Fungicide effects on N_2-fixing bacteria and N2-fixation in chickpea

Chao Yang[1,2], Chantal Hamel[1], Yantai Gan[1], Vladimir Vujanovic[2]

1. Semiarid Prairie Agricultural Research Centre, AAFC, Swift Current, SK, Canada
2. Food & Bioproducts Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Abstract

Fungicide application in field crops have unexpected non-target effects on the agro-ecosystem. Molecular methods (polymerase chain reaction – denaturing gradient gel electrophoresis and cloning technology) were used to test the effects of four fungicide application programs targeting Ascochyta blight (*Ascochyta rabiei*) on the N_2-fixing bacterial communities associated with two chickpea cultivars, and on chickpea nodulation. Treatments were replicated four times in complete blocks in the field, in 2008 and 2009. Results showed the richness of the N_2-fixing bacterial communities did not change significantly ($P > 0.05$, data didn't shown) with fungicide application, but different intensities of fungicide application selected different dominant N_2-fixing taxa, as revealed by Correspondence Analysis (CA) of DNA sequences. Genotypes of chickpea cultivars significantly affected both the richness and composition of the N_2-fixing bacterial communities, as revealed by results of CA. Both fungicide and crop genotype affected nodulation scores of chickpea based on ANOVA results ($P < 0.001$ for nodulation scores test and $P = 0.04$ for fixed N test), reflecting impacts on nitrogen fixation. Redundancy analysis (RDA) also revealed significant relationships ($P = 0.014$) among fixed nitrogen, nodulation scores and identified rhizosphere N_2-fixing bacteria. Based on these results, we conclude that both the foliar fungicide applications and chickpea genotype can affect the composition and function of N_2-fixing bacterial community in chickpea field.

Introduction

Nitrogen limits plant growth in many ecosystems (Fiore et al. 2010). Biological nitrogen fixation (BNF), performed by diazotrophic bacteria, makes an important contribution to soil nitrogen (Zhao et al. 2010; Zielke et al. 2005) and improves plant productivity. Much research was conducted to understand the mechanisms of BNF in diazotrophs (Bashan and de-Bashan 2010; Kessler and Leigh 1999; Oliveira et al. 2010; Petrova et al. 2000) because of the importance of their contribution to the biosphere. Diazotrophic bacteria can be divided in three functional groups, namely the free-living, the associative, and the symbiotic diazotrophs (Bürgmann et al. 2004). The symbiotic diazotrophs are largely associated with leguminous plants (Lindström et al. 2010) and N_2-fixing leguminous crops are widely used to input nitrogen in agroecosystems throughout the world. Chickpea could be an important source of nitrogen in Canadian wheat-based cropping systems. Fungicides are abundantly used in chickpea crops to control ascochyta blight, a devastating diseases of this plant (Gan et al. 2006), which may adversely affect agriculturally important microorganisms, including diazotrophs, and reduce the

performance of agroecosystems (Gaind et al. 2007). Thus, we used a PCR-DGGE protocol using nifH as a target, to define the effect of fungicide treatments on the diazotrophic bacterial community in field-grown chickpea rhizosphere, and to related fungicide-induced changes in diazotrophic community structure to the nodulation level, nitrogen fixation and yield of the crop.

Table 1 Timing and combinations of foliar fungicide treatments in chickpea field

Treatment	Growing Stage				
	Seedling	Vegetative	Early flower	Mid-flower	Podding
Control (C)	/	/	/	/	/
I	Headline® Duo	/	Headline® Duo	/	/
II	Headline® Duo	Bravo®	Headline® Duo	/	/
III	Headline® Duo	Bravo®	Headline® Duo	Bravo®	Bravo®

Note: Bravo® was applied at a rate of 1.0 kg ai.ha^{-1} chlorothalonil; Headline® Duo was applied at a rate of 100gms ai.ha^{-1} pyraclostrobin and 240 gms ai.ha^{-1} boscalid.

Fungicide applications were shown in Table 1. Based on PCR-DGGE analysis, 23 positive clones of *nifH* gene were selected at different positions on DGGE gel from all samples and sequenced. Although we found five more sequences in 2009 samples compared with sequences collected from 2008, results of MRPP analysis didn't show any significant effects coming from experimental year ($P > 0.05$, data didn't shown) on numbers of identified sequences. However, significant effects of cultivar ($P < 0.001$) were identified by the same analysis indicating selectivity effects of genotypes of chickpea on the diversity of their associated N_2-fixing bacteria.

Results of Correspondence Analysis (CA) shown composition of dominant N_2-fixing bacteria was modified by both fungicide treatment and cultivar (Fig. 1). Significant correlation was found between different N_2-fixing bacteria and combination of fungicide and chickpea cultivar in 2008 ($P = 0.014$, Fig. 1a). Although the similar trend of the relationship between identified bacteria and our experimental treatments described above still exist in 2009 (Fig. 1b), this association turned into non-significantly ($P > 0.05$), indicating the influence of experimental year on the composition of dominant N_2-fixing bacteria community, which may due to variations in weather conditions.

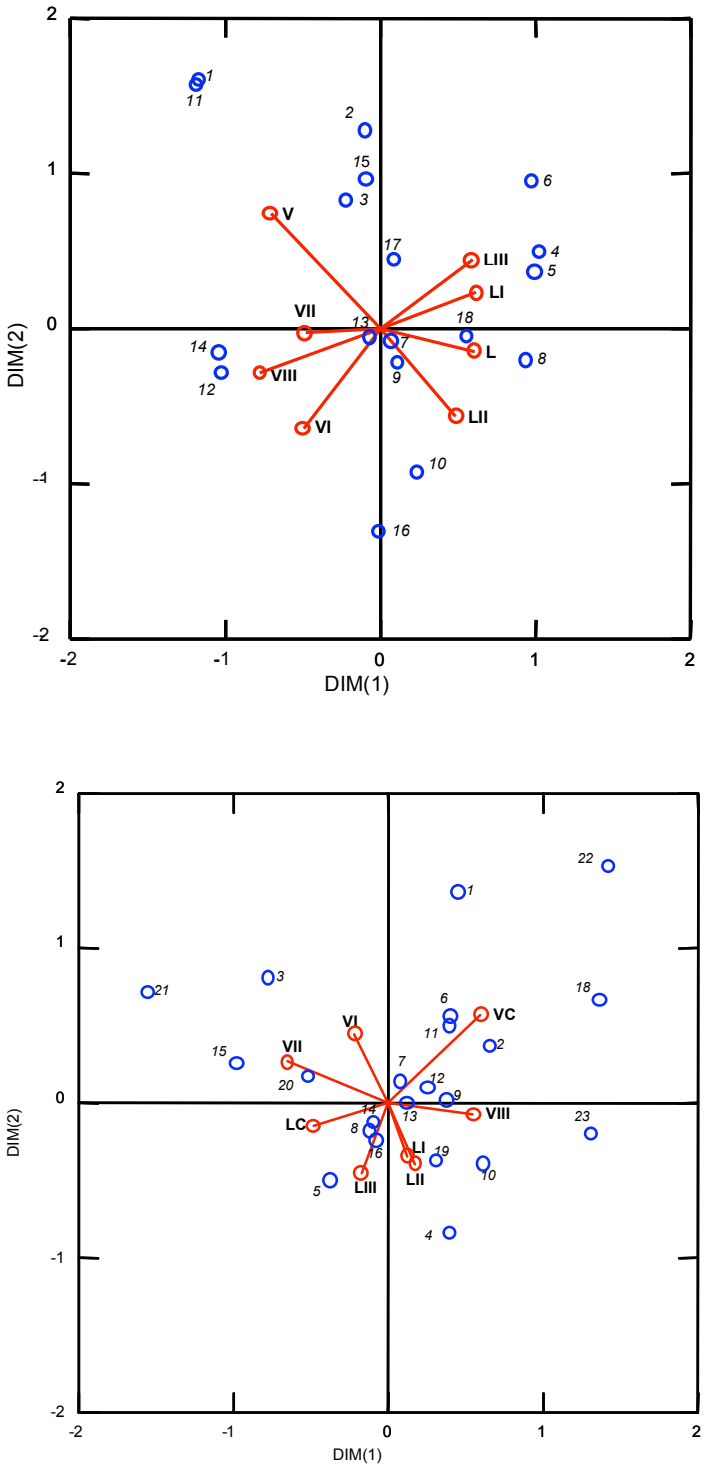

Fig. 1 Correspondence analysis (CA) of relationships between fungicide treatments and identified dominant N_2-fixing bacteria in the rhizosphere of both chickpea cultivars in 2008 (a) and 2009 (b) as revealed by nifH gene. (C: control; I, II and III: increasing levels of fungicide application intensity; V: Vanguard; L: Luna; Numbers are identified N_2-fixing bacteria shown in table 2. $P = 0.014$ in 2008, $P > 0.05$ in 2009, N = 32)

ANOVA results showed that chickpea nodulation scores significantly decreased ($P < 0.001$) with increase of fungicide application intensity of both years (Fig. 2), suggesting that fungicide use could restrict chickpea nodulation progress. A cultivar × fungicide interaction ($P < 0.001$) was detected showed that chickpea cultivars responded differently to fungicide treatments effects. Furthermore, interaction effects of year × fungicide ($P = 0.003$) and year × cultivar ($P < 0.001$) indicated year's effects on fungicide applications of both cultivars, which may due to variations in weather conditions.

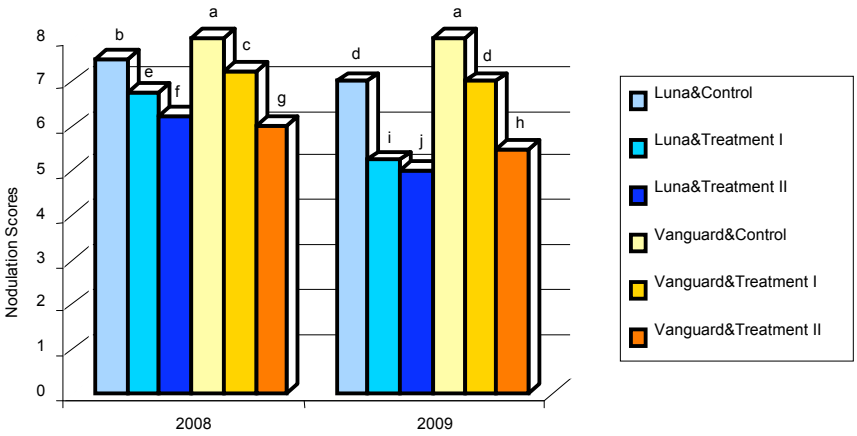

Fig. 2 Nodulation scores of two chickpea cultivars receiving different fungicide treatments in 2008 and 2009. A high score indicates extensive nodulation ($P < 0.001$, N = 48)

ANOVA results showed significant promoting effects of fungicide applications on fixed nitrogen was detected in our study (Fig. 3), suggesting fungicide usage could inhibit disease rate and brought better production of chickpea. However, nodulation scores was restricted by fungicide treatments (Fig. 2), indicating modification effects of fungicide on nodule activities in chickpea filed, which was also confirmed by Redundancy Analysis (RDA), as a significant negative relationship between nodulation scores and fixed nitrogen was found (Fig. 4).

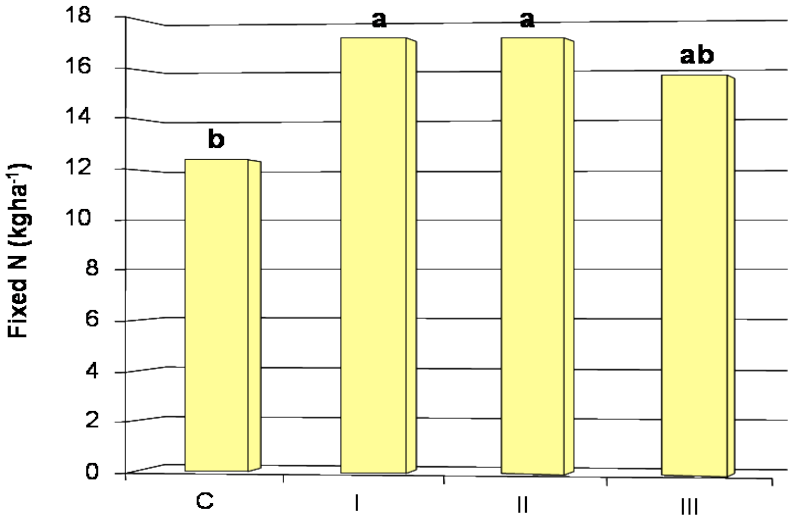

Fig. 3 Main effect of fungicide treatments on the amount of N2 fixed by field-grown chickpea. C: control; I, II and III: increasing intensity of fungicide application as explained in Table 1 (*P* = 0.04, n = 16)

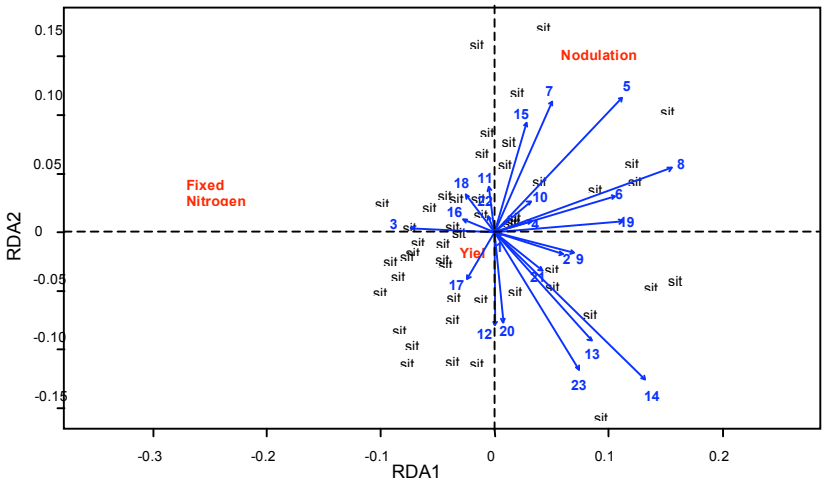

Fig. 4 Redundancy analysis (RDA) of the relationship between rhizosphere N_2-fixing bacteria richness, nodulation scores, fixed nitrogen and chickpea yield (sitx: experimental plot profiles; Numbers are identified N_2-fixing bacteria shown in table 2. *P* = 0.014, N = 48)