SUMMARY

Brown rot, caused by Monilinia fructicola, is the most important peach disease in Brazil. However, there is little information about the epidemiology and spatial patterns associated with this disease in organic orchards. This study characterized the spatial distribution of blossom blight and brown rot within trees under natural infection. The study was carried out in 2007 and 2008 in an organic orchard located in southern Brazil. The spatial pattern of diseased flowers (in a sample of 80 flowers per tree per year) and fruit (from 64 to 309 fruit per tree per year) within each peach tree was assessed using 16 quadrats per tree. The dispersion index (D) and the modified Taylor’s power law were used to characterize the spatial pattern of the disease. The conidia density of M. fructicola and the weather conditions were monitored. In both years of assessment, diseased flowers were distributed at random in 90% of the trees, and no correlation was found between the incidence of blossom blight from a given year and the incidence of blossom blight or brown rot in the previous year. The distribution of brown rot within the tree was aggregated in both years in 70% of the trees where disease incidence was lower than 90%, and a significant positive relationship was observed in disease incidence from one year to the other. Taylor’s power law was significant for blossom blight and brown rot but aggregation was verified only for brown rot. According to these results, blossom blight is not an important source of inoculum to fruits, and we suggest that the intensive chemical control during flowering in the subtropical regions of Brazil should be reviewed.

Key words: epidemiology, Prunus persicae, spatial distribution, aggregation.

INTRODUCTION

Brown rot, caused by Monilinia fructicola (G. Winter) Honey, is the most important peach disease in Brazil (May De Mio et al., 2008). Blossoms and fruits are susceptible to infection (Byrde and Willetts, 1977). Symptoms of the disease appear initially on the blossoms, which are covered with the grayish conidia of the fungus. At the base of infected flowers, small cankers can develop on the twigs around the flower stem; sometimes, the cankers encircle the twigs and cause twig blight (Agrios, 2005). Fruit symptoms appear when they approach maturity as small, circular, brown spots that rapidly increase and cover the entire fruit surface. Afterwards, fruits become completely rotted and dry up into a mummy (Agrios, 2005). Apothecia can develop from fruit mummies partially buried in the soil or debris on the orchard floor (Ogawa et al., 1995). In temperate regions, the inoculum from sexual reproduction has an important role in starting epidemics (Byrde and Willetts, 1977), but in Brazil, the sexual stage of M. fructicola is rare (Martins et al., 2005). In this country, the inoculum for primary infection probably comes from mummified fruit attached to trees from the same orchard or from neighbouring orchards. It is also possible that the cankers produced on twigs in the previous year contribute to the primary inoculum in the next year as described Everhart et al. (2011) for cherry trees.

Organic production systems represent 1.75% of Brazilian agriculture, but the domestic market for organic products in Brazil is continuously increasing. Only 4.4% of the area given over to organic agriculture is occupied by fruit and vegetables (IBGE, 2006). One of the main problems in organic peach production is the control of diseases and pests. In the southern region of Brazil, where the amount of rain is usually high during development of the fruit, the average brown rot incidence from 2005 to 2008 in organic orchards ranged from 20 to 98% (Negri et al., 2011; Keske et al., 2011). In organic systems, brown-rot control is based on winter treatments with lime sulfur or Bordeaux mixture, sanitation measures, such as the removal of diseased twigs, branches and fruit during pruning operations, and fruit protection, by paper bagging each fruit indi-
vidually (May De Mio et al., 2008). The most important sanitation measures to peach brown rot is the removal of mummified fruit (Byrde and Willets, 1977). This technique is important to suppress the primary inoculum produced inside the orchard, and bagging the fruit immediately after thinning is important to protect from the inoculum produced inside and outside the area (Monteiro et al., 2004).

Knowledge of pathogen dispersal patterns is essential for a better understanding of disease increase at a site and its subsequent spread (Mundt, 2009). Important in this regard are the concept of autoinfection, where the inoculum is produced inside an area, and alloinfection, where the inoculum comes from outside the area (Robinson, 1987). The relative importance of the origin of the primary inoculum, i.e. whether from inside or outside a peach orchard, can be partially inferred from the spatial pattern of diseased fruits in the trees. The level of aggregation of diseased fruits in the canopy under similar environmental conditions is dependent on the distance of the inoculum source and the pathogen dispersal mechanism. A high level of disease aggregation occurs when the pathogen is dispersed only over short distances (Madden and Boudreau, 1997; Ntahimpera et al., 1999). Splash-borne spores produce a much steeper dispersal gradient than wind-borne spores (McCartney et al., 2006), leading to a higher level of disease aggregation.

On tree cankers, M. fructicola produces conidio- phores bearing chains of conidia that are windblown, but may also be carried to susceptible parts of the tree by rainwater splashes that disperse them over short distances. Turbulence causes individual spores released from the same sources under the same wind conditions to follow different paths and travel different distances (McCartney et al., 2006). The probability that conidia dispersed by wind over long distances cause clustered diseased flowers or fruits in the branches of a tree is low, as reported by Spósito et al. (2008) for citrus black spot. Therefore, if diseased flowers or diseased fruit are randomly distributed within the tree canopy, external sources of inoculum are possibly more important than the internal ones. On the other hand, when fruits or flowers show an aggregated pattern, the internal inoculum sources, i.e. mummified fruits or cankers, predominate (Xu et al., 2001). As a consequence, sanitation measures should be reinforced to control the disease.

The objective of this work was to characterize the spatial pattern of blossom blight and peach brown rot within the trees in an organic orchard to determine the relative importance of inoculum for autoinfection or alloinfection.

**MATERIALS AND METHODS**

**Experimental design.** The experiment was carried out in 2007 and 2008 in a peach orchard with organic management. The trees of the early cv. Aurora were 10-year-old, trained in an open vase shape (four main scaffolds) and planted at a distance of 6.5 m between rows and 5 m along the row. The orchard was located in Brazil, at latitude 27° 11’ 07” S and longitude 49° 39’ 39” W. Rainfall (mm/day), relative humidity and temperature (°C) were recorded daily using a rain gauge and a thermohygrometer placed in the orchard.

Tree management followed the standard organic commercial practices, except that there were no fungicide treatments during the growing seasons. Winter treatments consisted of one spray of 2% Bordeaux mixture and two sprays of lime sulfur at 8 and 4% (from a stock solution of 32° Baume) and were applied in July during dormancy. Winter pruning was performed in July of each season, and thinning when the fruits were about 2 cm in diameter. The remaining fruits were maintained 8 to 10 cm from each other on the twigs. Fruit fly (Anastrepha fraterculus) was controlled with plastic traps containing orange juice at 50% dilution (2 litres) hung in every tree of the experiment. Peach moth (Grapholita molesta) was controlled by mating disruption using 80 dispensers with pheromones (Biolita, Bio Controle, Brazil) in the orchard.

**Disease assessment.** The spatial pattern of the disease in 10 trees randomly taken in the orchard was assessed at bloom, harvest and post-harvest in 2007 and 2008. Sixteen quadrats (0.5 × 0.5 m) were distributed in each tree with 4 quadrats per scaffold from the base to the top of the tree (0.5, 1.0, 1.5 and 2.0 m). A quadrat is defined as a square sampling unit of a predetermined size (Campbell and Madden, 1990).

Five opened flowers per quadrat (800 flowers per year) were collected from the orchard and brought to the laboratory in each sampling year. The flowers were placed in a gerbox-type plastic box (11 × 11 × 3.5 cm) lined with sterilized filter paper and humidified with 10 ml of sterile water, and incubated at 25°C in the dark for 3 days and at 4°C for 3 additional days (Luo and Michalides, 2001). Blossom blight incidence was quantified by observing the appearance of the pathogen sporodochia on the flowers.

The incidence of brown rot at harvest was determined by recording the number of rotten fruits per quadrat. The number of fruits per quadrat ranged from 4 to 19 in 2007 and 11 to 17 in 2008. At this time, undamaged and disease-free fruits were stored at room temperature (25±2°C) for 4 days when the disease was assessed. All fruits in each quadrat were assessed. The sum of diseased fruits at harvest and post-harvest was used to calculate the percentage of disease incidence in each quadrat. Disease occurrence was assessed in 3,041 and 3,861 fruits obtained from 10 trees naturally infected in 2007 and 2008, respectively.
Conidial density. Six wind catcher spore traps, made of vaseline-coated microscope glass slides placed vertically inside an open cylinder that moves with the wind direction, were placed at a height of 1.5 m from the ground, corresponding to the middle of the canopy. The slides were replaced weekly. For each slide, an area of 25 mm^2 was scanned with a microscope (400X), and the total number of *M. fructicola* conidia was counted. The conidia concentration in the air was calculated weekly from July (flowering) to November (harvest) of each year.

Data analysis. The data of disease incidence were used to estimate the binomial dispersion index: \( D = \frac{s^2}{p(1-p)/n} \), where \( s^2 \) is the observed variance (\( V_{\text{obs}} \)) among quadrats, \( p(1-p)/n \) is the variance of the binomial distribution (\( V_{\text{bin}} \)), \( n \) represents the number of flowers or fruit per quadrat and \( p \) represents the incidence of diseased flowers or fruits in the tree (Hughes et al., 1996). A \( \chi^2 \) test with \( N-1 = 15 \) degrees of freedom was applied to examine if the calculated \( D \) value was greater than 1 at a significance level of 5%. A large \( D (>1) \) combined with a small \( P (<0.05) \) suggests aggregation of symptomatic flowers or fruit. When \( D \) is not significantly different from 1, symptomatic flowers or fruits are distributed at random in all quadrats in the canopy (Madden and Hughes, 1995). Correlation analysis was made between \( D \) values and disease incidence in flowers and fruits.

The binary form of Taylor’s power law (Madden and Hughes, 1995) relates the observed variance (\( V_{\text{obs}} \)) and the expected binomial variance (\( V_{\text{bin}} \)) for a random distribution of binary data. In this case, the two variances are related by \( \log(V_{\text{obs}}) = \log(A) + b\log(V_{\text{bin}}) \), where \( A \) and \( b \) are parameters. Linear regression analysis was performed for all plots using the least-squares method of the Statistica software (version 8.0; Statsoft, USA). The significance of the relationship between \( \log(V_{\text{obs}}) \) and \( \log(V_{\text{bin}}) \) was determined by the F-test, and the appropriateness of the model was evaluated by the coefficient of determination (\( R^2 \)) and examination of the pattern of the residuals of regression; the data set was pooled for both years. A random spatial distribution of symptomatic flowers or fruits is inferred when \( b = A = 1 \). There is a constant level of aggregation for all incidence values when \( b = 1 \) and \( A > 1 \). When \( b > 1 \), the degree of aggregation varies according to the incidence (Madden and Hughes, 1995). The hypothesis of aggregation \( A > 1 \) and \( b > 1 \) was tested by a one-sided \( t \)-test using the estimated parameters and their standard deviations (Campbell and Madden, 1990).

The relationship between blossom blight and brown-rot incidence within each year and between brown-rot incidences in 2007 and 2008 on a quadrat basis were determined by linear regression, using software Statistica 8.0, which was also used for correlation analysis of the relationship between environmental variables and conidial density.

**RESULTS**

The incidences of blossom blight in 2008 and brown rot in 2007 and 2008 were high, but a high variation was observed between the trees and years (Table 1). The mean blossom blight incidences were 1.5 and 16% and ranged from 0 to 6.3% and from 0 to 33.8% in 2007 and 2008, respectively. The mean brown-rot incidences were 55.5% and 72.3% and ranged from 11.3% to

<table>
<thead>
<tr>
<th>Tree</th>
<th>blossom blight incidence (%)</th>
<th>D</th>
<th>brown rot incidence (%)</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>0.00</td>
<td>302</td>
<td>2.2*</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>0.00</td>
<td>309</td>
<td>11.3</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>0.00</td>
<td>198</td>
<td>43.1</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.00</td>
<td>183</td>
<td>45.9</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>2.5</td>
<td>117</td>
<td>62.9</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>0.00</td>
<td>92</td>
<td>90.2</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>6.3</td>
<td>221</td>
<td>80.5</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>1.3</td>
<td>215</td>
<td>85.6</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>1.3</td>
<td>66</td>
<td>98.5</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>3.6</td>
<td>191</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Mean \( \text{incidence} \) \( 1.5 \) a \( 55.5 \) A \( 16.0 \) b \( 72.3 \) A

\( ^{x} \) N is the sample size (5 flowers in 16 quadrats/tree).

\( ^{y} \) N is the number of peaches collected per tree for a total of 16 quadrats.

\( ^{z} \) Incidence of brown rot (%) in peaches sampled in 16 quadrats/tree. The sum of fruit with brown-rot symptoms at harvest and after 4 days of incubation was considered.

\( ^{a} \) Numbers followed by * indicate that \( D \) is significantly different from 1 by qui-square test (p< 0.05)

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Table 1. Blossom blight and brown-rot incidence in peach tree and the dispersion index (\( D \)) of the diseases within the tree in an organic orchard in south Brazil in two consecutive seasons: 2007 and 2008.
Spatial pattern of peach brown rot

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98.5% and from 48.5% to 94.1% in 2007 and 2008, respectively (Table 1).

Symptomatic flowers were randomly distributed within the trees in 2007 (D from 0 to 1) in all trees. In 2008, two of the ten trees showed an aggregation of flowers with symptoms with a D of 2.3 and 2.4 (Table 1). There was a significant correlation between D and blossom blight incidence when the data of both years were pooled (P≤0.05). The index of dispersion of diseased fruits ranged from 1 to 2.8 in 2007 and from 1.1 to 5.6 in 2008. In 2007, aggregation of diseased fruits was detected in 40% of the trees, while in 2008, aggregation was detected in 70%. However, in both years, the index of dispersion in two trees (9 and 10 in Table 1) indicated a random pattern of diseased fruits because disease incidence was approximately 90% (Table 1).

The regression analysis between the logarithm of binomial variance and observed variance for blossom blight was significant (P=2.17E-06), and the equation parameters were not different from 1, indicating a random pattern (Fig. 1A). For brown rot, the linear regression between the logarithm of binomial variance and observed variance was significant (P=1.13E-07). The slope was different from 1 but the logarithm of the intercept was not significantly different from 1, indicating that the aggregation of diseased fruits increases with the disease incidence (Fig. 1B).

There was a positive and significant relationship (P=4.9E-09) between brown-rot incidence in 2007 and in 2008, on a quadrat basis, but no relationship was observed between blossom blight and brown-rot incidence within each year or between brown rot in 2007 and

Fig. 1. Relationship between the logarithm of the theoretical variance of the binomial distribution (random) and the logarithm of the observed variance of the incidence of blossom blight (y=1.21x+0.46, R²=0.90) (A) and brown rot (y=1.38x+1.04, R²=0.81) (B), caused by Monilinia fructicola. The dashed line represents the binomial line (i.e., observed variance=theoretical binomial variance), and the solid line represents the linear regression line fitted to the data collected in ten plants in an orchard in Brazil. The analysis was performed with data from 2007 (closed circles) and 2008 (open circles) pooled together.

Fig. 2. The spore density (number of conidia/25mm²/week) of Monilinia fructicola collected in a wind catcher spore trap from blooming to harvest in an organic orchard in two consecutive seasons.
blossom blight in 2008 (data not shown).

The conidia density averaged 0.5 and 2.2 and ranged from 0 to 2.2 and from 0 to 12.8 conidia/25 mm²/week in 2007 and 2008, respectively. During the 2007 growing season, a low amount of conidia was observed. Two peaks were detected in September and November 2007. In 2008, the amount of trapped conidia increased from October (pre-harvest) to November (harvest) (Fig. 2). The amount of rain registered in the orchard in 2007 and 2008 was higher than the historical mean (Table 2). The number of rainy days was normal during the growing season in 2007 and increased from August to November in 2008. The temperatures during the growing seasons were in line with the historical mean (Table 2).

No relationship was observed between environmental variables and conidial density in the two years of assessment.

**DISCUSSION**

Based on our results, blossom blight in organic orchards seems to be largely a consequence of alloinfection *sensu* Robinson (1987). In both years of assessment, diseased flowers were distributed at random, and no correlation was established between the incidence of blossom blight from one year and the incidence of blossom blight or brown rot in the previous year on a quadrat basis. This behavior was not observed for brown rot. Autoinfection is likely to be the case in the fruits because aggregation of diseased fruits was observed in both years, and a significant positive relationship was observed between disease incidence from one year and the other.

According to Everhart *et al.* (2011), most of the blossom blight symptoms of sour cherry are due to the current year infections (alloinfection) for a three-dimensional analysis. In the majority of the cases, blossom blight symptoms were not aggregated within the tree. Differently from Everhart *et al.* (2011), who assessed blossom-blight symptoms in the trees, in our work the assessment was made on detached apparently healthy flowers induced to sporulate under favourable conditions. The distribution of diseased flowers was similar, regardless of the type of assessment (latent infection or not), suggesting that the primary inoculum originated outside the tree and was probably wind-dispersed.

The inocula produced on flowers were not related to brown rot infection, in accordance with Wilcox (1989), who reported that mummified sour cherry fruits were a more important source of *M. fructicola* inoculum for initiating fruit rot than blossom blight. In peach orchards from South Carolina (USA) blighted peach blossoms were also considered less important inoculum sources for ripening fruit than the aborted or thinned fruit on the ground (Landgraf and Zehr, 1982). A lack of correlation between flower and fruit infection by *M. fructicola* on peaches was also observed by Negri *et al.* (2011). All of these studies suggest that intensive chemical control during flowering, adopted in all peach-producing regions, is excessive or even unnecessary, if a careful removal of twig cankers is done. In the subtropical regions of Brazil, the situation is worse because flowering is uneven and can last for one month depending on the weather. Up to four fungicide sprays are usually applied when plants are blooming. The suppression of these sprays would reduce the cost of control and should be considered by Brazilian growers.

In our study, relatively few conidia were trapped during the blossom period in 2007 and 2008. However, the production of conidia increased sharply in the pre-harvest period. The same behavior was observed in cherry orchards in New York (Wilcox, 1989). The increase in conidia trapped in October in both years could be associated with the sporulation of twig cankers that was favored by the high humidity conditions. According to Everhart *et al.* (2011), it is reasonable to assume that twig cankers formed in the previous year would serve as the most important within-tree inoculum source. As a consequence, the inoculum produced by sporulating

**Table 2.** Number of days with rain, quantity of rain and mean temperature from blossom to harvest of the peach cv. Aurora in the years 2007 and 2008 in an organic orchard in southern Brazil.

<table>
<thead>
<tr>
<th>Month (bloom to harvest)</th>
<th>Number of days with rain (Mean 1995-2003)</th>
<th>Rain (Mean 1995-2003)</th>
<th>Mean temperature (°C) (Mean 1995-2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>9.8</td>
<td>7.0</td>
<td>10.0</td>
</tr>
<tr>
<td>September</td>
<td>11.9</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>October</td>
<td>13.7</td>
<td>11.0</td>
<td>15.0</td>
</tr>
<tr>
<td>November</td>
<td>11.7</td>
<td>11.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Means from 1985 to 2003 were obtained from the Ituporanga meteorological station, located at latitude of 27.22°S, longitude of 49.35°W and an altitude of 475 m.
Two important mechanisms of disease spread in the fruits within a tree are reported in the literature: fruit-to-fruit contact (Michailides and Morgan, 1997) and splash dispersal of conidia (van Leeuwen et al., 2000). Both mechanisms spread the inoculum over short distances, and this process facilitates the aggregation of diseased fruits within the tree. In our study, the level of aggregation of diseased fruits increased with disease incidence, as observed for *M. fructigena* in apple and pear (Xu et al., 2001).

Probably the inoculum for fruit infection is mostly rain-splash dispersed; thus, the proximity of fruits to twig cankers significantly influences disease incidence, which has practical implications for an effective control under organic orchards. Frequent and intensive pruning could help with removal of inoculum sources and contributes to controlling fruit infections. Finally, further trials are needed to assess the effect of the replacement of chemical control for shoot blight during flowering, and the effect of twig canker removal on brown-rot incidence. This management is especially important in organic systems, where the products recommended for disease control are restricted.

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**REFERENCES**


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