



## Mitigating Transgene Flow from Crops

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Two general approaches are used to deal with transgene flow: containment of the transgenes within the transgenic crop; or transgenic mitigation of the effects of the primary transgenic trait should it escape. Most containment mechanisms severely restrict gene flow only in one direction. Gene flow (leakage) is inevitable even in that direction, allowing spread through the population of undesired species, unless mitigated.

### Limitations to containing transgene flow

Several molecular mechanisms have been proposed to contain transgenes by preventing introgression to relatives *via* pollen. These containment strategies can either suppress gene outflow from the crop, or protect the crop from inflow from wild or weedy relatives.<sup>1</sup> The proposals to integrate the transgene in the plastid or mitochondrial genomes do not preclude the relative from pollinating the crop and then acting as the recurrent pollen parent. Claims of no paternal inheritance of plastome-encoded traits have been not substantiated, indeed maternal inheritance is leaky. Tobacco and other species typically transmit transplastomic traits *via* pollen at a frequency of  $10^{-3}$ – $10^{-4}$  in laboratory experiments. A large-scale field experiment utilized a *Setaria italica* (foxtail millet) with chloroplast-inherited atrazine resistance (bearing a nuclear dominant red leaf base marker) crossed with five different male sterile yellow- or green-leafed herbicide susceptible lines. Chloroplast-inherited resistance was pollen transmitted at a  $3 \times 10^{-4}$  frequency in >780,000 hybrid offspring.<sup>2</sup> Thus, chloroplast transformation is probably unacceptable for preventing transgene outflow, unless stacked with additional mechanisms.

Other molecular approaches suggested for crop transgene containment such as seed sterility, utilizing the "genetic use restriction technologies" (GURT), and recoverable block of function do not prevent transgene outflow from the crop in seed propagation fields, just inflow from the relative.

see 1

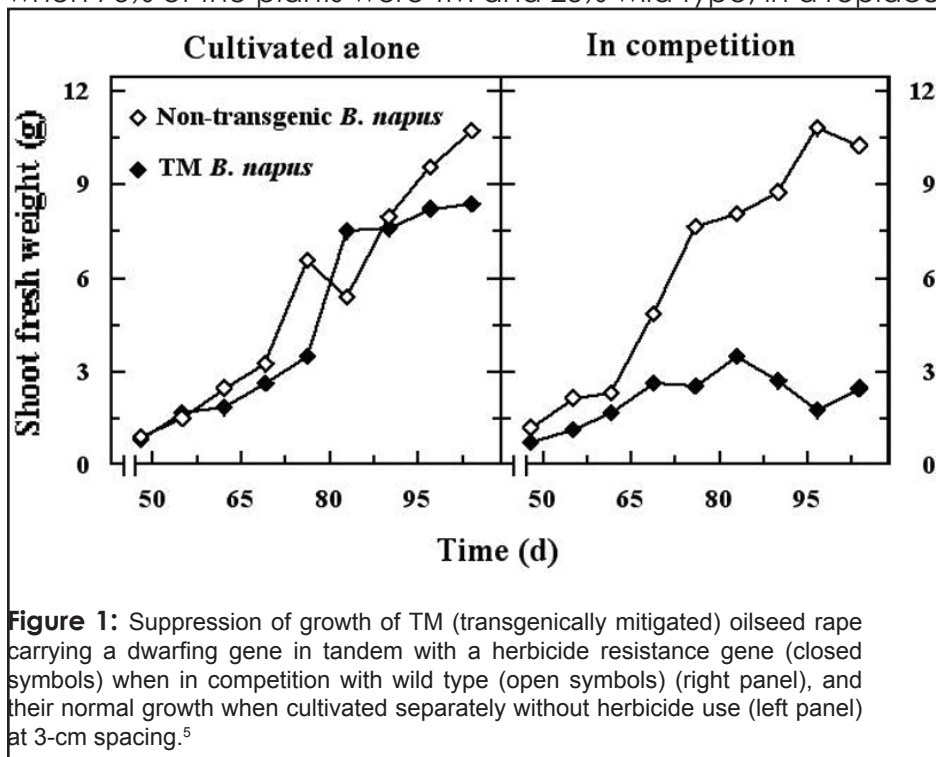
Risk can be reduced by stacking containment mechanisms together, compounding the infrequency of gene introgression, but once it occurs, the new bearer can disperse the transgenes with just a small fitness advantage throughout the population.<sup>1</sup>

### Mitigating establishment of 'leaked' transgenes

The spread of genes can be mitigated by maintaining the fitness of recipients below the fitness of the wild type. A concept of "transgenic mitigation" (TM) was proposed in which mitigator genes are tandemly linked to the desired primary transgene, which would reduce the fitness of hybrids and their rare progeny, considerably reducing risk.<sup>see 1</sup> This TM approach is based on the premises that: 1) tandem constructs act as tightly linked genes with exceedingly rare segregation from each other; 2) the TM traits chosen are neutral or favorable to crops, but deleterious to non-crop progeny; and 3) individuals bearing even mildly harmful TM traits will remain at very low frequencies in weed/wild populations because weeds typically have a very high seed output and strongly compete among themselves, eliminating even marginally unfit individuals. Thus, if the primary transgene of agricultural advantage is flanked in a tandem construct by TM gene(s) such as dwarfing, uniform seed ripening, non-shattering, anti-secondary dormancy, or non-bolting genes, the overall effect would be deleterious after introgression into relatives—the TM genes will reduce the competitive ability of the rare transgenic hybrids such that they cannot compete and persist in low frequencies in agroecosystems.



We used tobacco as a model to test the TM concept: a tandem construct was made containing an *ahas*<sup>R</sup> (acetolactate synthase) gene for herbicide resistance as the primary desirable gene, and the dwarfing  $\Delta gai$  (gibberellic acid-insensitive) mutant gene as a mitigator<sup>3</sup>. Dwarfing would be disadvantageous to the rare weeds introgressing the TM construct, as they could no longer compete with other crops or with fellow weeds, but is desirable in many crops, preventing lodging and producing less straw with more yield. The dwarf and herbicide resistant TM transgenic hybrid tobacco plants were more productive than the wild type when cultivated separately. They formed many more flowers than the wild type, which is indicative of a higher harvest index.<sup>3</sup> Conversely, the tobacco TM transgenics were weak competitors and highly unfit when co-cultivated with the wild type in ecological simulation competition experiments, and none set seeds at close spacing, even when 75% of the plants were TM and 25% wild type, in a replacement series.<sup>4</sup>



**Figure 1:** Suppression of growth of TM (transgenically mitigated) oilseed rape carrying a dwarfing gene in tandem with a herbicide resistance gene (closed symbols) when in competition with wild type (open symbols) (right panel), and their normal growth when cultivated separately without herbicide use (left panel) at 3-cm spacing.<sup>5</sup>

### Mitigation in a *Brassica* crop and related weed

We inserted the same construct into oilseed rape (*Brassica napus*) and tested the selfed progeny,<sup>5</sup> as well as hybrids and backcrosses with the weed *Brassica campestris* = *B. rapa*.<sup>6</sup> When cultivated separately, the dwarf transgenic oilseed rape grew slightly slower than the non-transgenic (**Fig. 1**), but produced > 50% more seed at the expense of the stem tissue (**Table 1**). When the TM transgenic oilseed rape plants were co-cultivated in competition with the wild type, they were suppressed and unable to grow



Biotype	Productivity grown alone	Relative fitness in competition with	
		<i>B. napus</i> NT	<i>B. rapa</i>
<i>B. napus</i> NT	320 <sup>b</sup>	-	4.3
<i>B. napus</i> TM	503 <sup>a</sup>	0.10	2.8
<i>B. rapa</i>	119 <sup>d</sup>	-	-
F <sub>2</sub> hybrids ( <i>B. rapa</i> X <i>B. napus</i> NT)	213 <sup>c</sup>	-	0.9
F <sub>2</sub> hybrids ( <i>B. rapa</i> X <i>B. napus</i> TM)	75 <sup>e,d</sup>	-	0.02
F <sub>2</sub> BC <sub>1</sub> [ <i>B. rapa</i> X (F <sub>1</sub> hybrids NT)]	63 <sup>e,d</sup>	-	0.2
F <sub>2</sub> BC <sub>1</sub> [ <i>B. rapa</i> X (F <sub>1</sub> hybrids TM)]	26 <sup>e</sup>	-	<0.01

normally (**Fig. 1**), and hardly set seed (**Table 1**) because they were so unfit to reproduce.<sup>5</sup> The TM hybrids with the weeds and their further backcrosses to the weeds were also exceedingly unfit and unable to compete with wild type weed.<sup>6</sup> In any rotational system, where the selector herbicide will not be used in the following crops, the TM offspring will be out-competed by non-transgenic cohorts and other species.

Thus, transgenic mitigation is clearly advantageous to a crop grown alone, while disadvantageous to a crop-weed hybrid living in a competitive environment. If a rare pollen grain bearing tandem transgenic traits hybridizes with a relative, it must compete with multitudes of wild type pollen to produce a hybrid. Its rare progeny must then compete with more fit wild type cohorts during self-thinning and establishment. A small degree of unfitness encoded by the TM construct would cause elimination of most progeny in all future generations as long as the primary gene provides no selective advantage and the linked gene confers unfitness.

Field studies are needed with crop/weed pairs to further evaluate risks with mitigation. The rare hybrid offspring from escaped pollen bearing transgenic mitigator genes should not pose a dire threat, especially to wild species outside fields, as the amount of pollen reaching the pristine wild would be minimal, and competition with the wild maximal.

### Special situations require special mitigators

The persistence of pharmaceutical transgenes in maize and their flow by pollen to neighboring fields could be mitigated by a tandem construct with "shrunken seed" loci (RNAi of sugar transformation to starch). Volunteer shrunken seeds and hybrids in nearby fields (discarded during harvest) cannot overwinter. Phytoremediation of soils could utilize the overexpression of cytokinin oxidase; the phenotypes have reduced shoot systems (unfitness to compete) but have faster growing, more extensive root systems, which are better for extracting toxic wastes.<sup>7</sup> Vegetatively propagated trees and other species could be rendered male and female sterile. Biennial and other bolting crops can be made non-bolting by interfering with gibberellic acid production, using the hormone to allow flower production for seed.<sup>1</sup>

Thus, transgenic mitigation systems can probably be conjured for any need to mitigate the effects of gene flow. The greatest security will be obtained when the gene of choice is flanked on either side by TM constructs, and is stacked with a containment system, or is in a male sterile background.<sup>8</sup>



## References

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