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Changes in functional structure of soil bacterial communities due to fungicide and insecticide applications in canola

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ABSTRACT

The fungicide vinclozolin and insecticide λ-cyhalothrin are widely used to control canola (*Brassica* spp.) diseases and insect pests, respectively, in Canada. We investigated non-target effects of these pesticides, applied at recommended rates, on soil microbial biomass, functional bacterial diversity and functional community structure of soil bacteria (by evaluating patterns of C substrate utilization) in canola rhizosphere and bulk soil at three locations in Alberta from 2002 to 2004. Experimental treatments were (a) untreated control, (b) vinclozolin fungicide foliar application, (c) λ-cyhalothrin insecticide foliar application, and (d) vinclozolin and λ-cyhalothrin applications. No significant pesticide effects on soil microbial biomass or functional bacterial diversity were observed, but the functional structures of soil bacteria were altered. In 1 of 12 cases, the control treatment had a different soil bacterial community structure from the 3 pesticide treatments. The fungicide treatment had different bacterial community structures from the control or insecticide treatments in 3 of 12 cases, the insecticide treatment had different community structures from the control or fungicide treatments in 4 of 12 cases, and the combined fungicide and insecticide treatment had different community structures from the other treatments in 3 of 12 cases. Therefore, evaluating soil bacterial functional structures revealed pesticide effects that were not detected when bacterial diversity or microbial biomass were measured in canola rhizosphere or bulk soil.

1. Introduction

Cabbage seedpod weevil (*Ceutorhynchus obstricus* (Marsham)) has become a major pest of canola (*Brassica* spp.) in western Canada (Dosdall et al., 2002), often warranting chemical control (Cárcamo et al., 2005). Two insecticides that are registered for use to control this pest in Canada are λ-cyhalothrin (Matador®) and deltamethrin (Decis 5EC®). The same insecticidal compounds can be applied to control other damaging insect pest infestations in canola, including lygus bugs (*Lygus* spp.), bertha armyworm (*Mamestra configurata* (Walker), and diamondback moth (*Plutella xylostella* (L.)) (Anonymous, 2008).

Scelerotinia stem rot (caused by *Sclerotinia sclerotiorum* (Lib) de Bary) is a major canola disease in western Canada (Turkington and Morrall, 1993; Bailey et al., 2003). Because cultural control measures such as crop rotation and sanitization are relatively ineffective (Williams and Stelfox, 1980), and management by canola host resistance has been difficult (Morrall and Dueck, 1982), effective stem rot control has been achieved by application of fungicides such as vinclozolin (Ronilan®) and iprodione (Rovral Flo®) (Anonymous, 2008). These fungicides also control alternaria blackspot, which is also an important disease affecting yield, pod shattering and green seed counts (Bailey et al., 2003) and is caused by *Alternaria brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire, and *A. raphani* (Groves and Skolko).

Information regarding non-target effects of these insecticides and fungicides on soil microorganisms in western Canada is required. Most field studies on fungicide or insecticide effects indicate that when they are applied at recommended rates, they usually have no significant effects or have transitory effects on soil microbial characteristics (Ahtiainen et al., 2003; Griffiths et al., 2006; Vig et al., 2008). In a study of the effects of 19 years of cumulative annual field applications of five pesticides, either singly or in combination at recommended or slightly above recommended rates, Hart and Brookes (1996) reported no measurable harmful effects on soil microbial biomass or its activity (C or N

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mineralization). These pesticides included two fungicides and two insecticides. Even in laboratory incubation studies, pesticides usually show significant effects on soil microbial characteristics only at unrealistically high concentrations (Anderson et al., 1981; Ahtiainen et al., 2003; Wang et al., 2004). Using molecular biological methods in a laboratory incubation study, Wang et al. (2004) showed that iprodione at a recommended concentration of 5 μg g^{-1} soil and incubated at 15 °C caused only temporary changes in the genetic structure of soil bacterial communities, but changes at 50 μg g^{-1} soil incubated at 30 °C were irreversible during the incubation period. Nonetheless, results of field studies cannot be generalized because responses depend not only on pesticide properties, but also on soil properties and environmental conditions.

The objective of this study was to investigate potential changes in soil bacterial community structure, diversity and microbial biomass in response to field application of vinclozolin fungicide and λ-cyhalothrin insecticide to canola.

2. Materials and methods

2.1. Treatments and field operations

The trial was conducted at Beaverlodge, Lacombe and Lethbridge in Alberta, Canada, from 2002 to 2004. At each location, the trial was conducted on a different field site each year. The soil properties at these sites are given in Table 1. Only selected treatments from a larger field trial were sampled for this study. Canola (*Brassica napus*, L., cv. Q2) was seeded from late April to early May at 150 seeds m^{-2} under no-till management system. Experimental treatments were (a) untreated control, (b) fungicide (vinclozolin as Ronilan® at 494 g ai ha^{-1}) foliar application, (c) insecticide (λ-cyhalothrin as Matador® at 10 g ai ha^{-1}) foliar application, and (d) fungicide and insecticide foliar applications (Table 2). The treatments were arranged in a randomized complete block design (RCBD) with four replicates. The pesticides were applied during early (20–30%) flowering stage. At the early green pod stage of canola, another fungicide (iprodione as Rovral Flo® at 498 g ai ha^{-1}) was applied and, depending on insect infestation, another insecticide (deltamethrin as Decis 5EC® at 7.4 g ai ha^{-1}) was also applied (Table 2). These second applications are not part of the treatments described here because soil samples for soil microbiological analysis were collected (at 100% flowering stage) before these applications. Nitrogen, P, K and S fertilizers were side-banded at seeding according to soil test recommendations. All plots received a pre-seed glyphosate herbicide treatment to control emerged weeds, and in-crop weeds were controlled with appropriate herbicides which were applied in all treatments.

From April to July (soil samples were collected in July), Beaverlodge received 148 mm of rainfall in 2002, 115 mm in 2003 and 160 mm in 2004. Normal (30-year average) rainfall at Beaverlodge for these months is 191 mm. Rainfall at Lacombe during the same period was 92 mm in 2002 and 208 mm in 2004; normal rainfall is 237 mm. At Lethbridge, April-to-July rainfall was 150 mm in 2003 and 210 mm in 2004; normal rainfall is 175 mm. Rainfall at Lethbridge in 2002 and at Lacombe in 2003 is not reported because soil microbiological data were not collected.

Table 1
Soil characteristics at the experimental sites.

Site	Soil type	Texture	pH (H_2O)	Organic matter (%)
Beaverlodge	Luvisol	Loam to clay loam	6.0–6.1	5.0–7.1
Lacombe	Phaeozem	Loam	7.3–7.5	9.3
Lethbridge	Phaeozem	Loam	7.8	3.6

2.2. Soil sampling

Samples were collected at 100% flowering stage (4.4) of canola (Harper and Berkenkamp, 1975). Plants were excavated from four random 0.5-m lengths of row from each plot. Loose soil was shaken off the roots, and the soil that adhered strongly to the roots was carefully brushed from the roots and kept as rhizosphere soil. Bulk (non-rhizosphere) soil (0–7.5 cm depth) was sampled from the middle of two adjacent crop rows near each of the four locations per plot. The four rhizosphere soil samples from each plot were combined into one sample, as were the four bulk soil samples. The samples were passed through a 2-mm sieve and stored at 4 °C until required for analysis.

2.3. Microbial community analysis

Community-level physiological profiles of soil bacteria were evaluated using the Biolog® method (Zak et al., 1994), which tests the ability of a microbial community to utilize different C substrates contained in a microplate. The procedure was adapted by colorimetrically standardizing inoculum densities in 1 g soil samples to about 10^3 cells mL^{-1} (Lupwayi et al., 2001a). Aliquots of 150 μL of the soil suspension were added to Biolog Ecoplate® microplates containing 31 substrates and a water control. The plates were incubated at 28 °C without shaking. Optical densities in the wells were read with an enzyme-linked immunosorbent assay (ELISA) plate reader at 590 nm after 48 h of incubation. The

Table 2
Fungicides and insecticides applied in the sampled treatments at each site.

Site	Year	Fungicide[a]		Insecticide[a]	
		Early flowering stage	Early green pod stage	Early flowering stage	Early green pod stage
Beaverlodge	2002	Vinclozolin	Iprodione	λ-Cyhalothrin	
	2003	Vinclozolin	Iprodione	λ-Cyhalothrin	Deltamethrin
	2004[b]	Vinclozolin	Iprodione	λ-Cyhalothrin	Deltamethrin
Lacombe	2002[c]	Vinclozolin	Iprodione	λ-Cyhalothrin	
	2003	n.a.[d]	n.a.	n.a.	n.a.
	2004	Vinclozolin	Iprodione	λ-Cyhalothrin	
Lethbridge	2002	n.a.	n.a.	n.a.	n.a.
	2003	Vinclozolin	Iprodione	λ-Cyhalothrin	Deltamethrin
	2004	Vinclozolin	Iprodione	λ-Cyhalothrin	Deltamethrin

[a] Only early flowering stage applications are relevant for this study because pod stage applications were done after soil had been sampled for microbiological analysis.
[b] At Lacombe in 2002, microbiological analyses were determined only in bulk soil.
[c] At Beaverlodge in 2004, microbiological analyses were determined only in rhizosphere soil.
[d] n.a. = not applicable because soil microbiological analyses were not determined.