Investigating the dynamics of *Streptomyces* species in Wisconsin potato systems for enhancing common scab control

By

Bryan J. Webster

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The dissertation was approved by the following members of the Final Oral Examination Committee:

Dr. Amanda J. Gevens, Assistant Professor, Plant Pathology
Dr. Russell Groves, Associate Professor, Entomology
Dr. Shelley Jansky, Associate Professor, Horticulture
Dr. Ann MacGuidwin, Professor, Plant Pathology
Dr. Douglas Rouse, Professor, Plant Pathology

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Investigating the dynamics of *Streptomyces* species in Wisconsin potato systems for enhancing common scab control

Bryan J. Webster

Under the advisement of Dr. Amanda J. Gevens

At the University of Wisconsin-Madison

Potato common scab (CS), a disease caused by multiple Gram-positive, filamentous *Streptomyces* bacteria, can impact tuber marketability due to to superficial, erumpent and/or severely pitted lesions. Symptoms are caused by the thaxtomin A phytotoxin, a pathogenicity factor associated with the *txtAB* gene; virulence genes *nec1* and *tomA* may also be present. Characterization of *Streptomyces* spp. isolates from CS-infected tubers sampled from Northern and Central Wisconsin revealed 16S rRNA homology with commonly reported phytopathogenic species in addition to five less common ribotypes. I demonstrated significant correlations between ribotype and location, cultivar, species, and CS lesion phenotype. Virulence genes *nec1* and *tomA* may influence thaxtomin A production based on a significant correlation using linear regression analysis ($P = 0.0003$). In my work on virulence factors *in vivo*, virulent ribotypes of *S. scabies* can produce specific lesion phenotypes. Netted lesions were significantly associated with five strains of *S. scabies* with the *txtAB*+, *nec1*-, *tomA*- virulence genotype, which may provide insight for targeting gene loci through breeding and resistance efforts.

Greenhouse trials in 2013-2014 assessed the impact that seed type and initial seed inoculum levels had on plant health and daughter tubers. At harvest, approximately 20% of daughter tubers from asymptomatic seed pieces (both cut and whole) exhibited CS.

Disease incidence significantly increased across both experimental trials when seed had 5 and ≥ 50% CS and was whole. Daughter tubers originating from cut seed pieces, when compared to whole seed, showed a reducted in CS—possibly due to the reduction in CS-infested peridermal surface area.

During 2009 to 2014, I evaluated the effects of fumigation and at-plant treatments for control of CS on ‘Yukon Gold’ potatoes in Antigo, Wisconsin. Microbial-based biopesticides did not statistically limit CS severity in 2009-2012, with the exception of *Bacillus subtilis* (Serenade Soil®) in 2010. Biochemical biopesticides did not provide significant CS control except for elemental sulphur (TigerSul®) in 2011. My data indicated that the fumigants metam sodium and chloropicrin, and the fungicide Blocker® (PCNB) resulted in increased yield and quality with reduced CS severity.

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Chapter 1

Characterization of plant pathogenic *Streptomyces* species from potato tubers from multiple growing regions in Wisconsin

Abstract

Potato common scab (CS) is a disease caused by a complex of Gram-positive, filamentous bacteria belonging to the genus *Streptomyces*. Plant pathogenic strains of *Streptomyces* cause disease by producing thaxtomin A from the *txtAB* gene along with two additional virulence genes, *nec1* and *tomA*, which may be present. To date, ten *Streptomyces* species carrying the *txtAB* gene have been described from various regions in the U.S. The present study focused on characterizing potato-pathogenic Streptomycete isolates from potato growing regions during 2012-2013. Isolates from CS-infected tubers sampled from primary potato-growing regions in Northern and Central Wisconsin were analyzed for phenotypic attributes and phylogenetic analysis based on 16S rRNA genetic regions. In total, 110 isolates were recovered from CS-symptomatic tubers expressing all lesion types (russeted/cracked, raised, and pitted scab) from 7 potato cultivars from 7 Wisconsin counties. Seventy-two out of 76 isolates were determined pathogenic based on *txtAB*+ status and confirmed with radish seedling assays. The topological arrangement of the phylogenetic tree constructed with 16S rRNA sequences of this isolate collection reflected homology among the most commonly reported species, *S. scabiei*, *S. stelliscabiei, S. europascabiei*, and *S. bottropensis.* The collection revealed over nine pathogenic *Streptomyces* ribotypes isolated from scab lesions. My findings suggested a change in the profile of the soilborne pathogen populations from 2006 to 2012-2013. Further characterization may provide insight into the mechanism of population change or pathogenicity and virulence factor shifts in order to improve our understanding of the pathogen in the soil environment.

Introduction

Potato common scab (CS) is a disease caused by a complex of Gram-positive, filamentous bacterial species belonging to the genus *Streptomyces* (Loria *et al.*, 2006; Wanner, 2009). *Streptomyces scabies* (Thaxt.) Waksman and Henrici is the primary pathogenic species responsible for causing CS challenges for potato producers across the globe by reducing crop yield and quality of product (Abdel-Rahman *et al.*, 2012; Bouchek-Mechiche *et al.*, 2000; Hill & Lazarovits, 2005; Loria *et al.*, 1997; Loria *et al.*, 2006; Park *et al.*, 2003; St-Onge *et al.*, 2008; Tanaka, 2004; Wanner, 2004; Zhao *et al.*, 2008). Of the several hundred bacterial species belonging to this genus, only a small number are able to infect developing potato tubers and tap roots of radish, parsnip, carrot, and beet (Goyer & Beaulieu, 1997; Stevenson *et al.*, 2001).

During tuber initiation, stolon ends become infected by one or more pathogenic strains, which induce formation of CS lesions via a pathogen phytotoxin that disrupts developing surface layers as tubers grow and mature (Fernie & Willmitzer, 2001; Tyner *et al.*, 1997; Wanner, 2009). The pathogenic strain of *Streptomyces* and the potato cultivar, in concert, have been shown to determine CS lesion type: netted/russeted, raised/erumpent, or pitted scab (Dees *et al.*, 2012; Lambert & Loria, 1989; Loria *et al.*, 2006). Successful CS management strategies have utilized an integrated approach that includes cultivar resistance, management of water during tuberization, management of soil pH to ≤ 5.2, and pesticides. However, these individual and/or combined approaches often provide inconsistent CS control (Lambert *et al.*, 2006; Powelson *et al.*, 1993; Wanner, 2009; Wilson *et al.*, 2001).

Plant pathogenic strains of *Streptomyces* cause disease by producing a phytotoxin called thaxtomin A (King *et al.*, 1989; Lawrence *et al.*, 1990). The gene responsible for biosynthesis of this toxin has been identified as *txtAB*, which is located on a chromosome along with two additional virulence genes, *nec1* and *tomA*, that are not needed for pathogenicity and may be present individually or together (Cullen & Lees, 2007; Kers *et al.*, 2005; Loria *et al.*, 2006) (Fig. 1). This chromosomal cluster, or pathogenicity island (PAI), can be transferred from one pathogenic species to another non-pathogenic species (Loria *et al.*, 2006). To date, ten *Streptomyces* species carrying the *txtAB* gene for pathogenicity have been described from various potato producing regions in the U.S. (Bouchek-Mechiche *et al.*, 2000; Wanner, 2006), Europe (Bouchek-Mechiche *et al.*, 2000; Flores-Gonzalez *et al.*, 2008), Japan (Miyajima *et al.*, 1998), Korea (Park *et al.*, 2003; Song *et al.*, 2004), and Canada (Goyer *et al*., 1996).

Genotypic analyses were carried out on isolates of pathogenic *Streptomyces* species from regions across New England, North Carolina, Florida, Idaho, Colorado, and multiple states in the Midwest, including Wisconsin (Wanner, 2009). Approximately seven potato growing regions in Wisconsin were sampled, resulting in 55 isolations collected during 2004 to 2006, with most isolates originating from Waushara, Langlade, Portage, Oneida, and Marquette Counties. Results of Wanner's 2009 survey generated a baseline profile of species, which serves as a reference for future assessments of population character. Because growers have recognized an increase in CS control fluctuations between fields and years, a re-evaluation of the *Streptomyces* species portfolio across Wisconsin may enhance our understanding of the current pathogen and resulting disease dynamics. The objective of this study was to characterize pathogenic

Streptomyces strains by evaluating phyletic relatedness of 16S rRNA, screening for pathogenicity, and noting cultivar and location for each isolate originating from multiple potato production regions of Wisconsin.

Materials & Methods

Pathogen isolation and culture maintenance

Bacterial strains were isolated from CS-infected tubers sampled from primary potato-growing regions in Northern and Central Wisconsin. An aggregated, representative field sample of approximately 25-100 lb with CS symptoms was randomly selected from potato grading lines at harvest or dug from fields using a W-shaped sampling pattern. Grower cooperators and/or extension agents also submitted smaller samples from different locations that consisted of 25-50 lb of CS-infected tubers. A random group of 10-15 infected tubers were selected from each sample location for pathogen isolations. Lesions were arbitrarily marked and sampled according to methods derived from Wanner (2007). Tubers were gently washed with tap water to remove organic debris and allowed to dry. Small (2 mm^2) excisions were taken from lesion margins, surface sterilized in 1.5% hypochlorite-dH_2O solution, rinsed in a sterile water bath, and allowed to dry in a laminar flow hood. Each excision was sliced into 1- to 2-mm layers and placed onto water agar in 90-mm Petri plates and incubated in darkness at 28°C for 14-21 days. Cultures were not sealed with Parafilm in order to encourage a dry microclimate and induce colony growth and sporulation. Data on location, potato cultivar, date, and lesion type each isolate originated from were collected and associated with all individual excisions.

After 3-5 days, colonies displaying characteristic aerial mycelia of *Streptomyces* spp. (Shirling & Gottlieb, 1966) were transferred to sterile water agar and allowed to mature for 1 week. Sub-cultures were made to segregate *Streptomyces* from non-target bacterial or fungal colonies. Isolations with persistent contaminants were excised,

transferred to a microcentrifuge tube and submerged in a 60°C water bath for 15 min. The contents were cooled and homogenized with a sterile pestle and plated onto water agar from a 10^2, 10^3, and 10^4 series dilution of the homogenate for re-isolation of pure colonies (Fig. 2).

Pure colonies were allowed to mature until sporulation of aerial mycelia was visible macro- and microscopically, which occurred after approximately two weeks of incubation. A sterile bacterial hoop dipped in sterile water was used to transfer *Streptomyces* spores, using a bacterial streaking pattern, onto solid yeast malt extract (YME) agar in 90-mm Petri dishes and incubated under the same conditions listed above. After three weeks, 8-mm plugs were transferred to a 20% glycerol solution in microcentrifuge tubes for long-term storage at -20°C. Additional transfers were made to oatmeal agar (OMA) in 90-mm Petri dishes and incubated under the previously described conditions in preparation for radish seedling assays.

Morphological characterization

Streptomyces spp. cultures on solid water agar in 90-mm Petri plates were visualized with an Axio Scope.A1 (Zeiss Imaging, Oberkochen, Germany) compound microscope (400x) for spore chain morphology according to the key from the International *Streptomyces* Project and cataloged as flexuous, rectus, retinaculate spiral, or simple spiral (Shirling & Gottlieb, 1966) (Fig. 3). The appearance of mycelial growth was distinctive from the growth of other organisms by refracting light under phase contrast conditions (Hopwood, 1960). *Streptomyces* spp. cultures on solid YME agar in 90-mm Petri plates were used to determine the color of sporulating colonies and any agar discoloration or pigment.

Radish pathogenicity assay

Plant pathogenic *Streptomyces* strains produce thaxtomin A when cultured on OMA, unlike cultures on water or YME media (Flores-Gonzales *et al.,* 2008). Mature OMA cultures of *Streptomyces* spp. isolates were combined with three 'Cherry Belle' radish seeds *in vitro* to determine pathogenicity, as previously described (Dees *et al.*, 2013; Flores-Gonzales *et al.,* 2008). Radish seeds were sterilized in 1% hypochlorite solution for 1 min, subsequently rinsed in two sterile distilled water (SDW) baths, and allowed to dry in a laminar flow hood. Three sterilized seeds were sunken into agar medium in each OMA plate containing a 14- to 20-day-old *Streptomyces* spp. isolate. Seeded cultures were incubated at room temperature (22°C) near a 12-hour fluorescent light source for 8 days. Control plates included seeds that were submerged into OMA without *Streptomyces* present, OMA with a *txtAB*-positive *S. scabies* isolate (WI06-19D, courtesy of L. Wanner, USDA), and into OMA with a *txtAB*-negative non-pathogenic isolate (WI06-23B, courtesy of L. Wanner, USDA). Seedling evaluations determined if an isolate was putatively pathogenic by showing hypertrophic, stunted, and/or blackened necrosis of hypocotyls and/or cotyledons. Diseased hypocotyls were < 2 cm in length; non-germinated seeds were colonized with visible sporulation. Putatively non-pathogenic cultures and control plates contained healthy seedlings of > 4 cm in length with no disease symptoms.

Genomic DNA extraction

Strains of *Streptomyces* isolates were grown on YME at 29°C in darkness for 14 days until sporulation occurred. Cells of mycelial mats were scraped and transferred to PowerBead tubes using the PowerSoil® DNA isolation kit (Mo Bio Laboratories, Inc.,

Carlsbad, CA) according to manufacturer's instructions with some modifications. PowerBead tubes containing isolate cells were vortexed for 5 min at 2400, transferred to a water bath at 60°C for 5 min, and vortexed again for 5 min at 2400 to ensure sufficient lysis of cells. Extracted DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA) and placed in storage at -20°C for genetic screening.

txtAB pathogenicity screen

Bacterial isolates were subjected to PCR analysis using *txtAB*-specific primers for detection of the operon responsible for thaxtomin A synthesis, as previously described by Wanner (2006). The primer set TxtAB1/TxtAB2 (5'-CACGTACGCGCAGTTCAATG-3'; 5'-AGATGATGTAGGCGGGACTC-3') was purchased from Integrated DNA Technologies (Coralville, IA). The 20-µl PCR reactions contained 1.0 µl of primers, 10.0 µl of PCR Master Mix (Promega Corp., Madison, WI), 7.0 µl of nuclease-free (NF) H_2O, and 1.0 µl of template DNA that was adjusted to concentrations of 2-25 ng/µl. PCR reaction conditions included an initial denaturation of 3 min at 94°C followed by 30 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 46°C, extension for 35 sec at 72°C, and a final extension for 7 min at 72°C. PCR product was held at 4°C until amplicons of the PAI region were separated by electrophoresis on 1% TAE gel and visualized with GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and Bio-Rad imaging and Quantity One 1-D analysis software (Bio-Rad Laboratories, Hercules, CA). A known pathogenic strain of *S. scabies* (WI06-19D, courtesy of L. Wanner, USDA) containing the *txtAB* or PAI operon was used as a positive control and a *txtAB*-negative non-pathogenic isolate (WI06-23B, courtesy of L. Wanner, USDA) was used as a negative control for all PCR reactions.

Analysis of 16S rRNA

Isolate DNA was subjected to polymerase chain reaction using a 16S-1F/16S-1R primer set (5'-CATTCACGGAGAGTTTGATCC; AGAAAGGAGGTGATCCAGCC-3') developed by Takeuchi & Sawada (1996) and reaction conditions previously described by Wanner (2013) to detect the 16S rRNA gene. The PCR mix (20-µl) contained 1.0 µl of primers, 10.0 µl of PCR Master Mix (Promega Corp., Madison, WI), 7.0 µl of NF H_2O, and 1.0 µl of template DNA. The PCR cycle consisted of an initial denaturation of 3 min at 95°C followed by 40 cycles of denaturation for 20 sec at 95°C, annealing for 30 sec at 50°C, extension for 2 min at 72°C, and a final extension for 5 min at 72°C. PCR product was held at 4°C until amplicons of the 1.5 kbp 16S region were separated by electrophoresis on 1% TAE gel and visualized with GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and Bio-Rad imaging and Quantity One 1-D analysis software (Bio-Rad Laboratories, Hercules, CA). DNA from pathogenic *S. scabies* species (WI06-19D) was used for comparison (courtesy of L. Wanner, USDA). Pooled PCR product from the DNA of each isolate was purified using QIAquick PCR purification kit (Qiagen Science, Germantown, MD) and submitted for Sanger sequencing at the University of Wisconsin Biotechnology Center. Partial sequence fragments (~750 bp) were assembled using EMBOSS: The European Molecular Biology Open Software Suite (Rice *et al.*, 2000). Assemblages were compared with highly similar sequences within the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (Zhang *et al.*, 2000).

Analysis of intergenic sequences

Although non-conserved regions of the 16S gene are sufficient for species identification of most plant pathogenic *Streptomyces*, there is high sequence similarity between *S. scabies* and *S. europascabiei*. Amplification of the intergenic transcribed spacer (ITS) region of the 16S operon was accomplished using the ITS-R/ITS-L primer pair (5'-GTCAAGTCATCATGCCCCTT; AAACTTGGCCACAGATGCTC-3') (Flores-Gonzalez *et al.*, 2008; Song *et al.*, 2004). Amplicons were exposed to the restriction enzyme *Hpy99I* (New England Biolabs, Beverly, MA) for digestion according to methods described by Flores-Gonzalez *et al.* (2008) and Song *et al.* (2004). Type strains with the restriction site present (*Hpy99I*+) include *S. scabies*, while strains without (*Hpy99I*-) are indicative of *S. europascabiei*. Known strains of *S. scabies* ATCC49173 (D63862) and *S. europascabiei* CFBP4497 (AJ007423) were used for comparison (courtesy of L. Wanner, USDA). The digested amplicons were separated on 1.5% agarose gel by electrophoresis with TAE buffer and visualized using GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and imaging as previously described.

Phylogenetic analysis

Assemblages of 16S sequences were aligned using Clustal W and MUSCLE software prior to constructing evolutionary trees with Molecular Evolutionary Genetics Analysis (MEGA) version 6.6. Phylogenetic constructs were based on neighbor-joining algorithms for analysis of the 16S rRNA gene sequences (Saitou & Nei, 1987; Tamura *et al.*, 2013). The sum of branch length shown = 1.28. Bootstrap values > 70 are shown and were generated from 1,000 test replicates trees with the associated taxa (Felsenstein,

1985). Sequenced strains used in this analysis are included as associative references and are described (Table 1). Footnotes indicate additional isolates with high 16S sequence similarity to the relative *Streptomyces* species based on independent phylogenetic analyses (data not shown). Species determinates were statistically analyzed for frequency by location (county of origin) with a contingency table-based chi-square test for independence of the species x location variables using the Crosstabulation feature in StatGraphics statistical analysis software package (Warrenton, VA). A bar and mosaic chart were generated to illustrate the composition of *Streptomyces* species per sample location.

Results

Pathogen isolation and culture maintenance

In total, 110 isolates were recovered from CS-symptomatic tubers expressing all lesion types, including russeted/cracked, raised, and pitted scab, from seven susceptible or intermediately susceptible potato cultivars from seven Wisconsin counties (Table 2). Chi-square statistical analysis revealed that the frequency of species is correlated to a particular county at the 90% confidence level (P-value < 0.0001) (Table 4). Eight non-potato *Streptomyces* strains from Wisconsin were included for comparison. Waushara and Langlade counties have significant potato production acres and accounted for 40 and 28% of the isolates, respectively (Table 3; Fig. 4). The remaining potato isolates were from the following counties, Portage (n=15), Oneida (n=10), Dane (n=5), Rusk (n=5), and Vilas (n=2).

Morphological characterization

Distinctive colonies of *Streptomyces* were visible on water agar using a dissecting microscope at 100-200x during the transfer process to isolate strains. Aerial mycelia of single colonies were characterized in 115 of the isolates with compound microscopy at 400x and a morphological key by Shirling & Gottlieb (1966). Aerial mycelia with spiral or corkscrew (uniform diameter) morphology were observed on 77 (70%) of the strain cultures (Fig. 5A). Thirty-two (29%) strains displayed retinaculum aerial mycelia, characterized by spirals or loops with irregular diameter, open loops, and straight hyphae with hooked ends (Fig. 5C). Three (0.03%) strains had flexuous or wavy aerial mycelia and three (0.03%) had rectus, or straight, mycelia (Fig. 5B).

Strain cultures typically developed sporulation in one to two weeks under incubation conditions of 28°C and darkness. Initial sporulation appeared white on the majority of strain cultures and became gray at maturity (14 days). Macroscopically, cultures grown on solid YME were variable with patterns of gray and white or solid gray sporulation. Seven of the strains differed in sporulation color with brown/dark gray or white with an orange-pink color. Sporulation with brown/dark gray appearance produced a dark brown pigment that altered the agar medium. White-orange sporulation produced an orange-pink pigment that altered the agar medium.

Radish pathogenicity assay

Radish seeds incorporated into *Streptomyces* cultures on OMA germinated after 3-4 days unless colonized by the culture strain, which inhibited healthy germination and would halt growth once the seed coat opened and the hypocotyl was visible. White sporulation that was characteristic of *Streptomyces* was observed on seed coats and was isolated onto water agar to confirm identity. Radish seedling hypocotyls that emerged from *txtAB*-positive strains became necrotic with black or brown tissue discoloration of hypocotyl and cotyledon, were stunted with a hypocotyl length of < 2 cm, and/or showed symptoms of hypertrophic or deformed growth (Fig. 6C). Healthy seedlings grown on solid water agar (Fig. 6A) and the *txtAB*-negative control strain WI06-23B (Fig. 6B) germinated with hypocotyl lengths of > 3-4 cm. Cotyledons were green and of normal growth on healthy seedlings. Strains with putative pathogenicity from radish seed assays were confirmed with *txtAB*-specific primers for detection of the PAI operon. Some strains lost viability in culture prior to being assayed with the radish seed procedure, but had genomic DNA extracted and were subjected to a *txtAB* primer-based pathogenicity screen

(Table 2). Of the 76 isolates screened, 71 were found to be pathogenic based on the assay previously described. Five of the strains screened had no apparent effect on hypocotyl emergence, elongation, and cotyledon development and were considered putatively non-pathogenic.

Genomic DNA extraction

Isolate DNA concentrations were assessed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). Isolate DNA with concentrations of < 2 ng/µl were loaded into an electrophoresis gel to assess quality. DNA extractions were repeated, if necessary. Concentrations > 50 ng/µl were diluted (50:50) with NF H_2O prior to PCR analyses.

txtAB pathogenicity screen

Of the 60 isolates screened with TxtAB1/TxtAB2 primers, four were negative for the *txtAB* gene, confirming the negative results from the radish seedling assay. The remaining 56 isolates screened with TxtAB1/TxtAB2 primers show gene fragments of approximately 385 bp that align with *S. scabies* 87-22 (Fig. 7). The non-pathogenic control isolate, WI06-23B, does not contain the PAI for biosynthesis of thaxtomin A and did not amplify gene fragments in this PCR analysis.

Analysis of 16S rRNA

Based on standard methods of determining *Streptomyces* species by sequencing the 1,530-nucleotide 16S region to compare variable regions that distinguish pathogenic species (Fig. 8), genetic screens of the 16S rRNA were carried out using PCR analysis (Fig. 9A). Five prime sequence regions were merged with the reverse compliment of the 3’ to create FASTA assemblies. Homologous sequence hits with a query cover of > 98%

were observed on 90% of the BLAST-analyzed isolate sequences. Highly similar sequence hits were observed between *S. scabies* and *S. europascabiei* and between *S. bottropensis* and *S. stelliscabiei*. Additional groupings of homologous sequences were observed in other BLAST results, which included: *S. ryensis, S. achromogenes*, and *S. rishiriensis*; and with *S. griseoruber* and *S. mirabilis*. Eleven of the isolate sequences were declared as unknown species if poor sequencing resulted in query matches of < 80% or did not produce any matches. Homologous sequence matches with published accession numbers were recorded and used for phylogenetic analysis (Table 1).

Analysis of intergenic sequences

The 16S region of *S. scabies* and *S. europascabiei* are nearly identical and require PCR analysis of the ITS region for species determination (Flores-Gonzalez *et al.*, 2008). Fifty-two (44%) isolate sequences had BLAST query matches with *S. scabies, S. europascabiei,* or both (Table 2). PCR amplification patterns from ITS-R/ITS-L primers confirmed ribosomal amplicons were in sufficient concentrations for restriction. Of the 52 isolates screened with the digestion enzyme, 46 were restricted (*Hpy99I* +) due to presence of the RFLP in the ITS region and classified as *S. scabies* (Fig. 9B). The remaining six isolates did not reveal fragmented amplicons (*Hpy99I*-) and were classified as *S. europascabiei*.

Phylogenetic analysis

Comparisons of ~1500 nt sequences of isolates produced sequence relatedness to type strains *S. scabies* (ATCC49173), *S. bottropensis* (ATCC25435), *S. stelliscabiei* (WI06-15D), *S. ryensis* (NBRC13834)/*S. achromogenes* (S22)/*S. rishiriensis* (GYB5), and *S. glauciniger* (D501) (Fig. 10). Fifty-two isolates are clustered with type strain *S.*

scabies (ATCC49173) (represented by 12-29H in Fig. 10). Fourteen isolate sequences are also clustered with *S. bottropensis* (ATCC25435) in the same subclade at a supported bootstrap value of 87% at the internal spacer shared with *S. europascabiei* (CFBP4497). Isolate 13-28H shares a phyletic line with *S. ossamyceticus* (NBRC13983). Representative isolate 12-17H and 15 additional isolates have 16S rDNA sequence relatedness to *S. stelliscabiei* (WI06-15D), and share its subclade with 12-32H and 13-612R. Possible descendants (12-20H and 13-609R) of this subclade have separated into two additional external nodes, or tips, at a 74% similarity. Eleven isolates (represented by 13-14H) have been clustered with a subclade that includes the closely related *S. ryensis* (NBRC13834), *S. achromogenes* (S22), and *S. rishiriensis* (GYB5). The isolate sequence of 13-4H is clustered with *S. griseoruber* (ICSSB1013). Sequences from isolates originating from radish are clustered with *S. recifensis* (ST100) with an internal node bootstrap value of 99%. The 16S rDNA of isolates 12-512R and 12-508R share an 89% similarity with *S. glauciniger* (D501) and share a phyletic line with 13-12H. Isolate 12-60H is included in this subclade and, together, shows 100% dissociation from other phyletic lines. 16S sequences of 12-528P and 12-103A are clustered with *S. aureus* (3184) with 100% similarity compared to other subclades.

Discussion

While *Streptomyces scabies* remains the primary dominant pathogenic species causing potato common scab, newly characterized and closely-related species are being identified as additional causal agents (Bouchek-Mechiche *et al.*, 2000; Dees *et al.*, 2012; Flores-Gonzalez *et al.*, 2008; Goyer *et al*., 1996; Miyajima *et al.*, 1998; Park *et al.*, 2003; Powelson *et al*., 1993; Song *et al.*, 2004; Wanner, 2006; 2009). In addition to the fluctuating microclimatic conditions in the soil biome, the composition and diversity of pathogenic species could be a significant factor in explaining why CS pressure often exhibits spatio-temporal variability (Wanner, 2009). With additional publications on *Streptomyces* populations and species characterization, we can further understand what factors are involved with disease development (Bouchek-Mechiche *et al.*, 1998; Flores-Gonzalez *et al.*, 2008; Kreuze *et al.*, 1999; Wanner, 2009). The present study focused on characterizing potato-pathogenic Streptomycete isolates from Wisconsin potato growing regions during 2012-2013. The locations sampled had baseline pathogen population data previously collected during 2004-2006 by Wanner *et al*. (2009).

Streptomycete isolates collected from Wisconsin during 2012-2013 reflect homogenous taxa with the most commonly reported species *S. scabiei*, *S. europascabiei*, and *S. bottropensis* (Manfio *et al.*, 2003; Wanner, 2009), based on the phylogenetic tree I constructed with 16S rRNA sequences. A notable subclade occupied by the ribotypes *S. achromogenes* subsp. *tomaymyceticus*, *S. ryensis,* and *S. rishiriensis* is comprised of 10 isolates from the present collection that show high genetic variation (99%) and is not positioned near the more commonly associated pathogenic species, which was also determined in the past by Wanner (2009). The repeated identification of this subclade

could imply that newly identified ribotypes are being reported as a result of increased sequencing or that newly pathogenic ribotypes are being discovered. The ribotype data also indicated a 99% genetically variable subclade of two isolates, originating from radish scab lesions, that showed 16S rRNA relatedness to *S. recifensis*, a species with no previous pathogenic description on potato (Sokolova *et al.*, 2004).

Streptomyces scabies was the prevalent species comprising more than 40% of my WI isolates, which concurs with previous work by Wanner (2009). Morphological evaluations revealed that of my 45 putative *S. scabies* isolates, 10 (22%) exhibited aerial mycelia that differed from the expected spiral morphology. This indicates that these isolates could belong to a closely related species to *S. scabies* with a nearly identical 16S sequence, as has been shown (Bukhalid *et al.*, 2002; Miyajima *et al.*, 1998). The second most predominant species, *Streptomyces stelliscabiei* (17%), was present in smaller sample groups from Dane and Rusk counties, suggesting that there could be a significantly higher presence of this ribotype in Wisconsin that may be further recognized with more intensive sampling. Because symptomatic potato samples were selected at random from grading lines or fields, the variety of species within a particular field location may give some indication of field-specific ribotype diversity, if present, as described by several other researchers (Flores-Gonzalez *et al.*, 2008; Loria *et al.*, 2006; Wanner, 2009). Sample sizes from Waushara and Langlade counties were larger than those from other locations. As a result, we see the widest range of *txtAB+* species from Waushara County, where the highest frequency (n=19) of isolates that included > 5 lesser-known ribotypes occured in addition to *S. scabies.* The presence of heterogenous ribotypes within different taxanomic clades and subclades from this single sampling

location may suggest that transference of the PAI from pathogenic to non-pathogenic *Streptomyces* has occurred between 2006 and 2012-2013.

I chose to characterize both pathogenic and non-pathogenic Streptomycete isolates to further identify species present in the tubersphere and in CS lesions. Of the four non-pathogenic isolates that were *txtAB*-negative, three did not have 16S rRNA sequence similarity to the four most related pathogenic ribotypes: *S. scabies, S. stelliscabiei, S. europascabiei,* and *S. bottropensis.* However, the remaining *txtAB*-negative strain was found to differ from type strains of *S. scabies* in morphology and in substrate pigmentation that is nearly identical to the characterization of *S. glauciniger* first described by Huang *et al.* (2004). In previous work, *S. glauciniger* expressed antimicrobial activity to other bacterial genera, but Huang *et al.* (2004) did not screen for the thaxtomin A-producing biosynthetic gene, *txtAB,* to determine pathogenicity. Interestingly, a more recent report shows that *S. glauciniger* has the potential for being a biocontrol agent against plant pathogenic fungi (Awad *et al.*, 2014).

The conserved 16S rRNA gene has been accepted as a standard means to differentiate genetic sequences among the members of this *Actinomycete* group (Dees *et al*., 2013; Flores-Gonzalez *et al*., 2008; Loria *et al.*, 2006; Wanner, 2009). But as research scientists attempt to better delineate between closely related strains, perhaps additional methods should become the standard and might include analysis of ribosomal ITS regions (Park & Kilbane, 2006), which is necessary for distinguishing between *S. scabies* and *S. europascabiei* (Dees *et al*., 2013; Flores-Gonzalez *et al*., 2008; Wanner, 2009).

The present collection reveals over nine pathogenic *Streptomyces* ribotypes isolated from scab lesions originating from over six Wisconsin counties. Approximately

22% of the isolates are of less-common ribotypes compared to a previous Wisconsin survey (Wanner, 2009), which suggests a change in the profile/species composition of Streptomycetes causing CS on potato tubers from similar sampling locations between 2006 and 2012-2013. Further characterization is needed in order to provide insight into the mechanisms of population change or PAI shifts to non-pathogenic *Streptomyces* species. Recognizing pathogenicity determinants, genetic variants, and population traits will improve our understanding of the pathogen in the soil environment. This approach may be our best course of action while comparative genomic studies seek to enhance our grasp of the host genetics responsible for resistance (Dees *et al.*, 2013). By combining these two areas where current knowledge gaps exist, we can advance the historically inconsistent management options for controlling potato CS.

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Table 1. GenBank accession numbers for *Streptomyces* species used in phylogenetic analyses.

Strain	Accession number
S. achromogenes S22	HQ850411
S. acidiscabies ATCC49003	AB026220
S. aureus 3184	EF371429
S. bottropensis ATCC25435	D63868
S. coralus CFCC3136	FJ883746
S. echinatus ISP5013	NR114823
S. europascabiei CFBP4497	AJ007423
S. glauciniger D501	KF317954
S. griseoruber ICSSB1013	AY094585
S. lannensis TA4-8	NR113181
S. mirabilis ATCC27447	AF112180
S. ossamyceticus NBRC13983	NR041156
S. recifensis st100	EU216596
S. reticuliscabiei CFBP4531	AJ007428
S. rishiriensis GYB5	JQ342915
S. ryensis NBRC13834	AB184517
S. scabies ATCC49173	D63862
S. stelliscabiei WI-6-15D	JF546730
S. turgidiscabies ATCC700248	AB026221

Table 2. Characterization of *Streptomyces* isolates from Wisconsin potato fields collected during 2012-2013. Species other than *S. scabies* and *S. europascabiei* were not analyzed with *Hpy99I*. Nt = isolate was not tested for pathogenicity screens.

Location (County)	Host (cultivar, if known)	Isolate	Colony Appearance	Aerial Mycelia Morphology	Phylogenetic Relatedness	ITS Analysis: *Hpy99I*	Radish Assay	*txtAB*
Waushara	carrot	12-car13	gray + white	spiral	*S. scabies*	n/a	nt	nt
Waupaca	potato (Innovator)	12-504R	gray + white	retinaculum	unknown	n/a	nt	nt
		12-508R	gray/brown (p)*	retinaculum	*S. glauciniger*	n/a	-	-
		12-512R	gray/brown (p)*	retinaculum	*S. glauciniger*	n/a	nt	nt
		12-14H	gray + white	spiral	*S. scabies*	+	+	+
Waushara	potato (Snowden)	12-17H	gray	spiral	*S. stelliscabiei*	n/a	+	+
		12-18H	gray	spiral	*S. stelliscabiei*	n/a	+	+
		12-20H	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		12-24H	white	spiral	*S. lannensis*	n/a	nt	nt
		12-25H	white	spiral	*S. lannensis*	n/a	+	+
		12-28H	gray + white	retinaculum	*S. bottropensis*	n/a	+	+
		12-29H	gray + white	spiral	*S. scabies*	+	nt	nt
		12-30H	gray + white	retinaculum	*S. bottropensis*	n/a	+	+
		12-32H	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		12-33H	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		12-34H	dk. gray + white	retinaculum	*S.ryensis/ achromogenes/ rishiriensis*	n/a	+	+
		12-35H	gray + white	spiral	*S. scabies*	+	+	+
		12-36H	gray	retinaculum	*S. scabies*	+	+	+
		12-38H	gray + white	retinaculum	*S. scabies*	+	nt	nt
		12-40H	gray + white	spiral	*S. stelliscabiei*	n/a	nt	nt
		12-41H	white	spiral	*S. scabies*	+	nt	nt
		12-42H	gray + white	flexuous	*S. stelliscabiei*	n/a	+	+
		12-44H	gray + white	retinaculum	*S. scabies*	+	+	+
		12-51H	gray + white	retinaculum	*S. scabies*	+	+	+
		12-60H	dk. gray (t)	spiral	*S. glauciniger*	n/a	+	+
Langlade	potato (Yukon	12-102A	gray + white	retinaculum	*S. stelliscabiei*	n/a	+	+
		12-103A	white (p)	spiral	*S. aureus*	n/a	+	+

	Gold)	12-104A	gray + white	retinaculum	*S. scabies*	+	+	+
		12-105A	gray	retinaculum	*S. scabies*	+	nt	nt
		12-110A	gray + white	retinaculum	*S. scabies*	+	+	+
		12-111A	gray + white	spiral	*S. scabies*	+	+	+
		12-112A	gray + white	retinaculum	*S. scabies*	+	+	+
		12-116A	gray + white	spiral	*S. scabies*	+	+	+
		12-118A	gray + white	spiral	*S. scabies*	+	+	+
		12-119A	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		12-121A	gray + white	spiral	*S. stelliscabiei*	n/a	nt	nt
		12-123A	gray + white	spiral	*S. scabies*	+	nt	nt
		12-124A	white	spiral	*S. scabies*	+	+	+
		12-125A	gray + white	spiral	*S. stelliscabiei*	n/a	nt	nt
		12-128A	dk. gray + white	retinaculum	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt
		12-129A	gray + white	retinaculum	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt
		12-132A	gray	spiral	*S. scabies*	+	+	+
		12-135A	gray + white	spiral	*S. scabies*	+	nt	nt
		12-136A	gray + white	spiral	*S. scabies*	+	nt	nt
		12-137A	gray	spiral	*S. europascabiei*	-	nt	nt
		12-140A	gray + white	retinaculum	*S. europascabiei*	-	+	+
		12-141A	brown (p)	unknown	unknown	n/a	nt	nt
		12-142A	gray + white	spiral	*S. europascabiei*	-	nt	nt
		12-145A	gray	spiral	*S. stelliscabiei*	n/a	nt	nt
Portage	potato (Norkotah)	12-523P	gray	spiral	*S. europascabiei*	-	nt	nt
		12-524P	gray + white	retinaculum	*S. bottropensis*	n/a	nt	nt
		12-525P	white (p)	flexuous	*S. scabies*	+	nt	nt
		12-526P	gray	spiral	*S. europascabiei*	-	nt	nt
		12-527P	white (p)	flexuous	*S. scabies*	+	nt	nt
		12-528P	dk. gray	spiral	*S. aureus*	n/a	nt	nt
Dane	beet	12-2A	unknown	spiral	*S. scabies*	+	nt	nt
		12-1B	unknown	spiral	*S. stelliscabiei*	n/a	nt	nt
		12-3B	unknown	spiral	*S. scabies*	+	nt	nt

		12-3C	unknown	spiral	*S. scabies*	+	nt	nt
		12-3D	unknown	spiral	*S. scabies*	+	nt	nt
Dane	radish	12-2N	unknown	unknown	*S. recifensis*	n/a	nt	nt
		12-2R	unknown	unknown	*S. recifensis*	n/a	nt	nt
Waushara	potato (Norkotah)	13-1H	gray	spiral	*S. scabies*	+	+	+
		13-2H	gray + white	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	+	+
		13-3H	white	spiral	unknown	n/a	+	+
		13-4H	unknown	unknown	*S. griseoruber*	n/a	nt	+
		13-5H	gray	spirals	*S. scabies*	+	+	+
		13-7H	gray + white	rectus	*S. ryensis/ achromogenes/ rishiriensis*	n/a	+	+
		13-9H	white	spiral	unknown	n/a	+	nt
		13-12H	dk. brown (p)	spiral	*S. glauciniger*	n/a	+	+
		13-14H	white	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt
		13-15H	gray + white	spiral	*S. scabies*	+	+	+
		13-20H	gray + white	spiral	*S. scabies*	+	-	-
		13-22H	gray	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	+	+
		13-24H	gray	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt
		13-28H	gray	spiral	*S. ossamyceticus*	n/a	nt	nt
		13-29H	gray	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt
		13-30H	white	spiral	unknown	n/a	-	-
		13-33H	white	spiral	unknown	n/a	-	nt
		13-34H	gray	spiral	*S. ryensis/ achromogenes/ rishiriensis*	n/a	nt	nt

		13-36H	gray	spiral	unknown	n/a	-	-
		13-55H	gray + white	spiral	*S. scabies*	+	nt	nt
		13-56H	gray + white	spiral	*S. scabies*	+	+	nt
		13-57H	gray	spiral	*S. scabies*	+	+	nt
		13-59H	gray	spiral	*S. scabies*	+	+	nt
Langlade	potato (Yukon Gold)	13-108A	white	spiral	*S. scabies*	+	nt	nt
Portage	potato (Norkotah)	13-252P	gray	spiral	*S. scabies*	+	+	+
		13-254P	white	retinaculum	*S. bottropensis*	n/a	+	+
		13-255P	gray + white	retinaculum	*S. bottropensis*	n/a	+	+
		13-257P	gray + white	retinaculum	*S. bottropensis*	n/a	+	nt
		13-258P	gray + white	retinaculum	*S. scabies*	+	+	nt
		13-259P	gray + white	retinaculum	*S. scabies*	+	+	+
		13-260P	gray + white	spiral	*S. scabies*	+	+	+
		13-262P	gray + white	spiral	*S. scabies*	+	+	+
		13-275P	gray + white	retinaculum	*S. bottropensis*	n/a	+	+
		13-277P	gray + white	spiral	*S. scabies*	+	+	+
		13-279P	gray + white	retinaculum	*S. bottropensis*	n/a	+	+
		13-281P	white	spiral	*S. scabies*	+	nt	nt
		13-282P	gray + white	spiral	*S. scabies*	+	+	+
		13-284P	gray + white	retinaculum	*S. bottropensis*	n/a	+	nt
		13-285P	gray + white	retinaculum	unknown	n/a	+	nt
Rusk	potato (Marcy)	13-301B	gray + white	spiral	*S. scabies*	+	+	nt
		13-302B	gray + white	spiral	unknown	n/a	+	nt
		13-303B	gray + white	spiral	*S. bottropensis*	n/a	+	nt
		13-305B	gray + white	spiral	*S. europascabiei*	-	+	+
		13-309B	gray + white	retinaculum	*S. bottropensis*	n/a	+	nt
Vilas	potato (Dk. Red Chieftan)	13-401E	gray + white	rectus	unknown	n/a	+	+
		13-402E	white	rectus	unknown	n/a	+	nt
Oneida	potato (Snowden)	13-603R	gray + white	spiral	*S. stelliscabiei*	n/a	+	nt
		13-604R	gray + white	spiral	*S. stelliscabiei*	n/a	+	nt

		13-609R	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		13-612R	gray	spiral	*S. stelliscabiei*	n/a	+	+
		13-613R	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		13-627R	gray + white	spiral	*S. stelliscabiei*	n/a	+	+
		13-629R	gray	spiral	*S. scabies*	+	+	nt
		13-630R	white	retinaculum	*S. bottropensis*	n/a	+	nt
		13-631R	gray + white	retinaculum	*S. bottropensis*	n/a	+	nt
		13-632R	gray	retinaculum	*S. bottropensis*	n/a	+	nt

Table 3. Contingency table of frequency of *Streptomyces* isolates by county. This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	S. scabies	Misc. spp.*	*S. stelliscabiei*	*S. bottropensis*	*S. europascabiei*	Row Total
Dane	4**	0	1	0	0	5
	3.6%	0.0%	0.9%	0.0%	0.0%	4.6%
	80.0%	0.0%	20.0%	0.0%	0.0%	
Langlade	15	5	5	1	5	31
	13.6%	4.6%	4.6%	0.9%	4.6%	28.2%
	48.4%	16.1%	16.1%	3.2%	16.1%	
Oneida	1	0	6	3	0	10
	0.9%	0.0%	5.6%	2.7%	0.0%	9.1%
	10.0%	0.0%	60.0%	30.0%	0.0%	
Portage	8	2	0	5	0	15
	7.3%	1.8%	0.0%	4.6%	0.0%	13.6%
	53.3%	13.3%	0.0%	33.3%	0.0%	
Rusk	1	1	0	2	1	5
	0.9%	0.9%	0.0%	1.8%	0.9%	4.6%
	20.0%	20.0%	0.0%	40.0%	20.0%	
Waushara	16	19	7	2	0	44
	14.6%	17.3%	6.4%	1.8%	0.0%	40.0%
	36.4%	43.2%	15.9%	4.6%	0.0%	
Column Total	45	27	19	13	6	110
	40.9%	24.6%	17.3%	11.8%	5.5%	100%

* Miscellaneous species (n < 5) include: *S. aureus, S. europascabiei, S. glauciniger, S. griseoruber, S. lannensis, S. ryensis, S. turgidiscabies, and undetermined species.*

** For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row.

Table 4. Chi-square test for independence between row and column categories for Table 3. Because the P-value is < 0.05, the observed frequency of species from a particular county is related at the 95% confidence level.

Test	***Statistic***	***Df***	***P-value***
Chi-square	60.659	20	< 0.0001

Figure 1. Genome containing the pathogenicity island (PAI) of *Streptomyces* spp. showing a conserved *txtAB* within the toxicogenic region and additional virulence genes upstream of the conserved core. Adapted from "Detection of common scab-causing *Streptomyces* in potato tubers and soil" by Wanner, L. A. (2013).

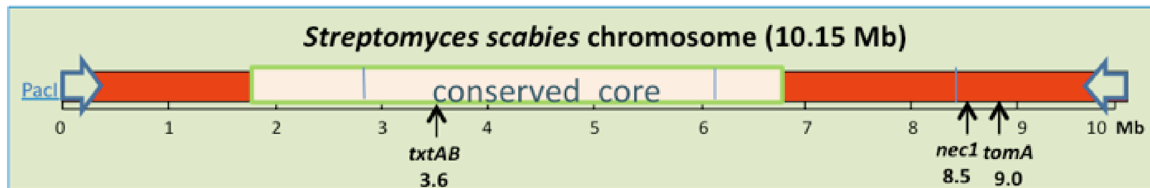


Figure 2. *Streptomyces* spp. on solid water agar in 90-mm Petri plate with serial dilutions (10^2, 10^3, and 10^4) per quadrant for pure colony selection. Culture incubation was at 28°C for 3-4 days in darkness.

Figure 3. Differentiation of aerial mycelia and spore morphology of Streptomycetes. Adapted from "Methods for Characterization of *Streptomyces* species," by E.B. Shirling and D. Gottlieb. 1966. International Journal of Systematic Bacteriology. Vol. 16:313-340.

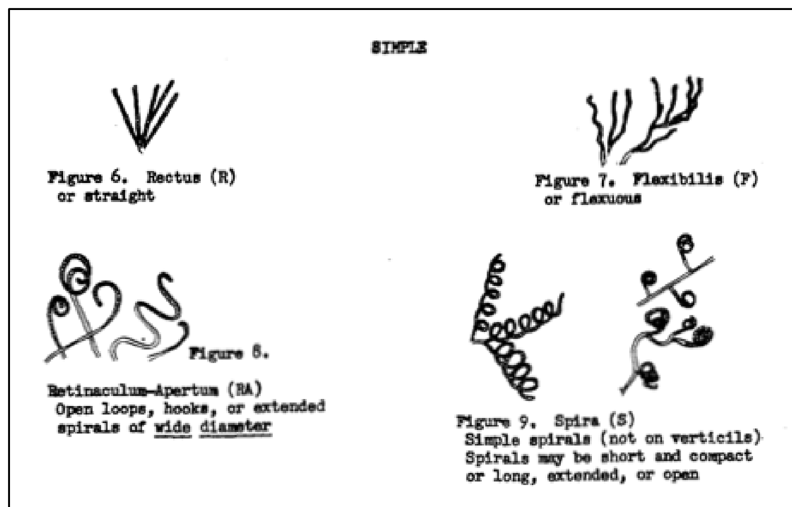
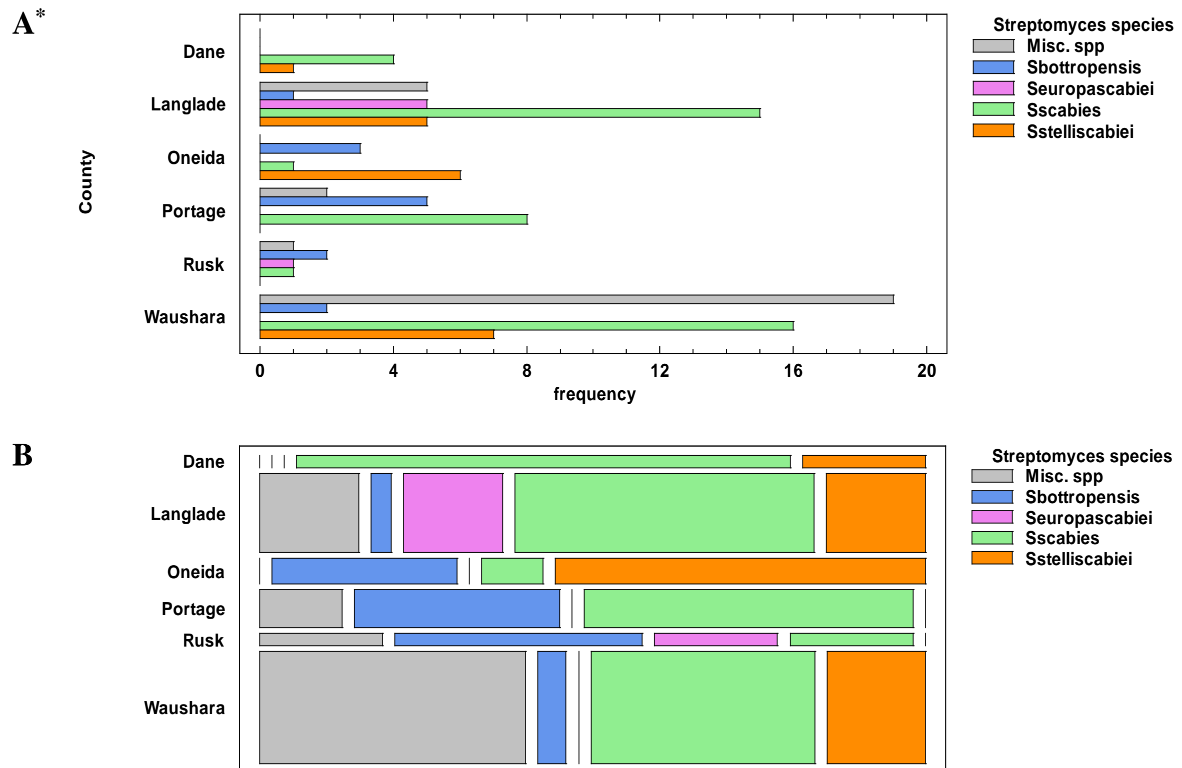


Figure 4. Bar (A) and mosaic (B) graphs depicting the composition of *Streptomyces* species isolated from common scab lesions on potato tubers sampled from six Wisconsin counties. The 'miscellaneous' species category included less common or unknown strains with putative pathogenicity.



* Charts A and B were generated from the Crosstabulation procedure using StatGraphics statistical analysis software, and were based on a two-way table showing frequency of occurrence of species by county.

Figure 5. Micrographs of mature aerial mycelia (7-14 d) of *Streptomyces* strains isolated from potato common scab lesions and grown on water agar. (A) Simple spiral morphology of aerial mycelia has uniform diameter of loops at 200x. (B) Straight, or rectus, aerial mycelia at 100x. (C) Retinaculate-spiral aerial mycelia have an irregular loop diameter, hooks or extended spirals at 100x. (D) Differentiation of conidial segments along spiral spore chain at 400x.

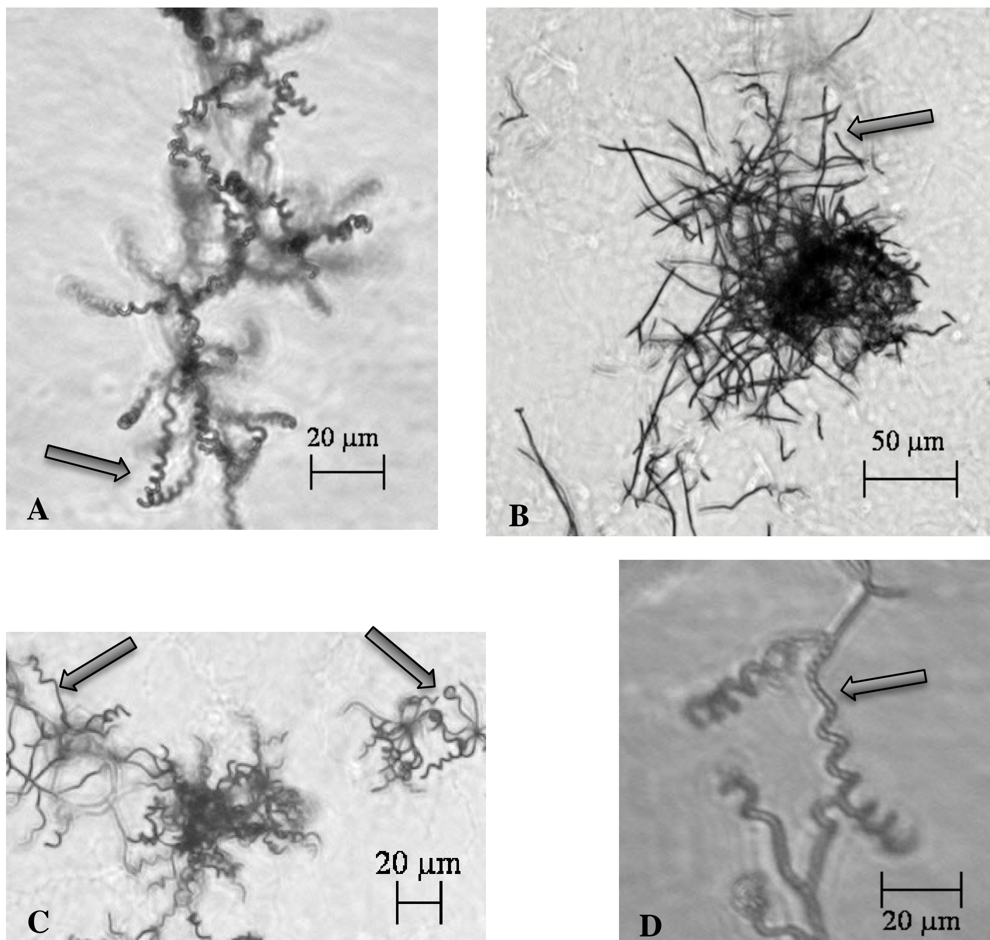
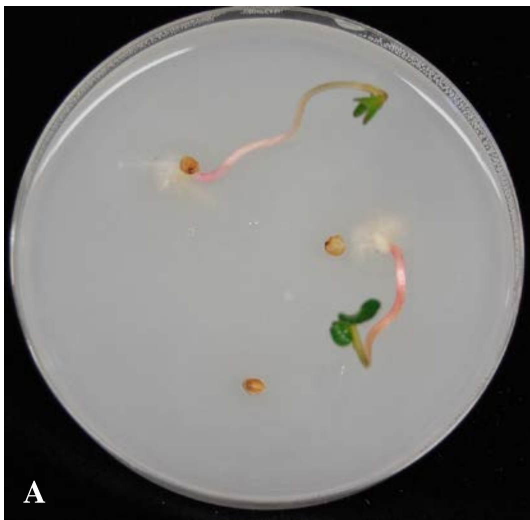


Figure 6. Radish seedling assay with *txtAB*+ isolates grown on oatmeal agar (OMA) to confirm pathogenicity, as described by Flores-Gonzalez *et al*. (2008). (A) Normal, healthy growth of control radish hypocotyls (2 of 3 germinated) on OMA in the absence of Streptomycetes. (B) Radish seedling growth in response to a non-pathogenic (*txtAB*-) isolate WI06-23B (courtesy of L. Wanner, USDA). (C) Stunted and necrotic growth of radish seedlings in the presence of a *txtAB*+ determinate strain of *Streptomyces stelliscabiei*.



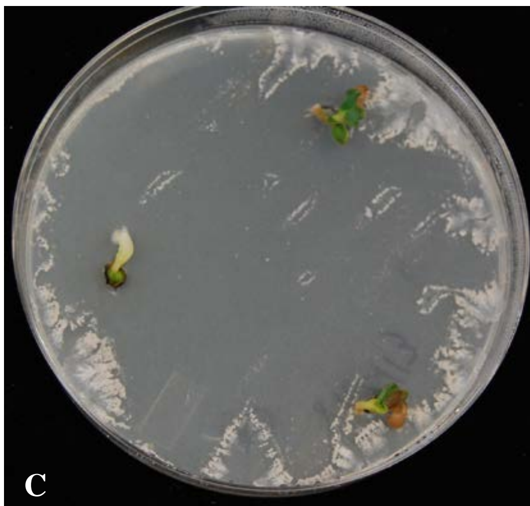
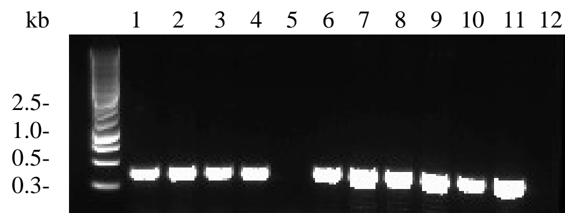


Figure 7. Electrophoresis PCR patterns from *Streptomyces* isolates screened for *txtAB* amplicon (~385 bp) and visualized on 1% Tris/acetic acid/EDTA (TAE) gel and imaged with Quantity One 1-D analysis software. A representative group of unknown isolates showing amplification of *txtAB* genes with TxtAB1/TxtAB2 primers. (A) Lane 1, highly virulent *txtAB*-positive *S. scabies* 87-22; lanes 2-4 and 6-11, *txtAB*-positive unknown *Streptomyces* strains; lane 5, *txtAB*-negative unknown *Streptomyces* strain; lane 12, DNA blank. (B) Lane 1, known *txtAB*-negative *S. scabies* WI06-23B (courtesy of L. Wanner, USDA); lanes 2 and 5, *txtAB*-negative unknown *Streptomyces* strain; lanes 3, 4, 6-11, *txtAB*-positive unknown *Streptomyces* strains; lane 12, DNA blank.

A



B

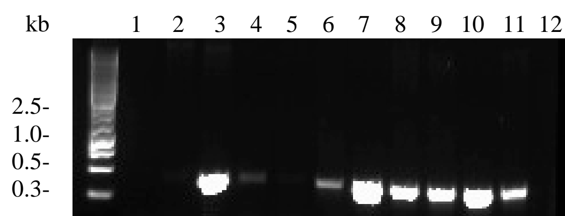


Figure 8. Taxonomically specific regions of the bacterial 16S ribosomal DNA are designated by the red boxes, which mark sequence variability between *Streptomyces* species. Adapted from "Detection of common scab-causing *Streptomyces* in potato tubers and soil" by Wanner, L.A. (2013).

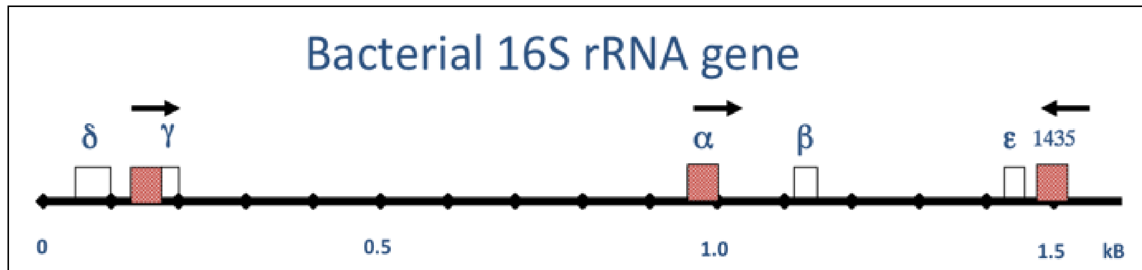
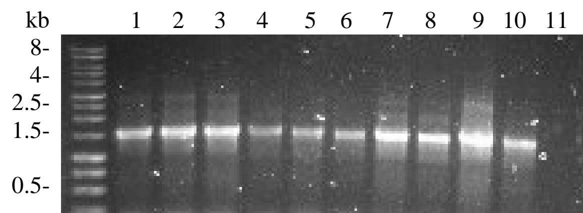


Figure 9. Electrophoresis PCR patterns from *Streptomyces* isolates and genetic screens on 1% Tris/acetic acid/EDTA (TAE) gel and imaged with Quantity One 1-D analysis software. (A) Amplicons (1.5 kb) of 16S rRNA sequence target region. Lane 1, known *S. scabies* isolate 87-22; lanes 2-10, unknown *Streptomyces* strains isolated from scab lesions on various tuber samples collected from Wisconsin fields; lane 11, DNA blank. (B) Intergenic transcribed spacer (ITS) regions of the 16S-23S operon (~700 bp) cut with *Hpy99I* restriction enzyme if restriction target region is present (note: additional *Hpy99I* target regions may exist among *S. scabies* to produce multiple restriction fragments) (Flores-Gonzalez *et al.*, 2008). Lane 1, *S. scabies* (87-22) is *Hpy99I*+ and contains amplicon fragments ≤ 700 bp; lane 2, *S. europascabiei* (WI06-13D.2) is *Hpy99I*- due to absence of restriction site. Lanes 3-7, putative *S. scabies* isolates based on ITS + *Hpy99I.* Lane 8, putative *S. europascabiei* isolate based on ITS + *Hpy99I.*

A



B

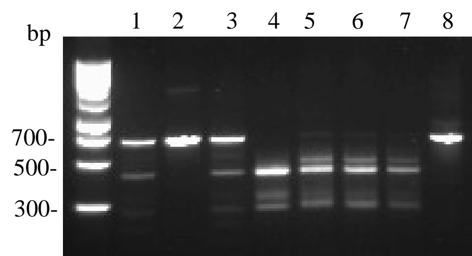
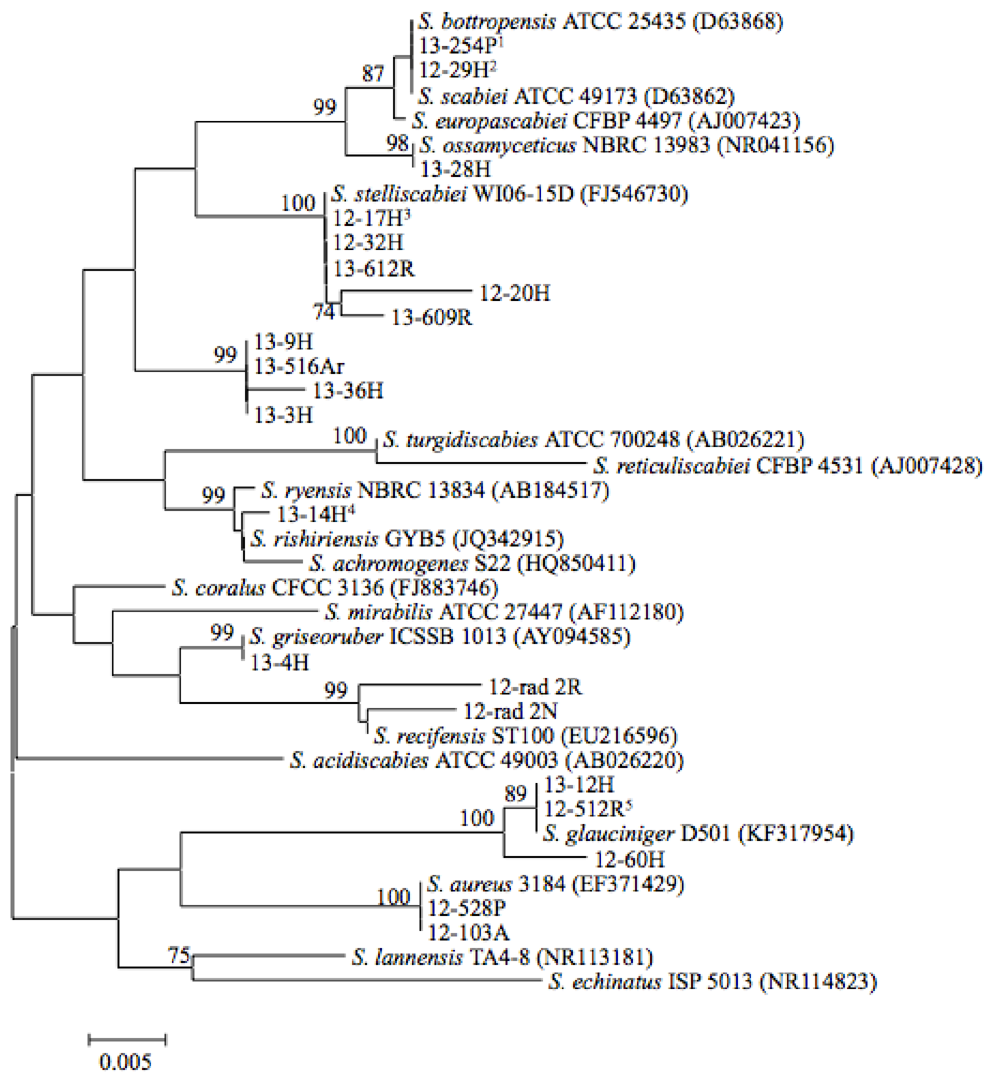


Figure 10. Phylogenetic relatedness between *Streptomyces* isolates based on neighbor-joining analysis of the 16S rRNA gene sequences (Saitou & Nei, 1987). The sum of branch length shown = 1.28. Bootstrap values > 70 are shown and were generated from 1,000 test replicate trees with the associated taxa (Felsenstein, 1985). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).



[1] *S. bottropensis* ATCC25435 (D63868): 12-28H, 12-30H, 12-524P, 13-254P, 13-257P, 13-275P, 13-279P, 13-284P, 13-303B, 13-309B, 13-630R, 13-631R, 13-632R.
[2] *S. scabiei* ATCC49173 (D63862): 12-29H, 12-car13, 12-35H, 12-36H, 12-38H, 12-41H, 12-44H, 12-51H, 12-104A, 12-105A, 12-110A, 12-111A, 12-112A, 12-116A, 12-118A, 12-123A, 12-124A, 12-132A, 12-135A, 12-136A, 12-137A, 12-140A, 12-142A, 12-523P, 12-525P, 12-526P, 12-527P, 12-beet 2A, 12-beet 3B, 12-beet 3C, 12-beet 3D, 13-1H, 13-5H, 13-20H, 13-55H, 13-56H, 13-57H, 13-59H, 13-108A, 13-252P, 13-258P, 13-259P, 13-260P, 13-262P, 13-277P, 13-281P, 13-282P, 13-301B, 13-305B, 13-401E, 13-629R.
[3] *S. stelliscabiei* WI06-15D (FJ546730): 12-beet 1B, 12-17H, 12-18H, 12-20H, 12-32H, 12-33H, 12-40H, 12-102A, 12-119A, 12-121A, 12-125A, 12-145A, 13-603R, 13-604R, 13-613R.
[4] *S. achromogenes* S22 (HQ850411)/*S. rishiriensis* GYB5 (JQ342915)/*S. ryensis* NBRC13834 (AB184517): 12-34H, 12-128A, 12-129A, 13-2H, 13-7H, 13-14H, 13-22H, 13-24H, 13-29H, 13-34H.
[5] *S. glauciniger* D501 (KF317954): 12-512R, 12-508R.

Chapter 2

Investigating correlations between pathogenicity and virulence factors of potato common scab-causing Streptomycetes and lesion phenotype

Abstract

Potato common scab (CS) is a disease caused by a complex of Gram-positive, filamentous *Streptomyces* bacteria. Of these, *S. scabies* has been the most predominant and recognized causal agent producing CS symptoms that are described as netted, raised, and pitted scab. Plant pathogenic strains of *Streptomyces* cause disease by producing thaxtomin A from the *txtAB* gene along with two additional virulence genes, *nec1* and *tomA*, which may be present. I characterized Streptomycetes from select commercial production regions of Wisconsin and determined correlations between *Streptomyces* ribotypes/strains and location, cultivar, species, and CS lesion phenotype. The pattern of virulence genes *nec1* and *tomA* among my Wisconsin isolates suggested that their presence may be influencing the role of thaxtomin A in producing disease phenotypes. Ribotypes or strains that were pathogenic (*txtAB*+) but were *nec1*- and *tomA*- produced low thaxtomin A (0.04 to 0.65 µg/g) compared to those that were *txtAB*+, *nec1*+, *tomA*+ (0.23 to 9.81 µg/g). Assigned virulence values based on the genotypic virulence of each strain revealed a significant correlation between quantity of thaxtomin A and virulence values using linear regression analysis (P-value=0.003). *In vivo*, virulence factor profiles of *S. scabies* isolates produced specific categorical disease phenotypes. The netted lesion type was significantly associated with five strains of *S. scabies* expressing the *txtAB*+, *nec1*-, *tomA*- virulence genotype. Further assessment of the differences in disease phenotypes from inoculations with other ribotypes that lack the *nec1* and *tomA* genes is warranted.

Introduction

Potato common scab (CS) is a disease caused by a complex of Gram-positive, filamentous *Streptomyces* bacteria. Of these species, *Streptomyces scabies* (Thaxt.) Waksman and Henrici has been the most predominant and widely recognized causal agent producing common scab symptoms that affect potato tuber quality and grade across the globe, especially in fresh market production, due to superficial and pitted lesions (Abdel-Rahman *et al.*, 2012; Bouchek-Mechiche *et al.*, 2000b; Hill & Lazarovits, 2005; Loria *et al.*, 1997; Loria *et al.*, 2006; Park *et al.*, 2003; St-Onge *et al.*, 2008; Tanaka, 2004; Wanner, 2004; Zhao *et al.*, 2008). The primary pathogenicity factor of CS-causing *Streptomyces* has been described as thaxtomin A, a phytotoxin that is synthesized by the gene *txtAB* (Healy *et al.*, 2000; Loria *et al.*, 2008). Without the production of thaxtomin A, scab does not occur on susceptible potato varieties (Goyer *et al.*, 1998; King *et al.*, 1991). Plant pathogenic Streptomycetes are saprophytic soilborne bacteria with complex pathogenicity strategies that are becoming better understood with enhanced genetic information. Molecular tools and analyses are providing insight into the transferrable pathogenicity island (PAI), which contains the *txtAB* gene, from pathogenic to previously non-pathogenic Streptomycetes as well as elucidating additional virulence genes for causing phenotypic disease symptoms (Lehtonen *et al.*, 2004; Loria *et al.*, 2006).

CS lesion phenotypes have been categorically described as superficial or netted lesions, raised or erumpent lesions, and pitted scab (Loria *et al.*, 1997). Multiple lesion phenotypes can be present on individual tubers, and can coalesce to create severe malformations. Pitted scab symptoms may range from small (< 5 mm) diameter pits to large gouges that leave up to 30% of the tuber disfigured or hollowed out, creating an

entry point for soilborne rot pathogens (Locci, 1994). Severe quality losses occur in potato when CS causes cracked peridermal lesions that are severely erumpent or deeply pitted (Delhaut & Stevenson, 2009). Upon infection by pathogenic species during tuber initiation, the thaxtomin A phytotoxin inhibits the biosynthesis of cellulose in developing tuber cells, resulting in malformations of the expanding phellem layers that cause the characteristic lesions (Bischoff, *et al.*, 2009; Khatri *et al.*, 2011; King *et al.*, 1989; King & Calhoun, 2009; Loria *et al.*, 2006).

In addition to thaxtomin A, two other genes have been identified as putative contributors to CS virulence and may or may not be present in a particular strain of pathogenic *Streptomyces*. The *nec1* gene was discovered by Bukhalid & Loria (1998) and adds to disease severity by inducing plant tissue necrosis of tubers, stolons, and roots of potato (Loria, 2003). Following the discovery of *nec1*, sequencing in the colonization region of the genome led to the finding of *tomA*, a gene homolog responsible for synthesizing a tomatinase enzyme that has been identified in phytopathogenic fungi and targets the antimicrobial glycoalkaloid tomatin as a possible way to disarm host response to infection (Kers *et al.*, 2005; Seipke & Loria, 2008). While *nec1* and *tomA*, together or individually, are not essential for disease, their presence with *txtA* in the PAI likely determine virulence or strain aggressiveness (Fig. 1). The PAI can be exchanged via horizontal gene transfer (conjugation) to non-plant pathogenic Streptomycetes to create a pathogenic species or strain, thereby altering the pathogenic diversity within a population of coexisting *Streptomyces* species in a field (Bukhalid *et al.*, 2002; Dees *et al.*, 2013; Kers *et al.*, 2005; Lerat *et al.*, 2009).

To date, there is limited conclusive information regarding reproducible factors, or combination of factors, that determine lesion type on specific potato cultivars over time (Wanner, 2009). In a study by Bouchek-Mechiche *et al.* (2000b), lesion phenotype was not tightly correlated with temperature, species, or potato variety using 19 isolates from potato growing areas in France. Certain species that they had isolated from netted scab, for instance, were able to cause other lesion phenotypes under higher temperature conditions. Other studies have investigated the effects of inoculum to determine if virulence and resulting scab severity was contingent on the amount of thaxtomin A produced by a particular Streptomycete strain (Keinath & Loria, 1991; Kinkel *et al.*, 1998). Kinkel *et al.* (1998) found a linear relationship between scab severity and inoculum density, but in terms of diseased surface area (%) and not lesion type. The relationship between diseased surface area and inoculum density became dissociated, however, when tested on different host cultivars and/or with different strains of *S. scabies* (Kinkel *et al.*, 1998). In a French study by Bouchek-Mechiche *et al.* (2006), superficial lesions (referred to as netted or russetted) were associated with a particular species (*S. reticuliscabiei*) because they had determined that *nec1* was absent in their *S. reticuliscabiei* strains but present in pathogenic strains of *S. scabies, S. europascabiei,* and *S. turgidiscabies.*

It is currently understood that numerous pathogenic species of *Streptomyces* can cause CS and that other virulence genes are somehow involved with disease development, in addition to the role of thaxtomin A and optimal environmental conditions (Loria *et al.*, 2006; Wanner, 2006; Wharton *et al.*, 2013). Despite advances in understanding the pathogen's virulence components under specific temperature

conditions, little is known of the determinants of specific lesion phenotypes (Keinath & Loria, 1991). The objectives of my research were to 1) determine if there was a correlation between lesion type and one or more of the following factors: species of the *Streptomyces* isolate, presence of virulence genes, potato cultivar, and field location (county) of the sample, and 2) evaluate reproducibility of lesion types with 24 PAI-characterized strains of *S. scabies* on a CS-susceptible cultivar in a controlled environment.

Materials & Methods

Streptomycete isolation

Bacterial strains were isolated from CS-symptomatic tubers sampled from concentrated potato production regions in Northern and Central Wisconsin during 2012-2013. An aggregated, representative field sample of approximately 11-45 kg (25-100 lb) of tubers with various lesion types was randomly selected from grading lines at harvest or dug from fields sampled in a W-shaped pattern. A random group of 10-15 CS-symptomatic tubers were selected from each sample location for isolations. Lesions were arbitrarily marked and sampled according to methods derived from Wanner (2007). Tubers were gently washed with tap water to remove debris and allowed to dry. Small (2 mm^2) excisions were taken from lesion margins, surface sterilized in 1.5% hypochlorite-dH_2O solution, rinsed in a sterile distilled water (SDW) bath, and allowed to dry in a laminar flow hood. Each excision was sliced into 1- to 2-mm layers and placed onto water agar in 90-mm diameter Petri plates and incubated in darkness at 28°C for 14-21 days. Plates were not sealed with Parafilm to encourage a dry microclimate and induce colony growth and sporulation. After 3-5 days, colonies displaying characteristic *Streptomyces* aerial mycelia (Shirling & Gottlieb, 1966) were transferred to sterile water agar in 90-mm diameter Petri plates and allowed to mature for 1 week. Sub-cultures were made to segregate *Streptomyces* from non-target bacterial or fungal colonies. Isolations with persistent contaminants were excised, transferred to a microcentrifuge tube and submerged in a 60°C water bath for 15 min. The contents were cooled and homogenized with a sterile pestle and plated onto water agar from a 10^2, 10^3, and 10^4 dilution series of the homogenate for re-isolation of pure colonies (Wanner, 2013). Axenic colonies were

allowed to mature until sporulation of aerial mycelia was visible microscopically at 400x magnification. A sterile bacterial loop dipped in sterile water was used to transfer *Streptomyces* spores, using a bacterial streaking pattern, onto solid yeast malt extract (YME) agar in 90-mm diameter Petri plates and incubated under the conditions previously stated. After three weeks, transfers were made to solid oatmeal agar (OMA) in 90-mm diameter Petri plates and incubated under the previously described conditions in preparation for radish seedling assays.

Radish pathogenicity assay

Plant pathogenic *Streptomyces* strains produce thaxtomin A when cultured on OMA (Flores-Gonzales *et al.,* 2008). Mature *Streptomyces* cultures on OMA were incubated with three 'Cherry Belle' radish seeds *in vitro* to determine pathogenicity, as previously described (Dees *et al.*, 2013; Flores-Gonzales *et al.,* 2008). Radish seeds were sterilized in 1% hypochlorite solution for 1 min, subsequently rinsed in two SDW baths, and allowed to dry in a laminar flow hood. Three sterilized seeds were sunken into agar medium in each culture plate containing a 14- to 20-day-old isolate. Seeded cultures were incubated at room temperature (22°C) near a fluorescent light source for 8 days. Control plates included seeds that were submerged into OMA without *Streptomyces* present, OMA with a *txtAB*-positive isolate (WI06-19D, courtesy of L. Wanner, USDA), and into OMA with a *txtAB*-negative non-pathogenic isolate (WI06-23B, courtesy of L. Wanner, USDA). Seedling evaluations determined if an isolate was putatively pathogenic by showing hypertrophic, stunted, and/or blackened necrosis of hypocotyls and/or cotyledons. Diseased hypocotyls were < 2 cm in length; non-germinated seeds were

colonized with visible sporulation. Putatively non-pathogenic cultures and control plates contained healthy seedlings of > 4 cm in length and were absent of disease symptoms.

Genomic DNA extraction

Strains of *Streptomyces* isolates were grown on solid YME agar on 90-mm diameter Petri plates at 28°C in darkness for 14 days until sporulation was evident. Mycelial mats were scraped and transferred to PowerBead tubes using the PowerSoil® DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to manufacturer's instructions with some modifications. PowerBead tubes containing isolate cells were vortexed for 5 min at 2400, transferred to a water bath at 60°C for 5 min, and returned to vortex for 5 min at 2400 to ensure sufficient lysis of cells. Extracted DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA) and placed in storage at -20°C for genetic screening.

txtAB pathogenicity screen

Bacterial isolates were subjected to PCR analysis using *txtAB*-specific primers for detection of the operon responsible for thaxtomin A synthesis, as was developed and previously described by Wanner (2006). The primer set TxtAB1/TxtAB2 (5'-CACGTACGCGCAGTTCAATG-3'; 5'-AGATGATGTAGGCGGGACTC-3') was purchased from Integrated DNA Technologies (Coralville, IA). The 20-µl PCR reactions contained 1.0 µl of primers, 10.0 µl of PCR Master Mix (Promega Corp., Madison, WI), 7.0 µl of nuclease-free (NF) H_2O, and 1.0 µl of template DNA that was adjusted to concentrations of 2-25 ng/µl. PCR reaction conditions included an initial denaturation of 3 min at 94°C followed by 30 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 46°C, extension for 35 sec at 72°C, and a final extension for 7 min at 72°C. PCR

product was held at 4°C until amplicons of the PAI region were separated by electrophoresis on 1% Tris/acetic acid/EDTA (TAE) gel and visualized with GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and Bio-Rad imaging and Quantity One 1-D analysis software (Bio-Rad Laboratories, Hercules, CA). A known pathogenic strain of *S. scabies* (WI06-19D, courtesy of L. Wanner, USDA) containing the *txtAB*, or PAI operon, was used as a positive control and a *txtAB*-negative non-pathogenic isolate (WI06-23B, courtesy of L. Wanner, USDA) was used as a negative control for all PCR reactions.

Analysis of 16S rRNA

Taxonomically-specific regions of the bacterial 16S ribosomal DNA reflect variability between *Streptomyces* species and are used for species identification based on 16S rRNA sequence similarity (Fig. 2). Isolate DNA was subjected to polymerase chain reaction using 16S-1F/16S-1R primer set (5'-CATTCACGGAGAGTTTGATCC; AGAAAGGAGGTGATCCAGCC-3') developed by Takeuchi & Sawada (1996) and reaction conditions previously described by Wanner (2013) to detect the 16S rRNA gene. The PCR mix (20-µl) contained 1.0 µl of primers, 10.0 µl of PCR Master Mix (Promega Corp., Madison, WI), 7.0 µl of NF H_2O, and 1.0 µl of template DNA. The PCR cycle consisted of an initial denaturation of 3 min at 95°C followed by 40 cycles of denaturation for 20 sec at 95°C, annealing for 30 sec at 50°C, extension for 2 min at 72°C, and a final extension for 5 min at 72°C. PCR product was held at 4°C until amplicons of the 16S 1.5 kbp region were separated by electrophoresis on 1% TAE gel and visualized with GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and Bio-Rad imaging and Quantity One 1-D analysis software (Bio-Rad Laboratories, Hercules, CA).

DNA from pathogenic *S. scabies* (WI06-19D) was used for comparison (courtesy of L. Wanner, USDA). Pooled PCR product from the DNA of each isolate was purified using QIAquick PCR purification kit (Qiagen Science, Germantown, MD) and submitted for Sanger sequencing at the University of Wisconsin Biotechnology Center. Partial sequence fragments (~750 bp) were assembled using EMBOSS: The European Molecular Biology Open Software Suite (Rice *et al.*, 2000). Assemblages were compared with highly similar sequences within the GenBank database using the Basic Local Alignment Search Tool (BLAST) algorithm (Zhang *et al.*, 2000).

Analysis of intergenic sequences

Although non-conserved regions of the 16S gene are sufficient for species identification of most plant pathogenic *Streptomyces*, there is high sequence similarity between *S. scabies* and *S. europascabiei*. Amplification of the intergenic transcribed spacer (ITS) region of the 16S operon was accomplished using the ITS-R/ITS-L primer pair (5'-GTCAAGTCATCATGCCCCTT; AAACTTGGCCACAGATGCTC-3') (Flores-Gonzalez *et al.*, 2008; Song *et al.*, 2004). Amplicons were exposed to the restriction enzyme *Hpy99I* (New England Biolabs, Beverly, MA) for digestion according to methods described by Flores-Gonzalez *et al.* (2008) and Song *et al.* (2004). Type strains with the restriction site present (*Hpy99I*+) include *S. scabies*, while strains without (*Hpy99I*-) are indicative of *S. europascabiei*. Known strains of *S. scabies* ATCC49173 (D63862) and *S. europascabiei* CFBP4497 (AJ007423) were used for comparison (courtesy of L. Wanner, USDA). The digested amplicons were separated on 1.5% agarose gel by electrophoresis with TAE buffer and visualized using GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and imaging described above.

Phylogenetic analysis

Assemblages of 16S sequences were aligned using Clustal W and MUSCLE software prior to constructing evolutionary trees with Molecular Evolutionary Genetics Analysis (MEGA) version 6.6. Phylogenetic constructs were based on neighbor-joining algorithms for analysis of the 16S rRNA gene sequences (Saitou & Nei, 1987; Tamura *et al.*, 2013). The sum of branch length shown = 1.28. Bootstrap values > 70 are shown and were generated from 1,000 test replicate trees with the associated taxa (Felsenstein, 1985). Sequenced strains used in this analysis are included as associative references and are described (Table 1). Footnotes indicate additional isolates with high 16S sequence similarity to the relative *Streptomyces* species based on independent phylogenetic analyses (data not shown).

Thaxtomin production & HPLC-MS analysis

Sixty *Streptomyces* isolates were selected based on the lesion type they were isolated from. The group of 60 was comprised of three groups of 20 isolates originating from each of the three CS lesion types: netted, raised, or pitted scab lesions. The 60 isolates were grown on solid YME in 90-mm diameter Petri plates for 2-3 weeks until maturity. Two 1.0-cm plugs were transferred to 50 ml of sterilized oatmeal broth (filtered through four layers of cheese-cloth to remove excess pulp) in 200-ml Erlenmeyer flasks. Inoculated filtrates were covered with foil and incubated at 28°C for 12 days on a shaker set to 200 rpm (Conn *et al.*, 1998; Kinkel *et al.*, 1998). Following incubation, each sample was transferred to a 50-ml sterile conical tube and centrifuged (3000 rpm for 10 min) to separate colony growth from the supernatant. The supernatant (20 ml) was vacuum-filtered and subjected to liquid extraction with HPLC-grade ethyl acetate. Two

consecutive extractions of 10 ml were carried out on each sample in a separation funnel and pooled prior to evaporation. The lower aqueous phase was drained before the thaxtomin-containing organic phase was collected in an amber-colored bottle and clamped into an N-evap nitrogen evaporator (Organomation Associates, Inc., Berlin, MA) for 4-6 hours to expedite evaporation (Conn *et al.*, 1998). Amber vials containing crude extracts were sealed and stored at -20°C for high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The colonies separated from aqueous media were dried at 55°C for 3 days and weighed to determine relative thaxtomin output per gram of colony growth (Goyer *et al.*, 1998). Crude thaxtomin extracts were quantified with reverse-phase HPLC using an Agilent 1100 binary pump system (Agilent Technologies, Santa Clara, CA) coupled to a 3200 Q Trap linear ion trap quadropole mass spectrometer (AB Sciex Instruments, Framingham, MA) and a 150 x 2 mm Polar-RP phase 4-µm column (Phenomenex, Inc., Torrance, CA). The thaxtomin extracts (solubilized in 1 µl methanol) were eluted at a flow rate of 250 µl/min with a 5-95% acetonitrile mobile phase gradient over 25 min and monitored at A380. Re-equilibration time between samples was 6 min. Purified thaxtomin A was used to establish a standard curve relating µg thaxtomin A to peak area measured at 380 nm. A calibration curve was created using pure thaxtomin A compound by subtracting the signal for endogenous thaxtomin to shift the y-intercept to zero in order to account for matrix effects from the liquid broth medium. MS/MS detection was set to positive mode at an ionspray voltage of 30. Final amounts of thaxtomin A produced under experimental conditions were converted from pmol concentrations and reported as µg thaxtomin A/g cell dry weight.

PAI-associated virulence gene analysis

Twenty-four *S. scabies* isolates were selected and subjected to PCR analysis using specific primers for detection of the *nec1* and *tomA* virulence genes (Bukhalid *et al.*, 1998; Kers *et al.*, 2005; Wanner, 2006). The primer sets Nf/Nr (5'-ATGAGCGCGAACGGAAGCCCCGGA-3'; 5'-GCAGGTCGTCACGAAGGATCG-3') and Tom3/Tom4 (5'-GAGGCGTTGGTGGAGTTCTA-3'; 5'-TTGGGGTTGTACTCCTCGTC-3') were purchased from Integrated DNA Technologies (Coralville, IA). The 20-µl PCR reactions contained 1.0 µl of primers, 10.0 µl of PCR Master Mix (Promega, Corp., Madison, WI), 7.0 µl of NF H_2O, and 1.0 µl of template DNA that was adjusted to concentrations of 2-25 ng/µl. PCR reaction conditions included an initial denaturation of 3 min at 94°C followed by 30 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 55°C, extension for 35 sec at 72°C, and a final extension for 7 min at 72°C. PCR product was held at 4°C until amplicons of the virulence gene region were separated by electrophoresis on 1% TAE gel and visualized with GelRed nucleic acid dye (Biotium Inc., Hayward, CA) and Bio-Rad imaging and Quantity One 1-D analysis software (Bio-Rad Laboratories, Hercules, CA). A known virulent strain WI06-19D (*nec1*+ and *tomA*+) of *S. scabies* (courtesy of L. Wanner, USDA) was used as a positive control and a non-pathogenic isolate WI06-23B (*nec1*- and *tomA*-) (courtesy of L. Wanner, USDA) was used as a negative control for all PCR screens.

Multivariate and statistical analyses

Sixty *Streptomyces* strains that consisted of 20 isolates originating from each of three lesion types (netted, raised, and pitted) were analyzed using phylogeny, virulence gene, and thaxtomin A output (µg/g) data. The one-way analysis of variance procedure

was used on quantitative data to determine statistically significant differences among species, isolate production of thaxtomin A, and virulence values based on a between-group component and a within-group component at the 95.0% confidence level with StatGraphics statistical analysis software package (StatPoint Technologies, Warrenton, VA). Linear regression analysis was conducted to show the relationship between thaxtomin A and assigned virulence values (presence of *nec1* and *tomA*) using the Simple Regression procedure in StatGraphics statistical analysis software package (StatPoint Technologies, Warrenton, VA).

Multivariate analyses of the 60 lesion-based isolates were also conducted by constructing 10 contingency tables that show frequency of occurrence of unique pairs of values for the following five qualitative isolate variables: species, lesion type of origin, location (county), virulence genes, and cultivar. For each two-way analysis, a Chi-square test for independence between row and column variables was carried out at the 95.0% confidence level using the Crosstabulation procedure in StatGraphics statistical analysis software package (StatPoint Technologies, Warrenton, VA).

Association of virulence factors with CS lesion phenotypes

An isolate subset of 24 strains of *S. scabies*, consisting of eight strains that were isolated from each of the three CS lesion types: netted, raised, and pitted scab was selected based on phyletic similarity to *S. scabies* (ATCC49173) for use in inoculation experiments to determine lesion phenotype on a susceptible cultivar. Each *S. scabies* strain was screened for presence of the PAI-associated virulence genes (*nec1* and *tomA*). A virulence gene value system was created to assess the presence or absence of *txtAB*, *nec1*, and *tomA* (Table 2b).

Four-week-old, disease-free tissue culture plantlets of 'Yukon Gold' were obtained from the University of Wisconsin Seed Potato Certification Program (Madison, WI) and planted into 3-gal pots containing a 2:1 mixture of sterile sand and field soil. Plants were maintained in a glass greenhouse at the University of Wisconsin-Madison at a temperature of 22-24°C and subjected to a 14-hr photoperiod to simulate field conditions. Each plant received approximately 500 ml of water every 4-5 days and the substrate was permitted to dry between watering to promote favorable CS conditions on progeny tubers. All plants were fertilized with Vigoro's 12-5-7 All Purpose Plant Food (Swiss Farms Products, Las Vegas, NV) at 30 and 60 days post-planting.

Inoculum preparation was adapted from Wanner (2013). Mature, 3-week-old *S. scabies* cultures (on YME) with visible sporulation were gently scraped with a sterile glass rod in plates flooded with 1.0 ml of sterile water. Spore suspensions were extracted from cultures and adjusted to 10^8 spores/ml using a hemacytometer and combined (1:1) with 20% glycerol in 2.0-ml microcentrifuge tubes and stored at -20°C. The thawed contents of each microcentrifuge tube were added to 100 ml of water and applied by drenching into soil of potted plants. Three replicate cultures and resulting microcentrifuge tube-suspensions were generated per strain for the 24 treatments. A known pathogenic strain WI06-27B (*txtAB*+, *nec1*- and *tomA*+) of *S. scabies* (courtesy of L. Wanner, USDA) was used as a positive control and a non-pathogenic isolate WI06-23B (*txtAB*-, *nec1*-, and *tomA*-) (courtesy of L. Wanner, USDA) was used as a negative control. An additional treatment consisting of non-inoculated plants was included. This experiment was repeated twice.

Tubers were harvested at 10 weeks (70 days) post trial initiation (PTI) and rated for CS. Disease severity ratings were calculated by using methods described by Wanner (2013), where lesion coverage (%) is multiplied by values assigned to lesions based on type and size (Table 2a). Multiple comparison procedures were used to identify statistically significant differences between treatment level means according to Tukey's honest significant difference (HSD) method at the 95.0% confidence level. Analysis of variance was used to determine if two replicated trials were significantly different at the 95.0% confidence level. Data from the two trials were pooled if they were not significantly different. Linear regression analysis was conducted to determine the relationship between inoculation-based CS severity and the virulence of 24 strains of *S. scabies* using the Simple Regression procedure in StatGraphics statistical analysis software package (StatPoint Technologies, Warrenton, VA).

Results

Streptomycete isolation

Of the 60 isolation attempts, a total of 58 Streptomycete isolates were excised from specific common scab lesion types on commercially-grown mature potato tubers from three Wisconsin potato production regions. Netted lesions generated 18 isolates, raised generated 20, and pitted lesions generated 20 isolates (Table 3).

Radish pathogenicity assay

Healthy seedlings grown on solid water agar, or with *txtAB*-negative control strain WI06-23B, germinated and resulted in healthy hypocotyls of > 3-4 cm in length. Radish seedling hypocotyls that emerged from a *txtAB*-positive strain became necrotic with black or brown tissue discoloration of hypocotyl and cotyledon, were stunted with a hypocotyl length of < 2 cm, and/or showed symptoms of hypertrophic or deformed growth. Seed coats also exhibited white pathogen sporulation. Strains with putative pathogenicity from radish seed assays were confirmed with *txtAB*-specific primers for detection of the PAI operon. Some strains lost viability in culture prior to being assayed with the radish seed procedure, but had genomic DNA extracted and were subjected to a *txtAB* primer-based pathogenicity screen.

Genomic DNA extraction

Concentrations of DNA from each isolate were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). Less than 2 ng/µl-samples were loaded into an electrophoresis gel to assess quality. DNA extractions were repeated, as necessary. Concentrations > 50 ng/µl were diluted (50:50) with NF H_2O prior to PCR analyses.

txtAB pathogenicity screen

Of the 58 isolates screened with TxtAB1/TxtAB2 primers, four were missing the *txtAB* gene, supporting the radish seedling assay results. The remaining 54 isolates had gene fragments of approximately 385 bp that align with *S. scabies* 87-22 (Fig. 3). The non-pathogenic control isolate, WI06-23B, did not contain the PAI for biosynthesis of thaxtomin A and did not amplify gene fragments in this PCR analysis.

Analysis of 16S rRNA

Genetic screens of the 16S rRNA were carried out using PCR analysis (Fig. 4a). Five-prime sequence regions were merged with the reverse compliment of the 3' to create FASTA assemblies. Highly homologous sequences with a query cover of > 98% were observed with 90% of the BLAST-analyzed isolates. Species pairings of *Streptomyces scabies* with *S. europascabiei* and *S. bottropensis* with *S. stelliscabiei* isolates were highly homologous with each other. Additional highly-similar sequences were observed between *S. ryensis, S. achromogenes*, and *S. rishiriensis*. Sequences that resulted in query matches of < 80% or did not produce any matches from the query search were declared undetermined. Highly similar sequence matches with published accession numbers were recorded and used for phylogenetic analyses.

Analysis of intergenic sequences

The 16S region of *S. scabies* and *S. europascabiei* are nearly identical and require PCR analysis of the internal transcribed spacer (ITS) region for species determination (Flores-Gonzalez *et al.*, 2008). Twenty-five (43%) isolate sequences had BLAST query matches with *S. scabies, S. europascabiei,* or both. PCR amplification patterns from ITS-R/ITS-L primers confirmed ribosomal amplicons were in sufficient concentrations for

restriction. Of the 52 isolates screened with the digestion enzyme, 46 were restricted (*Hpy99I* +) due to presence of the RFLP in the ITS region and classified as *S. scabies* (Fig. 4b). The remaining six isolates did not reveal fragmented amplicons (*Hpy99I-*) and were classified as *S. europascabiei*.

Phylogenetic analysis

Comparisons of ~1500 nt sequences of the 58 isolates had sequence relatedness to type strains *S. scabies* (ATCC49173), *S. bottropensis* (ATCC25435), *S. stelliscabiei* (WI06-15D), *S. ryensis* (NBRC13834)/*S. achromogenes* (S22)/*S. rishiriensis* (GYB5), and *S. glauciniger* (D501) (Fig. 5). Twenty-five isolates were clustered with type strain *S. scabies* (ATCC49173) (represented by 12-29H in Fig. 5). Six isolate sequences were clustered with *S. bottropensis* (ATCC25435) in the same subclade at a supported bootstrap value of 87% at the internal spacer shared with *S. europascabiei* (CFBP4497). Representative isolate 12-17H and 11 other isolates had 16S rDNA sequence relation to *S. stelliscabiei* (WI06-15D), and shared its subclade with 12-32H, and 13-612R. Possible descendants (12-20H and 13-609R) of this subclade have separated into two additional external nodes, or tips, at a 74% similarity. Four isolates (represented by 13-14H) were clustered with a subclade that included the closely related *S. ryensis* (NBRC13834), *S. achromogenes* (S22), and *S. rishiriensis* (GYB5). The isolate sequence of 13-4H was clustered with *S. griseoruber* (ICSSB1013). The 16S rDNA of isolates 12-512R and 12-508R had 89% similarity to *S. glauciniger* (D501), and shared a phyletic line with 13-12H. Isolate 12-60H was included in this subclade and collectively showed 100% dissociation from other phyletic lines. 16S sequences of 12-528P and 12-103A were clustered with *S. aureus* (3184) with 100% similarity compared to other subclades.

Thaxtomin production & HPLC-MS analysis

Twenty *S. scabies* isolates produced a range of thaxtomin A from 0.05-6.59 µg/g (Fig. 6). Among the 12 strains of *S. stelliscabiei*, thaxtomin A output ranged from 0.09-5.75 µg/g. The seven *S. bottropensis* isolates produced 0.28-5.28 µg/g of thaxtomin A. The remaining species, each comprised of 1-3 isolates, did not produce > 0.65 µg/g of thaxtomin A, with the exception of two isolates of *S. europascabiei.* These two isolates produced the highest quantity of thaxtomin A, with a minimum of 4.97 µg/g and a maximum of 9.81 µg/g. In some cases, non-pathogenic (*txtAB*-) strains produced thaxtomin A values of > 0 µg/g due to the residual matrix effects from the complexity of the culture broth medium.

One-way analysis of variance of thaxtomin A (µg/g) production based on *Streptomyces* species does not reveal a significant interaction at the 95.0% confidence level ($P = 0.4933$). Similarly, one-way analysis of variance of thaxtomin A (µg/g) produced from isolates originating from different lesion types does not reflect a statistically significant association ($P = 0.2720$).

PAI-associated virulence gene analysis

Four isolates were determined non-pathogenic because they lacked the PAI *txtAB* component, lacked the virulence genes *nec1* and *tomA* (Fig. 7), were non-pathogenic in the radish assay, and produced less than 0.06 µg/g of thaxtomin A (Table 3). The remaining 54 isolates were subjected to one-way analyses of variance between *Streptomyces* species x virulence values ($P < 0.0001$) and also between thaxtomin A (µg/g) x virulence values ($P = 0.0003$), which produced significant effects at the 95.0% confidence level (Table 4). Linear regression analysis of thaxtomin A (µg/g) x assigned

virulence values also produced a statistically significant association ($P = 0.0003$) between virulent strains and thaxtomin A production (Fig. 8).

Multivariate and statistical analyses

A total of 58 *Streptomyces* isolates were excised from netted (n=18), raised (n=20), and pitted scab (n=20) lesions on mature potato tubers. *Insignificant interactions*: P-values for variable interactions that were > 0.05 were observed with 5 of the 10 interactions tested (data not shown). The interaction between cultivar x location did, however, have a P-value < 0.0001, which reflects a clear and obvious association between cultivar and location but does not, however, contribute to disease or pathogen effects. The Chi-square tests did not reveal dependent associations with: *Streptomyces* species x virulence genes (P-value=0.9920); lesion type x virulence genes (P-value=0.8367); lesion type x cultivar (P-value=0.5806); location x virulence genes (P-value=0.8174); or virulence genes x cultivar (P-value=0.9735). *Significant interactions:* Chi-square tests for independence revealed significant relationships (Table 5) between the following variables: *Streptomyces* species x originating CS lesion phenotype (P-value=0.0144), *Streptomyces* species x location (P-value=0.0.0017), *Streptomyces* species x cultivar (P-value=0.0019), and lesion phenotype x location (P-value=0.0252) based on two-way analyses of contingency tables (Tables 6-10).

Association of virulence factors on CS lesion phenotypes

Virulence factors of *S. scabies* strains significantly impacted CS lesion phenotypes on progeny tubers of 'Yukon Gold' under greenhouse conditions (Fig. 9). Analysis of variance of CS severity and virulence of strains of *S. scabies* resulted in a significant interaction ($P < 0.0001$) (Table 11). Disease was not observed in non-

inoculated control treatments or with the non-pathogenic (WI06-23B) strain. Five isolates of *S. scabies* had DSVs that were < 5.0, which exhibited superficial netted scab lesion types (Fig. 10). The remaining isolates resulted in both raised and/or pitted lesions with DSVs ranging from 6.1-15.3. Evaluation of the presence of virulence genes *nec1* and *tomA* revealed that strains that produced netted scab phenotypes were *txtAB*+, *nec1*-, and *tomA*- (+ - -). Isolates that were positive for either *tomA* (+ - +) or *nec1* and *tomA* together (+ + +) produced a range of raised and pitted scab lesion phenotypes (Table 12).

Linear regression analysis to determine the relationship between CS severity and virulence of the infecting *S. scabies* isolate resulted in a statistically significant and strong relationship between the two variables with a correlation coefficient value=0.85 (Fig. 11). The R-squared statistic indicated that the fitted model accounted for 72.6% variability in CS severity.

Discussion

In my work with *Streptomyces scabies* isolates on 'Yukon Gold' under controlled conditions, the virulence factors *nec1* and *tomA* influenced CS lesion type. I showed that isolates of *S. scabies* that originated from each of the three lesion types reproduced raised and pitted scab lesions on daughter tubers when *nec1* and *tomA* were present. The netted lesion types occurred with strains of *S. scabies* with the *txtAB*+, *nec1*-, *tomA*- virulence genotype. Earlier studies that resulted in the determination of an association between netted scab and *S. reticuliscabiei* did not report on PAI-associated virulence genes (Bouchek-Mechiche *et al.*, 2000a,b; Pasco *et al.*, 2005). In 2006, Bouchek-Mechiche *et al.* (2006) reported that *S. reticuliscabiei*-associated netted scab lesions did not have the *nec1* gene. However, prior to my work, evaluations of PAI-associated lesion types had not been investigated in *S. scabies* since the tomatinase gene *tomA* was first described in 2008 (Seipke & Loria, 2008).

My work demonstrated significant correlations between *Streptomyces* ribotypes/strains and location, cultivar, species, and CS lesion phenotype. The primary potato-production regions of Wisconsin had just one or two predominating ribotypes based on a significant interaction between location x pathogen species. A similar association was also made between cultivar x species as a result of cultivar selection into a field with predominating ribotype(s) of *Streptomyces*. The correlation between lesion type and location corroborates with field reports of CS severity (superficial vs. pitted) being field-associated and reproducible in specific production fields over time (Lazarovits *et al.*, 2007; Wanner, 2004; 2009). However, the consistency of CS lesion phenotypes in specific locations over time is also influenced by cultivar (Bouchek-

Mechiche *et al.*, 2000b; Driscoll *et al.*, 2009; Hiltunen *et al.*, 2005; Tegg *et al.*, 2008; Wanner & Haynes, 2009; Wilson *et al.*, 2010). My associative analyses also indicated a relationship between CS lesion phenotype and *Streptomyces* species/ribotype, an association we can continue to understand with further characterization of the PAI status among population isolates.

The presence of the *txtAB* gene, which codes for production of thaxtomin A, is the pathogenicity factor for phytopathogenic *Streptomyces* (Goyer, *et al.*, 1998; Kim *et al.*, 1999; Wanner, 2009). The quantity of thaxtomin A produced by an isolate is believed to play a role in virulence among ribotypes (Kinkel *et al.*, 1998). The lack of significance between quantity of thaxtomin A, species/ribotype, or CS lesion phenotype among our 58 isolates (with nine *Streptomyces* biotypes/species represented) suggested that the presence of the phytotoxin may be dependent upon edaphic or other direct tubersphere features rather than on aggressiveness of individual ribotypes. In my work, thaxtomin A production may have been affected by the culturing process which includes an oatmeal broth (OMB) incubation. In the native pathosystem, thaxtomin A production is induced by the presence of suberin from stolons/tuber initiates, which is absent in OMB. In addition, the potato tuber periderm responds to infection with an arsenal of biochemicals that may also influence the mechanisms of thaxtomin A production (Lerat *et al.*, 2010). However, the pattern of existing virulence genes *nec1* and *tomA* among our 58 isolates suggested that their presence may be influencing thaxtomin A in producing specific lesion phenotypes. Ribotypes or strains that were pathogenic (*txtAB+*) but were *nec1-* and *tomA-* produced less thaxtomin compared to those that were *txtAB+*, *nec1+*, *tomA+*. Moreover, when I assigned virulence values based on the genotypic virulence of each

strain, I saw a significant correlation between quantity of thaxtomin A and virulence values.

Because there are multiple, complex factors involved in producing specific common scab lesion types, the disease is typically variable in incidence and severity in both research and commercial production fields. My work has focused on understanding the genotypic and phenotypic characters that are involved in CS lesion production through a reductive, empirical approach. From the characterization of 58 isolates selected from netted, raised, and pitted scab lesions on multiple cultivars from several potato-growing regions of Wisconsin, I determined significant correlations between the prevalence of species in particular counties planted with select cultivars when evaluating categorical lesion types. However, because more than one species can occupy a lesion and cannot definitively be associated as the direct causal agent (Wanner, 2009), these data suggest that the significance may be due to the prevalence of a population of certain ribotypes occupying the rhizosphere within a field. In addition, with knowledge of the transference of the PAI-associated virulence genes among closely-related species (Kers *et al.*, 2005), my data indicated that the determination of ribotype may be less important than the determination of virulence gene expression among field-associated populations. When I eliminated the multi-species variable with a glass-house inoculation experiment, it became evident that the profile of PAI-associated virulence genes among *S. scabies* strains had a significant impact on the production of netted lesion types. Further research on the difference in disease phenotypes from inoculations with other ribotypes that lack the *nec1* and *tomA* genes is warranted and may provide insight for targeting gene loci through breeding and resistance efforts.

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Table 1. Sequence identity accession numbers from GenBank for *Streptomyces* species used in phylogenetic analyses.

Strain	Accession number
S. achromogenes S22	HQ850411
S. acidiscabies ATCC49003	AB026220
S. aureus 3184	EF371429
S. bottropensis ATCC25435	D63868
S. coralus CFCC3136	FJ883746
S. echinatus ISP5013	NR114823
S. europascabiei CFBP4497	AJ007423
S. glauciniger D501	KF317954
S. griseoruber ICSSB1013	AY094585
S. lannensis TA4-8	NR113181
S. mirabilis ATCC27447	AF112180
S. ossamyceticus NBRC13983	NR041156
S. recifensis st100	EU216596
S. reticuliscabiei CFBP4531	AJ007428
S. rishiriensis GYB5	JQ342915
S. ryensis NBRC13834	AB184517
S. scabies ATCC49173	D63862
S. stelliscabiei WI-6-15D	JF546730
S. turgidiscabies ATCC700248	AB026221

Table 2a. Disease scale for determining severity of CS on harvested daughter tubers as adapted from Wanner, L. A. (2013). The overall severity rating is determined by multiplying the disease score that corresponds to tuber coverage (%) by the disease score that corresponds to lesion description (type and size).

Common scab severity			Common scab lesion phenotype		Disease severity rating
Disease score (Factor)	**Tuber coverage (%)**		**Disease score (Factor)**	**Lesion description**	
0	No scab		0	None	
1	1-10 %	**X**	1	Small superficial lesions, < 5 mm	**Product**
2	11-25%		2	Large, coalescing superficial lesions, > 5 mm	
3	26-50%		3	Small raised lesions, < 5 mm	
4	51-75%		4	Large, coalescing raised lesions, > 5 mm	
5	> 75%		5	Pitted lesions	

Table 2b. A virulence value scale assigning values to *Streptomyces* isolates based on the presence of the *txtAB*, *nec1*, and *tomA* virulence genes per strain.

Virulence value	*txtAB*	*nec1**	*tomA*
3	+	+	+
2	+	-	+
1	+	-	-
0**	-	-	-

* *nec1* genes were not detected without the presence of *tomA* in all isolates screened.
** non-pathogenic

Table 3. Location, species, pathogenicity, and virulence characterization of 58 *Streptomyces* strains from unique lesion types.

Lesion type	Location (county)	Cultivar	Isolate	Species (16S rRNA)*	txtAB	nec1	tomA	ThaxtominA (µg/g)	Radish assay
Netted	Waupaca	Innovator	12-508R	*S. glauciniger*	-	-	-	0.06	-
	Langlade	Yukon Gold	12-102A	*S. stelliscabiei*	+	+	+	0.24	+
			12-111A	*S. scabies*	+	+	+	3.94	+
			12-112A	*S. scabies*	+	+	+	5.81	+
	Waushara	Snowden	12-32H	*S. stelliscabiei*	+	-	-	0.09	+
			12-33H	*S. stelliscabiei*	+	+	+	3.56	+
			12-34H	*S. ryensis*	+	-	+	0.17	+
			12-35H	*S. scabies*	+	+	+	2.40	n/a
			12-36H	*S. scabies*	+	+	+	n/a	+
		Norkotah	13-1H	*S. scabies*	+	-	-	0.05	+
			13-2H	*S. ryensis*	+	-	-	0.37	+
			13-4H	*S. griseoruber*	+	-	-	n/a	n/a
			13-7H	*S. ryensis*	+	-	-	0.04	+
			13-12H	*S. glauciniger*	+	-	-	n/a	+
			13-30H	*undetermined*	-	-	-	0.05	-
	Portage	Norkotah	13-252P	*S. scabies*	+	+	+	1.01	+
	Vilas	Dk Red Chieftan	13-401E	*S. turgidiscabies*	+	-	-	0.16	+
	Oneida	Snowden	13-627R	*S. stelliscabiei*	+	+	+	0.87	+
Raised	Langlade	Yukon Gold	12-104A	*S. scabies*	+	+	+	2.39	+
			12-124A	*S. scabies*	+	+	+	0.23	+
			12-140A	*S. europascabiei*	+	+	+	9.81	+
	Waupaca	Innovator	12-14H	*S. scabies*	+	+	+	0.85	+
	Waushara	Snowden	12-17H	*S. stelliscabiei*	+	+	+	0.74	+
			12-18H	*S. stelliscabiei*	+	+	+	0.57	+
			12-20H	*S. stelliscabiei*	+	+	+	1.58	n/a
			12-28H	*S. bottropensis*	+	+	+	2.29	+
			12-30H	*S. bottropensis*	+	+	+	1.03	+
			12-51H	*S. scabies*	+	+	+	0.24	+
		Norkotah	13-3H	*undetermined*	+	-	-	0.20	+
			13-5H	*S. scabies*	+	+	+	n/a	+
			13-22H	*S. ryensis*	+	-	-	n/a	n/a
			13-36H	*undetermined*	-	-	-	n/a	-
	Portage	Norkotah	13-254P	*S. bottropensis*	+	+	+	0.28	+
			13-255P	*S. bottropensis*	+	+	+	2.07	+
			13-275P	*S. bottropensis*	+	+	+	4.43	+

			13-282P	*S. scabies*	+	+	+	2.61	+
	Rusk	Marcy	13-305B	*S. europascabiei*	+	+	+	4.97	+
			13-309B	*S. bottropensis*	+	+	+	5.28	n/a
Pitted	Waushara	Snowden	12-25H	*S. lannensis*	+	-	-	0.08	+
			12-42H	*S. stelliscabiei*	+	+	+	2.68	+
			12-44H	*S. scabies*	+	+	+	5.08	n/a
			12-60H	*S. glauciniger*	+	-	-	0.18	+
	Langlade	Yukon Gold	12-103A	*S. aureus*	+	-	-	0.65	n/a
			12-110A	*S. scabies*	+	-	-	0.07	+
			12-116A	*S. scabies*	+	+	+	1.79	+
			12-118A	*S. scabies*	+	+	+	3.03	+
			12-119A	*S. stelliscabiei*	+	+	+	5.75	+
			12-132A	*S. scabies*	+	+	+	0.55	+
	Waushara	Norkotah	13-15H	*S. scabies*	+	+	+	6.59	n/a
			13-20H	*S. scabies*	-	-	-	n/a	-
	Portage	Norkotah	13-259P	*S. scabies*	+	+	+	3.05	+
			13-260P	*S. scabies*	+	+	+	4.01	+
			13-262P	*S. scabies*	+	+	+	1.61	+
			13-277P	*S. scabies*	+	+	+	3.54	+
			13-279P	*S. bottropensis*	+	+	+	2.14	+
	Oneida	Snowden	13-609R	*S. stelliscabiei*	+	+	+	0.43	+
			13-612R	*S. stelliscabiei*	+	+	+	0.44	+
			13-613R	*S. stelliscabiei*	+	+	+	2.83	+

* Species with < 5 isolates have been grouped as 'miscellaneous' for statistical analyses.

Table 4. Analysis of variance of virulence values (presence of *nec1* and *tomA*) x *Streptomyces* species (1=*S. scabies*; 2=*S. stelliscabiei*; 3=*S. bottropensis*; 4=miscellaneous) and quantity of thaxtomin (µg/g) x virulence values (presence of *txtAB*, *nec1*, and *tomA*).

Source	**Sum of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
Species x virulence values					
Between groups	23.5892	3	7.86307	17.50	< 0.0001
Within groups	21.1167	47	0.449291		
Total (Corr.)	44.7059	50			
Thaxtomin x virulence values					
Between groups	56.3495	1	56.3495	15.53	0.0003
Within groups	177.814	49	3.62885		
Total (Corr.)	234.163	50			

Table 5. Chi-square tests for independence between row and column variables at the 95.0% confidence level based on five contingency tables that show frequency of occurrence of unique pairs of values for the following significant isolate variables: species, lesion type of origin, location (county), virulence genes (presence of *txtAB*, *nec1*, and *tomA*), and cultivar.

Isolate characteristics	**Statistic**	***Df***	***P-value*** [*]
Location x Cultivar	52.811	6	< 0.0001
Species x Location (county)	21.143	6	0.0017
Species x Cultivar	20.980	6	0.0019
Species x Lesion type	15.877	6	0.0144
Lesion type x Location	14.431	6	0.0252

* P-values < 0.05 indicate that isolate characteristic variables are dependent with contingency table values that are related at the 95.0% confidence level.

Table 6. Contingency table showing frequency of potato cultivars with common scab by location (county) of sample collection.

This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	Innovator	Russet Norkotah	Snowden	Yukon Gold	Row Total
Langlade/Oneida	0*	0	4	12	16
	0.00%	0.00%	7.27%	21.82%	29.09%
	0.00%	0.00%	25.00%	75.00%	
Portage	0	10	0	0	10
	0.00%	18.18%	0.00%	0.00%	18.18%
	0.00%	100.00%	0.00%	0.00%	
Waushara	2	12	15	0	29
	3.64%	21.82%	27.27%	0.00%	52.73%
	6.90%	41.38%	51.72%	0.00%	
Column Total	2	22	19	12	55
	3.64%	40.00%	34.55%	21.82%	100.00%

* For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row. Some expected cell counts < 5.

Table 7. Contingency table showing frequency of isolated *Streptomyces* strains by location (county of collection) of common scab sample. This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	S. bottropensis	*S. scabies*	*S. stelliscabiei*	Miscellaneous*	Row Total
Langlade/Oneida	0**	8	6	3	17
	0.00%	14.29%	10.71%	5.36%	30.36%
	0.00%	47.06%	35.29%	17.65%	
Portage	4	6	0	0	10
	7.14%	10.71%	0.00%	0.00%	17.86%
	40.00%	60.00%	0.00%	0.00%	
Waushara	2	9	6	12	29
	3.57%	16.07%	10.71%	21.43%	51.79%
	6.90%	31.03%	20.69%	41.38%	
Column Total	6	23	12	15	56
	10.71%	41.07%	21.43%	26.79%	100.00%

* Miscellaneous species with < 5 isolates includes: *S. aureus, S. europascabiei, S. glauciniger, S. griseoruber, S. lannensis, S. ryensis, S. turgidiscabies, and undetermined species.*

** For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row. Some expected cell counts < 5.

Table 8. Contingency table showing frequency of isolated *Streptomyces* strains from common scab-susceptible potato cultivars.

This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	S. bottropensis	*S. scabies*	*S. stelliscabiei*	Miscellaneous[*]	Row Total
Norkotah	4[**]	10	0	8	22
	7.55%	18.87%	0.00%	15.09%	41.51%
	18.18%	45.45%	0.00%	36.36%	
Snowden	2	4	10	3	19
	3.77%	7.55%	18.87%	5.66%	35.85%
	10.53%	21.05%	52.63%	15.79%	
Yukon Gold	0	8	2	2	12
	0.00%	15.09%	3.77%	3.77%	22.64%
	0.00%	66.67%	16.67%	16.67%	
Column Total	6	22	12	13	53
	11.32%	41.51%	22.64%	24.53%	100.00%

* Miscellaneous species with < 5 isolates includes: *S. aureus, S. europascabiei, S. glauciniger, S. griseoruber, S. lannensis, S. ryensis, S. turgidiscabies, and undetermined species.*

** For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row. Some expected cell counts < 5.

Table 9. Contingency table showing frequency of isolated *Streptomyces* strains by lesion it was isolated from. This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	S. bottropensis	*S. scabies*	*S. stelliscabiei*	Miscellaneous[*]	Row Total
Netted	0[**]	6	4	8	18
	0.00%	10.34%	6.90%	13.79%	31.03%
	0.00%	33.33%	22.22%	44.44%	
Pitted	0	11	5	4	20
	0.00%	18.97%	8.62%	6.90%	34.48%
	0.00%	55.00%	25.00%	20.00%	
Raised	6	6	3	5	20
	10.34%	10.34%	5.17%	8.62%	34.48%
	30.00%	30.00%	15.00%	25.00%	
Column Total	6	23	12	17	58
	10.34%	39.66%	20.69%	29.31%	100.00%

* Miscellaneous species with < 5 isolates includes: *S. aureus, S. europascabiei, S. glauciniger, S. griseoruber, S. lannensis, S. ryensis, S. turgidiscabies, and undetermined species.*

** For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row. Some expected cell counts < 5.

Table 10. Contingency table showing frequency of common scab lesion types by location (county of collection) of sample. This two-way table was generated from the Crosstabulation procedure in StatGraphics statistical analysis software.

	Langlade/Oneida	Portage	Rusk	Waushara	Row Total
Netted	5[*]	0	0	13	18
	8.62%	0.00%	0.00%	22.41%	31.03%
	27.78%	0.00%	0.00%	72.22%	
Pitted	9	5	0	6	20
	15.52%	8.62%	0.00%	10.34%	34.48%
	45.00%	25.00%	0.00%	30.00%	
Raised	3	4	2	11	20
	5.17%	6.90%	3.45%	18.97%	34.48%
	15.00%	20.00%	10.00%	55.00%	
Column Total	17	9	2	30	58
	29.31%	15.52%	3.45%	51.72%	100.00%

* For each cell, the first value is the count of frequency, the second value represents the percentage based on the entire table, and the third value is the percentage based on the other values in that row. Some expected cell counts < 5.

Table 11. Multivariate analysis of variance of common scab severity on 'Yukon Gold' potatoes inoculated with different virulent strains of *Streptomyces scabies* from two replicate greenhouse trials. Based on insignificant trial effects, severity data were pooled for treatment effects.

Source	**Type III Sums of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
Main Effects					
Trial	4.1408	1	4.1408	0.26	0.6139
Treatment	4312.52	26	165.866	10.25	< 0.0001
Interactions					
Trial x Treatment	468.798	26	18.0307	1.11	0.3385
Residual	1747.16	108	16.1774		
Total (Corr.)	6532.62	161			

Table 12. Effect of the presence of *txtAB* and virulence genes *nec1* and *tomA* on common scab on 'Yukon Gold' potatoes. Disease-free plants grown in pathogen-free soil were inoculated with 24 strains of *Streptomyces scabies* originating from each of three common scab lesion types (netted, raised, and pitted scab). Strains were selected based on phyletic similarity to *S. scabies* (ATCC49173) and evaluated for common scab on daughter tubers.

Isolate	Lesion of origin	*txtAB*	*nec1*	*tomA*	Lesion Severity* (Trial 1)	Lesion Severity (Trial 2)
12-29H	netted	+	+	+	P,P,P	P,P,P
12-35H	netted	+	+	+	P,P,P	P,N,P
12-112A	netted	+	+	+	P,P,R	P,P,P
12-123A	netted	+	+	+	P,P,P	P,P,P
12-525P	netted	+	-	-	N,N,N	N,N,N
12-527P	netted	+	-	-	N,N,N	N,N,N
13-1H	netted	+	-	-	N,N,N	N,N,N
13-252P	netted	+	+	+	P,R,R	P,P,R
12-41H	raised	+	-	-	N,N,N	N,N,N
12-104A	raised	+	+	+	P,P,P	P,P,R
12-105A	raised	+	+	+	P,P,P	P,P,0
13-5H	raised	+	+	+	P,P,P	P,P,P
13-258P	raised	+	-	+	P,P,P	P,P,P
13-281P	raised	+	-	+	P,P,R	P,P,P
13-282P	raised	+	+	+	P,P,P	P,P,R
13-301B	raised	+	-	-	N,N,N	0,N,N
12-132A	pitted	+	+	+	P,P,R	P,P,P
13-55H	pitted	+	+	+	P,P,P	P,P,R
13-56H	pitted	+	+	+	P,P,P	P,P,P
13-57H	pitted	+	+	+	P,P,P	P,P,R
13-259P	pitted	+	+	+	P,P,P	P,P,P
13-260P	pitted	+	+	+	P,P,R	P,P,P
13-262P	pitted	+	+	+	P,P,P	P,P,R
13-277P	pitted	+	+	+	P,P,P	P,P,R
WI06-23B	n/a	-	-	-	0,0,0	0,0,0
WI06-27B	n/a	+	-	+	P,P,R	P,P,R
Non-inoculated	n/a	n/a	n/a	n/a	0,0,0	0,0,0

* Maximum CS severity lesion observed, where: 0=no disease; N=netted lesions; R=raised lesions; P=pitted lesions.

Figure 1. Genome containing the pathogenicity island (PAI) of *Streptomyces* spp. showing a conserved *txtAB* within the toxicogenic region. Additional virulence genes lie upstream of the conserved core in the colonization region (*nec1* and *tomA*). Adapted from "Detection of common scab-causing *Streptomyces* in potato tubers and soil" by Wanner, L. A. (2013).

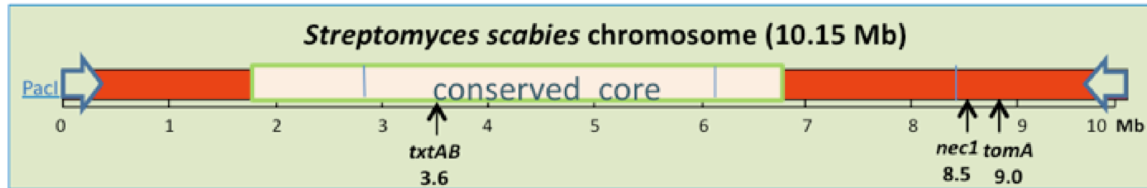


Figure 2. Taxonomically specific regions of the bacterial 16S ribosomal DNA are designated by the red boxes, which mark sequence variability between *Streptomyces* species. Adapted from "Detection of common scab-causing *Streptomyces* in potato tubers and soil" by Wanner, L.A. (2013).

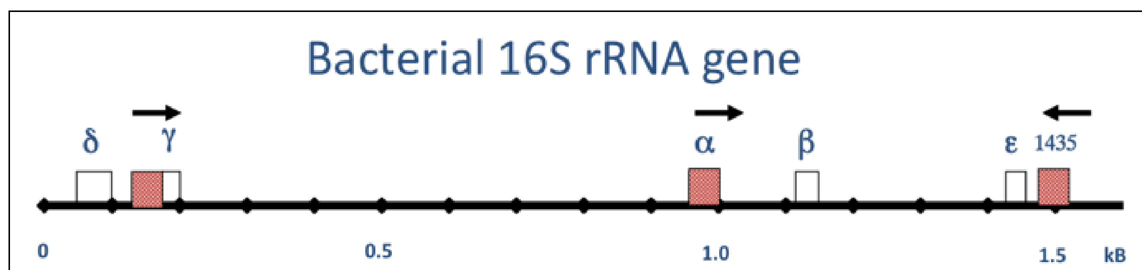
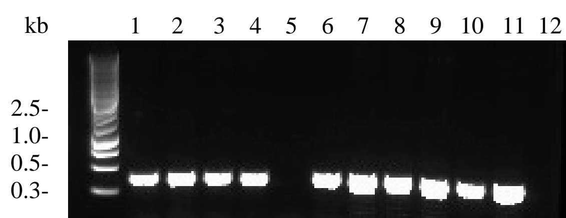


Figure 3. Representative group of *Streptomyces* spp. isolates showing amplification of *txtAB* genes (385 bp) with TxtAB1/TxtAB2 primers. (A) Lane 1, highly virulent *txtAB*-positive *S. scabies* 87-22; lanes 2-4 and 6-11, *txtAB*-positive unknown *Streptomyces* strains; lane 5, *txtAB*-negative unknown *Streptomyces* strain; lane 12, DNA blank. (B) Lane 1, known *txtAB*-negative *S. scabies* WI06-23B (courtesy of L. Wanner, USDA); lanes 2 and 5, *txtAB*-negative unknown *Streptomyces* strains; lanes 3, 4, 6-11, *txtAB*-positive unknown *Streptomyces* strains; lane 12, DNA blank.

A



B

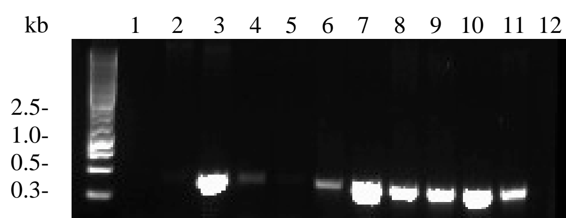
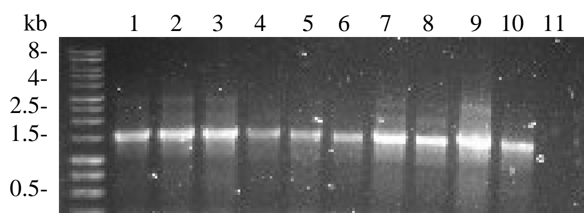


Figure 4. Electrophoresis PCR patterns from *Streptomyces* isolates and genetic screens on 1% Tris/acetic acid/EDTA (TAE) gel and imaged with Quantity One 1-D analysis software. (A) amplicons (1.5 kbp) of 16S rRNA sequence target region. Lane 1, known *S. scabies* isolate 87-22; lanes 2-10, unknown *Streptomyces* strains isolated from scab lesions on various tuber samples collected from Wisconsin fields; lane 11, DNA blank. (B) intergenic transcribed spacer (ITS) regions of the 16S-23S operon (~700 bp) cut with *Hpy99I* restriction enzyme if restriction target region is present (note: additional *Hpy99I* target regions may exist among *S. scabies* to produce multiple restriction fragments) (Flores-Gonzalez *et al.*, 2008). Lane 1, *S. scabies* (87-22) is *Hpy99I*+ and contains amplicon fragments ≤ 700 bp; lane 2, *S. europascabiei* (WI06-13D.2) is *Hpy99I*- due to absence of restriction site. Lanes 3-7, putative *S. scabies* isolates based on ITS + *Hpy99I.* Lane 8, putative *S. europascabiei* isolate based on ITS + *Hpy99I.*

A



B

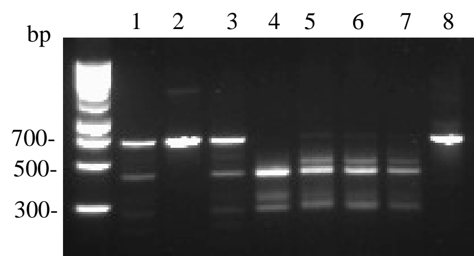
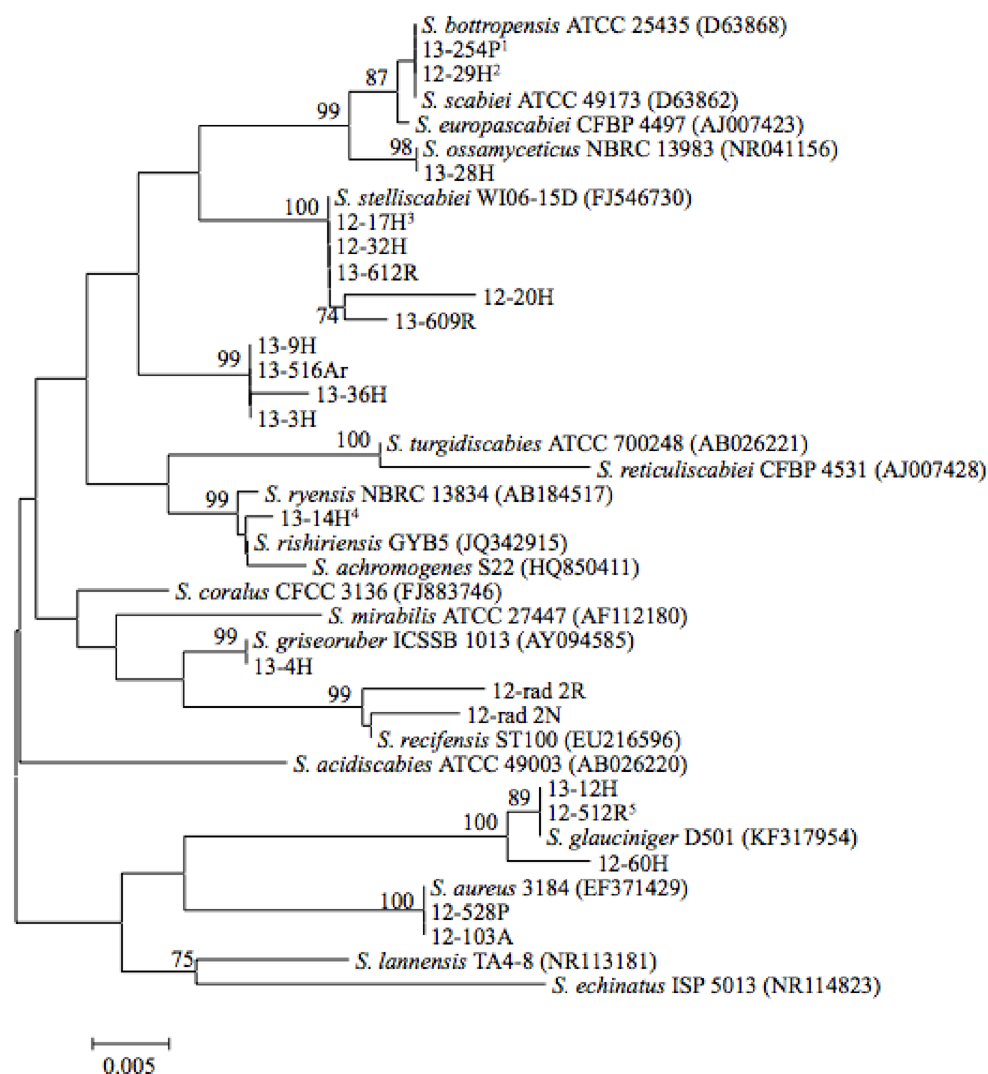


Figure 5. Phylogenetic relatedness between *Streptomyces* isolates based on neighbor-joining analysis (Saitou & Nei, 1987) of the 16S rRNA gene sequences. The sum of branch length shown = 1.28. Bootstrap values > 70 are shown and were generated from 1,000 test replicate trees with the associated taxa (Felsenstein, 1985). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).



[1] *S. bottropensis* ATCC25435 (D63868): 12-28H, 12-30H, 12-524P, 13-254P, 13-257P, 13-275P, 13-279P, 13-284P, 13-303B, 13-309B, 13-630R, 13-631R, 13-632R.
[2] *S. scabiei* ATCC49173 (D63862): 12-29H, 12-car13, 12-35H, 12-36H, 12-38H, 12-41H, 12-44H, 12-51H, 12-104A, 12-105A, 12-110A, 12-111A, 12-112A, 12-116A, 12-118A, 12-123A, 12-124A, 12-132A, 12-135A, 12-136A, 12-137A, 12-140A, 12-142A, 12-523P, 12-525P, 12-526P, 12-527P, 12-beet 2A, 12-beet 3B, 12-beet 3C, 12-beet 3D, 13-1H, 13-5H, 13-20H, 13-55H, 13-56H, 13-57H, 13-59H, 13-108A, 13-252P, 13-258P, 13-259P, 13-260P, 13-262P, 13-277P, 13-281P, 13-282P, 13-301B, 13-305B, 13-401E, 13-629R.
[3] *S. stelliscabiei* WI06-15D (FJ546730): 12-beet 1B, 12-17H, 12-18H, 12-20H, 12-32H, 12-33H, 12-40H, 12-102A, 12-119A, 12-121A, 12-125A, 12-145A, 13-603R, 13-604R, 13-613R.
[4] *S. achromogenes* S22 (HQ850411)/*S. rishiriensis* GYB5 (JQ342915)/*S. ryensis* NBRC13834 (AB184517): 12-34H, 12-128A, 12-129A, 13-2H, 13-7H, 13-14H, 13-22H, 13-24H, 13-29H, 13-34H.
[5] *S. glauciniger* D501 (KF317954): 12-512R, 12-508R.

Figure 6. Range of thaxtomin (µg/g) produced by *Streptomyces* species grown in oatmeal broth medium for 12 days at 28°C. The number of isolates within each species is indicated in parentheses.

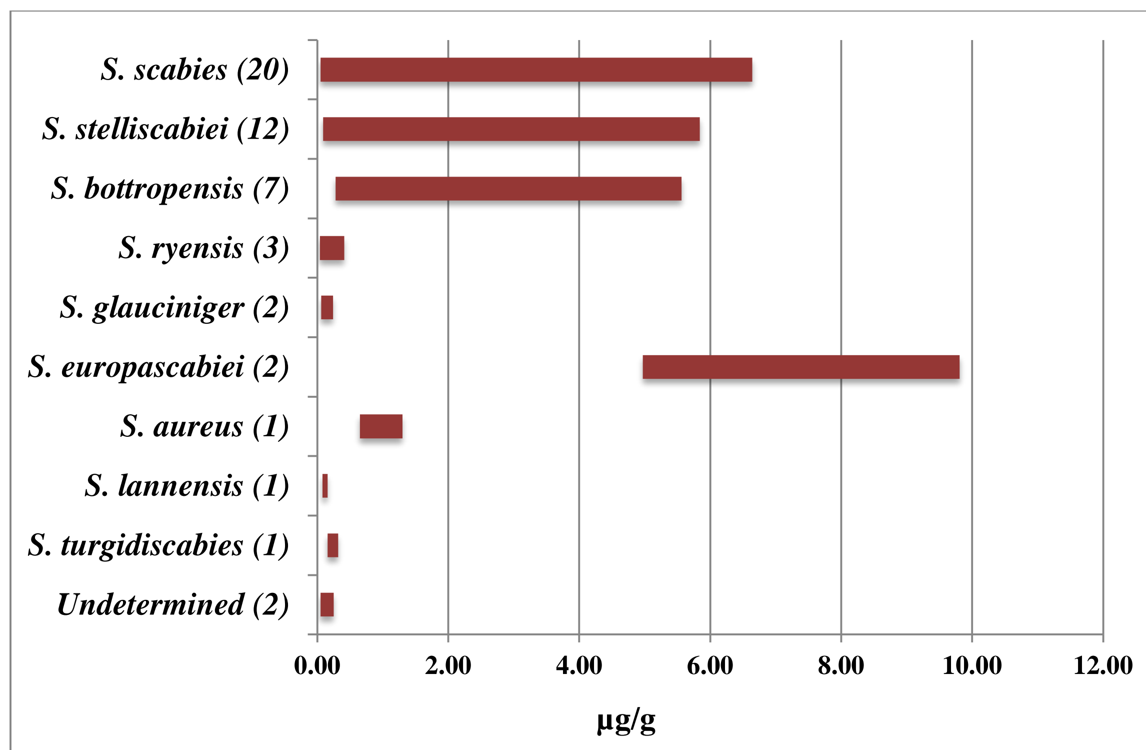
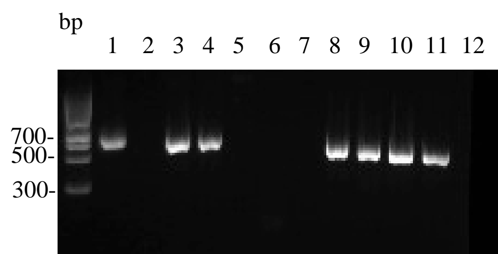


Figure 7. Electrophoresis PCR patterns from *Streptomyces* isolates screened for *nec1* (~700 bp) and *tomA* (~398 bp) amplicons and visualized on 1% Tris/acetic acid/EDTA (TAE) gel and imaged with Quantity One 1-D analysis software. Representative group of *Streptomyces* isolates showing amplification of *nec1* with Nf/Nr primers and *tomA* with Tom3/Tom4 primers. Known positive and negative control *Streptomyces* isolates were courtesy of L. Wanner, USDA. (A) Lane 1, *nec1*-positive *S. scabies* WI06-19D; lane 2, *nec1*-negative strain WI06-23B; lanes 3-4 and 8-11, *nec1*-positive *Streptomyces* strains; lanes 5-7, *nec1*-negative *Streptomyces* strains; lane 12, DNA blank. (B) Lane 1, *tomA*-positive *S. scabies* WI06-19D; lane 2, *tomA*-negative strain WI06-23B; lanes 3-5 and 7-10, *tomA*-positive *Streptomyces* strains; lane 6, *tomA*-negative *Streptomyces* strain; lane 11, DNA blank.

A



B

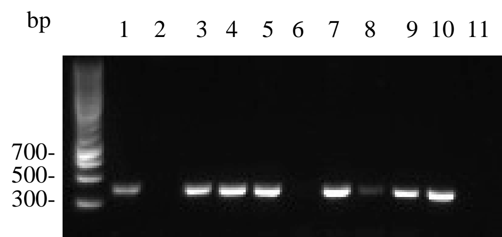
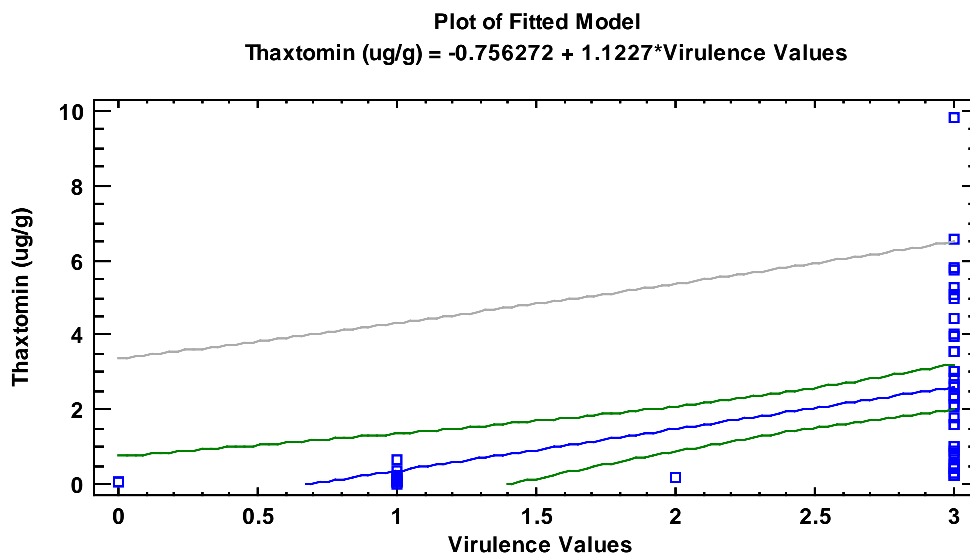


Figure 8. Linear regression analysis between quantities of thaxtomin A (µg/g) produced by *Streptomyces* strains and assigned virulence values (based on presence of *txtAB*, *nec1*, and *tomA*).



Parameter	**Least Squares Estimate**	**Standard Error**	**T Statistic**	***P-Value***
Intercept	-0.756272	0.752735	-1.0047	0.3200
Slope	1.1227	0.284906	3.94058	0.0003

Correlation Coefficient = 0.490553
R-squared = 24.0642%

Figure 9. Common scab severity on 'Yukon Gold' potatoes inoculated with 24 different strains of *Streptomyces scabies*. Mean DSVs are pooled from two replicated experiments with an insignificant effect between trials ($P = 0.6139$). Error bars represent standard error of the mean. Orange bars = isolates that originated from netted lesions; blue bars = isolates that originated from raised lesions; green bars = isolates that originated from pitted lesions.

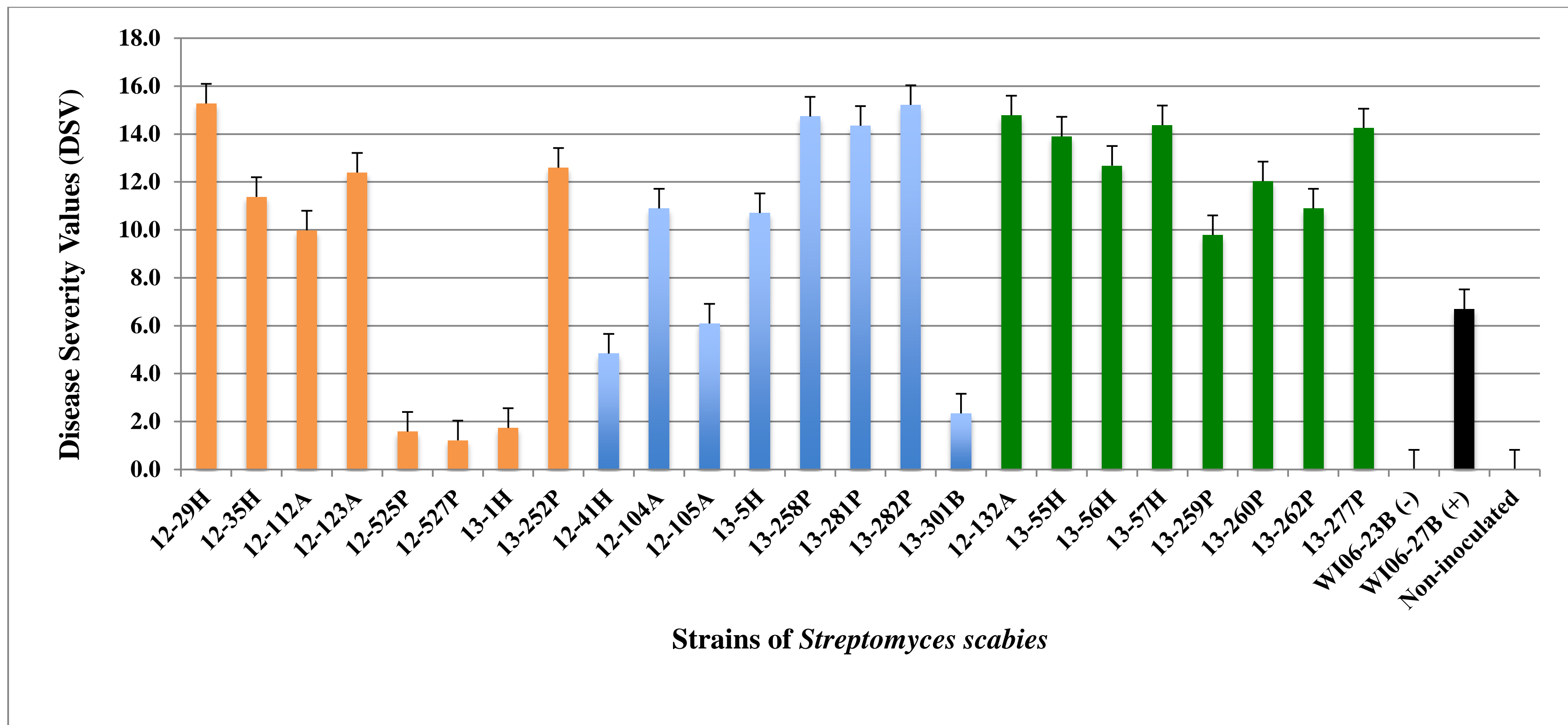
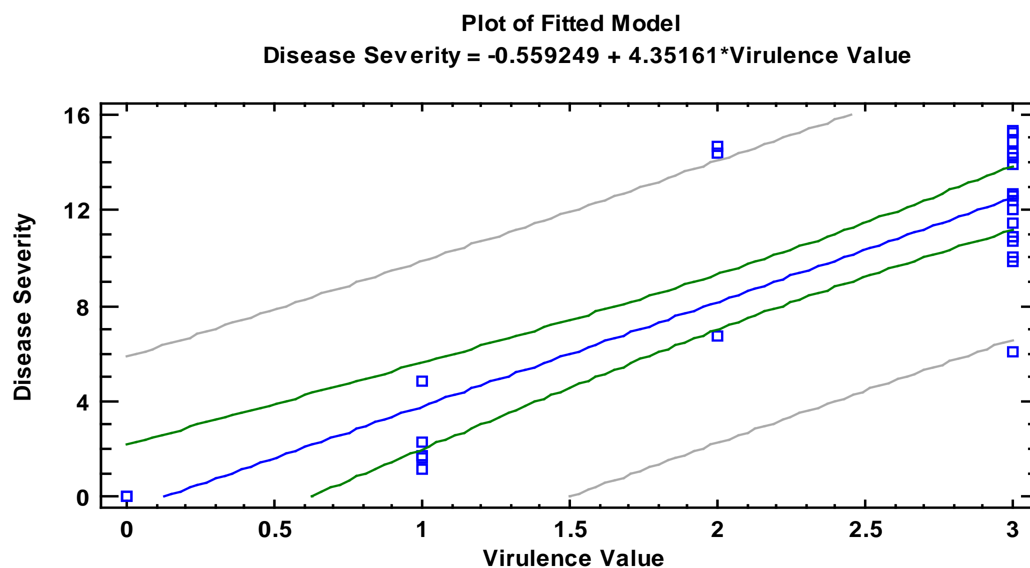


Figure 10. Common scab symptom types on ‘Yukon Gold’ potatoes inoculated with strains of *Streptomyces scabies* with differing virulence factors. (A) Healthy, asymptomatic tubers, (B) netted lesions, (C) raised lesions, and (D) pitted scab.

Figure 11. Linear regression analysis between common scab severity of 'Yukon Gold' daughter tubers and assigned virulence values based on presence of *txtAB*, *nec1*, and *tomA*.



Parameter	Least Squares Estimate	Standard Error	T Statistic	*P-Value*
Intercept	-0.559249	1.34069	-0.417135	0.6801
Slope	4.35161	0.534302	8.14448	< 0.0001

Correlation Coefficient = 0.852218
R-squared = 72.6275%

Chapter 3

Impact of seed-borne common scab on daughter tubers and plant health

Abstract

Common scab, caused primarily by the filamentous, soilborne bacterium *Streptomyces scabies*, creates significant challenges in the production of quality potato tubers in northern growing regions. Over the past several decades, substantial research has been conducted to improve our understanding of cultural and pesticide treatments for maintaining the health and yield of progeny daughter tubers. None of the previous studies, however, have addressed differences between the impact of CS from whole seed vs. cut seed pieces. A 12-week greenhouse trial was conducted with replication during 2013-2014 to assess the impact of seed type and CS severity on plant health and CS on daughter tubers. Cut and suberized 'Snowden' seed pieces (~2 oz.) exhibiting 0, 5, and ≥ 50% CS severity were sown in 3-gal pots containing a 50:50 mix of sterilized sand and field soil. Whole 'Snowden' seed (~2 oz.) with the same three levels of CS severity were sown under the same conditions. At harvest, approximately 20% of daughter tubers from asymptomatic seed pieces (both cut and whole) exhibited symptoms of CS. Disease incidence significantly increased for pieces that had 5 and ≥ 50% CS across both experimental trials. Disease severity also significantly increased on daughter tubers as the amount of CS originating on its seed piece increased. Plants grown from whole seed produced significantly more tubers, on average, when compared to cut seed. These results indicated that there is a reduction in CS on daughter tubers originating from cut seed pieces when compared to whole seed, possibly due to the reduction in CS-infested peridermal surface area. However, CS was noted on daughter tubers even when asymptomatic seed was planted, indicating that inoculum can be associated with periderm in absence of symptoms.

Introduction

Potato common scab (CS), caused primarily by the filamentous bacterium *Streptomyces scabies* (Thaxt.) Waksman and Henrici, is a serious disease affecting potato tuber quality and grade worldwide. CS is especially problematic in fresh market production areas due to visible superficial and pitted lesions (Loria *et al.*, 1997). In addition to potato, plant pathogenic *Streptomyces* species can also infect radish, carrot, beet, and other root crops (Stevenson *et al.*, 2001). In potato, tuber initiates of susceptible varieties can become infected by these soil-borne, and potentially seed-borne, bacteria which are present in most potato-producing soils (Khatri *et al.*, 2011; Pung & Cross, 2000; Wilson *et al.*, 2001). The window of susceptibility for CS infection and lesion development during the period of tuber initiation is a critical time for management if pathogenic *Streptomyces* populations are present and thriving in the rhizosphere (Khatri *et al*., 2010, 2011; Lapwood & Hering, 1970). As potato roots and stolons become established, tuber initials form on hooked stolon ends for an approximate two-week period during plant flowering (Fig. 1). The initiation stage is followed by bulking as tubers accumulate water, carbohydrates, and nutrients over the duration of the growing season (Fernie & Willmitzer, 2001; Gregory, 1956). Maturation, or tuber skin set, takes place at the end of the production season and is concurrent with vine senescence (Fernie & Willmitzer, 2001; Tyner *et al.*, 1997).

Khatri *et al.* (2011) has shown that CS is most pronounced when initial infection occurs during the tuber initiation phase and is minimized when initial infection occurs at or after maturation. As tuber initiates grow, phellem thickness and the number of cell layers increase at the infection site caused by a phytoxin produced by *S. scabies* (Khatri

et al., 2011). Common scab lesions become static once tubers reach maturity and are not known to increase in severity or produce new infections while in storage due to tuber skin set (Lulai & Freeman, 2001; Malakar-Kuenen & Tingey, 2006; Morris *et al.*, 1989; Tyner *et al.*, 1997). Cultivars having thicker periderm tissue (i.e., Russet potato types) are typically more resistant to CS, due to the rapid production of lignin in response to pathogen infection, compared to cultivars that have a thinner periderm and lower suberin content in the phellem (i.e., yellow or red round potato types) (Hammerschmidt *et al.*, 1984).

Potato cultivars are reproduced by vegetative propagation in most growing regions of the world, including North America where seed cutting and suberization are standard practices (Allen *et al.*, 1992). Seed tubers can be infected with numerous decay pathogens during the seed cutting process, which could delay emergence or reduce uniformity of plant stands (Allen *et al.*, 1992). Potato tubers that are > 2 oz. are cut and suberized prior to planting in typical seed production systems (Johnson, 1997; Nielson *et al.*, 1989). Whole, or "B," seed potatoes weighing < 2 oz. are left uncut and therefore require less labor-intensive steps by avoiding the precutting/suberization process. Cutting seed requires more preparation but is necessary for maximizing seed quantity (Johnson, 1997; Nielson *et al.*, 1989). Although seed cutting increases the risk for some diseases, there are benefits such as ease of seed-loading into planting equipment, more uniform plant stands, and increased yield (Johnson, 1997). Over the past several decades, potato growers and researchers have been interested in the impact of common scab-infected seed on yield and on the health of daughter tubers. A review of this topic by Pavlista (1996) asserted that rain and/or irrigation (Lapwood & Adams, 1975) and soil pH (McCreary,

1967) played greater roles in CS severity at harvest than the planting of CS-infected seed potatoes. This was widely accepted and corroborated by reports of CS disease pressure remaining low after multiple years of planting severely scab-infected seed in an effort to increase soil inoculum for research purposes (Adams & Hide, 1981; Houghland & Cash, 1956; Schaal, 1946). Conversely, Wilson *et al.* (1999) discussed seed treatment and tuber disinfestation work that showed significant contributions to field disease levels by seed-borne inoculum (Cairns *et al.*, 1936; Pung & Cross, 2000). Moreover, their research in 1999 established a direct relationship between disease severity in field and severity of disease on planted seed. The following year, disease-free seed pieces were overplanted in the same field and produced few CS symptoms on daughter tubers, suggesting that the previous year's seed inoculum did not contribute to overall field inoculum (Wilson *et al.*, 1999).

Recent work has focused on the impact of infected seed potatoes on CS incidence and severity on daughter tubers (Pavlista, 1996; Wang & Lazarovitz, 2005). Wang & Lazarovitz (2005) identified a direct correlation (r = 1.00) between high *Streptomyces* population densities in the root zone and disease incidence on daughter tubers. Their findings suggest that incorporation of diseased seed does impact pathogenic *Streptomyces* populations in the tubersphere even if continued planting of scab-infected seed in subsequent years does not increase overall scab pressure across the field. Surface-disinfested seed potatoes had very low populations of *Streptomyces* in the tubersphere and yielded highly marketable tubers with 0-5% CS (Wang & Lazarovits, 2005). The amount of scab on a seed piece can influence CS severity on progeny tubers within a growing season (Adams & Lapwood, 1978; Wang & Lazarovits, 2005; Wilson *et al.*,

1999). Therefore, it can be postulated that potato seed with few to no scab lesions would reduce or limit the introduction of additional inoculum into the tubersphere and result in little to no CS symptoms.

Because seed-borne inoculum is reportedly a key factor in CS symptom development (De & Sengupta, 1992, 1993; Keinath & Loria, 1991; Mishra *et al.*, 1991), and has been linked to the severity of CS on harvested tubers (Wang & Lazarovits, 2005; Wilson *et al.*, 1999), it could be proposed that whole seed potatoes may contain greater soil residue on the periderm in the absence of a cut side. If more surface area with soil residue contains inoculum, then whole seed may theoretically contain more inoculum of pathogenic *Streptomyces*. And, although cut seed requires greater pre-plant preparation and reduces the number of viable buds, or "eyes," for subsequent shoot development (Johnson, 1997), cut seed has less peridermal surface area compared to whole seed and could be introducing a lesser amount of inoculum into the rhizosphere by having less soil residue. To date, research has not addressed differences between the impacts of CS severity on agronomic traits of potatoes planted from either whole seed or cut seed pieces. The objective of this study was to investigate the influence of cut versus whole seed and seed-borne *Streptomyces* inoculum on plant health (emergence, canopy height, shoot number, and tuber number) and CS incidence and severity on daughter tubers in a controlled environment.

Materials & Methods

Seed potato source and experimental design

'Snowden' seed potatoes were obtained from a seed potato grower-cooperator in Rhinelander, WI. 'Snowden' is recognized as having moderate susceptibility to CS (Navarro, 2009). Whole seed potato tubers and cut seed pieces were categorized as 1) having no visible disease, 2) ≤ 5% surface coverage of common scab, and 3) ≥ 50% surface coverage of common scab (Table 1). A sand-soil mixture (50:50) was homogenized and autoclaved for 2.5 hours. Soil samples were randomly selected from a 24-pot experimental setup and plated on solid water, oatmeal (OMA), and yeast malt extract (YME) agar in order to verify the absence of viable *Streptomyces* from the soil sterilization process.

Six treatments with eight replications resulted in 48, 3-gal potted potato plants arranged in a completely randomized design. Twenty-four, 6-inch diameter plastic pots were sown with cut and suberized seed pieces in the soil-sand mixture previously described; the remaining 24 pots were sown with uncut B-sized seed. The study was conducted at the Walnut Street Greenhouse facility at the University of Wisconsin-Madison and was repeated. Both studies were maintained for approximately 12 weeks.

To prevent soil loss, cheesecloth was used to line the inner bottoms of pots. Each plant received approximately 500 ml of water every 4-5 days. The soil was allowed to fully dry between irrigation events to promote suitable conditions for CS development on developing tubers. The greenhouse was maintained at the ambient temperature range of 22-24°C and was subjected to a 14-hr photoperiod to simulate seasonal field conditions.

Plants were fertilized with Vigoro's 12-5-7 all-purpose plant food (Swiss Farms Products, Las Vegas, NV) at 30 and 60 days post-planting.

Evaluation parameters

Each plant was assessed for emergence, plant height, and number of shoots at 30, 60, and 90 days post-trial initiation (PTI). The total number of progeny tubers, yield (g), CS incidence (% of tubers with symptoms), and disease severity were measured at 90 days PTI. Disease severity ratings were calculated by using methods described by Wanner (2013), where lesion coverage (%) is multiplied by values assigned to lesions based on type and size (Table 2). Tubers of a size > 0.5 cm were accounted for in daughter tuber production, yield, and scab ratings. Tubers < 0.5 cm were omitted from evaluation. Multiple comparison procedures were used to identify statistically significant differences between treatment level means according to Tukey's honest significant difference (HSD) method at the 95.0% confidence level. Analysis of variance was used to determine if rating parameters from the two replicated trials were significantly different at the 95.0% confidence level. Data from the two trials were pooled if found to be highly similar (insignificantly different). Two-way analysis of variance (MANOVA) was also used to determine statistical differences among treatments with the main effects of initial inoculum level on seed (0, 5, or ≥ 50%) and of the seed type (cut or whole) as well as interaction effects between these variables at the 95.0% confidence level.

Results

No *Streptomyces* spp. colonies were observed on any agar media plates that contained samples of sterilized soil after 14 days of incubation. Plants originating from cut seed pieces with either 5 or ≥ 50% CS emerged 5-6 days earlier than those originating from whole seed, but not to a statistical degree ($P = 0.4715$). Of the cut seed treatments, plants emerged from asymptomatic seed 22 days PTI. Plants growing from seed of 5 and ≥ 50% CS emerged at 16 and 18 days, respectively. Plants originating from whole seed with 0, 5, and 50% CS emerged at 20, 22, and 24 days PTI, respectively.

Plant height, recorded at 30-day intervals, showed consistent increase across treatments (data not shown). At 30 days, plants ranged from 3.7 to 6.4 cm, with the shortest being those originating from cut and whole seed with ≥ 50% CS. At 60 days, plant height ranged from 10.8 to 13 cm; at 90 days, plant height increased to a range of 14.3 to 17.8 cm with the tallest plants belonging to asymptomatic cut and whole seed (17.8 and 17.1 cm, respectively), whole seed with 5% CS (16.9 cm), and whole seed with ≥ 50% CS (17.2 cm). No significant differences in plant height were observed among treatment levels at the 95% confidence level ($P = 0.2875$).

Shoot counts were taken at the final rating date prior to harvest (90 days PTI). While plant ratings reveal that shoot numbers ranged from 0 to 5 (data not shown), means among treatments show subtle differences among plants originating from different seed inoculum levels or from seed type ($P = 0.1285$). Averages became skewed when non-emerged plants were included, adjusting mean shoot counts to fall between 1.3 and 1.8.

Harvested plants were assessed for tuber production at 90 days PTI (Fig. 2). Numeric tuber data were pooled from two replicate trials based on an insignificant trial

effect according to analysis of variance ($P = 0.3470$). A two-way, multivariate analysis of variance (MANOVA) of mean number of tubers produced per plant resulted in a statistically significant difference between treatment levels based on seed type only ($P = 0.0015$) at the 95% confidence level (Table 3). Plants originating from whole seed produced significantly more daughter tubers compared to the cut seed treatments. Plants originating from whole seed produced ~6 tubers per plant, while plants originating from cut seed produced ~4.8 tubers per plant. An insignificant effect of initial inoculum (0, 5, and ≥ 50% CS) on tuber production was determined according to Tukey's HSD ($P = 0.5411$).

Yield values were assessed at 90 days PTI and were generally low due to early harvest of tubers (Fig. 3). Yield data from each trial are presented individually based on analysis of variance of trial effects that were significantly different ($P = 0.0085$). Two-way multivariate analysis of yield data from both replicated trials produced statistical differences ($P = 0.0319$ and $P = 0.0065$, respectively) between treatment levels based on seed type only (Table 3). Yield was significantly higher among the whole seed treatments when compared to cut seed treatments across both trials. Yield from whole seed treatments in trial 1 had a 12% yield gain compared to cut seed treatments. In trial 2, yield from whole seed treatments had a 6% yield gain compared to cut seed treatments. An insignificant effect of initial inoculum (0, 5, and ≥ 50% CS) on yield was determined for trials 1 and 2 according to Tukey's HSD ($P = 0.4626$ and $P = 0.6770$, respectively).

Common scab symptoms, quantified as percent disease incidence, were observed on progeny tubers from all treatments (Fig. 4). Disease incidence data from each trial are presented individually based on analysis of variance of trial effects that were significantly

different ($P = 0.0383$). Two-way multivariate analysis of disease incidence data from both replicated trials produced statistical differences ($P = 0.0014$ and $P = 0.0012$, respectively) between treatment levels based on initial inoculum only (Table 4). At the 12-week harvest date for each trial, approximately 23 and 18%, respectively, of daughter tubers that originated from asymptomatic seed showed CS symptoms. Disease incidence was nearly 47 and 32%, respectively, on daughter tubers when originating from seed with 5% initial inoculum. Of the daughter tubers produced from seed pieces with ≥ 50% initial inoculum, 49 and 42%, respectively, showed CS. Seed treaments with 5 and ≥ 50% initial inoculum produced daughter tubers with significantly more CS than asymptomatic seed in both trials.

Calculated disease severity values (previously described) for each treatment were pooled from two replicate trials based on an insignificant trial effect according to analysis of variance ($P = 0.2997$). Data were transformed (sq. root) to stabilize the variance among the irregular standard deviations (Table 4). A two-way, multivariate analysis of variance (MANOVA) of CS severity resulted in a statistically significant difference between treatment levels based on initial inoculum only ($P = 0.0079$). Asymptomatic seed produced tubers with the least amount of CS with a mean severity value that was statistically less than treatments with 5 and ≥ 50% initial inoculum (Fig. 5). CS severity increased as the amount of initial inoculum on its seed piece increased. As a result, seed that was planted with 5% initial inoculum produced tubers with 68% more CS severity than treatments that were planted with asymptomatic tubers (Fig. 6). Seed planted with ≥ 50% initial inoculum produced tubers with 75% more CS severity than treatments with no initial inoculum on seed pieces. The range of severity values (non-transformed) per

treatment is shown (Table 5). With the highest possible calculated severity value being 25, the severity values across two replicated trials from asymptomatic cut seed ranged from 0.0 to 2.9 and from asymptomatic whole seed ranged from 0.0 to 4.0. Cut and whole seed with 5% CS ranged from 0.2 to 8.5 and 0.0 to 8.3, respectively. Seed with ≥ 50% CS produced daughter tubers with CS severity that ranged from 0.8 to 3.8 for cut seed and from 0.3 to 5.0 for whole seed. The average ranking of these severity ranges for each treatment level was calculated from the median values using the Kruskal-Wallis test (Table 6). Based on the resulting P-value=0.01537, there were statistically significant differences among the medians (Fig. 7).

Discussion

My data reinforced the importance of seed potatoes as a source of *Streptomyces* spp. inoculum, as previously reported (Khatri *et al.*, 2011; Singh *et al.*, 1987; Wang & Lazarovits, 2004, 2005; Wilson *et al.*, 1999). I observed a significant effect of initial seed inoculum (0, 5, and ≥ 50%) on daughter tuber common scab. The severity of CS on seed potatoes, rather than cut or whole seed status, had the greatest influence on the resulting CS symptom expression. Roughly 20% of the daughter tubers generated from asymptomatic seed expressed disease symptoms. This result is not entirely unexpected due to the fact that hyphal-like growth and spores of pathogenic *Streptomyces* species can be found in soil debris in the tubersphere and on tuber periderm of even asymptomatic tubers (Khatri *et al.*, 2011; Loria *et al.*, 1997; Wang & Lazarovits, 2005; Wanner, 2009). Symptomatic tubers can harbor and contribute a greater quantity of inoculum than those without symptoms, as illustrated by my data which showed that when seed pieces increased in CS severity (5 and ≥ 50%), daughter tubers had increased CS incidence; this association was also determined by Wilson *et al.* (1999). Furthermore, seed with ≥ 50% CS has the potential to introduce a high level of inoculum into the rhizosphere, possibly causing tissue necrosis of stolons prior to tuber initiation contributing to reduced shoot and tuber production (Loria *et al.*, 1997; Wang & Lazarovits, 2005; Wilson *et al.*, 1999).

I observed a significant seed-type effect on tuber production. Because cut seed reportedly increase tuber quantity and yield (Johnson, 1997; Nielson *et al.*, 1989), our results were unexpected. My work showed an increase in tuber production from whole seed, which may have occurred due to a higher number of viable growth points per seed piece when compared to cut seed (Iritani, 1968). Even a small percentage of periderm

surface covered in CS lesions can limit the number of viable eyes, reducing plant stem and/or stolon number and subsequent tuber development (Adams & Hide, 1981). In combination with cut seed pieces, these characteristics could be responsible for the significant decrease in tuber quantity and yield that were produced from cut seed pieces compared to whole seed with the same amount of initial inoculum.

Overall, my data showed that whole seed produced tubers with greater CS incidence and severity when compared with tubers produced from cut seed pieces. This further suggests that whole seed may be infected and/or infested with more *Streptomyces* inoculum compared to cut seed with its reduced peridermal surface area, based on a previous study of periderm-associated inoculum (Wang & Lazarovits, 2005). My observations did not indicate a significant advantage in planting cut vs. whole seed with 0-5% visible CS when considering overall plant health in terms of emergence, development of shoots, and plant height. Because CS was detected on daughter tubers grown from asymptomatic seed, the potential exists for yield losses to occur on tubers produced with asymptomatic seed that originated from soils with populations of plant pathogenic *Streptomyces* species. Similar incidences of CS on daughter tubers from asymptomatic seed have been corroborated (Wang & Lazarovits, 2005; Wanner, 2006; Wilson *et al.*, 1999). Using seed potatoes with reduced peridermal surface area could minimize the introduction of pathogenic *Streptomyces* species in the rhizosphere, thereby reducing the severity of CS on the progeny tubers.

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Table 1. Description of seed treatments in 'Snowden' seed potato inoculum experiment during 2013-2014. Cut seed pieces were approximately 2-oz. in size and were suberized at 22°C for 36 hours prior to planting. Seed stock with common scab symptoms exhibited raised and/or pitted lesion types.

Trt number	**Diseased surface (%)**	**Cut seed pieces**	**Whole or 'B' seed pieces**
1	0; asymptomatic	+	**--**
2	≤ 5%	+	**--**
3	≥ 50%	+	**--**
4	0; asymptomatic	**--**	+
5	≤ 5%	**--**	+
6	≥ 50%	**--**	+

Table 2. Disease scale for determining severity of CS on harvested daughter tubers as adapted from Wanner, L. A. (2013). The overall severity rating is determined by multiplying the disease score that corresponds to tuber coverage (%) by the disease score that corresponds to lesion description (type and size).

Common scab severity			**Common scab lesion phenotype**		**Disease severity rating**
Disease score (Factor)	**Tuber coverage (%)**		**Disease score (Factor)**	**Lesion description**	
0	No scab		0	None	
1	1-10 %	**X**	1	Small superficial lesions, < 5 mm	**Product**
2	11-25%		2	Large, coalescing superficial lesions, > 5 mm	
3	26-50%		3	Small raised lesions, < 5 mm	
4	51-75%		4	Large, coalescing raised lesions, > 5 mm	
5	> 75%		5	Pitted lesions	

Table 3. Multivariate analysis of variance to determine two-way effects of initial seed inoculum and seed type with respect to mean number of tubers produced per plant and yield (at 90 days post-trial initiation). Output is from two replicate greenhouse trials. Based on insignificant trial effects, tuber data were pooled for MANOVA analyses.

Source	**Type III Sums of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
Number of tubers					
Main Effects					
Initial inoculum	1.96875	2	0.984375	0.62	0.5411
Seed type	18.1302	1	18.1302	11.48	0.0015
Interactions					
Initial inoc. x Seed type	8.76042	2	4.38021	2.77	0.0739
Residual	66.3437	42	1.57961		
Total (Corr.)	95.2031	47			
Yield (Trial 1)					
Main Effects					
Initial inoculum	9.82488	2	4.91244	0.79	0.4626
Seed type	31.0666	1	31.0666	4.98	0.0319
Interactions					
Initial inoc. x Seed type	8.5057	2	4.25285	0.68	0.5121
Residual	224.526	36	6.23682		
Total (Corr.)	275.288	41			
Yield (Trial 2)					
Main Effects					
Initial inoculum	0.695012	2	0.347506	0.39	0.6770
Seed type	7.23131	1	7.23131	8.19	0.0065
Interactions					
Initial inoc. x Seed type	0.157213	2	0.0786064	0.09	0.9150
Residual	37.071	42	0.882643		
Total (Corr.)	45.1545	47			

Table 4. Multivariate analysis of variance to determine two-way effects of initial seed inoculum and seed type with respect to common scab incidence and severity. Output is from two replicate greenhouse trials. Based on insignificant trial effects, common scab severity data were pooled for MANOVA analyses.

Source	**Type III Sums of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
CS Incidence (Trial 1)					
Main Effects					
Initial inoculum	5667.12	2	2833.56	7.97	0.0014
Seed type	1427.63	1	1427.63	4.02	0.0526
Interactions					
Initial inoc. x Seed type	1819.62	2	909.811	2.56	0.0913
Residual	12795.7	36	355.436		
Total (Corr.)	22594.0	41			
CS Incidence (Trial 2)					
Main Effects					
Initial inoculum	4673.45	2	2336.73	7.97	0.0012
Seed type	16.6852	1	16.6852	0.06	0.8126
Interactions					
Initial inoc. x Seed type	2.70542	2	1.35271	0.00	0.9954
Residual	12317.7	42	293.278		
Total (Corr.)	17010.5	47			
Severity					
Main Effects					
Initial inoculum	0.759378	2	0.379689	5.44	0.0079
Seed type	0.0468019	1	0.0468019	0.67	0.4174
Interactions					
Initial inoc. x Seed type	0.0845711	2	0.0422856	0.61	0.5502
Residual	2.93038	42	0.069771		
Total (Corr.)	3.82113	47			

Table 5. Disease severity ranges on 'Snowden' tubers were analyzed to demonstrate the irregular level of common scab that can occur when originating from seed pieces with varying levels of disease. The average ranking was calculated from the medians using the Kruskal-Wallis test. Based on the P-value=0.0154, there are significant differences among the medians at the 95% confidence level.

Treatments		Experiment 1		Experiment 2		Average Rank
		Min. Severity	Max. Severity	Min. Severity	Max. Severity	
1	Cut seed; Asymptomatic	0.0	0.6	0.0	2.9	10.13
2	Cut seed; 5% CS	0.5	8.5	0.2	4.0	26.75
3	Cut seed; ≥ 50% CS	0.8	2.5	1.2	3.8	32.63
4	Whole seed; Asymptomatic	0.0	3.8	0.0	4.0	19.75
5	Whole seed; 5% CS	0.7	3.0	0.0	8.3	27.25
6	Whole seed; ≥ 50% CS	0.3	2.7	0.5	5.0	30.5

Table 6. Kruskal-Wallis test for severity ranges on 'Snowden' tubers.

Trt	Sample Size	Average Rank
1	8	10.125
2	8	26.75
3	8	32.625
4	8	19.75
5	8	27.25
6	8	30.5

Test statistic=14.0375 P-value=0.01537

Figure 1. Tuberization stages from early initiate with a hooked end (far left) and subsequent bulking from accumulation of nutrients and water.

Figure 2. Mean number of ‘Snowden’ daughter tubers produced per plant at 90 days post-planting in response to varying levels of initial *Streptomyces* inoculum and cut vs. whole seed status. A two-way, multivariate analysis of variance (MANOVA) was used on pooled data from two replicated trials. A statistically significant difference was observed between seed type at planting using Tukey’s HSD (P = 0.05), indicated by bars without the same letter.

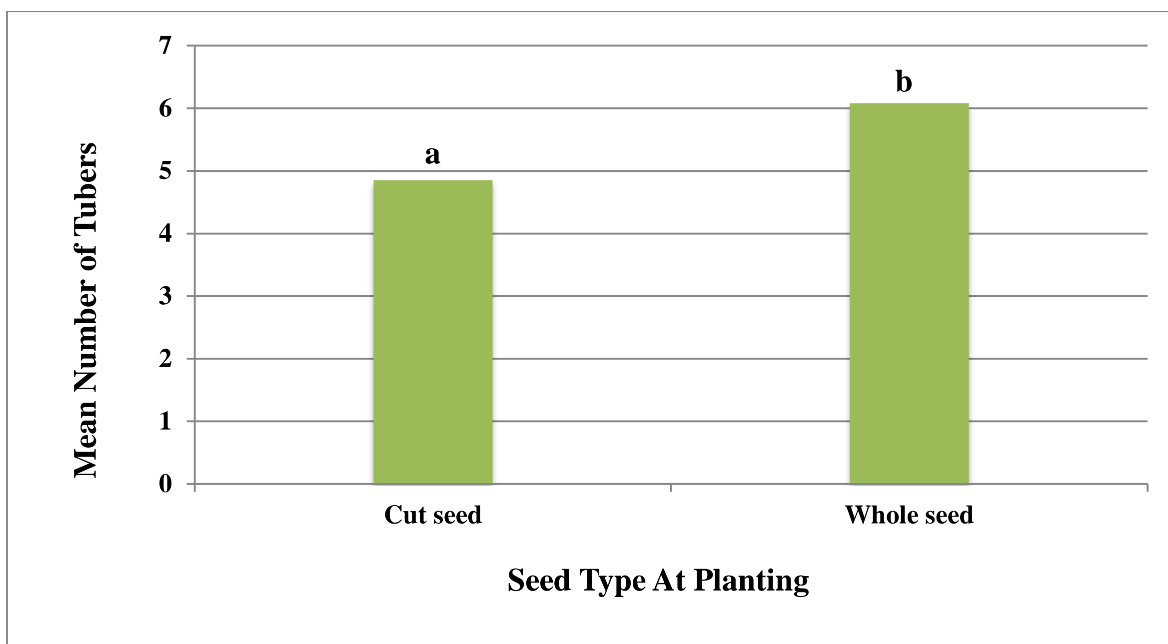


Figure 3. Yield values of 'Snowden' daughter tubers produced at 90 days post-planting in response to varying levels of of initial *Streptomyces* inoculum and cut vs. whole seed status. A two-way, multivariate analysis of variance (MANOVA) was used on each of two replicate trials. Statistically significant differences were observed between seed type using Tukey's HSD (P = 0.05), indicated by bars without the same letter.

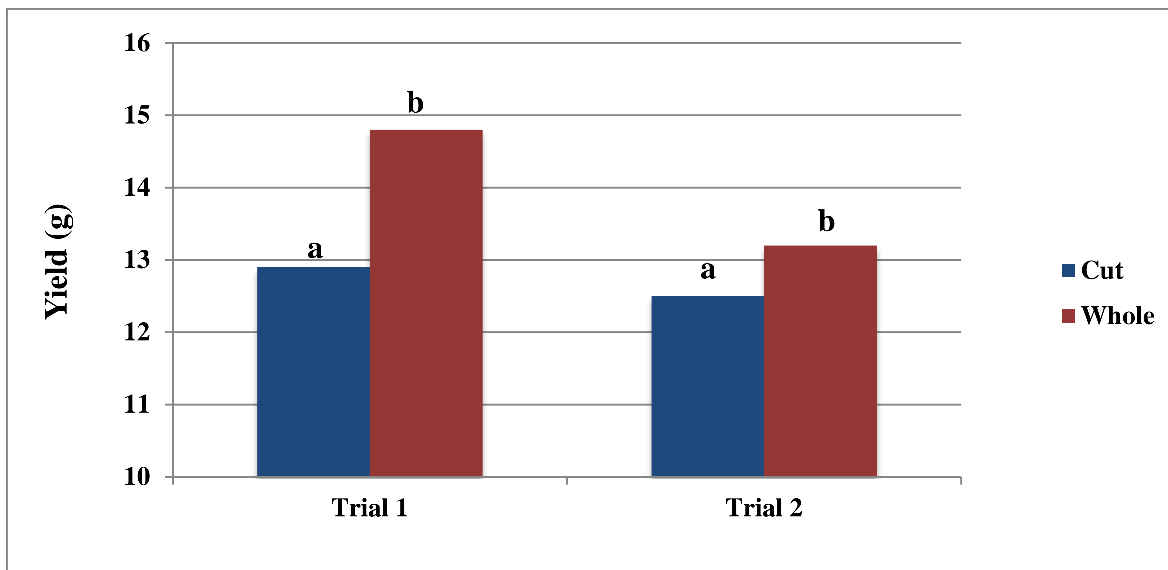


Figure 4. Common scab incidence ratings (%) on 'Snowden' daughter tubers produced at 90 days post-planting in response to varying levels of of initial *Streptomyces* inoculum and cut vs. whole seed status. A two-way, multivariate analysis of variance (MANOVA) was used on each of two replicate trials. Statistically significant differences were observed on seed with 0% initial inoculum according to Tukey's HSD (P = 0.05), indicated by bars without the same letter.

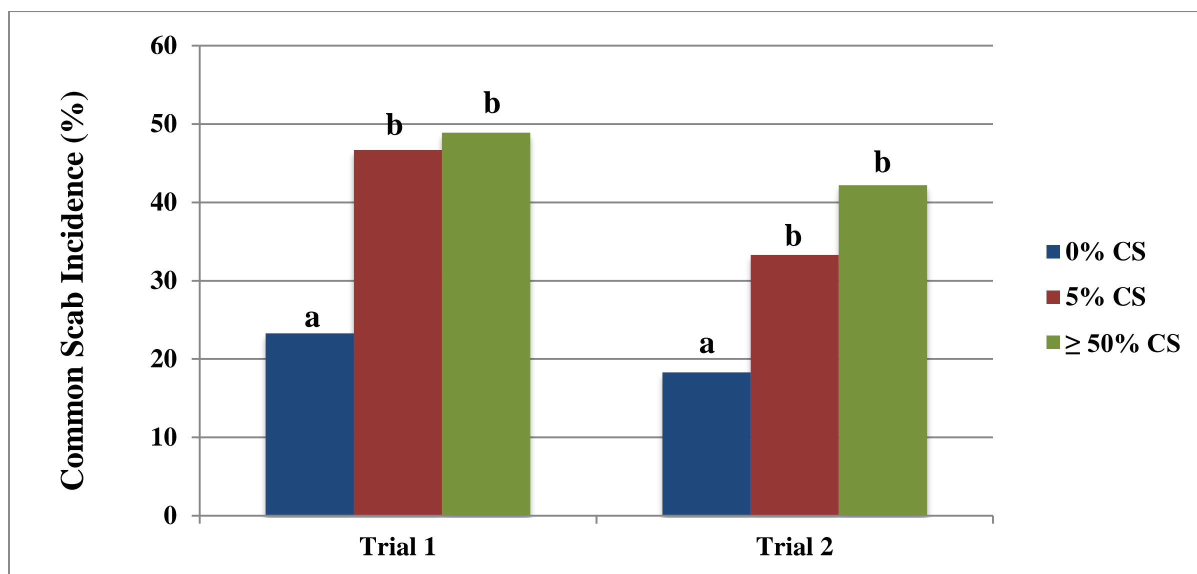


Figure 5. Common scab disease severity on ‘Snowden’ daughter tubers produced at 90 days post-planting in response to varying levels of of initial *Streptomyces* inoculum and cut vs. whole seed status. A two-way, multivariate analysis of variance (MANOVA) was used on pooled data from two replicate trials. Disease severity data were transformed by taking square root of the values in order to remove any dependence of the standard deviation on the mean. A statistically significant difference was observed on seed with 0% initial inoculum according to Tukey’s HSD (P = 0.05), indicated by bars without the same letter.

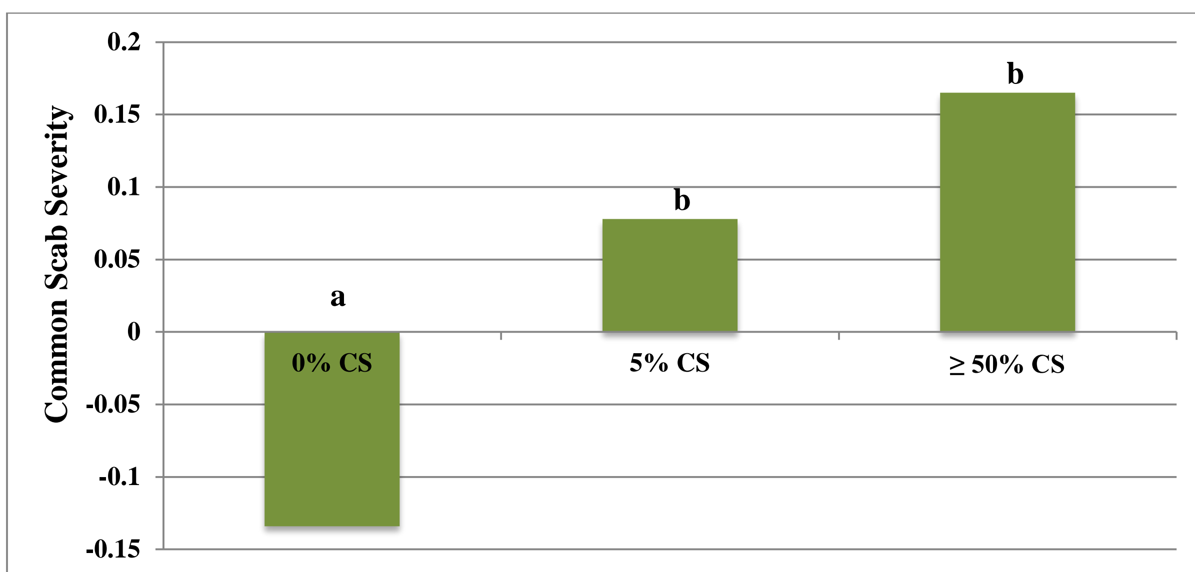


Figure 6. Levels of common scab severity on ‘Snowden’ tubers originating from seed pieces with 5% common scab grown under controlled conditions in a greenhouse on the campus of University of Wisconsin-Madison.

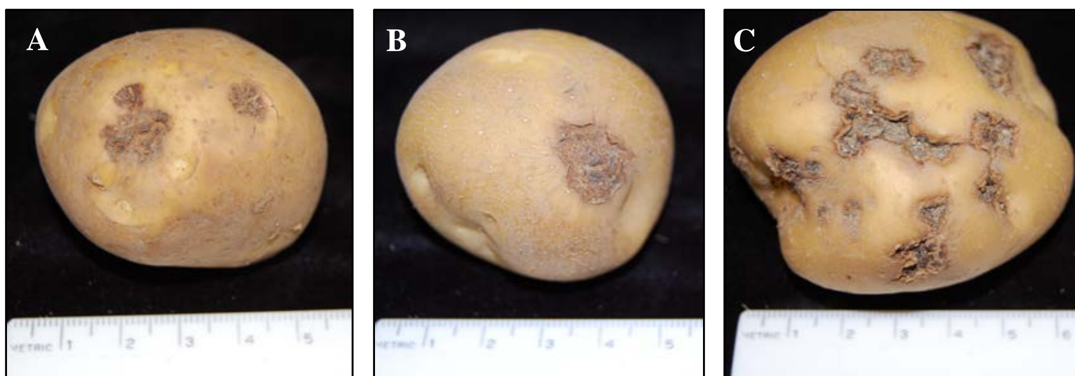
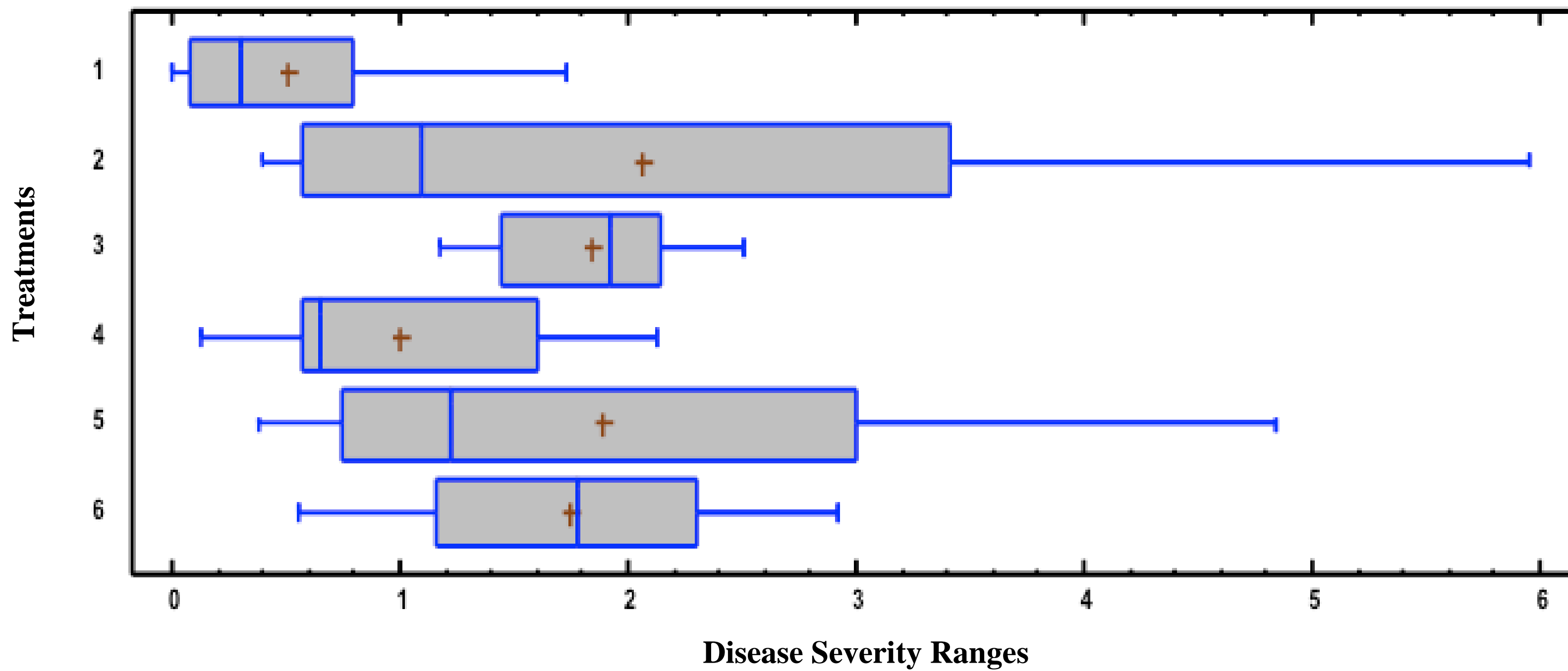


Figure 7. Box-and-whisker plot of CS disease severity ranges on 'Snowden' tubers. Vertical blue lines show the median value for each treatment. Boxes with red hash marks that do not overlap indicate treatment medians that are statistically different at each treatment level (P = 0.05). Error bars indicate standard deviation.



Chapter 4

Evaluating fumigation and at-plant treatments for the control of potato common scab in Wisconsin

Abstract

Potato common scab (CS) is a persistent disease that affects potatoes in most growing regions worldwide. Caused primarily by the soil-borne bacterium, *Streptomyces scabies*, this disease causes surface scab lesions and deep pits that render tubers unmarketable. Individually, cultural management approaches provide inconsistent control of CS. Integration of multiple strategies including cultivar resistance, careful irrigation management at tuberization, maintenance of soil pH below 5.2, soil fumigation, and at-plant fungicides have been inconsistently effective in mitigating losses to common scab. From 2009 to 2014, I evaluated fumigation and at-plant treatments for control of CS on susceptible 'Yukon Gold' in a scab disease nursery in Antigo, Wisconsin. Tubers were assessed for yield and disease severity using a scab index severity scale. Microbial-based biopesticide treatments did not statistically limit CS severity over four trial years (2009-2012), with the exception of *Bacillus subtilis* (Serenade Soil®) in 2010. Microbial-based biopesticide treatment yields above the untreated control were observed some years, but were not significant or reproducible. Biochemical biopesticides tested in 2011, 2012, and 2014 did not provide significant CS control except for elemental sulphur (TigerSul®) in 2011. Yields from the biochemical biopesticides were at or below yields from the untreated control plots. The variable performance of microbial biopesticides may be caused by the biological nature of the active ingredients, interactions with soil microbiota, and the flux of environmental conditions experienced between trialing years. Nematicide applications in 2014 did not significantly impact CS severity and produced yields that were numerically less than the untreated control. My data indicated that metam sodium, chloropicrin, and PCNB gave increased yield and quality with reduced

CS severity. Based on the reduction in CS severity and the increased yield in 2011, metam sodium could be effective in managing multiple potato pathogens that includes pathogenic *Streptomyces*. Chloropicrin provided CS suppression and improved yield with relative consistency.

Introduction

Potato producers worldwide face challenges with the control of common scab (CS), a disease that reduces crop yield and quality (Abdel-Rahman *et al.*, 2012; Bouchek-Mechiche *et al.*, 2000; Hill & Lazarovits, 2005; Loria *et al.*, 1997; Loria *et al.*, 2006; Park *et al.*, 2003; St-Onge *et al.*, 2008; Tanaka, 2004; Wanner, 2004; Zhao *et al.*, 2008). Potato tubers with extensive netted, raised, or pitted scab lesions are often unmarketable and are typically rejected for fresh market and seed sales (Delahaut & Stevenson, 2009; Hill & Lazarovits, 2007; Stevenson *et al.*, 2001). Of the major potato producing states, Wisconsin is ranked third with an annual farm gate value of $256 million produced on over 64,000 acres and marketed for tablestock, processing, and seed purposes (NASS, 2013). Although the economic impact of potato CS in the U.S. is not well documented, yield losses due to CS in Canada and other potato-producing countries range between $15 and 17 million annually (Dees & Wanner, 2012; Hill & Lazarovits, 2007). In 2002, it was estimated that CS-associated economic losses affected 82% of potato fields in the U.S. (Wanner & Kirk, 2015). Although CS has been a highly variable disease concern from year-to-year, it has become increasingly important for Wisconsin and Upper Midwestern producers due to one or more of the following factors: less land to commit to longer crop rotations, increased strain diversity and aggressiveness (Wanner, 2009), species variants that may transfer pathogenicity genes (Lerat *et al.*, 2009), increased spread from infected seed (Wilson *et al.*, 1999), and limited chemical or cultural management practices (Dees *et al.*, 2013).

Gram-positive, filamentous bacteria belonging to the *Streptomyces* genus cause this soil-borne disease (Loria, 2003). Of the several hundred species belonging to this

genus, only a small number are able to infect developing potato tubers and tap roots of radish, parsnip, carrot, and beet (Goyer & Beaulieu, 1997) (Fig.1). *Streptomyces scabies* has long been considered the primary causal agent until more recent genotyping has revealed that multiple species can be responsible for causing CS depending on geographic region, soil microbial populations, and soil pH (Bukhalid *et al.*, 1998; Clark *et al.*, 1998; Edwards *et al.*, 1989; Flores-Gonzalez *et al.*, 2008; Kreuze *et al.*, 1999; Lehtonen *et al.*, 2004; Song *et al.*, 2004; Wanner, 2006).

Potato tubers become infected by *Streptomyces* species during the tuber initiation phase. Symptoms develop into three distinct lesion types as tubers develop: russeted superficial lesions (Fig. 1B), raised corky patches that are erumpent (Fig. 1A), and pitted scabs that can vary from shallow to deep pits (Fig. 1A). Management strategies to control CS have utilized an integrated approach that includes cultivar resistance, management of water during tuberization (dry soil promotes disease), management of soil pH to ≤ 5.2, and pesticides. However, these individual or combined approaches provide inconsistent control of CS due to the dynamics of *Streptomyces* populations as a result of soil type, soil moisture, microbial activity, environmental fluctuations, and field history (Wanner, 2009; Wilson *et al.*, 2001). Pesticides including pentachloronitrobenzene, or PCNB (Blocker®), applied as a pre-plant soil treatment, and chloropicrin, applied as a soil fumigant prior to planting, have a history of use with some success in other regions. However, no treatment has consistently and effectively reduced CS (Dees & Wanner, 2012; Meng *et al.*, 2012; Wilson *et al.*, 1999, 2001). Some microbial-based biopesticides contain beneficial microorganisms that inhabit the soil and have been shown to provide

some CS reduction in controlled environments (Dees & Wanner, 2012). However, such biopesticides require further investigation in a field setting and under different soil types.

With the 2005 U.S. Environmental Protection Agency ban on methyl bromide use, there has been increased interest in chloropicrin as an alternative fumigant and as an enhancement to other fumigants, such as metam sodium, for fungal disease control in potato and vegetable systems (Hutchinson, 2005; Sydorovych et al., 2008). Chloropicrin was first registered as a broad-spectrum soil fumigant in the U.S. in 1975, but has been used specifically for nematode control since 1919 (Matthews, 1920). The fumigant was typically formulated with methyl bromide due to its effectiveness as a warning agent to reduce risk exposure (Duniway, 2002). Soil fumigation with chloropicrin-containing products is costly and less favored due to potential negative environmental and human health impacts (Dees & Wanner, 2012; Loria *et al.*, 1997; Sunseri, 2010). However, in recent years, commercial potato producers in Wisconsin have been interested in better understanding the impact of this fumigant, especially against soilborne diseases such as common scab, early dying disease, and powdery scab (Webster *et al.*, 2013).

My research aimed to evaluate all categories of pesticides for possible efficacy that included biopesticides (microbial-based and biochemical), conventional fungicides, nematicides, insecticides, and fumigants for the control of potato CS in a potato production field in Northern Wisconsin. The goal of this work was to provide data to further develop an integrated management program to preserve quality potato production while sustaining healthy soil and environmental sensitivity in Wisconsin and the Upper Midwestern region of the U.S.

Materials & Methods

This multi-year study was conducted during 2009 to 2014 in two CS field nurseries located at the University of Wisconsin Extension Langlade County Airport Research Station in Antigo, WI. Trials during the 2013-2014 growing seasons were conducted in a different scab nursery field from previous trial years. Fumigants were applied in the fall preceding the trial year, as standard for the commercial production region due to the limited window of moderate weather prior to potato planting in the spring. The remaining biopesticide, conventional fungicide, and nematicide treatments were applied in-furrow during planting of CS-susceptible 'Yukon Gold.' Nematicide treatments were also applied as foliar applications in some years (Table 2).

Approximately 2-oz seed pieces were cut mechanically from US#1 tubers and allowed to suberize prior to planting. A randomized complete block design with four replications was established in each trial year. Treatment plots consisted of four rows that were 20- to 40-ft long and spaced 36 in. apart with 12-in plant spacing within rows. Seed pieces were mechanically set into the open furrow. Immediately following seed set, treatments were applied with a CO_2 backpack sprayer equipped with a single TeeJet 8002VS flat fan nozzle calibrated to deliver 12 gal/A at a boom pressure of 40 psi into the open furrow on seed pieces and surrounding soil. Hilling discs were used to mechanically close the furrows immediately following pesticide application. To minimize soil compaction and damage to plants in rows used for foliar and yield evaluation, drive rows for pesticide application equipment were created alongside treatment plots.

In each trial year, the center two rows of each plot were harvested and graded into marketable (US#1, > 4-oz tuber, or 'A'), undersize (US#2, < 4-oz tuber, or 'B'), and cull

categories to determine overall yield. After undersized tubers were graded out, all remaining tubers were washed and 20 were arbitrarily selected for disease severity assessment. A disease severity value, or DSV, was determined using the following rating scale: 0 = no disease, 1 = 0-10% scabbed surface, 2 = 10-25% scabbed surface, and 3 = > 25% surface scab and/or pits present. An overall symptom severity index was calculated for each plot using the equation below, where the number of sampled tubers that fell into each class rating (#) was multiplied by its respective class number (1-3) and summed. The sum of these values equals a cumulative DSV, with a maximum = 60 DSVs.

DSV = (# of tubers*1) + (# of tubers*2) + (# of tubers*3)

Analysis of variance and a multiple comparison procedure to determine separation of treatment means were performed on disease severity and US#1 yield data using Fisher's least significant difference (LSD) at the 95% confidience level with StatGraphics statistical analysis software package (StatPoint Technologies, Warrenton, VA). Statistical results are reported if ANOVA $P < 0.05$ or if treatment means significantly differed from one another.

Results

Weather conditions and CS pressure in untreated control plots, 2009-2014

Because weather conditions can influence disease and the performance of some pesticides, temperature and precipitation were recorded during the trial seasons (Table 1). In 2011, I reported the greatest CS severity across the six years in the untreated control plots with 37.3 DSV and rainfall of 12.6 in. In 2009, the second highest CS severity in the untreated control plots was 31.8 DSV and the lowest rainfall accumulation (8.26 in). In descending order, the DSV values in the untreated control plots from 2010, 2013, and 2012 were 23.3, 20.5, and 15.2, respectively. The lowest recorded CS severity in the untreated control plots occurred in 2014 (DSV = 4) along with 17.09 in. rainfall. However, 17.2 in. rainfall also occurred in 2010, a year that produced CS DSV = 23.3 in untreated plots. DSV data from the untreated plots show high variability when compared to rainfall each trial year.

Microbial-based biopesticides

The mycorrhizal inoculant (Colonize®), harpin protein (Messenger®) + mycorrhizal inoculant (Colonize®), and the bio-stimulant/foliar nutrient product (Agzyme®) treatments were not statistically better in controlling CS than the untreated control in 2009 ($P = 0.9025$). The marketable yield was greater with the treatment of Agzyme® when compared to the control and the other biopesticides, but was not statistically significant ($P = 0.9307$). In 2010, microbial-based biopesticides *Bacillus subtilis* strain QST713 (Serenade Soil®) and another mycorrhizal product (MycoApply®) did not produce statistical differences among disease severity or yield values when compared to the untreated control (Table 3). However, according to multiple

range test comparisons, Serenade Soil® significantly reduced CS severity and increased yield compared to Quash® (Fig. 2,3). Serenade Soil® also resulted in a yield that was numerically greater than all other treatments at the 113 fl. oz. rate. Application of the lowest rate of Serenade Soil® (32.3 fl. oz.) resulted in the best CS severity control (DSV≈11). An evaluation of Serenade Soil® in 2011 did not result in similar control (Fig. 4; Table 4). When applied at 64 fl. oz. and 128 fl. oz., Serenade Soil® treatments resulted in DSVs of greater than 40 and 30, respectively. Additionally, Serenade Soil® treatments yielded approximately 60 cwt/A less than the untreated control in the 2011 study year (Fig. 5). In 2012, Serenade Soil® (128 fl. oz.) + Pic Plus® (117 lb) numerically limited CS severity by 5 DSVs compared to the untreated control (data not shown). The same application combination of Serenade Soil® (128 fl. oz.) + Pic Plus® (117 lb) produced a numerically higher yield (153.2 cwt/A) compared to the untreated control plots (122.5 cwt/A), though not statistically significant (Fig. 6). A lower rate of Serenade Soil® (64 fl. oz) combined with Blocker® (5 pt.) or with Pic Plus® (351 lb) did not numerically limit disease severity or produce greater yields when compared to the untreated controls. In 2013, Serenade Soil® did not significantly limit CS ($P = 0.9354$) or yield greater than the untreated control ($P = 0.5669$) at the 64 fl. oz., 128 fl. oz., or 64 fl. oz. + Bocker® (5 pt.) application rates. Similarly, our 2014 trial year did not observe significant CS control ($P = 0.8411$) or better yield ($P = 0.1652$) at 64 fl. oz. rate applied independently or in combination with Blocker® (75 fl. oz.) when compared to the untreated control.

Biochemical biopesticides

In 2011, the application of elemental sulfur (Tiger 90CR®) resulted in significant control of CS (approximate 21 DSV reduction), but resulted in a yield that was equivalent to the untreated control (approximately 220 cwt/A). The application of the plant defense activator Regalia SC® resulted in a reduction of CS, but severity values were not significantly different from the untreated control and yield was less than the untreated control. Similarly, a product containing iron, phosphite, plant oils, and a surfactant (AmegA®) did not significantly reduce CS when compared to the untreated control and yielded less than the untreated control (Fig. 4,5). In 2012, our re-evaluation of elemental sulfur (TigerSul® applied at 1000 lb.) did not significantly control CS or produce higher yield compared to the untreated control. The three application rates of Regalia® (29 fl. oz., 58 fl. oz. + Pic-C60 at 107 lb., and 7.3 fl. oz. + Pic C60 at 333 lb.) did not limit CS or yield greater than the untreated control. More specifically, the 29 fl. oz. rate of Regalia® produced a numerically lower yield than the control treatment (Fig. 6).

Statistically significant differences between TigerSul® (1000 lb) and Regalia® (7.3 fl. oz.; 58 fl. oz.) treatment applications were not observed when compared to the untreated control in our 2013 trial year for either CS severity ($P = 0.9354$) or yield ratings ($P = 0.5669$). However, Regalia® applied at 58 fl. oz. did produce a numerically higher yield (432.1 cwt/A) than the untreated control (403.5 cwt/A). A single application rate of Regalia® at 58 fl. oz. was repeated in 2014 but did not significantly limit disease ($P = 0.8411$) or yield ($P = 0.1652$) greater than the control.

Conventional fungicides

Treatments of azoxystrobin (Quadris 2F®) and metconazole (Quash 50WDG®) resulted in CS severities that were not statistically different from the untreated control in 2010 (Fig. 2). Quadris® treatment resulted in a numerical yield reduction (approximately 40 cwt/A) compared to the untreated control. Additionally, both application rates of Quash® (2 fl. oz.; 4 fl. oz.) resulted in significant yield reductions (75.3 and 84.4 cwt/A, respectively) compared to the untreated control (Fig. 3).

In 2011, the treatments containing pentochloronitrobenzene, or PCNB (Blocker®), reduced disease severity by approximately 14-26 DSVs when compared to the untreated control, but did not significantly increase yield (Fig. 4). Blocker® + naphthaleneacetic acid, or NAA, however, significantly limited disease severity by > 25 DSVs when compared to the untreated control. The application of the insecticide ethoprop (Mocap®) reduced disease by approximately 10 DSVs when compared to the untreated control, with a further reduction of approximately 15 DSVs when combined with Blocker®. The application of Mocap® + NAA resulted in nearly equivalent CS as the untreated control plots at approximately 37 DSVs, and yielded significantly less than the untreated control. The applications of Mocap® and Mocap® + Blocker® yielded less than the untreated control, though not to a statistically significant degree (Fig. 5).

In 2012, treatments of Blocker® at 10 pt., Blocker® at 5 pt. + Serenade Soil® (64 fl. oz.), and Blocker® at 10 pt. + NAA (0.33 oz.) did not significantly limit CS severity compared to the untreated control treatments ($P = 0.5975$). However, the latter treatment (Blocker® 10 pt. + NAA 0.33 oz.) did produce approximately 24 cwt/A more yield than the control treatment, though not enough to be statistically significant (Fig. 6). Quadris®,

applied at 11.7 fl. oz., numerically reduced CS severity by approximately 5 DSVs compared to the control treatment.

Fungicide applications in 2013 did not significantly reduce CS compared to the untreated control (*P* = 0.9354). Numerically, Blocker® at 10 pt. + NAA (now Rejuvenate®) at 0.33 oz. reduced CS by 4 DSVs lower than the control. Yields from applications of conventional fungicide treatments were not greater than the untreated control in this trial year (*P* = 0.5669).

Fungicide applications in 2014 did not significantly reduce CS compared to the untreated control (*P* = 0.8411). Numerical reductions in CS severity were observed with all fungicide treatments (with the exception of Rejuvenate® 0.33 oz.), but not more than 2 DSVs below the untreated control DSV rating of 4. No statistical differences in yield were observed among fungicide treatments (*P* = 0.1652). Quadris® applied at 11.7 fl. oz. produced a numerically higher yield of 352.3 cwt/A compared to the untreated control (337.7 cwt/A).

Nematicides

Nematicide applications in 2014 did not significantly impact CS severity (*P* = 0.8411). Compared to the untreated control, Vydate (2 gal in furrow; 4 pt. foliar), Nimitz 480 (7.2 pt. in furrow; 2 pt. foliar), and Nimitz 480 (7.2 pt. in furrow; 1 pt. foliar) limited CS severity with values that were lower than the untreated control (DSV = 4) by margins of 0.5, 1.2, and 0.5, respectively. Yields produced from treatment applications of nematicides were numerically less than the untreated control (*P* = 0.1652).

Soil fumigants

In our 2011 trial, fall-applied metam sodium (Vapam®) at 40 lb resulted in significant control of CS when compared to the untreated control (reduction of approximately 23 DSVs) (Fig. 4; Table 4). Additionally, Vapam® resulted in the highest yields of all of the treatments included in the trial (280 cwt/A) (Fig. 5). All three rates of chloropicrin (Pic Plus®) (117, 234, and 351 lb) and two out of three rates of chloropicrin + solvent (C60 Pic®) (167 and 333 lb) significantly reduced disease severity compared to the untreated control.

Fall-applied fumigant treatments in our 2012 trial resulted in CS severity values that were numerically lower than the untreated control (DSV = 15.3), with the exception of Pic Plus® at 351 lb + Serenade Soil® 64 fl. oz. which had a DSV = 18.5 (data not shown). Vapam® treatments produced the highest yield (184.1 cwt/A) compared to all other fumigation treatments, though not statistically different from them or significantly higher than the untreated control (122.5 cwt/A) (Fig. 6; Table 5).

Discussion

Control of potato common scab with pesticides in two Northern Wisconsin disease nurseries was highly variable during this multi-year investigation. The biopesticides (microbial-based and biochemical) and nematicide treatments provided variable, and often insignificant, CS control with yields at or below the untreated plots. PCNB, metam sodium, and chloropicrin produced increased yields with reduced CS severity; chloropicrin provided the most consistent CS suppression with improved yield. While location and potato cultivars remained consistent over the course of the study, year-to-year variability in rainfall and temperature likely influenced common scab.

Biopesticides, in my investigations, performed variably in controlling common scab during 2009-2014. However, biopesticides have offered some control of plant pathogens in agricultural production systems, such as fungal parasitism by *Trichoderma* spp. and Fusarium wilt suppression with *Gliocladium* and *Bacillus* spp. (Fravel, 2005; Howell, 2003; Köhl, *et al.*, 2011; Zhang *et al.*, 1996). While only occupying a small percentage of agrichemical sales, biopesticides offer disease control by utilizing different action mechanisms compared to conventional products, which allows them residence in a management program when alternated or combined with conventional pesticides (Fravel, 2005). The variability in disease control performance with microbial biopesticides, or biologicals, as shown in this 6-year study, can be attributed to the biological nature of the active ingredients, interactions with soil microbiota, and the flux of environmental conditions (Dees & Wanner, 2012). In my work, application of Serenade Soil® gave us better control than the untreated plots in 2010, but resulted in CS more severe than the

untreated in 2011. Similar findings have been reported in another potato-producing state that evaluated Serenade Soil® under field conditions (Kirk *et al.*, 2011).

Biochemical biopesticides provided some control of potato common scab in my multi-year investigations. In 2011, I included a few additional biochemical biopesticides, including elemental sulfur (Tiger 90CR®), extract of *Reynoutria sachalinensis* (Regalia SC®), and a product containing iron, phoshpite, plant oils, and a surfactant (AmegA®). Of the three biochemical biopesticides, only elemental sulfur significantly reduced CS severity by 20 DSVs when compared to the untreated plants. Furthermore, applications of sulfur resulted in yields that were higher than Blocker®, the conventional pesticide standard, and the other biopesticide treatments. It is theorized that the Tiger-Sul® treatment suppresses soil pathogens by reducing soil pH as local microorganisms oxidize this rapidly degrading form of sulfur (Tigersul.com). Suppression of CS by reducing soil pH has been previously reported with variable results (Bailey & Lazarovits, 2003; Davis *et al.*, 1976; Waterer, 2002). However, this may not be an effective management strategy if acidic-tolerant species are present in the field, such as *S. acidiscabies* and *S. turgiciscabies*, which survive at soil pH levels of 4.0 or above (Lambert & Loria, 1989; Miyajima *et al.*, 1998; Song *et al.*, 2004; St-Onge *et al.*, 2008).

The conventional fungicides Quadris® and Quash® were included in our trial in 2010 for the purpose of investigating potential effects of a strobilurin and a triazole on CS. Quadris® has become a commercial standard treatment at time of planting for potato Rhizoctonia control and Quash® is a relatively new fungicide with activity against foliar fungal pathogens (Atkinson *et al.*, 2011; Brewer & Larkin, 2005; Rideout *et al.*, 2013). Applications of Quash® at 2 fl. oz. and 4 fl. oz. did not limit common scab disease at or

below the untreated. This is likely due to the mode of action of metconazole, which inhibits the synthesis of sterols in eukaryotic fungi (Ware & Whitacre, 2004). Bacteria do not contain sterols, but utilize similarly structured hopenoids for putative cell membrane activity and, thus, are not necessary for growth of *S. scabies* (Seipke & Loria, 2009). Additionally, both rates of Quash® resulted in yields that were both significantly lower than the untreated plants. Further evaluation of metconazole as an in-furrow treatment is necessary to better understand its effects on potato yield and disease control. In 2011, I added a treatment that combined a conventional fungicide, pentochloronitrobenzene or PCNB (Blocker®), with naphthaleneacetic acid or NAA (Rejuvenate®), which is a synthetic rooting agent within the auxin family of plant hormones that can lower stem number and increase tuber bulking (Ware & Whitacre, 2004). There was an increase in yield with Rejuvenate® + Blocker® as a result of NAA restoring apical dominance and thereby increasing tuber bulking and marketable yield, which was further corroborated in recent potato trials (Larsen, 2014; Robinson, 2015).

While anecdotal reports have indicated an exacerbation of CS by metam sodium in other potato-producing states, this has not been supported in preliminary Wisconsin trials and warranted my interest in collecting data to address this contention. I evaluated metam sodium (Vapam®) during our 2011-2012 trials as a pre-plant, fall soil treatment to assess its role in CS incidence and severity (Racke, 1990; Smelt *et al.*, 1989). Fumigating soil with metam sodium (i.e., Vapam® or Sectagon®) prior to potato planting has been a standard management alternative in Wisconsin for control of root lesion nematodes (*Pratylenchus penetrans*) and *Verticillium dahliae*, causal agents of potato early dying disease (Bowden & Rouse, 1991; Nicot & Rouse, 1987; Saeed *et al.*, 1997). In other

potato producing regions, metam sodium has been reportedly effective in controlling *Rhizoctonia solani* by inhibiting sclerotial development and has been used to control two parasitic nematodes (*Meloidogyne chitwoodii* and *Paratrichodorus* spp.) in addition to potato early dying (Rowe & Powelson, 2002; Wicks *et al.*, 1996). While metam sodium does have some control of weed seeds at high rates (Hutchinson *et al.*, 2003), this is not one of the primary reasons for its use in Wisconsin potato production. Based on the reduction in CS severity (~23 DSVs less than the control) and the increased yield (~280 cwt/A) in the 2011 trial year, Vapam® could be effective in managing CS in Wisconsin.

Test plots fumigated with chloropicrin provided the most consistent CS suppression with improved yield compared to all other treatments tested in 2009-2014. Previously, chloropicrin reportedly reduced incidence of potato early dying and CS in 2004 and 2005 trials in Wisconsin (Rouse, 2006). Based on those results, I further evaluated multiple rates and formulations of chloropicrin during my field trials from 2011-2014. Pic Plus® contains 85% of the chloropicrin active ingredient with a proprietary solvent that allows for reduced volatility for slower gassing-off of the fumigant. This would, presumably, provide a protracted pesticidal activity with less application of the active ingredient (Hutchinson, 2005). A second chloropicrin product, Pic-C60®, was also evaluated and contains 60% active ingredient (Reddickfumigants.net). Efficacy results were similar in that three rates of Pic Plus® and two out of the three rates of Pic-C60® significantly reduced CS severity, as well as producing numerically increased yields with all of the chloropicrin treatments tested. Further investigation of CS control with metam sodium and chloropicrin fumigants took place in 2012 to assess residual impacts of a 2010-fall-fumigation on a 2012-potato-crop.

This work was conducted in response to grower requests to characterize the long-term disease management residual benefit of a single fumigation but did not limit disease or improve yield in the 2012 cropping year.

The reliance upon a single pesticide input to manage CS in potato often results in high variability of disease control. Improved understanding of the dynamics of pathogenic *Streptomyces* strains, such as strain aggressiveness and species profile within and among fields, could make possible a prescriptive, field-specific approach to CS management (Lazarovits *et al.*, 2007; Sturz *et al.*, 2004). My investigation of microbial biopesticides did not include non-pathogenic or antagonistic *Streptomyces* species, which have been shown to have some efficacy in managing CS and could further contribute to field-specific management (Doumbou *et al.*, 2001; Lorang *et al.*, 1995; McQueen *et al.*, 1985). My field studies found that chloropicrin provided CS suppression and increased yield with relative consistency, as has been reported in other field evaluations (Hutchinson, 2005; Dees & Wanner, 2012). While my studies have focused on pesticidal options for CS control, it is critical that management programs include cultural control approaches such as pH and irrigation management, as well as use of CS resistance in varietal selections. The integration of multiple cultural and chemical approaches to CS management remains important in sustaining production of healthy potato crops in production regions favoring CS.

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Table 1. Weather history for Langlade County Airport during 2009-2014, Antigo, Wisconsin.

Year	Rainfall (in.)	Mean Air Temp °F/°C	Growing-Degree Days (GDD_{50})	Planting Date	Harvest Date	CS DSV* (Untreated)
2009	8.26	58/14.4	1425	10 May	10 Oct	31.8
2010	17.2	62/16.7	1852	10 May	10 Oct	23.3
2011	12.6	61/16.1	1834	25 May	17 Sep	37.3
2012	11.09	63/17.2	1734	11 May	17 Sep	15.2
2013	12.17	62/16.7	1491	29 May	30 Sep	20.5
2014	17.09	61/16.1	1398	29 May	30 Sep	4.0

* Disease severity values were determined by the scale: 0 = no disease, 1 = 10% scabbed surface, 2 = 10-25% scabbed surface, and 3 = > 25% surface scab and/or pits present. Severity index was calculated using the equation,

DSV = (# of tubers*1) + (# of tubers*2) + (# of tubers*3)

The number of sampled tubers falls into each class rating (#) was multiplied by its respective class number (1-3) and summed. The sum = DSV.

Table 2. Treatment specifications for common scab field trials during 2009-2014 at the University of Wisconsin Extension Langlade County Research Farm on 'Yukon Gold' potato.

Trade name	Active Ingredient	Application Rate/Acre	Time of Application	2009	2010	2011	2012	2013	2014
Untreated control	N/A	N/A	N/A	X	X	X	X	X	X
Colonize	mycorrhizal inoculant	2.0 oz	At-plant	X	X				
Colonize + Messenger	mycorrhizal inoculant + harpin protein	2.0 oz + 3.0 oz	At-plant	X	X				
Agzyme	bio-stimulant and foliar nutrients	12.8 oz	At-plant	X					
Serenade Soil 1.34%	*Bacillus subtilis* strain 713	32.3 fl oz/113 fl oz/194 fl oz	At-plant		X	X			X
Quadris Flowable 2F	azoxystrobin	11.7 fl oz	At-plant		X	X	X	X	X
MycoApply	mycorrhizae	1.0 lb	At-plant		X				
Quash 50WDG	metconazole	2.0 fl oz/4.0 fl oz	At-plant		X				
Untreated control – No fumigation	N/A	N/A	N/A			X			
Vapam	metam sodium	40.0 lb	Sep 2010			X	X		
Blocker 4F	pentachloronitrobenzene (PCNB)	10.0 pt	At-plant			X	X	X	X
Blocker 4F + Mocap 15G	PCNB + ethoprop (15%)	10.0 pt + 20.5 lb	At-plant			X			
Mocap 15G	ethoprop (15%)	20.5 lb	At-plant			X			
Blocker 4F + Rejuvenate	PCNB + naphthalenic acid (NAA)	10.0 pt + 0.33 oz	At-plant			X	X	X	X
Rejuvenate	Naphthalenis acid (NAA)	0.33 oz	Seed trt					X	X
Blocker 4F + Serenade Soil	PCNB + *Bacillus subtilis*	5 pt + 4.4 oz	At-plant				X	X	X
Tiger-Sul 90CR	elemental sulfur	1000 lb	At-plant			X	X	X	
Regalia SC	*Reynoutria sachalinensis*	29.3 fl oz	At-plant			X	X		
Regalia 5SC	*Reynoutria sachalinensis extract*	0.5 fl oz	At-plant					X	
Regalia 5SC	*Reynoutria sachalinensis extract*	4.0 fl oz	At-plant					X	X
Pic Plus	chloropicrin (85%) + solvent	117 lb/234 lb/351 lb	Sep 2010			X			
Pic Plus	chloropicrin (85%)	234 lb a.i.acre	Sep 2010				X		
Pic Plus + Serenade Soil	chloropicrin (85%) + *Bacillus subtilis*	117 lb a.i./acre + 8.8 oz	Sep 2010/At-plant				X		
Pic Plus + Serenade Soil	chloropicrin (85%) + *Bacillus subtilis*	351 lb a.i./acre + 4.4 oz	Sep 2010/At-plant				X		
C60 Pic	chloropicrin (60%)	167 lb/250 lb/333 lb	Sep 2010			X			
C60 Pic + Regalia 5SC	chloropicrin (60%) + *Reynoutria sachalinensis* extract	167 lb a.i./acre +4.0 fl oz	Sep 2010/At-plant				X		
C60 Pic	chloropicrin (60%)	250 lb a.i./acre	Sep 2010				X		
C60 Pic + Regalia 5SC	Chloropicrin (60%) + *Reynoutria sachalinensis* extract	333 lb a.i.acre + 0.5 fl oz	Sep 2010/At-plant				X		
Mocap 15G + NAA	ethoprop (15%) + NAA	20.5 lb + 0.33 oz	At-plant			X			
AmegA SC	iron, phosphite, plant oils, and surfactant	10.0 pt	At-plant			X			

Table 3. Analysis of variance of common scab severity values and US#1 marketable yield from 'Yukon Gold' potatoes treated with biopesticides, conventional fungicides, and soil fumigation applications in 2010, Antigo, Wisconsin.

Source	**Sum of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
CS severity					
Between groups	872.225	9	96.9139	1.26	0.3000
Within groups	2313.75	30	77.125		
Total (Corr.)	3185.97	39			
Yield					
Between groups	42765.4	9	4751.71	1.86	0.0977
Within groups	76588.6	30	2552.95		
Total (Corr.)	119354.0	39			

Table 4. Analysis of variance of common scab severity values and US#1 marketable yield on 'Yukon Gold' potatoes treated with biopesticides, conventional fungicides, and soil fumigation applications in 2011, Antigo, Wisconsin.

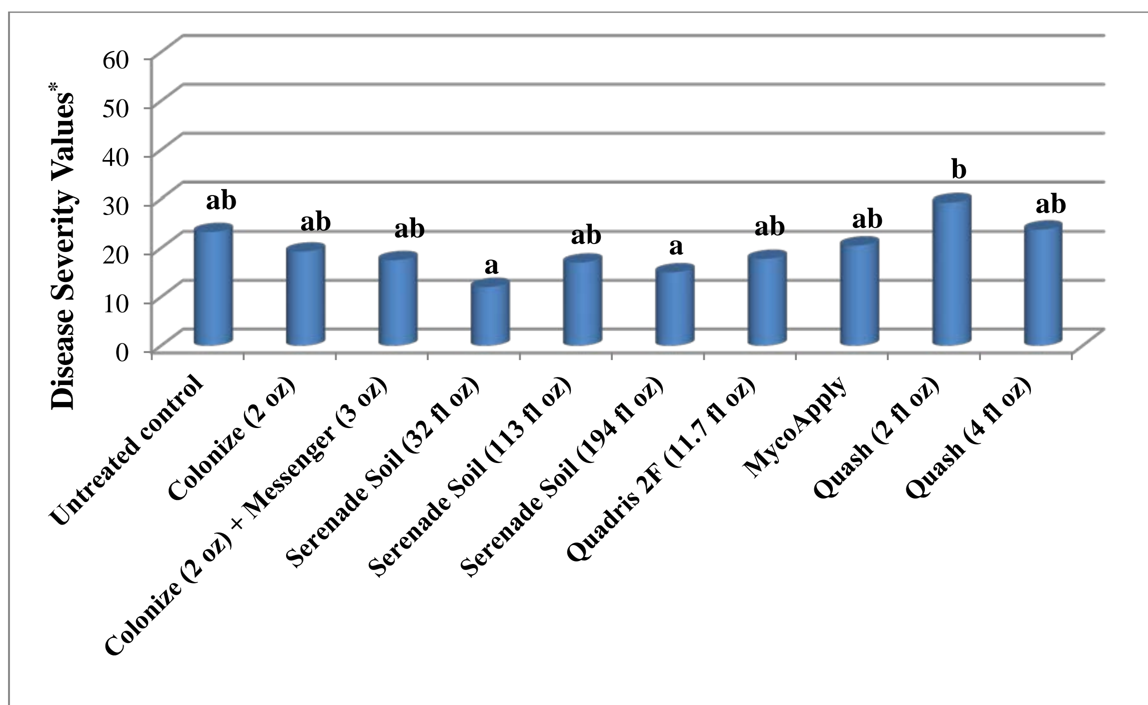
Source	**Sum of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
CS severity					
Between groups	10314.3	19	542.857	3.35	0.0002
Within groups	9554.42	56	161.939		
Total (Corr.)	19868.7	78			
Yield					
Between groups	53332.8	19	2806.99	4.89	0.0000
Within groups	32176.2	56	574.575		
Total (Corr.)	88862.8	78			

Table 5. Analysis of variance of US#1 marketable yield on 'Yukon Gold' potatoes treated with biopesticides, conventional fungicides, and soil fumigation applications in 2012, Antigo, Wisconsin.

Source	**Sum of Squares**	***Df***	**Mean Square**	**F-Ratio**	***P-Value***
Yield					
Between groups	27640.4	13	2126.18	0.88	0.5743
Within groups	100921.0	42	2402.89		
Total (Corr.)	128562.0	55			

Figure 1. Typical common scab lesions on potato and vegetable crops. A) Pitted common scab symptoms on ‘Russet Norkotah’ potato tuber. B) Superficial/netted scab symptoms on ‘Russet Norkotah’ potato tuber. C) Raised lesions showing hypertrophic growth and peridermal cracks on ‘Cherry Belle’ radish. D) Raised scab lesions girdling processing carrot root.

Figure 2. Common scab severity values from 'Yukon Gold' potatoes treated with biopesticide (microbial-based and biochemical) and conventional fungicides in 2010, Antigo, Wisconsin. Bars followed by the same letter are not significantly different (Fisher's LSD, *P*=0.05).

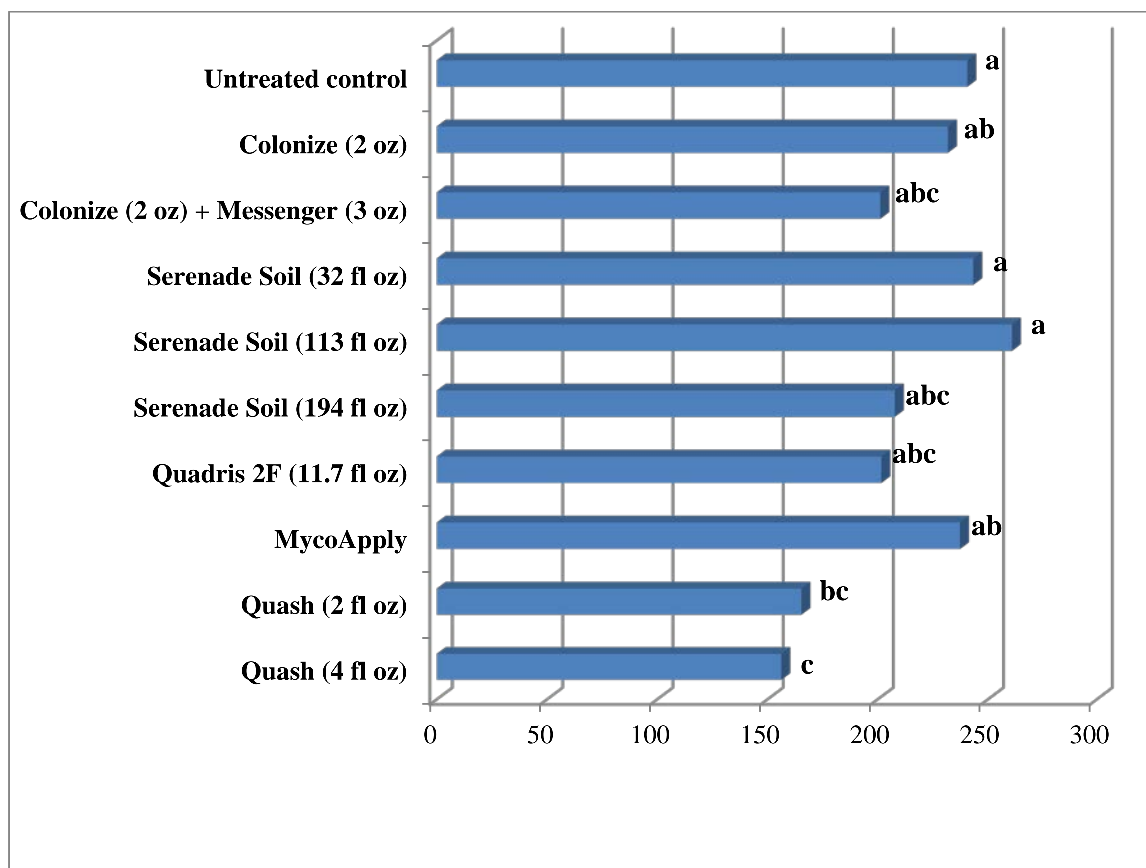


* Disease severity values were determined by the scale: 0 = no disease, 1 = 10% scabbed surface, 2 = 10-25% scabbed surface, and 3 = > 25% surface scab and/or pits present. Severity index was calculated using the equation,

DSV = (# of tubers*1) + (# of tubers*2) + (# of tubers*3)

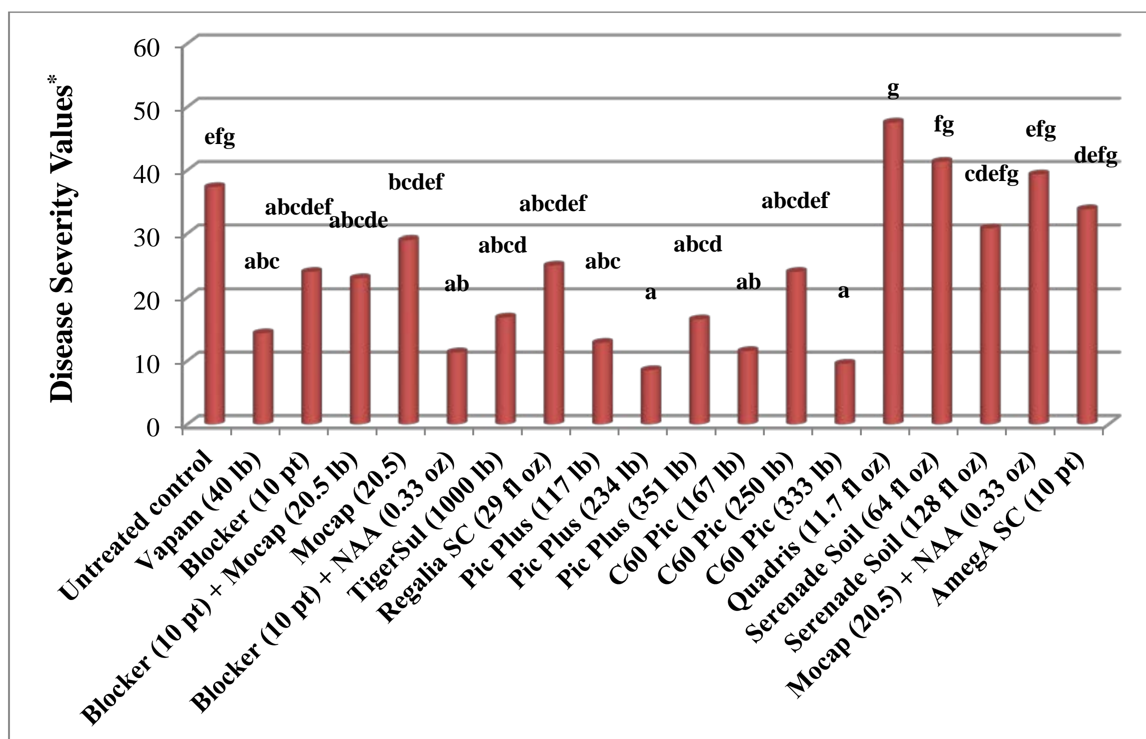
The number of sampled tubers falls into each class rating (#) was multiplied by its respective class number (1-3) and summed. The sum = DSV.

Figure 3. US#1 marketable yield[*] of 'Yukon Gold' treated with biopesticides (microbial-based and biochemical) and conventional fungicides in 2010, Antigo, Wisconsin. Bars followed by the same letter are not significantly different (Fisher's LSD, *P*=0.05).



* US#1 marketable yield is defined as the weight in cwt/acre or 100 lb per acre of potato tubers sized > 4 oz with damaged tubers removed.

Figure 4. Common scab severity values from 'Yukon Gold' potatoes with biopesticides (microbial-based and biochemical), conventional fungicides, and soil fumigation treatments in 2011, Antigo, Wisconsin. Bars followed by the same letter are not significantly different (Fisher's LSD, *P*=0.05).

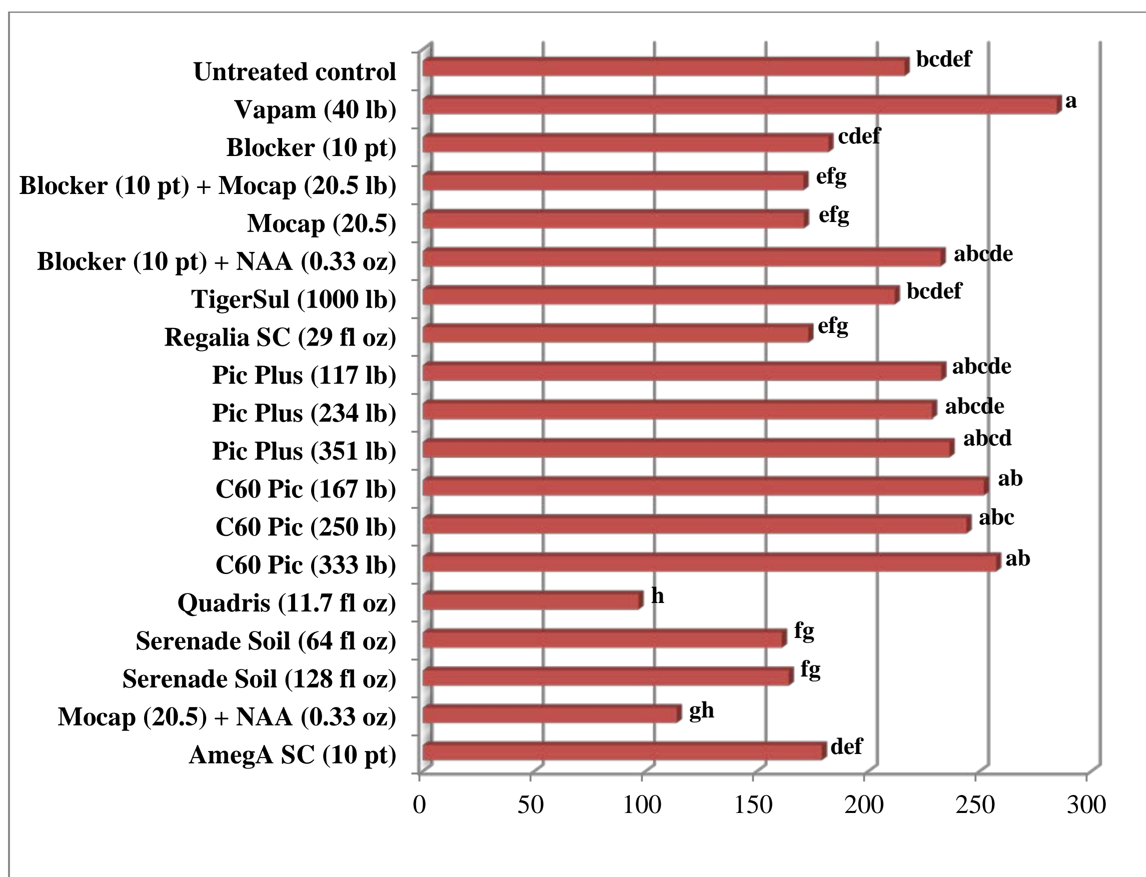


* Disease severity values were determined by the scale: 0 = no disease, 1 = 10% scabbed surface, 2 = 10-25% scabbed surface, and 3 = > 25% surface scab and/or pits present. Severity index was calculated using the equation,

DSV = (# of tubers*1) + (# of tubers*2) + (# of tubers*3)

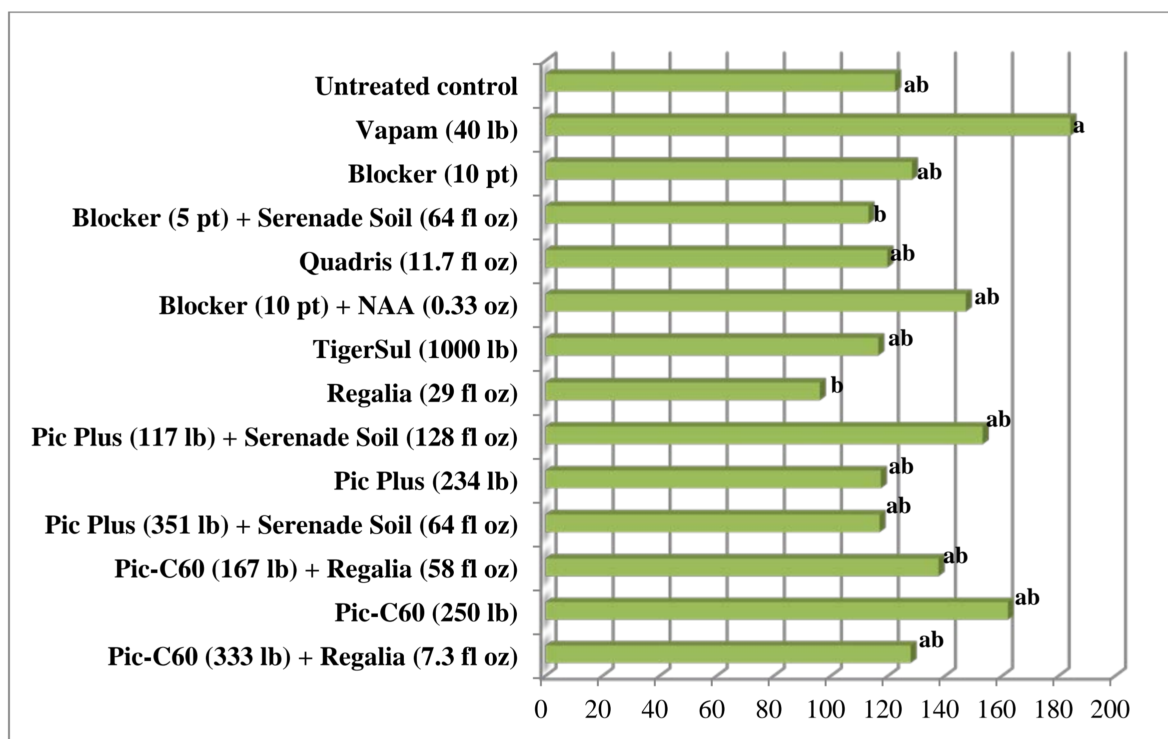
The number of sampled tubers falls into each class rating (#) was multiplied by its respective class number (1-3) and summed. The sum = DSV.

Figure 5. US#1 marketable yield[*] of 'Yukon Gold' potatoes treated with biopesticides (microbial-based and biochemical), conventional fungicides, and soil fumigation treatments in 2011, Antigo, Wisconsin. Bars followed by the same letter are not significantly different (Fisher's LSD, *P*=0.05).



* US#1 marketable yield is defined as the weight in cwt/acre or 100 lb per acre of potato tubers sized > 4 oz with damaged tubers removed.

Figure 6. US#1 marketable yield* of 'Yukon Gold' potatoes treated with biopesticides (microbial-based and biochemical), conventional fungicides, and soil fumigation treatments in 2012, Antigo, Wisconsin. Bars followed by the same letter are not significantly different (Fisher's LSD, *P*=0.05).



* US#1 marketable yield is defined as the weight in cwt/acre or 100 lb per acre of potato tubers sized > 4 oz with damaged tubers removed.