Genetic use restriction technologies (GURTs): strategies to impede transgene movement

Melissa J. Hills¹, Linda Hall², Paul G. Arnison³ and Allen G. Good¹

¹Department of Biological Sciences, University of Alberta, Edmonton, AB, T6H 2X6, Canada
²Department of Agriculture and Forestry, University of Alberta, Edmonton, AB, T6G 2P5, Canada
³Saponin Inc. 4420, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada

No clear consensus has emerged in the debate about the risks posed by transgenic crops and how to assess these risks accurately. In the meantime, interest is growing in strategies to impede transgene movement. This attention is being driven, in part, by expanding interest in using transgenic crops to produce pharmaceutical and industrial products. Potential strategies to impede transgene movement have been published in the scientific literature, and numerous patents have been submitted; however, the efficacy of such strategies has still to be evaluated in a field situation. In this review, we discuss some of the genetic strategies that could be used to restrict the spread of transgenomes, although at present many of these technologies are still largely at a theoretical stage of development.

Defining GURTs
Genetic use restriction technologies (GURTs) have been defined as a range of molecular strategies designed to impede transgene movement (http://www.biodiv.org/doc/meetings/cop/cop-07/information/cop-07-inf-31-en.pdf). This original definition has been expanded to comprise two major types of GURTs: T-GURTs (trait-GURTs) and V-GURTs (varietal-GURTs) (http://www.fao.org/waicent/FaoInfo/Agricult/AGP/AGPS/pgr/itwg/pdf/P1W7E.pdf). T-GURTs regulate the expression of a specific transgenic trait in plants while enabling plants to remain fertile and set viable seeds. These methods reduce the amount of the product and, therefore, the level of exposure, but not the frequency of the transgene in subsequent generations. By contrast, V-GURTs impede transgene movement, either by rendering the plant unable to develop properly, or produce functional pollen or seed, or by preventing the transmission of the transgene (Table 1), such that the occurrence or frequency of the transgene is significantly reduced in the subsequent generation.

In this review, we focus on molecular strategies designed to impede transgene movement (V-GURTs). See Refs [1,2] for reviews of trait restriction use technologies (T-GURTs). We evaluate different GURTs based on the specific plant processes they interrupt and concomitantly consider the technical challenges associated with their practical use in an agronomic setting. Finally, we address the question of which specific GURT technology can be used efficiently and effectively in different crops and different situations. We argue that it is only by understanding the crucial aspects of transgene spread during specific developmental stages that effective GURT strategies can be designed for different crops and products.

Applications of GURTs
GURTs have several different commercially valuable applications. GURTs can simultaneously restrict the transmission of a transgene and the use of proprietary germplasm. Restricting the use of proprietary germplasm (e.g. the terminator technology [3]; http://www.banterminator.org) has been criticized even though this method represents an effective way to limit gene flow to the environment [3]. GURTs represent a novel mechanism for companies to recapture the investment in innovations in plant breeding. In this sense, GURTs are similar to proprietary hybridization technologies that have existed for many decades [4]. There have been several recent reviews dealing with the potential welfare impacts and benefits of GURTs, based in part on the experience with maize hybrids [4–6]. GURTs might also be valuable to companies in addressing legal liabilities if the transgenic crop has the ability to cross with other commercial varieties or introgress into wild relatives [7–9].

If a GURT produces sterile seed, it has been argued that both the regulatory community and the activist sector should embrace the technology because it would enable the effects of transgene introduction to be effectively mitigated [10]. Regardless of whether GURTs are viewed positively or negatively, choosing a specific GURT for an application within a crop species will require a clear understanding of the role required of that specific GURT.

Reducing admixture
Gene flow is mediated by both pollen and seed, but seed has the potential to travel further and remain viable for extended time periods. Therefore, in principle, any technology that reduces the level of seed mixing or admixture (the unintentional adventitious presence of transgenic material in nontransgenic seed lots), or seed loss during harvesting could be useful in reducing the potential spread...
of the transgene. The removal of transgenic seed during seed cleaning could reduce the reintroduction of transgenes in harvested crop seed targeted for reseeding. Seed movement contributes to gene flow through feral or volunteer crops replenished from the seed bank and can provide further opportunities for gene flow. Technologies that would be useful as GURTs include any technology that would reduce seed shattering [11], or enable seeds containing the transgene to be separated mechanically by, for example, seed size, seed weight, or seed colour.

Several endogenous and introduced genes have been identified that affect seed size, weight and colour, in a variety of different species [12–14], and several of these genetic approaches have been patented [15,16]. However, most of these traits, including seed size are influenced by environmental parameters and are unlikely to be sufficiently robust to be considered a GURT. From a practical perspective, in most breeding programmes, ancillary traits such as seed size, or easily monitored visual clues such as seed colour, would only be considered if there were no negative yield or composition effects. Once seed containing a transgene has entered the seed bank (Figure 1), the regulation of seed, seedling and plant viability represent basic mechanisms of controlling the persistence of a transgenic trait. A variety of different approaches have been proposed to regulate seed viability, seedling establishment and plant growth.

**Table 1. Different V-GURTs used to prevent spread of specific transgenes**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Development stage</th>
<th>Natural (N) and transgene (T)</th>
<th>Species tested</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Reduced admixture</td>
<td>Seed</td>
<td>Mature pods</td>
<td>Arabidopsis, rice, Brassica</td>
<td>[11]</td>
</tr>
<tr>
<td>Seed shattering</td>
<td></td>
<td>N and T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed size</td>
<td></td>
<td>Mature seed</td>
<td>Brassica, Arabidopsis, tobacco</td>
<td>[13,14,16]</td>
</tr>
<tr>
<td>(2) Eliminate transgensics</td>
<td>Plant</td>
<td>Mature seed</td>
<td>Maize, Brassica</td>
<td>[12,15]</td>
</tr>
<tr>
<td>Seed colour</td>
<td></td>
<td>N and T</td>
<td>Tobacco, Brassica</td>
<td>[17–19,21]</td>
</tr>
<tr>
<td>Seed sterility</td>
<td>Germination</td>
<td>N and T</td>
<td>Tobacco, Arabidopsis</td>
<td>[21–24]</td>
</tr>
<tr>
<td>Conditional lethality</td>
<td>Vegetative growth</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced fitness</td>
<td>Vegetative growth</td>
<td>N and T</td>
<td>Arabidopsis, tobacco, Brassica</td>
<td>[25–28]</td>
</tr>
<tr>
<td>(3) Reduce gene movement</td>
<td>Pollen</td>
<td>Time to flowering</td>
<td>Brassica, Arabidopsis, wheat, barley</td>
<td>[29–33]</td>
</tr>
<tr>
<td>Flowering time</td>
<td></td>
<td>N and T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical flower structure</td>
<td>Flowering</td>
<td>T</td>
<td>Birch</td>
<td>[29]</td>
</tr>
<tr>
<td>Male sterility</td>
<td>Pollen viability</td>
<td>N and T</td>
<td>Many e.g. tobacco, Brassica, maize</td>
<td>[35–43]</td>
</tr>
<tr>
<td>Maternal inheritance</td>
<td>Chloroplast inheri</td>
<td>N and T</td>
<td>Soybean, Arabidopsis, tobacco</td>
<td>[44–46]</td>
</tr>
</tbody>
</table>

*Based on three specific biological processes that are affected, described in Figure 1.

Any technology that relies on an inducible or repressible system must be carefully considered within an agronomic context. Inefficient induction, or repression, and expression in the absence of induction, might limit the efficacy of such systems. Effective systems for inducible expression in plants are being developed [20]; however, these systems have been developed in the laboratory and their robustness in the field has yet to be evaluated. Furthermore, the feasibility of spraying the chemical required for induction or repression must be questioned. In particular, antibiotics, or vertebrate hormones, which are often used in inducible systems, are likely to be deemed as unacceptable in an agronomic context.

**Conditional selection and transgene mitigation**

Conditionally lethal factors, also known as suicidal genes, could be useful to control transgenic volunteers (Figures 1 and 3). The linkage of a conditional lethal gene to the novel trait provides a means of eliminating any unwanted volunteer plants carrying the transgene without affecting other plants. A conditional lethal gene can be one that converts an inert toxin (a protoxin that does not damage non-transgenic plants) to an active toxin that kills the transgenic plant. In addition to protoxins, specific organic analogues can be used as selective agents much as they are often used as selection systems for mutants in yeast.

---

*The epithet ‘terminator genes’ or ‘terminator technology’ was coined by an NGO, the Rural Advancement Foundation International. The original patent (US Patent 5 723 765) did not use the word ‘terminator’ and was titled ‘Control of plant gene expression’.*
Conditional lethality could also be useful as a safety measure in combination with another GURT.

One example of a conditional lethal system uses the Indole Acetomide Hydrolase (IAAH) gene from Agrobacterium \[21,22\]. This gene converts indole acetomide to indole acetic acid (IAA), or naphthalene acetomide to naphthalene acetic acid (NAA). Indole acetomide has no effect on plants that do not express IAAH, whereas plants expressing the hydrolase develop epinasty and might not recover. The inclusion of an auxin transport inhibitor maximizes the effect of toxic auxin production. An alternative system uses a marker gene, dao1, encoding D-amino acid oxidase \[23,24\]. This gene can provide both positive and negative selection depending on the substrate. D-alanine and D-serine are toxic to plants, but are metabolized by DAAO, providing a positive selection scheme. By contrast, D-isoleucine and D-valine provide a negative selection scheme because they become metabolized into toxic keto acids, 3-methyl-2-oxopentanoate and 3-methyl-2-oxobutyrate (3M-2-OB), respectively. These types of systems have the benefit of enabling transgenic plants to be selected while providing a specific removal system to reduce the transgene when selection pressure is applied. However, conditional lethality requires the availability of a suitable protoxin, toxin or analogue that is safe, functional and cost effective. Applying these compounds in field conditions such that they effectively eliminate transgenic plants might prove to be environmentally and technically challenging.

Another type of system is one where a gene of interest is linked to a gene that is selectively unfit, such that the tandem construct provides transgene mitigation (TM) by reducing the risk of gene establishment in volunteers or related weedy relatives. For example, a semi-dominant dwarfing gene \(D_gai\) was used to provide a selective disadvantage to plants containing the specific transgene \[25–28\]. It has been shown using tobacco and canola (Brassica napus) as model systems that when grown with wild-type segregants, the highest reproductive fitness of the TM transgensics was 17% for tobacco and only 12% for Brassica napus compared with that of the wild type \[26,27\]. These TM systems might be particularly useful in crops where volunteer plants are a problem in natural environments. To the best of our knowledge, no conditional lethal systems have been tested in the field.

Reduction of pollen-mediated gene movement

There are a variety of different approaches, both naturally occurring and imposed by recombinant means, designed to reduce gene flow or movement, including both intra- and inter-specific pollen-mediated gene flow. Traits that could act to reduce gene flow between congenitors or wild species include the modification of flowering time or the elimination of flowering \[29–33\]. Silver birch (Betula pendula) was successfully engineered not to flower, an application that might be particularly useful for transgenic trees \[29\].

Many genes that affect flowering time have been identified in Arabidopsis, Brassica, wheat and other species \[30,31\]. Although these genes could play a significant role in preventing intra- and inter-specific crossing, traits such as flowering time tend to be affected by the environment and seem unlikely to meet regulatory approval. Moreover, for some transgenic varieties, gene flow can be reduced much more simply by modifying seeding date, or by selecting a variety that naturally flowers at a different time than the non-transgenic varieties.

A variety of different male-sterility systems have been disclosed, most of these are similar to the Barnase/Barstar system \[34\] and require a tapetal- or pollen-specific promoter attached to a toxin gene. Toxin genes that have been used include RNases (such as Barnase) or another cytotoxic compound, or an antisense construct for a gene that is essential for male fertility \[35\]. For some applications, such as turfgrass \[36\], or any crop, which is harvested for biomass, a pollen toxic gene is all that is required.
Another system for the production of F1 hybrids requires two separate genetic components [18] (Figure 2b). The first is a gene that is inactive owing to a blocking sequence but that results in seedling death when expressed. A second gene encodes the recombinase, driven by a developmental-specific promoter. Hybrid (F1) seed is produced in a cross involving a parent containing the blocked lethal gene and a parent containing the recombinase gene that will be activated during seed development. Both parental types are capable of producing fertile seed if self-pollinated. In hybrid progeny, the recombinase is expressed late in embryo development, removing the blocking sequence, resulting in the death of the developing embryo [18]. Several male-sterility systems have been published [37–39] and numerous patents exist regarding engineering male sterility [35,40–43].

In the 'recoverable block of function' system, the DNA blocking sequence interrupts a specific physiological function, leading to cell death [38,39]. A second inducible sequence restores the blocked function. The benefit of this system is that the exogenous chemical is required to activate the 'blocked' functional construct, therefore, if cells are not exposed to the chemical, they will die. This addresses one of the main concerns with other inducible systems, that failure of the inducing chemical to induce the sterility gene could potentially allow 'escapes' (http://www.adonline.id.au/terminator/) [39].

Maternal inheritance
Another strategy could be to target the transgene to the organelle genome. In species with strict maternal
inhereditary, this strategy would prevent transgene escape via pollen flow. Chloroplasts have been successfully transformed in several species, including tobacco, tomato and cotton, and numerous agronomic traits have been introduced via chloroplast engineering [44–46]; however, using these techniques for the first time in an untested species can be challenging [45]. Although plastid genes are maternally inherited in most angiosperm plant species, low but non-zero transmission rates have been measured in some species including tobacco [47–49]. Furthermore, the phenomenon of gene transfer from the chloroplast to the nucleus is well documented [48,49], although the transgenes lacked the regulatory sequences necessary for expression in the nuclear genome [49].

An additional drawback of this approach is that it would not stop transgene escape via maternal inheritance. If any seeds remained to become volunteer plants, then the transgene could become incorporated within the cytoplasm of the wild population. In this case, if the gene had a selective advantage, it could increase in the population in a similar fashion to a nuclear gene [50].

Evaluating the risk of spread of transgenes

Risk assessment is an area of active research and has been reviewed in detail recently [50–54]. Risk assessment requires first hazard identification (for transgenic plants, this requires an evaluation of the components of the transgenic construct), which comprises exposure assessment (the probability or degree of exposure) and effects assessment (the probability that an adverse effect will occur, given exposure to the hazard) [52,53]. In this context, V-GURT [53,55] can reduce the production of a specific transgenic product, or degree of exposure, although they do not reduce the frequency of the transgene in the population. Much of the research on GURT has focused on measuring pollen flow between transgenic and non-transgenic plants. In recent years, it has become clear that hybridization between crops and their wild relatives is common, and that the widespread cultivation of a transgenic crop will increase the frequency of transgenes and the probability of transgene movement [52].

A mathematical analysis of this approach suggested that pollen leakage parameters larger that $10^{-3}$ have the potential to fail rather quickly, resulting in the movement of the transgene [50]. The likelihood of establishment and rate of spread of a transgene is governed both by the strength of selection and the migration rate [52,56]. Thus, even if hybridization between crops and wild species is a rare occurrence, a moderately advantageous transgene would be expected to increase rapidly in frequency in a population [57]. Although increased individual fitness does not necessarily translate into increased invasiveness, fitness remains the best predictor of allelic spread. Thus, the fitness effects of a gene in the wild are a far more important consideration than the overall rate of gene flow [57,58]. In this context, V-GURT can reduce pollen and seed-mediated movement of transgenes (Figure 1), and selectively unfit genes can reduce the fitness of feral crops and hybrids between crops and weedy relatives.

There has been widespread release of transgenic crops for the past 10 years but few environmental consequences have been documented [59], and herbicide resistance or insecticidal crops have a limited fitness advantage outside of the agro-ecological environmental. The number of potential traits that can be incorporated into crops and the range of plants used are likely to expand in the future. Traits could include increased stress tolerance, modified food quality and novel bio-industrial products that might increase the hazard or alter crop and hybrid fitness. A wider range of species, including trees, turf and aquatic plants might be developed with more potential to form feral populations. We suggest that V-GURT technology that would reduce the fitness of a plant carrying the transgene will be inherently more useful than GURT technologies that reduce gene flow (such as chloroplast transformation).

Conclusions

Although there has been significant public concern and discussion on GURT, many of the technologies have not been reduced to practice nor have they been tested in the field [51]. Moreover, their ability to effectively impede transgene movement and their environmental safety has yet to be substantiated. Although there are a variety of different GURT mechanisms that can be used to reduce transmission of a transgene, no strategy can completely stop gene movement. It is important to identify the basic processes that might result in the escape of the transgene or its persistence in an agricultural environment. For most commodity crops, processes such as seed admixture or the ability of a plant to produce volunteers are likely to be more important in maintaining transgenes in the agricultural environment than gene flow to wild relatives. Indeed, although there are several different approaches that block trait transmission by pollen, the importance of the movement of genes by pollen might be modest, depending on the crop, compared with the problems raised by the admixture of seed. In addition, any GURT will have to be evaluated based on the ecological impact of that GURT. For example, a GURT that disrupts normal pollen production could be viewed as having an ecological impact on those organisms that use pollen given that pollen supports many organisms

---

1 'Reduced to practice’ is a term used in patenting to indicate that the system works in a particular crop.
(e.g. insects) and provides an important source of nutrition for the ecosystem.

Although it is unlikely that any GURT will address a philosophical distrust of transgenic technologies, there are several paradoxical issues associated with GURTs. First, regulatory agencies prefer streamlined transgenic constructs, which might preclude using additional genes, even if they are safe [59]. Second, if the public’s concern is the consumption of any transgene, will the public accept an additional gene in their food? Any GURT must be evaluated within the context of risk analysis, which encompasses both scientific and ‘non-scientific’ considerations [60].

Acknowledgements
We thank Joseph Bothe for valuable editorial comments and Kofi Garbrah for assistance with the animated supplementary material. This research was funded by an NSERC Discovery grant and an NSERC Collaborative Research and Development grant to A.G.G.

Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2007.02.002.

References
15 Krehbers, E. et al. (1999) Use of anthocyanin genes to maintain male sterile plants, US Patent 5,880,331

www.sciencedirect.com
49 Huang, C.Y. et al. (2003) Direct measurement of the transfer rate of chloroplast DNA into the nucleus. Nature 422, 72–76

Endeavour

The quarterly magazine for the history and philosophy of science.

You can access Endeavour online on ScienceDirect, where you’ll find book reviews, editorial comment and a collection of beautifully illustrated articles on the history of science.

Featuring:

Information revolution: William Chambers, the publishing pioneer by A. Fyfe
Does history count? by K. Anderson
Waking up to shell shock: psychiatry in the US military during World War II by H. Pols
‘Higher, always higher’: technology, the military and aviation medicine during the age of the two world wars by C. Kehrt
Bully for Apatosaurus by P. Brinkman

Coming soon:

Environmentalism out of the Industrial Revolution by C. Macleod
Pandemic in print: the spread of influenza in the Fin de Siècle by J. Mussell
Earthquake theories in the early modern period by F. Willmoth
Science in fiction - attempts to make a science out of literary criticism by J. Adams
The birth of botanical Drosophila by S. Leonelli

And much, much more…

Endeavour is available on ScienceDirect, www.sciencedirect.com