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Peach Flower Reaction to Inoculation with *Monilinia fructicola* (Wint.) Honey

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Abstract

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is one of the main fungal diseases of stone fruit (*Prunus* spp.). Flower infection can cause significant damage to the trees, reducing the number of flowers, decreasing fruit set, and also providing a source of spore inoculum for fruit infection. The objective of this work was to study the reaction of peach flowers from different genotypes to *M. fructicola*; identify possible sources of resistance; and determine the frequency of distribution of seedlings from several progenies in different classes of infection incidence. Six cultivars, one selection, and six progenies, originated by crosses among them, were tested in 2001 and 2002. Flowers were inoculated with 0.1 ml of a $5x10^4$ spores/ml suspension and maintained under controlled environment at 24°C $\pm$ 2°C, 75 to 85% relative humidity, and 12 hour photoperiod. The percentage of infected blossoms (showing petals with necrotic spots) was evaluated 72 hours after inoculation. Cultivars Magno and Leonense were infected the least; eleven seedlings within the tested progenies also showed good resistance levels ($\leq$ 10%). However, further studies are needed for final conclusions. A transgressive inheritance was observed in the studied progenies. The broad sense heritability for blossom blight due to *M. fructicola* was relatively low (H = 0.30 – 0.42).

Introduction

Blossom blight caused by *Monilinia fructicola* (Wint.) Honey is considered a primary infection, which serves as an inoculum source for fruit infections that occur later in season (5, 7, 10, 11). The disease cycle starts after the tree breaks dormancy and develops when spores get in contact with and penetrate the flower organs of susceptible cultivars. Parts such as stigma, style, petals, and sepals can be the first infection points (2).

Breeding programs in mild winter regions of the world have been focused on releases of cultivars adapted to such conditions, i.e., low chill requirement cultivars. However, in southern Brazil these cultivars generally bloom early in the season. Several of these cultivars bloom in mid or late winter when environmental conditions (mainly the humidity) are favorable to the pathogen's development and consequently to the disease occurrence.

Chester (3) was probably the first researcher to artificially inoculate and infect peach flowers. The use of blossom blight resistant cultivars of stone fruit can reduce the incidence of brown rot in fruits. Dunegan & Goldsworty (6) observed a low incidence of fruit brown rot in orchards when blossom blight was controlled.

The objective of this research was to test flower susceptibility to infection by *M. fructicola* of several genotypes of peach, determine possible sources of brown rot resis-

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tance, and verify susceptibility or resistance in different peach progenies.

Materials and Methods

Plant material

In 2001, five cultivars released by Embrapa's peach breeding program in Pelotas, RS, Brazil ('Bolinha', 'Eldorado', 'Magno', 'Leonense', 'Linda'), seven progenies from 1996 crosses ('Magno' x 'Leonense'; selection Conserva 672 x 'Maciel'; selection Conserva 672 x 'Leonense'; selection Conserva 672 x selection A 334; selection Conserva 672 x 'Eldorado'; 'Leonense' x 'Bolinha') and one cross ('Bolinha' x 'Leonense') from 1997 were tested.

In 2002, six cultivars ('Leonense', 'Bolinha', 'Magno', 'Eldorado', 'Maciel' and 'Linda'), two selections (Conserva 536 and Conserva 672) and six progenies from crosses between some of them ('Magno' x 'Leonense'; selection Conserva 672 x 'Maciel'; selection Conserva 672 x 'Leonense'; selection Conserva 672 x selection A334; selection Conserva 672 x 'Eldorado' and 'Leonense' x 'Bolinha') made in 1996 were also tested.

Inoculum preparation

Culture mixed of isolates of *M. fructicola*, obtained from the collection of Embrapa Temperate Climate (Brazil), were incubated under controlled conditions at 25°± 2°C for 5 to 7 days in the dark. A spore suspension was prepared by adding sterile water in the cultures and adjusting to a concentration of $5x10^4$spores/ml using a hemacytometer. Six one-year twigs in 2001, and eight in 2002, were collected randomly from the four quadrants of the trees for each cultivar or selection. The fully opened flowers were discarded and the twigs surface sterilized with 0.125% of active chlorine solution for one minute and washed three times in distilled water. The twigs then were kept at 5° ± 1°C for 5 to 7 days and for 24 hours at room temperature (24° ± 2°C) in order to get more uniform developmental stage of the flower buds.

Inoculation of each flower bud was done by spraying 0.1 ml of the conidia suspension of *M. fructicola* using a hand atomizer De Villbiss; flower buds that served as controls were not inoculated; instead they were sprayed with sterilized distilled water.

The bases of the detached twigs were placed in water and covered with a transparent plastic bag bearing small holes which was sprayed inside with distilled water to create high humidity. The baskets containing the twigs were taken to a phytotron at 24°±2°C, high relative humidity (>90%), and 12 hour photoperiod. Percentage of infected flowers, based on the presence of necrotic lesions on the petals, was determined 72 hours after inoculation.

The experimental design was completely randomized and each plant was a replication. The number of replications was variable according to the availability of plants (Tables 1 and 2). The results were subjected to the variance analysis and the means were compared by Duncan multiple range test at 5% probability. The brown rot incidence was divided in 10 classes of according to the frequency of seedlings (Figures 1 to 13).

The broad sense heritability was calculated by using the average variability among plants of the same parent cultivar (propagated asexually), as the estimation of environmental variance. The total variance was obtained from the average variance of progenies. The genetic variance was obtained by the difference between the total and environmental variance.

Results and Discussion

In 2001, 'Bolinha' had the lowest incidence of infected flowers followed by 'Leonense', 'Magno', and 'Eldorado'; the selection Conserva 672 and 'Linda' had more than 85% infected flowers. In 2002, however, 'Magno' had the lowest incidence of flowers infected with brown rot (Table 1). The differences between the two years, mainly for 'Bolinha',

Table 1. Brown rot incidence on flowers of six cultivars and two peach selections in 2001 and 2002, evaluated at Embrapa Temperate Climate, Pelotas, RS, Brazil.

Genotype	Number of plants		Flowers with brown rot (%)*	
	2001	2002	2001	2002
'Bolinha'	2	16	25.0 f**	74.6 c**
Conserva 536	---****	3	---	42.6 g
Conserva 672	5	5	85.5 b	77.7 b
'Eldorado'	16	32	68.8 c	86.6 a
'Leonense'	8	14	34.8 e	57.7 f
'Linda'	9	12	88.8 a	61.9 d
'Maciel'	---	11	---	60.5 e
'Mango'	3	6	47.6 d	19.1 h
Total	43	99		
CV(%)***			32.3	22.2

* Flowers were inoculated by spraying a $5x10^4$ spores/ml suspension of *M. fructicola*.
** Means followed by the same letter in columns do not differ significantly according to Duncan's multiple range test at 5% probability.
*** CV (Coefficient of variation).
**** No data: flowers had already opened when the experiment started.

can be explained by the small number of plants of this cultivar used in 2001.

For the two years of evaluation, it was observed that 'Magno' and 'Leonense' presented the lowest infection percentages in relation to the others, while 'Linda' and selection Conserva 672 had the highest.

Adaskaveg et al. (1) evaluated flower resistance to *M. fructicola* of different peach genotypes using a conidiospore suspension of 2.0×10^4 spores/ml and kept the inoculated material for 48 hours in the laboratory under 20°C and in the field under temperature between 16° and 20°C. The percentage of infected anthers after 48 hours incubation was 10-30% lower in the resistant genotypes, cvs. Bolinha and Kakamas as compared to susceptible cvs. Starn, Loadel, Tufts, Flavorcrest. However, there were no significant differences after 72 hours of incubation. It is important to consider that the cultivars that the authors called resistant were based on previous work with fruits and not flowers.

In the present work, even after 72 hours incubation and using higher spore inoculum concentration (5×10^4 spores/ml) for flower inoculation, there were significant differences among the tested cultivars. Fortes (Pathologist, Embrapa Temperate Climate, personal comunication) found 'Bolinha' flowers susceptible to blossom blight after 72 hours of the inoculation with *M. fructicola*, using a concentration of 1.0×10^5 spores/ ml.

The results of the progenies studies are shown in Table 2. There were differences among progenies was well as years tested. The progeny obtained by crossing selection Conserva 672 x 'Leonense' showed the low-

Table 2. Evaluation of flower infection by *M. fructicola* and estimates of broad sense heritability (H) in seven peach progenies in 2001 and 2002 at Embrapa Temperate Climate, Pelotas, RS, in Brazil.

Cross	Number of Seedlings		Flowers with brown rot (%)*		Broad sense heritability (H)**	
	2001	2002	2001	2002	2001	2002
Cons. 672 x 'Maciel'	38	89	59.6 d	60.6 e	0.56	0.56
Cons. 672 x A.334	32	85	40.7 f	65.9 d	0.25	0.39
Cons. 672 x 'Leonense'	21	37	33.9 g	67.9 b	0.26	0.14
Cons. 672 x 'Eldorado'	27	28	75.3 b	75.8 a	0.06	0.26
'Leonense' x Bolinha'	22	24	61.7 c	56.9 f	0.41	0.53
'Mango' x 'Leonense'	20	7	44.7 e	66.1 c	0.34	0.66
'Bolinha' x 'Leonense'	19	-	82.6 a	-	0.22	-
Total	179	270	-	-	-	-
Mean	-	-	-	-	0.30	0.42

* Flowers were inoculated with a 5 x 10^4 spores/ml suspension of *M. fructicola*.
** H was calculated using for the environment variance the average variation of all the parent plants.
*** Means followed by the same letters in columns do not differ by Duncan's Multiple Range Test at 5% probability.

est infection level in 2001; however the same was not true in 2002. Progenies of selection Conserva 672 x 'Maciel' and 'Leonense' x 'Bolinha' were fairly good in both years while selection Conserva 672 x 'Eldorado' showed a high brown rot incidence in both years.

The average values of H estimated for blossom blight, in two years for the studied populations were low (H = 0.30 and 0.42), suggesting that the environmental influence is quite large.

The estimated values for the broad sense heritability were variable among progenies; however, the cross of selection Conserva 672 x 'Maciel' had consistent and high broad sense heritability (H=0.56) in both years. According to Nunes (8) the heritability value should be considered as referring to a certain population under specific conditions.

The results for the average broad sense heritability in the studied progenies differ in 2002 from the ones obtained in 2001 (Wilcoxon test). These differences could be accounted to the small number of populations studied.

Some genotypes (plants number 25, 35, 59, 85 of the cross Conserva 672 x 'Maciel'; plants 1, 9, 28 of 'Magno' x 'Leonense'; plant 33 of 'Leonense' x 'Bolinha'; plants 62, 98 of Conserva 672 x A.334; and plant 35 of Conserva 672 x 'Leonense') had good levels of resistance, with percentage of flowers infected equal or less than 10% (Table 3).

Comparison between the parent means with its progeny means showed no significant differences, suggesting that the resistance is a poligenic character and it is an additive type of inheritance. The only exception was in the progeny from a cross of 'Bolinha' x

Table 3. Genotypes with high levels of resistance to *Monilinia fructicola* (flower infection ≤ 10%) in flowers of five peach progenies in 2001 and three progenies in 2002 at Embrapa Clima Temperado, Pelotas, RS, Brazil.

Cross	Plant identification number	Average flower infection (%)*	
		2001	2002
Cons. 672 x 'Maciel'	25	9.1	---**
Cons. 672 x 'Maciel'	35	---	4.3
Cons. 672 x 'Maciel'	59	---	9.1
Cons. 672 x 'Maciel'	85	---	10.0
Cons. 672 x A.334	62	11.5	3.1
Cons. 672 x A.334	98	0	---
Cons. 672 x 'Leonense'	35	0	---
'Leonense' x 'Bolinha'	33	7.7	---
'Magno' x 'Leonense'	01	9.1	---
'Magno' x 'Leonense'	09	7.1	---
'Magno' x 'Leonense'	28	6.7	---

* Flowers were inoculated by spraying a 5 x 10^4 spores/ml suspension of *M. fructicola*.
** Not tested. Scarce and non uniform blooming.

'Leonense', in 2001 (the progeny of this cross was not tested in 2002). Renaud (9) stated that the flower susceptibility to *Monilinia laxa* was a dominant character. However, considering all the progenies tested in this work, it seems that the inheritance of flower susceptibility to *M. fructicola* does not have this pattern. In apricot, the resistance to *M. laxa* seems to be poligenic too (4).

In all the progenies it was possible to find individuals more susceptible and/or more resistant than either of the parents. The frequency distributions of the studied progenies are shown in Figures 1 to 13. There is one parent missing in Figures 1, 2 and 9 because cv.Maciel bloomed very early in 2001 and A.334 is not at Embrapa's collection (pollen was received from the University of Arkansas).

It is interesting to observe the significant numbers of individuals more resistant than the female parent (Figures 2 and 9). A similar situation was obtained with the progeny of selection Conserva 672 x c. 'Maciel' (Figures 1 and 8).

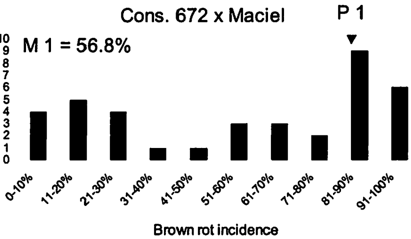
Conclusions

The cvs. Magno and Leonense showed the lowest percentages of blossom blight by *Monilinia fructicola* among the tested cultivars.

At least eleven seedlings among 179 tested in 2001 and 270 tested in 2002, had very low incidences of flower infection. However, further studies are needed before definite conclusions are made on the resistance to brown rot among these seedlings.

The broad sense heritability in 2001 and 2002 was low for the studied populations (30% and 42 % respectively) and highly variable from one progeny to another. However, it seems that it is possible to achieve higher resistance in some individuals even originating from moderately susceptible parents.

Figures 1 to 7. Frequency distribution of individuals of the studied progenies in classes, according to the percentage of flower infection by *Monilinia fructicola* after inoculation at Pelotas, RS, Brazil, in 2001 (where P1 = female parent and P2 = male parent; M1 and M2 = % average infection of the progeny and parents, respectively).



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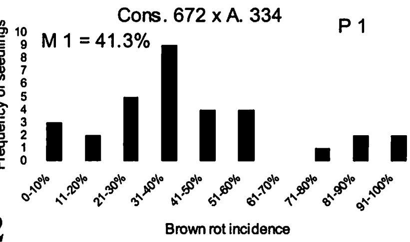

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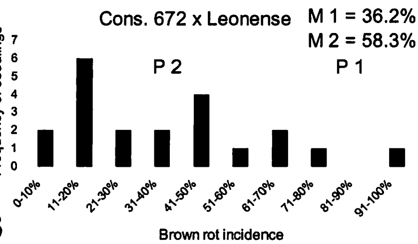

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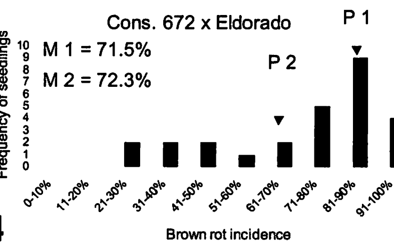

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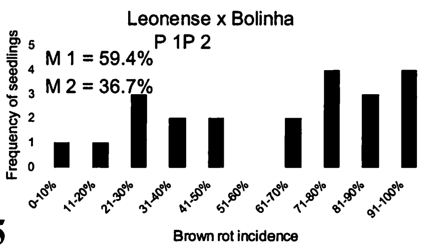

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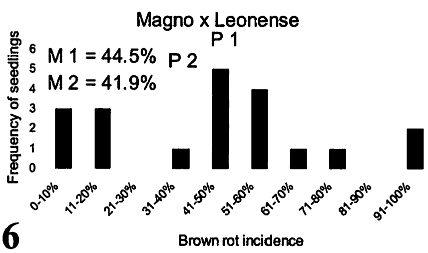

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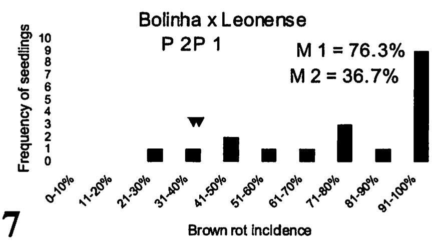

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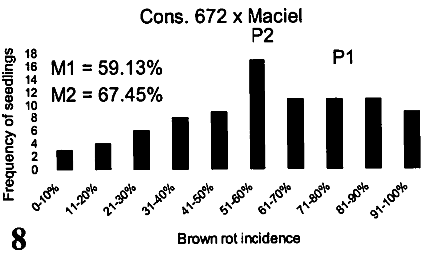
Figures 8 to 13. Frequency distribution of individuals of the studied progenies in classes, according to the percentage of flower infection by *Monilinia fructicola* after inoculation at Pelotas, RS, Brazil, in 2002 (where P1 = female parent and P2 = male parent; M1 and M2= % average infection of the progeny and parents, respectively).



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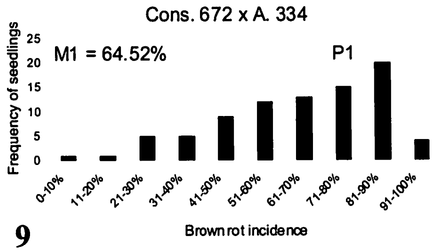

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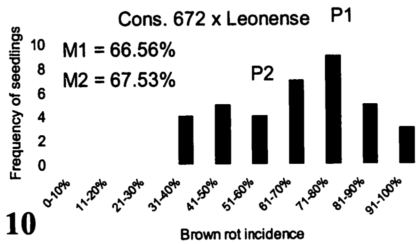

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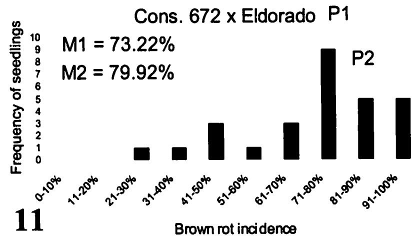

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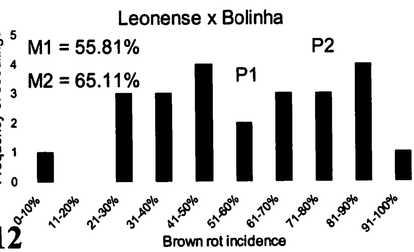

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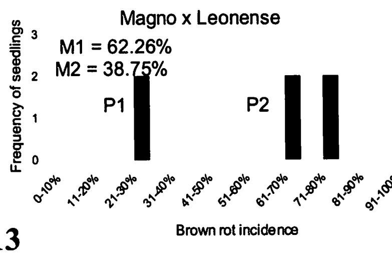

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