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Pyrosequencing reveals the impact of foliar fungicide application to chickpea on root fungal communities of durum wheat in subsequent year

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ABSTRACT

Frequent application of foliar fungicide is essential for chickpea production due to the susceptibility of this plant to ascochyta blight. Chlorothalonil, pyraclostrobin, and boscalid are commonly used to control the disease in Saskatchewan. While fungicides are meant to target specific fungal pathogens, they may impact non-target organisms and alter soil microbial community structure. The effects of the typical 5-time foliar fungicide application program to chickpea CDC Vanguard on the fungal communities associated with seminal and adventitious roots of the following durum wheat crop were studied in a 2 yr field experiment. Root fungal communities were characterized through analysis of the ITS1 region of root metagenomic DNA at the genus level. One hundred and seven fungal genera were detected in durum wheat roots. *Fusarium* was predominant in both years. A three-way interaction of fungicide application, root type and year on fungal community structure was detected. Unlike *Fusarium*, the relative abundances of the genera *Olpidium*, *Alternaria*, and *Cryptococcus* were greater in 2010, a very wet year. Fungicide application to chickpea increased the relative abundance of *Fusarium* in the seminal roots of a subsequent durum crop in 2009, but did not affect the relative abundance of *Fusarium* in 2010. We could not detect a significant impact of fungicide application to chickpea on durum wheat yield in the subsequent year. The effect of changes in root fungal communities on durum wheat grain yield is discussed.

Introduction

Agrochemicals are meant to control specific pests and pathogens in agriculture, but they may impact other components of the soil microbiota and compromise ecosystem functionality and sustainability (Chakravarty and Chatarpaul, 1990; Schreiner and Bethlenfalvay, 1996; Assaf et al., 2009; Lupwayi et al., 2009; Yang et al., 2012a; Mohiuddin

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and Mohammed, 2013). The effects of biocides on non-target microbial groups may be direct or indirect. Indirect effects occur through disturbance of other biological components of the ecosystem, such as plants, animals or other microbes (Chiocchio et al., 2000; Zambonelli and Iotti, 2001; Yang et al., 2011; Cruz et al., 2012). Many studies have documented non-target effects of agrochemicals on microbial communities. The structure of the soil bacterial community in maize monocultures was found to be altered by long-term application of herbicides (Seghers et al., 2003). High concentration of atrazine was reported to reduce soil bacterial biodiversity (Ros et al., 2006). Fungicide application can also change the structure of arbuscular mycorrhizal fungi (AMF) communities, reduce their production of spores, and the level of mycorrhizal root colonization (Schreiner and Bethlenfalvay, 1996; Assaf et al., 2009). Fungicide use can also adversely affect fungal endophytes in grasses (Hill and Brown, 2000; Wilson et al., 2008). Lupwayi et al. (2009) have shown stronger impacts of the fungicide vinclozolin and the insecticide l-cyhalothrin application on the functional diversity of soil bacteria than on community structure. A number of studies have revealed an increase in the population of specific components of the soil microbiota due to the application of agrochemicals (Lévesque et al., 1987; Lupwayi and Kennedy, 2007). This phenomenon is fairly well documented for the herbicide glyphosate, which favors *Fusarium*. For example, Lévesque et al. (1987) have shown an increase in the population of *Fusarium* spp. after application of glyphosate and Fernandez et al. (2009) have reported a positive association of *Fusarium avenaceum* and *F. graminearum* with glyphosate use, whereas the population of *Cochliobolus sativus* was simultaneously reduced. Nevertheless, due to the complexity of the soil microbiota and methodological limitations, our understanding of the non-target effects of agrochemicals on soil microbiota remains rudimentary (Torsvik et al., 1998; Sigler et al., 2000; Sigler and Turco, 2002; Kirk et al., 2004; Powell and Swanton, 2008). Recent advances in molecular microbial ecology have provided us with excellent tools to investigate the non-target effects of biocides on the soil microbiota.

Chickpea is frequently grown in rotation with cereals in Saskatchewan, Canada. It is highly susceptible to ascochyta blight, a disease caused by *Ascochyta rabiei*, and therefore receives up to six fungicide applications during the growing season (Gan et al., 2006; Agriculture and Agri-Food Canada, 2008; Armstrong-Cho et al., 2008). A few fungicides such as chlorothalonil, pyraclostrobin, and boscalid have been registered and are commonly used to control ascochyta blight in Saskatchewan (Armstrong-Cho et al., 2008). However, little is known about the non-target effects of these fungicides on the microbial components of the ecosystem. Yang et al. (2012a) studied the effects of fungicide application to chickpea on rhizospheric bacterial communities of subsequent durum wheat using pyrosequencing, and found a change in structure of bacterial communities. Soil fungi are more likely to be impacted by intense fungicide treatments, which in turn could compromise key functions and the productivity of the ecosystem (Jeffries and Rhodes, 1987; Reeleder, 2003; Robinson et al., 2005; Anderson and Parkin, 2007). The goal of this study was to determine the effects of fungicides commonly applied in chickpea production systems of Saskatchewan on root fungal communities of a subsequent durum wheat crop. Using pyrosequencing of the ITS1 region, fungal communities of seminal and adventitious roots were independently characterized to account for their differences in phenology and position in the soil.

Materials and methods

Experimental design and management

The experiment was conducted at the Semiarid Prairie Agricultural Research Centre in Swift Current, Saskatchewan (latitude 50° 18′ N; longitude 107° 41′ W). Chickpea grown in 2008 was followed by durum wheat in 2009, and the experiment was repeated in 2009–2010. The experiment was conducted in a randomized complete block design with four replicates where block was the random effect. Chickpea cultivar CDC Vanguard received two levels of fungicide treatments: (1) non-treated control and (2) five fungicide applications. Fungicide was applied starting 4–5 weeks after seeding and repeated every 10–14 d thereafter. Two products were used; Bravo (Syngenta Crop Protection Canada Inc., Guelph, ON, active ingredient (a.i.) chlorothalonil, 1 kg ha^{-1}) was applied three times and Headline Duo (BASF Canada Inc., Mississauga, ON, a.i. pyraclostrobin and boscalid 100 g ha^{-1} and 240 g ha^{-1}, respectively) was applied twice. Fungicides were applied in the following order: Headline Duo, Bravo, Headline Duo, Bravo and Bravo.

Average precipitation per month from Jan. to the end of Aug. in 2008, 2009 and 2010 was 46.38 mm, 28.38 mm, and 60.88 mm, respectively, according to Swift Current CDA weather station (latitude 50° 16′ N; longitude 107° 44′ W).

Durum wheat AC Avonlea was seeded on May 5th in 2009 and on May 13th in 2010 at a seeding rate of 113 kg ha^{-1}. Row spacing was 25.4 cm and plot size 2 m × 8 m. Seeds were treated with the fungicidal product VitaFlo 280 at 330 ml 100 kg^{-1}. Roundup Weathermax (Monsanto Canada Inc, Winnipeg, Manitoba, Canada) was applied not later than a day after seeding at a rate of 816.8 ml ha^{-1}. All plots received 56 kg N ha^{-1} and 22 kg P ha^{-1}. Crops were harvested on Aug. 28th in 2009 and on Sep. 27th in 2010. An intact area of 8 m × 1.27 m in each plot (four replicates) was harvested by a combine and grain yield (kg ha^{-1}) was calculated.

At the early heading stage, the fungal communities of the seminal and adventitious roots of durum wheat, after chickpea CDC Vanguard cultivar, were determined using pyrosequencing. Seminal and adventitious roots were analyzed separately to account for differences in time of emergence and the position in soil of the two root types.

Root sampling and processing

Thirty to forty durum wheat plants were dug up to a depth of about 20 cm in each plot at the early heading stage (early Jul.), using a shovel. Shoots were cut 4 cm above ground level. Roots were washed in tap water and dried at 60 °C for 72 hr. Before DNA extraction, a total of thirty two seminal and adventitious root samples (2 root types × 2 fungicide treatments × 2 yrs × 4 replicates) were surface sterilized (96 % ethanol for 30 s, 16.6 %