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Epidemiological studies and sustainable approaches for the control of strawberry powdery mildew

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ABSTRACT

Strawberry powdery mildew, caused by *Podosphaera aphanis*, is one of the most destructive diseases of strawberries worldwide, particularly under protected cultivation. Difficulties in detecting and managing early infections result in rapid development and yield losses up to 70%. Control still relies on frequent applications of chemical synthetic fungicides, due to the broad range of environmental conditions favorable to pathogen development and lack of effective alternatives. Although extensive research is available, existing knowledge remains fragmented and offers limited support for effective disease management. The first objectives of the thesis were (i) to revise existing literature and (ii) assess growers' challenges to identify current research gaps and practical constraints in the field. After analyzing the extensive literature, major gaps persist regarding key aspects of disease epidemiology and the development of effective management approaches. In particular, the main drivers of rapid disease spread under field conditions, the management of overwintering inoculum to limit early-season epidemic onset, and the effective integration of alternative natural fungicides into residue-minimizing strategies remain poorly investigated. To address these gaps, the experimental objectives were: (iii) to characterize the role of wind in the spatiotemporal dispersal of the disease in high tunnels; (iv) to quantify the role of overwintering inoculum and evaluate post-winter leaf removal for the control of early-season primary infection; and (v) to develop a management strategy by limiting synthetic fungicides to early-season applications and evaluating the performance of alternative products post-flowering. Disease spreading was evaluated in a two-year trial, where spatiotemporal colony development (along a 17-m high-tunnel, for 17 days) originated from a single source of inoculum, was compared under both natural and reduced wind conditions. Results showed that wind strongly influenced spatial spread and secondary hot-spot formation, yet *P. aphanis* dispersed efficiently even under reduced wind, advancing up to ~1.7 m day^{-1}. To quantify the role of overwintering inoculum in epidemic onset, the effect of post-winter leaf removal in an unsprayed plot was assessed by comparing non-sanitized plants with sanitized plants in which complete leaf removal was performed. In parallel, chasmothecium development in autumn and chasmothecium opening in spring were monitored according to temperature and leaf wetness and related to primary infection onset. Results showed that post-winter removal of infected/old leaves consistently reduced disease incidence by 50% and delayed onset. Chasmothecium development started after 8 and 16 cumulative hours in 2023 and 2024, at late-summer temperature <13 °C, while chasmothecium opening in spring started after 21 and 18 cumulative hours in 2023 and 2024 at temperatures >10° and at least 2 hours leaf wetness. Finally, to design a strategy to reduce the number and quantity of residues, we compared a standard chemical fungicide program with three integrated strategies each involving the substitution of synthetic fungicides after flowering with application of alternative products, namely an aqueous extract from germinated *Lupinus albus* L. seeds, potassium bicarbonate and sulfur. Results showed that limiting synthetic fungicides to the pre-flowering period and relying on natural products thereafter reduced residue numbers by up to 57% while maintaining disease control comparable to the standard program. Overall, this work showed that *P. aphanis* spreads rapidly in high tunnels even under minimal wind, implying that few initial foci can trigger fast epidemics. Combining rigorous post-winter leaf removal and synthetic chemical fungicides early in the season is therefore pivotal for reducing primary infection risk and delaying disease onset. When followed by the use of effective alternatives, low-residue strawberry production becomes achievable, supporting more sustainable and reliable disease management.

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most valuable small fruit crops worldwide, with global production exceeding 9 million tons and a steadily growing market demand (FAO, 2024). To meet off-season demand, production has shifted from conventional field systems to protected, substrate-based cultivation (Fig. 1), which decouples cropping from local seasonality but introduces new challenges for disease management and sustainability (Neri et al., 2012). Plastic tunnels and greenhouses partially control temperature, humidity and rainfall, thereby extending the production window and improving fruit quality. While these structures reduce diseases driven by prolonged leaf wetness, such as *Colletotrichum fragariae* A.N. Brooks (Wilson et al., 1990), *Botrytis cinerea* Pers. (Jarvis, 1964) *Mycosphaerella fragariae* (Tul. & C. Tul.) Lindau (Carisse et al., 2000), such protected conditions characterized by reduced solar radiation, absence of rainfall, high relative humidity and limited ventilation, promote strawberry powdery mildew (Amsalem et al., 2006; Asalf et al., 2021). Strawberry powdery mildew, caused by *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatsu, is one of the most destructive diseases affecting strawberry production worldwide (Menzel, 2022). The disease is often difficult to detect at early stages because initial infections develop mainly on the abaxial leaf surface (Sombardier et al., 2009). Once established, it progresses at exponential rate (Carisse et al., 2013b), causing yield losses of up to 70% (Dodgson et al., 2007; Peries, 1962;) and severe reductions in fruit quality (Peries, 1962). Because of the broad range of conducive conditions (15°<T<32°C, >55% relative humidity) (Amsalem et al., 2006) the risk of infection is frequently met from transplanting to the end of harvest.

To manage strawberry powdery mildew, farmers typically rely on intensive, calendar-based fungicide programs (Carisse et al., 2013a). While effective in the short term, repeated applications accelerated the selection of resistant *P. aphanis* populations, leading to loss of efficacy (Palmer and Holmes, 2021); as a result, conventional chemical control becomes increasingly unreliable with negative environmental and health impacts (Munoz-Leoz et al., 2011; Rjiba-Touati et al., 2023). Despite advances in research and the development of alternative control methods, growers still lack sustainable strategies that could serve as a viable substitute for chemical fungicides. For these reasons, IPM strategies (including better understanding of key epidemiological stages, cultural practices, natural products and physical methods) would be required to guarantee effective disease suppression while ensuring environmental sustainability and reducing the risk of residue accumulation on harvested fruit. Although research on strawberry powdery mildew is substantial, it remains highly fragmented. A structured synthesis of existing studies is therefore needed to consolidate current knowledge and highlight remaining research gaps.

Fig. 1 – Substrate-based strawberry production in high-tunnel growing system.

AIM OF THE THESIS AND SPECIFIC OBJECTIVES

The aim of this thesis is to provide strawberry growers and agronomists with evidence-based knowledge and practical, sustainable tools for managing strawberry powdery mildew under protected cultivation and minimize fungicide residues on fruits. To this end, the specific objectives are:

(i) To review existing knowledge on the epidemiology and methods for strawberry powdery mildew control and identify key research gaps requiring further investigation by undertaking a comprehensive literature review.

(ii) To assess growers' practices, knowledge and needs to address real on-farm challenges, by organizing a focus group.

(iii) To clarify the factors driving the rapid progression of the disease under high-tunnel conditions by characterizing the role of wind in the spatiotemporal dynamics of dispersal from a single inoculum source.

(iv) To reduce overwintering inoculum by validating infected/old-leaf removal as an early-season control method for primary infections;

(v) To validate low-residue integrated strategies under field conditions by designing a strategy that combines effective alternatives with optimized conventional programmes.

The first objective is addressed in the chapter "Epidemiology and control of strawberry powdery mildew: a review". The paper has been published in the Journal "Phytopathologia Mediterranea" in November 2023 (https://doi.org/doi.org/10.36253/phyto-14576). The second objective is addressed in the chapter "Grower insights into strawberry powdery mildew management: a focus-group analysis". The third objective is addressed in the chapter "The role of wind in the spatiotemporal distribution of strawberry powdery mildew in high-tunnel growing systems". The paper has been published in the Journal

"Aerobiologia" in May 2024 (https://doi.org/10.1007/s10453-025-09869-7). The fourth objective is addressed in the chapter "Sanitation practices targeting overwintering inoculum improve management of strawberry powdery mildew in high-tunnel production". The paper has been published in January 2026 by "Frontiers in Agronomy – Disease Management" (https://doi.org/10.3389/fagro.2026.1729740). The fifth objective is addressed in the chapter "Sequential use of pre-flowering chemical fungicides and post-flowering natural products effectively controls strawberry powdery mildew while maintaining low fruit residues". The paper has been published in February 2026 by "Crop Protection" (https://doi.org/10.1016/j.cropro.2026.107572).

Epidemiology and control of strawberry powdery mildew: a review

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Abstract

Strawberry powdery mildew, caused by *Podosphaera aphanis*, is an economically important disease for strawberry production. Typical symptoms are white mycelium on all aerial parts of affected plants, with young host tissues being the most susceptible. The pathogen overwinters on infected leaves, either as mycelium or chasmothecia, although the quantitative role of chasmothecia in epidemics are not fully understood. In spring, under favourable conditions, the fungus sporulates, disseminating conidia and causing polycyclic infections. The disease is mainly controlled using synthetic fungicides, but there is increasing interest in sustainable alternatives, including microbial biocontrol agents (e.g., *Ampelomyces quisqualis, Bacillus* spp.*, Trichoderma* spp.) and substances of plant or animal origin (e.g., *Equisetum arvense*, orange oil, chitosan, whey). Physical methods, (e.g. UV-C, ozone) are also promising alternatives to fungicides. All of these strategies should be combined with appropriate agronomic practices (e.g., overhead irrigation, canopy management) to create unfavourable environments for the pathogen. However, agronomic practices have never been assessed for *P. aphanis*. Disease forecasting models and DSSs, though available, are underutilized due to their complexity and lack of validation across locations.

This review presents the current state of knowledge on *P. aphanis* the available methods for control of strawberry powdery mildew, and highlights knowledge gaps relating to this host/pathogen relationship.

Keywords. *Podosphaera aphanis*, natural substances, biocontrol, agronomic practices, disease forecasting models.

Introduction

Strawberry powdery mildew (SPM), caused by *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatsu is a common disease, particularly in subtropical and tropical regions where strawberry (*Fragaria* × *ananassa* Duch) is grown (Nakzawa and Uchida, 1998; Amsalem et al., 2006; Gadoury et al.,2010; Carisse and Fall, 2021; Kasiamdari et al., 2021; Palmer and Holmes, 2021). Most strawberry cultivars are highly susceptible to the disease, and only very few are tolerant (Menzel, 2022). Strawberry powdery mildew is mostly managed by synthetic fungicides that are sprayed regularly from emergence of the first leaves to the end of the harvest season (Carisse et al., 2013a). This high use of fungicides fosters the build-up of resistant *P. aphanis* populations and has potentially negative impacts on animal and human health and the environment (Muñoz-Leoz et al.,2011; Rjiba-Touati et al., 2023). Due to increasing concerns relating to pesticides, consumers preferences have changed and are increasingly opting for food products free of pesticide residues (Rimal et al., 2001). As a result, agrarian systems are moving to sustainable and ecofriendly phytosanitary solutions, which foster research and development of innovative approaches to disease management (Deresa and Diriba, 2023).

Significant progress has been made to develop alternatives for management of SPM, and many publications confirm this strong scientific commitment. However, strawberry producers still lack effective methods for managing SPM that can be considered as viable substitutes for chemical fungicides (Deresa and Diriba, 2023).

The aim of this review is to summarize current knowledge on SPM, and to highlight gaps in understanding which, if clarified, could contribute to increased effectiveness of SPM management.

Methodology

This review is structured into the following sections: classification and morphology of *P. aphanis*, and the symptoms of SPM; epidemiology and the most significant stages of the disease cycle; conventional and alternative control methods for SPM; agronomic practices that must be integrated for effective disease control; and the most relevant predictive models, decision support systems (DSSs) and early detection systems for SPM. The review concludes by suggesting future research to improve SPM management.

The relevant literature was reviewed using Google Scholar, Scopus, and Web of Science searches, for reports published from 1962 to 2023. The following keywords were used alone and in combinations in the searches: *Ampelomyces quisqualis*, airborne inoculum, *Bacillus*, basic substances, bioassay, biochar, biological agent, biostimulants, chasmothecia, classification, cleistothecia, conidia, conidiophores, control, cultural practices, decision support system, detection, disease, distribution, environmental conditions, epidemiology, essential oils, field, fungicide, inorganic salts, irrigation, life cycle, low-toxicity compounds, machine learning, model, morphology, mycophagous mite, nutrition, overhead irrigation, overwintering inoculum, ozone treatment, plant extract, *Podosphaera aphanis*, predictive model, resistance, seaweed extract, *Sphaerotheca macularis*, symptoms, strawberry powdery mildew, *Trichoderma*, UV treatment, water stress.

The first search (46 papers) was carried out in order to select the first and the most cited records for the classification of *P. aphanis* (eight papers), its morphology (two papers), and the symptoms it causes (nine papers). A second search (27 papers) focused on the fungus life cycle (18 papers) and SPM epidemiology (11 papers). A third search (104 papers) aimed to identify the fungicides (nine papers) and the alternative products assessed for SPM control (95 papers) by focusing on classical and advanced solutions such as biological control (11 papers), inorganic salts (21 papers), plant extracts (16), seaweeds (10 papers), substances from animal origin (six papers), chitin and its derivatives (12 papers), UV-C (nine papers) and ozone technologies (six papers). The selected papers of the third search were analysed according to the research outcomes, carried out under field or laboratory conditions. When data on *P. aphanis* were lacking and alternatives for management of other powdery mildews could be useful indicators for future research, those alternatives were included in the review. A fourth search was carried out to identify agronomic practices useful for management of SPM, such as canopy management (eight papers), plant nutrition (four papers), overhead irrigation (two papers), genetic resistance (seven papers) or spray equipment (eight papers). In this fourth search, in cases where there was no literature available on SPM, literature related to other powdery mildews was analyzed. The fifth search included DSSs (17 papers), and early disease detection systems (six papers). Papers were not included when they showed low quality of experimental designs and data analysis, reported low powdery mildew severity in experimental controls (only for the efficacy trials), or were redundant due to other similar and previous results.

The pathogen and the disease

Podosphaera aphanis (*Erysiphaceae, Ascomycetes*) was first reported (sexual stage) in the United States of America (Geneva, New York) in 1886 (Arthur, 1886). In Europe, this fungus was identified a few years later (Salmon, 1900), when its asexual stage was also described. The causal agent of SPM was initially thought to be the same as hop powdery mildew, *Podosphaera macularis* (Wallr.) U. Braun and S. Takamatsu [formerly *Sphaerotheca macularis* (Wallr.) Magnus] (Jhooty and McKeen, 1965). In 1976, Liyanage and Royle discovered that powdery mildews of strawberry and hop were caused by two different pathogens. Recent taxonomic studies have described clear distinction between ascocarp appendages of *Podosphaera* and *Sphaerotheca* (Braun, 1982; Braun and Takamatsu 2000), which necessitated a change of the genus name of the agent of SPM to *Podosphaera* (Cook et al., 1997; Kirk et al., 2001).

Morphological characteristics, originally described in 1987 (Braun, 1987), were recently displayed with digital light microscopy (Iwasaki et al., 2021). The hyaline conidia of *P. aphanis* are ellipsoid–ovoid to doliiform–limoniform in shape and contain oil and fibrosin bodies. Their dimensions are 27–33 × 18–22 µm. The appressoria, which develop on germinated conidia, are 4 µm wide. Conidiophores (dimensions 84–129 × 8–11 µm) each produce six concatenated conidia. Chasmothecia (Fig. 1) are dark brown (100–125 × 65–80 µm) and are firmly attached to the surrounding mycelium. Each chasmothecium (Fig. 1) contains one ascus (dimensions 60–94 × 55–76 µm), which contains eight ellipsoid to subglobose ascospores.

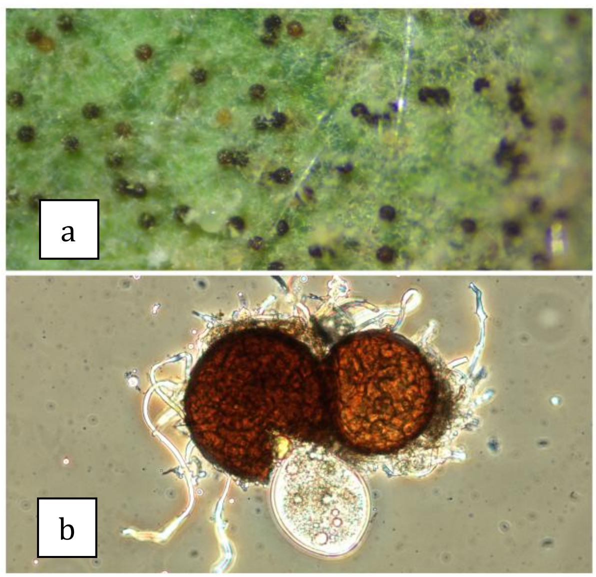

Fig.1 - a) Chasmothecia of *Podosphaera aphanis* on an abaxial surface of a strawberry leaf. b) Open chasmothecium and with released ascus.

Symptoms of strawberry powdery mildew

The typical symptoms of SPM are white powdery patches of mycelium and conidia, spread across all aerial parts (leaves, runners, flowers, fruit) of affected host plants (Fig. 2, a to i). Host tissues can be affected at all stages of development, although young organs (e.g., not fully expanded leaves, flowers, green berries) are more susceptible than older tissues (Carisse and Bouchard, 2010; Asalf et al., 2014). As the disease progresses, leaf edges curl upwards, and purple to reddish irregular blotches may develop on the leaf surfaces (Lambert et al., 2007) (Fig. 2, c and d). Round black chasmothecia may be visible on abaxial leaf surfaces, in late summer/autumn (Gadoury et al., 2010). Severe infections can cause strawberry yield losses of up to 30% (Carisse et al., 2013b), due to the white mycelium covering ripe and unripe fruit, fruit deformation (Fig. 2, g, h and i), hardening and dehydration, achene exposure (Fig. 2 g), and eventual fruit decay. Beside negative impacts on fruit quality, photosynthesis reduction, plant stunting and flower abortion are also associated with SPM (Peries, 1962a; Jhooty and McKeen, 1965; Gooding et al., 1981; Maas, 1998; Amsalem et al., 2006), although no data are available on the yield losses caused by these types of symptoms.

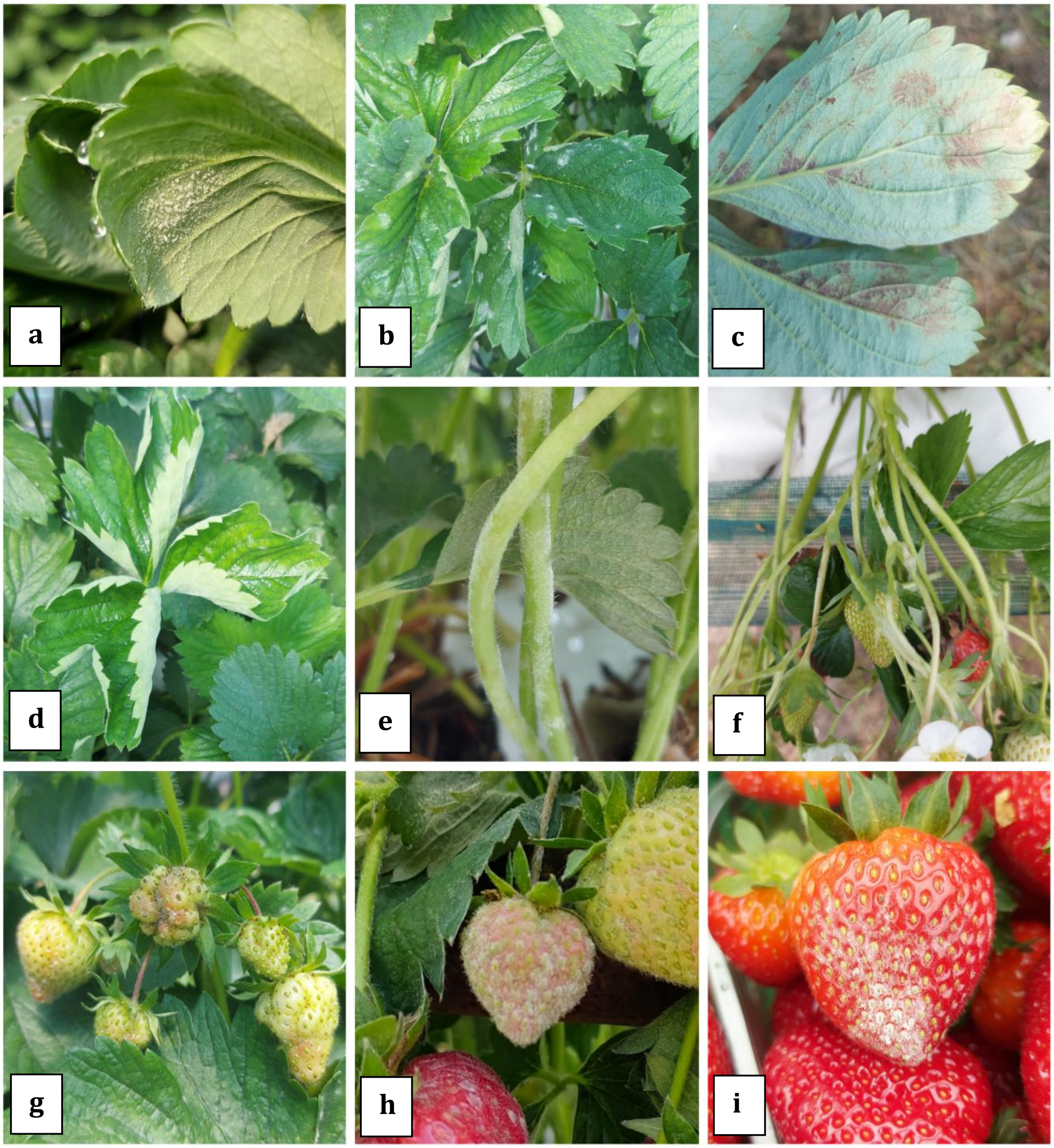

Fig. 2- Strawberry powdery mildew symptoms. a-b) white patches on the abaxial and adaxial leaf surface, c) red blotches on the leaf surface, c-d) leaf curling, e-f) white patches on leaf and flower petioles, g) fruit deformation, h-i) white mycelium and white patches on unripe and ripe fruits.

The disease cycle of Podosphaera aphanis

The disease cycle of strawberry powdery mildew (Fig. 3) has been extensively investigated. The pathogen overwinters as mycelium on living infected leaves, and sporulation recommences in spring, leading to conidium dissemination and consequent polycyclic infections (Gadoury et al., 2010; Iwasaki et al., 2021) (Fig. 3). Nevertheless, *P. aphanis* can also overwinter as chasmothecia, which developed in late summer/autumn (Gadoury et al., 2010; Jin et al., 2012) on the infected host leaves, in commercial fields or in the nurseries (Peries, 1962b). In spring, commonly from early March to late May in the northern hemisphere, mature chasmothecia release ascospores, which are responsible for the early infections on plants (Gadoury et al., 2010) (Fig. 3).

Asexual reproduction

Extensive research has been conducted on the processes of asexual reproduction during host vegetative growth, including laboratory and field studies on conidiation and polycyclic infections. These have provided insights into the dynamics of fungal development and dissemination. After infection, temperatures between 18 and 25°C at 97–100% relative humidity (RH) favour enlargement of the lesions, leading to conidiation (Miller et al., 2003; Amsalem et al., 2006). Conidiophores each develop from a generative cell that aft er a gradual upward elongation, produces conidium chains. Conidia are released when mature, following the individual order of development. Each time a conidium is released, the generative conidiophore cell starts to form a new conidium. The lifetime of conidiophores, from generative cell formation until the first conidium release, is approx. 125 to 150 h. At 22°C and 45–55% RH, with wind speed of 0.5 m s^{-1} (necessary for conidial detachment), each conidiophore releases an average of 38 progeny conidia within 96 h (Iwasaki et al., 2021). Within a colony lifetime (35 d after inoculation) each colony can release an average of 6.7×10^4 conidia (Ayabe et al., 2022).

Under laboratory conditions (22±1°C, 45–55% RH), conidia of *P. aphanis* germinate within 4–5 h after inoculation, with each conidium forming a germ tube that develops into an appressorium (Iwasaki et al., 2021). After successful host penetration, achieved by enzymatic and mechanical processes, a haustorium forms within the host epidermal cell, and typically invades the host plasma membrane 1 d after inoculation, and hyphal growth commences. Conidiophores develop 3–5 d after inoculation, and conidiation usually commences 6 d after inoculation (Peries, 1962a; Jhooty and McKeen, 1965; Iwasaki et al., 2021).

Conidia can germinate between 3 and 32–38°C (Jhooty and McKeen, 1965; Sombardier et al., 2009), and temperature influences the rate and speed of germination. For example, between 15 and 25°C germination of conidia varies between 85 to 88% (Amsalem et al., 2006), while only 1% germination was recorded at 5 or 35°C (Amsalem et al., 2006). At 5, 10, and 15°C, minima of, respectively, 25, 15 and 12 h were required for conidium germination, while between 18 and 30°C only 5 h were necessary (Peries, 1962a). Conidium germination rates are also influenced by different leaf surfaces, with is 20% greater germination on abaxial than adaxial surface (Maas, 1998; Sombardier et al., 2009). As for many powdery mildews, free water is detrimental to conidia and mycelium of *P. aphanis* (Peries, 1962a; Sombardier et al., 2009).

Sexual reproduction

Podosphaera aphanis is heterothallic, so initiation of chasmothecia begins when antheridium and ascogonium are formed from the mycelium of different mating types. Myceloid appendages extended from the outer chasmothecia wall are directed downward to the mycelium and tenaciously attached (Asalf et al., 2013). Initiation of ascocarps is regulated by temperature. The most favourable temperature for chasmothecium development is approx. 13°C, that occurs 10 to 14 days after inoculation (Asalf et al., 2013). Up to 400 chasmothecia per cm2 of leaf form after 14 d incubation at this temperature. However, chasmothecium development is largely suppressed at temperatures >13°C. For example, at 20C° the mean number of chasmothecia per cm2 was up to 21, and the incidences of leaves bearing chasmothecia at 9 or 12°C were much greater (respectively, 92 and 93%) than at 15, or 18°C, (respectively, 7 and 6%) (Asalf et al., 2013). Chasmothecia have different developmental stages: white, brown, and black when mature. Rupture of the ascus and ascospore release generally occurs at 22 to 25°C provided that the ascocarp remains in contact with a film of water (Gadoury et al., 2013).

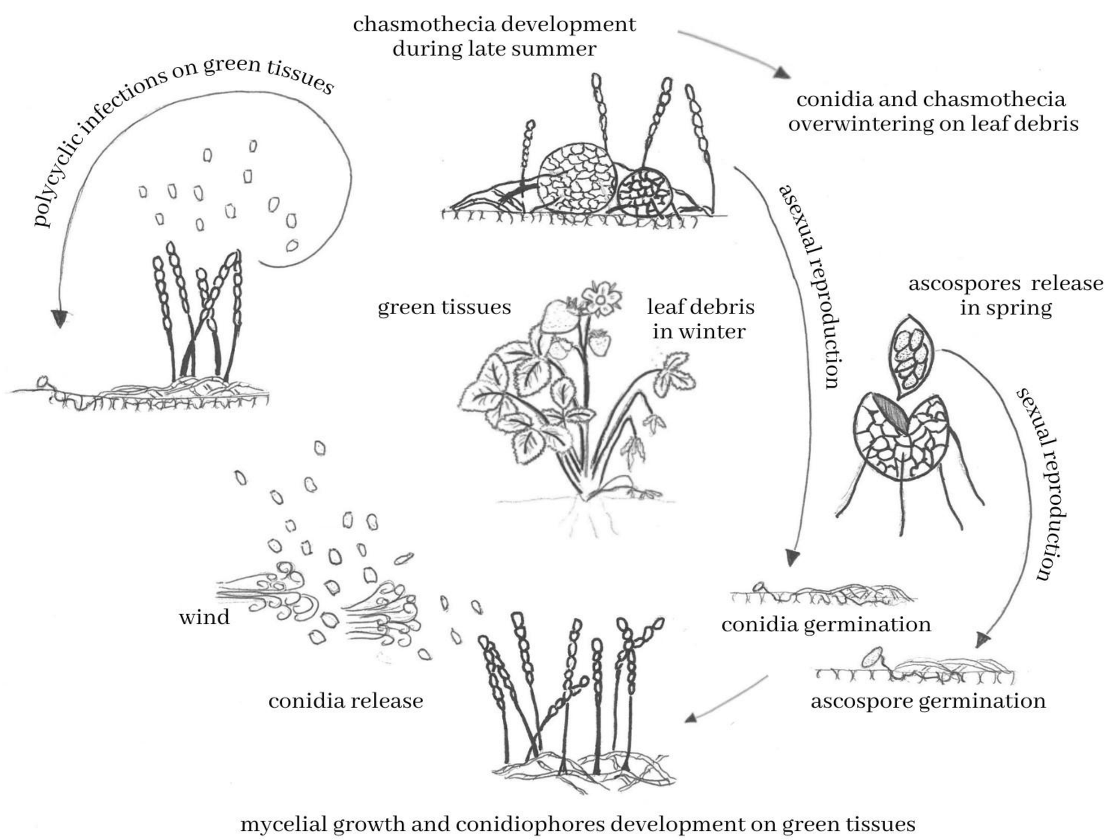

Fig.3 - Disease cycle of strawberry powdery mildew.

Epidemiology of strawberry powdery mildew

Environmental factors influencing the disease

Primary infections

The role and quantitative contribution of chasmothecia in initiation of SPM epidemics is not clear, and in some regions the asexual stage prevails over the chasmothecia, which are rare or absent (Howard and Albregts, 1982). This indicates a secondary role of chasmothecia in the SPM epidemiology. One possible reason is that geographically discontinuous distributions of mating types may prevent/reduce sexual reproduction (Gadoury et al., 2010). A second reason could be unsuitable temperatures for the ascocarp initiation (Gadoury et al., 2013). Temperature is a key environmental factor influencing ascocarp formation.

While the most favourable temperatures for the development of chasmothecia are well-documented, the conditions for chasmothecium survival during winter, and the related viability of ascospores, have been little studied. In a 4-year survey carried out in New York State and Norway, proportions of chasmothecia containing viable ascospores (i.e. positively reacting to fluorescein diacetate stain) consistently exceeded 80%. In contrast, ascospore germination on glass, investigated in the last two years of the same experiment, was highly variable, ranging from 42% to 98% (Gadoury et al., 2010). This variability underscores the need to explore the factors affecting ascospore germination and infection rates. Integration of such data into powdery mildew predictive models could increase prediction precision to assist commencement of disease control treatments at the beginning of strawberry production seasons. However, quantitative estimation of initial inoculum is a challenge for all forecasting models that have been developed in other powdery pathosystems (Gubler et al., 1999; Caffi et al., 2011).

Secondary infections

As with the majority of *Erysiphales*, *P. aphanis* releases conidia mainly during daytime (Blanco et al., 2004), and conidium release is affected by temperature and RH fluctuations. The release of conidia is directly correlated with increase in temperature and decrease in RH (Blanco et al., 2004). For example, Blanco et al. (2004) showed, in a 2-year experiment, that in the first year minimum conidium release occurred at 12°C and 86% RH, and maximum release was at 14°C and 73% RH. In the second-year release was at 13°C and 82% RH, and maximum release was at 18°C and 54% RH.

The quantity of conidia and the timing of their release are key for development of SPM epidemics (Willocquet et al., 1998; Van Maanen and Xu, 2003). As for other *Erysiphales*, populations of airborne SPM conidia depend on the quantity of infected organs in a crop. For example, there is a close correlation between the weekly average aerial concentration of conidia (conidia/m^3) and the weekly average number of diseased leaves and berries (Blanco et al., 2004; Van der Heyden et al., 2014). As the number of conidia in the air and the infected organs in a field are closely related, the number of conidia in the air and the first visible symptoms on plants are also closely related. If promptly recognized, the critical conidium concentration threshold at which crops must be treated could be crucial to avoid field disease outbreaks. First SPM symptoms likely to become visible after 7-14 d, when the airborne conidium concentration captured with an air sampler has recorded more than 500 conidia m^{-3} d^{-1} (Carisse and Bouchard, 2010).

Because SPM is a wind-borne disease, understanding patterns of conidium dispersal under field conditions is important for implementing disease control strategies. However, accurate models of pathogen spread over time from specific inoculum sources have not been developed, although there have been attempts to measure conidium dispersal. For example, after 3 d of exposure, the dispersal radius from infected plants used as inoculum sources was 1.2-1.5 m (Peries, 1962b). The dispersal of conidia from an infected source changes according to the environment. For example, dispersal is greater under plastic tunnels than in greenhouses (Willocquet et al., 2008), and is greater in open fields than in plastic tunnels (Carisse et al., 2013a). The hypothesis for these effects is that wind is less in greenhouses than in plastic tunnels, and less in tunnels than in open fields (Willocquet et al., 2008; Carisse et al., 2013a). In addition, this may explain why SPM dispersal in an open field is difficult to determine, because dispersal has heterogeneous patterns (Van der Heyden et al., 2014).

Agronomic factors influencing the disease

Cropping systems and crop cultivars

In response to growing market demand for strawberries, cropping systems have evolved from classical field production to highly complex approaches. The need to provide high-quality product throughout the year has led to progressive replacement of short-day (Junebearing) varieties with day-neutral (or everbearing) varieties, that produce fruit for the entire season. Both varieties June-bearing and everbearing are susceptible to powdery mildew, but the latter are more exposed to pathogen infections during their life cycle in summer (Maas, 1998). Whereas June-bearing varieties produce fruit until late spring, the growing season of day-neutral varieties coincides with optimal conditions for disease development in midsummer (Maas, 1998; Carisse and Bouchard, 2010; Blanco et al., 2004). Risk of infection is further enhanced where June-bearing and day-neutral varieties coexist. There is an overlapping production at the beginning of the season of new transplanted dayneutral plants with overwintering infected June-bearing varieties, which are sources of inoculum (Fall and Carisse, 2022). In subtropical regions, to ensure high yields, June-bearing varieties are planted in mid-summer for the first harvest, in late summer, and, after overwintering in the field, these crops each produce a second harvest in the following late-spring. The day-neutral varieties, on the other hand, are planted in mid-spring for a single growing season, that partially overlaps with the growing cycle of the June-bearing plants (Carisse and Fall, 2021).

As well as high susceptibility of everbearing strawberry varieties, the adoption of soilless production on raised beds in plastic tunnels or greenhouses gives environmental conditions that are conducive to powdery mildew (Xiao et al., 2001). Under coverage, SPM is not inhibited by rain and/or prolonged leaf wetness, which can stop conidium germination and eventually kill conidia in open fields. In addition, polyethylene/glass shading decreases sunlight intensity, favouring powdery mildew development because these pathogens are strongly photosensitive (Amsalem et al., 2006; Elad et al., 2007).

Plant water stress

Effects of plant water stress on *P. aphanis* infections has not been extensively studied, although for other powdery mildews host water stress reduces hyphal growth, slowing colonisation of new tissues, and also disrupts conidiation (Ayres and Woolacott, 1980; Caesar and Clerk, 1985). Xu et al. (2013) and Rossi et al (2020) showed positive correlations among plant hydration, disease susceptibility, and pathogen fitness. For example, 21 d after inoculation, water stressed plants showed slight reductions in disease severity on abaxial leaf surfaces compared to the well-hydrated plants (Rossi et al., 2020). Host water stress also affects conidium germination. Germination rates differed for conidia collected from plants grown at different soil moisture levels. Germination, assessed on water agar, increased linearly from 0 to 30% for conidia collected from plants grown in soil moisture levels ranging from 0 to approx. 53%.

Control methods and approaches

Fungicides

In the European Union, synthetic fungicides authorised for the control of SPM and categorised based on their modes of action (Frac Code List, 2022) (Table 1), belong to the following groups: hydroxy-(2-amino-) pyrimidines (A2), succinate dehydrogenase inhibitors (C2), quinone outside inhibitors (QoIs; C3), (C5), demethylation inhibitors (G1), and others with unknown modes of action (U). To reduce risks of selecting resistant pathogen populations, fungicides with different modes of action must be combined in appropriate disease management strategies (Palmer and Holmes, 2021). The majority of active substances authorised for the use against *P. aphanis* belong to few modes of action groups, resulting in recurrent use of a limited number of products with the same modes of action. Some of these fungicides, such as the triazoles (demethylation inhibitors) have favoured emergence of resistant *P. aphanis* populations (Sombardier et al., 2010; Palmer and Holmes, 2021).

Among the authorised active ingredients, only sulfur has multisite mode of action, which can be used to mitigate emergence of fungicide resistant *P. aphanis* populations (Peres and Mertely, 1969). However, although sulfur has low mammalian toxicity and has a long use history, including in organic farming, this chemical can negatively impact beneficial arthropods (Beers et al., 2009).

In addition to selection of resistant pathogen populations, some compounds, such as the triazoles (demethylation inhibitors; G1), are posing risks for animals and humans (Muñoz-Leoz et al., 2011; Rjiba-Touati et al., 2023). Also because of slow environmental degradation of these chemicals (EFSA, 2010), they have been associated with detrimental human health consequences, including infertility and disruptions in neurobehavioural functioning (Menegola et al., 2006; Zhang et al., 2016).

Table 1 **-** Fungicides authorised for use against strawberry powdery mildew in at least one European Union Member State (EU pesticide database, April 2023). The active ingredients are grouped according to the FRAC Code list, 2022.

Active substance	**Target Code**	**Group name**
Bupirimate	A2	Hydroxy- (2-amino-) pyrimidines
Boscalid	C2	Succinatedehydrogenase inhibitors
Cyflufenamid	U6	Phenylacetamides
Difenoconazole	G1	Demethylation Inhibitors
Fluopyram	C2	Succinatedehydrogenase inhibitors
Fluxapyroxad	C2	Succinatedehydrogenase inhibitor
Meptyldinocap	C5	Uncouplers of oxidative phosphorylation
Penconazole	G1	Demethylation Inhibitors
Pyraclostrobin	C3	Quinone outside inhibitors
Tetraconazole	G1	Demethylation Inhibitors
Trifloxystrobin	C3	Quinone outside inhibitors
Sulfur	M02	Inorganic

Bioprotection

There has been increased research to develop alternatives to synthesized fungicides. However, intrinsic bias often occurs, with tendency to publish positive results while ignoring the negative outcomes. This leaves an important gap in understanding of effectiveness of these alternatives, which may result increased expectation for efficacious products. When applied in the field, these products fail to control target diseases, either due to overestimation of efficacy or to lack of knowledge of factors that may reduce their effects, such as optimal concentrations, and timing and frequency of applications. This underlines the importance considering negative results which could contribute realistic evaluations. There are also discrepancies between published research and results obtained by the industry. While a range of commercial products have been officially authorised for the use in SPM control (and are therefore of proven efficacy), this often lacks robust confirmation in the scientific literature. Industry operators may not disclose efficacy data, which hinders the advancement of research efforts. This lack of information impedes collective progress in SPM control and raises concerns about the "robustness" of the effectiveness of these active substances. Several categories of alternatives to fungicides have been defined. Although their analysis is beyond the scope of the present review, the suggestion of Stenberg et al. (2021) is relevant: '*bioprotection can be used as an excellent umbrella term that encompasses protection provided by either living agents or non-living substances of biological origin* […] *with low impact on human health and the environment*'. In the present review we divide the alternatives into groups based on their nature and/or origins.

Inorganic salts

Several inorganic salts have been tested for efficacy in suppressing fungal pathogens, but when considering powdery mildews, potassium and sodium bicarbonates (Homma et al., 1981; Crisp et al., 2006a), potassium silicate (Menzies et al., 2019) and potassium phosphate (Reuveni et al., 1995; Reuveni and Reuveni, 1998) have been the most investigated. For SPM, there are fewer reports, and only potassium and sodium bicarbonates and silicates have been sufficiently assessed. For example, potassium and sodium bicarbonates (4 g L^{-1}) showed promising efficacy, in leaf bioassays, for control of *P. aphanis*, with, respectively, 87% and 84% reductions in hyphal biomass (Pertot et al., 2007). The promising laboratory results were not fully confirmed in the field, where prolonged applications of a combination of potassium bicarbonate and potassium silicate at 6 g L^{-1} gave 85% disease incidence compared to 88% for untreated controls (Gomez et al., 2017). A much greater rate of potassium bicarbonate (20 g L^{-1}), if integrated with fungicides, has given promising outcomes. Two applications of potassium bicarbonate were as effective as the systemic fungicide myclobutanil, suggesting a potential role for potassium bicarbonate in integrated disease management (Dodgson, 2007). Comparative studies assessing the effectiveness of inorganic salts versus conventional fungicides or their combinations, could improve their application strategies. Even if mode of action has yet to be fully defined (Deliopoulos et al., 2010), activity of bicarbonates likely occurs when the salts come into contact with the pathogen. This interaction inhibits sporulation and fungal development due to detrimental osmotic effects of K^+ imbalance, spore dehydration and increases in leaf surface pH (Ziv and Zitter, 1992; Kettlewell et al., 2000; Pertot et al., 2007). Bicarbonates need frequent applications to be effective, as is emphasized in label guidelines of commercial products (e.g., Karma 85, Certis Europe), that suggest 7-10 d interval between treatments. However, these repetitive treatments can possibly cause residual deposits and phytotoxicity.

Potassium silicate is another inorganic salt that has been extensively tested against SPM. Silicon (Si) is associated with beneficial effects on mechanical and physiological characteristics of plants, depending on whether it is applied to roots or to canopies. For example, potassium silicate 100 mg L^{-1} applied once to strawberry roots in hydroponics decreased disease severity by 17% (Kanto et al., 2004). This compound at 500 mg L^{-1}, applied at an average of 0.86 g m^{-2} d^{-1} during cultivation (Kanto et al., 2006) also suppressed SPM in soil by up to 15%. Kanto et al. (2007) demonstrated that hydroponic Si fertilization decreased disease severity and reduced fungal fitness. This was shown by reductions in germination of conidia collected from Si-treated plants compared to the controls. Germination rates were 49.7% for Si-treated plants and 67.2% for the controls. This protective role of root Si fertilization was attributed to Si accumulation in leaves, which hinders cuticle penetration by pathogens (Seal et al., 2018). This theory was supported by identification of Si transporters in strawberries, providing genetic evidence that strawberry is receptive for Si fertilisation (Ouellette et al., 2017). However, silicic acid is the only soluble form that plants can absorb to successfully store Si in leaves and decrease disease severity (Ouellette et al., 2017). Under a daily potassium silicate fertilization (1.7 mM Si) leaf accumulation can reach up to 3% Si on a dry weight basis (Ouellette et al., 2017). Silicon is also as an elicitor of plant resistance, and induces several defence-related reactions, such as the over-production of enzymes (e.g., polyphenoloxidase and peroxidase) and antifungal compounds (e.g., flavonoids and phytoalexins) (Wang et al., 2017).

Elicitation of resistance to SPM in strawberry has yet to be assessed. Although potassium silicate is promising when applied to plant roots, when applied to leaves this compound was less effective (Palmer et al., 2006; Jin, 2015; Gomez et al., 2017). Root applications influence various aspects of plant physiology and defence mechanisms, which may have greater disease suppression effects than foliar applications. The mode of action of foliar-applied potassium silicate for reducing powdery mildew has not been determined, but formation of physical barriers and osmotic effects on leaf surfaces may contribute to disease suppression (Bowen et al., 1992; Rodrigues et al., 2009).

Plant extracts

Plant extracts are complex mixtures containing bioactive compounds, that are obtained by physical processes such as distillation and extractions of/from leaves, stems, of fruit (SANCO, 2012). In the EU, plant extracts used as plant protection products are authorised according to Regulation (EC) No 1107/2009 (EU, 2009). Several plant extracts have shown promising results for suppressing powdery mildews, with various mechanisms including inhibition of spore germination and mycelium growth, and disrupting fungal reproductive structures (Marei et al., 2012; Silva et al., 2020). Among plant extracts, essential oils and aqueous extracts are promising groups for disease management.

Essential oils are concentrated hydrophobic liquids extracted from plants, by distillation with water or steam, mechanical processes (e.g., pressing or grinding), or dry distillation (ISO 9235, 2021). These oils contain volatile compounds (ISO 9235, 2021) that have diverse biological activities, including antifungal properties (Cavanagh, 2007; Ferraz et al., 2022). Among these, oils from *Thymus* spp. L., *Mentha* spp. L., *Melaleuca alternifolia* (Maiden and Betche) Cheel (tea tree oil) and *Citrus sinensis* (L.) Osbeck (orange oil) have been tested against several powdery mildew species (Reuveni et al., 2020; Mostafa et al., 2021; Frem et al., 2022), but limited information is available for SPM.

Orange oil is the only essential oil authorized in the EU and in at least one European Union Member State for control of SPM (European Pesticide Database, 2023). However, only a few papers report efficacy of orange oil against SPM (Prodorutti et al., 2019). For example, orange oil, if applied weekly, was as effective against SPM as most conventional fungicides. Disease severity was reduced from 81% (untreated control) to 14% by penconazole and 19% by orange oil (Prodorutti et al., 2019). The active component of orange oil is limonene, a volatile compound that disrupts fungal cell membranes and inhibits spore germination (Marei et al., 2012; Silva et al., 2020). Beside antifungal properties, orange oil has insecticidal properties, as is common with essential oils in general (Isman, 2020), possibly affecting beneficial insects and disrupting ecosystem balance. This highlights the importance of holistic pest management strategies that target pathogens but also do not generally impact the field environment.

Aqueous plant extracts may include secondary metabolites, phenolic compounds and enzymes that can directly affect pathogen physiology and growth (Tavares et al., 2021). For example, *Equisetum arvense* L., and *Salix* spp. L. cortex extracts can control some powdery mildew species, although with slight efficacy (Marchand et al., 2014; Frem et al., 2022). Among plant aqueous extracts, those from *E. arvense* are authorised in the EU as basic substances and in at least one European Union Member State for control of SPM (EU Pesticide Database, 2023). Although the mode of action is unknown, silicon as a major component of E. arvense reduces effects of excessive moisture on leaves and inhibits fungal growth. This involves creation of physical barriers of Si on leaf surfaces combined with osmotic effects that absorb excessive moisture favouring fungal proliferation (Bowen et al., 1992; Rodrigues et al., 2009).

<u>Seaweed extracts</u>

The first report of seaweed against powdery mildews was that of Stephenson (1966), and research on these extracts has expanded (Li et al., 2020; Elagamey et al., 2023). Abundant and common brown seaweeds such as *Ascophyllum nodosum* (L.) Le Jolis, *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria digitata* (Hudson) J.V. Lamouroux, are the most frequently used for their plant growth promoting activities (Khan et al., 2009). In the EU, most seaweed extracts used in agriculture are considered as fertilizers (EU, 2019). However, seaweeds have also been acknowledged as potential alternatives for plant protection products, due to their capacity to enhance plant disease resistance by interacting with secondary metabolism and defence-related processes (EIBC, 2012; OECD, 2017). For example, laminarin, a storage glucan extracted from L. digitata, is an authorised active substance for SPM in the EU, with demonstrated positive results in laboratory and field tests. In leaf assays, laminarin decreased *P. aphanis* conidium germination by 75% (Bajpai et al., 2019), while in greenhouse tests laminarin with a reduced chemical dosage, gave 1.7% SPM infestation, which was similar to the complete chemical scheme (Melis et al., 2017).

The laminarin mode of action against SPM not been investigated. However, its plant protection activity has been studied for several plant species and involves several key elements. The compound elicits production of defence compounds, such as phytoalexins (Aziz et al., 2003), and synthesis of pathogenesis-related proteins (Tziros et al., 2021). It may also directly interact with the pathogen, reducing conidium germination and fungal growth (Hu et al., 2012; de Borba et al., 2022).

Substances from animal origins

Cow's milk and whey have been studied for their plant growth-promoting activity (Sharratt et al., 1959; Ahmed Hashim, 2019), and as alternatives to synthetic fungicides. Fresh cow's milk, at concentrations greater than 10% in water, applied twice a week, was as effective (10% severity) as fenarimol and benomyl (9%) for reduce powdery mildew of zucchini squash, compared to the water control (56% severity), after 1 month since treatment (Bettiol, 1999). Similarly, 10% whey applied twice a week powdery mildew severity (caused by *Podosphaera xanthii* (Castagne) U. Braun and Shishkoff) by 71-94% in cucumber and 81-90% in zucchini, compared to experimental controls (Bettiol et al., 2008). Cow's milk and whey are already authorized in the EU as basic substances and in at least one European Union Member State for use against several powdery mildews (EU Pesticide Database, 2023).

Effects of milk and whey against powdery mildews may involve more than one mode of action. Electron spin resonance and scanning electron microscopy showed that fresh milk and whey applied to grape leaves infected by *Erysiphe necator* Schwein. led to the collapse of fungal hyphae and conidia within 24 h after treatments, likely because of release of free radicals, fatty acids, and lactoferrin by the milk microbial community (Crisp et al., 2006b). Despite high efficacy, the European Food Safety Authority has raised concerns about potential food allergies associated with lactose and milk proteins derived from the use of whey for plant protection (SANTE, 2021). Consequently, its application is restricted in the EU only to approved crops during plant growth stages devoid of fruit (EU Pesticides Database, 2023). Without additional safety data, milk/whey for SPM control could be authorized only in the EU at the beginning of crop growth, when disease outbreaks are commonly rare, making the alternative of little use for growers.

Chitin and chitin derivatives

Chitin, an amino polysaccharide, is a structural supporting component of fungal cell walls, and insect, nematode, and crustacean exoskeletons (Latgè, 2007). Chitin and chitin oligosaccharides have been assessed as plant protection agents (Li et al., 2020), because they are environmentally friendly and highly degradable (Yeul and Rayalu, 2013). These compounds have antimicrobial activities and elicit host defence mechanisms. When recognized by plant cells, they trigger several immune responses (Xing et al., 2015; Li et al., 2020), including lignification and cytoplasmic acidification (Barber et al., 1989).

Chitosan, the N-deacetylated derivative of chitin, is the most extensively studied among chitin fragments. Chitosan is a family of molecules with different sizes and compositions, so it has ductile chemical and physical properties (Aranaz et al., 2021). Chitosan stimulates plant defences and growth (Chakraborty et al., 2020) but also has filmogenic and fungicide properties against spore and mycelium growth (Martínez-Camacho et al., 2010; Meng et al., 2010). Chitosan is effective against several powdery mildew pathogens. *Sphaerotheca fuliginea* (Schltdl.) Pollacci on cucumber cotyledons in Petri dishes was inhibited by one preventive treatment of 2.5% chitosan (Moret and Muñoz, 2009). Similarly, a weekly foliar treatment of 0.5% chitosan on cutting roses decreased infections by *Podosphaera pannosa* (Wallr.) de Bary (Wulf et al., 2023). However, field studies with chitosan suggest it should be applied when pathogen levels are low (Wulf et al., 2023). Although chitosan has been authorised the EU as a basic substance for SPM control in at least one European country there are no reports of efficacy in scientific literature.

Chitosan fragments known as chitooligosaccharides (COS) have been tested in combination with pectin (oligogalacturonides, OGA) as elicitors of plant resistance in a formulation referred to as COS-OGA (Ferrari et al., 2013). Because of proven efficacy (van Aubel et al., 2014), COS-OGA has been authorised in the EU for the use against several powdery mildews, including SPM. However, no efficacy data are available for chitosan against SPM.

Microbial biocontrol agents

Microbial biocontrol agents (BCAs) are microorganisms that act against phytopathogens with various mechanisms (e.g., competition for resources, antibiosis, hyperparasitism, and induced resistance), and can control plant diseases (Köhl, et al., 2019). Several BCAs with different modes of action have been studied against SPM: *Ampelomyces quisqualis* Ces., *T. harzianum* Rifai, and *Bacillus spp*. Cohn are the most investigated. *Ampelomyces quisqualis* is a hyperparasite of several powdery mildew fungi (Sundheim, 1982; Falk et al., 1995). *Trichoderma spp*. strains are mycoparasites that can produce antifungal metabolites, and can induce host resistance (Vinale et al., 2008). *Bacillus spp*. produces many antimicrobial compounds and can induce resistance on plants (Pérez-García et al., 2011). The microbes commonly have good efficacy when applied under controlled laboratory/greenhouse conditions, but their efficacy decreases under commercial field conditions. For example, in vitro, *A. quisqualis* AQ10 and T. *harzianum* T39 decreased SPM hyphal biomass by, respectively, 46 and 74%, compared to untreated controls, but these organisms were not as effective as *B. amyloliquefaciens* (formerly *B. subtilis*) QST 713 Cohn that achieved results that were similar to those from to chemical pesticides (99% inhibition of hyphal biomass) (Pertot et al., 2007). However, under field conditions, the exclusive use of these microorganisms throughout crop growing seasons without integrating fungicides has been proven insufficient. Contrary to bioassay results, *T. harzianum* T39, in an integrated programme, had the greater activity than *A. quisqualis*. The average fruit incidence in the two locations was 25% for *T. harzianum* and 44% for *A. quisqualis* (Pertot et al., 2008). In contrast, their efficacy against leaf severity was variable across locations. Currently, *A. quisqualis* AQ10 and *B. amyloliquefaciens* QST 713 are authorized in the EU and in at least one European Union Member State for SPM control (EU Pesticide Database, 2023).

Inhibition of SPM conidiation (80.7% reduction) on leaf discs was also obtained combining *B. subtilis* ABiTEP GmbH FZB24 and *Metarhizium anisopliae* (Metschn.) Sorokīn (Sylla et al., 2013). However, no studies have reported assessments under field conditions. *Bacillus pumilus* Meyer and Gottheil QST2808 is also authorized in the EU and in at least one European Union Member State for SPM control (EU Pesticide Database, 2023). This microorganism, under field conditions, demonstrated high consistency against SPM compared to other tested BCAs. It showed better efficacy compared to a 14 d fungicide application regime, but not in comparison with a 7 d fungicide application schedule (Berrie and Xu, 2021). No data are available for efficacy of *B. pumilus* QST 2808 against *P. aphanis* under controlled conditions.

Understanding the epidemiology of SPM disease and the environmental conditions for survival and/or optimal growth of BCAs in the field are considered key factors for successful control strategies (Pertot et al., 2008). Variability in BCA efficacy under field conditions often stems from misuse of these living organisms, treating them as if they were synthetic fungicides, so use of BCAs is more complex than for chemical agents (Legein et al., 2020). Applying BCAs at specific stages of the pathogen cycle could be more strategic than frequent treatments during crop growth seasons, when environmental conditions may not be favourable for BCA growth. For example, *A. quisqualis* AQ10, when applied at the end of a crop growth season under suitable temperature and RH conditions can reduce inoculum for the following growing season. Ensuring BCA efficacy also includes assessing compatibility with conventional fungicides when developing integrated pest management programmes. For example, *A. quisqualis* is incompatible with commonly used chemicals against SPM, including penconazole, pyrimethanil, tebuconazole, cyprodinil, fosetyl-aluminium, azoxystrobin, and metalaxyl (Roberti et al., 2002). Research on biocontrol agents has a long history, but there has been little recent research focusing on SPM. The research community may have recognized that the previous approaches are not productive for addressing this issue.

Fungivorous biocontrol agents

While microbial BCAs have predominantly dominated biocontrol efforts against powdery mildews, fungivorous insect biocontrol agents, have recently emerged as potential contenders (IBMA, 2022). Pijnakker et al, (2022) reported that the mycophagous mite *Pronematus ubiquitus* McGregor gave promising results against tomato powdery mildew (*Oidium neolycopersici* L. Kiss), by decreasing disease severity to 4%, compared to 32% for untreated controls, eight weeks after mite release. The mites were in greater numbers where powdery mildew was severe. In addition, Pijnakker et al. (2022) suggested that for effective disease control this mite must be released preventatively. For SPM control, *P. ubiquitous* has not been assessed scientifically, but is currently being investigated by the industry, and is at first stages of market development (IBMA, 2022). Although the precise contribution of conidium nutrition and plant mediated effects on powdery mildew resistance, remain unclear, there is potential for determining these interactions. It is also important to develop understanding of whether *P. ubiquitus* is present in each territory of investigation, as potential field releases of alien mites may not be permitted (Heimpel and Cock, 2018).

Other control means

Crop canopy management

Plant canopies have important roles in the development of powdery mildew diseases, which are favoured by host vigour and high plant density in many host species (Jarvis et al., 2002). Dense canopies create microclimates (i.e., high humidity, low ventilation, low light penetration) that favour pathogen growth (Aust and Hoyningen-Huene, 1986; Keller et al., 2003), and suitable canopy management can reduce infection risks. Direct effects of canopy management on SPM control have not been validated in robust research. However, some studies indicate positive correlations between SPM severity and canopy density. For example, breeding for SPM resistance is leading to the selection of cultivars with reduced canopy densities due to consistent genetic correlations observed between host susceptibility and high canopy density (Kennedy et al., 2014). Although research on SPM is lacking, studies on other powdery mildews suggest practices that can be also tested for strawberry. For powdery mildew of hop (*P. macularis*) removal of highly susceptible climbing shoots and reductions in canopy density improved disease management and fungicide distribution (Gent et al., 2012; Gent et al., 2016). In grapevine, vertical trellis system and spring pruning reduced powdery mildew by up to 32% (Austin and Wilcox, 2011). Canopy thinning in strawberry crops has been assessed for yield optimization (Sønsteby et al., 2021) but has not been comprehensively investigated for SPM management. Similarly, removal of highly susceptible strawberry runners could reduce risks of SPM (Eccel et al., 2010), but this is yet to be precisely quantified.

Host nutrition

Balanced mineral nutrition is important for plant self defense, and when specific elements are either deficient or over-abundant, plants can become vulnerable to particular pathogens (Huber and Haneklaus, 2007). High nitrogen inputs have been associated with increased risk of fungal diseases. For SPM under experimental conditions, Xu et al. (2013) reported a 54% increase of nitrogen above fertigation standard (from 128 to 197 mg L^{-1}) applied from the beginning of bloom resulted in an 8% increase in disease severity. For deficiencies in the other macro- and micro-elements, there are no published reports relating to SPM susceptibility.

Some soil amendments may enhance plant defence against biotic stresses. For example, biochar can induce plant resistance by improving chemical and physical soil properties (e.g., water holding capacity, nutrient availability, soil texture), and by enhancing soil microbial activity such as plant growth promoting rhizobacteria (Schmidt et al., 2021). For strawberry, incorporation of 3% biochar into potting mixture resulted in high expression of defence-related genes and a related decrease of SMP (Harel et al., 2012).

Overhead irrigation

Although *P. aphanis* develops well under high RH (Amsalem et al., 2006), free water prevents conidium germination (Peries, 1962a). Water sprays on plant canopies can control SPM but could also promote pathogenic fungi that are favoured by a wet canopy, such as *Botrytis cinerea* Pers. and Colletotrichum spp. Corda. However, since micro-sprinklers are commonly used to spray water to reduce high temperature stress during summer (Liu et al., 2021), well-balanced overhead irrigation can be used to reduce SPM. For example, application of pulsed water mist has shown promising results: applications of 660 mL min^{-1} for 1 min four times a day was as effective as standard fungicide treatments, in high tunnel and open field conditions (Asalf et al., 2021). Overhead irrigation also reduces SPM severity when applied for long periods. For example, after 67 d of mist treatments, severity of powdery mildew decreased from 80 to 17% in high tunnels and from 73 to 22% in the open field, compared to untreated controls. Application of pulsed misting for 1 min four times a day did not increase in *B. cinerea* infections, indicating that if water was correctly applied, grey mould could be reduced (Asalf et al., 2021). Although overhead irrigation reduced the disease, procedures (i.e., frequency, volume, application methods) were not fully explored for maximizing efficacy.

Fungicide spray equipment

Spray equipment can also affect pest control (Ebert and Downer, 2006), and this is particularly the case for SPM because applied fungicide must reach the undersides of leaves, lower leaves and the fruit. This is particularly difficult when strawberry plants develop dense canopies. Low technology devices (i.e. hand-held and cannon sprayers) may not provide adequate and even fungicide distribution (Balsari et al., 2008; Bondesan et al., 2015). These devices are widely used in strawberry high-tunnels in Mediterranean regions (e.g., Italy and Spain) (Sánchez-Hermosilla et al., 2012; Cerruto et al., 2018), because they are inexpensive and easily adaptable to horticultural crops. Cannon sprayers also distribute plant protection agents at high pressure (>20 bar) and rates (1500–2500 L ha^{-1}), producing spray drift that can contaminate soil and may increase operator exposure (Sánchez-Hermosilla et al., 2011, 2012; Cerruto et al., 2018). In technologically advanced greenhouses, sprayers with increased efficiency, such as vertical booms (Braekman et al., 2010), or autonomous pesticide spraying robots (Abanay et al., 2022), have been associated with improved better canopy coverage and reduced application volumes compared with cannon and hand-held sprayers (Braekman et al., 2010).

Ultraviolet light

Light is an important factor for minimising fungal development and stress responses in plants, and ultraviolet light (UV) can suppress powdery mildews in several crop plant hosts (Gadoury et al., 1992; Suthaparan et al., 2012; Pate et al., 2020). For strawberry, the application of UV once or twice per week during night-time (60 s followed by 4 h dark period) resulted in up to 90% reduction of SPM incidence and severity compared to the controls (Janisiewicz et al., 2016). However, UV-based methodology is still at early commercial development and has various challenges. For example, UV technology is not adaptable to diverse rural growing systems, such as high-tunnels and open fields. In some cases, machines may not be able to access tunnels and/or move between benchtop rows. Application parameters (UV dose, light exposure durations, treatment frequencies) are not yet optimised and standardised. A range of doses spanning from 30 to 200 J m^2, administered once or twice per week, or at 10 d intervals, have been assessed (Van Delm et al., 2014; Janisiewicz et al., 2016; Suthaparan et al., 2012; Ledermann et al., 2021), without determining the best application schedule. Antifungal effects also only occur only irradiated host surfaces and UV light poorly penetrates crop canopies, and uniform light distribution is difficult in multi-layered crop canopies, giving limited SPM control on abaxial leaf surfaces (Delorme et al., 2020). Implementing UV light technologies is expensive: beside the initial costs that include purchase of UV equipment, installation, and the necessary modifications to the existing infrastructure, there are extra costs for electricity and frequent replacement of UV lamps (Rea et al., 2022). For these reasons, growers must carry out careful cost/benefit analyses when evaluating the feasibility of UV light for SPM control.

Ozone

Ozone (O_3) has antimicrobial activity and is rapidly decomposed in the environment. In the food industry O_3 is used to safely disinfect food, and as postharvest treatments to increase shelf-life of fruit and vegetables (Tzortzakis and Chrysargyris, 2017). For plant protection, O_3 has been tested against powdery mildews of several horticultural crops under controlled conditions, both as fumigant and as ozonated water (Hibben and Taylor, 1975; Rusch and Laurence, 1993; Khan and Khan, 1999; Fujiwara and Fujii, 2002; He et al., 2015). Effects of O_3 on powdery mildews and plants depends on concentration: at too low levels powdery mildews may be not harmed, while at too high levels host phytotoxicity may occur. For example, increasing concentrations of gaseous O_3 (from 50 to 200 ppb) applied intermittently (7 h d^{-1} for 7 d) on cucumber plants in closed-top chambers, decreased powdery mildew colonization from 70 to 23%. In addition, 50 ppb of O_3 increased the germination conidia collected from treated plants, while conidia exposed to greater concentrations (100 and 200 ppb) were smaller and had reduced germination compared to untreated controls. High O_3 concentrations (i.e., 200 ppb) can cause foliar necroses (Khan and Khan, 1999). Ozonated water gives similar results (Fujiwara and Fujii, 2002). Although some growers currently use ozonated water, its efficacy against SPM has not yet been assessed (Fujiwara and Fujii, 2004). Devices to spray ozonated water are available (e.g., MMBiozono, MMSpray, Italy; Mowat, Gr Gamberini, Italy), and are also tailored for strawberry production (e.g., GZO-D, ZonoSistem, Spain), but no definitive data are available (e.g. minimum exposure times, effective dosage) (Fujiwara and Fujii, 2004).

Genetic resistance to strawberry powdery mildew

Breeding for resistant varieties is an effective disease management strategy, provided that plants bear high-quality fruit, are well-suited to local cultivation regions and have adequate and long-lasting tolerance or resistance to pathogens. Resistance in strawberry to *P.aphanis* has low durability and is variable under different environmental conditions (Menzel, 2022). Whether this behaviour is related to unstable resistance genes or different virulence of SPM strains is unknown (Nelson et al., 1995). Several genes may control levels of infection, and under natural conditions inoculum density varies leading to differential elicitation of systemic resistance (Kennedy et al., 2013). In a plant breeding programme, beside inoculum level, other variables (e.g., climatic conditions, growing systems, time of season) may influence strawberry responses to pathogens, making comparison of results obtained in different breeding programmes challenging. Defining the optimum breeding methodology and conditions for development of resistant strawberry cultivars could be helpful (Menzel, 2022). Marker-assisted selection can accelerate cultivar improvement, but SPM resistance in strawberry is probably regulated by complex genetics with several additive genes involved. To date, several genes have been associated with SPM resistance (Menzel, 2022), including nine QTL genes (Cockerton et al., 2018; Sargent et al., 2019), seven TGA genes (Feng et al., 2020a) and 68 MLO sequences (Tapia et al., 2021).

Predictive models and Decision Support Systems

Reductions of fungicide use can also be achieved by optimizing, and thus reducing, numbers of spray applications, and predictive models and Decision Support Systems (DSSs) can help growers identify optimum timing of pesticide applications. Predictive models are based on empirical data collected from the field and/or under controlled conditions, and forecast disease development (Van Maanen and Xu, 2003). DSSs are interactive computer-based systems, which use predictive models, data analysis techniques, and recommend/support actions for farmers to manage diseases (Sprague and Carlson, 1982). Both of these tools are useful to schedule fungicide treatments, thereby avoiding unnecessary applications (Lazaro et al., 2021). For SPM, several predictive models (Carisse et al., 2013a, 2013b) and DSSs (Table 2) have been developed (Gubler et al., 1999; Eccel et al., 2010; Bardet and Vibert, 2011; Dodgson et al., 2021; Carisse and Fall, 2021; Fall and Carisse, 2022). However, the developed models, excepting that of Gubler et al. (1999), have not been validated in different locations. This decreases the reliability of the models, as agricultural conditions can vary widely from one region to another. Without validation it is therefore difficult to assess model robustness and accuracy in different environmental contexts.

Predictive models

Several models, as mentioned in the epidemiological section of this review, have been developed in Canada. For example, Carisse et al. (2013a) characterized a close relationship between SPM incidence and severity to define an economic loss threshold for fungicide interventions. In another model, Carisse et al. (2013b) described a strong positive linear relationship between seasonal crop losses, disease severity and daily mean airborne conidium concentration, to potentially define a severity and airborne conidium concentration threshold for fungicide interventions. Carisse and Bouchard (2010) defined windows of high leaf and berry susceptibility for June-bearing and everbearing strawberry cultivars.

<u>DSS developed by Carisse and Fall</u>

From these models, Carisse and Fall in 2021, modelled a DSS based on a decision tree forecast (the outcome of several algorithms that offered a model, following a subset of classification rules visualised and exemplified as a tree) (De Ville, 2013). This model forecasts risk of infection, firstly from airborne inoculum concentration and number of susceptible leaves, and then using mean RH, mean daily number of hours at temperature between 18 and 30°C, and mean daily number of hours at saturation vapour pressure between 10 and 25 mmHg during the previous 6 d. Carisse and Fall (2021) noted that the main characteristic of their prediction system was understanding that groups of variables can affect SPM e development, and that different combinations of these variables can result in similar disease severities. For example, low inoculum amounts and a limited number of susceptible leaves, but conducive weather, may yield the similar severities as scenarios of high inoculum, few susceptible leaves and less favourable weather conditions. The factor potentially hindering use of the model could be detection of airborne conidium concentrations, that Carisse and Fall (2021) suggested analysing manually, twice weekly using microscopy, for each strawberry field.

DSS developed by Fall and Carisse

Fall and Carisse (2022) developed a DSS according to a dynamic simulation model, which simulates the asexual life cycle of *P. aphanis* and its related severity. This model considers at which rate *P. aphanis* changes growth stage with time, according to weather conditions, simulating daily conidium production and resulting disease severity. In the model *P. aphanis* stages (initial inoculum, conidium germination dropout population, germinated conidia, cumulative proportion of diseased leaf area, secondary inoculum) are regulated and influenced by rate variables, such as sporulation rate, germination rate, lesion increase rate (defined by algebraic equations), that in turn are influenced by intermediate variables such as daily temperature, rainfall, RH and the number of leaves per plant (35,000 plant ha^{-1} in a 0.91 m row spacing field), estimated on a daily basis. The model is based on evidence that in May at least one lesion m^{-2} of strawberry field is sporulating, and the initial inoculum load in 1 ha of strawberry is assumed to be 1,000,000 conidia (Blanco et al., 2004; Carisse et al., 2013a, 2013b). According to the initial inoculum value, the model starts running each day, estimating inoculum load based on weather data and fungal development, thus predicting powdery mildew severity and related crop loss. According to disease severity, warning and action thresholds are simulated on a daily basis. For cost-effective management of SPM, crop managers in Quebec may tolerate 1% yield losses (warning) but not more than 5% losses (action).

UC Davis DSS

In California, a DSS developed by UC Davis (Gubler et al., 1999) was another attempt to forecast SPM epidemics, assessing risks and action thresholds. This model was developed for grape powdery mildew (caused by *E. necator*) and then applied for SPM (Hoffman and Gubler, 2002). The model focuses on forecasting ascospore infection to refine fungicide application timing at the start of each cropping season (Gubler et al., 1999; Hoffman and Gubler, 2002). This model assesses ascospore release according to leaf wetness and temperature, considering that at least 12–15 h of continuous leaf wetness at 10-15°C average temperatures are necessary for release. After the ascospore infection occurs, the model changes into the risk assessment phase, relying solely on temperature impacts on pathogen reproductive rate. To start an epidemic, the pathogen requires three consecutive days with at least 6 h between 21 and 30°C. If these conditions are not met, the index resets to zero; otherwise, the model initiates estimation of an infection index (from 0 to 100). Thresholds of action and frequency of intervention depends on risk. If the risk index remains low (<30), interval between treatments decreases (between 14 and 21 d). If risk index increases (>60), shorter intervals between applications are recommended (maximum, 7 d interval). The model was validated in 2002 under open field conditions, reducing 40% of fungicide treatments, compared to a calendar-based programme. However, after several tests in Quebec, the model did not accurately predict SPM at the beginning of the season, probably due to the wide range of favouring conditions. This resulted in similar numbers of fungicide applications prescribed as for calendar-based schedules (Bouchard, 2008).

Safeberry DSS

In Italy, Eccel et al. (2010) modelled the SafeBerry DSS, based on forecasted daytime temperatures over 3 d, and risk factors including daily disease incidence in a tunnel, type of sprayer, tunnel height, overhead irrigation, cultivar susceptibility, time of disease onset, time since last treatment, and presence of runners. Suitability of weather conditions for disease development was categorised according to day-time temperature as follows: low suitability (≤18 or >26°C), medium suitability (18 < T° ≤ 20 or 25 < T° ≤ 26°C), and high suitability (20 < T° ≤ 25°C). Outputs of this model are daily assessment of disease outbreak risk at the daily time/temperature during the previous 6 d and forecasted in the next 3 d, the favourability of temperature for disease in the next 3 d, and then a recommendation for action. The model includes two action possibilities: either 'Do not spray today' or 'Apply as soon as possible'. In the second scenario, a selection of recommended fungicides is provided, based on their modes of action, risks for pathogen resistance development, and timing restrictions prior to harvest. With this system in 2007, under tunnel conditions, up to 60% reductions in fungicide treatments were obtained.

DSS developed by Bardet and Vibert

In France, Bardet and Vibert (2011) developed a DSS that modelled five stages of the *P. aphanis* life cycle (inoculum dispersal, infection, mycelium growth, sporulation, and disease progression), as influenced by meteorological variables of temperature, RH, and precipitation. The model was based on evidence that conidium germination occurs between 5 and 32°C, mycelium growth is interrupted above 35°C, sporulation occurs between 7 and 28°C. and conidium dispersal occurs in low humidity conditions. The maximum threshold accepted by the model is set at RH <65% over a minimum duration of 8 h. At RH >85% for at least 5 h, germination occurs rapidly. The index risk separately considers the conditions favourable for infection, mycelium establishment with sporulation, and lesion development, and each stage has a 0 to 5 value. For example, 5 is assigned to infection under favourable conditions for the fungus (temperature between 20 and 26°C, and 85% < RH < 99%). For strawberry cultivars that are particularly susceptible, the model allows additional risk to be set. The model gives graphical representation of periods suitable for pathogen dissemination, infection and mycelium growth for a 4 d period. Fungicide treatments can appear on the graph provided their application dates and effectiveness duration are entered. The model was validated with experiments conducted in 2006 and 2007 under tunnel and glasshouse conditions, and in 2010 under tunnel conditions. This DSS is available for growers through the web platform Inoki (Ctifl, 2023).

Strawberry Powdery Mildew Forecasting Model DSS

In the United Kingdom, a DSS implementing 15 years of historical data was developed by Dodgson et al., (2021). The model is based on laboratory and field evidence that *P. aphanis*, under optimum conditions (>15.5 and <30°C, 60% RH), takes 144 h (disease conducive hours) to complete a cycle from conidium germination (6 h) to growth of elongating secondary hyphae and sporulation (138 h). The system then extends according to weather conditions (15.5°C, the minimum temperature for spore germination; 18°C, the minimum temperature for sporulation, at 60% RH). According to sensitivity analysis under field conditions, temperature is the main factor influencing fungal development and sporulation. Other secondary weather parameters with lower impacts on the prediction system, such as leaf wetness, were removed to simplify the rules of the forecast. Once one cycle is completed, a daily risk is predicted and used for guiding fungicide applications. The prediction system uses a 'traffic light' colour scheme indication to represent the progression of accumulated hours of conducive conditions. When 125 accumulated hours are reached, the line changes from yellow to red, indicating high risk of conidium production. A fungicide should normally be applied before the elapsed time reaches 144 accumulated hours, to prevent *P. aphanis* sporulation. When a fungicide application is made, the growers record this manually in the software, and reset the system to zero and the process repeats. Unlike the Carisse and Fall (2021) decision tree, the Dodgson et al. (2021) model does not essentially require accurate estimation of susceptible leaves and airborne inoculum, and these variables are deemed to be limiting. Instead, the Dodgson et al. (2021) forecast always assumes a standard presence of inoculum, and susceptibility for all crops. To effectively manage powdery mildew, growers are required to start each growing season with a clean-up spray treatment, as this was also confirmed by greater infection in crop where clean-up spray was neglected. Relying on these assumptions and the risk forecast, control of powdery mildew was demonstrated with 30% fungicide reduction. The model has been validated from 2009 to 2020 under tunnel conditions. To date, the Dodgson et al. (2021) online real-time web-based prediction system is used and sold with commercial licencing (Strawberry Powdery Mildew Forecasting Model, Agri-tech Service, United Kingdom).

Table 2 - Description of SPM DSSs.

Reference	Aim	Input drivers	Output	Validation	Treatment reduction	Commercial Application
Dodgson et al. (2021) DSS	Disease development forecast based on the number of hours with favourable conditions	T°, RH%	Daily risk predicted on cumulative h of conducive conditions, recommendation of action	2009-2020, under tunnels, UK	30% fungicide reduction	Agri-tech
Bardet and Vibert (2011) DSS	Disease development forecast based on favourable conditions for fungal stages	T°, RH% and rainfall	Graphical representation of disease progression and infection risk in 4-d period	2006-2007 under tunnels and glasshouse, 2010 under tunnels, France	Not available	Inoki
Eccel et al. (2010) SafeBerry DSS	Disease development forecast based on weather data, growing system, agronomic practices, host susceptibility.	T°, RH%, daily disease incidence, type of sprayer, tunnel height, overhead irrigation, cultivar susceptibility, time of disease onset, time since last treatment, presence of runners	Daily risk of disease outbreak and risk forecast in the next 3 d, recommendation of action	2007 under tunnels, Italy	60% fungicide reduction	Not available
Hoffman and Gubler (2002) DSS	Ascosporic infection and disease development forecast based on whether data	T° and leaf wetness	Treatment interval threshold according to risk index	2002 in open field, California, in 2008 in open field, Quebec	40% fungicide reduction in California, 0% in Quebec	Not available
Fall and Carisse (2022) DSS	Dynamic simulation of inoculum load and fungal development based on weather data	T°, rainfall, RH%, plant density, initial airborne inoculum concentration	Daily SPM severity, warning and action threshold and related crop loss	2006, 2007, 2008, 2015, 2016 and 2018 in raised beds open field, Quebec	Not available	Not available
Carisse and Fall (2021) DSS	Decision tree forecast of infection based on weather data	Airborne inoculum concentration, susceptible leaves, RH%, T°, vapour pressure	Daily infection risk, warning	2015, 2016, 2018 in raised beds open field, Quebec	Not available	Not available

New tools for early detection

Early disease detection is often complex and time-consuming, and for SPM, prompt recognition of the disease in the field is difficult (Carisse et al., 2013a). However, rapid development of advanced agricultural technologies, such as machine learning and vision, has helped capture of disease images, and, therefore, detection of pathogen presence and abundance in the field (Liu and Wang, 2021). Machine vision-based recognition may replace traditional naked eye identification with computing science. Robust models have recently been developed to detect SPM on strawberry leaves with high accuracy (>94%) (Shin et al., 2020, 2021).

At research level, analyses of volatile organic compounds released by diseased crops is another potential machine learning technique for disease detection. These compounds are potential biomarkers for warning and forecasting disease spreading in fields (Li et al., 2019). The approach is based on plant emission of unique profiles of Volatile Organic Compounds (VOCs) when attacked by a pathogen, which differ from profiles from undamaged plants, allowing interactive signalling with neighbouring plants and release of danger signals. Nearby undamaged plants may recognise this novel profile and activate physiological changes that enhance their readiness to future pathogen attacks (Effah et al., 2019). For powdery mildew detection, this has only been studied for *B. graminis*, where sensitivity and specificity of six wheat VOCs have been identified as possible biomarkers for disease detection (Hamow et al., 2021). Strawberry powdery mildew identification and detection by VOC analyses have not been assessed, although greenhouse-grown strawberry plants could be excellent candidates for VOC analyses.

Conclusions

This review has critically considered the extensive research on SPM, attempting to identify knowledge gaps that warrant further investigation. Given the similarities of SPM with the other powdery mildews, the available data on other species could be used to inspire future research. In addition, factors related to growers' approaches to plant protection strategies could be considered. For example, natural substances are used as supplementary and marginal tools in disease management spray programmes, which are still largely based on synthetic fungicides. To overcome this problem, data on natural substance efficacy under various environmental conditions should be generated and made available in the public domain. Exploring new more effective application methods may also increase farmer confidence on alternative products. Natural substances and antagonistic microorganism often have limited field persistence, and frequent and/or appropriate timing of applications are required. Exploration of solid set spraying systems, especially in greenhouses and tunnels, could provide valuable new direction for SPM management. Assessment of the impacts of agronomic practices on SPM, and validation of SPM forecasting models across diverse strawberry-producing regions also deserve research effort.

Genomic, transcriptomic, and metabolomic technologies could provide powerful tools for development of innovative plant protection strategies. Although promising, biotechnological tools remain underexplored for SPM control. These technologies could be useful for assisting the breeding for resistant host varieties. For example, naturally occurring or experimentally induced inactivation and/or mutation of MLO genes (e.g., by gene silencing and genome editing) may provide strong and long-lasting immunity/resistance to the fungus (Wan et al., 2020).

Transcriptomics and metabolomics can offer unique approaches for identifying host resistance traits (Castro-Moretti et al., 2020). When transcriptomic information is coupled with metabolomic analyses, plant defence mechanisms can be better understood (Wink, 1988), and this knowledge could guide targeted interventions. For example, metabolomics can guide selection or breeding of plant cultivars with increased levels of defence molecules. For example, SPM infections influence strawberry plant metabolism (Duan et al., 2022): alongside phenols, ten chitinases are upregulated in infected plants, indicating the role of chitinase in reaction to *P. aphanis* (Duan et al., 2022). For example, determining substances that can mimic pathogen effects on strawberry chitinase overexpression, or identifying cultivars that are can further overexpress these enzymes, could result in new tools for disease management, as has been demonstrated by some reported attempts (Feng et al., 2020b; Zhang et al., 2021; Yin et al., 2022). Alongside overexpression of plant defence related pathways, gene silencing with expression of RNAi constructs against host and/or pathogen target genes could be assessed (Capriotti et al., 2020). Through the utilization of host and/or pathogen RNA interference (RNAi), specific pathogen genes could be silenced by degrading their messenger RNAs. This process can hinder translation of the RNA into proteins, thereby disrupting pathogen ability to carry out normal biological processes (Zotti et al., 2018). RNAi-based fungicides are at early stages of development, but they have already been assessed against grape powdery mildew (*E. necator*), giving up to 64% reduction in conidium production compared to experimental controls (McRae et al., 2023). These new biotechnologies, although powerful, may be of limited use due to high costs (both for research and implementation), and because of existing restrictive regulations. Therefore, innovative investments and policy interventions are necessary to guarantee sufficient knowledge advancements from research on SPM.

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GROWER AND AGRONOMIST INSIGHTS INTO STRAWBERRY POWDERY MILDEW MANAGEMENT: A FOCUS-GROUP ANALYSIS

Unpublished research

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Abstract

Strawberry powdery mildew, caused by *Podosphaera aphanis*, represents a major disease for protected strawberry production in northern Italy. To identify research priorities for disease management under commercial conditions, a semi-structured focus group was conducted with growers and agronomists affiliated with a major Italian soft-fruit cooperative. Participants discussed disease perception, epidemiological drivers, conventional and alternative control strategies.

Powdery mildew was consistently identified as the most aggressive fungal disease, characterized by high unpredictability in epidemic onset and rapid development under protected conditions. Growers rely on frequent fungicide applications due to the wide range of favourable conditions for disease development, raising concerns about environmental and health side effects. In addition, uncertainties remain regarding pathogen epidemiology, particularly concerning the management of overwintering inoculum at the beginning of the season and the factors driving rapid spread of disease. While interest in alternatives to synthetic fungicides has increased, their adoption remains limited. Key constraints include the lack of validated strategies for biopesticides, the non-standardized structure of tunnels that restricts automation (e.g., UV-C treatments and automated spraying machinery), and limited labour availability for

the implementation of more labour-intensive cultural practices such as runner removal and canopy thinning.

The findings identify priority research areas in high-tunnel strawberry production, including improved understanding of *P. aphanis* overwintering and epidemic dynamics, identification of key epidemiological parameters to support earlier and more targeted control and evaluation of cultural practices that limit fungal development, such as infected leaf removal at the beginning of the season. In addition, this study highlights how the structural characteristics of small, fragmented farms, and limited resources for technological change, represent major constraints to the adoption of more effective and innovative powdery mildew management strategies.

Introduction

Strawberry production in northern Italy (Trentino Alto-Adige and Veneto) is strongly represented by Sant'Orsola Cooperative, the leading Italian producer of soft fruits, with more than 40 growers cultivating strawberries in high tunnels using peat-based soilless systems. Member farms are typically small to medium-sized (0.5–5 ha) and distributed across a wide altitudinal range (130–1,200 m a. s. l.). The cooperative oversees the entire supply chain, providing technical support to growers for nursery propagation, crop production, and managing post-harvest handling, storage and commercialization of the fruit. To meet market demand for extended and off-season supply, production relies on protected cultivation, characterized by tunnels varying in age, size and layout, and on the alternation of June-bearing (Elsanta) and everbearing cultivars (Murano, Vivara, Portola), enabling fruit production from May to October. June-bearing cultivars are typically transplanted in mid-July and produce a first crop in late summer; after overwintering in the field under polyethylene mulches, they produce a second crop harvested in mid-May. In contrast, everbearing cultivars are transplanted in early April and harvested continuously from late May to late October. Everbearing and June-bearing cultivars account for approximately 60% and 40% of the cultivated area, respectively.

Given its central role in providing technical support, the cooperative offers an appropriate context for assessing growers' perspectives and needs related to powdery mildew control. Focus groups are widely used in agricultural research to gather experiential knowledge, stakeholder perceptions and decision-making processes (Ates et al., 2017; Abubakar et al., 2025). A focus group is a structured group discussion, guided by a moderator, in which a small number (between six and eight) of participants (Krueger and Casey, 2000), share their experiences and opinions on a defined topic, allowing researchers to understand how a problem is perceived and managed (Rabiee, 2004). Particularly, in plant protection, this approach has been used to identify practical limitations, assess how growers perceive the reliability of control methods and understand factors influencing the adoption of integrated management strategies (Adam et al., 2015; Calliera et al., 2013).

The objective of this study was to identify research priorities to improve disease management by assessing the challenges faced by growers and agronomists in controlling strawberry powdery mildew under commercial high-tunnel production. A semi-structured focus group with 8 members of a major Italian soft-fruit cooperative was used to gather detailed insights on disease perception, epidemiological factors influencing strawberry powdery mildew under commercial conditions, conventional and innovative control methods.

Materials and Methods

Participant recruitment

The focus group was held on December 5, 2022. Growers were recruited through purposive sampling, in collaboration with the technical service of Sant'Orsola Cooperative. Selection criteria included: (i) farm size (≥ 0.5 ha of strawberry), (ii) geographical location (valley floors, mid-slope areas and high-altitude tunnels), (iii) years of experience with strawberry cultivation (ranging from 5 to 30 years), and (iv) grower age (ranging from 25 to 65). Growers were invited by telephone and all agreed to participate. Four agronomists, responsible for technical support to growers, were also invited (Table 1). Participation was voluntary, and informed consent was obtained verbally prior to the session.

Table 1 – Selection criteria of the focus group participants.

Role	Farm size (ha)	Location	Experience (years)	Age
Grower	0.6	Valley floor	12	38
Grower	3.2	Mid-slope	20	47
Grower	1.8	High-altitude	8	33
Grower	5.0	Valley floor	30	62
Agronomist	-	-	19	43
Agronomist	-	-	15	39
Agronomist	-	-	18	44
Agronomist	-	-	27	53

Development of the questionnaire

The session lasted 2 hours and followed a semi-structured format, in which the moderator used a guiding questionnaire, allowing participants to elaborate freely and introduce additional topics. The session was also attended by an observer taking detailed notes without contributing to the discussion.

The questionnaire was developed based on the literature review of *P. aphanis* epidemiology and management. Two thematic blocks were focused on (i) epidemiological factors influencing disease outbreak: such as cultivar susceptibility, microclimate in tunnels, overwintering inoculum, wind exposure, canopy density, nitrogen management, (ii) perceived efficacy of control methods they use, had used or heard of: such as chemical fungicides, biopesticides, physical or cultural methods and spraying equipment.

Data analysis

Notes taken by the observer during the session were transcribed immediately after the focus group, anonymized, and organized into preliminary macro-themes corresponding to the discussion blocks. Finally, the notes were iteratively reviewed to identify recurring concepts, emerging issues, and areas of consensus or divergence among participants. The focus group report was finalized at the beginning of the present PhD research.

Results

Perceived disease impact

All participants identify powdery mildew as the most aggressive fungal disease in strawberry production. They report that the pathogen primarily infects young, expanding tissues (emerging leaves, leaflets and stolons), which remain highly susceptible throughout the crop cycle. Early symptoms appear as circular powdery colonies on the abaxial leaf surface, often unnoticed until they progress to the adaxial side and cause leaf curling; after fungicide applications lesions may show purplish discolorations. Marked cultivar differences are noted: Vivara and Portola are perceived as more tolerant, with slower symptom development and reduced fruit susceptibility, whereas Murano and Elsanta are considered very susceptible. Symptom expression is also cultivar-dependent: Murano often showed early fruit infection before foliar symptoms, while Elsanta develops leaf symptoms earlier in the epidemic. In conducive seasons, fruit losses reach up to 80%, particularly when fungicide applications were delayed.

According to agronomists, disease onset varies by location, being delayed in mountain areas but earlier and faster in valley sites due to higher tunnel temperatures and humidity.

Epidemiological factors influencing outbreaks

By consensus, strawberry powdery mildew shows high unpredictability, with strong year-to-year variability in infection onset and epidemic development. In low-pressure seasons, weekly preventive sprays are sufficient, whereas in high-pressure years up to two applications per week are required to control the disease. Warm, humid conditions are the main drivers of disease development, conditions that under protected structures persist throughout the crop cycle and allow epidemics to initiate at any time without a predictable pattern. Growers report the need for predictive tools, (ideally a decision support system comparable to those available for apple scab), to anticipate infection risk and optimize fungicide timing.

Disease pressure occurs in both everbearing and June-bearing cultivars. Agronomists report that poorly managed June-bearing fields frequently act as the main source of early-season inoculum, which subsequently spreads to everbearing crops. Farmers report in contrast, outbreaks also occurring in isolated fields, indicating that infected nursery plants can introduce the pathogen independently of local inoculum sources. According to growers, powdery mildew is continually present in the production environment and able to trigger infection whenever conditions are favourable. According to both farmers and agronomists, key uncertainties remain regarding the pathogen's life cycle, including the role of the sexual stage, overwintering sites (senescent leaves, canopy debris, crown tissue or substrate), and dispersal pathways of conidia. After winter, June-bearing fields typically undergo coarse leaf removal, whereas everbearing crops are transplanted without sanitation. Agronomists report that crop vigour strongly influences disease susceptibility: well-nourished plants show lower infection levels, whereas nutrient-deficient plants are more susceptible. However, excessive nitrogen promotes dense canopy that favours powdery mildew.

Synthetic chemical fungicides

Given the unpredictability of disease onset, growers apply preventive sprays from transplanting (mid-April) until the end of the cropping cycle (mid-October). Once established, the disease is difficult to manage, as fungicides provide limited curative activity, reinforcing the need for early and continuous preventive coverage. This preventive strategy is also supported by agronomists.

The most widely used active ingredients include triazoles penconazole, difenoconazole, myclobutanil, tetraconazole, fluxapyroxad, penthiopyrad, fluopyram, trifloxystrobin. Agronomists report declining performance of some active ingredients, particularly penconazole, suggesting possible resistance linked to long-term repeated use.

Growers increasingly consider calendar-based programmes as environmentally unsustainable. New formulations such as difenoconazole + fluxapyroxad, difenoconazole + cyflufenamid, penthiopyrad and trifloxystrobin + fluopyram have improved control. However, highly effective products, especially trifloxystrobin + fluopyram , tend to be overused, increasing the risk of resistance. Sulfur remains effective but is underused due to irritation issues, off-odors, and potential disruption of beneficial arthropods.

Biopesticides

Grower and agronomist experience in the field with biopesticides and non-chemical strategies is limited. Most natural products show no measurable effect against *P. aphanis*, and their evaluation is hindered by poorly controlled on-farm trials, where untreated controls are typically absent. Consequently, it is impossible for growers to determine product efficacy and application timing.

According to agronomists, the mycoparasite *Ampelomyces quisqualis* (AQ10) provides moderate control under cool, humid autumn conditions, but its narrow environmental window and incompatibility with fungicides restrict its use. Potassium and sodium bicarbonates are applied early in the season or in rotation with synthetic fungicides, though efficacy is inconsistent and often requires high or frequent applications; repeated use may cause phytotoxicity, including leaf burn and ageing. Orange oil (Prevam) can be effective at high spray volumes or concentrations, but these conditions increase phytotoxicity risk, particularly under warm or slow-drying conditions, and the product is considered harmful to beneficial arthropods.

Foliar ortho-silicic acid provides little or no disease suppression under commercial conditions and may leave visible residues on fruit.

Physical methods

Some growers apply ozonated water twice weekly, reporting reduced fungicide inputs, but the absence of controls prevents attribution of efficacy to ozone. Although all participants expressed interest in UV-C treatments, adoption is constrained by practical limitations, operator safety concerns, and difficulties integrating such systems into older, non-standardized high-tunnel structures. Overhead irrigation, while already available in the field, is generally avoided due to concerns about increased *B. cinerea* incidence. According to two agronomists, however, overhead irrigation could represent a viable option if properly implemented with optimized irrigation timing to minimize the risk of *B. cinerea* development.

Cultural practices

Cultural practices such as canopy thinning (twice per season) and stolon removal (up to three times during the production season) are considered by agronomists essential for reducing humidity, improving air circulation and enhancing spray penetration. According to growers, labour demand, however, limits their consistent implementation. According to agronomists, excessive thinning may weaken plants and reduce yield, while stolon removal is regarded as effective without major drawbacks.

Spraying equipment

Air-blast cannon sprayers are widely used because of their low labour requirements; however, agronomists reported uneven spray coverage along tunnel length (15–30 m), resulting in untreated zones that may act as infection foci. Growers typically spray from the tunnel entrance, relying on airflow to carry the spray plume to the opposite end, which leads to declining coverage with distance. Although hand-held sprayers provide more uniform coverage, their high labour demand limits feasibility at scale. Consequently, application systems entering crop rows and delivering uniform canopy coverage are needed. Fixed or automated systems could improve both coverage and labour efficiency, but their adoption is constrained by sloping terrain, older tunnel structures, and high implementation costs.

Discussion

This focus group highlighted several limitations in the current management of strawberry powdery mildew under commercial high-tunnel production. Participant experiences align with published findings, particularly regarding the disease being one of the major constraints in protected cultivation, its rapid symptom development, and the difficulty of detecting early outbreaks (Carisse et al., 2013).

Growers rely on frequent fungicide applications, primarily fluxapyroxad, trifloxystrobin, fluopyram, difenoconazole, penconazole and cyflufenamid with no alternative options available due to inconsistent field performance and lack of validated application strategies. Among non-residue products, sulfur, potassium bicarbonate and orange oil were considered the most effective, although each has notable limitations: sulfur causes off-odors, operator irritation and affects beneficial arthropods, while potassium bicarbonate and orange oil present phytotoxicity risks and orange oil also harms beneficial organisms. These products therefore require careful integration into spray programs, alternating them with options that are neither phytotoxic nor disruptive to beneficial arthropods.

Widespread uncertainty about the basic epidemiology of *P. aphanis*, contributes to management strategies focused on calendar-based spraying rather than targeted interventions. Participants' interest in predictive tools highlights the need for decision-support systems tailored to protected strawberry production, especially given the high variability in epidemic onset caused by broadly favorable environmental conditions, However, existing studies in the literature are fragmented and often poorly validated, limiting their practical applicability (Aldrighetti and Pertot, 2023). Simple early-season indicators could help guide spray timing and delay initial infection (Caffi et al., 2011). Agronomists identified June-bearing overwintered crops as major early-season inoculum sources, while nursery plants were also considered potential contributors, indicating the need for sanitation measures that extend from nursery propagation to early-season management of overwintered plantings. Once the disease is present, growers stressed the need to understand its main drivers, perceived as unpredictable and constantly present, to support timely decisions and avoid unnecessary or poorly timed applications.

Participants identified agronomic factors, particularly canopy management, as important for disease control. Canopy thinning and stolon removal were considered beneficial, but their high labour demand leads to inconsistent implementation, and no evidence supports the efficacy of canopy thinning against strawberry powdery mildew (Aldrighetti and Pertot, 2023). Participants made no reference to breeding efforts for more tolerant cultivars, indicating that varietal choice is not currently considered for disease management, likely because current varietal choices are driven primarily by market traits (fruit quality, shelf-life, yield and continuity of production).

The widespread use of cannon sprayers, which produce uneven spray deposition along tunnel length (Bondesan et al., 2015), likely contributes to early infection foci and rapid epidemic onset, highlighting the need for application systems ensuring uniform canopy coverage. However, the predominance of older tunnel structures and the constraints of mountainous areas limit the feasibility of advanced technologies, such as UV-C systems, despite their proven effectiveness (Janisiewicz et al., 2016). The adoption of such technologies often requires costly infrastructure upgrades that growers, particularly those operating small and fragmented farms typical of mountain regions, consider economically unviable (Duglio et al., 2023). Consequently, structural and socio-economic constraints remain major barriers to the integration of innovative disease-management methods (Dhillon and Moncur, 2023). Although growers expressed interest in novel approaches, adoption was generally cautious, and even compatible, readily available options such as overhead irrigation were rarely used due to perceived limitations (Asalf et al., 2021). As a result, growers tend to rely on established chemical strategies, which are perceived as more reliable. Broader adoption of alternative tools will require improved technical support, targeted training, and incentives to reduce perceived economic and operational risks (Kinuthia et al., 2019).

Conclusion

This focus group revealed research priorities for improving powdery mildew management in protected strawberry production:

(i) improving understanding of *P. aphanis* epidemiology under high tunnels, particularly overwintering inoculum and early-season infection dynamics;

(ii) validating post-winter leaf removal, canopy thinning and stolon removal;

(iii) integrating effective alternatives to synthetic fungicides into reliable strategies;

(iv) replacing cannon sprayers with technologies that ensure more uniform canopy coverage;

(v) strengthening knowledge transfer to support the adoption of innovative approaches.

Contributions. All authors contributed to the conception and design of the study. The focus group was designed, organized, and conducted by A.A. and I.P. Development of the discussion guide and participant recruitment were carried out by A.A. A.A. served as the moderator of the focus group, while I.P. acted as non-participant observer. Data analysis was conducted by A.A. All authors contributed to the interpretation of the results, critically revised the manuscript, and approved the final version.

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The role of wind in the spatiotemporal distribution of strawberry powdery mildew in high-tunnel growing systems

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Abstract

Strawberry powdery mildew, caused by *Podosphaera aphanis*, is a major fungal disease affecting strawberry cultivation worldwide. Its rapid lifetime cycle and ability to spread under a wide range of favourable conditions make early detection and management particularly challenging. Understanding the impact of environmental factors on disease dispersal is crucial for improving forecasting and control strategies. This study investigated the spatiotemporal distribution of strawberry powdery mildew in a high tunnel with a specific focus on wind as a primary driver of inoculum release and dispersal. Disease spread was monitored from a single inoculum source, both under natural wind conditions and with minimized wind influence to assess pathogen dispersal efficiency under varying wind speeds. The infection rate was modelled using a Zero-Inflated Negative Binomial (ZINB) model according to airborne conidium concentration and distance from the inoculum. Results show that disease spread follows an exponential decay pattern with a dispersal rate of 1.65 m day^{-1}. Temperature and relative humidity significantly influence conidium release, with wind as the most critical factor driving pathogen dispersal. Wind contributes to the formation of heterogeneous infectious hotspots along the tunnel, shaping the spatial and temporal distribution of the disease. However, wind speed had no significant impact on quantitative disease progression, highlighting high pathogen dispersal efficiency even under low wind conditions.

Keywords: strawberry powdery mildew, wind, conidium release, patch distribution, dispersal efficiency

Introduction

Strawberry powdery mildew (SPM), caused by the Ascomycetes *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatsu is a disease present worldwide that can seriously threaten global strawberry production (Carisse et al., 2013a). The main symptoms of the disease consist of white, powdery patches of mycelium and conidia that can be found on all aerial parts of the plant (i.e. leaves, runners, flowers, fruits), with younger tissues being particularly vulnerable (Carisse and Bouchard, 2010; Asalf et al., 2014). Yield losses can reach up to 30%, due to several damaging effects: white mycelium covering ripe and unripe fruit, fruit hardening and dehydration, with deformation, exposure of achenes, and eventual fruit decay (Peries, 1962a; Carisse et al., 2013b). Given its substantial economic impact, extensive research has been conducted on the pathogen's life cycle and epidemiology, providing nowadays a robust understanding of this disease (Aldrighetti and Pertot, 2023).

To enhance fruit quality and extend strawberry production to the off-season, cultivation has transitioned from open-field methods to soilless systems under protective structures, such as plastic tunnels and greenhouses (Takeda, 2000; Menzel, 2025). However, these protected environments create conditions that favor disease, as they offer an ideal environment for its development. In fact, these protections, designed to mitigate fungal pathogens that thrive in high humidity, such as *Botrytis cinerea*, prevent rain and reduce leaf wetness, both factors which naturally inhibit *P. aphanis* conidium germination in open fields (Asalf et al., 2021). Furthermore, reduced sunlight intensity caused by polyethylene or glass covering promotes SPM, as powdery mildew (PM) pathogens are photosensitive (Amsalem et al., 2006; Elad et al., 2007). Strawberry powdery mildew can develop under a broad range of temperatures and relative humidity (RH) levels. Germination occurs optimally between 15°C and 25°C with 95% RH, declining rapidly as RH decreases, whereas it is suppressed below 5°C and above 35°C (Amsalem et al., 2006). Infection rate progresses optimally between 12°C and 22°C, stopping below at 8°C and above 32°C (Peries, 1962a; Amsalem et al., 2006; Sombardier et al., 2009). Under favourable conditions, pathogen's life cycle (germination, infection, conidiation) is remarkably rapid, completing within 5-6 days. At optimal conditions, conidia germinate within 4-5 hours, followed by hyphal formation within 24 hours. Conidiophores emerge approximately 5 days after inoculation, with conidium release starting 6 days after inoculation (Peries, 1962a; Jhooty and McKeen, 1965; Iwasaki et al., 2021). Within its lifetime (35 days after inoculation) each patch can release an average of 6.7×10^4 conidia (Ayabe et al., 2022). This rapid cycle combined with the wide range of favourable conditions, facilitates significant disease spread. Consequently, frequent applications of synthetic chemical fungicides are necessary from the emergence of the first leaves until the end of the season to manage the disease effectively.

The pathogen overwinters in two forms: as mycelium on overwintering green leaves (Gadoury et al., 2010; Iwasaki et al., 2021) and/or as chasmothecia, which develop in late summer/early autumn on infected leaves as temperature declines (Peries, 1962b; Gadoury et al., 2010; Jin et al., 2012). In spring, typically from early March to late May in the northern hemisphere, the overwintering mycelium and ascospores released from chasmothecia initiate primary infections on new plant growth (Gadoury et al., 2010). This leads to conidium production, dissemination and subsequent polycyclic infections (Gadoury et al., 2010; Iwasaki et al., 2021).

To manage SPM effectively, reducing the initial inoculum in the field, such as by removing old overwintering leaves, may be a key strategy to reduce SPM infections. Once established, timely interventions are crucial to prevent the rapid spread of the disease. However, field observations (Aldrighetti and Pertot, unpublished data) suggest that many farmers face difficulty detecting disease during the early phases of epidemics. This difficulty is likely tied to the localized nature of initial infections (Van Der Plank, 2013), which can lead to underestimations of inoculum source and its rate of spread. This knowledge gap underscores the need for further research into inoculum dispersal and infection dynamics under field conditions, which is crucial for developing effective management strategies (Aylor, 1999; Xu and Ridout, 1998).

In fungal pathogens, inoculum dispersal generally occurs in three phases: passive and/or active release, dispersal (normally through abiotic means of transport such as wind and rain) and deposition (Van Maanen and Xu, 2003). The efficiency of these phases directly influences new infections (de Vallavieille-Pope et al., 2000). For this reason, understanding how environmental factors impact these processes plays a key role in improving forecasting schemes. For many *Erysiphales*, including *P. aphanis*, conidium release is a complex and debated process (Adams et al., 1986). Active release of *P. aphanis* conidia mainly occurs during the daytime, often triggered by rising temperatures and decreasing RH (Blanco et al., 2004; Iwasaki et al., 2021). Passive conidium release is thought to be driven primarily by wind (Peries, 1962a; Fall and Carisse, 2022), as its natural turbulence significantly contributes to conidium dislodgment under field conditions (Aylor, 1990). However, the quantitative role of wind as a driver of conidium release under tunnel conditions remains undocumented.

Beyond conidium release, wind and turbulence play a crucial role in the dispersal of conidia, facilitating the spread of infections (Peries, 1965b; Willocquet et al., 2008; Prodorutti et al., 2024). However, unlike similar situations for pollutant concentration downwind to emission sources (Panofski and Dutton 1994, Rada et al. 2011), the connections of the onset of epidemics and the spatial distribution of the disease in the filed have never been directly associated with wind patterns. Hence, the exact extent to which wind influences the speed of disease spread and the spatiotemporal threshold for effective fungicides remains unclear.

This study aims to investigate the infection rate and spatial distribution of SPM, with a specific focus on wind as a primary driver for inoculum release and dispersal, and in particular by examining the progression of the disease under tunnel conditions. We assessed whether wind speed plays a key role in the rapid spread of infection, or if multiple inoculum sources within the field contribute more significantly to disease development. The specific objectives were to: (i) evaluate the influence of wind dynamics, temperature, RH and light intensity on conidium release under tunnel conditions and (ii) investigate the spatiotemporal development of SPM epidemics in relationship with wind, temperature, RH and light intensity. Over two growing seasons the concentration of airborne conidia, originating from a single infection focus, was monitored and related to the spatiotemporal distribution of infection patches. In the first growing season, dispersal and distribution were modelled under natural wind conditions. In the second season, wind influence was minimized within the tunnel to study disease spread under minimal wind conditions. This comparative approach aimed to clarify the impact of wind on disease spread patterns and provide new insights into SPM detection and management of early-stage epidemics strategies.

Materials and Methods

Experimental set-up

The experiments were carried out in 2023 and 2024, under a plastic-covered tunnel (Polyagri AG, Agripolyane, Italy; 9% of light transmission reduction) 17 m long and 8 m wide with a height of 3.90 m (Fig. 1a) located in Vigolo Vattaro municipality (46° 0' 13.223" N, 11° 11' 18.650" E, 682 m a.s.l.), Italy. The target area is located on the floor of a wide valley on the Vigolana Plateau (Fig. 2) an area where regular daily winds are known to occur under weather conditions associated with clear sky and calm upper wind (Giovannini et al., 2013; Laiti et al., 2013, 2014). When environmental conditions were predicted suitable for disease development (on June 9, 2023, and on May 13, 2024, respectively) according to field experience and established literature, refrigerated tray plants (cultivar SO.FR.12.04.16, available at Sant'Orsola variety collection) were transplanted in peat (Torba Silver® 20-40, Agrochimica, Italy) in suspended pots (four plants pot^{-1}). Fertigation (6 NO_3, 6 H_2PO_4, 5 K^+, 3 Ca^{+2}, 1.25 Mg^{+2} mmol L^{-1} in irrigation water at pH 5.5, electrical conductivity 1.5 mS cm^{-1}) was delivered with irrigation drippers (four pot^{-1}) and kept constant by an automatic system (Gravimetric, Spagnol, Italy), monitoring and adjusting nutrient concentrations, pH, and moisture levels. A total of 680 plants were arranged in five rows (136 plants row^{-1}) 17 m long, spaced 1.15 m apart (Fig. 1b) To guarantee the absence of sporulating PM foci in the tunnel all leaves were removed during transplanting, leaving only the bud. In 2023, tunnel perimeter was open from the base to 2.20 m high as it normally occurs in commercial cultivation. To study conidium release and symptoms distribution under minimised wind conditions, in 2024 the perimeter of the tunnel was enclosed with insect-proof nets (Biorete 25 mesh, Arrigoni, Italy) on the southeastern (S-E) and northeastern (N-E) sides and polyethylene film (Multisolar, Eiffel S.pa., Italy) on the S-W and northwestern (N-W) sides. Based on prevalent wind analysis carried out in the previous years (data not shown), the polyethylene film was selected to reduce the stronger prevailing upwind (S-W, N-W), while the insect nets to reduce weaker S-E/N-E wind while maintaining adequate ventilation. The tunnel is part of a block of six adjacent tunnels arranged in the S-E direction, with a shed located at the end of the block. The remaining sides of the tunnel are open. Maize is cultivated to the N and W direction, and an apple orchard is located to the S. The two seasons were designated as No Wind Fence (NoWF) and with Wind Fence (WF), respectively for natural wind conditions in 2023 and minimised wind conditions in 2024.

Weather monitoring

Weather data (wind direction, wind and gust speed, temperature, and RH) were recorded inside the tunnel at 5-minute intervals. Inside the tunnel, wind parameters were measured using a two-dimensional sonic wind sensor (600023/900028, METOS® by Pessl Instruments, Austria), while temperature and RH were monitored with a meteorological station (WSTATION, CET Electronics Sensors, Italy). The wind sensor and the meteorological station were placed at 1.90m in the tunnel centre. Macroclimatic conditions (i.e., air temperature, air RH, wind speed and direction, and solar radiation data were obtained as hourly averages from a nearby external meteorological station, located at a distance of about 1 km from the experimental tunnel, permanently operated by Fondazione Edmund Mach-Consulting and Services Center (https://meteo.fmach.it/meteo/dati.php).

Inoculation and sampling of airborne conidium concentration

Inoculation was carried out on June 13, 2023, and on May 20, 2024, corresponding to the phenological stage of one/two expanded leaves in the two years. Four eight-week-old, infected plants (50% SPM severity) were placed at the top of the central row in S-W direction, which corresponded to the prevailing upward wind determined during the two months prior. The plants served as the single inoculum source and were exposed for 17 days. Airborne conidium release from the inoculum source was measured with a cyclone-type volumetric spore sampler (Gabriele Marchi, Italy) with 250 L h^{-1} airflow rate was used to monitor airborne conidia under the tunnel. The sampler was positioned 35 cm above the canopy and 1 m from the inoculum, oriented toward the infected plants. Glass slides coated with a thin layer of vaseline (Vaselina Bianca F.U., Marco Viti, Italia) were also used to monitor airborne conidia. Glass slides were exposed 7 days, then collected and stored at 4°C until counting the number of conidia under a microscope at ×250 magnification. Conidial identification was performed based on morphological characteristics (ellipsoidal to ovoid shape, hyaline appearance, contained oil and fibrosin bodies, 27–33×18–22 μm in size), described by Iwasaki et al. (2021). Six daily counts were conducted at 4-hour intervals (0–4, 4–8, 8–12, 12–16, 16–20, 20–24 hours) and data were converted to conidia m^{-3} of air.

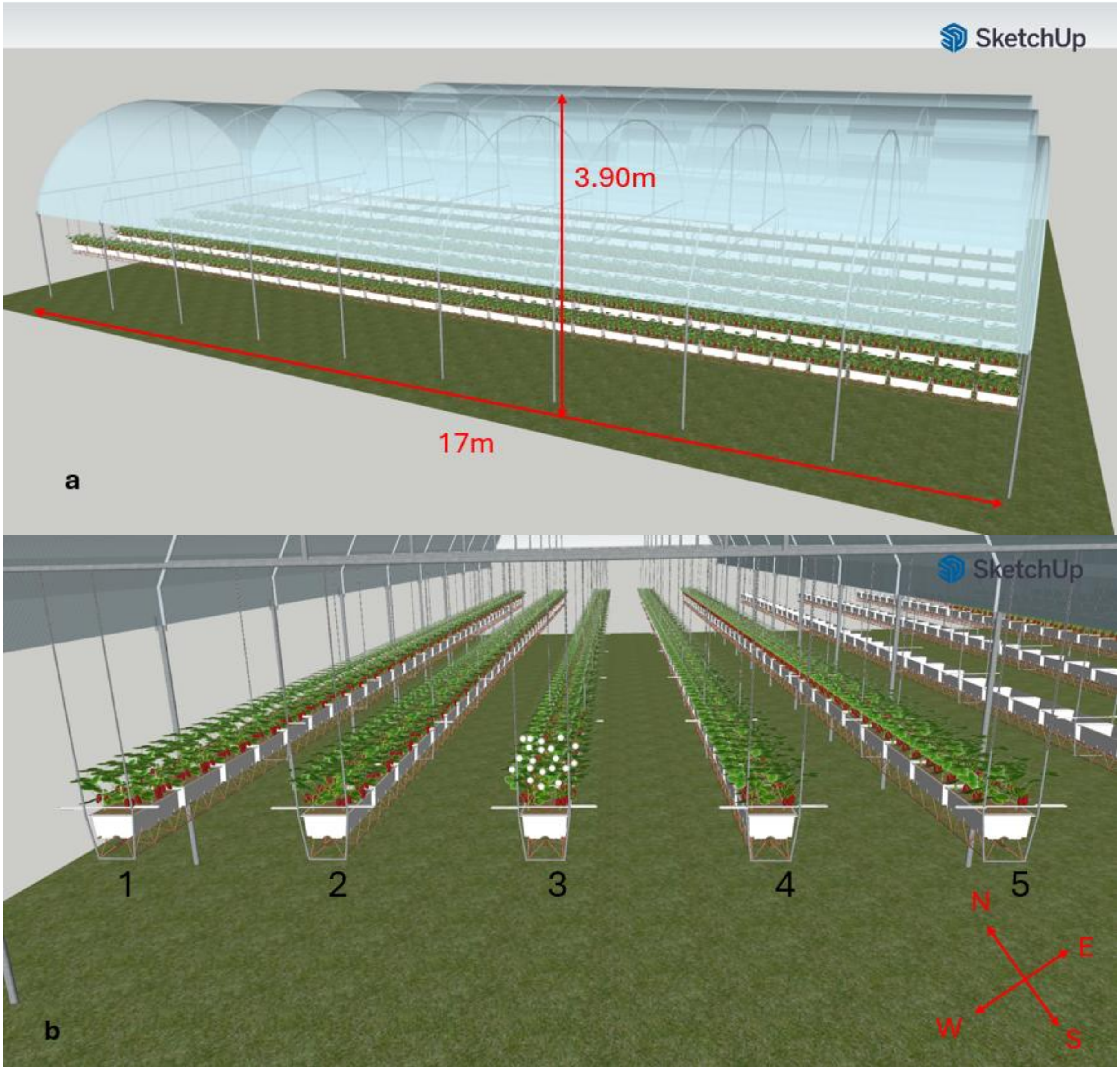

Fig. 1 - Experimental set-up in high-tunnel (a,b). Inoculum (plant with white patches) position on row 3 (b). Figures (a,b) were made with SketchUp (Trimble) https://www.sketchup.com.

Fig. 2 - Orography of Vigolana Plateau**.** Figure was taken from Google Earth https://earth.google.it.

Assessment of patch distribution

Following transplantation, each plant was numbered and georeferenced with x and y coordinates. The origin point (0,0) was set at a defined tunnel pole, with inter-row spacing of 1.15 m and plant spacing of 12.5 cm. Leaves of each plant were numbered on the adaxial surface with a permanent marker (N50, Pentel Pen, Japan), starting with the oldest leaf (numbered as “1”) and numbering new leaves sequentially as they emerged. New leaves were numbered as they emerged, providing z-coordinates (number of leaves per plant).

A georeferenced database of plant coordinates (x, y, z) was developed, interfacing with the open-source MySQL database (MySQL Database Software, 2024 Oracle, https://www.mysql.com/xy), built using the Django framework (Django Software Foundation, 2005–2024, https://www.djangoproject.com/). During observations, a map displaying georeferenced plants and their respective leaf numbers was used to record the number of SPM patches per leaf. To reduce this risk of operator-mediated contamination, all observations were carried out starting from the plants located furthest from the inoculum source and progressing toward those closest to it and more heavily infected. Additionally, we regularly disinfected our hands with disinfectant during assessment. Number of SPM patches were assessed 3, 7, 10, 14, and 17 days after inoculation in both years. Weather conditions and disease assessment procedures were maintained consistent with those used in the first year. Patch number was used as a proxy for the density of airborne conidium deposition on leaf surfaces.

Data analysis

Statistical analyses and plot creations were conducted using R version 4.1.1 (R Core Team, 2022). To define significant differences between NoWF and WF conducive conditions for SPM development, the Wilcoxon signed-rank test was carried out as data consisted of paired observations (days after inoculum exposure) and were non-normally distributed according to Shapiro-Wilk test.

To evaluate the influence of environmental factors (i.e., wind speed and direction, temperature, RH, light intensity) on airborne conidium concentration and infection rate expressed as patch number, two zero-inflated generalized linear models with a negative binomial distribution (ZINB) were fitted using the 'pscl' package (Zeileis et al., 2008).

The ZINB models were used for this analysis due to a high number of zeros in the response variables: 33% and 54% for the airborne conidia concentration and infection rate, respectively (Greene, 1994). We developed a mixture model comprising two generalized linear models (GLMs): one based on a Bernoulli distribution with logit link and the other on a Negative Binomial (NB) distribution with log link. This framework results in a ZINB distribution for the response variables, offering a practical interpretation of the zeros in the dataset. Specifically, the zeros are attributed to two distinct processes: "true" zeros, (e.g., conidium count is zero because conidia were not effectively released) represented by the count process (NB GLM), and "false" zeros, captured by the binomial process (Bernoulli GLM), (zeros, which the count process cannot explain, arising from underreporting or detection failures) (Zuur et al., 2009).

In the first model (ZINB1), we considered airborne conidium concentration as response variable and five possible explanatory variables both for the count model and the binomial model: (i) wind speed, (ii) wind direction, (iii) temperature, (iv) RH (v) light intensity. Given the strong collinearity between temperature and RH, and between temperature and wind speed, it was decided to analyse only the influence of wind speed, wind direction and light intensity.

In the second model (ZINB2), we considered as response variable the infection rate expressed as the number of patches and seven explanatory variables: (i) airborne conidium concentration over the previous 7 days responsible for new patches (i.e., 7-day lagged airborne conidium concentration) (ii) presence or absence of WF, (iii) distance of patches from inoculum, (iv) temperature, (v) RH, (vi) wind speed, (vii) wind direction. Given the strong collinearity, temperature, RH and wind speed and direction were dropped as covariates and only 7-day lagged airborne conidium concentration was kept since it resulted to be significantly correlated to the wind speed in the previous model (ZINB1). The residual distribution and fitness of the models were evaluated using ‘DHARMa’ and ‘performance’ packages (Hartig, 2017; Lüdecke et al., 2021). The Akaike's Information Criterion (AIC) (Sakamoto et al., 1986) was used to select the most parsimonious model, i.e. the one with the lowest AIC and significant coefficients.

To investigate the influence of wind on patch distribution patterns, based on classical results for the transport of passive tracers (Panofsky and Dutton, 1994), we adopted a heuristic approach and tested how an exponential decay model (1) fitted the data. To investigate the influence of wind on patch distribution pattern, the exponential decay model (1) was fitted to the data. The equation (1)

$$(1)\ \ y = a \times e^{-b} \cdot x + c$$

y is the number of patches leaf^{-1} plant^{-1}, x their distance downwind from the inoculum source, a is the initial patch density near the inoculum, b is the rate decay in patch density with distance and c is the number of patches persisting at greater distances.

Results

Wind

Given the position of the tunnel, located in complex terrain and in particular on the floor of a small valley, close to the slopes (Fig. 2), the meteorology of the area, especially in Spring and Summer, is characterized by periodic thermally driven circulations, which typically feature two phases corresponding to up-valley winds during daytime and down-valley winds during nighttime (Laiti et al. 2013, 2014; Giovannini et al. 2015, 2017). At each point within the mountainous area the phase and direction of the wind cycle depend on local topography, i.e. local sunrise and sunset times, slope angle and exposition to incoming solar radiation. Clearly, inside the tunnel, the main direction of the wind is determined by the tunnel structure itself and tunnel surroundings, as can be observed in the wind roses reported in Fig. 3. In NoWF wind frequently peaked at 3.6 m s^{-1}, predominantly from the S-W direction. In contrast, in WF wind intensity was much reduced, rarely exceeding 1.5 m s^{-1} and more frequently originating from N, N-E. The analysis of data from the nearby weather station shows that the daily mean wind speed, measured outside the tunnel at 10 m height, was 1.2 m s^{-1} in 2023 (NoWF) and 1.0 m s^{-1} in 2024 (WF). The distribution of wind speed and direction was similar during both years, with wind with higher intensity coming from the eastern and western sectors, as expected by the E-W orientation of the valley (Fig. S1).

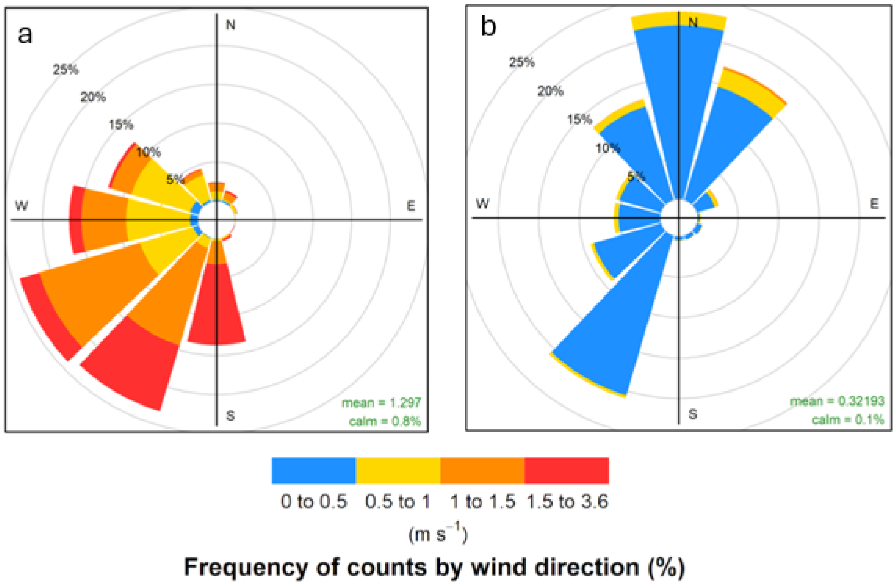

Fig. 3 - Wind roses characterizing the speed (m s $^{-1}$) and direction frequency (%) of wind under the tunnel with no wind fence (a) and with wind fence (b). One circle represents 5% for each direction and speed frequency. Colours represent wind speed from 0 to 3.6 m s^{-1}. The two-dimensional sonic wind sensor was mounted at 1.9 m height above ground.

Temperature, relative humidity and light intensity

In WF, a higher number of cumulated conducive hours (>15.5° C, < 30°C, > 60% RH) for SPM were recorded compared to NoWF (V = 31.5, p-value = 0.03) (Fig. 4). Daily mean RH under the tunnel in WF was higher (78% RH) with lower daily mean temperature (20.7° C) compared to NoWF conditions, (66% RH and 21.4° C, respectively). Daily mean temperature and RH outside the tunnel were 19.8° C and 68% in 2023 (NoWF) and 18.2° C and 82.6% in 2024 (WF). The average of hourly light intensity during the 17-day experiment was higher in 2023 (NoWF) compared to 2024 (NoWF) conditions, 0.95 MJ m^{-2} and 0.69 MJ m^{-2} respectively.

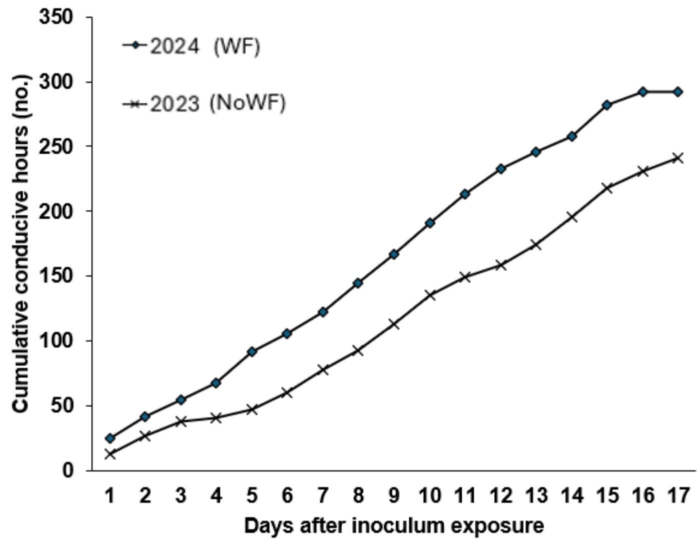

Fig. 4 Cumulated number of conducive hours > 15.5° C, < 30°C, > 50% RH in 2023 with no wind fence and 2024 with wind fence.

Conidium release

The daily observations (Fig. 5) in NoWF show that conidium dispersal increased between 12:00 and 24:00 (4^{th} – 6^{th} phase), coinciding with periods of higher wind speeds. In contrast in WF, the presence of the wind fence significantly reduced conidium flight, highlighting the dependence of conidium release and dispersal on wind dynamics. ZINB1 (Table 1) demonstrates a significant positive association between maximum wind speed and airborne conidium concentration (Estimate = 0.6758, $p < 0.001$), indicating that increased wind speed enhances conidium release. Conversely, wind direction and light intensity had no significant effect on conidium concentration. Even if temperature was dropped because of collinearity, its role in conidium release revealed a weaker but still significant positive association with airborne conidium concentration (airborne conidium concentration ~ temperature, Estimate = 0.0576, $p = 0.0015$). However, the effect of temperature was substantially less pronounced compared to wind speed, as indicated by the higher AIC values (818.587 for airborne conidium concentration ~ temperature, 797.060

for airborne conidium concentration ~ wind speed + wind direction + light intensity), highlighting that wind remains the primary driver of conidium dispersal. The zero-inflation component of ZINB1 revealed that neither wind speed and direction, nor light intensity significantly explained the probability of observing an absence of conidia ($p > 0.05$).

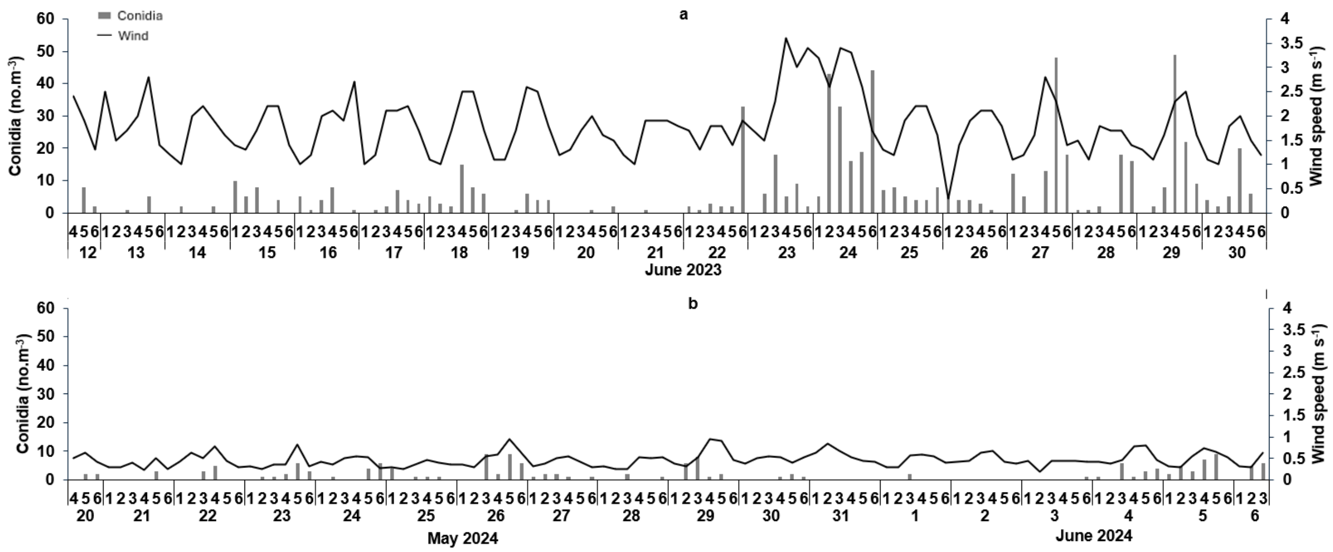

Fig. 5 - Wind speed and airborne concentration during the 17 day-experiments with no wind fence (a) and with wind fence (b). Grey bars indicate the conidium concentration for every daily phase (1-6). Black lines indicate the average wind speed.

Table 1- Summary of the selected best zero-inflated negative binomial regression model (ZINB1) for conidium concentration predictions based on wind speed, direction and light intensity.

Parameters of count model[a] **ZINB 1: airborne conidium concentration ~ wind speed + wind direction + light intensity**				
	Estimate	**se**	**Z**	***p* (>|z|)**
Intercept	-0.2008	0.5668	-0.354	0.723
wind speed	0.6830	0.1261	5.416	***
wind direction	0.0029	0.0022	1.319	0.187
light intensity	-0.0861	0.0932	-0.925	0.355

Parameters of binomial model[a]	**Estimate**	**se**	**Z**	***p* (>|z|)**
Intercept	-4.8593	3.2443	-1.498	0.1342
wind speed	-1.0413	0.7489	-1.390	0.1644
wind direction	0.0191	0.0106	1.791	0.0733
light intensity	-2.6079	3.0143	-0.865	0.3870

[a] Estimates of ZINB1 model, representing the effect size for each predictor, with standard errors (se), Z-value and significance levels: ***p < 0.001, **p < 0.01, *p < 0.05.

Temporal disease progression

In both conditions (NoWF and WF), initial patches were visible within 7 days of inoculum exposure (Fig. 6). Fourteen days post-inoculation, patch development on leaves increased linearly under both conditions, however in NoWF, a higher number of patches was observed compared to WF. Seventeen days after exposure, patch development in NoWF followed a rapid exponential growth pattern, whereas WF showed a slower linear increase. Airborne conidium concentration, in both conditions, mirrored the pattern of patch increase, suggesting a strong correlation between patch development and airborne conidia. Under WF conditions, the concentration of airborne conidia was significantly lower than in NoWF. However, 17 days after inoculum exposure, the number of patches was comparable between the two conditions (Fig. 6). The influence of wind was further quantified by analysing the number of patches/conidia ratio. Under NoWF, the ratio followed an exponential growth $y=a \cdot e^{bx}$ (R^2= 0.9974).

Conversely, under WF the ratio followed a power law $y=ax^k$ ($R^2=0.9956$), indicating a slower increase of the number of patches in relation with the number of conidia in the air.

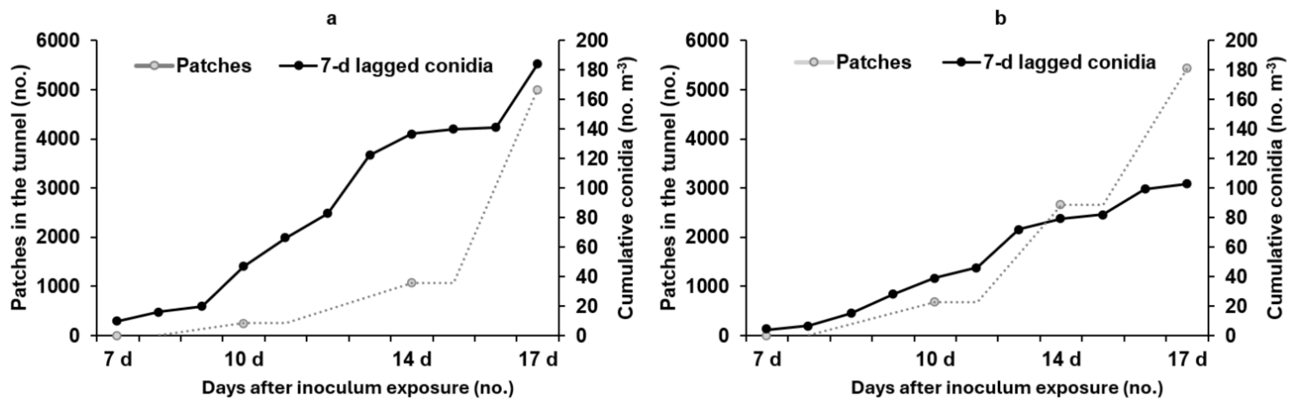

Fig. 6 - Progression of powdery mildew epidemic with no wind fance (a) and with wind fence (b). Patches in the tunnel are reported for every observation date. The cumulative number of airborne conidia is lagged by 7 days (one infection cycle), for comparing the timing of conidium release and resulting patch development (infection rate).

Spatial disease progression

In NoWF, patches were observed within 1.15 m of the inoculum after 7 days and spread throughout the 16.5 m tunnel by day 10, with a peak rate of 1.65 m day^{-1}. After 17 days, exponential growth occurred, with the highest concentration within 2.2 m of the inoculum. In WF, patches were initially restricted to 0.30 m from inoculum after 7 days, indicating a smaller dispersal radius. However, as observed in NoWF, by day 10, the infection had spread throughout the tunnel (16.5 m). After 17 days, the highest concentration of patches remained closer to the inoculum (4.5 m).

In NoWF, patch distribution was more homogeneous, indicating extensive conidium dispersal, whereas WF conditions resulted in localized patch development, consistent with reduced dispersal. While infection severity under WF conditions may appear higher compared to NoWF (Fig. 7), this is primarily due to the more localized distribution of patches rather than an actual increase in their number (NoWF Patches no.= 5006, WF Patches no.=5333, Fig. 6). The influence of wind on conidium distribution is visible, also 17 days after inoculum exposure: patch distribution in NoWF aligned with the prevailing wind direction in NoWF, with a higher patch density on row 4 and 5 (Fig. 8), a pattern which is not observed in WF, when wind speed and frequency were substantially reduced.

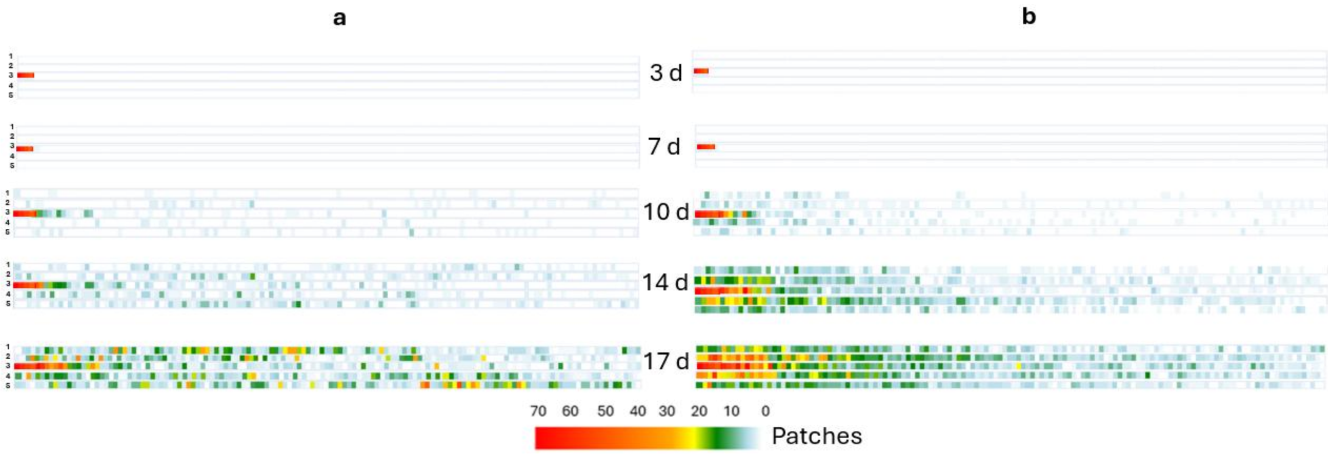

Fig. 7 - Spatial distribution of patches in the tunnel with no wind fence (a) and with wind fence (b), after 3, 7, 10, 14, 17 days from inoculum exposure. Each rectangle represents the tunnel, with lines representing 5 strawberry rows. Colours in the heatmap (from light blue to red) represent the number of patches per each plant (white represents no patches). Red rectangles at the beginning of tunnel represents inoculum plants. Tunnel direction is SW.

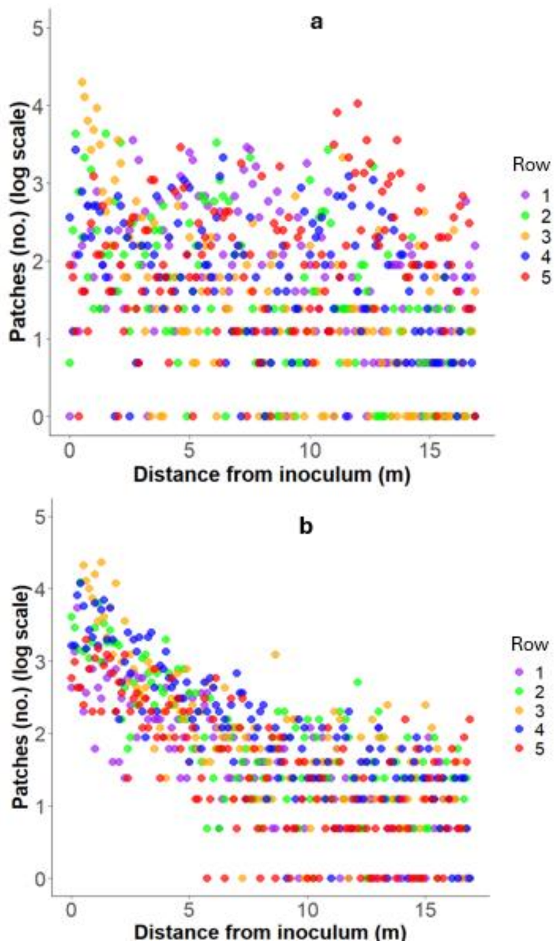

Fig 8 - Patch distribution from inoculum source with no wind fence after 17 days from inoculum exposure without wind fence (a), and with wind fence (b). Colours represent plant rows. Row numbers are reported in Fig. 1.

The spatial distribution of patches (Fig. 7) was quantitatively analysed using the exponential decay model (1) (Table 2, Fig. 9). The initial patch density *a* near the inoculum in NoWF was higher (16.5 patches leaf^{-1} plant), indicating that wind facilitated greater initial conidium dispersal, promoting more rapid patch growth. The decay parameter *b*, which represents the rate decrease in patch density with distance, was higher in NoWF (0.0179 m^{-1}), suggesting a steeper decline in patch density as distance increased due to wind-driven dispersal over longer distances. Conversely, in WF, the lower decay rate *b* (0.006504 m^{-1}) indicates a more gradual reduction in patch density with distance, likely due to limited conidium dispersal in absence of relevant wind. The asymptotic parameter *c*, which represents the number of patches persisting at greater distances, was lower in WF (0.7775 patches leaf^{-1} plant^{-1}) compared to NoWF (1.1205 patches leaf^{-1} plant^{-1}), suggesting fewer conidia were able to establish infections at higher distances under reduced wind conditions. Additionally, the R^2 residual was lower for the NoWF model (NoWF=0.4999, WF=0.8851).

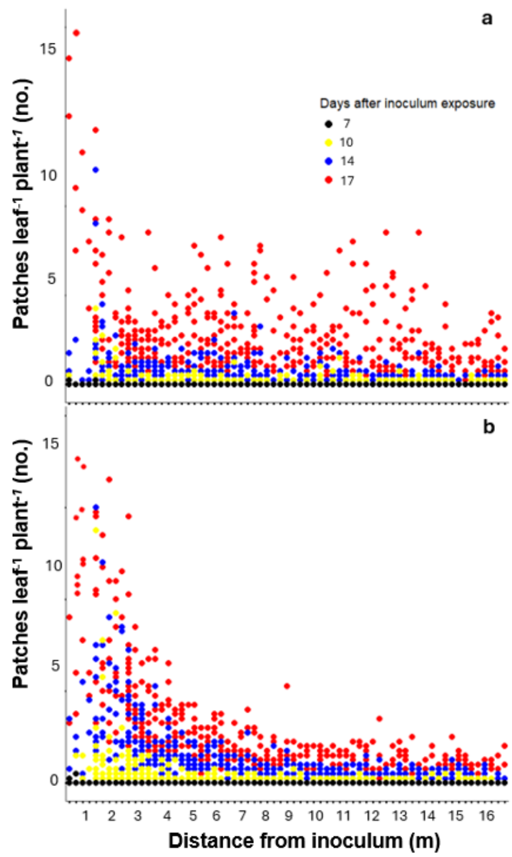

Fig. 9 - Patch distribution from inoculum source with no wind fence (a) and with wind fence (b) after 7, 10, 14, 17 days from inoculum exposure.

Table 2 - Exponential decay model fit results.

	a[a] (se)	b[a] (se)	c[a] (se)	R^2
No wind fence	16.5557*** (1.3004)	0.0179*** (0.0013)	1.1205***(0.0510)	0.4999
Wind fence	14.9401*** (0.5083)	0.0071*** (0.0025)	0.7775***(0.0454)	0.7851

[a]Estimates of exponential decay parameters. (se) is the standard error of the estimate. R^2 represents the squared correlation between the observed and predicted values. Significance levels: ***p < 0.001, **p < 0.01, *p < 0.05.

Table 3 Summary of the selected best zero-inflated negative binomial regression model (ZINB2) for the prediction of infection rate (number of patches) prediction based on distance, fence presence/absence, light intensity, 7-day lagged airborne conidium concentration.

Parameters of count model[a] ZINB 2: infection rate (number of patches) ~ distance from inoculum * WF/NoWF + light intensity + 7-day lagged airborne conidium concentration * WF/NoWF				
	Estimate[a]	**se[a]**	**Z[a]**	***p* (>|z|)[a]**
Intercept	-1.79177	0.19147	-9.358	***
distance	-0.32620	0.03024	-10.788	***
WF (NoWF as reference category)	2.91349	0.19153	15.212	***
light intensity	0.07867	0.04268	1.843	0.0653
7-day lagged airborne conidium concentration	2.36206	0.13512	17.481	***
distance*WF	-0.46391	0.04029	-11.513	***
WF*7-day lagged airborne conidium concentration	-0.59334	0.25959	-2.286	*

Parameters of binomial model[a]	**Estimate[a]**	**se[a]**	**Z[a]**	***p* (>|z|)[a]**
Intercept	-3.6180	1.5938	-2.270	*
distance	0.3916	0.1064	3.680	***
WF (NoWF as reference category)	-15.0536	555.3194	-0.027	0.9784
light intensity	3.4590	1.5778	2.192	*
7-day lagged airborne conidium concentration	-1.3062	0.6025	-2.168	*
distance*WF	0.3281	0.1777	1.847	0.0648
WF*7-day lagged airborne conidium concentration	-44.7843	1076.1734	-0.042	0.9668

[a] Estimates of ZINB2 model, representing the effect size for each predictor, with standard errors (se), Z-value and significance levels: ***p < 0.001, **p < 0.01, *p < 0.05.

The count component of the ZINB2 (Table 3) model revealed a significant negative association between the patch number and the distance from the inoculum (Estimate = -0.3262, $p < 0.001$), highlighting a reduced patch density as distance increased. Notably, the interaction between distance and WF further emphasized this trend (Estimate = -0.4639, $p < 0.001$), describing that under WF conditions, the infection rate decreased more rapidly with increasing distance. Wind fence conditions exhibited a positive association with patch number (Estimate = 2.9135, $p < 0.001$). In WF conditions, we still observed more favourable conditions for disease development. Seven-day lagged conidium concentration showed a robust positive effect on patch number (Estimate = 2.3621, $p < 0.001$). However, the interaction between WF and 7-day lagged conidium concentration (Estimate = -0.5933, $p = 0.0223$) reports that under WF conditions, the influence of conidium concentration on patch number decreased. In the binomial component (Table 3) distance from the inoculum showed a significant positive association with zero-inflation (Estimate = 0.3916, $p < 0.001$), indicating that plants farther from the inoculum source had a lower likelihood of infection. Light intensity also positively affected the probability of zero patches (Estimate = 3.4590, $p = 0.0284$), while 7-day lagged conidium concentration showed a significant negative effect (Estimate = -1.3062, $p = 0.0302$).

Discussion

This study investigated the influence of a wind fence on the release and dispersal of SPM conidia, by examining airborne conidium concentration originating from a single source of inoculum and the consequent spatiotemporal development of SPM patches under high-tunnel conditions. To assess whether wind dynamics may be considered the main driving factor for infection rate, we monitored the influence of wind on the relationship between airborne conidium concentration and the spatiotemporal development of patches, together with temperature, RH and light intensity.

Conidium release

Wind speed strongly influenced the concentration of airborne conidia. Under WF conditions, airborne conidium concentrations, measured at 1 m from the source, were significantly lower compared to NoWF conditions. However, dispersal still occurred efficiently, consistent with findings on other PM as barley PM (Pauvert, 1986), rose PM (Frinking, 1977) and tobacco PM (Cole and Geerligs, 1976). According to the ZINB2 model, higher concentrations of conidia observed 7 days prior infections strongly increase both the probability and the number of patches. However, according to ZINB2 under WF conditions, the influence of conidium concentration on patch number decreased. This indicates that the total number of conidia released may have been comparable across both conditions. In WF, reduced air movement may have prevented the sampler from detecting conidia originating from secondary infections that remained localized but still contributed to new patch development. These findings suggest that wind primarily influenced the movement of airborne conidia within the tunnel, but the pathogen demonstrated effective conidium release even at low wind speed (0.5 m s^{-1}). While this setup enabled the detection of airborne conidia released from the inoculum source under varying wind conditions, incorporating multiple traps along the tunnel's length would have provided a more detailed assessment of conidial dispersal patterns and a higher-resolution understanding of disease progression dynamics. This approach may be considered for future experimental designs to accurately trace the patter of conidia movement along the tunnel.

Beyond wind dynamics, conidium dispersal has been influenced by temperature and RH. In agreement with other findings (Blanco et al., 2004), higher temperatures and lower RH were associated with increased conidium release. However, wind speed resulted the main driving factor described by the highest impact on the ZINB1 model. In contrast, in our set-up light intensity had no influence on conidium release efficiency, contrary to other studies on *Erysiphales* (Adams et al., 1986) reporting higher conidium release at increasing infra-red light. Even under NoWF (natural wind speed, temperature increase and RH decrease), no airborne conidia were detected. This variability suggests that additional environmental or biological factors may play a significant role in conidium release. Factors such as conidium development and maturation could be critical. For example, as reported for SPM (Ayabe et al., 2022) and grape PM (Willocquet et al., 1998), the age of the patch can influence conidium dispersal. Young patches may exhibit slower conidiogenesis, while mature colonies tend to produce greater sporulation and form longer conidial chains, facilitating more efficient dispersal.

Temporal disease progression

In our study first visible symptoms appeared after 78 and 122 conducive hours in NoWF and WF, respectively. This is in line with previous laboratory studies that report *P. aphanis* visible colonies 5 days after inoculation at 20-22°C and 70-75% relative humidity (Amsalem et al., 2006; Iwasaki et al., 2021). Similarly, field-based decision support systems (Dodgson et al., 2021) report first visible symptoms after 138 cumulated conducive hours (>15° C, < 30° C, 60%) and sporulation after 144 (>18° C, < 30° C, 60%). The variability among studies suggests that *P. aphanis* can infect plants under a broad range of environmental conditions, and the time for symptoms appearance can be influenced by small differences in the environmental condition or sampling period. Differences in how environmental conditions accumulated over time may have influenced symptom development. This so-called "vintage effect" suggests that not only the quantity, but also the temporal pattern of favorable conditions can impact infection dynamics and should be considered when comparing epidemics across years (Madden et al., 2007). In addition, different local strains of *P. aphanis*, which may have adapted to local environmental conditions, may also be responsible of this slight variation, therefore calling for further investigation.

Wind reduction significantly influenced the temporal dynamics of disease progression, shifting the growth curve from an exponential to a linear pattern. Under NoWF, infection followed the typical Sshaped growth curve, with latent and exponential phases. This pattern is consistent with other field studies on *Erysiphales* (Waggoner and Rich, 1981; Lalancette and Hickey, 1986; Liu et al., 2015; Cao et al., 2021). The latent period lasted 13 days, with patch numbers increasing slowly until day 17, when the exponential phase began. This pattern also suggests the initiation of secondary infections, typical of polycyclic diseases as PM. Polycyclic infections are related to the exponential increase in conidial concentration, observed as early as 10 days after inoculation. In contrast, under WF, disease progression did not follow an exponential regression, but a linear regression, indicating a reduced secondary spread. Although during the latent period (after 10 and 14 days) patch number was significantly higher compared to NoWF, the disease progression stalled after day 17. This suggests that limited conidium dispersal within the tunnel, caused by reduced wind influence, minimized the establishment of secondary infections, which are crucial for exponential disease growth.

Despite the shift from exponential to linear growth under WF conditions, the temporal and spatial spread of the disease progressed at a similar rate in both NoWF and WF condition, with the disease reaching the entire tunnel length within 10 days. Disease spread was likely driven not only by the limited wind turbulence entering the tunnel but also by convective forces (Boulard et al., 2002). Convective forces are generated when differences within a close environment (as in WF conditions) in temperature and/or RH cause air to move, creating a circulation of air known as convection (Roy et al., 2002) that may be responsible for conidium dispersal. This indicates very high dispersal efficiency of *P. aphanis* even at low wind conditions.

Spatial disease progression

Wind played a pivotal role in shaping the spatial dispersal dynamics and severity of SPM, influencing both the distance and pattern of conidium dispersal. Airborne pathogens, including PM, generally exhibit an exponential decay pattern with a dispersal gradient heavily skewed toward short distance spread, though occasional long-distance dispersal events (characterized by long tails) can occur (Gregory, 1968; Fitt et al., 1987; Sackett and Mundt, 2005). In our study, patch development followed a similar exponential decay pattern only under WF conditions. Under NoWF, conidium dispersal deviated from the expected exponential decay, (as also described by the R^2 of the NoWF model), leading to the development of secondary infectious hotspot, which drove polycyclic infections randomly in the tunnel. Under NoWF, when laminar flow entered the tunnel and encountered disturbances such as obstacles as plants, it might have become turbulent, creating swirling patterns of air known as wind eddies (Davidson, 2015). Wind eddies might have caused conidia follow heterogeneous dispersal even when released from the same source. This is in line with Johnson et al., 2003 finding, reporting that dispersal characteristics tends to remain clustered near its source even after several generations, though new foci may emerge farther away.

Although wind contributed to a more heterogeneous pattern within the tunnel, the ZINB2 model indicated a higher infection rate under WF conditions. This was likely due to more favourable conditions (higher number of conducive hours > 15.5° C, < 30°C, > 50% RH).

The average of light intensity was higher in 2023 (NoWF) (1392 lux, daily average) compared to 2024 (NoWF) conditions (908 lux, daily average). However, as the ZINB2 count model reports, light intensity had no substantial impact on the infection rate (number of patches). Once the disease establishes in the field, other factors such as temperature and RH may contribute to sporulation and patch growth. This is further supported by the ZINB1 count model, which found no significant effect of light intensity on airborne conidium concentration. Our findings are in agreement with Amsalem et al., 2006 who reported that light had a negative effect on disease severity, only at high intensity 7000 lux compared to those at 1200 and 3800 lux. In contrast, according to the ZINB2 binomial model, light intensity had a strong negative effect on the probability of developing infection, suggesting that, as also supported by

Amsalem et al., 2006, light intensity may interfere with early infection stages, such as conidial germination.

It should be underlined that conidia are living organisms and cannot be considered as inert particles simply dispersed and deposited by airflow. Their contribution to disease gradients depends on multiple factors, including successful deposition on a suitable host and the presence of favourable conditions over time. As a result, the relationship between airborne conidium concentration and patch number may not always be straightforward, as observed under WF conditions, where air sampler data showed lower conidium concentrations while the number of patches remained stable. In addition, environmental variability between the two growing seasons does not allow for directly comparing disease incidence. In fact, even if NoWF and WF conditions had been tested in the same season, identical conditions would have been unattainable, as the presence of the wind fence would have inevitably influenced temperature and RH, further affecting disease development.

Management implications

The results of this study suggest that the dispersal rate from a single inoculum source may play a significant role in the rapid spread of SPM in the tunnels. While multiple inoculum sources may further promote disease development, the high dispersal efficiency observed from a single source emphasizes the importance of minimizing pathogen’s inoculum already at the beginning of the growing season. Early-season strategies to reduce inoculum could help in delaying disease onset and reduce its establishment in the field. Effective SPM management is most successful when implemented early in an epidemic, as treatments during the lag-phase offers the greatest changes to slow disease progression (Madden, 2007). Random sampling, while commonly employed, is not always the most efficient method for disease detection (Binns et al., 2000). Producers often locate PM “hotspots” in the field, where infections tend to be more severe or where symptoms typically appear first, such as areas with microclimates characterized by reduced sunlight and increased humidity (Austin and Wilcox, 2011). However, by the time visible symptoms appear, the disease may have already reached an advanced stage, limiting control efficacy. This challenge explains why farmers often relay on weekly spraying schedule. Indeed, the disease cycle is extremely short (7 days), and dispersal occurs at a rapid rate (1.65 m day^{-1}). This underscores the need for early monitoring and, more importantly, the development of reliable decision support systems, which are currently scarce and/or inadequately validated across various geographical regions (Aldrighetti and Pertot, 2023). Unfortunately, early diagnostic tools, such as molecular detection of airborne spores (Marimon et al., 2020) or real-time imaging (Shin et al., 2021) are still at experimental stage for SPM and are not yet available for routine monitoring of *P. aphanis* in protected cultivation systems.

In this study no pesticide sprays were applied, leaving open the question of how sprayers might influence disease spread. In fact, Willocquet et al. (1998) demonstrated that high-pressure pesticide treatments can generate strong airflow within the canopy, facilitating substantial conidium dispersal. Sprayers that release a high-velocity air-fungicide mixture may create turbulence that may increase conidia spread. Conversely, high-pressure spraying of preventive fungicides could enhance their efficiency by maximizing conidium contact.

Conclusions

Wind played a crucial role in shaping the spatial distribution of SPM once established in the tunnel, though it had no impact on the quantitative disease progression rate. This suggests that wind primarily contributes to spreading conidia rather than accelerating disease development within a tunnel. However, a limitation of these experiments is the potential confounding between wind and year effects, which limits their ability to quantitatively attribute variation in disease onset to specific environmental drivers. Future research could therefore include several comparative macroclimatic data and wind regime characterization across many years and different types of tunnels, as well as the use of multiple spore traps within the tunnel to spatially model spore dispersal patterns.

The most significant wind effect appears to be facilitating conidium transport over long distances, potentially enabling transmission to adjacent tunnels. However, to better understand this process, further research using controlled wind tunnel experiments could help determine the distances and wind speeds that most influence conidium dispersal. Also, use of suitable models (cf. e.g. Tomasi et al., 2019) simulating the transport of conidia under different realistic wind and weather conditions will be of utmost importance to provide different propagation scenarios and possibly predicting the situations more akin to the disease outburst in view of suitable interventions.

Statements and Declarations

Contributions. All authors contributed to the study conception and design. Material preparation and data collection were performed by A.A., meteorological analysis was performed by A.A., I.P., N.V., D.Z. and S.F. statistical analysis was performed by A.A., R.N. and R.R. N.V. prepared Fig. 3, while A.A. prepared Fig. 1, 2, 3, 4, 5, 6, 7, 8, 9 and all tables. The first draft of the manuscript was written by A.A. and I.P. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests. The authors declare that they have no competing interests.

Conflicts of interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Data availability

The raw data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary material

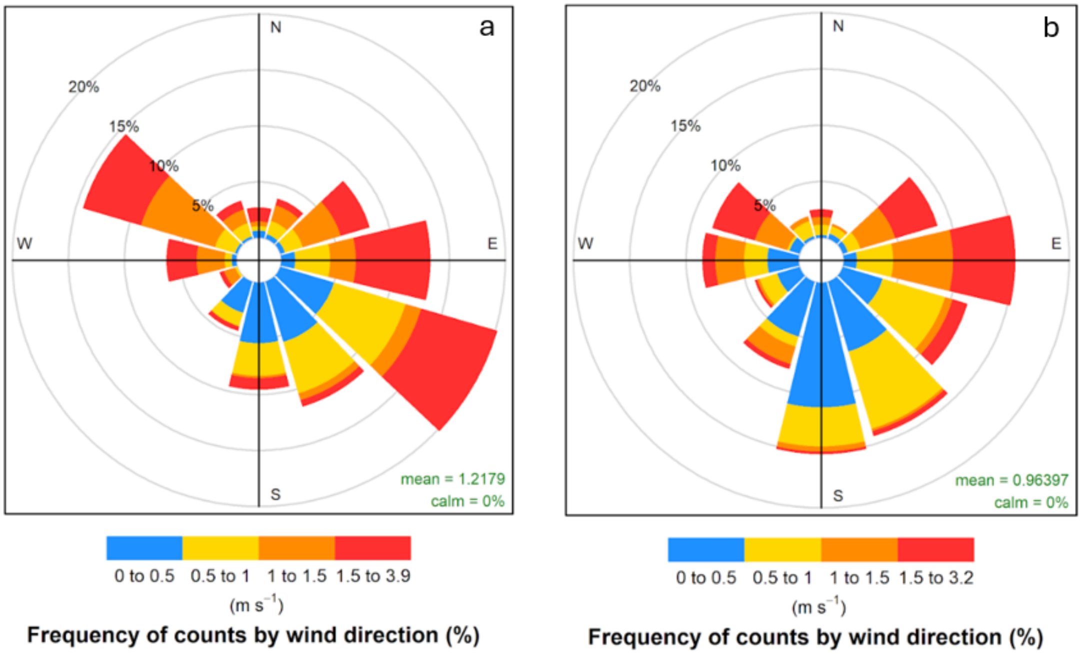

Fig. S1 - Wind roses characterizing the speed (m s^{-1}) and direction frequency (%) of wind outside the tunnel in 2023 (NoWF) (a) and 2024 (WF) (b). One circle represents 5% for each direction and speed frequency. Colours represent wind speed from 0 to 3.9 m s^{-1} (a), and from 0 to 3.2 m s^{-1} (b). The meteorological station, located 1 km distance from the experimental tunnel, permanently operated by Fondazione Edmund Mach-Consulting and Services Center (https://meteo.fmach.it/meteo/dati.php).

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SANITATION PRACTICES TARGETING OVERWINTERING INOCULUM IMPROVE MANAGEMENT OF STRAWBERRY POWDERY MILDEW IN HIGH-TUNNEL PRODUCTION

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Abstract

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

Introduction

Strawberry powdery mildew (SPM), caused by the Ascomycete *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatsu (2000) is a major fungal disease of strawberry (*Fragaria* × *ananassa*) worldwide (Peries, 1962; Menzel, 2022). Severe infections can reduce yields by up to 70% (Peries, 1962; Dodgson et al., 2008), due to flower abortion, fruit deformation and cracking, white mycelium and exposed achenes, postharvest decay, and reduced photosynthesis (Peries, 1962; Jhooty & McKeen, 1965; Carisse et al., 2013a, b). Young tissues are especially vulnerable (Carisse & Bouchard 2010; Asalf et al., 2014;). 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 (Carisse & Bouchard 2010; Asalf et al., 2014) 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 (Steffek et al., 2004; Hodgdon et al., 2024) where increasing consumer demand for year-round, high-quality berries offer growers access to premium markets (Samtani et al., 2019; Hodgdon et al., 2024;). To meet this demand, growers increasingly use protected systems such as polyethylene tunnels and greenhouses, with raised beds or suspended peat pots (Takeda, 1997; Neri et al., 2012; Mezzetti et al., 2018). 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 everbeares (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 (Peries, 1962; Gadoury et al., 2010;). 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 h (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 (MacHardy, 1996; Holb et al., 2006). 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 (Gadoury et al., 2009; Asalf et al., 2013b) 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.

Materials and Methods

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} NO_3^-, 6 mmol L^{-1} $H_2PO_4^-$, 5 mmol L^{-1} K^+, 3 mmol L^{-1} Ca^{2+}, and 1.25 mmol L^{-1} Mg^{2+}, 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.

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.

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) labelled 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).

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 (Rasband 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) 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 h to promote ascospore discharge, then examined and categorized as above.

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: (1):

$$(1)\ \text{Infection rate (IR)} = \frac{[\ln(f2) - \ln(f1)]}{(t2 - t1)}$$

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°C (hourly mean temperature < 13°). 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°C (hourly mean temperature > 10° 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.

Results

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% (χ^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 ± 2.4 sec plant^{-1}.

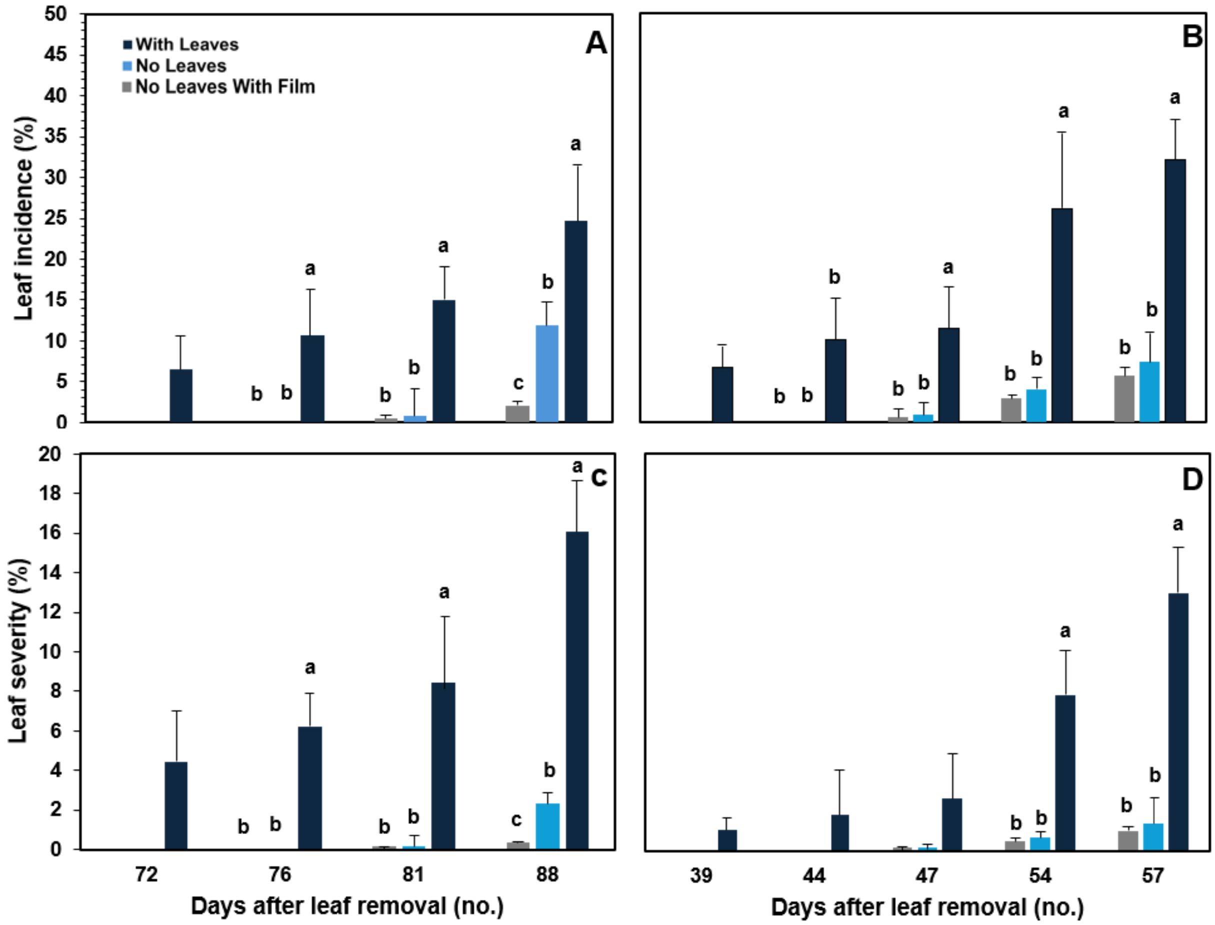

Fig. 1- Strawberry powdery mildew leaf incidence (a) and severity (c) in 2023 (72, 76, 81, 88 days after leaf removal), and leaf incidence (b) and severity (d) in 2024 (39, 44, 47, 54 and 57 days after leaf removal). "No Leaves With Film" corresponds to the plants where infected leaves were removed and 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.

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% (χ^2 = 23, $P < 0.001$) when infected leaves were removed (Fig. 2). Tukey's test confirmed the significant effect of the removal of infected leaves, with lower incidence observed after 69 and 74 DALR ($P < 0.05$). The film addition did not cause significant changes. Leaf removal required 24 ± 2.4 sec/plant.

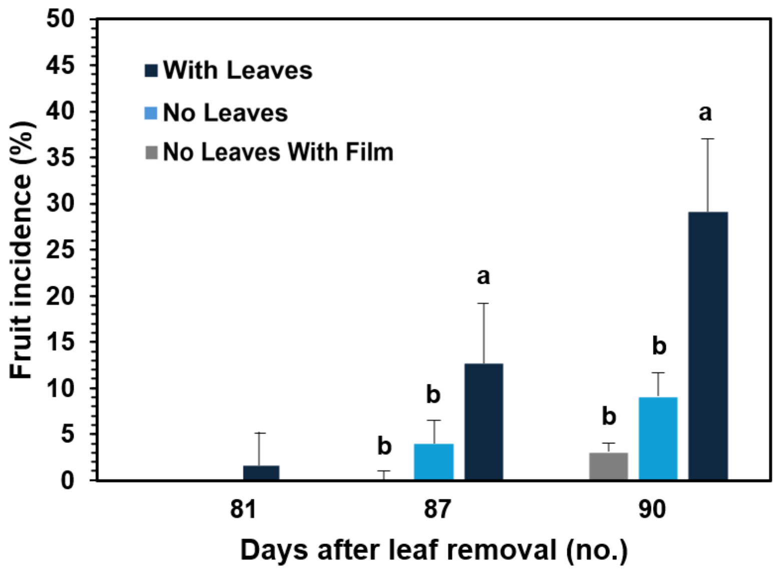

Fig. 2 - Incidence of strawberry powdery mildew on fruits (percentage of symptomatic fruits per plant) in 2023 assessed at 81, 87 and 90 days after removal of overwintered leaves and plant debris. Treatments are: No Leaves (leaves removed), With Film (leaves removed and pot substrate covered with plastic 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.

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 (Fig. 3). Infection increased over time in pots 2, 4, and 7, reaching 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 (Fig. 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 across all treatments, and no significant differences were observed in the number of newly developed leaves throughout the monitoring period (*P* = 0.2).

Fig. 3 - Heatmaps illustrate the progression of strawberry powdery mildew patches per plant in each of the randomized replicates 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.

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: Ap = −31.14, $p < 0.01$; 2024: Ap = −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).

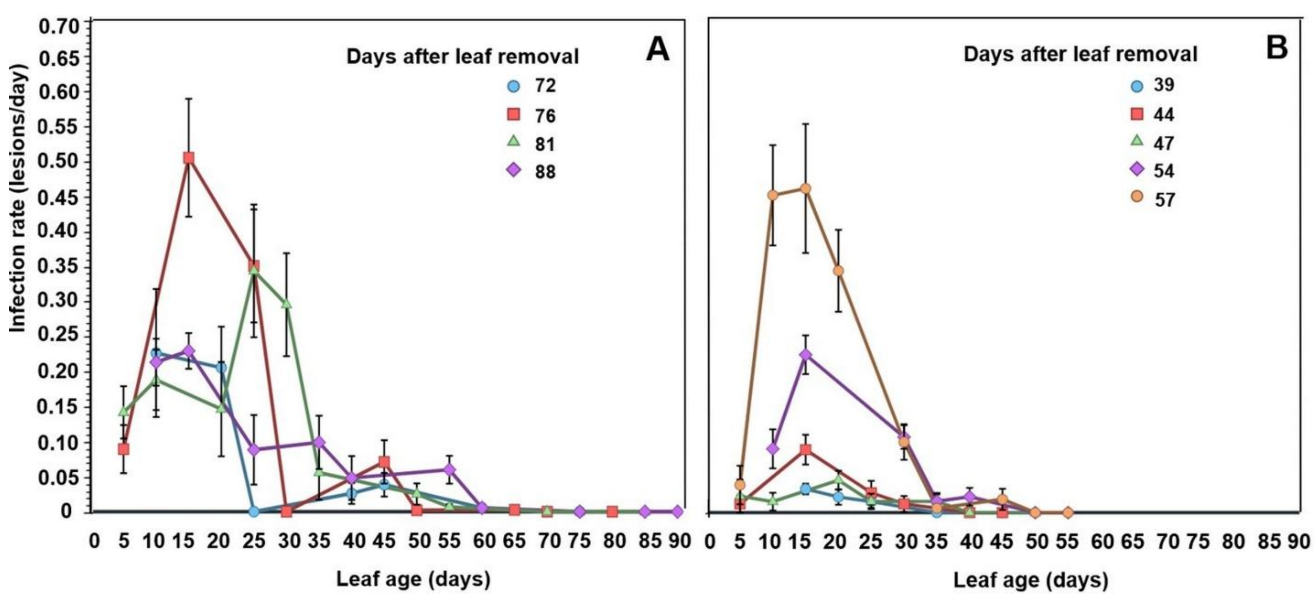

Fig. 4 - Infection rate by leaf age in 2023 (a) and 2024 (b), under high-tunnel conditions. Infection rate (IR) was calculated as $[\ln(f_2)-\ln(f_1)]/(t_2-t_1)$, where f_1 and f_2 are the number of lesions per leaf at times t_1 and t_2. Assessments were carried out twice a week. Leaf age is expressed 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.

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 h below the threshold of 13°C in 2022 and 16 h in 2023. Notably, temperatures in late summer 2022 were cooler, with a higher accumulation of hours below 13°C compared to 2023 (Fig. 5). This cooler period is associated with a significantly higher density of chasmothecia, which reached up to 13.1 chasmothecia/cm² of leaf area. In contrast, the warmer condition in 2023 corresponded with 87% of reduction in their density (1.9 chasmothecia/cm²). 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, t = 11.5, $p < 0.001$).

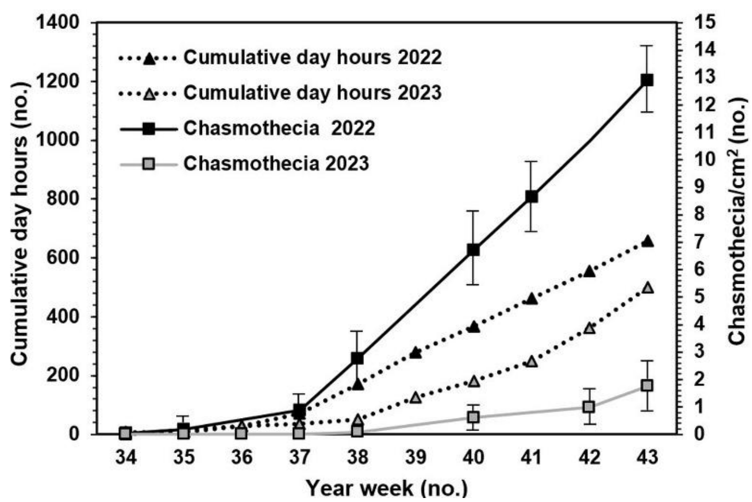

Fig. 5 - Chasmothecium formation of *Podosphaera aphanis* under high-tunnel conditions in 2023 and 2024 in relation to cumulative day hours with temperatures < 13°C. Chasmothecia were counted on 30 mildew-infected leaves collected every 10 days from mid-August to end October. Data are expressed as the number of chasmothecia/cm² of both the upper and lower leaf surfaces.

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.

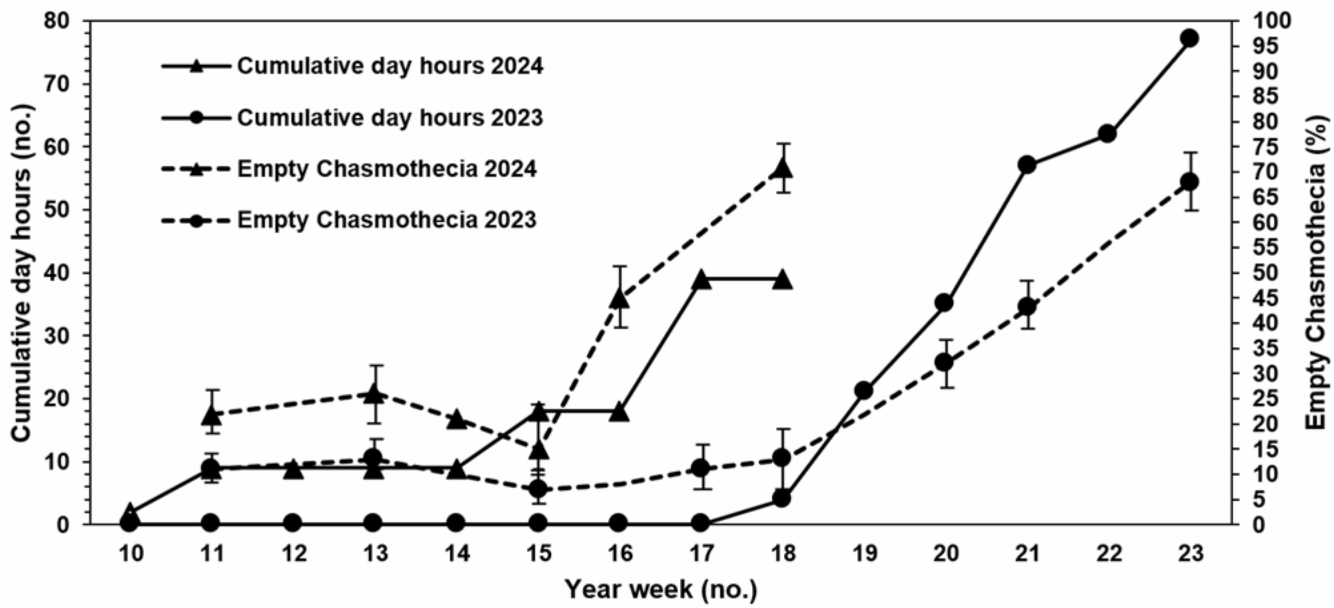

Fig. 6 - Proportion of empty chasmothecia of *Podosphaera aphanis* under high-tunnel in spring 2023 and 2024, shown in relation to cumulative day hours (≥ 10°C and ≥ 2 h leaf wetness from January 1st). Empty chasmothecia are used as proxy for ascospore discharge and were assessed in 10 cohorts (15 chasmothecia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

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

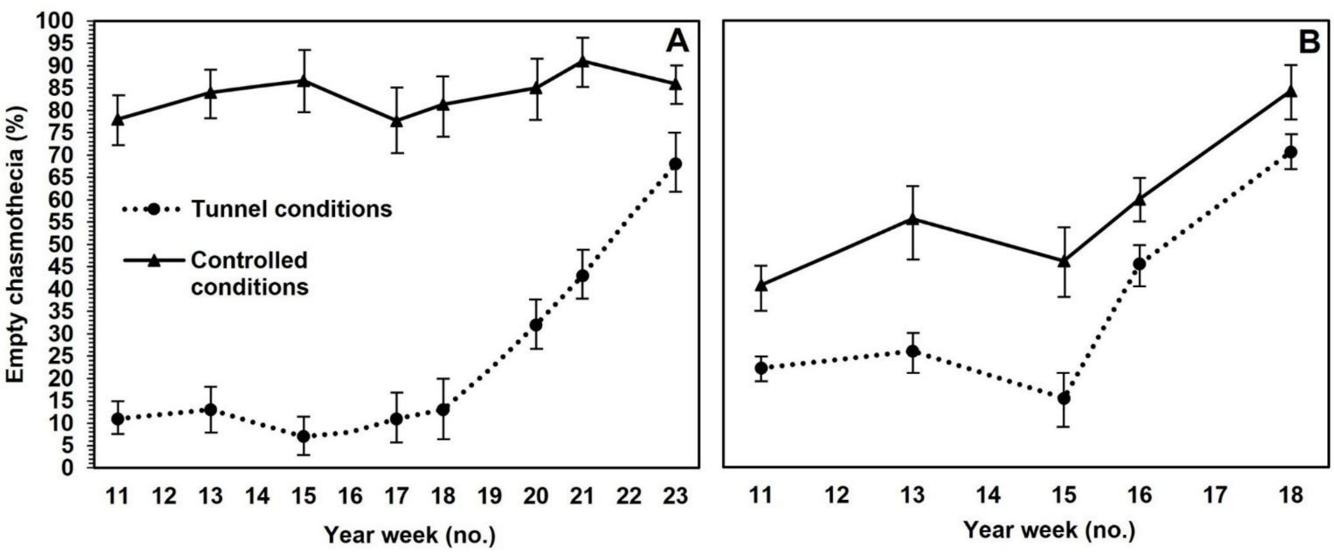

Fig. 7- Empty chasmothecia of *Podosphaera aphanis* under high-tunnel and controlled conditions in spring 2023 (a) and 2024 (b). Empty chasmothecia are used as proxy for ascospore discharge and assessed i) under tunnel conditions on chasmothecia (10 cohorts, 15 chasmothecia/cohort) collected in the tunnel every 10 days from infected leaves, ii) under controlled conditions on chasmothecia (10 cohorts, 15 chasmothecia/cohort) 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 h. Bars represent the standard error of the mean.

Discussion

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

Statements and Declarations

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Contributions

Anna Aldrighetti and Ilaria Pertot designed this study, analysed the data and wrote this manuscript. Anna Aldrighetti and Davide Zott performed the experiment in the field.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that no conflict of interest exists in the submission of this manuscript.

Financial interests

The authors declare they have no financial interests.

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SEQUENTIAL USE OF PRE-FLOWERING CHEMICAL FUNGICIDES AND POST-FLOWERING NATURAL PRODUCTS EFFECTIVELY CONTROLS STRAWBERRY POWDERY MILDEW WHILE RESULTING IN LOW RESIDUES IN FRUIT

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Abstract

Consumer concern about pesticide residues in food has increased markedly, and strawberries are among the most criticized crops, due to frequent pesticide applications resulting in multiple residues of synthetic active ingredients at harvest. Strawberry powdery mildew (caused by *Podosphaera aphanis*) is one of the major drivers of frequent chemical treatments, applied according to calendar-based schedules. To date, natural fungicides have not yet proven capable of fully replacing synthetic chemicals, underscoring the need for integrated well-designed strategies that ensure disease control while reducing residues on fruit. This study assessed a residue-reduction strategy in everbearing high-tunnel production, in which synthetic chemical fungicides were limited to the pre-flowering stage and replaced by natural fungicides (lupin seed extract, potassium bicarbonate, or sulfur) post-flowering. This integrated approach was compared with a standard calendar-based chemical strategy, and with two controls: synthetic fungicides up to pre-flowering followed by water, and an untreated water control. Residue analyses were conducted from the beginning of harvest. Results show that limiting synthetic fungicides to pre-flowering and applying natural products thereafter reduced up to 57% residue number and provided control comparable to the standard strategy, particularly with potassium bicarbonate or sulfur with up to 63% fruit incidence reduction. Lupin seed extract was slightly less effective under high disease pressure (15% higher fruit incidence) but still contributed to disease suppression. These findings demonstrate the feasibility of low-residue strawberry production and support the integration of natural fungicides into well-designed management strategies.

Keywords: Lupin seed extract, potassium bicarbonate, sulfur, strawberry powdery mildew, low-residue strawberry production

Introduction

In recent years, rising awareness of the negative impacts of pesticide use has intensified public concern over residues in food (Coulibaly et al., 2011; Grygorczyk et al., 2014), increasing demand for fruit and vegetables with minimal or no detectable residues (Chikte et al., 2024).

Strawberries (*Fragaria* × *ananassa* Duch.) are one of the crops most frequently associated with pesticide residues (Berlitz et al., 2022; Cross and Berrie, 2006; Keklik et al., 2025; Sánchez et al., 2019). Strawberry powdery mildew, caused by *Podosphaera aphanis* (Wallr.) U. Braun and S. Takamatsu is one of the major diseases contributing substantially to this issue, as its management relies heavily on fungicide applications (Keklik et al., 2025). The pathogen can develop under broad range of environmental conditions (typically temperature between 15-25°C and relative humidity ≥ 65%) (Amsalem et al., 2006; Miller et al., 2003) and cause yield losses up to 70% under conducive conditions (Dodgson et al., 2007). Strawberry cultivation is highly heterogeneous worldwide, but plastic tunnels and soilless culture, together with the adoption of everbearing cultivars producing fruits continuously from spring to autumn (Neri et al., 2012) are increasingly used to extend the production season and ensure a year-round supply (Menzel, 2025; Neri et al., 2012). Although agronomically advantageous, this prolonged production window requires a more intensive use of chemical fungicides.

Young tissues are highly susceptibile to the disease (Asalf et al., 2014; Carisse and Bouchard, 2010), although leaves develop increasing ontogenic resistance while growing (Asalf et al., 2014; Carisse and Bouchard, 2010). Fruits are highly vulnerable from petal fall and progressively become more resistant while ripening (Asalf et al., 2014). Because of the high susceptibility in the early growth stages, effective control measures must be implemented from the beginning of the season (Carisse et al., 2013), when even minimal inoculum can, under conducive conditions, initiate rapid and severe epidemic development (Aldrighetti et al., 2025). In addition, fungicide resistance management requires alternation and/or combination of fungicides with different modes of action (Yin et al., 2023). increasing the likelihood that multiple fungicide residues remain detectable in fruit at harvest.

Most published studies on strawberries have focused on dissipation kinetics to define good agricultural practices that ensure residues in fruits remain below maximum residue limits (Song et al., 2020; Stensvand and Christiansen, 2000), but not on minimizing total residues. Spraying fungicides close to harvest likely lead to higher risk to find detectable residues (Berrie and Cross, 2006). The persistence of some fungicides could be particularly pronounced when applications occur shortly after flowering. For example, as the fruit enlarges, cuticular wax deposition increases (Straube et al., 2024), which can further enhance the retention of lipophilic fungicides, such as azoxystrobin that exhibits a strong affinity for cuticular waxes (Hwang et al., 2019). In addition, studies linking fruit growth dynamics with residue dissipation have shown that faster fruit expansion is associated with reduced dissipation rates (Alister et al., 2017). Several studies have shown that fungicide residues may persist on fruit for 10–25 days or longer (Stensvand and Christiansen, 2000). This implies that due to the rapid flower-to-fruit development in strawberries, typically 20-40 days depending on the cultivar, the interval available for residue degradation is inherently short (Cao and Qu, 2022; Hernández-Martínez et al., 2023).

Among the most effective and currently used synthetic fungicides against strawberry powdery mildew are demethylation inhibitors succinate dehydrogenase, inhibitors, quinone outside inhibitors, and other molecules with unknown mode of action (e.g. bupirimate and cyflufenamid,) and multi-site contact fungicides, such as sulfur, which is frequently used both in conventional and organic production (Aldrighetti and Pertot, 2023) against strawberry powdery mildew (Onofre et al., 2021). Contrary to other synthetic fungicides, sulfur originating from exogenous applications cannot be analytically distinguished from endogenous sulfur as natural constituent element of plant tissues and at the concentrations normally used as fungicide poses no consumer health risk (Alvarez et al., 2023). For these reasons sulfur is normally exempt from maximum residue level requirements under EU and US regulatory frameworks.

In recent years, several natural alternatives to conventional fungicides for the management of strawberry powdery mildew have been investigated, but none of them demonstrated a comparable level of efficacy (Aldrighetti and Pertot, 2023). The activity of microbial biocontrol agents depends strongly on specific environmental requirements (e.g. temperature, relative humidity, etc.), which often do not coincide with those that favor powdery mildew development (Angeli et al., 2012; Kiss, 2003; Pertot et al., 2008). Consequently, although these agents show promising efficacy in laboratory assays and under optimal field conditions (Aldrighetti and Pertot, 2023), their performance is frequently reduced in the variable and suboptimal microclimate typically present in high tunnels. Some resistance inducers are available (e.g. COS-OGA, chitosan), but their efficacy against strawberry powdery mildew is commonly unsatisfactory under field conditions (Aldrighetti and Pertot, 2023). Several plant extracts have demonstrated good activity against *P. aphanis* in laboratory assays or after limited field applications but these results have not been confirmed in large-scale commercial use (Aldrighetti and Pertot, 2023); others negatively affects beneficial arthropods (Hollingsworth, 2005). Among plant extracts, lupin (*Lupinus albus* L.) seed extract has recently emerged as a promising low-risk active substance for the control of strawberry powdery mildew (European Pesticide Database, 2025), supported by its demonstrated efficacy against grapevine powdery mildew (Monteiro et al., 2015). The active component, a BLAD-containing oligomer (Banda de *L. albus* defensin), is a peptide complex derived from β-conglutin degradation during seed germination and exhibits broad antifungal activity through membrane disruption and metal-ion (Ca^{2+}, Mg^{2+}) chelation (Monteiro et al., 2015). Other lupin-derived metabolites, including quinolizidine alkaloids (e.g., lupanine, sparteine, multiflorine), may further contribute to antifungal activity by disrupting fungal cell membranes and inducing oxidative stress (European Commission, 2021; Saket et al., 2025). Among inorganic salts, potassium bicarbonate has been positively evaluated against several powdery mildew species, including *P. aphanis*, both in laboratory assays (Pertot et al., 2007) and under field conditions (Prodorutti et al., 2019). It acts by increasing leaf-surface pH and inducing osmotic disruption of fungal cells (Ziv and Zitter, 1992; Kettlewell et al., 2000).

Given the constraints of high tunnel production (the high disease pressure, the need for strong disease suppression during the early and most susceptible crop stages, the requirement of alternative synthetic fungicides with different mechanism of action to prevent resistance development, and the limited number of natural products with consistently robust field efficacy), restricting synthetic fungicide applications to the early phase of the production cycle may offer a strategic balance between disease management and residue reduction.

The aim of this study was to evaluate the feasibility of an integrated strategy capable of maintaining effective control of strawberry powdery mildew while achieving low or null synthetic fungicide residues at harvest. We hypothesized that replacing synthetic fungicides from flowering onwards with active ingredients exempt from maximum residue level requirements would minimize residues in the fruit. To test this hypothesis, we compared a standard full-season fungicide strategy with three integrated strategies in which synthetic chemical fungicides were applied only until flowering and subsequently replaced with sulfur, potassium bicarbonate, or lupin seed extract after flowering along with untreated control strategies.

Materials and methods

The experiment was carried out in 2025 in commercial-like plastic high tunnels (Polyagri AG, Agripolyane, Italy) located in Vigolo Vattaro, Trentino, Italy (46° 0′ 13.223″ N, 11° 11′ 18.650″ E, 682 m a.s.l.). The experiment was repeated; the first trial started on June 26 and the second on July 9. The two trials were performed using cold-stored mini-tray plants of the everbearing strawberry cultivar Murano (Mazzoni Nursery, Ferrara, Italy). A total of 132 strawberry plants per trial were transplanted into pots filled with a commercial peat-based substrate (Torba Silver® 20–40, Agrochimica, Italy). Plants were fertigated using four drippers per pot, providing a nutrient solution with the following composition: 6 mmol/L NO_3^-, 6 mmol/L $H_2PO_4^-$, 5 mmol/L K^+, 3 mmol/L Ca^{2+}, and 1.25 mmol/L Mg^{2+}, at pH 5.5 and electrical conductivity of 1.5 mS/cm. Fertigation was managed by an automated gravimetric system (Gravimetric, Spagnol, Italy), which controlled nutrient concentrations, pH, and substrate moisture to ensure optimal growing conditions.

Experimental design and active ingredients

The trials were organised in a randomized complete block design with four replicates per treatment. Each replicate consisted of two pots, with four plants per pot. A standard full chemical strategy commonly used by strawberry growers in high-tunnels (hereafter CHEM/CHEM) was compared with three management strategies where synthetic fungicides were replaced by alternative products after flowering, namely a plant extract from germinated *L. albus* seeds, (CHEM/LUP), potassium bicarbonate, (CHEM/BIC) and sulfur, (CHEM/SUL). Two controls sprayed with deionized water at the same time of the treatments of the strategies for the whole cycle (CONT) and only after flowering (CHEM/CONT) (Table. 1) were included. The standard full chemical strategy consisted of applications of conventional fungicides designed to ensure alternation of fungicide classes to reduce the risk of resistance development: fluxapyroxad + difenoconazole (0.6 ml/L; Dagonis, BASF, Germany; concentration of the active ingredients, 75 g/L + 50 g/L, respectively), fluopyram + trifloxystrobin (6 ml/L; Luna Sensation, Bayer Crop Science, Germany; concentration of the active ingredients in Luna Sensation, 21.4 g/L + 21.4 g/L, respectively), penconazole (0.5 ml/L; Topas 10 EC, Syngenta, Switzerland; concentration of the active ingredient, 100 g/L), bupirimate (10 ml/L; Nimrod, Adama, Israel; concentration of the active ingredient, 23.8 g/L;), and cyflufenamid + difenoconazole (1 ml/L; Cidely Top, Syngenta, Switzerland; concentration of the active ingredients, 38 g/L + 11.5 g/L, respectively). The three strategies substituting chemicals with the alternatives were identical to the CHEM/CHEM strategy until flowering, then were followed by application of *L. albus* seed extract (3 ml/L; Problad, Certis-Belchim, The Netherlands; concentration of *L. albus* extract 100%) potassium bicarbonate, (2 g/L; Karma®, Certis-Belchim, The Netherlands; concentration of potassium bicarbonate 85%) and 2 g/L sulfur (Veni Biosulfur, Koppert, The Netherlands, concentration of sulfur 50%). All treatments (fungicides, alternatives, water) were applied weekly in the morning with a backpack sprayer (Elektrischer Rucksack Sprüher, Sylstar, Germany), with a cone nozzle of 0.6 L/min, at a spray volume of 1,000 L/ha. At each treatment phenological stages, classified according to the BBCH scale for strawberry (Meier et al., 1994), were assessed as the average stage on 30 plants. The experiment lasted nine weeks, (referred as “week after transplanting” WAT). Within the integrated strategies, synthetic fungicides were applied at 0 and 1 WAT, and from 2 WAT (flowering stage) alternative products were applied. From the beginning of harvest, fruits were harvested three days after the weekly treatment, respecting the minimum safety interval.

Agronomic practices (e.g. runner removal) were carried out uniformly across all treatments. Eight weeks after transplanting, an assessment was conducted to assess potential negative effects of the tested strategies on predatory mite *Amblyseius andersoni* Chant, by randomly collecting ten leaves per replicate and examining mite presence under a stereomicroscope.

Table 1 - Disease management strategies tested against strawberry powdery mildew: standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT).

Management strategies[b]	Week after transplanting (WAT) - Phenological stage (BBCH)[a]									
	0-12	**1-51**	**2-63 (flowering)**	**3-71**	**4-83**	**5-87 (beginning of harvest)**	**6-89 (harvest)**	**7-89 (harvest)**	**8-89 (harvest)**	**9-89 (end of harvest)**
CHEM/CHEM	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	penconazole	bupirimate	cyflufenamid+ difenoconazole	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	penconazole	bupirimate	cyflufenamid+ difenoconazole
CHEM/LUPIN	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	lupin extract	lupin extract	lupin extract	lupin extract	lupin extract	lupin extract	lupin extract	lupin extract
CHEM/BIC	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate	potassium bicarbonate
CHEM/SUL	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	sulfur	sulfur	sulfur	sulfur	sulfur	sulfur	sulfur	sulfur
CHEM/CONT	fluxapyroxad+ difenoconazole	fluopyram+ trifloxystrobin	water	water	water	water	water	water	water	water
CONT	water	water	water	water	water	water	water	water	water	water

[a]Phenological stages refer to the BBCH scale for strawberry (BBCH 12 = two leaves unfolded, BBCH 51 = inflorescence emergence, BBCH 63 = ~20% flowering, BBCH 71 = fruit development, BBCH 83 = fruit colouring, BBCH 87 = main harvest).

[b]Commercial formulation of the active ingredients and suppliers: Dagonis, BASF, Germany (fluxapyroxad + difenoconazole), Luna Sensation, Bayer Crop Science, Germany (fluopyram + trifloxystrobin), Topas 10 EC, Syngenta, Switzerland (penconazole), Nimrod, Adama, Israel (bupirimate), Cidely Top, Syngenta, Switzerland (cyflufenamid + difenoconazole), Problad, Certis-Belchim, The Netherlands (lupin extract), Karma, Certis-Belchim, The Netherlands (potassium bicarbonate), Veni Biosulfur, Koppert, The Netherlands, (sulfur).

Disease and phytotoxicity assessment

Development of powdery mildew was assessed weekly from 0 WAT to 6 WAT and at the end of the experiment (9 WAT) by evaluating all leaves of each plant. To avoid errors due to possible spray drift between adjacent treatments, only the four central plants of each block were assessed. For each plant, disease incidence was calculated as the percentage of leaves affected, while disease severity was estimated as the percentage of leaf area covered by fungal mycelium and conidia. In order to assess the total impact of powdery mildew on leaves, in the entire growing cycle the area under the disease progress curve (AUDPC) was used as a proxy, calculated from leaf severity data according to the Shaner and Finney (1977) formula (1):

$$(1)\ AUDPC = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}(t_{i+1} - t_i)\right)$$

where y_i corresponds to disease severity (%) at assessment *i*, t_{i+1} corresponds to severity (%) at subsequent assessment *i* + 1, and ($t_{i+1} - t_i$) corresponds to the number of days between the two consecutive assessments. Fruit disease incidence (percentage of infected fruits) and severity (percentage of fruit surface covered by mycelium and conidia) were assessed weekly from the beginning (5 WAT) to the end of harvest (9 WAT).

Phytotoxicity was assessed weekly at 5 WAT and at the end of the experiment (9 WAT) by evaluating all leaves and fruits of each plant. Symptoms on leaves/fruits were classified on a 0-5 scale, where 0 = no symptoms; 1= very slight symptoms (small spots or discoloration barely noticeable/minimal bronzing); 2 = mild symptoms (hardening and bronzing/slight surface irregularities); 3 = moderate symptoms (small necrotic patches, curling or distortion/patchy russeting); 4 = severe symptoms (marginal necrosis and leaf deformation/extensive bronzing or russeting, limited cracking or deformation and fruits unmarketable); and 5 = very severe symptoms (Extensive necrosis covering most of the surface and leaf drop/severe cracking, deep lesion and deformities, fruit completely unmarketable).

Residue quantification at harvest

At the beginning of harvest (5 WAT, July 24 and August 11 for first and second trial, respectively) and at the end of the experiment (9 WAT, September 4 and September 17, respectively), approximately 250 g of fruits from each strategy and replicate were collected and immediately stored under cold conditions (5 °C). These sampling times were selected to assess pesticide residue levels at the beginning and at the end of the harvest period, in order to observe potential differences in residue dissipation. Within 24 h the fruit samples were analyzed by an accredited facility for multi-residue methods in food matrices (AgroBioLab Laboratory Srl; Bari, Italy), using a validated QuEChERS extraction followed by LC–MS/MS in accordance with European guidelines SANTE/11312/2021.

Data Analysis

Statistical analyses were performed using R software (version 4.4.1; R Core Team). Data were logit transformed. For each response variable (leaf incidence, leaf severity, fruit incidence, fruit severity) a combined ANOVA including trial and its interactions with treatment was performed to assess trial variability. When trial or trial × treatment effects were significant ($p < 0.05$), data were analyzed separately for each trial. To test differences among treatment in each observation one-way ANOVA, followed by Tukey's HSD test ($p < 0.05$) was performed. The area under the disease progress curve (AUDPC) for severity was calculated using 'agricolae' package (de Mendiburu and de Mendiburu, 2019). Due to AUDPC heterogeneous residual variance, Welch-Anova was performed, and treatment means were separated by Games-Howell test at $p < 0.05$. Differences in predatory mite counts among treatments were analyzed using Kruskal–Wallis test.

For residue data, concentrations of each active ingredient detected at harvest were log-transformed prior to analysis. When no significant differences between trials were detected ($p \geq 0.05$) in an ANOVA model including trial and its interaction with treatment, data from the two trials were pooled. Subsequently, pooled data were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). The percentage reduction in residue number of the integrated strategies compared to the full chemical strategy (CHEM/CHEM) was calculated as: (number of active ingredients detected in the CHEM/CHEM strategy − number of active ingredients detected in the integrated strategy) / number of active ingredients detected in the CHEM/CHEM strategy × 100.

Results

Efficacy assessment of powdery mildew management strategies

In both trials, powdery mildew symptoms appeared 3 WAT on CONT, coinciding with flowering onset. In trial 1 powdery mildew symptoms appeared on all the treated strategies one week later compared to the CONT, whereas no delays were observed in trial 2. Disease incidence on leaves significantly differed between trials ($p = 2.3\times10^{-2}$). In CONT, disease progressed rapidly and reached similar incidence levels in both trials (79.9% in trial 1 and 77.8% in trial 2) (Fig. 1). In contrast, CHEM/CHEM effectively suppressed disease development, reducing final incidence by approximately 60% (13.4% in trial 1 and 19.3% in trial 2). Across both trials, the integrated strategies did not differ from CHEM/CHEM and resulted in up to a 40% reduction in incidence compared with CONT at the final assessment at 9 WAT in trial 2 ($p = 3.4\times10^{-6}$). The disease in the standard strategy followed by water after flowering (CHEM/CONT) was significantly lower than in the CONT in trial 1 until 5 WAT ($p = 4.1\times10^{-4}$), but no significant differences were detected at any assessment in trial 2 (Fig. 1).

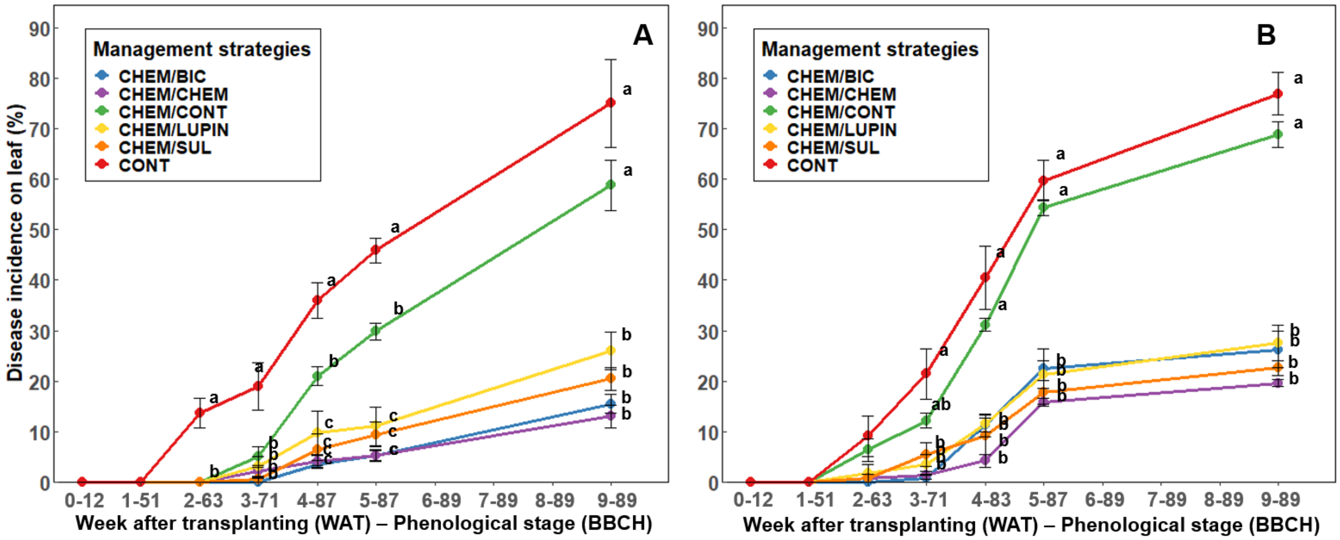
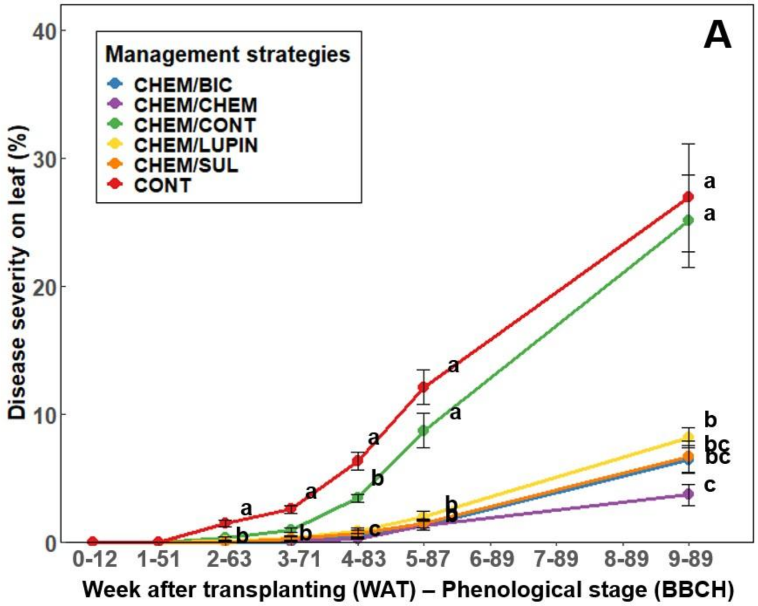


Fig. 1 - Incidence of strawberry powdery mildew on leaves under six management strategies in high-tunnel, in the first (A) and second trial (B): standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT). Data represent means of four replicates per treatment. Bars show means ± standard error. Different letters indicate significant differences among treatments (One-Way ANOVA, Tukey's HSD, α = 0.05).

Disease severity on leaves followed the same trend observed for incidence (Fig. 2) and did not differ between trials, therefore data of the two trails were pooled. The disease progressed rapidly in CONT, reaching 27% severity at 9 WAT. The three integrated strategies significantly reduced disease severity compared with CONT ($p = 6.3\times10^{-6}$) and showed no difference with CHEM/CHEM, except for CHEM/LUPIN at 9 WAT, which showed a higher severity ($p = 5.7\times10^{-3}$) (Fig. 2). The area under the disease progress curve (AUDPC) confirmed these results (Fig. 2). The integrated strategy supplemented with potassium bicarbonate (CHEM/BIC) and CHEM/SUL performed comparably to CHEM/CHEM ($p = 5.5\times10^{-1}$), whereas CHEM/LUPIN was significantly less effective ($p = 2.7\times10^{-3}$). The three integrated strategies and CHEM/CHEM all significantly reduced AUDPC compared with CONT and CHEM/CONT, which exhibited the highest values (246.4 ± 20.0) and did not differ from each other ($p = 8.1\times10^{-2}$).



Management strategies	AUDPC **B**
CHEM/CHEM	25.0 ± 5.2b
CHEM/BIC	35.4 ± 5.0bc
CHEM/SUL	40.2 ± 5.9bc
CHEM/LUPIN	51.3 ± 5.9c
CHEM/CONT	180.5 ± 13.0a
CONT	246.4 ± 20.0a

Fig. 2 - Severity on leaf (A) and area under the disease progress curve (AUDPC) (B) of strawberry powdery mildew under six management strategies in high-tunnel: standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT). Data represent means of four replicates per treatment. Bars show means ± standard error. Different letters indicate significant differences among treatments (A: One-Way ANOVA, Tukey's HSD, α = 0.05; B: Welch-Anova, Games-Howell test, $p < 0.05$).

At the beginning of harvest, powdery mildew symptoms were already present on fruits under all management strategies in both trials. Nevertheless, fruit incidence differed significantly between the two trials ($p = 7.02 \times 10^{-8}$). At 5 WAT in trial 1, CONT reached 9% incidence, which did not differ significantly from the treated strategies. In contrast, in trial 2, CONT already showed 68% incidence at the same stage, significantly higher than all treated strategies. Disease progressed markedly in the CONT, reaching at the final assessment 71% and 92% incidence in trials 1 and 2, respectively. In both trials, the integrated strategies significantly reduced fruit incidence compared with CONT at the last harvest, achieving reductions of up to 56% in trial 1 and 63% in trial 2. No significant differences were detected between the integrated strategies and CHEM/CHEM throughout the harvest period, except at the final harvest in trial

2, where CHEM/LUPIN showed 15% higher incidence than CHEM/CHEM ($p = 2.8×10^{-2}$). The water controls (CONT and CHEM/CONT) did not differ from each other in either trial (Fig. 3).

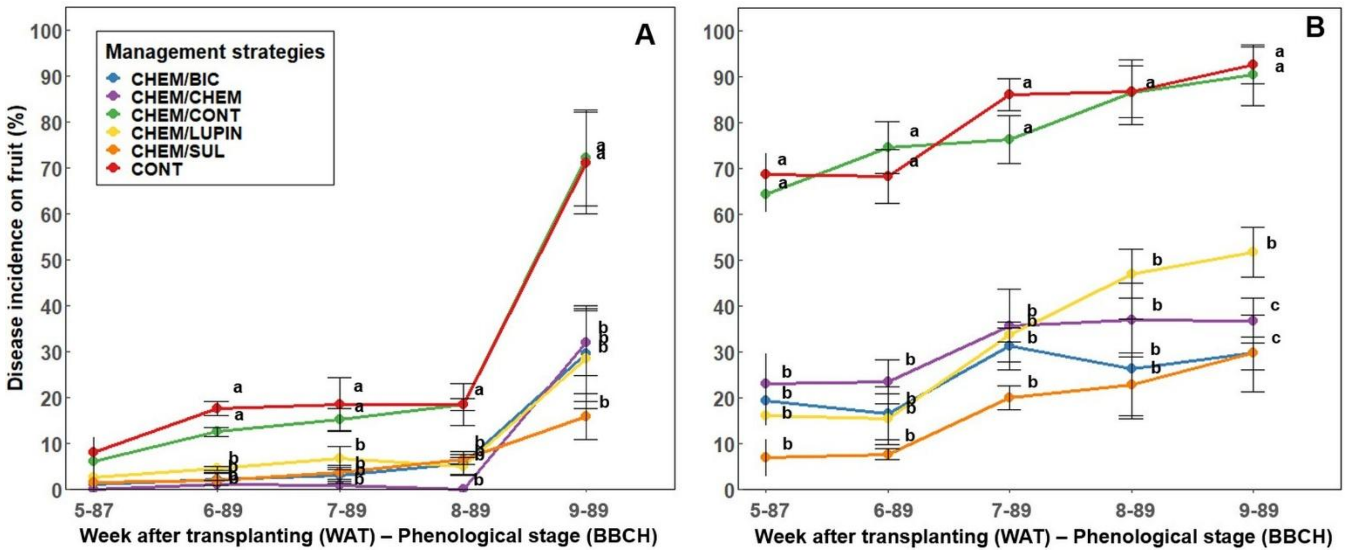


Fig. 3 - Incidence of strawberry powdery mildew on fruits from the beginning of harvest, under six management strategies in high-tunnel, in the first (A) and second trial (B): standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT). Data represent means of four replicates per treatment. Bars show means ± standard error. Different letters indicate significant differences among treatments (One-Way ANOVA, Tukey's HSD, α = 0.05).

Disease severity on fruit differed significantly between trials ($p = 2.9×10^{-5}$). In trial 1 disease severity on CONT fruit remained low (< 5%) until the last harvest and significant differences among strategies emerged only after three weeks from beginning of harvest (8 WAT) ($p = 3×10^{-2}$), when all the integrated strategies did not differ from CHEM/CHEM ($p = 1.6×10^{-1}$). In trial 2, CONT showed higher disease severity compared to trial 1 (from 14.5% to 47.1% by the final assessment). The three integrated strategies from the beginning of harvest suppressed fruit infection compared with CONT and CHEM/CONT, and did not significantly differ from CHEM/CHEM, except for the last harvest when

CHEM/LUPIN showed slightly lower efficacy than CHEM/CHEM ($p = 3.9 \times 10^{-3}$). In both trials the two controls (CHEM/CONT and CONT) did not differ from each other (Fig. 4).

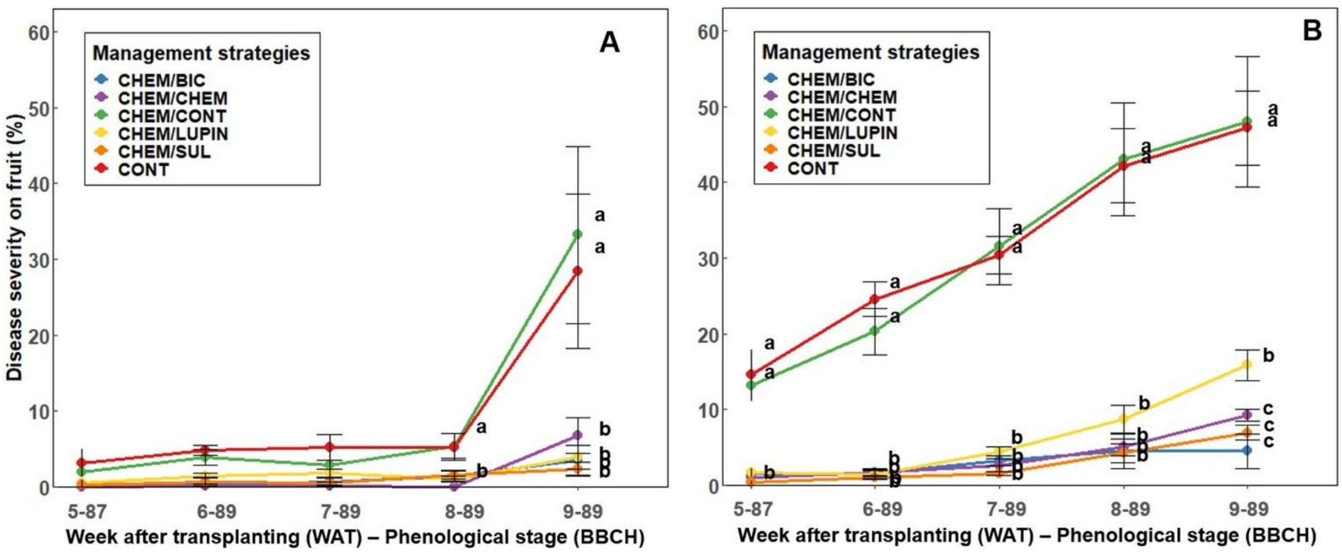


Fig. 4 - Severity of strawberry powdery mildew on fruits from the beginning of harvest under six management strategies in high-tunnel, in the first (A) and second trial (B): standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT). Data represent means of four replicates per treatment. Bars show means ± standard error. Different letters indicate significant differences among treatments (One-Way ANOVA, Tukey's HSD, $\alpha = 0.05$.

The assessment of beneficials revealed significant reduction in CHEM/SUL strategy (with an average of 0.4±0.06 mites/leaf) in the presence of predatory mites ($p = 3.5 \times 10^{-2}$), compared to the control 1.1±0.1 mites/leaf. No differences were observed among CONT (1.1±0.1 mites/leaf), CHEM/CONT (0.9±0.09 mites/leaf), CHEM/BIC (0.8±0.07 mites/leaf), CHEM/CHEM (1±0.1 mites/leaf), CHEM/LUPIN (0.8 ±0.06 mites/leaf).

Phytotoxicity symptoms differed among treatments. Sulfur resulted in low but increasing phytotoxicity scores along time (0.5 ± 0.05 at 5 WAT; 0.9 ± 0.09 at 9 WAT), mostly expressed as leaf hardening and mild curling. Potassium bicarbonate produced slightly higher scores (0.6 ± 0.06 at 5 WAT;

1.1 ± 0.1 at 9 WAT), with bronzing on young leaves and hardening/curling on older foliage. No phytotoxicity (score = 0) was recorded for CHEM/CHEM, CHEM/LUPIN, CHEM/CONT, CONT.

Residues quantification at harvest

Differences between the two trials were not significant ($p = 5.2\times10^{-2}$) and therefore, data were pooled for the statistical analysis. Residues were below the EU maximum residue level set under Regulation (EC) No 396/2005 in all strategies and time points (Table 2). At the beginning of harvest, all the active ingredients (bupirimate, cyflufenamid, difenoconazole, fluopyram, penconazole and trifloxystrobin,) were detected in CHEM/CHEM. In the integrated strategies three active ingredients (difenoconazole, fluopyram, trifloxystrobin) were consistently found across the integrated strategies (57% residue reduction). The concentration of difenoconazole in CHEM/CHEM differed from the integrated strategies ($p = 2.7\times10^{-2}$), whereas fluopyram and trifloxystrobin, showed no significant differences. For the other active ingredients, residue levels were consistently below 0.01 mg/kg across all strategies as expected because they were not applied; hence, these compounds were not considered for statistical analysis.

Table 2 - Mean residue concentrations of the active ingredients detected in strawberry fruit at the beginning (top) and end of harvest (bottom) under the six different disease management strategies in high-tunnel: standard full chemical (CHEM/CHEM); pre-flowering fungicides followed by *Lupinus albus* extract (CHEM/LUPIN), potassium bicarbonate (CHEM/BIC), sulfur (CHEM/SUL), or water (CHEM/CONT); water-only control (CONT). Different letters indicate significant differences among strategies (One-Way ANOVA, Tukey's HSD, α = 0.05)

		Residue concentration[a] (mg/kg) detected in fruits in each strategy					
Active Ingredient	**MRL[b]**	**CHEM/CHEM**	**CHEM/BIC**	**CHEM/LUPIN**	**CHEM/SUL**	**CHEM/CONT**	**CONT**
bupirimate	2	0.03±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
cyflufenamid	0.04	0.03±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
difenoconazole	2	0.7±0.04 a	0.03±0.02 b	0.04±0.03 b	0.03±0.01 b	0.03±0.02 b	n.d.
fluopyram	2	0.17±0.02	0.18±0.02	0.12±0.01	0.06±0.02	0.18±0.03	n.d.
fluxapiroxad	4	0.02±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
penconazole	0.5	0.05±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
trifloxystrobin	1	0.12±0.03	0.12±0.03	0.07±0.02	0.06±0.02	0.16±0.05	n.d.

Active Ingredient	**MRL[b]**	**CHEM/CHEM**	**CHEM/BIC**	**CHEM/LUPIN**	**CHEM/SUL**	**CHEM/CONT**	**CONT**
bupirimate	2	0.03±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
cyflufenamid	0.04	0.04±0.01	n.d.	n.d.	n.d.	n.d.	n.d.
difenoconazole	2	0.54±0.2 a	n.d. b	n.d. b	n.d. b	n.d. b	n.d.
fluopyram	2	0.08±0.02 a	0.02±0.01 b	0.02±0.01 b	0.02±0.01 b	0.02±0.01 b	n.d.
fluxapiroxad	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
penconazole	0.5	0.05±0.03	n.d.	n.d.	n.d.	n.d.	n.d.
trifloxystrobin	1	0.06±0.01 a	n.d. b	n.d. b	n.d. b	n.d. b	n.d.

[a] Values are means ± standard error of three replicates with two trials of each active ingredient. Values below the analytical limit of quantification (0.01 mg/kg) are reported as non-detectable (n.d.).
[b] Maximum Residue Level (MRL) allowed by European regulations (EC) No 396/2005.

At the end of harvest, in integrated strategies all active ingredients were consistently below or equal to EU maximum residue level set under Regulation (EC) No 396/2005. In CHEM/CHEM difenoconazole, trifloxystrobin, cyflufenamid, penconazole, fluopyram, and bupirimate were still detected. The concentration of difenoconazole, fluopyram and tryfloxystrobin in CHEM/CHEM was significantly higher than in the integrated strategies. After eight weeks from application (at BBCH 51), in the integrated strategies only fluopyram was still detectable (85% residue reduction) (Table 2).

Discussion

The present findings demonstrate that replacing synthetic chemical fungicides from flowering stage with substances exempted from residues limits, such as sulfur, potassium bicarbonate or lupin seed extract, can substantially reduce or eliminate detectable residues at harvest, while maintaining acceptable disease control.

Because infections can progress rapidly once initiated (Aldrighetti et al., 2025), applications were scheduled at 7-day intervals, consistent with the estimated 6-7-day infection cycle of *P. aphanis* under conducive conditions (Aldrighetti et al., 2025; Dodgson et al., 2021). Despite the frequent applications, the full chemical strategy did not fully control the disease and resulted in approximately 25% fruit incidence by the end of the experiment, confirming the difficulty in controlling strawberry powdery mildew once infections are established.

Among active substances exempted from residue limits, in our experiment sulfur confirmed its activity against strawberry powdery mildew control, even under high disease pressure (Aldrighetti and Pertot, 2023; Asalf et al., 2012; Onofre et al., 2021). However, the use of sulfur is not without drawbacks. Applications below 15°C are less effective (Juroszek et al., 2022), whereas temperatures exceeding 35°C for more than 2 h can cause phytotoxicity (Onofre et al., 2021), a condition that can easily occur under plastic tunnels in summer in temperate climates. In addition, frequent applications may negatively affect beneficial arthropod development (Angeli et al., 2005; James and Prishmann, 2010; Jepsen et al., 2007; Gesraha et al., 2019; 2021). Repeated applications may also leave visible deposits or off-flavors on fruit and cause leaf hardening, as also observed in our experiment. For these reasons, finding an alternative to sulfur with effective control and absence of negative side-effects could be highly helpful.

Potassium bicarbonate had consistently high efficacy among the two trials and comparable to the standard chemical strategy and the integrated strategy with sulfur. Its activity against other powdery mildew species (Deliopoulos et al., 2010; Holb and Kunz, 2016; Reuveni et al., 1996;) is well documented and this study confirmed its high efficacy within an integrated program also for strawberry powdery mildew control. However, these salts require frequent applications (7-10 days) to be effective, which in turn can increase the risk of visible residues and phytotoxicity due to its alkaline profile (Aldrighetti and Pertot, 2023). In our experiment, repeated applications led to mild leaf injury, with treated foliage appearing hardened than that of the untreated plants.

Lupin extract, evaluated here for the first time against *P. aphanis*, significantly reduced disease compared with the untreated control, although at the end of harvest its efficacy was slightly less effective than that of potassium bicarbonate and sulfur. In both trials, sulfur and potassium bicarbonate kept disease at comparable levels (never exceeding 30% incidence on leaf), despite the markedly higher disease pressure observed in trial 2, whereas lupin extract showed a significantly higher disease incidence on leaf, suggesting better performance on fruits under moderate disease pressure. Despite its lower efficacy on fruits under high disease pressure, in our experiment lupin extract demonstrated to be an interesting alternative option to sulfur and potassium bicarbonate because of its absence of negative effects on beneficial mites (Monteiro et al., 2015) and no phytotoxicity observed. In addition, this extract has also shown activity against strawberry grey mold *Botrytis cinerea* Pers. (European Pesticide Database, 2025), further supporting its role as a broader-spectrum tool within integrated strawberry disease management.

Residue analysis at the beginning of harvest clearly differentiated the standard chemical program from the integrated strategies with a 57% overall reduction at the first harvest (5 WAT) and an 85% overall reduction by the end of the crop cycle (9 WAT) compared with the standard chemical program. As expected, the standard program was the only one in which all seven synthetic fungicides were detected, although always at concentrations well below their respective MRLs. This pattern indicates that restricting synthetic fungicides to the early growth stages greatly limits their presence into the fruit. Difenoconazole exemplifies this effect particularly well. In fact, while it reached 0.7 mg/kg in standard chemical strategy, its levels in all integrated strategies were close to the limit of quantification and non-detectable at the beginning and end of harvest, respectively. Fluopyram was the only compound consistently detected across all integrated strategies, albeit at concentrations five- to tenfold lower than in the standard chemical program, highlighting its comparatively slow dissipation. In fact, it belongs to a class of fungicides with per- and polyfluoroalkyl-like structures, characterized by long-term environmental persistence (Rathod et al., 2022). Such reduction levels are relevant in the context of increasingly strict market requirements, where many retailers impose residue thresholds far below the EU maximum residue levels (Chickte et al., 2024). Dissipation of active substances may also depend on their chemical properties and/or systemic behavior within the plant (FAO, 2012; Liu et al., 2025; Tsuda et al., 2004). Systemic fungicides, such as difenoconazole, fluopyram and trifloxystrobin tend to degrade slowly because of their high lipophilicity and membrane permeability (Gikas et al., 2022, Rathod et al., 2022; Shi et al., 2025).

Depending on the season in which strawberry production takes place, fungicide applications occur under varying plant growing rates (Alister et al., 2017; Cao and Qu, 2022; Farha et al., 2016; Hernández-Martínez et al., 2023). Due to lower temperatures and shorter day length, spring and winter crops generally have longer growth cycles than summer plantings, (Cao and Qu, 2022; Chaiwong and Bishop, 2020; Hernández-Martínez et al., 2023). Consequently, the degradation dynamics of active substances applied at the same phenological stage, may vary with crop cycle length. Crop length further depends on the cultivar: June-bearing strawberries, characterized by a short and concentrated harvest period, typically require fewer fungicide applications, whereas everbearing cultivars produce over extended periods (Neri et al., 2012), frequently overlapping with environmental conditions favorable to powdery mildew development. In our study, the short summer crop cycle (36 days from transplanting to the first harvest) and the everbearing cultivar used likely limited the time available for residue dissipation while exposing plants to prolonged periods highly conducive to powdery mildew.

In the full chemical strategy, fungicides from different resistance mechanism classes were alternated or applied in mixtures to reduce selection pressure for resistance (Palmer and Holmes, 2021), reflecting standard regional practice. No decline in efficacy of the active substances used in this study has been reported to date in the region. However, reliance on multiple fungicides increases the likelihood of detecting a higher number of different residues in fruit, posing a challenge for strategies aiming at reducing the number of residues, rather than the quantity. This issue is particularly relevant for *P. aphanis*, as resistance to several active substances has already been documented in different production areas (Palmer and Holmes, 2021; Sombardier et al., 2010). Therefore, any approach aiming to reduce the number of applied active substances, and consequently residues, must be supported by regional sensitivity monitoring to ensure continued fungicide effectiveness under evolving resistance dynamics. In this context, the integration of multi-site, exempted from residue alternatives such as sulfur, potassium bicarbonate and lupin extract may help to reduce residues and manage resistance risks.

Conclusion

The present results demonstrate that substituting synthetic fungicides after flowering with substances exempted from residue limits, as sulfur, potassium bicarbonate, and lupin seed extract, can drastically lower residue load at harvest while still providing good suppression of *P. aphanis*. However, additional research will be necessary to generalize the integrated approaches and strengthen their robustness across the various production contexts. For example, the potential to enhance robustness by combining or alternating the three natural products deserves systematic evaluation over multiple climatic conditions and cultivars. These substances differ in physicochemical behavior and modes of action and different strategic combinations of them in relation to the phenological stage and/or the pressure of the disease could potentially also reduce the number of total applications required. In addition, integrating these products with agronomic and genetic strategies, for example including canopy ventilation primary inoculum reduction and resistant cultivars, may provide additive benefits for disease control and residue minimisation.

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CRediT authorship contribution statement

Anna Aldrighetti: Conceptualization, Data analysis, Investigation, Writing – original draft. Muhammad Waleed Tahir: Conceptualization, Data analysis, Investigation, Writing – review and editing. Ilaria Pertot: Conceptualization, Supervision, Writing – review and editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

We state that during the preparation of this work the authors used the tool "DeepL Write AI writing assistant" to support the final proof editing of the text for American English. After using this tool, the authors reviewed and edited the text as needed. They take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Discussion

The present study addressed key research gaps, identified in the literature and during the focus group with growers, concerning *P. aphanis* management in commercial high-tunnel strawberry systems, with the aim of improving understanding of epidemic onset and identifying more sustainable disease control strategies. Difficulties reported by growers and agronomists in controlling primary infections reflected broader gaps in understanding the early phases of epidemic development. Growers often perceive strawberry powdery mildew as unpredictable and *ubiquitous*, rapidly developing when environmental conditions become favorable. While strawberry powdery mildew has long been recognized as an aggressive disease, the spatial rate of epidemic dispersal had not previously been quantified. We therefore aimed to evaluate the spatiotemporal distribution of the disease originating from a single inoculum source and to identify the main environmental drivers (i.e., wind, temperature, relative humidity, and light intensity) that promote spore dispersal. Although the experiments have been carried out in two different seasons (under natural wind conditions in the first year and under reduced wind conditions in the second year) with different environmental conditions, our primary focus was on spatial distribution patterns and the rate of epidemic spread under contrasting wind regimes rather than on direct comparisons of disease severity between years. Despite differing climatic conditions, ZINB modelling consistently indicated a key role of wind in shaping dispersal patterns. Results showed that infection can spread rapidly (up to approximately 2 m per day) even under low wind conditions. In both years, a full infection cycle has been completed within seven days, and within ten days the pathogen dispersed throughout an entire tunnel even at 0.5 m/s wind. Under natural wind conditions, dispersal also led to multiple secondary foci that drove exponential epidemic growth, underscoring the role that wind has in accelerating disease development. Demonstrating to growers the rapid spread of the pathogen even from a single inoculum source, therefore provides field-based evidence that can effectively support recommendations for rigorous inoculum reduction after overwintering and early-season disease management.

Post-winter removal of infected and old leaves significantly reduced disease incidence and delayed epidemic development. Although this practice is labour-intensive (approximately 500 h ha^{-1}), early sanitation likely reduced heterogeneity among initial infection foci and enhanced the effectiveness of subsequent fungicide interventions. The removal of infected leaves, often difficult to reach with spray applications, may be relevant in production systems relying on cannon sprayers, whose limited spray penetration and uneven coverage are widely recognized as major constraints to effective disease control.

Despite the effectiveness of sanitation, considerable infection variability among plants was observed over the two experimental years, highlighting inherent complexity of infection dynamics under field conditions. The presence of old leaves and/or persistent morning dew may have contributed to microclimatic differences between treatments, leading to heterogeneous infection foci within the field. Although microclimatic conditions within the canopy were not directly monitored, the randomized block design helped to minimize spatial environmental heterogeneity in disease development.

While assessing sanitation practices, the analysis of primary infection dynamics revealed key epidemiological patterns. This study identified the leaf age cohort most epidemiologically relevant for disease build-up. Leaf infection rate peaked on leaves 10–15 days after unfolding, which can be distinguished within the dense strawberry canopy as leaves of intermediate height with lighter-colored and softer tissues. Focusing scouting efforts on newly expanded leaves, commonly considered the most susceptible tissues (Asalf et al., 2014), may result in an underestimation of ongoing infections, as the pathogen may not yet have had sufficient time to develop visible symptoms. Targeting this leaf cohort may therefore allow growers and advisors to reliably assess disease development in the field.

In parallel, the study qualitatively validated laboratory findings (Asalf et al., 2013; Gadoury et al., 2010) by quantifying thermal requirements for chasmothecium development under field conditions. Chasmothecium formation was first observed after 8 and 16 cumulative hours below 13°C in 2022 and 2023, respectively, indicating that relatively short periods of cool temperatures are sufficient to trigger sexual structure development in late summer. Chasmothecium density varied between years and was partially associated with late-summer temperatures, with a higher number of hours below 13°C in 2022 potentially contributing to increased abundance compared with 2023. However, temperature alone may not fully explain this variability, and additional biological factors, such as seasonal variation in the relative abundance of mating types, may also contribute (Gadoury et al., 2010). Although thermal thresholds provide useful parameters to estimate the risk and density of chasmothecium formation, our results show that lower chasmothecium density did not result in reduced disease intensity in the following spring, indicating that differences in overwintering inoculum may not lead to lower epidemic severity. Therefore, despite the relevance of pre-overwintering dynamics, growers should consistently assume a high risk of early infection.

When quantitative information on epidemiological dynamics and related environmental thresholds are limited, validating thermal requirements described in similar pathosystems allows rapid development of potential risk indicators. Under spring conditions, the present study quantified environmental thresholds for chasmothecium dehiscence and subsequent infection observations, based on *E. necator* environmental requirements (Gadoury and Pearson, 1990; Rossi et al., 2010). The cumulative number of favourable hours required for infection, defined as temperatures above 10°C combined with at least 2 h of continuous leaf wetness, was remarkably consistent between years (21 h in 2023 and 18 h in 2024). Although a broader range of environmental variables and longer-term observations would be required to fully characterize dehiscence dynamics, the close temporal concurrence between chasmothecium opening and infection may suggest a potential correlation. Further validation across seasons and environments will be required to assess the robustness of these indicators.

Beyond epidemiological considerations, growers highlighted the need for more sustainable disease management strategies capable of integrating conventional fungicides with alternative products, while at the same time reducing residues in fruit. In recent years, increasing attention has been focused on *zero-residue* and low residue crop protection strategies, driven by consumer concerns regarding pesticide residues and by downstream market requirements increasingly imposing residue limits well below legal thresholds (Chikte et al., 2024). This has led to the growing adoption of phenology-based disease management approaches that combine early-season applications of synthetic fungicides with the subsequent substitution of conventional products by residue-exempted or low-risk alternatives. While such strategies are increasingly promoted through technical advisory programs (Cinquemani, 2025) and certification frameworks (Chikte et al., 2024), only a limited number of peer-reviewed studies (Donna et al., 2025; Roškarič et al., 2023) have systematically evaluated their effectiveness in achieving *zero-residue* or low-residue levels at harvest under commercial conditions.

Our results demonstrated that substituting synthetic fungicides from flowering with residue-exempted products can substantially reduce residue load at harvest while effectively controlling the disease. Residue analyses clearly differentiated the standard chemical program from the integrated strategies, with a reduction (57%) in both the number and concentration of detectable residues over the harvest period. However, not all residues were eliminated, mainly due to the high persistence of active ingredients such as fluopyram, difenoconazole, and trifloxystrobin. This is consistent with previous findings from grapevine production where even though synthetic chemical fungicides have been substituted by exempted-residue products one month before harvesting, boscalid and Al-fosetyl residue levels remained above 0.01 mg kg^{-1} (Roškarič et al., 2023). Similarly, a study conducted on chickpea and soybean production systems demonstrated that environmental conditions across different growing seasons, as well as differences in agricultural practices, significantly influenced the degradation of active ingredients, even when pesticides were applied at comparable phenological stages (Donna et al., 2025). Considering the multiple variables affecting residue dynamics, including active ingredient persistence, environmental conditions, and agronomic practices, further research is warranted to develop *zero-residue* programs capable of ensuring consistent results under diverse production systems.

Although numerous studies have demonstrated the efficacy of inorganic salts, biological control agents, and botanical extracts against powdery mildew when integrated into conventional programs (McGrath and Shishkoff, 1999; Pertot et al., 2017), evidence of the efficacy of post-flowering use of residue-exempted products can be derived from efficacy trials rather than from experiments designed to test late-season substitution strategies. Among the residue-exempted products evaluated in our study, sulfur and potassium bicarbonate provided high levels of disease control, comparable to the standard chemical strategy. Nevertheless, both products showed relevant limitations when consequently applied, including mild phytotoxicity risks, and potential negative effects on beneficial arthropods in the case of sulfur, and mild phytotoxic effects associated with potassium bicarbonate. It should be noted that sulfur side effects may not represent a limiting factor in all production contexts. Strawberry production occurs in diverse cropping cycles (spring and autumn plantings) where high temperatures are not a major constraint, and they may differ in pest management strategies not relying on biological control. Formulation characteristics, such as particle size distribution and the type and concentration of surfactants or other co-formulants, may influence both antifungal efficacy and phytotoxic responses. Therefore, comparative evaluation of different commercial sulfur (e.g., Thiopron, Microtiol Dispers, and Kumulus Tecno) and potassium bicarbonate (e.g., Karma 85, Armicarb 85, Vitikappa) formulations would help identify products that maximize disease control while minimizing adverse effects under repeated applications.

In contrast, lupin seed extract, evaluated here for the first time against *P. aphanis*, showed moderate efficacy under high disease pressure but was characterized by the absence of phytotoxicity and negative effects on beneficial mites, supporting its potential role as a complementary tool within integrated disease management programs. Although lupin extract in integrated strategies warrants further investigation, it could reduce the risk of phytotoxic effects of consecutive applications of sulfur and potassium bicarbonate while maintaining disease control.

Our study also confirmed the complexity of controlling *P. aphanis* once infection is established. Even the full chemical strategy did not fully suppress disease development. This reduced efficacy may be associated with fungicide sensitivity within *P. aphanis* populations, not yet been assessed in the Trentino production area, but already documented for several active ingredients used in this study, including penconazole (Sombardier et al., 2010), used since the early 1980s (Morton and Staub, 2008), tryfloxystrobin and cyflufenamid (Palmer and Holmes, 2021). Although fungicides are often applied in mixtures, focus group discussions highlighted that the limited number of available products and the high frequency of applications lead to repeated use of the same active ingredients throughout the season. In particular, fluopyram (SDHI, FRAC 7) and trifloxystrobin (QoI, FRAC 11), identified as among the most effective by growers, are classified as high resistance-risk fungicides (FRAC code List, 2024).

Despite their efficacy, their slow degradation raises concerns within *zero residue* or low-residue strategies. However, reducing or excluding the use of highly effective fungicides could increase resistance risk by narrowing the range of modes of action and increasing reliance on fewer alternatives. Therefore, regional sensitivity monitoring is essential to maintain fungicide efficacy within low-residue strategies.

CONCLUSION

This doctoral project advances the epidemiological understanding and sustainable management of *P. aphanis* in protected strawberry systems. Nevertheless, the pathogen remains challenging, and further improvements in disease management are still required. Production systems differ widely, meaning that management priorities vary across contexts. In the context addressed by this project, short-term priorities should include: validating the field-based risk indicators developed in this study to support timely early-season interventions; conducting bioassays to assess fungicide sensitivity in local *P. aphanis* populations; evaluating the alternation of the three tested natural products to enhance strategy robustness; assessing complementary agronomic practices such as canopy thinning and runner removal during production; evaluating new spraying technologies. Automation may play a key role, particularly in the future when workforce availability is likely to decrease. However, the limited financial capacity of small and fragmented farms, as in the context of this study, may hinder the adoption of advanced technologies, potentially widening disparities within the sector. For these reasons, in the longer term, advanced biotechnological strategies, including molecular breeding, marker-assisted selection and RNA-based technologies, could support the development of cultivars and non-chemical options better suited to the constraints of protected strawberry systems.

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