

Resistance of Arabica coffee cultivars to leaf wounds and *Pseudomonas syringae* under field conditions

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Abstract

The aims of this study were: (a) to evaluate the resistance of coffee cultivars to *Pseudomonas syringae* (PS); (b) verify if there are coffee genotypes that present less wounds on the leaves; (c) to study the correlation between amount of wounds and PS severity. The field trial was installed in April 2014 at the IAPAR's experimental station (Londrina, Paraná, Brazil). 18 Arabica coffee cultivars were evaluated. The cultivars Mundo Novo IAC 376-4 and Catuaí Vermelho IAC 81 were the susceptible controls and IPR 102 was the resistant control. After 32 months of planting the field trial, resistance to PS was evaluated in December 2016. The evaluation of the PS severity was carried out under conditions of natural infections of *P. syringae* pv. *garcae* and *P. syringae* pv. *tabaci* based on the symptoms, using a grading scale from 1 to 5. The number of wounds on the leaves of the eight cultivars were counted. The results showed that IPR 102 was resistant to *Pseudomonas syringae*, whereas IPR 99, Arara, IPR 107, Acauã, Sabiá, Catucaiam 24137, Japy and Catuaí Vermelho IAC 81 were susceptible. Mundo Novo and Catucaí Amarelo 2SL were more susceptible than Catuaí Vermelho. IPR 106, Japiam, Catiguá MG 2, Catiguá MG 1 and IBC Palma 2 showed moderate resistance, while IPR 103 and Catucaiam 2015479 were moderately susceptible. The cultivars IPR 102 and IPR 106 presented resistance to wounding because they had lower wounds than other cultivars. Increased leaf wounds was associated with increased *P. syringae* severity.

Keywords: bacterial-halo-blight; bacterial-leaf-spot; *Coffea*; *garcae*; *tabaci*.

Abbreviations: BHB_Bacterial-halo-blight; BLS_bacterial-leaf-spot; HT_Híbrido de Timor; IAPAR_Instituto Agronômico do Paraná; PS_*Pseudomonas syringae*.

Introduction

BHB is a coffee disease caused by *Pseudomonas syringae* pv. *garcae* (Amaral et al., 1956). This disease occurs more frequently in regions of high altitude, mild temperatures, high rainfall, exposed to strong and constant winds, and sporadic frost (Zoccoli et al., 2011).

In these more favorable environmental conditions, the BHB causes production losses in important coffee producing states of Brazil such as Minas Gerais, São Paulo and Paraná (Moraes et al., 1975; Petek et al., 2006; Ito et al., 2008; Zoccoli et al., 2011). Besides Brazil, this disease has already been found in Kenya (Ramos and Shavdia, 1976; Ithiru et al., 2013), Ethiopia (Korobko and Wondimagegne, 1997), Uganda (Ramos and Shavdia, 1976) and China (Xuehui et al., 2013).

The BHB symptoms occur in leaves, young fruits and branches. The lesions on the leaves are irregular and of a brownish-brown color with yellowish halo around them. The lesions are more common on the edges of the leaves, where it is easier for bacteria to penetrate due to mechanical damages. However, they can extend throughout the leaf surface. In more severe attacks, necrosis of branches and young fruits may occur. Young plants are more susceptible and may suffer leaf shedding, die-back of the branches, over sprouting and delayed early development (Amaral et al., 1956). Recently, *P. syringae* pv. *tabaci* was reported as a pathogenic agent of the BLS, causing symptoms in coffee leaves very similar to that of the BHB (Destéfano et al., 2010; Rodrigues et al., 2017). In the state of Paraná, the

simultaneous occurrence of pathovars *garcae* and *tabaci* (Rodrigues et al., 2017) was identified.

The occurrence of these two bacterial diseases, especially the BHB, has caused significant losses in the coffee production, since the chemical control is not efficient and there are few cultivars identified with resistance.

The most suitable means of control is the use of resistant cultivars to *P. syringae* pv. *garcae* and *P. s.* pv. *tabaci*, since it is efficient and avoids the use of chemical control in coffee. Sources of resistance to BHB were identified in Arabica coffees from Ethiopia (Moraes et al., 1975; Mohan et al., 1978) and in other species such as *C. congensis* (Moraes et al., 1975), *C. eugenoides* and *C. stenophylla* (Mohan et al., 1978). Resistance to BHB was also observed in Arabica coffees with introgression of *C. canephora* genes such as in Icatu and HT derivatives (Mohan et al., 1978; Petek et al., 2006; Ito et al., 2008).

Few Arabica coffee cultivars were reported with a high level of resistance to BHB. Results obtained in field trials with natural occurrence of BHB showed that *C. arabica* cv. IPR 102 had a high level of resistance (Ito et al., 2008; Sera et al., 2017), while IPR 103, IPR 104, IPR 108 and IAPAR 59 showed an intermediate level of resistance (Ito et al., 2008). There are no studies reporting resistance to BLS in coffee.

Furthermore, there are no reports about the existence of coffee genotypes that present less wounds or injuries on the leaves under field conditions, which may represent a mechanism for the coffee genotypes to present a lower severity of the BHB and BLS. Although, there are reports that these bacteria penetrate through wounds caused by mechanical damages, agitation of leaves by wind, insects or other diseases (Zambolim, 2015), but there are still no studies on association between the amount of wounds and the PS severity.

The aims of this study were: (a) to evaluate the resistance of coffee cultivars to PS; (b) verify if there are coffee genotypes that present less wounds on the leaves; (c) to study the correlation between amount of wounds and PS severity.

Results and discussion

Assessment of the resistance to *Pseudomonas syringae*

By the evaluation of the PS severity, we observed that IPR 102 was the most resistant cultivar because it presented the lowest mean grade and differed from all other cultivars. IPR 106, Japiam, Catiguá MG 2, Catiguá MG 1 and IBC Palma 2 differed from the susceptible control Catuaí and showed moderate resistance. IPR 103 and Catucaiam 2015479 did not differ from cultivars with moderate resistance, but also did not differ from the Catuaí, indicating that they have moderate susceptibility. The cultivars IPR 107, Catucaiam 24137, IPR 99, Japy, Acauã, Sabiá and Arara did not differ from the susceptible controls Catuaí and Mundo Novo. The last cultivar and Catucaí Amarelo 2SL were the only ones that differed and showed more PS severity than moderately susceptible cultivars (Table 1). IPR 102 presented 100% resistant plants, indicating that shows resistance in homozygous condition.

Ito et al. (2008), studied young plants (10 months after planting) under field conditions with and observed that IPR

99 and IPR 107 were susceptible, IPR 102 was resistant and IPR 103 presented an intermediate resistance level.

Partial resistance to PS was found in cultivars derived from HT such as Catiguá MG 1, Catiguá MG 2 and IBC Palma 2 and those derived from Icatu x Catuaí as Japiam, IPR 103 and Catucaiam 2015479. IAPAR 59, which is derived from HT, has also been reported in previous studies as partially resistant (Petek et al., 2006; Ito et al., 2008). Mohan et al. (1978) also observed resistance in HT derivatives and in an Icatu genotype. It is possible that the sources of resistance of these cultivars are HT and Icatu, but we could not rule out the hypothesis that Villa Sarchí is a source of resistance as there are no studies reporting this. In IPR 103, Catucaiam 2015479 and IBC Palma 2, a predominance of plants with grade 3, was observed. This is indicating an intermediate resistance of the quantitative type. In Catiguá MG 1 plants with grades from 1 to 5 were observed, while in Catiguá MG 2 with grades from 1 to 4. Individual plants will be selected within these two cultivars to verify if it is possible to find lines with a higher level of resistance in the next generations.

Other HT-derived cultivars such as IPR 99, Arara, IPR 107, Acauã and Sabiá and those derived from Icatu x Catuaí such as Catucaiam 24137, Japy and Catucaí Amarelo 2SL were susceptible. Although Mundo Novo IAC 376-4 and Catucaí Amarelo 2SL did not differ from Catuaí Vermelho IAC 81, these two cultivars presented 80% of plants with grade 5 compared to 20% of Catuaí, indicating that they are more susceptible (Table 1). Mohan et al. (1978) studied seedlings at six months of age and with artificial inoculations of BHB in a greenhouse. It was also observed that Mundo Novo was more susceptible than Catuaí. Under field conditions, the adult plants of Mundo Novo IAC 376-4 and Catucaí Amarelo 2SL were more susceptible, probably because they have some morphological characteristics or plant architecture that increase the PS infection.

IPR 102 was the only cultivar that presented 100% of the resistant plants (grades 1 or 2), indicating that it has a high level of simultaneous resistance to pathovars *garcae* and *tabaci* of PS, under conditions of natural occurrence in the field.

Wounds on leaves

IPR 106 and IPR 102 presented the lowest amount of wounds on leaves and differed statistically from the other cultivars (Table 2).

This lower amount of wounds on IPR 106 may explain the difference of the results of our study, in which this cultivar showed moderate resistance, but was susceptible at younger ages (Ito et al., 2008). Therefore, IPR 106 may present adult-plant resistance under field conditions with natural infections, probably due to some morphological characteristics of the adult plant that provide less multiplication and dissemination of the pathogen such as plant architecture, leaf size, leaf shape, curling of leaf edge and intensity of plagiotropic branching. Some of these characteristics may promote a less favorable microclimatic condition for *Pseudomonas syringae* or a lower amount of leaf wounds, which facilitates the penetration of this bacteria.

Table 1. Means of PS severity and frequency of plants according to PS severity grading scale from 1 to 5, in Arabica coffee cultivars evaluated in Londrina (PR, Brazil), under field conditions with simultaneous occurrence of *Pseudomonas syringae* pv. *garcae* e pv. *tabaci*.

| Cultivar | Origin ⁽²⁾ | Frequency of plants (%) according to PS severity | | | | | PS ⁽³⁾ |
|---------------------------------|-----------------------|--|-------|-------|-------|-------|-------------------|
| | | 1 | 2 | 3 | 4 | 5 | |
| Catuaí Amarelo 2SL | Icatu x Catuaí | 0.00 | 0.00 | 0.00 | 20.00 | 80.00 | 4.80 a |
| Mundo Novo ⁽¹⁾ | Sumatra x Bourbon | 0.00 | 0.00 | 0.00 | 20.00 | 80.00 | 4.80 a |
| Arara | Sarchimor x Catuaí | 0.00 | 0.00 | 0.00 | 60.00 | 40.00 | 4.41 ab |
| Sabiá | Catimor x Acaiaí | 0.00 | 0.00 | 0.00 | 60.00 | 40.00 | 4.40 ab |
| Acauã | Sarchimor x MN | 0.00 | 0.00 | 66.66 | 33.33 | 0.00 | 4.33 ab |
| Catuaí V. IAC 81 ⁽¹⁾ | Caturra x MN | 0.00 | 0.00 | 0.00 | 80.00 | 20.00 | 4.20 ab |
| Japy | Icatu x Catuaí | 0.00 | 0.00 | 25.00 | 33.33 | 41.66 | 4.16 ab |
| IPR 99 | Sarchimor | 0.00 | 0.00 | 8.33 | 83.33 | 8.33 | 4.00 abc |
| Catucaiam 24137 | Icatu x Catuaí | 0.00 | 0.00 | 20.00 | 66.66 | 13.33 | 3.93 abc |
| IPR 107 | Sarchimor x MN | 0.00 | 0.00 | 33.33 | 41.66 | 25.00 | 3.91 abc |
| Catucaiam 2015479 | Icatu x Catuaí | 0.00 | 0.00 | 83.33 | 16.66 | 0.00 | 3.16 bcd |
| IPR 103 | Icatu x Catuaí | 0.00 | 0.00 | 76.66 | 13.33 | 0.00 | 3.13 bcd |
| IBC Palma 2 | Catuaí x Catimor | 0.00 | 33.33 | 50.00 | 16.66 | 0.00 | 2.83 cd |
| Catiguá MG 1 | Catuaí x HT | 25.00 | 16.66 | 25.00 | 16.66 | 16.66 | 2.83 cd |
| Catiguá MG 2 | Catuaí x HT | 11.11 | 44.44 | 22.22 | 22.22 | 0.00 | 2.55 d |
| Japiam | Icatu x Catuaí | 0.00 | 58.33 | 33.33 | 8.33 | 0.00 | 2.50 d |
| IPR 106 | Icatu | 13.33 | 66.66 | 20.00 | 0.00 | 0.00 | 2.06 d |
| IPR 102 | Icatu x Catuaí | 93.33 | 6.67 | 0.00 | 0.00 | 0.00 | 1.07 e |
| General mean | | | | | | | 3.51 |
| CV | | | | | | | 4.80% |

⁽¹⁾ Catuaí V. IAC 81 = Catuaí Vermelho IAC 81 (susceptible control); Mundo Novo = Mundo Novo IAC 376/4 (susceptible control). ⁽²⁾ MN = Mundo Novo; Sarchimor = Villa Sarchi x HT; Catimor = Caturra x HT. ⁽³⁾ PS severity grading scale from 1 to 5 (1 is more resistant and 5 is more susceptible). Means followed by the same letter do not differ by the Tukey test ($\alpha = 5\%$). Data transformed by $\sqrt{x+1}$.

Table 2. Means of amount of wounds on leaves (1st to 10th pair of leaves) of five branches of middle third per plant of Arabica coffee cultivars assessed in field trial.

| Cultivars | Wounds ⁽¹⁾ |
|------------------------|-----------------------|
| Sabiá | 27.18 a |
| Acauã | 27.01 a |
| Japiam | 26.76 a |
| IPR 103 | 25.19 a |
| Japy | 24.70 a |
| Catuaí Vermelho IAC 81 | 24.12 a |
| IPR 102 | 21.83 b |
| IPR 106 | 19.01 b |
| General mean | 24.47 |
| CV (%) | 7.65 |

⁽¹⁾ Means followed by the same letter do not differ by the Scott-Knott mean clustering test ($\alpha = 5\%$).

It is possible that IPR 102 have major or minor genes that respectively promote qualitative or quantitative resistance, combined with resistance promoted by the lower amount of leaf wounds.

Correlation between leaf wounds and PS severity

Although Zambolim (2015) reports that penetration of *Pseudomonas syringae* is facilitated by leaf wounds, there are no studies associating with the increased severity of this bacteria with wounds. In our study, a correlation of 0.592 ($\alpha = 1\%$) between the amount of wounds and the PS severity was observed, indicating that increased wounds led to increased PS severity.

Materials and methods

Trial and experimental design

The field trial was conducted in April 2014 at the IAPAR's experimental station (23° 22'S, 51° 10'W; asl = 585 m,

average annual temperature = 21.1° C), in Londrina, Paraná, Brazil. The experimental design was a randomized complete blocks design with three replicates of five plants per plot, spacing between rows of 2.50 m lines and between plants of 0.60 m.

Plant materials

The Arabica coffee cultivars IPR 99, IPR 106, IPR 107, IPR 107, Acauã, Arara, Catuaí Amarelo 2SL, Catucaiam 2015479, Catucaiam 24137, Japy, Japiam, IBC Palma 2, Sabiá, Catiguá MG1 and Catiguá MG2 were evaluated. The cultivars Mundo Novo IAC 376-4 and Catuaí Vermelho IAC 81 were the susceptible controls (Mohan et al., 1978; Ito et al., 2008; Zoccoli et al., 2011; Andreazi et al., 2015) and IPR 102 was the resistant control (Ito et al., 2008; Sera et al., 2017).

Resistance assessment

Resistance to PS was evaluated in December 2016, after 32 months of planting the field trial. An evaluation of the PS

severity was carried out under conditions of natural infections of *P. syringae* pv. *garcae* and *P. syringae* pv. *tabaci*, based on the symptoms described by Zambolim (2015), using a grading scale from 1 to 5 (Sera et al., 2017), in which: grade 1 = absence of necrotic lesions; 2 = from 0.01 to 3% leaves with small necrotic lesions (up to 0.5 cm), yellowish halo hardly visible to the naked eye; 3 = 3.01 to 15% leaves with small and medium lesions (up to 1 cm), with yellowish halo visible to the naked eye, with the possibility of 1% of large lesions (more than 1 cm); 4 = 15.01 to 30% leaves with small to large lesions; 5 = more than 30% of the leaves with small to large lesions, with the possibility of die-back of the branches. In this assessment, we assessed the leaves from the bottom third to the upper third of the plant, considering the first pair of fully expanded leaves to the tenth pair of fully expanded leaves. Plants with grades 1 and 2 were considered resistant and plants with grades 3, 4 and 5 as susceptible.

Identification of the pathovars of *Pseudomonas syringae*

A sample composed of 60 leaves of this experiment was sent to the Biological Institute, Campinas-SP, Brazil to identify pathogen.

The obtained isolates were submitted to the LOPAT tests (levan, oxidase, protopectinase in potato disks, arginine dihydrolase, and tobacco HR) and fluorescent pigment in BK medium (King et al., 1956). All strains presented the results, + - - - + to the respective tests and variable response to fluorescent pigment, indicating the inclusion of the obtained isolates in Group I of *P. syringae*.

Subsequently, the strains were subjected to biochemical (Lelliot et al, 1966;. Young and Triggs, 1994; Schaad., 2001; Rodrigues et al, 2017) and pathogenicity tests. The results determined the occurrence of *P. syringae* pv. *garcae*, causal agent of the bacterial-halo-blight and *P. syringae* pv. *tabaci*, causal agent of bacterial-leaf-spot, in the experimental field.

Evaluation of wounds on the leaves

The number of wounds on the leaves of the cultivars IPR 102, IPR 103, IPR 106, Acauã, Sabiá, Japy, Japiam and Catuaí Vermelho IAC 81 were counted by the naked eye, without the help of magnifying glass. The wounds on individual plants, five branches of the middle third of each plant and on the first pair of leaves fully expanded to the 10th pair, were counted. Leaf rips were considered as wounds, while disease and leaf miner (*Leucoptera coffeella*) lesions were not counted as wounds. The rips occurred mainly due to the friction between the leaves caused by the wind.

Statistical analysis

ANOVA, Bartlett's test of homogeneity of variances, Shapiro-Wilk normality test, and Tukey's test at 5% significance were performed using the R software version 3.3.0 (R Core Team 2016), package agricolae (Mendiburu 2015) for variable PS severity. Scott-Knott mean clustering test at 5% significance were performed using the R software version 3.3.0 (R Core Team 2016), package ScottKnott (Jelihovschi et al. 2014) for variable wounds on leaves. Data were transformed to $\sqrt{x + 1}$ for PS severity. The total amount of wounds of the

five branches of the middle third of each plant was used to analyze the data at the means of the plots.

To verify if there is an association between wounds and PS severity, the Spearman correlation was performed through program R version 3.3.0 (R Core Team, 2016), psych package (Revelle, 2016).

Conclusion

IPR 102 was resistant to *Pseudomonas syringae* under a natural occurrence of the pathovars *garcae* and *tabaci*, on field conditions in adult plants. IPR 106 and Japiam, derived from Icatu, Catiguá MG 2, Catiguá MG 1 and IBC Palma 2, derived from Híbrido de Timor, showed moderate resistance. Furthermore, we identified susceptibility in cultivars derived from Icatu such as Catucaiam 24137, Catucaí Amarelo 2SL and Japy, and some derived from Híbrido de Timor such as IPR 99, Arara, IPR 107, Acauã and Sabiá. Mundo Novo IAC 376-4 was more susceptible than Catuaí Vermelho IAC 81. IPR 103 and Catucaiam 2015479 also derived from Icatu were moderately susceptible. The plants with more leaf wounds presented more severity of *P. syringae*. The cultivars IPR 102 and IPR 106 presented resistance to wounding on the leaves of adult coffee plants.

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