Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

Part 1 - Summary Details

REPORTS

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **CLW2C**
Annual Report: ☐ Due 30-September
Progress Report: ☐ Due 31-January
Final Report: ☒ Due 30-September
(or within 3 months of completion of project)

Project Title: Travel to laboratories in USA

Project Commencement Date: Nov 2002 **Project Completion Date:** Nov 2002
Research Program: 4 Farming Systems

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TRAVEL REPORTS

1. A brief description of the purpose of the travel.

Taking advantage of a GRDC funded trip to attend the Annual meetings of Soil Science Soc of America held in Indianapolis, I requested CRDC funds to visit laboratories involved in research related to *Bt*-crops and soil biota i.e. laboratories of Dr. G. Stotzky at New York University and Dr. D.C. Coleman at the University of Georgia. Both for the CRDC and Dr. Gupta this would these visits would provide access to the knowledge of the work related to GM crops (including *Bt* and herbicide tolerant crops), in particular to latest techniques.

Prior to the Annual meetings Dr. Gupta visited laboratories at the Centre for Microbial Ecology, Michigan State University, East Lansing, in particular the group headed by Dr. J. Tiedje known for the development of molecular techniques to study soil microbial communities (GRDC funds). Once again this provided us with an opportunity to be exposed to latest techniques such as DNA microarrays for parallel analysis of (even quantify) many microbial genes.

2. What were the:

a) Major findings and outcomes

Annual meetings of Soil Science Society of America – Session 12:

A special symposium on the 'Environmental Impacts of Transgenic crops on soil biological functions was held with 6 key note speakers talking on various aspects of the topic covering both *Bt*-crops and Herbicide Tolerant crops (copies of the abstracts are attached with hard copy of the report). The symposium was first of its kind at the annual meetings and the symposium was well attended. Some poster papers covering the research on the impacts of *Bt*-corn on soil microbial communities were also presented.

- Persistence of *Bt* toxin in soils varied with soil type and environment. A sharp decline in the *Bt* protein levels during the early periods of decomposition (2-4 weeks) but measurable levels of *Bt* were observed even after 8-12 weeks. Soil type seems to have a significant impact on the level of persistence.
- Impacts of *Bt*-corn on soil microbial communities under field conditions: Almost all the work presented was from short-term studies and on phenotypic assessments i.e. both cultural and molecular techniques. Very little work was presented on the changes in biological functions it self and no information on the microbial-faunal interactions and soil-borne pathogens. GM corn (*Bt*-corn) was the test crop in most of the studies. Overall no definitive conclusions were presented. It was clear that it is necessary to use a functional approach coupled with efforts to include variability due to spatial patchiness of biota distribution in order to obtain meaningful information (i.e. useful for developing management options) on the impacts of GM crops on soil biota.
- Evidence for the accumulation of Glyphosate in the nodules of glyphosate-tolerant soybeans and a transient inhibition of nitrogenase activity was presented suggesting the potential for reduced nitrogen fixation in the glyphosate-tolerant soybeans. Since persistence of glyphosate in soils is less likely (due to its fast degradation by microbial activities) it may be unlikely that glyphosate applications in RR-cotton could influence nitrogen fixation by the legume rotational crops. A note of caution is that soil type and microbial activity levels influence glyphosate degradation rates.
- Very little work has been done on the long-term impacts of GM crops on and nutrient transformations both in terms of availability, efficiency of use of inputs and losses.
- Rhizosphere and root interior bacterial communities associated with HT canola were different compared to the conventional canola varieties. However seasonal and field

variability in microbial populations made it difficult to make conclusions about the direct impact of GM canola.

- A variety of methodologies were presented as suitable to assess the GM impacts on soil biological properties. No single method may give a definitive conclusion and a variety of techniques to determine phenotypic and genotypic assays need to be employed to assess the GM impacts on microbial community structure and function.
- A copy of the CD with abstracts from the annual meetings along with hard copies of GM crop related abstracts are enclosed with this report.

Visit to Dr. Coleman's lab at the University of Georgia:

- My discussions on their current research related to GM cotton revealed that they are taking a longer-term approach and the impact of RR cotton when compared to conventional cotton (HSB) on soil faunal composition (Mesofauna, Macrofauna and free living nematodes) was the major focus. Currently there have no ongoing work on soil microflora and functions. Following our discussions, Dr. Coleman indicated that they now plan to include aspects of soil fungi dynamics in their assessments.
- I feel that it is important to keep in touch with this group in order to learn about their findings regarding long-term impacts of GM cotton on soil biota.
- I presented a talk on the 'Diversity and functional importance of protozoa in agricultural soils'.

Visit to Dr. Stotzky's lab at New York State University:

- During the last 10-15 years, Dr. Stotzky's group have published a number of papers dealing with the persistence of *Bt*-protein (both bacterial and plant origin) in soil. Recently they published evidence for the exudation of *Bt*-protein by the GM corn roots in soil less and soil cultures under controlled environment conditions. My discussions with Dr. Stotzky were mainly focused on the methods used and the basis for their research approach soil biology investigations.
- Until now most of their work is phenotypic population based i.e. populations of bacteria, fungi, earthworms etc. and no effort to determine the likely impacts (or no impact) on functional groups of soil biota or biological functions. Dr. Stotzky does recognise that this approach had less chance to find transient effects and effects that may not be very large. Their work on the microbial community analysis using modern methods has started recently.
- Their work was entirely based on growth chamber studies. This type of work does provide insight into the mechanisms for the persistence of *Bt* in soils but may be less meaningful unless supported by field-based work. Our work on the *Bt*-cotton impacts is mainly field based with supporting glasshouse-based research investigating the likely mechanisms.
- Dr. Stotzky's work has indicated the potential for the longer term persistence of *Bt*-protein as a result of its binding with smectitic clays. Australian cotton is mainly grown on soils with large proportion of smectitic clays. It was evident that it may be necessary to investigate the potential for the longer-term persistence of *Bt*-protein following the cultivation of *Bt*-cotton varieties for more than one year. Since the degradation of *Bt*-protein in soil is microbially mediated, adoption of management practices that improve microbial activity may help reduce the persistence and build-up of *Bt*-protein in these soils.
- Discussions were also with Dr. Saxena, the main researcher for the majority of Dr. Stotzky's research during the last three years about methodology. There work is qualitative (using Envirologic sticks) and they seem to be not interested in absolute measurements. It was useful to learn that they used a concentration step from the

exudate media to the measurement using ELISA (this point was not indicated in the papers).

b) Other highlights

- Discussions with Dr. Franco Dazzo at the Michigan State University: Dr. Dazzo developed an image analysis program (CMEIAS), a computer-aided system to study bacterial morph types in microbial communities i.e. direct observation technique. In addition to the demonstration of its usefulness in various microbial ecology studies (Liu et al. 2001. Microbial Ecology. 41: 173-194), recently this technique was used to study the phyllosphere microbiota of GM Cotton. A brief look at the working of this program indicated that it would be a useful program for researchers in cotton biology research. I was informed that it is available, from the author, for other researchers who are interested for research purposes.

- My attendance at the Soil Science Society of America annual meetings (funded by Grains RDC) provided me with a valuable opportunity to interact and develop contacts with other researchers in USA who are involved GM crops-soil biota research i.e. Drs. J.S. Buyer and J. Theis. An initial contact has been established with a number of researchers and I plan to maintain the communication link to exchange ideas and results.

3. Detail the persons and institutions visited, giving full title, position details, location, duration of visit and purpose of visit to these people/places. (Note - please provide full names of institutions, not just acronyms.)

Dr. Stotzky, G., Department of Biology, New York University
Main Building, 10th Floor, 100 Washington Square East, New York, 10003
November 18th, 2002
Dr. Stotzky's lab is one of the principle laboratories that worked on the various aspects of *Bt*-toxin in soil systems. They have been involved in the development of methods to investigate the fate and persistence of *Bt*-protein in soils and studied the impacts of *Bt*-corn on soil biota. The main purpose of my visit to this laboratory was

- To learn more about their current research in relation to *Bt*-protein and *Bt* crops.
- To learn the finer points of methodology to detect very low levels of *Bt*-protein in soils. Dr. Stotzky's lab is well known for their research on the persistence of *Bt*-protein (both from soil bacteria *Bacillus thuringiensis* and *Bt*-corn in soils.
- To gauge the scientific standard of our research.

Dr. Coleman, D., Distinguished Research Professor,
Institute of Ecology, Ecology Annex
University of Georgia, Athens, GA 30602-2360
November 15 and 16th
Dr. Coleman is one of the pioneers soil ecology research and was primarily responsible for establishing evidence linking the detritus food web dynamics and the turnover of carbon, nitrogen and phosphorus.

- To learn about Dr. Coleman's current research on the dynamics of soil biota in Roundup Ready Ingard cotton farming systems. Dr. Coleman's lab is currently involved in a number of research projects studying the long-term changes in different soil biota communities in GM cotton farming systems compared to the conventional cotton varieties both in farmer paddock based trials and at the well known Horseshoe bend long tern trial.
- To gauge the scientific standard of our research and its relevance to cotton industry.

Duration of travel and visits to UG and NYU – 6 days

4. a) Are there any potential areas worth following up as a result of the travel?

There is very little current data on the long-term impacts (both beneficial and deleterious) of GM cotton technology on soil biota and biological functions. In addition to funding constraints, maintenance of plots or farmer fields under a single farming practice i.e. GM or conventional cotton systems is difficult. On going research at the University of Georgia's Horseshoe bend experimental site has the potential to shed light on the long-term changes in soil biota composition and associated ecosystem functions. I, therefore, have established a mutual understanding with Dr. Coleman to exchange ideas, approach to research and results in the fields of common interest. One of the main benefits from this interaction is our access to the information on the dynamics of soil fauna (especially mesofauna and macrofauna) in GM cotton soils.

Future developments from the research on the impacts of a variety of GM crops (e.g. *Bt* crops and Herbicide Tolerant crops) on soil biota and biological processes. A number of research groups in USA have started research on the impacts of GM Crops on soil biota.

- J. S. Buyer, USDA-ARS Beltsville, MD – influence of *Bt*-corn and rhizosphere microbial communities
- J.E. Theis, Cornell University – GM crops and soil microbial communities
- P.P. Motavalli, University of Missouri – GM crops and plant nutrient transformations
- Dr. J.J. Germida, University of Saskatchewan – Herbicide Tolerant crops
- R.M. Zablotowicz, USDA-ARS Stoneville, MS. – Roundup ready crops

Dr. Stotzky has extensive experience in the area of the persistence of *Bt*-protein in soils and has demonstrated the role of soil clays for the persistence of *Bt*-protein. If there were an opportunity it would be beneficial to both the researchers and cotton industry personnel to share Dr. Stotzky's knowledge on *Bt*-proteins and their significance in soil systems.

b) Any relevance or possible impact on the Australian Cotton Industry?

GM cotton varieties, both *Bt* and Herbicide Tolerant varieties, have become an integral part of Australian cotton industry and are likely to be during the next decade or more due to the likely benefits they could bring both in terms of economic and environment benefits and long-term health of cotton ecosystem. Like nearly all-new technologies, along with the benefits they could bring some problems may come if the technology is not properly used. A better understanding of all the relevant issues is needed so that a proper assessment can be made. It is impossible for Australian cotton industry to conduct research in all areas of GM crop technology, in particular long-term effects. Results from the research in USA could help identify specific issues that Australian cotton industry could focus under Australian soil and environment conditions.

5. How do you intend to share the knowledge you have gained with other people in the cotton industry?

Since the completion of this trip I have discussed what I learnt with a number of researchers involved in cotton soil biology research (e.g. Drs. Gary Fitt, David Nehl, Geoff Baker, Grant Roberts) through one to one contact over phone or in person. The CD with the abstracts of the SSSAM annual meetings may be made available to other researchers (copy enclosed).

Impact of Transgenic Bt-Corn on Microbial Community Composition in Three Soil Types. (S03-blackwood140410-Oral)

Authors:

- C.B.Blackwood - *Sustainable Ag. Sys. Lab, USDA-ARS, Beltsville, MD*
- J.S.Buyer - *Sustainable Ag. Sys. Lab, USDA-ARS, Beltsville, MD*

Abstract:

The effects of Bt toxins expressed by transgenic corn on soil microbial communities were investigated in a growth chamber experiment. Two lines of Bt-corn, expressing different Cry proteins, and their non-Bt isolines, were grown in pots containing three different agricultural soils. The soils ranged from a clay to a loamy sand, with 3.2 to 0.6% organic matter. After 5 weeks, the different soil types contained significantly different microbial communities as measured by PLFA profiles and total amounts of PLFA. Soil effects on bacterial and fungal metabolic potential, measured separately with Biolog assays, were also significant but accounted for very little of the total variability in the Biolog profiles. All 4 plant varieties, 2 Bt and 2 non-Bt, had very similar PLFA and Biolog profiles. Community composition of specific microbial divisions was also assayed by modified T-RFLP of the 16S ribosomal gene.

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Effects of CRW transgenic corn and tefluthrin on the soil microbial community: activity, diversity and abundance. (S03-thies161301-Poster)

Authors:

- M.H.Devare - *Cornell University*
- J.E.Thies - *Cornell University*
- C.M.Jones - *Cornell University*
- S.W.Culman - *Cornell University*
- C.E.Martinez - *Cornell University*

Abstract:

The effects of transgenic Bt corn resistant to the corn rootworm on the activity, diversity, and abundance of soil bacteria, fungi, and protozoa are being evaluated in a field trial planted to CRW (Bt) corn, a non-transgenic isoline (NoBt), and NoBt corn with the insecticide tefluthrin applied at planting. In 2001, soil samples were collected before planting, at anthesis and at harvest. For the latter two sampling points, each composite sample was separated into bulk and rhizosphere components. Biomass C in the rhizosphere from Bt plots was significantly higher than from NoBt plots at harvest. Short-term nitrification rate in NoBt plots without insecticide applied was lower than in those with insecticide. There were no other differences between treatments in biomass C, mineralizable N, soil respiration, short-term nitrification rate or in the abundance of protozoa at any sampling point. Data on diversity of soil bacterial and fungal communities evaluated by terminal-restriction fragment length polymorphism (T-RFLP) and internal transcribed spacer (ITS) analyses, respectively, and their abundance determined by direct counts using fluorescence microscopy will also be presented.

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Presentation Information:

Presentation Date: Tuesday, November 12, 2002
Presentation Time: 9:00-11:00 am
Poster Board Number: 2216

Methodologies to Assess the Impact of Genetically-Modified Crops on Soil Biological Properties Using Diagnostic Techniques. (S03-kremer165121-Oral)

Authors:

- R.J.Kremer* - *USDA-ARS, Columbia, MO*
- M.Fang - *University of Missouri, Columbia*
- P.P.Motavalli - *University of Missouri, Columbia*

Abstract:

Ecological assessment of genetically-modified (GM) crop impacts on microbial communities has received little attention. GM crops may release exudates into soil causing changes in rhizosphere microorganisms. How GM crops influence microorganisms is important in evaluating effects on soil biological processes. Because microbiological assays based on cultural methods reveal 1 - 10% of soil microflora, sensitive methods involving phenotypic and genotypic assays are necessary to better assess microbial diversity. Phenotypic assays including enzyme activities, fatty acid analysis and substrate utilization provide physiological and functional bases for characterizing microbial communities. Phylogenetic techniques based on PCR-amplified 16S rDNA fragments from soil microbial DNA, including DGGE, T-RFLP and SSCP describe diversity and composition of the soil microbial community. Reported impacts of GM crops on soil microorganisms are inconclusive, suggesting that influences of biotic and abiotic factors on interactions of GM crops and soil microorganisms require further investigation. Assessments of diagnostic techniques currently used for describing relationships of crop type and soil environmental factors with microbial community structure and function will be presented.

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Presentation Information:

Presentation Date: Wednesday, November 13, 2002
Presentation Time: 11:20 am

Impact of Genetically-Modified Crops and their Management on Plant Nutrient Transformations. (S03-motavalli191227-Oral)

Authors:

- P.P.Motavalli* - *University of Missouri*
- R.J.Kremer - *USDA-ARS*
- N.E.Means - *University of Missouri*

Abstract:

Alterations in plant nutrient transformations due to changes in soil physical, chemical and biological properties by the growth and management of genetically-modified (GM) crops may affect soil fertility and the fate of nutrients in the environment. Among the cited potential mechanisms for the impact of GM crops are changes in the composition and activity of soil microbial populations resulting from root exudates, residue inputs, gene transfer and pesticide applications. Other possible positive or negative impacts of GM crops on the environmental fate of nutrients include increased cultivated area in conservation tillage, cultivation of marginal and degraded lands, and changes in fertility and other cultural practices for specific crops. Little long-term research is currently available that has examined the potential agronomic and environmental impacts of GM crops and their management on nutrient transformations. A suggested strategy for future research on GM crops will be presented that stresses examination of long-term changes in soil nutrient transformations under varying soil and climatic conditions.

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Presentation Information:

Presentation Date: Wednesday, November 13, 2002
Presentation Time: 10:20 am

Keywords:

Genetically-Modified Crops, Plant Nutrients, Crop Management,

Impact of genetically modified crops on rhizosphere and root interior microbial communities. (S03-germida165120-Oral)

Authors:

- J.J.Germida* - *University of Saskatchewan*
- K.E.Dunfield - *University of Saskatchewan*

Abstract:

Non-target effects of transgenic plants on native flora and fauna including soil microorganisms are one of the least understood areas in the environmental risk assessments of genetically modified plants. Early studies showed that alterations in plant root exudates associated with transgenic plants could affect the composition of the rhizosphere microbial community. These changes could have either positive or negative effects on plant growth and health, and in turn, ecosystem sustainability. We assessed the impact of genetically modified canola plants on rhizosphere and root-interior microbial communities. This multiple field site, multiple field year study used CLPP, FAME and T-ARDRA to show that root interior and rhizosphere bacterial communities associated with a GM variety of canola were different from communities associated with conventional canola varieties, but these effects were dependent on sampling date and field site. Conclusions about the impact of transgenic plants on soil ecosystems were complicated by the dynamic nature of microbial communities and the major influence that field site and seasonal variability exert on biodiversity of soil microbial communities.

Release, Persistence, and Effects of Larvicidal Proteins from Bacillus thuringiensis in Soil. (S03-stotzky120831-Oral)

Authors:

- G.Stotzky - *New York University*

Abstract:

Larvicidal proteins produced by subspecies of Bacillus thuringiensis (Bt) and expressed in genetically engineered transgenic plants are released in root exudates throughout the growth of some plants (e.g., Cry1Ab protein from corn and rice and Cry3A protein from potato) but not of others (e.g., Cry1Ac protein from cotton, canola, and tobacco). The proteins in root exudates, as well as those released from biomass of all Bt plants or added in purified form, adsorbed and bound rapidly and tightly on surface-active particles (e.g., clays and humic acids) in soil, which significantly reduced the biodegradation of the proteins but not their larvicidal activity: e.g., purified protein persisted for 234 days, protein in root exudates persisted for 180 days, and protein in biomass persisted for three years, the longest times evaluated in soil in all cases. The biomass of Bt plants decomposed significantly slower than the biomass of isogenic non-Bt plants, both in soil and in the absence of soil but inoculated with a soil suspension. The slower decomposition of Bt corn may have been a result of the significantly higher content of lignin in Bt than in non-Bt corn. However, the content of lignin in the biomass of the other plants species, which was considerably lower than that of corn, was not significantly different between Bt and non-Bt plants. The vertical movement in soil of the protein, either purified, in root exudates, or from biomass of Bt corn, decreased as the amount of the clay minerals, kaolinite or montmorillonite, in the soil was increased. The protein was not taken up by non-Bt corn, carrot, radish, and turnip from soil in which Bt corn had been grown or into which purified protein or biomass of Bt corn had been incorporated. The proteins did not have any consistent statistically significant effects in soil on earthworms, nematodes, protozoa, culturable bacteria and fungi, and representative enzymes involved in the degradation of plant biomass or on bacteria, fungi, and algae in vitro.

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Implications of Glyphosate Resistant Transgenic Soybeans on the Bradyrhizobium Japonicum Symbiosis. (S03-zablotowicz143216-Oral)

Authors:

- R.M.Zablotowicz* - *USDA-ARS, Stoneville, MS*
- K.N.Reddy - *USDA-ARS, Stoneville, MS*

Abstract:

Glyphosate-resistant soybean expressing an insensitive 5-enolpyruvylshikimate-3-phosphate synthetase gene has revolutionized weed control and soybean production. Soybeans nitrogen fixing symbiont, B. japonicum, possess a glyphosate-sensitive enzyme and accumulate hydroxybenzoic acid intermediates of the shikimic acid pathway, causing injury or growth inhibition. In a series of greenhouse and field experiments glyphosate inhibited nodulation and leghemoglobin content of glyphosate-resistant soybean. Glyphosate accumulated in nodules, but inconsistent effects on nitrogenase activity were found in field studies. King et al (2001) demonstrated transient inhibition of nitrogenase activity following glyphosate application with the greatest effects under moisture stress. Hernandez et al. (1999), found the level of glyphosate inhibition of bacteroid nitrogenase activity was related to in vitro glyphosate sensitivity of the B. japonicum strains. These studies indicate the potential for reduced nitrogen fixation in the glyphosate-resistant soybean system, however consistent yield reductions due to reduced nitrogen fixation have not been demonstrated.

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Presentation Information:

Presentation Date: Wednesday, November 13, 2002
Presentation Time: 10:50 am

Keywords:

Glyphosate, Nitrogen fixation, Soybeans

DGGE Analysis for Soil Bacterial Diversity. (S03-deng194353-Poster)

Authors:

- H.Y.Sun* - *Oklahoma State University*
- S.P.Deng - *Oklahoma State University*
- W.R.Raun - *Oklahoma State University*

Abstract:

Experimental limitations have hindered the ability to characterize the diverse microbial community and to link a microbial community structure to its ecological functions. DGGE analyses of rDNA permit description of soil microbial fingerprints as well as identification of individual microorganisms inhabiting in the environment. We examined the DGGE fingerprints of the highly variable V9 region of 16S rDNA in six soils that were under different management practices. Microbial diversity and community structure were affected by soil management practices as evidenced by changes in the DGGE banding patterns. Based on similarity matching of 99 sequences obtained from one of the soils with those in the database, 84 showed similarity only to uncultured bacterial 16S rDNA or less than 95% matching. The ones matched with sequences in the database were mostly affiliated to Proteobacteria. Phylogenetic analyses revealed six major clusters and grouped into five major taxa: bacteroidetes, alpha proteobacteria, beta proteobacteria, gamma proteobacteria, and acidobacteria. Most of the sequences remain unclassified.

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The Impact of Bt Corn on Soil and Rhizosphere Microbial Communities. (S03-buyer075539-Oral)

Authors:

- J.S.Buyer - *USDA-ARS, Beltsville, MD*

Abstract:

The large-scale use of transgenic plants may have unintended ecological consequences. While nontarget effects on plants and macrofauna are heavily discussed in the scientific literature and popular press, nontarget effects on soil microbial communities have received relatively little attention. There is evidence that Bt corn residue is degraded more slowly than non-Bt residue, which could be a result of the toxin's insecticidal activity, a direct effect of the Bt toxin on microorganisms, or a result of increased lignification in the transgenic plant. While Bt toxins produced by transgenic cotton and corn have been detected in soil, and certain transgenic corn varieties may release the Bt toxin in root exudates, there is little evidence of any effect on soil or rhizosphere microbial community structure and function. The evidence for effects of other transgenic plants on microbial community structure will be reviewed, and the possibility of gene transfer from transgenic plants to microorganisms will be discussed.

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