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SCIENTIFIC ARTICLE
Phytopathology

Effect of pH soil and irrigation regimes on management of potato scab

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ABSTRACT

Potato scab caused by different species of phytopathogenic *Streptomyces* is considered one of the main bacterial diseases of economic crop importance worldwide. Several studies are being carried out in order to control the disease, but until now, there is no efficient way to do this. Some management strategies have been investigated including application of chemical and biological products and utilization of resistant cultivars of potato but there are few reports about the impact of pH and irrigation regimes on the disease. The present study aimed to evaluate the effects of these last two factors on the incidence and severity of potato scab caused by *S. scabiei*, *S. acidiscabies*, *Streptomyces* sp., *S. caviscabies* and *S. europaeiscabiei* in assays at pH 4.0, 4.5, 5.0, 5.5, 6.5 and 7.5; and irrigation regimes of once a week, alternate days and daily in greenhouse conditions. The experimental design for the pH tests was randomized blocks arranged in a 5x2 factorial scheme, with 5 replications and 3x2 for the irrigation regimes with 5 replications. The pH tests showed significant differences between the treatments and pH 4,0 - 4,5 presented lower incidence and severity of the disease for the most species tested but no significant differences were observed between the irrigation regimes. The soil acidification is considered a classic strategy for management of the disease and the results obtained herein corroborated this hypothesis.

Keywords: *Streptomyces*; potential of hydrogen; soil moistur; disease control.

INTRODUCTION

Potato (*Solanum tuberosum*) is the third most consumed food in the world after wheat and rice (DEVAUX et al., 2014; HAVERKORT; STRUIK, 2015). The crop can be affected by bacteria, fungi and viruses causing damage to all parts of the plant, with significant losses in production (FIERS et al., 2012).

Potato scab is an important bacterial disease caused by *Streptomyces* species that affect mainly the tubers causing necrotic lesions with a corky texture that may be from superficial to more extensive levels with different colors and aspects (DELLEMAN et al., 2005; LORIA, 2001). The disease external symptoms modify the appearance of potato tubers reducing their market value in natura or processed and in seed tubers production (LORIA et al., 1997). The severity and occurrence of symptoms may vary according to the environment, cultivar susceptibility, pathogen virulence and amount of inoculum (KEINATH; LORIA, 1991; LORANG et al., 1995; TÓTH et al., 2001). In Brazil, this disease is widespread throughout the country and has become a limiting factor in potato fields. Besides potato, phytopathogenic *Streptomyces* cause diseases in diverse root crops like radish, turnip, beet, carrot and sweet potato (GOYER; BEAULIEU, 1997; LABEDA; LYONS, 1992).

The description of potato scab occurred over 100 years ago (LORIA et al., 1997), but there is not yet a fully efficient control for the disease. According to DEES; WANNER (2012), the main control strategies of the disease include increased soil moisture during the tuberization period, soil acidification, crop rotation, biological control, healthy seeds, chemical control and use of resistant cultivars. However, these methods are not always reliable since contradictory results are

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presented in the literature and are not valid for all species associated to the disease (LAZAROVITS, 2010; PETERS et al., 2004; WATERER, 2002; WILSON et al., 2001).

The practices for altering the pH and soil moisture are considered classic measures regarding to potato scab control (LORIA, 2001). Maintenance or reduction of soil pH to values less than 5.2 generally suppress *Streptomyces scabiei*, causal agent of common scab (LAMBERT; LORIA, 1989a; LAMBERT et al., 2005). However, other pathogenic species such as *Streptomyces acidiscabies* (LAMBERT; LORIA, 1989b) and *Streptomyces turgidiscabies* (MIYAJIMA et al., 1998) tolerate soil pH values lower than 5.2. LACEY; WILSON (2001) found good control results for common scab with pH values lower than 5.0, but WIECHEL; CRUMP (2010) concluded that disease control was not related to the pH.

The increase of soil moisture, mainly in the period of tuberization, is considered a strategy to contain the disease since humid soils can provide a high growth rate of antagonists, greater availability of manganese and reduction the oxygen concentrations, conditions that can affect the growth rate of pathogenic *Streptomyces* (LEWIS, 1970). According to WILSON et al. (2001), non-irrigated soils in the tuberization period showed a higher degree of disease symptoms; however, LARKIN et al. (2011) reported that irrigation caused an increase in the number of potato scab symptoms.

Among controversies and a few reports on the role of soil pH and irrigation management, this work aimed to evaluate these two parameters in relation to the incidence and severity of potato scab caused by *Streptomyces* spp.

MATERIAL AND METHODS

Strains and growth conditions

Type strains of *S. acidiscabies* IBSBF 2110^T (= DSMZ 41668^T), *Streptomyces caviscabies* (GOYER et al., 1996) IBSBF 2051^T (= CFBP 4545^T), *Streptomyces europaeiscabiei* (BOUCHEK-MECHICHE et al., 2000) IBSBF 2023^T (= CFBP 4497^T) and two Brazilian strains, *S. scabiei* IBSBF 2950 and *Streptomyces* sp. IBSBF 2959, provided by the Phytobacteria Culture Collection of Instituto Biológico (IBSBF), Campinas, São Paulo, Brazil, were used in this study. *Streptomyces* strains were cultured in YME medium (4 g yeast extract, 10 g malt extract, 4 g dextrose, 18 g agar, per liter), pH 7.0 at 28 °C for 14 days. Then, the inoculum was scraped, added to 250 mL of sterilized Say 2X Solution (40 g sucrose, 2.4 g asparagine, 1.2 g K2HPO4, 20 g yeast extract, per liter), inoculated in sterilized vermiculite and maintained at 28 °C for 14 days. After bacterial growth, the vermiculite was added to the substrate in the proportion 1:12 in 2 L vessels.

pH and irrigation regimes assays

Potato seed minitubers (*S. tuberosum* L. ‘Agata’) were kindly provided by Solei Papa Tecnologia Company, Vargem Grande do Sul, São Paulo, Brazil. The assays were carried out in the winter (June to August) of 2018 in a greenhouse in the Laboratório de Bacteriologia Vegetal, Instituto Biológico, Campinas, São Paulo, Brazil. The temperatures in this period presented a minimum of 13.1 °C and a maximum of 26.8 °C. In addition, pH tests were also carried out with *S. acidiscabies* and *S. scabiei* species in the summer (December to March) with temperatures of 19.2 to 30.9 °C in order to compare the behavior of the pathogens in different temperatures.

Sterilized substrates (Sphagnum) at pH 4.0; 4.5; 5.5; 6.5, and 7.5, kindly provided from Carolina Soil Industrial Company, Santa Cruz do Sul, Rio Grande do Sul, Brazil, were tested. Before testing, the substrates were analyzed and presented the following characteristics: pH 4.0, N-ammonia 3.1 mg·L^{-1} (distillation), P 1.0 mg·L^{-1} (ICP-OES/Inductively Coupled Plasma Optical Emission Spectrometry), K 0.2 mg·L^{-1} (ICP-OES), Ca 7.8 mg·L^{-1} (ICP-OES), Mg 3.4 mg·L^{-1} (ICP-OES); pH 4.5, N-ammonia 3.5 mg·L^{-1} (distillation), P 0.4 mg·L^{-1} (ICP-OES), K 0.01 mg·L^{-1} (ICP-OES), Ca 2.3 mg·L^{-1} (ICP-OES), Mg 1.1 mg·L^{-1} (ICP-OES); pH 5.5, N-ammonia 0.3 mg·L^{-1} (distillation), P 0.3 mg·L^{-1} (ICP-OES), K < 0.01 mg·L^{-1} (ICP-OES), Ca 6.0 mg·L^{-1} (ICP-OES), Mg 4.9 mg·L^{-1} (ICP-OES); pH 6.5, N- ammonia 0.5 mg·L^{-1} (distillation), P 0.2 mg·L^{-1} (ICP-OES), K < 0.01 mg·L^{-1} (ICP-OES), Ca 8.2 mg·L^{-1} (ICP-OES), Mg 7.8 mg·L^{-1} (ICP-OES); and pH 7.5, N-ammonia 0.7 mg·L^{-1} (distillation), P 0.2 mg·L^{-1} (ICP-OES), K 0.4 mg·L^{-1} (ICP-OES), Ca 10.9 mg·L^{-1} (ICP-OES), Mg 4.7 mg·L^{-1} (ICP-OES). During the assays the substrate pH measurements were checked using the Pour Through method (LEBUDE; BILDERBACK, 2009).

The experimental design for pH tests was a randomized block with five replicates, in a 5×2 factorial arrangement. According to FAO (2008), soil with pH 5.2-6.4 is considered suitable for potato planting, so this study used the pH 6.5 as positive control and pots non-inoculated pots as negative control. The substrates were moistened with 100 mL of water/vessel on alternate days.

Irrigation assays, using sterilized substrate based on coconut fiber Golden Mix from Amafibra-Fibras e Substratos Agrícolas da Amazônia Company, Holambra, São Paulo, Brazil, consisted of once a week, alternate days and daily were and the experimental design established in a 3 x 2 factorial arrangement, with 5 replications. Until 45 days of planting, the irrigation was done on alternate days with 100 ml of water/vessel for all treatments, after this period the irrigation regimes were initiated with the beginning of the tuberization phase. Substrate without pathogen was used as control and an individual vessel containing one potato plant represented an experimental unit.

Experiments evaluation and statistical analysis

After senescence plants, the tubers were harvested and the disease incidence was calculated according to the diagrammatic scale of JAMES (1971), which evaluated the percentage of area of the tuber covered by lesions (1, 10, 25, or 50%). For the statistical analysis, scores from 1 to 4 were assigned according to the percentage of symptoms, respectively.

The severity of the disease was evaluated according to the scale of WANNER (2007), with a score 0 = without lesions, 1 = superficial lesions, <10 mm in diameter, 2 = superficial lesions> 10 mm in diameter, 3 = raised lesions <10 mm in diameter, 4 = raised lesions > 10 mm in diameter, 5 = deep lesions.

Statistical analysis was achieved using variance analysis (factorial ANOVA, $P \leq 0.05$) and Tukey's test using SISVAR software version 5.3 for Windows (Statistical Analysis Software, UFLA, Lavras, MG, Brazil). The data were transformed by $\sqrt{x}$ +0.5.

RESULTS AND DISCUSSION

pH assays

During the period of the assays, the pH of the substrates was verified and variations of no more than 0.5 were registered. Significant differences between the five values of pH inoculated with *S. acidiscabies*, *S. scabiei*, *Streptomyces* sp. or *S. caviscabies* were observed. The highest incidence values of potato scab lesions were observed at pH 6.5 and 7.5 for all species tested. Treatments at pH 4.0 showed disease suppression for all strains evaluated when compared with pH 6.5, considered positive control. At pH 4.5, lower incidence values of potato scab lesions were observed for *S. scabiei*, *Streptomyces* sp. and *S. caviscabieis*. Only *S. caviscabieis* was suppressed at pH 5.5 and *S. acidiscabies* showed the highest incidence values in all treatments (notes 2.0-2.5), except at pH 4.0 which showed incidence disease note of 1.0 (Fig. 1).

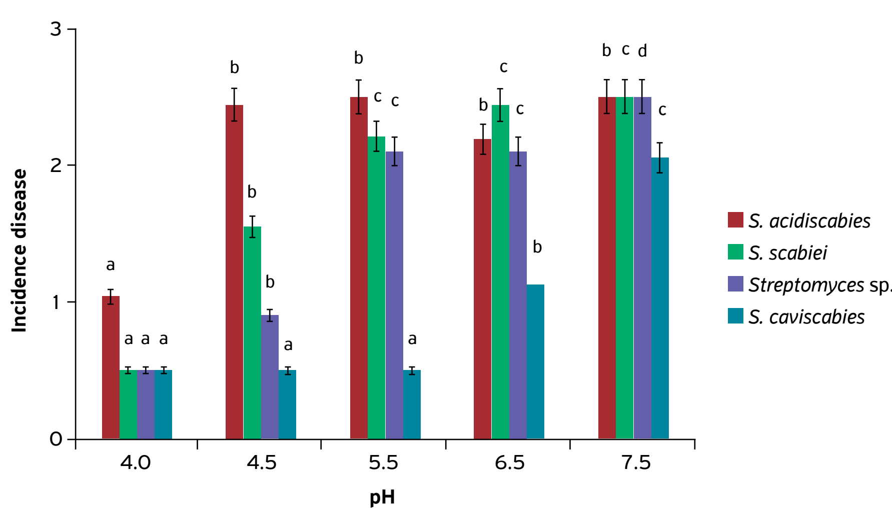

Figure 1. Incidence score of potato scab on different pH values of the substrates attributed to the symptoms caused by different species of *Streptomyces*. Means with the same lowercase letters in the same *Streptomyces* species were not significantly different according to Tukey's test at p = 0.05.
Source: Elaborated by the authors.

Streptomyces acidiscabies has been related to soils with pH less than 5.2; tolerating pH 3.8, in vitro tests (LAMBERT; LORIA, 1989a). Our results corroborated this data since this bacterial species was the only one that presented highest incidence value of potato scab lesions at pH 4.0.

The behavior of each strain tested was variable. At pH 4.0, *S. scabiei* showed similar results to the negative control (note 0.5) and at pH 4.5, this bacterial strain presented no significant differences when compared to *S. acidiscabies* and *Streptomyces* sp. (IBSBF 2959), but differences were observed when compared to the other treatments. Soils with pH values lower than 5.2 are generally considered suppressive for potato scab caused by *S. scabiei* (WAKSMAN, 1921; POWELSON et al., 1993).

LACEY and WILSON (2001) observed that 9 of 10 samples tested did not present disease symptoms in soil below pH 5.0, but in our study *S. scabiei* was able to cause disease symptoms from pH 4.5.

In tests with *Streptomyces* sp. (IBSBF 2959) strain, the highest incidence score was observed at pH 7.5 (note 2.5) while at pH 4.5, 5.5, and 6.5, the behavior of this strain was similar to *S. scabiei*, including at pH 4.0 which showed no differences with the negative control.

The use of soils with acidic pH can cause soil nutritional modification, with less distribution of some nutrients and increase of others. Low pH soils can cause an increase in the manganese levels (WATERER, 2002) and, according to THOMPSON and HUBER (2007), manganese increases the resistance of the tuber tissue to pathogen attack and inhibits the vegetative growth of *S. scabiei* before infection. New tests should be carried out in order to investigate the role of manganese on pathogenic process of *Streptomyces* strains.

S. caviscabies strain showed no significant differences in the values of disease incidence when compared to the negative control. The disease symptoms were verified in the pH 6.5 and 7.5, indicating that pH values below 6.5 may be a management strategy for soils contaminated with this bacterial species.

Our data demonstrated that pH of the substrate directly interfered in the development of potato scab symptoms in Brazil, under greenhouse conditions, and that pH values above 5.5 can increase the disease incidence. In contrast, WIECHEL and CRUMP (2010) evaluated the effect of soil pH on the disease symptoms in Australia for three years and concluded that the incidence of potato scab is not directly associated to pH factor. Only complementary studies with inoculations under field conditions to confirm if the symptoms caused by Brazilian strains can be suppressed at low pH values.

The evaluation of disease severity, using the scale of WANNER (2007), showed high averages in all pH values for *S. scabiei* strain, except at pH 4.0, indicating the high virulence degree of this bacterial species. *S.* cav*iscabies* strain was described associated with deep scab lesions (GOYER et al., 1996), but surprisingly under Brazilian conditions this species showed low severity value*s* and pH values close to 4.0 were considered the better to suppress the disease (Fig. 2).

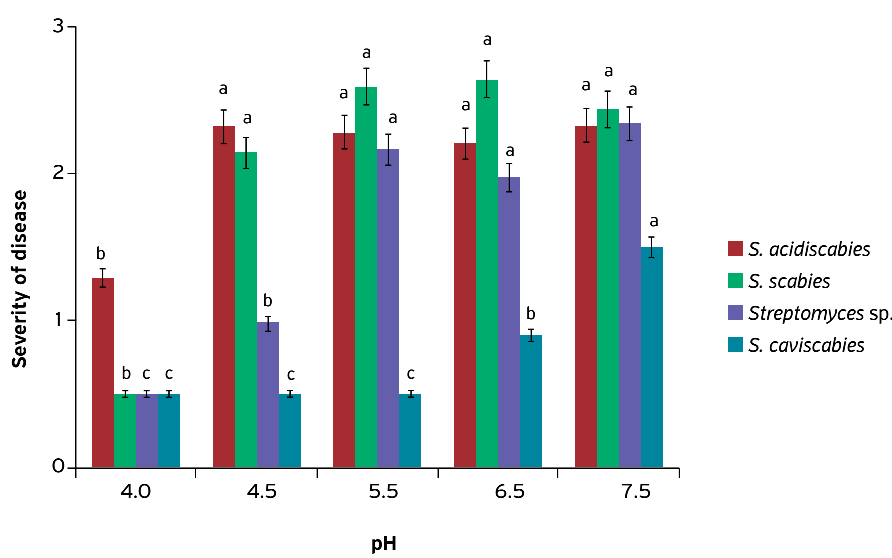

Figure 2. Severity score of potato scab on different pH values of the substrates attributed to the symptoms caused by different species of *Streptomyces.* Means with the same lowercase letters in the same *Streptomyces* species were not significantly different according to Tukey's test at p = 0.05.
Source: Elaborated by the authors.

Irrigation management

In the irrigation assays, no significant differences (P <0.05) were observed for *S. acidiscabies*, *S. scabiei* and *Streptomyces* sp. strains (Figure 3). The soil moisture can help in the development of antagonistic microorganisms that can move in soil through water films, colonizing the lenticels of potato tubers faster competing with *Streptomyces* (LEWIS, 1970). Since that the substrate used in the tests was sterilized, eliminating the presence of microorganisms, this may have been the reason why the moisture did not interfere in the disease, indicating that the presence of antagonistic microorganisms can be an important factor in the interaction pathogen-host-environment. According to LARKIN et al. (2011), the relationship between soil moisture and potato scab disease remains unclear and that this pathosystem is more complex than previously thought.

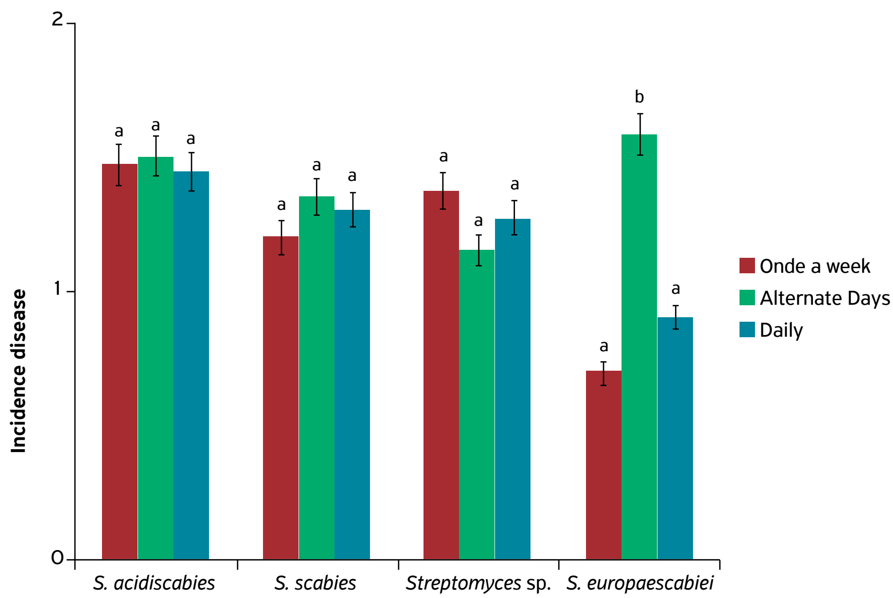

Figure 3. Score on the incidence of potato scab from plants grown on substrates inoculated with different irrigation regimes. Means with the same lowercase letters were not significantly different according to Tukey's test at α = 0.05.
Source: Elaborated by the authors.

In the *S. europaescabiei* assays, the highest disease incidence was observed with irrigation regime with alternate days (herein considered control of the treatments), while the decrease of incidence was verified with higher (dayly) or lower (once a week) humidity. JOHANSEN et al. (2014) also evaluated this bacterial species for three years on field and variations in the disease incidence between each year of planting were observed. During the first two years, higher disease incidence with dry soil conditions was detected (once a week regime), however in the third year the higher incidence was observed with irrigation on alternate days as verified in our study. These results indicate how complex the *Streptomyces* pathosystem can be and, therefore, only more studies on irrigation will can contribute for a better understanding this system.

CONCLUSIONS

The disease symptoms caused by different *Streptomyces* species at pH 4.0 were not verified, demonstrating that this practice may be an interesting strategy for potato scab management, however the soil acidification must be considered since it may lead to decreased tubers production.

The incidence and severity of disease caused by *S. caviscabies* were suppressed at pH 4.5 – 6.5, indicating that this range of pH can be used as an alternative practice in fields infected with this bacterial species.

The three irrigation regimes did not interfere in the incidence of potato scab for *S. acidiscabies*, *S. scabiei* and *Streptomyces* sp. strains, except for *S. europaescabiei*, which presented suppression of disease with higher or lower humidity.

AUTHORS' CONTRIBUTIONS

Conceptualization: Marques, H.M.C. **Data curation:** Marques, H.M.C. **Formal analysis:** Marques, H.M.C.; Appy, M.P. **Investigation:** Marques, H.M.C. **Supervision:** Destéfano, S.A.L. **Writing – original draft:** Marques, H.M.C. **Writing – review & editing:** Marques, H.M.C.; Destéfano, S.A.L.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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