SHORT COMMUNICATION

INOCULUM AND INFECTION DYNAMICS OF THE SOOTY BLOTCH AND FLYSPECK COMPLEX OF APPLES IN SOUTHERN BRAZIL

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SUMMARY

The objectives of this study were to assess the relationship of infection of apple by sooty blotch and flyspeck (SBFS) to environmental conditions during fruit developmental stages and to monitor the chronology of increase in SBFS incidence in an orchard of southern Brazil. Seven "infection windows" (IWs) were simulated by exposing developing fruit (cv. Fuji) for predetermined periods of time and covering them with fruit bags during the rest of the season. Check treatments included fruit that were either surface-disinfested and covered or disinfested and uncovered. SBFS incidence was recorded after harvest on the fruits from each treatment, after incubating them in a moist chamber, and correlation analysis was used to assess association with moisture-related variables during each IW. In a separate experiment, timing of disease appearance was monitored by sampling non-covered fruit weekly until harvest. SBFS signs were observed in the fruits from all IW and check treatments. Average incidence ranged from 15 to 65% across the IWs, whereas incidence was >95% in fruits that were not covered during the experiment. SBFS infections were detected at harvest in the partially covered check treatment, thus suggesting that inoculum was present before the fruit was first covered 31 days after petal fall. Linear relationships were observed between SBFS incidence, rainfall and leaf wetness duration recorded within an IW. The period between fruit inoculation and appearance of the disease in the field was about 49 days.

Key words: Malus domestica, epiphytic fungi, disease development, fungal ecology, organic productions.

Sooty blotch and flyspeck (SBFS) is a disease caused by a complex of more than 60 fungal species (Díaz Arias *et al*., 2010) that blemish the cuticle of apple fruit (Williamson and Sutton, 2000). Although SBFS has been studied in North America for more than 90 years (Colby, 1920; Hickey, 1960; Williamson and Sutton, 2000), studies elsewhere in the world are relatively recent (Grabowski and Wrona, 2004; Ivanovic *et al*., 2010). In South America, the disease was reported two decades ago from Brazil (Berton and Melzer, 1989) where is of increasing concern in organic apple production systems. Surveys in experimental blocks that did not receive fungicide sprays reported SBFS incidence and severity as high as 87% and 50%, respectively, with signs of the pathogens predominating in the peduncular region of the fruits (Spolti *et al*., 2011).

A widely used SBFS warning system was developed based on knowledge of the ecology of the fungi prevailing in North Carolina (USA) (Brown and Sutton, 1995). However, regional differences in environmental conditions emphasize caution when transferring this system to other parts of the world (Duttweiler *et al*., 2008). For example, neither the timing of fruit inoculation nor the duration of the incubation period has been clearly defined for all apple-producing regions. In North Carolina, macroscopically visible SBFS colonies may appear as early as 20 days after petal fall (Brown and Sutton, 1993). In Pennsylvania (USA) Hickey (1960) noted that SBFS incubation period varied from weeks to months depending on timing of inoculation and rainfall periods. In Poland, SBFS incubation periods ranged from 29 to 45 days (Grabowski and Wrona, 2004). Since inoculation timing and incubation of SBFS had not been studied previously in the Southern hemisphere, a study was carried out to (i) assess the availability of inoculum and the relationship of inoculation timing to environmental conditions at specific times during fruit developmental stages, and (ii) quantify progress of SBFS over time.

Experiments were conducted during the 2007-08 growing season in a 19-year-old orchard of cv. Fuji on MM-106 rootstock located near the municipality of Vacaria (Rio Grande do Sul, Brazil). Insecticides were sprayed according to local IPM recommendations (Valdebenito-Sanhueza *et al*., 2006) and no fungicides were applied in the plots. The area had a row of *Pinus* sp. trees as a windbreak located 15 m from the western edge of the apple orchard. The experiment was planned

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to assess inoculum availability and infection dynamics during simulating "infection windows" (IWs) by uncovering and re-covering apple fruits for periods of time during their development (Kim *et al*., 2002; Henríquez *et al*., 2008, Grabowski and Wrona, 2004; Mayr *et al*., 2010). One month after petal fall, 1200 1-year-old shoots containing small (3 cm diameter) symptomless fruits were selected arbitrarily and marked on a total of 30 apple trees. A total of 1000 fruit were marked, surface-disinfested by spraying with 70% ethanol, and sealed individually in a waterproof bag that had an opening at the bottom allowing air to penetrate (Kim *et al*., 2002).

Treatments were seven sequential IWs, varying in duration from 14 to 20 days, between initial fruit covering (1 December 2007) and harvest (17 March 2008). At the start of each IW, 100 fruits were uncovered, then re-covered at the start of the next IW (Fig. 1). Check treatments included 200 fruit each that were either surface-disinfested and covered from 1 December to 17 March or were not disinfested nor covered during the same period. The experiment was conducted using a randomized complete block design, with six apple trees per block and five replications. In each of the five blocks, 20 fruit were bagged (2 to 3 fruit per tree). Fruit from all treatments were harvested at the same time and incubated for 1 month in a moist chamber to minimize the potential effect of different incubation periods across the IWs. Least square difference and standard error were used to compare SBFS incidence among IWs.

In a separate experiment in the same orchard block, 25 trees were selected and the timing of appearance of SBFS colonies was monitored by weekly sampling of 100 arbitrarily selected fruit in the lower half of each tree from the second week of December until harvest. Sampled fruit were immediately taken to the laboratory for examination under a stereomicroscope. Fruit that lacked SBFS signs during initial examination were incubated for 30 days in a moist chamber at 100% relative humidity and 20°C under alternating 12 h daylight and darkness periods (Brown and Sutton, 1993), then re-examined. To determine the timing of first appearance of SFBS symptoms, another set of 250 non-bagged fruits in the lower portion of the trees was inspected using a hand lens (20X magnification) at 5-day intervals starting the first week of December (*ca*. 31 days after petal fall).

Moisture-related data [rainfall, relative humidity and leaf wetness duration (LWD)] were recorded hourly by a datalogger (Adcon A730SEN, Adcon Telemetry, Austria). LWD sensors were placed under the trees within the orchard at 1.5 m height. LWD sensors were not painted and were facing south at a 45° angle. Correlation analysis (Pearson`s correlation coefficient = r) was used to assess the association between moisture variables recorded during each IW and SBFS incidence recorded after harvest on the fruits from the same IW. For the second experiment, correlation analysis was also used to explore relationships between the disease (SBFS incidence by the time fruits were harvested or after 30 days of incubation in a moist chamber with not visible signs on the fruits) and moisture-related variables. These variables were: cumulative daily rainfall (mm); number of rainy days; rainfall amount per event; cumulative hours of rainfall (h); rain intensity (mm/h) and cumulative daily leaf wetness duration (LWD), excluding wetting periods of less than 4 h (Brown and Sutton, 1995). The experimental design was a randomized complete block, with five trees per block and five replications. Least square difference and standard deviations were used to compare incidence between infection win-

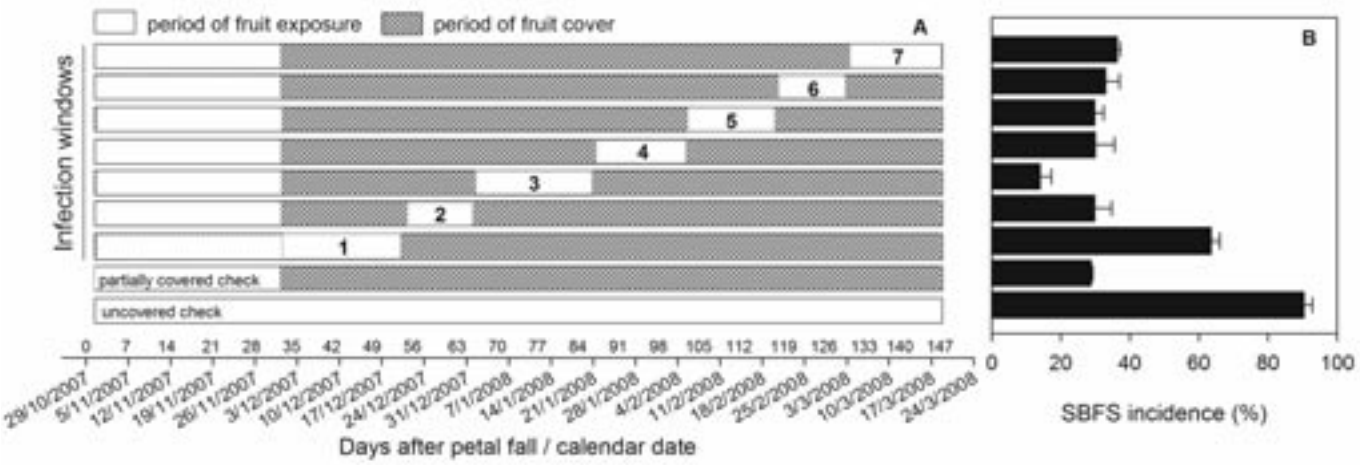

Fig. 1. (A) Timeline for sooty blotch and flyspeck (SBFS) infection windows established by removing fruit bags and subsequently re-covering the same apples (cv. Fuji) 14 to 20 days later. Fruit were initially covered on 1 December 2007 (31 days after petal fall on 29 October). (B) Mean and standard deviation (five replications) of SBFS incidence in each treatment determined at harvest after fruit incubation in a moist chamber for 30 days. Controls consisted of fruits that remained uncovered throughout the season and fruits that remained covered after 1 December.

dows. All statistical analyses were performed in SAS (version 9.2 SAS Institute, Cary, USA).

Sooty blotch signs, >90% of the fuliginous type (Batzer *et al.*, 2005), were observed on fruits from all treatments after incubating in the moist chamber. No visible signs were noticed by the naked eye when bags were removed to expose fruits on each IW. In fruits that were uncovered during a specific IW, average SBFS incidence ranged from 15 to 65%; the highest and lowest levels being observed in the first and third IW, respectively (Fig. 1). Overall, mean SBFS incidence for IW was similar to incidence on the fruits that were covered throughout the IW period (25%). In contrast, incidence was >90% in the fruits that were not covered during the experiment.

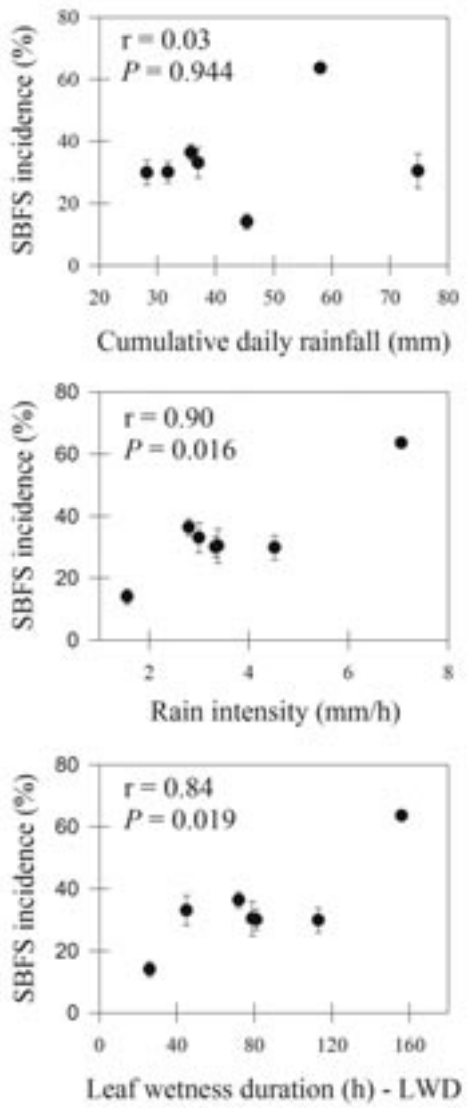

Fig. 2. Scatter plots and Pearson`s correlation coefficients (r) relating sooty blotch and flyspeck (SBFS) incidence to moisture-related variables during each of the seven infection windows (14 to 20 days each) from 1 month after petal fall (29 October) until harvest (17 March) of cv. Fuji. Mean (black circle) and standard deviation (vertical line) of five replicates (n=100 fruits per replication).

Correlation analysis showed a significant linear relationship between SBFS incidence and three weather-related variables recorded during an IW: rainfall amount per event (mm/rain) (r=0.90, *P*=0.016); rain intensity (mm/h) (r=0.91, *P*=0.005); and LWD (r=0.84, *P*=0.019). For the other variables, including cumulative RH (>90%), correlations were not significant ($P > 0.05$) (Fig. 2).

In the temporal progress study, no fruit from the first two assessments (December 3 and December 17) initially exhibited SBFS signs, but they became visible after incubation for 30 days in a moist chamber, and incidence exceeded 40% from the first assessment date (Fig. 3). Signs were visible on field-sampled apples under the stereomicroscope 65 days after petal fall, when incidence was 18%. Colonies were noted macroscopically in the field on the 20 of January. Mean SBFS incidence recorded in the field was 60% during the final assessment time, and nearly 100% for the same fruits after incubation in a moist chamber (Fig. 3). A significant positive correlation was found between SBFS incidence measured at harvest and after incubating fruits with no SBFS sign in a moist chamber (r=0.983, P<0.0001), as well as between the two SBFS measures and both cumulative daily rainfall (r≥0.9) and LWD (r>0.97) (Table 1).

The appearance of SBFS signs on fruits exposed during each of the IWs suggests that inoculation occurred after and/or prior to the time when fruits were first bagged. Furthermore, the fact that the disease was detected on fruits exposed before the first IW, suggests

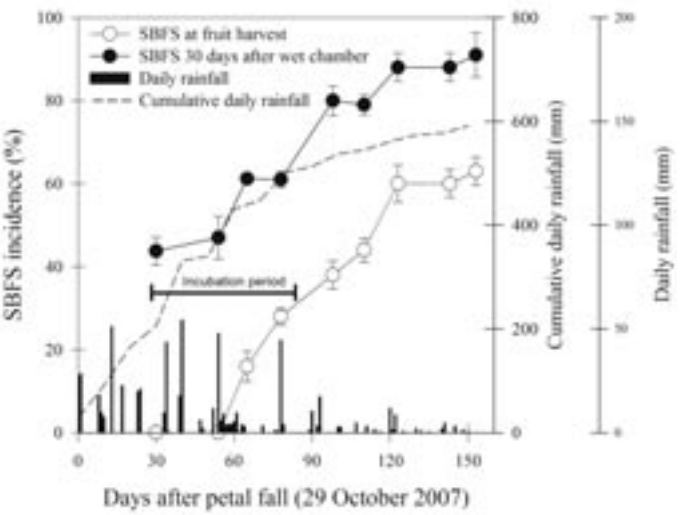

Fig. 3. Temporal progress of sooty blotch and flyspeck (SBFS) incidence on fruits (cv. Fuji) harvested at different times starting 31 days after petal fall. Measures were made at harvest, and again on the same apples after 30 days of incubation in a moist chamber at 21°C, using a stereomicroscope. Mean (circle) and standard deviation (vertical line) of five replicates (n=100 fruits per replication). Vertical bars and dashed line represent daily and cumulative rainfall, respectively. Horizontal bar represent the minimum length of incubation period (49 days) based on the date first SBFS signs observed in field.

Table 1. Variables matrix and Pearson`s correlation coefficient (r) for pairwise comparisons among incidence of sooty blotch and flyspeck (SBFS) on harvested apple fruits and on fruits incubated for a 30-day period in a wet chamber and moisture-related variables (cumulative rainfall and cumulative leaf wetness duration – LWD). *P*-values in parenthesis.

Variables	SBFS at harvest (%)	SBFS after moist chamber (%)	Cumulative daily rainfall (mm)
SBFS after moist chamber (%)	0.982 (<0.001)	-	0.975 (<0.001)
Cumulative daily rainfall (mm)	0.907 (0.001)	0.921 (<0.001)	-
Cumulative LWD (h)	0.972 (<0.001)	0.975 (<0.001)	0.927 (<0.001)

that the inoculum was already present on the fruits a month from petal fall and that disinfestation with ethanol did not eradicate all of it. The presence of inoculum during the earliest phase of fruit development is in agreement with observations from North Carolina, where the first SBFS infections occurred between 10 and 21 days after petal fall (Brown and Sutton, 1993).

The fact that the highest SBFS incidence was observed in the non-covered control fruits implies a cumulative pattern of deposition and successive infections during the entire period of fruit development. These findings are in accord with those of previous studies from Poland (Grabowski and Wrona, 2004) and Germany (Mayr *et al*., 2010) in which fruit bags were used. Mayr *et al*. (2010) noted that early-season infections resulted in higher disease levels than late-season infections, and that higher disease incidence in late- than early-maturing cultivars was related to longer exposure to secondary inoculum under disease-favorable environmental conditions.

Although it can be argued that fruit bagging after the exposure period would facilitate disease development because of the favourable microenvironmental conditions inside the bag, this probability was minimized by the fact that fruits from all IWs were incubated for a 30-day period in a moist chamber after harvest. However, if bagging had impacted disease development, fruits from the check treatment (bagged for 16 weeks) would have shown minimum or no SBFS signs. Hence, the primary effect of bagging was the prevention of deposition of new inoculum. We did not monitor the microenvironmental conditions inside the bags, but previous work has shown that temperature and relative humidity inside and outside a fruit bag were very similar (Zhang *et al*., 2003). During the present experiment, the mean daily maximum air temperature never exceeded 29°C and averaged 15 to 20°C, typical for the region and at levels that may not prevent or negatively affect the growth of most SBFS fungi (Batzer *et al*., 2010).

In our study, SBFS incidence resulting from potentially new deposition and infection during specific IWs was strongly associated with rainfall variables. These findings match those reported from Germany, where a linear relationship was observed between rain frequency during fruit exposure periods and final SBFS severity index (Mayr *et al*., 2010). The positive relationship between LWD and SBFS incidence may be due to the fact that crop wetness during the study period was usually associated with rain events. Correlation between cumulative daily rain and LWD during SBFS development in the second experiment was highly significant (r=0.92, *P*<0.001) (Table 1). Indeed, 65% of the wet periods during the 4-month period of the study were due to rainfalls (P. Spolti, unpublished information).

This close association of LWD with rainfall tallies with patterns found in North Carolina. In contrast, in the Upper U.S. Midwest wet hours were primarily associated with dew rather than rain, and the duration of periods of relative humidity (≥97%) predicted the timing of SBFS colonies appearance on apples more accurately than rain-associated variables (Duttweiler *et al*., 2008). Because fruits from all IWs were incubated evenly at the end of the experiment, correlation between SBFS and rainfall and LWD variables at specific IWs, which varied from 14 to 20 days in length, is likely to explain the increments in the number of fruits infected during the exposure period. The time difference between disease detection in the laboratory and its appearance in the field was about 49 days, suggesting that the incubation period was ≥50 days because fruits were not sampled early enough to establish the time of initial infection. This period exceeds that reported by Brown and Sutton (1993) from North Carolina, and Grabowski and Wrona (2004) from Poland (29 to 45 days).

These first observations of the SBFS dynamics in the Southern Hemisphere revealed that the key aspects of SBFS epidemiology, i.e. timing of inoculum onset and incubation period, parallel those observed elsewhere in the world and suggest that SBFS warning systems developed in other apple-growing regions could be adapted for use in Brazilian orchards.

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