Genetically engineered *Bt* rice – food safety concerns and environmental dangers

Cotter, J.

Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Exeter EX4 4PS, UK

April 2005

Technical Note: 05/2005

Genetically Engineered *Bt* Rice – food safety concerns and environmental dangers.

Janet Cotter

Greenpeace Research Laboratories Technical Note 05/2005

Introduction

Genetically engineered (GE) insect resistant *Bt* rice has not been approved for cultivation anywhere in the world. There is no environmental assessment, nor human food safety assessment available for any GE *Bt* rice. However, studies from other GE *Bt* crops such as maize (corn) and cotton give strong indications that *Bt* rice will have serious environmental consequences and there are human food safety concerns.

Food safety concerns of GE *Bt* rice include:

- Rice is the most important food for many people.
- No food safety assessment.
- Cry1Ac is a potential allergen.

Insect resistant GE *Bt* rice could lead to adverse effects on the environment if:

- non-target species are harmed, or
- the emergence of more troublesome weeds is encouraged;
- insects resistant to the introduced toxin evolve and require more intensive chemical control.
- genetic resources are contaminated.

What is GE insect resistant Bt rice?

GE insect resistant *Bt* rice varieties are developed to be resistant to certain pests such as leaffolder and yellow stem borer[1]. *Bt* cops are created by inserting a synthetic version of a gene from the naturally occurring soil bacterium, *Bacillus thuringiensis* (*Bt*) into the plant's own DNA, so the plant creates its own toxin to destroy pests.

There are several different types of GE *Bt* rice known to be under experimentation, either in the laboratory, or as field trials. These produce slightly different *Bt* toxins and include: Cry1Ab; Cry1Ac and those that contain both toxins "fused" together, Cry1Ab/Cry1Ac. These types of *Bt* toxins have slightly different properties. Most environmental studies have been conducted with the Cry1Ab toxin that is most commonly used in *Bt* maize. There are human food safety concerns concerning Cry1Ac. However, there is little to no information available at all on aspect of the human food safety or risk to the environment from fused toxins, Cry1Ab/Cry1Ac as it has not been used in any commercially grown GE food crops, anywhere in the word and there is no natural comparison. Monsanto's GE Bollgard cotton contain does contain a gene for a fused Cry1Ab/Cry1Ac[2], but it is not known if this is the same *Bt* protein produced in GE rice and cotton is not a food crop.

GE Bt rice and food safety

On average, rice provides 30% of calorie and 19% of protein intake in China[3]. Rice also forms an important part of the diet at all ages, including for babies where rice flour and gruel form a part of foods given during weaning[4]. GE maize and soybeans are largely used for animal feed and experiences with these crops cannot be used to assume the safety of GE rice.

Key questions for food safety are:

1) whether the genetic modification has resulted in potentially harmful unintended changes, and the implications of these if they occur; and
2) any toxic or allergic effects of the gene products introduced through genetic engineering.

No food safety assessment has been made for any *Bt* rice. There is limited information on Cry1Ab GE rice[5]. But there is no information available for the fused Cry1Ab/Cry1Ac toxin, its food safety is completely unknown.

For Cry1Ac, there is concern over its potential allergenicity. Research considering the immunogenicity of the Cry1Ac toxin[6] indicates that

- Cry1Ac protoxin is a potent immunogen.
- The protoxin is immunogenic by both the intraperitoneal (injected) and intragastric (ingested) route.
- The immune response to the protoxin is both systemic and mucosal.
- Cry1Ac protoxin binds to surface proteins in the mouse small intestine.

These research reports suggest extreme caution is required in the use of Cry1Ac GE rice.

Although the *Bt* toxins are likely to be at least partially degraded during cooking of rice before consumption, limiting their possible allergenic or toxic potential, there is, as yet, no data on this. This potential for allergenicity requires special caution and attention especially as rice is a staple food crop. The allergy concerns in relation to Cry1Ac or the fused protein in GE *Bt* rice could have regulatory consequences. For example, StarLink *Bt* maize was not allowed to be used in human food in North America because of the risk to cause allergies.

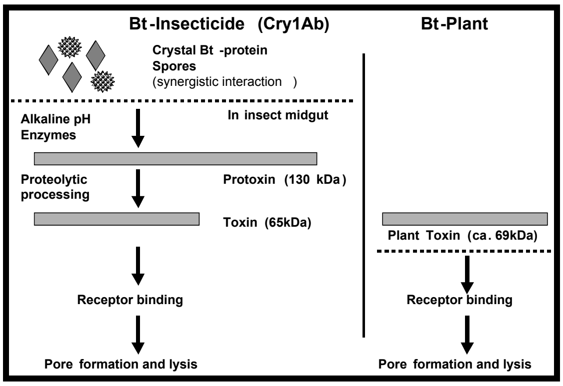
The FAO/WHO Codex Alimentarius, who are developing international standards for GE food safety testing have adopted a “decision tree” approach[7]. This means that, should any evidence of possible allergy be found (as is the case with Cry1Ac), a very thorough and detailed assessment on the allergenic risks would need to be performed according to the FAO/WHO guidelines. Therefore, Cry1Ac must be examined thoroughly as a potential allergen. This has not yet been done.

The human food safety of Bt GE rice is unknown. A thorough investigation for allergenicity is required.

Impacts on GE *Bt* rice on non-target organisms

In its natural form, *Bt* has been used by farmers practising organic and other sustainable growing methods since the 1950s as a spray to kill pests without damaging non-targeted insects or other wildlife. However, the *Bt* toxins produced by insect resistant rice are significantly different and have the potential to be harmful to beneficial predator insects.

Natural *Bt* sprays have little effect on non-target organisms because the bacterial "pro-toxin" is in an inactivated state and only becomes toxic when processed in the gut of certain (targeted) species of insect larvae. In contrast, many insect resistant plants contain an artificial, truncated *Bt* gene and less processing is required to generate the toxin. It is therefore less selective, and may harm non-target insects that do not have the enzymes to process the pro-toxin, as well as the pests for which it is intended (Fig. 1)[8].



Bt proteins from natural *Bt* sprays degrade relatively quickly in the field as a result of ultraviolet light and lose most toxic activity within several days to two weeks after application[9]. In *Bt* crops, however, the *Bt* toxin is produced throughout the entire lifespan of the plants.

Direct Effects:
GE *Bt* rice, like other *Bt* crops, could be harmful to non-target organisms if they either consume the toxin directly in pollen or plant debris, or indirectly by feeding on pests that have ingested the toxin. This could cause harm to ecosystems by reducing the numbers of important species, or reduce the numbers of beneficial organisms that would naturally help control the pest species.

The *Bt* toxins in GE rice are specifically toxic to Lepidoptera (butterflies and moths), but not all of these are pests. The potential for GE *Bt* crops to be directly toxic to non-target species was highlighted by research in the USA when it was demonstrated that pollen from one type of GE *Bt* maize (Bt176) was toxic to the much-loved Monarch butterfly[10].

More recently, it has been shown that long-term exposure even to relatively low levels of *Bt* in maize pollen causes adverse effects on larvae of the Monarch butterfly[11]. Importantly, these risks to non-target species were not identified until after commercialisation of *Bt* maize, and required several years of research for the long-term implications to be realized.

In Asia, the silkworm, *Bombyx mori,* is sensitive to the *Bt* toxin Cry1Aa, although much less so to Cry1Ab and Cry1Ac[12,13]. Research has shown that pollen is found on mulberry plants used for silkworms at levels that would be toxic if it contained Cry 1Aa *Bt* toxin[14]. In areas where silk production takes place using mixed rice/mulberry cropping, if Cry1Aa producing varieties of GE rice are introduced and the toxin is expressed in pollen, serious impacts on the silkworm could arise. In addition, long-term exposure to other *Bt* toxins such as Cry1Ab and Cry1Ac could quite possibly have harmful effects on silkworms similar to those observed to low level exposure of *Bt* to Monarch butterfly larvae. However, such long-term studies have not yet been performed.

GE Bt rice could have adverse effects on wildlife, particularly butterflies and moths.

Indirect effects:
Data from *Bt* maize indicate that the beneficial insects, lacewings, have increased mortality when fed on larvae of a maize pest, the corn borer, which had been fed on *Bt*[15]*.* Numbers of beneficial ladybeetles were found to be lower in *Bt* maize plots than in non-*Bt* maize. Ladybeetles feed on many food sources including on aphids, pollen, European corn borer eggs and other pest eggs[16], so have several routes of exposure to the *Bt* toxin. Non-target, beneficial species that may feed on rice pests could be similarly affected.

Changes in populations of both pests and of natural enemies have been documented in *Bt* cotton. Data from China show that use of *Bt* crops can exacerbate populations of other secondary pests, including aphids, lygus bug, whitefly, Carmine spider mite and thrips[17]. Studies there have shown significant reductions in populations of the beneficial parasites *Microplitis sp*. (88.9% reduction) and *Campoletis chloridae* (79.2% reduction) in *Bt* cotton fields[18].

Bt toxins from GE rice could be passed on higher up the food chain, affecting other organisms. GE Bt rice could also affect beneficial insects.

Impact of GE *Bt* Rice on soil health

Recent research has also show that *Bt* toxin is released from roots of *Bt* rice containing Cry1Ab and that the toxin persists as a result of binding to soil particles[19,20]. It is not clear whether the release of such toxins will aid in control of pest species or harm non-target species in soil.

Recent laboratory studies in China have identified changes in some soil enzyme activities and microbial communities when Bt-rice straws were incorporated in water-flooded soils[21,22]. It was considered that some change in the composition of the GE rice as a result of the introduction of the *Bt* gene was responsible, although the implications of the findings for soil fertility have not been tested. Lignin production in *Bt* maize has also been found to be unexpectedly increased which raised questions about its impact on degradation in soil[23].

Soil organisms play a crucial role in soil health, and therefore agriculture. An unknown number of species make up the soil food web and could be affected by *Bt* – yet tests have been conducted on very few, in very few soil types and ecosystems.

GE Bt rice may be problematic for long-term soil health, as it expresses proteins that are known to be toxic to certain insects.

Effects on Sustainable Agriculture

An additional environmental hazard of insect resistant crops is that targeted pests could develop resistance to the effects of *Bt*. This is because constant exposure to the *Bt* toxin produced by these plants encourages the survival of individual pests which have a genetic immunity to *Bt*. Over time, this could lead to the proliferation of resistant individuals to the extent that *Bt* would no longer be effective against the majority of the targeted pest population[24].

If widespread resistance were to occur, the insect resistant properties of the GE crops would become ineffective. The application of new and even more toxic chemical pesticides would therefore be almost inevitable.

Increased resistance would pose a serious threat to the use of Bt sprays, which are a useful tool in sustainable agriculture methods.

Potential for contamination

Wild rice:
Asia is the centre of origin of rice and, therefore, wild species with which cultivated rice (*Oryza sativa*) can outcross to (pollinate) are widely distributed. These wild species are sometimes weeds. Although rates of outcrossing are low compared to other crops, they are still considered significant[25]. The production of crosses between cultivated GE and wild rice is considered inevitable over time, where the two occur together. Insect-resistance in GE crops (e.g. *Bt* crops) is considered by scientists to be a fitness-enhancing gene, and thus likely to increase in frequency and spread throughout local populations[26]. The introduction of the *Bt* trait, which would improve the competitiveness of wild rice varieties could lead to their emergence as more problematic weeds. Such *Bt* wild rice may also swamp natural wild varieties and possibly lead to their extinction.

Although *O.rufigpon* is not present in central China, and is not a problem weed in rice fields, it does occur in the southern provinces of Guangdong, Guangxi, Hainan and Yunnan and it is considered endangered[27]. Therefore, GE rice could lead to negative impacts on this species and add to the already established need to protect these populations from gene flow from cultivated rice[28, 29].

The loss of wild species of rice through genetic contamination poses a considerable threat to efforts to conserve natural biodiversity and represents a serious loss of genetic resources and which can jeopardize breeding and food security in the future as breeding efforts depend on diverse genetic resources.

Weedy rice:
Where red rice is a problem, gene flow is inevitable over time, despite low outcrossing frequencies[30, 31]. The transfer of genes giving resistance to insect attack and disease may further increase the competitive advantage of red rice.

Non-GE rice:
Although rice is largely self-pollinating, pollen movement to 100 metres has been detected and is strongly influenced by wind speed and direction[32]. Therefore, some degree of cross pollination of neighbouring non-GE rice is almost inevitable.

Conclusions

1. The human food safety of Bt GE rice is unknown. A thorough investigation for allergenicity is required;
2. GE Bt rice could have adverse effects on wildlife, particularly butterflies and moths;
3. Bt toxins from GE rice could be passed on higher up the food chain, affecting other organisms. GE Bt rice could also affect beneficial insects;
4. GE Bt rice may be problematic for long-term soil health, as it expresses proteins that are known to be toxic to certain insects;
5. Increased resistance would pose a serious threat to the use of Bt sprays, which are a useful tool in sustainable agriculture methods;
6. The loss of wild species of rice through genetic contamination poses a considerable threat to efforts to conserve natural biodiversity and represents a serious loss of genetic resources.

References

[1] Tu, J., Zhang, G., Datta, K., Xu, C., He, Y. Zhang, O., Khush,G. and Datta, S.K. 2000. Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. Nature Biotechnology 18: 1101-1104.

[2] http://www.agbios.com/dbase.php?action=ShowProd&data=MON531%2F757%2F1076&frmat=LONG

[3] Rice Today, September 2002. Rice Facts. Essential food for the poor. http://www.irri.org/publications/today/pdfs/1-2/facts1-2.pdf <28th October 2004>

[4] Ministry of Health and Welfare, Japan (1999) Guideline for weaning (revised edition) Pediatrics International 41 (1): 115 - doi: 10.1046/j.1442-200x.1999.01037.x

[5] Wang, Z.H., Wang, Y., Cui, H.R., Xia, Y.W., Altosaar, I. & Shu, Q.Y. 2002. Toxicological evaluation of transgenic rice flour with a synthetic cry1Ab gene from *Bacillus thuringiensis*. Journal of the Science of Food and Agriculture 82: 738-744.

[6] Moreno-Fierros, L. García, N. Gutiérrez,R. López-Revilla, R.Vázquez-Padrón, RI..(2000). Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from *Bacillus thuringiensis* induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. Microbes Infect 2(8): 885-90; . Vázquez-Padrón, R.I, Moreno-Fierros, L. Neri-Bazán, L, de la Riva, G.A & López-Revilla, R. (1999). *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol 49: 578-584; Vázquez-Padrón, R.I Moreno-Fierros, L. Neri-Bazán, L, de la Riva, G.A & López-Revilla, R. (1999). Intragastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. Life Sciences 64(21): 1897-1912; Vázquez-Padrón, R. I., Moreno-Fierros, L. Neri-Bazán. L. Martínez-Gil, A.F., de la Riva, G.A. & López-Revilla, R.. (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. Braz J Med Biol Res 33: 147-155; Vázquez-Padrón, R. I., Gonzáles-Cabrera, J., García-Tovar, C. Neri-Bazán, L., López-Revilla, R., Hernández, M., Moreno-Fierros, L. & de la Riva.G.A. (2000). Cry1Ac protoxin from *Bacillus thuringiensis* sp. *kurstaki* HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Comms 271: 54-58.

[7] FAO-WHO 2001. Evaluation of Allergenicity of Genetically Modified Foods. Report of the joint FAO/WHO expert consultation on allergenicity of foods derived from Biotechnology, 22-25 January 2001, pp1-26.

[8] Hillbeck, A. 2001. Transgenic host plant resistance and non-target effects. In: *Genetically engineered organisms: assessing environmental and human health effects.* Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press. Hilbeck, A., M.S. Meier and A. Raps. 2000. Review on non-target organisms and *Bt* plants. Report prepared for Greenpeace International, Amsterdam, EcoStrat GmbH, Ecological Technology Assessment & Environmental Consulting, Zurich, Switzerland

[9] Hillbeck, A. 2001. Transgenic host plant resistance and non-target effects. In: *Genetically engineered organisms: assessing environmental and human health effects.* Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press.

[10] Losey J. E, Raynor, L. & Cater, M.E. (1999). Transgenic pollen harms monarch larvae. Nature 399: 214.

[11] Dively, G.P., R. Rose, M.K. Sears, R.L. Hellmich, D.E. Stanley-Horn, D.D. Calvin, J.M. Russo and P.L. Anderson. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab expressing corn during anthesis. *Environmental Entomology* 33: 1116-1125.

[12] Jenkins J.L & Dean ,D.H. (2001) Binding specificity of *Bacillus thuringiensis* Cry1Aa for purified, native *Bombyx mori* aminopeptidase N and cadherin-like receptors *BMC Biochemistry* 2001, 2:12. http://www.biomedcentral.com/1471-2091/2/12 <29th October 2004>.

[13] Wang Z-H Shu Q-Y, Cui H-R, Xu M-K, Xie X-B, Xia Y-W, (2002) The effect of *Bt* transgenic rice flour on the development of silkworm larvae and the sub-micro-structure of its midgut. Scientia Agricultura Sinica 35: 714-718.

[14] Fan, L-J., Wu, Y-Y., Pang, H-Q., Wu, J-G., Shu, Q-J., Xu, M-K., Lu, J-F. 2003. *Bt* rice pollen distribution on mulberry leaves near rice fields. Acta Ecologica Sinica 23: 826-833.

[15] Hillbeck, A., Baumgartner, M., Fried, P.M. & Bigler, F. 1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environmental Entomology 27: 480-487; Hillbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A. & Bigler, F. (1998) Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Crysoperla carnea* (Neuroptera: Chrysopidae). Environmental Entomology 27: 1255-1263.

[16] Wold, S.J., Burkness E.C., Hutchinson, W.D.,Venette, R.C. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringensis* toxin. Journal of Entomological Science 36: 177-187.

[17] Cui, J. and J. Xia. 1998. Effects of transgenic *Bt* cotton (with early maturity) on population dynamics of main pests and their natural enemies. *Acta Gossypii Sinica* 10: 255-262.

[18] Cui, J. and J. Xia. 1999. Effects of transgenic *Bt* cotton on the population dynamics of natural enemies. *Acta Gossypii Sinica* 11: 84-91

[19] Saxena, D. Stewart, CN, Altosaar, I. Shu, Q. & Stozky, G. 2004. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato and rice but not of *B. thuringiensis* canola, cotton and tobacco. Plant Physiology and Biochemistry 42: 383-387.

[20] Sun, C. Wu, Z., Zhang, Y. & Zhang, L. 2003. Effect of transgenic *Bt* rice planting on soil enzyme activities. Ying Yong Sgeng Tai Xue Bao 14: 2261-2264.

[21] Wu, W-X., Ye, Q-F, Hang, M, Duan, X-J, Jin, W-M. (2004) Bt-transgenic rice straw affects the culturable microbiota and dehydrogenase and phosphatase activities in a flooded paddy soil. Soil Biol Biochem 36: 289-295.

[22] Wu, W-X., Ye, Q-F. & Min, H. (2004) Effect of straws from Bt-transgenic rice on selected biological activities in water-flooded soil. Eur J Soil Biol 40: 15-22.

[23] Saxena, D. & Stotzky, G. 2001. *Bt* corn has a higher lignin content than non-*Bt* corn, American Journal of Botany. 88:1704-1706.

[24] See for example Andow, D.A. 2001. Resisting resistance to *Bt* corn. In: *Genetically engineered organisms: assessing environmental and human health effects.* Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press.

[25] Lu, B.-R., Song, Z. & Chen, J. 2003. Can transgenic rice cause ecological risks through transgene escape? Progress in Natural Science 13: 17-24. Song, Z.P., Lu, B-R., Zhu, Y.G. & Chen, J.K. 2003. Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. New Phytologis*t* 157: 657-665. Song, Z.P., Lu, B-R., Zhu, Y.G. & Chen, J.K. 2004. Pollen flow of cultivated rice measured under experimental conditions. Biodiversity and Conservation 13: 579–90.

[26] Stewart, Jr., C.N., J.N. All, P.L., Raymer, and S. Ramachandran. 1997. Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Molecular Ecology* 6: 773-779. Vacher, C., A.E. Weis, D. Hermann, T. Kossler, C. Young, M.E. Hochberg 2004. Impact of ecological factors on the initial invasion of *Bt* transgenes into wild populations of birdseed rape (*Brassica rapa*) *Theoretical and Applied Genetics* 109: 806-814.

[27] Gao, L. 2004. Population structure and conservation genetics of wild rice *Oryza rufipogon* (Poaceae): a region-wide perspective from microsatellite variation Molecular Ecology 13: 1009–1024.

[28] Song, Z.P., Lu, B.-R., Zhu, Y.G. & Chen, J.K. 2003. Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. New Phytologist 157: 657-665

[29] Song Z. P., Xu, X. Wang, B., Chen, J,K, & Lu, B-R. 2003. Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. Theoretical and Applied Genetics107:1492-1499.

[30] Messeguer, J. Marfa, V. Catala, MM, Guideroni, E. & Mele, E. 2004. A field study of pollen mediated gene flow from Mediterranean GM rice to conventional rice and the red rice weed. Molecular Biology 13: 103-112.

[31] Zhang, N. Linscombe, S. & Oard, J. 2003. Out-crossing frequency and genetic analysis of hybrids between tansgenic glufosinate herbicide-resistant rice and the weed, red rice. Euphytica 130: 35-45.

[32] Song, Z.P Lu, B-R & Chen JK*.* 2004. Pollen flow of cultivated rice measured under experimental conditions. *Biodiversity and Conservation* 13: 579–90.