regulates transgenic crops through the Plant Protection Act, which gives it the power to rule on genetic parts that come specifically from plant pests. Zinc-finger nucleases (ZFNs), for example, don't originate from pests, and, therefore, appear to fall outside the regulatory framework. In 2009, Vipula Shukla at Dow AgroSciences used ZFNs to produce herbicide resistance in corn without adding any foreign genes. Presumably, the seeds will be treated like any conventional breed. "Because the changes you introduce by those techniques are exactly like those you can make by classical mutagenesis, it shouldn't be subject to this horrendous regulation," Fedoroff says. Similar constructs called transcription activator–like effector nucleases could make gene editing even easier, they originate from the plant pest *Xanthomonas*, and might be captured under the current framework.

The USDA's Animal and Plant Health Inspection Service (APHIS) has yet to decide on its role in the process. "APHIS is currently considering the regulatory status of zinc-finger nucleases and transcription activator–like effector nucleases," says spokesman Richard Bell. The decision has the potential to change the entire industry (see p. 215).

Genomics and markers, however, are only part of the equation. "You can sequence a genome in a week or outline a biochemical pathway in gory detail, but the question then becomes, how do you use that information to help the engineering or the modification of the plant?" says Vipula Shukla, scientist at Dow AgroSciences.

The latest technology in gene manipulation adopted by seed companies has been zinc-finger nucleases (ZFNs). Dow AgroSciences licensed the technology from Sangamo of Richmond, California, under the name Exzact Precision Technology. Using ZFNs, genetic engineers can target and manipulate precise sequences of DNA. These are useful not just for gene insertion, but for cutting specific locations on the genome to disable or edit specific genes.

In 2009, Shukla and a team from Dow and Sangamo used ZFNs to target and disable a gene encoding an inositol pentakisphosphate kinase responsible for the storage of 75% of the phosphorus that is found in corn kernels and is an unhealthy component of animal feed. The team not only reduced seed phytate but conferred herbicide resistance too.

Because the technique forgoes inserting foreign genes into crop genomes, the technology raises the regulatory question—are ZFN-manipulated seeds bred or GM (**Box 1**)? "This increasingly gray area might not come under the regulated definitions of GM," says Dunwell. If so, they may eliminate the regulatory lag between development and commercialization (see p. 215).

Old problems, new traits

Getting pests under control still occupies plant researchers: the emergence of herbicide-resistant weeds has seed companies exploring new genes for combating them. So far, 21weeds have shown resistance to glyphosate, the active ingredient in Roundup—many of them have appeared in the years since the release of herbicide-resistant GM crops[5]. To counteract these new weed strains, crops with resistance to multiple pests have been produced (**Box 2**) and crops resistant to other herbicides, such as Dicamba (Monsanto) and acetolactate synthase (ALS, DuPont), are in late stages of development. Meanwhile, researchers are taking new approaches to supplementing *Bt*, such as RNA interference, to enable crops to ward off pests[6].

Beyond new suites for pest management and herbicide tolerance, the goals for the GM crops coming down the major seed companies' pipelines are to both increase yield and address abiotic stressors "As we're looking toward this next wave of traits, what's different and required is a deep understanding of the fundamental biology of how the plant works. We need to understand the relevant biochemical pathways, the energetics, how they use nutrients and, most importantly, how those biological components interact in the environment," Shukla says.

In that direction, research is now underway to identify the genes underlying crop architecture, leaf area and leaf angle, with a view to using genetic technologies to create new varieties that maximize photosynthesis. Root structure can also be altered to increase crop density by maximizing nutrient uptake while occupying the smallest area, says Flavell. In one example of this work, scientists at the Chinese Academy of Sciences linked the *OsSPL14* gene to the number of tillers, or shoots, at the base of rice. A mutation in the gene decreased the number of tillers and increased yield by 10%[7].

And then there's water stress. One need only look at the American Southwest to understand the focus on drought. The last year has been the driest period Texas has seen in 115 years. In the coming years, with population growth and biofuel crops competing for irrigation, there will be increasing strain on water resources. "Investigating plants to tolerate heat and drought—that's the most important thing we could be doing for the generation after next," says John Bedbrook, DuPont's vice president for agbiotech.

One of the most promising developments comes from an insertion of a gene originating from *Bacillus subtilis* that encodes a cold-shock protein. When bacteria are exposed to sudden cold, protein synthesis slows. As a result, the cell begins producing cold-shock proteins, chaperones that rescue misfolded mRNA to restore translation. This produces a new state of equilibrium and allows the cell to adapt to the new temperature. When plants undergo stress—from heat, cold or dryness—their metabolism slows, too. "They tend to hunker down," says Bob Reiter, biotech lead at Monsanto. By having a single gene that continually produces cold-shock proteins, the plant is prevented from slowing down.

In one Monsanto study, a transgenic corn strain with a cold-shock protein gene yielded 30.8% more grain under drought conditions than plants without the gene, although the germplasm, timing and severity of drought can affect the outcome. The gene has also proven to be effective in stress conditions brought on by heat and cold; rice with the transgene grew 35% taller than rice without[8]. Monsanto is planning large-scale field trials this year.

Performance Plants of Kingston, Ontario, Canada, has also developed a drought-tolerance gene technology called Yield Protection Technology (YPT), which it has licensed to several seed companies, including Syngenta based in Basel, Bayer CropScience and Scotts Miracle-Gro based in Marysville, Ohio. Canola with a promoter that downregulates the production of farnesyltransferase have a 26% increase in yield; in petunias, the modification nearly doubles the number of flowers per plant.

Box 2 Bumps in the road

2010 was a bad year for Monsanto. The company's newest corn cultivar—SmartStax—failed to meet its hype. The seed had been billed as a marvel of biotech. It combined eight gene inserts, which had never before been accomplished in a product, and pooled Monsanto's suite of technologies with those of Dow AgroSciences. The crops possessed above- and below-ground pest resistance and two types of herbicide tolerance. Despite these bells and whistles, when the first corn harvests in the US were tallied, SmartStax ears yielded 2.5% less than the company's cheaper, less-sophisticated seed with only three gene inserts.

Farmers were angry. They had paid $24 more per acre for the product[9]. The company tried to appease them by offering free credits for the next season, but the damage was done. Commentators around the country decried the company's health. "This may be the worst stock of 2010," Jim Cramer shouted on CNBC's *Mad Money*.

The incident was telling. Even though the destiny of agbiotech seems cast—gene technology speeding ahead and more farmers adopting it every year—these are uncertain times for the seed giants. The SmartStax story raises an important question that has yet to be answered. Will farmers pay a premium for the next generation of crops?

In 2010, Monsanto priced SmartStax seed too high and US farmers didn't go for it. They bought seed for only 3 million acres of SmartStax, instead of the 4 million that Monsanto had hoped for. But things might be picking up. Monsanto is reporting that SmartStax corn outperformed its competitors' products in 2011, and saw a 10-million acre increase for all its GM corn products.

For farmers, the seed advances boil down to the bottom line: "I believe the only meaningful word for all these technologies is yield," says Yafan Huang, of Performance Plants.

According to the company, suppression of farnesyltransferase triggers stomata to shut earlier and tighter in the drought cycle, allowing plants to hold onto moisture and recover sooner when finally watered. Farnesyltransferase is thought to dull the effects of the phytohormone abscisic acid, which modulates the size of stomata. Performance Plants uses RNA interference to downregulate the gene encoding farnesyltransferase. Yafan Huang, CSO of Performance Plants, expects YPT, the first of its suite of gene technologies, to enter the market in 2013.

Fortifying plants

Customers can expect to see food on their grocery shelves that have had trans fats removed or omega-3 fatty oils added. Soybeans engineered by Monsanto to produce oil with stearidonic acid omega-3 are used in foods ranging from yogurt to granola. Next year, DuPont will release a soybean strain that is high in oleic acid. "It's a soy oil that has a fatty-acid composition of an olive oil," says Bedbrook. To produce the variety, DuPont scientists expressed a 600-base-pair fragment of Δ12-desaturase (*FAD2)* gene, which caused gene silencing in the seed. These soybeans have 75% oleic acid, which is a monounsaturated, healthy oil, in the seed oil. The gene silencing prevented the formation of a second double-carbon bond on the oleic acid, and stopped the production of linoleic acid, a polyunsaturated fat.

These oil traits are early examples of seed companies adding nutritional benefits to their crops. In the aftermath of opposition in the developing world to Golden Rice—a strain that had elevated levels of β-carotene (vitamin A precursor), which is often lacking in diets in the developing world—HarvestPlus, a non-profit in Washington, DC, has used conventional breeding techniques to fortify staple crops in South Asia and Africa with vitamin A, zinc and iron.

Beyond food

Even with all the headway made in food crops, agbiotech is moving well beyond food. For nearly a decade, many small companies that license their technologies to the seed giants have been building an industry of crop alternatives to petroleum-based fuel. "In the big picture, there will have been a slice of time when fossil fuels were the source of energy, but of course for the thousands of years before it, the sun and the land were the sources of energy. So unless there's going to be some extraordinary source of solar voltaics or other innovations, I think the land is going to be realized as a substitute for fossil fuel," says Flavell.

Nearly all the companies are scrambling to find the right mixture to meet the rising demand. Ceres, for example, has added a salinity-tolerance gene to switchgrass to allow it to be grown for biofuels in marginal land without competing for resources with food crops. Mendel Biotechnology based in Hayward, California has invested in *Miscanthus giganteus*, a fast-growing, tall grass that has been genetically engineered to increase yield, and is now in the third year of field trials. Syngenta, for its part, has created a corn strain specifically for ethanol production that contains the gene encoding α-amylase, so that the enzyme begins the process of converting starch into sugar inside the plant. Today, ethanol manufacturers pour amylase into the corn slurry as a first processing step.

Looking down the road, higher-value products are also likely to be engineered into feedstocks. Cambridge, Massachusetts–based Metabolix, for example, has inserted a pathway in switchgrass, camelina and sugarcane to grow beads of polyhydroxyalkanoate within seeds to be extracted for the production of biodegradable plastic.

Alongside industrial applications, there is still the possibility that medicines within crops will also find their niche. New York-based Pfizer is developing a treatment for Gaucher's disease that is produced in GM carrot and tobacco cells. The cultures produce recombinant glucocerebrosidase, an enzyme required for metabolizing fat. The treatment is now under review at the US Food and Drug Administration, with a 1 May 2012 Prescription Drug User Fee Act date. Elsewhere, Calgary, Alberta, Canada–based SemBioSys has engineered safflower plants to produce insulin for diabetics. Phase 1/2 trials were completed in the UK in 2009 (where an approval pathway for biosimilars exists), in which the safflower-produced product was indistinguishable from Humulin (insulin), produced by Eli Lilly of Indianapolis. The company has a joint venture with the Chinese pharmaceutical giant Tasly of Tianjin, and is positioning the product for eventual registration in the US, Europe and China, according to Rick Pierce, president for US and international operations.

Amber waves

Jüergen Logemann, director of research at BASF Plant Science based in Raleigh, North Carolina, was addressing the US Congress a few years ago about the future of agriculture when an old farmer stood before the hearing committee. Logemann remembers the farmer's words: "For the last 30 years nobody cared what I was doing. I was poor. Now, suddenly I feel honored and valued. I feel like I've become king."

The farmer voiced a sentiment that resonates with agbiotech insiders, who feel the sector is on the cusp of the next generation of crop technologies. "You begin to rethink the economy of the rural areas," says Roger Beachy, one of the first plant genetic engineers and former director of the National Institute of Food and Agriculture in the US Department of Agriculture. "The rural areas become the factories to make all these raw materials. You value them in a different way than, 'oh, that's just making more corn.' It's not. It's economy."

The slew of new applications flowing from agbiotech have those in the industry thinking about society's connection to farmland in a new way. As crops are engineered to produce more grain or products such as medicine or fuel, and to do so under increasingly variable conditions, every acre of land, even marginal land, gains value. Seed companies still bear the burden of proving this dream to the public, especially when climate indicators and food shortages imply otherwise. But, as Flavell says, "with a little bit of optimism, we can believe that the role of the land is going to come back to hold a new position in the way that perhaps cities did in the past hundred years."

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Is it Time to Adjust the Current Regulatory Risk Assessment for GM Food and Feed?

Marcel Kuntz and Agnès E. Ricroch

In 1987, a National Academy of Science (USA) report entitled Introduction of Recombinant DNA-Engineered Organisms into the Environment had already stated that "there is no evidence that unique hazards exist in the use of recombinant DNA techniques or in the transfer of genes between unrelated organisms" and "that the risk[s]...are the same in kind as those associated with...other genetic techniques." In addition, in 1989 and 1990, scientists (including 16 European Nobel Prize Laureates) had warned against a legislation targeting the process (transgenesis) and not the product itself (its traits). Despite these recommendations by scientists, the European Union (EU) institutions have adopted regulations on "genetically modified organisms" (GMOs) that, in fact, essentially target recombinant DNA techniques (excluding mutagenesis for example). Since then, regulations on the marketing of "genetically modified" (GM) crops have been strengthened continuously due to political pressure, not just in the EU but also in other countries such as India, for example. The "precautionary principle" has acquired the status of a doctrine in EU regulation[1].

In this review, which focuses exclusively on food/feed safety aspects of GM lines, we first summarize the regulatory and risk perception context for GM food marketing in the EU. Many European citizens remain unconvinced by the safety of GM food[2] despite the fact that a thorough, lengthy, and costly evaluation of GMOs is imposed before marketing. This context has prompted new studies by public research laboratories, using alternative evaluation techniques (i.e., not part of the regular evaluation process), namely large scale profiling of GM varieties and long-term animal feeding studies, whose conclusions are discussed here.

A brief overview of the European regulatory context for GM food

In 1990, in order to implement a specific regulatory regime, the European Commission (EC) published twin Directives on contained use and deliberate release of GMOs into the environment[3,4,5]. In contrast with, for example, the Food and Drug Administration (FDA, USA) policy statement addressing food "derived from any new plant variety, whether developed by traditional breeding techniques or cellular or molecular techniques"[6], the EC's proposal followed a process-based approach, creating special and distinct regulations for the approval and marketing of GMOs (i.e., obtained essentially via transgenesis). In 1997, the regulatory structure of Directive 90/220 was supplemented by Regulation 258/97, the so-called Novel Foods Regulation and Novel Food Ingredients Regulation, which established an evaluation procedure (based on an internationally accepted concept) demanding that GM foods remain "substantially equivalent" (or nutritionally equivalent) to existing foods (to their conventional counterparts) in terms of "*their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein*" (article 3).

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The new EC Directive 2001/18 on the release of GMOs into the environment, adopted in March 2001, introduced new concepts, namely traceability and labelling of GMOs as well as a threshold for permitted traces of GM ingredients (art. 21). In contrast, historically, the FDA has not considered that plant breeding techniques should be material subject to labelling.

In the EU, a so-called "safeguard clause" allows a Member State to provisionally restrict or prohibit the use and/or sale of that product on its territory (art. 23) if it can provide justifiable reasons to consider that a GMO constitutes a risk to human health or the environment. This clause has been used abusively by several Member States[7].

An important difference with regard to food/feed safety between USA and Europe is that the FDA approach gives more emphasis on post-market regulation while Europe's policy imposes a heavy pre-market regulation. Nevertheless, in both cases, safety assessment is structured, step-wise, and comparative; in addition, public consultation procedures have been established.

In 2002, the Council and European Parliament adopted EC Regulation 178/2002 pursuant to which a new risk assessment agency, namely the European Food Safety Authority (EFSA), was created. This European agency, however, does not fully replace the Member State scientific agencies, especially since political authorities have asked EFSA to interact with them. EFSA provides its opinion after reviewing the applicant's file and usually requests additional data from the applicants, which delays its final opinion. Authorization is then subject to a vote by Member States, or to an EC decision if Member States do not reach a "qualified" majority vote (they never do). Any authorization of a GM variety is limited to ten years.

A matter of risk perception rather than demonstrated risk

To understand the reasons for this regulatory burden in the EU, it is important to place the EU food safety regulation within the context of the aftermath of the Bovine Spongiform Encephalopathy (BSE) crisis that struck in 1996, first in the UK and then in other countries. The first GM crops were commercially introduced in the USA and in Europe within the relative time frame of this "mad cow" crisis, that is to say, at a time of major consumer distrust in "industrial" food safety, in agroindustries, and also in politicians. In April 1996, within a month of the ban on British beef, the EC approved the import of GM soy products, which were actually imported from the USA to the EU, starting from November 1996. When some non-governmental pressure groups protested against replacing feed that had been banned in response to the BSE crisis with GM soy feed, they got huge media and public attention.

However, stricter regulations and application of the "precautionary principle" have failed to convince GMO opponents and consumers that EU regulations are robust regarding food and feed safety. It may even have convinced consumers that, since the regulation has changed, it *must* mean that GMOs are intrinsically risky.

Although it is unlikely that, in such a context, additional research will lead to a rapid change in public perception, but considering that such additional research has already been performed, we decided to review the relevant scientific literature in order to generate some knowledge from these data. We reviewed the scientific publications 1) to check whether new "omics" profiling techniques reveal unintended effects in plants due to the genetic modification, and 2) whether long-term studies as well as multigenerational feeding studies can detect the consequences of potential unintended effects in animals (that were not revealed by the current toxicological tests).

What can be learned from the use of new 'omics' techniques on the safety of GM plants?

The first question we addressed was: Might the improvement of a plant variety through transgenesis lead to unintended effects (i.e., beyond the effect anticipated from the new transgenic trait) and, if so, could it impact consumers' health? We examined recently published high-throughput profiling studies and their conclusions about the effect of the genetic modification itself, compared with environmental and intervariety variation. These included 44 studies for major crops and also studies using *Arabidopsis thaliana* as a reference plant[8]. Metabolomics was the prevalently used technique, but transcriptomics and to a lesser extent proteomics were also used.

We first considered the profiling of GM crop lines with new agronomic traits, but without deliberate modifications to metabolic pathways. None of these published "omic" assessments points to new safety concerns about marketed GM cultivars. Our survey did reveal some differences with respect to the comparator conventional lines: comparing various conventional lines consistently showed more differences. Thus, these non-targeted profiling studies consistently indicated that transgenesis has fewer unintended impacts than conventional breeding.

This is not surprising since GM lines have been selected, from the laboratory to the field, by a process based on phenotypic and compositional equivalence with a close comparator, and not based only on the suitable expression of a new trait. In addition, the new trait is often introgressed into elite lines, which efficiently eliminates unwanted genomic changes that might have occurred in the original transformant.

"Omic" profiling also indicates that environmental factors (such as field location, sampling time during the season or at different seasons, and mineral nutrition) consistently exert a greater influence than transgenesis on plant gene expression or composition. Interestingly, one study[9] showed that transcriptome alteration was greater in mutagenized plants than in transgenic plants (unlike transgenic lines, mutagenized lines are not subjected to food safety assessment in the EU). We then examined data on GM lines with altered metabolic traits. Available data indicated that such intended modifications do not necessarily exhibit pleiotropic changes, although some do occur when certain pathways are modified, which is not unexpected.

In summary, although of varying quality and despite being exploratory in nature, these "omic" data do not indicate, when taken together, that more food safety testing is necessary for GM crops and, in our opinion, rather suggest that, apart from specific cases, their risk assessment should be lessened.

What can be learned from long-term and multigenerational feeding studies with GM diet?

Safety assessment of whole food/feed follows an integrated approach using various tests, including toxicity tests, such as a 90-day feeding trial on rodents. This test is designed to detect any possible toxicological effects of the GM diet compared with the control diet. These repeated-dose 90-day oral toxicity studies in rodents have been performed according to the OECD Test guideline n°408 (1998). According to an EFSA statement[10] "animal feeding trials with rodents or other (target) animal species (e.g., broilers) […are] not deemed necessary on a routine basis." It seems contradictory with the observation that such tests are routinely included in the pre-marketing application files examined by EFSA. Others, especially GMO opponents, often argue that 90-day rodent feeding studies may be insufficient to reveal the presence of late effects in animals. Therefore, we decided to evaluate whether long-term studies, namely those performed for longer than 90 days, as well as multigenerational studies, provide different findings than 90-day studies and whether unintended effects are detected.

Our systematic review has collected data concerning the effects of diets containing GM maize, potato, soybean, rice, or triticale on animal health[11]. We examined 12 long-term studies (of more than 90 days, up to 2 years in duration) and 12 multigenerational studies (from 2 to 5 generations). These 24 international studies were conducted by independent institutes from the USA, Brazil, Japan, and Norway, among others. The particularly interesting aspect of this broad range of studies is not only the fact that they originate from different countries, but also the variety of animals tested: chickens, mice, rats, goats, cows, and salmon. These animals were fed 33% of currently marketed transgenic plants (maize, soybean), and rice, triticale, and potatoes in their diet at the rate set by the OECD in 1998. We also referenced the 90-day studies using GM feed for which long-term or multigenerational study data were available.

Examination included biochemical analyses, histological examination of specific organs, hematology, and the detection of transgenic DNA. The statistical findings and methods have been critically analyzed for

each study, and it was found that not all methods match international recommendation for GM food assessment. However, none of these 24 studies suggest any health hazards. In general, there were no statistically significant differences within parameters observed. When some small differences were observed, these fell within the normal variation range of the considered parameter or were unlikely to be linked to the genetic modification, as the authors often themselves stressed. Thus, these studies do not find a biological or toxicological impact of long term consumption of a GM diet.

Taken together, these studies do not provide any evidence that long term or multigenerational feeding tests are needed on a routine basis. Thus, if required at all (if doubts about the nutritional equivalence still exist), a 90-day feeding study performed on rodents can be considered sufficient (apart from specific cases). All these toxicological studies converge and thus confirm that the marketed GM varieties are nutritionally equivalent to their non-GM counterparts and can be safely used in food and feed. This conclusion also implies that the pre-marketing assessments requested by toxicologists were robust. There seems little point to further increase the rigor of assessments, as is the case in EFSA's recently published (2011 12) general guidance for carrying out 90-day feeding studies, notably by increasing test animal numbers in order to comply with debatable statistical considerations.

Conclusions

The above-mentioned 1987 opinion of the National Academy of Science (USA) is now largely confirmed by present day experience: 1) 15 years of cultivation of increasingly larger area of GM crops (from 1.7 million hectares of GM crops in 1996 to 148 million hectares in 2010) without identification of health problems; 2) the data on pre-market GM food safety assessment; and 3) additional research by academic laboratories, for example, as summarized here using large scale "omic" profiling or long-term feeding studies on animals.

Thus, on a scientific basis, it may be time to simplify the assessment of food products derived from plants obtained by modern biotechnology (at least those with agronomic traits and no deliberate metabolic changes), and therefore reduce their costs. However, the GM food scare has never been science-based, but rather originates from a different rationality.

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European Food Safety Authority Issues Opinion Insect-Resistant, Herbicide Tolerant Soybean for Food and Feed

EFSA Panel on Genetically Modified Organisms Panel Members

Abstract

This scientific opinion is an evaluation of a risk assessment for placing on the market the genetically modified (GM) insect-resistant and herbicide-tolerant soybean MON 87701 × MON 89788 for food and feed uses, import and processing. Soybean MON 87701 × MON 89788 was produced by conventional crossing methods, and the F1 plant is hemizygous for all newly introduced traits. The soybean contains the Cry1Ac and CP4 *epsps* genes conferring resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides. No biologically relevant differences were identified in the composition or agronomic and phenotypic characteristics of soybean MON 87701 × MON 89788, as compared with its comparator, except that it expresses the Cry1Ac and CP4 EPSPS proteins. The safety assessment identified no concerns regarding the potential toxicity and allergenicity of soybean MON 87701 × MON 89788. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering its intended use as food and feed, environmental risks associated with an unlikely but theoretically possible horizontal gene transfer from soybean MON 87701 × MON 89788 to bacteria have not been identified. Potential interactions of soybean MON 87701 × MON 89788 with the biotic and abiotic environment were not considered to be an issue owing to the low level of exposure. The monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87701 × MON 89788. In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 × MON 89788 addresses the scientific comments raised by Member States and that the soybean MON 87701 × MON 89788, as described in this application, is as safe as its comparator with respect to potential effects on human and animal health and the environment, in the context of its intended uses.

Summary

Following the submission of an application (EFSA-GMO-NL-2009-73) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of insect-resistant genetically modified (GM) soybean MON 87701 × MON 89788 (Unique Identifier MON-877Ø1-2 × MON-89788-1) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-73, additional information supplied by the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from applications for placing on the market under European Union regulatory procedures the single soybean events MON 87701 and MON 89788 was taken into account. The scope of application EFSA-GMO-NL-2009-73 is for food and feed uses and for import and processing of soybean MON 87701 × MON 89788 within the EU in the same way as any non-GM soybean, but it excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87701 × MON 89788 with reference to the intended uses and appropriate principles described in its guidance documents of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA 2006) and for the risk assessment of GM plants containing stacked transformation events (EFSA 2007). The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analysis of the composition and phenotypic and agronomic characteristics was undertaken, and the safety of the new proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of the environmental impacts and the post-market environmental monitoring plan was also undertaken.

The single soybean events MON 87701 and MON 89788 were the subject of separate earlier risk assessment evaluations by the EFSA GMO Panel. The EFSA GMO Panel concluded that they are unlikely to have any adverse effect on human and animal health and the environment, in the context of their intended uses (EFSA 2008, 2011a). The placing on the market of products containing, consisting of, or produced from genetically modified soybean MON 89788 was authorised pursuant to Regulation (EC) No

1829/2003. No new genes, in addition to those occurring in soybean MON 87701 and MON 89788, have been introduced in soybean MON 87701 × MON 89788. Soybean MON 87701 × MON 89788 was produced by conventional crossing of the single soybean events to combine in the same stack resistance against certain lepidopteran target pests and tolerance to glyphosate-based herbicides.

Molecular analysis has confirmed that soybean MON 87701 and MON 89788 inserts are present and that their structures are retained in soybean MON 87701 × MON 89788. The result of the updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert–plant DNA junctions did not reveal a safety concern. The overall levels of the Cry1Ac and CP4 EPSPS proteins were comparable to those of the corresponding single soybean events MON 87701 and MON 89788.

The EFSA GMO Panel compared the composition and phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 with its comparator (A5547), assessed all statistically significant differences identified, and came to the conclusion that no biologically relevant differences were identified in the composition or phenotypic and agronomic characteristics of soybean MON 87701 × MON 89788 as compared with its comparator (A5547) and that the composition fell within the range of non-GM soybean varieties, except that soybean MON 87701 × MON 89788 expressed the CP4 EPSPS and Cry1Ac proteins. A small increase in final stand count in soybean MON 87701 × MON 89788 was observed, but no safety issues were identified linked to this increase. The risk assessment included an analysis of data from analytical and bioinformatics studies, as well as in vitro and in vivo studies. The EFSA GMO Panel concluded that soybean MON 87701 × MON 89788 is as safe as its comparator and that the overall allergenicity of the whole plant has not changed.

Potential interaction between the soybean events with respect to an effect on human and animal health were the focus of the assessment on food/feed issues. On the basis of the known functional characteristics and modes of action of the newly expressed proteins (Cry1Ac and CP4 EPSPS), the EFSA GMO Panel considers it unlikely that interactions between these proteins would occur that would raise any safety concerns. Thus, the Panel is of the opinion that soybean MON 87701 × MON 89788 is as safe and as nutritious as its comparator and commercial soybean varieties, in the context of its intended uses.

The application EFSA-GMO-NL-2009-73 concerns food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean MON 87701 × MON 89788. There are no indications of an increased likelihood of the establishment and spread of feral soybean plants in the event of the accidental release into the environment of viable soybean MON 87701 × MON 89788 grains during transport and processing for food and feed uses, except under conditions of infestation by the specific lepidopteran pests or the application of glyphosate-based herbicides. Taking into account the scope of the application, both the rare occurrence of feral soybean plants and the low levels of exposure to the environment indicate that the risk to target and non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 × MON 89788 to environmental bacteria does not raise concern owing to the lack of a selective advantage in the context of its intended uses. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of soybean MON 87701 × MON 89788. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 × MON 89788 addresses the scientific issues indicated by the guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that soybean MON 87701 × MON 89788 is as safe as its comparator with respect to potential effects on human and animal health or the environment in the context of its intended uses. In addition, the EFSA GMO Panel is of the opinion that crossing of single soybean events MON 87701 and MON 89788 to produce soybean MON 87701 × MON 89788 does not result in interactions between the events that would affect the safety of soybean MON 87701 × MON 89788 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses.

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Both Expiring and Healthy Patents Breed Challenges for AgBiotech

Phill Jones

The AgBiotech industry experiences countless court battles as companies enforce patent rights against each other and against farmers. Ironically, the expiration of patents will inflict strife as well.

The patent on a pioneer genetically engineered (GE) biotech trait, Monsanto's first-generation Roundup Ready® 1 (RR 1) soybean technology, is set to expire in 2014. Consequently, farmers could save seed from their 2014 GE RR 1 soybean harvest for planting in 2015. Seed producers will be able to use or even stack the RR 1 trait without paying royalties to Monsanto. Patent expiration could mean cost savings for farmers and more freedom to grow GE plants that contain the RR 1 trait without fear of a patent infringement suit. Yet any advantages offered by patent expiration come with a cost.

"In the near future, the last of the Roundup Ready soybean patents will expire," says Roger A. McEowen, professor in agricultural law at Iowa State University (Ames, Iowa). "That expiration will be followed by the expiration of other patents on biotech crops and expiring approvals in overseas markets like the European Union and China. Those expirations could lead to the planting of so-called 'generic' versions of Roundup Ready seeds that lack approval in overseas markets, complicating the export process and potentially disrupting billions in trade."

Ensuring open markets for GE products is expensive. In its efforts to maintain global regulatory approvals for a product, Monsanto may spend about $1–1.5 million per year. Monsanto has promised to continue global regulatory approvals through 2021 for RR 1 soybeans, removing the risk of a loss of GE soybean exports to the EU and China. The company will not pay the bill indefinitely as its patents expire, however. The AgBiotech industry must devise a way to transfer registrations to an organization that will maintain the necessary global regulatory approvals.

Thomas Redick, an attorney with the Global Environmental Ethics Counsel, explained that there is no legal framework for handling regulatory requirements of AgBiotech GE traits as they come off patent. An industry-wide agreement would help technology providers to transfer regulatory data from the patent holder to an organization, such as a seed company or consortium of seed companies, which would sustain necessary regulatory requirements, including export approvals for the biotech trait.

"The answer to regulatory approval is simple in concept but perhaps complex in implementation," say Redick and Western Michigan University business professor Norman W. Hawker. "The longstanding pesticide industry practice of 'data access and compensation' provides one possible solution to potential trade disruption. US law has given biocide innovators certain rights to 'data compensation' that provide an analogous legal remedy and procedure." According to this type of scheme, companies interested in marketing a generic version of a genetic event can buy rights to proprietary health and safety information held by the former patent owner. In this way, generic companies need not perform their own health and environmental studies.

The American Seed Trade Association is designing procedures to help farmers maintain access to quality GE seed and to international markets for their GE products. "[W]e are again working with (the Biotechnology Industry Organization) to create an accord, a binding agreement to transfer data packages between the original patent holder and those utilizing the event once it goes off patent," ASTA chairman and president John Nelsen told the *Western Farm Press*. "A guidance document is also being developed to help companies who are considering entering the 'generic' market, in addition to outreach and educational documents focusing on regulatory obligations and responsibilities, and the intellectual property of seed."

Pioneer Hi-Bred Sues Monsanto Over Patented Method for Growing Corn

Phill Jones

During October 2011, DuPont's Pioneer Hi-Bred International sued Monsanto Co, claiming that Monsanto infringed two patents that claim enhanced germination of corn seeds. Pioneer's patented methods increase the vigor of maize seeds by defoliating maize plants at a particular time after pollination but before harvest. Specifically, US Patent No. 5,518,989 claims a "method for treating a stand of maize plants, comprising the steps of (A) reducing functional leaf area in substantially all of said plants, wherein said reducing is effected at between about 600 and about 850 (growing degree days) after pollination of said plants, and then (B) harvesting said stand, such that a seed assemblage is obtained from said stand that is characterized by a level of seed vigor that is enhanced relative to the level of seed vigor in a seed assemblage harvested from a comparison stand of maize plants not subjected to said reducing of functional leaf area." In its US Patent No. 6,162,974, Pioneer claimed maize seed assemblages and stands of maize plants that have enhanced seed vigor due to the application of the methods claimed in the '989 patent.

According to Pioneer, Monsanto or its agents used the methods of the '989 patent to produce seeds, at least at its Constantine, Michigan, site. Pioneer also asserted that Monsanto has been aware of Pioneer's patents, at least since the time that two of Pioneer's inventors began work at Monsanto. Furthermore, Pioneer contends that Monsanto is deliberately inducing or contributing to infringement of the patents by causing growers in Constantine to defoliate and harvest maize plants in an infringing manner.

Monsanto denied Pioneer's allegations and asserted a counterclaim that Pioneer/DuPont's patents are invalid for failure to satisfy various patentability requirements. "[T]his filing appears to be another in a series of frivolous claims initiated by DuPont against our business and aimed at distracting us from our mission of investing in and delivering new product offerings to farmers around the world," Monsanto retorted in a press release. "We will defend our business against this latest attack."

The case should be decided – at least, in the district court – later this year.

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Identification of Acyanogenic Forage Sorghum by a Combination of Biochemical Screening and TILLING

Cecilia Blomstedt

Introduction

Sorghum is an important cereal crop world-wide that is grown for both human and animal consumption. As a C4 plant, sorghum has high water-use efficiency, is tolerant to drought and high temperatures, and can grow on marginal land that may not be suitable for other crops. These are important attributes in the context of current global climate change and limited availability of fertilizers and arable land. However, when its cells are disrupted by herbivory, sorghum releases toxic hydrogen cyanide (HCN) from the degradation of the cyanogenic glucoside dhurrin by ß-glucosidase. The release of HCN has both advantages and disadvantages. The plant benefits because cyanogenic glucosides defend against insect attack and are involved in nitrogen turnover and storage used for growth[1]. However, dhurrin-containing sorghum plants are highly toxic during early growth (up to 0.5m tall), and the toxicity of adult plants may increase following environmental stress or the application of high nitrogen fertilizers. It is estimated that in Australia alone the reluctance of farmers to use stressed sorghum as animal fodder decreases the value of the crop by up to $25 million p.a. Therefore the production of zero or low cyanogenic lines has been a goal for many years.

In sorghum, cyanogenesis has been extensively studied at the molecular and biochemical levels, and the genes encoding the three key biosynthetic enzymes have been identified: two cytochrome P450s (CYP79A1 and CYP71E1); and one UDP-glucosyltransferase (UGT85B1) (**Fig. 1**)[2]. The first step in this pathway is the conversion of tyrosine to an oxime intermediate by CYP79A1. The second cytochrome P450 (CYP71E1) converts the oxime to a hydroxynitrile, which is stabilized by glycosylation by UGT85B1 (**Fig. 1**). These enzymes form a metabolon that allows labile and toxic intermediates to be channeled into dhurrin synthesis and prevents undesirable metabolic cross-talk[3]. To generate and identify mutations in the dhurrin biosynthetic enzymes with the aim of producing zero or reduced cyanogenic lines, we targeted CYP79A1, utilizing a Targeted Induced Local Lesions in Genomes (TILLING) program[4]. Naturally-occurring acyanogenic individuals have been identified in a number of cyanogenic species, such as white clover, and indicate that it may be possible to generate acyanogenic plants without detrimental effects.

Targeted Induced Local Lesions IN Genomes (TILLING)

To generate a mutagenized population to screen for acyanogenic plants, we treated more than 50,000 sorghum seeds with EMS, resulting in the germination and growth of 16,300 M1 plants that were self pollinated. The second generation of 4,200 M2 plants was screened, using the Feigl-Anger assay[6], for the inability to produce HCN. Individual lines with elevated HCNp were also selected for potential commercial use as biofumigants. This initial screen identified 264 putative mutant lines, which were then analyzed for HCN production by a quantitative assay,

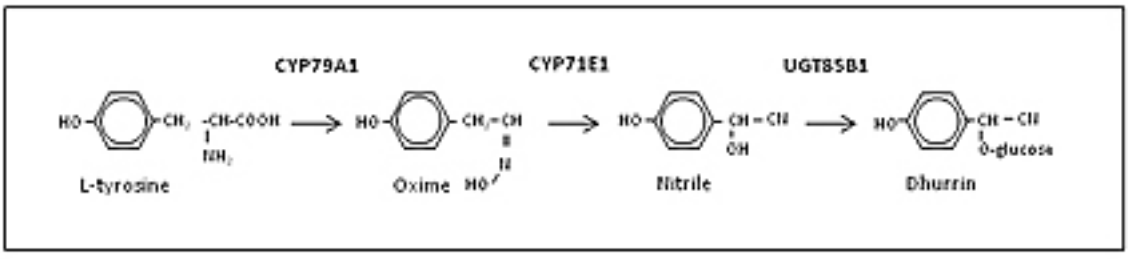

Figure 1. The biosynthetic pathway of dhurrin in forage sorghum

and the DNA was extracted for TLLING analysis. This resulted in the identification of nine putative CYP79A1 mutant lines with a decreased or absent hydrogen cyanide potential (HCNp). One line with an elevated HCNp was also identified. These individual lines were characterized in greater detail[4].

Sequencing showed that all lines were independently derived and that the mutations were the expected G:C to A:T transitions due to EMS treatment. One line was totally cyanide deficient (*tcd1*) in both shoot and root tissue throughout all stages of growth and development. Molecular modeling, based on the solved crystal structure of relevant P450s, indicated that the *tcd1* mutation, a proline to leucine amino acid change, is believed to interfere with the structural organization of the CYP79A1 protein and prevent substrate binding and subsequently a loss of catalytic activity. The presence of dhurrin and the biochemical activity of CYP79A1 were assayed in microsomal preparations from 4-day-old etiolated seedlings of the *tcd1* line, which confirmed the complete loss of CYP79A1 activity and dhurrin production. Western blot analysis showed that the non-functional CYP79A1 protein was present in the microsomal preps. In the elevated cyanide potential (*ecp1*) line, molecular modeling of the amino acid sequence of the mutated CYP79A1 gene that contained an E145K amino acid change suggested that this mutation may increase substrate affinity, leading to the observed increase in dhurrin production.

In addition to mutant lines with characterized mutations in the CYP79A1 gene, we also identified three lines that had very low HCNp in the leaves of adult plants but show no mutations in either the *CYP79A1* or *UGT85B1* structural gene sequences. While these mutants produce shoot tissue that is essentially acyanogenic at adult stages, there is no substantial reduction in the dhurrin content in young plant tissue (seedlings and microsomal preparations from 4-day-old etiolated seedlings) or in the roots of adult plants compared with levels found in non-mutated parent plants. These three mutant lines were designated adult cyanide deficient category (*acdc*) mutants 1–3. We postulate that low dhurrin levels in the adult leaves of *acdc* mutants could result from mutations in adult leaf-specific regulatory genes controlling the expression levels of biosynthetic genes and/or degradation pathways. This may indicate that root accumulation is not dependent on biosynthetic capacity in leaves.

The individual mutant plants generated and characterized in this study have been grown under field conditions for up to five generations. All lines remained healthy and grew well, showing no susceptibility to insect or fungal attack (**Fig. 2A**). The adult *acdc* mutants showed no change in obvious morphology or phenotypic characteristics following the drop in dhurrin content compared to non-mutated plants (**Fig. 2B**). The *tcd1* mutant that produced no dhurrin in any tissue grew slightly slower during early development, but at approximately the 4–5 leaf stage, the plants showed no major difference compared to non-mutated plants (**Fig. 2C**). This may indicate that, during early sorghum development, HCN may be important as a source of nitrogen for growth.

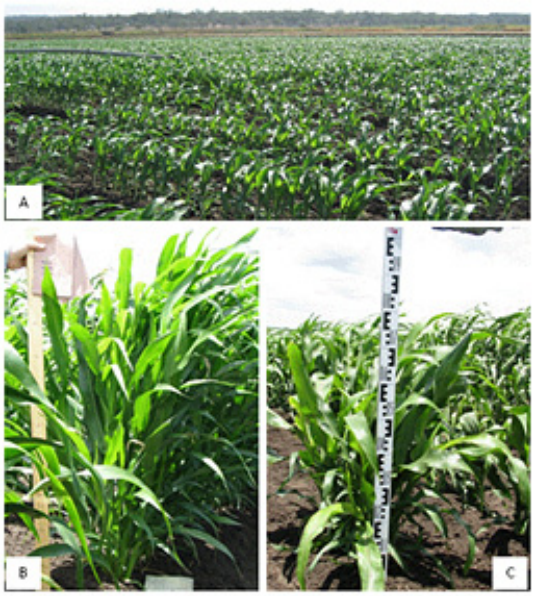

Figure 2. Sorghum mutant lines growing in the field in Queensland, Australia (~9 weeks old) (A) The M5 generation of selected mutant lines of specific interest, including the tcd1 and acdc1-3 mutants, growing in the field for seed collection to be used in future experiments. (B) acdc1 mutant ~0.8m tall. (C) tcd1 mutant ~0.6m tall.

While environmental factors such as drought and high nitrogen may increase the dhurrin content in adult sorghum plants to toxic levels, preliminary experiments indicate that dhurrin production is not induced by drought in any tissue of the *tcd1* mutant and not in the shoot tissue of adult *acdc* plants. The accumulation of dhurrin at normal levels in young *acdc* plants but not in adult tissue suggests differential regulation of dhurrin synthesis or breakdown at various developmental stages. The availability of the *acdc* mutants may assist in dissecting the molecular pathways

regulating cyanogenesis in response to developmental and environmental signals.

Conclusion

In this study, we used the combination of a biochemical screen and a TILLING approach to successfully identify sorghum plants in which cyanogenesis has been substantially altered without using transgenic approaches. We have produced several viable lines, including acyanogenic or low HCNp lines, as well as lines that accumulate high HCNp in the adult stages. In addition to being of agronomic value, these lines provide an excellent resource for increasing our understanding of the molecular mechanisms involved in cyanogenesis and factors that affect its regulation.

Since the publication of these results, the desired characteristic—the inability of these forage sorghum plants to produce dhurrin—has been introgressed into commercial elite hybrids to provide a benefit to the agricultural industry. Analysis of these introgressed lines are on-going to ensure that the absence of HCNp is maintained and also to ensure a continued selection for positive morphological characteristics.

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Tiptoeing around transgenics

New techniques for manipulating plant genomes are yielding plants touted as nontransgenic. Will that relieve regulatory burden? Emily Waltz investigates.

Last August, it came to light that the US Department of Agriculture (USDA, Washington, DC) has been quietly informing crop trait companies that plants made with certain novel approaches to genetic modification would not require regulatory oversight. In a letter dated 26 May 2010, the USDA informed Indianapolis-based Dow AgroSciences that genetically modified (GM) corn developed using a zinc-finger nuclease (ZFN) technique would fall outside of the agency's authority. Six years earlier, in correspondence dated 24 March 2004, the USDA informed Cibus Genetics in Annapolis, Maryland, that plants made with the company's chimeric DNA-RNA oligonucleotide-directed repair technology would also not warrant review. These letters effectively give a green light for the two companies to begin field trials and commercialize GM plants without further review, much as for new varieties created by mutagenesis or conventional breeding. The letters were retrieved through Freedom of Information Act (FOIA) requests submitted by industry experts and reviewed by *Nature Biotechnology*.

The two techniques exemplify a host of new approaches to creating GM plants that, although not always developed with this intent, may allow companies to avoid burdensome regulations designed for the technology of a previous era. As opportunities to, in effect, tiptoe around regulations have appeared, industry players have begun capitalizing on them. "Every time we get together with companies—particularly small companies—the question is posed: How else can we circumvent these regulations?" says Alan McHughen, a biotechnologist and Jefferson Science Fellow in Washington, DC. "It has always been a question," he says, and now companies are putting their theories into practice.

Clear sailing for targeted mutagenesis

In the US, the USDA's regulatory domain over GM plants arises from decades-old statutes that give it authority to regulate 'plant pests' (as defined in the Coordinated Framework for the Regulation of Biotechnology of 1986, available here: http://usbiotechreg.nbii.gov/). Genes taken from plant pests are commonly used in the construction of transgenic plants. For example, the use of the cauliflower mosaic virus 35S promoter has been commonplace as a means to constitutively activate transgenes, and the plant pest *Agrobacterium tumefaciens* has been the workhorse delivery system for shuttling foreign genes into plant genomes. What's more, non–plant-derived genes have been transferred into plants to confer desired traits (the *Agrobacterium* sp. 5-enoylpyruvylshikimate-3-phosphate synthase gene to confer glyphosate resistance) or for selection purposes (antibiotic-resistance genes). Because the creation of nearly all GM plants thus far has involved these tools, the USDA has maintained its authority to regulate essentially all GM plants (see p. 211).

But advanced technologies are quickly supplanting the old methodologies[1]. The technologies used by Dow and Cibus, for example, fall outside the USDA's authority because neither involves genetic material originating from plant pests. Instead of adding foreign DNA, the companies edit or alter plant genes through site-specific mutagenesis techniques.

In the Cibus approach, chemically synthesized chimeric single-stranded DNA oligonucleotides direct the modification of an existing gene—similar to work pioneered by Eric Kmiec at the University of Delaware with chimeric DNA-RNA oligonucleotides. The oligonucleotides complement plant genes except for a single base pair. When introduced into a plant, the oligo hybridizes with the plant gene, creating a single mismatch; this is recognized as an error and repaired by the plant cell's DNA-repair enzymes using the oligonucleotide as a template. The chimeric oligonucleotide itself is digested by nucleases in the cell within hours, and the plant is left with a gene that codes for a desired trait. Cibus distinguishes its products from those made through more traditional genetic modification. According to Peter Beetham, senior vice president of research at Cibus, "They're not genetically modified and they're not transgenic," a message repeated in videos on the company website. Of course, this all depends on how one defines 'genetically modified'.

The USDA's 2004 letter to Cibus says that the agency has no authority to regulate Cibus' technology, a position that followed an extensive review of the technology by the agency, according to Beetham. The company hopes to market its first product, herbicide-tolerant canola, in the US in 2012, he says. However, the product is pending approval by the Canadian Food Inspection Agency, which regulates the environmental release of plants with novel traits, including those created through biotech, mutagenesis or conventional breeding techniques. It is unclear how the technique would be treated under European regulations; some independent researchers argue that it would be excluded from European regulation because mediating genome changes by means of oligonucleotides is equivalent to mutagenesis, which is not regulated in the EU[2].

Dow's product relies on ZFN technology, another site-specific approach. ZFNs are engineered proteins that can be designed to make DNA double-stranded breaks at specific genomic locations. Using a cell's own repair machinery to repair the break, specific gene modifications can be made. Dow used the technology to delete sections of the gene for inositol-1,3,4,5,6-pentakisphosphate 2-kinase, which catalyzes the final step in phytate biosynthesis in corn seeds. The method reduces the corn's level of phytate, an antinutritional component of feed grain.

The USDA's letter to Dow effectively gives the company a green light to begin experimental field trials and commercialize reduced-phytate corn without further review by the agency. Dow has not yet initiated field trials of the corn and has no plans to do so, according to Brad Shurdut, global lead for regulatory and government affairs at Dow. The company consulted with the USDA provisionally in case it should decide to move forward with development, Shurdut says.

Dow will continue to consult with the USDA on new ZFN products in its pipeline, Shurdut says. The company has many applications for the technology, including some that involve foreign DNA, so some products would be likely

Fast track to market. Rather than the three to ten years normally required for a seedling plum to produce fruit, FasTrack plum lines carrying the early-flowering gene produce fruit less than a year after being planted from seed. (Source: Agricultural Research Service, Washington, DC.)

to fall within the agency's authority. "I don't see zinc fingers being categorically exempt" from regulatory oversight, Shurdut says. "USDA has been very explicit that they will make decisions on a case-by-case basis." Over time, if a particular ZFN application can be shown to make specific, repeatable changes in a plant, Shurdut hopes to see the regulatory burden reduced. "We think that the extent of regulation should depend on the nature of the final product, not on the process employed in its development," adds Garry Hamlin, a spokesperson for Dow.

The USDA's decisions in the Dow and Cibus cases are of great interest to industry players developing these as well as other targeted GM technologies, such as transcription activator–like effector nucleases and meganucleases. "Targeted mutagenesis companies are seeking clarification on whether their products are or are not going to be regulated," says Scott Thenell, a regulatory consultant at Thenell & Associates in Walnut Creek, California, who filed one of the FOIA requests on behalf of a client. "These letters are some of the first examples to help clarify that question," he says. The topic "is of great interest to me and my clients and we have particular views on how products of the technology should be regulated."

Now that the letters have become public, more companies will likely seek similar passes from the USDA for products created with targeted mutagenesis techniques, says Drew Kershen, a law professor at University of Oklahoma in Norman. The increased interest may force the USDA, and other authorities globally, to come up with formal regulatory plans for these techniques. "It's going to force regulators to rethink the rules," he says. "All the regulatory agencies are going to have to face the reality that science is moving on."

Jennifer Kuzma, an associate professor in the Science, Technology and Environmental Policy program at the University of Minnesota in Minneapolis, had also filed a FOIA request for the letters. She says the USDA should be consulting with experts in a more public way before granting exemptions to specific companies. "These decisions are really under the radar," she says. "They're not being debated openly, and that is a concern." She suggests that the agency convene an advisory committee composed of academics and stakeholders to openly discuss the new techniques and how they should be regulated.

The USDA has had to give regulatory passes to some old techniques as well. In July 2011, the agency found that herbicide-tolerant bluegrass, made by Scotts Miracle-Gro of Marysville, Ohio, fell outside of its authority because no plant pests were used. The gene for herbicide resistance, *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase), came from thale cress (*Arabidopsis thaliana*), and other genetic elements came from corn and rice. And instead of using *Agrobacterium* to deliver genes, the company used a gene gun, which blasts DNA into plant cells on pellets made of gold. None of these technologies is new, but Scotts' calculated effort to combine them with the intent of bypassing federal oversight exposed a critical weakness in USDA's regulations.

Stealth genetic engineering

Another USDA decision spurring surprisingly little academic debate is its announcement in June that it would deem the progeny of a new transgenic corn line as "nontransgenic." The line, a type of corn used to increase the volume of female parent seed for hybrid seed corn production, belongs to Johnston, Iowa–based Pioneer Hi-Bred.

In common hybrid seed production systems, inbred male lines of corn pollinate inbred female lines. To prevent the females from pollinating themselves, seed companies physically remove the pollen-producing tassel on the female plants—a labor-intensive part of hybrid seed corn production. Pioneer has created, through conventional breeding, a female parent that cannot self-pollinate. This ensures that the female plant will be pollinated by its male counterpart in the field and eliminates the need for detasseling. But it also puts a wrench into the production of inbred female parent seed. So Pioneer developed a transgenic helper line of seed corn, called a maintainer, to increase female parent seed production. The maintainer line contains a cassette of genes that restores fertility and prevents functional transgenic pollen from being produced. The cassette also includes a color marker gene that makes the seed fluoresce and appear pink under ultraviolet light.

In designated production fields, the maintainer line is planted alongside sterile female parents for pollination. The progeny of the two do not contain the cassette of transgenes, and those progeny go on to be used in hybrid seed corn production. To ensure that none of the progeny going into hybrid production contain the transgenes, the seeds are scanned under ultraviolet light. The company deemed the process 'seed production technology', or SPT.

In June, the USDA approved, or deregulated, the maintainer line, and upon Pioneer's request, deemed the commercial progeny of the corn "nontransgenic." "We are very excited about the way USDA worked with us," says Tracy Linbo, global biotech affairs and regulatory lead at Pioneer. The designation of "nontransgenic" from the USDA is important to Pioneer for international commerce purposes, says Bridget Anderson, a spokesperson for Pioneer. The company would like commercial grain from hybrids produced with the SPT process to be exported without additional regulatory review by importing countries. The company has spoken with regulators in Canada, Japan, Mexico, Taiwan and South Korea, and all have verbally agreed that grain produced with the SPT process is nontransgenic, Linbo says. "We described the science to them," she says. "We want to make sure they know it's not transgenic."

Transgenic or not, the product is still the progeny of genetic engineering, and consumers should be made aware of this, says Michael Hansen, a senior scientist at the Consumers Union in Yonkers, New York. "They are trying to play with terminology," he says. "All these new technologies are ways to weasel around a very narrow definition of transgenic," he says. "I would consider that misleading to the public." Hansen says he reviewed Pioneer's analysis of the efficiency of its SPT process and is concerned that transgenic material could find its way into the seeds. "It's not a foolproof system," he says. His organization plans to alert the public, organic growers and concerned groups in importing countries that SPT seeds are offspring of genetically engineered plants.

Whether or not transgenic material pops up in Pioneer's seeds, consuming the progeny of a GM plant will likely still conflict with the values of those opposed to GM organisms, says Kuzma. "The people who are concerned or more wary of these technologies—my sense is that those people are not going to care that the gene is not in the progeny," she says.

Manipulating flowering

The USDA's ruling on SPT corn may be of particular interest to developers of transgenic early-flowering breeding systems, which yield nontransgenic progeny. In this breeding scheme, plants are genetically engineered to flower early, and once they do, the transgene is outcrossed through conventional breeding.

Scientists at the Agricultural Research Service (ARS) in Kearneysville, West Virginia, a research arm of the USDA, have applied the breeding scheme to plums. The plums are transformed with an early-flowering gene from poplar (*Populus trichocarpa* flowering locus T1, *ptFT1*). The gene shortens the tree's juvenile period to less than a year—a useful trait for tree breeders who would normally have to wait up to six years for the tree to flower and reach the reproductive stage.

Once the plant flowers, it is crossed with nontransgenic varieties with desirable traits, such as disease resistance and fruit quality. Markers are used to pick out those that have

the right traits. "We keep going until we have a population with the qualities that we want," says Ralph Scorza, a research horticulturist at the ARS who is developing the plums. At the end of the breeding process, Scorza and his team select those that do not contain the ptFT1 transgene. Outcrossing ptFT1 is necessary for growing robust trees: if the gene is left in the trees, "they're bushy, and the branches are weepy," Scorza says. "But when the gene is out of there the plants grow fine." Developing a tree that is nontransgenic wasn't Scorza's goal, but rather a consequence of the entire system, he says. "It's a side aspect."

Scorza calls the system "FasTrack," and he sees it as both a means to speed up breeding of new varieties and a research tool to study gene function. Regulators at the USDA on 27 October informed Scorza that plum cultivars resulting from his FasTrack breeding system will fall outside of the agency's regulatory authority, as long as those cultivars do not contain transgenes or pieces of transgenes.

German researchers developing a similar early-flowering breeding program in apple are unsure of how European regulators will view their product[3]. "We were discussing exactly this point at our last project meeting," says Matthias Fladung, deputy director of the Institute of Forest Genetics at Johann Heinrich von Thuenen Institute in Grosshansdorf, Germany. Fladung says his group has asked German and European regulators in an official way whether the apples would be considered GM organisms, and is waiting for a response.

Keeping transgenes down

In a different kind of creative tree-development scheme, scientists are studying chimeric grafting, in which transgenic rootstocks are joined with nontransgenic scions: the branches and upper portions of trees. Through traditional recombinant DNA methods, genes for disease resistance or other useful traits are introduced into a rootstock, and the rootstock is grafted to a nontransgenic scion. The junction is like a skin graft: it is wrapped in tape and kept moist, until the vascular systems of the two ends grow together.

This approach raises the question, Does the transgenic material in the rootstock make its way past the graft junction and up to the branches where edible fruit is produced? Guido Schnabel, a plant scientist at Clemson University in Clemson, South Carolina, is investigating this question in plum trees. In a 2010 study, Schnabel found that *Gastrodia* anti-fungal protein-1 (*GAFP-1*), a gene from an orchid that confers pest and disease resistance in plum tree rootstocks, was not migrating into the grafted shoot or leaves[4]. The resulting plums, then, would not contain the *GAFP-1* transgene. "It would be good to know if the consumer would accept something like this," Schnabel says. But he cautions: "Although our studies have shown no recombinant gene transfer to the canopy, we cannot exclude the possibility that over time that might happen." The transgene may also be transferring at a rate lower than the detection threshold, he says.

All in the family

One way to get around foreign genetic material altogether is to source material for a putative genetic modification from a sexually compatible species, a category called cisgenics or intragenics. J.R. Simplot in Boise, Idaho, has developed a cisgenic potato low in acrylamide, a compound that has been linked to health issues and to bruising, a cosmetic defect. All the genetic material to confer this trait came from potato. "We didn't use transgenic approaches because there was no need to do so," says Caius Rommens, director of R&D at J.R. Simplot. "The most important potato issues, both in terms of food quality and sustainable agriculture, can be solved through cisgenics," he says.

The company has petitioned the USDA to approve the potato, and a decision is pending. Although all the genetic material came from potato, the USDA has regulatory authority over the product because the plant pest *Agrobacterium* was used as a transformation method.

Companies developing cisgenics may also soon get a regulatory break from the US Environmental Protection Agency (EPA). Normally, GM plants with an insect-resistance trait must be reviewed by the EPA as well as the USDA. (Plants containing material harmful to pests, so-called plant-incorporated protectants, are regulated like pesticides.) But EPA is considering exempting cisgenic plants from its review process[5]. In March 2011, the EPA shared a draft of the proposed rule change with two other federal agencies. "The initial steps in rulemaking require vetting with other agencies and costing it out," says Doug Gurian-Sherman, a senior scientist at the Union of Concerned Scientists and a former risk assessor at the EPA. "That is a substantial commitment and is not done unless the agency is very serious about moving forward." If the EPA exempts cisgenics, the USDA is likely to follow suit, he says.

Going the cisgenics route might pay off in the marketplace as well. Educated consumers are more likely to choose cisgenic food over transgenic food, Simplot researchers have found through their own studies and a review of the literature. "The closer we can stay to breeding, the easier," says Rommens. "The consumer prefers genes from inside the species."

Consumers beware?

But foreign DNA isn't the only thing that concerns consumers, say some researchers. "The concern over GM organisms is not restricted to the inserted gene or its presence in the plant," says Hansen at Consumers Union. "There is also concern about the unintended effects that could occur as a result of insertional mutagenesis." As in plant mutagenesis, which is not covered by regulation, many GM techniques can result in random insertions of the transgenic DNA into the host plant's genome, which can cause unintended mutations that often can't be detected. Some of these changes can hang around for generations of plant breeding. "Where the gene is inserted into the genome makes a big difference," adds Gurian-Sherman.

Targeted approaches address that issue to some degree. In their ZFN research, Dow scientists have not, to date, identified unintended changes from the use of the technology in their assessments in their assessments carried out by deep sequencing genes related to the target sequence, which are the most likely to be hit, says Hamlin at Dow. However, "any form of cell reproduction, whether uncontrolled, in-nature or assisted by man, has the inherent ability to introduce random genetic changes," he says. "Biology does not offer any gold standard for flawless reproduction of cells."

But the differences between the targeted approaches and the older, less precise methods of genetic modification may be lost on the masses. "I don't think the public is going to make that distinction," Kuzma says. "People are concerned about choice and access to information and having trust in the people who oversee the regulation of the technology." She notes that the USDA's letters to Dow and Cibus were forced out of the agency by FOIA requests—not the most public-friendly way to go about regulation.

Emily Waltz, Nashville, Tennessee

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Now that the letters have become public, more companies will likely seek similar passes from the USDA for products created with targeted mutagenesis techniques, says Drew Kershen, a law professor at University of Oklahoma in Norman. The increased interest may force the USDA, and other authorities globally, to come up with formal regulatory plans for these techniques. "It's going to force regulators to rethink the rules," he says. "All the regulatory agencies are going to have to face the reality that science is moving on."

Jennifer Kuzma, an associate professor in the Science, Technology and Environmental Policy program at the University of Minnesota in Minneapolis, had also filed a FOIA request for the letters. She says the USDA should be consulting with experts in a more public way before granting exemptions to specific companies. "These decisions are really under the radar," she says. "They're not being debated openly, and that is a concern." She suggests that the agency convene an advisory committee composed of academics and stakeholders to openly discuss the new techniques and how they should be regulated.

The USDA has had to give regulatory passes to some old techniques as well. In July 2011, the agency found that herbicide-tolerant bluegrass, made by Scotts Miracle-Gro of Marysville, Ohio, fell outside of its authority because no plant pests were used. The gene for herbicide resistance, *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase), came from thale cress (*Arabidopsis thaliana*), and other genetic elements came from corn and rice. And instead of using *Agrobacterium* to deliver genes, the company used a gene gun, which blasts DNA into plant cells on pellets made of gold. None of these technologies is new, but Scotts' calculated effort to combine them with the intent of bypassing federal oversight exposed a critical weakness in USDA's regulations.

Stealth genetic engineering

Another USDA decision spurring surprisingly little academic debate is its announcement in June that it would deem the progeny of a new transgenic corn line as "nontransgenic." The line, a type of corn used to increase the volume of female parent seed for hybrid seed corn production, belongs to Johnston, Iowa–based Pioneer Hi-Bred.

In common hybrid seed production systems, inbred male lines of corn pollinate inbred female lines. To prevent the females from pollinating themselves, seed companies physically remove the pollen-producing tassel on the female plants—a labor-intensive part of hybrid seed corn production. Pioneer has created, through conventional breeding, a female parent that cannot self-pollinate. This ensures that the female plant will be pollinated by its male counterpart in the field and eliminates the need for detasseling. But it also puts a wrench into the production of inbred female parent seed. So Pioneer developed a transgenic helper line of seed corn, called a maintainer, to increase female parent seed production. The maintainer line contains a cassette of genes that restores fertility and prevents functional transgenic pollen from being produced. The cassette also includes a color marker gene that makes the seed fluoresce and appear pink under ultraviolet light.

In designated production fields, the maintainer line is planted alongside sterile female parents for pollination. The progeny of the two do not contain the cassette of transgenes, and those progeny go on to be used in hybrid seed corn production. To ensure that none of the progeny going into hybrid production contain the transgenes, the seeds are scanned under ultraviolet light. The company deemed the process 'seed production technology', or SPT.

In June, the USDA approved, or deregulated, the maintainer line, and upon Pioneer's request, deemed the commercial progeny of the corn "nontransgenic." "We are very excited about the way USDA worked with us," says Tracy Linbo, global biotech affairs and regulatory lead at Pioneer. The designation of "nontransgenic" from the USDA is important to Pioneer for international commerce purposes, says Bridget Anderson, a spokesperson for Pioneer. The company would like commercial grain from hybrids produced with the SPT process to be exported without additional regulatory review by importing countries. The company has spoken with regulators in Canada, Japan, Mexico, Taiwan and South Korea, and all have verbally agreed that grain produced with the SPT process is nontransgenic, Linbo says. "We described the science to them," she says. "We want to make sure they know it's not transgenic."

Transgenic or not, the product is still the progeny of genetic engineering, and consumers should be made aware of this, says Michael Hansen, a senior scientist at the Consumers Union in Yonkers, New York. "They are trying to play with terminology," he says. "All these new technologies are ways to weasel around a very narrow definition of transgenic," he says. "I would consider that misleading to the public." Hansen says he reviewed Pioneer's analysis of the efficiency of its SPT process and is concerned that transgenic material could find its way into the seeds. "It's not a foolproof system," he says. His organization plans to alert the public, organic growers and concerned groups in importing countries that SPT seeds are offspring of genetically engineered plants.

Whether or not transgenic material pops up in Pioneer's seeds, consuming the progeny of a GM plant will likely still conflict with the values of those opposed to GM organisms, says Kuzma. "The people who are concerned or more wary of these technologies—my sense is that those people are not going to care that the gene is not in the progeny," she says.

Manipulating flowering

The USDA's ruling on SPT corn may be of particular interest to developers of transgenic early-flowering breeding systems, which yield nontransgenic progeny. In this breeding scheme, plants are genetically engineered to flower early, and once they do, the transgene is outcrossed through conventional breeding.

Scientists at the Agricultural Research Service (ARS) in Kearneysville, West Virginia, a research arm of the USDA, have applied the breeding scheme to plums. The plums are transformed with an early-flowering gene from poplar (*Populus trichocarpa* flowering locus T1, *ptFT1*). The gene shortens the tree's juvenile period to less than a year—a useful trait for tree breeders who would normally have to wait up to six years for the tree to flower and reach the reproductive stage.

Once the plant flowers, it is crossed with nontransgenic varieties with desirable traits, such as disease resistance and fruit quality. Markers are used to pick out those that have

the right traits. "We keep going until we have a population with the qualities that we want," says Ralph Scorza, a research horticulturist at the ARS who is developing the plums. At the end of the breeding process, Scorza and his team select those that do not contain the ptFT1 transgene. Outcrossing ptFT1 is necessary for growing robust trees: if the gene is left in the trees, "they're bushy, and the branches are weepy," Scorza says. "But when the gene is out of there the plants grow fine." Developing a tree that is nontransgenic wasn't Scorza's goal, but rather a consequence of the entire system, he says. "It's a side aspect."

Scorza calls the system "FasTrack," and he sees it as both a means to speed up breeding of new varieties and a research tool to study gene function. Regulators at the USDA on 27 October informed Scorza that plum cultivars resulting from his FasTrack breeding system will fall outside of the agency's regulatory authority, as long as those cultivars do not contain transgenes or pieces of transgenes.

German researchers developing a similar early-flowering breeding program in apple are unsure of how European regulators will view their product[3]. "We were discussing exactly this point at our last project meeting," says Matthias Fladung, deputy director of the Institute of Forest Genetics at Johann Heinrich von Thuenen Institute in Grosshansdorf, Germany. Fladung says his group has asked German and European regulators in an official way whether the apples would be considered GM organisms, and is waiting for a response.

Keeping transgenes down

In a different kind of creative tree-development scheme, scientists are studying chimeric grafting, in which transgenic rootstocks are joined with nontransgenic scions: the branches and upper portions of trees. Through traditional recombinant DNA methods, genes for disease resistance or other useful traits are introduced into a rootstock, and the rootstock is grafted to a nontransgenic scion. The junction is like a skin graft: it is wrapped in tape and kept moist, until the vascular systems of the two ends grow together.

This approach raises the question, Does the transgenic material in the rootstock make its way past the graft junction and up to the branches where edible fruit is produced? Guido Schnabel, a plant scientist at Clemson University in Clemson, South Carolina, is investigating this question in plum trees. In a 2010 study, Schnabel found that *Gastrodia* anti-fungal protein-1 (*GAFP-1*), a gene from an orchid that confers pest and disease resistance in plum tree rootstocks, was not migrating into the grafted shoot or leaves[4]. The resulting plums, then, would not contain the *GAFP-1* transgene. "It would be good to know if the consumer would accept something like this," Schnabel says. But he cautions: "Although our studies have shown no recombinant gene transfer to the canopy, we cannot exclude the possibility that over time that might happen." The transgene may also be transferring at a rate lower than the detection threshold, he says.

All in the family

One way to get around foreign genetic material altogether is to source material for a putative genetic modification from a sexually compatible species, a category called cisgenics or intragenics. J.R. Simplot in Boise, Idaho, has developed a cisgenic potato low in acrylamide, a compound that has been linked to health issues and to bruising, a cosmetic defect. All the genetic material to confer this trait came from potato. "We didn't use transgenic approaches because there was no need to do so," says Caius Rommens, director of R&D at J.R. Simplot. "The most important potato issues, both in terms of food quality and sustainable agriculture, can be solved through cisgenics," he says.

The company has petitioned the USDA to approve the potato, and a decision is pending. Although all the genetic material came from potato, the USDA has regulatory authority over the product because the plant pest *Agrobacterium* was used as a transformation method.

Companies developing cisgenics may also soon get a regulatory break from the US Environmental Protection Agency (EPA). Normally, GM plants with an insect-resistance trait must be reviewed by the EPA as well as the USDA. (Plants containing material harmful to pests, so-called plant-incorporated protectants, are regulated like pesticides.) But EPA is considering exempting cisgenic plants from its review process[5]. In March 2011, the EPA shared a draft of the proposed rule change with two other federal agencies. "The initial steps in rulemaking require vetting with other agencies and costing it out," says Doug Gurian-Sherman, a senior scientist at the Union of Concerned Scientists and a former risk assessor at the EPA. "That is a substantial commitment and is not done unless the agency is very serious about moving forward." If the EPA exempts cisgenics, the USDA is likely to follow suit, he says.

Going the cisgenics route might pay off in the marketplace as well. Educated consumers are more likely to choose cisgenic food over transgenic food, Simplot researchers have found through their own studies and a review of the literature. "The closer we can stay to breeding, the easier," says Rommens. "The consumer prefers genes from inside the species."

Consumers beware?

But foreign DNA isn't the only thing that concerns consumers, say some researchers. "The concern over GM organisms is not restricted to the inserted gene or its presence in the plant," says Hansen at Consumers Union. "There is also concern about the unintended effects that could occur as a result of insertional mutagenesis." As in plant mutagenesis, which is not covered by regulation, many GM techniques can result in random insertions of the transgenic DNA into the host plant's genome, which can cause unintended mutations that often can't be detected. Some of these changes can hang around for generations of plant breeding. "Where the gene is inserted into the genome makes a big difference," adds Gurian-Sherman.

Targeted approaches address that issue to some degree. In their ZFN research, Dow scientists have not, to date, identified unintended changes from the use of the technology in their assessments in their assessments carried out by deep sequencing genes related to the target sequence, which are the most likely to be hit, says Hamlin at Dow. However, "any form of cell reproduction, whether uncontrolled, in-nature or assisted by man, has the inherent ability to introduce random genetic changes," he says. "Biology does not offer any gold standard for flawless reproduction of cells."

But the differences between the targeted approaches and the older, less precise methods of genetic modification may be lost on the masses. "I don't think the public is going to make that distinction," Kuzma says. "People are concerned about choice and access to information and having trust in the people who oversee the regulation of the technology." She notes that the USDA's letters to Dow and Cibus were forced out of the agency by FOIA requests—not the most public-friendly way to go about regulation.

Emily Waltz, Nashville, Tennessee

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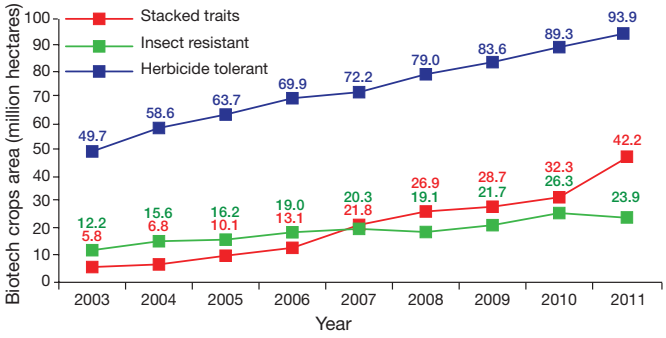
Existing agbiotech traits continue global march

Andrew Marshall

Developing countries grew close to 50% of the world's transgenic crops, with China, India, Brazil, Argentina and South Africa contributing 44%. A total of 16.7-million farmers used transgenic seed last year, up from 1.3 million in 2010. Stacked-trait crops continue to show rapid growth. Very few novel traits were deregulated, but Brazilian authorities did approve a virus-resistant bean. Europe witnessed the lowest number of field trials since 1991, when records began.

Global area by transgenic trait

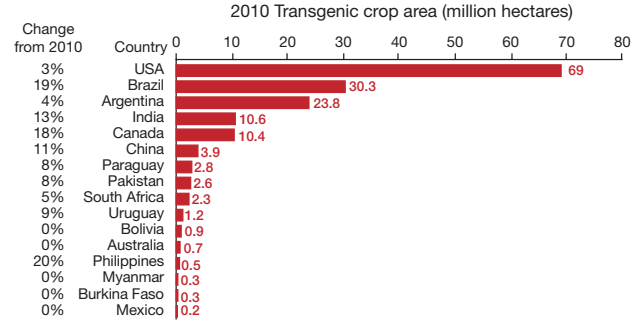
Substantial growth in plantings of crops with two or more stacked traits.



Source: International Service for the Acquisition of Agri-Biotech Applications.

Global area of transgenic crops by country

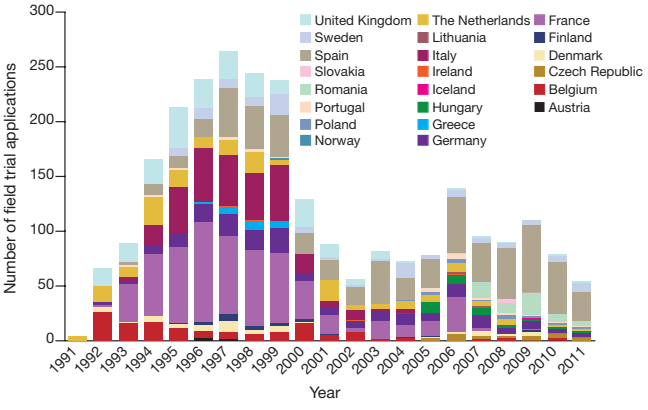
Transgenic acreage expanded rapidly in Brazil, India and Canada, with China close behind and Mexico now outstripping Spain. Turkey imported transgenic crops for the first time.



Source: International Service for the Acquisition of Agri-Biotech Applications.

EU transgenic crop field trials

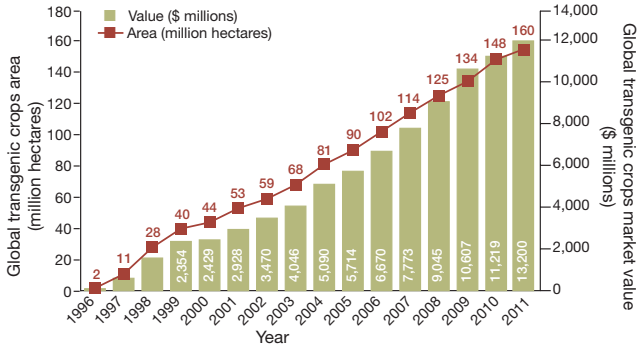
Only 55 field trials in the EU, the lowest number since 1991.



Source: European Commission Joint Research Centre; http://gmoinfo.jrc.ec.europa.eu/.

Historical global area and value of transgenic crops

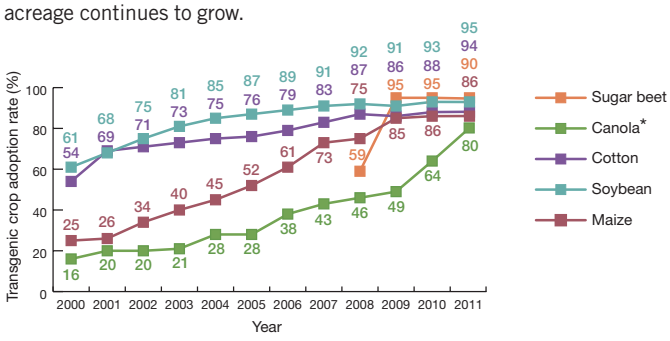
Transgenic acreage grew 8% in 2011, now representing 36% of the global seed market.



Source: International Service for the Acquisition of Agri-Biotech Applications. Value data are explicitly from seeds and licensing revenue rather than from 'crops' themselves.

Transgenic crop adoption rate in the US

Transgenic maize, soybean, cotton and sugar beet consolidated; canola acreage continues to grow.



*Canola adoption rates are based on global data rather than US data.
Source: International Service for the Acquisition of Agri-Biotech Applications; National Agricultural Statitstics Service.

Transgenic crop and/or traits receiving approval

Country	Crop	Company	Decision
US	Maize	Syngenta	MON 87460-4. 'Drought-resistant' transgenic maize line expressing the *Bacillus subtilis* cold shock protein B (*cspB*) and neomycin phosphotransferase (*nptII*) marker
US	Soybean	Monsanto	MON 87705-IR162-4. Transgenic soybean line with stacked traits of improved fatty acid profile and glyphosate resistance. Contains DNA segments to suppress D12 desaturase (*FAD2*) and acyl-ACP thioesterase (*FATB*) genes together with CP4 5-enol-pyruvylshikimate-3-phosphate synthase
Brazil	Bean	Brazilian Agricultural Research Corporation (EMBRAPA)	EMBRAPA 5.1. Transgenic bean resistant to bean golden mosaic virus via expression of a fragment of viral *AC1* gene generating sense and antisense arms.

Source: http://www.aphis.usda.gov/biotechnology/not_reg.html.

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nature biotechnology

Agnostic about agriculture

Averting a global food crisis will require the deconstruction of several hurdles to the deployment of new strategies in plant breeding.

Last October, just 12 years after the 6 billionth person was born, the United Nations declared that 7 billion people now inhabit the earth. Of these 7 billion, close to a billion are chronically undernourished and another billion are malnourished. The world's population will swell to 9 billion in the next 50 years, during which the human race will consume twice as much food as it has since the beginning of agriculture, 10,000 years ago. As the rate of population growth outstrips the rate of yield growth for crop staples, the world faces a food crisis that will require unprecedented intellectual, financial and material investment. It will also require the full deployment of every plant breeding technology currently available, including the generation of crops via transgenesis. But even more importantly, it will necessitate a reemphasis on innovation, greater diversification of the agrochemical and agbiotech industry, streamlining and harmonization of regulatory oversight, and an end to the political grandstanding that has characterized the agbiotech debate so far.

The world's burgeoning population is not the only threat to world food security. Changing lifestyles in developing countries, competition from subsidized biofuels, marginalization of land by soil erosion and salinity, deterioration of natural resources and dwindling of groundwater levels also contribute: not to mention climate change. Meeting these challenges will involve improving local access to resources and good farm practice; enhancing soil, water, nutrient and pest management; providing microcredits; and strengthening local markets, among other measures.

Crop improvement will also be key, necessitating the deployment of the best plant breeding technologies currently available. This issue of *Nature Biotechnology* brings together several articles highlighting how these novel technologies, such as zinc-finger endonuclease genome engineering, oligonucleotide-directed mutagenesis and RNA-dependent DNA methylation, might help in the future. None of those approaches provide a panacea for world food demand, but each may be part of the solution. And yet several factors currently stand in their way.

One obstacle is the level of investment in agriculture R&D. In 2012, the research budgets of the US Department of Agriculture and European Commission under the Common Agricultural Policy are only $2.3 billion and €4.5 billion, respectively… chicken feed compared with the US National Institutes of Health budget of $31.2 billion. Private R&D funding levels are also less than ideal. A December report from the USDA's Economic Research Service (ERS) highlights consolidation in the agrochemical market, which has not only reduced the number of companies in the sector and expanded their individual size but also slowed increases in R&D investment. In 2008, only 30 agbiotech startups were active, with less than one per year founded between 2004 and 2009. None was started in 2008 or 2009. Multinational agrochemical companies account for 70% of total R&D spend in seed biotech, other (non-multinational) seed companies 26%, and agbiotech startups only 4%.

With so little competition in research, it is unsurprising that the output of new traits from the agricultural sector is underwhelming. Of 160 million hectares of transgenic crops planted by 16.7 million farmers in 29 countries last year, most were based on decades-old technology: *Bacillus thuringiensis* (*Bt*)-toxin maize, soybean and cotton, glyphosate-resistant cereals and/or stacked varieties. And although there are new varieties with improved tolerance to biotic or abiotic stresses—a drought-resistant maize strain was just approved in December—these are coming to market at a glacial pace.

Which raises the key problem: regulation. In Europe, since the mid-1980s, regulators have shifted from evidence-based risk assessments to implementation of rules that specifically discriminate against transgenic products and emphasize the precautionary approach. Those rules kick in when a transgene is involved anywhere in crop development, even if the final product doesn't contain foreign DNA. This is all the more disturbing given that regulators are currently trying to assess which additional new plant breeding techniques are captured within this framework (see p. 231). Stateside, the Environmental Protection Agency is proposing expanded rules to codify data requirements for plant incorporated protectants, suggesting that it, too, is moving toward the precautionary principle.

This continued regulatory expansion is perturbing, especially given that current rules were initially instigated only because data on the risks of genetic modification were deemed insufficient. The fact that we now know better seems not to count for anything. There is no scientific uncertainty about whether crops generated via transgenesis are riskier than conventionally produced varieties. They simply are not! And thus regulatory oversight should be reined in, not ramped up.

Overburdensome regulation adds to the time and cost of new crop development—on average, 4 years and €6.8 million per variety in Europe. Paradoxically, it also reinforces the corporate monopolies that many transgenic technology opponents rail against—only multinationals have pockets deep enough to navigate the regulatory system. It also sets a poor example to governments in developing countries that look to the West for guidance on how to implement their own regulatory frameworks. In turn, a lack of clear regulation in developing countries stymies local efforts to bring crops with novel traits to market and spreads uncertainty as to whether products will be excluded from the European market.

Policymakers need to wake up and recognize that the lack of incentives for innovation in both the private and public sectors is compromising the world's ability to combat hunger. As product development can take decades in agriculture, action needs to be taken now to deregulate proven technologies and shift regulation to assessment of the crop traits themselves. Stopgap, Band-Aid solutions will not be enough. When food shortages come—and they will, even to regions where food availability is currently high—coming generations will ask why more was not done to deploy the full range of plant breeding technologies available.

To our knowledge, every claim of a negative consequence to health or the environment from the use of these crops has failed to withstand scrutiny. Indeed, one of the signal benefits of the explosive uptake by farmers around the world, wherever they have been allowed access, is that they have brought life to the vision of the future first articulated by Rachel Carson[11] when she described the new paradigm she hoped for in the relationship between humans and our environment. In 1962, Carson wrote: "A truly extraordinary variety of alternatives to the chemical control of insects is available. Some are already in use and have achieved brilliant success. Others are in the stage of laboratory testing. Still others are little more than ideas in the minds of imaginative scientists, waiting for the opportunity to put them to the test. All have this in common: they are *biological* solutions, based on understanding of the living organisms they seek to control, and of the whole fabric of life to which these organisms belong. Specialists representing various areas of the vast field of biology are contributing—entomologists, pathologists, geneticists, physiologists, biochemists and ecologists—all pouring their knowledge and their creative inspirations into the formation of a new science of biotic controls"[11].

It is not sufficient, then, merely to catalog a handful of innovations in plant breeding technologies that could help magnify farmer's abilities to meet the exploding demands for food, feed and fiber that are foreseen over the next few decades. The size of the challenge means that impediments must not be tolerated, especially if we want to leave land for uses other than humans to live on or raise food—if we want to set land aside for biodiversity, for wilderness. It is imperative that the impediments now obstructing innovations in these critical areas be examined, and those that cannot be justified must be removed[12].

The fact is that the new breeding technologies will make their contributions to improving yield and sustainability primarily as they are integrated with technologies that were on the cutting edge not long ago, but are now quite conventional. These include recombinant DNA technology, as well as *Agrobacterium*- and particle bombardment-mediated transformation, all of which continue to be discriminated against by the European approach to regulation. By any honest reckoning, the level of scrutiny to which crops improved through biotech are subjected is completely unwarranted by the body of knowledge acquired over three decades of experience with such crops, including 15 years in commercial production. This is true around the world, but nowhere is the chasm between regulatory regime and the implications of facts and experience greater than in Europe. Although Europe is sufficiently wealthy to buy its food, the indirect effects of European regulations and attitudes have had a unconscionably inhibitory effect on the introduction of biotech crops in less developed countries in most need of them, particularly on the African continent[13].

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