



Managing scab diseases of potato and radish caused by *Streptomyces* spp. using *Bacillus amyloliquefaciens* BAC03 and other biomaterials



Qingxiao Meng^a, Linda E. Hanson^{a,b}, Dave Douches^a, Jianjun J. Hao^{a,c,*}

^a Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48823, USA

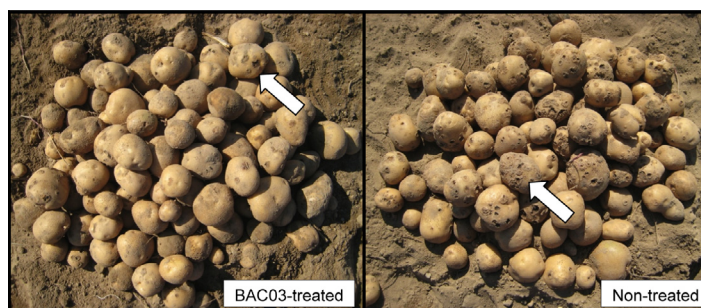
^b USDA-ARS Research Plant Pathology, East Lansing, MI 48823, USA

^c School of Food and Agriculture, University of Maine, Orono, ME 04469, USA

HIGHLIGHTS

- Chestnut tissues, essential oils, horseradish, and *Bacillus* strain BAC03 reduced scab in condition-controlled trials.
- The efficacy by chestnut tissues, oregano essential oils, and horseradish varied depending on locations and seasons.
- BAC03 significantly reduced the scab diseases and may increase the plant growth confirmed by field trials.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 20 June 2013

Accepted 11 September 2013

Available online 18 September 2013

Keywords:

Common scab
Streptomyces
 Biological control
 Field evaluation
 Biomaterials

ABSTRACT

Streptomyces spp. cause scab in plants like potato and radish. To effectively control this disease, biologically based materials were examined for their efficacies. In greenhouse or growth chamber tests, potting mix was infested with *Streptomyces scabies* (10^6 CFU cm^{-3}), followed by applying different products. *Bacillus amyloliquefaciens* strain BAC03 (10^9 CFU cm^{-3}) reduced disease severity and potentially enhanced growth of radish; chestnut tissues at 50% in volume, oregano and clove essential oils ($0.1 \mu\text{g cm}^{-3}$), and ground horseradish ($>0.18 \text{ g m}^{-3}$) significantly reduced the severity of common scab in potato and/or radish. In two Michigan fields in 2011 and 2012, chestnut tissues (1.15 kg m^{-2}), oregano essential oil (1.5 ml m^{-2}), and ground horseradish (0.38 g m^{-2}) were incorporated into the soil 2 weeks before planting. BAC03 (10^6 CFU ml^{-1} , 1 L m^{-2}) was drenched into the root zone soil 4 times at 2 week intervals starting 1 month after planting. BAC03 significantly reduced disease severity by 17–57% relative to control in two locations over 2 years. BAC03 also enhanced potato tuber weight by 33% and 26% in 2011 in two locations. The rest of the treatments caused significant disease reduction, but were less effective compared to BAC03, and efficacy varied depending on location and year. Therefore, BAC03 can be a good biological control agent for potato common scab management.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Streptomyces scabies is the major species of pathogenic *Streptomyces* spp., which are distributed worldwide and cause potato common scab (Loria et al., 1997; Wanner, 2006), and scabby dis-

ease in radish and other crops (Goyer and Beaulieu, 1997). The economic losses in most potato production areas are significant due to lack of effective management strategies (Dees and Wanner, 2012; Wanner and Haynes, 2009). Since most of the necrotic lesions are formed on the potato tuber surface, cosmetic damage contributes more than yield reduction in economic losses (Loria et al., 1997). The pathogen can survive as a saprophyte for long periods in the absence of the host, and different species of the pathogen having distinct genetic backgrounds may respond differently, making dis-

* Corresponding author. Address: School of Food and Agriculture, University of Maine, Orono, ME 04469, USA.

E-mail address: jianjun.hao1@maine.edu (J.J. Hao).

ease management more difficult (Hao et al., 2009; Hiltunen et al., 2009; Jiang et al., 2012; Loria et al., 2006; Wanner, 2007).

Many approaches have been used to manage common scab, but few are practical or effective (Hiltunen et al., 2009; Larkin, 2008; Larkin and Griffin, 2007). Using cultivars resistant to common scab is considered the most effective method, but availability of resistant germplasm is limited (Douches et al., 2009; Wanner and Haynes, 2009). Lower soil pH at 5.2 and increasing soil moisture can reduce the disease severity, but may reduce potato yield, and these methods are not always applicable (Dees and Wanner, 2012; Hiltunen et al., 2009). Use of pentachloronitrobenzene is partially effective, however it can cause environmental concerns due to its long half-life in soil (Davis et al., 1976). Therefore, additional or alternative strategies for managing common scab are needed.

Among cultural practices, organic soil amendments, green manures, and crop rotation have been shown to reduce common scab (Abbasi et al., 2006; Sakuma et al., 2011; Singhai et al., 2011). Addition of biological materials has provided promising results for disease suppression by promoting activity and diversity of resident microbial organisms, increasing plant-beneficial organisms, and promoting antagonists toward pathogens (Larkin, 2008). Many biological materials have been studied for potato common scab management, such as fish emulsion (Abbasi et al., 2006); soymeal, meat and bone meal (Lazarovits et al., 1999); lopsided oat green manure (Sakuma et al., 2011); and mustard green manure (Larkin and Griffin, 2007). In addition, several groups of plant growth promoting rhizobacteria (PGPR) displayed promising activities for biological control of common scab, such as *Pseudomonas* spp. (Singhai et al., 2011), *Bacillus* spp. (Han et al., 2005; Schmiedeknecht et al., 1998), and non-pathogenic *Streptomyces* spp. (Hiltunen et al., 2009).

To seek additional effective biological control options, we have examined various materials in laboratory and greenhouse trials (Bi et al., 2012; Hao et al., 2012; Meng et al., 2012a). Extracts of chestnut tissue displayed a strong antimicrobial activity against many plant pathogens, which probably is associated with antimicrobial compounds such as flavonol glycoside and several terpenoid substances (Hao et al., 2012). Clove and oregano essential oil has shown a high level of suppressive activity against *Phytophthora capsici* (Bi et al., 2012). Additionally, ground horseradish tissue inhibited the growth of several *Streptomyces* spp. in vitro (Meng, unpublished). *Bacillus amyloliquefaciens* strain BAC03 (Patent No.: US 7,615,366 B2) was isolated from a soil naturally suppressive to potato common scab (Meng et al., 2012b), and has shown good biological control activity (Meng et al., 2012a).

We tested these materials for their ability to manage common scab under controlled and uncontrolled conditions. The objectives of this study were to evaluate 4 biologically derived materials, including chestnut tissue, clove and oregano essential oil, horseradish, and *B. amyloliquefaciens* strain BAC03, for their ability to suppress scab either in potato or radish under greenhouse and field conditions.

2. Materials and methods

2.1. Preparation of bacterial inoculum and essential oils

Streptomyces scabies ATCC49173 (from the American Type Culture Collection, and isolated from potato tuber) was cultured in oatmeal broth at 28 °C for 4–5 days in an incubator shaker at 180 rpm (Loria et al., 1995). The concentration of *S. scabies* in the liquid culture was determined by dilution plating on yeast malt extract (YME; EMB Chemical Inc., Gibbstown, NJ, USA) agar. *Bacillus amyloliquefaciens* strain BAC03 was cultured in tryptic soy broth

(TSB; EMB Chemical Inc., Gibbstown, NJ) at 28 °C for 48 h at 180 rpm. The concentration was determined by dilution plating on tryptic soy agar (TSA) plates.

Commercial oregano essential oil (OE; Aura Cacia Inc., Urbana, IA, USA) and clove essential oil (CE; Aura Cacia Inc., Urbana, IA) were dissolved in dimethyl sulfoxide (DMSO; Krackeler Scientific Inc., Albany, NY, USA) as stock solutions (0.8 mg ml⁻¹) and stored at 4 °C in the dark.

2.2. Biological control activities of biomaterials tested in greenhouse

2.2.1. *B. amyloliquefaciens* BAC03 on scab and plant growth of radish

Radish ('Cherry Belle', Burpee Inc. Warminster, PA, USA) seeds were pre-germinated in a Petri dish with moist filter paper overnight at 25 °C. After germination, seedlings were transplanted in a 1 L pot containing potting mix (ASB Greenworld Inc., New Brunswick, VA, USA) in a greenhouse (Michigan State University greenhouse facility, East Lansing, MI). Growth conditions in the greenhouse were around 18–22 °C with a 14-h photoperiod, supplemented by light at 200 μmol m⁻² s⁻¹. *Bacillus amyloliquefaciens* strain BAC03 liquid culture was drenched into potting mix to make a final concentration of 10⁵ CFU cm⁻³ 1 week post seedling emergence. A liquid culture of *S. scabies* strain ATCC49173 was added to the potting mix by drenching to give a final concentration of 10⁶ CFU cm⁻³ 1 week post seedling emergence. There were 4 replicates for each treatment. Radish leaves and roots were harvested and weighed 6 weeks post planting. Disease severity was determined by measuring the lesion severity using the 0–5 scale of Wanner (2004), where 0 = no disease and 5 = the whole root is covered by scab lesions. This study was conducted two times.

2.2.2. Effect of chestnut tissues on potato common scab in growth chamber

Chestnut tissues (shell/pellicle complex) were obtained from chestnut peeling process using a commercial brulage peeling line (Boema S.P.A., Neive, Italy), located at the Michigan State University Roger's Reserve, Jackson, MI. Potato seed tubers ('Snowden') were surface-disinfested with 1% NaClO for 5 min and rinsed with sterile distilled water 3 times. Potato seed tubers were air dried at room temperature and planted in 3.78 L pots containing mixtures of potting mix and chestnut tissues at 0:1, 1:1, and 1:0 (chestnut tissues:potting soil = vol:vol), with 4 replicates each. Growth chamber settings were 16 h of light and 8 h of dark, with light intensity of 200 μmol m⁻² s⁻¹, and constant temperature of 18 °C. One week after seedling emergence, a liquid culture of *S. scabies* strain ATCC49173 was drenched to the potting mix to give a final concentration of 10⁶ CFU cm⁻³ potting mix.

Potato tubers were harvested 4 months after planting, examined for lesions, and given a severity rating using a 0–5 scale (Hao et al., 2009), where 0 = no symptoms, 1 = 1 to 10% surface area with superficial or raised lesions, 2 = 11–25% surface area with superficial or raised lesions, 3 = 26–50% surface area with superficial or raised lesions, 4 = more than 50% surface area with superficial or raised lesions or 6–25% pitted lesion area and 5 ≥ 50% surface area with superficial or raised lesions or >25% pitted area. The average disease score was calculated as Σ (score × number of tubers with that score)/total number of tubers evaluated. The experiment was conducted twice.

2.2.3. Effect of oregano and clove essential oils on scab in potato and radish

Potato seed tubers ('Snowden') and germinated radish seedlings ('Cherry Belle'), prepared as described above, were sown in potting mix, immediately followed by drenching with clove or oregano essential oils, which resulted in final concentrations in potting mix of approximately 0.1 μg cm⁻³. DMSO was used as a control.

One week after seedling emergence, a liquid culture of *S. scabies* strain ATCC49173 was drenched to the potting mix to give a final concentration of 10^6 CFU cm^{-3} potting mix. Disease was rated 6 weeks after radish planting and 4 months after potato planting, with the criteria mentioned above (Hao et al., 2009; Wanner, 2004). The experiments were conducted twice.

2.2.4. Effect of ground horseradish on potato common scab

Horseradish root (J.R. Kelly Inc., Collinsville, IL) purchased from fresh market was ground using a blender (Waring Laboratory Inc., Torrington, CT). The ground horseradish tissue was mixed into potting mix at 0, 0.18, 0.33, 0.91, and 1.8 g m^{-3} (dry weight) with 4 replicates each. One week after seedling emergence, a liquid culture of *S. scabies* strain ATCC49173 was added as described above. Disease ratings were conducted 4 months after planting at harvest (Hao et al., 2009). The experiment was conducted twice.

2.3. Field trials

Field trials were conducted at the Montcalm Research Center (N43°21.148', W85°10.510', designated as MRC) at Lakeview, MI, and Michigan State University Soil Science Research Center (N42°42.942', W84°27.953', designated as MSU) at East Lansing, MI in 2011 and 2012. The field trial was divided into 4 blocks (replications), with 12 rows (0.6 by 7.5 m per row) in each block as two-row treatment plots. The plots were arranged in a randomized complete block design. Twenty-two seed tubers were planted in each row with 30 cm spacing. Spacing between rows was 30 cm. Potatoes were planted on 3rd and 27th June in 2011, as well as 22th May and 5th June in 2012. Scab-free (by visual evaluation for lesions) potato ('Snowden') seed tubers were planted along the furrow. Pesticides and fertilizers were applied following standard procedures used in commercial production.

Oregano essential oil (Aura Cacia Inc., Urbana, IA) was dissolved in DMSO and added to water to obtain a final concentration of 0.35% (vol/vol) for OE and 1.15% (vol/vol) for DMSO. Horseradish was cut into small pieces and ground by using a blender as above. The final concentration was adjusted to 0.17 kg L^{-1} (dry weight/water). Chestnut tissue (1.15 kg m^{-2} , dry weight), ground horseradish (38 g m^{-2} , dry weight), and oregano essential oil (1.5 ml m^{-2}) were incorporated into the soil with a rotor tiller (Earthquake Inc., Cumberland, WI, USA) 2 weeks before planting. Soil was then covered with a plastic sheet (Husky Inc., Grand Prairie, TX, USA) for 2 weeks, followed by planting potato seed tubers. A diluted BAC03 culture suspension [10^6 colony forming unit (CFU) ml^{-1}] in TSB was applied 4 times after planting at 2 weeks intervals starting 1 month after planting. The BAC03 liquid culture was manually applied to each potato plant by slowly drenching in areas close to plant roots (0.25 m in diameter around the main stem, which resulted in a concentration of 1 L m^{-2} at 10^6 CFU ml^{-1}). For a control treatment, the same volume of TSB media was drenched using the same method.

Potatoes were harvested on 19th September and 10th October in 2011, as well as 28th September and 22th October in 2012. Fifty tubers were randomly selected from each plot for evaluation. Disease lesions on potato tubers were rated using the scale mentioned earlier (Hao et al., 2009). For yield assessment, all potatoes in a two-row (3 m row^{-1}) plot were manually harvested and weighed.

2.4. Statistical analysis

Data were analyzed using SAS software (version 9.2, SAS Inc., Cary, NC, USA). Procedure GLM was used for the analysis of variance, and Fisher's least significant difference (LSD) multiple comparisons were performed for mean separation of yield where ANOVA showed significant differences. Non-parametric data anal-

ysis was used for disease analysis, and Kruskal–Wallis test was performed to compare mean values. Regression was performed for the relationship between horseradish concentration and disease score. Effect of horseradish on scab severity was analyzed using Spearman's rank correlation.

3. Results

3.1. Effect of biomaterials tested in greenhouse or growth chamber

Application of BAC03 dramatically increased the fresh weight of radish leaves (126%) and roots (166%). Although addition of the pathogen (*S. scabies*) alone was able to increase fresh weight of leaves and roots of radish to certain degree, this increase was much less (14 and 10%, respectively) than with BAC03 plus the pathogen (Table 1). In the aspect of disease suppression, *B. amyloliquefaciens* BAC03 significantly ($P < 0.05$) reduced the severity of radish scab by 70%. The average disease scores were 0.3 and 1 for BAC03 treatment and control, respectively (Table 1).

To determine whether mixtures of chestnut tissues affect potato common scab, a disease assay was conducted in the growth chamber. Disease severity on potato tubers was significantly reduced when chestnut tissues were incorporated in potting soil, and negatively correlated ($R^2 = 0.96$) with the amount of chestnut material applied (Table 2). This effect was observed in both trials.

In radish, oregano and clove essential oils significantly reduced disease severity ($P < 0.05$), and no disease was observed in potato under the treatment of oregano essential oil (Fig. 1). Based on the results of greenhouse trials, oregano essential oil was selected for further study in the field. Horseradish reduced common scab in the greenhouse. As the concentration of ground horseradish increased in potting soil, the severity of common scab decreased, and no disease was observed in the treatment with 1.8 g m^{-3} of horseradish (Fig. 2).

3.2. Field trials

There were interactions between year, location, and treatment ($P < 0.05$), therefore the data were analyzed individually for each year and location. In 2011 field trial at MRC, BAC03, oregano essential oil, and horseradish significantly reduced scab severity compared to the controls, with BAC03 and horseradish having the greatest efficacy ($P < 0.05$), reducing disease severity by 49% in BAC03 treatment relative to TSB control, and 37% in horseradish treatment compared to the non-treated control. The disease severity under chestnut-tissue treatment was 1.1, lower than that in the TSB control, but not significantly different from the non-treated control ($P < 0.05$) (Fig. 3A). In the MSU trial, BAC03 had significant reduction in common scab severity (57%), with a disease score of 0.4 ($P < 0.05$). The effects of chestnut tissue, oregano essential oil, and horseradish were not significant (Fig. 3B). In the 2012 trial at both MRC and MSU, BAC03 was the only treatment that significantly reduced the disease severity of potato common scab by 17% and 43%, respectively (Fig. 3C and D; Fig. 4).

In the 2011 trial at MRC, *B. amyloliquefaciens* strain BAC03 was the only treatment that displayed a yield promotion, with a relative efficacy of 33% compared to the TSB control (Table 3). The average tuber yields from treatments with chestnut tissue, oregano essential oil, and horseradish were not significantly different from the control ($P < 0.05$) (Table 3). Similar results of potato yield were observed at MSU, namely only BAC03 significantly increased the tuber weight by 26% ($P < 0.05$) (Table 3). In 2012 at MRC, potato tuber yield from the plot treated with BAC03 was higher than those from two other treatments, but not from the control (Table 3). On the MSU farm, there were no significant differences in yield ob-

Table 1
Effect of *Bacillus amyloliquefaciens* BAC03 on scab (*Streptomyces scabies*) and plant fresh weight of radish in greenhouse trials.

Treatment	Disease score	Fresh weight (g plant ⁻¹)	
		Leaf	Root
Non-treated	0 c **	5.7 ± 0.4 c	10.4 ± 0.4 c
<i>Streptomyces scabies</i> (10 ⁶ CFU cm ⁻³) *	1.0 ± 0.2 a	6.5 ± 0.4 b	11.4 ± 0.5 b
<i>Streptomyces scabies</i> (10 ⁶ CFU cm ⁻³) and BAC03 (10 ⁵ CFU cm ⁻³)	0.3 ± 0.2 b	12.9 ± 0.9 a	27.7 ± 0.8 a

* One week post seedling emergence, liquid culture of *Streptomyces scabies* strain ATCC49173 was added to the mixture of potting mix by drenching to give a final concentration of 10⁶ CFU cm⁻³.

** Values are means (average of 16 measurements) ± standard deviations. Multiple comparisons were performed using Fisher's least square difference test at a significance level $\alpha = 0.05$. Values followed by same letters in a column are not significantly different. Disease severity was determined by measuring the lesion severity using the 0–5 scale of Wanner (2004), where 0 = no disease and 5 = the whole root is covered by scab lesions. This study was conducted two times.

Table 2
Effect of chestnut tissues on potato common scab in greenhouse trials.

Ratio of chestnut tissue in potting mix (%)	Disease score	
	24 May 2010	10 July 2010
0*	1.3 ± 0.1 a **	2.4 ± 0.3 a
50	0.7 ± 0.1 b	1.0 ± 0.2 b
100	0.3 ± 0.1 c	0.3 ± 0.1 c

* One week post seedling emergence, liquid culture of *Streptomyces scabies* strain ATCC49173 was added to the mixture of potting mix and chestnut tissues by drenching to give a final concentration of 10⁶ CFU cm⁻³.

** Values are means (average of 20 measurements) ± standard deviations. Multiple comparisons were conducted in each trial using Fisher's least square difference test. Means with same letters were not significantly different at $\alpha = 0.05$. Severity of common scab was evaluated using a 0–5 scale (10), where 0 = no disease and 5 = 50% surface area of the tuber covered by scab lesions.

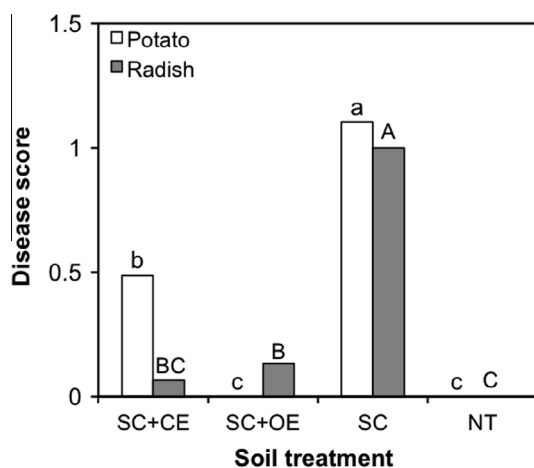


Fig. 1. Effect of oregano essential oil (OE, 0.1 $\mu\text{g cm}^{-3}$ soil) and clove essential oil (CE, 0.1 $\mu\text{g cm}^{-3}$ soil) on scab in potato (open bars) and radish (filled bars). One week after seedling emergence, potting mix was infested with *Streptomyces scabies* (SC, 10⁶ CFU cm⁻³) except non-treated control (NT). Mean values were separated by lower-case (for potato) or capitalized (for radish) letters at significance level $\alpha = 0.05$.

served among treatments with any of the different biomaterials (Table 3).

4. Discussion

In this research, different biologically-based materials were assessed for their efficacies in suppressing scab caused by *Streptomyces* spp. in potato or radish. The results indicated that although application of chestnut tissue, oregano essential oil, and horseradish showed disease inhibition in the greenhouse or growth chamber condition, their efficacies varied in the field depending on years

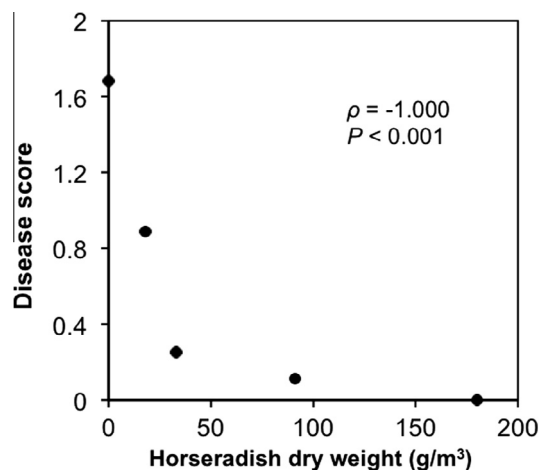


Fig. 2. Effect of ground horseradish on potato common scab tested in the greenhouse. Ground horseradish was incorporated in potting mix at various concentrations (g m⁻³). For regression analysis, the concentration was transformed to $[1/(\text{concentration} + 0.13)]$. A liquid culture of *Streptomyces scabies* strain ATCC49173 was added to the potting mix by drenching to give a final concentration of 10⁶ CFU cm⁻³ potting mix 1 week after seedling emergence. Data represents two combined trials. Severity of common scab was evaluated using a 0–5 scale (Hao et al., 2009), where 0 = no disease and 5 = 50% surface area of the tuber covered by scab lesions.

and locations. *Bacillus amyloliquefaciens* BAC03 had a consistent effect on disease reduction, with the efficacy ranging from 17% to 57% in all field trial over 2 years.

Field soil is a complex environment that contains many factors we cannot completely control. Development of potato common scab is not only affected by the pathogen population, but also by physical and other biological factors, such as weather conditions, soil properties, plant varieties, and cultivation strategies (Lazarovits et al., 2007). In this study, varied disease pressures and effects of disease management in different years and locations reflect this complexity. For example, the average disease scores at MRC farm were 1.2 and 1.75 (in the control) in 2011 and 2012, respectively. There also was a difference in disease pressure between different locations, for example the average disease scores in the control were 1.75 and 0.6 at MRC and MSU farm, respectively in 2012. Furthermore, in comparison to scab only caused by *S. scabies* under the greenhouse condition, the background of *Streptomyces* spp. in the field condition are more complex and could include multiple species of pathogenic *Streptomyces* spp. (Wanner, 2006). All above factors could affect the outcomes of disease development under natural conditions and make disease management more difficult. Similar results have been observed by other researchers. For example, Abbasi et al. (2006) reported treatment with 1% fish emulsion reduced scab severity in the field in 2004, but not in 2005, and concluded that fish emulsion was not effective in soils with high dis-

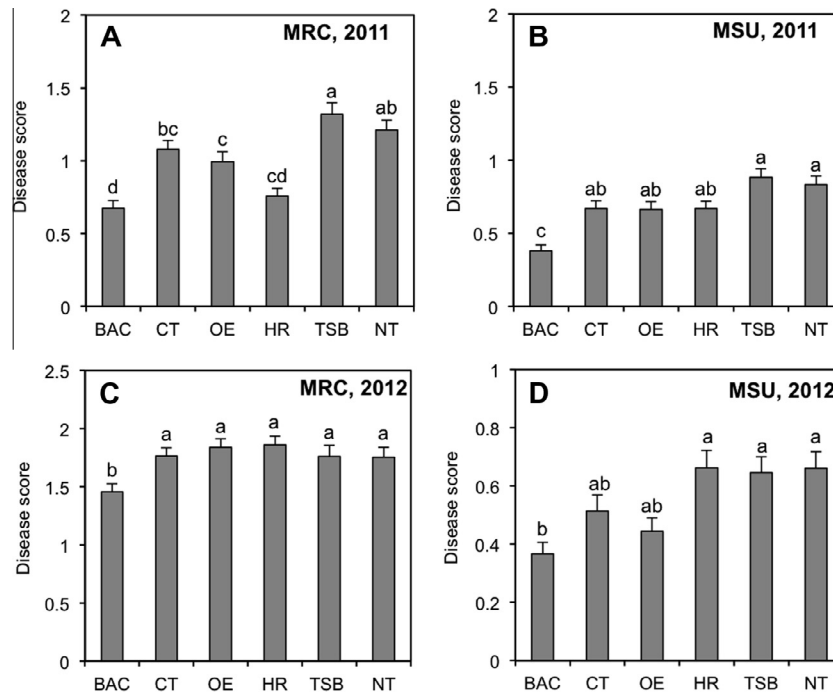


Fig. 3. Effects of biologically based materials on potato common scab ratings in field trials at Montcalm Research Center (MRC) and Soil Science Research Center on MSU campus (MSU) in 2011 and 2012. Treatments included chestnut tissues (CT, 1.15 kg m⁻²), ground horseradish (HR, 0.38 g m⁻²), and oregano essential oil (OE, 1.5 ml m⁻²) applied and mixed into the soil 2 weeks before potato planting, or a liquid culture of *Bacillus amyloliquefaciens* strain BAC03 [BAC, 1 L m⁻², 10⁶ colony forming units (CFU)] in tryptic soy broth (TSB), and TSB only as a control. Severity of common scab was evaluated using a 0–5 scale (Hao et al., 2009), where 0 = no disease and 5 = 50% surface area of the tuber covered by scab lesions. Bars on each value show standard errors. Values of the bars with the same letters were not significantly different.

Table 3

Effects of *Bacillus amyloliquefaciens* (BAC03), chestnut tissue, ground horseradish, and oregano essential oil on potato yield at Montcalm Research Center (MRC), Lakeview, and Michigan State University Soil Science Research Center (MSU), East Lansing in Michigan in 2011 and 2012.

Year	Location	Treatment	Rate of application	Yield (kg m ⁻²)
2011	MRC	BAC03 (10 ⁶ CFU ml ⁻¹)	1 L m ⁻²	2.7 ± 0.4 a [*]
		Chestnut tissue	1.15 kg m ⁻²	1.9 ± 0.2 abc
		Ground horseradish	38 g m ⁻²	2.6 ± 0.7 ab
		Oregano essential oil	1.5 ml m ⁻²	2.3 ± 0.3 abc
		Tryptic soy broth (TSB)	1 L m ⁻²	1.8 ± 0.5 bc
		Non-treated	–	1.7 ± 0.7 c
	MSU	BAC03 (10 ⁶ CFU ml ⁻¹)	1 L m ⁻²	7.3 ± 0.4 a
		Chestnut tissue	1.15 kg m ⁻²	5.6 ± 1.2 bc
		Ground horseradish	38 g m ⁻²	6.2 ± 1.3 abc
		Oregano essential oil (0.35% v/v)	1.5 ml m ⁻²	7.0 ± 0.6 ab
		TSB	1 L m ⁻²	5.4 ± 0.7 c
		Non-treated	–	5.5 ± 1.2 bc
2012	MRC	BAC03 (10 ⁶ CFU ml ⁻¹)	1 L m ⁻²	4.4 ± 0.3 a
		Chestnut tissue	1.15 kg m ⁻²	3.4 ± 0.2 b
		Ground horseradish	38 g m ⁻²	3.6 ± 0.3 b
		Oregano essential oil (0.35% v/v)	1.5 ml m ⁻²	3.8 ± 0.3 ab
		TSB	1 L m ⁻²	4.0 ± 0.5 ab
		Non-treated	–	3.9 ± 0.5 ab
	MSU	BAC03 (10 ⁶ CFU ml ⁻¹)	1 L m ⁻²	2.4 ± 0.2 ^{ns}
		Chestnut tissue	1.15 kg m ⁻²	2.1 ± 0.4
		Ground horseradish	38 g m ⁻²	2.1 ± 0.3
		Oregano essential oil (0.35% v/v)	1.5 ml m ⁻²	1.9 ± 0.3
		TSB	1 L m ⁻²	2.0 ± 0.1
		Non-treated	–	1.9 ± 0.5

^{ns} No significant differences.

* Multiple comparisons were performed for each year using Fisher's least square difference test at significance level $\alpha = 0.05$. Values followed by same letters for a given location year are not significantly different.

ease pressure (Abbasi et al., 2006). Lazarovits et al. (1999) reported amendment with soymeal, as well as meat and bone meal had an ability to inhibit potato scab in the first 2 years, while no effect was observed in the third year. In addition, application of these biomaterials had a negative impact on potato yield. Therefore,

developing a commercial product with consistent efficacy for common scab management is a challenging job.

Treatments with oregano essential oil, chestnut tissues, and horseradish all have demonstrated positive results of antimicrobial and biocontrol activities in the laboratory and under controlled

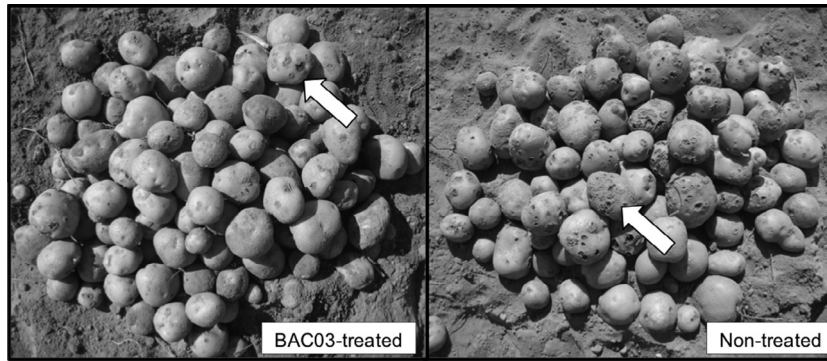


Fig. 4. Comparison of potato tubers harvested at MRC where soil was naturally infested with *Streptomyces* spp. Left panel: soil treated with *Bacillus amyloliquefaciens* strain BAC03 in tryptic soy broth, and right panel: soil treated with tryptic soy broth only. White arrow indicates scab lesions.

environment in this and previous work (Bi et al., 2012; Hao et al., 2012). They could be an addition for disease management, especially organic agricultural production. Chestnut tissues are by-products of the peeling processing. Using them for disease control can add value to the production, and the economics can be improved. While the field efficacies of these 3 products were less effective compared to BAC03, and most importantly they are not as consistent as BAC03, some efficacy was observed. We would not doubt the contribution of these 3 biomaterials in disease management, but further studies are needed to optimize their efficacies by understanding application conditions.

Among the biomaterials tested in this study, *B. amyloliquefaciens* strain BAC03 has shown a promising result in reducing potato common scab, as well as enhancing the yield of potato and radish either in greenhouse test or field trial. The field results for disease control were significant and consistent in two locations for 2 years. This is an important step for the commercialization of BAC03, and extremely important in potato production where effective strategies of common scab management are lacking. *Bacillus* spp. are a well known group of bacteria for biological control and plant growth promotion (Lugtenberg and Kamilova, 2009). In a previous study, we demonstrated antimicrobial activities against *Streptomyces* spp. in the lab, and a non-ribosomally synthesized peptide (LCI) was characterized as a major antimicrobial substance (Meng et al., 2012a). Strain *Bacillus* sp. sunhua was reported to inhibit *S. scabiei* in vitro because of macrolactin A and iturin A (Han et al., 2005), but without field validation. Schmiedeknecht et al. (1998) reported application with several *Bacillus* strains reduced common scab up to 70% in the greenhouse, and up to 67% in the field. Moreover, the plants appeared more vigorous than non-treated plants, and the yields of potato tubers were higher (up to 16%), although the bacteria mentioned above are still at research stage.

In conclusion, this research has indicated BAC03 can be a good biological control agent for potato common scab management. Horseradish, oregano essential oil, and chestnut tissues may also help in potato production. Further investigation on all of these biomaterials is needed for optimization of application, spectrum of other activity against other soilborne pathogens, and, for BAC03 effects on plant growth promotion.

Acknowledgments

This project was partially supported by Michigan Potato Industry Commission, and Michigan State University Project GREENE. The authors are grateful to Joe Coombs, Greg Steere, and Matt Zuehlke in Michigan State University Potato Breeding Center and Bruce Sackett in Montcalm Research Center for providing technical sup-

port in the field. We thank Y. Bi, H. Liu, and J. Huck for assistance in the lab and field.

References

- Abbasi, P.A., Conn, K.L., Lazarovits, G., 2006. Effect of fish emulsion used as a preplanting soil amendment on Verticillium wilt, scab, and tuber yield of potato. *Can. J. Plant Pathol.* 28, 509–518.
- Bi, Y., Jiang, H., Hausbeck, M.K., Hao, J.J., 2012. Inhibitory effects of essential oils for controlling *Phytophthora capsici*. *Plant Dis.* 96, 797–803.
- Davis, J.R., McMaster, G.M., Callihan, R.H., Nissley, F.H., Pavek, J.J., 1976. Influence of soil moisture and fungicide treatments on common scab and mineral content of potatoes. *Phytopathology* 66, 228–233.
- Dees, M.W., Wanner, L.A., 2012. In search of better management of potato common scab. *Potato Res.* 55, 249–268.
- Douches, D.S., Coombs, J., Hammerschmidt, R., Kirk, W.W., Long, C., 2009. Kalkaska: a round white chip-processing-potato variety with common scab resistance. *Am. J. Potato Res.* 86, 347–355.
- Goyer, C., Beaulieu, C., 1997. Host range of streptomycete strains causing common scab. *Plant Dis.* 81, 901–904.
- Han, J.S., Cheng, J.H., Yoon, T.M., Song, J., Rajkarnikar, A., Kim, W.G., Yoo, I.D., Yang, Y.Y., Suh, J.W., 2005. Biological control agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. *J. Appl. Microbiol.* 99, 213–221.
- Hao, J.J., Meng, Q.X., Yin, J.F., Kirk, W.W., 2009. Characterization of a new *Streptomyces* strain, DS3024, that causes potato common scab. *Plant Dis.* 93, 1329–1334.
- Hao, J.J., Liu, H.W., Donis-Gonzalez, I.R., Lu, X.H., Jones, A.D., Fulbright, D.W., 2012. Antimicrobial activity of chestnut extracts for potential use in managing soilborne plant pathogens. *Plant Dis.* 96, 354–360.
- Hiltunen, L.H., Ojanpera, T., Kortemaa, H., Richter, E., Lehtonen, M.J., Valkonen, J.P.T., 2009. Interactions and biocontrol of pathogenic *Streptomyces* strains co-occurring in potato scab lesions. *J. Appl. Microbiol.* 106, 199–212.
- Jiang, H.H., Meng, Q.X., Hanson, L.E., Hao, J.J., 2012. First report of *Streptomyces stelliscabiei* causing potato common scab in Michigan. *Plant Dis.* 96, 904.
- Larkin, R.P., 2008. Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato. *Soil Biol. Biochem.* 40, 1341–1351.
- Larkin, R.P., Griffin, T.S., 2007. Control of soilborne potato diseases using Brassica green manures. *Crop Prot.* 26, 1067–1077.
- Lazarovits, G., Conn, K.L., Potter, J., 1999. Reduction of potato scab, Verticillium wilt, and nematodes by soymeal and meat and bone meal in two Ontario potato fields. *Can. J. Plant Pathol.* 21, 345–353.
- Lazarovits, G., Hill, J., Patterson, G., Conn, K.L., Crump, N.S., 2007. Edaphic soil levels of mineral nutrients, pH, organic matter, and cationic exchange capacity in the geocaulosphere associated with potato common scab. *Phytopathology* 97, 1071–1082.
- Loria, R., Bukhalid, R.A., Creath, R.A., Leiner, R.H., Olivier, M., Steffens, J.C., 1995. Differential production of thaxtomins by pathogenic *Streptomyces* species in vitro. *Phytopathology* 85, 537–541.
- Loria, R., Bukhalid, R.A., Fry, B.A., King, R.R., 1997. Plant pathogenicity in the genus *Streptomyces*. *Plant Dis.* 81, 836–846.
- Loria, R., Kers, J., Joshi, M., 2006. Evolution of plant pathogenicity in *Streptomyces*. *Annu. Rev. Phytopathol.* 44, 469–487.
- Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63, 541–556.
- Meng, Q.X., Jiang, H.H., Hanson, L.E., Hao, J.J., 2012a. Characterizing a novel strain of *Bacillus amyloliquefaciens* BAC03 for potential biological control application. *J. Appl. Microbiol.* 113, 1165–1175.
- Meng, Q.X., Yin, J.F., Rosenzweig, N., Douches, D., Hao, J.J., 2012b. Culture-based assessment of microbial communities in soil suppressive to potato common scab. *Plant Dis.* 96, 712–717.

- Sakuma, F., Maeda, M., Takahashi, M., Hashizume, K., Kondo, N., 2011. Suppression of common scab of potato caused by *Streptomyces turgidiscabies* using lopsided oat green manure. *Plant Dis.* 95, 1124–1130.
- Schmiedeknecht, G., Bochow, H., Junge, H., 1998. Use of *Bacillus subtilis* as biocontrol agent. II. Biological control of potato diseases. *J. Plant Dis. Prot.* 105, 376–386.
- Singhai, P.K., Sarma, B.K., Srivastava, J.S., 2011. Biological management of common scab of potato through *Pseudomonas* species and vermicompost. *Biol. Control* 57, 150–157.
- Wanner, L.A., 2004. Field isolates of *Streptomyces* differ in pathogenicity and virulence on radish. *Plant Dis.* 88, 785–796.
- Wanner, L.A., 2006. A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. *Phytopathology* 96, 1363–1371.
- Wanner, L.A., 2007. A new strain of *Streptomyces* causing common scab in potato. *Plant Dis.* 91, 352–359.
- Wanner, L.A., Haynes, K.G., 2009. Aggressiveness of *Streptomyces* on four potato cultivars and implications for common scab resistance breeding. *Am. J. Potato Res.* 86, 335–346.