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## Reação de diferentes genótipos de pessegueiro quanto à podridão parda em flores

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### Abstract

Brown rot is the main disease of peach trees. Contamination of flowers can cause significant losses in production, which requires effective control. The aim of this work was to test different flower peach genotypes for blossom blight in two production cycles. The work was carried out at the Universidade Tecnológica Federal do Paraná – *Campus Dois Vizinhos*. The experimental design was entirely randomized, with four replications of four branches, considering each genotype as treatment. The collected branches were prepared with the removal of old opened flowers and damaged ones. The flower buds and newly opened flowers were inoculated individually with 0.15 mL of *Monilinia fructicola* conidial suspension ( $1.0 \times 10^5$  spores mL<sup>-1</sup>). Branches of the control treatment were sprayed with 0.15 mL of distilled water. The flowers were examined 72 hours after inoculation, and the infected flowers percentage was evaluated. The peach genotypes evaluated in the two production cycles differ statistically for blossom blight incidence, in both cycles. The results demonstrate that there are different susceptibility degrees for blossom blight, being ‘*Cascata 1070*’ and ‘*Cascata 1055*’ genotypes with the lower susceptibility to disease.

**Keywords:** Blossom blight; *Monilinia fructicola*; resistance to diseases; divergence genetic; breeding peach.

### Resumo

A podridão parda é a principal doença do pessegueiro. A contaminação das flores pode causar perdas significativas na produção, o que torna necessário o efetivo controle para evitar que continuem presentes no pomar. O objetivo deste trabalho foi testar o comportamento de genótipos de pessegueiro quanto à podri-



dão parda em flores em dois ciclos produtivos. O experimento foi conduzido na Universidade Tecnológica Federal do Paraná – Campus Dois Vizinhos. Foi utilizado o delineamento experimental completamente casualizado, com quatro repetições de 4 ramos com flores, considerando cada genótipo como tratamento. Os ramos coletados foram preparados com a eliminação de flores velhas e danificadas. Os botões florais e as flores recém-abertas foram inoculados, individualmente, com 0,15 mL de suspensão conidial ( $1,0 \times 10^5$  esporos mL<sup>-1</sup>) de *M. fructicola*. No tratamento controle, foram pulverizados com 0,15 mL de água destilada. As flores foram examinadas 72 horas após a inoculação e avaliou-se visualmente a percentagem de flores infectadas. Os genótipos de pessegueiro avaliados diferiram significativamente quanto à incidência de podridão parda nas flores em ambos os ciclos. Houve diferentes graus de suscetibilidade à podridão parda em flores. Os genótipos ‘Cascata 1070’ e ‘Cascata 1055’ foram os que apresentaram menor suscetibilidade a ela.

**Palavras-chave:** Queima de flores; *Monilinia fructicola*; resistência a doenças; divergência genética; melhoramento do pessegueiro.

## Resumen

### Reacción de diferentes genotipos de melocotón para la pudrición parda en flores

La podredumbre parda es la principal enfermedad del melocotonero. La contaminación de las flores causa pérdidas importantes en la producción, así es necesario un control eficaz. En este trabajo, se objetivó evaluar el comportamiento de genotipos de melocotón para la pudrición parda en flores en dos ciclos productivos. El ensayo se realizó en la Universidad Tecnológica Federal do Paraná - *Campus Dois Vizinhos*. Se utilizó un diseño completamente al azar, con cuatro réplicas de cuatro ramas con flores y se consideró cada genotipo como tratamiento. Las ramas recogidas fueron preparadas con la eliminación de flores viejas y dañadas. Los botones florales y las flores recién abiertas fueron inoculados individualmente con 0.15 mL de suspensión de conidios ( $1.0 \times 10^5$  mL<sup>-1</sup>) de *Monilinia fructicola*. En el tratamiento de control, fueron rociados con 0,15 mL de agua destilada. Las flores se las examinaron 72 horas después de la inoculación y se evaluó visualmente el porcentaje de flores infectadas. Los genotipos de melocotón evaluados difirieron significativamente en la incidencia de pudrición parda en las flores en ambos ciclos. Hubo diferentes grados de susceptibilidad a la pudrición parda en las flores. Los genotipos “Cascata 1070” y “Cascata 1055” fueron los menos susceptibles a enfermedad.

**Palabras clave:** Marchitez de las flores; *Monilinia fructicola*; resistencia a enfermedades; divergencia genética; mejora del melocotonero.

## Introduction

The peach tree is a temperate fruit tree that has been cultivated in humid subtropical climate regions (BARON-MONTÉL *et al.*, 2019). This climate is highly favorable to increase the incidence and severity rate of some diseases, especially those caused by biotic agents, as fungi, that can affect the growers significantly, both for decreasing the production as it to prejudice the fruit quality (WAGNER JÚNIOR *et al.*, 2008).

Among the most damage diseases for the peach tree, the brown rot stands out, and it can be caused by three species from the genera *Monilinia*, *M. fructicola* (G. Winter) Honey, *M. laxa* (Aderhold & Ruhland) Honey e *M. fructigena* (Aderh. & Ruhl.) Honey (GARCIA-BENITEZ *et al.*, 2017). Although Souza *et al.* (2008) reported the existence of *M. laxa* in Brazil, the *M. fructicola* continue to be the main specie will cause of this disease.

The main symptoms of this disease are the burning flowers, chancre and branches injuries and the fruit rot (VILLARINO *et al.*, 2016). The infected flower shows brown petals spots, it may also demonstrate conidia on the stamens, anthers or other organs, that it can lead the flowers death, which remains to the stalk adhered indefinitely, becoming an inoculums source for fruits (BYRDE; WILLETTS, 1977), what it contributes for disease dissemination.

According to Martini and Mari (2014), many of fruit infections have begun in the bloom, where after to pass to the fruit, occurring pathogen manifestations during maturation, so leading to damages in harvest

and post harvest.

Then, the flowers contamination can cause meaningful yield lost, reduction in the flowers number, the fruit set and it serves as inoculum source for subsequent fruit infections (LANDGRAF; ZEHR, 1982; SHOLBERG *et al.*, 1981).

Accordingly, it becomes necessary the effective control of brown rot since the flowering stage, avoiding continued spread in the orchard. Normally, people have adopted the use of chemical control, that increase the production costs and not guarantee efficiency to reduce disease in the orchard (MAY-DE-MIO *et al.*, 2008), mainly when there are appropriate conditions (humidity and temperature) the damages occur in equal proportions, with or without this type of control.

An alternative to try be efficient the control of brown rot could be with use resistant varieties, with posterior adoption of sanitary practices and cultural control, which it would eliminate or reduce the fungicides application. Thus, it can reduce production costs (NEGRI *et al.*, 2011; OBI *et al.*, 2017) and possible soil and water contaminations.

This way, to turn this alternative possible, it is necessary to realize evaluations in different genotypes peach tree to brown rot in flowers. This would possible selection of these with certain levels of tolerance or resistance, using them in orchards or as parents in peach breeding programs. The aim of this work was to test the reaction of different peach genotypes to brown rot in flowers.

## Material and Methods

The work was carried out in the Phytosanitary Laboratory of the Universidade Tecnológica Federal do Paraná – *Campus Dois Vizinhos*, during two productive cycles.

In the laboratory conditions, the incidence of brown rot evaluation was verified in flowers genotypes peach using the detached branches method. The analyzed genotypes belong to the peach collection implanted in the UTFPR – *Campus Pato Branco* experimental area, in Pato Branco – PR city, Brazil (latitude 26° 10' 39" S, longitude 56° 41' 21" W, and an average altitude of 750 m).

The plants of each genotype were conducted in vase systems, with spacing 5 x 4 m between plants and lines, respectively. The management practices were done according to general recommendation to the culture, without chemical products utilization to flowering diseases control. The peach genotype collection was implanted in September 2003 and 2004.

In the first productive cycle the flowers reactions from nine genotypes ('Atenas', 'Tropic Snow' and Libra and, the selections 'Conserva 977', 'Conserva 844', 'Conserva 655', 'Cascata 967', 'Cascata 962', and 'Conserva 688') to brown rot, were evaluated. In the second cycle were evaluated 16 genotypes, ('Atenas', 'Tropic Snow' and 'Olímpia' and, the selections 'Conserva 977', 'Conserva 844', 'Conserva 655', 'Cascata 967', 'Conserva 1153', 'Conserva 1187', 'Conserva 1396', 'Conserva 1434', 'Cascata 1303', 'Cascata 1070', 'Conserva 1186', 'Cascata 1055' and 'Conserva 871').

The experimental design was completely randomized, considering each genotype peach as treatment, using four repetitions and four branches per plot. The fungi isolated in the first cycle was obtained from Embrapa Clima Temperado, Pelotas – RS State, Brazil. In the second cycle the isolated was obtained from the UTFPR - *Campus Pato Branco* Peaches Collection fruits and of the commercial orchard from Southeast regions of Paraná State, Brazil. After the collected from both materials, they were transferred to Petri® dishes in laboratory, with PDA medium (potato, dextrose, agar) and in chamber B.O.D. incubated to 25 ± 2 °C, during five to seven days in dark. Through successive dilutions was adjusted the *M. fructicola* suspension concentration to 1,0 x 10<sup>5</sup> spores mL<sup>-1</sup>, using a Neubauer chamber (WAGNER JÚNIOR *et al.*, 2005).

The collected branches were prepared with the exclusion of open and damaged flowers. The buds and the newly opened flowers were inoculated, individually, with 0,15 mL from *M. fructicola* conidial suspension, using a plastic sprinkler.

After inoculation, the branches were preserved distilled water in 180 mL plastic glasses. The branches were then protected with clear plastic bags (34,5 x 49,0 cm) punctured and moistened with distiller water. They were put in plastic boxes, and it kept at room temperature.

The flowers were examined 72 hours after the inoculation, it being visually it evaluated the percentage of flowers infected. It was considered those that showed necrotic spots on petals (WAGNER JÚNIOR *et al.*, 2005).



The data were evaluated by variance analysis and to the Scott & Knott test ( $p \leq 0,05$ ). The incidence data were prior transformed in  $\arcsine \sqrt{x/100}$ , for not showing normality according to Lillieford test. The medium “t” test was realized among the genotypes evaluated in both productive cycles. The genotypes studied in both productive cycles (first and second) were also evaluated as grouping analysis through the “nearest neighbor” method and optimizing grouping by “Tocher” method, using as dissimilarity measure the distance of Mahalanobis, to both methods (CRUZ *et al.*, 2004). All the analyses were realized using GENES® program (CRUZ, 2006).

## Results and Discussion

The peach genotypes evaluated in the two productive cycles had significant differences to brown rot incidence. So, we observed that the genotypes showed different susceptibility and/or tolerance levels to disease in flowers (Table 1).

**Table 1:** Brown Rot incidence (%) in 19 genotypes Peach Tree Flowers inoculated with *M. fructicola*, in the two productive cycles.

Genotypes	Brown Rot Incidence (%)	
	First cycle	Second cycle
Tropic Snow	63.11 c*	62.11 a
Atenas	73.64 c	63.30 a
Conserva 977	100.00 a	65.06 a
Conserva 844	92.07 b	59.94 a
Conserva 655	89.20 b	62.44 a
Cascata 967	89.28 b	45.04 b
Cascata 962	86.33 b	-
Conserva 688	85.95 b	-
Libra	80.04 c	-
Conserva 1153	-	69.00 a
Conserva 1187	-	52.25 a
Conserva 1396	-	68.59 a
Conserva 1434	-	54.10 a
Cascata 1303	-	51.03 a
Cascata 1070	-	24.40 c
Conserva 1186	-	63.05 a
Cascata 1055	-	30.10 c
Conserva 871	-	54.69 a
Olímpia	-	37.69 b
CV (%)**	14.00	14.55

\* Averages followed by the same letter in the column do not differ each other, by the Scott & Knott ( $p \leq 0,05$ ) test grouping.

\*\* CV (Variation coefficient)

In the first productive cycle occurred a generation of three groups by the Scott & Knott test, one in the susceptibility percentage between 63 and 80,04% constituted by the genotypes ‘Tropic Snow’, ‘Atenas’ and ‘Libra’, another more susceptible with incidence between 85,95 to 92,07% formed by ‘Conserva 844’, ‘Conserva 655’, ‘Cascata 967’, ‘Cascata 962’, ‘Conserva 688’ and the last highly susceptible with the ‘Conserva 977’ genotypes (100% of brow rot incidence in flowers). Thus, in the present cycle was verified that the analyzed genotypes are not tolerant to brown rot in flowers (Table 1). The pathogen *M. fructicola* may show different incidence levels at different stages of peach blossom opening. In a study, May-De-Mio *et al.* (2008) report that the highest incidence phase of the disease was detected in a fully open flower.

Studying the incidence and severity of peach genotypes, Santos *et al.* (2012) found that higher levels of resistance to *M. fructicola* may occur in the Conserva 930 and Jubileu genotypes when compared to the cultivar Bolinha, used as resistance pattern. The same number of groups was also obtained when was analyzed the

brown rot in flowers in the second productive cycles, even a greater number of genotypes evaluated. In this cycle, we had a less susceptible group, grouping the genotypes 'Cascata 1070' and 'Cascata 1055' and, the group with moderate susceptibility composed by 'Olímpia' e 'Cascata 967' (Table 1).

The "Cascata 1070" genotype, although not significantly differ from 'Cascata 1055' showed the lower incidence average (24.4%) and so it may be considered with a certain tolerance to brown rot in flowers. However, this result was based just in one productive cycle, it is necessary to analyze it for one more cycle to obtain more conclusive results to the reaction to *M. fructicola* in flowers, since that results from a unique cycle are generally insufficient to a reliable evaluation of resistance. In some southern regions, the flowering of peach tree has not uniformity, with a cycle that can vary up to one week from one crop to another, what it can be very dependent on climatic conditions and the removal of leaves from the orchard after harvesting the fruits (BIASI *et al.*, 2004).

Others analyzed genotypes in the second productive cycles, 'Atenas', 'Tropic Snow', 'Conserva 977', 'Conserva 844', 'Conserva 655', 'Conserva 1153', 'Conserva 1187', 'Conserva 1396', 'Conserva 1434', 'Cascata 1303', 'Conserva 1186' and 'Conserva 871', fit in the group with greater susceptibility, with superior incidence of 50% (Table 1).

However, we could observe in second productive cycles a lower incidence of brown rot in flowers when comparing to first cycle, with genotypes had similar behavior, like 'Tropic Snow' and 'Atenas'. In the flower, the fungus can use the stigma to reach the ovary, reaching the peduncle and penetrating the branch (DUTRA *et al.*, 2019). When the pathogen arrives in the branches, cancers, subsequent ringing and death of the terminal part appear (MAY-DE-MIO *et al.*, 2014). The selection 'Cascata 967' was the only one that showed superior incidence of 50% in one cycle (first) and inferior to this in the other (second).

The reduction obtained in the second productive cycle incidence can be related to the type of inoculum utilized, once in the first cycle was used other isolated and time of inoculation. Santos *et al.* (2012) observed that lesion sizes up to 72 h after inoculation with *M. fructicola* were smaller than 96 h, but some cultivars with 24 h already had lesions. In addition, physical characteristics such as thickness and density of the cuticular layer may offer more or less resistance to pathogen penetration, due to its composition and compounds present in the cuticle trigger pathogen attack signaling mechanisms (STANGARLIN, 2011). The grouping by Tocher method, based in the Mahalanobis distance allowed the individualization of three groups in the first cycle and of four groups in second cycles (Tables 2 and 3, respectively). We observed that the grouping realized by Tocher Method compared with the Scott & Knott test was similar to some genotypes.

In Table 2, we observed that the 'Tropic Snow' cultivate was individualized in just one group, separating from 'Atenas' and 'Libra' genotypes by the Tocher Method, it being the latter grouping with the genotypes susceptible to the disease. The 'Conserva 977' genotype was also individualized in a unique group, since it was the most susceptible to the disease. So, we verified that the resultant grouping from the conglomeration analysis by the Tocher method, based on the Mahalanobis distance, allowed individualize the most susceptible genotype and the most tolerant to brown rot in flowers.

**Table 2:** Grouping resulting from the conglomeration analysis by Tocher method, based on Mahalanobis distance, among nine peach tree genotypes from Peach Collection from the experimental area of UTFPR, Pato Branco, PR, in first productive cycle.

Group	Genotypes
I	Conserva 655; Cascata 967; Conserva 844; Cascata 962; Conserva 688 (8) Libra (9) e Atenas (2);
II	Tropic Snow (1);
III	Conserva 977 (3);

In the second cycle, as a greater number of genotypes we analyzed, there was a formation of one more group comparing to the previous cycle, it being formed two groups with certain tolerance to brown rot in flowers (Groups III and IV). The IV group formed by the genotypes ('Cascata 1070' and 'Cascata 1055') was the one with greater tolerance to the disease with incidence results lower than 30%. The III group, composed by 'Olímpia' and 'Cascata 967' was another that also showed certain tolerance, with incidence between 37% and 45% (Table 3). This grouping was identical to those realized by the Scott & Knott test in the same cycle (Table 1). These results can be compared with those obtained by Wagner Júnior *et al.* (2008), where the 'Conserva

672' genotype showed a slower progression of disease infection, it being considered, therefore, as a genotype with tolerance reaction to *M. fructicola*. In addition, the 'Bolinha', 'Eldorado', 'Leonense', 'Maciel', 'Magno' and 'Linda' peach varieties were considered as tolerant to the disease, by the authors.

**Table 3:** Grouping resulting from the conglomeration analysis by Tocher method, based on Mahalanobis distance, among 16 Peach genotypes from Peach Collection from the experimental area of UTFPR, Pato Branco, PR, in second productive cycle.

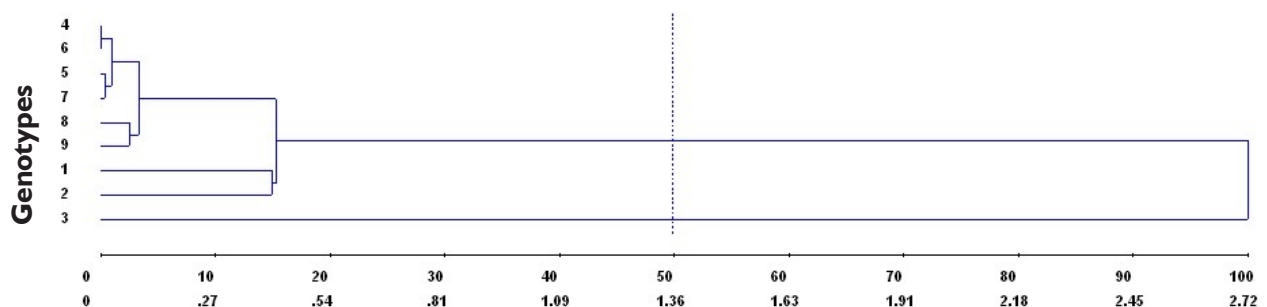
Group	Genotypes
I	Atenas (2); Conserva 1186 (13); Conserva 655 (5); Tropic Snow (1); Conserva 977 (3); Conserva 844 (4); Conserva 1396 (9) e Conserva 1153 (7)
II	Conserva 1434 (10); Conserva 871 (15); Conserva 1187 (8) e Cascata 1303 (11);
III	Cascata 967 (6) e Olímpia (16);
IV	Cascata 1070 (12) e Cascata 1055 (14).

The genotypes with incidence between 51% and 55% ('Conserva 1434', 'Conserva 871', 'Conserva 1187' and 'Cascata 1303') fit in group II, it being possible to consider them as susceptible to the disease. The other genotypes with incidence above 55% were grouped in the group I, forming so, a group with a higher level of susceptibility ('Atenas', 'Conserva 1186', 'Conserva 655', 'Tropic Snow', 'Conserva 977', 'Conserva 844', 'Conserva 1396' and 'Conserva 1153') (Table 3).

The grouping formed by the Tocher Method in the second cycle, proved to have more criteria in comparison to Scott & Knott test since it has individualized more the previously formed groups (Tables 1 and 3).

However, comparing the results obtained in the first cycle by Tocher Method (Table 2) with the grouping method "nearest neighbor" (Figure 1) we could observe that the last method formed lower number of groups. In this cycle, by the "nearest neighbor" method there was a formation of only two groups, individualizing in just one group the most susceptible genotype ('Conserva 977'), keeping the other in another group. In a study of Assmann *et al.* (2010), the genotype ('Conserva 977') showed susceptibility to the fungus *Tranzschelia discolor*, an etiological agent of peach leaf rust. They showed that grouping using the "nearest neighbor" method did not separate those with moderate susceptibility and with the highest incidence. Chemical and physical factors are thought to account for differences often observed in disease resistance among different fruit developmental stages (PRUSKY, 1996).

**Figure 1:** Grouping Method: Simple Linkage – Nearest Neighbor: Genetic dissimilarity dendrogram among nine peach tree genotype (first productive cycle) obtained by the Nearest Neighbor Method based on the percentage of brown rot in flowers incidence, using the widespread Mahalanobis distance. In the X axis were represented the distance percentages among the population and in the Y axis were represented by the nine genotypes.



Genotypes Legend: 1- Tropic Snow; 2- Atenas; 3- Conserva 977; 4- Conserva 844; 5- Conserva 655; 6- Conserva 967; 7- Cascata 962; 8- Conserva 688; 9- Libra.

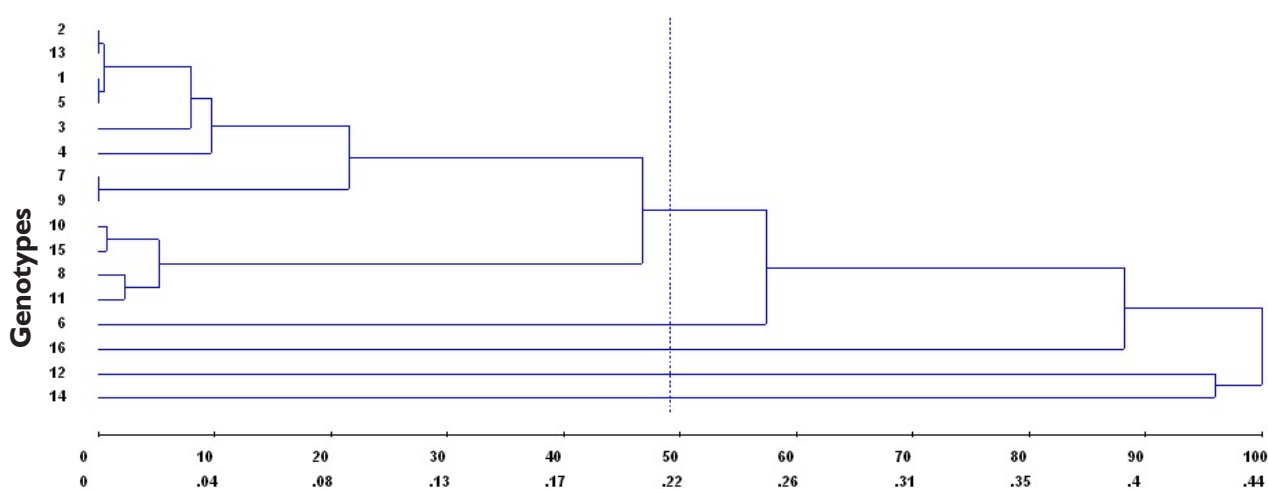
In the dendrogram formation by the 'nearest neighbor' method, in the first cycle, we considered the greatest distance, 2,72 (obtained by D2) as 100% of distance, obtained between 'Tropic Snow' and 'Conserva 977' genotypes (Figure 1). To the second cycle we considered as greatest distance the value 0,44, obtained between

'Tropic Snow' and 'Cascata 1070' genotypes.

It is assumed that, both distances obtained with 'Tropic Snow' variety (first and second cycles) is due to the fact that it is originated from Florida University and it does not show parentage with anyone the selections that showed greater distance, explaining the greater genetic diversity.

Analyzing the results, in the second cycle, the grouping by the 'nearest neighbor' method (Figure 2) showed the formation of one more group (5) compared with the obtained by Tocher Method. However, by the 'nearest neighbor' method there were the formation of four groups with an unique genotype (group 1 – 'Cascata 1055'; group 2 – 'Cascata 1070', group 3 – 'Olímpia' and group 4 – 'Cascata 967'), using in this one just those with lower susceptibility to brown rot in flowers (incidence < 45%). And the others, with incidence above 45%, fit in a single group.

**Figure 2: Genetic dissimilarity dendrogram among 16 peach tree genotype (second productive cycle) obtained by the Nearest Neighbor Method based on the percentage of brown rot in flowers incidence, using the widespread Mahalanobis distance. In the X axis were represented the distance percentages among the population and in the Y axis were represented by 16 genotypes.**



Genotypes Legend: 1-Tropic Snow; 2- Atenas; 3- Conserva 977; 4- Conserva 844; 5- Conserva 655; 6- Conserva 967; 7- Conserva 1153; 8- Conserva 1187; 9- Conserva 1396; 10- Conserva 1434; 11- Cascata 1303; 12- Cascata 1070; 13- Conserva 1186; 14- Cascata 1055; 15- Conserva 871; 16- Olímpia.

Although the 'Cascata 1055' and 'Conserva 1153' genotypes had in common the same paternal parent (Granada variety), they showed different susceptibility levels and were distinctly grouped, what may indicate maternal inheritance. This hypothesis can be raised once 'Conserva 655' and 'Conserva 871' genotypes showed the same ancestral too ('Diamante' variety), and they were grouped as those with greater susceptibility to brown rot, according to the 'nearest neighbor' grouping, it has in its maternal genealogy the same cultivate ('Taquari Precoce'). Wagner Júnior (2003) studied the heritability to brown rot suggested that there isn't a cytoplasmic inheritance to this disease in flowers, in other words, maternal effect. So, it is necessary the realization of new works that prove which effect is involved in the resistance of peach tree to this disease.

## Conclusion

The results obtained showed that there were different levels of susceptibility to brown rot in flowers, with 'Cascata 1070' and 'Cascata 1055' genotypes showed lower susceptibility to brown rot in flowers, what can be potential to use in orchards as parents in future breeding programs.

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