Revista Ceres
ISSN: 0034-737X
ceresonline@ufv.br
Universidade Federal de Viçosa
Brasil

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Revista Ceres, vol. 63, núm. 4, julio-agosto, 2016, pp. 486-491
Universidade Federal de Viçosa
Viçosa, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=305246992008

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Calcium and potassium contents in nutrient solution on Phoma leaf spot intensity in coffee seedlings[1]

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10.1590/0034-737X201663040008

ABSTRACT

Coffee is one of the main export commodities of Brazilian agribusiness. Phoma leaf spot [*Phoma tarda* (Stewart) Boerema & Bollen] is one of the most important coffee fungal diseases in Brazil. Therefore, this study aimed to evaluate the intensity of Phoma leaf spot in coffee seedlings supplied with different rates of Ca^{+2} and K^{+}. The study was conducted under controlled conditions in a growth chamber, at the Department of Phytopathology – UFLA, from February 2010 to December 2011. The assay was repeated twice under the same conditions. The nutrient solutions consisted of five concentrations of K^{+} (3, 4, 5, 6, 7 mmol L^{-1}) and Ca^{+2} (2, 4, 6, 8 and 10 mmol L^{-1}). The experiment was arranged in a randomized block design, with 25 treatments and three replicates, with two plants per plot. The areas under incidence progress curve (AUIPC) and severity (AUSPC) were calculated. At the lowest rate of Ca^{2+} (2 mmol L^{-1}) and highest K^{+} (6 and 7 mmol L^{-1}), approximately, the AUIPC was the smallest. For the AUSPC, the lowest rates of Ca^{+2} and K^{+} resulted in the lowest severities. Supply of Ca^{+2} and K^{+} in nutrient solution reduced AUIPC and AUSPC of Phoma leaf spot, and these nutrients can be recommended for the management of the disease.

Key words: incidence; severity; *Phoma tarda* (Stewart) Boerema & Bollen; *Coffea arabica* L.; mineral nutrition.

RESUMO

Intensidade da mancha de Phoma em mudas de cafeeiro sob doses de cálcio e de potássio em solução nutritiva

O café é um dos principais itens de exportação do agronegócio brasileiro. A mancha de Phoma, ocasionada pelo fungo *Phoma tarda* (Stewart) Boerema & Bollen, é uma das mais importantes doenças do cafeeiro no Brasil. Por essa razão, este trabalho teve como objetivo avaliar a intensidade da mancha de Phoma do cafeeiro em mudas supridas com doses de Ca^{+2} e de K^{+}. O experimento foi conduzido, sob condições controladas, em câmara de crescimento, no Departamento de Fitopatologia da UFLA, de fevereiro de 2010 a dezembro de 2011. O ensaio foi repetido duas vezes sob as mesmas condições. As soluções nutritivas foram compostas por cinco doses de K^{+} (3, 4, 5, 6 e 7 mmol L^{-1}) e de Ca^{+2} (2, 4, 6, 8 e 10 mmol L^{-1}). O delineamento foi em blocos casualizados, com 25 tratamentos, três repetições e duas plantas por unidade experimental. As áreas abaixo da curva de progresso da incidência (AACPI) e da severidade (AACPS) foram calculadas. Na menor dose de Ca^{+2} (2 mmol L^{-1}) e nas maiores de K^{+} (6 e 7 mmol L^{-1}), aproximadamente, observou-se menor AACPI. Para a AACPS, as menores doses de Ca^{+2} e de K^{+} resultaram em menores severidades. Os suprimentos de Ca^{+2} e de K^{+} em solução nutritiva reduziram as AACPI e AACPS da mancha de Phoma do cafeeiro e o uso desses nutrientes pode ser recomendado no manejo da doença.

Palavras-chave: incidência; severidade; *Phoma tarda* (Stewart) Boerema & Bollen; *Coffea arabica* L.; nutrição mineral.

Submitted on November 29th, 2013 and accepted on February 26th, 2016.

[1] This article is part of the first author's master dissertation.

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INTRODUCTION

Coffee is one of the main export commodities of Brazilian agribusiness (Santos *et al*., 2014). The most cultivated coffee species are Arabica (*Coffea arabica* L.) and Robusta or Conilon (*Coffea canephora* L.) (Mohammed & Jambo, 2015). Arabica coffee accounts for 70% of Brazilian production, and the state of Minas Gerais is the largest producer, accounting for over 50% of national production (Companhia Nacional de Abastecimento, 2015). However, pests and diseases can reduce the productivity of Arabica coffee.

Phoma leaf spot, caused by the fungus *Phoma tarda* (Stewart) Boerema & Bollen, is one of the most important coffee diseases in Brazil (Salgado *et al*., 2009) and has caused significant loss of quality and productivity of coffee crops (Lorenzetti *et al*., 2015). Symptoms observed are tip and branch dieback, necrosis of rosettes, mummified berries and leaf spots. Among the control methods, it is recommended the planting in areas less subject to cold winds, installation of windbreaks, balanced fertilization with nitrogen (N), calcium (Ca) and micronutrients, as well as chemical control when necessary (Pozza *et al*., 2010).

Nutrients are involved in plant defense strategies acting as integral components, activators, inhibitors, and regulators of synthesis or metabolism. Therefore, nutritional imbalance is one of the main causes for the occurrence and susceptibility of plants to pests and diseases (Fancelli, 2008), considering also the environmental conditions, because nutrition is also affected by temperature, humidity, and changes in soil conditions.

It is believed that nutrients, including Ca and K, may contribute to the control of plant diseases, by increasing its resistance to fungal attacks. The mechanisms involved in mineral nutrition may be the formation of mechanical barriers such as thicker cell walls and the synthesis of compounds involved in the plant natural defense such as phytoalexins, antioxidants and flavonoids. K is essential for the synthesis of proteins, starch and cellulose of plants. Cellulose is the main component of cell walls and deficiency of this component can make them permeable, resulting in high concentrations of sugar and amino acids in leaves, as it is observed in calcium deficiency (Spann & Schumann, 2013), hence plants become less resistant to diseases.

Several studies have shown how nutrients can affect the management of plant diseases (Downer *et al*., 2013; Obasa *et al*., 2013; Veresoglou *et al*., 2013; Manching *et al*., 2014; Mohamed *et al*., 2014; Vrandeèiæ *et al*., 2014) and the importance of Ca and K for increasing plant resistance (Spann & Schumann, 2009; El-Bramawy & Shaban, 2010; Silva & Rodrigues, 2013; Spann & Schumann, 2013; Wang *et al*., 2013; Sharf *et al*., 2014).

In this context, the objective of this work was to evaluate the combination of different rates of Ca^{2+} and K^+ in nutrient solution on Phoma leaf spot intensity in coffee seedlings.

MATERIAL AND METHODS

The experiment was conducted under controlled conditions in a growth chamber with average temperature of 18 ± 2 °C (< 23 °C) (Pozza *et al.* , 2010) and photoperiod of 12 h (Lima *et al*., 2010), at the Plant Pathology Department of the Federal University of Lavras, Minas Gerais, from February 2010 to December 2011. The experiment was repeated twice under the same conditions.

Seedlings of Red Catuaí IAC 62, with three pairs of leaves, were purchased and transferred to Hoagland's solution (Hoagland & Arnon, 1950) for adaptation to the nutrient solution, at 20, 50, 75 and 100% of the ionic strength. This plants remaining in each concentration for 15 days, in 4-L containers, with continuous aeration so that seedlings received (O_2) for 24 h/day from a compressor-driven air system. Seedlings of uniform size were selected and transferred to 4-L plastic containers with Hoagland solution supplemented with the treatments, in which they remained with their root system immersed for four months. The nutrient solutions were calculated with basis on this solution, and Ca^{2+} and K^+ were balanced using the following sources: NH_4NO_3, $Ca(NO_3)_2$, KNO_3, K_2PO_4 and $CaCl_2$.

The treatments consisted of five rates of Ca^{2+} (2, 4, 6, 8 and 10 mmol L^{-1}), according to Garcia Jr. *et al*. (2003) with modifications, in combination with five rates of K^+ (3, 4, 5, 6, 7 mmol L^{-1}) following the methodology of Lima *et al*. (2010) with modifications. The experiment was arranged in a 5 x 5 factorial randomized block design, with three repetitions per treatment, and two plants in the same pot per repetition.

When needed, the containers were completed with deionized water; pH of solutions was monitored daily with pH meter and kept between 5.0 and 5.5, ideal range for coffee plants. The solution was replaced when depleted of K^+ and NO_3^- ions, which was measured by a Horiba Cardy Compact Ion Meter®. The solutions were replaced when depletion reached 70% of the initial concentration in all treatments (Pozza *et al*., 2001).

The CML 720 isolate of *P. tarda* from the Mycological collection of Universidade Federal de Lavras was used in the inoculation. Spores, produced from pycnidia in sporulating lesions were transferred to Petri dishes with malt-agar extract (MA) at 2% and incubated for 12 days in BOD, at a 20 °C and 12 h photoperiod (Lima *et al*., 2010).

The spore suspension was prepared by adding 20 mL of distilled water (initial volume) to Petri dishes with the pure fungal colonies in 2% MA. Spores were collected by scraping the medium surface with a blade, then filtered with a gauze, and the concentration adjusted to 2 x 106 spores ml^{-1} in a Neubauer chamber.

The seedlings were inoculated through minor injuries using Carborudum® abrasive in the adaxial face of leaves. After inoculation, plants were covered with plastic bags and incubated for 72 h in a growth chamber (Lima *et al*., 2010), with average temperature of 18 °C (Pozza *et al*., 2010).

Weekly evaluations initiated 12 days after inoculation, amounting to seven procedures in total. To estimate the incidence, the number of leaves with symptoms was counted in relation to the total number of leaves per plant. Severity was assigned with the percentage of diseased leaf area, as follows: 1.3, 2.5, 6, 7.5, 12, 20, 30 and 50%, according to the diagrammatic scale proposed by Salgado *et al*. (2009).

From these evaluations, the area under the disease progress curve was calculated for incidence (AUIPC) and severity (AUSPC) in each treatment, according to Shaner & Finney (1977), and surface graphs were generated using the software Statistica 5.0 (StatSoft, 1995).

The joint analysis of the two assays over time was carried out to test difference between them. The statistical analysis, with the complete variance analysis, was performed with the software SISVAR® 4.6 (Build 6.1) (Ferreira, 2000). The variables found significant by the F test were fitted to linear regression models and points of maximum and minimum of each equation were obtained.

RESULTS AND DISCUSSION

The joint analysis of the assays showed no significant differences between the two experiments. Therefore, the statistical analysis was carried out with the average of data from the two experiments.

The supply of Ca^{2+} and K^{+} had significant interaction for AUIPC (Figure 1) and AUSPC (Figure 2) of Phoma leaf spot. The AUIPC was smaller when combining, approximately, the lowest rate of Ca^{2+} (2 mmol L^{-1}) with the highest rates of K^{+} (6 and 7 mmol L^{-1}). Ca^{+2} rates above 4 mmol L^{-1} with K^{+} rates below approximately 4 mmol L^{-1} accounted for the greatest AUIPC (Figure 1).

The AUSPC was smaller with the lowest rates of Ca^{2+} and K^{+}. The increase in K^{+}, even with the highest Ca^{2+} rates, resulted in higher intensity of the disease (Figure 2).

For coffee and other crops, a number of authors also studied the interaction between nutrients in the reduction of disease intensity, including Ca^{2+} and/or K^{+}. In a study on AUIPC and AUSPC of Cercospora leaf spot in coffee in nutrient solution, the two variables showed reduction with increasing K^{+} rates up to 4.79 mmol L^{-1} and increasing Ca^{+2} rates (Garcia Jr. *et al* . 2003), but the variables increased with increasing rates of K^{+} and decreasing N rates (Pozza *et al*., 2001).

Lima *et al*. (2010) reported that the intensity of Phoma leaf spot (AUSPC and AUIPC) in coffee seedlings in nutrient solution increased with increasing N rates and K^{+} rates above 6.59 mmol L^{-1}.

Increased contents of P and K reduced both the final severity and the progress rate of soybean rust, in the vegetative stages and cultivars tested (Balardin *et al*., 2006), in addition, the lowest N rate combined with the highest K^{+} rate provided the lowest severity levels in two maize cultivars (Carvalho *et al*., 2013).

The interaction between these two nutrients was also observed in other pathosystems. There was significant interaction between Ca^{2+} and K^{+} for the number of eggs per female of the soybean cyst nematode. There was no significant effect of calcium alone on any nematode variables (Pinheiro *et al*. 2009).

The supply of calcium reduced the AUSPC of soybean rust for all potassium rates, and the lowest severity was recorded with the combination 8 mmol L^{-1} of K^{+} and 11 mmol L^{-1} of Ca^{+2} (Pinheiro *et al*., 2011).

Lower contents of K^{+} and higher contents of Ca^{+2} in coffee leaves during the cherry filling stage provided greater nutritional balance and increased resistance to Cercospora leaf spot and rust (Santos *et al*., 2008).

The different behavior between AUIPC and AUSPC in the response surface was influenced by the differences in the phases of the host-pathogen interactions in association with the evaluation of these variables. Incidence, or the number of lesions, is associated with the successful penetration of the pathogen, with its ability to germinate, the recognition of pathogen-host relationship and breaking of physical barriers of resistance such as the wax layer and the cell wall. On the other hand, severity, or the increase in the lesion area caused by pathogen colonization, depends on both the disruption of the cell wall, because the wax layer has already been penetrated, and the interaction with the chemical barriers of resistance, as enough time may have passed to activate the genes accounting for this kind of defense (Schumann & D'arcy, 2006).

The balance between the cations, at lower rates of Ca^{2+} and K^{+}, was essential to provide smaller AUIPC and AUSPC, since the increase of either one can influence the behavior of the disease, especially the excess rates, which provided greater AUIPC and AUSPC.

Low Ca^{2+} rates may hinder the formation of physical resistance barriers such as wax layer and cell wall, or even prevent them to be formed with the adequate thickness or strength to stop penetration or increase the latent and incubation periods of the infection. On the other hand, excess Ca^{2+} in the nutrient solution may inhibit K^+ uptake and other nutrients that are essential to metabolic pathways for the synthesis of lignin, tannins and phenolics (Graham & Webb, 1991).

K is important for the development of the cell wall and can directly correlate with the ability of a pathogen to penetrate the plant tissue. The host susceptibility to the pathogen is linked to deficiency of this nutrient because of the role it plays in the metabolism of the plant (Spann & Schumann, 2009).

However, it is necessary to define what deficiency in each pathosystem is. In this study, the highest K^+ rates combined with the lowest Ca^{+2} provided the smallest AUIPC. Thus, the increases in K^+ or Ca^{2+} rates interfered with the availability of both, because of the competition between the two nutrients. Contrariwise, for AUSPC, both the lowest K^+ and the lowest Ca^{2+} rates resulted in less severity.

Mineral nutrients are important factors in plant-disease interactions and are essential for the growth and development of plants and micro-organisms. The way each of them affects the response of a plant to disease, i.e., positively or negatively, is particular to each system (Spann & Schumann, 2013).

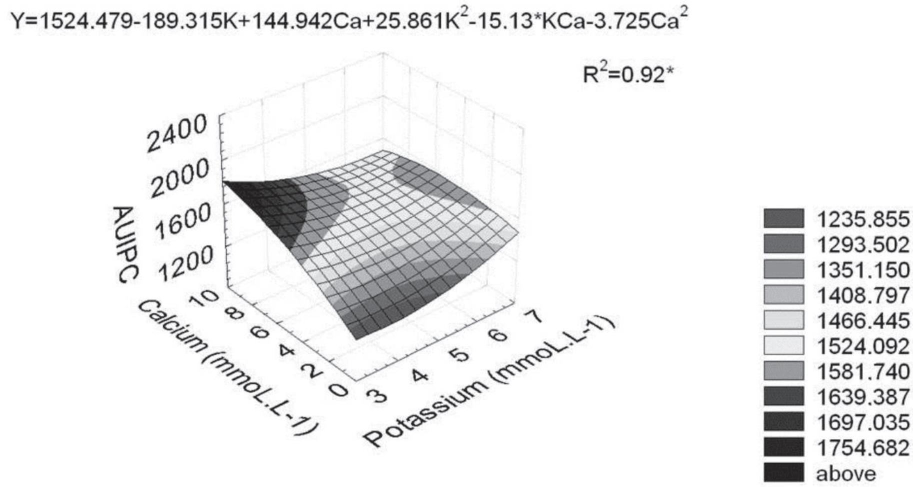

Figure 1. Area under incidence progress curve (AUIPC) of Phoma leaf spot in coffee seedlings(*Coffea arabica*), as a function of Ca^{2+} and K^+ in nutrient solution.

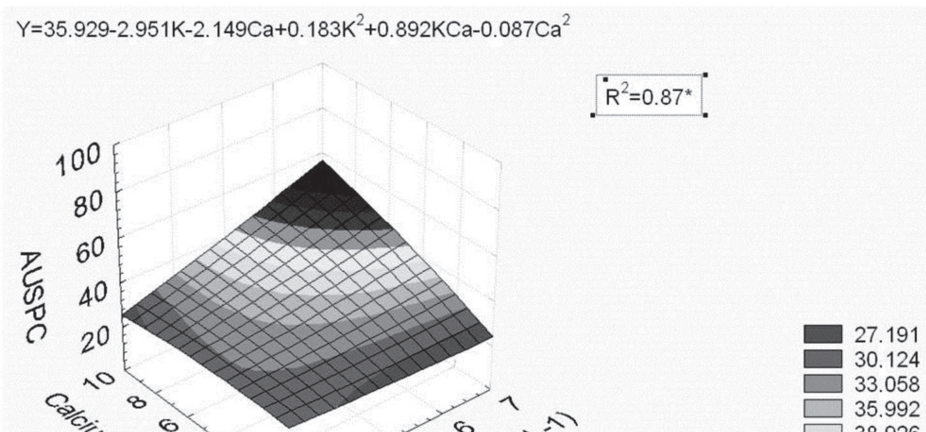

Figure 2. Area under severity progress curve of Phoma leaf spot (AUSPC) in coffee seeldings (*Coffea arabica*), as a function of Ca^{2+} and K^+ in nutrient solution.

CONCLUSION

The supply of Ca^{+2} and K^{+} in nutrient solution reduced AUIPC and AUSPC of Phoma leaf spot in coffee seedlings.

The highest K^{+} rates (6 and 7 mmol L^{-1}) combined with the lowest Ca^{2+} (2 mmol L^{-1}) rate resulted in smaller AUIPC.

The lowest K^{+} rates combined with the lowest Ca^{2+} rate resulted in smaller AUSPC.

The use of these two nutrients can be recommended for the management of the Phoma leaf spot in coffee seedlings.

ACKNOWLEDGEMENTS

The authors thank the Foundation for Research of the State of Minas Gerais - FAPEMIG for the financial support and the scholarships.

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