

Can crop transgenes be kept on a leash?

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Debates about the benefits and risks of genetically modified (GM) crops need to acknowledge two realities: (1) the movement of transgenes beyond their intended destinations is a virtual certainty; and (2) it is unlikely that transgenes can be retracted once they have escaped. Transgenes escape via the movement of pollen and seeds, and this movement is facilitated by the growing number of incidents involving human error. Re-examination of our risk management policies and our assumptions about containment is essential as genes coding for pharmaceutical and industrial proteins are being inserted into the second generation of GM food crops. Even the best designed risk management can be foiled by human error, a reality that is underestimated by most GM crop-risk analyses. Thus, our evaluation of risk should assume that whatever transgene is being examined has a good chance of escaping.

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Genetically modified organisms (GMOs) are now a part of everyday life in the US, with ingredients from GM crops present in the majority of our processed foods (Hopkin 2001). GM crops are also a major feature of our landscape. In 2003, GM crops were grown on 42.8 million hectares within the US alone, an area larger than the entire state of California (James 2003). Genetic engineering promises society everything from crops with improved agronomic and nutritional qualities to frivolities, such as colored lawns and fluorescing pet fish (Figure 1). The possibilities seem to be limited only by our imaginations (Dunwell 1999). Thus far, however, commercially available GMOs have been almost exclusively limited to crops of major economic importance (eg corn, soybean, cotton, and canola), and the commercially introduced traits have been primarily agronomic (eg insect or herbicide resistance; Figure 2).

Different degrees of confinement are warranted for different types of GM crops, depending primarily on the nature of the genetically altered traits and the breeding system of the crops and related species. For those GM

varieties that have been deregulated by the US Department of Agriculture (ie approved for widespread commercial production), confinement is typically not expected. However, there are special cases in which there has been an intent to locally segregate or contain transgenes even for deregulated varieties. For example, some commercially approved GM crops are restricted from being grown in particular states where there are concerns regarding hybridization with weedy relatives; thus, GM cotton can be grown in all states except Florida and Hawaii (EPA 2000). In another well known case, it was assumed that potential risk could be avoided by requiring that seeds remain segregated according to their allowed use; for example, StarLink corn was intended as animal feed but not as human food. The accumulated experiences regarding containment of crops with altered agronomic properties – both before and after their deregulation – provide clear lessons about our ability to contain transgenes.

A second body of evidence regarding containment comes from the last 4 or 5 years of experience with crops that are engineered to cheaply and efficiently produce pharmaceutical and industrial proteins (Giddings *et al.* 2000; see Table 1 for examples of pharmaceutical proteins currently in development). For these varieties there are no realistic expectations of deregulated production – their cultivation will, in all likelihood, forever be limited to “confined” field trials. Examples of the confinement measures for the cultivation of these crops include geographic isolation, scouting for and destroying escaped plants that sprout in subsequent seasons (volunteer plants), and the dedication of equipment for use only on the regulated crop. Inexpensive production of drugs, vaccines, and enzymes would provide benefits to society, but these crops may also represent new risks and they certainly pose new challenges to our ability to contain transgenes while growing plants outdoors.

The issue of containing transgenes has become a flashpoint in the current debate about biotechnology. If trans-

In a nutshell:

- The movement of transgenes beyond their intended destinations is a virtual certainty
- It is unlikely that transgenes can be retracted once they have escaped
- Human error can foil even the best designed strategies for risk management
- Evaluation of risk should assume that transgenes have a good chance of escaping
- The second generation of GM plants includes traits which could put humans, as well as ecosystems, at risk following transgene escape

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Figure 1. These transgenic GloFish illustrate one end of the spectrum for novelty and frivolity of GM organisms that are currently on the market.

genes can be contained, then regulations can be much more permissive about which traits are allowed in crop plants; on the other hand, if transgenes will inevitably escape and spread widely, despite our best intentions (or predictions) of containment, then we need to be much more cautious about what traits are allowed – not only for widespread commercial release, but also in plants that would be grown in small, presumably contained, plots.

Twenty years of accumulated experience with biotechnology provides us with a wealth of examples and evidence that bear on the question of transgene containment. In this review we provide information to support and emphasize two critical points: (1) the movement of transgenes beyond their intended destinations is a virtual certainty; and (2) it is unlikely that transgenes can be retracted once they have escaped. These points support the need for caution in considerations of the release of GM crops.

■ Genes move a lot, and often to unintended places

The movement of transgenes follows many different routes. The most obvious one is via pollen, which can be carried long distances by either wind or pollinators (eg Rieger *et al.* 2002; Chilcutt and Tabashnik 2004). Genes can also escape after a crop has been harvested and plowed under because volunteer and feral crop populations can appear in subsequent years and act as potential sources for the reintroduction of transgenes (Gulden *et al.* 2003). Genes also travel great distances when, knowingly or unknowingly, humans transport crop seeds over huge distances, including between continents.

One of the best documented examples of far-ranging gene spread involves canola (*Brassica napus* L.). Canola has been genetically engineered to tolerate glyphosate herbicide (Roundup Ready canola) and, separately, to tolerate glufosinate herbicide (Liberty Link canola). With

the unconfined commercial release of GM canola in Canada, transgene movement from canola crop to canola crop was predicted (CFIA 1995), but the speed and extent of movement surprised everyone. By 1998, after only two seasons of commercial cultivation of GM herbicide-tolerant canola types in western Canada, volunteer canola plants carrying GM resistance traits were found in many fields where farmers were not intentionally growing these GM varieties (Hall *et al.* 2000). More importantly, even though the original GM canola possessed either glyphosate tolerance or glufosinate tolerance, individual plants of volunteer canola appeared that possessed both forms of resistance.

Hybridization with other species can be an important additional route for transgene escape. Most crops hybridize with non-crop species in at least some part of their global ranges (Ellstrand *et al.* 1999). Some, such as canola, readily hybridize with related weed species, and we should expect many such hybrids to be created every year. Based on pollen dispersal data, distributions of canola fields, and distributions of weedy mustard populations, Wilkinson *et al.* (2003) estimate that tens of thousands of canola–weed hybrids are produced each year in the UK alone (Figure 3).

Hybridization and gene flow are often controlled by age-old agronomic practices for maintaining seed purity. The most common isolation technique depends on geographic isolation combined with buffers and windbreaks grown around field trials of GM crops (USDA 2003a). Unfortunately, isolation can be broken because pollen flow can cross barriers and surprisingly large distances (Rieger *et al.* 2003; Friesen *et al.* 2003). For example, Reiger *et al.* (2003) studied the movement of canola pollen and detected pollen-mediated gene flow nearly 3 km from a source field. In addition, Watrud *et al.* (2004) found that gene flow from GM creeping bentgrass occurred over 21 km from the source.

Because classical isolation techniques do not provide complete containment, genetic engineers have argued that they can devise technological solutions to the problem of gene movement. The three most familiar and feasible technical solutions are (1) transformation of chloroplasts rather than nuclear DNA (Daniell *et al.* 1998), because chloroplasts are primarily maternally inherited in most species; (2) the controversial “terminator technology” in which plants are genetically engineered to produce sterile seeds (controversial because this interferes with farmers’ ability to save seed), and (3) cytoplasmic male sterility, which involves mitochondrial genes that prevent production of functional pollen. The National Research Council (NRC 2004) recently reviewed these and other

bioconfinement tools and concluded that no method is likely to be completely effective. The NRC suggested that rather than relying on bioconfinement, we should put care into selecting host species for certain traits. All GM crops do not warrant the same level of concern. Even among the pharmaceutical crops, there may be certain pharmaceutical proteins that are completely benign to humans and the environment. However, it might be appropriate to restrict plants producing the more high-risk proteins to contained facilities or to disallow their production in food plants entirely (NRC 2004). Clearly, many transgenic proteins will fall in between these two extremes, and the degree of confinement required should depend upon the level of risk to human and environmental health, as well as the risk to public confidence.

■ Human error and transgene movement

Recent experiences with GM crops suggest that containment will inevitably fail, frequently as a result of human error. Examples include accidental commingling of GM with non-GM seeds or food products, accidental release of unapproved transgenes into commercial seed, and the failure of industry and growers to follow USDA protocols for field trials. Yet risk assessment applied to GM crops has tended to overlook the importance and apparent ubiquity of human error and its consequences for transgene escape. A brief summary of a few recent mishaps highlights important issues that should be considered in future risk assessments.

Several major biotech firms were recently fined for violations of safety protocols during field trials of GM crops not yet approved for commercial production. In December 2002, Dow AgroSciences was fined for not establishing proper barriers and windbreaks around a GM cornfield (Gillis 2002a). In that same month, Pioneer Hi-Bred (a subsidiary of DuPont) was fined for growing GM corn in a field that was too near to another corn field, potentially allowing cross-pollination (Gillis 2002a). Pioneer was fined again in March 2003 for failing to report detected contamination among the neighboring corn fields within the allotted time (Gillis 2003a). Similarly, in October 2003, Monsanto was fined for violations that occurred in 2001 field trials of GM corn and cotton (Gillis 2003b).

A disturbing example of human error involved corn genetically modified to produce a vaccine that prevents diarrhea in pigs. In November 2002, the USDA discovered that ProdiGene had failed to comply with federal regulations in two field trials, conducted in Nebraska and Iowa (Gillis 2002b, 2003c). In both locations, ProdiGene failed to destroy volunteer corn plants in the subsequent

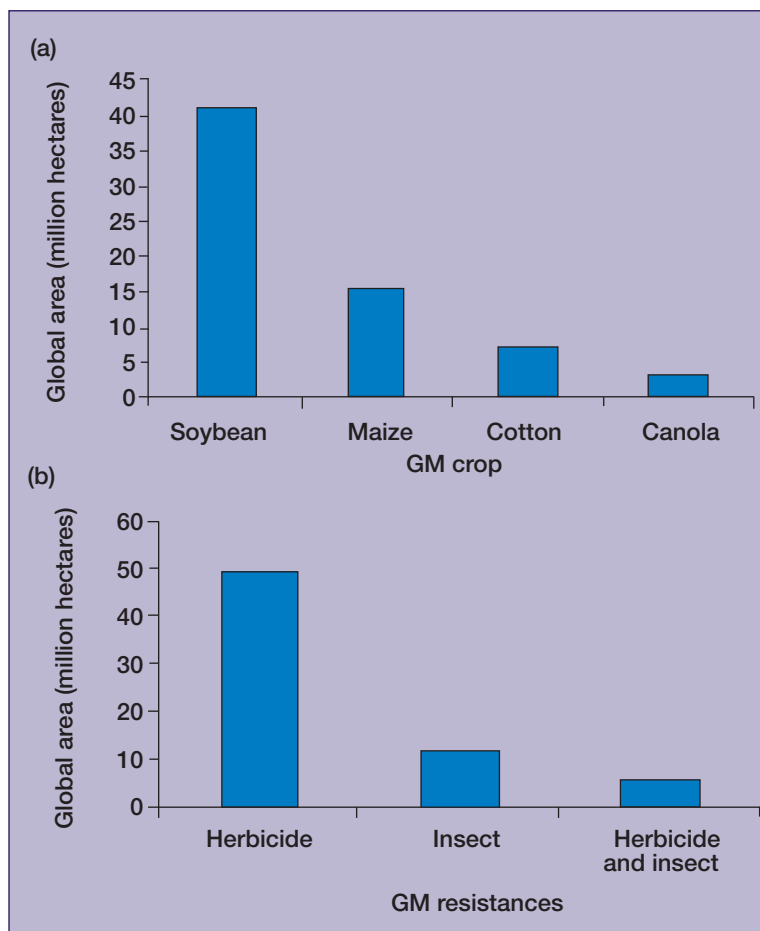


Figure 2. Global area planted with crops genetically modified for improved agronomic properties in 2003. Areas are shown (a) by crop and (b) by trait. Total worldwide area planted with GM crops in 2003 was 67.7 million hectares. Data are from James (2003).

growing season. In Nebraska, the volunteer corn had been shredded and mixed among soybeans at a grain elevator, necessitating the destruction of 500 000 bushels of soybeans. In Iowa, 155 acres of corn surrounding a test site had to be destroyed because of possible contamination, via pollen, from volunteer plants. ProdiGene was fined \$250 000 – one of the largest fines ever levied by the US against a biotech company for a violation of containment regulations.

A particularly puzzling mishap involved transgenes that seem to have escaped industry control and entered the commercial market prior to federal approval (Pollack 2002). In a letter to the USDA (November 9, 2001), Monsanto admitted that small quantities of a non-approved type of GM herbicide-tolerant canola, called GT200, could be present within commercial canola sold in the US. Monsanto requested that the USDA grant retroactive approval of GT200. Although it had never been sold in North America and was not found in commercial canola in the US, GT200 was detected in Canadian canola. Monsanto could not explain how the transgene came to be present in Canadian canola. Aventis CropScience was similarly concerned that some

Table 1. Examples of pharmaceutical crops currently in development

Company/institution	Protein	Crop	Examples of potential uses
Meristem Therapeutics	Lipase	Rice	Supplement digestive enzymes in cystic fibrosis patients and others
	Various monoclonal antibodies	Tobacco	Therapeutic proteins to treat cancer and various infectious diseases
	Human serum albumin	Tobacco	Expand blood volume following severe bleeding
	Lactoferrin	Maize	Defense protein that can treat numerous infections
	Collagen	Tobacco	Wound dressings, tissue engineering and artificial skin, wrinkle and scar treatments
Prodigene	Trypsin	Maize	Protease used in insulin production, cell culture, manufacture of vaccines, and wound care
	Aprotinin	Maize	Protease inhibitor used in cell culture, protein purification, and wound care
Ventria	Lactoferrin	Rice and barley	See above
	Lysozyme	Rice	Small enzyme that attacks the protective cell walls of bacteria; can treat numerous infections
Planet Biotechnology	Antibody to <i>Streptococcus mutans</i>	Tobacco	Prevent tooth decay (<i>Streptococcus mutans</i> is the bacteria that causes tooth decay)
Chlorogen Inc	Human serum albumin	Tobacco	See above
Iowa State University	<i>E coli</i> LT-B subunit protein	Corn	Vaccine against <i>E coli</i> infection

This list is not exhaustive but is meant to illustrate the diversity of pharmaceutical proteins that are in development and the variety of crops that are being used to produce them.

of its GM canola may also have been inadvertently released prior to federal approval.

Seed purity has long been an important issue for agronomists and plant breeders. Recently, Friesen *et al.* (2003) and Downey and Beckie (2002) tested non-GM canola seedlots that were grown in western Canada and found that after only 6–7 years of commercial production of GM canola, the majority of tested seedlots contained at least trace amounts of genetically engineered herbicide-tolerance traits. In fact, 97% (32 of 33) of the seedlots tested by Friesen *et al.* (2003), and 59% (41 of 70) of the seedlots tested by Downey and Beckie (2002) had foreign transgenes present at detectable levels (above 0.01%). The contamination could have resulted from inadvertent mechanical mixing of certified seedlots during harvest or handling, or contamination (possibly from pollen-mediated gene flow) occurring in earlier generations of pedigreed seed production (ie Breeder or Foundation seed). This high level of contamination in pedigreed seed is noteworthy and disturbing because it shows that even stringent segregation systems were not sufficient to deliver pure non-GM canola seed to farmers in western Canada.

Finally, a probably harmless, yet embarrassing human error was committed by researchers at the University of California (UC). It was recently discovered that the Charles M Rick Tomato Genetics Resource Center at UC Davis had unknowingly distributed seeds of tomatoes containing an approved GM trait to researchers in 14

countries over the past 7 years (Lee and Lau 2003). At least one research project was derailed by receiving this seed. The university has been attempting to recall the approximately 30 seed samples, and has apologized to the recipients.

Although in isolation none of the above examples are terribly alarming, taken together they reveal a worrisome pattern; smart, highly trained, and conscientious people make mistakes, and those mistakes may be repeated and go unnoticed for years. Moreover, although most field trials are performed properly, the rules established to prevent the spread of transgenes from experimental GM varieties are occasionally neglected. The door for transgene escape is occasionally flung wide open. These and similar incidents should serve as a wake-up call to industry, to regulators, and to the public. Transgene movement beyond their intended destination is, for all practical purposes, a foregone conclusion. Unless regulatory oversight and enforcement are improved, containment will fail (Taylor and Tick 2003). As a result, regulatory policies that assume risk control is possible through containment should be re-examined.

■ No turning back

Unlike most of the agricultural technologies introduced in the past, the decision to introduce transgenic crops on a broad scale may be irreversible. Even persistent pesti-

cides will eventually break down if we simply stop using them, but models from theoretical population genetics suggest that transgenes can persist in the environment for very long time periods. Transgenes that have a selective advantage (eg resistance traits for a herbicide that is used frequently) can easily persist in a gene pool for many generations (Van Acker *et al.* 2003). Even selectively neutral or slightly detrimental genes can persist for long periods, especially if gene flow is ongoing (Ellstrand *et al.* 1999). Although there have been no field experiments that directly assess whether we can remove or recall transgenes once they have escaped into natural gene pools, two lines of evidence suggest that it would be extremely difficult to perform a recall once a transgenic organism becomes widespread. First, although the presence of transgenes will not necessarily make a plant more invasive or harmful, the many failed attempts to eradicate non-native species should alert us to the potential difficulty of eradicating living organisms in general, once they are released or have escaped into the environment. Efforts to eradicate non-native species are often prohibitively expensive and typically involve spraying large quantities of highly toxic compounds that also affect non-target species, including humans (Myers *et al.* 2000). The US, for example, spends about \$45 million each year to control a single non-native plant, purple loosestrife (*Lythrum salicaria*; Pimentel *et al.* 2000). Despite these efforts, purple loosestrife continues to spread rapidly and is now present in 48 states (Pimentel *et al.* 2000).

Second, we need only look to the well known story of StarLink corn to see just how hard it can be to recall a transgene once it has become widespread. Engineered to express insecticidal cry9 protein, StarLink corn was approved for animal feed but not human consumption. It did not, however, remain segregated – in 2000, cry9 was discovered in a wide variety of processed foods. Despite a massive recall of food products and extraordinary efforts to recover StarLink seed, the cry9 transgenes still persisted at detectable levels in US corn supplies 3 years later (USDA 2003b). The lingering presence of StarLink demonstrates that once a transgene makes its way into the general food supply, it may take many years and enormous effort to get rid of it (Figure 4). In contrast to



Figure 3. A field of canola (*Brassica napus L*) in bloom.

StarLink corn, it is expected that transgenes coding for pharmaceutical and industrial proteins will never become widespread in the first place. However, the StarLink experience shows that should there ever be a massive containment failure, by which an undesirable transgene somehow manages to gain a strong foothold in the seed supply, it may not be possible to subsequently eradicate that gene.

Even with minimal uncertainty regarding the human and environmental safety of GM traits, it would generally be prudent to maintain some non-GM seed lineages for cases where we want to establish cropping systems that are free of GM traits. Unfortunately, recent tests performed on traditional seed varieties of corn, soybeans, and canola in the US, and of canola in Canada, have found pervasive transgenic contamination (Friesen *et al.* 2003; Mellon and Rissler 2004). Although the levels of contamination were generally low (typically less than 1%

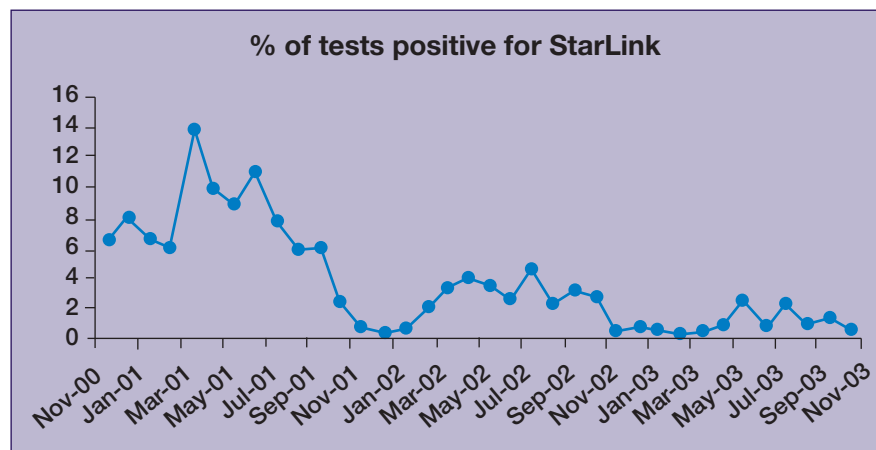


Figure 4. Persistence of StarLink transgenes in US corn. Because the tests for StarLink are performed on a voluntary basis and not on a statistical sampling of the total US corn crop, the values may not accurately reflect the overall levels of contamination. What is important to note, however, is the lingering presence of StarLink in the samples, despite extensive efforts to recall the transgene. Data from USDA (2003b).



Figure 5. With increased soil cultivation (tillage) comes increased risk of erosion – a trend that can be slowed and possibly reversed by the adoption of no-till practices.

of individual seeds contain transgenes, although levels of contamination were occasionally much higher), the findings have some unsettling implications for the future. Contamination such as that uncovered by a Union of Concerned Scientists study (Mellon and Rissler 2004) shows just how hard it may be to obtain GM-free seeds in the future.

■ Does transgene escape matter?

It is not certain whether escape of transgenes, in and of itself, constitutes risk, but escape does enhance the possibility of risk. A particularly well-documented demonstration of possible environmental consequences comes from Snow *et al.* (2003). Commercial sunflowers readily hybridize with weedy sunflowers. Snow and colleagues showed that if a transgene coding for an insecticidal compound moves into weedy sunflowers, the weeds experienced reduced herbivory and produced more seeds. Thus, a problem weed could be made even worse by transgene escape.

Concerning human health, GM foods that are currently on the market do not appear to have caused any great harm, but a recent review by Pryme and Lembcke (2003) highlights a striking lack of published, independent studies examining effects of GM food and feed on mammals. In contrast to commercialized varieties, there could be serious health consequences if transgenes coding for certain pharmaceutical and industrial proteins were to escape into crops being grown for food or feed – this potential for harm is precisely why the USDA requires strict confinement measures during the cultivation, processing, and transport of these varieties.

Movement of deregulated transgenes into non-GM crop fields can also have important effects on broad agricultural practices. The clearest example of this concerns reduced tillage farming. In reduced tillage cropping systems, non-selective glyphosate herbicide (Roundup) is

used instead of tillage to control weeds prior to crop seeding. Reduced tillage provides substantial and measurable economic benefits to farms, in addition to broader environmental benefits (Lafond *et al.* 1992; McRae *et al.* 2000; Derksen *et al.* 2002; Agriculture and Agri-Food Canada 2003; Figure 5). Although the adoption of reduced tillage practices was well underway before GM glyphosate-resistant crops (eg Roundup Ready canola and soy) were first introduced in Canada, the adoption of Roundup Ready crops has facilitated further adoption of reduced tillage practices. Unfortunately, the widespread movement of transgenes conferring glyphosate resistance now threatens the viability of reduced tillage practices. The ubiquitous appearance of Roundup-tolerant volunteer canola in western Canada

(Friesen *et al.* 2003) makes glyphosate a selective herbicide; reduced tillage farmers (even those not growing Roundup Ready canola) must now add a second herbicide to the pre-seeding glyphosate treatment. This adds cost and reduces the economic feasibility of reduced tillage cropping. It also adds herbicide load on the environment (Van Acker *et al.* 2003).

Finally, transgene escape has important implications for those farmers and organizations that are hoping to avoid or minimize the occurrence of GM traits on their land or in their crops and thus, for food processors and consumers who wish to keep certain traits out of food products. If a farmer wants to be certified as organic, or a food provider wants to reassure the public that some food is GM-free, there must be frequent testing and discarding of contaminated seed or grain lots. All this will cost money and create additional costs for entire production systems; currently, where the service is available, genetic purity testing for individual seedlots costs approximately \$500.

■ GM crops on the horizon

Although widespread planting of transgenic crops such as herbicide-resistant canola may have environmental consequences, it is typically thought that human health is not at risk. This may change if pharmaceutical and industrial crop production becomes widespread. In 2002, the USDA approved 20 permits for field trials (130 acres on 34 sites) involving plants engineered to produce pharmaceutical proteins (USDA 2003a; see also USDA 2005). A great deal of attention is paid to developing and enforcing confinement protocols for the production of pharmaceutical crops. However, the history of mistakes with previous GM plants suggests that similar mistakes could occur with pharmaceutical crops, where the consequences of lost containment may be more dire. The National Research Council (2004) states that an “organism that is typically grown to produce a common and widespread

food product probably would be a poor choice as a precursor for an industrial compound, unless that organism were to be grown under stringent conditions of confinement. Alternative non-food host organisms should be sought for genes that code for transgenic products that need to be kept out of the food supply.” However, of the over 200 field trials conducted to date for GM crops producing pharmaceutical or industrial products, over 75% involved corn – a wind-pollinated and out-crossing food crop (UCS 2003). Moreover, even *Nature Biotechnology*, a journal that most would label as pro-biotechnology, challenged the wisdom of using corn for pharmaceutical production. The editorial board of *Nature Biotechnology* (2004) wrote: “It seems that an industry in which the PhD is the intellectual norm is either incapable of learning a simple lesson from the past or cannot bring itself to act appropriately, despite what it has learned previously”. Pharmaceutical crops make the issue of containment and human error a matter of public health as well as an environmental concern.

■ Conclusions

GM crops are here to stay, and they may produce numerous societal benefits, including inexpensive production of drugs and more nutritious foods. Advocates of biotechnology often point to the precision of the technology and our mastery of DNA as reassuring. Perhaps it is, but we will never be able to master and fully eliminate human error. The inevitability of mistakes, and therefore of transgene escape, must be factored into our policies, regulations, and risk assessments for GM plants. There is therefore a pressing need for the development of models that simulate the entire lifecycle of those transgenic crops that do require confinement – from the time when seeds leave a company’s custody until they are planted in a field, harvested, processed, and successfully shipped to a processing facility. These models should include detailed information about all possible routes for transgene escape, including biological processes such as long-distance pollen dispersal, seed movement by animals, and viability of seeds following consumption by animals. Just as importantly, these models must include routes of transgene escape that result from human error, such as a failure to perform appropriate scouting for volunteers, inadvertent mixing of GM and non-GM products, inadequate cleaning of equipment, and violations of procedures for chain of custody. There are many possible points at which containment could be breached, and data are needed to estimate probabilities at each of these points. These models, if properly parameterized, should help regulators to identify where to build in redundancies to improve confinement, and many of these redundancies will need to be aimed at human error. However, we must keep in mind that even the best designed risk management with redundancy and enforcement can still be foiled by human error. We should not have confidence in our ability to keep GM

plants on a tight leash. Rather, total containment can never be assured or assumed, and our evaluation of risk should be predicated on the idea that transgenes always have some chance of escaping. Re-examination of our risk management policies and of our assumptions about containment is essential as we move into the second generation of GM crops, some of which will have the potential for serious adverse effects.

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