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Aerial dispersal of *Venturia inaequalis* ascospores with under-canopy sprinkler irrigation for apple scab management

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Abstract Sprinkler irrigation systems can release ascospores of *Venturia inaequalis*, the cause of apple scab, from infected leaves on the ground under conditions unsuitable for infection, and thus reducing the primary inoculum. Under-canopy irrigation was carried out for two hours in the middle of the day over overwintered apple leaves heavily infected with scab, either in a wind-protected enclosure or in a wind-exposed orchard. Ascospores were captured with rotating-arm spore traps at heights ranging from 0.3 m to 3.0 m above the ground. Ascospores dispersed above the irrigated layer and were detected at all heights above the sprinklers. Wind played a critical role in spore transport, evident from the set-up where wind interference was minimised by a wind fence, resulting in higher airborne spore numbers across all measured heights compared with the orchard exposed to unrestricted wind conditions. Furthermore, vertical temperature gradients significantly correlated with spore distributions, particularly where negative gradients at heights between 0.3 m and 0.05 m and positive gradients at heights between 1.0 m and 0.3 m led to spore retention within the irrigated zone. The findings highlight that ascospores, dispersed above the irrigated layers, could settle on susceptible tissues. It thus becomes imperative to ensure a rain-free period of at least 24 h post-irrigation and, if a rainfall shortly occurs after irrigation, the application of curative fungicides becomes essential following unexpected rain. Reliable weather forecasts are therefore crucial in determining the effectiveness of under-canopy irrigation to reduce apple scab incidence.

Keywords Apple · Ascospore distribution · Wind · Disease control · *Malus × domestica* · Sustainability

Introduction

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint, is one of the most important diseases of apple (*Malus × domestica*) worldwide. Symptoms of the

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disease can occur on leaves, fruit, petioles, sepals, pedicels and young shoots (MacHardy, 1996). The main economic impact stems from the scab-like lesions that may deform and crack the fruits.

Venturia inaequalis overwinters mainly in the infected leaves lying on the soil of the orchard, where the development of pseudothecia takes place (MacHardy, 1996). In particular, during late winter and spring, the pseudothecia progressively mature and develop asci and ascospores (Biggs & Stensvand, 2014). Mature ascospores are released in spring when pseudothecia have been moistened by rain or heavy dew, and are dispersed by wind. If suitable conditions of wetness duration and temperature are met, ascospores germinate and start the colonization of the plant tissues (primary infection). Subsequently, conidia developed in scab lesions may cause several cycles of secondary infections during the growing season (MacHardy, 1996; Mills, 1944; Philion et al., 2020; Stensvand et al., 1997). The maturation and discharge of ascospores typically starts from bud break of the apple tree and lasts 6–10 weeks, with a peak commonly between the stages of pink bud and full bloom (MacHardy, 1996).

Aerial dispersal of propagules depends on different factors: liberation, escape, transport, survival and deposition (Mahaffee et al., 2023). Ascospores of *V. inaequalis* are actively discharged from pseudothecia during rainfall. When pseudothecia are moistened by rain, the asci expand through the ostiole, and the increase of hydrostatic pressure inside the ascus causes rupturing of the exotunica and release of ascospores from the tip of the ascus (Aylor, 2017; MacHardy, 1996). In still air, most ascospores are ejected a distance less than 10 mm, and about half of them reach no more than 3.5 mm (Aylor & Anagnostakis, 1991). Ascospores are mostly released in daylight hours (Brook, 1969, 1975; Gadoury et al., 1998; MacHardy & Gadoury, 1986; Rossi et al., 2001), and the peak of the release commonly occurs in the middle of the day (MacHardy & Gadoury, 1986).

The average dimensions of ascospores of *V. inaequalis* are 6×13 μm (Aylor and Kiyomoto, 1993). They have an aerodynamic diameter (the theoretical diameter of a nonspherical particle having the same terminal settling velocity as an equally dense, spherical particle of such diameter) of about 8.2 μm, and their settling speed in still air is about 0.002 m/s (Gregory, 1973). The estimated mass of an ascospore of *V. inaequalis* is around 0.3 ng (Aylor, 2017). With these characteristics, ascospore motion from the leaf litter on the ground to the apple growing tissues mainly depends on turbulence and mean wind (Aylor, 1998, 2017). Due to the small size and mass of these propagules, even low flow velocities (e.g., upward air motions from a sun heated surface) are sufficient to overcome the force of gravity, once they are released from the laminar boundary layer of the leaf (Fischer et al., 2010; Mahaffee et al., 2023). Airborne ascospores can therefore land on susceptible leaves and fruits by either wet deposition (washout by rain) or dry deposition (impaction and sedimentation; Aylor, 1998, 2017).

Primary infections of apple scab depend quantitatively on ascospore concentration in the air surrounding susceptible tissues (Aylor, 1998; Aylor & Kiyomoto, 1993). Aerial ascospore concentration decreases rapidly with height above the ground and with downwind distance from the source of infection and this dispersion of spores is controlled by wind shear, turbulent diffusion, and rain washout (Aylor, 1998). In a study using rotating arm samplers (Rotorods) carried out in apple orchards during daylight rain events, only 6% of the ascospores detected at 0.15 m above the ground were found at 3.0 m height in the turfed inter-row (Aylor, 1995). The steep decrease of ascospore concentration with height was due to a rapid increase of wind speed and turbulence intensity. In another study with rotating arm samplers, aerial concentration of ascospores of *V. inaequalis* within the tree canopy and their deposition decreased with increasing height, while ascospore concentration in rainwater was highest at the tree level, from 1 to 3 m above the ground (Carisse et al., 2007). The vertical dilution of aerial ascospores of *V. inaequalis* was modelled by Rossi et al. (2003) on the basis of the height above the ground only, and it was estimated by integrating an exponential decay function modified from Aylor and Kiyomoto (1993). A high correlation resulted between model estimates and the vertical pattern of ascospore numbers reported by Aylor (1995).

Regarding the horizontal ascospore dispersal, MacHardy (1996) reported that most airborne ascospores of *V. inaequalis* are deposited at a distance less than 100 m from their source. Kaplan (1986) found a steep spore dispersal gradient from the source: at 5–6 m from the source, the airborne concentration of ascospores was reduced by 99%. Moreover, the spatial distribution of ascospores in a commercial apple orchard during major periods of ascospore release did not result in a uniform distribution but rather in a patchy aggregation (Charest et al., 2002).

Chemical control of apple scab mainly relies on fungicide applications targeting primary infections caused by ascospores (Aylor, 1998). Sprinkler irrigation of overwintering leaves lying on the ground during periods of dry weather may be a sustainable method to reduce the primary inoculum of apple scab, by promoting the release of ascospores in periods with minimal risk of infection. Indeed, Prodorutti et al. (2024) showed that irrigation applied on a sunny day 24–48 h ahead of forecasted rain caused a significant release of ascospores of *V. inaequalis* on the day of irrigation. Two hours of irrigation reduced the incidence of apple scab on leaves and fruits by more than 50% on average (Prodorutti et al., 2024). However, it remains uncertain whether this effect arose from ascospores failing to reach the canopy or if they reached it but underwent a loss of viability throughout the day. In fact, studies on ascospore discharge, dispersal and deposition of *V. inaequalis* in apple orchards were all carried out during rain events (Aylor, 1995; Carisse et al., 2007; Charest et al., 2002; Kaplan, 1986; Rossi et al., 2003).

The present research aimed to fill some of this gap in knowledge and investigate the dispersion of ascospores above ground following under-canopy irrigation, which holds significant practical implications for apple scab management. In fact, this approach is based on inducing the release of ascospores with an irrigation applied under conditions that are not suitable for infection (e.g., on a sunny day). However, if ascospores can disperse in the plant canopy above the irrigated zone and land on susceptible apple tissue, and if unexpected rain occurs shortly after the irrigation, curative fungicides might be needed to mitigate the infection risk.

Materials and Methods

To study the ascospore dispersal under conditions of either absence of wind and tree canopy interference, or under real orchard conditions, two experimental set-ups were implemented in open fields in the Trentino province in Northern Italy. In the first experimental set-up, located in San Michele all'Adige (46.190056N latitude, 11.134666E longitude), the dispersal of ascospores of *V. inaequalis* was assessed in absence of apple trees and minimizing the wind by surrounding the testing area with a wind barrier (NO WIND). In the second set-up carried out in Cles (46.361280N latitude, 11.040625E longitude), the assessment was carried out in a commercial apple orchard under natural wind conditions (WIND). In the NO WIND set-up, the experimental site was a grass meadow located at least 100 m away from apple orchards. In the WIND set-up, the experimental site was in a seventeen-year-old apple orchard with spindle training system (cv. Golden Delicious grafted on rootstock M9) with rows approximately north–south oriented, with the distance of plants in the row and the inter-row distance being 1.0 m and 3.2 m, respectively. The height of the apple plants was 2.8–3.0 m. The inter-row was covered with permanent grass, which was regularly mowed, and the rows were weeded.

On 4 November 2020, just before leaf fall, apple leaves with scab symptoms were collected from trees of cv. Golden Delicious in orchards in Borgo Valsugana and San Michele all'Adige, where no fungicides were applied during the growing season. A mixture of the leaves from the two locations were immediately placed in a single layer on the ground with a density of approximately 150 leaves/m^2 (plot sizes were 3.0×2.0 m in NO WIND and 1.4×0.8 m in WIND), above a white non-woven fabric of permeable polypropylene (Ortoclima, Tenax s.p.a., Lecco, Italy) which prevented earthworms from degrading the leaves, and then covered with a wire mesh (10×10 mm) to keep the leaves in place, as described in Prodorutti et al. (2024).

Four trials were carried out in each experimental set-up in spring 2021, at the time of ascospore release of *V. inaequalis* (MacHardy, 1996): on 30 March, 20 April, 25 April, and 10 May in NO WIND, and on 10 April, 24 April, 28 April, and 4 May in WIND. During that time of the season the weather in the area is typically characterized by clear-sky days, with strong incoming solar radiation during daytime and outgoing longwave radiation during night time (Laiti et al., 2014a, 2018), favouring large diurnal temperature ranges, as well as the development of daily-periodic local breezes, such as valley winds (Falocchi et al., 2019; Giovannini et al., 2015, 2017; Laiti et al., 2014b), sometimes alternating with perturbations associated with instabilities producing isolated showers or even thunderstorms.

Custom built rotating-arm impaction spore samplers (Rotorod type) were placed at different heights above the leaf litter on the ground (0.3, 1.0, 1.5, 2.0, 2.5, 3.0 m). Each sampler had two plastic sticks (1.65 mm square cross section × 20.0 mm long, 83.0 mm apart and rotated at 2400 rpm) where spores were captured (Carisse et al., 2007). Before starting the trials, the front side of the sticks in the rotating direction was covered with a thin layer of silicon grease (High vacuum grease, Dow Corning corporation, Midland, MI, USA), to catch airborne spores.

Data loggers (Tinytag Plus 2 TGP-4500, Gemini Data Loggers Ltd, Chichester, UK) with temperature (T) and relative humidity (RH) sensors were placed at the same heights as the samplers. One spore sampler and one data logger per height were used. Samplers and data loggers were fixed to a metallic structure consisting of a pole with horizontal rods of 1.2 m length (Fig. 1). Samplers and data loggers were placed at the opposite side of the rods, and the pole was placed in the middle of the leaf litter. Horizontal rods were arranged to avoid overlapping of samplers and data loggers at the different heights. An additional data logger for temperature measurement (Tinytag Plus 2 TGP-4020 with a thermistor probe, Gemini Data Loggers Ltd, Chichester, United Kingdom) was placed on the leaf

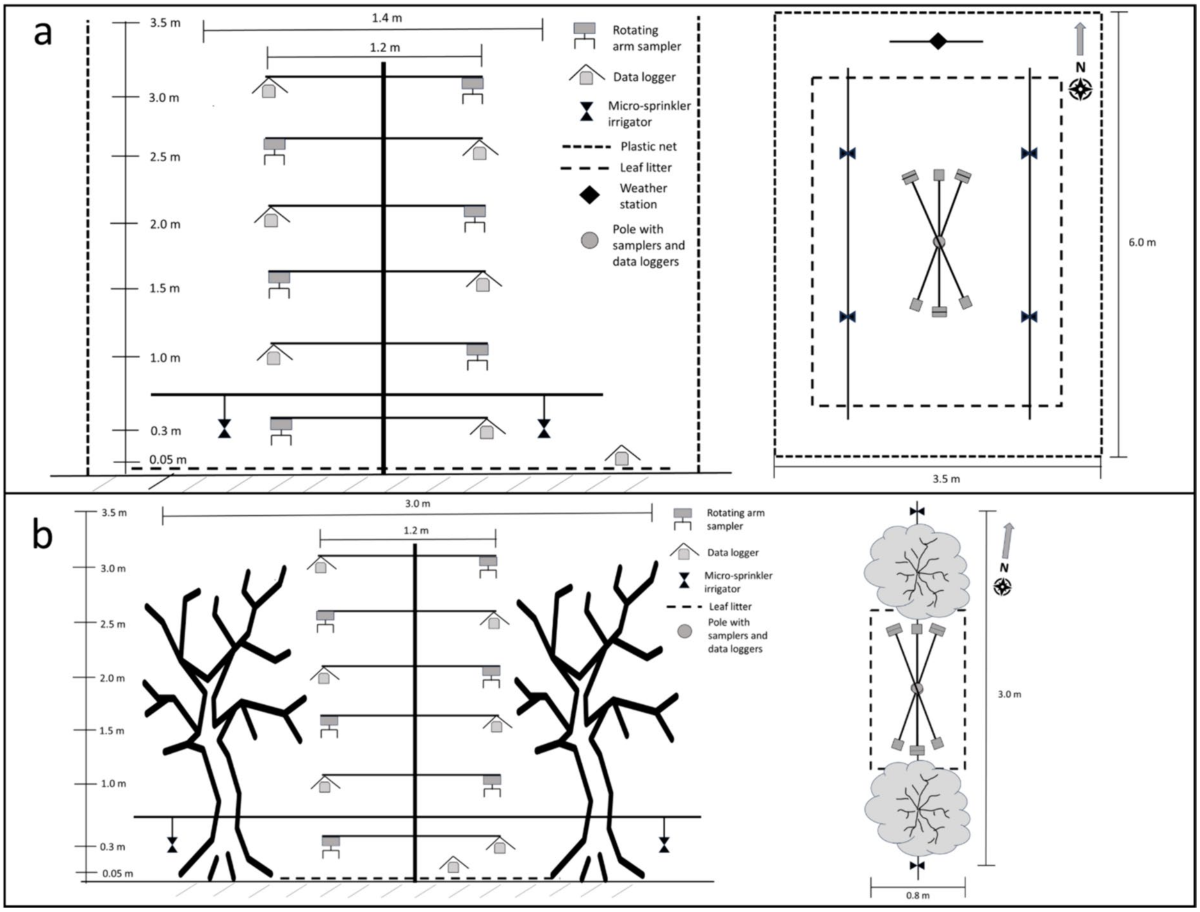


Fig. 1 **a**) Lateral and overhead view of the experimental setups with a wind fence in San Michele all'Adige (NO WIND) and **b**) exposed to natural wind in an orchard in Cles (WIND). The dimensions of the fence, of the structure bringing data loggers and rotating arm samplers and of irrigator distance are also indicated

litter at 0.05 m height from the ground. At 0.05 m only the T sensor was used, because the RH sensor would have been wetted by irrigation at the ground level. Data loggers were covered with a plastic roof (an insect monitoring trap appropriately modified to hold the data logger) to avoid direct exposure to sunlight and wetting from irrigation, and they were placed at the different heights just before starting and removed at the end of each trial. Data of T and RH were registered with time laps of 1 min, and 15 min and 4 h averages were calculated. The vertical T gradients (dT/dz) and the vertical RH gradients (dRH/dz) were calculated for each subsequent height (z_i and z_{i-1}) as (T_i–T_{i-1}) / (z_i-z_{i-1}) and (RH_i-RH_{i-1}) / (z_i-z_{i-1}), respectively.

In NO WIND, the wind barrier (anti-rain plastic net, Scudonet, Tessitura Boscato s.r.l., Marano Vicentino, Italy) was placed all around the leaf litter to close the sides and avoid the direct effect of wind. The net was 3.5 m tall, with a side length of 6.0×3.5 m. The barrier was north–south oriented on the longer side (Fig. 1a). A structure with concrete poles and steel cords was set up to support the net. Immediately before starting each irrigation, the net was opened and spread around the entire perimeter of the litter, and the lower part was fixed to the ground. In WIND, the pole with rotating samplers and data loggers was placed in a row between two apple trees (Fig. 1b) and approximately in the middle of a plot (300 m^2) that was kept untreated with fungicides the previous growing season. No plastic net was placed around the pole and the leaf litter, to carry out the trials under natural wind conditions.

In each experimental set-up, a micro-sprinkler irrigation system (01 KR, Ecorain Irrigation Systems s.r.l., Rubano, Italy) was used. Sprinklers released a water volume of 35 L/h and were placed at 0.3 m from the ground. In NO WIND, four sprinklers were placed above the leaf litter to homogeneously wet the entire litter surface (Fig. 1). In WIND, the under-canopy irrigation system was composed of micro-sprinklers set up along the rows, at 3.0 m from each other and covered a surface of 1500 m^2. The irrigation intensity was about 4 mm/h and 3 mm/h in NO WIND and WIND, respectively. The height of wetting during irrigations was 0.5 m in both locations. The lower spore sampler (at 0.3 m) was therefore placed in the irrigated zone while the other samplers were above the irrigated zone.

The irrigations were applied according to Prodorutti et al. (2024): i) the weather is dry and sunny on the day of irrigation, ii) no rain has occurred in the previous two or more days, and iii) no rain is forecast in the next 24–48 h. Irrigation was carried out for two hours, from 11 am to 1 pm (Central European Time, CET).

To collect all ejected ascospores from infected leaves on the ground, the rotating samplers worked in both locations for four hours (from 11 am to 3 pm, CET), i.e., during the two hours of irrigation plus two hours afterwards. Ascospores of *V. inaequalis* trapped on the sticks of the rotating arms at the end of each experiment were counted under a light microscope (200×magnification), and the total numbers per height and the percentage on the total number of spores trapped per height and per day were calculated.

Weather data were recorded during the experiments by weather stations (m TMF 500, Nesa s.r.l., Vidor, Treviso, Italy) located at the experimental sites. In NO WIND one weather station was placed within the area surrounded by the plastic net and another one was outside the net perimeter, exposed to natural climatic conditions. In WIND the weather station was located at the edge of the orchard. In both sites an anemometer was positioned at 3 m above ground.

Factor Analysis of Mixed Data (FAMD) was applied for a multivariate exploratory data analysis by using FactoMineR (Lê et al., 2008) packages of the R language (version 4.3.2; R Core team, 2023). The tidyverse packages (Wickham et al., 2019) of the R language were used to handle data and generate plots. Regression analyses were carried out by using the software Statistica version 14.0.1.25 (TIBCO Software Inc.).

Results

Under-canopy sprinkler irrigation triggered the release of ascospores of *V. inaequalis* from the infected leaves on the ground (Prodorutti et al., 2024). In the experimental set-up (NO WIND and WIND), T, RH, dT/dz, dRH/dz, percentage of spores and height from the ground explained 70.7% of the data distribution in the FAMD analysis (Fig. 2). The data appeared clearly separated for the two experimental set-ups and for the different heights, especially in

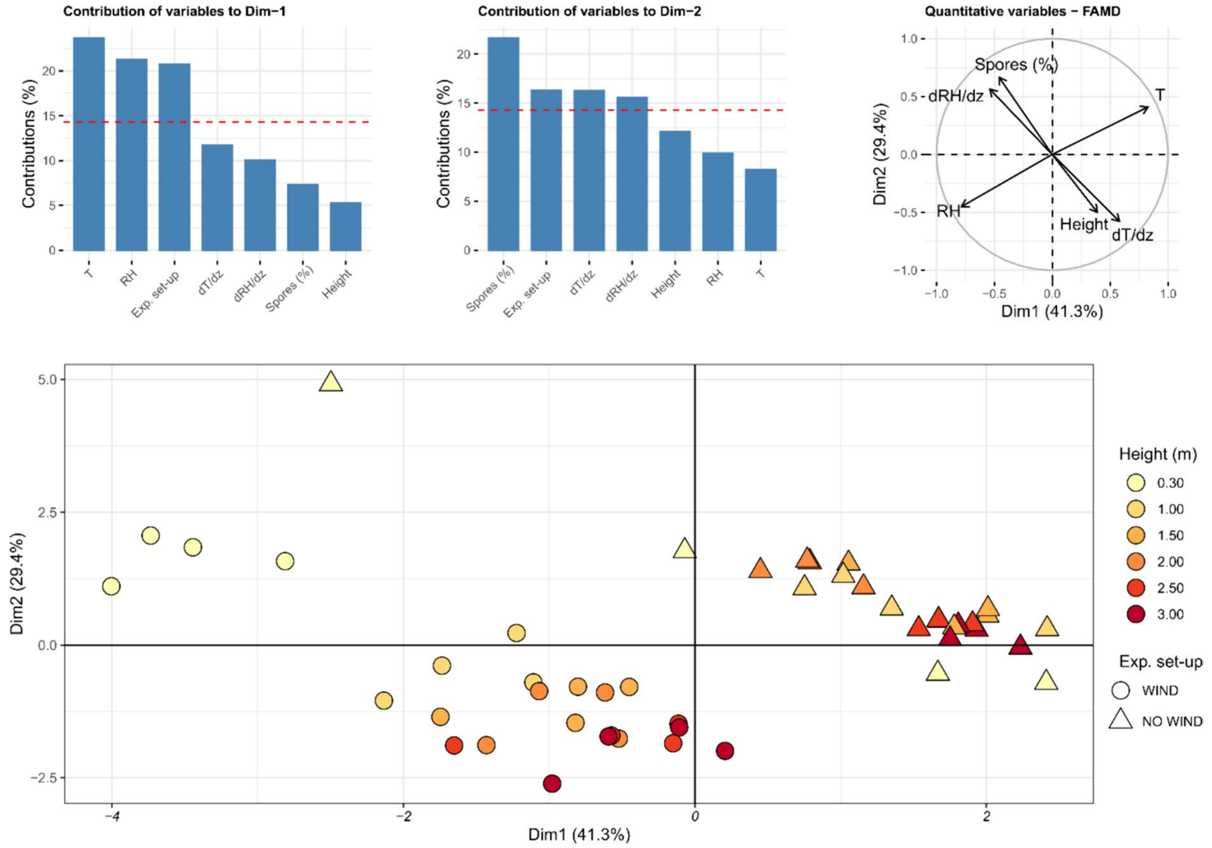


Fig. 2 Factor Analysis of Mixed Data (FAMD) of all factors involved in the study: percentage of ascospores of *Venturia inaequalis* (Spores %), temperature (T), relative humidity (RH), T and RH vertical gradients (dT/dz and dRH/dz, respectively), height (m) and experimental set-up (WIND and NO WIND). Contribution of the variables to dimension 1 (Dim 1) and dimension 2 (Dim 2) and the factor map of quantitative variables are presented. Values of T, RH, and vertical gradients for T and RH are averages recorded during the four hours of the trials

WIND. The percentage of spores was negatively correlated with height and dT/dz (opposite sign vectors) and positively correlated with dRH/dz. Average T and RH in the 4 h trials did not correlate with the percentage of spores (Fig. 2).

Temperature patterns in WIND (average T from 11 am to 3 pm), measured from 0.05 to 3.0 m, were similar in the four trials (Fig. 3a): a higher T close to the ground (0.05 m), a lower T at 0.3 m and similar (approximately steady) values from 1.0 to 3.0 m. Overall, the lowest temperatures were recorded on 10 April (10–12 °C), on a cloudy day with a low global radiation (200–300 W/m^2). Temperatures ranging, at all heights, from 16 to 19 °C were recorded in the other three experimental days, under sunny or partly cloudy conditions (global radiation 600–900 W/m^2). Conversely in NO WIND, the T pattern was variable in the four trials (Fig. 3b): on 30 March and 20 April, similarly to WIND, a higher average T was measured close to the ground (0.05 m) compared to at 0.3 m, and the decrease of T from 0.05 to 0.3 m was more evident on 30 March. On 25 April and 10 May, temperatures were higher at 0.3 m compared to 0.05 m (about + 3 and + 2 °C, respectively). Slight overall differences were observed between 1.5 and 3.0 m. Temperatures were always higher in NO WIND than WIND, reaching average values around 28 °C on 10 May. In NO WIND, the trials were carried out in sunny days, with a global radiation ranging from 700 to 1000 W/m^2.

Overall, the RH was slightly decreasing from 0.3 to 3 m from the ground, both in WIND and NO WIND (Fig. 4). Generally, a higher RH was measured in WIND in comparison to NO WIND, which coincided

Fig. 3 Temperatures (T) recorded during the four trials (marked with dates) with under-canopy sprinkling to release ascospores of *Venturia inaequalis,* in presence of natural wind (WIND, **a**) and in absence of wind (NO WIND, **b**) at different heights from the ground. Data represents the average temperature from 11 am to 3 pm

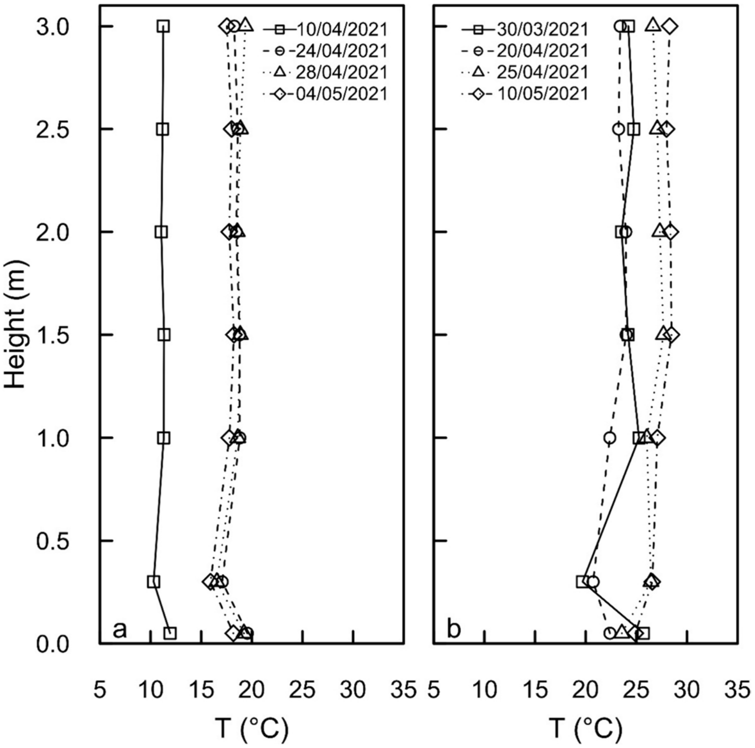
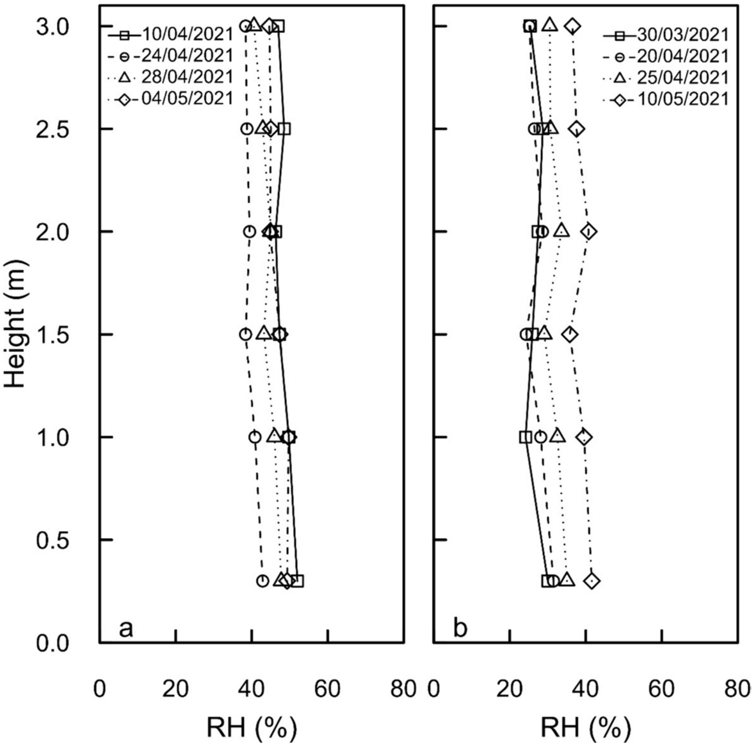


Fig. 4 Relative humidity (RH) recorded during the four trials (marked with dates) with under-canopy sprinkling to release ascospores of *Venturia inaequalis,* in presence of natural wind (WIND, **a**) and in absence of wind (NO WIND, **b**) at different heights from the ground. Data represents the average relative humidity from 11 am to 3 pm



with lower T in WIND compared to NO WIND. In particular, in WIND the average RH in the four-hour trials was around 50% at 0.3 m (range 43–52%) and decreased by 4–7% at 3 m. In NO WIND, the four-hour average RH values ranged from 30 to 42% at 0.3 m, with a decrease of 5–6% at 3 m height.

In both experimental set-ups, ascospores were trapped by the spore samplers at all heights above ground, from 0.3 m to 3.0 m (Fig. 5). The total number of spores recorded in NO WIND were 1302, 2181, 2836 and 1234 on 30 March, 20 April, 25 April, and 10 May, respectively, while in WIND they were 39, 32, 40 and 54 on 10 April, 24 April, 28 April, and 4 May, respectively.

In WIND, the pattern of spore trapping at the different heights above ground was similar in the four trials, with an overall higher percentage of spores at 0.3 m and progressively fewer spores with increasing height. Only on 24 April, a slightly higher number of spores (3 spores) was noted at 1.0 m compared to 0.3 m. At 0.3 m the proportion of the total number of spores ranged from 31 to 52% (Fig. 5a).

In NO WIND, the spore distribution pattern varied among the trials (Fig. 5b). On 30 March, the pattern was similar to WIND, with the highest percentage of spores at 0.3 m. Conversely, on 25 April and 10 May less than 10% of the spores were trapped at 0.3 m, while 24 and 25%, respectively, were trapped at 1.0 m. On 20 April the spore trapping ranged from 13% at 0.3 m to 21% at 2.0 m. Overall, from 1.0 m to 3.0 m, the percentage of spores was slightly decreasing and at 3.0 m the quantity ranged from 9 to 15%.

In NO WIND, 68 – 97% of the spores were trapped above the irrigated zone (≥ 1.0 m). In WIND, less than 50% of spores were observed at heights ≥ 1.0 m on 4 May, while in the other trials the number of spores ranged between 54 and 69% at those heights.

When pooling the data of the four trials, in WIND the percentage of spores at the various heights was best fitted with the exponential decay function $y = 69.1253*\exp(-0.0116*x)$, where y is the percentage of spores and x is height from the ground. The log transformed data of spore percentages follow a linear regression ($y = 4.2359–1.1567*x$, $r = -0.90$, $P < 0.0001$). Conversely in NO WIND, a linear regression between the percentage of spores and height from the ground was not significant ($r = -0.15$, $P = 0.49$). Neither did the log transformed data of the percentage of spores show a significant regression with height for NO WIND ($r = 0.07$, $P = 0.75$).

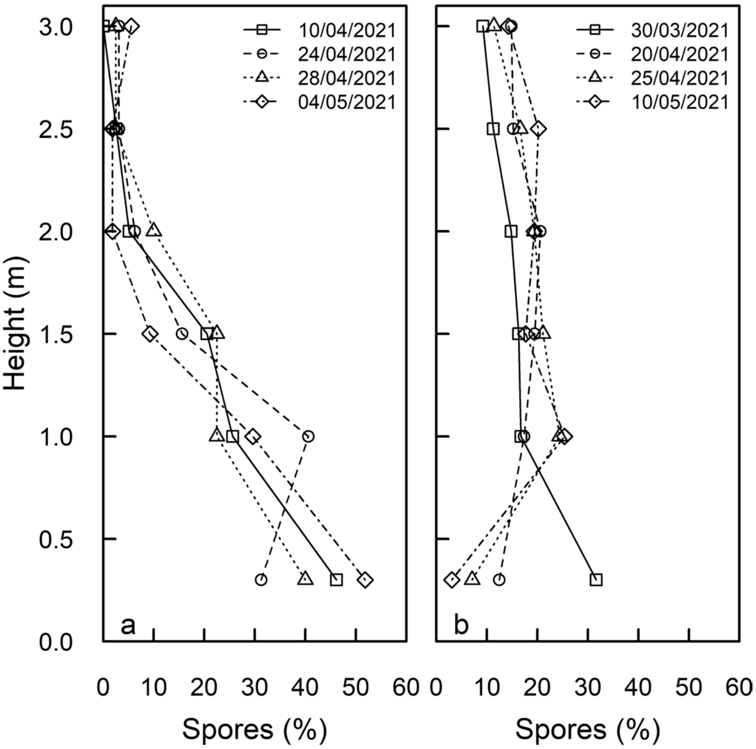


Fig. 5 **a**) Vertical distribution of the percentage of ascospores of *Venturia inaequalis* in presence of natural wind (WIND) and **b**) without wind (NO WIND) in each of four trials (marked with dates). Irrigation was carried out from 11 am to 1 pm and spore trapping from 11 am to 3 pm

Excluding the data at 0.3 m, the percentage of spores and their log transformed data in NO WIND showed instead a significantly decreasing linear trend ($y = 25.2709 – 3.9925*x$, $r = -0.69$, $P < 0.001$ and $y = 3.3119 – 0.2458*x$, $r = -0.69$, $P < 0.001$, for % spores and log transformed data, respectively).

Both in WIND and NO WIND, dT/dz was significantly correlated with the percentage of spores ($r = -0.55$, $P < 0.01$ and $r = -0.57$, $P < 0.01$, respectively) while dRH/dz was not ($P > 0.05$), and since dT/dz could influence the vertical movement of spores, we delved deeper into the analysis of dT/dz in relation to the different spore patterns.

The dT/dz values between 0.3 and 0.05 m and between 1.0 and 0.3 m from the ground were consistent with the spore patterns at the lower heights (Fig. 3, Fig. 5, Fig. 6,). In fact, a negative dT/dz between 0.3 and 0.05 m and a positive dT/dz between 1.0 and 0.3 m corresponded to a higher or slightly different percentage of spores in the irrigated layer (0.3 m) compared to 1.0 m. These conditions occurred in the four trials in WIND and on 30 March and 20 April in NO WIND. On 25 April and 10 May in NO WIND, there was instead a positive dT/dz at 0.3–0.05 m and a thermal gradient close to zero at 1.0–0.3 m. At these conditions the percentage of spores was lower at 0.3 m compared to the other heights. Above 1.0 m the values of dT/dz were close to zero both in NO WIND and WIND (Fig. 6).

The dataset of percentage of spores and dT/dz from NO WIND and WIND was merged for the regression analysis. At 0.3 + 1.0 m, there was a significant linear regression between the percentage of spores and dT/dz, with a negative angular coefficient ($y = 25.0663 – 0.8673*x$, $r = -0.56$, $P < 0.05$). No correlation resulted between the same data relating to heights from 1.5 to 3.0 m ($r = 0.17$, $P > 0.05$).

Wind speed, in the four trials in WIND, was approximately 0.8–1.0 m/s during the first two hours and slightly increased to 1.5–1.7 m/s from 1 to 3 pm. The wind direction ranged, overall, between 180 and 200°, corresponding to a southerly wind, which was also the direction of the tree lines in the orchard. On 10 April the wind speed and direction were more

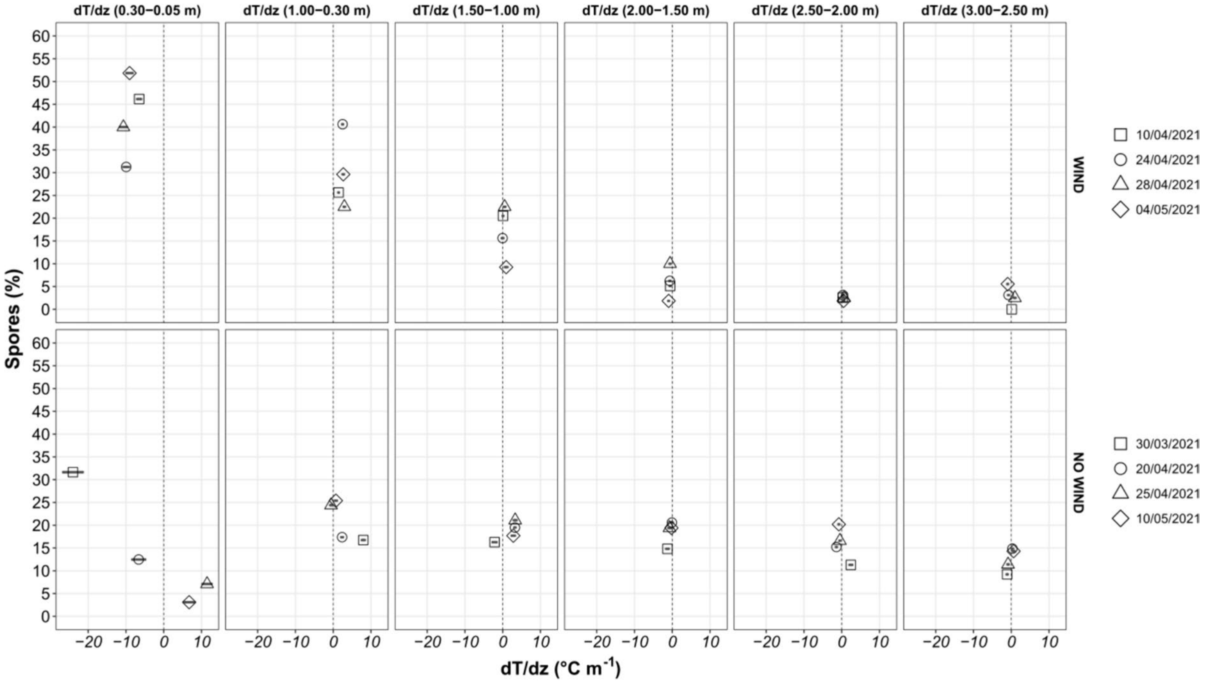


Fig. 6 Vertical temperature gradients (dT/dz, average of the four-hour trial) in comparison with the percentage of ascospores of *Venturia inaequalis* at different heights, in presence of natural wind (WIND, top) and in absence of wind (NO WIND, bottom). Different symbols represent the four trials (marked with dates) carried out in each experimental set-up, and bars represent the standard error of dT/dz. Irrigation was carried out from 11 am to 1 pm and spore trapping from 11 am to 3 pm

variable, not exceeding 1.5 m/s and with an average direction of 156° (Fig. 7).

In the testing area protected by the fence in NO WIND, the wind speed was mostly absent or very low (0.1–0.2 m/s) during all trials. Only on 10 May was it slightly higher (0.3–0.5 m/s) for 45 min (Fig. 8). Outside the fence, under natural conditions, wind speed was generally low until noon (0.5–1 m/s) and increased to 2.5–3.0 m/s in the following hours. The wind speed showed an irregular trend only on 20 April, remaining mostly below 1 m/s. Wind direction was almost constant on 25 April and 10 May (average direction of 232° and 213°, respectively). On 30 March and 20 April, the direction was around 30° from 11 am to 1 pm and changed to around 230–260° in the last 1–2 h of the trial (Fig. 9).

Discussion

In this study, we confirmed that ascospores of *V. inaequalis* can move above the irrigated layer of 0.5 m, and more spores were detected at heights above the sprinklers (measured at 1 to 3 m) than below (measured at 0.3 m). Furthermore, with the current irrigation rates (3–4 mm/h,) our results suggest that the washout of ascospores by the sprinkler irrigation is relatively limited, which is in agreement with Aylor (1995), who stated that the removal of ascospores of *V. inaequalis* from the air by rain had little effect on the vertical profiles of ascospore concentration.

The experimental set-up influenced the vertical distribution of the ascospores released by under-canopy irrigation. The wind fence led to a greater concentration of airborne spores at all heights above the ground, with a notable increase in the proportion of spores dispersing upward. This is in contrast to the natural orchard conditions, where airborne ascospores declined rapidly with height, following an exponential decay pattern, similar to that previously observed during rain events with ascospores of *V. inaequalis* (Aylor, 1995, 1998; Carisse et al., 2007; Rossi et al., 2003). Indeed, under natural rainy conditions, airborne spores are typically carried horizontally by the wind in the downwind direction and vertically diffused by wind turbulence, resulting in a decrease in spore concentration with distance from the source both horizontally and vertically (Aylor, 1995; Rossi

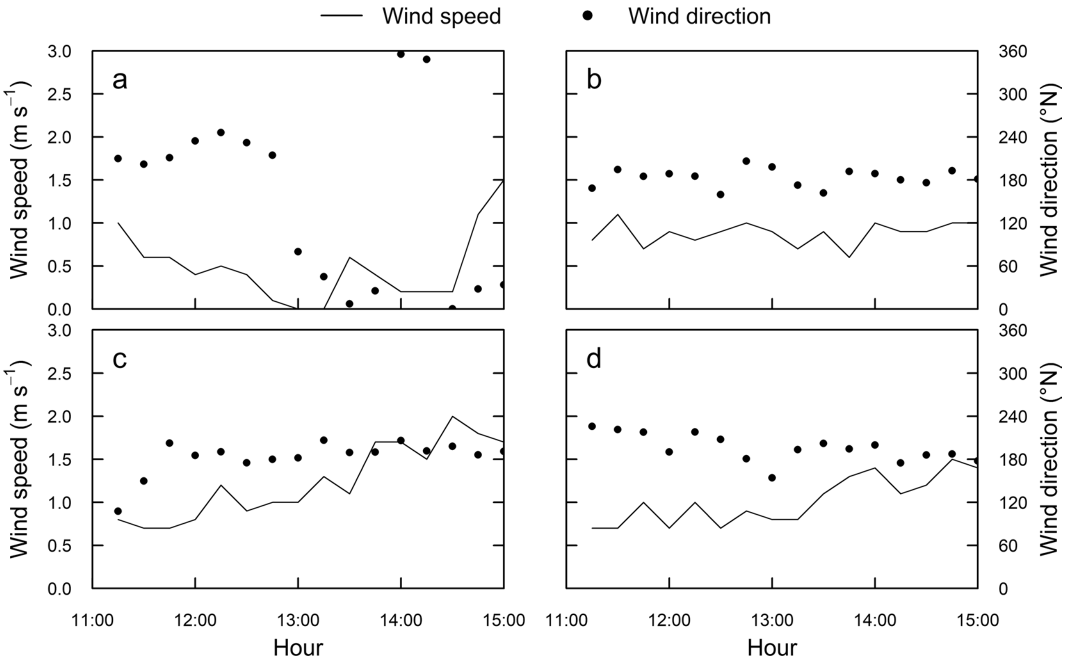


Fig. 7 Average wind speed (m/s) and wind direction (0° = north) measured every 15 min at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in presence of natural wind in Cles (WIND). Lowercase letters represent the trial dates: **a**) 10 April; **b**) 24 April; **c**) 28 April; **d**) 4 May

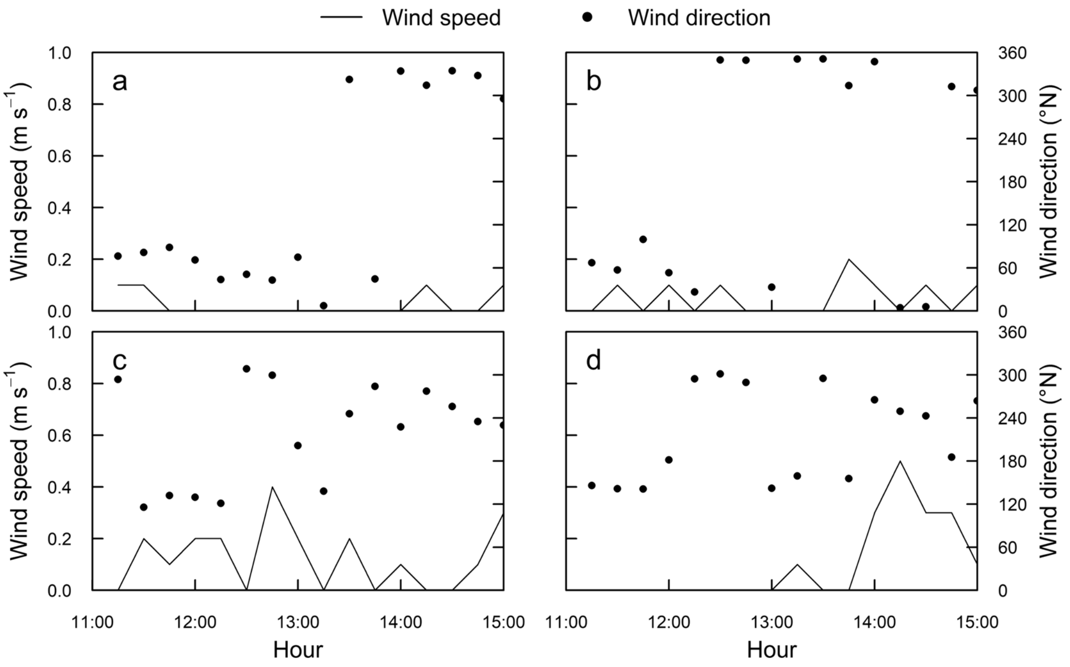


Fig. 8 Average wind speed (m/s) and wind direction (0° = north) measured every 15 min at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in San Michele all'Adige inside the testing area protected by the wind fence (NO WIND). Lowercase letters represent the trial dates: **a**) 30 March; **b**) 20 April; **c**) 25 April; **d**) 10 May

et al., 2003). The sources of *V. inaequalis* infected leaves used to make the litter were the same in the two set-ups. Even if there was a difference in the size of the leaf litter (larger area in NO WIND compared to WIND), the density of the leaves per unit of surface was identical. Because the aim of this study was to investigate ascospore dispersal at different heights under controlled conditions with minimal wind and under real orchard conditions, we focused on the relative portion of released spores rather than absolute spore counts, therefore there was no implication of using different plot sizes in the analysis. The difference in numbers of captured spores can be attributed to the effect of wind, effectively removing spores from their source in the orchard. Moreover, it should be considered that the spores trapped in the orchard could also originate from natural inoculum overwintering in leaf litter on the ground of the irrigated area. Leaf litters were prepared by collecting heavily scab-infected apple leaves from untreated plots, which were then laid out in a single layer on the ground. An initial check carried out on 2 March confirmed comparable numbers of maturing pseudothecia between the two sets of leaves.

Our study demonstrates that in the orchard environment, under-canopy sprinkler irrigation has a similar effect on the vertical dispersion of ascospores as rain, clearly suggesting that wind plays a dominant role in their transport. When wind was removed within the testing area, this resulted in only a small decrease of ascospore numbers above the irrigated layer. In fact, even at a height of 3.0 m, significant numbers of ascospores were detected. In particular, such a distribution pattern of ascospores may be attributed to advection performed by secondary motions induced by the upper wind within the volume surrounded by the fence, which can be considered as a cavity flow (Shankar & Deshpande, 2000), similar to what occurs in enclosed street-canyon environments (Giovannini et al., 2013). In other words, the wind blowing over the top of the fence likely induced a secondary circulation cell within the volume surrounded by the fence and intensified turbulence within the lateral boundaries.

The vertical temperature gradients (dT/dz) showed consistent and significant correlations with spore distribution patterns at lower heights, suggesting their influence on spore diffusion in these air layers.

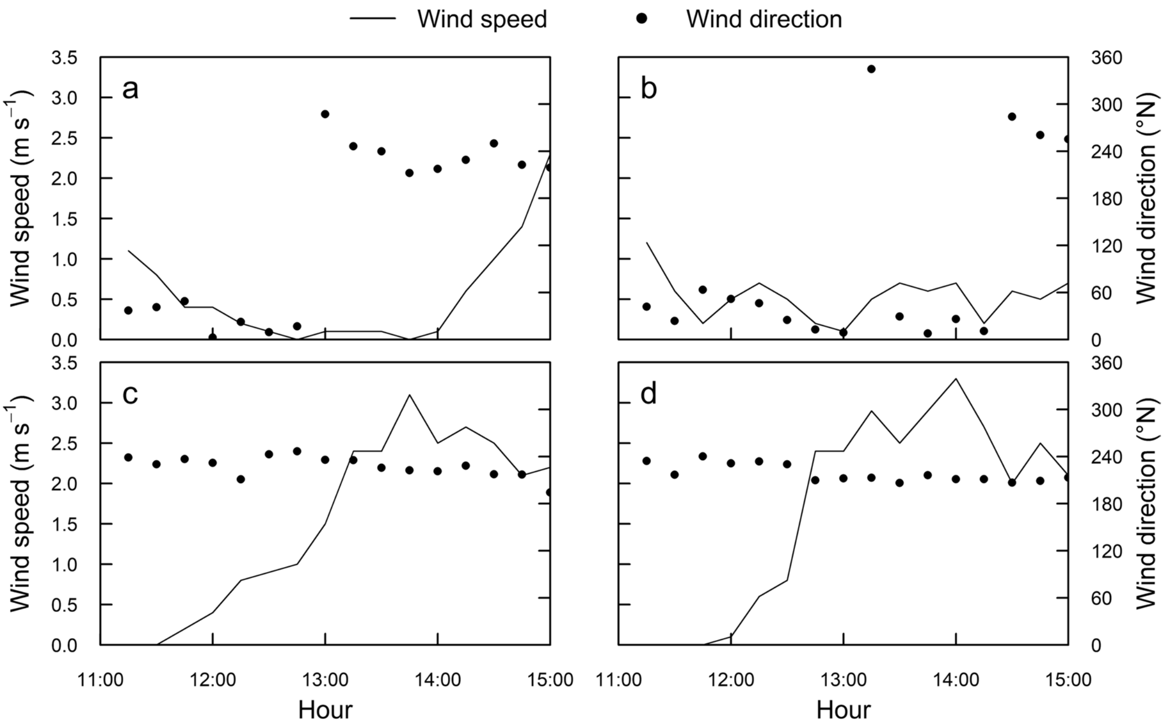


Fig. 9 Average wind speed (m/s) and wind direction (0° = north) measured every 15 min at 3.0 m above the ground from 11 am to 3 pm in the four trial dates in San Michele all'Adige, in the open field outside the fence. Lowercase letters represent the trial dates: **a**) 30 March; **b**) 20 April; **c**) 25 April; **d**) 10 May

Specifically, when dT/dz was negative between 0.3 m and 0.05 m and positive between 1.0 m and 0.3 m, the temperature in the irrigated layer was lower compared to both the ground and the layer above the irrigation. Under these conditions, spores ejected from the ground's leaf litter tended to be trapped within the irrigated zone, primarily due to the warmer and drier layer above. Conversely, when dT/dz was positive between 0.3 m and 0.05 m and near zero between 1.0 m and 0.3 (as observed in the last two trials in NO WIND on April 25th and May 10th), the airborne ascospores more readily transcended through the irrigated layer. Additionally, higher wind speeds and temperatures in these two trials likely enhanced turbulence and convective motions within the fence, possibly further explaining the reduced spore counts at 0.3 m in comparison to the layers above. Overall, the results of the present study suggest that irrigation carried out in the middle of sunny days could decrease the upward spore diffusion due to the decrease of T in the irrigated zone, compared to the ground and the layer above the irrigation. Under natural conditions, during daylight hours on sunny days, air in contact with the ground surface is warmer than the air layers above, and therefore warmer air tends to rise upwards due to its lower density. The study did not assess the potential role of splash dispersal in ascospore movement, which remains an open area for future research.

Airborne ascospores beyond the irrigated layer may either settle on susceptible apple tissues through dry deposition (sedimentation and impaction), disperse out of the orchard via wind and turbulent diffusion, or they may also settle on the ground. Young apple leaves and fruits are particularly susceptible to infections of *V. inaequalis* (MacHardy, 1996). For example, 3 to 5 days old, unfurled leaves of vegetative apple shoots are the most susceptible to infections, and a low number of ascospores (5 to 10 spores) is sufficient to cause a scab lesion at this stage (Aylor, 2017; Aylor & Kiyomoto, 1993; Philion et al., 2020). Susceptibility declines quickly with leaf age (Aylor, 2017; MacHardy, 1996); however, in spring in concomitance with the primary infections of ascospores of *V. inaequalis*, rapid shoot growth results in the continuous emergence of new, young susceptible leaves. Generally, most of fungal spores remain within the crop where they are released (MacHardy, 1996), and it is estimated that less than 10% of released fungal spores move beyond the boundary of a crop (Gregory, 1973). Low wind speed, as occurred during our trials, creates conditions for a higher persistence and concentration of airborne spores within the orchard (Aylor, 1998). These findings hold significant practical implications on the use of under-canopy irrigation to promote ascospore discharge. If accurate weather forecasts enable irrigation to be implemented 24–48 h prior to rainfall, ascospores are then ejected under conditions unsuitable for apple scab infections and consequently subjected to a fast decay without causing disease (Prodorutti et al., 2024). Nevertheless, if a significant portion of ascospores is dispersed above the irrigated layers as evidenced by this study, and possibly settle on susceptible tissues, it becomes imperative to schedule irrigations based on trustable weather forecasts, ensuring a rain-free period of at least 24 h post-irrigation. In the event of rainfall shortly after irrigation, ascospores deposited on plants may remain viable, enhancing the risk of apple scab infection and, consequently, the application of a curative fungicide treatment becomes essential following unexpected rain. Conflicting and unclear data exist regarding the viability of ascospores on leaves under dry conditions and the required length of a dry period to consider successive wet periods as separate events. A dry period ranging from 4 to 24 h might be necessary to separate wet periods (MacHardy, 1996). It was also suggested that successive wet periods may be considered a single event if the intervening dry period is less than 12 h under sunny conditions or less than 24 h regardless of weather conditions (MacHardy, 1996).

Under-canopy sprinkler irrigation offers a sustainable approach to diminish the occurrence of apple scab during the primary season (Prodorutti et al., 2024). However, its effectiveness may be even more pronounced in regions characterized by infrequent and isolated rainfall. In such areas, where weather forecasts are likely to be more accurate, irrigation can be more effective in facilitating ascospore discharge and depletion than in regions experiencing frequent and heavy rainfall.

In conclusion, this is the first study shedding light on the vertical dispersal patterns of *V. inaequalis* ascospores following under-canopy irrigation, which is a promising approach to management of apple scab. The different set-ups allowed understanding the effect of wind on ascospore dispersal. Wind played a critical role in spore transport, evident from the set-up where wind interference was minimised by a wind fence. The effect of other factors besides wind, such as dT/dz, was moreover highlighted by comparing the ascospore dispersal in a wind-protected enclosure with a wind-exposed orchard. Our study underscores that irrigation targeting spore release should be done in the warmest part of the day, when both higher wind speeds can contribute to lower spore concentration in the canopy and the overwintering leaves on the ground (representing the primary inoculum source) can dry quickly, thus avoiding the maturation of pseudothecia. However, attention must be paid if a rainfall shortly occurs after irrigation, and in that case an application of a curative fungicide may become essential.

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Data availability The data supporting this study are available from the corresponding author on reasonable request.

Declarations

Competing interest The authors declare no conflict of interest.

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