# Microbial Biomass and Diversity in Roundup-Ready Corn Rhizosphere

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# Abstract

The effects of Roundup-Ready (RR) corn, grown in monoculture or in rotation with canola, on microbial biomass and diversity in corn rhizosphere were investigated at Lethbridge from 2002 to 2004. In monoculture, weeds in RR corn or conventional corn were controlled by applying either Roundup or other herbicides. In rotations, RR corn rotated with RR canola or Liberty-Link (LL) canola was compared with conventional corn rotated with conventional canola. Microbiological properties in corn rhizosphere were measured at tasseling stage. In monoculture, RR corn affected microbial biomass only in 2003, when application of Roundup increased microbial biomass in RR corn rhizosphere, but not in conventional corn rhizosphere. The diversity of bacteria in RR corn rhizosphere was greater than that in conventional corn rhizosphere, regardless of Roundup application, only in 2002. RR corn in rotation did not affect microbial biomass or bacterial diversity. Therefore, RR corn affected microbial biomass or diversity in only one of three years when it was grown in monoculture, but there were no effects when corn was grown in rotation with canola. When effects were significant, the rhizosphere of RR corn had greater microbial biomass or diversity that that of conventional corn.

# Introduction

Genetically-modified (transgenic) crops have been in commercial use in Canada since 1995, when herbicide-resistant canola was introduced (Beckie et al. 2006). Use of herbicide-resistant crops has encouraged adoption of reduced tillage practices, which reduce soil degradation (Ammann 2005; Cerdeira and Duke 2006). However, growing these crops can have non-target effects on other flora and fauna. These crops can affect soil microorganisms due to differences in the amount and composition of root exudates (Saxena et al. 2002), gene transfer from the transgenic crop (de Vries et al. 2004), and effects of management practices for transgenic crops, e.g., pesticide applications (Sessitsch et al. 2004), tillage, and fertilizer application (Motavalli et al. 2004). These crops can also affect soil microorganisms due to differences in the amounts and composition of decomposing crop residues (Saxena and Stotscky 2001; Hopkins and Gregorich 2005). The objective of this study was to evaluate the effects of growing Roundup-Ready (RR) corn on soil microbial biomass and diversity in the rhizosphere of corn.

### Methodology

From 2000, RR corn and conventional corn were grown in monoculture with or without their respective pesticides at Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB. RR corn was also grown in rotation with RR canola and LL canola, and equivalent rotations consisting of conventional crops were included as controls. The following list contains the treatments, which were replicated four times:-

- 1. RR corn in monoculture [Roundup (glyphosate) applied].
- 2. RR corn in monoculture (no Roundup).
- 3. Conventional corn (herbicides applied atrazine, bromoxynil, nicosulfuron, EPTC or MCPA as required).
- 4. Conventional corn (no herbicides).
- 5. RR canola RR corn RR canola Bt corn (transgenic cultivars in rotation).
- 6. Canola corn canola corn (conventional cultivars in rotation).

At tasseling growth stage of corn from 2002 to 2004, rhizosphere soil was sampled by excavating plants from 0.5 m row length at four different locations in a plot, shaking off loose soil, and carefully collecting (by brushing) soil adhering to plant roots. The soil was sieved through a 2 mm sieve and stored at 4°C until required for analysis.

Soil MBC was measured using the substrate-induced respiration (SIR) method (Horwath and Paul 1994). Functional bacterial diversity was evaluated by the Biolog<sup>TM</sup> method (Zak et al. 1994), which tests the ability of a microbial community to utilize different C substrates contained in a microplate. On the basis of the patterns of utilization of the substrates by the bacteria from each soil, Shannon index (H') of functional diversity was calculated as follows (Magurran 1988; Zak et al. 1994), using PC-ORD software (McCune and Mefford 1997):-

$$H' = -\Sigma p_i (\ln p_i)$$

where  $p_i = Ratio of activity (i.e., optical density reading) on the$ *i*th substrate to the sum of activities on all substrates.

Data from Treatments 1 to 4 were analysed as a 2 x 2 factorial, i.e., two RR traits (RR vs. conventional corn) and two Roundup applications (Roundup vs. not Roundup). Data from rotation treatments (Trt 5, RR corn vs. Trt 6, conventional corn) were analysed separately.

#### Results

#### Microbial biomass C

In monoculture, RR corn affected microbial biomass C only in 2003 (Table 1), when application of Roundup increased microbial biomass in RR corn rhizosphere, but not in conventional corn rhizosphere (Fig. 1). In rotation, RR corn did not affect microbial biomass C in any year (Table 1).

Treatment	P > F		
	2002	2003	2004
Monoculture			
RR trait	0.263	0.057	0.567
Roundup	0.331	0.348	0.155
Interaction	0.365	0.039* (Fig.	0.593
		1)	
Rotation	0.261	0.802	0.221

**Table 1.** Summary of Effects of Roundup-Ready (RR) Corn on Microbial Biomass C in Corn Rhizosphere. Corn was Grown either in Monoculture or in Rotation with Canola.

#### 2003 Rhizosphere



**Figure 1.** Microbial biomass C in the rhizosphere of RR corn and conventional corn grown in monoculture in 2003. RR = Roundup Ready, S.E. = standard error. Weeds in conventional corn "+ Roundup" treatment were controlled with atrazine, bromoxynil, nicosulfuron, EPTC or MCPA as required.

#### Functional diversity

The diversity of bacteria in RR corn rhizosphere was greater than that in conventional corn rhizosphere, regardless of Roundup application, only in 2002 (Table 2 and Fig. 2). In rotation, RR corn did not affect microbial biomass or bacterial diversity in any year (Table 2).

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Treatment		P > F	
	2002	2003	2004
Monoculture			
RR trait	0.013* (Fig. 2)	0737	0.371
Roundup	0.482	0.487	0.493
Interaction	0.401	0.846	0.801
Rotation	0.843	0.074	0.953

**Table 2.** Summary of Effects of Roundup-Ready (RR) Corn on the Functional Diversity of Bacteria in Corn Rhizosphere. Corn was Grown either in Monoculture or in Rotation with Canola.

#### 2002 Rhizosphere



**Figure 2.** Functional diversity of bacteria in the rhizosphere of RR corn and conventional corn grown in monoculture in 2002. RR = Roundup Ready, S.E. = standard error.

#### Discussion

In the few cases that the Roundup-resistant trait or herbicide affected soil microorganisms in the rhizosphere of corn grown in monoculture, it increased microbial biomass or diversity. Haney et al. (2000) and Ratcliffe et al. (2006) also observed that Roundup applied at higherthan-recommended rates increased microbial biomass or activity. This increase may be due to soil microorganisms metabolizing (a) the carbon (and N and P) contained in Roundup, or (b) the carbon from decomposing weeds killed by the herbicide. In most other studies, Roundup applied at recommended rates has been observed to have no significant effects on soil microbial biomass (Olson and Lindwall 1991; Haney et al. 2000; Lupwayi et al. 2004). In a Roundup-resistant corn-soybean cropping system, Liphadzi et al. (2005) also found no Roundup-related differences in soil microbial biomass. Shifts in bacterial community structure in the rhizosphere of herbicide-resistant canola varieties have been reported (Siciliano et al. 1998; Siciliano and Germida 1999; Dunfield and Germida 2001, 2003), but the changes did not persist into the following growing season (Dunfield and Germida, 2003).

There were no significant effects when corn was grown in rotation with canola, probably due to the reduced frequency of RR corn in rotation compared with corn in monoculture. The results reported here are from corn rhizosphere and may be different than effects in bulk soil.

### Conclusions

RR corn affected microbial biomass C or diversity in only one of three years when it was grown in monoculture, but there were no effects when corn was grown in rotation with canola. When effects were significant, the rhizosphere of RR corn had greater microbial biomass or diversity that that of conventional corn.

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### References

- Ammann, K. 2005. Effects of biotechnology on biodiversity: herbicide-tolerant and insectresistant GM crops. TRENDS in Biotech. 23: 388-394.
- Beckie, H. J., Harker, K. N., Hall, L. M., Warwick, S. I., Legere, A., Sikkema, A., Clayton, G. W., Thomas, A. G., Leeson, J. Y., Seguin-Swartz, G. and Simard, M. J. 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86: 1243-1264.
- Cerdeira, A. L. and Duke, S. O. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. J.Environ. Qual. 35: 1633-1658.
- **Dunfield, K. E. and Germida, J. J. 2001.** Diversity of bacterial communities in the rhizosphere and root-interior of field-grown genetically modified *Brassica napus*. FEMS Microbiol. Ecol. **38**: 1-9.
- **Dunfield, K. E. and Germida, J. J. 2003.** Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified canola (*Brassica napus*). Appl. Environ. Microbiol. **69**: 7310-7318.
- Haney, R. L., Senseman, S.A., Hons, F. M. and Zuberer, D. A. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48: 89-93.
- Hopkins, D. W. and Gregorich, E. G. 2005. Decomposition of residues and loss of the  $\delta$ -endotoxin from transgenic (*Bt*) corn (*Zea mays* L.) in soil. Can. J. Soil Sci. 85: 19-26.
- Horwath, W. R. and Paul, E. A. 1994. Microbial biomass. Pages 753-773 in R. W. Weaver, S. Angle, P. Bottomly, D. Bezdicek, S. Smith, A. Tabatabai and A. Wollum, eds. Methods of soil analysis. Part 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, Wisconsin.

- Liphadzi, K. B., Al-Khatib, K., Bensch, C. N., Stahlman, P. W., Dille, J. A., Todd, T., Rice, C. W., Horak, M. J. and Head, G. 2005. Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system. Weed Sci. 53: 536-545.
- Lupwayi, N. Z., Harker, K. N., Clayton, G. W., Turkington, T. K., Rice, W. A. and O'Donovan, J. T., 2004. Soil microbial biomass and diversity after herbicide application. Can. J. Plant Sci. 84: 677-685.
- Magurran A. E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, New Jersey.
- Motavalli, P. P., Kremer, R. J., Fang, M. and Means, N. E. 2004. Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J. Environ. Qual. 33: 816-824.
- McCune, B. and Mefford, M. J. 1997. PC-ORD. Multivariate analysis of ecological data, Version 3.0. MjM Software Design, Gleneden Beach, OR. 47 pp.
- Olson, B. M. and Lindwall, C. W. 1991. Soil microbial activity under chemical fallow conditions: effects of 2, 4-D and glyphosate. Soil Biol. Biochem. 23: 1071-1075.
- Ratcliffe, A. W., Busse, M. D. and Shestak, C. J. 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl. Soil Ecol. 34: 114-124.
- Saxena, D. and Stotscky, G. 2001. Bt corn has higher lignin content than non-Bt corn. Amer. J. Bot. 88: 1704-1706.
- Saxena, D., Flores, S. and Stotscky, G. 2002. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biol. Biochem. 34: 133-137.
- Sessitsch, A., Gyamfi, S., Tscherko, D., Gerzabek, M. H., Kandeler, E. 2004. Activity of microorganisms in the rhizosphere of herbicide treated and untreated transgenic glufosinate-tolerant and wild-type oilseed rape grown in containment. Plant Soil 266: 105-116.
- Siciliano, S. D. and Germida, J. J. 1999. Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol. Ecol. 29: 263-272.
- Siciliano, S. D., Theoret, C. M., de Freitas, J. R., Hucl, P.J. and Germida, J. J. 1998. Differences in the microbial communities associated with different cultivars of canola and wheat. Can. J. Microbiol. 44: 844-851.
- de Vries, J. and Wackernagel, W. 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant Soil 266: 91-104.
- Zak, J. C., Willig, M. R., Moorhead, D. L. and Wildman, H. G. 1994. Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem. 26: 1101-1108.