GM: Toxic effects of Bt varieties

There is now so much evidence of harmful toxic effects associated with BT varieties that all past approvals by the EC must be revisited. The approvals procedures for GM varieties "in the pi [peline" must also be stopped instantly. If they are not, that would be interpreted by any reasonable person as criminal negligence by EFSA and by Commissioners Dimas and Kyprianou in particular.

Note the cases of "toxic shock" leading to animal deaths:

Cows ate GM maize and died (BT176) http://www.i-sis.org.uk/CAGMMAD.php

25% death rate among sheep which grazed on Bt cotton plants in India http://www.gmwatch.org/archive2.asp?arcid=6499 http://www.gmwatch.org/archive2.asp?arcid=6494

Note also the effects on human health arising from contact with Bt varieties: http://www.i-sis.org.uk/full/MILTBTFull.php http://www.gmwatch.org/archive2.asp?arcid=6472

Some Australian research not widely reported.......... It refers to Cry1Ac toxin and backs up the findings of the Hungarian team including Bela Darvas, whose findings for MON810 maize are appended at the base of the page.

From Gupta and Watson:

Quote: " ..... We have shown that different plant parts of Bt cotton (leaves, stubble and roots) contain large concentrations of Bt toxin and therefore have the potential to be a reservoir of Bt toxin in agricultural fields of Australia."

Quote: "........... our results suggest that Bt toxin has the potential to enter the soil system throughout the Bt cotton growing season, through both a root release process and root turnover. Levels of Bt toxin entering the soil system could therefore be significantly higher than previously suggested ...."

Quote: " ..... roots with Bt toxin are in constant contact with the soil system (including soil biota) and Bt toxin levels in fine roots were found to be as high as that in younger leaves. In view of the results reported above (large concentrations of Bt toxin in Bt cotton roots and demonstrated root release), more detailed investigations on the environmental fate of the root-derived Bt toxin, binding to soil components and build up, and movement beyond the rhizosphere and root zone, are warranted."

The concerns of the authors shine through, although they are careful (so as to keep their paymasters happy) not to flag up "harm" or even the potential for harm. We can see the heavy hand of CSIRO here, in playing down the significance of the findings. But this research is very relevant, given the new revelations about sheep deaths in India among animals grazing on Bt cotton plants.

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Ecological impacts of GM cotton on soil biodiversity Below ground production of Bt by GM cotton and Bt cotton impacts on soil biological processes Dr Vadakattu VSR Gupta and Dr Stephanie Watson Consultancy report by CSIRO Land and Water, August 2004

http://www.deh.gov.au/settlements/publications/biotechnology/gm- cotton/summary.html

Summary This research programme focuses on the impacts of genetically modified (GM) cotton crops (Bacillus thuringiensis cotton; Bt cotton for short) on soil biodiversity and ecosystem function. The experimental work was based upon the need to establish the risk of production and release of Bt toxin by below ground plant parts of cotton and its potential persistence in the soil where Bt cotton crops are grown in Australia. In addition, the potential impacts of new gene products may affect key soil biological processes essential for a number of ecosystem functions.

Genetic modification of organisms (plants, microbes and animals) to incorporate useful traits is a powerful technology for the future development of sustainable agricultural systems. Transgenic cotton varieties modified to express the Cry1Ac insecticidal toxin (Bt cotton) that is toxic to some insect pests are now grown in Australia. However, little experimental data (especially quantitative) is available on the environmental consequences of sustained expression and/or presence of Bt toxin in various parts of Bt cotton plants. In addition very little is known about the potential for the persistence of Bt toxin released from Bt cotton plants in Australian cotton soils.

Soil biota mediate or regulate a variety of functions essential for plant growth and productivity, soil resource structure, and ecosystem health. Soil biota are diverse in terms of their physiological nature, size and environmental requirements. The composition and metabolic capabilities of the soil microbial and faunal communities underpin the occurrence and rates of many soil processes. Microbial- faunal (micro-, meso- and macrofauna) interactions play a critical role in a variety of biological functions both in the rhizosphere (the zone directly surrounding and influenced by roots) and the soil near decomposing plant residues. Plant residues are the primary source of metabolic energy (carbon) in Australian soils and the majority of biota populations and biota-mediated processes are concentrated in the rhizosphere and near crop residues. Therefore any change to the quality of crop residues and rhizosphere inputs will potentially modify the dynamics of soil biota composition and activity (Gupta et al., 1998 and 1999). Genetically modified plants, through (1) the products of introduced genes, (2) modified rhizosphere chemistry, or (3) altered crop residue quality, have the potential to significantly change the microbial dynamics, soil biodiversity and essential ecosystem functions such as nutrient mineralisation, disease incidence, carbon turnover and plant growth. While reduced pesticide use associated with Bt cotton varieties is clearly beneficial, very little is known on the potential non-target effects of Bt cotton plants on the functional groups of soil biota and associated biological processes that are critical for sustained cotton productivity and essential for ecosystem health.

It is known that the Bt cotton varieties produce Bt toxin in above ground plant parts such as leaves (particularly young leaves), flower buds etc. but no information is available on the production of Bt toxin in the below ground plant parts. In addition it has been assumed that "during the life of the plant the Bt-endotoxin in Ingard cotton is enclosed within plant cells and it would only enter the soil environment after the above ground plant material is ploughed in" (NRA, 1996).

In this project we measured the levels of Bt toxin production in different plant parts of cotton, especially below ground parts, and also evaluated the mechanisms through which the Bt toxin enters the soil environment. We found that in controlled environments (glasshouse and growth chamber) and field experiments, Bt cotton varieties expressed Bt genes and produced measurable amounts of Bt toxin in different parts of the cotton root system (tap, secondary and fine roots, and root hairs). Our results indicate that the levels of Bt toxin in roots are similar to those observed in leaves whereas the levels of Bt toxin in stems were the lowest. For example, Bt toxin levels in the leaves of cotton variety Sicot 289i ranged from 2,900 to 20,300 ppb and in the roots from 4,900 to 18,700 ppb.

The general decrease over time of Bt toxin levels in leaves is generally accepted (especially in Ingard varieties) to be due to the ageing of the various plant tissues and gradual breakdown of Bt toxin within these tissues. However, we found that as the plants grew older, the levels of Bt toxin in roots of 8-week old Bt cotton (Sicot 289i) were higher (4,900 and 7,000 ppb dry weight in taproot and fine roots, respectively) than that in leaves (2,900 ppb dry weight). We also found that in most situations Bt toxin levels in the fine roots were higher than other parts of the root system and plant-related reductions in this part of the root system were smaller compared to other plant parts. This higher level of Bt toxin below ground can be attributed to the continued growth of new root systems through the later stages of the cotton season.

We observed the presence of Bt toxin in the roots of Bt cotton varieties grown in three different soils (Avon, SA, Waikerie, SA and Narrabri, NSW). The results show that Bt toxin was produced in every major part of Bt cotton plants (leaves, stems, and roots), that root Bt toxin production was comparable (or higher in the later stages of cotton plant) to that in cotton leaves and that the above observations held true for all three soil types. In these experiments we did not find any detectable levels of Bt toxin in the conventional non-Bt cotton plants.

We also found that the roots of Bt cotton varieties release Bt toxin, both in vitro (solution culture) and by soil-grown plants, through presumably passive release from the roots or as cell lysates, and the levels of release (cell-free) of Bt toxin from roots were significantly increased (> 6-fold) following any damage to root system (eg fine roots). The non-Bt cotton cultivars, as expected, released no detectable Bt toxin. We found Bt toxin release from plants that were 2 to 12 weeks old and found no evidence for the presence of Bt toxin from roots of non-Bt cotton varieties.

Root hairs and sloughed epidermal cells contribute a significant amount of root material in the rhizosphere of actively growing plants. We found that the sloughed epidermal cells and fine-root hair fragments from Bt cotton (Sicot 289i) plants contained large concentrations of Bt toxin (eg 1317 ppb/g wet weight) whereas non-Bt control (Sicot 189) cells/fine-root hairs showed no Bt toxin. Thus, our results suggest that Bt toxin has the potential to enter the soil system throughout the Bt cotton growing season, through both a root release process and root turnover. Levels of Bt toxin entering the soil system could therefore be significantly higher than previously suggested on the basis of contributions of Bt toxin to soil from above-ground cotton material only (NRA, 1996)*.

* A more recent assessment by the Australian Pesticides and Veterinary Medicines Authority, for Bollgard II cotton, does consider the contribution of Bt toxin from root sources (APVMA 2003).

Unlike the Bt toxin from leaves and other above ground plant parts, which may enter soil only after defoliation (leaves) and cotton harvest (stems), roots with Bt toxin are in constant contact with the soil system (including soil biota) and Bt toxin levels in fine roots were found to be as high as that in younger leaves. In view of the results reported above (large concentrations of Bt toxin in Bt cotton roots and demonstrated root release), more detailed investigations on the environmental fate of the root-derived Bt toxin, binding to soil components and build up, and movement beyond the rhizosphere and root zone, are warranted. Results from our initial work found detectable levels of Bt toxin in the rhizosphere of Bt cotton varieties by using both immunological tests and insect bioassays.

Leaf material, in general, constitutes a major component of the easily decomposable part of crop residues and therefore supports larger populations of soil biota and higher levels of biological activity. Results from leaf decomposition experiment have shown that detectable levels of Bt toxin were observed in decomposing leaves throughout an 8-week field incubation experiment. The implications of the presence of Bt toxin for the composition of soil biota (soil fauna and microflora) during the main period of leaf material decomposition are unknown. Therefore, there is a clear need for further detailed investigations on the impacts of both leaf- and root- derived Bt toxins on soil biodiversity and associated biological functions.

Microbial growth indicators measured in this study (decomposition rates, substrate induced respiration, and respiration quotients) suggest that microbial population growth on Bt cotton leaf litter might be different than for non-Bt varieties. Microscopic examination revealed an apparent increase in fungi and fungal spores on the Bt cotton residues compared to the non-Bt residues. Experiments did not indicate whether these changes were likely to be detrimental, neutral or beneficial in an agricultural situation. These experiments need to be repeated over multiple seasons before firm conclusions can be drawn.

In summary, our work clearly demonstrates the evidence for avenues, other than through leaves, for Bt toxin to enter the soil system throughout the cotton growing season. This is contrary to the previous assumption that "it only enters after the above ground plant material is ploughed in" (NRA 1996). Our results indicate that rhizosphere-inhabiting soil biota are continuously exposed to Bt toxin produced in the roots (through root releases and root turnover) throughout the growing season and then further exposed as crop residues decompose after harvest.

In Australian soils, the rhizosphere environment is one of two key zones where the majority of soil biota reside, the other zone being the soil around decomposing crop residues. Populations of different groups of microbiota are generally higher (>10-fold) in rhizosphere soils compared to that in bulk soil, and rhizosphere biological activity accounts for >60% of overall soil biological activity. Implications of our results on Bt toxin production and release below ground by Bt cotton varieties are that the input from Bt cotton roots has previously been significantly underestimated and the impacts of this hitherto unknown, toxin input are yet to be fully investigated.

We have shown that different plant parts of Bt cotton (leaves, stubble and roots) contain large concentrations of Bt toxin and therefore have the potential to be a reservoir of Bt toxin in agricultural fields of Australia. Our findings showing large concentrations of Bt toxin (above soil background) in decomposing Bt cotton leaf residues even after the decomposition of >40% of leaf residue indicate that Bt toxin from dead leaves is not easily degraded by soil microorganisms, which one would expect for such a protein substance. If more Bt toxin enters the soil environment than is degraded by microbes, eaten by insect larvae or inactivated by sunlight there is potential for the toxin to accumulate if it is bound and protected by soil particles (clays, minerals and humic acids). Could accumulation of active Bt toxin constitute a hazard to non-target organisms and impact the biodiversity and functionality of the organisms inhabiting the soil?

Soil fungi associated with decomposing crop residues could be either non-pathogenic species or species that cause soilborne plant diseases. Crop residues are the primary source of carbon for soil biota populations in Australian soils hence the composition of biota associated has a great significance in regulating the essential biological functions in the ecosystem. Observations on differences in microbial populations, including a possible increase of fungi on decomposing Bt-cotton, require further investigation over more than one season before differences can be confirmed. If the observed trend is real, its significance is not yet clear since the changes to soil biota could be detrimental, neutral or beneficial to agricultural soil ecosystems.

Finally, consideration of the environmental fate of Bt toxin from Bt crops has sometimes focussed only on the expression of the Bt gene in above-ground plant parts, but our results suggest further investigation into the environmental fate of the root-released Bt toxin in soil is required, both during the cotton season and following the harvest of the cotton crop.

This project is based with the CSIRO Land and Water group in Adelaide with collaborative links for fieldwork with researchers at Australian Cotton Research Institute (ACRI) research farm in Narrabri, NSW (Mr. Grant Roberts, CSIRO Plant Industry).

Australian Government, Department of Environment and Heritage. 2005. Summary of the Ecological Impacts of GM Cotton on soil biodiversity report.

Note: the material presented in this document is in preparation for publication in the scientific literature.

The Hungarian team found the following for MON810 maize:

"The Bt maize produces 1500-2000 times as much Bt-toxin as is released through a single treatment in conventional crop protection, with the chemical called DIPEL, which contains Bt toxin." II. "Other experiments have found that the residues of Bt plants are slower to decompose than their isogenic lines. Some 8% of the toxin produced by the plant remained in the field after harvesting. Indeed, a substantial share of this active toxin quantity could be identified in the soil 11 months later." III. "In the soil of the field under the transgenic plant, the entire biological activity was lower than in the control field." IV. "The caterpillars thriving on herbs in and on the edges of maize fields, hatching during the pollination period, are the most substantially affected by the Bt toxin produced by MON 810.. Shades of Bt176 maize and dead cows etc, and now the news of a 25% death rate among sheep consuming the foliage of Bt cotton in India??