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Biological Control of Fusarium Crown and Root Rot of Wheat by *Streptomyces* Isolates – It's Complicated

Mark Winter,^{1,†} Peter L. Samuels,² Lindsey K. Otto-Hanson,² Ruth Dill-Macky,² and Linda L. Kinkel²

¹Department of Crop Sciences, Göttingen University, 37077 Göttingen, Germany; and ²Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108

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ABSTRACT

The predominant causal agents of Fusarium crown and root rot (FCR) of wheat, along with *Fusarium pseudograminearum*, are *F. graminearum* and *F. culmorum*. Members of the Gram-positive bacterial genus *Streptomyces* have been shown to inhibit isolates of the genus *Fusarium*, and *Fusarium* spp. isolates are also able to inhibit *Streptomyces* isolates in vitro. However, little is known about these complex antagonistic interactions and the potential for inhibitory *Streptomyces* to reduce FCR of wheat. The aim of this study was to analyze whether inhibitory *Streptomyces* isolates affect FCR of wheat and reduce root and stem base colonization by *Fusarium culmorum*. We enriched sterilized potting soil with spore suspensions of two *Streptomyces* isolates, inoculated the soil with *F. culmorum*-colonized wheat straw, and planted pre-germinated wheat seedlings. At 4 weeks, *F. culmorum*-inoculated plants had significant FCR symptoms on roots and showed reduced fresh weight of roots and above-ground plant biomass compared with the non-inoculated controls. Enrichment of soil with an inhibitory *Streptomyces* isolate reduced *F. culmorum* DNA in roots and stem bases by 75% compared with inoculation with *F. culmorum* alone. Interestingly, co-inoculation of *F. culmorum* with a non-inhibitory

Streptomyces isolate led to the highest levels *F. culmorum* DNA in stem base tissue and greatest *Streptomyces* densities (CFU per g of soil) in the rhizosphere. In vitro assays revealed that *F. culmorum* showed a strong inhibitory activity against the pathogen-inhibitory *Streptomyces* isolate but not against the non-inhibitory isolate. In vitro tests with a larger set of 17 *Streptomyces* and five *Fusarium* spp. isolates revealed that there was little variation among *Fusarium* spp. isolates in capacities to inhibit the collection of *Streptomyces* isolates. In contrast, the sensitivity to inhibition by pathogenic *Fusarium* spp. isolates varied widely among *Streptomyces* isolates. The results of this study suggest the potential of *Streptomyces* isolates for biocontrol of FCR of wheat, while highlighting the specificity of *Streptomyces*–*Fusarium* interactions. Broader understanding of the variation in susceptibility within *Fusarium* spp. populations to *Streptomyces* inhibition and vice versa are needed to advance the potential for successful biological control.

Keywords: antagonism, *Fusarium culmorum*, Fusarium foot and root rot, *Fusarium graminearum*.

Fusarium crown and root rot (FCR) of wheat is a soilborne disease that occurs in most wheat-growing areas worldwide (Moya-Elizondo 2013). Significant yield losses attributed to FCR are reported from dryland wheat-growing regions including the Pacific Northwest of the United States, Australia, and the Middle East (Fouly and Pedersen

1996; Poole et al. 2013; Wildermuth et al. 1997). With global climate change predicted to increase the frequencies and intensities of droughts, and changes in rainfall patterns across many wheat production regions, the prevalence of FCR is expected to increase in areas where it is currently reported and expand into other wheat production areas (Moya-Elizondo 2013). The predominant causal agents of FCR are, along with *Fusarium pseudograminearum* O'Donnell and Aoki, the ascomycetous fungi *F. culmorum* (W.G. Smith) Sacc., and *F. graminearum* Schwabe (Burgess et al. 1993; Chekali et al. 2011; Paulitz et al. 2002; Smiley and Patterson 1996). The primary sources of inoculum for FCR infections are soilborne chlamydospores and infested plant debris in the upper (~10 cm) soil surface layer (Cook 1980). Stem base and root infections of wheat by *F. culmorum* and *F. graminearum* can take place as early as the seedling stage and may result in seedling blight (Scherer et al. 2013). Early infection is initiated by germinating mycelium invading the

[†]Corresponding author: M. Winter; mark.winter@agr.uni-goettingen.de

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rhizodermal root tissue and infecting the coleoptile (Beccari et al. 2011; Duthie and Hall 1987). In later stages, the pathogen colonizes the whole stem base up to the third node (Beccari et al. 2011; Clement and Parry 1998; Cook 1981). Infection of the crown of the plant results in impaired vascular tissue function and increases lodging, and both symptoms contribute to yield losses (Clement and Parry 1998; Smiley and Patterson 1996). In addition to their pathogenic lifestyle, *Fusarium* species are considered to be ubiquitous saprophytes, and can play important roles in nutrient cycling and are strongly interacting with the soil microbial community (King et al. 2011; LeBlanc et al. 2017; Li et al. 2008).

The genus *Streptomyces* consists of several hundred species of Gram-positive filamentous bacteria in the phylum *Actinobacteria*. *Streptomyces* are omnipresent in soil and the plant rhizosphere and are recognized for their significant ability to produce highly diverse antibiotics in remarkable quantities (Challis and Hopwood 2003; Watve et al. 2001). In diverse crop production systems, *Streptomyces* have been shown to suppress soilborne diseases caused by plant pathogenic fungi, bacteria, and nematodes (Samac and Kinkel 2001; Wiggins and Kinkel 2005a, 2005b). This suppression is achieved through multiple mechanisms, including antibiosis and competition for nutrients and space, induced resistance in the host plant, quorum quenching, degradative enzymes, and the production of nitrous oxide (Cohen and Mazzola 2006; Cohen et al. 2005; Mahadevan and Crawford 1997; Mahmoudi et al. 2011).

Although *Streptomyces*, *F. culmorum*, and *F. graminearum* are members of the microbial community in many agricultural soils, information on their interactions, including reciprocal antagonistic inhibitory activity, is scarce. A recent study showed that members of the genus *Streptomyces* possessed strong inhibitory activity against various coexisting *Fusarium* species, and *Fusarium* spp. isolates were also able to inhibit *Streptomyces* isolates in vitro (Essarioui et al. 2017). The authors found that the occurrence of strong *Fusarium*-antagonistic *Streptomyces* and *Streptomyces*-antagonistic *Fusarium* phenotypes is influenced by the richness of the plant community and resource competition, and showed that *Fusarium* spp. and *Streptomyces* isolates develop a competitive and/or antagonistic relationship in soil. However, little is known about these complex interactions in agricultural settings, including the potential for inhibitory *Streptomyces* isolates to reduce FCR of wheat. In this study we analyzed the effects of *Streptomyces* isolates on FCR of wheat and specifically whether *Streptomyces* can reduce root and stem base colonization by *F. culmorum*, as measured by the levels of *F. culmorum* DNA in these tissues. Further, we analyzed *Streptomyces* and *Fusarium* spp. isolate inhibitory interactions and whether reciprocal interactions play a role in biocontrol. The specific objectives of this work were (i) to characterize the inhibitory interactions and resource competition between *Streptomyces* isolates and isolates of *F. culmorum* and *F. graminearum*, and (ii) to analyze whether the enrichment of soil with a *Fusarium*-antagonistic *Streptomyces* isolate can effectively reduce soilborne colonization of wheat roots and stem bases by *F. culmorum*. Together, we wanted to evaluate whether in vitro inhibitory interactions and resource competition between *Streptomyces* and *Fusarium* spp. isolates can explain biological control of FCR of wheat by *Streptomyces* under controlled conditions. This research highlights the complex isolate-specific interactions between *Streptomyces* and *Fusarium* spp. isolates and the potential to control a prevalent disease using pathogen suppressive *Streptomyces*.

MATERIALS AND METHODS

***Fusarium* isolates.** Pathogenic *F. culmorum* isolates 10212001, 10286002, 10281010, and the *F. graminearum* isolate 10100004

selected from the collection of the Small Grains Pathology Laboratory, Department of Plant Pathology at the University of Minnesota, U.S.A., and *F. graminearum* isolate 02-15 from the fungal collection of the USDA-ARS Cereal Disease Laboratory, St. Paul, MN, U.S.A., were used in this work. Isolates were selected based on their capacity to cause varying levels of FCR in wheat in growth chamber experiments (Winter et al. 2018).

***Streptomyces* isolates.** To study the interaction between the five pathogenic *Fusarium* spp. isolates and *Streptomyces* during FCR of wheat, nine *Streptomyces* isolates were obtained from wheat rhizospheres collected from four non-tilled fields (two isolates per field for three fields and three for one field) after harvest in October 2015. Briefly, stubble of wheat plants, including roots and attached soil (three to five stubbles per field) were collected in the Red River valley in northwest Minnesota. Rhizosphere soil samples were obtained within 24 h after sampling by shaking the attached soil from wheat roots, air-drying soil overnight under two layers of sterile cheesecloth, and storing soil in sealed plastic bags in the refrigerator prior to processing. For *Streptomyces* isolation, 5 g of soil was suspended in 50 ml of sterile distilled water in a 125 ml Erlenmeyer flask and shaken for 1 h on a reciprocal shaker (175 rpm, 4°C). After sedimentation for 10 min, the supernatant was serially diluted to 1:100 and 1:1,000, and 100 µl was spread using a Drigalski spatula onto the surface of 1% water agar (WA) medium in Petri plates. Plates were incubated at 28°C for 3 days. Colonies with typical *Streptomyces* appearance, *Streptomyces* exhibit characteristic powdery sporulation on WA medium, and the vast majority of isolates with this morphology are in the genus *Streptomyces* (more than 95% based on own 16S sequence analysis, data not shown), were transferred to Petri plates containing oatmeal agar (OA) (Lorang et al. 1995). Plates were incubated at 28°C for 10 days, and isolates were harvested with sterile cotton swabs into 20% glycerol at 1.0×10^8 colony forming units (CFU) per ml and stored in 2 ml aliquots at -20°C. Additionally, eight *Streptomyces* isolates, 3211.1, 1231.5, 3212.5, 2233.5, 2311.3, GS4-24, GS4-17, and GS8-22, with known inhibitory potential toward *Fusarium* spp. isolates (L. Kinkel, University of Minnesota, St. Paul, MN) were used in the inhibitory assays (Supplementary Table S2). This set includes isolates from both native prairie soils and agricultural fields.

Inhibitory potential of *Streptomyces* isolates against isolates of the species *F. culmorum* and *F. graminearum*. The inhibitory potential of *Streptomyces* isolates against *Fusarium* spp. isolates (all possible pairwise combinations) was tested in an in vitro assay on OA medium as previously described (Essarioui et al. 2017). Briefly, Petri plates containing OA were inoculated in the center with a plug (7 mm diameter) cut from within a *Fusarium* spp. colony grown on potato dextrose agar medium (PDA). Three *Streptomyces* colonies were dotted onto the plate (4 µl at a density of 1.0×10^8 CFU per ml) at equal distances from each other and 2.75 cm from the *Fusarium* agar center plug, and the plates were incubated at 28°C for 5 to 7 days, or until mycelial growth reached the edge of the plate. Inhibition of each *Streptomyces* isolate against a *Fusarium* spp. isolate was quantified as the average distance (mm) between the outer edge of the *Streptomyces* colony for an isolate and the outer edge of the cleared inhibition zone surrounding the colony, as measured in two directions at right angles to each other. Each *Streptomyces*-*Fusarium* combination was replicated three times, whereby the three dotted *Streptomyces* colonies were arbitrarily assigned to different plates. *Streptomyces* isolates were considered as inhibitors when the mean inhibition zone was ≥ 2 mm and non-inhibitors when the mean inhibition zone was < 2 mm.

Inhibitory potential of *F. culmorum* and *F. graminearum* isolates against *Streptomyces* isolates. *Fusarium* inhibition of *Streptomyces* was evaluated in vitro in a manner similar to that

described in Essarioui et al. (2017). Briefly, starch casein agar medium (SCA; 10 g of starch, 0.3 g of casein, 0.02 g of CaCO_3 , 2.0 g of KNO_3 , 2.0 g of NaCl , 2.0 g of K_2HPO_4 , 0.05 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 g of ZnCl_2 , and 15 g of agar per liter of deionized H_2O) was first inoculated by spreading a spore suspension of a *Streptomyces* isolate over the entire surface of the SCA medium (100 μl at 1.0×10^6 CFU per ml per plate) (Becker and Kinkel 1999). For each *Fusarium* spp. isolate, three agar plugs (PDA, 7 mm diameter), taken from the growing edge of a 7- to 10-day-old culture were placed 2.75 cm from the center of the plate, and placed equidistant with respect to each other. Plates were inverted and incubated overnight at 4 to 5°C in order to allow antimicrobial compounds produced by *Fusarium* to diffuse into the medium. Plates were subsequently incubated at 28°C for 3 to 4 days. Inhibition zones were measured as described above.

***Streptomyces* and *Fusarium* resource use characterization.** Resource use profiles were evaluated for *Fusarium* spp. isolates ($n = 5$) and *Streptomyces* ($n = 9$) isolates obtained from the field soil samples using Biolog SF-P2 microplates (Biolog, Inc., Hayward, CA) as described previously with a slight modification (Essarioui et al. 2017; Schlatter et al. 2009). Briefly, *Fusarium* inoculum (macroconidia) was produced on mung bean agar (Dill-Macky 2003). Macroconidia were swabbed using a sterile cotton swab into 0.2% carrageenan and adjusted to a concentration of 1.0×10^5 conidia per ml for inoculation of Biolog microplates. Plates were incubated for 3 days at 28°C, after which optical density (OD) was measured at 590 nm using a Synergy H1 Reader (BioTek Instruments, Winooski, VT). The OD for the water control was subtracted from the OD indicating growth on each of the 95 substrates tested, the resulting wells with an adjusted OD ≤ 0.005 were considered to have an OD value of 0. All remaining wells with non-zero OD readings were considered nutrients used for growth. In order to assess overall growth phenotypes for each isolate, total growth, average growth, and niche width were assessed. Total growth was calculated as the sum of ODs across used nutrients for each isolate. Average growth was quantified as the average OD across the number of used nutrients for a given isolate. Finally, niche width for each isolate was defined as the number of nutrients used out of the 95 possible nutrients included in the SF-P2 Biolog plates. In order to interrogate resource competition between isolates, escape from competition and effective competition were evaluated for all pairwise asymmetrical *Fusarium*–*Streptomyces* combinations, to account for the potential impact of one isolate on another, and vice versa. Escape was defined as the proportion of total growth of one isolate (Isolate A) for which there is no apparent resource competition with another isolate (Isolate B):

$$\text{Escape}_{\text{A with respect to B}} =$$

$$\left(\frac{\text{When } (\text{Growth}_{\text{A}} - \text{Growth}_{\text{B}}) \geq 0, \sum \text{Growth}_{\text{A}} - \text{Growth}_{\text{B}}}{\sum \text{Growth}_{\text{A}} \text{ across all nutrients}} \right) \times 100$$

Effective competition was defined as the proportion of an isolate's total growth for which an isolate will compete directly with another isolate:

$$\text{Effective Competition}_{\text{A with respect to B}} = 100 - \text{Escape}_{\text{A with respect to B}}$$

Growth chamber experiments. For growth chamber experiments, a highly aggressive isolate of *F. culmorum* (in terms of wheat stem base infection; 10212001) and two *Streptomyces* isolates showing high (S05) and low (S06) inhibition of *Fusarium* spp. isolates in in vitro assays were used. *Streptomyces* isolate stocks were produced by inoculating OA media in Petri plates with 100 ml

of *Streptomyces* stock suspension, as described previously, and incubating them for 14 days at 28°C. Colonies were transferred with a sterile cotton swab into 20% glycerol. The concentration of CFUs was determined by serial dilution on WA (1%). Spore suspensions of the respective *Streptomyces* isolates were added to soil (3:1 ratio of potting soil to sand) at 8.0×10^5 CFU per g of soil. Soil was incubated in plastic tubs (40 cm $\times$ 25 cm $\times$ 14 cm) for 24 h at 28°C. Subsequently, the *Streptomyces*-enriched soil was inoculated with the fungal pathogen, *F. culmorum*, by incorporating wheat straw colonized with *F. culmorum* isolate 10212001 into soil (7.2 g of straw per 1 kg of soil). *Fusarium*-colonized wheat straw was produced as follows: 30 g of ground wheat straw (1 to 2 cm long pieces) and 20 ml of water were autoclaved (121°C for 30 min) on two consecutive days in plastic boxes (10.5 cm $\times$ 10.5 cm $\times$ 9.5 cm). Subsequently, the wheat straw was inoculated with 10 ml spore suspension (2.5×10^5 spores per ml) of *F. culmorum* per box and incubated at 21°C for 4 weeks. Spore suspensions of *F. culmorum* used to inoculate the straw were produced as previously described by Winter et al. (2013). Additionally, potting soil was inoculated with *F. culmorum* or isolate S05 or S06 of *Streptomyces* alone. A non-inoculated treatment served as a control, resulting in six different treatments.

The hard red spring wheat variety 'Samson' (WestBred, Monsanto, St. Louis, MO, released in 2007) was used for growth chamber experiments. Seeds were surface sterilized under constant agitation in 70% EtOH for 30 s and subsequently washed twice in sterile deionized water. Subsequently, the surface disinfested seeds were placed on autoclaved (15 min at 121°C) moist gravel (100 g) in glass Petri dishes and covered with aluminum foil for germination at 21°C for 4 days prior to planting. Pre-germinated wheat seedlings, two seedlings per pot (20.5 cm [depth] $\times$ 4 cm [diameter], Deepot D33L, Stuewe & Sons, Inc, OR), were then planted into the differentially treated potting soils. Each treatment consisted of 30 plants (15 pots).

Plants were grown in a growth chamber (Conviron, Winnipeg, Canada) under a 16:8 h day/night cycle at 21/16°C. After 2 weeks the number of surviving plants in each treatment was assessed as the survival rate (%). Four weeks after planting, 24 plants per treatment were harvested. Plants were cut off just above the crown roots, fresh weight of above-ground plant material was determined and disease symptoms of FCR was visually assessed. For symptoms, disease severity on culms was rated using a 0 to 3 scale, where 0 = no lesion visible, 1 = lesion covering up 50% of stem surface area, 2 = lesion covering 51 to 100% of stem surface area, and 3 = stem base rotten. Leaf sheaths were removed and the bottom 5 cm of the stem base was excised, plant tissues from the same cone were combined to one sample, flash-frozen in liquid nitrogen, and stored at –20°C until used for the quantification of *F. culmorum* DNA by qPCR. Roots were gently removed from pots, loosely attached potting soil was removed by hand, and rhizosphere soil samples were obtained by shaking off the tightly attached soil from roots. Rhizosphere soil samples were used to determine the *Streptomyces* density in the soil by completing a serial dilution plating of a soil suspension as described above. Roots were subsequently rinsed under a faucet fitted with a spray nozzle to remove any remaining soil. After patting dry with paper towels, disease symptoms and root fresh weight were assessed.

Disease severity on roots was visually rated as the percentage of diseased root tissue. Briefly, brownish discolored root tissue was considered as diseased and assessed in relation to the healthy (not discolored) root tissue. Subsequently, roots were flash-frozen in liquid nitrogen and stored in –20°C until quantification of *F. culmorum* DNA by qPCR. Both genomic DNA extraction and quantification of fungal DNA of *F. culmorum* in stem base and root tissue was conducted as described in Winter et al. (2018).

Data analyses. Statistical analyses were performed with the computer software Statistica (StatSoft, Tulsa, U.S.A.). Data of disease severity rating at the stem base were ordinally scaled and thus the univariate non-parametric Kruskal-Wallis test was used to evaluate differences in stem base symptoms. Disease rating data from roots, fungal biomass quantifications, and *Streptomyces* densities were log-transformed with the formula $\log(x + 1)$ prior to analysis of variance (ANOVA) to obtain a suitable distribution of the residuals. Log-transformed data and the plant fresh weights were analyzed for significant differences with Tukey's posthoc test ($P \leq 0.05$). A generalized linear model (GLM) and honestly significant difference (HSD) posthoc test ($P \leq 0.05$) for unequal sample sizes were carried out to evaluate differences in effective competition between *Fusarium* spp. isolates against *Streptomyces* isolates S05 and S06, and vice versa, and in resource use (total growth, average growth, mean niche width) between *Fusarium* spp. isolates and *Streptomyces* isolates S05 and S06. A GLM and an HSD posthoc test ($P \leq 0.05$) for unequal sample sizes were carried out to evaluate differences between *Streptomyces* densities in rhizosphere soil after subtracting background *Streptomyces* densities measured in the treatment inoculated with 10212001 alone, considered as a naturally occurring *Streptomyces* population, from measured *Streptomyces* densities in all treatments. Tukey's posthoc test ($P \leq 0.05$) was performed using a GLM to analyze differences in inhibition between *Fusarium* versus *Streptomyces* and vice versa. The coefficient of variation (CV, %) of *Fusarium* inhibition against single and all *Streptomyces* isolates tested was calculated as the ratio of the standard deviation to the mean.

RESULTS

Inhibitory potential of *Streptomyces* isolates from wheat rhizospheres. Out of the nine *Streptomyces* isolates tested, two (S02 and S07) exhibited no inhibition of any *Fusarium* spp. isolate, and five isolates showed only limited inhibition (inhibition zones ≤ 2 mm) of individual *Fusarium* spp. isolates (Table 1). Thus, in general, resistance of *Fusarium* spp. isolates to inhibition by individual *Streptomyces* isolates was very high. However, one *Streptomyces* isolate (S05) inhibited the growth of all *Fusarium* spp. isolates with inhibition zones ≥ 3 mm (Table 1).

Inhibitory potential of *Fusarium* spp. isolates against *Streptomyces* isolates. We tested the inhibitory potential of *F. culmorum* ($n = 3$) and *F. graminearum* ($n = 2$) isolates against 17 *Streptomyces* isolates. There was little variation among *Fusarium* spp. isolates in their capacities to inhibit *Streptomyces* isolates (mean inhibition zones ranged from 2.4 to 3.5 mm) (Table 2). Nevertheless, there were significant species effects (one-way ANOVA, $P = 0.027$): *F. graminearum* displayed greater mean inhibition to *Streptomyces* isolates than *F. culmorum*. The mean CV (%) of inhibition zones (*Fusarium* spp. against *Streptomyces* isolates) among *Fusarium* spp. isolates was 102%, and varied from 82 to 118% for individual isolates. There was considerable variation in sensitivity of different *Streptomyces* isolates to individual *Fusarium* spp. isolates. *Fusarium* spp. isolates demonstrated low inhibition (< 2 mm zone) against six *Streptomyces* isolates (35%), whereas inhibition was considerable (≥ 2 mm zone) against the majority of tested *Streptomyces* isolates (11 isolates [65%]).

Conversely, sensitivity to inhibition by *Fusarium* spp. isolates varied widely among *Streptomyces* isolates (mean inhibition zones ranged from 0 to 10.7 mm) (Table 2). The mean CV of inhibition zones for individual *Streptomyces* isolates inhibited by *Fusarium* spp. isolates was 47% and varied from 0 to 131%. The highest variation in sensitivity was found among the isolates from wheat fields. Isolates S03 and S09 were not inhibited in growth by any of the tested *Fusarium* spp. isolates, whereas S10 and S02 were highly sensitive to *Fusarium* spp. isolates (Table 2). Isolate S02 showed by far the highest sensitivity, especially to *F. graminearum* isolate 10100004, with a mean inhibition zone of 16.2 mm (Table 2).

Inhibitory activity of *F. culmorum* isolate 10212001 against *Streptomyces* isolates S05 and S06, and vice versa. *F. culmorum* isolate 10212001 exhibited strong inhibitory activity against *Streptomyces* isolate S05 but not against isolate S06 (Fig. 1). Conversely, *Streptomyces* isolate S05 inhibited the growth of *F. culmorum* isolate 10212001 in vitro, but *Streptomyces* isolate S06 did not inhibit this isolate. The two *Streptomyces* isolates displayed significant differences in both inhibition against 10212001 and resistance to inhibition by 10212001 (Fig. 1). Likewise, the *F. culmorum* isolate inhibited the *Streptomyces* isolate with strong inhibitory potential against *Fusarium* spp. isolates, but not the non-inhibitory *Streptomyces* isolate (Table 1, Fig. 1).

TABLE 1
Mean inhibition zones of nine *Streptomyces* isolates against five *Fusarium* spp. isolates in in vitro assays^z

<i>Fusarium</i> isolates	Inhibitory potential of <i>Streptomyces</i> against <i>Fusarium</i> (mean inhibition zone [mm] $\pm$ standard deviation)								
	<i>Streptomyces</i> isolates								
	S02	S03	S04	S05	S06	S07	S08	S09	S10
10212001	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	4.0 $\pm$ 1.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.8 $\pm$ 1.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
10286002	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	3.5 $\pm$ 1.5	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.2 $\pm$ 0.3	1.0 $\pm$ 0.0	0.0 $\pm$ 0.0
10281010	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.5 $\pm$ 0.5	4.3 $\pm$ 0.6	3.5 $\pm$ 0.9	0.0 $\pm$ 0.0	2.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
10100004	0.0 $\pm$ 0.0	1.2 $\pm$ 0.3	0.0 $\pm$ 0.0	3.2 $\pm$ 0.3	0.7 $\pm$ 1.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
02-15	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	3.3 $\pm$ 1.5	0.5 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	2.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Mean	0.0 c $\pm$ 0.0	0.2 bc $\pm$ 0.5	0.3 bc $\pm$ 0.6	3.7 a $\pm$ 1.1	0.9 b $\pm$ 1.5	0.0 c $\pm$ 0.0	0.8 bc $\pm$ 0.9	1.0 b $\pm$ 0.7	0.4 bc $\pm$ 0.5

^z Petri plates (9 cm diameter) containing oatmeal agar (OA) were inoculated in the center with an agar plug cut out from a 5-day-old *Fusarium* spp. colony. Three *Streptomyces* spp. colonies were dotted, 4 μ l at a density of 1.0×10^8 CFU per ml, at equal distances from the *Fusarium* spp. overgrown agar plug and incubated until the *Fusarium* colony reached the plate edge. The average inhibition of *Streptomyces* against *Fusarium* spp. was measured as the distance (mm) between the edge of the *Streptomyces* isolate and the outer edge of the cleared inhibition zone, as measured in two directions at right angles to each other. Each *Streptomyces*-*Fusarium* combination was replicated three times. *Streptomyces* isolates were considered as inhibitors when the mean inhibition zone was ≥ 2 mm and as noninhibitors when the mean inhibition zone was < 2 mm. Different letters indicate significant differences between the mean inhibitory potential of *Streptomyces* isolates against *Fusarium* spp. according to Tukey test ($P \leq 0.05$) performed within a generalized linear model.

Resource use profiles and effective competition of *Fusarium* spp. isolates against S05 and S06 and vice versa. *Fusarium* spp., inhibitory, and non-inhibitory *Streptomyces* isolates did not differ in niche width, average growth, or total growth (Supplementary Table S1).

Overall, there was no significant difference in the effective competitive impact of *Fusarium* on *Streptomyces* versus *Streptomyces* on *Fusarium* (one-way ANOVA, $P = 0.951$ within a GLM). Effective competition was highest for *Fusarium* spp. isolates against *Streptomyces* isolate S05, which was 33% higher than for *Fusarium* spp. isolates against isolate S06 (Fig. 2). In contrast, there was no difference between S05 and S06 with respect to either isolates effective competition against the tested *Fusarium* spp. isolates.

Growth chamber experiments. Inoculation with *F. culmorum* (isolate 10212001) led to significant colonization of wheat plants, resulting in 16% mortality (Table 3). When *F. culmorum* isolate 10212001 was inoculated together with either the non-inhibitory (S06) or inhibitory (S05) *Streptomyces* isolate, the mortality rate was higher than in the absence of *Streptomyces* (23% for 10212001 + S05 and 26% for 10212001 + S06, Table 3). The highest disease ratings on roots and stem bases were observed when *F. culmorum* 10212001 was co-inoculated with *Streptomyces* S06. Wheat root biomass was reduced after inoculation with isolate 10212001, though differences were significant only in contrast to inoculation with *Streptomyces* isolate S06 alone (Table 3). Above-ground plant material was significantly reduced in the *F. culmorum*-inoculated plants compared

with the control and the treatments inoculated with either *Streptomyces* isolates (S05 and S06) alone (Table 3).

Despite greater mortality rates, co-inoculation of *F. culmorum* and *Streptomyces* isolates did not affect root or above-ground plant biomass of the surviving plants. DNA quantifications in root and stem base tissue showed that inoculation with *F. culmorum* (10212001) led to significant fungal colonization of plant tissue. Co-inoculation of *F. culmorum* with *Streptomyces* isolate S05 significantly reduced the colonization of root and stem base tissue by *F. culmorum*. In contrast, when *Streptomyces* isolate S06 was inoculated together with *F. culmorum*, pathogen DNA levels were not significantly reduced in root tissue and were higher in stem base than in any other tissues. Control, S06-, and S05-enriched soil plants had low *F. culmorum* DNA quantities in roots and stem bases. *Streptomyces* populations were present in the rhizosphere of plants in all treatments. Enrichment of potting soil with isolates S05 and S06 significantly increased *Streptomyces* densities, with the highest densities observed in the isolate S06-enriched treatment. Notably, dual inoculation of *Streptomyces* isolates and *F. culmorum* significantly increased the densities of *Streptomyces* in the rhizosphere compared with inoculation with *Streptomyces* alone, with increases of sixfold and fourfold recorded for S05 and S06, respectively (Table 3, Fig. 3). *Streptomyces* densities in soil and *F. culmorum* DNA levels in plant tissue (fungal colonization) were not correlated.

TABLE 2
Inhibitory potential of three *Fusarium culmorum* and two *F. graminearum* isolates against 17 inhibitory and non-inhibitory *Streptomyces* isolates, coefficient of variation (CV) of mean inhibition zones of individual *Fusarium* against *Streptomyces* isolates (columns), and CV of mean inhibition of *Fusarium* spp. against individual *Streptomyces* isolates (rows)²

Inhibitory potential of <i>Fusarium</i> spp. against <i>Streptomyces</i> spp. isolates (mean inhibition zone [mm] ± standard deviation)								
<i>Streptomyces</i> isolates	Inhibitory to <i>Fusarium</i> (yes/no)	<i>F. culmorum</i> isolate			<i>F. graminearum</i> isolate		Mean	CV (%)
		10212001	10286002	10281010	10100004	02-15		
1231.5	Yes	3.0 ± 1.2	2.1 ± 0.5	1.7 ± 0.3	2.1 ± 0.8	2.2 ± 0.1	2.2 ± 0.7 cdefg	32.9
2233.5	Yes	2.3 ± 0.5	2.1 ± 0.3	4.2 ± 0.1	2.1 ± 1.0	3.1 ± 0.3	2.8 ± 0.9 cde	33.8
2311.3	Yes	2.8 ± 0.4	2.7 ± 0.5	3.1 ± 0.2	2.0 ± 0.5	3.5 ± 0.2	2.8 ± 0.6 cde	22.2
3211.1	No	5.4 ± 0.6	3.5 ± 0.5	6.0 ± 0.6	4.5 ± 1.2	1.8 ± 0.4	4.2 ± 1.6 c	38.6
3212.5	Yes	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	0.6 ± 0.5 fgh	84.5
GS 4-17	Yes	1.0 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	1.0 ± 0.0	1.5 ± 0.2	0.8 ± 0.5 efgh	66.3
GS 4-24	No	2.4 ± 0.4	1.8 ± 0.3	0.0 ± 0.0	2.1 ± 0.0	2.6 ± 0.2	1.8 ± 1.0 defgh	55.0
GS 8-22	Yes	1.4 ± 0.3	2.5 ± 0.2	2.4 ± 0.2	3.4 ± 0.5	3.2 ± 0.1	2.6 ± 0.8 cdef	30.3
S02	No	8.2 ± 1.0	8.9 ± 1.7	6.2 ± 1.3	16.2 ± 2.7	13.9 ± 0.6	10.7 ± 4.1 a	38.7
S03	No	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 h	0.0
S04	No	2.6 ± 0.6	1.8 ± 0.2	2.0 ± 0.4	3.1 ± 0.3	3.6 ± 1.1	2.6 ± 0.9 cdef	32.6
S05	Yes	3.6 ± 0.5	3.8 ± 0.1	2.0 ± 0.1	4.9 ± 0.2	4.2 ± 1.0	3.7 ± 1.1 cd	28.9
S06	No	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.3	1.4 ± 0.2	0.5 ± 0.6 gh	131.0
S07	No	2.7 ± 0.6	0.9 ± 0.4	8.9 ± 1.1	1.2 ± 0.3	1.8 ± 0.1	3.1 ± 3.1 cd	101.4
S08	No	4.2 ± 0.2	4.4 ± 0.3	1.1 ± 0.1	5.0 ± 0.3	5.9 ± 0.1	4.1 ± 1.7 c	40.8
S09	No	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 h	0.0
S10	No	6.2 ± 0.4	8.1 ± 0.6	2.6 ± 0.2	7.9 ± 0.9	10.1 ± 0.4	7.0 ± 2.7 b	37.9
	Mean	2.6 ± 2.3 a	2.6 ± 2.6 a	2.4 ± 2.6 a	3.3 ± 3.9 a	3.5 ± 3.5 a		
CV (%)		82.4	99.2	110.0	117.5	100.6		

² Inhibition zones were assessed in vitro in a plate on oatmeal agar medium after 3 to 4 days of incubation. Mean inhibition zones in columns and rows followed by the same letter are not significantly different according to Tukey test ($P \leq 0.05$) performed within a generalized linear model, $n = 3$.

DISCUSSION

Biocontrol of *Fusarium* crown and root rot by *Streptomyces* isolates. This work illustrates the potential but also challenges using *Streptomyces* isolates for biological control of FCR of wheat. Specifically, *Streptomyces* isolates showed inhibitory potential toward *Fusarium* spp. isolates in vitro and one isolate (S05) tested reduced wheat stem base and root colonization with *F. culmorum* by 75% under controlled conditions, compared with inoculation with *F. culmorum* alone. Conversely, a *Streptomyces* isolate classified as

Fig. 1. Mean inhibition zones (bars, mm) $\pm$ standard deviation (error bars) of *Streptomyces* isolates against the *Fusarium culmorum* (Fc) isolate 10212001 (left) and Fc 10212001 against *Streptomyces* (right). Inhibition zones were assessed in an in vitro plate assay. Different lowercase and uppercase letters indicate significant differences in inhibition zones between *Streptomyces* isolates against 10212001 and 10212001 against *Streptomyces* isolates, respectively, according to Tukey test ($P \leq 0.05$) performed within a generalized linear model, $n = 3$.

Fig. 2. Mean effective competition (EC) of *Fusarium* spp. isolates in relation to inhibitory *Streptomyces* isolate S05 and non-inhibitory *Streptomyces* isolate S06 and vice versa. In order to interrogate resource competition between isolates, EC was evaluated in a pairwise, asymmetric manner that would account for the impact one isolate would have on another and vice versa. For the calculation of EC, see methods. The genus had no significant effect on EC (one-way analysis of variance [$P = 0.951$] within a generalized linear model [GLM]). Different lowercase and capital letters indicate significant differences in EC between *Fusarium* against *Streptomyces* isolates and *Streptomyces* against *Fusarium* spp. isolates, respectively, according to Tukey test performed within a GLM, $n = 5$.

non-inhibitory to *Fusarium* spp. isolates in vitro (S06) showed no effect on *F. culmorum* colonization of roots and stem bases of wheat. The in vitro results were mirrored by the greenhouse experiments conducted in this study. It may be that inhibition of *F. culmorum* by *Streptomyces* occurs by similar mechanisms in vitro and in soil in planta, suggesting that the in vitro inhibition assay may serve as a useful diagnostic tool for identifying candidate biocontrol strains that may be effective in reducing FCR of wheat.

Antagonistic activity of *Streptomyces* against pathogens may be achieved through different mechanisms, including antibiosis, nutrient competition, production of degradative enzymes, induced resistance, nitrous oxide production, and quorum quenching (Cohen and Mazzola 2006; Cohen et al. 2005; Mahadevan and Crawford 1997; Mahmoudi et al. 2011). In our competitive plate assays, we tested antagonism by antibiosis, whereby secondary metabolites diffuse through the agar medium. Differences in densities of *Streptomyces* in treatments with and without co-inoculation of *F. culmorum* and *Streptomyces* isolates suggest that competition was of minor importance for antagonism. Both tested *Streptomyces* isolates, inhibitory and non-inhibitory, co-inoculated with *F. culmorum* significantly increased *Streptomyces* densities in the rhizosphere, but only S05 reduced plant colonization. In contrast, the non-inhibitory *Streptomyces* isolate S06 achieved the highest densities in the rhizosphere when co-inoculated with *F. culmorum*, and did not inhibit *Fusarium* colonization. There are several possible explanations for the observed effect of *F. culmorum* on *Streptomyces* densities. Two likely explanations are that *F. culmorum* changed the habitat (e.g., *F. culmorum* activity may have made nutrients accessible for *Streptomyces*) or stimulated *Streptomyces* proliferation through the production of secondary metabolites. Noteworthy, the well-known and -investigated trichothecene mycotoxins produced by *F. culmorum* during wheat root colonization do not inhibit *Streptomyces* isolates (unpublished data). However, subinhibitory concentrations of antimicrobial metabolites have been reported to act as signaling molecules by altering nutrient utilization and gene expression (Fajardo and Martínez 2008; Vaz Jauri et al. 2013), they may be prime candidates for triggering sporulation or altering the growth dynamics of

Fig. 3. Mean *Streptomyces* density (CFU per g of soil) in wheat rhizosphere soil among plants inoculated with the *Fusarium culmorum* isolate 10212001 and/or *Streptomyces* isolates S05 and S06 4 weeks after incubation. Densities reflect numbers after subtracting background *Streptomyces* densities, measured in the treatment inoculated with 10212001 alone. Different letters indicate significant differences between treatment means according to Tukey's honestly significant difference test for unequal means ($P \leq 0.05$), performed within a generalized linear model, $n = 12$ and $n = 8$ for 10212001 + S06, respectively.

Streptomyces. It is likely that secondary metabolites of *Fusarium* spp. might act as signaling molecules, and thus enhance the antagonistic activity of *Streptomyces* populations, which we did not test in our study. This should be addressed in future research to explain the highly complex interactions between *Fusarium* spp. and *Streptomyces* isolates.

To our knowledge, this is the first study to show that a *Streptomyces* isolate, identified as inhibitory in vitro, can protect wheat roots and stem bases from *F. culmorum* colonization. However, co-inoculation of this strain (S05) with *F. culmorum* did not reduce FCR disease and/or did not influence plant biomass of wheat. The results of this study differ from previous research showing that seed-coating with *Streptomyces* can significantly reduce seedling blight, disease incidence and severity of seedborne FCR of wheat (Berg et al. 2001; Roberti et al. 2000). However, in the latter and in this study, the practical potential of *Streptomyces* for the control of FCR in the field remains unknown. We believe that coating of wheat seeds with antagonistic *Streptomyces* isolates is unlikely to be efficient for biological control of FCR in the field. Chlamydospores and *Fusarium*-colonized plant debris in the first 10 cm of soil are considered the primary source of inoculum for FCR (Cook 1980), which initiate the infection of the coleoptile and the stem base of wheat. Therefore, enriching the soil with antagonists seems to be more promising to control FCR of wheat. However, a major challenge for large-scale biological control of FCR in the field will be the establishment of effective methods to enrich the soil with antagonistic microbial populations, including *Streptomyces*. Rice grains (Soares et al. 2007) or wheat bran (El-Tarabily et al. 2000) may serve as suitable substrates for mass production of antagonist inoculum, but have not yet been evaluated for FCR suppression of wheat. Further studies are needed to investigate whether inhibitory *Streptomyces* populations can be established in the field to prevent wheat root and stem base colonization by *F. culmorum* and yield losses caused by FCR of wheat.

***Streptomyces*–*Fusarium* spp. interactions are highly isolate-specific.** The two *Streptomyces* isolates used in growth chamber experiments not only differed in their inhibitory activity against *Fusarium* spp. isolates but also in their resource utilization and resource use overlap with *Fusarium* spp. isolates, which was evaluated by comparison of individual resource use phenotypes

obtained after incubation in monoculture on biology plates. The assumption that resource similarity found in in vitro assays using Biolog plates is related to resource competition in soil was based on the findings by Kinkel et al. (2014) showing that resource competition data obtain in Biolog assays shed light on species interaction dynamics in soil. Among the five *Fusarium* spp. isolates, average *Streptomyces* niche overlap with *Fusarium* was higher for isolate S05 than for isolate S06. High effective competition (niche overlap) between coevolved microbes is related to high inhibitory potential among sympatric *Streptomyces* and *Fusarium* populations (Essarioui et al. 2017; Kinkel et al. 2014). In keeping with this finding, *Streptomyces* isolate S05 and *F. culmorum* isolate 10212001 showed both high niche overlap and strong reciprocal inhibition, which might indicate coevolution of S05 and *F. culmorum*. As stated earlier, direct competition might not have been responsible for antagonism, but it is likely that high niche overlap induced the production of antimicrobial compounds against *Fusarium* in S05. Conversely, *Streptomyces* isolate S06 and *F. culmorum* isolate 10212001 failed to demonstrate antagonism for each other and competed less for nutrients. This might explain why isolate S06 was able to proliferate strongly and where the stem bases in treatments enriched by this isolates showed elevated colonization by *F. culmorum* in terms of fungal DNA during co-inoculation. The *Streptomyces* isolates were obtained from two different wheat fields in Minnesota, which also varied in the soil type and characteristics and cropping history, which have been shown to influence the frequency of antagonistic *Streptomyces* phenotypes in soil (Bakker et al. 2010; Bailey and Kassen 2012; Essarioui et al. 2017; Kinkel et al. 2011; Otto-Hanson et al. 2013; Schlatter et al. 2009). In our study, we found highly specific inhibitory interactions between *Fusarium* spp. and *Streptomyces* isolates that were only in few cases related to niche overlap between isolates, and were not consistently reciprocal. The lack of recent coevolutionary interactions between *Fusarium* spp. and *Streptomyces* isolates investigated here may account for the absence of specific relationships between inhibitory and resource use phenotypes among *Fusarium*–*Streptomyces* pairs.

Interestingly, each *Streptomyces* isolate demonstrated fairly uniform resistance to inhibition by different *Fusarium* spp. isolates, whereas each *Fusarium* spp. isolate exhibited widely varying resistances to *Streptomyces* antagonism. It may be that inhibitory

TABLE 3

Survival rate of seedlings, mean disease symptoms on roots, mean disease ratings on stem bases, fresh weights (FW) of roots and above-ground plant material (referred as stem base), *Fusarium culmorum* DNA content, and *Streptomyces* density in rhizosphere soil 4 weeks after inoculation of wheat seedlings with *F. culmorum* (isolate 10212001), and/or amendment with an inhibitory (S05) or non-inhibitory (S06) *Streptomyces* isolate^z

Inoculation/amendment treatment	Survival rate (%)	Roots			Stem base			
		Disease rating (%)	FW (g)	<i>F. culmorum</i> DNA content (pg per mg FW)	Disease rating (0 to 3 scale)	FW (g)	<i>F. culmorum</i> DNA content (pg per mg FW)	<i>Streptomyces</i> density (CFU per g of soil)
<i>F. culmorum</i>	85.7	3.8 a	0.50 c	49.40 a	0.29 ab	1.22 b	133.34 a	4.10E+04 d
<i>F. culmorum</i> + S05	76.7	1.8 ab	0.55 bc	11.15 b	0.46 ab	1.09 b	31.86 bc	5.66E+06 b
<i>F. culmorum</i> + S06	63.3	4.0 a	0.57 abc	31.23 ab	0.79 a	1.19 b	416.46 ab	1.41E+07 a
S05	100	1.4 ab	0.68 ab	0.57 d	0.00 b	2.26 a	9.18 c	1.05E+06 c
S06	100	0.0 b	0.76 a	4.52 c	0.13 ab	2.15 a	38.66 bc	3.11E+06 b
Control	100	1.6 ab	0.66 abc	1.82 d	0.00 b	2.19 a	13.70 c	1.35E+05 d

^z Different letters within a column indicate significant differences according to Kruskal-Wallis test (disease ratings for stem bases considered separately) and Tukey test ($P \leq 0.05$). Data for disease ratings in roots, DNA, and *Streptomyces* densities were transformed with the formula $\log(x + 1)$ prior to analysis of variance, $n = 12$ and $n = 8$ for 10212001 + S06, respectively.

activity of *F. culmorum* and *F. graminearum* is based on only a few metabolites that are differentially bioactive toward different *Streptomyces* isolates. High diversity in resistance among *Streptomyces* to multiple antibiotic compounds has been shown in several studies (Davelos et al. 2004; Fujisawa and Weisblum 1981; Phillips et al. 1994). However, knowledge on the mechanisms of *Fusarium* inhibition of other microbes, especially soil bacteria, is scarce. It has been shown that fusaric acid, which can be produced by many *Fusarium* species (Bacon et al. 1996; Brown et al. 2015), suppresses the production of antifungal metabolites by the ubiquitous soil bacterium *Pseudomonas* (Duffy and Dfago 1997; van Rij et al. 2005). Fusaric acid is predominantly produced by *Fusarium* species in the section Liseola, including *F. moniliforme*, *F. solani*, and *F. oxysporum* (Bacon et al. 1996), and not by *F. culmorum* and *F. graminearum* (Brown et al. 2015; Hansen et al. 2015; Vanheule et al. 2016). The latter two *Fusarium* species are not only the predominant causal agents of FCR of wheat, but are also considered to play key roles in nutrient cycling and in shaping soil microbial communities (King et al. 2011; LeBlanc et al. 2017; Li et al. 2008). Presumably, these impacts are based in part on their inhibitory activities against indigenous soil microbes. Further studies are needed identifying the bioactive metabolites produced by *F. culmorum* and *F. graminearum* during their interactions with soil bacteria. In conclusion, *Streptomyces* isolates can effectively reduce the colonization of wheat roots and stem bases by the fungal pathogen *F. culmorum*, which may be the starting point of identifying practical biocontrol for FCR in the field. Our study supports a previous finding (Essarioui et al. 2017) that *Fusarium* can also effectively inhibit *Streptomyces*, though it appears that this relationship is not consistently reciprocal. A better understanding of *Fusarium*–*Streptomyces* interactions in soil mediated by both antagonism and effective competition are needed.

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LITERATURE CITED

- Bacon, C. W., Porter, J. K., Norred, W. P., and Leslie, J. F. 1996. Production of fusaric acid by *Fusarium* species. *Appl. Environ. Microbiol.* 62:4039-4043.
- Bailey, S. F., and Kassen, R. 2012. Spatial structure of ecological opportunity drives adaptation in bacterium. *Am. Nat.* 180:270-283.
- Bakker, M. G., Glover, J. D., Mai, J. G., and Kinkel, L. L. 2010. Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes. *Appl. Soil Ecol.* 46:35-42.
- Beccari, G., Covarelli, L., and Nicholson, P. 2011. Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium culmorum*. *Plant Pathol.* 60:671-684.
- Becker, D. M., and Kinkel, L. L. 1999. Strategies for quantitative isolation of *Streptomyces* from soil for studies of pathogen ecology and disease microbiology. Pages 349-362 in: *Recent Research Developments in Microbiology*, Research Signpost, Trivandrum, India.
- Berg, G., Marten, P., Minkwitz, A., and Brckner, S. 2001. Efficient biological control of fungal plant diseases by *Streptomyces* sp. DSMZ 12424. *JPD* 108:1-10.
- Brown, D. W., Lee, S. H., Kim, L. H., Ryu, J. G., Lee, S., and Seo, Y. 2015. Identification of a 12-gene fusaric acid biosynthetic gene cluster in *Fusarium* species through comparative and functional genomics. *Mol. Plant-Microbe Interact.* 28:319-332.
- Burgess, L. W., Backhouse, D., Summerell, B. A., Pattison, A. B., Klein, T. A., Esdaile, R. J., and Ticehurst, G. 1993. Long-term effects of stubble management on the incidence of infection of wheat by *Fusarium graminearum* Schw. Group 1. *Aust. J. Exp. Agric.* 33:451-456.
- Challis, G. L., and Hopwood, D. A. 2003. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc. Natl. Acad. Sci.* 100(suppl. 2):14555-14561.
- Chekali, S., Gargouri, S., Paulitz, T., Nicol, J. M., Rezgui, M., and Nasraoui, B. 2011. Effects of *Fusarium culmorum* and water stress on durum wheat in Tunisia. *Crop Prot.* 30:718-725.
- Clement, J., and Parry, D. 1998. Stem-base disease and fungal colonisation of winter wheat grown in compost inoculated with *Fusarium culmorum*, *F. graminearum* and *Microdochium nivale*. *Eur. J. Plant Pathol.* 104:323-330.
- Cohen, M. F., and Mazzola, M. 2006. Resident bacteria, nitric oxide emission and particle size modulate the effect of *Brassica napus* seed meal on disease incited by *Rhizoctonia solani* and *Pythium* spp. *Plant Soil* 286:75-86.
- Cohen, M. F., Yamasaki, H., and Mazzola, M. 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. *Soil Biol. Biochem.* 37:1215-1227.
- Cook, R. J. 1980. *Fusarium* foot rot of wheat and its control in the Pacific Northwest. *Plant Dis.* 64:1061-1066.
- Cook, R. J. 1981. *Fusarium* diseases of wheat and other small grains in North America. Pages 40-52 in: *Fusarium: Diseases, Biology, and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park.
- Davelos, A. L., Kinkel, L. L., and Samac, D. A. 2004. Spatial variation in frequency and intensity of antibiotic interactions among streptomycetes from Prairie Soil. *Appl. Environ. Microbiol.* 70:1051-1058.
- Dill-Macky, R. 2003. Inoculation methods and evaluation of *Fusarium* head blight resistance in wheat. Pages 84-210 in: *Fusarium Head Blight of Wheat and Barley*. K. J. Leonard and W. R. Bushnell, eds. American Phytopathological Society, St. Paul, MN.
- Duffy, B. K., and Dfago, G. 1997. Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* 87:1250-1257.
- Duthie, J., and Hall, R. 1987. Transmission of *Fusarium graminearum* from seed to stems of winter wheat. *Plant Pathol.* 36:33-37.
- El-Tarabily, K. A., Soliman, M. H., Nassar, A. H., Al-Hassani, H. A., Sivasithamparan, K., and McKenna, F. 2000. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathol.* 49:573-583.
- Essarioui, A., LeBlanc, N., Kistler, H. C., and Kinkel, L. L. 2017. Plant community richness mediates inhibitory interactions and resource competition between *Streptomyces* and *Fusarium* populations in the rhizosphere. *Microbiol. Ecol.* 74:157-167.
- Fajardo, A., and Martnez, J. L. 2008. Antibiotics as signals that trigger specific bacterial responses. *Curr. Opin. Microbiol.* 11:161-167.
- Fouly, H., and Pedersen, W. 1996. What root rotting fungi in the "Old" and "New" agricultural lands of Egypt. *Plant Dis.* 80:1298-1300.
- Fujisawa, Y., and Weisblum, B. 1981. A family of r-determinants in *Streptomyces* spp. that specifies inducible resistance to macrolide, lincosamide, and streptogramin type B antibiotics. *J. Bacteriol.* 146:621-631.
- Hansen, F. T., Gardiner, D. M., Lyse, E., Fuertes, P. R., Tudzynski, B., and Wiemann, P. 2015. An update to polyketide synthase and non-ribosomal synthetase genes and nomenclature in *Fusarium*. *Fungal Genet. Biol.* 75:20-29.
- King, B. C., Waxman, K. D., Nenni, N. V., Walker, L. P., Bergstrom, G. C., and Gibson, D. M. 2011. Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi. *Biotechnol. Biofuels* 4:4.
- Kinkel, L. L., Bakker, M. G., and Schlatter, D. C. 2011. A coevolutionary framework for managing disease-suppressive soils. *Annu. Rev. Phytopathol.* 49:47-67.
- Kinkel, L. L., Schlatter, D. C., Xiao, K., and Baines, A. D. 2014. Sympatric inhibition and niche differentiation suggest alternative coevolutionary trajectories among *Streptomyces*. *ISME J.* 8:249-256.
- LeBlanc, N., Essarioui, A., Kinkel, L. L., and Kistler, H. C. 2017. Phylogeny, plant species, and plant diversity influence carbon use phenotypes among *Fusarium* populations in the rhizosphere microbiome. *Phytopathology* 107:150-157.
- Li, L., Li, X.-Z., Tang, W.-Z., Zhao, J., and Qu, Y.-B. 2008. Screening of a fungus capable of powerful and selective delignification on wheat straw. *Lett. Appl. Microbiol.* 47:415-420.
- Lorang, J. M., Liu, D., Anderson, N. A., and Schottel, J. L. 1995. Identification of potato scab inducing and suppressive species of *Streptomyces*. *Phytopathology* 85:261-268.
- Mahadevan, B., and Crawford, D. L. 1997. Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. *Enzyme Microb. Technol.* 20:489-493.

- Mahmoudi, E., Tabatabaei, B. E. S., and Venturi, V. 2011. Virulence attenuation of *Pectobacterium carotovorum* using N-Acyl-homoserine lactone degrading bacteria isolated from potato rhizosphere. *Plant Pathol. J.* 27:242-248.
- Moya-Elizondo, E. A. 2013. *Fusarium* crown rot disease: Biology, interactions, management and function as a possible sensor of global climate change. *Cienc. Investig. Agrar.* 40:235-252.
- Otto-Hanson, L. K., Grabau, Z., Rosen, C., Salomon, C. E., and Kinkel, L. L. 2013. Pathogen variation and urea influence selection and success of *Streptomyces* mixtures in biocontrol. *Phytopathology* 103:34-42.
- Paulitz, T. C., Smiley, R. W., and Cook, R. J. 2002. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, U.S.A. *Can. J. Plant Pathol.* 24:416-428.
- Phillips, L., Wellington, E. M. H., and Rees, S. B. 1994. The distribution of antibiotic resistance patterns within streptomycetes and their use in secondary metabolite screening. *J. Ind. Microbiol.* 13:53-62.
- Poole, G. J., Smiley, R. W., Walker, C., Huggins, D., Rupp, R., and Abatzoglou, J. 2013. Effect of climate on the distribution of *Fusarium* spp. causing crown rot of wheat in the Pacific Northwest of the United States. *Phytopathology* 103:1130-1140.
- Roberti, R., Flori, P., Pisi, A., Brunelli, A., and Cesari, A. 2000. Evaluation of biological seed treatment of wheat for the control of seed-borne *Fusarium culmorum*. *JPD* 107:484-493.
- Samac, D. A., and Kinkel, L. L. 2001. Suppression of the root-lesion nematode (*Pratylenchus penetrans*) in alfalfa (*Medicago sativa*) by *Streptomyces* spp. *Plant Soil* 235:35-44.
- Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., and Pasquali, M. 2013. *Fusarium culmorum*: Causal agent of foot and root rot and head blight on wheat. *Mol. Plant Pathol.* 14:323-341.
- Schlatter, D., Fubuh, A., Xiao, K., Hernandez, D., Hobbie, S., and Kinkel, L. 2009. Resource amendments influence density and competitive phenotypes of *Streptomyces* in soil. *Microbiol. Ecol.* 57:413-420.
- Smiley, R., and Patterson, L.-M. 1996. Pathogenic fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Dis.* 80:944-949.
- Soares, A. C. F., Sousa, C. D. S., Garrido, M. D. S., and Perez, J. O. 2007. Production of streptomycete inoculum in sterilized rice. *Sci. Agric.* 64: 641-644.
- van Rij, E. T., Girard, G., Lugtenberg, B. J. J., and Bloemberg, G. V. 2005. Influence of fusaric acid on phenazine-1-carboxamide synthesis and gene expression of *Pseudomonas chlororaphis* strain PCL1391. *Microbiology* 151:2805-2814.
- Vanheule, A., Audenaert, K., Warris, S., van de Geest, H., Schijlen, E., and Höfte, M. 2016. Living apart together: Crosstalk between the core and supernumerary genomes in a fungal plant pathogen. *BMC Genomics* 17:670.
- Vaz Jauri, P., Bakker, M. G., Salomon, C. E., and Kinkel, L. L. 2013. Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil streptomycetes. *PLoS One* 8:e81064.
- Watve, M. G., Tickoo, R., Jog, M. M., and Bhole, B. D. 2001. How many antibiotics are produced by the genus *Streptomyces*? *Arch. Microbiol.* 176: 386-390.
- Wiggins, B. E., and Kinkel, L. L. 2005a. Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. *Plant Soil* 268:271-283.
- Wiggins, B. E., and Kinkel, L. L. 2005b. Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. *Phytopathology* 95:178-185.
- Wildermuth, G., Thomas, G., Radford, B., McNamara, R., and Kelly, A. 1997. Crown rot and common root rot in wheat grown under different tillage and stubble treatments in southern Queensland, Australia. *Soil Tillage Res.* 44: 211-224.
- Winter, M., Koopmann, B., Doell, K., Karlovsky, P., and von Tiedemann, A. 2013. Mechanisms regulating grain contamination with trichothecenes translocated from the stem base of wheat (*Triticum aestivum* L.) infected with *Fusarium culmorum*. *Phytopathology* 103:682-689.
- Winter, M., Samuels, P. L., Dong, Y., and Dill-Macky, R. 2018. Trichothecene production is detrimental to early root colonisation by *Fusarium culmorum* and *F. graminearum* in *Fusarium* crown and root rot of wheat. *Plant Pathol.* 68:185-195.