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Potassium phosphite reduction of *Candidatus* Liberibacter spp. population on leaves of 'Ponkan' tangerines tree with huanglongbing

Rener Luciano de Souza Ferraz[1*], Marcelo de Andrade Barbosa[2], Maria José Araújo Wanderley[3], Patrícia da Silva Costa[4], Ivomberg Dourado Magalhães[5], Aldair de Souza Medeiros[5], Hugo Miranda Faria[2], Vinicius Marchioro[2], Alberto Soares de Melo[6] and Franklin Alves dos Anjos[7]

[1]Academic Unit of Agricultural Engineering, Federal University of Campina Grande, Paraiba, Brazil.
[2]Department of Soils and Fertilizers, São Paulo State University, São Paulo, Brazil.
[3]Department of Agriculture, Federal University of Paraiba, Paraíba, Brazil.
[4]Department of Animal Science, Federal University of Campina Grande, Paraíba, Brazil.
[5]Department of Plant Production, Federal University of Alagoas, CEP: 57072-900, Maceió, Alagoas, Brazil.
[6]Department of Agrarian Sciences, State University of Paraíba, Paraiba, Brazil.
[7]Federal Institute of Education, Science and Technology of Alagoas, Brazil.

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The objective of this work was to evaluate the population variation dynamics of *Candidatus* Liberibacter spp. under phosphite applications. The 'Ponkan' tangerines (*Citrus reticulata* Blanco cv. Ponkan) with symptoms and a high degree of huanglongbing (HLB) severity were used for the test. 'Ponkan' tangerine plants were treated with potassium phosphite leaf applications at a dose of 2.5 L ha^{-1}. Applications frequency was intervals of 25 days with a total of two applications. There was variation in bacterium *Candidatus* Liberibacter spp. population, depending on the application of phosphite. Phosphites have an indirect action on pathogens control, stimulating the formation of phytoalexins, a natural self-defense substance of plants. Indirect effects of these products were reported in citrus seedlings presenting symptoms of phosphorus deficiency, which when treated with foliar applications of potassium phosphite overcame these symptoms, restoring plant growth. *Candidatus* Liberibacter spp. population in 'Ponkan' (*Citrus reticulata* Blanco cv. Ponkan) leaves was reduced and the phosphorus and potassium contents increased with 2.5 L ha^{-1} of potassium phosphite by means of leaf application.

Key words: *Citrus reticulata*, leaf nutrition, huanglongbing, phosphorus, potassium.

INTRODUCTION

Citrus crops are an important activity worldwide with special relevance for Brazilian economy. Citrus were introduced

*Corresponding author. E-mail: ferraz340@gmail.com.

in Brazil by the first colonizing expeditions, probably in Brazilian State of Bahia. Citrus crop expansion is attributed to the emergence of new varieties
and to the development of cultivars more adapted to cultivated ecosystems (Lopes et al., 2011).

Brazil is the largest world producer of citrus plants and the largest exporter of sweet orange juice, which is the main product of the agro-industrial complex of Brazilian citrus growing sector.

Citrus crops are distributed in all regions of Brazil, but with a notable concentration in the Southeast region, especially in São Paulo State, whose orchards are mainly based on sweet orange trees, followed by tangerines and acid lime trees (Cunha Sobrinho et al., 2013). Based on the above, it is important to alert that citrus activity is threatened by the occurrence of the most destructive disease in the world, which is pointed out as the protagonist of the decline of the world citrus cultivation.

This disease is known as greening (huanglongbing, HLB), caused by the bacterial pathogen *Candidatus*.

The long asymptomatic phase of this disease consists of an obstacle in the decision-making process for strategies to control the pathogen (Aksenov et al., 2014).

Among the main damages caused by huanglongbing disease irregular leaf yellowing, fruit drop and deformity, as well seed abortion and significant reductions in fruits qualitative aspects, such as increased acidity and reduction of soluble solids content can be observed (Choi et al., 2013).

In this sense, Pourreza et al. (2014) reported that such problems can have a significant influence on the quality of the raw material supplied to the citrus industry, reflecting directly on the economic sustainability of the productive and industrial chain. This scenario denotes the importance of search for alternatives for controlling or reducing this disease by management of the vector *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) or reduction and eliminating the bacteria. In this perspective, the use of phosphite may be a viable alternative, especially because the action of phosphite has been reported many times against different pathogens of the most varied cultivated plants (Dianese and Blum, 2010).

These compounds are not phytotoxic and have high fungicidal activity, and can act directly by inhibiting the development of the pathogens, also indirectly activating the host plant defence system (Costa et al., 2014).

The objective of this work was to evaluate *Candidatus* Liberibacter spp. population dynamics under phosphite application via the leaves in 'Ponkan' (*Citrus reticulata* Blanco cv. Ponkan) to develop a strategy to reduce damages caused by huanglongbing on citrus plants.

MATERIALS AND METHODS

Experimental area

The experiment was carried out from April to May 2014, in an orchard, in the municipality of Pirangi (Sao Paulo State, Brazil) (21° 5' 28" S, 48° 39' 20" W), at 511 m altitude. Temperature, relative humidity, solar radiation and rainfall were obtained with the objective of characterizing climatic conditions during the period the research was conducted (Figure 1).'Ponkan' plants (*Citrus reticulata* Blanco cv. Ponkan) with symptoms and a high severity degree of huanglongbing disease were used for the experimental test. First, we found twelve plants with characteristics of disease; thereafter, leaves from symptomatic branches were collected to confirm the presence of the bacterium *Candidatus* Liberibacter spp. These branches were evaluated using a quantitative analysis of polymerase chain reaction (qPCR), and the branch was reserved for subsequent foliar biomass collections (Wang et al., 2006).

Treatments application

The plants were treated with foliar applications of potassium phosphite at a dose of 2.5 L ha^{-1}. Two applications occurred (each 25-day intervals) using a gasoline and oil (two-stroke) coastal farm sprayer, with a capacity of 10 L of mixture and horizontal of 14.5 m action radius. The application of mixture was on the lower part of the leaves with time of two minutes of application for each plant. This time was previously calculated for complete coverage of plant top.

Bacterial population

Twenty-five days after treatment and first collection of leaves, a new collection of plant material and new treatment was performed, and this new collection was performed after another 25 days. The collected material was sent to Sylvio Moreira Citrus Center in Cordeirópolis, State of São Paulo, to perform qPCR (Wang et al., 2006) to monitor bacterium population variation dynamics by means of Ct (Cycle Threshold) value, where Cts <29 denote positive reaction with expressive amount of nucleic acid (NA) of the target bacterium. Cts between 30 and 37 indicate moderate amounts of NA; Cts between 38 and 40 characterize minimal qPRC reactions denoting minimal NA amounts of the target bacterium in the sample.

Phosphorus and potassium contents

Ten leaves of the total collected in each evaluation were separated, conducted in a forced air circulation oven, and maintained at temperature of 65°C until reaching a constant mass. Subsequently, dried leaves were pre-processed manually in a sterile container and conducted to a grinding mill to obtain a homogeneous sample.

The samples were sent to Laboratory of the Department of Soils and Fertilizers of the Paulista State University, Campus of Jaboticabal, S.P., Brazil, to carry out the analyzes according to the method established by Bataglia et al. (1983). For determining macronutrients, such phosphorus (P) and potassium (K), nitroperchloric digestion was used. These nutrients are part of potassium phosphite nutritional composition and were evaluated to confirm their absorption by the plant and correlation with the bacterial population.

Statistical analysis

The data obtained from response variables were submitted to analysis of variance ($p<0.5$). The means of each evaluation were compared by Tukey test (Barbosa and Maldonado Jr, 2015). The correlation between the bacterial population and phosphorus and potassium contents was investigated by means of Principal

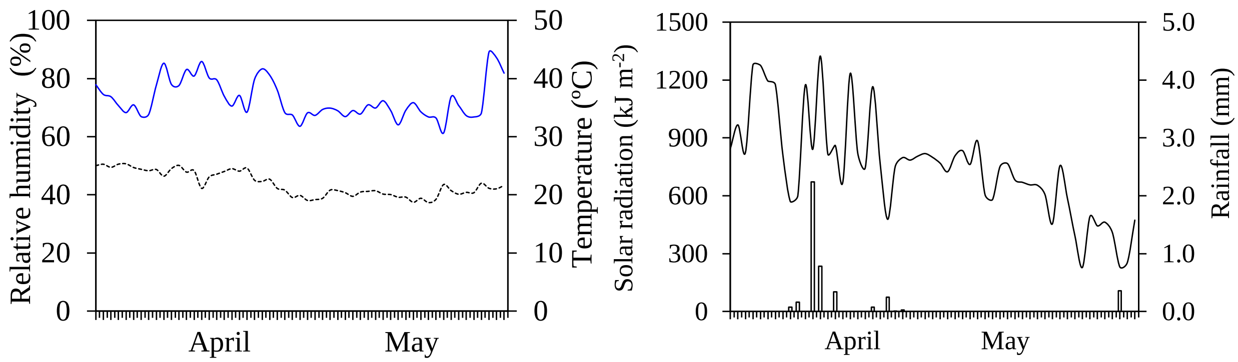

Figure 1. Graphical representation of climatic variables quantified from April to May. Pirangi, SP, Brazil. 2014. —— Relative humidity; **------** Average air temperature; □ Rainfall; and —— Solar radiation.

Table 1. Analysis of variance table for bacterial population (Ct), phosphorus content (Pg kg^{-1}) and potassium (kg kg^{-1}) in 'Ponkan' tangerine leaves under potassium phosphite applications in Pirangi, SP, Brazil during 2014.

Source of variance	Degrees of freedom	Mean squares		
		Ct	**P**	**K**
Apllications	2	14.34**	0.28**	2.97**
Residual error	9	1.51	0.01	0.16
CV (%)		6.33	6.39	4.24

**: Significant by test F ($p < 0.01$).

Component Analysis (PCA). For this, the data were standardized with means zero and unit variance (Hair et al., 2009).

RESULTS

A significant effect of the potassium phosphite applications on bacterial (Ct), phosphorus (P) and potassium (K) content in 'Ponkan' tangerine leaves is shown in Table 1. According to the results, there was variation in the population of the bacterium *Candidatus* Liberibacter spp. according to phosphite application. At the time of the first evaluation a Ct average value of 18.4±1.2 was found, indicating a high concentration of bacterium nucleic acids in the sample. In a new evaluation, after 25 days of treatment, the Ct mean value (18.3±07) remained stable. In the third evaluation, there was an increase of 14.8% in the value of Ct (21.6±2.2) when compared to the value observed in the first evaluation, and an increase of 15.3% in relation to the value of Ct verified in the second evaluation (Figure 2).

'Ponkan' tangerine leaves had a phosphorus content of 1.63 ± 0.10 g kg^{-1} in the first application of potassium phosphite on dose of 2.5 L ha^{-1}. Those value increased up 1.73±0.11 g kg^{-1} in the second application after 25 days, although the difference between the contents of the first and second application was not significant, varying in 5.8%. In the third application, there was a significant increase in P content which reached 2.13±0.10 g kg^{-1}, representing a percentage gain of 23.5 and 18.8% in relation to the first and second applications, respectively (Figure 3).

There was no significant difference in the potassium content of 'Ponkan' leaves in the first (8.8±0.78 g kg^{-1}) and second applications (9.4±0.20 g kg^{-1}), and percentage change of 6.4% was recorded. On the other hand, the contents of this nutrient increased by 10.5% with the third potassium phosphite application, reaching 10.5±0.15 g kg^{-1} (Figure 4).

By means of principal component analysis, it was verified that the three original variables (Ct, P and K) are in the same dimension (CP_1) with 83.15% of representativeness of total variance, which indicates the existence of an expressive correlation between these variables and the first main component. In fact, for the bacterial population, represented by Ct, a high correlation was verified (r = 0.86); and also to phosphorus (r = 0.94) and potassium contents, which had a correlation coefficient of r = 0.93 (Figure 5).

DISCUSSION

Phosphites have indirect action on pathogens control, stimulating phytoalexins production, a natural substance

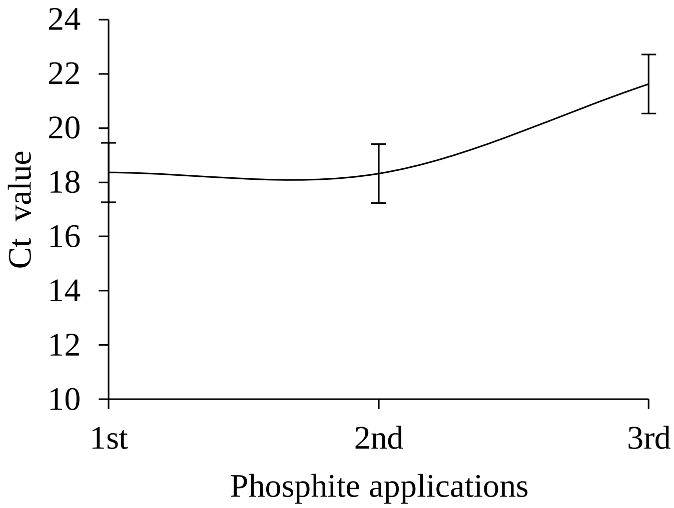

Figure 2. Ct values obtained from qPCR analyzes in 'Ponkan' tangerine leaves under applications of potassium phosphite in Pirangi, SP, Brazil during 2014.

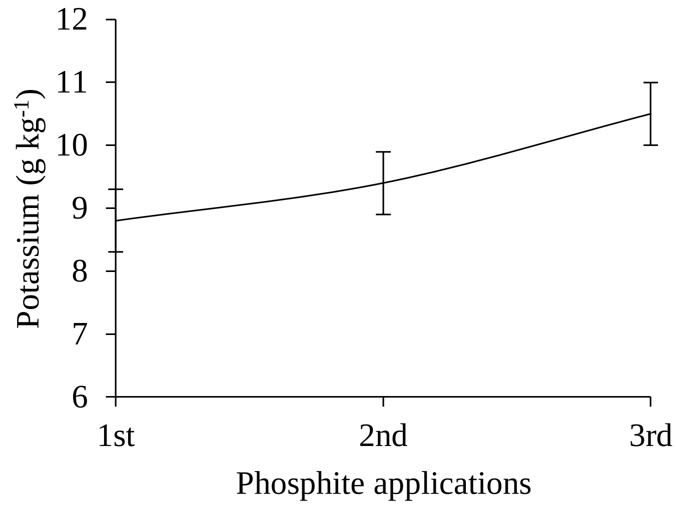

Figure 4. Potassium content in 'Ponkan' tangerine leaves under potassium phosphite applications in Pirangi, SP, Brazil during 2014.

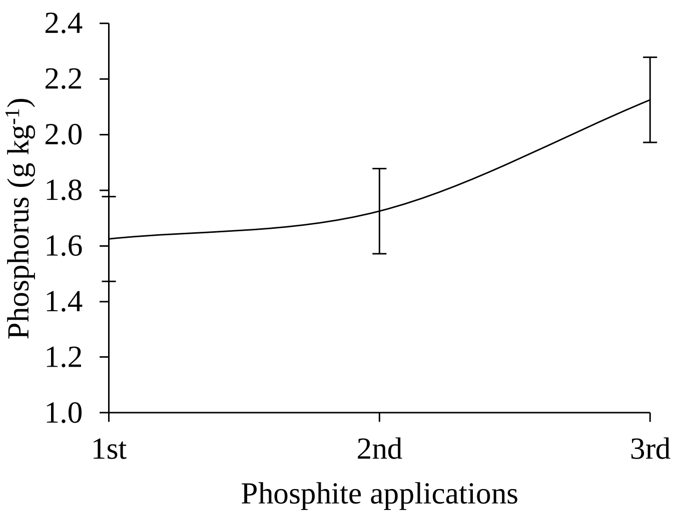

Figure 3. Phosphorus content in 'Ponkan' tangerine leaves under potassium phosphite applications in Pirangi, SP, Brazil during 2014.

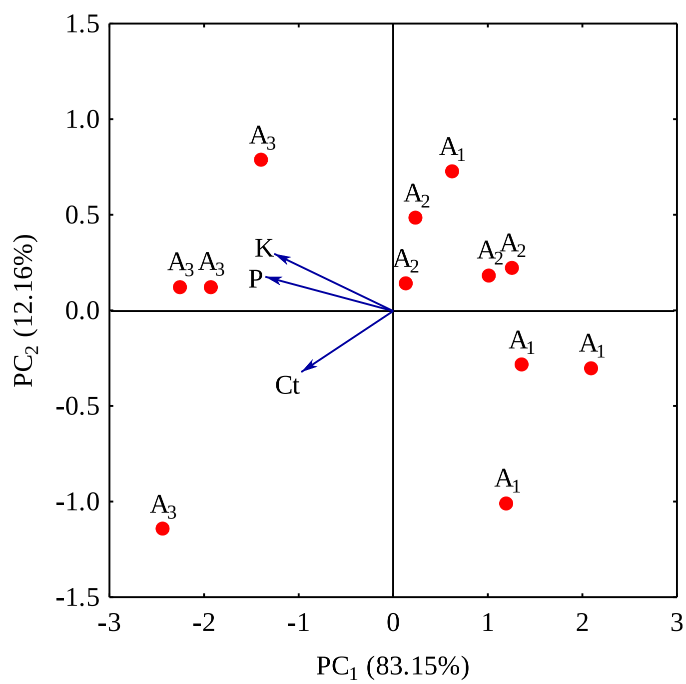

Figure 5. Two-dimensional projection of phosphorus and potassium contents and bacterial population in 'Ponkan' tangerine leaves under potassium phosphite applications in Pirangi, SP during 2014.

of self-defence of plants (Dercks and Creasy, 1989). The indirect effects of these products were reported by Lovatt (1990) who observed that citrus seedlings presenting symptoms of phosphorus deficiency, treated with foliar applications of potassium phosphite, exceeded these symptoms, restoring plant growth. Foliar applications of phosphite during pre-flourishing in 'Valencia' orange plants increased flower numbers, productivity and soluble solids content in fruits (Albrigo, 1997). Stansly et al. (2013) evaluated an orchard for four years with a significant contribution in the plant production when the foliar program was employed, which is most commonly adopted by farmers in Florida together with the intensive control of the vector.

Several studies show differences in the improvements provided by the phosphite application. In fact, Gottwald et al. (2012), working with nutritional treatments containing potassium phosphite, did not observe effect of the nutritional treatments on the bacterium *Candidatus*

Liberibacter spp. when compared to the control treatment in citrus plants. Ratifying this information, Johnson et al. (2013) also showed that the bacterial population in plants that received the nutritional management was similar to those that received the traditional nutrition.

It is important to note that in this research, there occurred population dynamics variation of *Candidatus* Liberibacter spp., Ct values that presented average of close to 20.0. Ct values that were close to this order are often found in leaves diagnosed with HLB in Florida (USA), and the range of 18.0 <Ct <30.0 is more common for more than 90% of the samples analyzed (McCollum et al., 2013). Therefore, we may infer that new researches with greater number of applications and evaluations are preponderant for better elucidation of the population dynamics of the bacterium in citrus plants.

In this study, the increases in P levels recorded are due to the supply of this nutrient via foliar and possible residual effect in the soil, as well as consequent root absorption, providing plants greater capacity in absorption and distribution of the nutrient, as far as new applications are applied (Boaretto et al., 2003). Increasing P is so important, because this nutrient is an essential cellular constituent for nucleic acids, phosphoproteins, phospholipids and ATP synthesis (Hammond and White, 2008). Increases of P contents in citrus were reported by Zhao et al. (2013) that verified the reestablishment of P levels in plants with severe HLB symptoms under foliar phosphorus applications. In a complementary sense, successive applications can be adopted by virtue of mobility and consequent translocation to plant organs where it will be used (Taiz and Zeiger, 2013).

Potassium acts as a regulator of the cellular osmotic potential, besides acting as an enzymatic activator on photosynthetic process and cellular respiration. Generally, due to its easy mobility in the plant, the nutrient is translocated to younger leaves, which justifies its foliar supplementation because part of this nutrient is compromised in older leaves as a result of disease severity. Increasing K content may also reflect improvements in the production quality, since this element influences the size, thickness of the bark, acidity and soluble solids content in fruits (Mattos Jr. et al., 2004; Marini et al., 2005; Taiz and Zeiger, 2013).

Potassium supplementation via foliar and its consequent increase in leaves is benefical for the plant, even under infection caused by *Candidatus* Liberibacter spp., because potassium is directly linked to stomatic movements, especially in the regulation and turgidity maintenance of guard cells, in a way that stoma opening depends on water absorption by these cells. This process is possible starting from K^+ ions accumulation; a fact that contribute to increased osmotic potential and to favour water entering in stomatal cells, facilitating stomatal orifice opening (Brodribb and Holbrook, 2003; Chaerle et al., 2005; Lemos et al., 2012; Morgan et al., 2016).

Conclusion

Candidatus Liberibacter spp. population in leaves of 'Ponkan' tangerine (*Citrus reticulata* Blanco cv. Ponkan) was reduced and phosphorus and potassium contents increased with application of 2.5 L ha^{-1} of potassium phosphite on leaves. Potassium phosphite is an alternative for reducing the bacterial population in orchards infected by huanglongbing. However, the Ministry of Agriculture, Livestock and Supply (Map) published in the Official Gazette of the Union, normative instruction obliging citrus farmers throughout the country to eradicate plants with symptoms of huanglongbing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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