



OPEN ACCESS

EDITED BY

Omer Frenkel,
Agricultural Research Organization (ARO),
Israel

REVIEWED BY

Abazar Ghorbani,
Nanjing Forestry University, China
Johanna Bac-Molenaar,
Wageningen University and Research,
Netherlands

*CORRESPONDENCE

Anna Aldrighetti
✉ anna.aldrighetti@unitn.it

RECEIVED 21 October 2025

REVISED 16 January 2026

ACCEPTED 19 January 2026

PUBLISHED 05 February 2026

CITATION

Aldrighetti A, Zott D and Pertot I (2026)
Sanitation practices targeting overwintering
inoculum improve management of strawberry
powdery mildew in high-tunnel production.
Front. Agron. 8:1729740.
doi: 10.3389/fagro.2026.1729740

COPYRIGHT

© 2026 Aldrighetti, Zott and Pertot. This is an
open-access article distributed under the terms
of the [Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication
in this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Sanitation practices targeting overwintering inoculum improve management of strawberry powdery mildew in high-tunnel production

Anna Aldrighetti^{1*}, Davide Zott² and Ilaria Pertot¹

¹Centre Agriculture Food Environment – C3A, University of Trento, San Michele all'Adige, Italy,

²Department of Agronomy, Food, Natural Resources, Animals and Environment – DAFNAE, University of Padova, Legnaro, Italy

Strawberry powdery mildew, caused by *Podosphaera aphanis*, is a major disease of strawberry, capable of leading to yield losses of up to 70%. Its management typically relies on frequent chemical fungicide applications due to the extended infection window of the pathogen. *Podosphaera aphanis* overwinters as mycelium and chasmothecia on infected plant material, which may act as primary inoculum in spring. This two-year study investigated the effectiveness of post-winter removal of infected leaf and plant debris in disease control and quantified the role of chasmothecia in early season infection under high-tunnel conditions. Complete removal of infected tissues delayed disease onset and reduced severity by up to 88%. In the two years, chasmothecium formation increased in late summer following 8 and 16 cumulative hours below 13 °C, respectively. In spring, ascospore infection events occurred after the accumulation of 21 and 18 cumulative hours with temperatures above 10 °C combined with at least two consecutive hours of leaf wetness, consistently with previous findings. The efficacy of the sanitation practice was further supported by excluding the possibility that chasmothecia and/or chasmothecia on debris fragments potentially fallen into the substrate could trigger infection. In addition, we investigated the timing of ascospore-derived infections by monitoring disease progression on individual leaves. Infection rate was quantified across different leaf ages to assess their role in epidemic development and their potential contribution to secondary inoculum. The highest infection rate was observed on 15-day-old class age, with a crucial role in supporting early pathogen development. These results indicate the importance of sanitation practices, such as overwintering infected leaf removal in reducing early-season infections and informing more targeted control strategies.

KEYWORDS

chasmothecia, infected leaf removal, infection rate, overwintering inoculum, strawberry powdery mildew

1 Introduction

Strawberry powdery mildew (SPM), caused by the Ascomycete *Podosphaera aphanis* (Wallr.) Braun and Takamatsu (2000) is a major fungal disease of strawberry (*Fragaria × ananassa*) worldwide (Menzel, 2022; Peries, 1962). Severe infections can reduce yields by up to 70% (Dodgson et al., 2008; Peries, 1962), due to flower abortion, fruit deformation and cracking, white mycelium and exposed achenes, postharvest decay, and reduced photosynthesis (Jhooty and McKeen, 1965; Peries, 1962). Young tissues are especially vulnerable (Asalf et al., 2014; Carisse and Bouchard, 2010). Leaves rapidly develop ontogenic resistance after unfolding, whereas fruits are highly susceptible after petal fall but gradually acquire resistance during ripening (Asalf et al., 2014). While previous studies (Asalf et al., 2014; Carisse and Bouchard, 2010) evaluated age-related leaf susceptibility by inoculating leaves at different stages and evaluating infection after inoculation, none has explored how disease progresses over time on individual leaves of different ages, nor how these leaves contribute to their role in sustaining secondary infections and contributing to overall epidemic development.

Strawberry is a high-value crop in Europe and the US (Hodgdon et al., 2024; Steffek et al., 2004) where increasing consumer demand for year-round, high-quality berries offers growers access to premium markets (Hodgdon et al., 2024; Samtani et al., 2019). To meet this demand, growers increasingly use protected systems such as polyethylene tunnels and greenhouses, with raised beds or suspended peat pots (Mezzetti et al., 2018; Neri et al., 2012; Takeda, 1997). These conditions, while shielding crops from adverse weather conditions, favor SPM by enhancing conidial germination through reduced spore wash-off, limited leaf wetness, and low light intensity, factors that in open fields normally inhibit infection (Amsalem et al., 2006; Asalf et al., 2021). To ensure continuous production, growers combine short-day and day-neutral cultivars, which differ in flowering response and cropping period (Mezzetti et al., 2018). Both are susceptible (Menzel, 2022), though everbears (day-neutral) face higher disease pressure during their extended harvest in summer (Blanco et al., 2004; Carisse and Bouchard, 2010).

Disease dynamics are also influenced by the transplanting system. In annual replanting, infected nursery transplants are often the main inoculum source (Peries, 1962; Stensvand et al., 2024). Outbreak risk increases in perennial or multi-season cycles, where June-bearing plants established in midsummer and overwintered under straw or polypropylene can accumulate high inoculum if not properly managed postharvest (Caffi et al., 2011). Although these systems represent a minority of global strawberry cultivation, they persist in continental climates (e.g., Central Europe, Northeastern US, Southern Canada), where short-day cultivars often outperform day-neutrals (Samtani et al., 2019).

Strawberry powdery mildew overwinters as mycelium on living tissues and as chasmothecia (fruiting bodies), on senescent leaves and plant debris (Gadoury et al., 2010; Peries, 1962). Chasmothecia formation under laboratory condition was triggered by temperatures below 13 °C when compatible mating types were

present, as required by the heterothallic nature of *P. aphanis* (Asalf et al., 2013a). Most remain attached to host tissue, but about 20% may detach after mechanical disturbance (Gadoury et al., 2010). The fate of detached structures and their role in initiating spring infections remain unclear. Under both greenhouse and field conditions, chasmothecia form in late summer, with ascospore discharge and infection occurring with spring plant growth (Gadoury et al., 2010). However, how field conditions affect their formation, maturation, their discharge, as well as the extent to which ascospore-derived primary infections contribute to epidemic development, remain poorly understood. For grapevine powdery mildew *Erysiphe necator* Schw. chasmothecia opening and ascospore discharge occur at temperature >10 °C combined with continuous leaf-wetness periods ≥2 hours (Gadoury and Pearson, 1990; Rossi et al., 2010). Whether similar environmental conditions regulate chasmothecia opening and ascospore discharge in *P. aphanis* remains to be determined.

Sanitation practices targeting overwintering inoculum are widely used against other fungal pathogens that overwinter through fruiting bodies or sclerotia. For example, *Venturia inaequalis* (Cooke) G. Wint., the causal agent of apple scab, overwinters in fallen leaves as pseudothecia, which release ascospores in spring. Leaf removal, composting, or urea application to accelerate leaf decomposition can greatly reduce primary inoculum (Holb, 2006; MacHardy, 1996). Likewise, in *Botrytis cinerea* Pers.:Fr., the causal agent of gray mold in strawberries, sanitation targeting overwintering sclerotia lowers early-season pressure (Elad et al., 2007). For SPM, in the last decades leaf removal has been frequently suggested by agronomists (AHDB Horticulture, 2020) and researchers (Asalf et al., 2013b; Gadoury et al., 2009) as a potential strategy to reduce overwintering inoculum and delay epidemics. However, its effectiveness under field conditions has never been experimentally tested, and its impact on early-season infections remains uncertain.

The aim of this study was to assess whether sanitation practices based on post-winter leaf removal are sufficient to suppress primary infections of *P. aphanis*, and to exclude the possibility that alternative inoculum sources, such as detached chasmothecia remaining in the peat substrate, contribute to the initiation of spring epidemics. To further characterize how primary infections evolve and lead to secondary spread, we tracked disease development on individual leaves over time, focusing on the role of leaf age in secondary inoculum buildup. Finally, we monitored how environmental factors (temperature, relative humidity, and leaf wetness) may influence chasmothecia formation, maturation, and ascospore release under field conditions.

2 Materials and methods

2.1 Experimental set up in high tunnel

Trials were conducted in a commercial-like plastic high tunnel (Polyagri AG, Agripolyane, Italy; 17.5 m long, 4.5 m wide) located in Vigolo Vattaro, Italy (46° 0' 13.223" N, 11° 11' 18.650" E, 682 m a.s.l.).

A total of 112 cold-stored tray plants of the strawberry cultivar SO.FR.12.04.16 (Sant'Orsola cultivar collection) were transplanted on May 5, 2022, and May 13, 2023, into suspended pots filled with peat substrate (Torba Silver® 20–40, Agrochimica, Italy), four plants per pot. Fertigation was supplied via four drippers per pot with a nutrient solution containing 6 mmol L⁻¹ NO₃⁻, 6 mmol L⁻¹ H₂PO₄⁻, 5 mmol L⁻¹ K⁺, 3 mmol L⁻¹ Ca²⁺, and 1.25 mmol L⁻¹ Mg²⁺, at pH 5.5 and electrical conductivity of 1.5 mS cm⁻¹. The system (Gravimetric, Spagnol, Italy) automatically monitored and adjusted nutrient concentrations, pH, and substrate moisture to maintain optimal growing conditions.

To ensure a uniform disease background, SPM inoculum was introduced by randomly placing four infected plants (≈20% severity) in the tunnel on June 18, 2022, and June 13, 2023. No fungicides, insecticides, or acaricides were applied in either season to allow natural disease progression (average plant severity = 24.0 ± 3.2% standard error). Sensors (WSTATION, CET Electronics Sensors, Italy) for temperature (C°) and RH (%) were positioned 1.9 m above ground (≈0.5 m above the canopy), whereas leaf wetness (min hour⁻¹) sensors were placed at canopy level. Temperature and relative humidity data were recorded with three measurements hour⁻¹. Because plants were grown under protected cultivation, rainfall was not measured.

2.2 Infected leaf and plant debris removal and role of detached chasmothecia in the peat substrate in primary infection initiation

On October 23, 2022, and on October 25, 2023, all pots were removed from suspended rows and overwintered on the ground beneath polypropylene tissue (TNT gr. 30, Novagryl, France) without fertigation. Just prior budbreak (March 9, 2023, and March 15, 2024), plants were uncovered and randomly assigned to one of the three following treatments (seven replicate pots per treatment, four plants each): i) manual removal of all leaves and debris, retaining only buds newly emerging from the crown (No Leaves); ii) as above, with pots additionally wrapped in stretch film (Film estensibile, Polycomm, Italy) covering only the substrate surface and leaving only the crown exposed, with the irrigation drippers placed below the film. The bottom of the pot was left uncovered to ensure normal drainage (No Leaves With Film); iii) no removal of leaves and plant debris, serving as untreated control (With Leaves). To estimate the feasibility of the sanitation practice, the time required to clean each pot was recorded and averaged across replicates. All plants were examined under a stereomicroscope (90×) to check for overwintered mycelium within buds and for the presence of chasmothecia, to determine whether infections could arise from mycelial sources or exclusively via ascospore-driven primary infections. The experiment was conducted in an area with no other strawberry plants to avoid external airborne inoculum, in a randomized block design with treatments separated to prevent direct cross-contamination.

2.3 Disease onset and infection rate

Each plant within each replicate and treatment was assigned a unique identifier. Newly expanded leaves, corresponding to leaf stage 4 of [Asalf et al. \(2014\)](#) were sequentially numbered on the adaxial surface with a black marker (Pentel Pen N50, Pentel Co. LTD., Japan) at weekly intervals, starting with the first emerged leaf (March 16, 2023, and March 22, 2024) labeled as “1” and continuing numerically as new leaves were formed. Observations were expressed as days after leaf removal (DALR). After symptoms onset (72 DALR in 2023 and 39 DALR in 2024) the following parameters were recorded every 3–4 days: SPM leaf incidence (percentage of symptomatic leaves per plant) and severity (percentage of symptomatic area of both leaf sides per plant). The number of SPM patches per leaf was assessed as a proxy to track ascospore infections at early infection stage. The SPM incidence on fruits (percentage of symptomatic fruits per plant) was recorded on May 29, June 6, June 9, 2023 (81, 87, 90 DALR). Due to early and severe infections in 2024 (onset on April 22, 39 DALR) fruit assessment was not feasible as incidence reached ~100% across treatments by the beginning of harvest (79 DALR).

2.4 Chasmothecium formation and maturation and ascospore discharge

To monitor late-summer chasmothecium formation, 30 mildew-infected leaves (approximately two-week-old leaves) were collected randomly from the 112 plants every 10 days from mid-August to the end of October both in 2022 and 2023. Leaf area was measured using the Image-J software ([Schneider et al., 2012](#)) and chasmothecia were counted under a stereomicroscope (90× magnification). Data were expressed as the number of chasmothecia cm⁻² on both the upper and lower leaf surfaces. To monitor chasmothecia maturity and ascospore discharge in spring, from March 9 to May 3 in 2023, and from March 15 to June 3 in 2024, 10 cohorts (15 chasmothecia cohort⁻¹) were collected every ten days from infected plants (With Leaves) in the tunnel, examined under a microscope (200× magnification) and classified as either ‘containing ascospores’ or ‘empty’. To determine whether chasmothecia remaining closed in the field reflected immaturity or suboptimal environmental conditions for release (discharge readiness), additional cohorts (ten per sampling date; 15 chasmothecia per cohort) were collected from infected leaves and placed on water-soaked cotton in Petri dishes at 22 ± 1 °C for 24 hours to promote ascospore discharge, then examined and categorized as above.

2.5 Statistical analysis

Statistical analyses were performed in R (version 2024.12.1). To account for repeated dependent measurements over time, leaf disease incidence was analyzed using generalized linear mixed

model (GLMM) with a binomial distribution and logit link function (lme4 package). For the binomial model, incidence was specified as the number of infected leaves out of the total number of assessed leaves per plant at each assessment. Leaf disease severity was analyzed using linear mixed-effects model (LMM) after data log-transformation (lme4 package). In both models, treatment, time (DALR), and their interaction were included as fixed effects, while pot was included as random effect. Fruit disease incidence was analyzed using the same leaf incidence modeling approach, based on the number of infected fruits out of the total number of assessed fruits per plant at each assessment, with treatment and time included as fixed effects and pot as random effect. Model diagnostics were evaluated by examining overdispersion and deviance and Pearson residuals for GLMM, and by residual plots (normal Q-Q plots and residuals versus fitted values) for LMM. The significance of fixed effects was assessed on the fitted mixed-effects models using analysis of variance (F-test) for LMM and likelihood ratio test for GLMM. *Post-hoc* pairwise comparisons among treatments at each assessment were performed using Tukey's adjusted tests (emmeans package). Differences in leaf number among treatments at each sampling date were tested with the Kruskal-Wallis non-parametric test. Infection rate (IR) was calculated from patch number per leaf using the following formula:

$$\text{Infection rate (IR)} = \frac{\ln(f_2) - \ln(f_1)}{(t_2 - t_1)}$$

where f_1 and f_2 are the number of patches at two consecutive observations t_1 and t_2 . To allow a standardized comparison of infection dynamics leaf ages were grouped into 5-day age classes (e.g., 0–5, 5–10 days). The IR expresses the relative rate of increase in lesion number day^{-1} . A constant of 0.1 was added to each lesion count to avoid undefined values for zero counts. The Mack-Wolfe test (PMCMRplus package) was used to assess whether the IR follows an umbrella-shaped trend across leaf ages and identify the age class with the maximum disease peak. A generalized linear model (GLM) with a Gaussian distribution was analyzed to validate the association between chasmothecium formation (number cm^{-2}) and cumulative hours with temperature $< 13^\circ\text{C}$ (hourly mean temperature $< 13^\circ$). A generalized linear model (GLM) with a binomial distribution and logit link function was analyzed to validate the association between empty chasmothecia in spring and cumulative hours $> 10^\circ\text{C}$ (hourly mean temperature $> 10^\circ$ from 1 January), combined with continuous leaf-wetness periods ≥ 2 hours (hourly mean leaf-wetness = 60 min), which are the conditions for ascospore discharge in grapevine powdery mildew (Gadoury and Pearson, 1990; Rossi et al., 2010). In both models, 'year' was included as a fixed factor to account for inter-annual variability. Model diagnostics were assessed by inspecting residual plots (normal Q-Q plots and residuals versus fitted values) for the Gaussian GLM, and by evaluating overdispersion, deviance and Pearson residuals for the binomial GLM. When measurements were missing within an hour, hourly mean values were calculated using the available readings.

3 Results

3.1 Infected leaf and debris removal and role of detached chasmothecia in the substrate in primary infection initiation

In both years SPM mycelium and colonies were not detected in strawberry buds. Overall leaf incidence and severity were comparable between the two years of analysis. The removal of infected leaves significantly reduced disease incidence in both years 2023 ($\chi^2 = 384.5$, $p < 0.001$) and 2024 ($\chi^2 = 525.96$, $p < 0.001$) (Figure 1). At the final disease assessment (88 DALR in 2023 and 57 DALR in 2024), incidence in the untreated control with infected leaves reached 26% and 24%, respectively, while incidence in the treatment without infected leaves was 12% in 2023 and 9% in 2024. These values correspond to a reduction in disease incidence of approximately 54% in 2023 and 63% in 2024. Tukey's test indicates a significantly higher incidence on the treatment with infected leaves in the last three assessments 76 DALR in 2023, and in the last two assessments 54 DALR in 2024, respectively. The application of the plastic film had no effect, except for the last assessment on disease incidence (88 DALR) in 2023.

The removal of infected leaves also significantly reduced disease severity compared to the control with infected leaves, in both 2023 ($F = 78.51$, $p < 0.001$) and 2024 ($F = 135.77$, $p < 0.001$) (Figure 1). At the final assessment (88 DALR in 2023 and 57 DALR in 2024) leaf disease severity in the control with infected leaves reached 16% and 13%, respectively, while severity in the treatment without infected leaves remained as low as 2% in 2023 and 1% in 2024. These values correspond to a reduction in disease severity of approximately 88% in 2023 and 92% in 2024. In 2023, Tukey's test revealed significant differences between treatments with and without infected leaves after 76 DALR, while in 2024 significant difference was observed after 54 DALR ($p < 0.05$). The film addition caused significant difference in disease leaf severity only in the last assessment 88 DALR in 2023 ($p < 0.05$).

In both years, disease onset was delayed where the infected leaves were removed, by 9 days in 2023 and 8 days in 2024. Fruit incidence also showed a significant response with a decrease of 70% ($\chi^2 = 107.214$, $p < 0.001$) when infected leaves were removed (Figure 2). Tukey's test confirmed the significant effect of the removal of infected leaves, with lower incidence observed after 87 and 90 DALR ($p < 0.05$). The film addition did not cause significant changes. Leaf removal required $24 \pm 2.4 \text{ sec plant}^{-1}$.

3.2 Development of disease onset and infection rate

In 2023, the presence of the infected leaves led to an early onset of PM, with early patches appearing in pots 2 and 4 at 72 DALR (Figure 3). Infection increased over time in pots 2, 4, and 7, reaching

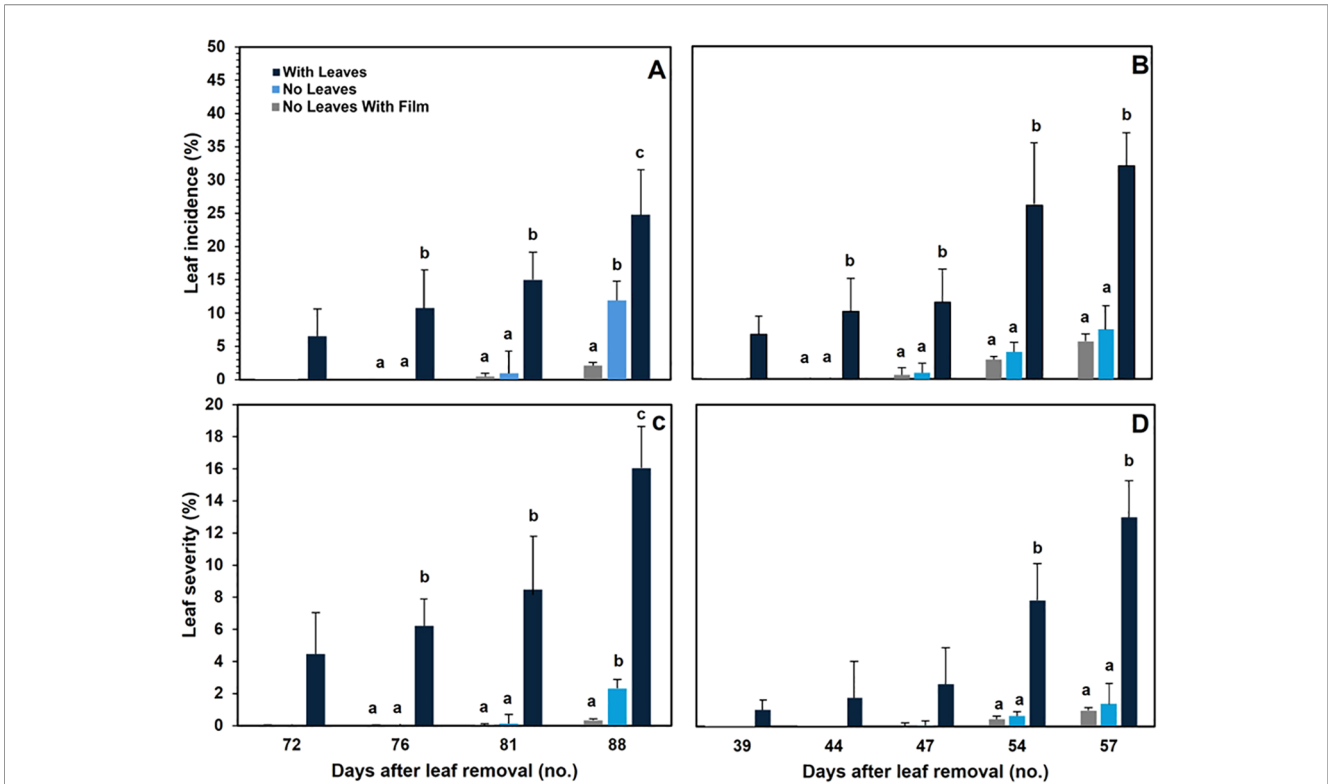


FIGURE 1 Strawberry powdery mildew leaf incidence (A) and severity (C) in 2023 (72, 76, 81, 88 days after leaf removal), and leaf incidence (B) and severity (D) in 2024 (39, 44, 47, 54 and 57 days after leaf removal). "No Leaves With Film" corresponds to the plants where infected leaves were removed and a plastic film was covering the substrate, "No Leaves" to the plants where infected leaves were removed, "With Leaves" to the plants where the infected leaves were not removed (untreated control). Letters indicate significant differences between groups according to the Tukey's adjusted test ($p < 0.05$) performed for each sampling time, while bars represent standard error. No letters indicate no significant difference between groups.

peak severity by 88 DALR. In contrast, patch development in pots 1, 3, 5, and 6 remained more localized. Without infected leaves independently from film addition a low number of patches developed in both years. In 2024 (Figure 3), patch distribution was more homogeneous across pots. Infected leaves led to

moderate/severe patch development, particularly from 47 DALR, with the highest intensity observed at 54 and 57 DALR in pots 2, 4 and 6. Without infected leaves and with plastic film patches developed, but at a lower intensity compared to treatment with infected leaves. Leaf development followed a similar progression

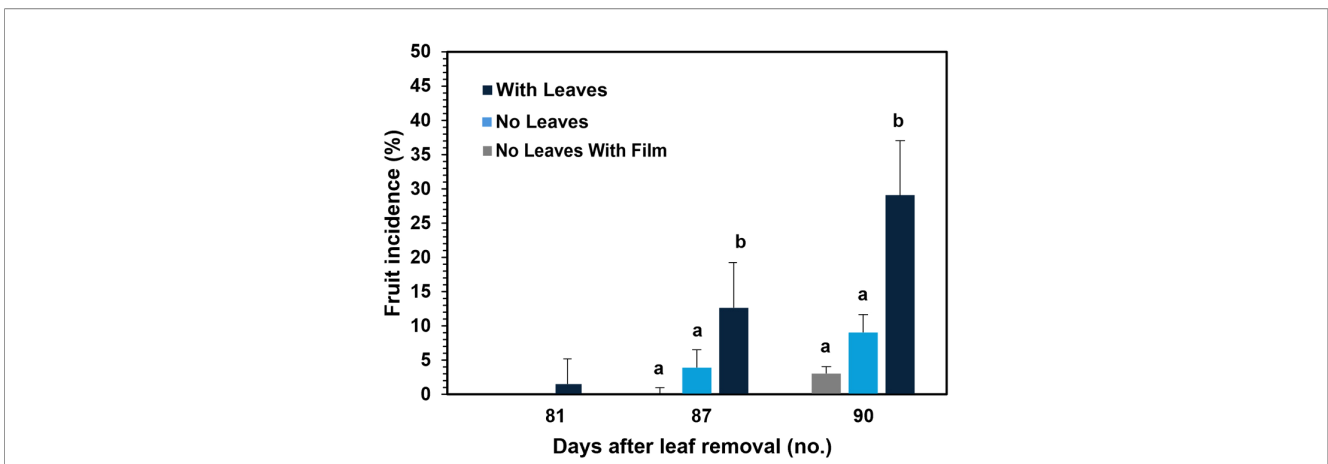


FIGURE 2 Incidence of strawberry powdery mildew on fruits (percentage of symptomatic fruits per plant) in 2023 assessed at 81, 87 and 90 days after removal of overwintered leaves and plant debris. Treatments are: No Leaves (leaves removed), With Film (leaves removed and pot substrate covered with plastic film) and With Leaves (untreated control). Different letters indicate significant differences between treatments at each sampling time (Tukey's adjusted test, $p < 0.05$), bars represent standard error. The absence of letters indicates no significant differences.

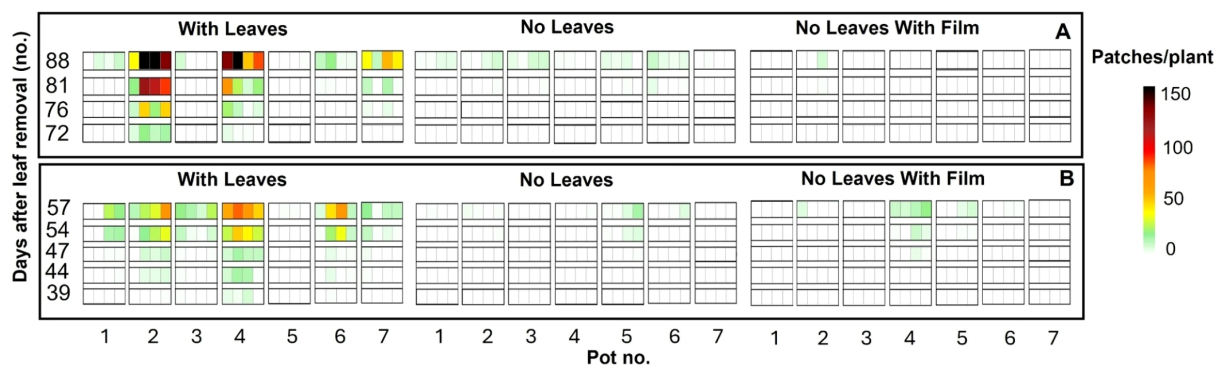


FIGURE 3 Heatmaps illustrate the progression of strawberry powdery mildew patches per plant in each of the randomized replicate in 2023 72, 76, 81, 88 days after leaf removal (a) and in 2024 39, 44, 47, 54, 57 days after leaf removal (b). In each treatment, the evolution of patches in each of the 7 replicated pots with 4 plants are shown and each plant is represented by a single square. The color gradient from white to black indicates patch number plant⁻¹.

across all treatments, and no significant differences were observed in the number of newly developed leaves throughout the monitoring period ($p = 0.2$).

Leaf age significantly affected IR, with younger leaves being more susceptible to disease than older leaves (Figure 4). A significant umbrella-shaped relationship between IR and leaf age was detected in both years by the Mack-Wolfe test (2023: $A_p = -31.14$, $p < 0.01$; 2024: $A_p = -35.10$, $p < 0.01$). In 2023, $IR > 0.1$ lesions day⁻¹ was displayed between 10–35 days old leaves, peaking 0.50 lesions day⁻¹ at 15 days-old after 76 DALR. In 2024, $IR > 0.1$ lesions day⁻¹ was observed in leaves aged 10–30 days, peaking at 0.50 lesions day⁻¹ in 10–15 days old leaves after 57 DALR. In 2023, the highest IR was registered at the second observation after 76 DALR, while in 2024 at the last observation after 57 DALR. No lesions developed on leaves older than 60 and 50 days in 2023 and 2024, respectively (Figure 4).

3.3 Chasmothecium formation in late summer

In both 2022 and 2023, chasmothecia began to form in late August, coinciding with the seasonal decrease of temperatures. Their formation started after a cumulative total of 8 hours below the threshold of 13 °C in 2022 and 16 hours in 2023. Notably, temperatures in late summer 2022 were cooler, with a higher accumulation of hours below 13 °C compared to 2023 (Figure 5). This cooler period is associated with a significantly higher density of chasmothecia, which reached up to 13.1 chasmothecia cm⁻² of leaf area. In contrast, the warmer condition in 2023 corresponded with 87% of reduction in their density (1.9 chasmothecia cm⁻²). The generalized linear model showed a positive relationship between the number of cumulative hours below 13 °C and chasmothecium formation (Estimate = 0.0034 ± 0.0003, $p < 0.001$).

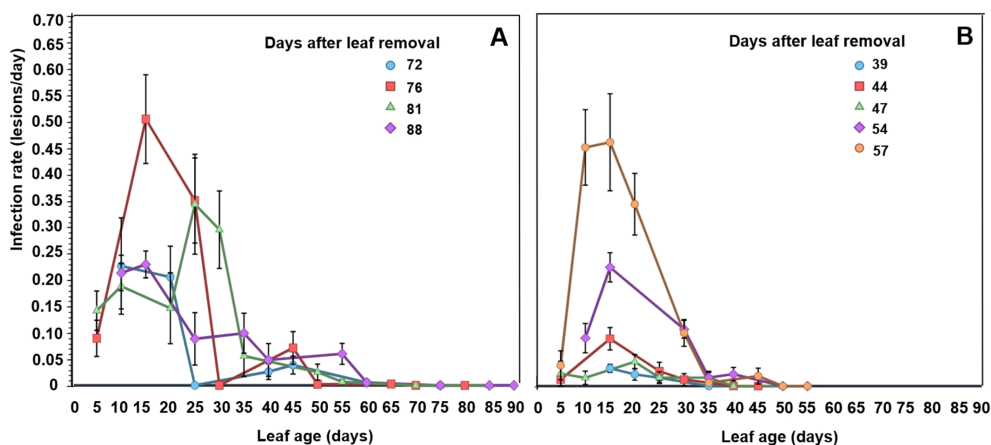


FIGURE 4 Infection rate by leaf age in 2023 (A) and 2024 (B), under high-tunnel conditions. Infection rate (IR) was calculated as $[\ln(f_2) - \ln(f_1)] / (t_2 - t_1)$, where f_1 and f_2 are the number of lesions per leaf at times t_1 and t_2 . Assessments were carried out twice a week. Leaf age is expressed as days after leaf expansion. Colors represent infection rate at different observations expressed as days after leaf removal. Bars represent standard error of the mean.

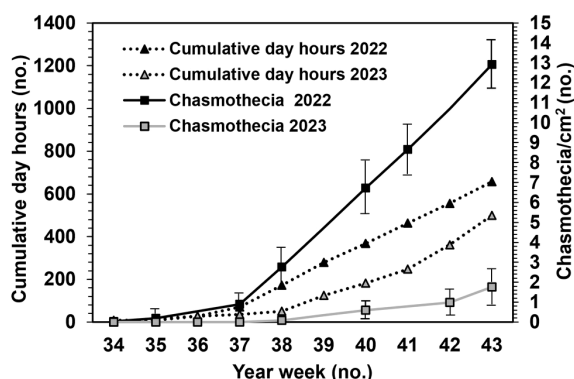


FIGURE 5 Chasmothecium formation of *Podosphaera aphanis* under high-tunnel conditions in 2023 and 2024 in relation to cumulative day hours with temperatures < 13 °C. Chasmothecia were counted on 30 mildew-infected leaves collected every 10 days from mid-August to end October. Data are expressed as the number of chasmothecia cm⁻² of both the upper and lower leaf surfaces.

3.4 Chasmothecium maturation and ascospore discharge

Empty chasmothecia, as proxy for ascospore discharge has been positively associated with environmental conditions (Estimate = 0.0049 ± 0.0008, *p* < 0.001), specifically with cumulative day hours with temperatures above 10 °C combined with continuous leaf-wetness periods ≥ 2 hours (Figure 6). Year had no significant effect (*p* = 0.28). In 2023, conducive conditions for ascospore discharge started in week 11, while in 2024 in week 18 (Figure 6). In 2023, infection onset was delayed by four weeks compared to 2024, likely due to cooler spring temperatures and lack of leaf wetness. By mid-March, 10% of chasmothecia in 2023 and 20% in 2024 were already empty. Thirty percent and 45% of chasmothecia opened once cumulative hours exceeded 21 hours in 2023 (week 15) and 18

hours in 2024 (week 19), respectively. Symptoms occurred one week after first openings in week 20 in 2023 and in week 16 in 2024, respectively.

Under controlled conditions (22 ± 1 °C), 80% of chasmothecia collected in 2023 and 50% collected in 2024 opened within 24 h, in mid-March (Figure 7). Under high-tunnel both years showed similar final opening rate, with approximately 70% of chasmothecia discharging ascospores (Figure 7) in end-May in 2023 and end-April in 2024.

4 Discussion

Understanding the overwintering dynamics of *P. aphanis* is essential for developing effective disease management strategies. A

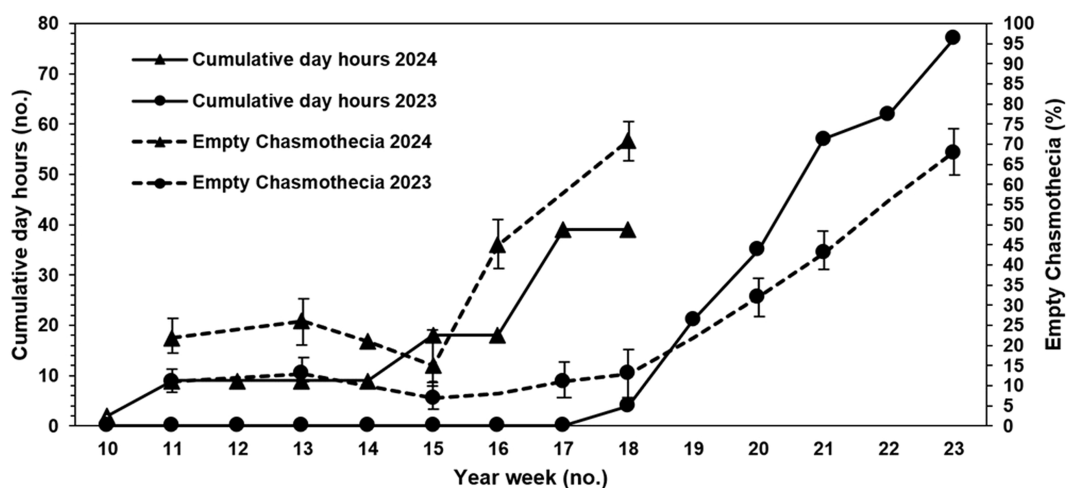


FIGURE 6 Proportion of empty chasmothecia of *Podosphaera aphanis* under high-tunnel in spring 2023 and 2024, shown in relation to cumulative day hours (>10 °C and ≥ 2 hours leaf wetness from 1 January). Empty chasmothecia are used as proxy for ascospore discharge and were assessed in 10 cohorts (15 chasmothecia cohort⁻¹) collected every 10 days from infected leaves in the tunnel. Bars represent the standard error of the mean.

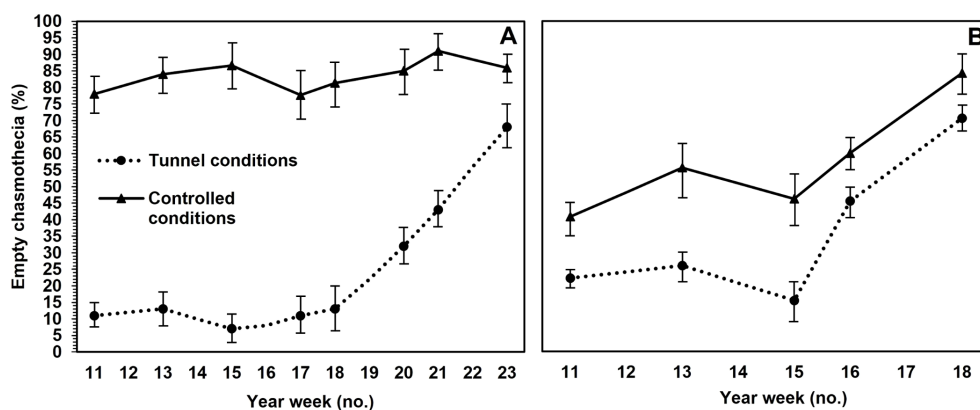


FIGURE 7

Empty chasmothecia of *Podosphaera aphanis* under high-tunnel and controlled conditions in spring 2023 (A) and 2024 (B). Empty chasmothecia are used as proxy for ascospore discharge and assessed i) under tunnel conditions on chasmothecia (10 cohorts, 15 chasmothecia cohort⁻¹) collected in the tunnel every 10 days from infected leaves, ii) under controlled conditions on chasmothecia (10 cohorts, 15 chasmothecia cohort⁻¹) collected in the tunnel every 10 days from infected leaves and placed in Petri dishes on water-soaked cotton at 22 ± 1 °C for 24 hours. Bars represent the standard error of the mean.

single infection focus can rapidly lead to serious epidemics (Aldrighetti et al., 2025), suggesting the importance of thorough removal of overwintering inoculum. Our findings demonstrated that removing infected leaves and plant debris reduced disease incidence by up to 70% on fruits and 63% on leaves, significantly delaying symptom onset (by 9 days in 2023 and 8 days in 2024). The delayed onset and lower disease severity in sanitized pots indicate that these late infections were primarily driven by conidium dispersal from the nearby infected plants, rather than by residual inoculum. We can therefore hypothesize that in the absence of external inoculum sources, disease development would have been further delayed and severity reduced. Despite the presence of high inoculum levels, the variability in disease severity among replicates indicates that primary infections under field conditions may be highly heterogeneous, resulting in heterogeneous foci. The presence of old leaves and/or morning dew may have created microclimatic differences between treatments, adding complexity to disease dynamics in the field.

Leaf age negatively affected IR, consistent with ontogenic resistance already described for SPM (Amsalem et al., 2006; Asalf et al., 2014; Carisse and Bouchard, 2010). Young leaves were more susceptible, with the highest IR (up to 0.50 lesions day⁻¹) between 10 and 15 days after unfolding and declining as leaves aged. The low IR on newly expanded leaves in our study can be explained with insufficient time for pathogen establishment at the time of assessment, as SPM symptoms typically appear 6–7 days after inoculation under field conditions (Dodgson et al., 2021). This apparently looks in contrast with previous studies (Asalf et al., 2014; Carisse and Bouchard, 2010). However, they assessed disease severity after the incubation period on leaves simultaneously inoculated at the various developmental stages. In contrast we assigned to each leaf a precise chronological age post-unfolding and we monitored IR over time, quantifying which leaf cohorts are more epidemiologically relevant for pathogen spread.

Unlike *E. necator* in grapevine, which overwinters in dormant buds and may initiate spring infections through flag shoots (Pearson and Goheen, 1988), in our study *P. aphanis* colonies were never found in the crown and in the newly emerged leaves at budbreak. This absence is in line with previous findings of Peries (1961), who reported no evidence of SPM mycelium in dormant strawberry buds. Excluding the role of mycelium in the bud as overwintering strategy of the pathogen, chasmothecia appeared to be the primary source of inoculum at the beginning of the growing season. While chasmothecia of *P. aphanis* are typically adherent to senescing leaves (Gadoury et al., 2010), physical degradation of plant material during overwintering, along with mechanical forces (Gadoury et al., 2010) may have led to the incorporation of viable inoculum into the substrate. In both years of our study, the application of the plastic film to prevent contact between the plant and the substrate did not alter the timing of early-season infections. This suggests that chasmothecia potentially present on small plant debris in the substrate did not play a major role in initiating the epidemic. As reported for other *Erysiphales*, chasmothecia that may have fallen on the substrate could have been degraded (Cortesi et al., 1995; Gadoury and Pearson, 1988). These findings demonstrate that removing infected leaves is sufficient to suppress disease onset. The use of plastic film did not increase disease development except for a slight increase in leaf severity observed at the final assessment in 2023. Although temperature and RH within the plant canopy were not measured, this result suggests that the plastic film did not substantially alter the microclimatic conditions under this treatment.

In our experiment, chasmothecium formation has been positively correlated with late-summer temperatures, validating previous laboratory studies (Asalf et al., 2013a; Gadoury et al., 2010), which reported optimal development under constant temperatures around 13 °C. In our trials, chasmothecia began to form after 8 and 16 cumulative hours below 13 °C in 2023 and 2024,

respectively. Their density was higher in 2023 and increased linearly with lower temperatures than 13 °C. However, despite the low number of chasmothecia formed in 2024, disease severity showed only a slight variation between years, suggesting that high disease severity is not necessarily correlated with high overwintering inoculum levels. By mid-March, chasmothecia formed under the high-tunnel were already mature and capable of discharging ascospores when exposed to favorable laboratory conditions (constant temperature of 22 °C and a water film), with 75% and 50% of chasmothecia opened in 2023 and 2024, respectively. Under high-tunnel conditions, ascospore discharge occurred when favorable environmental conditions were met, by late May in 2023 and late April in 2024, with 70% of chasmothecia releasing ascospores in both years. These results are consistent with those of Gadoury et al. (2010), who reported that 50% of chasmothecia opened by mid-April, with discharge (20%) starting as early as mid-March.

The coincidence between ascospore discharge and the onset of primary infections in both years provides further support for a central role of chasmothecia as the primary inoculum source for disease initiation in spring. Although direct measurements of airborne conidia were not performed and early infection events may have remained below the detection threshold, epidemic onset may be best explained by ascospore-mediated infection. Interestingly, the cumulative number of favorable hours required for infection (>10 °C combined with at least 2 hours of continuous leaf wetness) was remarkably consistent between years (21 hours in 2023 and 18 hours in 2024). This stability implies that monitoring cumulative hours of favorable conditions could serve as a reliable tool to anticipate the timing of the first infections, thereby improving the accuracy of disease warning systems and the efficiency of early-season management strategies. Even under protected conditions, leaf wetness was sufficient to trigger chasmothecia opening and ascospore release. Notably, these conditions are those reported for grapevine powdery mildew (Gadoury and Pearson, 1990; Rossi et al., 2010), suggesting common epidemiological drivers across pathosystems.

Considering that ascospore discharge in *P. aphanis* requires both free water on the ascocarp wall (Gadoury et al., 2010) and increasing temperatures, the removal of infected leaves should be carried out as early as possible in spring. Particularly for multi-season cultivation systems, during the first sunny days in spring, when plants are still on the ground and covered with polypropylene tissue, if RH increases and promotes dew formation, this can hydrate chasmothecia and promote ascospore release (Stensvand et al., 1998). Furthermore, nighttime plant coverings used for frost protection may create a moist microenvironment favorable to primary infection.

Although labor-intensive, this practice may still be justified for a high-value crop like strawberry. Leaf removal in the plurennial June-bearing cultivar required up to 500 hours ha⁻¹, reflecting the

high plant vigor and dense foliage of these plants. A comprehensive economic evaluation is anyhow warranted in each specific production context, since the potential benefit, for example reduced fungicide use and related residues on fruits, could offset the additional labor costs.

In conclusion, leaf removal plays a crucial role in SPM management by effectively reducing overwintering inoculum, thus delaying epidemic onset and lowering the need of fungicide applications. Scaling up this practice could provide insights into its economic viability as part of a broader integrated pest management strategy. Because leaf removal may impact plant physiology, future research should also consider yield quality and quantity over the entire production. Although the number of leaves did not significantly differ among treatments, the contribution of older leaves in early-season photosynthesis and resource allocation deserves further attention, as their removal may affect negatively fruit quality.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

AA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DZ: Data curation, Investigation, Writing – original draft, Writing – review & editing. IP: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Acknowledgments

The main author gratefully acknowledges Sant'Orsola Cooperative and in particular Gianluca Savini for his support, Loris Osti and Michele Bertoldi for their valuable technical assistance in strawberry production.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial

intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- AHDB Horticulture (2020). *Control of strawberry powdery mildew* (Kenilworth, UK: Agriculture and Horticulture Development Board). Available online at: https://projectblue.blob.core.windows.net/media/Default/Imported%20Publication%20Docs/AHDB%20Horticulture%20ControlStrawberryMildew_2638_190220_WEB.pdf (Accessed September 19, 2025).
- Aldrighetti, A., Vendrame, N., Nieri, R., Zardi, D., Farina, S., Rosà, R., et al. (2025). The role of wind in the spatiotemporal distribution of strawberry powdery mildew in high-tunnel growing systems. *Aerobiologia* 2, 1–18. doi: 10.1007/s10453-025-09869-7
- Amsalem, L., Freeman, S., Rav-David, D., Nitzani, Y., Szejnberg, A., Pertot, I., et al. (2006). Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. *Eur. J. Plant Pathol.* 114, 283–292. doi: 10.1007/s10658-005-5804-6
- Asalf, B., Gadoury, D. M., Tronsmo, A. M., Seem, R. C., Cadle-Davidson, L., Brewer, M. T., et al. (2013a). Temperature regulates the initiation of chasmothecia in powdery mildew of strawberry. *Phytopathology* 103, 717–724. doi: 10.1094/PHYTO-09-12-0252-R
- Asalf, B., Gadoury, D. M., Tronsmo, A. M., Seem, R. C., Dobson, A., Peres, N. A., et al. (2014). Ontogenic resistance of leaves and fruit, and how leaf folding influences the distribution of powdery mildew on strawberry plants colonized by *Podosphaera aphanis*. *Phytopathology* 104, 954–963. doi: 10.1094/PHYTO-12-13-0345-R
- Asalf, B., Gadoury, D. M., Tronsmo, A. M., Seem, R. C., and Stensvand, A. (2013b). “Increased knowledge of pathogen biology and epidemiology for integrated disease management: The case of strawberry powdery mildew,” in *NJF Seminar on IPM in Nordic and Baltic Berry Crops*, vol. 9. (NJF Report, Copenhagen, Denmark), 5–6.
- Asalf, B., Onofre, R. B., Gadoury, D. M., Peres, N. A., and Stensvand, A. (2021). Pooled water mists for suppression of strawberry powdery mildew. *Plant Dis.* 105, 71–77. doi: 10.1094/PDIS-04-20-0735-RE
- Blanco, C., de Los Santos, B., Barrau, A., Arroyo, F. T., Porras, M., and Romero, F. (2004). Relationship among concentrations of *Sphaerotheca macularis* conidia in the air, environmental conditions, and the incidence of powdery mildew in strawberry. *Plant Dis.* 88, 878–881. doi: 10.1094/PDIS.2004.88.8.878
- Braun, U., and Takamatsu, S. (2000). Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences: some taxonomic consequences. *Schlechtendalia* 4, 1–33.
- Caffi, T., Rossi, V., Legler, S. E., and Bugiani, R. (2011). A mechanistic model simulating ascospore infections by *Erysiphe necator*, the powdery mildew fungus of grapevine. *Plant Pathol.* 60, 522–531. doi: 10.1111/j.1365-3059.2010.02395.x
- Carisse, O., and Bouchard, J. (2010). Age-related susceptibility of strawberry leaves and berries to infection by *Podosphaera aphanis*. *Crop Prot.* 29, 969–978. doi: 10.1016/j.cropro.2010.03.008
- Cortesi, P., Gadoury, D. M., Seem, R. C., and Pearson, R. C. (1995). Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevines. *Plant Dis.* 79, 15–19. doi: 10.1094/PD-79-0015
- Dodgson, J., Hall, A. M., and Parker, S. (2008). *Control of strawberry powdery mildew under protection*. HDC Factsheet 17/08 (Kenilworth, UK: HDC Horticulture).
- Dodgson, J. L. A., Liu, B., Wileman, H. J., Mutasa-Gottgens, E. S., and Hall, A. M. (2021). Development and evaluation of a decision prediction tool for the reduction of fungicide applications for the control of strawberry powdery mildew epidemics. *bioRxiv*. doi: 10.1101/2021.08.04.455115
- Y. Elad, B. Williamson, P. Tudzynski and N. Delen (Eds.) (2007). *Botrytis: Biology, pathology and control* (Dordrecht, Netherlands: Springer).
- Gadoury, D. M., Asalf, B., Heidenreich, M. C., Herrero, M. L., Welsler, M. J., Seem, R. C., et al. (2010). Initiation, development, and survival of cleistothecia of *Podosphaera aphanis* and their role in the epidemiology of strawberry powdery mildew. *Phytopathology* 100, 246–251. doi: 10.1094/PHYTO-100-3-0246
- Gadoury, D. M., and Pearson, R. C. (1988). Initiation, development, dispersal and survival of cleistothecia of *Uncinula necator* in New York vineyards. *Phytopathology* 78, 1413–1421. doi: 10.1094/Phyto-78-1413
- Gadoury, D. M., and Pearson, R. C. (1990). Ascocarp dehiscence and ascospore discharge in *Uncinula necator*. *Phytopathology* 80, 393–401. doi: 10.1094/Phyto-80-393
- Gadoury, D. M., Seem, R. C., Wilcox, W. F., and Peres, N. A. (2009). Recent advances in epidemiology of strawberry powdery mildew. *IOBC/WPRS Bull.* 41, 85–92.
- Hodgdon, E. A., Conner, D. S., McDermott, L. G., Pritts, M. P., Handley, D. T., Orde, K. M., et al. (2024). A current view on strawberry production practices and trends in the Northeastern United States and Canada. *HortTechnology* 34, 574–584. doi: 10.21273/HORTTECH05457-24
- Holb, I. J. (2006). Effect of sanitation treatments on leaf litter, ascospore production of *Venturia inaequalis* and scab incidence in apple orchards. *Eur. J. Plant Pathol.* 115, 293–307. doi: 10.1007/s10658-006-9013-8
- Jhoo, J. S., and McKeen, W. E. (1965). Influence of host leaves on germination of the asexual spores of *Sphaerotheca macularis* (Wallr. ex Fr.) Cooke. *Can. J. Microbiol.* 11, 539–545. doi: 10.1139/m65-071
- MacHardy, W. E. (1996). *Apple scab: Biology, epidemiology, and management* (St. Paul, MN: American Phytopathological Society).
- Menzel, C. M. (2022). A review of powdery mildew in strawberries: resistance of species, hybrids and cultivars to the pathogen is highly variable. *J. Hortic. Sci. Biotechnol.* 97, 273–297. doi: 10.1080/14620316.2021.1985402
- Mezzetti, B., Giampieri, F., Zhang, Y. T., and Zhong, C. F. (2018). Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. *J. Berry Res.* 8, 205–221. doi: 10.3233/JBR-180314
- Neri, D., Baruzzi, G., Massetani, F., and Faedi, W. (2012). Strawberry production in forced and protected culture in Europe as a response to climate change. *Can. J. Plant Sci.* 92, 1021–1036. doi: 10.4141/cjps2011-276
- R. C. Pearson and A. C. Goheen (Eds.) (1988). *Compendium of grape diseases* (St. Paul, MN: American Phytopathological Society Press).
- Peries, O. S. (1961). Overwintering of *Sphaerotheca humuli* on strawberry plants. *Plant Pathol.* 10, 168–170. doi: 10.1111/j.1365-3059.1961.tb00006.x
- Peries, O. S. (1962). Studies on strawberry mildew, caused by *Sphaerotheca macularis*. I. Biology of the fungus. *Ann. Appl. Biol.* 50, 211–224. doi: 10.1111/j.1744-7348.1962.tb06004.x
- Rossi, V., Caffi, T., and Legler, S. E. (2010). Dynamics of ascospore maturation and discharge in *Erysiphe necator*, the causal agent of grape powdery mildew. *Phytopathology* 100, 1321–1329. doi: 10.1094/PHYTO-05-10-0149
- Samtani, J. B., Rom, C. R., Friedrich, H., Fennimore, S. A., Finn, C. E., Petran, A., et al. (2019). The status of the strawberry industry in the United States. *HortTechnology* 29, 11–24. doi: 10.21273/HORTTECH04135-18
- Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Steffek, R., Bylemans, D., Nikolova, G., Carlen, C., Faby, R., Daugaard, H., et al. (2004). Status of sustainable strawberry production within Europe. *Acta Hort.* 649, 247–250. doi: 10.17660/ActaHortic.2004.649.46

Stensvand, A., Amundsen, T., Semb, L., Gadoury, D. M., and Seem, R. C. (1998). Discharge and dissemination of ascospores by *Venturia inaequalis* during dew. *Plant Dis.* 82, 761–764. doi: 10.1094/PDIS.1998.82.7.761

Stensvand, A., Wang, N. Y., Le, V. H., Da Silva, C. D., Asalf, B., Grieu, C., et al. (2024). Aerated steam eradicates powdery mildew from strawberry transplants. *Eur. J. Plant Pathol.* 168, 199–205. doi: 10.1007/s10658-023-02744-6

Takeda, F. (1997). Strawberry production in soilless culture systems. *Acta Hort.* 481, 289–296. doi: 10.17660/ActaHortic.1999.481.31