eISSN 1981-1829

Ciência e Agrotecnologia
Science and Agrotechnology

# *Pseudomonas coronafaciens* pv. *garcae* and *P. amygdali* pv. *tabaci* isolated from coffee plants cause diseases in different species

## *Pseudomonas coronafaciens* pv. *garcae* e *P. amygdali* pv. *tabaci* isolados de plantas de café causam doenças em diferentes espécies

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### ABSTRACT

Bacterial halo blight and bacterial leaf spot have similar symptoms in coffee plants, and the etiological agents *Pseudomonas coronafaciens* pv. *garcae* (*Pcg*) and *P. amygdali* pv. *tabaci* (*Pat*) have very similar colony morphologies and biochemical characterizations, which makes it difficult to provide a correct diagnosis. To date, *Coffea arabica* is the only known natural host of *Pcg*, while *Pat* affects a wide range of host plants. However, no studies have yet been conducted to test the pathogenicity of *Pcg* strains in *Pat* hosts or *Pat* strains from different hosts in coffee plants. The objectives of this study were to evaluate the virulence level among *Pcg* strains and to perform cross-inoculation tests to confirm the hosts specificity for *Pcg* and *Pat*. In the virulence tests, there was variation in aggressiveness among *Pcg* strains. The reference isolate (CFBP 1634) and seven *Pcg* strains considered more aggressive were selected for cross-inoculation testing on different *Pat* hosts. All the *Pat* strains, regardless of the host of origin, caused disease in the coffee seedlings and *Pcg* strains caused disease in *Phaseolus vulgaris*, *Cucumis sativus*, *Carica papaya*, *Aster* sp., *Coffea arabica*, *Celosia plumosa* and *Desmodium incanum*. Therefore, there is no host specificity for *Pcg* and *Pat*, using artificial inoculation. These results are important for the beginning of the knowledge of the host range of *Pcg* and may contribute to the development of strategies to manage the disease.

**Index terms:** Bacterial halo blight; coffee bacterial leaf spot; artificial inoculation.

### RESUMO

A mancha aureolada e a mancha bacteriana apresentam sintomas semelhantes em plantas de café, e os agentes etiológicos *Pseudomonas coronafaciens* pv. *garcae* (*Pcg*) e *P. amygdali* pv. *tabaci* (*Pat*) apresentam morfologias de colônias e caracterizações bioquímicas muito semelhantes, o que dificulta o diagnóstico correto. Até o momento, *Coffea arabica* é o único hospedeiro natural conhecido de *Pcg*, enquanto *Pat* afeta uma ampla gama de plantas hospedeiras. No entanto, ainda não foram conduzidos estudos para testar a patogenicidade de isolados de *Pcg* em hospedeiros de *Pat* ou isolados de *Pat* de diferentes hospedeiros em plantas de café. Os objetivos deste estudo foram avaliar o nível de virulência entre isolados de *Pcg* e realizar testes de inoculação cruzada para confirmar a especificidade dos hospedeiros para *Pcg* e *Pat*. Nos testes de virulência, houve variação na agressividade entre isolados de *Pcg*. O isolado de referência (CFBP 1634) e sete isolados de *Pcg* considerados mais agressivos foram selecionados para testes de inoculação cruzada em diferentes hospedeiros de *Pat*. Todos os isolados de *Pat*, independentemente do hospedeiro de origem, causaram doença nas mudas de café e os isolados de *Pcg* causaram doença em *Phaseolus vulgaris, Cucumis sativus, Carica papaya, Aster* sp., *Coffea arabica, Celosia plumosa* e *Desmodium incanum*. Portanto, não há especificidade de hospedeiro para *Pcg* e *Pat*, de acordo com a inoculação artificial. Esses resultados são importantes para o início do conhecimento da taxa de hospedeiros de *Pcg* e podem contribuir para o desenvolvimento de estratégias de manejo da doença.

**Termos para indexação:** Mancha aureolada; mancha bacteriana do cafeeiro; inoculação artificial.

## Introduction

Brazil is the world's largest producer and exporter of coffee, with an estimated production of 58.81 million bags, and it is the second-largest consumer of this commodity; the State of Minas Gerais is the largest Brazilian producer (Associação Brasileira da Indústria de Café - ABIC, 2023; Companhia Nacional de Abastecimento - CONAB, 2024). However, the bacterial diseases, such as bacterial halo blight (BHB), bacterial leaf spot (BLS) and bacterial leaf blight (BLB) caused by *Pseudomonas coronafaciens* pv. *garcae* (*Pcg*, syn. *P. syringae* pv. *garcae*, Amaral, Teixeira & Pinheiro, 1956; Dutta et al., 2018), *P. amygdali* pv. *tabaci* (*Pat*, syn. *P. syringae* pv. *tabaci*, Wolf & Foster, 1917; Gomila et al., 2017) and *P. cichorii* (*Pch*, Robbs et al., 1974), respectively, cause significant reductions in coffee production in some countries (Badel & Zambolin 2019; Raimundi et al., 2021).

Agricultural Sciences

Ciênc. Agrotec., 49:e000925, 2025
http://dx.doi.org/10.1590/1413-7054202549000925

Editor: Renato Paiva

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*Received in January 20, 2025 and approved in April 9, 2025*

Among the bacterial diseases, BHB is widely distributed in the main *Coffea arabica* producing regions of Brazil and its incidence has also increased in regions where *Coffea canephora* is being cultivated, a species that accounts for 25% of the Country's coffee production (Rodrigues et al., 2020). According to Raimundi et al. (2021), there is a prevalence of *Pcg* in coffee production fields in the State of Minas Gerais.

Bacterial leaf spot was first reported in coffee nurseries in São Paulo State by Rodrigues-Neto et al. (2006). *Pseudomonas amygdali* pv. *tabaci* produces symptoms very similar to those of *Pcg*, which are primarily characterized by dark brown leaf spots surrounded by a yellow halo, both in nursery seedlings and in plants in the field (Destéfano et al., 2010; Pozza, Carvalho & Chalfoun, 2010; Raimundi et al., 2021). *Pseudomonas* pathovars deliver phytotoxins that contribute to symptom development during the interaction with their hosts, such as tabtoxin, coronatine, phaseolotoxin, tagetitoxin and syringomycin (Hwang et al., 2005; Badel & Zambolin, 2019). The tabtoxin produced by these pathogens is responsible for the formation of the chlorotic halo around the lesions in the leaves of the host plants due to its intervention in nitrogen metabolism. Although this toxin is not essential for pathogenicity, it induces similar symptoms in plants and may favor increased virulence in some strains (Barta & Willis, 2005).

In addition to the similarity in the symptoms caused by these bacteria in coffee plants, the morphology of the colonies as well as the biochemical characterization of these bacteria are very similar, making the discrimination of these diseases a major challenge for plant pathologists. Regarding biochemical characterization, according to Schaad, Jones and Chun (2001), identification of *Pseudomonas* species is mainly based on the LOPAT (Levan + Oxidase + Potato Rot + Arginine + Tobacco Hypersensitivity) tests. In the LOPAT tests, *Pcg* is distinguished from *Pch* by being Levan positive and oxidase negative. In addition to LOPAT, *Pch* does not hydrolyze gelatin, does not use trehalose, sucrose, sorbitol and cellobiose, and uses L-tartrate, while *Pcg* hydrolyzes gelatin, produces acid from trehalose, D-sorbitol, cellobiose and sucrose, and does not produce acid from L-tartrate. The biochemical differentiation between *Pcg* and *Pat* is made only through the use of the sugars Trigonelline and L(+) tartrate by *Pat*, which are not used by *Pcg* (Bradbury, 1986; Schaad, Jones, & Chun, 2001). This leads to incorrect diagnosis of the disease, and the importance of BLS in Brazil has probably been underestimated (Badel & Zambolin, 2019; Raimundi et al., 2021). Furthermore, the simultaneous occurrence of both bacteria was observed in the same coffee plant in the Paraná State (Rodrigues et al., 2017a). However, *Pcg*, *Pat* and *Pch* can be clearly distinguished using *rpoD* locus-based phylogenetic analysis and by repetitive element-polymerase chain reaction (rep-PCR). This methodology can help in the correct diagnosis (Raimundi et al., 2021)

*Pseudomonas amygdali* pv. *tabaci* has a wide host range, while to date, studies have reported that coffee is the only natural host of *Pcg*. Studies aiming to test the pathogenicity of *Pcg* strains on *Pat* hosts and *Pat* strains from different hosts on coffee plants have not yet been conducted. Such studies would be interesting from an epidemiological point of view because they could clarify the origin of the bacterial inoculum and prevent the spread of the disease from one crop to another, contributing to the control of the pathogen population in an integrated management program against bacterial diseases in coffee plants. In this context, the objectives of this study were to verify the diverse virulence of *Pcg* strains and to perform cross-inoculation tests to confirm the host specificity of *Pcg* and *Pta*.

## Material and Methods

### Biochemical characterization

The bacterial strains were biochemically characterized by Gram's reaction, oxidase and arginine dihydrolase assays, gelatin hydrolysis, esculin hydrolysis, fluorescent pigment production in King's B medium and the use of sorbitol, sucrose, adonitol and lactose according to Schaad, Jones and Chun (2001) and Barta and Willis (2005).

### Virulence test of *Pseudomonas coronafaciens* pv. *garcae* strains

For the virulence test, 73 *Pcg* strains obtained from leaves and stems collected from diseased seedlings and/or coffee plants in Minas Gerais State, Brazil (Raimundi et al., 2021), (Table 1) and the *Pcg* reference isolate (CFBP 1634), obtained from the "*Collection Française de Bactéries Phytopathogènes*" (CFBP), were grown in 90-mm-diameter Petri dishes containing King's B (KB) media (King, Ward, & Raney, 1954) incubated at 28 °C (Mariano & Souza, 2016). After 48 h, bacterial suspensions were prepared in sterile saline solution (0.85% NaCl) and calibrated to $A_{600nm}$ = 0.4 (approximately $1 \times 10^9$ CFU $mL^{-1}$) with a spectrophotometer (McTavish et al., 2024).

The inoculations were performed on healthy coffee seedlings of the *Coffea arabica* cv. Catuaí Vermelho IAC 99, which is susceptible to bacterial halo blight (Ito et a., 2008). Seedlings with four pairs of true leaves were used for inoculation with a multi-needle set immersed in the bacterial suspension (Zoccoli, Takatsu & Uesugi, 2011). The experiment was conducted using a completely randomized design with three replicates, with each replicate represented by one seedling and the first and second pairs of leaves being inoculated. The same procedure was performed in control plants, which were inoculated with saline solution (NaCl 0.85%). After the inoculation, the seedlings were kept in a humidity chamber for 72 h at 23 °C, with relative humidity higher than 70% and a photoperiod consisting of 12 h of light. The severity of the disease was assessed based on the area occupied by the lesions using the diagrammatic scale proposed by Belan et al. (2014). The first assessment was performed five

days after inoculation, and seven assessments were performed in total. The mean severity of two pairs of inoculated leaves was used to calculate the Area Under the Disease Progress Curve (AUDPC) (Simko & Piepho, 2012) for each isolate. The AUDPC was calculated for each treatment using severity versus assessment days, using the formula proposed by Shanner and Finney (1977) (Equation 1):

$$AACPD = \sum_{i}^{N-1} \frac{(Xi + Xi + 1)(Ti + 1 - Ti)}{2} \quad (1)$$

Where $N$ is the number of assessments, Xi is the severity, and ($Ti$+1–$Ti$) is the number of days between consecutive assessments.

The data obtained were subjected to analysis of variance (ANOVA) to detect significant differences ($p<0.05$). Those with different means were then subjected to the Scott-Knott mean test. All statistical analyses were performed with the "R" software version 3.1.3.

Symptomatic leaves were collected, and fragments of the lesions were subjected to a bacterial streaming test to confirm the bacterial etiology of the disease. The isolation of the bacterium present in the samples with the confirmed bacterial etiology in KB was then performed (Mariano & Souza, 2016). The experiment was conducted in a growth chamber and was repeated twice.

## Host specificity evaluated by cross-inoculation tests

The *Pat* reference strain obtained from the eight different hosts (*Nicotiana tabacum* (IBSBF 1972), *Phaseolus vulgaris* (IBSP 703), *Cucumis sativus* (IBSBF 758), *Carica papaya* (IBSBF 1822), *Desmodium incanum* (IBSP 974), *Aster* sp. (IBSP 1662), *Celosia plumosa* (IBSBF 1437) and *Coffea arabica* (IBSBF 2249) (Table 2) were inoculated in healthy coffee seedlings of the cv. Catuaí Vermelho IAC 99, which contained four pairs of true leaves.

The *Pcg* reference isolate (CFBP 1634) and seven of the most virulent *Pcg* strains (UFLA 59, UFLA 60, UFLA 126, UFLA 131, UFLA 134, UFLA 149 and UFLA 158) were inoculated in healthy seedlings of the *Carica papaya*, *Phaseolus vulgaris*, *Cucumis sativus*, *Desmodium incanum*, *Aster* sp., *Celosia plumosa*, *Nicotiana tabacum* and *Coffea arabica*.

**Table 1**: *Pseudomonas coronafaciens* pv. *garcae* strains used on virulence test.

| Strains [a] | Cultivate | Origin[b] |
|---|---|---|
| UFLA 06; UFLA 20; UFLA 21; UFLA 97; UFLA 98; UFLA 99; UFLA 106; UFLA 107; UFLA 109 | Mundo Novo 376/4 | Nepomuceno |
| UFLA 77; UFLA 78; UFLA 79; UFLA 80; UFLA 85; UFLA 86; UFLA 87; UFLA 88; UFLA 89 A; UFLA 89C; UFLA 90; UFLA 91A; UFLA 101; UFLA 102; UFLA 103; UFLA 104; UFLA 105 | Catuaí Vermelho/99 | |
| UFLA 43, UFLA 44, UFLA 46, UFLA 48; UFLA 52; UFLA 53; UFLA 54; UFLA 58; UFLA 59; UFLA 60; UFLA 61 | Mundo Novo 376/4 | Três Pontas |
| UFLA 114; UFLA 115; UFLA 116; UFLA 121; UFLA 117; UFLA 118; UFLA 119; UFLA 120; UFLA 127; UFLA 130; UFLA 157; UFLA 158 | Catuaí Vermelho/99 | |
| UFLA 81; UFLA 82; UFLA 83 | Catuaí Vermelho/99 | Patos de Minas |
| UFLA 84 | Catuaí Vermelho/99 | Lavras |
| UFLA 112 | Catuaí Vermelho/99 | Varginha |
| UFLA 113 | Catuaí Vermelho/99 | Vargem Grande |
| UFLA 122; UFLA 123 | Catuaí Vermelho/99 | Nova Rezende |
| UFLA 125 | Acaiá | São Sebastião do Paraíso |
| UFLA 126 | Mundo Novo 376/4 | Santo Antônio do Amparo |
| UFLA 154 | Catucaí 2SL | |
| UFLA 131; UFLA 132; UFLA 133; UFLA 134 | Mundo Novo 376/4 | Patrocínio |
| UFLA 138; UFLA 139 | Catuaí Vermelho IAC 144 | Elói Mendes |
| UFLA 148; UFLA 149 | Catuaí Amarelo IAC 62 | Ijací |
| UFLA 150; UFLA 151 | Catuaí Vermelho IAC 144 | Muzambinho |
| UFLA 152; UFLA 153; UFLA 156 | Mundo Novo 376/4 | Santana da Vargem |

[a] UFLA= Federal University of Lavras collection of plant pathogenic bacteria, Lavras, MG. [b] Municipalities in the Minas Gerais State, Brazil.

The inoculum was prepared by culturing each isolate on 90-mm-diameter plates containing KB and incubated at 28 °C. After 48 h, bacterial suspensions were prepared in sterile saline solution (0.85% NaCl) and calibrated to $A_{600nm}$ = 0.4 (approximately 1 × $10^9$ CFU $mL^{-1}$) with a spectrophotometer (McTavish et al., 2024).

The inoculation methods were injection, with multiple needles and spraying the bacterial suspension (approximately 1 × $10^9$ CFU $mL^{-1}$) (Mariano & Souza, 2016). Each isolate was inoculated into three plants of each species, which were tested for each inoculation method, for a total of nine plants inoculated per isolate. The same procedure was performed in the negative control plants inoculated with saline solution (0.85% NaCl) only.

The seedlings were maintained in a humidity chamber for 24 h before inoculation and for 72 h after inoculation, at 23 °C and in a relative humidity higher than 70%, and then placed in a greenhouse. The experiment was monitored daily to track the appearance of symptoms. Assessments of disease incidence were performed, i.e., using the qualitative method and the presence or absence of symptoms. Treatments were arranged in a complete randomized design and were experiments repeated twice.

### Confirmation of identity of the bacterial re-isolated

At the end of the assessments, the symptomatic leaves were collected, and fragments of the lesions were subjected to a bacterial streaming test to confirm the bacterial etiology of the disease. The isolation of the bacterium present in the samples with a confirmed bacterial etiology was then performed. Repetitive extragenic palindromic polymerase chain reaction (REP-PCR) was also performed to compare the genetic profile of the reisolated colonies with the respective reference strains. For the REP-PCR, primers corresponding to the REP region, REPIR-I (5' IIICGICGICATCIGGC 3') and REP-2I (5' ICGICTTATGIGGCCTAC 3') (Louws et al., 1994), were synthesized by Invitrogen Brazil, LTDA. The amplification protocol of Louws et al. (1994) was used. The reaction was performed in a volume of 25 µL containing 2.5 µL of 10X Taq buffer; 2.0 µL of $MgCl_2$ (25 mM); 1 µL of dNTP (10 mM); 2 µL of each primer (10 µM); 0.5 µL of Taq DNA polymerase enzyme (5 U/µL) (Invitrogen), 2 µL of DNA (50 ng/µL) and 13.0 µL of nuclease-free water. The amplification was performed in a Therm-1000 Axygen MaxyGene thermocycler using the following cycles: initial denaturation at 95 °C for 6 min; 30 cycles consisting of denaturation at 94 °C for 1 min, annealing for 1 min at 44 °C and extension at 65 °C for 1 min, with a final extension cycle at 65 °C for 16 min. The amplified rep-PCR products were visualized on a 1.5% agarose gel in 1X TBE buffer stained with GelRed Nucleic Acid Gel Stain (Biotium®).

## Results and Discussion

### Biochemical characterization

The 73 strains pathogenic to coffee plants were Gram negative, arginine dihydrolase negative, hydrolyzed esculin and were negative for oxidase. Only strains UFLA 21, UFLA 48, UFLA 60, UFLA 61, UFLA 79, UFLA 85, UFLA 98 e UFLA 102 were negative for gelatin hydrolysis, the remaining strains were positive. All strains produced acid from sorbitol and sucrose, except for UFLA 44, that failed to produce acid from sorbitol. Adonitol and lactose was not used by any isolate (Raimundi et al., 2021). Since the biochemical characteristics of these bacteria, *Pcg*, *Pat* and *Pch*, are very similar, the repeated extragenic palindromic polymerase chain reaction (REP-PCR) was also performed to compare the genetic profile of the reisolated colonies with the respective reference strains for correct identification.

**Table 2**: Strains used in the cross-inoculation test.

| Strains [a] | Species/pathovars | Host of origin |
|---|---|---|
| CFBP 1634 | *P. coronafaciens* pv. *garcae* | *Coffea arabica* |
| UFLA: 59; UFLA 160; UFLA 126; UFLA 131; UFLA134; UFLA 149; UFLA 158 | *P. coronafaciens* pv. *garcae* | *Coffea arabica* |
| IBSBF 2249 | *P. amygdali* pv. *tabaci* | *Coffea arabica* |
| IBSBF 1972 | *P. amygdali* pv. *tabaci* | *Nicotiana tabacum* |
| IBSBF 1822 | *P. amygdali* pv. *tabaci* | *Carica papaya* |
| IBSBF 1662 | *P. amygdali* pv. *tabaci* | *Aster sp.* |
| IBSBF 1437 | *P. amygdali* pv. *tabaci* | *Celosia plumosa* |
| IBSBF 758 | *P. amygdali* pv. *tabaci* | *Cucumis sativus* |
| IBSBF 974 | *P. amygdali* pv. *tabaci* | *Desmodium incanum* |
| IBSBF 703 | *P. amygdali* pv. *tabaci* | *Phaseolus vulgaris* |

[a] CFBP = French Collection of plant pathogenic bacteria, IBSBF = Phytobacteria Culture Collection of the Biological Institute, Campinas, SP, Brazil. UFLA= Federal University of Lavras collection of plant pathogenic bacteria.

## Virulence test of *Pseudomonas coronafaciens* pv. *garcae* strains

All 73 *Pcg* strains were able to infect coffee plants, however, there were differences in the size of the lesions with a significant difference ($p < 0.05$) between the strains in terms of the Area Under the Disease-Progress Curve (AUDPC) according to a Scott-Knott test (Table 3). The more virulent strains group presented AUDPC values that were 10 to 20 times higher than the less virulent group of strains. The first symptoms of the disease were observed starting seven days after inoculation and were characterized as necrotic spots surrounded by a yellowish halo (Figure 1). The symptoms of bacterial halo blight were not observed in the control treatment. The bacterial colonies reisolated from the symptomatic leaves were similar to the inoculated *Pcg* colonies, completing Koch's postulates.

Among the 35 strains considered most virulent 63% were from the *Coffea arabica* cv. Catuaí Vermelho IAC 99, 28% were from cv. Mundo Novo 376/4 and 3% of these strains were from cultivars Catuaí Amarelo IAC 62, Catuaí 2SL and Acaiá. Among the 21 moderately virulent strains, 52% were from cv. Mundo Novo 376/4, 38% cv. Catuaí Vermelho IAC 99 and 5% cv. Catuaí Amarelo IAC 62 and cv. Catuaí Vermelho IAC 144. Regarding the 17 strains considered less virulent, 41% were from cv. Catuaí Vermelho IAC 99, 35% were from cv. Mundo Novo 376/4 and 24% were from cv. Catuaí Vermelho IAC 144. Considering the total of strains tested here, the majority (36 strains) are from cv. Catuaí Vermelho IAC 99, 28 strains are from cv. Mundo Novo 376/4, four strains are from cv. Catuaí Vermelho IAC 144, two strains are from cv. Catuaí Amarelo IAC 62, one isolate was from cv. Catuaí 2SL and one isolate was from cv. Acaiá.

Studies carried out in Kenya and Brazil reported similar results (Ithiru et al., 2013; Rodrigues et al., 2017b; Maciel et al., 2018; Mwangi et al., 2018). Aggressiveness variation among *Pcg* strains to the coffee seedlings may have been caused by different factors, such as phytohormones and polysaccharide production, the expression of virulence genes and the production of toxins (Prasannath, 2013). Most *Pcg* strains produce tabtoxin in different quantities (Lydon & Patterson, 2001). Although tabtoxin is not essential for pathogenicity, it may influence the level of phytobacteria virulence, leading to increased disease severity (Barta & Willis, 2005).

Rodrigues et al. (2017b) suggested the possibility that *Pcg* bacterial mutants were adapting to the coffee crop, and that there was an occurrence of intraspecific variability in the pathogen (Raimundi et al., 2021; Alves et al., 2022). The virulence of the strains differed depending on the cultivar/variety from which they were obtained. This differential reaction in relation to the virulence of the strains, when noted in different genotypes (cultivars), can be explained by the ability of the pathogen to supplant the defense mechanisms of the host plant (Ithiru et al., 2013; Rodrigues et al., 2017b). According to studies conducted in areas naturally infested with bacterial halo blight in the Paraná state, to date, only the IPR 102 cultivar is resistant to the disease, whereas the cultivars IPR 103, IPR 104, IPR 108 and IPR 59 are partially resistant (Petek et al., 2006; Ito et al., 2008; Sera, Sera & Fazuoli, 2017).

## Host specificity evaluated by cross-inoculation tests

All the *Pat* reference strains, independent of the host of origin and the inoculation method (injection, multineedle and spraying of the bacterial suspension) caused disease in the coffee seedlings, which showed symptoms of brown leaf spots surrounded by a yellow halo (Figure 2).

Regardless of the inoculation method (injection, multineedle and spraying), eight *Pcg* strains obtained from coffee plants (CFBP 1634, UFLA 59, UFLA 60, UFLA 126, UFLA 131, UFLA 134, UFLA 149 and UFLA 158) caused disease in *Phaseolus vulgaris*, *Cucumis sativus*, *Carica papaya*, *Aster* sp. and *Coffea arabica*. However, disease symptoms on *Celosia plumose* were observed only with inoculation by the injection method, and disease symptoms on *Desmodium incanum* were observed only with the spray method. In none of the three inoculation methods tested did *Pcg* induce disease symptoms in *Nicotiana tabacum*.

Initial symptoms were observed seven days after inoculation and consisted of oily, gray-yellow lesions that subsequently became necrotic and were surrounded by a yellowish halo (Figure 3 A-U). *Nicotiana tabacum* showed a hypersensitive reaction when inoculated with the *Pcg* strains (Figure 3 V-X).

The bacterial etiology was confirmed by REP-PCR, in which the band pattern was similar between the colonies obtained from the reisolation and the respective *Pat* reference strains (IBSP 1972, IBSP 703, IBSP 758, IBSP 1822, IBSP 974, IBSP 1662, IBSP 1437 and IBSP 2249) thus fulfilling Koch's postulates. There was no bacterial exudation or reisolation of bacteria from lesions on *N. tabacum* leaves. REP-PCR also showed differences in the band patterns of *Pat* strains from *Aster* sp. and *Celosia plumosa* in relation to the strains from other hosts, demonstrating intrapathovar genetic diversity (Figure 4).

In comparison with the two species, can we say that *Pcg* has a restricted host range while *Pta* has no specificity. *Cucumis sativus, Carica papaya, Desmodium incanum, Aster* sp. and *Celosia plumosa*, which are natural hosts of *Pat*, also showed symptoms when inoculated with *Pcg*. Other studies also show that *Pcg* can induce symptoms in other crops through artificial inoculation, including *Citrus* sp., *Coffea eugenioides, C. excelsa, Ligustrum lucidum, Solanum lycopersicum, Olea europaea, Phaseolus vulgaris, Solanum paniculatum* var. *autilobum, Solanum tuberosum* (Bradbury, 1986) and *Avena sativa* (Barta & Willis, 2005). In this study, to fullfil Koch's, REP-PCR used to confirm the presence of *Pcg* in the observed symptoms and thus confirm the pathogenicity of the bacteria to different hosts. Therefore, the lack of host specificity for *Pcg* and *Pat*, both coffee and the natural host plants of *Pat*, indicates that these plant species may serve as reservoirs of inoculants for these pathogens, allowing their survival for a long time and making their control difficult.

**Table 3:** Area under the Disease-Progress Curve (AUDPC) averages for coffee seedlings (*Coffea arabica*) of 'Catuaí Vermelho IAC-99' assessed for bacterial halo blight caused by *Pseudomonas coronafaciens* pv. *garcae*.

| Strains [a] | AUDPC | Strains | AUDPC |
|---|---|---|---|
| UFLA 59 | 2.20 a | UFLA 58 | 1.20 b |
| UFLA 126 | 2.10 a | UFLA 104 | 1.13 b |
| UFLA 60 | 2.09 a | UFLA 119 | 1.08 b |
| UFLA 131 | 2.04 a | UFLA 48 | 1.08 b |
| UFLA 149 | 2.02 a | UFLA 80 | 1.08 b |
| UFLA 134 | 1.98 a | UFLA 148 | 1.00 b |
| UFLA 158 | 1.98 a | UFLA 152 | 0.93 b |
| UFLA 115 | 1.95 a | UFLA 79 | 0.93 b |
| UFLA 78 | 1.95 a | UFLA 84 | 0.91 b |
| UFLA 97 | 1.91 a | UFLA 151 | 0.89 b |
| UFLA 112 | 1.90 a | UFLA 46 | 0.82 b |
| UFLA 77 | 1.89 a | UFLA 132 | 0.79 b |
| UFLA 89C | 1.86 a | UFLA 122 | 0.72 b |
| UFLA 118 | 1.85 a | UFLA 86 | 0.70 b |
| UFLA 87 | 1.82 a | UFLA 53 | 0.69 b |
| UFLA 154 | 1.81 a | UFLA 43 | 0.68 b |
| UFLA 125 | 1.80 a | UFLA 133 | 0.65 b |
| UFLA 117 | 1.80 a | UFLA 116 | 0.54 b |
| UFLA 82 | 1.73 a | UFLA 61 | 0.49 b |
| UFLA 44 | 1.72 a | UFLA 52 | 0.38 b |
| UFLA 153 | 1.71 a | UFLA 156 | 0.35 b |
| UFLA 90 | 1.71 a | UFLA 150 | 0.13 c |
| UFLA 83 | 1.70 a | UFLA 139 | 0.33 c |
| UFLA 157 | 1.66 a | UFLA 114 | 0.32 c |
| UFLA 130 | 1.64 a | UFLA 89A | 0.30 c |
| UFLA 123 | 1.63 a | UFLA 88 | 0.28 c |
| UFLA 127 | 1.63 a | UFLA 101 | 0.27 c |
| UFLA 120 | 1.57 a | UFLA 98 | 0.26 c |
| UFLA 113 | 1.57 a | UFLA 99 | 0.25 c |
| CFBP 1634 | 1.57 a | UFLA 91A | 0.24 c |
| UFLA 20 | 1.56 a | UFLA 105 | 0.23 c |
| UFLA 85 | 1.49 a | UFLA 06 | 0.21 c |
| UFLA 102 | 1.46 a | UFLA 138 | 0.20 c |
| UFLA 109 | 1.43 a | UFLA 54 | 0.18 c |
| UFLA 103 | 1.36 a | UFLA 121 | 0.16 c |
| | | UFLA 107 | 0.15 c |
| | | UFLA 21 | 0.14 c |
| | | UFLA 106 | 0.12 c |

[a] CFBP = French Collection of plant pathogenic bacteria, IBSBF = Phytobacteria Culture Collection of the Biological Institute, Campinas, SP, Brazil. UFLA= Federal University of Lavras collection of plant pathogenic bacteria. Means followed by same letter in the column belong to the same group, according to Scott-Knott clustering, $p < 0.05$. a = greatest disease severity; b = moderate disease severity; c = lower disease severity.


**Figure 1:** Symptoms in coffee seedlings caused by *Pseudomonas coronafaciens* pv. *garcae* strains A) UFLA 105, B) UFLA 58 and C) UFLA 120 14 days after inoculation.

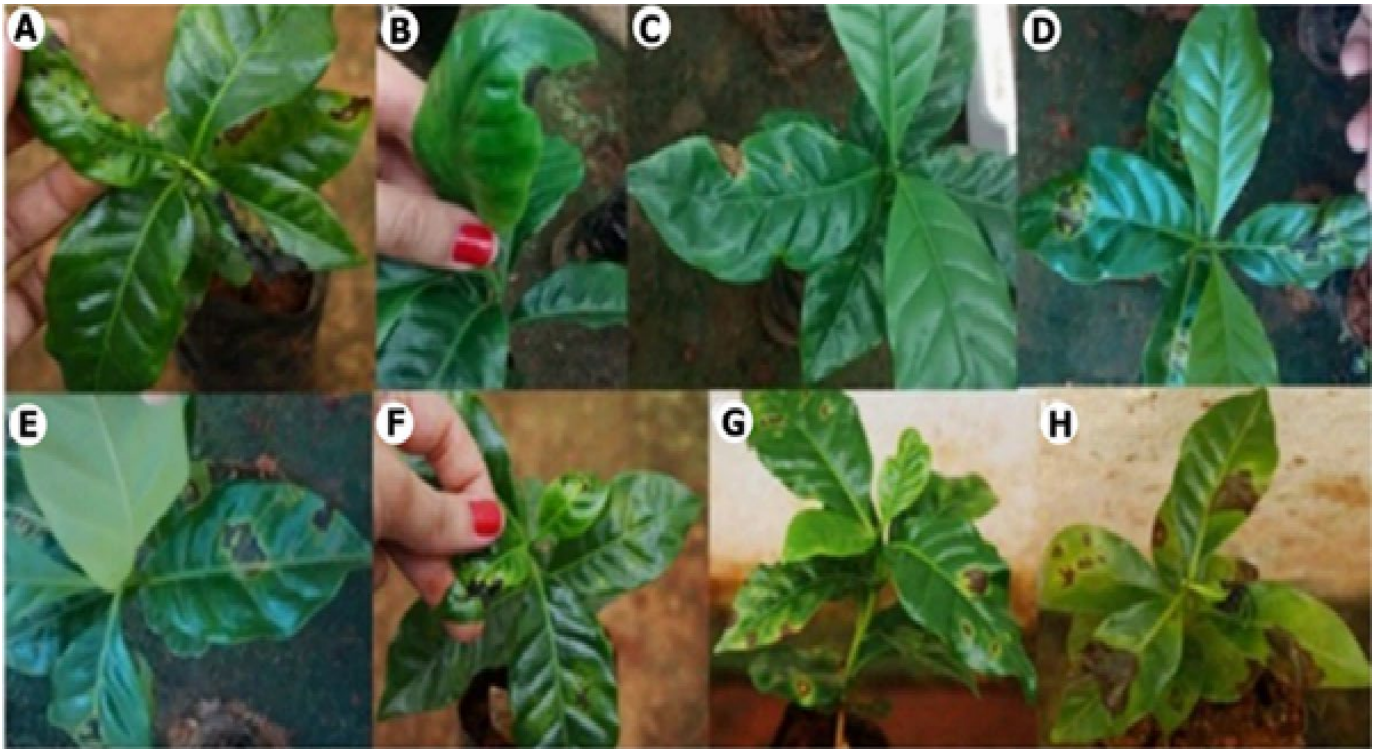


**Figure 2:** Symptoms in coffee seedlings caused by *Pseudomonas amygdali* pv. *tabaci* from the following hosts/inoculation method used: A) *Coffea arabica*/spraying/ (IBSP 2249), B) *Aster* sp./spraying/(IBSP 1662), C) *Celosia plumosa*/spraying/(IBSP 1437), D) *Phaseolus vulgaris*/multineedle/(IBSP 703), E) *Carica papaya*/multineedle/(IBSP 1822), F) *Cucumis sativus*/multineedle/(IBSP 758), G) *Nicotiana tabacum*/Injection/(IBSP 1972) and H) *Desmodium incanum*/Injection/(IBSP 974).

This is the first report in which *Pcg* was able to induce symptoms in cucumber (*C. sativus*), papaya (*C. papaya*), common bean (*P. vulgaris*), *D. incanum, Aster* sp. and *C. plumosa*, which are natural hosts of *Pat*, and it is the first report of *Pat* originating from these species causing symptoms in coffee. These bacterial strains are difficult to control due to a lack of information on the dynamics of the diseases, the reduced number of registered specific products and the development of pathogen resistance to antibiotics (Zoccoli, Takatsu, & Uesugi, 2011; Rodrigues et al., 2013; Patrício & Oliveira, 2014; Belan et al., 2016). Therefore, the results obtained in the present study are important from an epidemiological point, breeding of resistant cultivars using simultaneous *Pat* and *Pcg*, since the mixed infections by *Pcg* and *Pat* may occur in the same plant material (Rodrigues et al., 2017a), and provide knowledge for developing control strategies, including elimination of alternatives hosts.

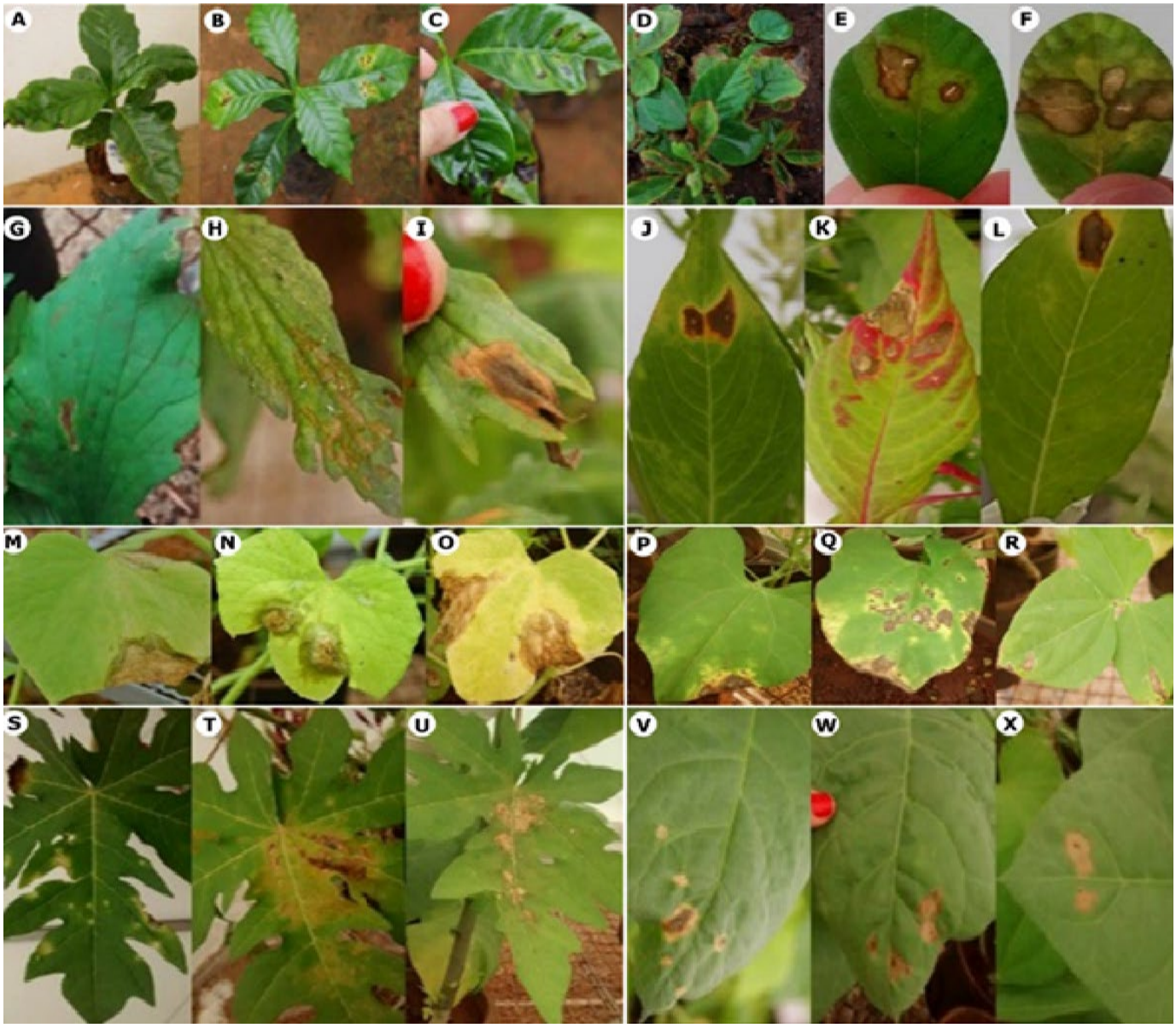


**Figure 3:** Symptoms caused by *Pseudomonas coronafaciens* pv. *garcae* strains. A-C) *Coffea arabica*: A) UFLA 59 (spraying), B) UFLA 60 (multineedle) and C) UFLA126 (injection) in coffee plants; D-F) *Desmodium incanum*: (spraying method) D) UFLA 149, E) UFLA 59, F) Pcg (CFBP 1634); G-I) *Aster* sp.: G) UFLA 131 (spraying), H) UFLA 134 (multineedle), I) UFLA149 (injection); J-L) *Celosia plumosa*: ( injection metho) J) UFLA 134, K) (CFBP 1634), L) UFLA 126; M-O) *Phaseolus vulgaris*: M) UFLA 60 (spraying), N) UFLA 126 (multineedle), O) UFLA149 (injection); P-R) *Cucumis sativus*: P) UFLA 158 (spraying), Q) Pcg (CFBP 1634) (multineedle), R) UFLA 59 (injection); S-U) *Carica papaya*: S) (CFBP 1634) (spraying), T) UFLA 134 (multineedle), U) UFLA 60 (injection); V-X) Symptoms of HR (hypersensitivity reaction) caused by *Pseudomonas coronafaciens* pv. *garcae* strains in *Nicotiana tabacum* leaves using each of the following methods: V) UFLA 126 (spraying), W) UFLA 149 (multineedle), X) UFLA 158 (injection).

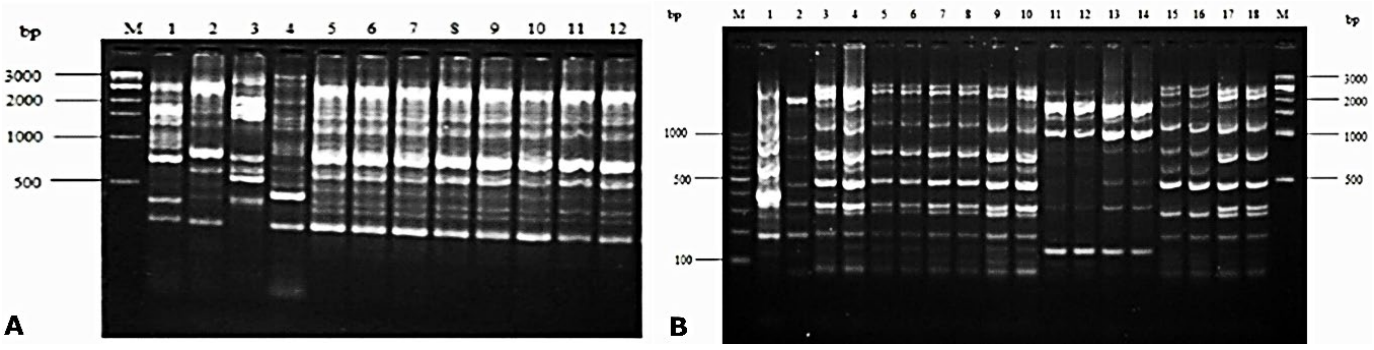


**Figure 4**: Analysis of PCR products in agarose gel when using *primers* REPIR and REP2I: A) Comparing the genetic profiles of the reference strains: M) 1 kb Molecular Weight Marker, 1) *Pseudomonas cichorii* (CFBP 2101), 2) *Pseudomonas coronafaciens* pv *garcae* (Pcg) (CFBP 1634), 3) *Pseudomonas amygdali* pv. *tabaci* (Pat) (IBSBF 2249), 4) *Burkholderia andropogonis* (IBSPF 166), 5) (CFBP 1634), with Pcg colonies reisolated from 6) *Aster* sp., 7) *Celosia plumosa*, 8) *Phaseolus vulgaris*, 9) *Carica papaya*, 10) *Cucumis sativus*, 11) *Desmodium incanum* and 12) *Coffea arabica*; B) Comparing the genetic profiles of Pat colonies that were reisolated from the coffee plants and their respective reference strains: M) 100 bp Molecular Weight Marker, 1) Pch (CFBP 2101), 2) Pcg (CFBP 1634), 3) and 4) IBSP 2249, 5) and 6) IBSP 703, 7) and 8) IBSP 1822, 9) and 10) IBSP 758, 11) and 12) IBSP 1662, 13) and 14) IBSP 1437, 15) and 16) IBSP 1972, 17) and 18) IBSP 974, and M) 1 kb Molecular Weight Marker.

## Conclusions

*Pcg* strains from coffee plants in Minas Gerais varied in aggressiveness. *Pcg* strains caused disease in *Cucumis sativus*, *Carica papaya*, *Desmodium incanum, Aster* sp. and *Celosia plumosa* after artificial inoculation. *Pat* strains from natural hosts *Coffea arabica, Phaseolus vulgaris*, *Cucumis sativus*, *Carica papaya*, *Desmodium incanum, Aster* sp., *Celosia plumosa* and *Nicotiana tabacum* caused symptoms in coffee plants after artificial inoculation. These results are important for developing control strategies including cultivars resistant to *Pcg* and *Pat* simultaneously and elimination alternative hosts.

## Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, the Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and the Instituto Nacional de Ciência e Tecnologia do Café – INCT Café, for providing scholarships to the authors and for supporting and funding this study. The authors would also like to thank Ana Maria dos Santos for her technical assistance.

## Author Contribution

Conceptual idea: Raimundi, M. K.; Souza, R. M.; Methodology design: Souza, R. M.; Resende, M. L. V.; Data collection: Raimundi, M. K.; Alvarenga, A. S.; Ribeiro, D. H.; Data analysis and interpretation: Raimundi, M. K.; Souza, R. M.; Guimarães, S. S. C.; Writing and editing: Raimundi, M. K.; Souza, R. M.; Guimarães, S. S. C.

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