In planta reduction of maize seedling stalk lesions by the bacterial endophyte *Bacillus mojavensis*

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Abstract: Maize (*Zea mays* L.) is susceptible to infection by *Fusarium verticillioides* through autoinfection and alloinfection, resulting in diseases and contamination of maize kernels with the fumonisin mycotoxins. Attempts at controlling this fungus are currently being done with biocontrol agents such as bacteria, and this includes bacterial endophytes, such as *Bacillus mojavensis*. In addition to producing fumonisins, which are phytotoxic and mycotoxic, *F. verticillioides* also produces fusaric acid, which acts both as a phytotoxin and as an antibiotic. The question now is Can *B. mojavensis* reduce lesion development in maize during the alloinfection process, simulated by internode injection of the fungus? Mutant strains of *B. mojavensis* that tolerate fusaric acid were used in a growth room study to determine the development of stalk lesions, indicative of maize seedling blight, by co-inoculations with a wild-type strain of *F. verticillioides* and with non-fusaric acid producing mutants of *F. verticillioides*. Lesions were measured on 14-day-old maize stalks consisting of treatment groups inoculated with and without mutants and wild-type strains of bacteria and fungi. The results indicate that the fusaric-acid-tolerant *B. mojavensis* mutant reduced stalk lesions, suggesting an in planta role for this substance as an antibiotic. Further, lesion development occurred in maize infected with *F. verticillioides* mutants that do not produce fusaric acid, indicating a role for other phytotoxins, such as the fumonisins. Thus, additional pathological components should be examined before strains of *B. mojavensis* can be identified as being effective as a biocontrol agent, particularly for the control of seedling disease of maize.

Key words: Bacillus mojavensis, bacterial endophyte, biological control, *Fusarium verticillioides*, fumonisin, fusaric acid.

Résumé : Le maïs (*Zea mays* L.) est sujet à l'infection par *Fusarium verticillioides* à cause des phénomènes d'auto-infection et d'allo-infection, résultant en maladies et en la contamination des grains de maïs par les fumonisines, des mycotoxines. Des tentatives de contrôle de ce champignon à l'aide d'agents de contrôle biologique comme les bactéries, notamment les endophytes bactériens tel que *Bacillus mojavensis*, sont actuellement en cours. Cependant, le champignon produit aussi de l'acide fusarique qui agit tant comme phytotoxine qu'antibiotique, comme les fumonisines qui sont phytotoxiques et mycotoxiques. La question qui se pose maintenant est de savoir si *B. mojavensis* peut réduire le développement des lésions lors du processus d'allo-infection chez le maïs, simulé par l'injection du champignon dans l'entrenoeud du maïs. Des souches mutantes de *B. mojavensis* tolérantes à l'acide fusarique ont été utilisées dans une étude en chambre de croissance afin de mesurer le développement de lésions sur la tige du maïs, indicatives de la fusariose, à la suite de l'inoculation de la souche sauvage de *F. verticillioides* et d'un mutant ne produisant pas d'acide fusarique. Les lésions ont été mesurées sur des tiges de maïs de 14 jours chez les groupes inoculés ou non avec les souches mutantes ou sauvages de bactéries et de champignons. Les résultats indiquent que la souche mutante de *B. mojavensis* tolérante à l'acide fusarique réduisait les lésions de la tige, suggérant que cette substance ait agi comme antibiotique in planta. De plus, le développement des lésions survenait aussi chez le maïs infecté par des souches mutantes de *F. verticillioides* ne produisant pas d'acide fusarique, indiquant que d'autres phytotoxines comme les fumonisines aient joué un rôle. Ainsi, des composantes pathologiques additionnelles doivent être examinées avant que des souches de *B. mojavensis* puissent être identifiées comme agents de contrôle biologique efficaces, particulièrement pour le contrôle des maladies des semis de maïs.

Mots-clés : Bacillus mojavensis, endophyte bactérien, contrôle biologique, *Fusarium verticillioides*, fumonisine, acide fusarique.

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Introduction

Fusarium verticillioides (Saccardo) Nirenberg (Holomorph: *Gibberella moniliformis* Wineland; synonym *Fusarium moniliforme*) is a symptomless biotrophic endophyte during most of its association with maize (*Zea mays* L.), which was first suggested by Foley (1962) and substantiated by Munkvold et al. (1997) and Bacon et al. (2001). It was further established that this endophytic infection moved from plant to kernel (Kedera et al. 1994) and from kernel to plant

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(Bacon et al. 2001; Oren et al. 2003) and back to kernel (Bacon et al. 2001). We have also determined that owing to the endophytic state of *F. verticillioides*, there is autoinfection of maize (Bacon and Hinton 1996) as well as alloinfection, which is accomplished via the fungus entering wounds on roots or from insects entering maize ears and decaying maize silks (Munkvold et al. 1997; Sobek and Munkvold 1999). Under less than ideal culture conditions, the fungus becomes a pathogen, rapidly killing plants, particularly young seedlings. Fusaric acid (5-butylpicolinic acid) was discovered and implicated in the pathology of seedling blight by Yabuta et al. (1937), which was substantiated by Gaumann (1957), and in the wilt disease of other plants (Tamari and Kaji 1954; Heitefuss et al. 1960; Drysdale 1984). Fusaric acid is produced by *F. verticillioides* and other *Fusarium* species (Bacon et al. 1996).

Fusarium verticillioides also colonizes injured or senescing tissue as saprophytes (Bacon and Hinton 1996), producing fumonisin mycotoxins, particularly, in damaged ear and kernels (Munkvold et al. 1997). Fumonisins are a group of homologous mycotoxins produced by several *Fusarium* species, particularly *F. verticillioides*, and are associated with maize and maize debris (Magan and Olsen 2004). The fumonisins are responsible for toxicoses in livestock and poultry (Riley et al. 1993), associated with human esophageal cancer (Marasas et al. 1979), and currently suspected as risk factors for human neural tube defects (Waes et al. 2005).

Plant resistance and fungicides are two major approaches used to control diseases by *F. verticillioides* and its mycotoxins. However, an emerging approach designed to utilize biological control of the endophytic infection of *F. verticillioides* involves the use of several biocontrol bacteria, especially endophytic bacteria (Bacon et al. 2001; Lodewyckx et al. 2002; Cavaglieri et al. 2005), although there are compounding biotic and abiotic environmental factors that reduce the efficiency of such organisms. We are using the bacterial endophyte *Bacillus mojavensis* as a biocontrol agent for reducing the fumonisin content of maize but find that this bacterium is sensitive to fusaric acid that is produced by *Fusarium* species, such as *F. verticillioides.* Fusaric acid also functions as a bactericide (Schnider-Keel et al. 2000; Bacon et al. 2004), which has been suggested as a deterrent to the successful use of *B. mojavensis* and other biocontrol agents under field conditions (Bacon et al. 2004). However, *B. mojavensis* is not affected by the fumonisins (C.W. Bacon and D.M. Hinton, unpublished).

To test the utility of *B. mojavensis* for biocontrol purposes, it is important to know if toxins, such as fusaric acid or the fumonisins produced by *F. verticillioides*, are interactive with the biocontrol bacterium intended as a protectant for specific maize diseases. We produced mutants of the bacterium that are tolerant to fusaric acid but also show attributes for biocontrol of the parental strain (Bacon et al. 2007), and we isolated non-fusaric acid producing mutants of *F. verticillioides* to test for this toxin on this bacterium and its interaction with the pathology observed on maize seedlings. As an indicator of in planta interaction, the current objective was to develop an in vitro model to measure the ability of these bacterial mutants to reduce the lesions produced during alloinfection by non-fusaric acid producing mutants of *F. verticillioides* on seedlings of susceptible cultivars of sweet and field maize. Another objective was to indirectly determine the pathological contribution of *F. verticillioides* infection to the apparent seedling pathology observed in the absence of fusaric acid by the in planta colonization of maize by a non-fusaric acid producing strain of *F. verticillioides.*

Materials and methods

Bacteria

Nutrient agar (Difco, Detroit, Michigan) was used for routine laboratory maintenance of all bacteria. The bacteria used in this study included *B. mojavensis* RRC101, the patented endophytic strain (ATCC 55732) shown to confer disease protection and reduce the level of fumonisin mycotoxin in maize seedlings (Bacon et al. 2001). The rifampin mutant, RRC112rif, was derived from RRC101 as described earlier (Bacon et al. 2007). The *B. mojavensis* fusaric acid resistant mutant, RRC112fa, was developed from the RRC112rif mutant (aqueous suspension of 112rif cells (10^3 cfu·mL^{-1}) by following standard UV mutagenesis procedures, except the height of the UV source was 12 cm from the aqueous solution of bacterial spores, as this species demonstrated strong UV resistance when irradiated at the higher distances usually recommended. Following selected time periods, 1 mL of irradiated cells was plated onto potato–dextrose agar (PDA) medium amended with graded concentrations of fusaric acid (Sigma Chemical Co., St. Louis, Missouri); surviving colonies were removed and plated onto similar concentrations of fusaric acid, which was repeated over 12 serial transfers on the fusaric acid amended PDA medium to ensure stability of fusaric acid resistant colonies. These surviving colonies were selected, tested, and compared with its original parent RRC101 for inhibitory and endophytic and molecular genetic qualities (Bacon et al. 2006; Olubajo and Bacon 2008) with and without fusaric acid and labeled as being resistant to fusaric acid. In addition to these strains, 10 strains of *B. mojavensis* were used for comparisons with the mutants and biocontrol strains for their biocontrol potential for reducing *F. verticillioides* lesions produced in the sweet maize variety Early Sunglow. These strains, along with their desert origins, included NRRL B14699 (Mojave), B14701 (Mojave), B14702 (Mojave), B14703 (Gobi), B14704 (Gobi), B14705 (Gobi), B14706 (Gobi), B14708 (Gobi), B14710 (Gobi), and B14712 (Gobi).

Fungi

All fungi were maintained at 4 °C on silica gel and grown on PDA at 26 °C. The fungi included *F. verticillioides* RRC Patgus, a virulent strain to sweet and field maize; RRC408, a virulent wild-type strain isolated from diseased maize; UV28, a non-fusaric acid producer generated from RRC408; and MRC826, a symptomless endophytic strain that infects most field maize cultivars and is associated with livestock and poultry toxicities and with fumonisin production. The mutant UV28 was derived from RRC408 and was produced by exposing aqueous suspensions of conidia to UV light on PDA medium, and chemically screened for its inability to produce fusaric acid on ground autoclaved maize following prior procedures for analysis of this toxin (Bacon et al. 2004). This mutant was characterized as being identical to the wild type, RRC408, in all phenotypic traits measured, including the pro-

duction levels of the fumonisin mycotoxins on autoclaved maize with the exception that it produced trace quantities of fusaric acid (Bacon et al. 2004).

Maize

The maize cultivar used was Early Sunglow, a 60RM (maturing at approximately 60 days) sweet maize that is highly susceptible to *F. verticillioides* (Glenn et al. 2008) and is compatible with culture conditions in the greenhouse or plant growth room. This cultivar also was used for the seedling virulent test, since it developed lesions characteristic of stalk rot described for this fungus. The highly susceptible field hybrid, 3140, obtained from Pioneer Hi-Breed Internationals, Inc. (Des Moines, Iowa, USA), was also used in this study. However, MRC826 was a symptomless endophyte when cultured with this cultivar and others rated resistant under ideal plant growing conditions.

Maize culture and pathogenicity assay

Soil used for maize culture was a synthetic mix (Redi-Earth, pH 5.5–6.0), placed in 20 cm plastic pot, and these pots were sterilized for 30 min at 121 °C, which was followed by a second autoclaving for 20 min the following day. Maize seeds were surface and internally sterilized following prior procedures (Bacon et al. 1994). These sterilized seeds were subjected to our pathogenicity assay described earlier (Bacon et al. 1994; Bacon and Hinton 2007) using the following procedure. Seeds of each cultivar were inoculated with bacteria prior to planting with a 5 mL aqueous suspension of washed bacterial inoculum (10^6 cfu·mL^{-1}), prepared from 48 h nutrient broth cultures grown at 25 °C. This inoculum was placed over disinfected seeds, mixed to cover all seed, and air-dried (approximately 12 h under sterile ventilation). Control seeds of each cultivar were similarly sterilized and treated with sterile water, except bacteria were omitted. The bacterial inoculated sterilized and control seeds were planted in sterile soil, and the germinated seeds were thinned to 10 plants per pot. Inoculated and noninoculated control groups consisted of seeds with or without bacteria. All plants were grown for 35 days under aseptic conditions in a plant growth light room at 32 °C under a 16 h light (cool white, high output fluorescent tubes, an average of 254 μmol·m^{-2}·s^{-1}) and an 8 h dark regime at 29 °C. Plants were watered daily and fertilized as needed with a water-soluble fertilizer.

After 35 days, stalks of each treatment group were injected 5 cm above the soil surface with a sterile syringe containing 0.1 mL of an aqueous spore suspension (1×10^8 spores·mL^{-1}) of *F. verticillioides* Patgus or the non-fusaric acid producing mutant UV28. Each fungal inoculum was prepared from 10- to 14-day-old cultures of the fungi grown on PDA. Injected control groups consisted of the control inoculated plants as indicated above injected or not injected with water. All plants were covered with plastic bags, placed in a tray of distilled water to deliver approximately 90% relative humidity for 48 h. The bag was removed and the plants grown for an additional 7 days under standard growth room conditions. Lesion development was determined by a modified procedure of Hooker (1956), Drepper and Renfro (1990), and Jardine and Leslie (1999) using stalks that were cut longitudinally with a razor blade 1 cm above the inoculation puncture extending down through 1 or 2 internodes and measuring the length of necrotic lesions produced during the stalk inoculations. Lesion length was a measure of the zone of discoloration determined as the distance from either end of the necrotic zone. Both stalk diameters and lesion lengths were measured with an electronic digital recording caliper (0.02 mm accuracy).

Table 1. Percent reduction and lesion length in the field maize *Zea mays* 'Pioneer 3140' infected with and without *Bacillus mojavensis* strains and *Fusarium verticillioides* strains.

Fungal or bacterial treatment	Mean lesion length (cm)*	% Lesion reduction†
Control, water inoculated	0.00	—
B. mojavensis RRC112fa	0.00	—
F. verticillioides 408	0.86b	—
B. mojavensis RRC112fa + *F. verticillioides* 408	0.60a	30
F. verticillioides UV28	0.97b	—
B. mojavensis RRC112fa + *F. verticillioides* UV28	0.57a	41
F. verticillioides MRC826	0.93b	—
B. mojavensis RRC112fa + *F. verticillioides* MRC826	0.55a	41

*Values are the means of 12 plants, repeated twice. Means within the same column followed by the same letter are not significantly different from each other (Fisher's least significant difference test, $P \leq 0.05$).

†Percentage lesion reduction expressed within each maize cultivar obtained for each of the three fungal strains with and without the biocontrol bacterium.

Companion treatment groups of plants were simultaneously harvested, washed free of soil and separated into roots and stalks, and used to determine the effects of the bacterium on plant growth characteristics. The lengths of roots and shoots were measured; dry weights of the plants determined after drying at 60 °C, and the results of growth were reported as an average of eight or more measurements. The stalk diameter was measured 5 cm above the soil level, at the point from which disease should develop. The experimental design for all experiments was a randomized block design with four replications, and each experiment was conducted twice. The Fisher's least significant difference test was used to assess significant differences between pairs of means.

Results

The Early Sunglow sweet maize – *F. verticillioides* pathosystem used here is a convenient model to determine the role of fusaric acid in the interaction of a bacterial endophytic biocontrol agent with this fungal pathogen (Figs. 1 and 2). Lesions were produced by the fungus on the field maize, and the results were similar to or in some instances produced more pronounced necrotic lesions than those obtained in the sweet maize trial (Table 1 and Fig. 2). *Bacillus mojavensis* produced a 30%–41% reduction in lesion length among maize inoculated with the three strains of *F. verticillioides* tested. There was no correlation in the fusaric acid production and the mean lesion lengths. The non-fusaric acid producing mutant, *F. verticillioides* UV28, produced the longest and most intensely colored necrotic lesion (not shown) compared with those strains of fungi that produced fusaric acid. Further, the mean lesion lengths were statisti-

Fig. 1. Effect of *Bacillus mojavensis* 112fa on lesion production in 45-day-old stalk sections of *Zea mays* 'Early Sunglow'. (A) Control: water injected but noninfected with *Fusarium verticillioides* RRC Patgus; (B) infected with *F. verticillioides*, without *B. mojavensis*; and (C) infected with *F. verticillioides* and *B. mojavensis*. Arrows indicate necrotic lesions.

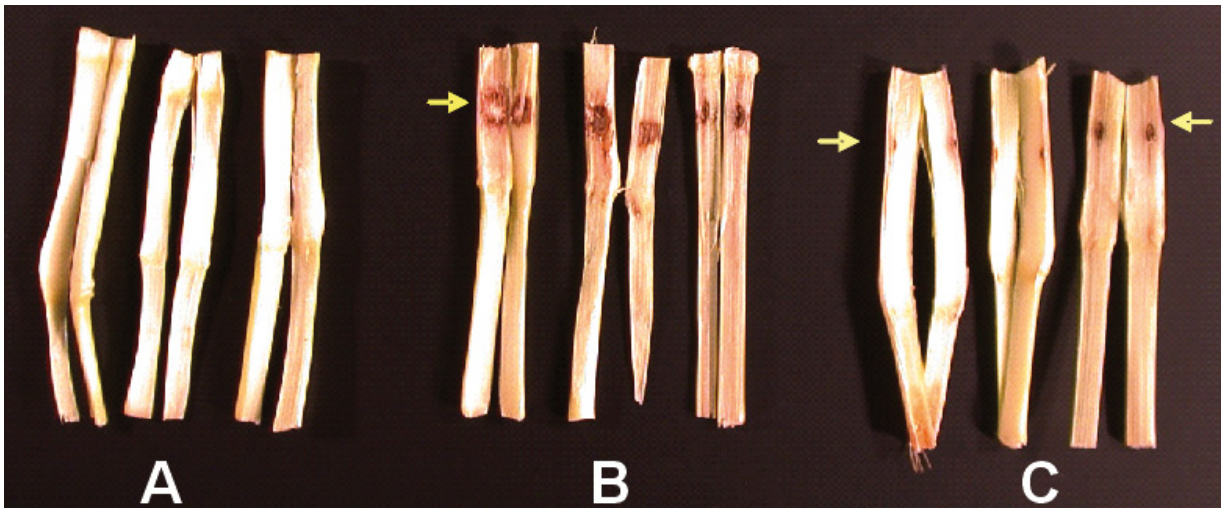
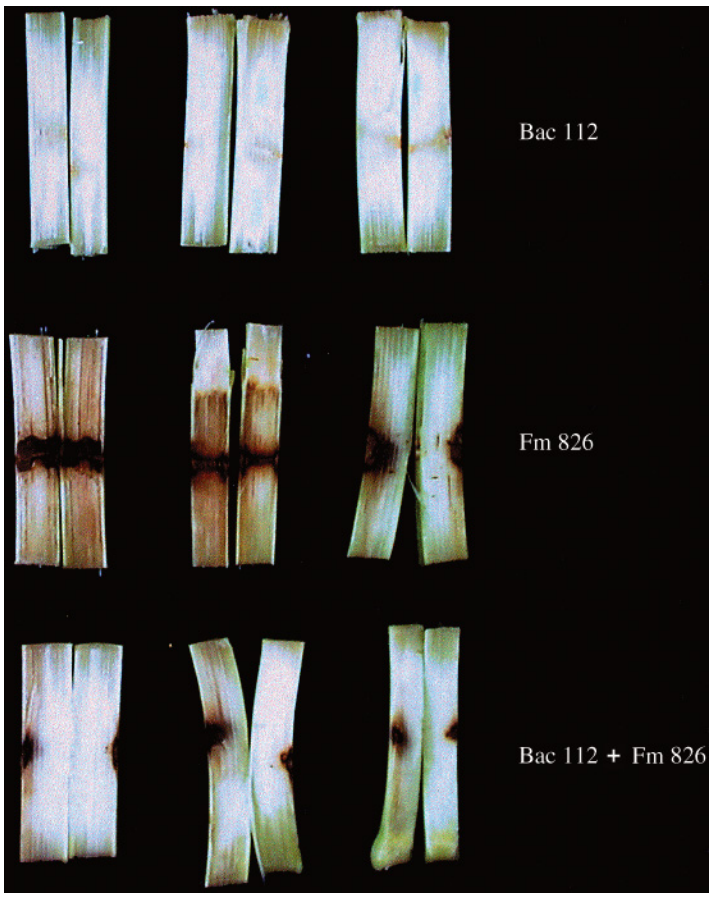


Fig. 2. Effect of *Bacillus mojavensis* 112fa on lesion production in 45-day-old stalk sections of the field maize *Zea mays* 'Pioneer 3140'. Stalks were infected with *B. mojavensis* 112fa, Bac 112; *Fusarium verticillioides*, Fm 826; and a mixed inoculum of *B. mojavensis* and *F. verticillioides* (Bac 112 + Fm 826).



cally similar to that of the wild-type strain but statistically different from that of the parental type RRC408 when this strain was co-inoculated with the bacterium (Table 1).

A test of 10 other strains of *B. mojavensis* showed extreme differences in their ability to reduce the lesions produced by the pathogenic isolate of *F. verticillioides* (Table 2). One

Table 2. Percentage lesion length reduction by strains of *Bacillus mojavensis* on 34-day-old *Zea mays* 'Early Sunglow' maize seedlings infected by *Fusarium verticillioides* Patgus.

Strain*	% Lesion reduction†	Origin
RRC101 (ATCC 55732)	58	Maize (Italy)
NRRL B14699	25	Mojave Desert
NRRL B14701	45	Mojave Desert
NRRL B14702	0	Mojave Desert
NRRL B14703	18	Gobi Desert
NRRL B14704	26	Gobi Desert
NRRL B14705	45	Gobi Desert
NRRL B14706	47	Gobi Desert
NRRL B14708	39	Gobi Desert
NRRL B14710	24	Gobi Desert
NRRL B14712	58	Gobi Desert

*RRC, Russell Research Center, USDA, ARS, Athens, Georgia; NRRL, Northern Regional Research Laboratory (National Center for Agricultural Utilization Research, USDA, ARS, Peoria, Illinois, USA).

†Expressed as a percentage of lesion length produced by *Fusarium verticillioides* Patgus without bacteria.

Table 3. Lesion length reduction in *Zea mays* 'Early Sunglow' maize infected with and without *Bacillus mojavensis* RRC112fa and two strains of *Fusarium verticillioides.*

	Mean lesion length (cm)†	
Fungal or bacterial treatment*	Experiment 1	Experiment 2
No fungus (control group)	0.00	0.00
B. mojavensis RRC112fa	0.00	0.00
F. verticillioides Patgus	1.25c	1.10c
F. verticillioides UV28	0.67b	1.16c
B. mojavensis RRC112fa + *F. verticillioides* Patgus	0.55b	0.77b
B. mojavensis RRC112fa + *F. verticillioides* UV28	0.35a	0.48a

**Bacillus mojavensis* RRC112fa, a fusaric acid resistant mutant; *F. verticillioides* UV28, a non-fusaric acid producing mutant; *F. verticillioides* Patgus, a pathogenic isolate.

†Values are the means of 10 plants, repeated twice. Means within the same column followed by the same letter are not significantly different from each (Fisher's least significant difference test, $P \leq 0.05$).

strain, NRRL B14702, was ineffective in reducing the lesion size, while another strain, NRRL B14712, was equally effective as the patented isolate RRC101.

In experiments 1 and 2, the average length of lesions (Fig. 1) produced by the virulent strain *F. verticillioides* Patgus on sweet maize with *B. mojavensis* RRC112fa was reduced significantly ($P = 0.05$) by 44% and 63%, respectively (Table 3). This represents a 54% reduction in lesion length over both experiments. In groups inoculated with the non-fusaric acid producing mutant (*F. verticillioides* UV28) there was a significant reduction in the first experiment but not in the second, but in both experiments when these mutant-inoculated groups were co-inoculated with the fusaric acid tolerant strain *B. mojavensis* RRC112fa, there were significant reductions observed in both experiments.

There was a significant reduction in lesion length by the bacterium regardless of its ability to tolerate fusaric acid

Fig. 3. The effects of the patented strain *Bacillus mojavensis* RRC101 (101), its rifampin mutant RRC112rif (112rif), and the fusaric acid tolerant mutant (112fa), on reduction of lesions caused by *Fusarium verticillioides* RRC Patgus (Patgus) on *Zea mays* 'Early Sunglow'. Values were computed to test the significant difference between the bacterial treatment groups and the control (non-bacterial group). Bars with different letters are significantly different ($P < 0.0001$).

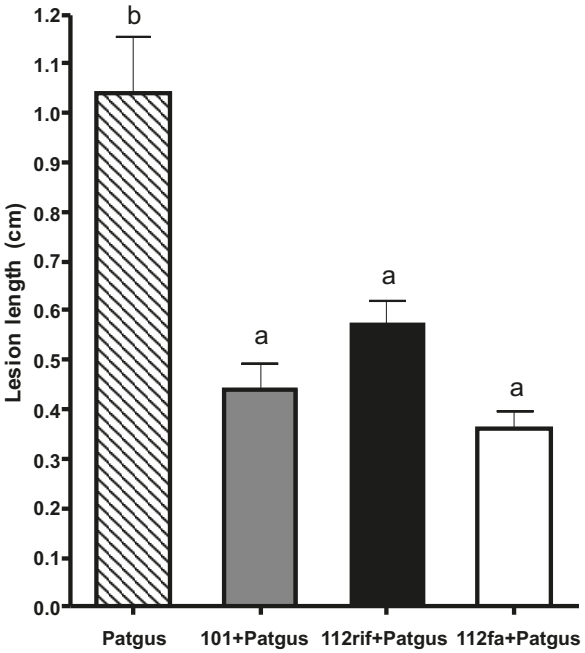


Table 4. Stalk diameters of 35-day-old plants of the sweet maize *Zea mays* 'Early Sunglow' inoculated with *Bacillus mojavensis* strains and *Fusarium verticillioides*.

Treatment	Stalk diameter (mm)*
Control plants, not inoculated	5.71a
F. verticillioides Patgus	6.03a
F. verticillioides UV28	5.27b
B. mojavensis RRC112fa	5.66a
B. mojavensis RRC112fa + *F. verticillioides* Patgus	5.77a
B. mojavensis RRC112fa + *F. verticillioides* UV28	5.93a

*Means within the same column followed by the same letter are not significantly different from each other according to Fisher's least significance difference test ($P \leq 0.05$).

(Fig. 3). The fusaric acid tolerant mutant, 112fa, and the parental types all reduced lesion length similarly when co-inoculated with *F. verticillioides* Patgus. The fusaric acid tolerant mutant reduced lesion length by approximately 70%, which however, was not significantly different from reduction of lesion length when the other strains of this bacterium were used to protect maize.

The effects of *B. mojavensis* RRC112rif on the growth rate of maize was significantly higher than that of the controls

(data not shown). Maize infected with strains of *F. verticillioides* varied in their effects on stalk diameter (Table 4). Strain *F. verticillioides* UV28 significantly reduced the diameter of maize stalks compared with the other treatment groups. However, when the bacterium was co-inoculated with this strain, there was no significant reduction in stalk diameter compared with control. All other plant parameters measured among the treatment groups were not significantly different (data not shown).

Discussion

In the experiments described here, conditions were established to be effective in inducing necrotic lesion development in maize. Seedling blight via alloinfection was reduced by the application of *B. mojavensis* using lesion size development as the criterion. The results of this study indicate that this endophytic bacterium has the potential to control *F. verticillioides*-induced seedling blight. This pathogen and other *Fusarium* species are common in fields routinely used for maize culture where infective propagules are contaminants of crop residue in soils. Lesions of this disease appeared more severe on the cultivar of field maize rated susceptible to fusaria ear rot than that on the sweet maize cultivar. However, since the same strain of fungi was not used for both cultivar tests, it is erroneous to make such a comparison, which was an objective of this work. There are apparent physiological and genetic differences in the susceptibilities of sweet maize cultivars and field maize cultivars to the fungus in planta and to the fumonisins in vitro (Williams et al. 2007; Glenn et al. 2008; Zitomer et al. 2010), and the observations in this work appear to support those data. The data do indicate that sweet and field cultivars of maize are susceptible to this pathogen, and the lesions produced are reduced by the endophytic bacterium, using our model system.

The role of fusaric acid in disease induction is not clear. Necrotic lesions in plants have been reported from the direct application of purified fusaric acid to plants. Fusaric acid and related compounds cause tomato plants to develop rapid chlorosis that develop into necrotic lesions (Barna and Györgyi 1992). Higher concentrations than those used in our work are needed for necrosis in tobacco leaves, appearing to affect older tobacco leaves rather than younger ones (Sarhan and Hegazi 1988). Other mechanisms may also operate along with the infection process, such as the production of cutinases that have been established as being required for necrosis by some species of this genus (Shaykh et al. 1977). While lesion development was observed in this work, general signs of wilt were not evident in any of the intact plants of the treatment groups during this brief observation period. Disease expression, e.g., wilt and death, might also require abiotic stresses, but such conditions were not a part of these experiments. However, these severe disease signs have been induced in maize infected with *F. verticillioides* (Bacon and Hinton 1996).

Yabuta et al. (1937) was the first to suggest that fusaric acid is a phytotoxin and inhibits the growth of rice seedlings, while Gaumann (1957) established that fusaric acid is a wilt toxin of tomato. Fusaric acid is produced by most *Fusarium* species (Bacon et al. 1996), and the involvement of this toxin in plant and animal diseases is very broad, ranging from a hypotensive effect in humans to a potentiator of specific classes of toxins and a weak mycotoxin (see reviews by Gaumann 1958; Wang and Ng 1999). Since *F. verticillioides* mutants devoid of fusaric acid were effective in producing disease symptoms characteristic of seedling blight, our work does not clearly identify a role for this acid as a maize phytotoxin. However, a role in the infection process, i.e., the dissolution of maize cell wall integrity thereby facilitating hyphal entry into host cells, cannot be dismissed. Similar conclusions have been reached by others (Gaumann 1958; Kuo and Scheffer 1964; Marrè and Vergani 1993). Quite possibly fusaric acid might serve the purpose of cellular lysis, providing entry of pathogens into plants (Tamari and Kaji 1954; Drysdale 1984; Wang and Ng 1999; Yates et al. 2003) and enabling induction of kernel rot. In the present study, the fungus was inoculated via a needle into maize stalks, simulating an alloinfection process, which in these experiments does not necessitate the need for hyphal infection and associated mechanisms such as fusaric acid production.

In earlier studies (Bacon et al. 2001, 2006), we established that this bacterium reduced the amount of the fumonisin mycotoxin produced by *F. verticillioides*, indicating reduced secondary metabolism by this fungus as well as reduced seedling blight in maize and wheat (Bacon et al. 2001; Bacon and Hinton 2007). The inhibitory effects of fusaric acid on biocontrol bacteria (Schnider-Keel et al. 2000; Bacon et al. 2004, 2006) emphasize its antibiotic action that can effectively prevent the growth of gram-negative and gram-positive bacteria in culture (Toyoda et al. 1988; Schnider-Keel et al. 2000; Landa et al. 2002; Bacon et al. 2006). In this current work, studies were performed in planta, but the results were not that clear. Lesion reduction was observed in maize previously inoculated with *B. mojavensis*. Further, our results indicated a range of lesion lengths for all the *F. verticillioides* strains, regardless of their ability to produce fusaric acid. Therefore, all strains of *F. verticillioides* should be considered potential pathogens of maize, which is a conclusion reached by others (Jardine and Leslie 1999; Glenn et al. 2008).

The simultaneous production of the fumonisins and their potential influence on lesion development should not be ignored, and it is suggested that their in planta production might be confounding the pathological observation under field conditions. The results from the non-fusaric acid producing mutants suggest the involvement of alternative toxins in this blight disease. The role of the fumonisins as phytotoxins was contradictory initially (Abbas and Boyette 1992), but current viewpoints have changed. There are correlations of the fumonisins with strains of the fungus and maize seedling blight (Desjardins et al. 1995; Glenn et al. 2008), and it has been demonstrated that purified fumonisin, B_1, produces necrosis in maize seedlings (Van Asch et al. 1992; Lamprecht et al. 1994; Williams et al. 2006; Zitomer et al. 2010). Further, the ability of this toxin to cause metabolic disruptions of ceramide biosynthesis in plants (Williams et al. 2007), and the correlation at the gene level with seedling blight pathology (Glenn et al. 2008), strongly support the conclusion that the fumonisins may in fact be the major toxins responsible for blight of maize and may account for the results observed in this study. Nevertheless, the conclusion reached by Jardine and Leslie (1999) was that under their

conditions, stalk rot and the ability to produce fumonisins were not correlated, although fumonisins might play a role in aggressiveness of strains. This suggests that both fungal strain genetics and other predisposing factors might account for the variation in disease expression. We feel that one abiotic factor used here, moisture, might account for some of the variation observed under field conditions.

This work identified another strain of *B. mojavensis* (NRRL B14712), an isolate from the Gobi Desert, as being just as effective as the patented strain RRC101 in reducing the lesion size caused by infection with *F. verticillioides*. This work also illustrates the broad differences in maize seedling protection by strains of this bacterium, for example, strain NRRL B14702 was not effective in reducing lesion development in maize seedlings. All strains used here have been shown to be endophytic (Bacon and Hinton 1996, 2007), but not all strains produce a diffusible inhibitor on nutrient agar that is antagonistic to *F. verticillioides* and other fungi. For example, strain NRRL B14712, which is demonstrated here as being just as effective in lesion reduction as the patented strain RRC101, is not antagonistic to *F. verticillioides* on plate tests. This strain when cultured on nutrient agar produced a diffusible zone of inhibition of <0.1 mm, whereas RRC101 produces a diffusible zone of inhibition of >18 mm, which was rated very antagonistic to *F. verticillioides* (Bacon et al. 2006). This suggests that biocontrol of *F. verticillioides* by each strain may not occur by the same process and that biocontrol mechanisms operating within strains of this endophytic species are varied and complex. Additional studies under controlled laboratory conditions along with field studies using mutants and wild-type strains of bacteria and non-fumonisin producing fungi should define the extent of biocontrol by *B. mojavensis* over *F. verticillioides* for complete suppression of at least seedling disease in maize. Further, the interrelation of fumonisin mycotoxin with the pathology in the absence of fusaric acid suggests that fumonisin mycotoxins are responsible for seedling and ear rot.

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