# Fungicide Application Effects on Diversity Components of Chickpea Rhizospheric Bacterial Community

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**Key words**: chickpea, fungicide, rhizosphere, bacteria, cultivar

**Abstract**

Molecular (polymerase chain reaction – denaturing gradient gel elecrophoresis) methods and correspondent analysis have been used in order to test changes of the diversity of bacterial communities in chickpea rhizospheric soil under different cultivars and fungicide treatments. Results showed that chickpea genotypes influence their microbial environment differently. Besides, fungicide applications could negatively affect the diversity of dominant bacterial DNA sequences, and this effect increased with the number of fungicide application on chickpea aerial parts.

**Introduction**

Chickpea (*Cicer arietinum L.*) is the third most important food legume in the world, which has been planted in many arid and semiarid regions because of its resistance to drought. Since chickpea is very easily infected by some fungi pathogens such as ascochyta blight (*Ascochyta rabiei*) which may cause very serious loss for farmers, thus, fungicide application is a common practice in chickpea production (Gan et al. 2006) to prevent the disease. Since some bacterial species have similar chemical binding sites as fungi, these fungicide applications might influence soil bacteria communities (Navas-Cortes et al. 1995; Pethybridge et al. 2005; Shtienberg et al. 2006). In this study, molecular fingerprinting (Cloning, PCR and DGGE) methods were applied to test if fungicide application strategies could affect chickpea associated rhizospheric bacterial communities under the field-grown condition**.**

**Objective**

To discuss influences of fungicide application on the diversity components of the rhizosphere bacteria associated with chickpea in the field.

**Method**

Four fungicide treatments: (FI) Headline Duo applied at seedling and early flower stage, (FII)

Headline Duo applied at seedling and early flower stage and Bravo applied at vegetative stage, (FIII) Headline Duo applied at seedling and early flower stage and Bravo applied at vegetative, mid-flower and podding stage, and (FC) no fungicide use, were applied onto two chickpea cultivars, Vanguard and Luna, in Swift Current SK, Canada, in 2008. The field experiment had a complete randomized block design with four replicates.

Bacterial diversity was analyzed after PCR (polymerase chain reaction) amplification. Raw DNA extracted from soil (UltraClean Soil DNA Isolation Kit, MO BIO Lab. Inc) was amplified with universal bacterial primers 968f / R1401 and DGGE specialized primers 968f / R1401-GC in a nested protocol with our optimized conditions: 94 ℃ 4 min – (94 ℃ 30 sec, 65 ℃ 30 sec, 72 ℃ 1 min) with 30 cycles -- 72 ℃ 10 min -- 4 ℃ keep till gel test. 40 µl of PCR products were analyzed by DGGE (denaturing gradient gel electrophoresis) using a Dcode Universal Mutation Detection system (Bio-Rad Laboratories) at a constant temperature of 60 ℃ and 65 V for 16 hours. Then we sank the gel into SYBR Safe DNA gel stain solution for 1 hour to visualize positive bands. Visualized bands of all samples on DGGE gel were compared with the markers made using positive clones of DNA sequences found in our samples.

Identified bands' sequences have been edited by BioEdit 7.0.9.0. Statistical analyses (Correspondent analysis) were done using the R program (www.**r**-project.org/).

**Results and Discussion**

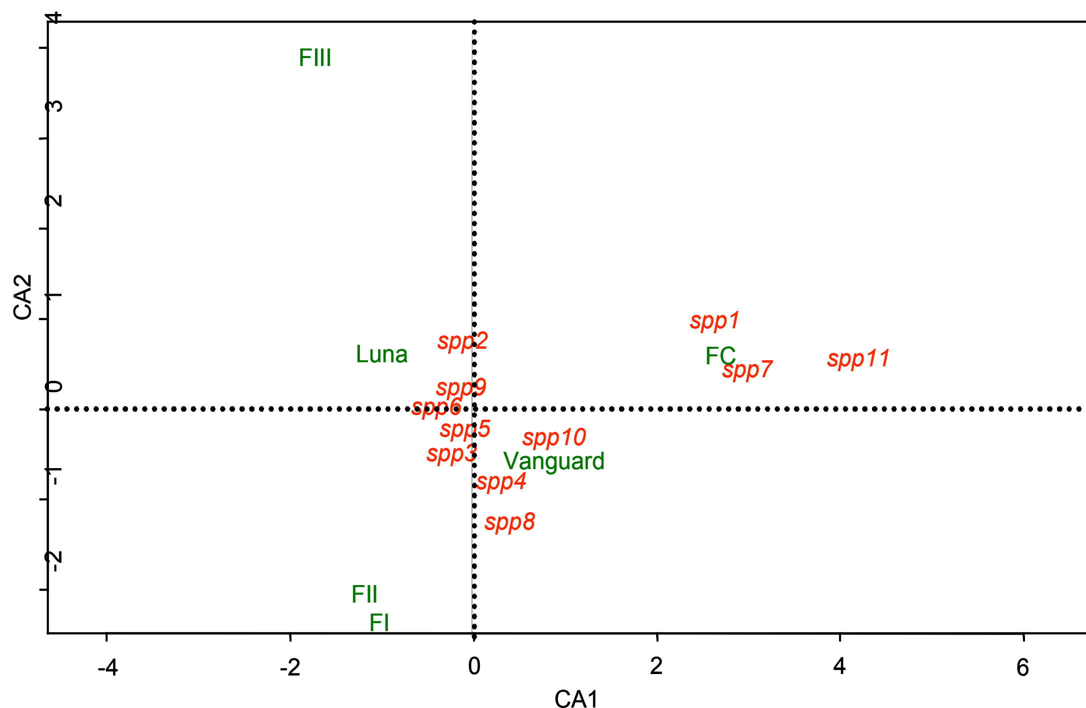


**Figure 1.** Correspondent analysis (CA) of relationship between fungicide treatments and identified rhizospheric bacteria on both chickpea cultivars

The diversity of rhizospheric bacterial communities associated with field-grown chickpea differed between fungicide treated (FI, FII and FIII) and control plots (FC). Figure.1 showed that fungicide treatment I and II gathered together, and this absence of difference between these two treatments suggests that applying Bravo at chickpea vegetative growth stage, between two applications of Headline Duo, has little impact on the rhizospheric bacterial community of the plant.

Sequences *spp3, spp4, spp5, spp6, spp8* and *spp9* showed up at almost all fungicide treatment plots of both chickpea cultivars (Table 1) indicated that these species grew stable in chickpea rhizospheric soil. Meanwhile, *spp1, spp7* and *spp11* closely associated with controls indicated these species were sensitive with chemicals we used in chickpea growth. *Spp2* which has been found in most Luna cultivar plots, and *spp10* which has been found in most of Vanguard plots, showed cultivar select effects on their rhizosphere associated bacteria.

**Table 1.** Distribution of Identified Bacterial Species in Each Cultivar and Fungicide Treatment

| Treatment | Bacterial Richness | Identified bacterial species | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | YCB1 | YCB2 | YCB3 | YCB4 | YCB5 | YCB6 | YCB7 | YCB8 | YCB9 | YCB10 | YCB11 |
| Vanguard.C. | 11 | € | € | € | € | € | € | € | € | € | € | € |
| Vanguard.I. | 7 | | | € | € | € | € | | € | € | € | |
| Vanguard.II. | 7 | | | € | € | € | € | | € | € | € | |
| Vanguard.III. | 5 | | € | | € | € | € | | | € | € | |
| Luna.C. | 9 | | € | € | € | € | € | € | € | € | € | |
| Luna.I. | 7 | | € | € | € | € | € | | € | € | | |
| Luna.II. | 7 | | € | € | € | € | € | | € | € | | |
| Luna.III. | 5 | | € | € | | € | € | | | € | | |

***Note***: Roma number I. II. III. Mean three fungicide treatments, C. means control, as we present at method part.

Sum in all, fungicide application decreased the diversity of dominant bacteria in field-grown chickpea rhizosphere, and this impact of fungicide application on diversity was negatively related with the number of fungicide application on chickpea aerial parts.

Besides, applying one more Bravo at chickpea vegetative stage between applications of Headline Duo at seedling and early flowering stages, had seemingly no impact on diversity changes of rhizosphere bacteria of both chickpea cultivars as the richness of bacteria communities in these treatments didn't change. But changes in rhizospheric bacterial diversity components with different chickpea cultivars highlight the influence of chickpea genotypes on its microbial environment.

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