Relationship Among Concentrations of *Sphaerotheca macularis* Conidia in the Air, Environmental Conditions, and the Incidence of Powdery Mildew in Strawberry

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ABSTRACT

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Atmospheric concentrations of *Sphaerotheca macularis* conidia were monitored for 2 years on a strawberry crop in Huelva (southwestern Spain). The presence of airborne conidia was determined to assess the role of weather conditions on conidial release. The relationship between airborne conidia and incidence of powdery mildew on fruit was also studied. Concentrations of conidia were estimated with a Burkard volumetric spore sampler. The presence of conidia was related to temperature, relative humidity, and rainfall, with a positive correlation for the first factor and a negative correlation with the other two. The presence of conidia in the air was positively correlated with disease incidence. A diurnal pattern of conidia release was observed.

Additional keywords: aerobiology, epidemiology

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most important crops in Huelva (southwestern Spain), with more than 7,000 ha cultivated in 2001 (1). Powdery mildew is caused by the obligate parasite *Sphaerotheca macularis* (Wall. ex Fries) Jacz f. sp. *fragariae* (Peries). It is one of the most common diseases on the aerial parts of the plant and has been reported worldwide (18). In southwestern Spain, it was first confirmed in 2001 (5).

Symptoms of powdery mildew appear on leaves, flowers, and fruits. Leaves curl upward with spoon-like shapes and white mycelium on the surface (21). Flowers and peduncles can be covered by white mycelium (18). Infected fruit become hard, covered by white mycelium, and unmarketable (10,11).

Control of powdery mildew relies on the application of protectant and systemic fungicides, beginning early in the flowering period. The disease can be controlled by foliar applications of sulfur (16). Aerobiological studies could lead to a better understanding of the biology and epidemiology of *S. macularis* and contribute to the development of a disease management program to avoid unnecessary fungicide applications without increased risk of disease.

The aim of this work was to determine the relationship of environmental condi-

tions with the presence of conidia of *S. macularis* in the air during two strawberry crop seasons (2000–2001 and 2001–2002). The relationship between aerial conidia and incidence of powdery mildew on ripe fruit was also investigated.

MATERIALS AND METHODS

Sampling was carried out in an experimental strawberry farm located near Moguer (Huelva District, southwestern Spain) for two growing seasons from October to May in 2001 and 2002 in the same plot. The soil (90% sand, 2.8% slime, and 7.2% clay) had never been previously fumigated, and strawberry had not been grown there for 10 years prior to the start of this work. The plot consisted of treated-plant and untreated-plant subplots of two cultivars (Andana and Camarosa) in a randomized complete block with four replicates. Each subplot was 1.5 × 12.5 m and had three raised beds. Treatments were foliar applications of sulfur and fungal proteins (NBT Co. Ltd., Sevilla, Spain).

Each year in the last week of October, strawberries were planted. Plants were grown in an intensive annual system on drip-irrigated raised beds (30 cm high and 50 cm wide) with black plastic mulch. Plants were spaced 25 cm apart within each of two rows per plot per raised bed, and the rows were spaced 25 cm apart. Beds were covered with small, polyethylene tunnels (microtunnels) from late November to early March for both seasons (17).

Concentrations of airborne conidia were studied with a 7-day volumetric spore sampler (Burkard Mfg. Co. Ltd., Rickmansworth, Hertfordshire, England) located at the center of the field between two micro-

tunnels and operated at a constant flow rate of 10 liters of air per min. The sampling orifice was 40 cm above the beds. Melinex tape coated with petroleum jelly was used in the spore sampler. The tape was replaced weekly and cut into 48-mm long segments, each representing a single day. Each tape segment was mounted on a glass slide with glycerin jelly (50 ml of glycerin, 7 g of jelly, 1 g of phenol, and 42 ml of distilled water) and stained with acid fuchsin (5 ml of glycerin jelly solution plus 0.5 ml of acid fuchsin) (7).

Each tape segment was examined at 2-mm intervals in two longitudinal transects to the direction of tape movement with a light microscope (×400). Counts were corrected to compensate for the area sampled and recorded as the number of conidia per cubic meter of air sampled per day (19). Distribution of conidia was calculated as the mean number of conidia recorded each hour of the day during the whole season. A weekly average of conidia was calculated and compared with weekly data for disease incidence.

With the help of an airborne spore and pollen atlas (9), *S. macularis* conidia were identified by their morphological characteristics: size (20 to 23 × 13 to 20 µm), barrel shape when turgid, and presence of granules inside the conidia (2).

Meteorological data were obtained from an automatic weather station (CR10X data logger, Campbell Scientific Ltd., Leicestershire, U.K.; Humidity and Temperature Sensor HMP 45C, Vaisala Oyj, Helsinki, Finland; and Tipping Bucket rain gauge ARG100, Campbell Scientific Ltd.). The weather station, part of the Meteorological Data Service Network (Junta de Andalucía), was located 5 km from the experimental plot. Hourly temperature and relative humidity data were used to calculate daily mean temperature (T) and daily mean relative humidity (RH). Total daily rainfall (R) was also measured. The correlation between meteorological factors and conidial concentration was examined with Spearman's Correlation Test (Statistix 7; Analytical Software Ltd., La Jolla, CA), which is a general test used for microorganism infection data (6).

All ripe fruits were collected once a week in untreated subplots. Incidence of powdery mildew was expressed as the mean percentage of symptomatic fruit (10,11). Since the effect of the cultivar on disease incidence was not significant, the

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Fig. 1. A, Daily total airborne conidia of *Sphaerotheca macularis*; B, rainfall; C, mean daily relative humidity; and D, mean air temperature at the Moguer experimental plot during the 2000–2001 season. Concentrations of airborne conidia were determined with a Burkard volumetric spore sampler operated continuously. Arrows indicate dates of planting and initiation of harvest.

Fig. 2. A, Daily total airborne conidia of *Sphaerotheca macularis*; B, rainfall; C, mean relative humidity; and D, mean air temperature at the Moguer experimental plot during 2001–2002 season. Concentrations of airborne conidia were determined with a Burkard volumetric spore sampler operated continuously. Arrows indicate dates of planting and initiation of harvest.

mean was calculated for the two cultivars. The correlation between disease incidence and weekly mean conidial concentration was examined using Spearman's Correlation Test. Conidial data were not transformed because the distribution was not normal (6).

RESULTS

Seasonal periodicity and influence of meteorological factors. In both years, conidia were first detected early in October, were collected throughout the season, and their numbers increased as the crop matured (Figs. 1 and 2).

During the 2000–2001 season, the concentration of conidia in sampled air ranged from 0 to 336 conidia/m³ until the first day of harvest (day 107). During the harvest period (day 107 to day 205), the concentration of conidia ranged from 0 to 4,587 conidia/m³ (Fig. 1A). Minimal concentration of conidia (zero) was recorded in 78 nonconsecutive days during the season. For these days, average T was 12.5°C (Fig. 1A and D), average RH was 86.4% (Fig. 1A and C) and rainfall was >5 mm for 25.3% of these days (Fig. 1A and B). The highest concentration of conidia (4,587 conidia/m³) was reached with a T of 14.4°C, 73% RH, and no rainfall (Fig. 1). Conidial concentration over 2,000 co-

Table 1. Spearman's rank correlation between number of conidia trapped in the air and meteorological factors and the weekly mean conidia concentration in the air and weekly mean disease incidence on the untreated subplots during the 2000–2001 and the 2001–2002 season in an experimental plot in Moguer (Huelva District, southwestern Spain)^a

Season	Temperature	RH	Rainfall	Disease incidence (% symptomatic fruit)
2000–2001	0.48 ***	–0.49 ***	–0.28 ***	0.73 **
2001–2002	0.40 ***	–0.44 ***	–0.31 ***	0.64 *

^a * = significant at $P = 0.05$, ** = significant at $P = 0.01$, and *** = significant at $P = 0.001$.

nidia/m³ was registered on 20 nonconsecutive days (Fig. 1A). During this period, average T was 15.4°C (Fig. 1D), average RH was 67% (Fig. 1C), and no rainfall occurred (Fig. 1B).

During the 2001–2002 season, the concentration of conidia in sampled air ranged from 0 to 725 conidia/m³ until the first day of harvest (day 129) and varied between 0 and 3,056 conidia/m³ during the harvest period (day 129 to day 224) (Fig. 2A). No conidia were trapped on 37 nonconsecutive days during the season. For these days, average T was 12.0°C (Fig. 2A and D), average RH was 82.1% (Fig. 2A and C), and rainfall was >5 mm during 18.4% of the days (Fig. 2A and B). The highest concentration of conidia (3,056 conidia/m³) was reached with a T of 18.0°C, 54% RH, and no rainfall (Fig. 2). Conidial concentration >2,000 conidia/m³ was regis-

tered for nine nonconsecutive days (Fig. 2A). During this period, average T was 16.2°C (Fig. 2D), average RH was 61.7% (Fig. 2C), and rainfall <5 mm was registered on only 2 days (Fig. 2B).

The concentration of conidia over the crop was higher during the second season (2001–2002) than during the first (2000–2001) (Figs. 1A and 2A).

There was a significant and positive correlation between temperature and number of conidia trapped. The correlations between rainfall and conidia and RH and conidia were negative and significant (Table 1).

Diurnal periodicity. The number of airborne conidia trapped generally followed a diurnal pattern (Fig. 3). Conidia concentration was greatest between 1100 and 1900 h during the 2000–2001 season, with a peak between 1300 and 1500 h. During

Fig. 3. Diurnal periodicity of *Sphaerotheca macularis* airborne conidia trapped from the air over a strawberry experimental plot in Moguer, Spain. Values given are the average of conidia trapped at the same hour of the day during the season 2000–2001 and 2001–2002. Concentrations of airborne conidia were determined with a Burkard volumetric spore sampler that was operated continuously.

Fig. 4. Weekly incidence of powdery mildew on strawberry fruit in an experimental plot in Moguer, Spain during the A, 2000–2001 and B, 2001–2002 seasons. Disease incidence was estimated as the mean percentage of affected fruit harvested from untreated subplots. The number of conidia of *Sphaerotheca macularis* in the air is shown as weekly mean. Conidia were trapped in the plot by a Burkard volumetric spore sampler that was operated continuously.

the second season, most conidia were trapped between 0900 and 2000 h, with the peak between 1300 and 1500 h.

Disease incidence. During the first season, the incidence of powdery mildew on fruit varied depending on the number of conidia trapped during the previous week. Thus, an increase in the number of conidia trapped led to a higher disease incidence 1 week later. Disease incidence was high from days 120 to 149 without corresponding high conidia concentration (Fig. 4A). During this period, rainfall was >5 mm, whereas T was higher than 15°C and RH was over 90% (Fig. 1B, C, and D). During the first 2 months of the harvest period in the second season, this pattern was not so clearly observed, but for the rest of the season, the pattern was similar to that observed during the first season (Fig. 4B). In the second season, powdery mildew incidence and number of conidia trapped weekly were lower than those measured for the first season (Fig. 4). The correlation between disease incidence and number of conidia was positive and significant (Table 1).

DISCUSSION

After the planting date, the number of conidia trapped over the crop increased, indicating that the plants may have been an important source of inoculum. As Duhart et al. (8) reported in research carried out in nurseries, as much as 22% of plants exported to production fields were infected with *S. macularis*. When the harvest season started, the number of conidia trapped increased.

Rainfall decreased the concentration of conidia on the sampled air over the crop. Raindrops apparently washed conidia from the air, and may also have inhibited the sporulation of colonies on the leaf surface (20,26).

Optimal temperatures reported for powdery mildew vary depending on the crop and conditions where it is grown (12). For *S. macularis* on strawberry, optimal temperature range for germination is approximately 15 to 25°C (24). In our study, the period of peak conidia concentration corresponded with periods of T approximately 15°C.

A negative correlation was found between RH and the conidia trapped. Although *S. macularis* needs high RH for germination of conidia and RH fluctuations for liberation of conidia (2,15), free water on the leaf surface could inhibit the growth of the mycelium and the formation and germination of conidia (14).

As with the majority of the Erysiphales, the conidial maturation process is a photosensitive process. Therefore, conidia produced at night remain immature until they are exposed to daylight and then are released once maturation is completed (3).

Positive correlations between conidial presence and disease incidence indicates

that the presence of higher conidia levels leads to a higher incidence of the disease if weather conditions permit the establishment of the pathogen on susceptible tissue. When one of the three meteorological factors measured was suboptimal for conidia germination and mycelium development, disease incidence decreased. Nevertheless, when meteorological conditions were highly favorable for disease development, disease incidence was high without high conidia concentration, as was observed during the first season (days 120 to 149). During this period, T and RH were optimal for disease development (12), but rainfall probably caused a reduction of conidia trapped (20,26). Thus, meteorological factors could be considered responsible for the increase in the number of conidia trapped during the harvest period, and consequently for the increase of powdery mildew incidence.

For the management of powdery mildew, predictive models have been developed in other crops. Chellemi and Marois (4) developed a model for grape powdery mildew based on the estimation of inoculum germination rates and weather parameters. Sall (22) used the infection rate, the portion of infected tissue, and the length of the infection. For apple powdery mildew, Xu (25) developed three sub-models to describe the influence of weather on conidial production, dispersion, and germination. Other researchers have considered the presence of airborne conidia over the crop on their model construction. Jeger (13) used cumulative spore catches and atmospheric conditions to predict disease incidence for apple powdery mildew, and Smith and Davies (23) worked out an empirical model for barley powdery mildew as a function of temperature and conidia trapped. The current work, carried out over a strawberry crop, could be the basis for development of a

predictive model of powdery mildew occurrence in strawberry production, which would be a tool to improve crop management and the efficiency of fungicide applications.

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