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Terminator: The Sequel

Despite the fact that governments re-affirmed and strengthened the United Nations' moratorium on Terminator technology (a.k.a. genetic use restriction technology [GURTs]) in March 2006, public and private sector researchers are developing a new generation of suicide seeds – using chemically induced "switches" to turn a genetically modified (GM) plant's fertility on or off.

Issue: Under the guise of biosafety, the European Union's 3-year Transcontainer Project is investing millions of euros in strategies that cannot promise fail-safe containment of transgenes from GM crops, but could nonetheless function as Terminator, posing unacceptable threats to farmers, biodiversity and food sovereignty. Terminator technology – genetic seed sterilization – was initially developed by the multinational seed/agrochemical industry and the US government to maximize seed industry profits by preventing farmers from re-planting harvested seed. Researchers are also developing new techniques to excise transgenes from GM plants at a specific time in the plant's development, and methods to kill a plant with "conditionally lethal" genes. This new generation of GURTs will shift the burden of trait control to the farmer. Under some scenarios, farmers will be obliged to pay for the privilege of restoring seed fertility every year – a new form of perpetual monopoly for the seed industry.

Impact: Whether intended or not, new research on molecular containment of transgenes will ultimately allow the multinational seed industry to tighten its grasp on proprietary germplasm and restrict the rights of farmers. Industry and governments are already working to overturn the existing moratorium on Terminator technology at the UN Convention on Biological Diversity (CBD). In the months leading up to the CBD's 9th Conference of the Parties (Bonn, Germany 19-30 May 2008), industry will argue that global warming requires urgent introduction of transgenic crops and trees for biofuels – and that Terminator-type technologies offer a precautionary, environmental necessity to prevent transgene flow. Ironically, society is being asked to foot the bill for a new techno-fix to mitigate the genetic contamination caused by the biotech industry's defective GM seeds.

Players: Taxpayer-financed research on biological containment of GM crops subsidizes the corporate agenda. A handful of multinational seed corporations control biotech seeds and the proprietary seed market as a whole has seen unprecedented corporate concentration. In 2006, the world's top 4 seed companies – Monsanto, DuPont, Syngenta and Groupe Limagrain – accounted for half (49%) of the proprietary seed market.

Policy: Governments keep trying to find ways to make GM seeds safe and acceptable and they keep failing. They should stop trying. There is no such thing as a safe and acceptable form of Terminator. The EU should discontinue funding for research on "reversible transgenic sterility," and re-assess funding for other research projects undertaken by Transcontainer. Rather than support research on coexistence to bail out the agbiotech industry, the EU should instead fund sustainable agricultural research that benefits farmers and the public. National governments should propose legislation to prohibit field-testing and commercial sale of Terminator technologies. Governments meeting at the 9th Conference of the Parties to the Convention on Biological Diversity in Bonn, Germany must strengthen the moratorium on GURTs by recommending a ban on the technology.

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Introduction

Responding to almost a decade of popular protest, governments meeting at the UN Convention on Biological Diversity in March 2006 (Curitiba, Brazil) re-affirmed and strengthened the existing moratorium on Terminator technology (a.k.a. genetic use restriction technologies – GURTs).[1] The moratorium recommends against the field-testing or commercialization of seeds that have been genetically engineered to produce sterile seeds at harvest. Terminator technology was initially developed by the multinational seed/agrochemical industry and the US government to prevent farmers from re-planting harvested seed in order to maximize seed industry profits. Suicide seeds threaten food sovereignty because over 1.4 billion people depend on farm-saved seed.

What are GURTs? Genetic Use Restriction Technology is a broad term that refers to genetic engineering technologies that use a number of interactive or interdependent genes in combination with an environmental or chemical inducer (such as heat-shock or ethanol) to switch on or off the expression of a plant's genetic traits. In the case of varietal GURTs, or V-GURTs, the reproductive viability of the entire plant is under the control of the company/institution that sells the seed. T-GURTs, or trait-specific GURTs, are designed to restrict the use of particular transgenes (or events), such as those for herbicide tolerance, insect resistance, and other traits. In the "official" language of the United Nations, the term GURT is used to refer to Terminator.

Despite the fact that governments meeting in Brazil overwhelmingly supported the moratorium on Terminator, researchers in the public and private sector are expanding and refining research on a new generation of GURTs. Current research on biological containment seeks to prevent engineered genetic traits (transgenes) in GM plants from spreading to non-GM plants and wild relatives – a growing problem for the biotech industry and for society. (It is also a major stumbling block in industry's quest to develop pharmaceutical crops and GM trees). However, the same technology that is being developed to prevent the spread of pollen and transgenes from GM plants can also be used to control the plant's reproductive viability and/or prevent farmers from saving and re-planting harvested seeds. This report examines some of the new research to develop molecular systems for controlling transgenes that are simultaneously advancing Terminator 2.0.

In the US and Europe, taxpayer money is being used to develop a new suite of sophisticated molecular technologies to solve the biotech industry's contamination problem. Society is being asked to foot the bill for a new techno-fix to mitigate the genetic contamination caused by the biotech industry's defective GM seeds. Under the guise of "environmental security" for GM crops, industry will use the new generation of Terminator technologies to tighten its grasp on proprietary germplasm and biologically restrict the rights of farmers to re-plant harvested seeds. Under some scenarios, farmers will be obliged to pay for the privilege of restoring seed fertility every year.

The first generation of Terminator patents (late 1990s) described molecular techniques that were largely theoretical and relatively primitive compared to current research on technically advanced GURT technologies. Today, researchers continue to develop chemically induced "switches" that turn a plant's fertility on

or off, but they are also developing techniques to excise transgenes from a GM plant at a specific time in the plant's development, and methods to kill a plant with "conditionally lethal" genes. The new generation of GURTs aims to shift the burden of trait control to the farmer – requiring her/him to buy a new, proprietary inducer (most likely a chemical) to switch desirable genetic traits – including fertility – on or off. The key point is that viability of the crop would still be controlled by the corporation that sells the seed.

> "One enduring lesson from agricultural biotech is that it is a huge mistake to underestimate biosafety concerns. A corollary is that Nature will always find a way; Murphy's law implies that no matter how unlikely it seems that genes will flow, they eventually will." – C. Neal Stewart, Jr., *Nature Biotechnology*, March 2007[1]

Biotech's Achilles Heel: Today it is generally acknowledged that gene flow from some genetically modified organisms (GMOs) to non-GMO plants and wild relatives has the potential to harm ecosystems or threaten the food supply. GM contamination is the Achilles Heel of agricultural biotechnology. Plants are living organisms that operate in a dynamic, evolutionary context – they are not machines – and neither industry nor government regulators have been able to contain or control GMOs. The stakes are higher today because commercial firms are conducting open-air field-tests of GM plants engineered to produce drugs or industrial chemicals in food and field crops. As far as the public and food industry are concerned, there is zero tolerance for allowing contamination of the human food chain with transgenes from industrial or pharmaceutical crops.

> According to the GM contamination register, from 1996-2006 there were 146 publicly documented contamination events involving 42 countries on six continents.[1]

GMOs can inadvertently contaminate crops and/or food supplies by two major routes.[2] Pollen from GM crops can cross-pollinate with related crop plants or wild or weed relatives nearby. GMO contamination may also occur when transgenic seeds or plant parts physically mix with non-GMO crops during the process of seed production, harvest, storage, transport or processing. GM volunteers – plants that survive in the field from a previous crop season – can also cause unwanted contamination by either pollen or seeds.

In the United States, crops not approved by regulatory authorities for human consumption have contaminated fields, or have been discovered in the food supply. When unauthorized GMOs are discovered in export shipments, some importing governments have denied them entry. These incidents have cost hundreds of millions of dollars in product recalls, lost revenues for farmers, and a giant headache for the biotech industry – which is still struggling to convince wary consumers of the benefits of GM foods. For example, even seven years after the StarLink "taco debacle" in 2000, the biotech and food industries are still testing for the presence of StarLink contamination. StarLink refers to a GM maize variety sold by Aventis (now owned by Bayer) that entered the food supply although it had never been authorized for human consumption. The total cost for StarLink testing and product loss has exceeded a staggering $600 million to date.[3]

In the absence of a global monitoring system, GeneWatch UK and Greenpeace maintain a GM Contamination Register that records publicly documented contamination events.[4]

Ag biotech's future commercial success depends on finding a plausible techno-fix to prevent leaky genes from escaping. If governments can be convinced that biological containment of GMOs is technically possible, it will open the floodgates to new markets for GM crops, and commercial-scale production of GM pharmaceutical plants (plants engineered to produce drugs), and GM industrial crops (plants engineered to produce chemical compounds for industrial use), as well as GM trees. A new generation of GM biofuel crops is also being aggressively promoted as the most efficient route to biofuel production, especially in the global South. ETC Group believes that the rush to plant energy crops will shift marginal land away from food production and adversely affect soil, water, biodiversity, land tenure and the livelihoods of peasant farmers and indigenous peoples.

Who benefits from publicly financed research on biocontainment and Terminator seeds? A handful of multinational seed corporations control the global biotech seed market. With 2006 revenues of $4,028 million, Monsanto – the world's largest seed company – accounts for one-fifth of the global proprietary seed market. The top 3 companies – Monsanto, DuPont and Syngenta – account for $8,552 million – or 44% of the total proprietary seed market.[5] Why should public funds be used to develop a new techno-fix to rescue the Gene Giants' defective technology?

Publicly financed research on biological containment of GM crops subsidizes corporate Gene Giants. Genetic trait control technology – especially genetic switches to turn fertility on and off – will be promoted as an environmental security measure. Industry will argue that its seeds offer an additional level of biosafety protection, and it won't be long before regulators will require that all transgenic crops be engineered to contain fertility switches or other biocontainment technologies – ostensibly to prevent promiscuous transgenes from contaminating related plants or weeds growing nearby.

This report examines three areas of current research to develop molecular systems for controlling transgenes (biocontainment) that are simultaneously advancing Terminator technologies.

1) Recoverable block of function (reversible transgenic sterility)
2) Gene Excision
3) Conditional Lethality Genes

Each section examines how these technologies work and how they may fail. What are the potential implications for farmers and food sovereignty, biodiversity and the environment?

1. European Union's Transcontainer Project: Bringing Terminator Back to Life Using "Zombie" Seed Technology (aka Recoverable Block of Function)

Brussels Sprout-less? Despite the fact that European consumers have overwhelmingly rejected genetically modified foods, the European Commission is supporting a 3-year, €5.38 million (US$6.8 million) "Transcontainer Project" involving 13 research partners – both public and private.[6] Launched in May 2006, the goal of the Transcontainer Project is to develop GM crops and trees for Europe that are "biologically contained" (to prevent the spread of transgenes and the contamination of conventional or organic plants growing nearby). Transcontainer research supports the goal of "co-existence" – the controversial idea that GM crops and non-GM crops can peacefully co-exist (or, that "acceptable" levels of GM contamination can be negotiated). In other words, it is a publicly funded initiative to help the biotechnology industry overcome the European public's rejection of GM foods and crops.

The Transcontainer Project involves multiple research objectives to achieve biological containment.[7] These include, 1) Chloroplast transformation; 2) Controllable Flowering; and Controllable Fertility. Fact sheets describing the research in each of these areas are available on the Transcontainer website.[8]

This report does not attempt to provide an analysis of all the research objectives that fall under the Transcontainer umbrella. Critical analyses of various molecular strategies for biocontainment of transgenes have been analyzed elsewhere.[9] Although several of Transcontainer's research objectives merit a strong critique, in this report we focus on just one of the programme's research objectives: "To develop a seed lethal transgene containment system based on Recoverable Block of Function (RBF) in oilseed rape [canola]." This research is being conducted at the University of Milan, Italy, under the direction of Professor Martin Kater. The RBF system was originally developed by a Finnish research group, UniCrop, Ltd.[10] Kater reports that his group is testing the first constructs in oilseed rape, and that his system "is far more promising" than the Finnish system. The University of Milan researchers are applying for a patent on their new RBF system.[11]

Reversible Transgenic Sterility: The Transcontainer website states that the project's research on reversible sterility of GM crops "will only partially resemble" Terminator because its GM plants will include a mechanism to allow farmers to restore the fertility of the crops and because its purpose is not to restrict the farmers' ability to re-plant proprietary seed. "Reversible transgenic sterility" is a method by which the plant's fertility can be lost or regained by design. Plants are engineered with sterility as the default condition, but sterility can be reversed with the application of an external stimulus that restores the plant's viability. In order for to bring the "zombie" seed back from the dead, the farmer or breeder must use an external stimulus (such as a proprietary chemical) to restore the seed's fertility.

Piet Schenkelaars is a biotech industry consultant who handles all communications for the EU's Transcontainer Project. Given the political sensitivity surrounding Terminator technology, the Transcontainer Project coordinators apparently decided it was important to

hire an information gatekeeper. Responding to questions posed by ETC Group, Schenkelaar maintains that reversible sterility is a benefit for farmers because it could prevent the spread of transgenes in the environment, and prevent volunteer plants in the next growing season.[12] In the real world, however, corporate Gene Giants will use this design to transfer the burden of trait control to the farmer who will be obliged either to buy an external chemical inducer (likely a proprietary product) to restore the seeds' fertility every single generation or buy new seed every season.

In another email vetted by Schenkelaars, the Transcontainer researchers in Milan acknowledge that the practical applications of reversible sterility may be very different when it is adopted by commercial firms: **"We are an academic group who is trying to invent new systems and give proof of concept. When our system is efficient and breeding companies would be interested to use it, they might change the constructs including another induction system."**[13]

The Transcontainer Project addresses the UN moratorium on field trials and commercial use of GURTs, but claims that the Project's research does not run counter to the moratorium because GM crops developed by Transcontainer "will only be tested in laboratories or greenhouses and they will not be tested in the field or commercialised within the scope of the project." In other words, the Project's after-life is up for (corporate) grabs. The Transcontainer website notes that the CBD also encourages further research on the environmental and socio-economic impacts of GURTs – a subject which will be examined by Transcontainer researchers. According to Schenkelaars, studies on the socio-economic impact of the RBF system will be undertaken by Dr. Justus Wesseler at the Wageningen University in the Netherlands.

Table 1: Patents on Reversible Genetic Sterility

Patent / Application #	Assignee	Inventor	Date Published (or Granted) / Filed	Description
EP1303628B1	UniCrop, Ltd. (Finland)	Kuvshinov, Koivu, *et al.*	4 Oct. 2006 / 16 July 2001	DNA construct for controlling transgene segregation and escape in a sexually reproducing transgenic plant comprises a recoverable block of function system
WO06005807A1	UniCrop, Ltd.	Kuvshinov, Koivu, *et al.*	19 Jan. 2006 / 5 July 2005	Use of phytoene synthase for controlling transgene escape
US6849776	UniCrop, Ltd.	Kuvshinov, Koivu, *et al.*	1 Feb. 2005 / 14 July 2000	Molecular control of transgene segregation and its escape by a recoverable block of function (RBF) system
US20050039229A1	UniCrop, Ltd.	Kuvshinov, Koivu, *et al.*	17 Feb. 2005 / 15 July 2004	Double recoverable block of function
WO0206498A1	UniCrop, Ltd.	Kuvshinov, Koivu, *et al.*	24 Jan. 2002 / 16 July 2001	Molecular control of transgene segregation and its escape by a recoverable block of function (RBF) system

How do "Zombie" seeds work?

What is Recoverable Block of Function? Recoverable Block of Function (RBF) to create reversible seed lethality is a genetic engineering scheme whereby genes are put into the crop plant in order to cause seeds to fail to germinate, thus preventing seeds from passing on their genes to the next generation. The purpose of seed lethality in this system, according to Transcontainer, is to block the germination of seeds that bear genetically engineered traits (such as herbicide tolerance, insect resistance, pharmaceutical production, etc.). In the RBF system, the gene causing lethality would be physically linked to the "genetic trait of interest" – on the same piece of DNA – so that they are inherited together. The gene causing lethality, including its promoters and other regulatory elements, is called the *Blocking Construct* (BC).

Having a BC alone is a dead end, literally. Breeders would be unable to multiply seeds, and farmers would be unable to re-plant harvested seeds. So another piece of DNA containing a gene that makes a product capable of canceling lethality is also engineered into the crop. This is called the Recovering Construct (RC) – and it's what makes it possible to bring the seed "back from the dead." The RC can be turned on when desired, using an environmental or chemical trigger, for example. Some of the RBF systems are triggered by heat shock, alcohol applications or antibiotics. Ethanol is the trigger currently being developed for oilseed rape by Transcontainer researchers.[14] If the RC is not intentionally turned on, the BC will act alone and seeds will not germinate. Thus the default position for RBF is seed death. A breeder or farmer must take a specific action to recover seed viability and the action must be repeated in every generation to maintain seed viability. Although the RBF system itself may not be designed with the intent to restrict seed use *per se*, it does so nevertheless.

Can genes escape through pollen? For crops such as oilseed rape/canola where the seed is the harvested part, robust fertilization is required, so functional pollen is necessary. Pollen of a seed-lethal RBF variety is capable of normal fertilization of the ovules within the crop, and also of any sexually compatible varieties or wild relatives it contacts. This is because the lethal factor is designed for production at the end of seed development and/or during germination and is "off" in the rest of the plant, including pollen. The RBF genes are present, and will be transferred to any seed fertilized by the RBF pollen. As these seeds develop, the BC genes will activate when the seeds mature and germinate, and thus there should be no further gene escape because contaminated seeds will die unless rescued by activating RC. If it functions as designed, the RBF system would thus prevent the spread of engineered traits beyond a single generation. However, that does not rule out the spread of negative impacts. For example, if RBF seed cross-pollinates with sexually compatible plants growing in a neighboring farmer's field, the progeny of those plants will fail to germinate when re-planted. The unsuspecting farmer would have no way of knowing that a percentage of her/his seeds will be sterile until those seeds failed to germinate after being planted. Even if cross-pollination occurs infrequently, the negative impacts could be significant. For small farmers, a small loss may be their profit margin. For wild plants, a small loss might mean extinction in a marginal habitat, and could also endanger animals depending on the plant for food.

There are many possible configurations of the RBF system.[15] Some might offer a higher degree of biological containment through the seed than others. However, all have in common a few characteristics that merit public concern.

First, the BC makes something that is lethal to seeds. In some cases this will be a toxin from a bacterium, fungus or other source. Will this toxin enter the food supply, and how will it be evaluated for health effects? The current techniques and standards for testing GM crops are inadequate to establish unequivocally the safety of these toxins in food for humans, much less for wild animals that may depend on the margins of agriculture for their survival.

The same concern applies to the RC, if the farmer or breeder restores the fertility of his/her seeds using the necessary stimulus. The seeds will contain the product of the recovery gene, and it will be part of the food supply, so it will also need to be evaluated for safety.

Whether the aim is to restrict seed use or not, the result of RBF is that the burden will be placed on the farmer to apply an external inducer to restore seed fertility – of the right strength, at the right moment in the growth of the crop – and this will have to be repeated every generation.

Because the seeds of that generation – and that generation only – would be rescued if the RC were activated, the whole seed-lethality system should remain intact if those seeds are planted: The BC would still cause the next batch of seeds to die, and the RC would still be present and could be activated by the inducer again. Although this feature theoretically functions for the purpose of biocontainment, it simultaneously strengthens the Terminator properties of RBF.

Recoverable block of function is a V-GURT Terminator, where the reproductive viability of the entire plant is under the control of the company/institution that sells the seed. It obliges the farmer to do something specific in order to have fertile seeds to replant. The Finnish scientist who pioneered the RBF system in the 1990s, and whose work is being further developed by Transcontainer researchers in Milan, classifies his work as a V-GURT technology.[16] A 2004 report by the US National Research Council also refers to RBF as a V-GURT.[17]

Box 1: Why Would Farmers Choose to Buy Terminator or Zombie Seeds?

It may seem strange that farmers would knowingly buy sterile seed when it will mean increased cost every season – farmers will have to buy new seed or buy and apply the chemical required to restore seed viability. The biggest seed companies will do whatever they can to get farmers on the sterile-seed platform, however, because it enables them to reduce competition and capture a bigger share of the market. Gene Giants will coerce farmers to choose sterile seed by ensuring that the latest technology (i.e., genetic traits) is available only on that platform. The companies will even acknowledge – as a selling point for sterile seed – the very real threat of GM contamination and the need to contain gene flow. Sterile seed platforms will be promoted (and in some cases required) as safer, more responsible seed technology. The Gene Giants may keep prices low, initially, as an extra enticement. Once farmers are on the platform and the competition has been destroyed, companies can start pricing the seed (in the case of Terminator) or pricing the chemical that restores seed viability (in the case of Zombie) as high as they want. Zombie is a dream scenario for the Gene Giants because it will be cheaper for them to sell farmers an industrial chemical for bringing seeds "back to life" (rather than pay the seed-multiplication, warehousing and distribution costs required to sell new seed every planting season). Ironically, the Gene Giants will argue that availability of multiple sterile-seed platforms offers more "choice" to farmers!

How Zombie Technology May Fail

There are several potential weaknesses in the RBF system that could affect the functioning of the BC or the RC.

EcoNexus, a public interest research organization based in the UK, observes that the use of a V-GURT system for gene containment "can only be as good as its weakest parts," and "none of the components tested for any of the possible V-GURTs systems are 100% reliable or effective."[18] In a 2006 briefing submitted to the Convention on Biological Diversity, EcoNexus and the Federation of German Scientists provide a detailed review of inherent weaknesses in the design and performance of V-GURTs – all of which apply to RBF technology. Readers who wish to review these issues in more detail should refer to this document: *V-GURTs (Terminator Technology): Design, Reality and Inherent Risks.*[19]

Potential problems with Transcontainer's RBF system include, for example:

Insufficient inducer: Activation of the recovery function is likely to be the least reliable step in the RBF process. Problems include incomplete penetration of the inducer into seed tissues; issues of the inducer changing the ecology of the crop (ethanol is toxic to many microorganisms, for example); and weather causing delays that could

result in missing the window for activating the Recovery Construct (RC). **Gene silencing** can cause interference with the expression of transgenes in a GM plant (especially under stress conditions) and unexpectedly change the traits or behaviour of the organism. In the case of RBF, for example, silencing of the blocking construct (BC) could disrupt the RBF system and result in viable seed (allowing transgene escape).

Segregation of the different genetic components: The GM "trait of interest" and the RBF genes must remain in close proximity, on the same strand of DNA, and remain linked during reproduction. If any of the transgenes involved in the RBF system were to become separated from each other, the system would fail. If segregation occurs, transgenes could escape and the system failure would be difficult to detect.

A 2005 paper by the Finnish researchers who first developed reversible transgenic sterility provides a glimpse of the state of the art for RBF, and points to many of its potential weaknesses, including gene silencing, mutations and segregation.[20] In order to avoid potential problems that could result in seed viability and possible escape of transgenes, the researchers engineered the RBF system in tobacco to contain double "back-up" systems. A discussion of this paper, and the technical challenges of designing a foolproof containment system using RBF, is discussed in more detail in the Appendix.

Box 2: Transcontainer's Support for Zombie Seed Technology (RBF) is a winning strategy for corporate seed industry

1. Public funding for Zombie Seed technology subsidizes the corporate research agenda. Transcontainer aims to promote public acceptance of GM crops. It also opens the door to an oxymoronic Terminator – a kinder, gentler and environmentally safe suicide seed.

2. The burden of genetic trait control will be transferred to the farmer, who will be obliged either to buy an external chemical inducer (likely a proprietary product) to restore the seeds' fertility every single generation or buy new seed every season. The profit-making appeal of the Zombie approach could prove irresistible for the Gene Giants: in the future, farmers could be *encouraged to save harvested seed* and rely on the application of a chemical inducer to restore fertility – a scenario that would reduce industry's seed multiplication and transport costs. Ultimately, it would mean lower costs and greater profits for the seed company.

3. By claiming that Zombie seed technology (RBF) means a high degree of environmental security for GM crops, industry and governments hope to win new markets for biotech – including pharma and biofuel crops and GM trees. This is a concern because molecular systems for controlling transgenes will not be failsafe – and may introduce additional biosafety hazards. To function as a viable containment system, Zombie seeds must meet the virtually zero contamination standard to prevent contamination of the food and feed supply (and to prevent contamination of related wild relatives and weeds in the environment).

4. Although the Transcontainer Project's research is limited to European crops, the reality is that any advances in genetic trait control technology will ultimately be adopted indiscriminately to further commercial, proprietary goals. If RBF-Terminator systems linked to seed lethality are commercialized, farmers everywhere (including the global South) will be obliged to pay for the privilege of restoring seed fertility.

The Bottom Line on Zombie Technology

RBF systems linked to seed lethality are clearly V-GURTs because the seeds are genetically programmed to die during development or germination. Breeders and farmers can recover viable seeds by taking some action pre-ordained by the design of the RBF system, and this recovery action must be undertaken in every generation to maintain seed viability. The RBF system has many potential weaknesses because of the complexity involved in making both the Blocking Construct and the Recovery Construct in a living plant virtually failsafe.

A system that would require farmers to restore fertility of their crops year after year undoubtedly seeks to increase seed industry profits by assuring repeat business (either for the chemical inducer or new seeds). Although Transcontainer researchers in the EU claim that the RBF system is not designed to restrict seed use *per se*, it does so nevertheless. It is important to keep in mind that RBF will be linked to a proprietary engineered trait, which will also be protected by patents. The act of saving and re-using RBF seeds will therefore be restricted by legal as well as biological mechanisms.

The EU Transcontainer Project is advancing research on a V-GURT system. If the project succeeds in convincing government regulators that RBF is a viable strategy for containing transgenes, the researchers will have developed a Terminator technology using public money. If RBF doesn't work precisely and consistently but ends up in the field, anyway, the Project will have paved the way for unprecedented levels of contamination (involving GM biofuel, pharma and industrial crops), with potentially disastrous results for biodiversity, the environment and human health. For Transcontainer researchers to divorce themselves from this reality is to ignore the real world implications of their research and the reality of corporate controlled biotech seeds.

2. The Exorcist: Technologies to delete transgenes from GM plants

The biotech industry is increasingly interested in gene excision (that is, cutting out or deleting transgenes) as a strategy for both biocontainment and restricting access to proprietary germplasm. Recent publications and patent activity describe methods to delete transgenes from a GM plant during some stage of its life.

"Out, damn'd spot! Out, I say!" For more than 15 years, patent applications have been filed on methods for excising transgenic DNA from plants. Most of the early work focused on deleting only those foreign genes introduced into the plant for selection purposes. (In the process of producing transgenic plants, a "marker" gene is coupled with a foreign gene that is associated with a desired trait, such as insect resistance. Genetic engineers insert the linked genes into plant cells at the same time. Testing for the presence of the marker gene reveals whether or not the "gene of interest" has also been transferred successfully.) Once marker genes have served their function during initial development, they are no longer needed. Marker genes, particularly antibiotic resistance marker genes, have raised safety concerns, which explains the early motivation to produce transgenic plants whose marker genes could be excised.[21]

Footloose and GM-free? Instead of limiting gene-excision to marker genes,

recent research is devoted to snipping *all* transgenic DNA out of a transgenic plant at some stage in its development – before the plant flowers and produces pollen, for example, or before it becomes food or when expression of the transgene is no longer desired. As with RBF, research on Exorcist technologies reflects the biotech industry's belated acknowledgement that co-existence of GM and non-GM crops has been unworkable. While excising transgenic DNA will not reverse all the effects of the genetic engineering (see below), it is probable that companies will push to have their products declared "GM Free" for the purposes of labeling consumer products, particularly in Europe where the public has roundly rejected GM foods.

Biotech proponents hope that Exorcist can cleanse GM plants as well as the industry's image. C. S. Prakash, perhaps the world's best-known biotech booster, says of the industry's leaky gene problems: "Most problems raised by science can be solved by additional science itself."[22] The game plan is clear: all that's needed to correct a techno-fix that turned out to be faulty is a new and improved techno-fix.

Like Zombie methods, Exorcist technologies are "dual use" GURTs: (1) assuming 100% effectiveness, they could work as a biocontainment strategy to prevent the escape of transgenes and (2) even at levels of effectiveness considerably lower than 100%, they could work as a biological method to enforce patents (by restricting access to proprietary genes and traits).

Exorcist as a "Democratic Terminator?"
Given the UN moratorium on Terminator, it has become socially unacceptable to openly pursue research on suicide seeds. Exorcist offers a proposed workaround solution to the Terminator "taboo." In the opinion of Ludmila Mlynarova and Jan-Peter Nap, two researchers in the Plant Sciences Group at Wageningen University in the Netherlands, Exorcist could be an "interesting alternative" to Terminator.[23] They acknowledge that, while farmers could save Exorcist seeds, in theory, the practice of seed-saving will be "subject to the national seed laws and intellectual property systems."[24] They also point out that with gene-excision technologies, farmers, breeders and seed producers are all faced with additional work to maintain, develop or test for the transgene-of-interest in the seed. (In this way, Exorcist is like RBF.) The Wageningen researchers conclude that Exorcist "could be considered a different and 'democratic' application of terminator technology: both producers and growers will have to take additional steps to maintain the trait-of-interest, whereas undesired spread is 'terminated.'"[25] Other researchers dabbling in GM-exorcism put it more plainly: Excision methods "would have both technology restriction and biosafety utilities without seed sterility."[26]

How Does "Exorcist" Work?

Exorcist builds on the basic method for excising marker genes, but a larger package of genetic material called a "gene cassette" can be snipped out of the plant. The general process works like this: Genetic engineers insert the gene cassette – it's helpful to think of the cassette as a group of cars in a toy train set – into the genome of a plant. The cassette can be made up of lots of "cars," including: marker genes, genes associated with desired traits, a sequence of DNA that expresses a protein capable of initiating the excision process and "promoters" that jumpstart everything by activating the protein-expressing DNA. The promoter, and therefore the whole excision process, can be activated by a chemical stimulus or by a

developmental stimulus, depending on how it's engineered. The cassette of foreign DNA is flanked on both ends – the first and last cars of the toy train – by more foreign DNA, a pair of recognition sequences (excision sequences). They determine where the excision will take place – all the genetic material between the two excision sequences will be removed from the plant, though one of the two excision sequences will stay behind. When the chemical or developmental stimulus activates the promoter, the promoter activates the protein-expressing DNA. The protein activates excision at the sites within the borders of the excision sequences.

Yeah, it does, kind of: "Although it may sound silly to transform plants with transgenes with the aim to remove all incoming DNA, the crucial issue is *where* in the life cycle of the crop the transgenes are removed." – Ludmila Mlynarova & Jan-Peter Nap, Wageningen University[27]

In March 2007 researchers at the University of Connecticut (USA) described a "GM-gene-deletor" system in *Plant Biotechnology Journal* that has piqued interest in gene excision technology.[28] The system builds on a patent application the same research group filed in 2001, which described methods for removing all (or almost all) of the DNA that was originally engineered into a plant.[29] Their excision process, like the one described above, can be triggered by an external environmental or chemical trigger. It can also be designed to occur at a particular developmental stage, without the need for external activation. By timing the excision to take place before reproduction, for example, the pollen and seeds would no longer contain the engineered trait, which would not be passed on. Theoretically, farmers could save seeds, and those seeds would not contain the engineered trait. The University of Connecticut researchers claim that they were able to engineer tobacco plants that excised the transgenes from pollen and seeds in all of the progeny seedlings tested – more than 25,000 of them.

How Exorcist May Fail

Gene excision technology is complex and involves dozens of elements that need to work in coordination. In its current state the risk of failure is high. In the University of Connecticut experiments with GM tobacco discussed above, the excision was controlled by pollen- and/or seed-specific promoters, rather than by promoters that respond to external signals. (This method is known as *auto-excision* – the promoters were produced automatically in pollen and seeds, and the transgenic DNA was excised in only those parts of the plant.) Thus the plants were not changed into completely "GM-free" plants though their engineered genes were not passed on via sexual reproduction. The tobacco lines were propagated by cuttings in order to maintain their engineered traits in future generations. Although this could be useful in some crops that are vegetatively propagated (some fruits, potatoes, and sugarcane, for example), for most applications, plant breeders would need to cross plants without the traits auto-excising during reproduction. Therefore, external triggers – allowing excision to be activated and deactivated at will – will be necessary. It remains to be seen whether heat shock, hormones or external chemical triggers would be able to replicate the excision rates claimed in this study using developmentally regulated promoters. What's more, these tobacco studies were done in greenhouses under controlled environmental conditions. Experience with engineered crops over the last ten years has shown that weather, nutrient levels and diseases can affect transgene

expression. Will there be more mistakes in excision in the presence of nutrient deficiencies, drought or unusual weather? In the age of climate change, linking excision activation to heat shock seems a particularly risky business. How will changes in transgene expression be evaluated?

In order to claim transgene containment, the biotech industry acknowledges that excision must be failsafe – it must function in 100% of the pollen and seeds of the plants. Researchers have estimated that just one mistake in one thousand could be enough to allow escape of the transgene to wild relatives or neighboring varieties within as few as 10 generations.[30] In fact, the researchers concluded that one mistake in a thousand [10^{-3}] "might seem small, but it might not be small enough. There may be a genuine need for smaller leakage parameters."[31] In another recent study by researchers at Wageningen University in the Netherlands, success of one excision technique activated during pollen development was measured in samples ranging from 100-17,000 seeds (tobacco and *Arabidopsis thaliana* [thale cress]).[32] The transgene was detected in only 0.027% of the seed. While this rate of failure may seem negligible, if the rate is extrapolated to the scale of commercial agriculture, and using canola (edible rapeseed) seeds as the example, this failure rate could amount to nearly 3,000 transgenic plants volunteering in the field per hectare (among the estimated 10 million canola seeds that escape during harvest).[33]

The most recent patent application (see Table 2, below) for excision of transgenic DNA in crops, issued to Pioneer Hi-Bred (DuPont), is similar in concept to the method developed at the University of Connecticut, but is based on bringing together two different lines of transgenic plants. When the different lines mate, the engineered traits from one line are designed to activate engineered traits in the other line to cause DNA excision in a wide variety of configurations. Thus the trigger for excision is ultimately the crossing of the two lines. There can also be a requirement for an additional external trigger as in other GURTs, but it is not necessary. This means that plant breeders could maintain the transgenic DNA as long as they keep the lines separated from one another (to avoid activating the excision process).

But Pioneer's invention is not limited to excision via the crossing of two transgenic lines. In fact, the patent is quite broad and bold in its claims. The patent abstract states, "By matching promoters, responsive to various inducers, plant tissues or plant developmental states with the recombinase systems [the excision machinery], stop fragments and transgenes, virtually any trait may be expressed or excised at any plant development stage or in any plant generation."

Table 2: Patents and Patent Applications on New Methods to Excise Transgenic DNA

Patent / Application #	Assignee	Inventor	Dates Published / Filed	Description
WO0136595A3 See also: US20060253934A1	Pioneer Hi-Bred (DuPont)	Yadav, Narendra S., USA	2006-11-09 / 2006-07-21	Methods for conditional transgene expression and trait removal in plants – virtually any trait may be expressed or excised at any plant development stage or in any plant generation
WO0210415A3 See also: EP1307570A2 US20020124280A1	University of Connecticut	Li, Yi *et al.*	2002-02-07 / 2001-07-27	Methods for the controlled, automatic excision of heterologous DNA from transgenic plants and DNA-excising gene cassettes for producing non-transgenic food; may permit crops produced from transgenic plants to be co-mingled with non-transgenic crops for marketing purposes
US20040143874A1	The Rockefeller University	Moller, Simon Geir	2004-07-22 / 2004-01-13	Inducible site-specific recombination for the activation and removal of transgenes in transgenic plants
WO0216609A3	BASF Plant Science GmbH	Mankin, Luke	2002-02-28 / 2001-08-27	Self-Excising Polynucleotides and uses thereof – useful for producing transgenic plants, removing transgenes from these plants or crops (e.g. food commodities), and restricting the distribution of transgenes within the environment
WO0216624A1	Institute of Molecular Agrobiology, Singapore	Sundaresan, Venkatesan *et al.*	2002-02-28 / 2000-08-25	Reduction of transmission of transgenes in plants; DNA construct useful for excising transgene from plant at specific time
WO0229071A3 See also: US20020078476A1	Maxygen, Inc.	Stemmer, Willem, P. C.	2002-04-11 / 2001-10-05	Methods and compositions relating to the generation of partially transgenic organisms (i.e., a transgenic plant capable of producing a non-transgenic agricultural product).
WO02064801A1	Unicrop, Ltd.	Kuvshinov, Koivu, *et al.*	2002-08-22 / 2002-02-14	Molecular control of transgene escape by a repressible excision system

Caveats to Cutting Out DNA:
Verification that transgene-escapes are not occurring is a big problem with Exorcist technologies. Since plants generally look the same with and without transgenic DNA, a lot of expensive and time-consuming testing on a regular basis could be required to check whether the excision was working properly under real field conditions. And, of course, in the event that some genes have escaped, it will be impossible to "recall" them.

Jack Heinemann, professor of gene ecology at the University of Canterbury, New Zealand, points out that excising transgenes at target sites does not entirely reverse the effects of the original transgenic gene insertion.[34] This makes

sense, of course: if a human body is penetrated by a bullet, and the bullet removed hours later in surgery, the surgery will not have returned the body to its pre-shot state. With Exorcist, one of the excision sequences bordering the gene cassette is left behind after the genetic material in the cassette is excised. If the sequence left behind is lodged in an active gene crucial to the plant's health, it could act like a mutation in that gene. In any case, the sequence left behind becomes part of the plant's chromosome – it will be inherited by future generations and could cause disruptions further down the line.[35] The University of Connecticut researchers acknowledge the potential for adverse environmental and health effects from this non-expressed DNA sequence but they conclude that these effects "should be minimal or relatively easy to determine."[36]

Given the thousands of genes of unknown or incompletely known function in a plant and their nuanced interrelationships, and the likelihood that the piece of DNA that has been left behind will be randomly inserted in the genome of the plant, how will environmental and health effects be determined? Such studies are complex and long-term, and these long-term studies have yet to be done thoroughly on the engineered crops currently being grown.[37]

The Bottom Line on Exorcist Technology

Proponents of Exorcist technology point out that gene excision technology – if it works as designed – wouldn't be a Terminator technology because the progeny are fertile. Farmers would be able to save harvested seed, and that seed would no longer contain GM traits. However, even in its current imperfect state, where the potential for transgene escape has not been eliminated, it's important to remember that Exorcist could function as an effective, biological patent protection. In practice, the company retains control of the engineered traits, by determining when the excision takes place and by what means. Depending on the particular method of Exorcism, gene-excision would either be out of farmers' control (i.e., auto-excision) or it would be their responsibility (burden) to make sure it happened: They would have to apply the external chemical to stimulate the promoter and begin the excision process.

Regardless, if the technology fails and the transgenes are not completely excised, it is the farmer who could be charged with infringing the patent on proprietary genes. How would the farmer prove that he/she is a victim of technology failure and not culpable of patent infringement or liable for contamination?

It's unclear how soon Exorcist technologies could be commercialized. Many of the schemes envisioned are quite complex, involving dozens of elements that need to work in coordination. This will likely take years of experimentation, though experience with commercial biotech thus far makes clear that the industry doesn't necessarily have to get its product right to get it to market.

3. Conditional Lethality: Pull-the-Plug Plants

The US National Research Council's 2004 report, *Biological Confinement of Genetically Engineered Organisms*, points out that no single bioconfinement technique will achieve complete success on its own. The report notes that the use of multiple techniques, with different strengths and weaknesses, will decrease

the probability of failure (because the failure of one method could be backed-up by the integrity of another).[38]

In the event that all else fails, companies are pursuing extreme biocontainment methods as an ultimate back-up plan – ways to pull the plug on transgenic plants, terminating them and their transgenic DNA.

"Because methods can fail, a single confinement method will not necessarily prevent transgene escape." US National Research Council, *Biological Confinement of Genetically Engineered Organisms*, 2004.

How does "Pull the Plug" Work?

"Pull-the-Plug plants" are engineered so that the trait of interest and a conditionally lethal gene are juxtaposed and inserted together into the plant's DNA. If the lethal gene is triggered, the plant dies, taking the GM trait down with it. If it's not triggered, the plant lives and can pass on its foreign genes – both the gene associated with the trait of interest and the lethal gene – to the next generation. The conditionally lethal gene can code for a toxin itself and have a promoter that is triggered by a chemical or environmental stimulus, or it can code for an enzyme that transforms an applied chemical into a toxin. Dead plants don't transfer genes,[39] so this method should be completely "effective" as a biocontainment strategy for pollen- and seed-mediated gene flow assuming the trigger works efficiently and is activated before any gene escape takes place. The concept of conditional lethality is to create the opposite of an herbicide-tolerant plant – an "herbicide-susceptible" plant, with the herbicide selected to be specific to the engineered plant (and ideally, not lethal or harmful to other plants, the environment, people or other animals). Pull-the-Plug plants differ from Zombie (RBF) technology because the default position is not automatic death: For Pull-the-Plug plants to commit suicide, the promoter must be triggered.

The Pull-the-Plug patent issued to Dow Agrosciences in the table below[40] suggests an unusual twist on the idea of engineering a plant to self-destruct (if that idea weren't twisted enough). The inventors outline a plan to use a conditionally lethal gene to sicken – but not kill – plants containing transgenes, as a way to identify visually which individual plants contain the genes and which do not. If the plant looks deformed after being given a sub-lethal dose, then it can be assumed the plant has the engineered DNA and can either be selected to live and "rescued" by discontinuing the trigger, or it can be destroyed. It may be though that diseases could confound the identification of plants with transgenes: Are the plants looking sickly because of the sub-lethal effects of the triggered gene, or do they have a virus, for example?

Table 3: Patents and Patent Applications on "Conditionally Lethal" Plants

Patent / Application #	Assignee	Inventor	Date Published (or Granted) / Filed	Description
US6753459	Dow Agrosciences; National Research Council of Canada	Keller, Wilfred A. *et al.*	2004-06-22 / 2001-06-22	Gene construct comprising a conditionally lethal gene functional in a plant cell, useful for producing a transgenic plant which can be removed from a growing environment [and identified visually]
US20040154054A1 See also: US6743968	Dellaporta, Stephen L. *et al.*	Yale University	2004-08-05 / 2004-03-17	Genetic construct for the control of transgenes in transgenic plants comprises a sex-specific promoter operatively linked to a suicide gene that selects against male or female gametes containing the suicide gene
EP0658207B1	Bright, Simon, W. J. *et al.*	Syngenta Limited	2002-09-25 / 1993-07-29	Recombinant plant genome containing gene cascade requiring a chemical inducer to produce a mature plant – allows control of expression of plant genes, and production of non-viable protectable plants

Why Pull the Plug? Ostensibly, pull-the-plug plants are being developed as a strategy for last-resort biocontainment, which could boost the plausibility of co-existence and the perception that GM plants could be biosafe. The Dow patent highlights the utility of being able to remove an unwanted plant from a growing environment. If pharma crops are rigged with conditionally lethal genes, for example, soaking a field with the chemical trigger after harvest would presumably provide added assurance that no living plants were inadvertently left behind (which, of course, doesn't address the problem of gene flow while the plants were alive).

There is also the more sinister possibility that conditionally lethal genes smuggled into the crops of an enemy population would allow the chemical trigger to be used as an anti-crop biological weapon to attack a strategic crop. Is it far-fetched to suggest that this application of a Terminator technology could be an anti-crop weapon? Unfortunately, history shows that research on biological warfare against crops is not uncommon. According to Simon Whitby of the University of Bradford (UK), because of the potential devastation that can be inflicted on a country by destroying its crops, all state-supported biological weapons programs in the 20th century included research on anti-crop measures.[41] While this could be accomplished quite thoroughly with conventional herbicides, pull-the-plug plants could be introduced unobserved, perhaps during peacetime, allowing the technology-holder to exploit the ability to sicken plants rather than kill them outright.

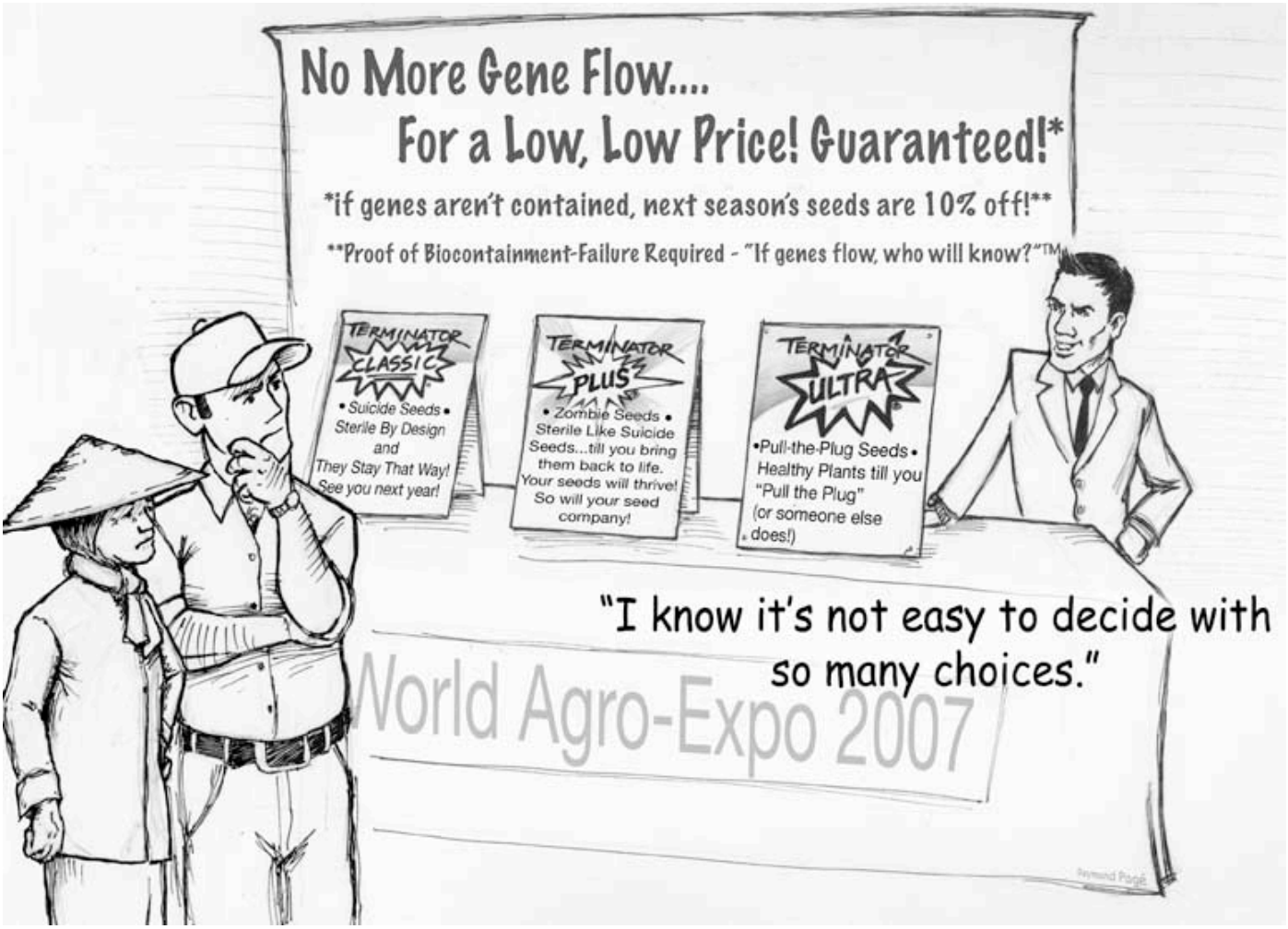
How Pull-the-Plug May Fail

In the field there are the usual problems of using chemical triggers: incomplete penetration leading to some plants escaping, the difficulty of finding a chemical trigger that really could be environmentally benign when used on a commercial scale and getting the weather

to cooperate with the treatment schedule. As with other GURT technologies, there is also the problem of gene silencing and gene mutations (see Zombie technology section, above); if the lethal gene is silenced or undergoes mutation, it would result in transgene escape.

The Bottom Line on Pull-the-Plug Technology

Pull the Plug, like Zombie and Exorcist, is also a dual-use GURT – it "works" as both a biocontainment strategy – admittedly, an extreme one – and as a patent protection strategy. Companies could pull the plug on plants they believe are being grown without the proper licensing agreements. Biotech companies have previously resorted to draconian measures to ferret out farmers suspected of possible patent infringement– (i.e., hiring private police to investigate potential violators, establishing toll-free hotlines to encourage farmers to snitch on their farm neighbors, etc.). Now companies could threaten a farmer suspected of patent infringement with triggering the lethal gene or they could simply apply a chemical trigger to get positive or negative confirmation of their suspicions. Pulling the plug could be a lot less labor intensive than relying on informants or taking farmers to court. Conditional lethality also expands the possibilities for biological warfare against crops.



Conclusion and Recommendations

Industry and governments are already working to overturn the existing moratorium on Terminator technology at the UN Convention on Biological Diversity. In the months leading up to the CBD's 9th Conference of the Parties (Bonn, Germany 19-30 May 2008), industry will argue that global warming requires urgent introduction of transgenic crops and trees for biofuels – and that Terminator-type technologies offer a precautionary measure and environmental necessity to prevent transgene flow. Research on molecular biocontainment strategies is already being promoted as a biosafety solution for transgenic crops, trees and pharmaceutical-producing plants.[42] According to its website, the results of the EU-funded Transcontainer Project "will contribute to an informed decision whether the [CBD] moratorium should be continued or modified in the context of supporting EU coexistence measures."

Governments and industry are investing millions of dollars in molecular biocontainment strategies that cannot promise fail-safe containment of transgenes, but could nonetheless function as Terminator technologies that pose unacceptable threats to farmers, biodiversity and food sovereignty. The solution to a flawed technology is not a new techno-fix. Ironically, society is being asked to foot the bill for another new and untested technology – one that is designed to maximize seed industry profits – in an attempt to reel in the genetic contamination problem caused by the very same companies. There is no such thing as a safe and acceptable form of Terminator. New research on molecular containment of transgenes will ultimately allow the multinational seed industry to tighten its grasp on proprietary germplasm, restrict the rights of farmers and dictate the conditions under which seeds and plants are viable. Under some scenarios, farmers will be obliged to pay for the privilege of restoring seed fertility every year – a new form of perpetual monopoly for the seed industry.

ETC Group offers the following recommendations:

- The technical understanding and political debate surrounding GURTs/Terminator must be expanded and updated to include technical advances in the technology. With V-GURT or Terminator technologies the reproductive viability or vigor of the plant is controlled by the corporation that sells the seed. New, molecular strategies for biocontainment are attempting to shift the burden for containing transgenes to farmers and society.

- Governments and civil society must not succumb to the technological imperative and the argument that molecular containment strategies will provide a viable solution to prevent the escape of transgenes. Those who are working to resist GM crops must not accept biological containment strategies as a techno-fix for GM contamination. If GM plants are not safe, they are unacceptable and should not be planted.

- Public financing should not be used to support Terminator technology. The European Commission should discontinue funding for research on Zombie seeds (Recoverable Block of Function), and re-assess funding for other research projects

undertaken by Transcontainer. Rather than funding research on coexistence to bail out the agbiotech industry, the EU should instead fund sustainable agricultural research that benefits farmers and the public.

- Plants engineered to contain conditionally lethal genes (pull-the-plug genes) have the potential to be used as anti-crop weapons, to sicken or kill an enemy's crops. Patents on this technology should be revoked and future applications denied on the grounds that they violate public morality (*ordre public)*.
- National governments should propose legislation to prohibit field-testing and commercial sale of Terminator (V-GURT) technologies.
- Governments meeting at the 9th Conference of the Parties to the Convention on Biological Diversity in Bonn, Germany must strengthen the moratorium on GURTs by recommending a ban on the technology.

Appendix: Will Recoverable Block of Function (RBF) work well enough to contain transgenes?

Will RBF work well enough to contain transgenes? Many problems will need to be overcome, and although researchers are addressing the points where the technology might fail, they have a long way to go before this system can be relied upon to keep transgenes from wandering. The EU-funded Transcontainer Project is using the RBF system first developed by Finnish researchers with UniCrop, Ltd. The Transcontainer researchers have not yet published their work, but claim that it is far more promising than the Finnish system. The latest paper from Viktor Kuvshinov's research group (UniCrop) provides the most recent published information on the state of the art for RBF, and points to many of its potential weaknesses.[43]

In their experiments, tobacco plants are used as a model because they are easy to manipulate in tissue culture. The goal of the experiments is to engineer tobacco with the components of RBF to prevent transgenes from escaping via seeds. In particular, the researchers attempted to engineer the plants in a way that would minimize some of the problems they foresaw with the method: gene silencing and mutations resulting in seed viability and thus possible escape of the transgene.

Here is how Kuvshinov's team set up the most important parts of the experiment:

First, the researchers chose the BC which would be responsible for killing seeds if they contained transgenic DNA, in this case a gene that codes for the toxin barnase. However, genes mutate. If the barnase gene mutated so that the toxin no longer killed the seeds, the adjacent transgene for a trait of interest could spread via those seeds. Also, plants have ways of recognizing foreign genes and turning them off—gene silencing—that would also allow seeds with transgenes to live. So Kuvshinov's team decided to reduce these two possible problems by engineering the tobacco plants with two genes for barnase that had different DNA sequences but coded for the same barnase protein, which is possible because there are several codons for each amino acid. Each barnase gene had a unique promoter sequence, as well, so that one was more strongly expressed during seed development, and the other during germination. The likelihood of mutations occurring in two genes and disabling both toxins is much lower than for one gene, and the different DNA sequences make silencing less likely. They also arranged these barnase genes in a way that would minimize silencing.

If the tobacco plants behaved as expected with these barnase genes inserted, they would grow normally, make flowers, and then as the seeds developed the barnase gene with the seed-specific promoter would turn on and the seeds would be non-viable. If for some reason that gene failed, the second barnase gene programmed to make toxin during germination would act as a back-up. The result would be seeds that would die and be unable to pass on transgenic DNA.

If breeders or farmers wanted to get viable seeds they would have to inactivate the barnase toxin with the RC part of the package. In this case, RC is a gene for barstar, a protein that inactivates barnase. This gene was fitted with a promoter sequence from a heat shock protein, allowing it to be turned on by treating the tobacco plants with high temperatures as the seeds were forming. They only used one barstar gene, presumably

because the researchers were more concerned with biocontainment than with recovery of viability. Mutations or silencing of the barstar gene would make it difficult to recover viable seeds, but would not lead to gene escape.

The transgenic trait in the experiment used to determine the success of containment was a commonly used gene for an enzyme that forms a blue color when a particular substrate is added. Tissues containing the transgene can be easily identified by their blue color.

Putting the pieces together, when they engineer tobacco plants with the two barnase genes, the barstar gene, and the color trait, they expect to obtain plants that grow normally until seeds form. Then the seeds will either die as they mature or when they germinate because of barnase, unless the plants are subjected to high temperatures as the seeds develop. Heat should cause barstar to be made, inactivating the toxin, and thus restoring fertility.

The question is: will this work efficiently enough to kill all of the transgene-containing seeds for confinement, and will the barstar be able to rescue enough of them to be useful to farmers and breeders? (Although the purported reason for using the specific arrangements of genes was to discourage mutations and silencing, they did not actually determine if these problems were lessened in this study.)

The authors conclude that yes, it is possible to use this double-BC system to kill seeds efficiently, and that a single RC can rescue enough seeds to be useful. However, a few aspects of the study deserve comment.

First, only a few of the tobacco plants containing this complex transgenic DNA insert functioned as expected. Most of the plants behaved erratically. Many of them were stunted or otherwise looked strange, as if the barnase gene was being expressed outside of the seed even though the promoters should have kept expression to seeds. Some plants formed seeds that were viable, even without being heat shocked, as if they were not making enough barnase. Of those that behaved as expected, the levels of barnase and barstar varied a lot.

This kind of variability in the first plants regenerated from the genetic engineering process is common. Researchers simply sort through the plants for the ones that have the amount and pattern of gene expression they want, and then grow those to see if they maintain the desired expression from generation to generation. However, it is always possible that the process of genetic engineering can cause changes in the way the plants grow and develop that are missed in the screening.

Kuvshinov et al. were able to find some tobacco plants containing their transgenic constructs that did indeed grow normally until seed development, and then all of their transgene-containing seeds failed to germinate. Seed sample sizes ranged from 100-1200 seeds, which is not enough to determine if the method works well enough in an agricultural setting where many millions of seeds are formed in a field. They were also able to use heat shock to restore fertility, in some cases to 90% or more. However, they do note that this method of rescuing seeds may not confine genes if the plants are exposed to hot temperatures in the fields.

To conclude, the researchers did make some progress in proving their concept of RBF, but they have a long way to go before it will function as designed in agriculture. Heat shock will probably not be a good inducer of RC given the normal range of temperatures in many parts of the world, the barnase/barstar system may not work as well in large seeds as it does in the very small tobacco seeds, efficiency will have to be very high to contain genes in agricultural situations where many more seeds are produced, and their strategy for reducing mutations and silencing has yet to be experimentally tested for those effects, although they have shown that the large, complex insert can function in the plant. In addition, promoters for BC will have to be optimized for each species.

Endnotes:

[1] In 2000 the Conference of the Parties to the Convention on Biological Diversity first adopted the *de facto* moratorium on field trials and commercial use of GURTs. The moratorium was re-affirmed in 2006. The text of the decision is available here: http://www.biodiv.org/decisions/default.aspx?m=COP-8&id=11037&lg=0

[2] Genes may transfer by other processes, such as horizontal gene transfer conducted by viruses or parasites (see Heinemann, J.A. and Bungard, R.A. 2005. Horizontal Gene Transfer. *In* 2nd Ed. Encyclopedia of Molecular Cell Biology and Molecular Medicine (Meyers R.A. ed). Wiley-VCH, p. 223-243).

[3] According to Jeffrey Barach, Vice-President and Center Director, GMA/Food Products Association. Comments made during Agrifood Nanotechnology Workshop, Michigan State University, April 2, 2007.

[4] http://www.gmcontaminationregister.org/

[5] According to seed industry consultants, the Context Network, the value of the global proprietary seed market was $19.6 billion in 2006. For more information on top 10 seed companies, based on 2006 seed revenues: http://www.etcgroup.org/en/materials/publications.html?pub_id=615

[6] Transcontainer is a European Commission-funded Specific Targeted Research or Innovation Project (STREP) in the Sixth Framework Programme Priority 5: Food Quality and Safety. Background on Transcontainer: http://www.transcontainer.wur.nl/UK/

[7] Part of Transcontainer's work involves development of biocontainment systems that control fertility. Of the six technical objectives (Fact Sheet: Transcontainer & Controllable Fertility), five involve male-sterility. Male-sterility reduces contamination by cross-pollination, but viable seeds can pass on genes, so it is not a totally contained system. One of their objectives is to combine male-sterility with seedless fruits for more complete transgene containment. However, male-sterility alone is invaluable for production of hybrid seeds, and this is one of Transcontainer's goals—"to determine the utility of pollen transgene containment technologies in hybrid breeding systems." Hybrid seed production is, of course, very lucrative for the seed industry because the seeds command high prices and have to be purchased every season if farmers want to gain the varietal benefits. However, hybrid seeds are not sterile seeds.

[8] http://www.transcontainer.wur.nl/UK/Fact+sheets/

[9] See for example, Union of Concerned Scientists, *A Growing Concern: Protecting the Food Supply in an Era of Pharmaceutical and Industrial Crops*, 2004; US National Research Council of the National Academies, *Biological Confinement of Genetically Engineered Organisms*, National Academies Press, 2004, pp. 8-9. See also links on Transcontainer website: http://www.transcontainer.wur.nl/UK/Literature+links/

[10] http://www.unicrop.fi/control.html

[11] Email correspondence from Piet Schenkelaars, Schenkelaars Biotechnology Consultancy, to ETC Group, March 2, 2007.

[12] Email correspondence from Piet Schenkelaars to ETC Group, March 2, 2007.

[13] *Ibid*.

[14] *Ibid*.

[15] See, for example, US Patent 6,849,776 filed July 14, 2000; issued February 1, 2005. Kuvshinov, V. et al., UniCrop Ltd. "Molecular control of transgene segregation and its escape by a recoverable block of function (RBF) system."

[16] Viktor Kuvshinov, Ph.D., is the original developer of RBF strategies for containing transgenes. In an email to ETC Group, 28 February 2007, he writes: "RBF should belong to V-GURT."

[17] The RBF system is briefly described in the section on V-GURTs in *Biological Confinement of Genetically Engineered Organisms*, National Research Council, Washington, DC: The National Academies Press, pp. 72-75.

[18] EcoNexus, "V-GURTs (Terminator) as a biological containment tool?" June 2005. www.econexus.info
[19] Available on the EcoNexus website: www.econexus.info/pdf/ENx-CBD-GURTs-2006.pdf
[20] Kuvshinov, V., A. Anisimov, B.M. Yahya, and A. Kanerva, 2005. Double recoverable block of function—a molecular control of transgene flow with enhanced reliability. *Environ. Biosafety Res.* 4: 103-112.
[21] See, for example, patent WO9301283A1 filed by the US Department of Agriculture in 1992 and US5792924, "Biologically safe plant transformation system," issued to the Regents of the University of California in 1998.
[22] From C. S. Prakash, "The Genetically Modified Crop Debate in the Context of Agricultural Evolution," *Plant Physiology*, May 2001, Vol. 126, pp. 8-15. The quote continues: "For example, appropriate promoters may ensure that pollen will not express genes toxic to beneficial insects, while gene expression strategies, such as sterile pollen, could reduce the risk of gene flow."
[23] Ludmila Mlynarova & Jan-Peter Nap, "Transgenic Plants that Make Non-Transgenic Pollen," *ISB News Report*, August 2006, on the Internet:
http://www.isb.vt.edu/news/2006/news06.aug.htm#aug0603
[24] *Ibid*.
[25] *Ibid*.
[26] Luo, K., H. Duan, D. Zhao, X. Zheng, W. Deng, Y. Chen, C. N. Stewart Jr., R. McAvoy, X. Jiang, Y. Wu, A. He, Y. Pei and Y. Li, 2007. 'GM-gene-deleter': fused *loxP-FRT* recognition sequences dramatically improve the efficiency of FLP or CRE recombinase on transgene excision from pollen and seed of tobacco plants. *Plant Biotechnology Journal* 5: 263-274.
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