Notas Científicas

Pyoverdine use for the control of passion fruit bacterial blight

Carla Maria Cavalcanti Ribeiro[1], Giovanni Ribeiro de Souza[2],
Daniel Augusto Schurt[2] and Bernardo de Almeida Halfeld-Vieira[3]

[1]Universidade Federal de Roraima, Avenida Capitão Ene Garcez, nº 2.413, Aeroporto, CEP 69310-000 Boa Vista, RR, Brazil. E-mail: ribeiro_cmc@yahoo.com.br [2]Embrapa Roraima, Rodovia BR-174, Km 8, Distrito Industrial, Caixa Postal 133, CEP 69301-970 Boa Vista, RR, Brazil. E-mail: giovanni.souza@embrapa.br, daniel.schurt@embrapa.br [3]Embrapa Meio Ambiente, Rodovia SP-340, Km 127,5, s/nº, CEP 13820-000 Tanquinho Velho, Jaguariúna, SP, Brazil. E-mail: bernardo.halfeld@embrapa.br

Abstract – The objective of this work was to evaluate the effect of a bacterial filtrate containing pyoverdine on the population dynamics of *Xanthomonas axonopodis* pv. *passiflorae*, and the severity of passion fruit bacterial blight. The treatments were: King's B medium solution; King's B medium solution with 2 μmol L^{-1} Fe^{2+} supplementation; and King's B medium filtrate containing two concentrations of the siderophore pyoverdine produced by *Pseudomonas* sp. The filtrate containing pyoverdine at Abs$_{363}$ = 0.231 (highest concentration) reduces the number of cells of *X. axonopodis* pv. *passiflorae*, and at Abs$_{363}$ = 0.115 and Abs = 0.231 significantly reduces the severity of bacterial blight.

Index terms: *Passiflora edulis*, *Pseudomonas, Xanthomonas axonopodis* pv. *passiflorae*, phylloplane, siderophore.

Uso de pioverdina no controle da mancha bacteriana do maracujazeiro

Resumo – O objetivo deste trabalho foi avaliar o efeito de um filtrado bacteriano com pioverdina sobre a dinâmica populacional de *Xanthomonas axonopodis* pv. *passiflorae*, e a severidade da mancha bacteriana do maracujazeiro. Os tratamentos foram: solução do meio B de King; solução do meio B de King suplementado com 2 μmol L^{-1} de Fe^{2+}; e filtrado do meio B de King cultivado com duas concentrações do sideróforo pioverdina, produzida por *Pseudomonas* sp. O filtrado com pioverdina à Abs$_{363}$=0,231 (maior concentração) reduz o número de células de *X. axonopodis* pv. *passiflorae*, e à concentração Abs$_{363}$=0,1155 e Abs$_{363}$=0,231 reduz significativamente a severidade da mancha bacteriana.

Termos para indexação: *Passiflora edulis*, *Pseudomonas*, *Xanthomonas axonopodis* pv. *passiflorae*, filoplano, sideróforo.

Passion fruit, *Passiflora edulis*, is native to Brazil and is widely cultivated for therapeutic use, cosmetic products and, particularly, for juice production. Most passion fruit species are native to Central and South America. The primary biodiversity areas are located in Colombia and Brazil (Ramaiya et al., 2014).

One of the most important passion fruit diseases is bacterial blight, which is caused by *Xanthomonas axonopodis* pv. *passiflora*e, and represents severe economic losses to farmers (Meletti, 2011).

Genetic improvement is one of the most promising methods for controlling the disease in commercial crops (Nakatani et al., 2009; Meletti, 2011); however, it is not efficient to prevent bacterial development (Munhoz et al., 2011).

Therefore, biological control should be investigated. Iron is an essential micronutrient for both plants and microorganisms, including bacteria. In iron-limited environments, some bacteria can overcome iron restriction using siderophores, which are iron-chelating compounds. Pyoverdines are water-soluble siderophores produced by fluorescent *Pseudomonas* spp. with a high affinity for iron (Expert & O'Brian, 2012), which limits iron availability to pathogens. Halfeld-Vieira et al. (2015) observed the potential of pyoverdine-producing phylloplane bacteria in disease management. However, the effect of these compounds on the population density of *X. axonopodis* pv. *passiflorae* on the phylloplane, and how this potential alteration could affect disease severity remains unknown.

The objective of this work was to evaluate the effect of a bacterial filtrate, containing pyoverdine, on the populational dynamics of *X. axonopodis* pv.

passiflorae, and the severity of bacterial blight on passion fruit.

An isolate of *X. axonopodis* pv. *passiflorae* (*Xap*) from São Paulo state (*Xap*-SP) was sequentially cultured in 523 medium (Kado & Heskett, 1970), to establish a *Xap* strain resistant to rifampicin. Increasing antibiotic concentrations were used to select a mutant strain resistant to rifampicin at 200 mg L^{-1} concentration (Jacques et al., 2005).

The in vivo experiment was carried out using the antagonist isolate *Pseudomonas* sp. 29RR, which was used in a previous study to control passion fruit bacterial blight. This antagonist strain produces pyoverdine that inhibits the *Xap*-SP growth by restricting iron availability in the culture medium, instead of restricting it by antibiosis (Halfeld-Vieira et al., 2015).

First, the 29RR isolate was cultured in test tubes with King's B medium for 48 hours at 27°C (King et al., 1954). Three treatments (T) were performed: T1, King's B medium; T2, King's B medium supplemented with 2 µmol L^{-1} Fe^{2+}; and T3, King's B medium surface cultured with 1 mL of the 29RR bacterial culture. Erlenmeyer flasks were kept in the incubator for 72 hours at 27°C; after that, 100 mL of sterile distilled water was added to each flask, and the flasks were agitated in an orbital shaker at 180 rpm for 60 min. The pyoverdine diffusion in the culture media containing bacteria was assessed in a dark chamber at a 375 nm wavelength. The solution was centrifuged at 10,000 rpm for 15 min, and filtered through a nitrocellulose membrane of 0.22 µmol L^{-1} pore size. The solution absorbance was Abs_{363} = 0.231, and then a twofold dilution was performed to Abs_{363} = 0.1155. These two bacterial filtrate concentrations were used in the experiment. The control treatments were prepared without bacterial cultures, and consisted only of the culture medium with or without 2 µmol L^{-1} Fe^{2+}, obtained from $FeSO_4.7H_2O$.

Forty-two passion fruit plants 'BRS Gigante-Amarelo', at the stage with five expanded leaves, were treated inside a temperature-controlled (28±2°C) greenhouse. The plants were treated with the 29RR filtrate until the dripping point. Twenty-one plants were treated with the solution at Abs_{363} = 0.231, and the other 21 were treated with the solution at Abs_{363} = 0.115. The control groups consisted of two extra sets of 21 plants each. After drying, plants from all treatments were inoculated with a solution containing 1.0x10^7 CFU mL^{-1} of *Xap*-SP resistant to rifampicin (200 mg L^{-1}). Plants were maintained for 24 hours in a wet chamber, inside the greenhouse.

After this period, the number of CFUs cm^{-2} of leaf was calculated as follows: an area of 9 cm^2 from basal leaves was collected from each plant. Samples were transferred to 125 mL Erlenmeyer flasks containing 20 mL of 0.85% NaCl, and 0.05% tween-20 solution. Then, the samples were shaken in an orbital shaker, at 170 rpm, for 30 min. Three leaves were used in each treatment, and each sample from a distinct plant was considered a replicate. After serial dilutions, 100 µL of the solution was added and spread using a Drigalski spatula onto a Petri dish with 523 medium containing cycloheximide (50 mg L^{-1}) and rifampicin (200 mg L^{-1}). The number of CFUs cm^{-2} of leaf was calculated after a 72-hour incubation at 27°C. Leaf samples were collected for seven consecutive days after the inoculation of *Xap*-SP (adapted from the study by Lanna Filho et al., 2010). Regression analyses were performed using SigmaPlot v.12 (Madden et al., 2007). The rates of decline in the population of *Xap*-SP in relation to time were obtained by linearization of growth curves, and analyzed by Student's t-test to compare treatments.

Twelve plants were selected from each treatment, in order to evaluate the effect of pyoverdine on disease severity. Disease severity was assessed 19 days after inoculation of *Xap*-SP. Data were analyzed by proc GLM from the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA), and the means were compared by Fisher's LSD test, at 5% probability.

All treatments reduced the *Xap*-SP population during the study period (Figure 1). However, only the treatment with the higher concentration of pyoverdine promoted a significantly higher reduction of the bacterial population. This treatment also reduced the population density of *Xap*-SP faster than the other treatments.

Pyoverdine action on disease control does not occur simply by reducing the population of resident *Xap*-SP, since the treatment with the lower concentration of pyoverdine (Abs_{363} = 0.115) did not reduce the population density significantly, in comparison with the control group (Figure 1). The lack of iron on the phylloplane may have influenced infectivity, reducing disease severity in the pyoverdine treatments (Figure 2).

The limited availability of iron has been associated with gene expression modulation, virulence, antibiotic
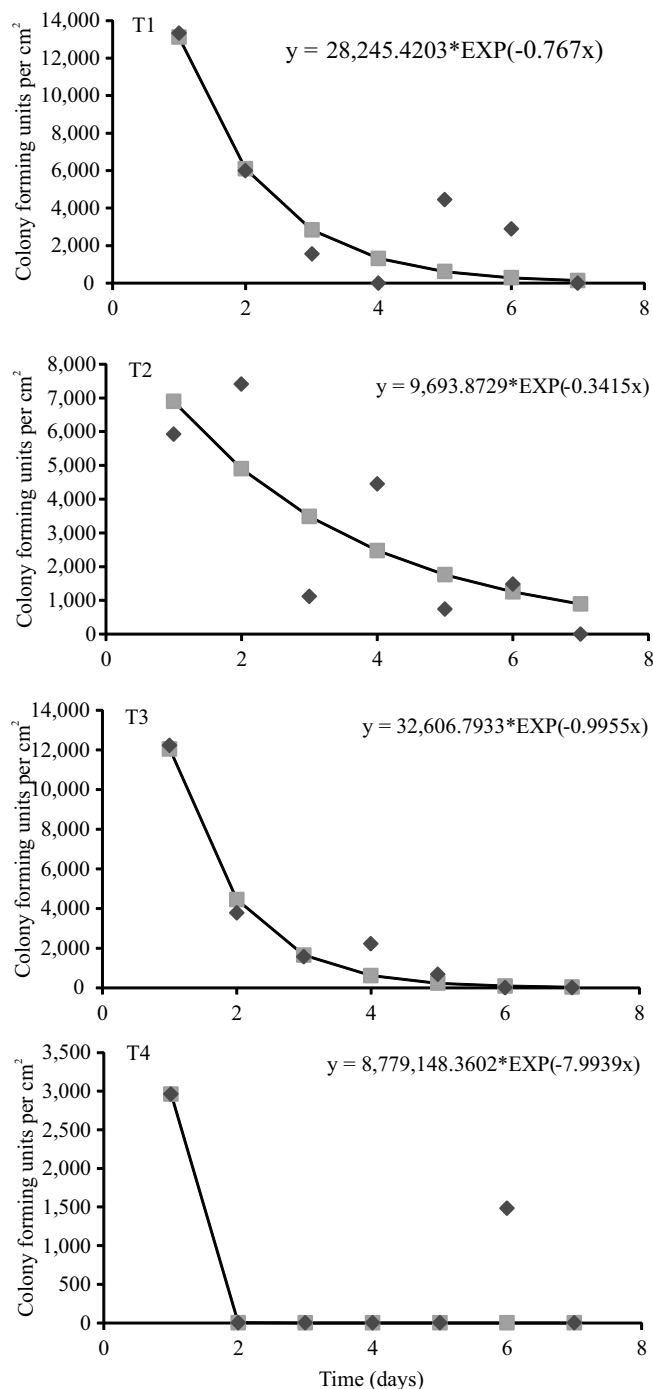
Figure 1. Population dynamics of *Xanthomonas axonopodis* pv. *passiflorae* on the passion fruit phylloplane ('BRS Gigante Amarelo'), in relation to time: T1, leaves were sprayed with a solution obtained from 523 medium with 2 μmol L^{-1} Fe^{2+} supplementation; T2, leaves were sprayed with a solution obtained from 523 medium without the supplementation of 2 μmol L^{-1} Fe^{2+}; T3, leaves were sprayed with a pyoverdine solution at Abs$_{363}$ = 0.1155; and T4, leaves were sprayed with a pyoverdine solution at Abs$_{363}$ = 0.231.
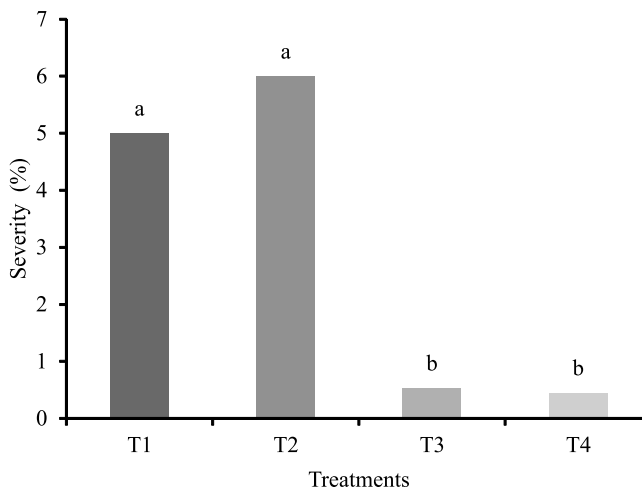

Figure 2. Effect of the isolate 29RR of *Pseudomonas* sp. culture filtrate on the severity of passion fruit bacterial blight caused by *X. axonopodis* pv. *passiflorae* (*Xap*-SP), inoculated in passion fruit plants ('BRS Gigante Amarelo') at the stage with five expanded leaves. Treatments: T1, aqueous solution of King's B medium; T2, aqueous solution of King's B medium with 2 μmol L^{-1} Fe^{+2} supplementation; T3, aqueous filtrate solution with pyoverdine at Abs$_{363}$ = 0.1155; and T4, aqueous filtrate solution with pyoverdine at Abs$_{363}$ = 0.231. Means with the equal letters, in the columns, do not differ by Fisher's LSD test, at 5% probability.

production, and toxic compound synthesis (Karamanoli et al., 2011), and these factors may interfere with the disease severity.

The filtrate treatment with the highest concentration of pyoverdine (Abs$_{363}$ = 0.231) reduced the number of cells of *X. axonopodis* pv. *passiflorae*. Moreover, both pyoverdine concentrations (Abs$_{363}$ = 0.1155 and 0.231) significantly reduced the severity of passion fruit bacterial blight.

Acknowledgments

References

EXPERT, D.; O'BRIAN, M.R. (Ed.). **Molecular aspects of iron metabolism in pathogenic and symbiotic plant-microbe associations**. Dordrecht: Springer, 2012. 86p. DOI: 10.1007/978-94-007-5267-2.

HALFELD-VIEIRA, B. de A.; SILVA, W.L.M. da; SCHURT, D.A.; ISHIDA, A.K.N.; SOUZA, G.R. de; NECHET, K. de L. Understanding the mechanism of biological control of passionfruit bacterial blight promoted by autochthonous phylloplane bacteria. **Biological Control**, v.80, p.40-49, 2015. DOI: 10.1016/j. biocontrol.2014.09.011.

JACQUES, M.-A.; JOSI, K.; DARRASSE, A.; SAMSON, R. *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* is aggregated in stable biofilm population sizes in the phyllosphere of field-grown beans. **Applied and Environmental Microbiology**, v.71, p.2008-2015, 2005. DOI: 10.1128/AEM.71.4.2008-2015.2005.

KADO, C.I.; HESKETT, M.G. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. **Phytopathology**, v.60, p.969-976, 1970. DOI: 10.1094/Phyto-60-969.

KARAMANOLI, K.; BOULIGARAKI, P.; CONSTANTINIDOU, H.-I.A.; LINDOW, S.E. Polyphenolic compounds on leaves limit iron availability and affect growth of epiphytic bacteria. **Annals of Applied Biology**, v.159, p.99-108, 2011. DOI: 10.1111/j.1744-7348.2011.00478.x.

KING, E.O.; WARD, M.K.; RANEY, D.E. Two simple media for the demonstration of pyocyanin and fluorescein. **Journal of Laboratory and Clinical Medicine**, v.44, p.301-307, 1954.

LANNA FILHO, R.; ROMEIRO, R. da S.; ALVES, E. Bacterial spot and early blight biocontrol by epiphytic bacteria in tomato plants. **Pesquisa Agropecuária Brasileira**, v.45, p.1381-1387, 2010. DOI: 10.1590/S0100-204X2010001200007.

MADDEN, L.V.; HUGHES, G.; BOSCH, F. van den. **The study of plant disease epidemics**. St. Paul: American Phytopathological Society, 2007. 432p.

MELETTI, L.M.M. Avanços na cultura do maracujá no Brasil. **Revista Brasileira de Fruticultura**, v.33, p.E83-E91, 2011. Número especial. DOI: 10.1590/S0100-29452011000500012.

MUNHOZ, C.F.; WEISS, B.; HANAI, L.R.; ZUCCHI, M.I.; FUNGARO, M.H.P.; OLIVEIRA, A.L.M.; MONTEIRO-VITORELLO, C.B.; VIERA, M.L.C. Genetic diversity and a PCR-based method for *Xanthomonas axonopodis* detection in passion fruit. **Phytopathology**, v.101, p.416-424, 2011. DOI: 10.1094/PHYTO-06-10-0169.

NAKATANI, A.K.; LOPES, R.; CAMARGO, L.E.A. Variabilidade genética de *Xanthomonas axonopodis* pv. *passiflorae*. **Summa Phytopathologica**, v.35, p.116-120, 2009. DOI: 10.1590/S0100-54052009000200006.

RAMAIYA, S.D.; BUJANG, J.S.; ZAKARIA, M.H. Genetic diversity in *Passiflora* species assessed by morphological and ITS sequence analysis. **The Scientific World Journal**, v.2014, Article ID 598313, 11p., 2014. DOI: 10.1155/2014/598313.