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Scientists Discover New Route for GM-gene 'Escape'

Genetically modified genes can jump species via wounds, yes horizontal gene transfer happens, and at high frequencies; it is the greatest, most underestimated hazard from GMOs released into the environment *Dr. Mae-Wan Ho*

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Gene "escape" a misnomer for horizontal gene transfer

Scientists at Bristol University in the UK announced the discovery of [1] "a previously unknown route" whereby "GM genes may escape into the natural environment." "Escape" is a misnomer. There is no need for the GM (genetically modified) genes to "escape", when genetically modified organisms (GMOs) have been released in great abundance and with gay abandon into the environment over the past 17 years. At issue is how fast and how widely the GM genes can *spread*, and what dire consequences could arise.

The "escape" referred to is horizontal gene transfer – the spread of GM genes by infection and multiplication (literally like a virus) regardless of species barriers; hence the rate of spread is much more rapid, and the extent virtually unlimited. New combinations of genetic material are created at unprecedented speed; affecting species the most that reproduce the fastest, i.e., bacteria and viruses that cause diseases. Horizontal gene transfer and recombination is indeed a main route for generating new strains of bacteria and viruses that cause diseases. Genetic modification and release of GMOs into the environment is nothing if not greatly facilitated horizontal gene transfer and recombination. It has created highways for gene trafficking in place of narrow by-ways and occasional footpaths that previously existed.

Some of us have long considered horizontal gene transfer to be *the* most serious hidden and underestimated hazard of genetic engineering, and have alerted regulators accordingly, time and again, since GMOs were first released (see for example [3, 4] (Gene Technology and Gene Ecology of Infectious Diseases, ISIS scientific publication; Genetic Engineering Dream or Nightmare, ISIS publication). The recent "emergency" warning sent by a senior US Department of Agriculture scientist to US Secretary of Agriculture on a suspected pathogen "new to science" associated with GM crops may prove to be a case in point [5] (Emergency! Pathogen New to Science Found in Roundup Ready GM Crops? *SiS* 50).

Plant wounds hotspots for gene trafficking

The researchers at Bristol University showed that plant wounds, that could be created by insect bites, abrasion and other mechanical damage, are hotspots for gene trafficking due to

the wound hormones produced by the plant. Under such circumstances, the soil bacterium *Agrobacterium tumefaciens*, which causes crown gall disease in plants, could enlarge its host range to infect fungi, and insert foreign genes into the fungi's genome [2]. This has large implications on the safety of GMOs already widely released into the environment.

A. tumefaciens is probably unique among natural plant pathogens in carrying out trans-Kingdom horizontal gene transfer during an infection, and it is this ability that has been widely exploited for creating GM crops, grown on an estimated 134 million hectares worldwide in 2009, and "jumped' another 10 percent in 2010, according to industry-funded International Service for the Acquisition of Agri-biotech Applications (ISAAA) [6].

Research commissioned by the UK Department of the Environment, Food and Rural Affairs (DEFRA) in the 1990s had already revealed that it is very difficult, if not impossible to get rid of the *Agrobacterium* vector used in creating the transgenic plant [7], and the bacterium is likely to remain dormant even after the transgenic plants are transplanted into the soil. Hence, it is expected to facilitate horizontal gene transfer, in the first instance, to wild-type *Agrobacterium* in the soil, and further afield.

Disease-causing strains *of A. tumefaciens* have an extrachromosomal Ti (tumour-inducing) plasmid that enables the horizontal transfer of a segment of the Ti plasmid, the T-DNA, into the plant cell genome when the bacterium's virulence (disease causing) system is activated by hormones produced by the wounded plant. This feature is exploited in creating genetically modified organisms (GMOs), by disarming the bacterium, and incorporating the virulence genes in a 'binary' vector that has to be used in conjunction with the disarmed *Agrobacterium* strain.

In the 1990s, it was shown that the range of organisms transformed by *Agrobacterium* could be extended if the wound hormone acetosyringone was used to induce the virulence system.

The researchers at Bristol University reasoned that as *A. tumefaciens* is a soil-dwelling pathogen that often infects plants through wounds, it is conceivable that the bacterium could encounter numerous species of microorganisms, including pathogenic fungi that the same method to gain entry into the plant. The wound sites are likely to be exuding wound hormones such as acetosyringone, so the bacteria are primed for T-DNA transfer.

Experiments confirmed their suspicion in full

They carried out their investigation using the wilt-causing fungus *Verticillium albo-atrum*, a strong candidate for encounters with *Agrobacterium* in the plant, as it has a similar wide host range in plants, infecting both root and crown. Previous lab experiments have shown that *V. albo-atrum* cannot be transformed by *Agrobacterium* in the absence of acetosyringone. So, if it is presented with *Agrobacterium* on plant tissue, and transformation does occur, it must be the plant that supplies the wound hormone.

Peeled and sliced potato tubers and carrots, leave- and stem-sections from tobacco plants were used as the plant tissues for testing. After sterilization, they were inoculated with both *A. tumefaciens* and *V. albo-atrum* and left at room temperature in a covered agar dish for a minimum of 8 days and a maximum of 42 days.

Successful transformants of *V. albo-atrum* were obtained from every kind of plant tissue. 2 out of 17 potato slices, 1 out of 15 carrot slices; 14 out of 42 dishes each with 3-5 leaf pieces, and 10 out of 31 stem sections (without agar plate, so as to be as close to the natural condition as possible). These transformants were confirmed with molecular genetic analyses.

Implications on risk assessments of GMOs still understated

The researchers concluded [2]: "This work therefore raises interesting questions about whether the host range of *A. tumefaciens* in nature is greater than just plants. It is possible that evidence of such events could be looked for retrospectively in the increasing number of genome sequences becoming available....

"In addition, the result may well have implications for the risk assessment of GM plants generated via *Agrobacterium*-mediated transformation, as *Agrobacterium* can survive within plant tissue through transformation and tissue culture and can therefore be found within regenerated transgenic plants..."

This is an understatement of a serious risk that has been known almost since the first release of *Agrobacterium*-transformed GMOs into the environment.

The risks are far greater than admitted

We have repeatedly drawn attention to the possibility of facilitated horizontal gene transfer from GMOs created with *Agrobacterium* vector, which is even stronger than originally envisaged due to other discoveries made since then. I reproduce what we wrote in 2008 [8] (Horizontal Gene Transfer from GMOs Does Happen, *SiS* 38), which repeats an earlier account [9] (Living with the Fluid Genome , ISIS publication) (see Box).

Agrobacterium vector a vehicle for facilitated horizontal gene transfer [8, 9]

"We have ..provided evidence strongly suggesting that the most common method of creating transgenic plants may also serve as a ready route for horizontal gene transfer [9, 10].

"Agrobacterium tumefaciens, the soil bacterium that causes crown gall disease, has been developed as a major gene transfer vector for making transgenic plants. Foreign genes are typically spliced into the T-DNA - part of a plasmid of *A. tumefaciens* called Ti (tumour-inducing) – which ends up integrated into the genome of the plant cell that subsequently develops into a tumour.

"But further investigations revealed that the process whereby *Agrobacterium* injects T-DNA into plant cells strongly resembles *conjugation*, the mating process between bacterial cells.

Conjugation, mediated by certain bacterial plasmids requires a sequence called the origin of transfer (*oriT*) on the DNA that's transferred. All the other functions can be supplied from unlinked sources, referred to as 'trans-acting functions' (or *tra*). Thus, 'disabled' plasmids, with no trans-acting functions, can nevertheless be transferred by 'helper' plasmids that carry genes coding for the trans-acting functions. And that's the basis of a complicated vector system devised, involving *Agrobacterium* T-DNA, which has been used for creating numerous transgenic plants.

"It soon transpired that the left and right borders of the T-DNA are similar to *oriT*, and can be replaced by it. Furthermore, the disarmed T-DNA, lacking the trans-acting functions (*virulence* genes that contribute to disease), can be helped by similar genes belonging to many other pathogenic bacteria. It seems that the trans-kingdom gene transfer of *Agrobacterium* and the conjugative systems of bacteria are both involved in transporting macromolecules, not just DNA but also protein.

"That means transgenic plants created by the T-DNA vector system have a ready route for horizontal gene escape, via *Agrobacterium*, helped by the ordinary conjugative mechanisms of many other bacteria that cause diseases, which are present in the environment.

"In fact, the possibility that *Agrobacterium* can serve as a vehicle for horizontal gene escape was first raised in 1997 in a study sponsored by the UK Government [7, 12], which found it extremely difficult to get rid of the *Agrobacterium* in the vector system after transformation. Treatment with an armoury of antibiotics and repeated subculture of the transgenic plants over 13 months failed to get rid of the bacterium. Furthermore, 12.5 percent of the *Agrobacterium* remaining still contained the binary vector (T-DNA and helper plasmid), and *were hence fully capable of transforming other plants.*

"Agrobacterium not only transfers genes into plant cells; there is possibility for *retro*transfer of DNA *from* the plant cell *to Agrobacterium* [13]. High rates of gene transfer

are associated with the plant root system and the germinating seed, where conjugation is most likely [14]. There, *Agrobacterium* could multiply and transfer transgenic DNA to other bacteria, as well as to the next crop to be planted. These possibilities have yet to be investigated empirically.

"Finally, *Agrobacterium* attaches to and genetically transforms several human cell lines [15, 16] (Common plant vector injects genes into human cells *ISIS News* 11/12). In stably transformed HeLa cells (a human cell line derived originally from a cancer patient), the integration of *T-DNA* occurred at the right border, exactly as would happen when it is transferred into a plant cell genome. This suggests that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transforming plants cells.

"The possibility that *Agrobacterium* is a vehicle for horizontal transfer of transgenic DNA remains unresolved to this day."

Agrobacterium transfers genes into human cells

It is also worth reiterating our comment on the scientific paper [15] documenting that *Agrobacterium* can transfer genes into human cells [16].

"The paper shows that human cancer cells along with neurons and kidney cells were transformed with the *Agrobacterium* T-DNA. Such observations should raise alarm for those who use *Agrobacterium* in the laboratory.

"The integrated T-DNA will almost certainly act as a mutagen as it integrates into human chromosomes. Cancer can be triggered by activation of oncogenes (ie, cancer genes) or inactivation of cancer-suppressing genes. Furthermore, the sequences carried within the T-DNA in the transforming bacterium can be expressed in the transformed cells (the viral promoter CaMV has been found to be active in HeLa cells [17]) ….

"It is clear that little has been done to prevent environmental escape of the transforming bacteria or to quantify such releases. In conclusion, a study of cancer incidence among those exposed to *Agrobacterium tumefaciens* in the laboratory and in the field is needed. It would be worthwhile to screen workers for T-DNA sequences."

To conclude

The discovery by the Bristol University researchers barely scratches the surface of the hidden hazards of GMOs from horizontal gene transfer. It is high time for a global ban to be imposed on further environmental releases of GMOs, and all those responsible for releasing them should be brought to book.

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