THE ENVIRONMENTAL IMPACT OF GENETICALLY MODIFIED CROP PLANTS ON THE MICROBIOLOGY OF THE RHIZOSPHERE

A joint project through
Dept of Medical Biotechnology
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South Australia

and

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SUMMARY

The effect of genetically modified crop plants on the microbiology of the rhizosphere was investigated using the single-gene *Bt* cotton as a case study. The project compared the rhizosphere microbiota of four Ingard® [1]cotton plant varieties that were closely matched with their non-GM parental strains. The plants were grown in three different Australian soils, ie, a vertisol from a cotton-growing region, and two soils, a fine sandy loam and a red sand from South Australia that had not been exposed to cotton.

At the time of the commencement of the project, the only commercially available genetically modified plants were cotton and carnations. The cotton industry in Australia is worth $1.5b annually, and care of the soil and the dynamics of its living microbial consortia needs to be understood for optimum management to enable agricultural sustainability.

The general outline of the thesis incorporated four main sections:

1. Experimental setup and analysis of the soils and plants to be used, quantification of the Cry1A(c) plant-produced *Bt* protein, and its persistence in the soil environment.
2. Measurement of the selected microbial populations of bacteria, fungi, AM-fungi, protozoans and nematodes, by counting and estimation by dilution and most-probable number methods.
3. Assessment of selected metabolic pathways to determine the effects on the soil microbial community by chemical and other biochemical methods
4. An overall analysis between different group ratios of expression of each of the variables tested, and the summary of the risk analysis and conclusion.

The outcome of this work was the acquisition of scientific data to produce an environmental impact report. The findings of this study showed that generally the microbial populations and the products of major metabolic pathways correlated more closely within the non-GM and GM plant rhizospheres of the paired trials than those of separate trials, indicating that soil and plant cultivar had a stronger environmental effect. The results obtained from the paired trials did not show that there were consistent effects on the rhizosphere soil microbiota that could be attributed to the presence of the Cry1A(c) *Bt* plant protein on the selected strains of cotton plants. The results from the tests of the paired trials correlate highly with previously published work that the risk factors of genetically modified cotton plants on the microbiology of the rhizosphere soil were found be negligible and not consistent across trials.

[1] ® Monsanto Co. St Louis, MO.

… the PhD

God give us strength.
Strength to hold on and strength to let go.

Amen.

Leunig, 2004

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Diana Walter

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The work was undertaken in a CSIRO laboratory. Funding for the equipment and consumables was provided by student grants from Flinders University and through a CSIRO academic top-up, so institutional bias was minimised. With reference to the current plant genetic modification debate and personal interest, it is important to state that I am an independent scientist and this work was carried out in an academic setting. There were no links to any biotechnology companies, nor to any environmental groups.

ABBREVIATIONS[2]

ACU	Analytical Chemistry Unit, CSIRO, Adelaide.
A-M fungi	arbuscular-mycorrhizal fungi
Bt	*Bacillus thuringiensis*
cfu	colony forming units
Cry1A(c)	The gene that has been inserted into the genetically modified cotton plants, which is transcribed to the insecticidal protein, or the resultant *Bt* protein after transcription.
DGGE	Denaturing Gradient Gel Electrophoresis
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
GM	Genetically modified. More correctly referred to as transgenic or genetically engineered crops (Conway & Tonniessen, 1999), but adopted by common usage. In this study, it refers to the plant strain which has been engineered to express the Cry1A(c) protein toxic to *Lepidoptera*, and the names of the cotton plant strains have been suffixed with an "i" indicating the presence of the recessive gene.
GMAC	Genetic Modification Advisory Committee
MIR	Mid-infrared
Suffix 'i'	Indicates the strain of the genetically modified cultivar of the corresponding non-GM parent plant with the inserted gene.
ICP	Insecticidal crystal protein
MPN	Most probable number
OGTR	Office of the Gene Technology Regulator
P	Phosphate, especially in relation to arbuscular-mycorrhizal fungi
PLFA	Phospholipid Fatty Acid(s)
ppm	parts per million, or 1×10^6
ppb	parts per billion, or 1×10^9
SPSS	Statistical software. SPSS Inc, 233 S Wacker Drive, 11th Floor, Chicago, Illinois. USA.

The abbreviations for the standard chemical symbols have been used throughout.

[2] The formatting of latin abbreviations in this thesis followed the protocol of the Commonwealth of Australia Style Manual for Editors and Writers. Where not specifically mentioned, The Concise Macquarie Dictionary 1982 style was used.

DEFINITIONS

Active site[3]	A protein encoded by a portion of the 2.4Kb *BamH*1-Kpn I DNA fragment … containing fewer amino acids than the protein resulting from transcription and translation of the entire DNA fragment, wherein said protein possesses the same spectrum of insecticidal activity against lepidopteran insects as the toxin protein of Baccillus [sic] *thuringiensis* var. *kurstaki.*
Adsorption	A process by which molecules, or ions are retained on the surfaces of solids by chemical or physical binding.
Bulk soil	Refers to the soil taken from the bulk soil container before the plants were grown, and 'non-rhizosphere soil' refers to the soil taken from the container, but kept under the same conditions of moisture and addition of nutrient as the plants in each trial. It did not contain living plant roots, as all pots were weeded by hand.
Duplicate	See 'Replicate' below.
Exudates[4]	Compounds of low molecular weight which leak from root cells into either the intercellular spaces and then to the soil via the cell junctions or directly through the epidermal cell walls into the soil. The release of these compounds is not metabolically mediated. The process of trans-membrane transport is passive.
Genetically modified plant	Within this work, the term genetically modified plant has been taken to mean a plant, or plant product which contains the protein produced under the control of the gene insertion, and its promoters, whether or not the plant is viable (alive). Since the presence of the protein is proof of the efficacy of plant gene DNA transcription, the detection of the *Bt* protein in the plant tissue has been taken as proof of the modification of Ingard cotton. This definition varies from the definition by the OGTR received by personal communication on 18/12/03 which stated that a genetically modified plant had to be living, and that the modified characteristics within a dead plant was therefore known as a GM-product.
Microflora	Bacteria, including actinomycetes and fungi.

[3] US Patent & Trademark Office, US Patent No. 6,197,747, March 6, 2001, to the assignee Monsanto Co.

[4] A.D. Rovira, R.C. Foster and J.K. Martin. 1979 Note on terminology: Origin, nature and nomenclature of the organic materials in the rhizosphere. In: *The soil-root interface.* J.L. Harley & R. Scott Russell eds. p 1-3. Academic Press. London.

Microfauna	Protozoa, nematodes and other invertebrates generally < 200 microns long.
Microhabitat	Clusters of microaggregates which may be composed of several microsites (e.g., aerobic and anaerobic). Approximately the size of one cohesive soil crumb.
Non-rhizosphere soil	See 'Bulk soil' above.
Plasticity	In soil: the quality that allows soil to be moulded like plasticine. In plants: the response to environmental influences whereby the plant adapts to its surroundings. In microbial populations: the resultant mix in populations which may not return to its former components after an event.
Phenotype	Observable form which may differ between similar microorganisms.
r-strategist	A member of a population with a high rate of intrinsic increase.
Redundancy	The replacement of a soil process by a similar or different type of microbe, to ensure that the function is continued. This has been likened to an insurance-effect to take over important functions by decreasing species (Björn Sohlenius: available http://www.abo.fi/fak/mnf/biol/nni/lec_bsohls.htm).
Replicate	The population numbers or result of an assay extracted from separate pots within each separate paired trial, incorporating the arithmetic mean of the duplicates. The term 'duplicates' refers to the number or result obtained from two samples taken from within the same pot.
Secretions	Compounds of low and high molecular weight mucilages which are exported across the cell membrane by active transport.
Standard error of the mean (SEM)	Calculated by dividing the standard deviation by the number of replicates (Zar, 1984, p. 86).
Variability	The difference in the microbial populations with regard to spatial distribution within a soil microsite, or difference attributable to separate trials.
Variance	The unevenness of data away from the arithmetic mean of computation of a single statistical unit.