Subject category: Note - Soilborne pathogens / Agents pathogènes telluriques

CANADIAN GOLDENROD RESIDUES AND EXTRACTS INHIBIT THE GROWTH OF *STREPTOMYCES SCABIEI*, THE CAUSAL AGENT OF POTATO COMMON SCAB

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Accepted 11 November 2017

Abstract: Common scab is one of the most important diseases affecting potato crops worldwide. Using fresh residues and/or bio-products of Canadian goldenrod (*Solidago canadensis*) may offer an alternative to harmful conventional fumigants. In this study, we aimed to: i) conduct a preliminary investigation of the utilization of *S. canadensis* to reduce common scab severity (Experiment 1), and ii) determine the allopathic potentials of *S. canadensis* extracts on *Streptomyces scabiei* (also known as *S. scabies*), the most important soil pathogen responsible for causing common scab in North America (Experiment 2). Compared to control plants, preliminary results showed that adding 1.2 kg of fresh *S. canadensis* residue per m^2 reduced scab severity by about 45 % (Experiment 1). Furthermore, hexane and dichloromethane extracts of *S. canadensis*, at a concentration of 200 $\mu g \cdot mL^{-1}$, inhibited the growth of *S. scabiei* by about 97 % (Experiment 2). These results were comparable with those using tetracycline (2.5 $\mu g \cdot mL^{-1}$), a known inhibitor of *S. scabies*. Both experiments suggested that *S. canadensis* may represent a new approach for controlling potato common scab. More studies are required to better understand the mechanisms involved in *S. canadensis* induced reduction of common scab in order to standardize the approaches.

Keywords: Allelopathic, common scab, Canadian goldenrod, *Solidago canadensis, Streptomyces scabies.*

Résumé : La gale commune est une maladie tellurique importante chez la pomme de terre et l'utilisation de résidus et/ou extraits de verge d'or du Canada (*Solidago Canadensis*) pourrait représenter une alternative prometteuse aux pesticides (fumigants) utilisés pour combattre la maladie. Les objectifs de cette recherche étaient i) effectuer une expérience préliminaire afin de mesurer les effets de l'incorporation de résidus frais de *S. canadensis* sur la sévérité de la gale commune (expérience 1) et ii) déterminer les potentiels allélopathiques des extraits de S. *canadensis* sur *Streptomyces scabiei*, un important agent pathogène causant la maladie de la gale commune (expérience 2). Nos résultats préliminaires issus de l'expérience 1 montrent qu'ajouter 1.2 kg m^{-2} de *S. canadensis* (résidus frais) permet de réduire significativement de 45 % la sévérité de la gale commune. Les extraits de *S. canadensis* effectués avec l'hexane et le dichlorométhane et à des concentrations de 200 µg mL^{-1} permettent d'inhiber à 97 % la croissance de *S. scabiei*, résultats comparables à la tétracycline (2.5 µg mL^{-1}), un antibiotique connu pour inhiber la croissance de *S. scabiei*. Les résultats de cette étude montrent clairement et pour une première fois le potentiel d'utilisation de *S. canadensis* comme moyen de lutte contre la maladie de la gale commune chez la pomme de terre. D'autres recherche seront toutefois nécessaires pour bien comprendre et cibler les mécanismes impliqués afin de standardiser et d'optimiser cette nouvelle et prometteuse approche.

Mots clés : Allélopathie, gale commune, verge d'or du Canada, *Solidago canadensis, Streptomyces scabiei.*

Introduction

Common scab is one of the most widespread and important diseases affecting potato crops worldwide. In Canada, losses associated with common scab were estimated at $15–17 million for 2002 alone (Hill & Lazarovits 2005). This disease is caused by filamentous gram-positive bacteria (Actinobacteria) of the genus *Streptomyces* (Locci 1994), with *Streptomyces scabiei* (Thaxter) Lambert and Loria being the most important causal agent in North America (Wanner 2006; Wanner 2007; St-Onge et al. 2008). Effective means of reducing potato common scab are still needed, as inconsistent results have been reported with nearly all current methods, including reducing soil pH (pH <5.5), introducing crop rotation and cover crops, and improving soil fertility and irrigation management (Dees & Wanner 2012). Soil fumigation can mitigate the symptoms of common scab (Braun et al. 2017). However, as most of the soil fumigants have negative environmental impacts and are very expensive, their use in Canada has been strongly restricted over the last decade, increasing the need to develop more environmentally-friendly alternatives.

Canadian goldenrod (*Solidago canadensis* L.) is a plant species originally from north-eastern America that produces many allelopathic chemicals that negatively affect the germination, growth, and reproduction of many other plants (Sun et al. 2006; Abhilasha et al. 2008). The allelochemicals produced by *S. canadensis* include flavonoids (Krepinsky & Herout 1962; Apáti et al. 2003), phenolic acids (Kalemba 1992), sesquiterpenes (Bohlmann et al. 1980), diterpenes (Anthonsen et al. 1969; Reznicek et al. 1991), and saponins (Reznicek et al. 1991). These allelochemicals likely have a marked effect on soil microorganisms, favoring positive or negative feedbacks related to crop protection. Previous studies have demonstrated that *S. canadensis* allelochemicals may significantly reduce specific soil-borne diseases on tomato associated with *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn (Zhang et al. 2009b; Zhang et al. 2011). However, the same authors also showed that *S.*

canadensis may alter soil fertility by significantly reducing soil nitrification processes ($N\text{-}NO_3^-$) six-fold and by decreasing soil phosphorus content by about 11 times (Zhang et al. 2009a).

Using bio-extracts of *S. canadensis* may offer an alternative to harmful conventional fumigants. To our knowledge, the use of *S. canadensis* (either whole plant and/or extracts) to reduce common scab on potatoes has never been tested. Our study objectives were to: i) conduct a preliminary investigation of the utilization of *S. canadensis* to reduce potato common scab symptoms and minimize decreases in soil fertility (Experiment 1); and ii) assess the allopathic potential of *S. canadensis* extracts on *S. scabiei* (Experiment 2).

Material and methods

Experiment 1

A greenhouse experiment was carried out at the Université du Québec à Chicoutimi during the summer of 2014. A full factorial experiment was designed, with the first factor being with/without a mineral fertilizer application and the second factor with different rates of *S. canadensis* (0, 0.7, 0.9, 1.0, and 1.2 kg of fresh biomass per m^2). There were five replicates, providing a total of 50 experimental units (EU), where each of the EU comprised a 12 L capacity pot (25 cm diameter × 25 cm high).

The soil used for this experiment was collected from a local potato field (48° 36′ 52″ N; 71° 17′ 47″ W) that had a history of serious common scab. The soil was a Bourget loam containing about 45 % sand and 50 % silt (Raymond 1971). The initial chemical properties of this soil are presented in Table 1. The soil was thoroughly mixed by placing it on a tarp and using a rake to homogenize the soil before potting. All fertilized pots received the equivalent of 135 kg ha^{-1} of N, 200 kg ha^{-1} of P_2O_5, and 160 kg ha^{-1} of K_2O to match the fertilizer requirements for potato in Quebec (Pellerin 2010). We used calcium ammonium nitrate, ammonium phosphate, and potassium chloride as sources for N, P, and K, respectively. All fertilizers were granular and thoroughly mixed within the soil (about 10 L of soil per pot).

Above ground biomass (stems and leaves) of *S. canadensis* was collected from a local abandoned field (48° 34′ 20″ N; 71° 21′ 50″ W) at the end of June 2014, since the leaves of *S. canadensis* have more allelopathic chemicals than any other parts of the plant (Sun et al. 2006). The *S. canadensis* biomass was immediately cut into ~1 cm pieces using a coffee grinder and then manually incorporated (as fresh residues) into the potting soil in accordance with the respective treatment concentrations. The latter two steps were performed as fast as possible to minimize the loss of volatile metabolites. Water was added to all pots to provide soil moisture at about 80 % of field capacity. All EU were randomly distributed throughout the greenhouse.

A moderately resistant dark red potato ‘Chieftain’ that is widely used among producers in the region (Quebec Parmentier, Saint-Ambroise, Quebec) was planted at a 12 cm depth (one single tuber per EU) 10 days after fertilization and the incorporation of *S. canadensis*. Day and night temperatures were maintained at ca. 24 and 12 °C, respectively. Artificial light was used in order to keep photoperiod times at 15 hours per day. Soil moisture was kept constant during the growing period at 70 to 80 % of field capacity (watering from 2 to 7 times per week, depending on the plant development stage). Relative humidity in the air varied from 60 to 80 % throughout the experiment. Plants were grown for 72 days in the greenhouse.

At harvest, each potato plant was separated into three parts: i) above ground (stems and leaves), ii) roots, and iii) tubers. The specific gravity of each tuber was determined using a device that weighed the tuber in air and in water (Fong & Redshaw 1973). The scab severity was determined by taking the average of all tubers from one plant. The scab severity from each EU was determined based on the percent surface area infected (0 to 100 % scale) and was calculated for each tuber using the following equation:

$$\text{Scab severity, \%} = \frac{a + b + c + d + n}{A + B + C + D + N} * 100$$

where lowercase is the sum of surface area covered by the scab lesions (in mm^2) on each tuber, and uppercase is the total surface of each tuber (in mm^2). An electronic Vernier scale was used to measure lengths, whereas simple two-dimensional geometrical shapes (circles, squares, triangles, or rectangles) were used to compose and calculate surface area covered by each of the scab lesion. Two main geometric shapes (sphere or ellipsoid) were used to estimate the total surface of each tuber. Thereafter, all parts of the plant were dried at 60 °C for 7 days to obtain dry biomass.

Experiment 2

Extracts were obtained from air-dried and powdered aerial parts of *S. canadensis* (100 g) using a Soxhlet extractor with successive soakings with hexane, dichloromethane, and methanol. Tissues were left in each solvent for 24 hr. After filtration, the solvents were evaporated under vacuum. The antibacterial activity of *S. canadensis* extracts was evaluated on strain *S. scabiei* EF-35 (Faucher et al. 1992). Briefly, *S. scabei* was cultured in Yeast Mold Extract Broth medium (YMEB) until an optical density of 0.1 was reached. Then, bacterial cells were diluted 1/5 in YMEB medium and a volume of 100 µL of this dilution was seeded in 96-well microplates. The extracts were first dissolved in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 80 $mg{\cdot}mL^{-1}$ and then diluted by a factor of 1/200 and 1/800 in YMEB to obtain a concentration of 400 $\mu g{\cdot}mL^{-1}$ and 100 $\mu g{\cdot}mL^{-1}$, respectively. A volume of 100 µL of these two prepared concentrations was then added to the microplates containing bacteria, to obtain final concentrations for the extracts of 200 $\mu g{\cdot}mL^{-1}$ and 50 $\mu g{\cdot}mL^{-1}$. The final concentrations (0.25 % and 0.0125 %) of DMSO diluted in YMEB were not found to be toxic against *S. scabiei* (data not shown). Tetracycline (2.5 $\mu g{\cdot}mL^{-1}$) was used as a positive control. The microplates were then incubated at 30 ˚C for 24 hr. After this, a volume of 50 µL of resazurin (62.5 $\mu g\ mL^{-1}$) was added to each well (O'Brien et al. 2000) and the microplates were incubated for another hour at 30 ˚C.

The fluorescence was performed using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Each condition was replicated six times, and the growth inhibition was calculated by comparing the fluorescence of bacteria-filled wells with the untreated negative controls, after subtraction of the blank. All results were triplicates of three representative experiments.

Statistical analysis

Generalized linear models were used for variance analysis. Normality and homogeneity of the variance were verified before any analyses took place. The least significant difference (LSD) was used when generalized model showed significant differences among *S. canadensis* inputs (Experiment 1), whereas Student-Newman-Keul (SNK) was used as a post-hoc test to detect differences between the treated and untreated bacteria microplates (Experiment 2). All statistical analysis were performed using SPSS for Windows software, Version 21.0, Released 2012 (IBM Corp. 2012).

Results and discussion

Experiment 1

Preliminary results show that scab severity was significantly reduced with *S. canadensis* residues (Table 2); adding 1.2 kg of fresh *S. canadensis* residue per m^2 reduced the coverage of lesions on the tubers by ~20 % (Fig. 1). In relative terms, this represents a 45 % reduction of disease severity. In comparison, a recent study from Eastern Canada showed a disease reduction of 30 % when using conventional fumigants and 36 % when combined with mustard meal (crushed *Brassica juncea* at 1065 kg ha^{-1}) (Al-Mughrabi et al. 2016). Furthermore, unlike the latter study, *S. canadensis* inputs did not significantly affect (positively or negatively) tuber yields, specific gravity, nor biomass production (Table 2). Therefore, there is no evidence that adding *S. canadensis* residues up to 1.2 kg m^{-2} alter soil fertility.

Adding mineral fertilizers significantly reduced scab severity by about 16 % (Tables 2 and 3). Moreover, adding mineral fertilizers strongly improved soil fertility by increasing above ground, root, and tuber biomass each by about 2-fold, but also helped to produce healthier plants that were more resistant to common scab disease (Table 3). Indeed, a soil that provides good growing conditions (e.g. abundant nutrients) will favor plant health, an effect known as a part of the soil suppressiveness to scab (Janvier et al. 2007), as recently demonstrated for mustard meal (Al-Mughrabi et al. 2016). However, the lack of significant interactions between the fertilizer and *S. canadensis* residues (MF × SC: non-significant, Table 2) may suggest that the processes involved in the disease suppression are not the same among these two factors. Other disease control mechanisms such as bio-fumigant and/or microorganism control effects should therefore be investigated (Experiment 2).

Experiment 2

We assessed the effects of hexane, dichloromethane, and methanol extracts from *S. canadensis* on the growth of *S. scabiei*. Bacteria were incubated in the presence or absence of extracts (50 and 200 $\mu g\ mL^{-1}$) or tetracycline (2.5 $\mu g\ mL^{-1}$), a positive control antibiotic. Tetracycline inhibited *S. scabiei* growth by about 99 %. Interestingly, the results showed that hexane and dichloromethane extracts at a concentration of 200 $\mu g\ mL^{-1}$ significantly inhibited the growth of *S. scabiei* by about 97 % in comparison with 86 % for methanol extracts (Fig. 2). At the concentration of 50 $\mu g\ mL^{-1}$, dichloromethane was found the most effective extract inhibiting *S. scabiei* survival by about 82 % in comparison with 57 % and 50 % for hexane and methanol extracts, respectively (Fig. 2). These results confirm that *S. canadensis* contains active compounds (allelochemicals) that operate against *S. scabiei*.

Antimicrobial activity of extracts of *S. canadensis* has been already reported (Deepa & Ravichandiran 2010), but our study is the first that specifically shows antimicrobial activity again *S. scabiei*. Although experiment 1 presents preliminary data (conducted once and unreplicated experiment), both experiments suggest that *S. canadensis* is a new and promising avenue for controlling potato common scab. More studies are needed to clearly identify the molecules involved in these processes and to better understand their efficacy under field conditions (e.g. competition and/or mutualism among soil microorganisms) to better control and standardize the use of *S. canadensis*.

Funding

The authors thank the Fonds de développement de l'Université du Québec à Chicoutimi (FUQAC : Fonds de soutien à la recherche sur la qualité des sols agricoles nordiques) for financial support.

Acknowledgments

The authors are grateful to Catherine Dussault and Karl Girard-Lalancette for their help with biological assays. Samuel Morissette and Propur Inc. are thanked for providing soils and potato seeds. We thank Dre C. Beaulieu for providing the *S. scabiei* EF-35 strain. We also want to thank the anonymous reviewers for their careful reading and their many insightful comments and suggestions.

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Table 1. Chemical properties of the soil used for Experiment 1.

Soil property, unit	Value
Soil pH	6.1
Soil organic matter, %	3.9
Soil cation exchange capacity (CEC), meq 100 g^{-1}	7.6
P_2O_5, mg kg^{-1}	11.2
K_2O, mg kg^{-1}	74.1
Mg, mg kg^{-1}	88.0
Ca, mg kg^{-1}	625
Al, mg kg^{-1}	2,030
Fe, mg kg^{-1}	52

Table 2. *F* values and probabilities obtained from generalized linear models testing the effects of the mineral fertilizer (MF) and *Solidago canadensis* (SC) inputs on response variables.

Source of variation	df	$AGBM_{dry}$	RBM_{dry}	Tuber $yield_{dry}$	Tuber specific gravity	Scab severity
		F value (*P*)				
Mineral fertilizer (MF)	1	**138.4 (<0.001)**	**23.4 (<0.001)**	**48.1 (<0.001)**	3.7 (0.061)	**14.2 (0.001)**
Solidago canadensis (SC)	4	1.4 (0.262)	1.5 (0.210)	0.9 (0.467)	0.8 (0.544)	**3.0 (0.030)**
MF × SC	4	1.1(0.394)	0.7 (0.572)	0.7 (0.590)	0.8 (0.515)	0.8 (0.552)

df: degrees of freedom; AGBM: aboveground biomass; RBM: root biomass.

Numbers in **bold** are significant ($P < 0.05$).

Table 3. Mineral fertilizer (MF) applications significantly affecting above-ground biomass (AGBM), root biomass (RB), tuber yield, and scab severity.

Property, unit	Not fertilized	Fertilized
	Average (STD)	
$AGBM_{dry}$, $g{\cdot}plant^{-1}$	10.72 (1.66)	19.98 (3.64)
RBM_{dry}, $g{\cdot}plant^{-1}$	3.97 (1.19)	5.62 (1.25)
Tuber $yield_{dry}$, $g{\cdot}plant^{-1}$	22.02 (5.67)	36.05 (8.18)
Scab severity, %	44.86 (17.40)	29.88 (12.00)

STD: standard deviation from the mean of 25 sample size per treatment (n=50).

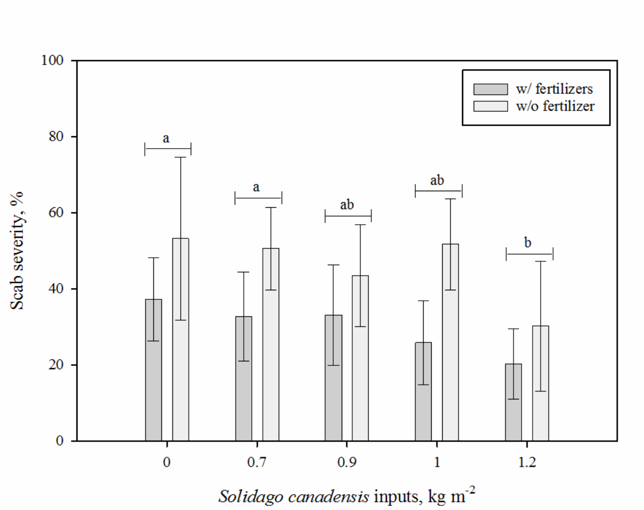

Fig. 1. The effect of *Solidago canadensis* inputs (kg of fresh stems and leaf residues per m^2) on the scab severity (coverage of lesions; 0 to 100 % scale). Letters indicate significant differences between combined averages using a least significant difference (LSD) post-hoc test. Error bars represent the standard deviation from the mean (n=5). There was a significant difference between fertilized (w/ fertilizers) and unfertilized (w/o fertilizer) treatments (Table 2).

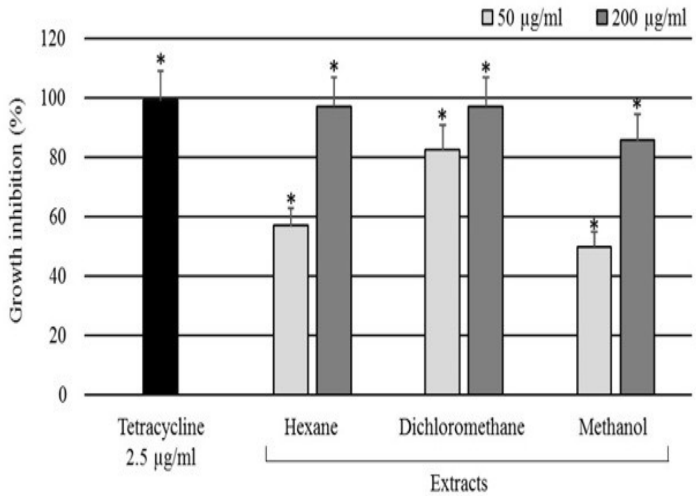

Fig. 2. Growth inhibition of *Streptomyces scabiei* due to *S. Solidago canadensis* extracts after 24 hr of treatment at concentrations of 50 µg·mL^{-1} (light gray column) and 200 µg·mL^{-1} (dark gray column). Tetracycline (2.5 µg·mL^{-1}) was used as positive control (black column). * Significantly different from untreated bacteria based on Student-Newman-Keuls post-hoc test ($P < 0.05$). Error bars are standard deviations from the mean. These results are triplicates representative of three different experiments.