Plant Pathology (2014) **63**, 519–529 Doi: 10.1111/ppa.12138

Spatiotemporal characterization of citrus postbloom fruit drop in Brazil and its relationship to pathogen dispersal

G. J. Silva-Junior[ab], M. B. Spósito[a], D. R. Marin[b], P. J. Ribeiro-Junior[c] and L. Amorim[a]*

[a]*Plant Pathology Department, University of Sao Paulo, Piracicaba, SP, 13418-900;* [b]*Research and Development Department, Fundecitrus, Araraquara, SP, 14801-970; and* [c]*Statistics Department, Federal University of Parana, Curitiba, PR, 81531-990, Brazil*

Citrus postbloom fruit drop (PFD) is caused by *Colletotrichum acutatum* and *C. gloeosporioides*. These pathogens attack the flowers and cause premature fruit drop and the retention of fruit calyces. This study was designed to characterize the spatial and temporal dynamics of PFD in commercial citrus-growing areas to better understand the disease spread. Experiments were carried out in three young orchards (500 trees each) in two municipalities in Sao Paulo State, Brazil. Symptoms of PFD on the flowers and presence of persistent calyces were assessed in each of three orchards for three years. Logistic, Gompertz and monomolecular models were fitted to the incidence data over time from the trees with symptoms. The spatial pattern of diseased trees was characterized by a dispersion index and by Taylor's power law. An autologistic model was used for the spatiotemporal analysis. The logistic model provided the best fit to the disease incidence data, which had a fast progress rate of 0·53 per day. During the early epidemic of PFD, the spatial pattern of diseased trees was random, which suggested that inoculum spread was due to mechanisms other than rain splash. As the disease incidence increased (up to 12·6%), the spatial pattern of diseased trees became aggregated. The rapid rate of disease progress and the distribution of PFD suggest that dispersal of the pathogen is possibly related to a mechanism other than splash dispersal, which is more typical of other fruit diseases caused by *Colletotrichum* spp.

Keywords: blossom blight, *Citrus sinensis*, *Colletotrichum acutatum*, logistic model, spread of inoculum

Introduction

Postbloom fruit drop (PFD), caused by *Colletotrichum acutatum* (Brown *et al.*, 1996; Peres *et al.*, 2005) and *C. gloeosporioides* (Lima *et al.*, 2011; McGovern *et al.*, 2012), is an important disease of citrus in São Paulo State, where 80% of Brazilian citrus production occurs (Neves *et al.*, 2010). PFD is known to affect flowers and leads to abscission of fruitlets. When the disease incidence is high, fruit drop may reach staggering proportions. This phenomenon was observed in South Brazil in the 1970s when the production of sweet orange was reduced by 95% (Porto, 1993). Although PFD symptoms have been reported in Central America since the 1950s, the cause was not determined until the late 1970s (Fagan, 1979). In Brazil, PFD was first reported in 1979 (Porto, 1993), and in Florida in 1983 (McMillan & Timmer, 1989). It is established that PFD is caused by *C. acutatum*. However, *C. gloeosporioides* isolates have recently been found associated with PFD symptoms in Brazil (Lima *et al.*, 2011) and Bermuda (McGovern *et al.*, 2012). These pathogens cause salmon- to brown-coloured elliptical lesions on petals that expand rapidly over petal tissue in favourable weather, causing blossom blight. Most of the fruitlets formed on PFD-infected branches abort prematurely, hence the name postbloom fruit drop. The premature fruit drop that is caused by PFD is also characterized by retention of the peduncles and calyces on the plant. However, the correlation between symptoms in flowers and retention of calyces is not always significant (Timmer & Zitko, 1993). The symptoms caused by both pathogens are indistinguishable and there is no evidence of dispersal differences between *C. acutatum* and *C. gloeosporioides*. In strawberry, these two *Colletotrichum* species were dispersed by splash in a similar manner under controlled conditions (Ntahimpera *et al.*, 1999).

The pathogen survives in the absence of citrus flowers as appressoria on symptomless vegetative tissue (Zulfiqar *et al.*, 1996) and on weeds (Frare & Amorim, 2012). However, under field conditions, attempts to detect the pathogen on symptomless tissue or on weeds by any method, including molecular diagnostic tools, have failed (G. J. Silva-Junior, Universidade de Sao Paulo, Piracicaba, Brazil, unpublished data). On diseased citrus petals, the pathogen forms acervuli and produces abundant conidia that are protected by a mucilaginous matrix. These conidia are dispersed to healthy flowers by rain splash (Timmer *et al.*, 1994; Peres *et al.*, 2005). The pathogen infects petals intra- and intercellularly and also through the stomata. Distinct tissues of the petals are colonized, including the vascular system, particularly the xylem (Marques *et al.*, 2013).

*E-mail: lilian.amorim@usp.br

Published online 19 October 2013

Plant Pathology

In most citrus-growing areas, PFD epidemics are sporadic. The disease occurs suddenly, even in areas without previous PFD epidemics (Timmer *et al.*, 1994). PFD outbreaks were reported in Florida in 1988, 1993, 1994 and 1998 (Timmer & Brown, 2000) and in São Paulo in 1977, 1990 and 1993 (Porto, 1993). Disease progress curves were not quantified, but in both regions, disease incidence was associated with above-average rainfall during the flowering period. However, the origin of the primary inoculum and initial spread of the epidemics are unknown. Epidemics with high disease progress rates rarely occur in nature but when they occur, they are mostly associated with pathogens that have a short latent period and the ability to disperse over long distances. Despite a short latent period in citrus flowers, which is *c.* 5 days for cv. Valencia sweet orange (Zulfiqar *et al.*, 1996; Lima *et al.*, 2011), *C. acutatum* is limited by dispersal primarily through rain splash (Fitt *et al.*, 1989; Bailey & Jeger, 1992; Madden, 1997). That a pathogen with such short-distance dispersal capacity can cause such explosive epidemics is controversial. PFD gradients in 3–4-year-old sweet orange orchards in Florida were steep at the beginning of the epidemics, but became shallower as the epidemic progressed, potentially as a result of secondary foci (Agostini *et al.*, 1993). Nonetheless, the incidence of the disease in these experiments did not exceed 30% of flowers. Thus, the explosive nature of the disease was not observed.

The spread of plant diseases is a direct consequence of inoculum dispersal. However, spatial patterns of disease progress may differ from those of inoculum dispersal (McCartney *et al.*, 2006). Disease patterns often result from many individual dispersal events from a number of sources over different time frames. Environmental and biological factors that affect infection and disease development can influence the spatial pattern of diseased plants (McCartney *et al.*, 2006). However, the spatial distribution of plants with and without symptoms is helpful for understanding the disease dispersal mechanisms in different environments (Upton & Fingleton, 1985). The spatial pattern of an infected population is rarely uniform, and the aggregation of diseased plants can be quantified by one or more aggregation indices (Madden & Hughes, 1995).

Currently, PFD is controlled by fungicide sprays during bloom (Goes *et al.*, 2008). In southwestern São Paulo State, many citrus farms contain between 0·5 and 5 million trees (Neves *et al.*, 2010). In these areas, spraying for PFD control must be conducted day and night to achieve the weekly recommended spray frequency. The success of PFD control is associated with the time of spraying and knowledge regarding the disease development during favourable conditions. To optimize PFD control, it is necessary to determine the origin of the primary inoculum and the spatial and temporal disease progress. When the primary inoculum comes from within the orchard, PFD control can be enhanced by spraying eradicant fungicides before blooming. Conversely, if the primary inoculum originates from outside the orchard, control must be performed throughout the blooming period using protective fungicides. The distribution of diseased trees within the orchard also affects the strategy for control of PFD. If diseased trees are aggregated, fungicide sprays can be directed to the foci of disease. If the distribution pattern of PFD affected trees is random, chemical control should be applied evenly throughout the area. However, the origin of the primary inoculum as well as the spatial and temporal progress of PFD under field conditions is unknown. Characterizing the disease progress over time and space is essential for understanding how disease develops in plant populations and how disease control measures affect disease epidemics (Madden *et al.*, 2007). Thus, the goal of this study was to characterize the spatial and temporal progress of PFD epidemics in commercial citrus-growing areas and to infer the pathogen dispersal process. The hypothesis was that short distance (rain splash) dispersal, typical of anthracnose diseases, is predominant in dissemination of the *Colletotrichum* spp. causing PFD. When an aggregated pattern of diseased plants occurs associated with a low disease incidence and the rate of temporal progress is similar to other anthracnose diseases, this hypothesis is accepted. Alternatively, when random patterns of diseased plants prevail and high disease progress rates are observed, long distance or both short and long distance dispersal mechanisms are likely to occur.

Materials and methods

Field trials

Field trials were conducted from 2008 to 2010 in three different orchards located in southwest Sao Paulo State, Brazil. In each orchard, 500 trees that did not receive fungicide sprayings during the flowering period were selected. Surrounding trees were sprayed according to standard farm practice (from one to four sprays per season). The trees were arranged in 20 rows with 25 trees in each row. In field trial 1 (22°49·562′S, 49°22·024′W, 575 m a.s.l.) *Citrus sinensis* cv. Valencia (sweet orange) grafted on cv. Swingle citrumelo (*Citrus paradisi* cv. Duncan × *Poncirus trifoliate*) at a spacing of 3·0 × 7·0 m was planted in April 2006 (Fig. 1). In field trial 2 (22°47·010′S, 49°23·524′W, 645 m a.s.l.) *C. sinensis* cv. Natal grafted on *Citrus limonia* cv. Rangpur (lime) at a spacing of 2·8 × 6·5 m was planted in April 2007. In field trial 3 (23°36·898′S, 49°14·290′W, 661 m a.s.l.) cv. Valencia grafted on cv. Swingle at a spacing of 2·8 × 6·5 m was planted in December 2007. Trial 3 was surrounded by orchards of young citrus.

During the experimental period, the temperature, rainfall and wetness period were recorded by meteorological stations (Davis Hayward), one of them located in trial 1, which was 5·2 km from trial 2, and another located 6·3 km from trial 3.

Disease assessment

In all assessments, flowerless trees, trees bearing only flower buttons, trees with open and symptomless flowers, and trees with flowers with symptoms were counted. The frequency and timing of the assessments varied in each of the three years (Table 1). In each trial, the incidence of persistent calyces was assessed once at 3–4 months after petal fall. Persistent calyces were removed from the trees after assessment.

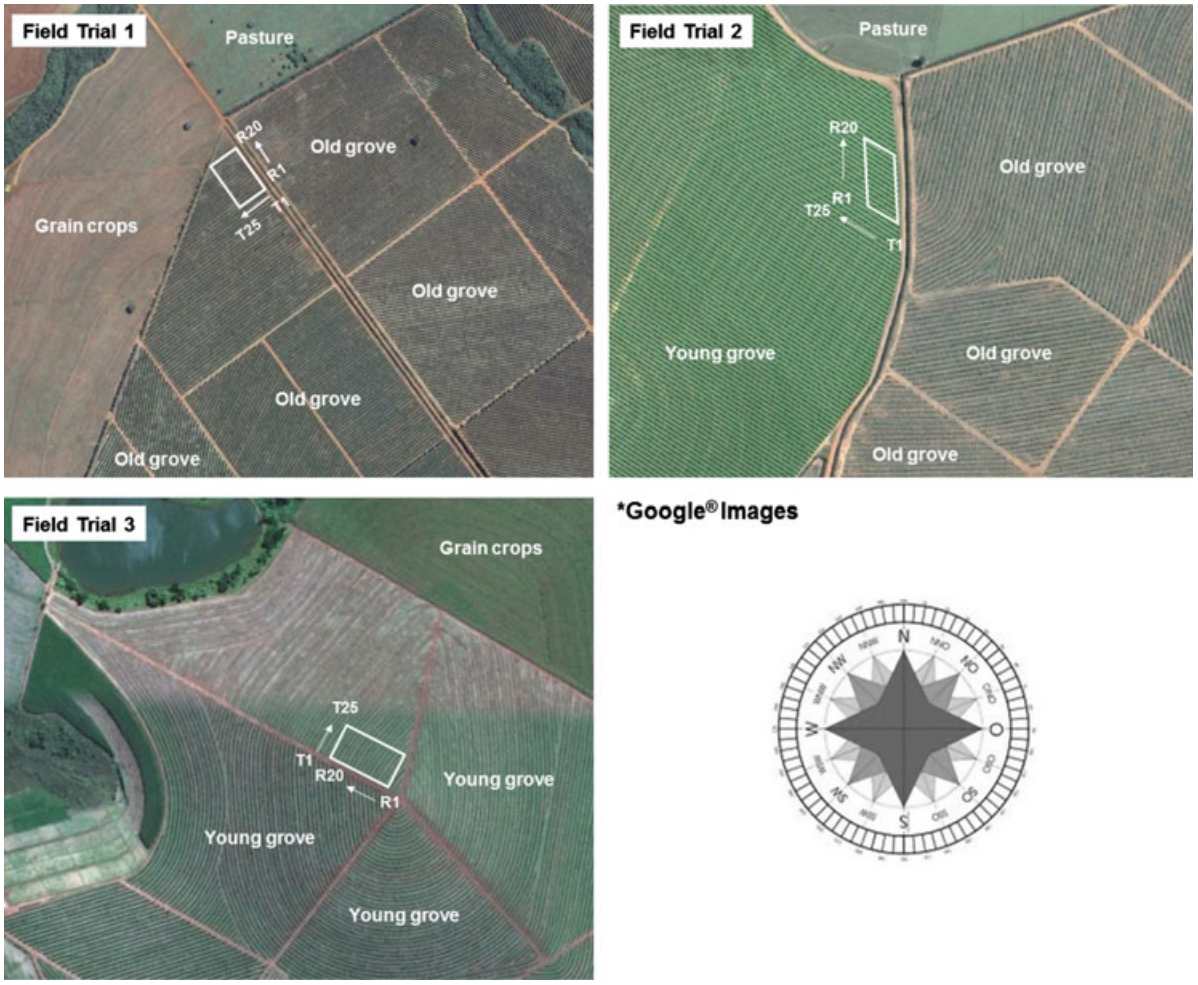

Figure 1 Map of experimental areas. Field trials 1 and 2 were located in the municipality of Santa Cruz do Rio Pardo, SP, and trial 3 was located in Taquarituba, SP. Each field trial was composed of 20 rows (R1 to R20) with 25 trees in each row (T1 to T25).

Table 1 Assessment dates of citrus postbloom fruit drop in three trials from 2008 to 2010

Trial	2008	2009	2010
1	9, 18, 26 Sep 3 Oct	5, 11, 18, 27 Aug 1, 8, 14, 18, 24 Sep	13, 22 Oct 3, 8 Nov
2	8, 17, 25 Sep 2 Oct	12, 28 Aug 9, 15, 21, 25 Sep	5, 11, 16, 23, 31 Aug 13, 22 Sep
3	No flowers[a]	7, 10, 12, 17, 19, 21, 24, 26, 31 Aug 2, 7, 9, 11, 14, 16, 18, 21 Sep	3, 10, 20, 25 Aug 3, 13, 22 Sep

[a]No bloom occurred as a result of the tree age (9 months).

Temporal analysis of epidemics

Logistic $[y(t) = y_{max}/(1 + ((y_{max} - y_0)/y_0)*\exp(-r*t))]$, Gompertz $[y(t) = y_{max}*(\exp(-(-\log(y_0/y_{max}))*\exp(-r*t)))]$ and monomolecular $[y(t) = y_{max} - (y_{max} - y_0)*\exp(-r*t)]$ models were fitted to the disease incidence with non-linear regression analysis using STATISTICA v. 6 (Statsoft). Here, $y(t)$ is the proportion of diseased trees at time t, y_0 is the initial inoculum, r is the disease progress rate, y_{max} is the curve asymptote, and t is the time in days. The best-fit model was selected based on the coefficient of determination (R^2), the distribution of residuals and the standard error of the y_{max}, y_0 and r parameters that were estimated by each model fit (Madden *et al.*, 2007). The equation parameters were compared using a t-test. If the asymptotes differed from 1, the rate parameter was multiplied by the observed asymptote ($r*y_{max}$) to adjust the rate for each of the epidemics (Madden *et al.*, 2007).

The coincidence of trees with symptoms between consecutive years was assessed with a chi-square test ($P = 0{\cdot}05$). This test determined whether a tree with symptoms in a given year showed symptoms in the first assessment of the following season. The test was performed using the incidence data from trial 2 in 2008–2009 and from trial 3 in 2009–2010. Correlation analysis between the number of rainy days and disease incidence in each season was performed.

Spatial analysis of epidemics

Maps showing the cumulative incidence of trees with symptoms (flowers with symptoms and persistent calyces) were made for

each assessment in the three trials. The incidence of trees with PFD symptoms in quadrats of 2 × 2 and 3 × 2 (2 or 3 trees per row × 2 rows) was used to determine the dispersion index ($D = V_{obs}/V_{bin}$, where V_{obs} is the observed variance and V_{bin} is the binomial variance). The quadrat 2 × 2 was chosen to test aggregation under the smallest hierarchical level and the quadrat 3 × 2, to test the aggregation in a square area. Additionally, these quadrat sizes were chosen for comparative purposes as the spatial distribution of other citrus diseases has been analysed using these quadrat sizes (Bergamin Filho *et al.*, 2000; Laranjeira *et al.*, 2004; Bassanezi & Laranjeira, 2007). The modified Taylor's power law [$\log(V_{obs}) = \log(A) + b\ \log(V_{bin})$] (Madden & Hughes, 1995) was also determined for pooled epidemic data. The significance of the dispersion index was determined with a chi-square test ($P = 0{\cdot}05$). D values equal to 1·0 indicated that trees with symptoms for a given assessment date were randomly distributed (null hypothesis). Conversely, D values that were significantly > 1·0 indicated aggregation (Madden *et al.*, 2007). The significance of the relationship between log (V_{obs}) and log(V_{bin}) in Taylor's power law was determined by an F-test ($P = 0{\cdot}05$). The equality of the b parameter to 1·0 was tested with a t-test ($P = 0{\cdot}05$) by using the b parameter and its standard error. Values of b that were > 1·0 (alternative hypothesis) indicated aggregation, which varied with disease incidence. Moreover, $b = 1{\cdot}0$ with $\log(A) > 0$ indicated aggregation of diseased trees, and $b = 1{\cdot}0$ with $\log(A) = 0$ indicated that diseased trees occurred randomly. Values of $b < 1$ indicated regularity (Madden & Hughes, 1995).

Spatiotemporal autologistic analysis

The autologistic model (Besag, 1972) accounted for the spatial structure of the disease by building covariates from the disease status of the neighbouring trees. For this analysis, trees were rated with binary scores as follows: 1 for trees with symptoms (diseased) and 0 for flowerless or symptomless trees (healthy). The autologistic model was adapted by Krainski *et al.* (2008) from the R statistical environment for statistical analysis (R Development Core Team, 2013) to account for the temporal patterns that occur when estimating the probability of disease in a tree at an arbitrary location as a function of the disease status of the neighbouring trees at another time. Comparing a sequence of models with different covariates for disease status within and across rows at current and previous observation times allows detection of the relevant spatial and temporal patterns that were associated with the spread of PFD (Krainski *et al.*, 2008).

Using this model, covariates were built for each assessment date by considering the following: (i) the disease status of the immediate neighbour trees within the row, (ii) in the column (across row), and (iii) in both. For such analysis, the selected models (Table 2) included covariates that were built based on the incidence of PFD in neighbouring trees on a previous date ($t - 1$) for trials 1 and 3 in 2008 and 2009, respectively.

Table 2 Autologistic models used to fit the citrus postbloom fruit drop incidence data

Status of the neighbouring tree	Linear predictor[a]
Within the row in the previous assessment	$\beta_0 + \beta_1 W_{(t-1)}$
Across rows in the previous assessment	$\beta_0 + \beta_1 A_{(t-1)}$

[a]W and A are covariates that denote the status of the neighbouring (healthy = 0 or diseased = 1) trees within and across rows, respectively on the previous ($t - 1$) assessment.

Results

Temporal analysis of epidemics

In 2008, PFD only occurred in trees from trial 1 (Fig. 2a). Four days of rain occurred during the flowering period and 25·2% of the trees had symptoms of PFD. In trial 2, bloom was scarce and no flowers with symptoms were observed (Fig. 2b). In trial 3 no bloom occurred as a result of tree age; they were planted 9 months prior to the disease assessment (Fig. 2c).

In 2009, disease incidence reached 100% in both trials 1 and 2 and there was frequent rain during bloom (Fig. 2d,e). In trial 1, the disease incidence increased from 5 to 73% within 9 days. In trial 2, the disease incidence increased from 13 to 98% within 12 days. In trial 3, one tree with symptoms was observed following the first rainy day. This incidence (0·2% of trees) remained unchanged for the following 20 days, but final disease incidence in trial 3 was 7·0% at the end of the bloom period (Fig. 2f).

In 2010, bloom was delayed in trial 1 because of a prolonged dry season that lasted from July to September. The bloom period started in October and was short as a result of high temperatures (data not shown). The first trees with symptoms were observed after rainy days, and the final disease incidence was 47·2% (Fig. 2g). In this year no disease was detected in trials 2 and 3 (Fig. 2h,i).

In trials 1 and 2, the number of rainy days was positively correlated with the incidence of diseased trees at the end of the blooming period for all seasons ($r = 0{\cdot}99$; $P < 0{\cdot}05$). In trial 3 the correlation between rainy days and PFD incidence was not significant as a result of the late blooming in 2009.

The logistic model showed a better fit to observed data when compared to the Gompertz and monomolecular models for all epidemics (Table 3). The asymptotes were between 0·3 and 0·98 and were significantly higher in 2009 than in the other seasons. The rates of disease increase were high (from 0·22 to 0·55 per day). However, these rates were affected by the variable asymptote. When the rates were scaled the minimum value was 0·07 per day for trial 1 in 2008 and the maximum was 0·53 per day for trial 2 in 2009. In all epidemics, the initial inoculum was lower than 0·001.

There was no significant correlation ($P > 0{\cdot}05$) between the incidence of trees with symptoms at the end of a blooming season and the incidence of trees with symptoms at the beginning of the following blooming period in Trial 3 (Table 4). In trial 2, 30·8% of trees with symptoms that were observed in the final 2008 assessment had symptoms in the first 2009 assessment. The observed number of co-symptomatic trees, eight, is larger than expected from a random selection. Overall, 87·3% of trees that showed symptoms in the first 2009 assessment did not show PFD symptoms in the previous year.

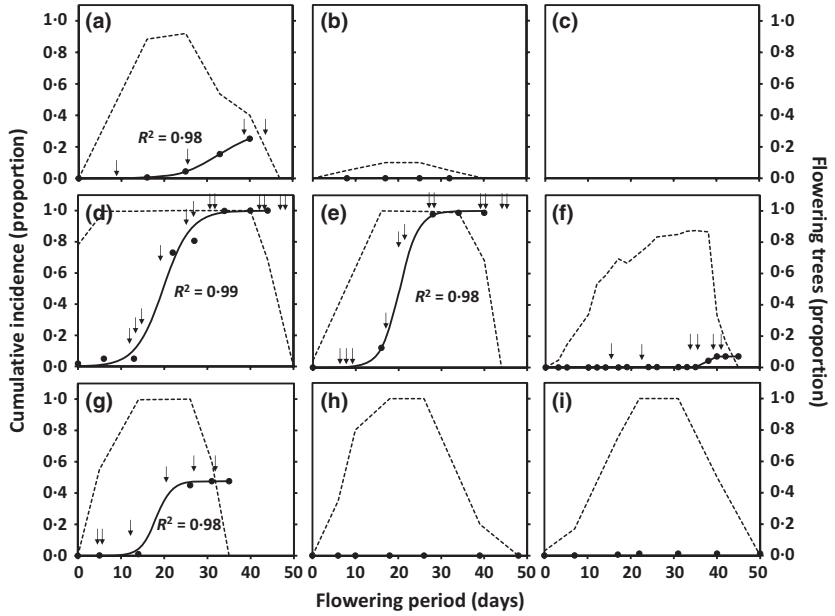

Figure 2 Cumulative PFD incidence (proportion) of trees with flowers with symptoms (dark circles) and proportion of flowering trees (dotted line) in 2008 (a, b, c), 2009 (d, e, f) and 2010 (g, h, i) in trials 1 (a, d, g), 2 (b, e, h) and 3 (c, f, i) in São Paulo State, Brazil. Solid lines indicate the logistic model fitted to the observed incidence of postbloom fruit drop. Arrows indicate rainfall events.

Table 3 Parameters and respective errors estimated by non-linear regression of logistic, Gompertz and monomolecular models fitted to the incidence of trees with symptoms of citrus postbloom fruit drop in field trials 1 and 2 from 2008 to 2010

		Estimated parameters						
Year/trial	Model[a]	R^2	y_{max}	y_{max} SE	y_0	y_0 SE	r	$r.y_{max}$[b]
2008/1	Logistic	0·98	0·30	0·001	<0·001	<0·001	0·22	0·07
	Gompertz	0·97	0·58	–	<0·001	–	0·06	0·03
	Monomolecular	NS						
2009/1	Logistic	0·99	0·97	0·046	0·001	0·002	0·34	0·33
	Gompertz	0·98	1·00	–	<0·001	–	0·17	0·17
	Monomolecular	0·81	1·00	–	<0·0	0·194	0·05	0·05
2009/2	Logistic	0·98	0·98	<0·001	<0·001	<0·001	0·54	0·53
	Gompertz	0·98	1·00	-	<0·001	–	0·26	0·26
	Monomolecular	0·99	0·99	0·006	<0·0	93·14	0·32	0·32
2010/1	Logistic	0·99	0·48	0·001	<0·001	0	0·55	0·26
	Gompertz	0·95	0·48	–	0	–	0·23	0·11
	Monomolecular	0·77	0·48	–	<0·0	0·280	0·07	0·03

[a]Logistic, Gompertz and monomolecular models were fitted to the observed incidence of trees with symptoms of postbloom fruit drop on flowers using $y(t) = y_{max}/(1 + ((y_{max} - y_0)/y_0)*\exp(-r*t))$, $y(t) = y_{max}*(\exp(-(-\log(y_0/y_{max}))*\exp(-r*t)))$ and $y(t) = y_{max} - (y_{max} - y_0)*\exp(-r*t)$ respectively, where y is the proportion of trees with disease, t is time in days, y_{max} is the asymptote, y_0 is the initial inoculum, r is the disease progress rate, R^2 is the coefficient of determination and SE is the standard error of the mean.
[b]Scaled rate, which is the transformed rate based on y_{max} (Madden *et al.*, 2007).

Table 4 Number of postbloom fruit drop citrus trees with and without symptoms in the final assessment in 2008 and first assessment in 2009 for trial 2 and final assessment in 2009 and first assessment in 2010 for trial 3

	Trial 2				Trial 3		
	First assessment in 2009				First assessment in 2010		
Final assessment in 2008	With symptoms	Symptomless	Total	Final assessment in 2009	With symptoms	Symptomless	Total
With symptoms	8	18	26	With symptoms	1	87	88
Symptomless	55	419	474	Symptomless	6	406	412
Total	63	437	500	Total	7	493	500

Spatial analysis of epidemics

The spatial distribution of trees with symptoms on five assessment dates for the three trials is shown in Figures 3, 4 and 5. In all trials, the incidence of trees with persistent calyces was higher than the incidence of trees that showed flowers with symptoms. A random pattern of PFD distribution ($D = 1$) was observed in trial 1 for the three assessed years at the onset of the epidemics (Fig. 3). In this area, disease distribution became aggregated when the incidence of diseased trees reached 25%. In trial 2, PFD was only observed in 2009 and an aggregated pattern of disease distribution existed at all assessment dates when symptoms were present (Fig. 4). In trial

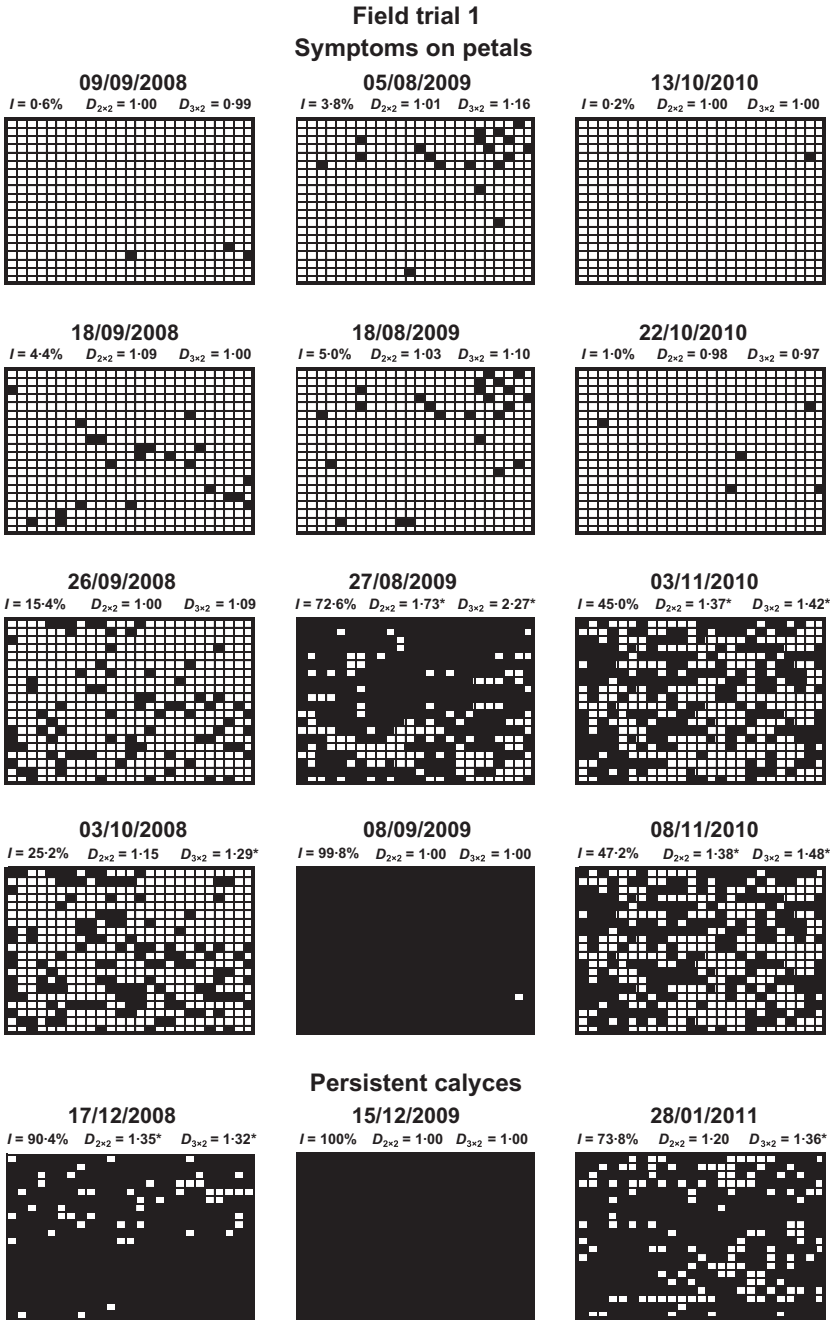

Figure 3 Spatial distribution of trees with postbloom fruit drop (petals with symptoms or persistent calyces) in field trial 1 in Santa Cruz do Rio Pardo, SP, in 2008, 2009 and 2010. Maps show four assessments of petal symptoms during the flowering period and one assessment of persistent calyces after flowering. Black and white quadrants indicate trees with and without (no flowers or no diseased petals) symptoms, respectively. *I* is the incidence of diseased trees (as a percentage), $D_{2\times2}$ and $D_{3\times2}$ is the dispersion index for two quadrat sizes and *indicates the aggregation of diseased trees ($D > 1$).

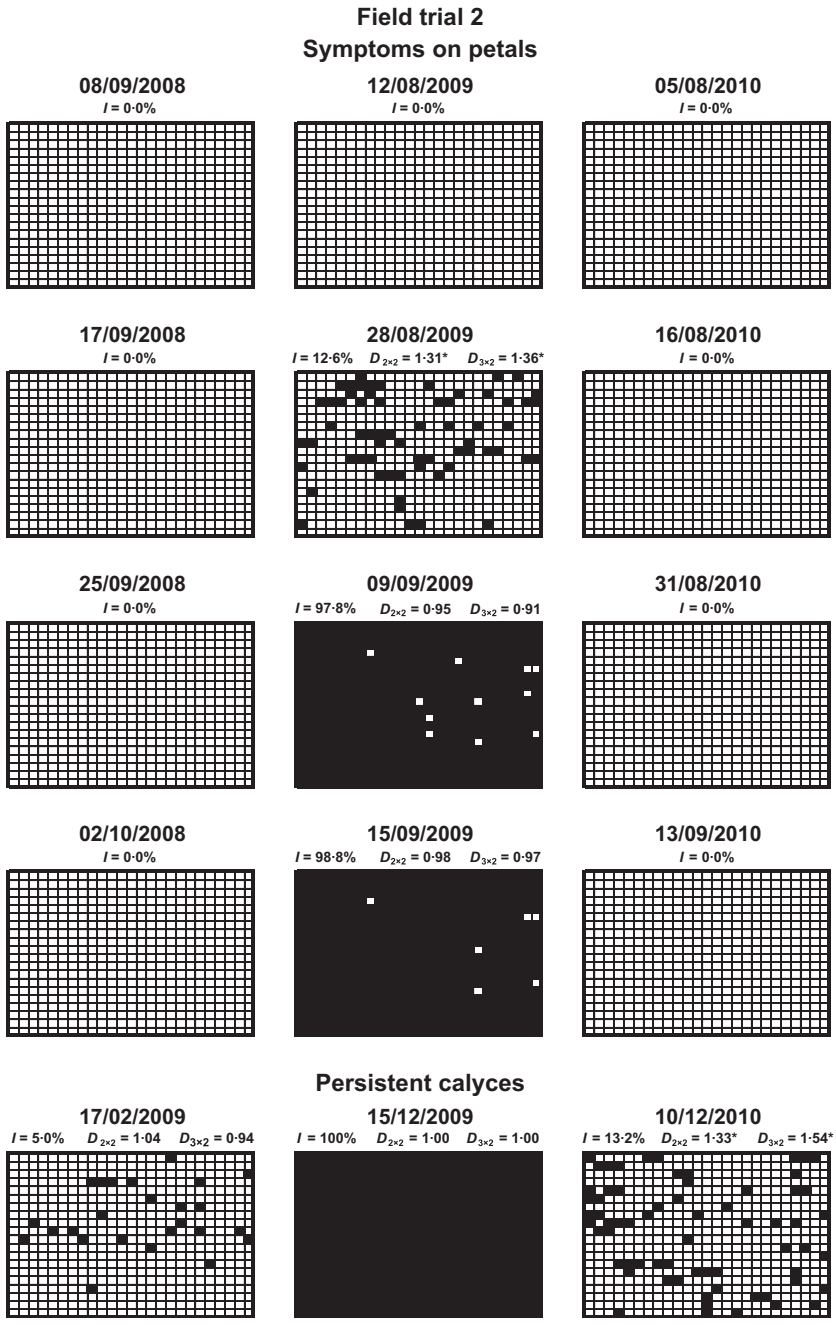

Figure 4 Spatial distribution of trees with postbloom fruit drop (petals with symptoms or persistent calyces) in field trial 2 in Santa Cruz do Rio Pardo, SP, in 2008, 2009 and 2010. Maps show four assessments of petal symptoms during the flowering period and one assessment of persistent calyces after flowering. Black and white quadrants indicate trees with and without symptoms (no flowers or no diseased petals), respectively. *I* is the incidence of diseased trees (as a percentage), $D_{2\times2}$ and $D_{3\times2}$ is the dispersion index for two quadrat sizes and * indicates the aggregation of diseased trees ($D > 1$).

3, where incidence of trees with flowers showing symptoms did not exceed 7%, the distribution of diseased trees was always random (Fig. 5).

The regression parameters of Taylor's power law applied to the pooled data for quadrats 2 × 2 (Fig. 6a) and 3 × 2 (Fig. 6b) indicated aggregation of diseased trees ($\log(A) > 0$ and $b > 1$).

Spatiotemporal autologistic model

The likelihood of a tree becoming diseased relied significantly ($P < 0{\cdot}05$) on the number of neighbouring trees with symptoms within-row in the previous assessment only in the fourth assessment of trial 1 in 2008, when disease incidence was 25·2%. When a given tree was

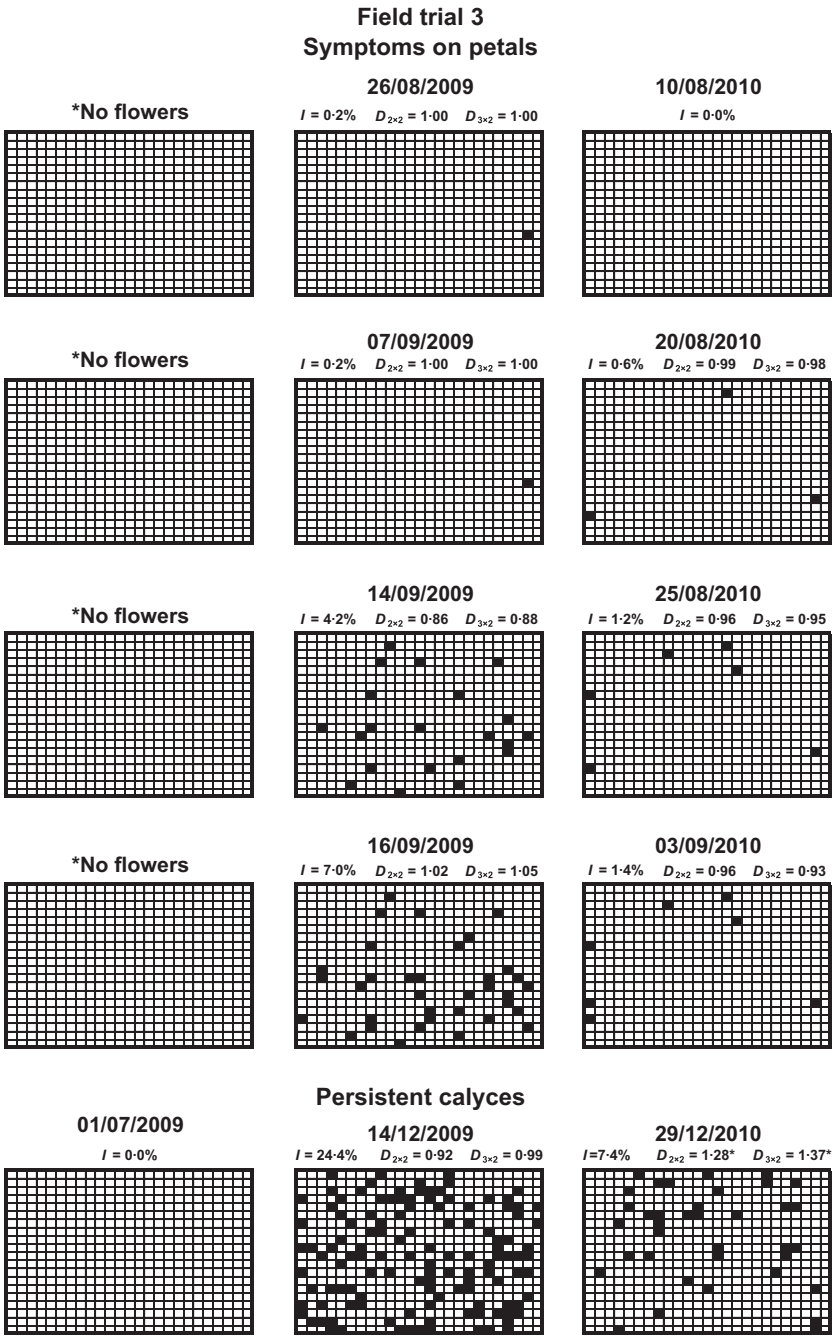

Figure 5 Spatial distribution trees with postbloom fruit drop (petals with symptoms or persistent calyces) in field trial 3 in Taquarituba, SP, in 2008, 2009 and 2010. The maps show four assessments of petal symptoms during the flowering period and one assessment of persistent calyces after flowering. Black and white quadrants indicate trees with and without symptoms (no flowers or no diseased petals), respectively. *I* is the incidence of diseased trees (as a percentage), $D_{2\times2}$ and $D_{3\times2}$ is the dispersion index for two quadrat sizes and *indicates the aggregation of diseased trees ($D > 1$).

bordered by 0, 1 or 2 trees with symptoms in the third assessment, the probabilities of this tree becoming infected in the next assessment were 0·273, 0·512 and 0·746, respectively (Table 5). The distributions of the diseased trees showed no correspondence with neighbouring trees with symptoms across-row in previous assessments (data not shown).

For trial 3 in 2009, the trees with symptoms in the last assessment (7·0% incidence) showed the same likelihood ($P > 0·05$) of being infected irrespective of the presence

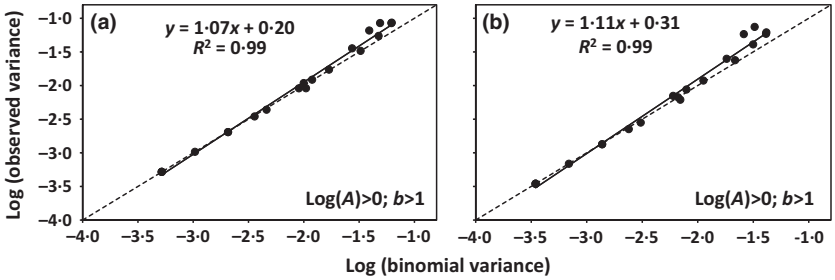

Figure 6 Relationship between the logarithm of the observed variance (V_{obs}) and the logarithm of the binomial variance (V_{bin}) for postbloom fruit drop incidence in sweet orange trees for the pooled data of five orchards/years using quadrat sizes 2 × 2 (a) and 3 × 2 (b). The solid line represents log (V_{obs}) = b log (V_{bin}) + log(A) fitted to the data by linear regression. The dashed line represents the binomial fit.

Table 5 The likelihood of a tree developing symptoms of postbloom fruit drop, as a result of the disease status of the immediate neighbouring trees within a row in the previous assessment in four periods in trial 1, 2008

	Number of neighbouring diseased trees			
Assessment (PFD incidence)	0	1	2	*P*
1 (0·006)	–	–	–	–
2 (0·044)	0·070	0·500	0·930	0·079
3 (0·154)	0·197	0·325	0·486	0·226
4 (0·252)	0·273	0·512	0·746	0·037[a]

[a]Values of $P < 0·05$ indicates significant differences in the probability that a tree develops symptoms as a result of the 0, 1 or 2 immediate neighbour trees within a row being diseased in the previous assessment.

of neighbouring diseased trees in the previous assessment, which showed 4·2% of trees with symptoms of PFD (data not shown).

Discussion

Rapidly developing epidemics of PFD occurred in 2009 in two trials where the disease was observed in the previous year. Frequent consecutive days of rain occurred during the flowering period in 2009. Conversely, in 2008 and 2010 the total rain events were fewer than in 2009 (four and six, respectively). The number of rainy days during the flowering period was correlated to final incidence of PFD. Similarly in Belize, PFD increase was related to prolonged wetness periods accompanied by at least two consecutive days of rain, regardless of rainfall totals (Denham & Waller, 1981). Generally, disease epidemics that are caused by splash-dispersed pathogens, such as *Colletotrichum* spp., are associated with rain events (Madden, 1997). Rapidly developing disease progress curves for anthracnose diseases may occur in areas with a high density of short crops, such as dry beans (Ntahimpera *et al.*, 1997) and yams (Sweetmore *et al.*, 1994). However, in perennial crops such as coffee, progress of anthracnose is slower (Mouen Bedimo *et al.*, 2007). Thus, the occurrence of explosive epidemics caused by *Colletotrichum* spp. in a perennial crop, such as citrus, is uncommon.

In this study, rapidly developing epidemics occurred at first or second blooming when trees were 2 and 3 years' old, respectively. Analysis of the PFD epidemics with the autologistic model and the index of dispersal showed that, for most of the field trials, diseased trees were randomly distributed at the beginning of the epidemic. This suggested that part of the initial inoculum was coming from outside the orchard. Disease spread is the direct consequence of propagule dispersal although spatial patterns of disease may be quite different from the dispersal patterns that cause them (McCartney & Fitt, 1998). However, under homogeneous environmental conditions, the spatial distribution of diseased trees can be related to the dispersal pattern of the inoculum and a random distribution of diseased trees is usually associated with pathogen dispersal over long distances (Burdon *et al.*, 1989). An aggregated distribution of trees with PFD symptoms was observed only when the disease incidence reached 12·6%. Pathogens disseminated over short distances by rain splash generally produce an aggregated pattern of plants with symptoms at the beginning of epidemics; at low disease incidences, the nearest plants to the source of infection are more likely to become diseased (Madden, 1997).

Taylor's power law analysis for both quadrat sizes also suggested that long distance or both short and long distance dispersal mechanisms were occurring during a PFD epidemic. The regression parameter values of Taylor's power law estimated for PFD were similar to those estimated for citrus variegated chlorosis, caused by *Xylella fastidiosa*, a sharpshooter-vectored bacterium (Laranjeira *et al.*, 2004). The PFD parameter values were also similar to those estimated for citrus canker (*Xanthomonas citri* subsp. *citri*) after the introduction of the citrus leaf miner (*Phyllocnistis citrella*) in Brazil, which have altered the spatial pattern of the disease from aggregate to moderately aggregate or even random (Bergamin Filho *et al.*, 2000). In contrast, the PFD parameter values were lower than those observed for citrus leprosis (log(A) = 0·70 and b = 1·23 for 3 × 3 quadrat size), caused by *Citrus leprosis virus* and transmitted by the mite, *Brevipalpus phoenicis*. The vector disseminates the disease around

the primary foci and generates a highly aggregated disease pattern (Bassanezi & Laranjeira, 2007).

Although the monomolecular model has been demonstrated as suitable for describing epidemics with random distribution of primary infection (Madden *et al.*, 1987), the best fit for the PFD progress curves was obtained with the logistic model. In general, the monomolecular model is used to describe monocyclic diseases and the logistic or Gompertz models are used for polycyclic diseases. However, the biological nature of the disease cycle cannot be ascertained from a statistical model that fitted to the data (Madden *et al.*, 1987). In Brazil, the estimated PFD progress rates varied between trials and years, and reached up to 0·53 per day. This rate was 2- and 5-fold greater than the rates observed for yam anthracnose and coffee berry disease, respectively (Sweetmore *et al.*, 1994; Mouen Bedimo *et al.*, 2007). As the main dispersal mechanism for *Colletotrichum* spp. occurs over short distances by splash droplets, the explosive epidemics of PFD could be explained by inoculum dispersal mechanisms over long distances, such as transport by insects or windblown raindrops (Peña & Duncan, 1989; Agostini *et al.*, 1993).

Overall, PFD of citrus shows an epidemiological behaviour that is different to other fruit anthracnose diseases that are predominantly spread through splash-dispersed spores. The distribution of diseased trees and the progress rates with time indicate a high dispersal efficiency that is potentially related to an alternative mechanism for inoculum dissemination. Further studies are needed to determine if insects, such as bees, are responsible for long distance spread of PFD.

Acknowledgements

The authors would like to thank Cia Agroterenas Citrus (J Aprígio Tank Junior and Márcio A. Soares) and Castel Franco (Dr Lourival C. Monaco) for supporting this work and the agencies FAPESP (2008/54176-4 and 2008/54599-2) and CNPq (authors' scholarships) for financial support. They also acknowledge Dr E. T. Krainski, from UFPR, for his contributions on the use of the autologistic model.

References

Agostini JP, Gottwald TR, Timmer LW, 1993. Temporal and spatial dynamics of postbloom fruit drop of citrus in Florida. *Phytopathology* **83**, 485–90.

Bailey JA, Jeger MJ, 1992. *Colletotrichum: Biology, Pathology and Control*. Wallingford, UK: CAB International.

Bassanezi RB, Laranjeira FF, 2007. Spatial patterns of leprosis and its mite vector in commercial citrus groves in Brazil. *Plant Pathology* **56**, 97–106.

Bergamin Filho A, Amorim L, Laranjeira FF, Gottwald TR, 2000. Epidemiology of citrus canker in Brazil with and without the Asian citrus leafminer. In: Dixon WN, Levy L, Gottwald TR, eds. *Proceedings of the International Citrus Canker Research Workshop, 2000*. Fort Pierce, USA: USDA, 6.

Besag JE, 1972. Nearest-neighbour systems and the auto-logistic model for binary data. *Journal of the Royal Statistical Society B* **34**, 75–83.

Brown AE, Sreenivasaprasad S, Timmer LW, 1996. Molecular characterization of slow-growing orange and key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. *Phytopathology* **86**, 523–7.

Burdon JJ, Jarosz AM, Kirby GC, 1989. Pattern and patchiness in plant–pathogen interactions – causes and consequences. *Annual Review of Ecology and Systematics* **20**, 119–36.

Denham TG, Waller JM, 1981. Some epidemiological aspects of post-bloom fruit drop disease (*Colletotrichum gloeosporioides*) in citrus. *Annals of Applied Biology* **98**, 65–77.

Fagan H, 1979. Postbloom fruit drop, a new disease of citrus associated with a form of *Colletotrichum gloeosporioides*. *Annals of Applied Biology* **91**, 13–20.

Fitt BDL, McCartney HA, Walklate PJ, 1989. The role of rain in dispersal of pathogen inoculum. *Annual Review of Phytopathology* **27**, 241–70.

Frare G, Amorim L, 2012. Survival of *Colletotrichum acutatum*, the causal agent of citrus postbloom fruit drop, on weeds. *Phytopathology* **102**, S4.41–2.

Goes A, Garrido RBO, Reis RF, Baldassari RB, Soares MA, 2008. Evaluation of fungicide applications to sweet orange at different flowering stages for control of postbloom fruit drop caused by *Colletotrichum acutatum*. *Crop Protection* **27**, 71–6.

Krainski ET, Ribeiro Junior PJ, Bassanezi RB, Franciscon L, 2008. Autologistic model with an application to the citrus 'sudden death' disease. *Scientia Agricola* **65**, 541–7.

Laranjeira FF, Bergamin Filho A, Amorim L, Gottwald TR, 2004. Dinâmica espacial da clorose variegada dos citros em três regiões do Estado de São Paulo. *Fitopatologia Brasileira* **29**, 56–65.

Lima WG, Spósito MB, Amorim L, Gonçalves FP, Melo Filho PA, 2011. *Colletotrichum gloeosporioides*, a new causal agent of citrus post-bloom fruit drop. *European Journal of Plant Pathology* **131**, 157–65.

Madden LV, 1997. Effects of rain on splash dispersal of fungal pathogens. *Canadian Journal of Plant Pathology* **19**, 225–30.

Madden LV, Hughes G, 1995. Plant disease incidence: distributions, heterogeneity, and temporal analysis. *Annual Review of Phytopathology* **33**, 529–64.

Madden LV, Louie R, Knoke JK, 1987. Temporal and spatial analysis of maize dwarf mosaic epidemics. *Phytopathology* **77**, 148–56.

Madden LV, Hughes G, van den Bosch F, 2007. *The Study of Plant Disease Epidemics*. St Paul, MN, USA: APS Press.

Marques JPR, Amorim L, Spósito MB, Appezzato-da-Glória B, 2013. Histopathology of postbloom fruit drop caused by *Colletotrichum acutatum* in citrus flowers. *European Journal of Plant Pathology* **135**, 783–90.

McCartney HA, Fitt BDL, 1998. Dispersal of foliar fungal plant pathogens: mechanisms, gradients and spatial patterns. In: Jones DG, ed. *The Epidemiology of Plant Diseases*. Dordrecht, The Netherlands: Kluwer, 138–60.

McCartney HA, Fitt BDL, West JS, 2006. Dispersal of foliar plant pathogens: mechanisms, gradients and spatial patterns. In: Cooke BM, Jones DG, Kaye B, eds. *The Epidemiology of Plant Diseases*. Dordrecht, The Netherlands: Springer, 159–92.

McGovern RJ, Seijo TE, Hendricks K, Roberts PD, 2012. New report of *Colletotrichum gloeosporioides* causing postbloom fruit drop on citrus in Bermuda. *Canadian Journal of Plant Pathology* **34**, 187–94.

McMillan RT, Timmer LW, 1989. Outbreak of citrus postbloom fruit drop caused by *Colletotrichum gloeosporioides* in Florida. *Plant Disease* **73**, 81.

Mouen Bedimo JA, Bieysse D, Cilas C, Nottéghem JL, 2007. Spatio-temporal dynamics of arabica coffee berry disease caused by *Colletotrichum kahawae* on a plot scale. *Plant Disease* **91**, 1229–36.

Neves MF, Trombin VG, Milan P, Lopes FF, Cressoni F, Kalaki R, 2010. *O Retrato da Citricultura Brasileira*. São Paulo, Brazil: CitrusBR.

Ntahimpera N, Dillard HR, Cobb AC, Seem RC, 1997. Influence of tillage practices on anthracnose development and distribution in dry bean fields. *Plant Disease* **81**, 71–6.

Ntahimpera N, Wilson LL, Ellis MA, Madden LV, 1999. Comparison of rain effects on splash dispersal of three *Colletotrichum* species infecting strawberry. *Phytopathology* **89**, 555–63.

Peña JE, Duncan R, 1989. Role of arthropods in the transmission of postbloom fruit drop. *Proceedings of the Florida State Horticultural Society* **102**, 249–51.

Peres NA, Timmer LW, Adaskaveg JE, Correll JC, 2005. Lifestyles of *Colletotrichum acutatum*. *Plant Disease* **89**, 784–96.

Porto OM, 1993. Queda anormal de frutos jovens de citros. *Laranja* **14**, 341–56.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. [http://www.rproject.org]. Accessed 4 January 2013.

Sweetmore A, Simons SA, Kenward M, 1994. Comparison of disease progress curves for yam anthracnose (*Colletotrichum gloeosporioides*). *Plant Pathology* **43**, 206–15.

Timmer LW, Brown GE, 2000. Biology and control of anthracnose diseases of citrus. In: Prusky D, Freeman S, Dickman MB, eds. Colletotrichum*: Host Specificity, Pathology, and Host–Pathogen Interactions*. St Paul, MN: APS Press, 300–16.

Timmer LW, Zitko SE, 1993. Relationships of environmental factors and inoculum levels to the incidence of postbloom fruit drop of citrus. *Plant Disease* **77**, 501–4.

Timmer LW, Agostini JP, Zitko SE, Zulfiqar M, 1994. Postbloom fruit drop, an increasingly prevalent disease of citrus in the Americas. *Plant Disease* **78**, 329–34.

Upton G, Fingleton B, 1985. *Spatial Data Analysis by Example: Point Pattern and Quantitative Data*. Chichester, UK: John Wiley.

Zulfiqar M, Brlansky RH, Timmer LW, 1996. Infection of flower and vegetative tissues of citrus by *Colletotrichum acutatum* and *C. gloeosporioides*. *Mycologia* **88**, 121–8.